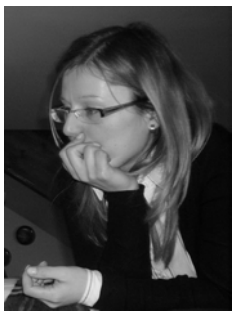
SLN and NLC as viscoelastic enhancers for topical drug delivery



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Introduction

Hydrogels based on polyacrylate polymers are an important class of biomaterials potentially suitable for controlled release of drugs, particularly for topical and dermatological applications. The skin delivery of several drugs can be improved by the addition of penetration enhancers to the semi-solid systems. Examples of such substances are the dimethyl sulfoxide [1, 2], cetyl alcohol [3, 4], olesan oil [1] and oleic acid [3, 5]. However, these substances might be related to sub optimization of the rheological/mechanical properties of the developed topical drug delivery systems due to a decrease of the viscosity. To improve such properties of the semi-solid systems, and further and enhance the drug penetration within the skin, nanoparticles composed of solid lipids have been exploited. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are composed of a solid matrix once they are derived from o/w emulsions simply replacing the liquid lipid (oil) by a lipid being solid both at room and body temperature. Being the particle matrix is in a solid state, its melting point which varies between 50ºC (122ºF) and up to almost 100ºC (212ºF) can be adjusted by the choice of the lipid. SLN are the first generation of the lipid nanoparticles, consisting of a matrix which is produced solely from a solid lipid. The lipid can be a highly pure lipid e.g. tristearin or a less defined mixture of acylglycerols, such as Compritol® or Imwitor®. NLC are produced from a lipid blend, consisting of a mixture of a solid lipid with a liquid lipid (oil). This mixture is chosen in order to obtain a particle matrix which is in a solid state at the anticipated melting point. Relatively high amounts of oil can be incorporated when choosing a mixture with a high melting lipid, e.g. blending the oil with a wax of high melting point (80-90ºC).
This paper characterizes the interaction of SLN and NLC with polyacrylate polymers regarding their dynamic mechanical properties. Lipid nanoparticles have been dispersed into freshly prepared hydrogels and, besides the obtained increment of the hardness and stiffness, this approach has also modified the rheological behavior and consequently the processability of the developed product. Advantages of reduction of costs can also be pointed out. The solid matrices interact with the polymer hydrogel thermodynamically according to their surface potentials (zeta potential) and hydrodynamically through flow field interactions.

Materials and methods

Materials

Glyceryl tripalmitate was obtained from Sasol GmbH (Witten, Germany). This lipid consists of a high content of microcrystalline triacylglycerols (approx. 90%) and monocarboxilic acids (approx. 10%). It is a glycerol ester of selected, even-numbered and unbranched fatty acids of natural origin, is free from antioxidants and other stabilizing agents. Miglyol®812 (Caelo, Hilden, Germany) was the liquid lipid selected for the production of NLC and consists of medium chain triacylglycerols (C_8-C_{10}), having a density between 0.945 and 0.955 g/cm^3. The surfactant used to stabilize the aqueous dispersions of lipid nanoparticles was Tyloxapol® obtained from Sigma-Aldrich (Deisenhofen, Germany), which is a polymer of 4-(1,1,3,3-tetramethylbutil)-phenol with ethylene oxide and formaldehyde. Carbopol®934 (polyacrylate) was obtained from BF Goodrich (Ohio, USA). The water used in all experiments was Purified Water (European Pharmacopoeia, 4th ed.)

obtained from a MilliQ Plus, Millipore (Schwalbach, Germany). It is mainly characterized by an electrical resistivity of 18 MΩ and a total organic content equal or lower than 10 ppb.

Methods

Production of aqueous SLN or NLC dispersions

Placebo SLN and NLC have been produced applying the hot high pressure homogenization technique [6, 7]. SLN and NLC composed of 20% (m/m) of lipid phase and 5% (m/m) of surfactant were prepared as described in detail elsewhere [7-9]. Briefly, glyceryl tripalmitate was melted at 85ºC and in case of NLC 30% of solid lipid (related to the lipid phase) has been replaced my liquid lipid. A pre-emulsion was formed after dispersing the hot lipid phase in an aqueous surfactant solution using an Ultra-Turrax T25 (Staufen, Germany) at 8000 rpm for 1 min. The obtained pre-emulsion was passed through an APV Micron Lab 40 high pressure homogenizer (APV Systems, Unna, Germany), at 85ºC and applying a pressure of 500 bar. The obtained aqueous dispersions were filled in siliconized glass vials, which were immediately sealed and stored at room temperature (20ºC).

Production of hydrogels-loaded SLN or NLC

Hydrogels-loaded SLN or NLC were composed of 5% glycerol, 50% SLN or NLC aqueous dispersion, a sufficient amount of the gel-forming polymer and purified water. Briefly, the gel-forming polymer, glycerol and water were weighed in a beaker and stirred with a high speed stirrer (Cito Unguator Konictzko, Bamberg, Germany) at approximately 1000 rpm for 5 min. This initial high-shear mixing was followed by low-shear planetary mixing during the neutralization gelling process performed by addition of Trizma®Pre-set crystals pH 7.0 until reaching the pH of 6.5. Finally, the aqueous SLN or NLC dispersion was added to the freshly prepared hydrogels under continuous stirring at 1000 rpm for 3 min.

Particle size and zeta potential analysis

The particle size analysis of SLN and NLC dispersions was performed by photon correlation spectroscopy (PCS) with a Zetasizer 4 (Malvern Instruments, UK) and by laser diffractometry (LD) using a Coulter®LS 230 (Coulter Electronics, Germany). PCS yields the mean diameter of the bulk population and polydispersity index (PI). The LD data were evaluated using the diameters d50%, and d95% of the volume distribution. By laser Doppler anemometry (LDA) the Zetasizer 4 was used to measure the electrophoretic mobility, which was converted to the zeta potential using the Helmoltz-Smoluchowski equation. Lipid dispersions were previously diluted with bidistilled water adjusted to a conductivity 50 µS/cm with a solution of 0.9% NaCl.

Rheological analysis

The rheological properties of the formulations were studied by continuous shear investigations, which were performed in order to evaluate the shear rate as a function of shear stress. This study started applying 0 Pa up to a maximum shear stress of 50 Pa and the resulting shear rate was measured. Rheological measurements were carried out 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). Analysis was performed within the range of 20±0.1ºC.

Results and discussion

Freshly prepared aqueous SLN and NLC dispersions have been incorporated into polyacrylate-based hydrogels. Two different formulations were developed (Table I) and stored at different temperatures (4ºC, 25ºC and 40ºC) in laminate foil tubes. This optimized packaging material avoids light exposure once it gradually decreases the consistency of polyacrylate gels.

Table I: Composition of SLN- and NLC-based hydrogels %(m/m).

Composition	SLN-based hydrogels	NLC-based hydrogels
Carbopol®934	0.50%	0.50%
Trizma®	0.15%	0.15%
Glycerol	5.00%	5.00%
Glyceryl tripalmitate	9.00%	6.50%
Myglyol®812	-	2.50%
Tyloxapol®	2.50%	2.50%
Water ad	100%	100%

The obtained Z-ave diameters before dispersing SLN and NLC within the hydrogel were 220-250 nm and the PI were 0.100-0.250. LD analysis showed LD50% and LD90% values between 0.140-0.200 μm and 0.350-0.480 μm, respectively. After dispersing SLN and NLC, no major changes in particle size parameters were recorded.

SLN and NLC dispersions were also electrostatically stabilized revealing zeta potentials between -25 and -30 mV. In electrostatically stabilized aqueous dispersions, the range of the electrostatic repulsion is expressed by the Debye length, being nearly equal to 1 nm in the dispersions. Due to the fact that the effective volume fraction of nanoparticles with adsorbed surfactant is considerably larger than the effective volume fraction of the nanoparticles in an electrostatically stabilized system, the systems will exhibit higher viscosities if also stabilized by surfactant molecules.

During the rheological analysis the intimate contact between embedded lipid nanoparticles within the polyacrylate gels give rise to many non-linear rheological features, which strongly manifest themselves at high concentrations. Therefore, the presence of SLN and NLC enhances the non-linear behavior of gels and can contribute with new non-linear effects especially at high concentrations. These non-linear responses are, for example, shear thinning and yield stress, which appear when the nanoparticles can form individual clusters that are held together by adhesive forces. These forces have been tested using continuous shear investigations. Table II depicts the complex viscosity recorded during these tests for samples stored at different temperatures.

Table II: Complex viscosity [mPa.s] of SLN- and NLC-based hydrogels stored at three different temperatures recorded during a shear rate interval from 0 s^{-1} to 100 s^{-1}.

Semi-solid formulation	Shear rate variation	Storage temperature/Complex viscosity [mPa.s] 4º	25º	40ºC
SLN-based hydrogels	0 s^{-1}	197000	625000	172000
	100 s^{-1}	3850	823	6610
	0 s^{-1}	75500	6980	189000
NLC-based hydrogels	0 s^{-1}	187000	179000	118000
	100 s^{-1}	3390	3110	2500
	0 s^{-1}	130000	113000	91700

Flow curves revealed the occurrence of pseudoplastic behavior in all systems at different storage conditions. This shear thinning behavior occurs when viscosity decreases with increasing shear rate (Table II), while yield stress appears in dispersions that do not flow until that critical stress values is

overcome. The probability of direct interaction between the particles increases as the concentration of solid lipid content increases (SLN versus NLC). The formation of separate individual clusters due to particle adhesion during shear rate variation is also possible even at small concentrations, affecting the flow properties of the viscoelastic materials. Adhesion has been shown to be reversible and, as expected, these non-linear effects were enhanced in SLN-based hydrogels due to the more irregular geometry of SLN in comparison to the spherical-like shape particles of NLC (data not shown).

Conclusions

In dispersed systems, thixotropy arises when shear stress values measured by progressively increasing the shear rate are higher from those measured when one progressively decreases it. Thixotropy is a kind of viscoelasticity that has a very long relaxation time caused by flow induced changes in structure that are generally erased after hours of quiescence. This study shows that the developed formulations have thixotropy at relatively low shear rates. However, the amount of thixotropy is higher for SLN-based hydrogels in comparison to NLC-based hydrogels. When the topical gels experience high shear rates, the network structure between neighboring microgel particles as well as the entanglements between long polymer chain segments break down, Therefore, the shear stresses or viscosities in decreasing shear rate curve are lower. The lower curves represent apparent shear stresses of the up curve and the higher curve represent the down curve. The rheological properties were scaled with respect to the solid lipid content (SLN versus NLC) and superimposed onto the results obtained for nanoparticles-free hydrogels. Therefore, it can be concluded that the dynamic mechanical properties evaluated in this study show the suitability of lipid nanoparticles as viscoelastic enhancers for topical formulations.

References

1. Baboota, S., Shakeel, F., Kohli, K., *Formulation and evaluation of once-a-day transdermal gels of diclofenac diethylamine.* Methods Find. Exp. Clin. Pharmacol., 2006. **28**(2): p. 109-114.
2. Bugaj, A., Juzeniene, A., Juzenas, P., Iani, V., Ma, L.W., Moan, J., *The effect of skin permeation enhancers on the formation of porphyrins in mouse skin during topical application of the methyl ester of 5-aminolevulinic acid.* J. Photochem. Photobiol. B., 2006. **83**(2): p. 94-97.
3. Mehdizadeh, A., Ghahremani, M.H., Rouini, M.R., Toliyat, T., *Effects of pressure sensitive adhesives and chemical permeation enhancers on the permeability of fentanyl through excised rat skin.* Acta Pharm., 2006. **56**(2): p. 219-229.
4. Zesch, A., Bauer, E., *Quantitative aspects of the percutaneous uptake of wool wax alcohols (cetyl alcohol) and paraffins (octadecane) from different ointment bases.* Derm. Beruf. Umwelt., 1985. **33**: p. 15-20.
5. Lopes, L.B., Ferreira, D.A., de Paula, D., Garcia, M.T., Thomazini, J.A., Fantini, M.C., Bentley, M.V., *Reverse hexagonal phase nanodispersion of monoolein and oleic Acid for topical delivery of peptides: in vitro and in vivo skin penetration of cyclosporin a.* Pharm. Res., 2006. **23**(6): p. 1332-1342.
6. Müller, R.H., Mehnert, W., Lucks, J.-S., Schwarz, C., zur Mühlen, A., Weyhers, H., Freitas, C., Rühl, D., *Solid lipid nanoparticles (SLN) - An alternative colloidal carrier system for controlled drug delivery.* Eur. J. Pharm. Biopharm., 1995. **41**: p. 62-69.
7. Müller, R.H., Mäder, K., Gohla, S., *Solid lipid nanoparticles (SLN) for controlled drug delivery - A review of the state of art.* Eur. J. Pharm. Biopharm., 2000. **50**: p. 161-177.
8. Mehnert, W., Mäder, K., *Solid lipid nanoparticles - Production, characterization and applications.* Adv. Drug Deliv. Rev., 2001. **47**: p. 165-196.
9. Müller, R.H., Mehnert, W., Souto, E.B., *Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for dermal delivery*, in *Percutaneous Absorption*, L. Bronaugh, Editor. 2005, Marcel Dekker, Inc.: New York, Basel, Hong-Kong. p. 719-738.