

8 Summary

This thesis investigates the possibilities and limits of the SolEmuls[®] technology on the basis of three drugs which have a model character due to their physical and chemical characteristics, but are also of relevance for therapy. By co-homogenisation of emulsions and drug powder, the SolEmuls[®] technology facilitates the integration of the drug into the interface of the emulsion without using organic solvents. Therefore, by means of this technology emulsions can be loaded with drugs which are insoluble in water and in oil. A good example of this phenomenon is the drug amphotericin B, which is the focus of this study, along with xenon and omeprazole. Xenon is only slightly soluble in oils and it was assumed that the interfacial layer also plays an important role for loading the emulsions with Xenon. The best *in vivo* results were obtained by using highly dispersed xenon emulsions with a large interface. Xenon is also an excellent and well tolerated anaesthetic with high potential in therapy. Omeprazole, also only slightly soluble in oils, was chosen as third model drug; the aim being to produce higher concentrated emulsions by additionally localising the drug into the lecithin layer. The ability of the interface to stabilise the drug was investigated. From this perspective, omeprazole is an ideal model drug because of its chemical lability and its coloured degradation products which allow a macroscopical screening on the basis of the colour of the emulsions.

The first amphotericin B emulsions were produced by Dr. Sven Schmidt, the work was continued in this thesis. Several parameters with relevance for physical and chemical stability of the amphotericin B emulsions were systematically investigated, the number of homogenisation cycles used for producing the emulsions by high pressure homogenisation being very important. It was found that the emulsions produced with several cycles were physically stable, but there was no clear dependency regarding chemical stability. Typically, the drug powder or the Nanosuspension is added to a pre-formed parenteral o/w emulsion. Here the alternative *de novo* production was investigated, though using this method no further chemical stability could be produced. Another important parameter is the fineness of the raw material. The high streaming velocities of the fluid in the homogenisation gap lead to a fast dissolution of the drug powder; this is the physical principle of the SolEmuls[®] technology. Further decreasing the particle size, and therefore also the surface area, also accelerates the dissolution rate. Using a fine raw material, e.g. a nanosuspension, fewer homogenisation

cycles are necessary and therefore advantageous for later potential industrial production. The amphotericin B nanosuspensions contained fine particles, which, however, had a tendency to aggregate. In this work it was therefore investigated, if aggregation disturbed the emulsion production process. Optimised amphotericin B nanosuspensions with glycerol media were produced, a glycerol content of 60% being optimal. This work shows that potential aggregation is not important as a production factor. In this thesis, different contents of amphotericin B were observed both immediately after production and after various storage times. To clarify this finding, the influence of various temperatures on the emulsions was investigated, e.g. after production, over a period of 7 hours or during sterilisation.

The physical and chemical long-time stability is an important criterion for potential application. Amphotericin B emulsions were investigated over a period of 6-12 months. The emulsion itself was found to be stable, but during storage the drug precipitated out of the lecithin layer and amphotericin B crystals formed. Therefore, an amphotericin B emulsion of 1 mg/mL is stable for a short time and a concentration of e.g. 0.5 mg/mL could lead to a long-time stable emulsion.

The effect of the variation of the lecithin layer composition, for example by using soya lecithin or hydrated lecithins, on the chemical stability was also intensively studied. It was determined that the chemical composition of the lecithin layer greatly influences the chemical stability of amphotericin B. With soya lecithin, almost all amphotericin B degraded during sterilisation, whereas the usually used egg lecithin led to a drug content of up to 98% after the sterilisation process. The influence of the lecithin layer on chemical stability is clear, but under identical production parameters variations of the amphotericin B content still occurred during sterilisation and storage. This phenomenon remains to be more fully clarified.

Tolerability, i.e. potential toxicity, and biological effectiveness are important for applications of the amphotericin B emulsions. *In vitro* and *in vivo* studies were performed and the biological effectiveness was demonstrated for the sterile and non-sterile emulsions. No acute toxicity was seen *in vivo*. This result was expected because of the use of the fat emulsions in the parenteral nutrition and the described reduction of side effects due to the fat emulsions.

Finally, the enclosed content of amphotericin B in the fat emulsions was studied by means of centrifugation test. This centrifugation test was not suitable for determining the insoluble drug content, at least not under the conditions used, since the centrifugation pressure drives the drug out of the interface. Therefore, the microscopical examination of the undiluted

emulsions, up to now the standard procedure for determining larger particles in parenteral emulsions, remains the best method.

In vivo studies of highly dispersed emulsions with the model drug Xenon had already yielded some successful results. These emulsions were produced from pre-formed parenteral emulsions by additional high pressure homogenisation followed by loading the emulsion with xenon at a pressure of 2 bar. Here it is demonstrated –in contrast to the working hypothesis of Prof. Dr. Georgieff’s group - that the size of the interface and the composition of the lecithin layer had no effect on the loading capacity. The solubilities of xenon in the single phases were found to be additive, if the phases only mix weakly with each other. The usually important interface in the SolEmuls[®] technology plays only a secondary role for the xenon emulsions. For fluids which mix with each other (e.g. the oil LCT and ethanol or water and ethanol), there is no additive effect. The additivity of these solutions deviates from the ideal case; unfortunately, the deviation is negative, meaning that a mixture of the components leads to a lower solubility than would be theoretically expected. Thus, the ideal emulsions for loading xenon consist of single components with a high xenon solubility, which should be stabilized with a well tolerated emulsifier e.g. lecithin.

Based on these findings, 7 emulsions were used for *in vivo* tests in pigs, e.g. Abbolipid[®] 10% with 4% of tween[®] 80. This emulsion also showed the best clinical effect. In general, the major problem remains the loss of xenon in the lung passage. During the *in vivo* studies an apnoe of 2 min was used to reduce the problem, but this can later not be performed therapeutically. In this study, targeting with apolipoprotein E did not transport a sufficient amount of xenon to the brain. The loss during the lung passage was found to be too high. Although the systems were optimised galenically, they cannot be employed in therapy because of the given anatomic conditions (loss through the lung passage).

In the final part of this thesis, the effect of a possible chemical stabilisation of a very labile drug, in this example omeprazole, was investigated. Emulsions with 1 mg/mL, 2 mg/mL and 3 mg/mL were loaded with omeprazole. In theory, 1 mg/mL should dissolve completely in the oil phase. The other emulsions were “oversaturated” by dissolving the omeprazole in the lecithin layer, with crystal formation in the 3 mg/mL emulsion. The 2 mg/mL emulsion showed only few crystals over the investigation period. It follows that in case of omeprazole, the used lecithin did not show a clear stabilisation effect. In contrast to this result, the same lecithin showed very good stabilisation effect in the case of amphotericin B. In conclusion, it is clear that there is a drug specific interaction with the stabilising molecules, meaning that

determining the optimal chemical composition of the stabiliser layer must be performed for every drug. The ideal case would involve controlled development in combination with spectral studies for monitoring molecular interactions.