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Research paper

Intravenous itraconazole emulsions produced by SolEmuls technology

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Dedicated to Prof. Dr. Dr.h.c. Bernd W. Müller on the occasion of his 60th birthday.

Abstract

Itraconazole is a drug which is poorly soluble in both the water and oil phases of emulsions. Incorporation in parenteral emulsions was performed applying the SolEmuls® Technology, i.e. localising the drug in the interfacial lecithin layer of the emulsions by homogenising a hybrid dispersion of oil droplets and drug nanocrystals in water. The maximum loading capacity of the emulsion system was found to be 10 mg/ml; at 20 mg/ml the loading capacity was exceeded leading to remaining drug nanocrystals in the emulsion. Incorporation of itraconazole into the lecithin layer led to an enhanced dispersion effect, i.e. with increasing drug concentration the droplet size of the emulsions decreased. Physical long-term stability of the optimum emulsion with 10 mg/ml could be shown over a period of 3 months at room temperature.

Keywords: Itraconazole; Emulsions; High pressure homogenisation; Poorly soluble drugs; Particle size; SolEmuls

1. Introduction

Itraconazole is an antimycotic drug used in the treatment of systemic fungal infections. The commercial product Sporanox® IV is used for treatment of histoplasmosis, blastomycosis and refractory aspergillosis by intravenous injection. Itraconazole is a poorly soluble drug; dissolution of itraconazole in Sporanox® was achieved by complexation with 2-hydroxypropyl-β-cyclodextrin. The problem of the treatment is the toxicity and side effects caused by the formulation itself, i.e. the toxicity of the cyclodextrin. The side effects occurring (e.g. Splenomegaly, ataxia) under Sporanox® treatment limit the maximum applicable dose to 200 mg/50 ml, infused over 1 h and twice a day. In some cases this is not sufficiently high for efficient treatment, i.e. the complete eradication of Aspergillosis.

As an alternative, improved and better tolerated formulation, drug nanosuspensions of itraconazole were produced by high pressure homogenisation [1]. Nanosuspensions are defined as ultra-fine suspensions of pure drug nanoparticles stabilised with a surfactant or surfactant mixtures. Preparation can be performed by ball or pearl milling [2] or alternatively by piston-gap homogenisation in water [3] or in non-aqueous media (e.g. glycerol) or mixtures of water with water miscible liquids (e.g. isotonic water–glycerol mixtures) [4]. In general, preparation by homogenisation is preferable because of having a low product contamination by the production equipment, typically below 1 ppm [5]. Nanosuspensions produced by pearl milling can contain contamination from the milling material [6], figures of 70 ppm were reported at conferences or in the literature [7–9]. After intravenous injection the drug nanocrystals dissolve in the relatively large liquid volume of the blood; blood components with solubilising activity can further increase the dissolution velocity which is a priori high because of the large surface area of the particles. Depending on the compound specific dissolution pressure, the drug particles can dissolve so fast that one yields the pharmacokinetic of an injected drug solution [10]. Alternatively, in case of slower dissolution, a fraction of the injected dose can accumulate in the macrophages of the

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Reticula endothelial system (RES), mainly in the liver, by passive targeting and be exploited to treat RES or liver diseases [11]. In case of surface-modified drug nanocrystals, an active targeting to the brain can be achieved via Apolipoprotein E mediated uptake [12,13]. The company Baxter also produced itraconazole nanosuspensions stabilised with a water miscible organic solvent, applying their so-called 'NANOEDGE™' Technology (high pressure homogenisation of aqueous suspensions). In comparative studies with Sporanox® it could be shown that the drug nanosuspensions showed good tolerability. In addition, higher treatment efficacy was found in vivo due to higher doses administered. Sporanox® has a dose limitation due to the occurrence of toxic side effects. Laser diffractometry (LD_{50}) in rats was <40 mg/kg for Sporanox®, >320 mg/kg for the NANOEDGE™ formulation [14].

Drug nanosuspensions are a novel formulation for intravenous delivery. In general the regulatory authorities require more intensive proof of safety when injecting suspensions of rigid particles than when administering flexible oil droplets of a drug-loaded parenteral emulsion. Therefore due to easier registration, a parenteral emulsion appears to be the system of first choice, of course only when an emulsion with a sufficiently high drug-load can be produced. Simultaneously the process needs to be economic to have a competitive product deemed affordable by the health systems.

The history of the technology goes back to the cheap AmBisome® replacement done by the hospital pharmacists. They injected the solution of Amphotericin B (Fungizone®) into Intralipid bottles and shook it, assuming that the drug would diffuse into the emulsion. However, they did not realise that they were actually performing 'via humida paratum' (v.h.p) leading to the precipitation of the drug crystals and not its diffusion. Formulation approaches developed afterwards also proved inefficient, a fact demonstrated by the low number of such products on the market. The 'Solvent Approach' is one of them. According to this approach, one dissolved the drug and the lecithin in an organic solvent, evaporated the organic solvent and used the drug-lecithin blend to form o/w emulsions [15]. The process was not practical due to the requirement of a second step during production of the emulsions.

Itraconazole is a drug which is simultaneously poorly soluble in water and in organic media, e.g. oils of parenteral emulsions. Therefore production of itraconazole emulsions is not possible by applying the simple production process as used for emulsions loaded with diazepam, etomidate or propofol (i.e. dissolving the drug in the oil and preparing the emulsion). Incorporation is only possible by localising the drug in the lecithin layer at the oil–water interface. This paper describes the production of such itraconazole emulsions using the recently developed SolEmuls® Technology [16,17] which is a technology basically combining ultra-fine drug suspensions/nanosuspensions with the traditional parenteral emulsions. Increasing concentrations of itraconazole were incorporated to study the effect of drug concentration on the mean size and size distribution of emulsion droplets, the incorporation capacity of the emulsion system including dissolution of the drug and distribution into the interface during the SolEmuls process.

2. Material and methods

Lipofundin MCT 20% was kindly provided by B. Braun Melsungen (Melsungen, Germany). Itraconazole was obtained from Chemo Iberica S.A. (Madrid, Spain), kindly arranged by Technology Catalysts Inc. (Falls Church, VA, USA).

High pressure homogenisation was performed using a discontinuous Micron LAB 40 from APV Systems GmbH (Unna, Germany). Homogenisation conditions were 1500 bar (21,300 psi) up to 20 homogenisation cycles at 45°C.

Particle size analysis was performed by laser diffractometry using the Coulter LS 230 from Coulter Electronics (Krefeld, Germany). The diffractometer yields a volume distribution, characterisation parameters were the diameters D 50–99% (e.g. a diameter of 99% means that 99% of the particles of the volume distribution are below the given size in micrometers). In addition photon correlation spectroscopy (PCS) was employed for high resolution analysis of the bulk population of the emulsion droplets. The measuring range is approximately 3 nm to 3 μm. Due to the high sensitivity and good reproducibility of PCS, at optimal measuring conditions a size difference of 3–4% can be considered significant (e.g. 10 nm with an emulsion diameter of approximately 250 nm), whereas this is not possible with LD data. Therefore PCS is a high resolution analysis to detect small differences. The equipment used was a Zetasizer 4 from Malvern Instruments (Malvern, UK).

The same Zetasizer was used to measure zeta potential (ZP). The measuring medium was distilled water with its conductivity adjusted to 50 μS/cm by addition of NaCl. Adjusting the conductivity to this fixed value avoids fluctuations of the ZP due to differences in the conductivity of the distilled water [18]. Measurements were performed at a field strength of 20 V/cm, conversion of the electrophoretic mobility to the ZP was performed using the Helmholtz–Smoluchowski equation.

LD can only detect the particle size, but not differentiate between oil droplets and potentially similarly sized drug nanocrystals. Therefore light microscopy using a Leitz microcope (Wetzlar, Germany) was additionally employed for detection of non-dissolved crystals. Polarised light was used, magnifications were 630× to search for drug particles larger than 1 μm. Oil immersion and magnification of 1000× were employed to detect drug nanocrystals with a size of a few hundred nanometers. The detection limit of the light microscope is about 0.2 μm, the use of polarised light enabled easy detection of particles in the range of

200–300 nm (of course only detection but no precise size measurement). This is similar to the principle used in normal light microscopy in combination with laser light; the presence of even very small particles close to the detection limit of the microscope can be detected by the reflection of the laser light. The emulsions were not diluted prior to microscopic examination to increase the probability of finding even a few non-dissolved drug crystals. Typically, a screening of 20 microscopic fields was performed. Analysis of undiluted emulsions to detect a few other particles (in this case larger oil droplets) is a method routinely employed to characterise parenteral emulsions [19].

3. Results and discussion

3.1. Production of emulsions

The basic principle of the SolEmuls technology is that drug powder is added to an emulsion by high speed stirring yielding a 'hybrid dispersion' of oil droplets and drug particles in water. There are basically two different methods. In method A the drug particles are added to a pre-formed emulsion, e.g. parenteral emulsions such as Lipofundin or Intralipid. Then the mixture is homogenised until the drug crystals are dissolved. In method B a de novo production is performed, that means pre-emulsion is produced by dispersing oil in surfactant solution, then the drug particles are added to the water phase of this pre-emulsion and subsequently the homogenisation process is performed. The result of both processes is the same, an ultra-fine emulsion having the drug located in the interfacial lecithin layer. The drug can be added as ultra-fine powder (e.g. micronised drug by jet-milling), alternatively the drug can be added as drug nanocrystals (e.g. aqueous nanosuspension produced by high pressure homogenisation). Addition as drug nanocrystals will accelerate dissolution of the drug and partitioning to the interfacial layer.

The itraconazole powder analysed by LD showed a mean diameter of a micronised powder, the diameter *D* 50% was 11 μm, but contained a pronounced fraction of larger particles, i.e. 99% of the particles were below 180 μm (Fig. 1, right).

To minimise the homogenisation cycles of the emulsion required for achieving complete dissolution of the drug, the itraconazole powder was transformed into a nanosuspension. Itraconazole was dispersed in a surfactant solution containing 0.5% Tween 80. Dispersion was performed by high speed stirring using an ultra-turax at 8000 rpm for 1 min. The obtained pre-suspension was then homogenised at 1500 bar. Fig. 1 (left) shows the LD size distribution of the obtained nanosuspension, the PCS diameter was 360 nm and the polydispersity index 0.320, the latter indicating a slightly broad distribution.

The nanosuspension contained 1% drug, that means 10 mg of drug/ml suspension. To yield a drug concentration of 1 mg/ml emulsion, 4.0 ml of the concentrated nanosuspension was added to 40 ml emulsion. Addition of such a small amount of concentrated emulsion led only to a negligible dilution of the emulsion that means it still

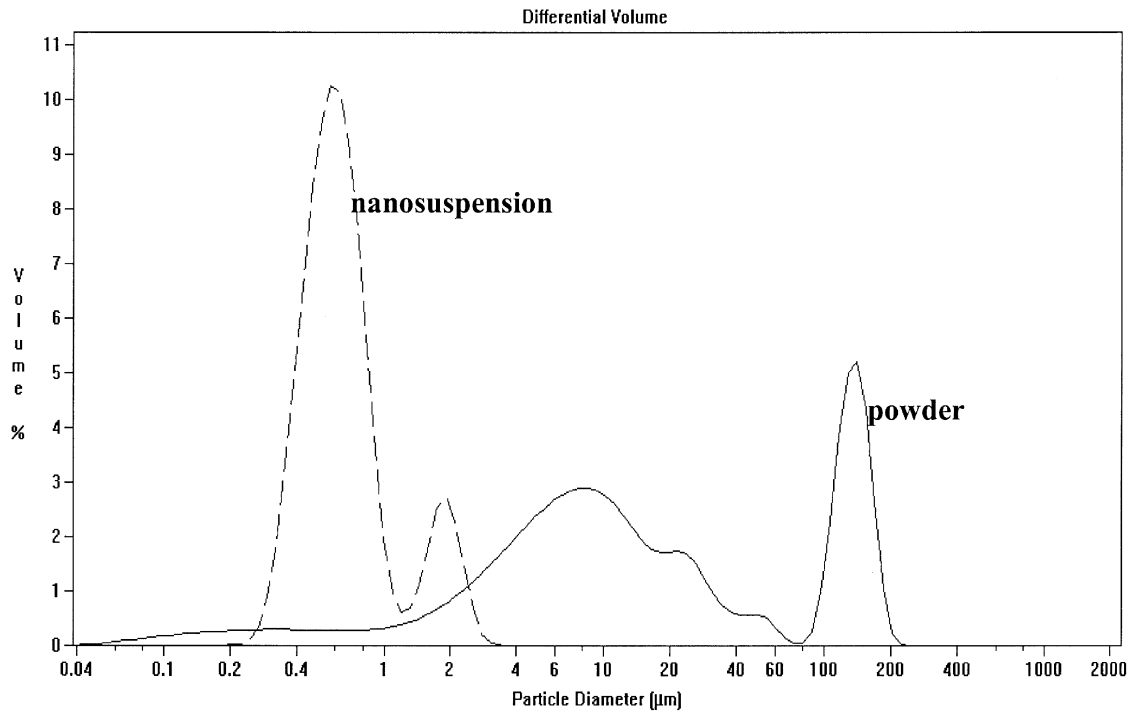

Fig. 1. LD size distribution of the drug powder (right) and after transfer to a nanosuspension (left).

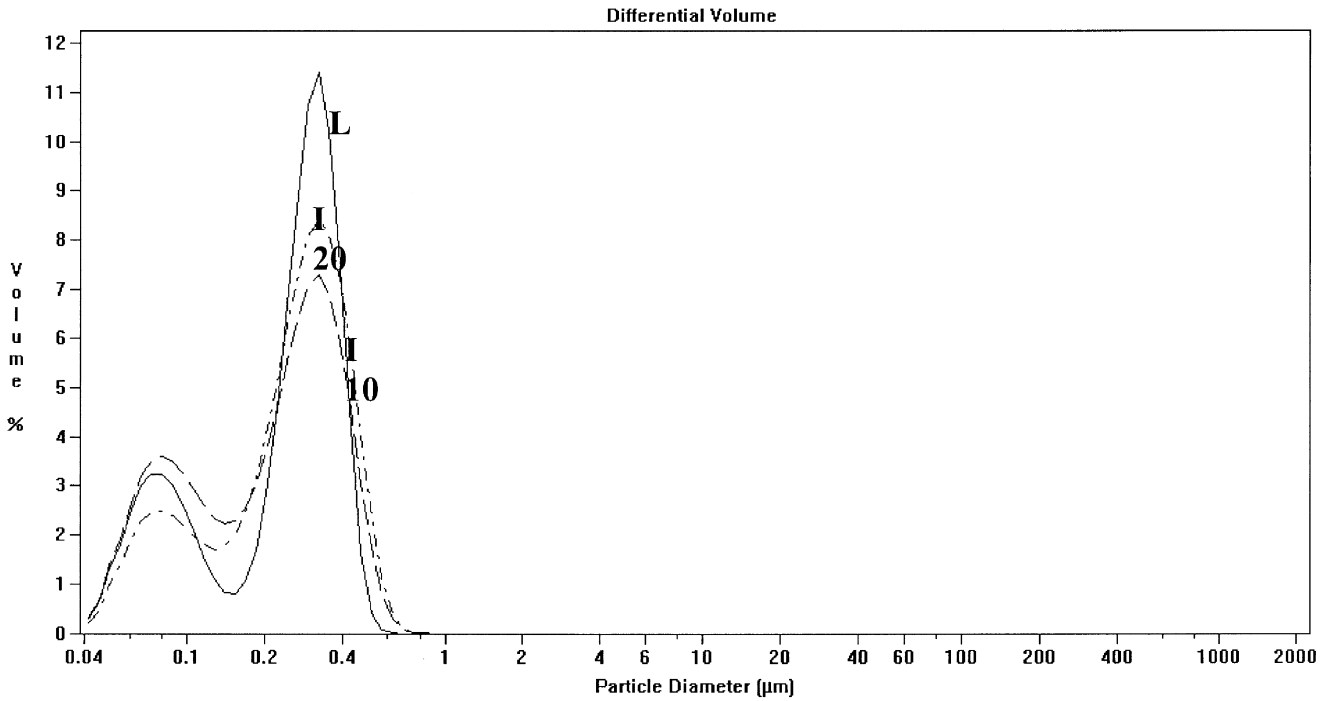

Fig. 2. LD size distribution of unloaded Lipofundin MCT emulsion (L) and the itraconazole-loaded emulsion (1 mg/ml) after 10 (I 10) and 20 (I 20) homogenisation cycles.

contains approximately 18% oil. To obtain concentrations of 5 and 10 mg/ml itraconazole, five and ten times higher quantities of nanosuspensions were added, respectively. The obtained hybrid dispersions were then subjected again to high pressure homogenisation at 1500 bar.

3.2. Effect of cycle numbers on the emulsion droplet size

The hybrid dispersions were homogenised applying 20 homogenisation cycles, samples were drawn after 1, 5, 10, 15, 20 homogenisation cycles and analysed by PCS and LD. Fig. 2 shows the LD size distributions of the original Lipofundin MCT emulsion (L) and the itraconazole-containing emulsion (1 mg/ml) after 10 and 20 homogenisation cycles (I 10 and I 20).

There is only a slight shift to the left of the homogenised emulsions compared to the original one; practically very little difference could be seen between the homogenised emulsions after 10 and 20 cycles. Fig. 3 shows the LD diameters 50 and 99% of the original emulsion Lipofundin

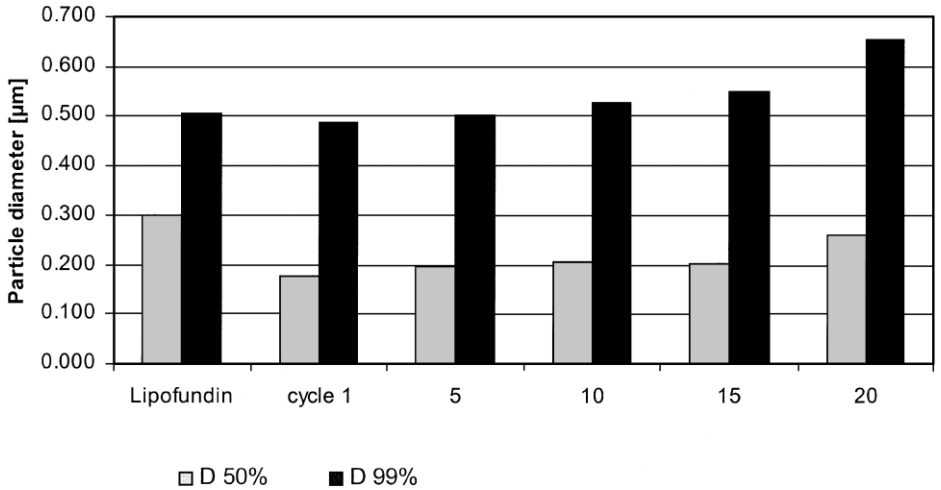

Fig. 3. LD diameters 50 and 99% of the unloaded Lipofundin emulsion (left) and the itraconazole-loaded emulsion (5 mg/ml) as a function of homogenisation cycles.

and the drug-loaded emulsion (5 mg/ml) as a function of the cycle numbers.

There is a slight decrease in *D* 99% from the unloaded emulsion to the drug-loaded emulsion after one homogenisation cycle, a pronounced decrease by 0.1 μm in the *D* 50%. LD diameters show no or little change during the first five homogenisation cycles, that means the maximum dispersitivity at the given power density (pressure) has already been reached after one homogenisation cycle. A slight increase was observed from 10 to 20 cycles, indicating that obviously too much energy was put in the system, causing some very limited coalescence. However, this is of no relevance to the product because the emulsions are still extremely finely dispersed.

This datum largely concurs with the PCS data. There is a decrease in PCS diameter from the original emulsion to the homogenised itraconazole-loaded emulsion after cycle 1, the diameter decreases from 300 to 228 nm. There is an increase at cycle 15 and 20, the PCS diameter being 250 nm diameter at cycle 20. Considering the low standard deviation of PCS (typically $<1\%$) this is a significant increase. The polydispersity index stays around 0.070 (± 0.010) indicating little change of the width of the size distribution.

3.3. Analysis for drug crystals

Light microscopy was employed to detect non-dissolved drug crystals. The high pressure homogenisation process enhances drug dissolution due to three different mechanisms:

1. the homogenisation process leads to further size diminution of the crystals, increasing their surface area and thus accelerating the dissolution velocity;
2. the dissolution pressure (and consequently saturation solubility) increases with decreasing particle size, this leads to an increasing concentration gradient $(C_s - C_x)/h$ in the Noyes–Whitney equation also leading to a higher dissolution velocity (C_s, saturation solubility; C_x, bulk concentration; h, diffusional distance);
3. the high streaming velocity in the homogenisation gap leads to a fast and efficient removal of dissolved drug in the vicinity of the particle surface, as a result the diffusional distance h decreases and the concentration gradient increases, again accelerating the dissolution velocity dc/dt.

Light microscopy analysis should reveal how many homogenisation cycles are required for dissolution of the drug. Analysis was performed using the undiluted emulsion to increase the probability of detecting crystals (dark field, polarised light). At a concentration of 10.0 mg/ml crystals were still detectable after one homogenisation cycle, after five homogenisation cycles detection of crystals was not possible (Fig. 4).

From these data, five homogenisation cycles appeared to be sufficient in pharmaceutical production for incorporation of itraconazole in the interfacial layer at a concentration of 10.0 mg/ml. For a concentration of 1.0 mg/ml complete dissolution was achieved after one homogenisation cycle. The concentration of 1.0 mg/ml is well above the solubility of itraconazole in the oil and water phase, being 6.8 and 1.8 μg/ml, respectively.

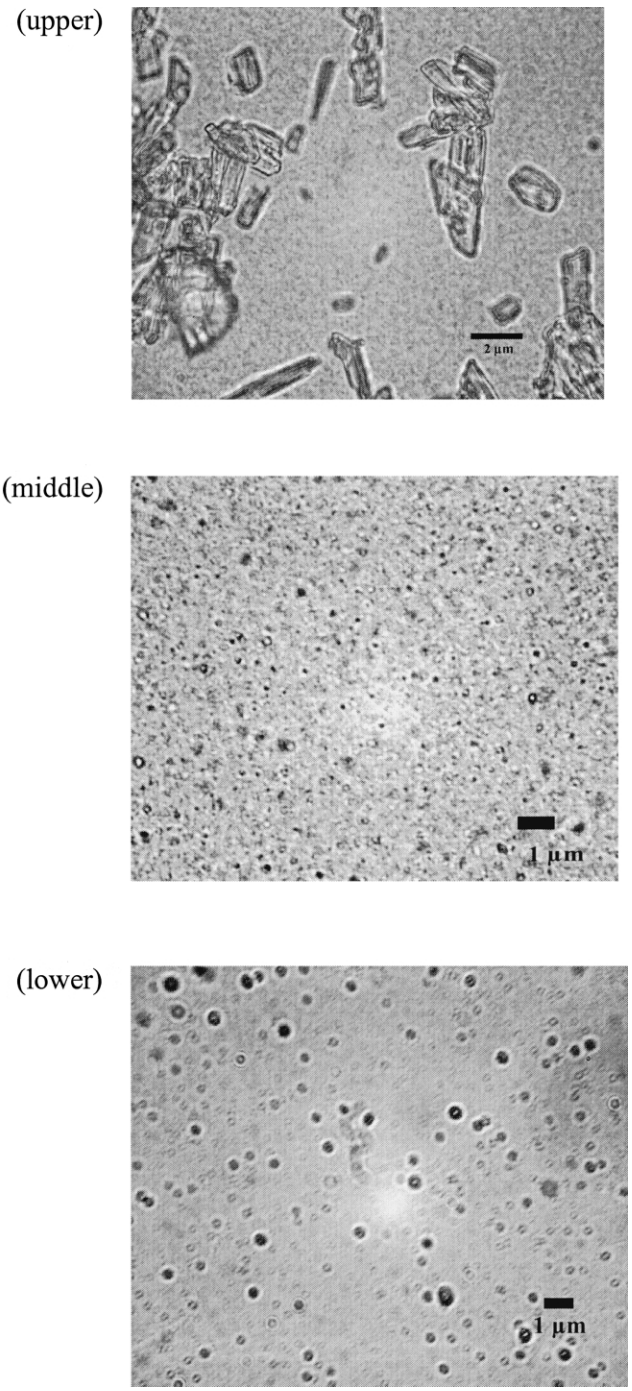

Fig. 4. Polarised light microscopy graphs of emulsion with added drug nanosuspension prior to homogenisation (upper), after one homogenisation cycle (middle) and after five homogenisation cycles (lower).

3.4. Effect of drug concentration on the emulsion

To achieve an acceptably low injection volume, a higher drug concentration in the emulsions is required. One dose of Sporanox® injection contains 200 mg in 50 ml. At a concentration of 1 mg/ml emulsion, this would correspond to an injection volume 200 ml. To reduce the injection volume, attempts were made to incorporate higher drug concentrations of 5 and 10 mg/ml into Lipofundin emulsion (corresponding to 40 and 20 ml injection volume). Homogenisation parameters were identical, i.e. 1500 bar and 20 homogenisation cycles.

Also for these two higher concentrations a similar behaviour in size reduction was found as observed with 1 mg/ml. There was a slight decrease in LD and PCS diameters from the original emulsions to the drug-loaded emulsions after one homogenisation cycle, then little change or slight increase at cycles 15 and 20 was observed (data not shown).

However, a comparison of the diameters of the bulk populations with increasing drug concentrations, revealed a significant decrease in the bulk diameter (Fig. 5).

The bulk diameter decreased from 254 to 225 nm with increasing drug-load. This is a clear difference; the increase was surprising because primarily no effect on particle size, but rather destabilisation of the stabiliser layer with subsequent size increase is reported in the literature. Destabilisation by incorporating drugs can occur due to reduction of ZP [18] and subsequent reduced electrostatic repulsion and thus destabilisation [20]. In addition, drug incorporation into the interfacial layer can reduce the rigidity of the layer, that means increasing the fluidity, e.g. as reported also for certain ions such as calcium [21]. Increased fluidity of a film (i.e. the film is less viscous) promotes fusion of the stabilising films of two colliding emulsion droplets, thus promoting coalescence and formation of large droplets. For Amphotericin B it has been reported that incorporation of the drug molecules seems to occur rather as clusters in the interfacial layer [22]. Such a patch-wise arrangement is less likely to lead to reduced interfacial tension and improved dispersion properties of such a mixture. Such arrangements lead rather to destabilisation of disperse systems. It is known that incorporation of surfactants of soap into the thin bath foam also takes place patch-wise. These areas can have a more rigid structure, leading to distortions of the flexible film and foam destruction [23]. A good example from daily life is the dropping of a solid piece of soap into a foam bath. Shortly after this event the foam starts to disintegrate and disappears rather quickly. Based on these considerations and the observed dispersion effect with increasing drug concentration, molecular dispersion of itraconazole in the lecithin layer is considered to be likely (similar to mixed surfactants). This would resemble the mechanism described for surfactant mixtures, e.g. Lanette N. Lanette N is a 9:1 mixture of Lanette O (cethyl stearyl alcohol) and Lanette E (cethyl stearyl sulphate). Lipofundin emulsion contains 1.2% of lecithin that means 12 mg of lecithin in relation to 1, 5 and 10 mg itraconazole, respectively. The strongest decrease in size was observed when moving from 5 to 10 mg/ml itraconazole concentration; that means approximately 1:1 mixture of lecithin to drug seems to have optimal dispersion properties.

Of course, after these findings emulsions were prepared containing 20 mg/ml itraconazole. However, these emulsions were found to contain detectable drug nanocrystals even after 20 homogenisation cycles. This indicates that the loading capacity of the emulsion system was exceeded. Of course, it is also possible to administer such hybrid dispersions via the intravenous route. The only prerequisite is that these hybrid dispersions are physically stable, that means no droplet coalescence occurs and also no crystal growth due to the Ostwald ripening effect. Ostwald ripening is unlikely due to the homogeneous size of both drug

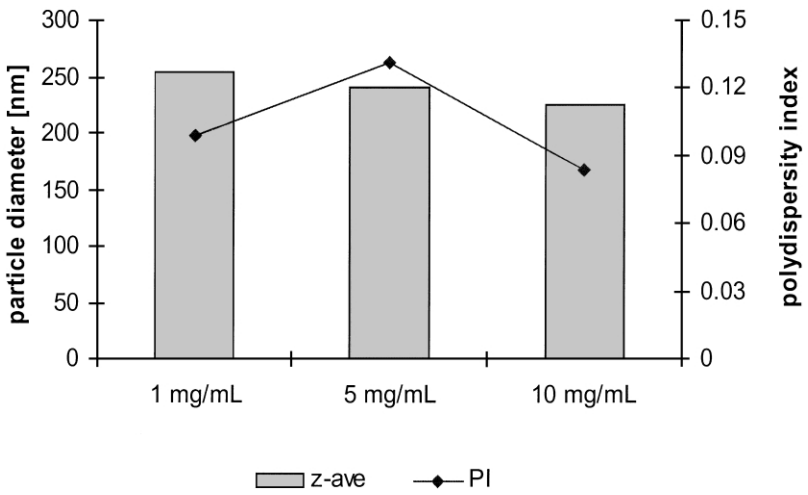

Fig. 5. PCS diameters (*z*-average) and polydispersity indices (PI) of itraconazole-loaded emulsions at increasing drug concentrations.

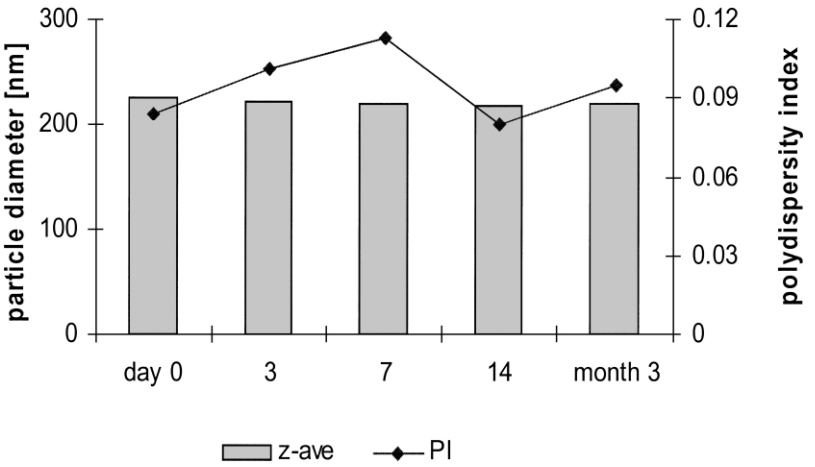

Fig. 6. PCS diameters (*z*-average) and polydispersity indices (PI) of the itraconazole emulsion (10 mg/ml) as a function of storage time at room temperature.

nanocrystals and oil droplets. This should create similar dissolution pressures for the drug and very little difference in saturation solubility in the vicinity of emulsion droplets and drug crystals. In addition, the solubility of the drug in the aqueous phase is extremely low, thus minimising ripening effects. The aim of this study was to produce real emulsion systems not hybrid dispersions. Therefore, the stability of the hybrid dispersions was not studied in detail.

Dissolution of drug crystals was again followed by light microscopy. Depending on the drug concentration, drug nanocrystals can be detected after one homogenisation cycle; crystals were no longer detectable after five homogenisation cycles when dealing with the highest concentration of 10 mg/ml, leading to complete dissolution in the emulsion. As stated above, no complete drug dissolution was found for the 20 mg/ml concentration even when applying 20 homogenisation cycles.

3.5. Physical long-term stability of the emulsions

Physical stability of the optimum emulsion at 10 mg/ml was monitored for a period of 3 months (data available until now). The emulsions were stored at room temperature and particle size analysis performed by LD and PCS. The LD characterisation diameters showed no or very little change. The PCS diameter, as the most sensitive parameter for changes in the bulk population, showed no increase, measuring 225 nm at the day of production and 220 nm after 3 months storage (Fig. 6).

The PCS diameters of the emulsions for parenteral nutrition are typically in the range from 200 to 400 nm [19], therefore intravenous administration of the itraconazole emulsion is no problem. A critical parameter of course is the content of droplets in the low micrometer range, as they are outside the detection limit of the PCS (>3 μm). However, LD diameters including the very sensitive diameter *D* 99% were all very well below 5 μm, thus i.v. administration is also no problem with regard to the content of larger microdroplets.

The change in polydispersity index as a function of time is not considerable, being 0.084 on the day of production (day 0) and 0.095 in month 3. The slight increase from a value of 0.084 on day 0 to a value of 0.113 on day 7 can be attributed to a few large oil droplets, thus not affecting the physical stability of the emulsion. A polydispersity index of 0.100 still indicates a relatively narrow distribution; values up to 0.250 are reported for parenteral fat emulsions [24].

4. Conclusion

Itraconazole emulsions could be produced with a drug-load as high as 10 mg/ml. This concentration is remarkable because the solubility in MCT oil is only 6.8 μg/ml and in water 1.8 μg/ml, that means in oil and water phase of the emulsion approximately 2.0 μg/ml. Incorporated were 10 mg/ml, that means a factor 5000 higher than the solubility in the two phases. In addition to Amphotericin B, ketoconazole, nimesulide [25], etc. itraconazole is another drug which could be successfully incorporated in parenteral emulsions using the SolEmuls® Technology. These data support the use of SolEmuls as a general formulation principle for incorporating drugs into parenteral emulsions, i.e. drugs which are poorly soluble in water and simultaneously in oils.

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