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Rheological and *in vitro* release behaviour of clotrimazole-containing aqueous SLN dispersions and commercial creams

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Clotrimazole is a wide spectrum local imidazolic antifungal agent used in several dermatological creams, having e.g. 1% (m/m) such as Canesten® and Fungizid-ratiopharm® cream. In the present work, a new system based on solid lipid nanoparticles (SLN™) containing the identical concentration of drug has been developed. A comparative study between the rheological properties of the referred creams and the developed aqueous SLN dispersions was carried out. The influence of incorporation of SLN in a standard hydrophilic cream on its flow curves was also assessed. In addition, the release of clotrimazole from the two commercial creams, as well as from aqueous SLN dispersions was studied. Concerning the rheological investigations, all tested commercial creams revealed very low shear rates and no yield points. Lipid nanoparticles having a mean diameter of approx. 200 nm have been incorporated into a hydrophilic cream, in a concentration of 20%, 30% or 40% (m/m). The hydrophilic cream containing 20% of SLN showed a dilatant-like character; however, increasing the percentage of incorporated lipid nanoparticles to 30% and 40% the formulation changed to a more pseudoplastic character, showing yield values of 28 Pa and 39 Pa, respectively. For *in vitro* release studies, Franz diffusion cells with a cellulose acetate membrane were used to measure the release of clotrimazole from two different commercial formulations in comparison to the aqueous SLN dispersion. After 6 h the amount of drug released was higher than 48% when delivered from both investigated commercial formulations and not higher than 25% when delivered from the aqueous SLN dispersion. The percentage of drug released determined after 24 h was more than 50% for Canesten® cream and Fungizid-ratiopharm® cream and not higher than 30% for the developed SLN formulation showing its prolonged release character.

1. Introduction

Clotrimazole or (1-2-chlorphenyl-diphenylmethyl)-1-4-imidazole (CZ) is an azole-type antifungal agent that is known to have topical activity against pathogenic dermatophytes (Greenberg 2002) and yeasts (Henry 2000). It has a local antifungal effect, especially on the mucous membranes and, therefore it is widely used for the treatment of mycotic infections of the genitor-urinary tract (Kast 2002). It shares with econazole and miconazole the first choice status for topical treatment of Tinea pedis, Tinea crusis and Tinea corporis due to any of the afore-mentioned organisms, candidiasis due to *Candida albicans* and Tinea versicolor due to *Pityrosporon obiculare*, as well as for local treatment of oropharyngeal candidiasis (Vazquez 2000).

However, the clinical use of CZ has some practical disadvantages mainly due to poor water solubility and side effects, such as allergic skin rash and urticaria. Therefore, the development of a novel type of delivery system could lead to significant advantages in the clinical use of this drug. With the aim of using innovative ways to administer CZ, possible overcoming or alleviating the solubility and allergic problems associated with its usage, we compared topical formulations based on solid lipid nanoparticles (SLN™) and commercial formulations containing this antifungal agent with regard to rheological properties and release profiles.

There are two essential features that justify the attention devoted to lipid nanoparticles, such as SLN: (i) their small particle size which ensures a close contact to the skin and mucosa (Jenning 1999), and (ii) their controlled release behaviour that makes possible a therapeutic effect of the drug for longer periods of time, thus increasing the amount of encapsulated agents penetrating into this route (Jenning 2000). Moreover, as lipid nanoparticles they are adhesive showing occlusive (Jenning 1999; Wissing 2001; Wissing 2003) and targeting properties particularly for lipophilic drugs (Maia 2000). However, the stability of these colloidal systems can be compromised under the drastic environmental that they are exposed after topical administration (Müller 2002).

The specific aim of the present work was, firstly to assess the rheological properties of a hydrophilic cream that has been used as vehicle for SLN, in comparison to commercial creams available on the pharmaceutical market and, secondly to investigate the release profile of CZ from the aqueous SLN dispersions comparing to the same commercial creams mentioned above. For these purposes, tripalmitine SLN containing 1% (m/m) of CZ were prepared and incorporated in a hydrophilic cream, i.e. Unguentum emulsificans aquosum, in order to obtain a final formulation containing 0.5% of drug.

2. Investigations, results and discussion

2.1. Characterization of the developed formulations

The solid character of SLN after dispersion into the cream has been confirmed by differential scanning calorimetry analysis (data not shown). Table 1 shows the obtained particle size parameters of SLN before and after incorporation into the hydrophilic cream. Previously to particle size analysis of SLN incorporated into hydrophilic cream, samples were diluted with bidestilled water to weak opalescence, a procedure that did not alter the size distribution obtained as could be verified beforehand. As shown in Table 1, a mean particle size of approx. 200 nm could be obtained for clotrimazole-loaded SLN prepared with 20% of glyceryl tripalmitate in the suspension. LD measurements revealed that 95% of the nanoparticles were smaller than 650 nm, showing a considerable small particle size and a narrow distribution. With regard to incorporation of SLN into the hydrophilic cream, size analysis by LD led to a superposition of the size distribution of the emulsion droplets and the SLN. LD data of the SLN-containing o/w hydrophilic cream are also shown in Table 1 (right). Due to the relatively weak scattering intensity of 200 nm particles versus 2–5 μm droplets ($I \approx R^3/r^6$), separate analysis of the SLN inside of the emulsion was not possible. In a previous study, a rather complex separation procedure was developed for a special cream system (Dingler 1998). This cannot be transferred directly to any of these creams. Therefore, a simple approach was taken, i.e. transferring the microscopic analysis method for parenteral fat emulsions (Müller 1993). The samples were placed undiluted on a cover slip and analysed microscopically to screen for aggregates of approx. 1–5 μm. In polarised light irregularly shaped crystalline SLN aggregates can be differentiated from spherical oil droplets. No major aggregation was observed (i.e. less than 2 particles $>$ 1 μm/microscopic field).

2.2. Rheological behaviour of the formulations

Creams, such as hydrophilic cream, are dispersions of two immiscible liquids, the oil of the internal phase and water (external or continuous phase). The presence of emulsifying molecules at the interface of oil and water decreases the interfacial free energy forming a homogeneous and stable cream. Incorporation of lipid nanoparticles might affect the stability or structure of the semi-solid system. Rheological behaviour of such systems provides qualitative and quantitative information of the internal structure of the cream. In addition, it relates also to the spreading/application behaviour during administration to the skin (e.g. yield value should not be too low). Therefore, the prepared SLN-containing cream has been evaluated in comparison to commercial creams such as Canesten® and Fungizid-ratiopharm® cream. Fig. 1 represents the plots of shear rate as a function of shear stress for commercial creams selected for the present study, as well as hydrophilic cream containing 20% (m/m) of aqueous SLN dispersion.

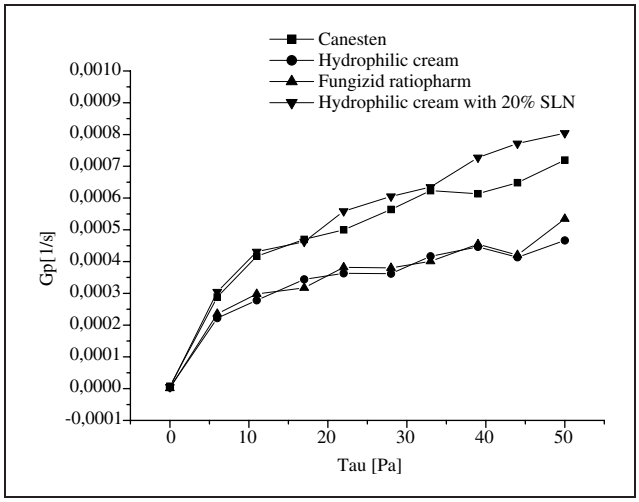

Fig. 1: Shear rate as a function of shear stress of Canesten® cream, Fungizid-ratiopharm® cream, hydrophilic cream and hydrophilic cream containing 20% (m/m) of aqueous SLN dispersion

As observed in Fig. 1, every formulation behaved similarly to dilatant systems, revealing extremely low shear rates with the increase of the shear stress. The most linear shear rate under increasing shear stress was Canesten® cream. The presence of 20% of SLN into hydrophilic cream slightly increased the recorded values of shear rate.

Fig. 2 shows the influence in the shear rate of the increase of SLN concentration into hydrophilic cream. Incorporation of 30% and 40% of aqueous SLN dispersion into hydrophilic cream resulted in shear-thinning and pseudoplastic semi-solid systems, revealing the presence of a yield value in both situations. The yield value of conventional creams is usually 9 Pa; however, the tested commercial creams did not show this typical behaviour (Fig. 1). Incorporation of 30% of SLN led to a yield value of 28 Pa and the presence of 40% of SLN increased this latter value to 39 Pa (Fig. 2). At steady-state, lipid nanoparticles confer some rigidity to the system but, as the flow starts the structure begins to break down and viscosity decreases. Viscoelastic and shear-thinning properties, as well as the presence of a yield value are optimal properties for prediction of the stability of semi-solid systems such as creams, since these later maintain their consistency during

Table 1: PCS diameter, PI and LD diameters of the developed formulations

Evaluated parameters	Pure aqueous SLN dispersion	Hydrophilic cream	SLN-containing hydrophilic cream
PCS diameter (n = 10)	201.4 nm ± 4.7	–	–
PI (n = 10)	0.220 ± 0.022	–	–
d50% (n = 3)	0.273 μm ± 0.007	1.312 μm ± 0.106	1.932 μm ± 0.039
d90% (n = 3)	0.561 μm ± 0.003	3.891 μm ± 0.271	4.529 μm ± 0.161
d95% (n = 3)	0.647 μm ± 0.005	4.754 μm ± 0.156	5.773 μm ± 0.186

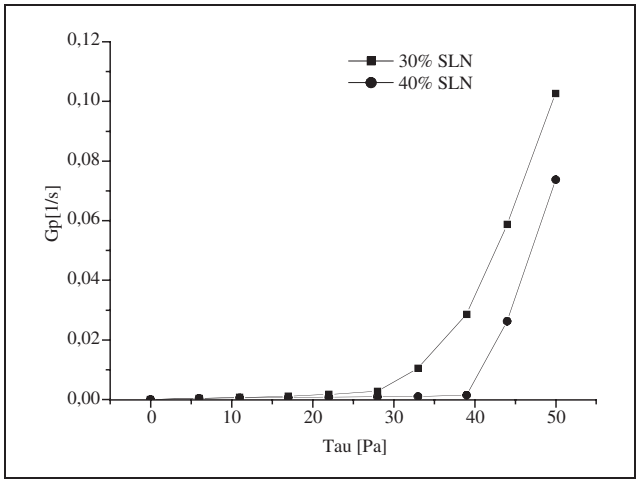

Fig. 2: Shear rate as a function of shear stress of hydrophilic cream containing 30% and 40% (m/m) of aqueous SLN dispersion

storage time and are afterwards easy to apply (Försyer 1997). These results are probably due to the creation of a kind of gel network structures by SLN. Such a model was proposed for aqueous lipid particle dispersions with 30% to 50% of lipid content. The particles form a network similar to an Aerosil® gel, like peals on a necklace (Junginger 1992). The increase of SLN concentration had clearly the highest yield value point (Fig. 2). Commercial creams and SLN-free hydrophilic cream did not show yield points at the applied shear stress range.

The observed differences between SLN-free and SLN-loaded formulations at low shear rates indicate that interparticle forces, as well as the type of the interactions between lipid nanoparticles (i.e. electrostatic versus steric), control the flow behaviour of such systems. During shear flow investigations, SLN can come closer and form pearl-like structures due to van der Waals attractive forces. Higher resistance to flow and higher degree of shear-thinning behaviour observed in Fig. 2 are due to breakdown of the structures with imposed shear stress. The presence of compact, organized and strong pearl-like network structures inside the hydrophilic cream can have significant beneficial effects on the stability and rheological properties of such topical formulations.

2.3. In vitro release profile of clotrimazole

For the design of the release studies static Franz diffusion cells have been chosen once they are more suitable and less time consuming than dialysis bags when the aim is to assess the release of drugs from colloidal carriers. In addition, in these devices the release occurs via passive diffusion through the lipid particles to the interface with the diffusion membrane and across this membrane (treated with isopropyl myristate) placed between the donor compartment and acceptor medium. Furthermore, the acceptor medium is under constant stirring and it allows withdrawing of samples at specific intervals and the measured amount can be plotted against the time. However, the choice of the acceptor medium can create problems in particular if this phase in supposed to be representative of the skin. Therefore, a solution of 100 mM acetate buffer, pH 6.0 with 35% (v/v) of dioxane has been chosen, where CZ shows good solubility ($> 70\%$ m/v).

The amount of CZ penetrated into the acceptor compartment was determined against an appropriated linear calibration curve at 243 nm. The assay was linear ($R^2 > 0.996$) in the concentration range of 25 and 150 μg/ml. According to Fick's second law of diffusion, the total amount of CZ (Qt) appearing in the acceptor solution in time t is expressed as follows (Mei 2003):

$$Q_t = AKLC_0 \left[\left(\frac{D_t}{L^2}\right) - \left(\frac{1}{6}\right) - \left(\frac{2}{\pi^2}\right) - \sum \left(\frac{(-1)^n}{n^2}\right) \exp\left(\frac{D^n 2\pi^2 t}{L^2}\right) \right] \quad (1)$$

where A is the effective diffusion area, C_0 is the initial concentration of drug which remains constant in the semi-solid formulation, D is the diffusion coefficient, L is the thickness of the diffusion membrane and K is the partition coefficient of the drug between membrane and formulation. At steady-state, Eq. (1) can be simplified in the following equation:

$$\frac{Q_t}{A} = KLC_0 \left[\left(\frac{D_t}{L^2}\right) - \left(\frac{1}{6}\right) \right] \quad (2)$$

The flux of drug through the diffusion membrane is calculated as the amount of diffused drug divided by A versus time. Therefore, from Eq. (2) the flux, J, can be determined as follows:

$$J = C_0 \frac{KD}{L} = C_0 K_p \quad (3)$$

where K_p is the permeability coefficient.

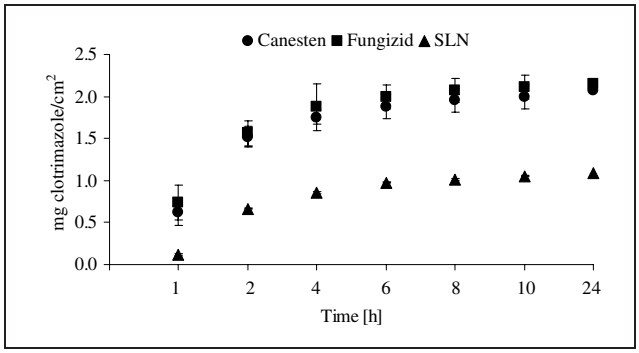

Fig. 3: *In vitro* release of CZ from commercial formulations and aqueous SLN dispersion (20% SLN) in 100 mM acetate buffer, pH 6.0 with 35% (v/v) of dioxane, at 37 °C

Fig. 3 shows the amount per cm^2 of CZ released from the studied formulations as a function of time. The release profile was followed for 24 h and the recorded values correspond to the average of three independent tests, i.e. three Franz cells for each tested formulation. It is clearly visible that CZ was faster released from the tested commercial formulations than from the aqueous SLN dispersion. After 1 h the cumulative amount of CZ was higher than 0.6 mg/cm^2 for both commercial creams, while at the same period aqueous SLN dispersion showed a value lower than 0.15 mg/cm^2. SLN could retard CZ release by the fact that drug molecules are entrapped in the lipid matrix. The recorded J value for aqueous SLN dispersion was 45.6 ± 0.7 μg/cm^2/h. For commercial creams, i.e. Canesten® and Fungizid-ratiopharm® cream, the obtained values were 86.3 ± 0.6 μg/cm^2/h and 89.5 ± 0.7 μg/cm^2/h, respectively. To summarize, the percentage of drug released after 24 h was more than 50% for both commercial formulations and not higher than 30% for the SLN formulation developed (Fig. 3).

In order to improve the therapeutic efficacy of CZ a sustained release of this drug over a period of several hours might be highly beneficial. The release of encapsulated

Table 2: The regression equations of *in vitro* release of CZ from lipid nanoparticles, according to different release models

Release model	Regression equation	R^2
Zero order	$Q_1 = Q_2 + K_0 t$	0.01628
First order	$\log Q_1 = \log Q_0 + \frac{K_1}{2.303} t$	0.23323
Higuchi	$Q_t = K_H \sqrt{t}$	0.97527
Weibull	$\log [-\ln (1 - (Q_1/Q_\infty))]_1 = b \times \log t - \log a$	0.93262

CZ should differ from the drug in the commercial creams because of the solid matrix of the former and subsequently drug immobilization (Westesen 1997). Therefore, a modified release profile of the drug is guaranteed. This observation allows asserting that the selected excipients have different influences on drug release profiles, emphasizing the importance of SLN for the development of a new sustained delivery system for this antifungal agent.

The quantitative analysis of the values obtained in the release studies can easily be analysed using mathematical models that express the results as a function of some characteristics of the formulation. The obtained release profile of CZ from aqueous SLN dispersion closely resembles to the ones obtained with commercial creams, however with lower J values, i.e. the curves show the same shape (Fig. 3). At periodic intervals, the concentration of CZ has been determined. Table 2 shows the regression equations of *in vitro* release of CZ from lipid nanoparticles, according to different release models.

From Table 2 the most suitable model describing the CZ release from lipid nanoparticles is the Higuchi model, which is in accordance to the literature (Higuchi 1961, 1963). A R^2 value close to 1 (approx. 0.9753) has been obtained. The Higuchi model has been adjusted to the CZ release profile from aqueous SLN dispersion and it is shown in Fig. 4.

In the first 2 h 17% of CZ was measured in the acceptor medium, while in the following 8h the cumulative amount of drug released increased to 27% (Fig. 4). The longest release time was up to 10 h. The results demonstrate a burst effect followed a slow and continuous release. The release of CZ from lipid nanoparticles was faster in the first hours probably because the drug was adsorbed onto the nanoparticle surface rather than entrapped into the nanoparticle core. The cumulative CZ release of the new SLN formulation is about 2-fold lower after 24 h than from the commercial formulations. This is an indication for the excellent suitability of this vehicle for CZ. The Higuchi model describes the dissolution of drugs in suspension from ointment bases, but it is also in accordance to other dissolution profiles obtained from many other pharmaceutical dosage forms.

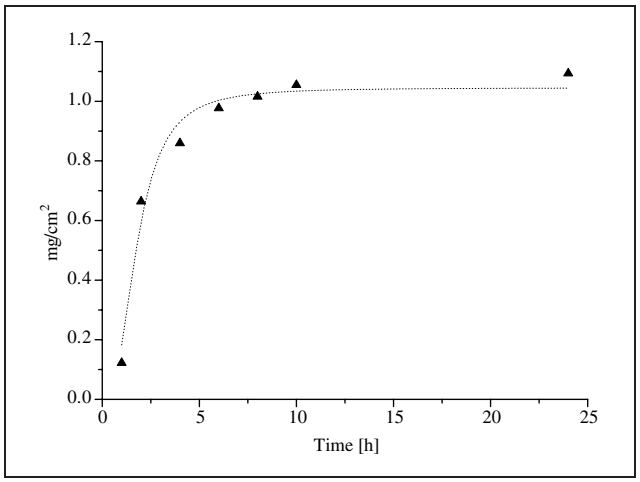

Fig. 4: Comparison between the release profile of CZ obtained from SLN (▲) and the theoretical Higuchi model (. . .)

2.4. Conclusions

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 have been assessed. Under polarised light no major SLN aggregation has been detected. SLN remained in their solid state after 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. The differences observed in flowability of the investigated formulations proved that SLN have a considerable influence on the three-dimensional structure of semi-solid formulations. The semi-solid formulations developed were simple to manufacture and have shown suitable mechanical properties for topical purposes.

In vitro release investigations showed that SLN exhibit a modified release profile for clotrimazole. In comparison to commercial formulations, a prolonged residence time of drug in the vehicle could be obtained for a period of 24 h. Furthermore, a release profile that follows the Higuchi model has been characterized for these systems. This model might be appropriate for topical/dermatological therapy when an immediate effect of the active ingredient is required the site of action followed by a continuous supply.

3. Experimental

3.1. Reference materials

Clotrimazole was kindly supplied by Caelo GmbH (Hilden, Germany). Glyceryl tripalmitate (Dynasan® 116) 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. The emulsifying agent used to stabilize the aqueous SLN dispersions was Tyloxapol® (Caelo GmbH, Hilden, Germany), a polymer of 4-(1,1,3,3-tetramethyl)-phenol with ethylene oxide and formaldehyde. 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.

Fungizid-ratiopharm® cream (batch number C26808) labelled to contain 10 mg/g of clotrimazole and Canesten® cream (batch number CCTGT3) labelled to contain 10 mg/g of clotrimazole (Bayer, Germany). A hydrophilic cream, i.e. Unguentum emulsificans aquosum (batch number 0000095141) was purchased from a local pharmacy (Apotheke im Kaufzentrum, Siemensdamm, Berlin).

3.2. Preparation of aqueous SLN dispersions

Aqueous SLN dispersions composed of 20% (m/m) of lipid phase and 5% (m/m) of surfactant were prepared as described in detail elsewhere (Müller 2000; Mehnert 2001; Müller 2005). Briefly, Dynasan® 116 was melted at 90 °C and 5% of CZ (related to the lipid phase) was added to the melted lipid and dissolved. A pre-emulsion was formed after dispersing the hot lipid phase in a surfactant aqueous 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 90 °C 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 (20 °C).

3.3. Preparation of SLN-containing hydrophilic cream

Freshly prepared aqueous SLN dispersion was incorporated into the hydrophilic cream using a high speed stirrer (Cito Unguator Konietzko, Bamberg, Germany) at approx. 1000 rpm for 3 min, in a concentration of 20%, 30% or 40% (m/m) of dispersion in the cream.

3.4. Particle size analysis

The particle size analysis of SLN dispersion 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%, d90% and d95% of the volume distribution.

3.5. Rheological measurements

The rheological properties of the formulations were studied by continuous shear investigations, which were performed in order to evaluate the shear rate (D) as a function of shear stress (τ). 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 at 20 ± 0.1 °C 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°).

3.6. In vitro release studies

In order to investigate the drug release profile from SLN and commercial creams, static Franz diffusion cells were used. These cells consist of donor and acceptor chambers between which a membrane is positioned (Franz 1975). The area for diffusion was 0.64 cm^2 and the acceptor chamber volume was approx. 5.5 ml. Cellulose nitrate membranes (Sartorius, Germany) with an average pore size of 0.1 µm were selected and they have been previously treated with isopropyl myristate in order to mimic skin lipophilicity. The acceptor chamber was maintained at 32 °C, in order to ensure the surface skin temperature at the membrane.

The acceptor medium consisted of a solution of 100 mM acetate buffer, pH 6.0 with 35% (v/v) of dioxane. An appropriate volume of aqueous SLN dispersion (containing 1% of CZ) or of respective cream was applied to the donor compartment. Samples (250 µl) were collected over 24 h and analysed by spectrophotometric determination at 243 nm. After each sample taking, Franz cells were filled up with acceptor medium, in order to ensure the sink conditions during the experiment. For each formulation, the release studies were performed in triplicate.

UV spectrophotometric quantifications of CZ were carried out using an Uvikon 940 double-beam spectrophotometer (Kontron Instruments, Eching, Germany). Validation of the method was performed regarding linearity, precision, accuracy, selectivity, sensitivity and stability.

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