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The use of SLN® and NLC® as topical particulate carriers for imidazole antifungal agents

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Two different imidazole antifungal agents have been used as model drugs to be incorporated into solid lipid nanoparticles (SLN®) and nanostructured lipid carriers (NLC®), once they are very well established as anti-mycotics for the treatment of topical fungal infections. Because of the high mucoadhesive properties and the strong *in situ* gelling properties of polyacrylic acid polymers, hydrogels prepared with those macromolecules might be a promising vehicle for imidazole-loaded lipid nanoparticles, such as the above-mentioned SLN and NLC. Thus, in this study Carbopol®934 has been selected for the preparation of semi-solid formulations based on SLN and NLC. Formulations have been stored at three different temperatures before and after particle incorporation into polyacrylate hydrogels. The particle size and the chemical stability of incorporated model drugs have been monitored by HPLC analysis for two years. On the day of production 91.7% and 98.7% of clotrimazole, but only 62.1% and 70.3% of ketoconazole have been recovered from SLN and NLC, respectively. More than 95% of clotrimazole but less than 30% of ketoconazole were detected in the developed formulations after a shelf life of two years. Those values showed to be higher than those obtained with reference emulsions of similar composition and droplet sizes. By rheological measurements a pseudoplastic behaviour with thixotropic properties has been characterized for all semi-solid systems.

1. Introduction

During the past thirty years considerable attention has been focused on the development of novel and controlled release drug delivery systems. Many of those systems are based on hydrogels where the drug is solubilized, e.g. by surfactants. The hydrophilic polymers used as gellifying agents are usually cross-linked by means of covalent bonds. Placed in an aqueous environment, these hydrogels are able to swell rapidly and retain large volumes of water in their swollen structure. Hydrogels prepared with polyacrylic acid polymers have been used extensively for the controlled release of numerous drugs (Changez et al. 2004, 2005). These formulations are highly biocompatible, being useful for both hydrophilic and lipophilic drugs, as well as vehicles for drug-loaded nanoparticles such as SLN and NLC (Souto et al. 2004). In order to obtain a controlled release profile, drugs can be dispersed in the hydrogel network using surfactants or, otherwise, be previously incorporated into carriers where they show higher solubility. In this work we have considered the second approach and for the preparation of those carriers we have used high melting lipophilic molecules, such as triacylglycerols of fatty acids. Calorimetric and X-ray diffraction studies (Aquilano et al. 1994), as well as TEM investigations (Cavalli et al. 1992), support the view that these carriers are characterized by a solid matrix. Advantages of the solid matrix are related to the increase of the physical stability of the incorporated drug in opposite to emulsions, which can suffer from reduction of the zeta potential leading to aggregation, drug expulsion and eventually breaking of the emulsion.

The introduction of clotrimazole into the therapy of vaginal *Candida* and *Torulopsis* infections has significantly shortened the previously customary duration of treatment and reduced the frequency of dosage of the active ingredient (Chang et al. 2002). With regard to ketoconazole, it is also a synthetic azole antifungal, known as a broad-spectrum agent structurally related to other imidazole-derivative antifungals (Fernández-Torres et al. 2000). It is wildly used for the treatment of different kinds of tinea infections, as well as for the treatment of cutaneous candidiasis caused by *Candida albicans*.

It may be assumed that the topical application of the new formulations assayed in the present study would allow obtaining a good therapeutic response. Naturally, an improved therapeutic profile of drug substances, while most desirable and necessary, must be weighed against the cost of such therapy. SLN and NLC can lead to a substantial cost reduction, particularly if there is a corresponding reduction of drug degradation, i.e. increase of drug stability when incorporated into those lipid carriers. Advantages of using particulate carriers in dermatological drug delivery are also related to the fact that being of natural sources (biodegradable and physiological lipids) they can decrease skin irritation and allergic reactions.

There is also the problem that in particular circumstances some drugs appear to be bound or retained in the skin, being the rate-limiting step through the skin rather than release of drug from the delivery system. The use of SLN and NLC will promote diffusion of drug into the skin due to the occlusive effect of these carriers (Wissing and Müller 2002). Together with their adhesive properties these carriers appear as a suitable system to be investigated for the delivery of antifungals.

2. Investigations, results and discussion

From the regulatory point of view, the introduction of a new product to the pharmaceutical market has two aspects: the regulatory issues concerning the production line itself and finally the product. The production lines used for SLN and NLC are already in use for the production of emulsions for parenteral nutrition (Müller et al. 1995). They are accepted by the regulatory authorities and therefore *a priori* no major hurdles should occur. The production lines available are made of materials to be qualified and suitable for pharmaceutical production. The unit itself is able to be qualified and validated (Schneppe 1998; Müller et al. 2000). In addition, the product itself needs to fulfil the regulatory criteria, for example contamination from the production unit or contaminants being in an acceptable range. This is the case for the homogenizers used for SLN and NLC production, metal contamination (Fe) is typically below 1 ppm (Krause et al. 2000). The SLN and NLC are prepared by hot high pressure homogenization (HPH), which means the obtained final product is an aqueous dispersion. However, such an aqueous suspension of lipid nanoparticles is only in some cases the desired final formulation for the market. Creams, lotions or hydrogels will be more convenient formulations for the patient. A major advantage of SLN and NLC technology is that it a novel technology can be combined with these traditional topical dosage forms. Highly concentrated particle dispersions can be added during the production process of lotions, creams and gels or simply admixed to the formulations (Müller et al. 2005), as done in the present study. In this work, aqueous SLN and NLC dispersions were previously prepared and then dispersed in polyacrylate-based hydrogels in a following phase of product development.

2.1. Macroscopic appearance and pysicochemical characterization of the dispersions

Table 1 shows the composition of the drug-loaded SLN and NLC formulations developed for the present investigation. Concerning the macroscopic appearance of the dispersions, a milky appearance has been observed once they are prepared with approximately 15% to 20% of lipid phase. Ketoconazole-loaded lipid nanoparticles have been stored under light protection, and only at room temperature (25 °C) and at 4 °C, once they were shown to be sensitive under light exposure and high temperatures of storage (Souto and Müller 2005).

For the physicochemical characterization the zeta potential and particle size parameters of the lipid particles were monitored during one year and are shown in Table 2.

The physicochemical characteristics of the developed systems were quite different. Clotrimazole-loaded particles showed a narrow size distribution and higher stability during the evaluated storage time. On the contrary, ketoconazole-loaded particles showed higher long-term instability, despite the particles had higher zeta potential values. The higher zeta potential values are due to the charged stabilizer used, i.e. sodium deoxycholate. The SLN/Ke are stabilized simultaneously by steric and electrostatic forces. From these considerations they should possess rather a higher stability. However, the zeta potential is below the critical value (30 mV) to be sufficient for sole stabilization by electrostatic repulsion. At the same time, the steric barrier provided by poloxamer® 188 was obviously not sufficient to avoid particle growth over the period of one year.

The particle sizes of SLN and NLC were also analysed after their incorporation into hydrogels. In this case only Dynasan116-based SLN and NLC formulations were used for the preparation of semi-solid systems, which have been stored at three different temperatures for a period of three months. Table 3 shows the obtained results.

The semi-solid systems prepared showed a white appearance after dispersing the lipid particles in the hydrogels, which was maintained during the storage time at three different temperatures. The values of the zeta potential of clotrimazole-loaded SLN and NLC increased significantly. However, variations of particle size parameters could be discarded. This is in agreement with the theory, which says that increased zeta potential provides increased stability by electrostatic repulsion. Thus, no size increase should occur. The increase of zeta potential values can be explained by adsorption of negatively charged Carbopol molecules onto the surface of the lipid nanoparticles.

2.2. Assessment of chemical stability of entrapped drugs into the carriers

In this early phase of development an optimization of the drug loading capacity of the carriers seems to be necessary, because it affects the amount of drug that might penetrate according to the applied dose. Therefore, the stability of the drug incorporated into SLN and NLC was assessed by means of HPLC analysis during one year of storage at 4 °C and at 25 °C (Tables 4 and 5).

For both imidazole agents, their chemical stability was shown to be higher for NLC formulations in comparison to SLN formulations in all situations, i.e. storage temperature and shelf life. In addition, the shelf life at lower stor-

Table 1: Composition of the developed imidazole-containing SLN and NLC formulations % (m/m)

Composition	SLN/Co	NLC/Co	SLN/Ke	NLC/Ke
Drug	1.00% Clotrimazole	1.00% Clotrimazole	0.75% Ketoconazole	0.75% Ketoconazole
Solid lipid	19.00% Dynasan® 116	13.50% Dynasan® 116	14.25% Compritol® 888	10.50% Compritol® 888
Liquid lipid	–	5.50% Miglyol® 812	–	4.50% Tocopherol
Surfactant	5.00% Tyloxapol	5.00% Tyloxapol	2.50% Poloxamer® 188	2.50% Poloxamer® 188
Co-surfactant	–	–	0.125% sodium deoxycholate	0.125% sodium deoxycholate
Water ad	100%	100%	100%	100%

The symbols Co and Ke stand for samples containing clotrimazole and ketoconazole, respectively

Table 2: Zeta potential and particle size parameters of developed SLN and NLC formulations stored at three different temperatures (4, 25 and 40 °C) for one year

Size parameters	Age	°C	SLN/Co	NLC/Co	SLN/Ke	NLC/Ke
ZP (mV)	Day 1	4	−14.3 ± 0.1	−10.4 ± 0.1	−26.3 ± 0.9	−20.2 ± 1.5
		25	−12.3 ± 0.9	−8.4 ± 1.1	−19.8 ± 0.0	−19.3 ± 0.0
		40	−12.3 ± 1.6	−8.4 ± 1.1	–	–
	Year 1	4	−9.4 ± 0.5	−9.8 ± 0.5	−18.2 ± 0.3	−16.4 ± 1.1
		25	−10.8 ± 0.6	−11.5 ± 0.1	−10.6 ± 0.9	−1.6 ± 0.0
		40	−14.7 ± 0.5	−17.4 ± 0.7	–	–
PCS (nm)	Day 1	4	214.4 ± 0.3	188.5 ± 1.6	189.8 ± 4.8	326.5 ± 5.8
		25	201.4 ± 4.7	182.3 ± 1.9	217.1 ± 1.7	238.8 ± 9.4
		40	198.9 ± 2.2	188.6 ± 2.0	–	–
	Year 1	4	172.7 ± 6.8	204.2 ± 15.5	257.1 ± 0.058	1978.8 ± 151.669
		25	149.8 ± 4.2	188.1 ± 9.6	386.8 ± 3.200	>3 μm
		40	159.9 ± 4.2	183.6 ± 7.6	–	–
PI	Day 1	4	0.241 ± 0.024	0.170 ± 0.021	0.134 ± 0.042	0.213 ± 0.036
		25	0.220 ± 0.021	0.182 ± 0.021	0.202 ± 0.055	0.235 ± 0.041
		40	0.216 ± 0.030	0.231 ± 0.008	–	–
	Year 1	4	0.225 ± 0.085	0.190 ± 0.096	0.293 ± 0.011	0.876 ± 0.137
		25	0.213 ± 0.056	0.215 ± 0.120	0.292 ± 0.124	1.0 ± 0.0
		40	0.210 ± 0.076	0.218 ± 0.110	–	–
LD (50%) (μm)	Day 1	4	0.164 ± 0.002	0.183 ± 0.001	0.109 ± 0.007	0.247 ± 0.031
		25	0.273 ± 0.007	0.189 ± 0.003	0.223 ± 0.018	0.344 ± 0.013
		40	0.204 ± 0.001	0.174 ± 0.003	–	–
	Year 1	4	0.123 ± 0.001	0.255 ± 0.012	0.274 ± 0.016	39.130 ± 2.503
		25	0.097 ± 0.000	0.214 ± 0.003	2.482 ± 0.157	31.370 ± 1.432
		40	0.096 ± 0.001	0.193 ± 0.011	–	–
LD (90%) (μm)	Day 1	4	0.417 ± 0.005	0.420 ± 0.001	0.256 ± 0.054	0.412 ± 0.012
		25	0.561 ± 0.003	0.431 ± 0.001	0.439 ± 0.009	0.479 ± 0.004
		40	0.412 ± 0.001	0.407 ± 0.003	–	–
	Year 1	4	0.307 ± 0.004	0.447 ± 0.029	0.554 ± 0.012	154.500 ± 10.611
		25	0.170 ± 0.001	0.422 ± 0.021	4.561 ± 0.032	43.379 ± 8.323
		40	0.170 ± 0.002	0.407 ± 0.018	–	–

Table 3: Zeta potential and particle size parameters of developed polyacrylate hydrogels formulations stored at three different temperatures (4, 25 and 40 °C)

Size parameters	Age	Temp (°C)	SLN/Co-hydrogel	NLC/Co-hydrogel
ZP (mV)	Day 1	4	−31.9 ± 0.7	−24.5 ± 1.2
		25	−30.2 ± 0.5	−32.6 ± 3.2
		40	−25.6 ± 0.6	−30.7 ± 0.9
	Month 3	4	−26.3 ± 0.8	−19.8 ± 2.1
		25	−24.8 ± 0.3	−27.1 ± 1.4
		40	−20.8 ± 0.7	−24.9 ± 1.1
PCS (nm)	Day 1	4	243.5 ± 19.0	231.4 ± 10.1
		25	183.1 ± 6.6	211.9 ± 9.1
		40	269.7 ± 8.3	206.8 ± 8.5
	Month 3	4	241.2 ± 27.2	243.8 ± 27.7
		25	176.8 ± 9.3	206.1 ± 14.7
		40	258.4 ± 15.9	196.8 ± 13.3
PI	Day 1	4	0.321 ± 0.082	0.441 ± 0.013
		25	0.461 ± 0.074	0.445 ± 0.027
		40	0.210 ± 0.095	0.455 ± 0.027
	Month 3	4	0.350 ± 0.108	0.403 ± 0.104
		25	0.533 ± 0.032	0.428 ± 0.032
		40	0.264 ± 0.087	0.390 ± 0.049
LD (50%)	Day 1	4	0.199 ± 0.047	0.306 ± 0.009
		25	0.240 ± 0.011	0.371 ± 0.010
		40	0.307 ± 0.009	0.373 ± 0.016
	Month 3	4	0.164 ± 0.001	0.272 ± 0.001
		25	0.215 ± 0.004	0.359 ± 0.002
		40	0.300 ± 0.001	0.354 ± 0.001
LD (90%)	Day 1	4	1.599 ± 1.991	1.608 ± 0.078
		25	3.184 ± 0.144	2.181 ± 0.088
		40	0.519 ± 0.002	3.444 ± 0.192
	Month 3	4	0.434 ± 0.001	0.910 ± 0.020
		25	3.052 ± 0.013	2.025 ± 0.025
		40	0.515 ± 0.000	3.160 ± 0.028

Table 4: Amount of clotrimazole detected by HPLC analysis immediately after preparation (day 0), after one month and one year of storage at 4 °C and at 25 °C

Storage temperature	SLN/Co			NLC/Co		
	Day 0	Month 1	Year 1	Day 0	Month 1	Year 1
4 °C		87.6%	87.1%		94.9%	94.5%
25 °C	91.7%	84.2%	83.7%	98.7%	92.2%	91.7%

Data are expressed as percentage of clotrimazole detected in dissolution medium with regard to the theoretical amount used for the preparation of drug-loaded SLN and NLC. ($y = 71.963x - 0.025$; $R^2 = 0.9995$)

Table 5: Amount of ketoconazole detected by HPLC analysis immediately after preparation (day 0), after one month and one year of storage at 4 °C and at 25 °C

Storage temperature	SLN/Ke			NLC/Ke		
	Day 0	Month 1	Year 1	Day 0	Month 1	Year 1
4 °C		48.8%	44.8%		57.3%	54.1%
25 °C	62.1%	20.5%	15.6%	70.3%	32.0%	22.6%

Data are expressed as percentage of ketoconazole detected in dissolution medium with regard to the theoretical amount used for the preparation of drug-loaded SLN and NLC. ($y = 1.3064x + 1.4148$; $R^2 = 0.9999$)

age temperatures could also contribute to the increase of chemical stability. After one year of storage at 4 °C approximately 95% of clotrimazole and 54% of ketoconazole remained in the NLC formulations. These results impair the importance of matrix morphology and of storage temperature on chemical stability of imidazoles, especially for labile drugs such as ketoconazole. SLN are composed

of a more ordered matrix which might be related to drug expulsion during storage. Therefore, only approximately 84% of clotrimazole and 16% of ketoconazole were found in SLN formulations after one year of storage at room temperature. On the contrary, in the NLC matrix there are many imperfections which are able to accommodate the drug avoiding the decrease of drug loading during shelf life.

2.3. Correlation of the rheological parameters of semi-solid systems

The consistency of a dermatological formulation affects the release of a drug. Dispersions of lipid nanoparticles under shear flow experience different types of forces such as hydrodynamic forces (including the viscous drag force and particle-particle interaction through flow field induced by neighbouring particles), colloid chemical forces (including electrostatic, stearic, and London-van der Waals attractive forces), and forces due to gravitational, inertial, electro-viscous, and thermal or molecular collision effects. For the evaluation of these properties the applied stress, as well as the duration of the stress application, must be previously defined in order to avoid the destruction of the structure, so that measurements can provide information on the inter-molecular and inter-particle forces in the material (Martin 1993). To gain some insight into the influence of storage temperature and nature of lipid matrix, shear stress variation was recorded at a pre-defined shear rate between $0\ s^{-1}$ to $100\ s^{-1}$ for semi-solid systems composed of Dynasan®116 based lipid nanoparticles stored at three different temperatures (Figs. 1 and 2).

During all experiments, the temperature has been accurately maintained at 20 ± 0.1 °C. It is important that the temperature does not change during the rheological analysis to avoid obtaining false positive results in the test for thixotropy. From Figs. 1 and 2 it can be stated that all systems show thixotropy, which may be defined as an isothermal and comparatively slow recovery, on standing of a material, of a consistency lost through shearing. In complex systems such as SLN- and NLC-loaded hydrogels in which a loose network connects together the lipid nanoparticles, thixotropy proceeds from structural breakdown and re-aggregation. At a steady-state or at very low shear rates, the three-dimensional structure provides the system with some rigidity and the system behaves as a gel. Once the device starts stressing the sample, the structure begins to disrupt as the points of contact break and the particles start to align. The sample starts flowing, and its consistency decreases progressively with time as the shear stress and shear rate increase. Thus, the system suffers transformation from a gel-network to a sol-network. When the applied stress is decreased or removed, the internal structure of the system starts to reform but with a time lag, as the particles which build the network need a period in which contact to each other. Therefore, the shapes of rheograms which are obtained for thixotropic systems are highly dependent on the rate at which the shear conditions are changed and the time during which the sample is under the applied shear rate. Furthermore, the recorded flow curve will also depend on the previous shear history of the sample, including the way in which the viscometer has been loaded with the test sample. Using a rotational viscometer, in conjunction with an X−Y plotter, the shear rate has been steadily increased to obtain readings for the up curve. At a shear rate of $100\ s^{-1}$ (preset maximum value achieved after a set sweep time), the shear rate has been steadily decreased to obtain the down curve.

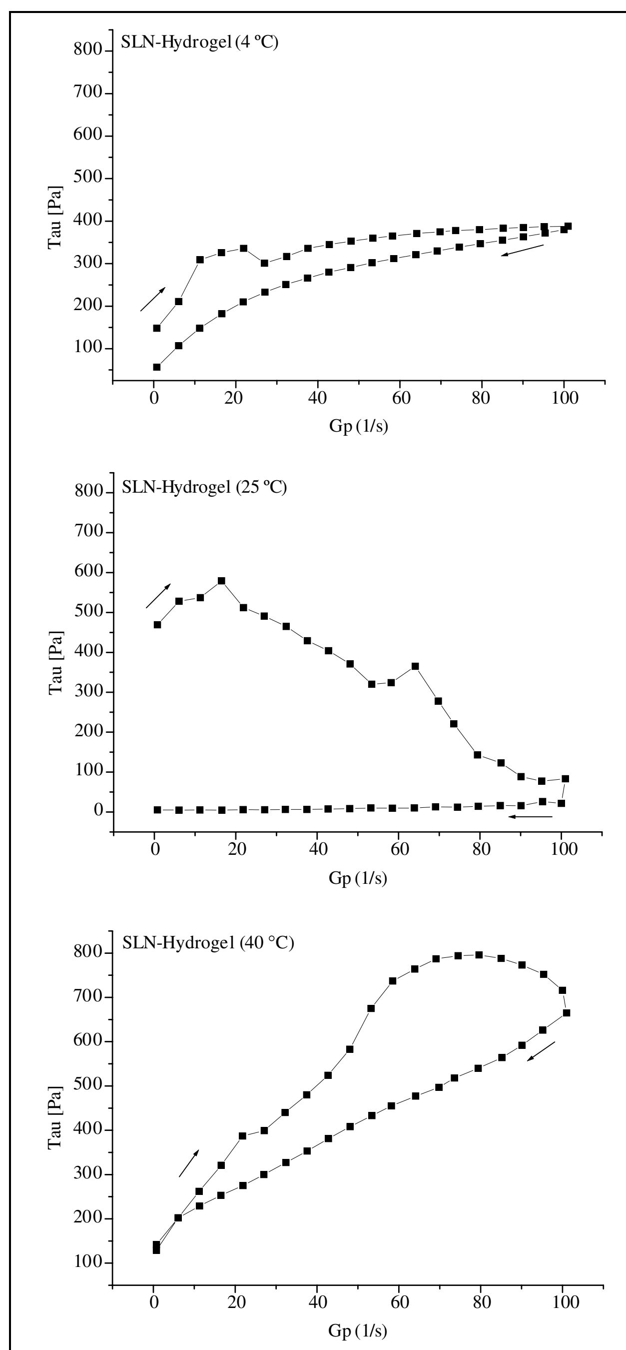

Fig. 1: Shear rate (1/s) versus shear stress [Pa] of SLN-loaded hydrogels after 1 week of storage at three different temperatures

Regarding the SLN-loaded hydrogels (Fig. 1), the hysteresis loops of the rheograms were significantly different depending on the storage temperature of the hydrogels. Samples stored at 25 °C and at 40 °C showed the highest areas of which measures the extent of thixotropy in the body under the conditions of the test. This means that there is a higher structural breakdown in those samples. At 4 °C the sample shows smaller differences between shear stress values at low shear rates, indicating a more ordered structure of particles entrapped in the gel network.

Concerning the NLC-loaded hydrogels (Fig. 2), also the up curve does not coincide with the down curve, meaning that the samples also show thixotropy, however with lower structural breakdown than SLN-loaded hydrogels, once the areas are much smaller. The observed differences of the registered shear stress values at low shear rates indicate

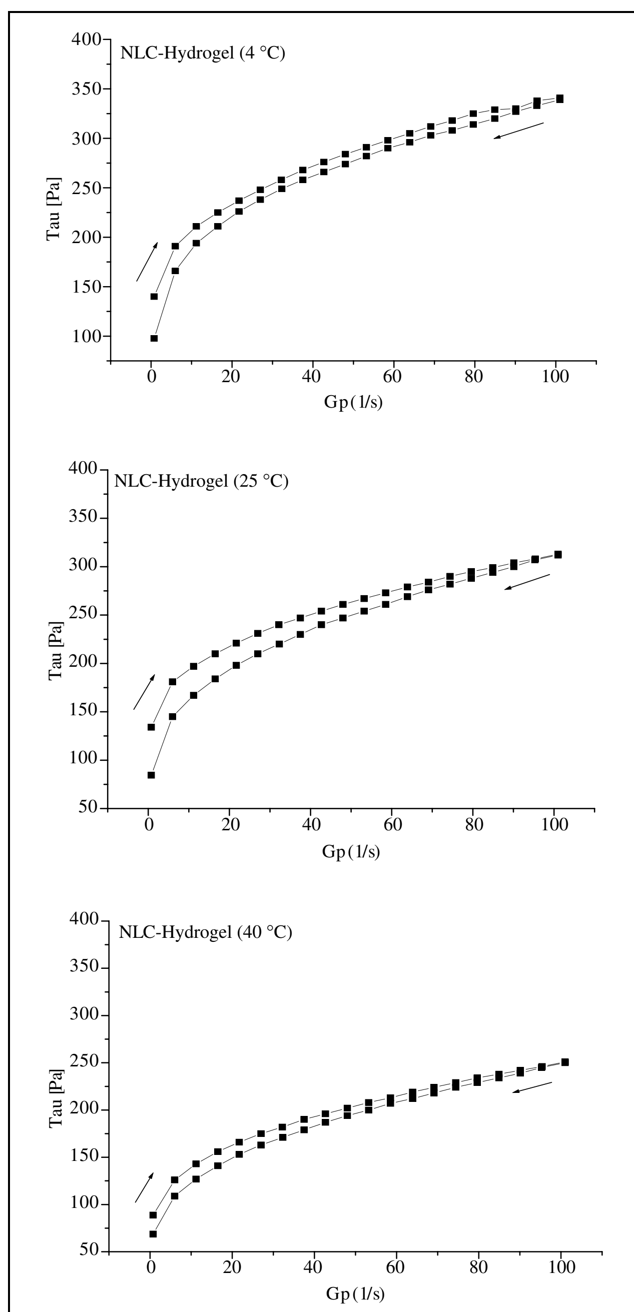

Fig. 2: Shear rate (1/s) versus shear stress [Pa] of NLC-loaded hydrogels after 1 week of storage at three different temperatures

that forces between entrapped particles and also the type of the interactions (electrostatic versus steric) between them control the flow behaviour of semi-solid systems at low shear rates.

This test also allowed the assessment of the variation of complex viscosity as a function of the applied shear rate. Table 6 depicts the resultant viscosity values collected from $0\ s^{-1}$ to $100\ s^{-1}$ (up curve) and back to $0\ s^{-1}$ (down curve). The differences on the rheological behaviour of both developed semi-solid systems (SLN and NLC-loaded hydrogels) could also be explained based on the lipid content of the formulations. According to Krieger (1972), the increase of lipid content in a formulation increases its viscosity described by the following equation:

$$\eta = \eta_0 \left(1 - \frac{\varphi}{p}\right)^{-[\eta]\,p} \qquad (1)$$

Table 6: Complex viscosity [mPa.s] of SLN- and NLC-based hydrogels stored at three different temperatures (4, 25 and 40 °C)

Semi-solid formulation	Shear rate variation	Storage temperature/Complex viscosity (mPa.s)		
		4 °C	25 °C	40 °C
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

Recorded during a shear rate interval from $0\ s^{-1}$ to $100\ s^{-1}$ (up curve) and back to $0\ s^{-1}$ (down curve)

where η is the viscosity of the dispersion, η_0 is the viscosity of the dispersion medium, $[\eta]$ is the intrinsic viscosity, ϕ is the volume fraction of the disperse phase and p is the volume fraction of disperse phase at most dense packing. It is clear that SLN-based systems have higher viscosity than NLC-based systems, once the former have higher solid lipid content (9.50% versus 6.75%). The semi-solid systems behave quite different according to the nature of the lipid matrix dispersed in the hydrogel. In spite of both systems show thixotropy (Figs. 1 and 2), the behaviour of SLN-based semi-solid systems was more dependent on the storage temperature than NLC-based semi-solid systems.

SLN-based hydrogels revealed higher dependency upon the applied shear rate than the NLC-based hydrogels, particularly the samples stored at 25 °C (Table 6). At the end of the assay the complex viscosity values became lower for the SLN-based hydrogels than for the NLC-based hydrogels.

2.4. Conclusions

In conclusion, it has been shown that lipid nanoparticles are suitable for chemically stabilized imidazole agents. However, lipid nanoparticles-based formulations need to be optimized concerning the entrapment of labile drugs, such as ketoconazole. Particularly for clotrimazole, an appropriate lipid nanoparticles-based system could be developed having physicochemical stability for at least one year. In aqueous NLC dispersions prepared with tripalmitin and Miglyol approximately 95% of clotrimazole was recovered after one year at room temperature. In the system developed for ketoconazole delivery (glycerol behenate and α-tocopherol) only 54% of drug was recovered after one-year at the same temperature. These results emphasize the effect of the nature of the lipid as well as of the drug in the chemical stability of the system. It is important to notice that ketoconazole is highly unstable. Compritol (glycerol behenate) could, however, slightly stabilize the drug specially when using the antioxidant as liquid lipid for NLC production.

Concerning the development of more suitable formulations for topical administration, loading of creams and gels with aqueous SLN and NLC dispersions might be an interesting approach to overcome some drawbacks related to rheological characteristics of such nanoparticle systems. The literature reports that lipid nanoparticles can be incorporated not only into simple o/w and w/o emulsions but also into multiple emulsions. There are two different ways of incorporating lipid nanoparticles into creams: (i) the cream is produced with reduced water content and a highly con-

centrated lipid nanoparticles dispersion is admixed to the cream; and (ii) a part of the water phase of the cream is replaced by a highly concentrated nanoparticle dispersion; then the usual production method of the cream is applied. In creams, the majority of lipid nanoparticles remains in the water phase, with only partial association to the surface of oil droplets being observed (Dingler et al. 1997). However, there was no dissolution of the lipid nanoparticles into the oil phase of the creams as shown by DSC measurements. The melting enthalpy of lipid nanoparticles in the cream remained unchanged during storage (Müller and Dinger 1998a). Thus, lipid nanoparticles have proved to be physically stable in creams, even when they are added to the water prior to the production of the cream (Müller and Dinger 1998b). To produce hydrogels, a highly concentrated lipid nanoparticle dispersion can be admixed to a gel with reduced water content. Alternatively, the gel-forming excipient can be added to the dispersion containing all ingredients of the final gel formation.

An improved method for preparation of semi-solid systems is the one-step-production of topical lipid nanoparticle hydrogels. Admixing SLN or NLC to hydrogels or replacing a part of the water phase limits the total amount of lipid nanoparticles that can be incorporated in such a semi-solid system. The results presented in this paper show systems having thixotropic properties, which depend on the storage temperature and on the nature of the dispersed lipid phase. Samples stored at 4 °C and particularly NLC-based formulations showed to be more suitable for topical purposes.

3. Experimental

3.1. Materials

Clotrimazole was supplied by Caelo GmbH (Hilden, Germany) and ketoconazole was a kind gift from Chemo Iberica S.A. (Madrid, Spain). Glyceryl tripalmitate (Dynasan®116) was obtained from Sasol (Witten, Germany) and glyceryl behenate (Compritol®888 ATO) from Gattefossé GmbH (Weil am Rhein, Germany). The liquid lipids Miglyol®812 and α-tocopherol and the emulsifying agent Tyloxapol® (4-(1,1,3,3-tetramethylbutil)-phenol with ethylene oxide and formaldehyde) were purchased from Sigma-Aldrich (Deisenhofen, Germany). Poloxamer®188 was supplied by BASF AG (Ludwigshafen, Germany) and sodium deoxycholate by Fluka (Buchs, Switzerland). Carbopol®934 (polyacrylate) was purchased from BF Goodrich, (Ohio, USA). The water used in all experiments was Purified Water (European Pharmacopoeia, 4th ed.) obtained from a MilliQ Plus, Millipore system (Schwalbach, Germany).

3.2. Preparation of SLN and NLC

Lipid nanoparticles were prepared by hot high pressure homogenization (HPH) developed by Müller and Lucks (1996), a methodology that attributes the decrease of particle size to cavitation and turbulences leading to series in the nanometer range. Briefly, a pre-emulsion was prepared by melting the lipid components at a temperature 5–10 °C above the melting point of the solid lipids (Dynasan®116 > 63 °C and Compritol®888 ATO > 69 °C) and dispersing this melt under high-speed stirring in a hot aqueous surfactant solution of identical temperature using an Ultra-Turrax (IKA, Staufen, Germany). The obtained pre-emulsion was homogenized at the same temperature using a Micron LAB 40 (APV Homogenizers, Lübeck, Germany) applying 500 bar and three homogenization cycles. After homogenization the prepared o/w nanoemulsion was cooled at room temperature, the lipid recrystallized forming the aqueous SLN or NLC dispersions. For the production of the drug-loaded formulation, a specific amount of lipid phase (5% m/m) has been replaced by drug in the formulation previously to the preparation of the pre-emulsion. The production has been performed in the same way as the drug-free lipid nanoparticles.

3.3. Zeta potential and particle size analysis

Zeta potential measurements were performed in distilled water ($n = 3$, standard deviation < 5%) adjusted to a conductivity of 50 μS/cm by addition of NaCl (Müller 1996), using a Zetasizer IV (Malvern Instruments, UK). The electrophoretic mobility was converted to a zeta potential using the Helmholtz-Smoluchowski equation. This process was done by the software included within the system. Each SLN and NLC formulation was measured five times in triplicate over a period of one year. The mean particle size was assessed by photon correlation spectroscopy (PCS) also using the Malvern Zetasizer IV (Malvern Instruments, UK), which gives the so-called "z-average" as the size of the bulk population and the polydispersity index (PI), i.e. a measure for the width of the particle size distribution. The laser diffractometry (LD) analysis was performed using the Coulter®LS 230 (Beckmann-Coulter, Krefeld, Germany) to measure the diameters LD 50% and LD 90% of the volume distribution of the developed SLN and NLC before and after incorporation into polyacrylate hydrogels. Prior to particle size analysis the semi-solid SLN and NLC formulations were diluted with double-distilled water by shaking to weak opalescence.

3.4. Preparation of polyacrylic acid hydrogels

In order to prepare the hydrogel formulations, the gel-forming polymer (Carbopol®934) was dispersed in distilled water containing 10% of glycerol adjusting the pH to 6.5 with Trizma®Pre-set crystals pH 7.0 (tris(hydroxyl-methyl) aminomethane) from Sigma-Aldrich (Deisenhofen, Germany). 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. Methyl paraben (Sigma-Aldrich, Deisenhofen, Germany) was used as preservative of the semi-solid systems. It has been added to the water phase during the preparation of the hydrogels.

3.5. HPLC analysis

HPLC analysis was performed according to USP XXIV using a Kroma System 2000 (Kontron Instruments, Berlin, Germany) running in the isocratic modus. The system consisted of a HPLC pump 220, an Auto-sampler T360 and a UV detector 430. A water bath Haake W90 (Haake, Karlsruhe, Germany) was used for the control of the temperature. UV detection was performed using a cartridge column Nucleosil-120 C18 (3 μm) having a length of 100 × 4 mm ID (Knauer, Berlin, Germany). As test conditions a mobile phase consisting of methanol/water 8 : 2 (v/v) was used, with an injection volume of 1 μl, flow rate of 1.5 ml/min, pressure of 14.8 MPa, at room temperature. The Kontron HPLC software was used for the analysis of the results, i.e. integration of the peaks. The retention time for clotrimazole was 11.4 min and for ketoconazole was 9.3 min. For both drugs, the wavelength of maximum absorption was 254 nm and 220 nm, respectively. Concentrations of clotrimazole and ketoconazole were determined against appropriate calibration curves.

3.6. Rheological analysis

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.

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 stress [Pa] as a function of the shear rate [s^{-1}]. This study started applying a shear rate of 0 s^{-1} up to a maximum of 100 s^{-1} and the resulting shear stress [Pa] was measured.

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