9 Summary of the thesis

The overall aim, to develop an improved in vitro formulation of cylosporine was realised in four steps:

First step:
Evaluation and validation of size analysis

Laser diffractometry (LD) is used as a frequent size analysis for suspensions in the nanosized range. According to light scattering theory, the real refractive index (RI) and the imaginary refractive index (IRI) need to be put into the software for proper analysis. However, these parameters are difficult to assess. Therefore scientists world-wide are using "simplified" approaches. This thesis investigated, if they are valid or create non-sense results (at least non-sense results in the majority of cases, ignoring when by luck the right result was obtained due to the compensation of different artefacts).

Very often, because the RI and IRI are not known, scientists are using the Fraunhofer model to analyse nanosized material. According to the theory, this model is only valid for particles 5 - 6 times larger than the wavelength of the light wavelength used for analysis, i.e. in the case of a helium neon laser of 750 nm being appr. 4µm. This existing and well known theory does not seem to affect the scientists in their choice of the analytical model used; many people are still using just Fraunhofer. The results of the thesis clearly show, these Fraunhofer analysis results might even lead into a wrong direction.

In order to enhance the sensitivity for very small particles by laser diffractometry, scientists tend to employ a high resolution mode (e.g. PIDS in case of the LS 230), which is an additional but different technique than pure laser diffractometry. The selection pleases the analyser, because it gives nice results of very small particles. However, the particles are only so small because the software seems to be programmed this way that it overestimates small particles whereas it ignores larger particles, which clearly leads to the non finding of larger particles within a polydisperse sample.

Even worse, scientists select the Mie analysis mode, but press the "automeasuring button". By doing this, the software selects the standard parameters for RI and IRI stored in the computer. Of course, the resulting size distributions are far away from reality. As a nice example the "magic" RI of 1.456 should be cited. This is the RI determined for parenteral fat emulsions, stored in the software of our LD system, but also being published world-wide as a refractive index. This led to the fact that in our group - by selecting the auto measuring button -, this index was also used for drug nanosuspensions. Of course, the LD results were false.

Meanwhile even in literature research this “magic value” is found as being valid for drug nanosuspensions of various drugs.

The invalidity of this approach was clearly shown in the model simulations performed and by the application of light microscopy, performed in parallel to the LD measurements. Light microscopy is often forgotten as a very important characterisation method. People tend to use methods as highly sophisticated (and expensive) as possible, because they think these methods are being better because of their complexity and expensiveness. However, “seeing is believing” as emphasised by the marketing people of the microscopy companies. Indeed, microscopy was found to be the best and most reliable method to prove the existence or absence of aggregates and/or large crystals within a sample and to check the validity of the LD analysis.

The LD analysis results were proven to be wrong by detecting large particles in non-diluted nanosuspensions by light microscopy. Using undiluted suspensions increases the likelihood of finding large aggregates and crystals.

To overcome the problems of the unknown optical parameters a method to determine the real refractive index was established by measuring the refractive index increment dn/dc by using an instrument with a precision of 6 digits behind the point (at the first glance this precision sounds ridiculous but it proved top be necessary for a precise measurement). The determination with a manual Abbé refractometer is not sufficient. In addition, the determination of the Becke line was performed. However, this method is not precise enough.

As a summary, a method was established to determine refractive indices and to perform accurate size measurements with suspensions in the nanosized range. One of the pre-requisites for developing the in vitro improved cyclosporine nanosuspension was fulfilled. In addition, publication of these data should improve LD analysis world-wide.

Second Step:
Evaluating the soundness of the existing screening procedures for obtaining optimised nanosuspension established and used by now

The existing procedure to screen for optimised stabiliser combinations was to prepare a stock nanosuspension with only one stabiliser and admixing concentrated solutions of the co-stabilisers (mixing ratio 1:1, e.g. 2ml stock plus 2ml). This accelerated the screening because 20 different stabiliser combinations could be generated from producing just one stock nanosuspension of 40 ml (instead performing 20 single homogenisations for each stabiliser mixture). In this thesis nanosuspensions were prepared according to the “established dilution

procedure" in the research group. Alternatively the same formulations were prepared homogenising each formulation.

The stability tests revealed that the separately homogenised nanosuspensions showed smaller crystal sizes and sometimes much better physical stability. It has been ignored that diluting the nanosuspensions with unsaturated stabiliser solution lead to dissolution effects of the nanocrystals. Of course the smallest crystals dissolve first and faster (higher saturation solubility) leaving behind the larger crystals. Thus the mean size increased. It could be shown that an annealing process by high energy input, that means homogenising the nanosuspension again after admixing of the co-stabiliser solution, avoided this instability. However, by doing this, the work load is very little reduced compared to preparing the formulations separately.

Based on these finding the standard operation procedure (SOP) for the screening procedure for optimised nanosuspensions had to be re-written in the research group.

Third step:
Coming up with a novel concept for an improved oral cyclosporine nanosuspension

The nanosuspension developed by Runge (dating back to 1996) showed an extremely poor bioavailability. The problem could definitely not be solved by just trying different other stabilisers without a new rational behind.

The new rational to be tried in this thesis was to investigate if surfactants or steric stabilisers, being simultaneously p-glycoprotein inhibitor, were able to efficiently physically stabilise cyclosporine drug nanosuspensions.

The basic approach of this thesis was to critically question if the previous approaches taken were correct at all or were the optimum approach (do not believe anything, check and verify it). Therefore it was investigated if the nanosuspension production procedure used by now in the research group (to homogenise without any temperature control) was suitable for cyclosporine. The result proved the lack of suitability of the previously used production technology for cyclosporine. Aggregation phenomena were observed above 30°C and very pronounced above 50°C. Mechanisms are the reduced solubility of cyclosporine above 30°C and potential structural changes in the molecule with increasing temperature.

Using the optimised production procedure, cyclosporine nanosuspensions were produced using different stabilisers, focussing on stabilisers with inhibitory effect on p-glycoprotein (e.g. TPGS). As nanosuspensions showing highest stability, the formulations with TPGS could be identified. These formulations are is suitable for an in vivo test, i.e. with the potential to lead to an increased oral bioavailability.

The stability of the cyclosporine nanosuspensions was investigated using the "old", i.e. previously used laser diffraction analysis in comparison to the optimised procedure developed in the first part of this thesis. The result again proved the unsuitability of the previous sizing approach. Based on this measuring procedure, all prepared nanosuspensions would have been judged as being perfectly stable. The new laser diffraction procedure was able to detect the particle aggregates in the unstable systems and clearly identified the TPGS formulation as the one with highest stability.

Fourth step:
"Thinking out of the box" (in German: Querdenken) about other formulations than necessarily nanosuspensions or lipid nanoparticles

As outlined in the section aims of the thesis, the research group focuses only on two approaches to solve delivery problems, the drug nanocrystals and the solid lipid nanoparticles/nanostructured lipid carriers (SLN/NLC). Of course this focus narrows the spectrum and it must not be forgotten, that other delivery systems, different from those above might be more suitable for certain actives. For cyclosporine it is reported that the bioavailability can be increased with the presence of lipid compounds during administration, which is obviously not the case for nanosuspensions.

Therefore alternative formulation approaches containing a lipophilic phase were investigated. Also here focus was put on the addition of p-glycoprotein inhibitors and small sized final formulations. Self emulsifying drug delivery systems (SEDDS) seemed to be a promising alternative. SEDDS are well described in the literature. The novel approach was to use a lipophilic compound which simultaneously has inhibitory effects on p-glycoprotein. Peppermint oil was identified to be appropriate for this.

The development of SEDDS containing peppermint oil only and no cyclosporine were successful. SEDDS with cyclosporine dissolved in peppermint oil could be produced but the droplet sizes generated were considered as being too large for an efficient bioavailability enhancement in the gut.

Another approach was the development of a stabiliser free formulation. The ratio behind was to avoid a possible entrapment of cyclosporine into the micelles of the stabiliser and a decrease in bioavailability therefore, as it is reported in the literature for many compounds.

The formulation developed is based on peppermint oil containing dissolved cyclosporine. The oily solution was simply absorbed into Aeroperls 300 and leads to a flowing powder (Cycloperls), which can be further processed (e.g. tablets, capsules).

Despite being surfactant-free the Cycloperls released the peppermint oil in very fine droplets, being distinctly smaller than the SEDDS droplets. From this it appears a worthwhile system for in vivo testing. The Aeroperls could be sufficiently loaded and remained as a flowing powder, suitable for capsule filling.

The overall conclusion:

A more reliable laser diffractometer analytical procedure was developed to assess with higher accuracy the size of nanocrystals, the prerequisite for a sound formulation development.

The screening procedure for optimised nanosuspension formulations was distinctly improved and the mechanism, leading to artefacts in the previous procedures, could be identified.

A next generation approach for drug nanocrystals was realised by the SmartCrystal® technology, by combining nanocrystal technology with the inhibition strategy for p-glycoprotein.

On top, an even simpler approach was realised by the development of the stabiliser free Cycloperls. In this formulation cyclosporine is dissolved in an oil with inhibitory effect on p-glycoprotein (peppermint oil), the oily solution is simply absorbed into porous Aeroperls.

In this thesis two alternative cyclosporine formulations were developed– SmartCrystal®s and Cycloperls. The formulations are physically stable and should theoretically show an improved oral bioavailability, which should be proved in in vivo tests.