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Solubilizing Poorly Soluble Antimycotic Agents by Emulsification via a Solvent-Free Process

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ABSTRACT

The purpose of this study was to formulate itraconazole and ketoconazole as oil/water emulsions for parenteral delivery by using a solvent-free homogenization process, namely SolEmuls (solubilization by emulsification) technology. The drugs were incorporated in the commercial emulsion Lipofundin MCT 20%, composed of a medium-chain triglyceride/long-chain triglyceride (MCT/LCT) oil phase (1:1) and stabilized with 1.2% lecithin. Different parameters such as drug-loading capacity, long-term physical stability, and completeness of drug dissolution were investigated. Up to 10.0 mg/mL complete drug dissolution was achieved with itraconazole; at 20 mg/mL hybrid dispersion was obtained. Itraconazole-loaded emulsions were physically stable for 9 months (data up to now). Ketoconazole showed physical instability in the Lipofundin emulsion, which was stabilized with only 1.2% lecithin. Stabilization of ketoconazole-loaded emulsions was achieved using additionally Tween 80 as steric stabilizer. Higher concentrations of ketoconazole (ie, 10.0 mg/mL concentrated ketoconazole emulsions) were also produced with additional 2.0% Tween 80. Ketoconazole-loaded emulsions, 1 mg/mL, which were stabilized with 2.0% Tween 80, were stable for a period of 6 months. It can be concluded, after formulating amphotericin B and carbamazepine with SolEmuls technology, that SolEmuls was also applicable to the antimycotic agents itraconazole and ketoconazole, yielding IV-applicable emulsions with cost-effective production technologies.

KEYWORDS: nanoemulsions, itraconazole, ketoconazole, stability, high-pressure homogenization

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INTRODUCTION

Many drugs have an aqueous solubility of less than 100 mg/L (100 ppm) and some, even lower than 1 mg/mL.[1] Drugs that are considered to be poorly soluble have solubility values of approximately 100 µg/mL[2] and some even lower (eg, itraconazole with a water solubility of 1.8 µg/mL).[3] Poor solubility causes subsequently poor bioavailability after oral administration. The alternative is IV injection, but for this the drugs need to be "solubilized." There are various approaches to solubilizing drugs such as inclusion complexes with cyclodextrins,[4] formation of mixed micelles, and liposome formulations. However, these approaches have proved to be inefficient, as evidenced by the low number of marketed products. Some of the partly successful formulations possess additional physical stability problems such as aqueous dispersion (eg, AmBisome—the liposomal product of amphotericin B). AmBisome needs to be lyophilized and reconstituted prior to administration. Thus, the optimal solution to this problem has not yet been developed.

Of course, the problem involves not only an innovative formulation approach, but also an exclusive technology that provides the pharmaceutical industry with an automated and industrial-scale production possibility. In recent years, the pharmaceutical industry has been increasingly focused on patented formulation technologies (ie, those developed by the small entrepreneur pharmaceutical companies) rather than on developing new chemical entities (NCEs). However, developing a creative formulation concept is alone not enough; the industry also needs to be provided with a simple and an automated production process.

One possible formulation approach for IV injectables is the use of parenteral oil/water (o/w) emulsions.[5] Apart from solubilizing drugs, often there is also a reduction of side effects (eg, amphotericin B in its emulsion formulations in comparison to nonliposomal marketed products).[6,7] To reduce costs, hospital pharmacists have injected the solution of amphotericin B (eg, Fungizone) into the intralipid bottles,

assuming that the drug would diffuse into the emulsion. However, the drug precipitated as large crystals, which did not fully dissolve even after intensive shaking for a period of 18 hours with a frequency of 2800 rpm.[8] The basic reason for the precipitation of drug crystals is the low dissolution velocity and the low saturation solubility of the drug in the aqueous phase.

To incorporate drugs such as amphotericin B in o/w emulsions, the "solvent approach" was developed by Davis et al.[9] This approach involved dissolving the drug and the lecithin in an organic solvent, evaporating the organic solvent, and using the drug-lecithin blend to form the emulsion. However, the solvent approach faced some complications regarding industrial application because of the solvent removal process and the requirement for production of the emulsions under sterile conditions.

As an alternative, a new concept called "SolEmuls technology" was developed.[10] The technology uses a solvent-free high-pressure homogenization process to incorporate poorly soluble drugs in a commercial emulsion (eg, Lipofundin and Intralipid). Alternatively, a de novo production of the emulsion can be performed. The basic advantage of this technology is its applicability to drugs that are poorly water soluble and simultaneously poorly oil soluble (eg, amphotericin B). It is also of note that high-pressure homogenization is a process that is currently being employed in industry (eg, parenteral emulsion production). Therefore, carrying the production from bench side to industrial scale is relatively easy to perform. Another advantage of the technology is the use of already registered oils (the use of MCT and LCT oils already present in the commercial emulsions) making expensive toxicology studies redundant.

When starting from a preformed emulsion, either a jet-milled powder or, preferentially, a drug nanosuspension is taken and admixed to a preformed emulsion such as Lipofundin. In the de novo production, the powder or nanosuspension is admixed to a prepared coarse pre-emulsion. When homogenizing the drug powder with an emulsion, 2 effects are achieved simultaneously:

Drugs like amphotericin B and carbamazepine, which are poorly soluble in water and simultaneously poorly soluble in the registered oils (eg, the solubility of amphotericin B in water is 0.1 mg/mL) have already been successfully localized in the interface of o/w emulsion. This study describes the applications of SolEmuls technology to the poorly soluble antimycotics itraconazole and ketoconazole, investigates the limitations of this technology (ie, maximum-loading capacity and physical stability), and describes solutions to overcome them.

MATERIALS AND METHODS

Lipofundin MCT 20% was kindly provided by B. Braun Melsungen (Melsungen, Germany). Ketoconazole and itraconazole were obtained from Chemo Iberica S. A. (Madrid, Spain), kindly arranged by Technology Catalysts Inc (Falls Church, VA). The surfactant Lutrol F 68 (Poloxamer 188) was obtained from BASF (Ludwigshafen, Germany) and Tween 80 was purchased from Merck (Darmstadt, Germany).

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

Particle size analysis was performed by laser diffractometry (LD) using the Coulter LS 230 from Beckmann-Coulter (Krefeld, Germany). The diffractometer yields a volume distribution; characterization parameters were the diameters D 50%, D 90%, D 95%, and D 99% (eg, a diameter 99% means that 99% of the particles of the volume distribution are below the given size in micrometers). The calculation of the LD data was done by using the Mie theory, taking a real refractive index of 1.46 and an imaginary refractive index of 0.01.[11] In addition, photon correlation spectroscopy (PCS) was employed. The measuring range of PCS is approximately 3 nm to 3 µm. The equipment used was a Zetasizer 4 from Malvern Instruments (Malvern, UK).

The same Zetasizer was used to measure the zeta potential (ZP). The measuring medium was distilled water having its conductivity adjusted to 50 µS/cm by addition of NaCl. Adjusting the conductivity to this fixed value avoids fluctuations of the ZP caused by differences in the conductivity of the distilled water.[12] 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 detect the particle size but cannot differentiate between oil droplets and potentially the similarly sized drug nanocrystals. Therefore, light microscopy using a Leitz microscope (Wetzlar, Germany) was additionally employed for detection of nondissolved crystals. Polarized light was used; magnifications were 630-fold to search for drug particles larger then 1 µm. Oil immersion and magnification of 1000 times was employed to detect drug nanocrystals with a size of a few hundred nanometers. Detection limit of the light microscope is approximately 0.2 µm; the use of polarized light enabled easy detection of particles in the range of 200 to 300 nm (of course—only detection—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 nondissolved drug crystals. Typically, a screening of 20 microscopic fields was performed. Analysis of undiluted emulsions to detect few larger particles (in this case larger oil droplets) is a method routinely employed to characterize parenteral emulsions.[13]

Scanning electron microscope (SEM) photos of the bulk powders were taken using an SEM (S-2250N, Hitachi, Tokyo, Japan) with an accelerating voltage of 25 kV, secondary electron detector, and high vacuum operation. The samples were coated with a gold-palladium mixture in a SCD 050 Sputter coater (BAL-TEC AG, Balzers, Principality of Lichtenstein) yielding a film thickness of 5 nm.

RESULTS AND DISCUSSION

Itraconazole Emulsions

Itraconazole is an antimycotic that is used in the eradication of *Candida albicans*. The IV market product of itraconazole is Sporanox I.V. Sporanox I.V. is known to lead potentially to ataxia and splenomegaly as side effects and thus has a dose limitation (once daily and a maximum of 20 mg/kg, every day). In Sporanox I.V., itraconazole forms a complex with 2-hydroxypropyl-β-cyclodextrin. The problem of the treatment is the toxicity and side effects caused by the formulation itself, which results in the toxicity of the cyclodextrin. The side effects that occur (eg, splenomegaly, ataxia) under Sporanox treatment limit the maximum applicable dose to 200 mg/50 mL, infused over 1 hour, twice a day. In some cases, this is not sufficiently high for efficient treatment (ie, complete eradication of aspergillosis). An improved formulation alternative for itraconazole with fewer side effects is needed.

Figure 1A shows the SEM photo of the bulk powder of itraconazole having needle-shaped crystals with an LD diameter of D 50%, 11.0 μm. The powder was first nanonized by producing a nanosuspension. The drug powder (0.4 g) was dispersed in an aqueous surfactant (0.5%) solution (40 mL) by high-speed stirring and subsequently homogenized at 1500 bar, applying 20 homogenization cycles. This process resulted in a nanosuspension with a PCS diameter of 360 nm and a polydispersity index of 0.350. Lipofundin MCT 20% was admixed to add 40.0 mL with increasing amounts of this nanosuspension, and the resulting predispersion was homogenized at 45°C applying 1500 bar homogenization pressure.

Increasing concentrations of itraconazole emulsions were produced and investigated in terms of their physical stability at room temperature. Dissolution of the drug molecules into the interface was investigated for all concentrations via light microscopy as defined in materials and methods.

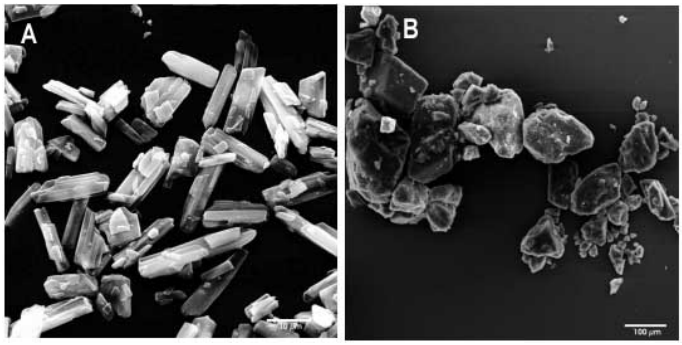

Figure 1. SEM pictures of itraconazole powder (A) and ketoconazole powder (B), bars 10 μm and 100 μm, respectively.

The lowest concentration used was 1 mg/mL. The light-microscopy pictures (polarized light) of the itraconazole-loaded emulsion after 1 cycle at 1500 bar showed no drug crystals, indicating that even after just 1 cycle at 1500 bar complete dissolution of the drug was achieved. However, 1 cycle appeared to be insufficient for a complete dissolution at higher concentrations (ie, at 10 mg/mL, itraconazole-loaded emulsions); samples after 5 homogenization cycles did not have any visible crystals under polarized light, whereas samples after 1 homogenization cycle had crystals.

The produced emulsions had a particle diameter of 231 nm with a polydispersity index of 0.091 at 1 mg/mL (cycle 15). At the higher concentration of 10.0 mg/mL, the particle size was reduced, being 223 nm with a polydispersity index of 0.110 at 10 mg/mL (cycle 15). Similar results were previously obtained with another drug (ie, carbamazepine) following this method.[14] The only explanation to this finding is that the drug behaved as a cosurfactant, and drug incorporation into the lecithin improved the emulsifying properties, leading to smaller drug-loaded oil droplets.

The itraconazole-loaded emulsions of both concentrations showed a good physical stability for a period of 9 months (data obtained up to now). The PCS diameter was 255 nm on the day of production and 256 nm after 9 months with a polydispersity index of 0.094, which is a clear indication of a narrow distribution. The LD diameters, D 50% and D 99%, were 0.278 μm and 0.524 μm, respectively, on the day of production and showed no change after 9 months of storage at room temperature. Light-microscopy investigation was done periodically to investigate a recrystallization effect of the drug-loaded emulsions. After 9 months, no drug crystals were observed under polarized light (observing 20 microscopic fields at 1000 magnification, oil immersion), indicating absence of recrystallization.

Itraconazole, 10 mg/mL, was incorporated into Lipofundin MCT 20% emulsion. Homogenization parameters were identical (ie, 1500 bar and 20 homogenization cycles). The bulk diameter decreased from 254 nm to 225 nm with increasing drug load. This is a clear difference. The polydispersity index was still below 0.150, clearly indicating a narrow size distribution.

Ketoconazole Emulsions

Ketoconazole is another antimycotic drug used for the treatment of esophageal candidiasis and systemic fungal infections. Gastrointestinal disturbances are the most common side effects following oral administration. Ketoconazole is also used topically. After topical administration of ketoconazole, irritation, dermatitis, or a burning sensation is reported.[15] In addition, ketoconazole shows hepatotoxicity and therefore cannot be administered to patients with preexisting liver disease. Because of the low solubility values of ketoconazole, it is sensible to formulate an emulsion via SolEmuls technology.

In comparison to itraconazole, ketoconazole is a more roughly shaped powder (Figure 1B). The LD diameters, D 50% and D 99%, of ketoconazole coarse powder were 28 µm and 250 µm, respectively. Ketoconazole was processed the same way as itraconazole (ie, producing the ketoconazole nanosuspension first [yielding a PCS diameter of 750 nm and a polydispersity index of 0.380], admixing the Lipofundin MCT 20% to the nanosuspension, and further homogenizing the predispersion to yield the drug-loaded emulsion).

Concentrated ketoconazole emulsions, 1 mg/mL, were produced via high-pressure homogenization. Figure 2 shows the particle diameter of the emulsions as a function of cycle numbers (1 mg/mL). There is a slight increase in particle diameter after cycle 1 (ie, 254 nm for cycle 1 and 274 nm for cycle 5). Then, the particle size remains constant between cycle 5 and cycle 20. The polydispersity index remains in the range 0.06 to 0.09. This finding indicates a narrow size distribution. For parenteral emulsions, values up to 0.250 are reported.[16] The LD diameters of these emulsions (stabilized with only 1.2% Lecithin), D 50% and D 99%, were 0.322 µm and 0.587 µm, respectively, at cycle 20. The calculation of the LD data was done using the Mie theory accompanied by the polarization intensity differential scattering (PIDS) approximation.[17] PIDS enables high resolution of the submicron range of the particle size distribution of the emulsion.

Ketoconazole showed a different behavior in comparison with other drugs (eg, carbamazepine[18] and amphotericin B[19]). Ketoconazole-loaded emulsions were only stable for 3 days.

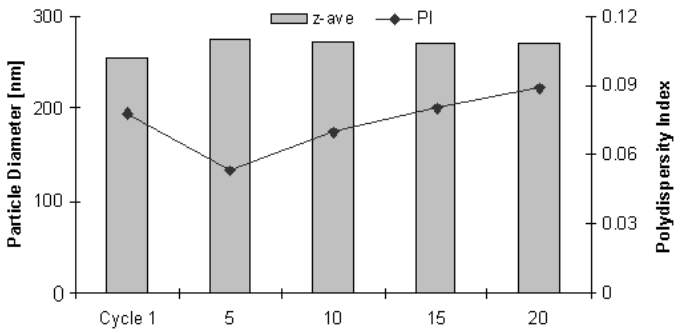

Figure 2. PCS diameters (z-ave) and polydispersity indices (PI) of ketoconazole emulsions (1 mg/mL) as a function of homogenization cycles (1.2% lecithin).

Stabilization of the ketoconazole emulsions was attempted using different concentrations of poloxamer 188 added to 1.2% lecithin, which is already present in Lipofundin MCT 20%. Emulsions were prepared with 1.2% lecithin content by homogenizing the ketoconazole nanosuspension with Lipofundin MCT 20% and adding 0.3%, 0.5%, 0.8%, 1.0%, 1.5%, and 2.0% Poloxamer 188 to the homogenized emulsions. The batch prepared for the stability study was prepared with only 10 homogenization cycles, because cycle 10 was the most stable sample by the previous batch (emulsions with 1.2% egg lecithin). The same process was repeated with Tween 80, and the physical stability of the ketoconazole-loaded emulsions with both stabilizers was monitored over a period of 6 months.

Table 1 shows the ZP and the pH values of the Poloxamer-188- and the Tween-80-stabilized ketoconazole emulsions. There is no distinct change in pH and ZP values with increasing amount of surfactants, and both formulations show ZP values in the range of -52 mV to -40 mV, which is an indication of good long-term stability by electrostatic repulsion.

Table 2 shows the PCS characterization data of ketoconazole emulsions stabilized with different concentrations of Poloxamer 188 and Tween 80 added to 1.2% lecithin, on the day of production. In general, the PCS diameters do not change for both surfactants (added to 1.2% lecithin) except for 2.0% Poloxamer. There was a distinct increase in PCS diameters from 298 nm at 1.5% Poloxamer to 348 nm at 2.0% Poloxamer added to the emulsion. This increase in diameter was accompanied by a simultaneous increase in the polydispersity index. However, the Tween-80–stabilized emulsions did not show any change in particle size as a function of added surfactant concentration.

Ketoconazole emulsions stabilized with 2.0% Tween 80 were stable as a function of storage time over a period of 6 months. The PCS diameter stayed constant during this time and the polydispersity index was in the

Table 1. The pH and Zeta Potential Values [mV] of the Ketoconazole-Loaded Emulsions Stabilized with Poloxamer 188 and Tween 80

% of Poloxamer 188/ Tween 80 Added	Poloxamer 188 pH	[mV]	Tween 80 pH	[mV]
0% (1.2% lecithin)	5.23	-50.8	5.23	-50.8
0.3%	5.25	-50.1	5.21	-45.8
0.5%	5.22	-50.9	5.25	-45.4
0.8%	5.33	-51.7	5.22	-43.8
1.0%	5.19	-50.8	5.24	-43.9
1.5%	5.30	-51.2	5.20	-44.0
2.0%	5.53	-45.9	5.20	-43.9

Table 2. Photon Correlation Spectroscopy Diameters and Polydispersity Indices of Ketoconazole Emulsions Stabilized With Different Concentrations of Poloxamer 188 and Tween 80 on the Day of Production (Pressure 1500 bar; 10 Cycles)*

% of Poloxamer 188/ Tween 80 Added	Poloxamer 188 z-ave (nm)	PI	Tween 80 z-ave (nm)	PI
0% (1.2% lecithin)	265.9	0.110	265.9	0.110
0.3%	290.4	0.054	281.7	0.065
0.5%	288.3	0.059	284.6	0.087
0.8%	290.6	0.069	285.2	0.087
1.0%	289.3	0.067	285.1	0.075
1.5%	298.5	0.086	285.4	0.082
2.0%	324.1	0.271	284.5	0.068

*z-ave indicates photon correlation spectroscopy diameter; and PI, polydispersity index.

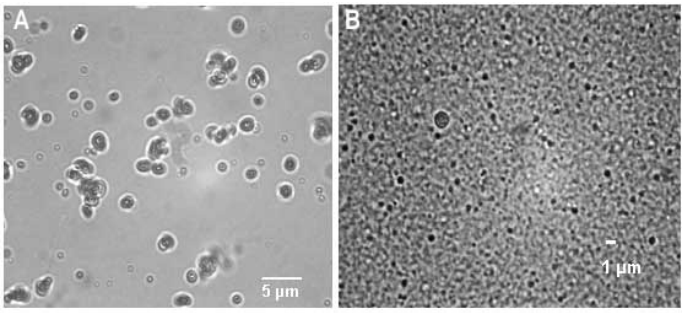

Figure 3. Light-microscopy pictures of ketoconazole-loaded emulsion stabilized with only 1.2% lecithin (A) and emulsion stabilized with addition of 2.0% Tween 80 after 6 months storage at room temperature (B).

range of 0.087 to 0.073. After 6 months, the emulsions were again inspected under polarized light, scanning 20 microscopic fields. Microscopic analysis of the ketoconazole emulsions stabilized with 2.0% Tween 80 revealed 3 pictures out of 20 microscopic fields with a single or only a few detectable emulsion droplets of approximately 1 to 2 µm in size; no drug crystals were observed. Figure 3 shows the photomicrograph of the unstable ketoconazole-loaded emulsion (only lecithin, Figure 3A) and stabilized with additional 2.0% Tween 80 (Figure 3B) taken 6 months after production. As can be seen, the number of oil droplets ≥1 µm is much less; no drug crystals are detectable in the Tween-80–stabilized emulsion. Therefore, the stabilization of the emulsions was successful, yielding a product applicable for parenteral administration.

Table 3, upper, shows the PCS diameters and the polydispersity indices of the 10.0 mg/mL ketoconazole-loaded emulsions. These emulsions were also stabilized with 2.0% Tween 80 and are currently under investigation in terms of their physical stability. After 20 homogenization cycles, the emulsion reached a PCS diameter of 150 nm, with a polydispersity index of 0.150. The LD diameter, D 50%, did not show a considerable difference as a function of homogenization cycles, but there is a clear increase in LD diameter, D 99%, at cycle 15. This is a result of too much energy given to the emulsion after 10 homogenization cycles. Basically, this is in agreement with the homogenization results of the 1.0 mg/mL ketoconazole-loaded emulsions, which were stabilized with only 1.2% lecithin. The most stable emulsion was cycle 10; emulsions produced with 15 and 20 homogenization cycles were the ones that have coalescenced first. As with the other drugs, the drug-concentration effect was observed with ketoconazole-loaded emulsions as

Table 3. Photon Correlation Spectroscopy Diameters and Polydispersity Indices of Ketoconazole-Loaded Emulsions (10 mg/mL) and Laser Diffractometry Diameters, D 50% and D 99%, as a Function of Homogenization Cycles*

Cycle	PCS z-ave (nm)	PI	LD D 50% (μm)	LD D 99% (μm)
1	154.4	0.172	0.102	0.284
5	140.0	0.159	0.095	0.248
10	139.5	0.156	0.094	0.250
15	136.5	0.135	0.096	0.332
20	142.4	0.141	0.096	0.298

*PCS z-ave indicates photon correlation spectroscopy diameter; PI, polydispersity index; and LD, laser diffractometry.

well. The PCS diameter decreased from 254 nm for 1 mg/mL (cycle 1) to 150 nm at 10.0 mg/mL (cycle 1). This is a very clear decrease.

CONCLUSION

It could be shown that—apart from amphotericin B and carbamazepine[14]—SolEmuls technology is applicable to itraconazole and ketoconazole. In case a drug destabilizes the emulsion (ketoconazole), physical stability problems can be easily solved by adding a steric stabilizer. It is also very important to note that high-pressure homogenizers used for SolEmuls technology are accepted by the regulatory authorities in the production of IV pharmaceuticals and can be run on large scale thus avoiding regulatory hurdles.

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