6 CONCLUSIONS

The success of a new developed pharmaceutical formulation is related to the fact that it is able to deliver the active substance to the target organ at therapeutically relevant levels, with negligible discomfort and side effects, increasing the patient compliance to the therapeutics. Regarding this respect, the route of administration is of major relevance. Topical administration of active substances offers several attractions compared to traditional routes, e.g. it avoids the hepatic first-pass metabolism, it has the potential of long-term controlled release with avoidance of the topical peak-through plasma profiles associated with frequent dosage regiments, it is easy to administer and it has the possibility for treatment withdrawal if that is the case [374]. Nevertheless, topical administration route is the main target when the skin itself is the damaged organ.

Despite the substantial potential of transdermal and dermal drug delivery, only relatively few drugs are yet commercially available as topical formulations. The main limitation lies in the barrier function of the skin, which is considered one of the most impermeable epithelia of the human body to exogenous substances. Therefore, the major concerns when developing a new formulation for topical administration of active substances is to provide a sufficient increase in drug penetration into the skin, without inducing significant irreversible alterations to the skin barrier functions.

Conventional formulations intended for topical and dermatological administration of drugs, such as creams, foams, pessaries and gels, are considered to reside for a relatively short period of time at the targeted site. During the recent decades several studies have suggested that novel drug delivery systems based on lipid nanoparticles have the potential of increasing cutaneous drug delivery of both hydrophilic and lipophilic drugs compared to the above mentioned conventional vehicles. These lipid-based systems, well kown as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), are composed of a solid matrix of physiological nature which might thereby fulfil the many promising aspects of the topical route and should in addition provide a controlled and prolonged release of drugs. Their lipid based composition and small particle size contribute to the enhancement of penetration of compounds incorporated into these particles. With aging the stratum corneum barrier becomes fragile and the recovery is delayed [375]. Lipids play a crucial role for the water impermeability barrier function of the skin. Thus, the use of lipids as drug delivery systems

has synergistic effect once they can improve skin barrier homeostasis as the same time as they deliver the drug for pharmacological treatment. To improve the efficiency of these systems, they rely on their occlusive nature to increase the permeability of the active substances.

The aim of the present thesis has been to review the recent literature with respect to the use of SLN and NLC as colloidal carriers for several chemically different drugs, and to discuss the influence of their composition, components and structure on the drug delivery potential for antifungal agents as model drugs. In order to maintain locally a certain drug level and enable lower dosing frequency and lower amount of administered drug, imidazoles-loaded SLN and NLC formulations have been proposed in the present thesis as newly controlled and prolonged delivery systems.

In the present work it has been shown that lipid nanoparticles are suitable for chemically stabilize imidazole agents, i.e. clotrimazole and ketoconazole. However, lipid nanoparticles-based formulations need to be optimized concerning the entrapment of labile drugs, such as ketoconazole. Based on experiences with hard fats nanoparticles, two different types of lipid nanoparticles have been investigated concerning their suitability for imidazole antifungal agents. The structure and mixing behaviour of these particles have been characterized and practical implications on controlled release properties have been tested, in comparison to commercial formulations containing one model drug in the same concentration as the developed SLN and NLC formulations. Based on solubility studies, a suitable oil has been successfully incorporated in a matrix of a solid long chain acylglycerol for the production of NLC. The crystal order was therefore disturbed however, the carrier remained solid and the oil inside the particle remained in a liquid state.

Concerning the development and characterization of clotrimazole-loaded SLN and NLC dispersions, appropriate lipid nanoparticles-based systems, composed of Dynasan®116 (tripalmitin) as solid lipid and Miglyol®812 as oil, could be developed having physicochemical stability of at least one year. Immediately after production (day 0) SLN and NLC revealed a mean particle size lower than 250 nm and they remained in their colloidal size range (< 1 μm) during two years of storage with ZP values between -20 mV and -10 mV. By imaging analysis SLN showed their spherical shape which was transformed into a platelet-like structure during storage. The high chemical stability of clotrimazole to be processed by HPH has been confirmed by thermal analysis (TGA and DSC) and by X-ray diffraction (WAXS). DSC and WAXS studies have shown that clotrimazole was maintained entrapped and solubilized in the lipid matrix of SLN and NLC, being the selected lipids suitable

materials for this model drug. By HPLC analysis it was confirmed the high loading capacity of these lipid nanoparticles in comparison to a reference emulsion of identical composition. Immediately after production the percentage of drug recovered was 91.7% for SLN and 98.7% for NLC. During shelf life, only a slight decrease of the amount of drug recovered to 90% for SLN and 95% for NLC has been observed after two years at room temperature. From the reference emulsion under the same storage conditions only 50% of drug has been recovered. The storage at 4ºC slightly increased the percentages of drug recovered from the systems. The rheological characterization of the aqueous SLN and NLC dispersions revealed systems with elastic properties, which are dependent on the structure of the lipid matrix, i.e. presence/absence of oil. The storage conditions, i.e. temperature and shelf life, also influenced the viscoelastic properties of those systems. In general, the storage at room temperature increased the magnitude of the viscoelastic parameters. Nevertheless, the systems maintained their predominant elastic component.

According to these results, it seems that Dynasan®116-based SLN and NLC stabilized with the non-ionic surfactant Tyloxapol® are promising vehicles for clotrimazole. In order to optimize a suitable topical formulation for the delivery of this active substance, the developed aqueous SLN and NLC dispersions have been further entrapped into Carbopol®934-based hydrogels and these semi-solid systems have been physicochemically characterized as well.

In the systems developed for ketoconazole delivery, Compritol®888 ATO (glycerol behenate) and α-tocopherol have been selected as solid and liquid lipid, respectively for SLN and NLC production. The lipid nanoparticles have been stabilized using a suitable surfactant/co-surfactant system composed of Poloxamer®188 and sodium deoxycholate. Immediately after production SLN and NLC showed a mean particle size between 190 nm and 360 nm. Their colloidal size range was dependent on the storage conditions, i.e. temperature and light exposure. Particle sizes higher than 3 μm have been measured. Due to the labile character of ketoconazole, samples were not stored at 40ºC. Dark conditions could only optimize the chemical stability of drug. Particles remained negatively charged, with the exception of ketoconazole-loaded NLC stored at 25ºC. By imaging analysis SLN showed their irregular spherical shape one week after production. The presence of drug crystals outside the lipid matrix was observed after one year of storage. The lower chemical stability of ketoconazole has been assessed by thermal analysis and by X-ray diffraction. DSC and WAXS studies have shown that ketoconazole was first solubilized in the lipid matrix of glycerol behenate-based SLN and NLC, however, due to the fact that this lipid crystallizes mainly in the β'

polymorphic form, and being somehow more unstable than tripalmitin, the drug is expelled from the lipid matrix with storage time. Although being composed of a mixture of mono-, di- and triacylglycerols, it has been shown that Compritol®888 ATO consists of a very small amount of α form which tends to disappear under thermal stress due to its high thermodynamic instability. It crystallizes mainly in its β' modification, which is also highly sensitive to high temperatures. However, using an appropriate surfactant composition, SLN and NLC could be obtained by HPH. By HPLC analysis the loading capacity of glycerol behenate-based SLN and NLC for ketoconazole has been assessed, in comparison to a reference emulsion of identical composition. Immediately after production the percentage of drug recovered was 62.1% for SLN and 70.3% for NLC. During shelf life, a decrease of the amount of drug recovered to 21.7% for SLN and 37.7% for NLC has been observed after two years at room temperature under dark conditions. After two years at the same storage conditions only 10.2% of drug has been recovered from the reference emulsion. The storage at 4ºC slightly increased the percentages of drug recovered from the systems, i.e. to 15.6%, 41.9% and 51.4% for the reference emulsion, SLN and NLC, respectively. 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®888 ATO (glycerol behenate) could, however, slightly stabilize the drug especially when using the antioxidant (α-tocopherol) as liquid lipid for NLC production. The rheological analysis of the aqueous SLN and NLC dispersions differed significantly whether the matrix is SLN or NLC. Aqueous SLN dispersions are composed of a more weak structure, which cannot be totally linked to elastic-like properties, whereas aqueous NLC dispersions showed a tan δ lower than 1 during the applied frequency range, being therefore more elastic-like systems. The storage conditions, i.e. temperature, light exposure and shelf life, as well as the presence of drug also influenced the viscoelastic properties of those systems.

The ideal candidate formulation for the controlled delivery of antifungals to the topical and dermatological route should exhibit a variety of characteristics. These include ease of application into the skin and retention within its packaging, controlled drug release, ease of manufacture and eventual clearence from the application site. In addition, a vehicle for incorporation of SLN and NLC should provide as well adequate pH value, stability and rheological characteristics. Due to good physical, chemical and biological properties of hydrophilic polymer gels, a polymer of acrylic acid (Carbopol®934) has been chosen as an appropriate vehicle for incorporation of Dynasan®116-based SLN and NLC dispersions.

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 nanoparticles systems. The scientific 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 concentrated lipid nanoparticle 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. 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. 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. 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 semi-solid system.

In the present thesis, the compatibility of lipid nanoparticles, namely Dynasan®116-based SLN and NLC, with Carbopol®934-based hydrogels has been proven. Therefore, it is worthy to use these gels as vehicles for incorporation of the above mentioned lipid nanoparticles. SLN and NLC have been dispersed into hydrogels under conditions which preserve their original structure and particle size. By PCS analysis a mean particle size lower than 260 nm was obtained and by LD it was detected that 90% of the particles remained lower than 3 μm. The porosity of the hydrogel network allowed therefore intact lipid nanoparticles remain stable for at least three months (PI < 0.550) at three different temperatures under optimized packaging. The particles remained negatively charged during this shelf life and storage conditions. After incorporation into the hydrogel network DSC and WAXS analyses demonstrated that SLN could maintain their solid lipid character (RI > 70% at 25ºC) during storage. On the contrary, NLC revealed an amorphous matrix with a very low RI, between 4-

6%, during storage at 25ºC. For these latter systems the RI could be increased when stored at 4ºC.

For the mechanical characterization of semi-solid formulations, empty Carbopol hydrogels and those containing SLN and NLC were tested for basic rheological and texture properties. The results show systems having thixotropic properties, which depend on the storage temperature and on the nature of the dispersed lipid phase. Samples stored at 4ºC and particularly NLC-based formulations showed to be more suitable for topical purposes. In addition, by rheological and texture analyses, semi-solid formulations were shown to have a pseudoplastic-like behaviour typical from commercially available topical and dermatological formulations, as well as good adhesive properties and appropriate consistency and strength intended for administration purposes. By correlating the empirical tests used for the analysis of the texture with the rheological properties described for the semi-solid formulations it could be demonstrated that these formulations follow the Bingham model (R^2 = 1), which defines the shear-thinning and pseudoplastic-like behaviour. A schematic three-dimensional model network has been proposed for the entrapment of lipid nanoparticles into Carbopol hydrogels.

Comparison studies of the *in vitro* release profile of clotrimazole from aqueous SLN dispersions with commercial available clotrimazole creams (Canesten® cream and Fungizid® ratiopharm cream) have been performed using Franz diffusion cells. The controlled release ability of the lipid nanoparticles has been confirmed, i.e. the obtained drug flux for SLN was approximately 46 μg/cm^2/hr, whereas for Canesten® and Fungizid® ratiopharm creams the obtained values were, respectively, 86 μg/cm^2/hr and 90 μg/cm^2/hr. It could also be mathematically demonstrated that SLN displayed a clotrimazole release rate based on the Higuchi model (R^2 = 0.97527). This model describes a first burst effect of drug followed by a slow and continuous release. In the first 2 hr approximately 17% of incorporated drug has been released and in the following 8 hr the cumulative amount of drug released increased to 27%.

Dynasan®116-based SLN dispersions have also been incorporated into an o/w hydrophilic cream (20% SLN m/m) intended for topical administration and their particle size, as well as rheological properties have been analysed. By LD analysis the inner oil droplets of the cream having approximately 6 μm were measured. Under polarised light no major SLN aggregation has been detected. SLN remained in their solid state after their incorporation in the hydrophilic cream. Flow investigations showed that the increase of SLN concentration to 30%

and 40% resulted in shear-thinning and pseudoplastic systems with yield values of 28 Pa and 39 Pa, respectively.

To summarise, the aqueous SLN and NLC dispersions, as well as the semi-solid formulations developed and described in the present thesis were simple to manufacture and have shown suitable release and mechanical properties for topical and dermatological purposes.

A significant development in the pharmaceutical technology field is that newly drug delivery systems are increasingly becoming a combination of drug formulations and sophisticated devices. This approach brings together technologies from both science and engineering, leading to interdisciplinary challenges. The constant research on newer, more sophisticated drug delivery systems and the increased competition among pharmaceutical companies are leading to more collaboration, partnering with and acquisitions of drug delivery companies in the short-term. These partnerships will open new markets, using a broad range of emerging technologies. In a quest for increased market shares and revenues, companies will have patient compliance and convenience.

Despite the critical role that process design plays in the development of new drug systems, it is important to recognize that it does not occur alone. In addition to process design, successful scale-up depends on a number of factors that are distinct yet intimately related, including the characteristics of the bulk drug and the excipients, formulation and dosage form, and process control. Only when each of these factors is properly addressed can the product development program result in a quality product that can be manufactured consistently with a validated procedure.