Cystic Fibrosis (CF) is the most frequent lethal inherited disease in the white population. In CF patients, the pulmonary complications are responsible for the majority of morbidity and virtually all mortality. Life expectancy has improved dramatically over the last decades due to a higher efficacy of the antibiotic treatment but also due to improved mucolytic therapy, airway physiotherapy and improved nutritional strategies including the use of pancreatic enzyme preparations. Gene therapy is a promising new therapeutic strategy but not yet available and probably will not be for the majority of the adult patients in short time.

The optimization of antibiotic treatment has been subject of research in our Cystic Fibrosis center for several years. To date, there is no consensus what strategy of antibiotic therapy is to be preferred. The inhalation of antibiotics seems an interesting option to reach the microorganisms in the airways. Limited efficacy of the inhalation of antibiotics and limited knowledge of the pharmacokinetics after inhalation provided a rationale for a number of studies described in this thesis. Nebulizers are the only devices currently used in CF to inhale antibiotics. A drawback of inhalation therapy with nebulizers is the low deposition efficiency of the drug in the airways. Furthermore, because it is time consuming, patient compliance is relatively low. Reasons why a dry powder inhaler, being a more efficient and convenient alternative, was developed and investigated.

In chapter 1 a general introduction is given. The actual knowledge of inhalation therapy is summarized and objectives of the studies are defined. Subsequently, general aspects of inhalation therapy with nebulizers are discussed in Part 1 (chapters 2 and 3) of this thesis. In chapter 2 the technical aspects of nebulization therapy are described and two basic types of nebulizers, the jet and the ultrasonic nebulizer are discussed. Furthermore, the two main parameters to evaluate the performance of nebulizers are defined: the droplet size distribution of the aerosol and the drug output rate.

The choice of the type of nebulizer for nebulization of a certain drug solution should initially be based on laboratory evaluation. The major part of the mass or volume distribution should preferably correspond with aerodynamic particle diameters in the range of 1 to 5 micrometer. The intended drug output must be realized within a reasonable nebulization time (less than 30 min). The relation between in vitro and in vivo deposition is only partly understood and to date it has not been possible to predict drug delivery from in vitro studies on nebulizers only. Therefore, patients’ studies should be performed before a drug solution for nebulization can be recommended for clinical practice.
Summary

Furthermore, the mechanical properties of nebulizers are likely to change during use. Incorrect cleaning, maintenance and disinfection procedures may change the nebulizer performance in time. Reason why the performance of a nebulizer should be checked periodically.

In chapter 3 cleaning and disinfection methods for nebulizers are reviewed. Based on the existing methods the conclusion is drawn that a general cleaning and disinfection method for all common nebulizers is feasible.

The studies described in Part 2 (chapters 4 to 7) of this thesis started with a practical question: Which nebulizer should be used for inhalation of tobramycin and how should the apparatus be used in practice?

Fourteen commercially available jet and ultrasonic nebulizers were investigated with the aim to select the most suitable type of apparatus for the inhalation of a 10% tobramycin solution.

Two different techniques for measurement of particle size distribution were evaluated: laser diffraction and cascade impactor analysis. To standardize the measurements, a special adapter was constructed to connect the devices to the laser diffraction apparatus. The final selection of the nebulizers was based on particle size distribution, output and stable performance during nebulization.

It was concluded that 3 jet nebulizer compressor combinations (Porta-Neb Sidestream, Porta-Neb Ventstream and Paribo Pari LC+) have a reasonable output and an acceptable particle size distribution for the administration of a 10% tobramycin solution. The Sidestream is less suitable for the administration of antibiotics because of a higher loss of drug to the environment during exhalation. In practice, the Ventstream and the Pari LC+, known as so called “breath assisted, open vent nebulizers”, resulted to be the only nebulizers suitable for further investigations with tobramycin. The Ventstream was used in further experiments.

The practical question evolved to further investigations aiming to improve the antibiotic inhalation therapy in CF patients. Tobramycin and other aminoglycosides exhibit rapid, concentration dependent bacterial killing. However, a layer of viscid mucus and alginate-like slime limits the diffusion of antibiotics in CF patients. Therefore, a high concentration at the site of infections is of paramount importance.

However, it is likely that not all sites of infection are reached by current inhalation therapy with antibiotics and in case of exacerbations intravenous administered antibiotics are added. If effective serum concentrations could be reached by inhalation therapy as well, this method of administration may be an alternative for intravenous treatment.
In *chapter 5* the development of a highly concentrated tobramycin solution for inhalation is described. Several tobramycin solutions, ranging from 5 to 30% (m/v), were compared after aerosolization with a Porta-Neb Ventstream jet nebulizer combination and a Medix Sonix 2000 ultrasonic nebulizer. Laser diffraction and cascade impactor analysis were used for the particle size characterization of the aerosolized solutions. The output rate was determined in volume and mass output per minute.

From the output rate measurements it was concluded that a 20% tobramycin solution is the optimal and maximal concentration to be aerosolized. The jet nebulizer combination was found to be the most suitable. Using the jet nebulizer combination and the 20% solution, it is possible to administer a dose of 1000 mg tobramycin by inhalation within 30 minutes.

Based on the *in vitro* results, the nebulization of a high dose of tobramycin aiming at a therapeutic serum concentration was further investigated in CF patients.

The aim of the pilot study described in *chapter 6* was to assess the pharmacokinetics of tobramycin under optimal and standardized aerosol circumstances that were concluded from *chapter 4*. Six patients were studied after inhalation of 600 mg tobramycin. A jet nebulizer combination loaded with a 10% solution of tobramycin in water was used. The maximum serum levels of tobramycin ranged from 0.77 mg/L to 3.63 mg/L (mean 1.70 mg/L). The pharmacokinetic data were best described by a two-compartment model. Compared to intravenous administration, a prolonged terminal half life was found, which could be explained by the slow absorption of tobramycin from the site of administration (flip-flop model).

Despite standardized aerosol conditions, considerable interpatient variability was observed. However, the relatively low serum levels allow a further increase of the dose.

In *chapter 5* it was found that 1000 mg tobramycin (as a 20% solution) is the highest dose that can be nebulized with currently available devices within a reasonable time.

In the study described in *chapter 7* the pharmacokinetics of tobramycin after inhalation of 1000 mg (as a 20% solution) with a Porta-Neb Ventstream jet nebulizer combination were investigated in five patients. The maximum serum concentrations ranged from 1.78 to 3.25 mg/L (mean 2.42 mg/L). Again a prolonged terminal half-life was observed. The observed prolonged residence time of tobramycin in the lung after inhalation of a high dosage is favorable regarding its clinical effect.
To date, the exact airway deposition pattern after inhalation is not known. Due to obstructions by mucus not all sites of infection will be reached. Inhaled antibiotics most likely will reach well ventilated segments of the airways whereas systemically administered antibiotics may reach the well perfused regions. Therefore, in treatment of exacerbations sufficiently high serum concentrations are also required. It is assumed that peak serum levels of at least 8 mg/L are required for clinical efficacy. Such concentrations cannot be reached with current inhalation therapy only. After inhalation of 1000 mg a tobramycin peak concentration of 3.25 mg/L was found at maximum.

The deposition and consequently the concentration of tobramycin in the airways is important as the serum level is only an indirect parameter for the efficacy of the inhalation therapy. The higher administered dose gave a significant increase of the area under the curve (AUC) reflecting the higher deposition of tobramycin in the airways. To evaluate the consequence of the higher deposition, a pharmacokinetic inhalation model was constructed as a first step to elucidate the concentration-time profile of tobramycin in the airway compartment and the resulting serum concentration. High tobramycin concentrations (> 100 mg/L) were calculated for the airway compartment. A high concentration of tobramycin in the target area is critical for the success of the therapy. The simulated tobramycin concentrations of the airway compartment were well above minimal inhibition concentration (MIC) levels of *Pseudomonas aeruginosa*. These concentration data are in accordance with measured sputum levels after inhalation of tobramycin or gentamicin as reported in several studies.

The inhalation model may also be used to answer the question whether exacerbations can be treated with inhaled tobramycin. Using the Porta-Neb Ventstream combination an inhalation time of at least 1.5 hour is necessary to deliver 200 mg in the target area, which will lead to therapeutic serum concentrations. Such long inhalation times are not acceptable for clinical treatment.

Finally, the model offers a Bayesian prior in therapeutic drug monitoring to predict accumulation of serum levels and prevent toxicity. Toxic tobramycin trough levels are not expected with a lungdose of 44 or 67 mg twice a day, which can be attained with a nebulizer dose of 600 and 1000 mg, respectively.

Next to tobramycin, colistin is another antipseudomonal drug used by inhalation in CF patients. In Part 3 of this thesis studies with colistin are described.
To date, no pharmacokinetic data or serum levels are available after inhalation of colistin. Pharmacokinetic data of colistin are limited to intravenous administration. These data are mainly based on microbiological assay techniques. In case of inhalation therapy lower serum levels are to be expected. Therefore, a sensitive analytical method with an acceptable coefficient of variation is needed for the determination of colistin in patient samples after inhalation. The development and validation of the method are described in chapter 8.

Nebulization of a drug solution has some drawbacks, e.g. the long inhalation time, the low pulmonary deposition, and the therapeutic efficacy. To improve patient compliance, a dry powder inhaler (DPI) provides a possible alternative for the nebulization of aqueous solutions of antibiotics. Both the powder formulation and the inhaler device contribute to its performance.

In chapter 9 we describe the development of a colistin dry powder dosage form for inhalation. Furthermore, a new inhaler device, which operates adequately at a wide range of inspiratory flows, was constructed.

It is possible to increase the lung deposition of colistin inhalation from 10% to about 40% of the inhaler device nominal dose. Furthermore, by using a dry powder inhaler instead of a nebulizer the patient comfort is increased by the reduced time needed to administer the dose.

The test inhaler can be loaded with doses of 12 mg. Assuming a deposition efficiency of 40%, approximately 5 mg colistin will reach the airways. From literature data, the mean bioavailability of a nebulizer is assumed to be approximately 10% of the nebulizer dose. To date, an aqueous solution of 160 mg of colistin is used in maintenance therapy and approximately 16 mg will be deposited in the airways. This means that 3 inhalations of the powder formulation are required in order to mimic the deposition of the nebulization of 160 mg colistin as a solution.

We concluded that the new formulation in combination with the test inhaler may contribute to the efficacy of the antibiotic therapy in CF patients. It is very likely that the patient compliance will benefit from the increase in comfort. These assumptions were investigated in a clinical study described in chapter 10.

The aim of the study described in chapter 10 was to assess the feasibility of the new formulation in combination with a new inhaler device in healthy volunteers. Furthermore, the feasibility of the dry powder inhaler as compared to the nebulization of colistin was investigated in CF patients. The test inhaler used in chapter 9 was improved with a dose system. The serum concentra-
tions of colistin after the inhalation of a nebulized solution and after inhalation of a dry powder were used as an indirect parameter to compare the deposition efficiency of both inhalation systems in patients.

Volunteers and patients were asked to inhale a dose of 25 mg colistin as a dry powder mixture with lactose (colistin content 83.3%). The patients were also asked to nebulize 160 mg colistin as a solution.

The individual pulmonary function parameters (FEV1 and IVC scores) were not significantly different before and after inhalation of the dry powder by the volunteers nor after nebulization of the solution by the patients. In some CF patients a decrease in pulmonary function and cough was observed after inhalation of the dry powder. The pulmonary function recovered slowly.

In patients the peak serum concentrations ranged from 18 to 64 μg/L after inhalation of 160 mg colistin as a solution and from 77 to 159 μg/L after inhalation of 25 mg colistin as dry powder. Furthermore, the AUC tends to be higher after inhalation of the dry powder, indicating an even better deposition for the dry powder inhaler than expected.

The new DPI formulation provides a potential effective alternative for nebulized colistin. The convenience of the device is a major advantage and the dosage form was highly appreciated by the patients. It will improve patient compliance and may allow for a higher dosing frequency. The observed decrease in pulmonary function and cough in some patients are a serious drawback and are not acceptable for long term use. However, there are several possibilities to optimize the dry powder inhaler.

This study shows that the dose of 25 mg colistin can be drastically reduced. A dose of 10 mg colistin sulfate will suffice to mimic exposure of the currently used nebulization of 160 mg. The powder formulation can be further improved by optimizing the particle size distribution of the drug. This may lead to greater accuracy of deposition in specific regions of the airways. Finally, a lower dose in combination with a bronchodilator pretreatment may overcome the observed side effects. These improvement strategies are interesting subjects for future studies.