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Research paper

Semisolid SLN™ dispersions for topical application: influence of formulation and production parameters on viscoelastic properties

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Abstract

Aqueous solid lipid nanoparticle (SLN) dispersions with a high lipid content up to 35% and viscous to semisolid consistency were produced by a high pressure homogenization process. Despite their high lipid content and viscosity these dispersions preserved their colloidal size range. The SLN dispersions were compared to nanoemulsions and microparticle dispersions with regard to particle size, viscoelastic properties and formation of a semisolid gel structure. Viscoelastic measurements including oscillation stress sweep tests and oscillation frequency sweep tests demonstrated that the existence of a solid particle matrix with a particle size in the nanometer range is a prerequisite to form a semisolid dispersion having the appropriate consistency for topical application. Striking differences were observed between solid lipid micro- and nanodispersions of the same composition. Particle size reduction resulted in an 80-fold increase of the elastic modulus. Particle size distribution, the physical state of the dispersed lipid phase and the emulsifier concentration have been identified as further key factors for the viscoelastic properties and gel structure of the lipid nanodispersions. By conducting oscillation measurements it was possible to relate the stability of lipid dispersions to specific rheological parameters therefore providing a sensitive tool in stability assessment. Changing the production process from a 40 ml batch to a 2 l batch turned out to have an influence on the colloidal structures of semisolid SLN dispersions. Consistency increased but particle size and ratio of elastic to viscous properties stayed in the same range.

Keywords: Solid lipid nanoparticles; High pressure homogenization; Rheology; Oscillatory testing; Topical application; Stability

1. Introduction

Aqueous dispersions of solid lipid nanoparticles (SLN) [1] represent an alternative drug carrier system to emulsions and polymeric nanoparticles [2,3]. They are particularly promising as drug carriers for topical application [4]. Occlusion properties due to film formation on the skin surface which reduce transepidermal water loss (TEWL) have been reported [5]. Occlusion can enhance the penetration of drugs through the stratum corneum by increased hydration [6]. Apart from a nonspecific occlusion effect on penetration, penetration might also be affected by the SLN carrier itself [7]. Furthermore, stabilization of chemically unstable drugs by incorporating them into a lipid matrix might be possible [8]. Scaling-up of the production process to medium and large scale is easily accessible as demonstrated for batch sizes up to 50 kg [9].

To get a topical dosage form having the desired semisolid consistency, the SLN dispersion has to be incorporated into commonly used dermal carriers like hydrogels or creams. Major disadvantages thereof are the limited SLN- and therewith—limited drug load, several time consuming production steps and possible incompatibilities with the ingredients of the gel or the cream. A new one-step production process delivering a semisolid formulation including solid lipid nanoparticles has recently been developed that voids these disadvantages [10]. It was found that despite the semisolid consistency the lipid dispersions preserved their colloidal particle size even after long storage times. By carrying out viscoelastic measurements it could be shown that these semisolid dispersions form a gel-like structure with a prevailing elastic component similar to standard dermal preparations [11].

The aim of the present study is to elucidate the observed phenomena and to investigate the influence of key properties of the lipid dispersion (particle size, physical state of the lipid, emulsifier) on the rheological properties.

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2. Materials and methods

2.1. Materials

The lipid Precifac® ATO (cetylpalmitate) was provided by Gattefossé (Weil a.R., Germany). The surfactant sucrose stearic acid ester S1670 by Mitsubihi-Kagaku Foods Corporation, was a gift from Syntapharm (Mülheim-Ruhr, Germany). The liquid wax Cetiol®A (Lauric acid hexylester) was obtained from Cognis Deutschland GmbH (Düsseldorf, Germany). The materials were used as received. Water was used in double-distilled quality.

2.2. Preparation of lipid dispersions

Different SLN dispersions consisting of 30–35% Precifac ATO, 2–5% sucrose fatty acid ester as emulsifier and double-distilled water added up to 100% (all m/m%) were produced by the hot homogenization technique with a high pressure homogenizer APV Micron Lab 40 (APV Deutschland GmbH, Lübeck, Germany). The lipid was heated up to 85°C and dispersed in the hot surfactant solution (85°C), using an Ultra-Turrax T25 (Janke & Kunkel GmbH and Co KG, Staufen, Germany) at 9500 rpm for 1 min. This pre-emulsion was then homogenized at 85°C by high pressure homogenization applying three homogenization cycles at 500 bar. The nanoemulsions were produced in the same manner as the SLN dispersions except that the solid wax was replaced by the liquid wax, hexyllaurate. The microparticle dispersions were produced by Ultra-Turrax at 9500 rpm for 2 min. Lipid and surfactant solution were also heated up to 85°C prior to processing. The 2 l batches were produced with a Micron LAB 60 (APV Deutschland GmbH, Lübeck, Germany) modified to the needs of a GMP production [12]. The preemulsion was prepared in the feeding vessel using a dissolver disc at 600 rpm for 2 min before being processed by the homogenizer for three homogenization cycles in the discontinuous mode [12]. Pressures applied were 500 bar at the first valve and 50 bar at the second valve. The samples were left to equilibrate for 24 h prior to further analysis.

2.3. Particle size analysis

Particle sizes were analyzed by photon correlation spectroscopy (PCS) with a Zetasizer 4 (Malvern Instruments, UK) and laser diffraction (LD) with a Mastersizer E (Malvern Instruments, UK). PCS yields the mean particle size and the polydispersity index (PI) as a measure of the width of the distribution. The LD data were evaluated using volume distribution. A diameter 90% value of 1 μm indicates that 90% of all particles possess a diameter of 1 μm or less. Prior to particle size analysis the semisolid SLN dispersions were diluted with double-distilled water to weak opalescence. All measurements were done in triplicate.

2.4. Viscoelastic measurements

The viscoelastic measurements were performed with a rheometer Rheo Stress RS 100 (Haake, Karlruhe, Germany) equipped with a cone-and-plate test geometry (plate diameter 20 mm, cone angle 4°). Unless otherwise indicated all measurements were carried out at a temperature of 20 ± 0.1°C. Oscillation stress sweep tests were carried out at a constant frequency of 1Hz in a stress range of 100 Pa. Oscillation frequency sweep tests were performed over a frequency range from 0.1 to 10 Hz at a constant stress amplitude of 1 and 5 Pa, respectively.

3. Results and discussion

3.1. Influence of particle size and particle size distribution on gel structure

In order to study the influence of particle size on viscoelastic properties and the formation of a semisolid gel structure, two lipid dispersions of identical composition were produced by different production methods resulting in distinct particle sizes (see Table 1). Both dispersions consisted of 35% dispersed lipid phase and 5% emulsifier. One solid lipid dispersion (formulation A) was produced using an Ultraturrax revealing a mean particle size in the micrometer range. The diameter 50% measured by laser diffraction was 7.71 ± 0.26 μm. The other solid lipid dispersion (formulation B1) was produced by high pressure homogenization yielding a nanodispersion with a diameter 50% of 0.30 ± 0.01 μm. To get comprehensive information on the rheological state of the dispersions oscillation measurements yielding information about viscous and elastic properties of the investigated system were performed. Oscillation tests are dynamic methods for determining the viscoelastic properties of the tested material in its rheological ground state [13]. In contrast to continuous shear techniques, oscillatory techniques do not disrupt static structures. When performing oscillation measurements

Table 1
Composition and physical properties of investigated formulations

Formulation		Composition	Physical state of the lipid	Particle size (LD diameter 50%)
A	Microparticle dispersion	35% cetylpalmitate 5% sucrose stearic acid ester	Solid	7.71 μm
B1	SLN dispersion	35% cetylpalmitate 5% sucrose stearic acid ester	Solid	0.30 μm

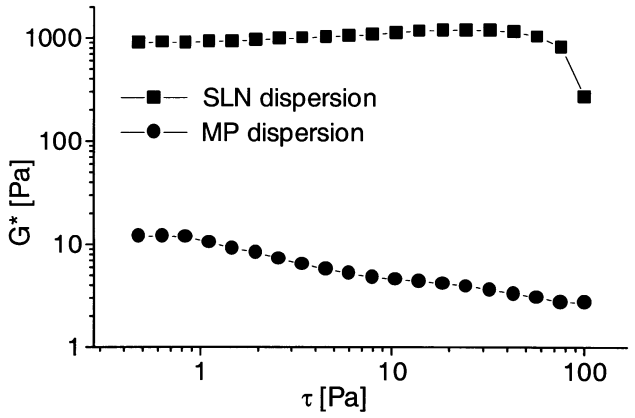

Fig. 1. Stress sweep data from the SLN dispersion (formulation B1) and the microparticle dispersion (formulation A). Complex modulus G* measured as a function of stress (τ) at a frequency of 1 Hz.

first the linear viscoelastic region has to be determined by a stress sweep at a constant frequency. A stress sweep is a dynamic test where the complex modulus G* is measured as a function of stress at a constant frequency. The range of stress over which G* is independent of the applied stress is called the linear viscoelastic region. Over the linear region the structure of the dispersion remains intact. Despite the same composition the two dispersions show a completely different behavior. The viscoelastic region of the nanodispersion extends to a stress of almost 60 Pa compared to the dispersion in the micrometer range whose complex modulus G* already decreases at a stress of 2 Pa as demonstrated in Fig. 1. The lipid dispersion with the mean particle size in the micrometer range therefore reveals a much weaker and sensitive structure compared to the lipid nanodispersion. A value of 1 Pa was chosen as the stress amplitude in the subsequent studies since it lies in the viscoelastic region for both tested systems.

To get information about the viscous and elastic behavior of an investigated system and the network structure formed by particle-particle interactions an oscillation frequency sweep test has to be performed. A frequency sweep test is a dynamic test measuring the response of a system as a function of frequency at a constant stress amplitude. It reveals the storage modulus G' (elastic response), the loss modulus G'' (viscous response) and the complex viscosity η^*. In Table 2 the viscoelastic parameters G', G'' and phase angle of the SLN dispersion (formulation B1) are compared to G', G'' and phase angle of the dispersion with mean particle size in the micrometer range (formulation A). A significant increase of both moduli can be observed with decreasing particle size of the dispersion. The storage modulus G' increases about eighty fold from 4.9 ± 1.0 to 390.8 ± 54.4 Pa when decreasing the particle size to the nanometer range. Also the loss modulus G'' increased from 8.1 ± 0.8 to 37.5 ± 6.5 Pa. The G' and G'' values of the solid lipid nanoparticle dispersion indicate the formation of a viscoelastic gel whereas the microparticle dispersion does not form a gel. Simultaneously, the phase angle decreased from 58.6 ± 2.7° for the microparticle dispersion to the very low value of 5.5 ± 0.2° for the nanodispersion. Since it is measure of the proportion of elastic to viscous fractions in a system, the decrease of the phase angle indicates the change of a more viscous to a more elastic system by decreasing the particle size. For solid lipid dispersions, the particle size in the nanometer range is therefore required for the formation of a semisolid gel structure with a prevailing elastic component.

Table 2
Influence of particle size on viscoelastic parameters G' (storage modulus), G'' (loss modulus) and phase angle ($n = 3$)

	G' (Pa)	G'' (Pa)	Phase angle (°)
Formulation A (microparticle dispersion)	4.9 ± 1.0	8.1 ± 0.8	58.6 ± 2.7
Formulation B1 (SLN dispersion)	390.8 ± 54.5	37.5 ± 6.5	5.5 ± 0.2

The changes in viscoelastic behavior can be attributed to the presence of different particle-particle interaction since there are different particle sizes and particle size distributions. Decreasing the particle size is accompanied by a huge increase of surface. Therefore, the number of contact points increases and so particle-particle interactions are more pronounced, leading to a three-dimensional network structure. Another important factor is that viscosity is also a function of the width of the distribution [14]. By increasing the polydispersity, the viscosity can be reduced. The lipid microparticle dispersion shows a bimodal size distribution with particles in the micro- and nanometer range having an influence on the packing of the particles in the dispersion. The smaller particles may fit between the voids of the larger particles thereby reducing the interactions between the latter [14].

3.2. 3.2. Influence of the physical state of the dispersed phase on gel structure

In addition to particle size, the impact of the physical state of the lipid on the viscoelastic properties was investigated. The viscoelastic properties of a solid lipid nanodipersion (cetylpalmitate based nanosuspension: formulation B2) was compared to a liquid lipid nanodispersion (hexyllaurate nanoemulsion: formulation C; see Table 3). Both dispersions consisted of the same amount of dispersed lipid phase (30% w/w) and emulsifier (5% w/w) and were produced by the same production process (high pressure homogenization at 85°C, 500 bar and three production cycles). Mean particle size measured by photon correlation spectroscopy was 179 ± 0 and 200 ± 0 nm for the nanoemulsion and nanodispersion, respectively. Both dispersions are comparable in particle size and show a narrow particle size distribution (polydispersity index of about 0.1). As Table 4 demonstrates, replacement of the dispersed liquid lipid by a solid lipid resulted in dramatic increase of elastic modulus G' and loss

Table 3
Composition and physical properties of investigated formulations

Formulation		Composition	Physical state of the lipid	Particle size (PCS diameter)
B2	SLN dispersion	30% cetylpalmitate 5% sucrose stearic acid ester	Solid	200 nm
C	Nanoemulsion	30% hexyllaurate 5% sucrose stearic acid ester	Liquid	179 nm

modulus G''. G' which is a measure of the elasticity of the tested material and a direct measure of particle-particle interactions increased about a hundred fold from 0.9 ± 0.1 Pa for the nanoemulsion (formulation C) to 86.9 ± 14.8 Pa for the nanosuspension (formulation B2). The nanoemulsion did not form a semisolid gel structure in contrast to the nanosuspension. This fact could be explained by the different particle shapes resulting from the different physical state of the used matrix material. In contrast to spherical droplets of the nanoemulsion, solid lipid nanoparticles made from the solid wax cetylpalmitate display a different appearance. Solid cetylpalmitate nanoparticles tend to crystallize in a platelet-like structure with a step-like surface showing layers of defined height which was found by transmission electron microscopy (TEM) and atomic force microscopy (AFM) [15]. These non-spherical particles therefore provide a much higher degree of contact points increasing the possibility of particle-particle interactions. As a consequence the formation of a three-dimensional network structure with a semisolid consistency is more pronounced. Such 'platelet-based' gel structures are known for example from Montmorillonite gels [16].

3.3. Influence of emulsifier concentration on stability and gel structure

Apart from internal phase content, particle size, particle shape, etc., the emulsifier and its concentration usually also have an influence on rheological properties of cream systems and aqueous and oily suspensions [17,18]. Increasing the emulsifier concentration leads to an increase of viscoelastic moduli and viscosity [18,19].

To investigate the influence of the emulsifier concentration on the stability of semisolid systems derived from solid lipid nanoparticles the following studies were carried out. Semisolid SLN dispersions with 30% internal lipid phase and different emulsifier concentrations ranging from 2 to 5% were produced as described above. They were analyzed with respect to particle size and viscoelastic properties.

Table 4
Influence of physical state of the dispersed phase on viscoelastic parameters G' (storage modulus) and G'' (loss modulus) ($n = 3$)

	G' (Pa)	G'' (Pa)
Formulation B2 (SLN dispersion)	86.9 ± 14.8	11.7 ± 3.4
Formulation C (nanoemulsion)	0.9 ± 0.1	1.6 ± 0.1

The resulting dispersions revealed mean particle sizes measured by PCS at day 1 after production which increased slightly with decreasing surfactant concentration (Table 5). SLN dispersions with surfactant concentrations up to 3% remained stable concerning particle size over a period of 90 days. No particle growth could be detected. In contrast, the SLN dispersion with only 2% surfactant showed increasing particle growth with storage time. It would be desirable for product development to detect this physical instability at an earlier stage. This is possible by carrying out oscillation frequency sweep tests. As Fig. 2 demonstrates, the frequency sweep curve of the unstable lipid dispersion with an emulsifier concentration of 2% shows a completely different behavior compared to the curves of the stable dispersions. The values of the storage moduli of the stable dispersions with 3, 4 and 5% emulsifier are steady over the full frequency range indicating the ability of the dispersion to resist structural changes under stress. On the contrary the dispersion with 2% emulsifier which showed distinct particle growth on storage displayed different storage moduli depending on the applied oscillation frequency. The values of the storage modulus are increasing with increasing frequency indicating a less stable structure. Specific rheological tests like oscillation frequency tests can therefore be used as a sensitive tool to predict stability of dispersed systems like semisolid SLN dispersions.

3.4. Influence of production scale up on particle size and gel structure

Successful production scale up of liquid SLN dispersions has already been demonstrated. SLN dispersions with 10–20% dispersed lipid phase could be produced reproducibly in different batch sizes up to 50 l by high pressure homogenization [9]. In the following the production scale up of the newly developed semisolid SLN dispersions by high

Table 5
Influence of emulsifier concentration on particle size of 30% lipid dispersion measured by PCS (data with standard deviations)

Day	Particle size (nm)			
	5%	4%	3%	2%
1	216 ± 4	224 ± 0	249 ± 0	311 ± 3
14	221 ± 1	228 ± 1	247 ± 2	320 ± 3
30	223 ± 1	227 ± 3	246 ± 1	335 ± 4
90	222 ± 2	229 ± 1	249 ± 2	587 ± 3

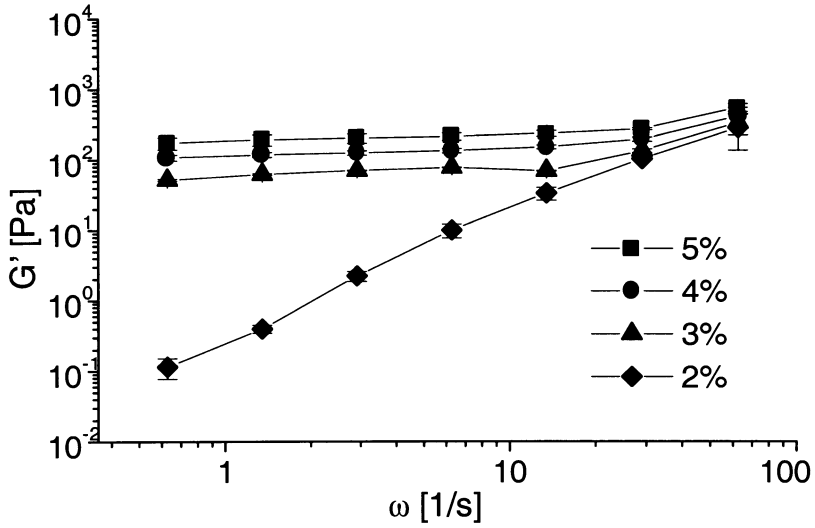

Fig. 2. Oscillation frequency sweep data of 30% lipid dispersions stabilized with different emulsifier concentrations. Storage moduli G′ measured as a function of frequency.

pressure homogenization technique was carried out. Semisolid SLN dispersions with 35% lipid content of different batch sizes were produced using two different homogenizers. A 40 ml batch was produced with a lab scale homogenizer Micron LAB 40 and compared to a 2 l batch produced with a LAB 60 homogenizer with respect to particle size and viscoelastic properties. Both homogenizers are piston gap homogenizers and have a similar construction. However, they are different in terms of thermoregulation, maximum production capacity, maximum pressure and the number of valves and pistons [9]. Concerning mean particle size measured by PCS, a small decrease from 266 ± 3 to 230 ± 1 nm can be noticed when changing from the LAB 40 (40 ml batch) to the LAB 60 (2 l batch) homogenizer (Table 6). This was also found for liquid SLN dispersions [9] and is most likely due to improved temperature control and a second homogenization valve of the LAB 60 compared to the LAB 40. In contrast to the LAB 40 homogenizer, the LAB 60 homogenizer can be completely thermoregulated and is equipped with a second homogenization valve which creates a back pressure and redisperses coalesced droplets or aggregates. As demonstrated in Table 6 there is also a difference in the viscoelastic parameters G′ and G″. The storage modulus and the loss modulus of the 2 l batch are higher than the moduli of the 40 ml batch. This effect might be caused in part by the different cooling rate of the two batches which is a function of the batch size. The 40 ml batch cools faster to room temperature than the 2 l batch. Colloidal structures which determine viscoelastic properties of a product vary according to the cooling rate this has also been shown for emulsions [20]. Despite the differences in the absolute values of the G′ and G″ moduli the phase angles being a measure of relation of elastic and viscous components reveal similar values indicating a higher prevalence of elastic behavior in both systems (7.0 ± 2.7° and 4.5 ± 0.2° for 40 ml and 2 l batch, respectively).

Table 6
Influence of batch size on particle size (PCS diameter) and viscoelastic parameters G′ (storage modulus), G″ (loss modulus) and phase angle ($n = 3$)

	Mean size (nm)	G′ (Pa)	G″ (Pa)	Phase angle (°)
40 ml batch	266 ± 3	401.6 ± 70.9	51.2 ± 3.8	7.0 ± 2.7
2 l batch	230 ± 1	1310.8 ± 97.1	101.8 ± 2.2	4.5 ± 0.2

4. Conclusion

It could be shown that physical properties like particle size, particle size distribution and physical state of the dispersed lipid phase have a great impact on the formation of a semisolid gel structure within lipid particle dispersions. By employing viscoelastic measurements it could be demonstrated that the existence of a solid particle matrix with a particle size in the nanometer range is a prerequisite to form a semisolid dispersion having the appropriate consistency for topical application. Moreover, the emulsifier concentration was found to have an influence on gel structure of these semisolid dispersions and viscoelastic parameters proved to be a sensitive tool to assess the stability of dispersed systems. It could also be demonstrated that a production scale up of semisolid SLN dispersions is possible and that similar to semisolid emulsions the production process has an influence on the colloidal structures which determine the viscoelastic properties thereof.

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