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Research paper

Evaluation of the physical stability of SLN and NLC before and after incorporation into hydrogel formulations

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Abstract

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 these systems have to be incorporated into commonly used dermal carriers, such as creams or hydrogels, in order to have a proper semisolid consistency. For the present investigation four different gel-forming agents (xanthan gum, hydroxyethylcellulose 4000, Carbopol®943 and chitosan) were selected for hydrogel preparation. Aqueous dispersions of lipid nanoparticles—solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC)—made from tripalmitin were prepared by hot high pressure homogenization and then incorporated into the freshly prepared hydrogels. NLC differ from SLN due to the presence of a liquid lipid (Miglyol®812) in the lipid matrix. Lipid nanoparticles were physically characterized before and after their incorporation into hydrogels. By means of rheological investigations it could be demonstrated that physical properties of the dispersed lipid phase have a great impact on the rheological properties of the prepared semisolid formulations. By employing an oscillation frequency sweep test, significant differences in elastic response of SLN and NLC aqueous dispersions could be observed.

Keywords: Solid lipid nanoparticles; Nanostructured lipid carriers; Hydrogels; Rheology; Topical administration

1. Introduction

Solid lipid nanoparticles (SLN™) and nanostructured lipid carriers (NLC™) are colloidal lipidic systems that have been proposed for several administration routes, such as parenteral, oral and topical route [1,2]. Both carrier types are based on solid lipids, however, they can be distinguished by their inner structure. SLN consist of pure solid lipids and NLC contain a certain percentage of additional liquid lipid leading to imperfections in the crystal lattice. Concerning topical administration, these systems possess occlusive properties due to film formation on the skin surface. They reduce the transepidermal water loss [3] and therefore enhance the penetration of drugs through the stratum corneum by increased hydration [4]. It has also been reported that the occlusion factor of SLN and NLC is related to their particle size, i.e. it increases with the decrease of the mean particle diameter [5]. Lipid aqueous dispersions with small nanoparticles and narrow size distributions are preferably obtained by emulsification of the molten matrix lipid in a hot aqueous phase with adequate emulsifying agents and subsequent crystallization of the dispersed lipid matrix [6]. Therefore, our systems were prepared by hot high pressure homogenization technique (hot HPH). Concerning liquid dispersions of lipid nanoparticles, they usually need to be incorporated in convenient dosage forms to obtain a topical application form having the desired semisolid consistency. However, when incorporated into a semisolid dosage form such as hydrogels, the physicochemical characteristics of SLN and NLC can be modified as a result of interactions between the components of the final formulation, which can be evaluated using rheological measurements [7,8].

Semisolid systems are characterized as materials that retain their shape when unconfined, but flow or deform when an external force is applied. The present study focuses on the preparation and physical characterization of

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SLN and NLC aqueous dispersions before and after their incorporation into hydrogels concerning their particle size, zeta potential and rheological properties.

2. Materials

The following materials were used from the indicated sources without further purification procedures. For SLN and NLC preparation, Dynasan®116 (glyceryl tripalmitate) was obtained from Contensio Chemicals GmbH (Witten, Germany), Tyloxapol® from Sigma Aldrich (Deisenhofen, Germany) and Miglyol®812 from Caelo (Hilden, Germany). For hydrogels preparation, Carbopol®934 (polyacrylate) was purchased from BF Goodrich, USA, and xanthan gum, hydroxyethylcellulose 4000 (HEC), chitosan and glycerol were purchased from Sigma Aldrich (Deisenhofen, Germany). The water used for all experiments was purified water obtained from a MilliQ Plus (Millipore, Schwalbach, Germany).

3. Methods

3.1. Preparation of aqueous SLN and NLC dispersions and hydrogels

The preparation of aqueous SLN and NLC dispersions was carried out according to Müller et al. [9]. Briefly, aqueous dispersions of NLC and SLN, composed of 20% (w/w) of lipid phase and 5% (w/w) of emulsifying agent (Tyloxapol®), were produced using the hot HPH technique. The lipid phase of SLN consisted of 100% Dynasan®116 and the lipid phase of NLC consisted of 70% Dynasan®116 and 30% Miglyol®812. The lipid was melted above its melting point and dispersed under high-speed stirring using an Ultra-Turrax (Jahnke and Kunkel GmbH and Co KG, Staufen, Germany) in the hot aqueous surfactant solution of identical temperature (90 °C). The obtained pre-emulsion was then homogenized at 90 °C with a Micron LAB 40 (APV Systems, Germany) applying 500 bar and three cycles. After homogenization the produced O/W nanoemulsion was cooled down to 20 °C, the lipid recrystallized forming SLN or NLC aqueous dispersions.

In order to prepare four different hydrogel formulations, the correspondent gel-forming polymer was dispersed in distilled water containing 10% of glycerol. Aqueous SLN or NLC dispersions and hydrogels were mixed in a high speed stirrer (Cito Unguator Konietzko, Bamberg, Germany) at approximately 1000 rpm for 5 min, to yield gels containing a final concentration of 5% lipid nanoparticles. The hydrogels composed of Carbopol®934 and chitosan were adjusted to pH 6.5 and 4, respectively. As reference the aqueous SLN and NLC dispersion were used. The SLN- and NLC-loaded hydrogels were stored at room temperature for 3 months.

3.2. Particle size analysis

The particle size analysis of SLN and NLC was performed by photon correlation spectroscopy (PCS) and laser diffractometry (LD). The PCS yielded the mean diameter of the main population and polydispersity index (PI) as a measure for the width of the particle size distribution. The equation used to determine the PI value is proprietary of Malvern and while it is directly proportional to the size variance, values lower than 0.1 are regarded as monodisperse for objects in the size range of 100 nm [1,9]. For PCS measurements, all the samples were diluted with bidistilled water to suitable concentration and analysed with a Malvern Zetasizer 4 (Malvern Instruments, UK). Prior to particle size analysis by PCS, the semisolid SLN or NLC dispersions were diluted with double-distilled water to weak opalescence. For the LD analysis the diameters 50, 90 and 99% were used. For example, D 99% means that 99% (volume distribution) of the measured particles are below the given value. LD was performed using a Coulter®LS 230 (Beckmann-Coulter Electronics, Germany). Prior to particle size analysis by LD the semisolid SLN and NLC formulations were diluted with double-distilled water by shaking to weak opalescence. All measurements were performed in triplicate.

3.3. Zeta potential

The surface charge of SLN and NLC before and after incorporation into hydrogels was determined by measurement of the zeta potential (ξ) of the lipid nanoparticles calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For the ξ measurements a Malvern Zetasizer 4 (Malvern Instruments, UK) was used. The field strength was 20 V/cm on a large bore measuring cell (4 mm). Samples were diluted with bidistilled water adjusted to a conductivity of 50 μS/cm with a solution of 0.9% NaCl (if not otherwise stated).

3.4. X-ray diffraction

X-ray investigations were performed by wide-angle X-ray scattering (WAXS, 2 Theta 4–40°) on a Philips PW 1830 X-ray generator (Philips, Amedo, The Netherlands) with a copper anode (Cu Kα radiation, 40 kV, 25 mA, $\lambda = 0.15418$ nm), using a Goniometer PW18120 as a detector. SLN and NLC aqueous dispersions were previously transformed into a paste using locust bean gum as a thickening agent, and then mounted into a specific device before the measurement by WAXS. The data used were typically collected with a step width of 0.02° and a count time of 60 s.

3.5. Rheological measurements

The rheological measurements were 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 4°). If not otherwise indicated all measurements were carried out at a temperature of 20 ± 0.1 °C.

For the rheological characterization of the aqueous SLN and NLC dispersions an oscillation frequency sweep test was performed over a frequency range from 0 to 10 Hz at constant stress amplitude of 5 Pa. The rheological properties of the developed hydrogels containing SLN and NLC were studied by continuous shear investigations, which were performed in order to evaluate the shear rate [1/s] as a function of shear stress [Pa]. This study started applying 0 Pa up to a maximum shear stress of 50 Pa and the resulting shear rate was measured.

4. Results and discussion

4.1. Characterization of the investigated formulations

For the production of aqueous SLN and NLC dispersions containing 20% of lipid matrix, the formulations given in Table 1 were chosen.

For the present investigation, four different hydrogel types were prepared using an optimal stabilizer combination of water, gel-forming polymer and glycerol as hydrating agent. The aqueous SLN and NLC dispersions were admixed to the freshly prepared hydrogels. Table 2 shows the final composition of the investigated SLN- and NLC-containing hydrogels.

4.2. Particle size analysis

The results obtained after particle size analysis are shown in Figs. 1 and 2. PCS results (Fig. 1) prove the colloidal particle size of lipid nanoparticles before and after incorporation into the four different hydrogels. The PI is higher for xanthan gum (approx. 0.3–0.4) and chitosan (approx. 0.4) hydrogels. After analysis of the obtained LD results of the formulations stored at room temperature for 90 days (Fig. 2), particles with diameters above 700 nm could be excluded. For all tested formulations, 50% of the particles were below 200 nm after 3 months at 20 °C. The incorporation into hydrogels did not result in particle aggregation.

Table 1
Composition of the investigated SLN and NLC formulations [% (w/w)]

Formulation	Dynasan®116 (%)	Miglyol®116 (%)	Tyloxapol® (%)	Water (%)
A (SLN)	20	–	5	75
B (NLC)	14	6	5	75

4.3. Zeta potential (ξ)

The measurement of the ξ allows predictions about the stability of colloidal aqueous dispersions [10]. Usually, particle aggregation is less likely to occur for charged particles with high ξ (>|30| mV) due to electric repulsion [11]. In general, lipid nanoparticles are negatively charged on the surface [12].

The determination of ξ was performed in aqueous SLN and NLC dispersions stored at room temperature. The ξ and standard deviation (SD) values of SLN and NLC before and after their incorporation into hydrogels are shown in Table 3.

SLN and NLC were negatively charged when incorporated into xanthan gum, HEC and Carbopol®934 hydrogels. The opposite was observed after incorporation into chitosan hydrogels. These results are due to the cationic character of this bioadhesive polymer. The positively charged amino groups are capable of neutralizing the negative charge at the surface of these particles and change the ξ. As a consequence of the charge reversal to the positive value of ξ, the particles are highly instable around the point of zero charge and aggregation is facilitated, as it could be observed after particle size analysis. In addition, one needs to use acetic acid in order to start gel formation of chitosan, which can also be a reason for the observed ξ.

Concerning Carbopol®934 hydrogels, their carboxylic groups have to be neutralized with NaOH in order to exhibit gel-forming properties. This neutralizing agent could enhance aggregation because of the action of sodium ions as electrolyte, which can reduce the ξ of the particles [13]. As a consequence of this lower ξ value aggregation may occur. This phenomenon is well known for lipid nanoemulsions [14], as well as for SLN when incorporated into polyacrylate hydrogels [15]. However, the tested lipid nanoparticles made from Dynasan®116 and Tyloxapol® revealed no sensitivity to exposure to sodium ions.

Fig. 3 compares the ξ values of the aqueous SLN and NLC dispersions before and after their incorporation into hydrogels, measured on day 1 and after 90 days of storage at room temperature.

Fig. 3 reveals that during storage time, the ξ value of the surface of lipid nanoparticles remains practically unchanged (e.g. B_1, A_3, B_3) or slightly decreases (e.g. A_1, A_2). In comparison to SLN formulations with the same lipid content, NLC formulations show lower ξ values.

In general terms, SLN and NLC aqueous dispersions can be incorporated into hydrogels consisting of more or less uncharged polymers without significant changes in the particle size characteristics and ξ values [16]. In the case of gel-forming polymers with very polar groups, like chitosan, possible interactions between negative surface charge of lipid nanoparticles and polar groups of this polymer must be taken into account.

Table 2
Final composition of the investigated (A) SLN- and (B) NLC-loaded hydrogels formulations [% (w/w)]

Formulation	Dynasan®116 (%)	Miglyol®116 (%)	Tyloxapol® (%)	Gel-forming agent	Glycerol (%)	Water (%)
A_1	10	–	2.5	1% Xanthan gum	5	81.50
A_2	10	–	2.5	1.75% HEC 4000	5	80.75
A_3	10	–	2.5	0.5% Carbopol®934	5	82.00
A_4	10	–	2.5	1% Chitosan	5	81.50
B_1	7	3	2.5	1% Xanthan gum	5	81.50
B_2	7	3	2.5	1.75% HEC 4000	5	80.75
B_3	7	3	2.5	0.5% Carbopol®934	5	82.00
B_4	7	3	2.5	1% Chitosan	5	81.50

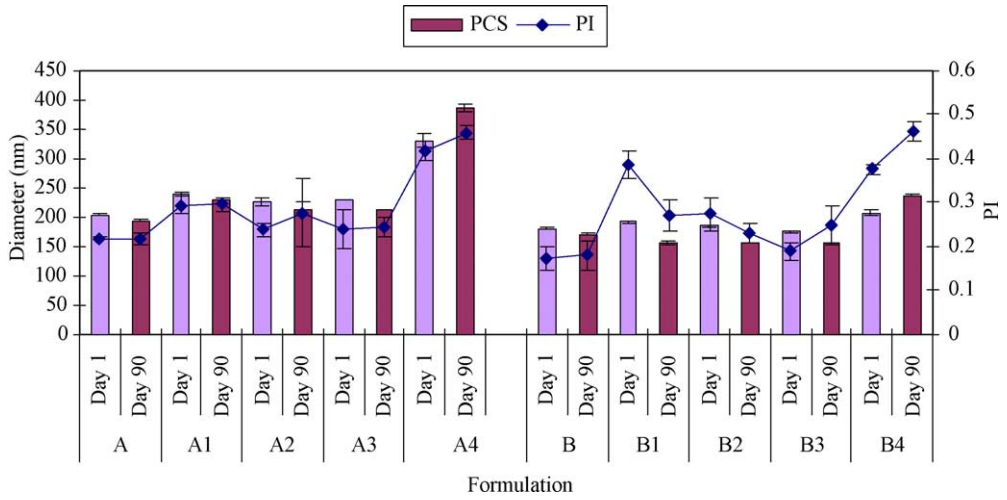

Fig. 1. PCS diameters (nm) and PI of SLN and NLC formulations before and after incorporation into different hydrogels measured after 1 and 90 days of storage at room temperature. (Composition of formulations: Table 2.)

4.4. X-ray diffraction

X-ray diffraction has been used for the study of molecular structure and polymorphism of lipid nanoparticles [17,18]. Therefore, these measurements were performed after 7 days of storage at room temperature, in order to compare the crystalline nature of the investigated lipid particles.

Fig. 4 shows the X-ray diffractograms of SLN and NLC aqueous dispersions made from tripalmitin. The plots have been displaced vertically for better visualization. The units of intensity are arbitrary.

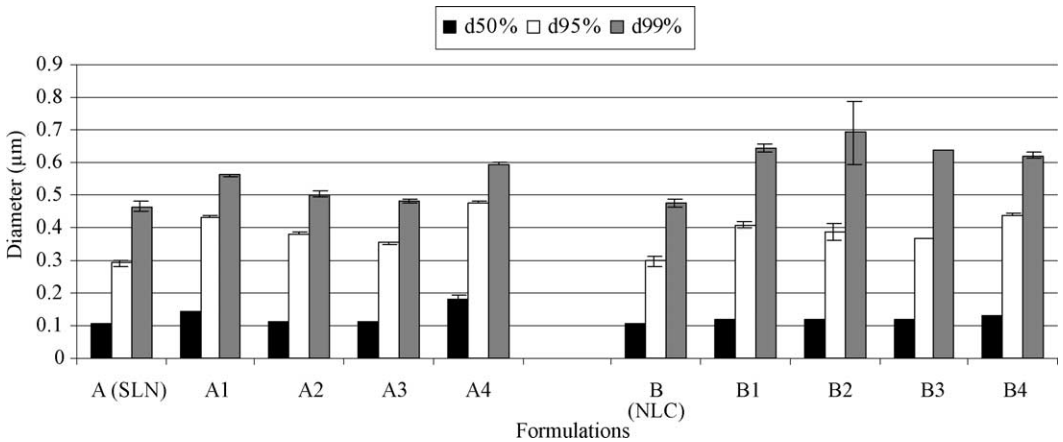

Fig. 2. LD diameters (μm) of SLN and NLC formulations before and after incorporation into different hydrogels after 90 days of storage at room temperature. (Composition of formulations: Table 2.)

Table 3
ξ (mV) and SD values of SLN and NLC before and after their incorporation into hydrogels, measured on day 1 of storage at 20 °C (composition of formulations: Table 2)

Formulation	ξ	SD	Formulation	ξ	SD
A (SLN)	−23.4	0.608	B (NLC)	−16.8	0.153
A_1	−30.0	1.054	B_1	−17.5	0.404
A_2	−14.4	0.252	B_2	−10.8	0.361
A_3	−15.0	0.058	B_3	−12.2	0.603
A_4	+39.5	0.451	B_4	+36.9	0.321

The SLN curve shows the characteristic peaks for β′/β modifications (identified with arrows), which are displayed in the wide angle regions pointing to the crystalline nature of these particles. As expected, these polymorphic forms are not visible in X-ray pattern of NLC aqueous dispersion. Although, these later display a thermal event (melting endotherm) in DSC heating runs (data not shown). Note that, whereas X-ray diffraction data only allow differentiation between crystalline data and amorphous material, DSC data can be used to differentiate between amorphous solids and liquids [19]. In comparison to SLN with the same lipid content, NLC formulations evidence lower lipid crystallinity in agreement with the obtained DSC data. These X-ray crystallization studies confirm that the transition rates of liquid oils are lower than solid lipids. These differences may be due to the more ordered structure of SLN in comparison to NLC.

4.5. Rheological measurements

Rheological measurements are useful for the characterization of the viscoelastic properties of aqueous SLN and NLC dispersions and of SLN and NLC-containing hydrogels. Therefore, an oscillation frequency sweep test was carried out for all tested SLN and NLC dispersions. In addition, continuous shear investigations were performed in the tested hydrogel formulations in order to evaluate the shear rate as a function of shear stress.

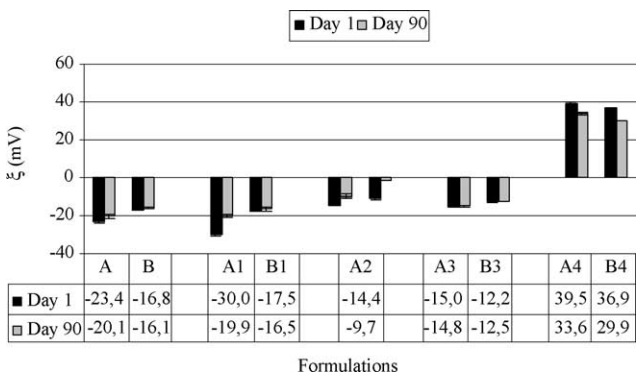

Fig. 3. Comparison between the ξ values of the aqueous SLN and NLC dispersions before and after their incorporation into hydrogels, measured on day 1 and after 90 days of storage at 20 °C. (Composition of formulations: Table 2.)

4.5.1. Oscillation frequency sweep test

Fig. 5 shows the results of the oscillation frequency sweep test of aqueous SLN dispersion after 7 days of storage at room temperature. It can be observed that the storage modulus G' (elastic component) is far greater than the loss modulus G'' (viscous component) over the measured frequency range, indicating the presence of a gel-like structure. The loss modulus G'' shows weak dependence on the applied frequency, i.e. it remains practically the same over the frequency range, while the storage modulus G' is more dependent on the applied frequency, i.e. it increases from 7000 to 9000 Pa with the increase of the applied frequency. It could be observed that the viscosity decreases with increasing frequency.

Concerning aqueous NLC dispersion (Fig. 6), the storage modulus G' was closer to the loss modulus G'' over the measured frequency range and both are dependent upon the applied frequency. The increase of the frequency was followed by an increase in viscosity.

These differences observed on the viscoelastic properties of lipid dispersions may be due to the physical state of the dispersed phase (Fig. 4). Note that both solid lipid aqueous dispersions were prepared with the same content of lipid phase (20% w/w) and emulsifying agent (5% w/w), and are comparable in particle size and PI (Fig. 1). As Figs. 5 and 6 demonstrate, by displacing 30% of liquid lipid (in NLC) by solid lipid (in SLN) a significant increase of elastic modulus G' and loss modulus G'' can be observed. Studies performed by Lippacher showed that SLN dispersions possess higher elastic properties than emulsions of comparable lipid content [20]. In fact, if the storage modulus G' is higher by about one order of magnitude than the loss modulus G'', and if both parameters show weak dependence on the applied frequency, the system is more elastic than viscous in the investigated frequency range [20]. This means that SLN systems are more elastic than NLC systems of compared lipid content. The decrease in viscosity observed with increase in applied frequency is also typical for viscoelastic solids and can be found for standard topical dosage forms [21].

4.5.2. Continuous shear rheometry

Conventional SLN and NLC aqueous dispersions contain about 10–20% (w/w) of lipid matrix and 80–90% (w/w) of water [22,23]. As a result, liquid solid lipid dispersions possess a low viscosity (approximately 100 mPa s) and a yield value of practically zero [15]. Therefore, the liquid solid lipid dispersions usually have to be incorporated in convenient topical dosage forms like hydrogels or creams to obtain a topical application form having the desired semisolid consistency [21,24]. Incompatibilities with ingredients from the hydrogel or cream may occur due to interactions between the gel forming polymer, emulsifying

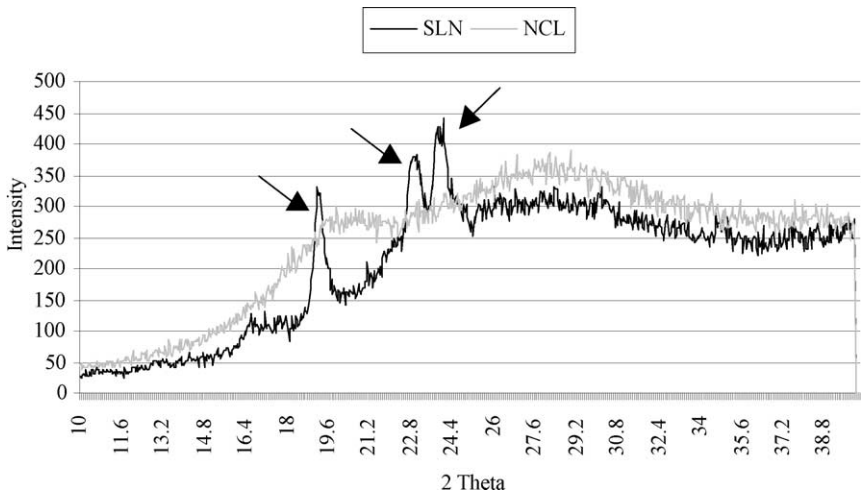

Fig. 4. X-ray patterns of aqueous SLN and NLC dispersions after 7 days of storage at 20 °C.

agents, lipid and drug. These interactions can affect the semisolid consistency of the topical formulation and, therefore, its rheological status, which is a very important physical parameter in the development of a potential new drug delivery system for topical use. The rheological behaviour of hydrogels-loaded with SLN and NLC was evaluated after 1 week of storage at room temperature. Fig. 6 shows the flow curves of the four different hydrogels containing SLN and NLC aqueous dispersions.

The flow curves of the gels are shown in Fig. 7. The incorporation of lipid particles into xanthan gum hydrogels (A_1, B_1) resulted in flow curves with plastic characteristics, where the shear rate increases with increasing shear stress. Ascending and descending flow curve overlap and show no time effects like, e.g. thixotropy. The lipid particles in the semisolid system tend to align with increasing shear stress which is alleviating the flow. This phenomenon was more emphasized for NLC-containing hydrogels, while SLN-containing hydrogels revealed less dependency upon the applied shear stress. The incorporation of SLN and NLC into HEC hydrogels (A_2, B_2) also showed flow curves with plastic characteristics, although the magnitude of the shear rate is much lower in comparison to xanthan gum hydrogels.

SLN- and NLC-containing polyacrylate hydrogels (A_3, B_3) revealed a significant increase in yield value especially SLN formulations. Unlike NLC formulations, the ascending and descending curve of SLN-containing polyacrylate hydrogels did not overlap, showing thixotropy. NLC formulations revealed more dependency upon the applied shear stress. Concerning chitosan hydrogels (A_4, B_4), they revealed a more Newtonian behaviour with a yield value of approximately zero.

To sum up, increasing the lipid content of the system leads to different flow characteristics. Flow curves of pure hydrogels reveal a weaker and more sensitive structure, in comparison to flow curves of SLN- and NLC-containing hydrogels (data not shown). Thixotropy could be observed with systems having higher lipid content. HEC and chitosan hydrogels are more liquid and they might not have a good consistency for topical administration. Since lipid nanoparticles and xanthan gum and Carbopol®934 hydrogels interact such that rheological properties are affected, it can be envisaged that the combination of these

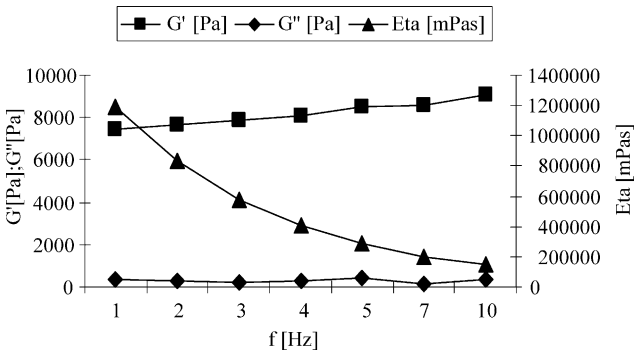

Fig. 5. Storage modulus (G'), loss modulus (G'') and complex viscosity (η) of SLN dispersion as a function of the frequency at constant stress amplitude of 5 Pa, measured after 7 days of storage at 20 °C.

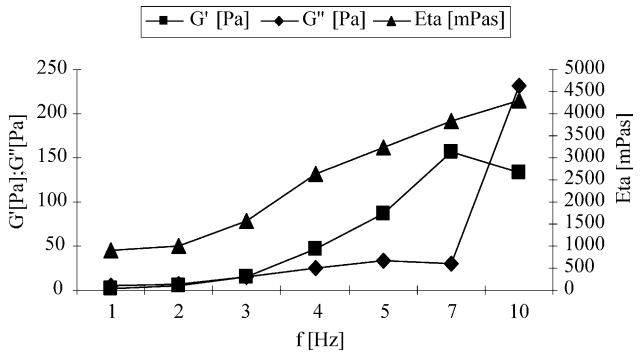

Fig. 6. Storage modulus (G'), loss modulus (G'') and complex viscosity (η) of NLC aqueous dispersion as a function of the frequency at constant stress amplitude of 5 Pa, measured after 7 days of storage at 20 °C.

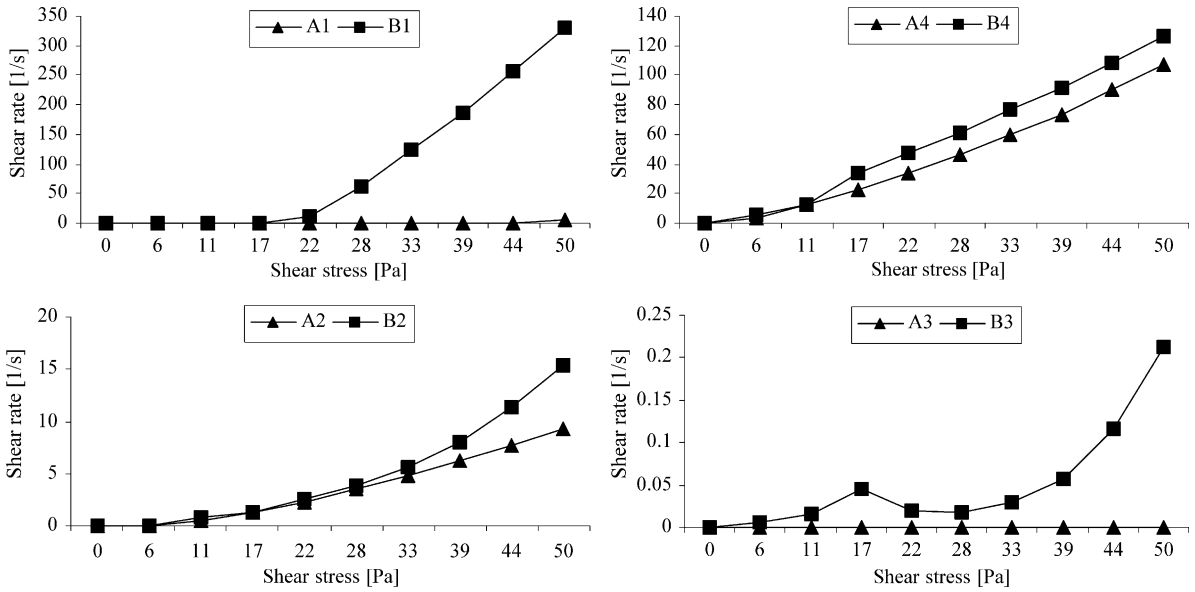

Fig. 7. Shear rate of SLN- and NLC-containing hydrogels as a function of shear stress, measured after 7 days of storage at 20 °C. (Composition of formulations: Table 2.)

gel-forming agents in certain ratios comprises a novel semisolid system appropriate for topical administration [25]. In comparison to xanthan gum hydrogels, lipid nanoparticles containing Carbopol®934 hydrogels revealed less dependency upon the applied shear stress.

5. Conclusions

From a physical point of view, SLN and NLC dispersions can be considered as promising systems for topical administration. The size distribution of SLN and NLC before and after incorporation into hydrogels revealed a unimodal profile. By means of WAXS investigations, the presence of a less ordered matrix in NLC structure could be demonstrated in comparison to SLN matrix; although, this is not an impediment for dermal administration. Because the rheological investigations provide information about application of semisolid formulations and their performance on skin, these studies were conducted both on SLN and NLC aqueous dispersions, as well as on SLN- and NLC-loaded hydrogels. Physical characterization by means of oscillation sweep tests and continuous rheometry show that these systems are complex and their performance is dependent on the structure of the gel-forming polymer used for hydrogel preparation. Moreover, increasing the solid lipid content of the dispersed phase an increase of the elastic component was observed.

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