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Characterisation of an in vitro dissolution method for assessment of novel pulmonary drug delivery systems

With a focus on controlled release systems

IRÈS VAN DER ZWAAN



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Abstract

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Pulmonary drug delivery has been used for decades to treat local diseases like asthma. When using the pulmonary route to deliver drugs, several important lung features are being used, such as a large surface area available for absorption, high organ vascularization, and a thin blood-alveolar barrier. Pulmonary drug delivery systems on the market are formulations with a rapid release, which leads to a high drug concentration initially and a prompt decline in concentration shortly thereafter. This could cause unfavourable adverse effects or toxicity to the lung tissue at the onset of the release and could also result in decreased efficacy. To overcome these challenges, there is a need to develop controlled release drug delivery systems to improve the therapeutic effectiveness of inhaled drugs. When a drug is inhaled, the drug particles will deposit in the lung, and the drug needs to dissolve in the lung fluids before the drug is available for uptake locally or in the systemic circulation. The absorption inhaled drug thus depends on the dissolution of the drug particles in the lung fluid. As a result, it could be possible to prolong the duration of the drug effect, by prolonging the time it takes for the dissolution of the drug particles. Due to this, in vitro methods analysing the dissolution of the drug particles in the lung are of high relevance for the development of novel pulmonary drug delivery systems. It is therefore of high importance that the dissolution profiles that are measured are well understood. The overall aim of this thesis was to evaluate and characterise an in vitro dissolution method (Transwell system) for assessment of novel pulmonary drug delivery systems, with a focus on future controlled release systems. A developed mechanistic model was used to analyse experimental dissolution data and to predict which process was the rate limiting step in the obtained profiles. The developed mechanistic model provided the same rank order as the Weibull fit, however the model provided additional detailed understanding of the used dissolution process and setup. In addition, two novel controlled release drug delivery systems, mesoporous silica particles and hyaluronic based hydrogels, were successfully analysed using this in vitro dissolution system. Both delivery systems showed a promising aerosolization and control over the release profiles. Finally, the micellar contribution to diffusion of poorly soluble inhaled drugs during in vitro dissolution was defined and validated using the obtained in vitro dissolution profiles. Physiologically based biopharmaceutics modelling tools were successfully established for Bud, BDP and FP using the diffusivity values taking into account the micellar contribution of the surfactant.

Keywords: Pulmonary drug delivery, in vitro dissolution, mechanistic model, controlled release, physiologically based biopharmaceutics modelling

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Dance for yourself. If someone understands, good. If not, no matter.
- Louis Horst

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I. Frenning, G., **van der Zwaan, I.**, Franek, F., Fransson, R., Tehler, U. (2020) Model for the analysis of membrane-type dissolution tests for inhaled drugs. *Molecular Pharmaceutics*, 17:2426-2434.
- II. **van der Zwaan, I.**, Franek, F. Fransson, R., Tehler, U., Frenning, G. (2022) Characterization of membrane-type dissolution profiles of clinically available orally inhaled products using a Weibull fit and a mechanistic model. *Molecular Pharmaceutics*, 19(9):3114-3124.
- III. **van der Zwaan, I.** Pilkington, G. A. Frenning, G., Pitcairn, G. R., Feiler, A. Mesoporous silica particles for pulmonary drug delivery: multiple routes towards controlled release. (*Submitted*)
- IV. Nikjoo, D.*, **van der Zwaan, I.***, Rudén, J., Frenning, G. Engineered microparticles of hyaluronic acid hydrogel for controlled release of salbutamol sulfate. (*Manuscript*)
* Contributed equally to the work
- V. **van der Zwaan, I.**, Franek, F., Westergren, J., Tehler, U., Frenning, G., Fransson, R. Determination of micellar contribution to diffusion of poorly soluble inhaled drugs during dissolution and extrapolating the micellar contribution in PBBM. (*Manuscript*)

Author's contribution to the publication: Paper I – Investigation and writing, Paper II, III and V – Conceptualization, methodology, validation, investigation and writing, Paper IV – was in collaboration with Dariush Nikjoo, I assessed aerodynamic performance, drug release, quantification, discussed results and conclusions and co-authored the manuscript.

Additional papers not included in this thesis

- a. Nikjoo, D., van der Zwaan, I., Brülls, M., Tehler, U., Frenning, G. (2021) Hyaluronic acid hydrogels for controlled pulmonary drug delivery – A Particle engineering approach. *Pharmaceutics*, 13 (11), 1878.
- b. van der Zwaan, I., Frenning, G. (2023) A new modelling approach for dissolution of polydisperse powders. *Int. J. Pharm*, 633, 122626.

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Abbreviations

A	Surface area of the solid-liquid interface
$AAFE$	Absolute average fold error
AFE	Average fold error
ACI	Andersen Cascade Impactor
API	Active Pharmaceutical Ingredient
B-17-MP	Beclomethasone 17-monopropionate
BDP	Beclomethasone dipropionate
Bud	Budesonide
b	Shape parameter
C	Bulk concentration
COPD	Chronic Obstructive Pulmonary Disease
C_b	Concentration dissolved free monomer
C_s	Solubility
c_s	Non-dimensional solubility/inverse dose number
D	Diffusion coefficient of drug in dissolution medium
D_{API}	Diffusivity of API in water
$Diameter_{API}$	Diameter of the API
DPI	Dry Powder Inhaler
D_{SDS}	Diffusivity in SDS
$Diameter_{SDS}$	Diameter of an SDS molecule
d_{10}	10th percentile
d_{50}	Mass median diameter
d_{90}	90th percentile
ED	Emitted dose
$F_{dissolution}$	Predicted theoretical dissolution profile
FP	Fluticasone propionate
FPF	Fine Particle Fraction
FSI	Fast Screening Impactor
$f_{stirring}$	Stirring factor (Nielsen)
HAGA	Hyaluronic acid hydrogel particles
HASS	Hyaluronic acid hydrogel particles loaded with Salbutamol
h_{stag}	Thickness of the stagnant layer
MMAD	Median mass aerodynamic diameter
mACI	modified Andersen Cascade Impactor
NGI	Next Generation Impactor

OBS	Observed
PBBM	Physiologically Based Biopharmaceutics Modelling
PBPK	Physiologically Based Pharmacokinetic modelling
PBS	Phosphate-buffered Saline
PIDS	Polarization Intensity Differential Scattering
PK	Pharmacokinetics
PRED	Predicted
pMDI	pressurised Metered Dose Inhaler
R	Particle radius
S_0	Initial concentration of solid drug in the donor compartment
S_b	Solubility in the bulk of freemonomer
S_{buffer}	Solubility of the drug in the buffer/aqueous phase
S_{SDS}	Solubility of the drug in the specific SDS volume fraction
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscopy
SLF	Simulated Lung Fluid
TFA	Trifluoroacetic acid
t_{63}	Time at which 63% of the fraction is dissolved
t_{abs}	Characteristic absorption time
t_{diff}	Characteristic diffusion time (t_{abs} in Paper IV-V)
t_{diss}	Characteristic dissolution time
UPLC-UV	Ultra Performance Liquid Chromatography coupled with UV
UV	Ultraviolet
u	Fraction absorbed drug
σ	Shape parameter in lognormal particle-size distribution

Introduction

Pulmonary drug delivery

Inhalation therapy has its origin at least 4000 years ago, when traditional Ayurvedic medicines were used to treat lung problems by smoking several herbal preparations.¹ However, despite the long history in inhalation therapies and the successful symptomatic treatment of local lung diseases, the pulmonary route is still an administration route with great potential and many challenges.

When using the pulmonary route to deliver drugs, several important lung features are being used, such as a large surface area available for absorption, high organ vascularization, and a thin blood-alveolar barrier.²⁻⁴ Besides these features, delivery via the lung provides the possibility of a rapid onset of action and avoidance of first pass metabolism.² In addition, pulmonary delivery systems are convenient for patients and can be used to treat local and systemic diseases.^{3,5} This results in a drug delivery route that potentially has several advantages over other drug delivery routes of administration.^{2,3} In addition, the pulmonary drug delivery route could be suitable for small molecules, peptides, proteins, microRNAs, and vaccines, which makes it a drug delivery route with numerous opportunities.⁶⁻⁸

However, pulmonary drug delivery has several disadvantages that make the development of successful novel drug delivery systems challenging. The size of the drug particles is of high importance to successfully deliver the drug to the lungs. The optimal aerodynamic size range for drug particles used in a pulmonary formulation should be between 1 and 5 μm . This allows particles to reach the lower lung and avoid contact with the upper airways.⁹ Besides the particle size, mucociliary clearance and cough in the upper airways and rapid clearance by the alveolar macrophages in the lower airways are processes that potentially could challenge the development of pulmonary delivery systems.¹⁰ Despite these challenges, several orally inhaled drugs are available on the market, and in recent decades these drugs have successfully provided symptomatic relief for millions of patients suffering from lung diseases, such as chronic obstructive pulmonary disease (COPD) and asthma.¹¹⁻¹³ These commercially available drugs are generally available in three different types of devices, Dry Powder Inhalers (DPI), pressurized Metered Dose Inhalers

(pMDI), and nebulizers. DPIs contain dry powder formulations, while pMDIs and nebulizers contain suspensions and solutions.^{14,15}

Controlled release formulations

Pulmonary drug delivery systems on the market are formulations with a rapid release, which leads to a high drug concentration initially and a prompt decline in concentration shortly thereafter. This could cause unfavourable adverse effects or toxicity to the lung tissue at the onset of the release and could also result in decreased efficacy.² To overcome these challenges, there is a need to develop controlled release drug delivery systems to improve the therapeutic effectiveness of inhaled drugs. A controlled release drug delivery system would avoid high peak concentrations at the onset of drug release and would provide a longer therapeutic effect due to a prolonged release of the drug, with drug concentrations staying within the therapeutic window. Additionally, by controlling the drug release of the formulation, the dosing frequency can be reduced, thereby improving patient compliance, and the drug can be released at a specific target.^{5,16} Even though a pulmonary controlled release drug delivery system would be highly valuable, there is currently no pulmonary controlled release formulation available on the market.⁵

Regardless of the lack of pulmonary controlled release formulations on the market, a tremendous amount of research has been done to develop possible novel formulations. Several different formulation approaches have been used and developed, including micelles, liposomes, polymeric nanoparticles, solid lipid nanoparticles, dendrimers, large porous particles, swellable microparticles, PEGylated conjugates, and atomic layer deposition.^{5,17-21} Two different novel controlled release delivery strategies will be studied in this thesis; amorphous mesoporous silica particles and hyaluronic acid hydrogels.

Amorphous mesoporous silica particles have been studied for several decades as pharmaceutical delivery systems, mainly for different delivery routes, such as topical drug delivery.^{22,23} More recently, however, these mesoporous silica particles have been studied for inhalation.^{24,25} One of the main advantages of these silica particles is the ability to tune both particle and pore size. Moreover, the particles allow for high drug loading and can enhance the release of poorly water-soluble drugs due to encapsulation in the silica matrix.^{23,26,27}

Hydrogels have been widely used for several different clinical applications, such as tissue engineering and drug delivery, due to their ability to form matrix networks from water-soluble natural or synthetic polymers.²⁸ Due to the low toxicity, biodegradability, and high biocompatibility, hydrogels could be of

interest when developing novel drug delivery systems. Hydrogels have therefore been studied intensely mainly for other delivery routes, such as the oral route. However, developing hydrogels for pulmonary drug delivery can be beneficial, as hydrogels have mucoadhesive properties and can prolong the residence time in the lungs.²⁹ Additionally, the porous matrix structure of the hydrogels can be modified, which can be useful in the development of a controlled release formulation.^{28,30}

Lung anatomy and physiology

The human lungs are a complex organ that are built to facilitate gas exchange between the air and blood, but are also highly efficient in removing particles.³¹ The lungs can be visualized as branches of a tree, the so called tracheobronchial tree, where every branch divides to form two smaller airways (Fig. 1). The tracheobronchial tree is divided into 23 generations, or branches, from the trachea down to the alveolar sacs. These generations can be divided into two regions, the central region, transporting gases to and from the external environment, and the peripheral region, where the gas exchange takes place.³¹⁻³⁴

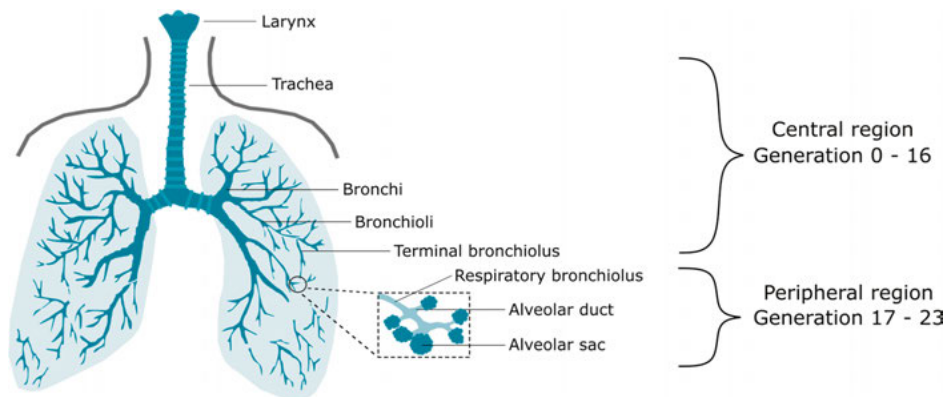


Figure 1. Schematic figure of the human tracheobronchial tree including the corresponding generations.

Central region

The central region, also referred to as the conducting zone, consists of the trachea, bronchi, bronchioles, and terminal bronchioles, defined as generation 0 to 16.³¹ The central region of the lungs contains large epithelial cells, a thick mucus layer, and a relatively large amount of lung lining fluid (Fig. 2). The central region of the lung contains a thick layer of epithelial cells, and drug

permeation over this layer typically is the limiting factor for drug absorption.^{35,36} In the central region, particles are cleared by the mucociliary escalator, which moves particles up to the pharynx to eventually be swallowed.^{34,37}

Peripheral region

The peripheral region, also referred to as the respiratory zone, consists of the respiratory bronchioles, alveolar ducts, and alveolar sacs, defined as generation 17 to 23.³¹ The peripheral region contains a thin layer of epithelial cells, a thin mucus layer, and a small amount of lung lining fluid (Fig. 2). For the peripheral region, the limited amount of lung lining fluid causes the solubility and dissolution of a drug to be the limiting factor for absorption.^{35,36} Particles in the peripheral region are cleared by phagocytosis.³⁷

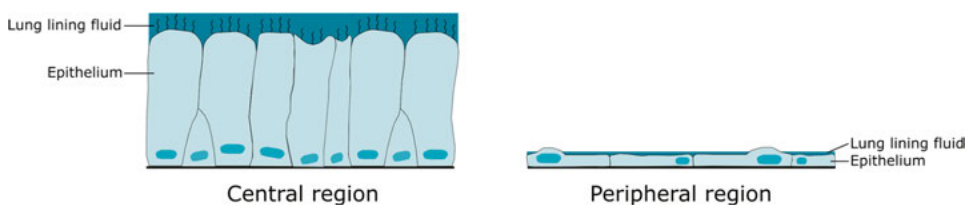


Figure 2. Schematic figure of the lung epithelium in the central and the peripheral lung regions.

Absorption of inhaled drugs from the lung

Lung absorption is affected by several different important processes. To successfully predict the absorption of inhaled drugs, these processes, such as deposition and dissolution of the particles, need to be well understood.

Deposition

The deposition of an inhaled drug is influenced by many different factors including inhalation manoeuvre and device (e.g. rate of inhalation, inhaled volume, and device design), as well as lung physiology and drug product (e.g. aerodynamic particle size, shape, and density).^{31,34} As mentioned earlier, the optimal aerodynamic particle size is between 1-5 μm .⁹ However, depending on if the particles are smaller or bigger within this size range, the particles end up in different lung regions. Particles with a bigger aerodynamic particle size end up in the central lung region, and particles with a smaller aerodynamic particle size end up in the peripheral region.^{36,38} Due to the anatomical differences between the two lung regions, as described before, the deposition of drugs in the different regions could influence the absorption profiles of orally inhaled drugs.

Dissolution

When a drug is inhaled, the drug particles will deposit in the lung, and after deposition, the drug has to dissolve in the lung fluids before the drug is available for uptake locally or in the systemic circulation (Fig. 3).^{39,40} The absorption of the inhaled drug thus depends on the dissolution of the drug particles in the lung fluid.⁴¹ As a result, it could be possible to prolong the duration of the effect of a drug, by prolonging the time it takes for the dissolution of the drug particles.⁴² Due to this, in vitro methods analysing the dissolution of the drug particles in the lung are of high relevance for the development of novel pulmonary drug delivery systems and can be useful to establish in vitro – in vivo correlations.³⁹ It is therefore of high importance that the dissolution profiles that are measured are well understood.

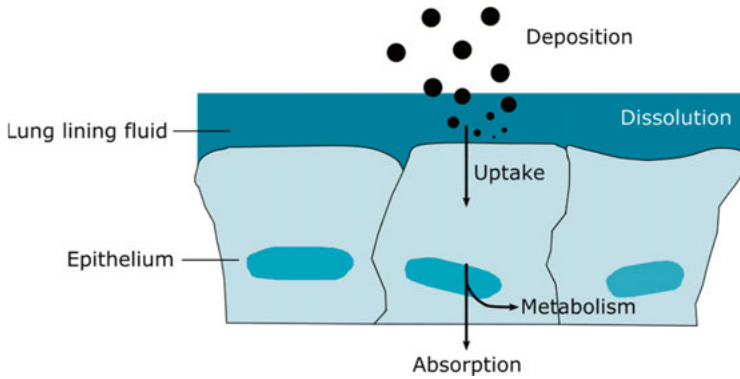


Figure 3. Schematic overview of the path of drug particles that deposit in the lung until absorption.

Dissolution is defined as the rate of mass transfer from a solid to a solvent and can be described by the Noyes-Whitney/Nernst-Brunner equation,^{43–46}

$$\frac{dM}{dt} = \frac{D \times A \times (C_s - C)}{h_{stag}} \quad \text{Eq. (1)}$$

where dM/dt is the dissolution rate, D the diffusion coefficient, A the surface area of the solid-liquid interface, C_s solubility of the drug in the dissolution medium, C the concentration of the drug in the bulk and h_{stag} the thickness of the stagnant layer (sometimes also referred to as a hydrodynamic diffusion layer). It can be seen that the dissolution rate increases with increasing surface area, diffusivity and increased solubility. The dissolution rate decreases with increasing stagnant layer thickness, which is dependent on temperature and rate of stirring.⁴³

Pulmonary dissolution models

In vitro models

Predictive and discriminating in vitro methods are important in order to ascertain whether a promising pulmonary drug delivery system has been achieved and/or to successfully establish an in vitro-in vivo correlation. Although dissolution testing for solid and semi-solid dosage forms (e.g. tablets, capsules) is already a standardized method to aid formulation development and to assess bioequivalence of formulations, no such standardized method is available for inhaled powders.⁴⁷⁻⁴⁹ Setting up a standardized method and reproducing the lung in an in vitro setup is a challenging task, due to the uniqueness of some lung features. These features, such as lung surfactant and an extremely small volume of aqueous fluid are difficult to reproduce, in turn making the development of a standardized in vitro dissolution method difficult.⁴⁹ As no standardized method is available, several different approaches have been developed and studied, such as the Franz diffusion cell, the Transwell system, the USP 2 paddle apparatus, and several flow-through systems.^{41,42,50-54} Most of the methods used are systems that have a donor and an acceptor compartment separate by a membrane (Fig. 4).

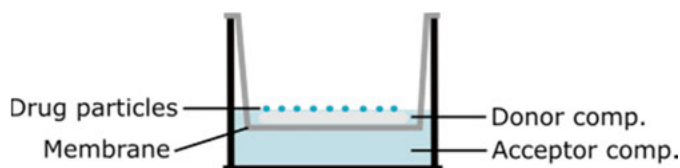


Figure 4. Schematic representation of a membrane-type dissolution method (Transwell system) including a donor compartment, where the drug particles dissolve, a membrane, separating the donor and acceptor compartment, and an acceptor compartment, where sampling takes place.

One of the reasons to use a membrane-type setup is that the drug particles used in the dissolution experiment need to be well dispersed prior to the dissolution experiment to mimic lung deposition. This can be done using an impactor such as the next generation impactor (NGI) or Andersen Cascade Impactor (ACI). These impactors can be used to disperse the particles and to collect the dose with the correct aerodynamic particle size on a collection filter.⁵⁵ The collection filters can be transferred and directly used in the dissolution experiments. As a result, most of the proposed in vitro dissolution setups for orally inhaled drugs contain a membrane (to hold the collection filter) that separates the donor and acceptor compartment. Due to this membrane, the dissolution profiles obtained from these methods do not only reflect dissolution of the drug, but could also be influenced by the diffusion of the drug over the membrane from the donor to the acceptor compartment.⁴¹ The dissolution profiles obtained

from these membrane-type dissolution methods can be defined as effective dissolution profiles. The effective dissolution profiles represent the dissolved drug concentration in the acceptor compartment.

Dissolution medium

Currently, there is no standard dissolution medium that is used for orally inhaled products. Several different media were used in the past years, ranging from simple buffers to more complicated media mimicking the lung lining fluid. Nowadays, dissolution media that are commonly used contain some amount of surfactant, synthetic or natural, to mimic the lung lining fluid, as it has been shown that using only water or salt buffers resulted in a slow dissolution compared to that *in vivo*.⁵⁶ A previous study of Hassoun et al. compared the dissolution rate determined in a simple buffer (without any surfactant) and in a PBS buffer with 0.5% SDS (a commonly used medium)^{39,51,57} to a simulated lung fluid (SLF) containing several lipids, proteins, ions and antioxidants. Comparing the dissolution rate in the two different media to the SLF showed that the drug indeed dissolved slower in the buffer solution than in SLF. However, using the buffer with surfactant resulted in a faster dissolution of the drug than in SLF.⁵⁸ This showed that it is challenging, however very important, to define a standardized dissolution medium for orally inhaled drugs.

Analysis of *in vitro* dissolution profiles

The Weibull distribution function can be used for a simple and more standard analysis to compare and rank different dissolution profiles.⁵⁰ However, to obtain more understanding and insight in to the dissolution profiles, several mechanistic models have been developed and used to analyse *in vitro* dissolution data. A previously developed mechanistic model by May et al.⁵⁹ was used to analyse dissolution data based on the Noyes-Whitney/Nernst-Brunner equation, and polydispersity of the drug particles was taken into account in that model. However, the model by May et al.⁵⁹ did not take into account the possible effect of the membrane that separates the two different compartments in the dissolution setup. Recently, mechanistic models taking into account the diffusion over the membrane, as well as the dissolution, have been investigated.⁶⁰

Pulmonary absorption models

Analysing and understanding dissolution profiles obtained from *in vitro* dissolution methods are important steps; however, to obtain a good *in vitro* – *in vivo* correlation, all processes need to be taken in to account. *In silico* models

can be used to simulate and predict the lung absorption for orally inhaled products. One in silico tool that has been used for this is physiologically based pharmacokinetic modelling (PBPK). PBPK models are compartmental models built up by several mechanistic descriptions to describe the lung biopharmaceutics and pharmacokinetics (PK).^{61,62} Physiologically based biopharmaceutics modelling (PBBM), a subfield of PBPK modelling, uses PBPK modelling principles with physicochemical properties of the drug and formulation characteristics to predict in vivo drug release, absorption and PK of a drug product.^{63,64}

Aims of the thesis

The overall aim of this thesis was to evaluate and characterise an in vitro dissolution method for assessment of novel pulmonary drug delivery systems, with a focus on future controlled release systems.

The specific aims for the papers included in this thesis were as follows:

- I. To develop a mechanistic model that could analyse dissolution data obtained from a membrane-type in vitro setup by taking into account dissolution and diffusion over the membrane.
- II. To analyse experimental dissolution profiles using the developed mechanistic model and a semi-empirical model based on the Weibull distribution to get a better understanding of the dissolution profiles obtained by a Transwell system.
- III. To investigate the potential of amorphous mesoporous silica particles as a novel modified release pulmonary drug delivery system by comparing the dissolution profiles, obtained from a Transwell system, of several different formulations.
- IV. To determine the potential of Salbutamol loaded hyaluronic acid hydrogels as a novel pulmonary controlled release formulation by determining the dissolution profiles, obtained from a Transwell system, and fine particle fraction of three different formulations.
- V. To investigate micellar contribution to diffusion of poorly soluble inhaled drugs during dissolution and to extrapolate the influence of micellar diffusion on human lung absorption using PBBM.

Methods

Materials

Investigated formulations

Different drugs and formulations were used throughout this thesis. Clinically available formulations were used for the determination of the dissolution profiles in **Paper II** and **V**. For **Paper II** and **V**, three different drugs were used namely, Budesonide (Bud; Pulmicort Turbuhaler, 400 µg/dose), Beclomethasone dipropionate (BDP; Beclomet Easyhaler, 200 µg/dose) and Fluticasone propionate (FP; Flutide Diskus, 500 µg/dose). In **Paper III**, the release profiles of amorphous mesoporous silica formulations loaded with two different drugs, Budesonide and CMPD-X, were investigated. Several mesoporous silica formulations were used with different structural characteristics. The three Budesonide loaded formulations had three different average particle diameters (2.4, 3.9, and 6.3 µm) and a similar pore size (11.1 – 11.6 nm). The CMPD-X loaded particles had a similar particle diameter (2.2 – 2.4 µm) but a different pore size (11.6, 7.1, and 2.3 nm). For **Paper IV**, a hyaluronic acid hydrogel formulation loaded with Salbutamol was investigated. Three different hydrogel formulations were used with 20, 33 or 50% Salbutamol loaded in the formulations, later referred to as HASS (4:1), HASS (4:2) and HASS (4:4), respectively.

Solubility determination

The shake flask method was used to determine the solubility of the used drugs. An excess of drug was added to the preferred medium and the preparation was placed on a shaking table (Heidolph Unimax 1010) for 72 h. Two samples were taken, one 24 h after the start of the experiment and one after 72 h at the end of the experiment. The taken samples were centrifuged for 15 min at 14500 rpm (Centrifuge 5430, Eppendorf, Germany) before quantification by UPLC-UV.

Particle size determination

The particle size distribution of the formulations was determined by laser diffraction (Coulter LS230, Coulter Corp, Miami, USA). Suspensions of the

drugs were made in water with a few drops of 2% Tween 20 to avoid agglomeration. The suspensions were sonicated in a sonication bath (Ultrasonic Cleaner Branson, B5210E-MT, Danbury, U.S.A) for 10 min, directly prior to the start of the measurement. The particle size distribution was determined with a dispersant refractive index of 1.332, an imaginary index of 1.0 and a particle refractive index of 1.333. To calculate the particle size distributions the Fraunhofer theory was used, in which PIDS data was included. Both the mass median diameter d_{50} and the span, $(d_{90} - d_{10})/d_{50}$ where d_{90} and d_{10} are the 90th and 10th percentiles, were determined.

Dose collection

Dose collection of the formulations was done using a modified Andersen Cascade Impactor (mACI; Fig. 5), for **Paper II, III and V**. The mACI is similar to a normal ACI until stage 1, however, after Stage 1 the normal stages were replaced by five hollow stages to allow the particles to sediment on the collection filters in the Filter Stage.⁵⁰ The cut-off diameter after Stage 1, with a flow rate of 60 L/min, was 4.4 μm , this means that the fraction that was collected on the Filter Stage had an aerodynamic diameter below 4.4 μm . Therefore for all formulations a flow rate of 60 L/min was used with a suction time of 0.3 sec. A sedimentation time of 20 minutes was used per dose, after which the filters on the Filter Stage were collected and used for the dissolution experiments.

For **Paper II and V**, clinically available formulations were used to deposit the drug on the collection filters in the Filter Stage. Depending on the strength of the device a different number of doses were actuated to obtain around 10 μg of drug on each collection filter. For **Paper III**, the formulations were directly weighed into an inhaler. The inhaler that was used, was a screenhaler device⁶⁵ coupled with a Turbuhaler® mouthpiece, which was connected to the throat of the mACI prior to the start of the experiment. The amount of the formulation that was weighed into the screenhaler device was determined to match the total amount of drug in the dissolution experiment, approximately 10 μg .

Dose collection in **Paper IV** was not executed by using the mACI. Due to the limiting amount of formulation that was available, the formulation was directly weighed in on the collection filters. A similar amount of formulation was weighed in on the collection filters, approximately 150 μg .

Scanning electron microscopy

To validate the deposition and dispersion of the formulations on the collection filters in the Filter Stage, scanning electron microscopy (SEM) images were

taken. A Leo/Zeiss 1550 microscope (Jena, Germany) was used to visualize the particles. For **Paper II** and **III**, the SEM holders were placed in the mACI in the Filter Stage to mimic the dispersion of the formulations on the collection filters that were used for the dissolution experiments. In **Paper III**, additional images were obtained to visualize the formulations before dispersion as well. Before analysis, all SEM holders were coated with a thin layer of Au/Pd under argon using a sputter coater (Polaron, Quorum Technologies Ltd., Newhaven, United Kingdom).

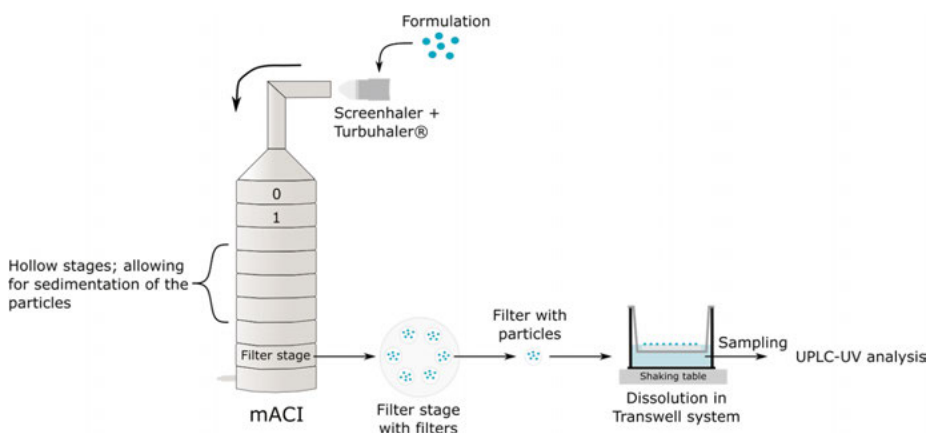


Figure 5. Schematic diagram of experimental procedure including deposition using mACI followed by dissolution using a Transwell system.

Dissolution media

For the dissolution experiments, different media were used throughout this thesis. For **Paper II**, PBS with 0.2% SDS and 0.5% SDS were used. For **Paper III**, PBS with 0.5% SDS and simulated lung fluid (SLF; Gamble's solution; pH 7.4) was used and prepared according to Marques et al.⁶⁶ The dissolution medium in **Paper IV** was water and the dissolution medium in **Paper V** was PBS with 0.5% SDS. All buffers were filtered with a 0.2 μm syringe filter before use.

In vitro dissolution method

A Transwell system was used to determine the effective dissolution profiles of the different drugs. A Corning Transwell system (Sigma-Aldrich, Germany) with 24 mm inserts and polycarbonate membranes with a pore size of 8 μm , **Paper II, III** and **V**, and 0.4 μm , **Paper IV**, was used. The collection

filters that were obtained from the mACI were placed in the donor compartment of the Transwell system and 0.7 mL of dissolution media was added on top to start the dissolution experiment. The acceptor compartment contained an additional 2.3 mL of dissolution medium. The Transwell system was placed on a shaking table (Heidolph Unimax 1010), with a shaking speed of 150 rpm, during the total duration of the experiment. Samples of 200 μ L were taken from the acceptor compartment between 2 and 180 min and replaced by fresh buffer to maintain the same volume over time. After the final sampling point, 3 mL of methanol was added for an additional 30 min to determine the total amount of drug in the experiment.

To determine the diffusion of the drug from the donor to acceptor compartment through the polycarbonate membrane, a solution was pipetted in the donor compartment. The amount of the API solution matched the amount as used for the dissolution (around 10 μ g). As described for the dissolution experiments samples were taken over a time period of 2-120 min and replaced with fresh dissolution media.

All samples taken were quantified by using UPLC-UV. However, for **Paper IV** the samples were centrifuged, at 14500 rpm for 15 min, using a centrifugal filter unit to remove excess of hyaluronic acid hydrogel prior to quantification.

Determination of fine particle fraction

To determine the fine particle fraction (FPF) of the formulations in **Paper IV** a Fast Screening Impactor (FSI; Copley Scientific, UK) was used.⁶⁷ The formulations were weighed into the screenhaler device, a similar device as used for the mACI. The FSI was run with the following settings, a flow rate of 60 L/min and a suction time of 4 sec. The amount of drug was determined at the three stages of the FSI: the throat, the preseparator, and the Filter stage. To determine the amount in the throat, preseparator, and on the filter, 20 mL of water with 0.03% trifluoroacetic acid (TFA) was added to the different stages. After incubating for 20 min, a sample was taken from the three different stages. Before quantification, the samples were centrifuged using a centrifugal filter unit for 15 min at 14500 rpm.

By determining the total amount of drug on each stage it is possible to determine the FPF. The Filter stage represents the FPF, meaning that all the particles that have deposited on the Filter Stage have an aerodynamic diameter below 5 μ m and thus represent the fraction that reaches the lung. The FPF of all formulations was determined by using the following formula,

$$FPF = \frac{\text{Filter stage}}{ED} \times 100, \quad \text{Eq. (2)}$$

in which FPF is the fine particle fraction in percent, Filter stage represents the amount of drug deposit on the filter and the emitted dose (ED) represents the total sum of drug that was present on all the three stages.

Quantification method

For quantitative analysis, the samples were analysed using ultra performance liquid chromatography coupled with a UV detector (UPLC-UV).

A Waters Acquity UPLC-UV I-Class system with a BEH C18 column (2.1 × 50 mm) with 1.7 μm particle size was used to analyse the samples. Mobile phase A consisted of 0.03% TFA in water and mobile phase B consisted of 0.03% TFA in acetonitrile. The method that was used to quantify Budesonide was adapted from Franek et al.,⁵⁰ with a starting mobile phase composition of 65:35 (A:B) to 20:80 in 1.33 min and back to 65:35 with a total run time of 1.8 min. The flow rate for Budesonide was 0.6 mL/min and the wavelength was set to 254 nm. To quantify Beclomethasone dipropionate an isocratic method with a mobile phase ratio of 45:55 (A:B), a flow rate of 0.8 mL/min, run time of 1.20 min and a wavelength of 241 nm was used. For Fluticasone propionate an isocratic method with a mobile phase ratio of 50:50 (A:B), a flow rate of 0.9 mL/min, run time of 1.10 min and a wavelength of 237 nm was used. To quantify CMPD-X a gradient method was used with a mobile phase ratio of 90:10 (A:B) to a ratio of 0.1:99.9 (A:B) in 2 min and back to a ratio of 90:10 (A:B), with a total run time of 2.7 min. The wavelength used for CMPD-X was set to 343 nm and the flow rate was 0.5 mL/min. To quantify Salbutamol an isocratic method was used with a mobile phase ratio of 97:3 (A:B), a total run time of 1.5 min, flow rate of 0.8 mL/min and wavelength was set to 224 nm. Injection volume was 2 μL for all samples, the temperature of the column was 40 °C and the temperature of the sample compartment was 18 °C.

Quantification was done using a standard curve with an external standard. Validation of the UPLC-UV methods was done by determination of inter- and intraday variation of standard curve samples in the range of 0.05 to 10 μg/mL.

Data analysis

The dissolution data was analysed using two different approaches, one was by using a Weibull fit, and the other approach used a mechanistic model to determine the time course of the dissolved drug concentration in the donor compartment, later referred to as the corrected Transwell dissolution.⁶⁸

Weibull function

The effective dissolution profiles (fraction dissolved and released drug u vs. time t) were fitted to the Weibull distribution function,⁵⁰

$$u = 1 - e^{-(t/t_{63})^b}, \quad \text{Eq. (3)}$$

where the scale and shape parameters are denoted by t_{63} and b , respectively. The t_{63} , where 63% of the total fraction was dissolved, was used as a comparison value between the different profiles.

Analysis using mechanistic models

Besides only using the Weibull function to analyse the effective dissolution profiles, a mechanistic model was used to determine the corrected Transwell dissolution profiles (Fig 6). In **Paper I**, a mechanistic model was developed that took both the dissolution and the effect of the membrane into account.⁶⁸ The mathematical model was based on the Noyes-Whitney/Nernst-Brunner equation, considering dissolution, coupled with Fick's law, to take into account diffusion through the membrane. The dissolution of the drug is assumed to take place in a membrane-type dissolution setup, assuming that both compartments contain the same dissolution medium. It was assumed that all drug was in solid form at the initial stage and that sink conditions prevailed.

Several input parameters were needed to analyse dissolution data with the mechanistic model and to predict the rate limiting process in the profile. One of the input parameters that had to be defined was the c_s , the non-dimensional solubility, which essentially is an inverse dose number for the drug in the donor compartment.⁶⁹ c_s was defined as C_s/S_0 , in which C_s is the solubility of the drug in the dissolution medium and S_0 is the initial solid concentration in the donor compartment. This implies that having a c_s value below 1, only a fraction of the solid drug can be dissolved in the donor compartment, and with a c_s value higher than 1 it implies that all solid drug can be dissolved in the donor compartment. The c_s was determined per drug, in each media used, by

determining the solubility using a shake flask method and using the extracted total amount that was present in the Transwell system.

To improve the fit of the mechanistic model (**Paper I**) the model was extended to polydisperse powders (**Paper II**). The shape parameter σ was needed for the extended version of the mechanistic model. By fitting a lognormal distribution of experimentally obtained particle size distributions the σ could be extracted and used for the model calculations. Prior to **Paper V** the model has been further optimised considering a radius-dependent thickness of the stagnant layer.⁷⁰

t_{abs} and t_{diss} were the predicted characteristic absorption time (referred to as characteristic diffusion time in **Paper I** and **II**) and characteristic dissolution time, respectively. Both of these parameters could be determined based on experimental data or could be determined using the model itself. Determination of the dissolution and the absorption time provides indications of the rate limiting process in the dissolution profile and could subsequently be used to determine the corrected Transwell dissolution profiles. The corrected Transwell dissolution profiles displayed the time course of the dissolved drug concentration in the donor compartment, in contrast to the effective dissolution profiles that displayed both dissolution and the diffusion over the membrane (Fig. 6).

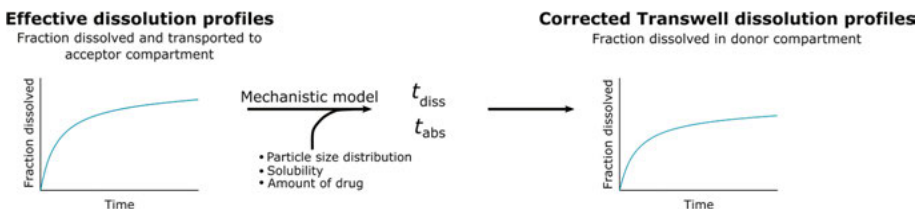


Figure 6. Schematic overview of data analysis process using the developed mechanistic model, defining effective and corrected Transwell dissolution profiles.

Physiologically based biopharmaceutics modelling

For **Paper V**, Lung-Sim, an in house PBBM tool from AstraZeneca, was used. By taking into account the different important factors that play a role during the administration of inhaled drugs, e.g. airway deposition, dissolution, and permeability, Lung-Sim can predict the drug absorption in the lungs.^{71,72} The lung structure and airway dimensions in the Lung-Sim model were based on the Weibel lung model, modified by Boger and Fridén, with a functional residual capacity scaled to 3300 mL.^{33,61} It was shown that the tracheobronchial epithelium layer is leakier than the alveolar epithelium layer; therefore the permeability value for the alveolar region was scaled 10 times lower compared to the tracheobronchial region.^{61,73}

Pharmacokinetic input parameters

The pharmacokinetic input parameters for all three drugs were obtained from literature data. For Bud and FP intravenous and inhaled profiles were obtained from Thorsson et al.⁷⁴, and for BDP these profiles were obtained from Daley-Yates et al.⁷⁵ BDP is a prodrug that instantly metabolizes in the lung to Beclomethasone 17-monopropionate (B-17-MP); the plasma concentrations are therefore determined based on the metabolite, B-17-MP.⁷⁵ The pharmacokinetic study and the dissolution experiments were done using similar products for both Bud and FP. Unfortunately, due to lack of literature data for BDP the products used were not identical, the pharmacokinetic study was done with a pMDI product and the dissolution experiments were done using a DPI product. A two- or three-compartment model was fitted to the intravenous plasma profiles to determine the PK parameters, such as rate of distribution and plasma clearance.

Particle-size and deposition input parameters

The particle-size distributions of all three products obtained in **Paper II** were used as input parameters. To determine the lung deposition in Lung-Sim, literature data was used. For Bud, impactor and scintigraphy data from Ball et al. was used, and for BDP and FP literature MMAD values were used as input parameters to determine the deposition.⁷⁶⁻⁷⁸

Diffusivity input parameter

Three different diffusivity parameters were used as input parameters for the PBBM. The first diffusivity value that was used, was the diffusivity of the API in water (D_{API} ; no surfactant/micelles present) calculated using the Stoke-Einstein equation. The other two diffusivity values that were used as input in the model were determined within the presence of surfactant and were calculated using the following equation,

$$D = D_{API} + D_{SDS} \times \frac{S_{SDS}}{S_{buffer}} \quad (4)$$

in which D_{API} is the diffusivity of the drug in water, D_{SDS} the diffusivity in SDS, S_{SDS} the solubility of the drug with the specific SDS volume fraction and S_{buffer} the solubility of the drug in the buffer. The D_{SDS} is defined as follows,

$$D_{SDS} = D_{API} \times \frac{Diameter_{API}}{Diameter_{SDS}} \quad (5)$$

in which $Diameter_{API}$ is calculated using the molar mass and the density of the API and for the $Diameter_{SDS}$ a diameter of 4 nm was used, based on literature data.^{79,80} Two different diffusivity values were determined with different amount of surfactant; 0.2% and 0.5% (Table 1).

Table 1. Calculated diffusivity values for Bud, BDP and FP in water, 0.2% SDS and 0.5% SDS.

Drug	Water (10^{-9} m ² /s)	0.2% SDS (10^{-9} m ² /s)	0.5% SDS (10^{-9} m ² /s)
Bud	0.64	2.74	5.94
BDP	0.67	17.3	41.7
FP	0.62	9.16	21.1

To validate the obtained diffusivity values a theoretical dissolution tool was used, which predicted a dissolution profile according to Fick's law,

$$F_{dissolution} = 4 \times \pi \times R \times D \times (S_b - C_b) \times f_{stirring} \quad (6)$$

where R is the particle radius, D is the diffusivity, S_b the solubility in bulk of the free monomer, C_b the concentration dissolved free monomer and $f_{stirring}$ the stirring factor (Nielsen).⁸¹

Statistical evaluation

The average fold error (AFE) and the absolute average fold error (AAFE) were used to determine the similarity between the observed and the predicted data.

$$AFE = 10^{(1/n) \sum \log(PRED/OBS)} \quad (7)$$

$$AAFE = 10^{(1/n) \sum \log(|PRED/OBS|)} \quad (8)$$

The AFE indicates whether the model over- or underpredicts compared to the observed data. The AAFE indicates the absolute value of error, an AAFE value of 1 indicates a perfect fit between the predicted and observed data and an AAFE value of 2 indicates an average 2-fold difference between the predicted and observed data.

Results & Discussion

In the following chapter the main findings of **Paper I-V** are summarized. This includes characterisation of the developed mechanistic model, the Transwell system, potential novel formulation in the Transwell system and establishing PBBM tools using micellar contribution.

Validation of mechanistic model

Analysing dissolution profiles with a mechanistic model that takes the different processes present during the experimental data collection into account is extremely valuable. Therefore a mechanistic model was developed that took into account not only the dissolution but also the diffusion that is caused by the membrane in the in vitro dissolution setup.

Data from recently published dissolution studies were used to validate the mechanistic model and to see if it was possible to determine the rate limiting process during the experiments. Sakagami et al.⁸² determined dissolution profiles of orally inhaled drugs using a Transwell based dissolution system. The c_s parameter was determined based on data from the study, and the absorption time, t_{abs} , was determined based on the drug that had an extremely fast dissolution time. By having these parameters set, t_{diss} could be determined by fitting the mechanistic model to the experimental data. The agreement between the experimentally obtained data and the model calculations was satisfactory, and showed a difference in extracted t_{diss} for all three drugs (Fig. 7). Therefore it could be concluded that the difference between the profiles could indeed be caused by their differences in solubility and dissolution rate.

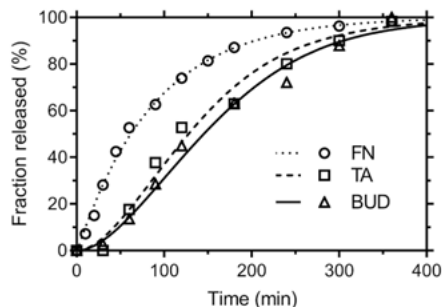


Figure 7. Sakagami et al.⁸² experimental data in symbols and predicted model calculations in lines.

Eedara et al. recently studied a novel type of dissolution setup, which consists of a donor compartment that was separated by a dialysis membrane from a flow through cell.⁵⁴ In comparison to the previous data set from Sakagami et al. the input parameters that were determined for Eedara et al. were the c_s and the t_{diss} , instead of the t_{abs} . The reason for this was that in the experimental setup the flow rate in the flow through cell was changed using the same drug, meaning that the solubility did not influence the dissolution profiles. This showed again good agreement between the experimentally obtained dissolution profiles and the predicted model calculations (Fig. 8).

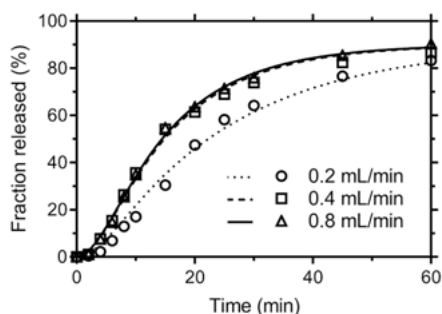


Figure 8. Experimental data from Eedara et al.⁵⁴ (symbols) and model calculations (lines).

Analysing experimental dissolution data using the developed mechanistic model showed a good agreement between the model and the experimentally obtained data. The model enabled extraction of the t_{diss} or t_{abs} and could predict which process was the rate limiting step in the dissolution profiles.

Characterisation of Transwell system

After successful validation of the developed mechanistic model with literature data, further validation with experimentally obtained dissolution data was performed. The dissolution profiles of three different clinically available orally inhaled drugs were experimentally determined using a Transwell system and analysed using two different approaches. One approach was the more simplistic and standard Weibull fit which results in a t_{63} value, which represents the time it takes for 63% of the fraction to be dissolved and transported across the membrane. The other approach used the previously described mechanistic model with an extension that takes into account the polydispersity of the drug particles.

Table 2. Experimentally determined solubility, mass median diameter and span of Budesonide (Bud), Beclomethasone dipropionate (BDP) and Fluticasone propionate (FP). Standard deviations within parentheses.

	Bud	BDP	FP
Solubility PBS + 0.03% SDS ($\mu\text{g/mL}$)	26 (0.3)	0.2 (0.1)	0.1 (0)
Solubility PBS + 0.2% SDS ($\mu\text{g/mL}$)	399 (2)	23 (1)	4.7 (0)
Solubility PBS + 0.5% SDS ($\mu\text{g/mL}$)	878 (5)	52 (1)	13 (0)
Mass median diameter (μm)	2.1 (0.0)	3.3 (0.2)	3.0 (0.0)
Span (μm)	2.5 (0.6)	2.5 (0.1)	2.2 (0.0)

The drugs used were selected based on the wide range of solubility values, with the most soluble drug being Budesonide, to Beclomethasone dipropionate, and the least soluble being Fluticasone propionate (Table 2). All drugs were used in their original device and dispersed using the mACI. To verify that using different devices and different number of actuations gave a similar deposition pattern, SEM pictures were taken of the mACI collection plate (Fig. 9). This showed that all three different formulations are dispersed in a similar way irrespective of the difference in device and number of doses.

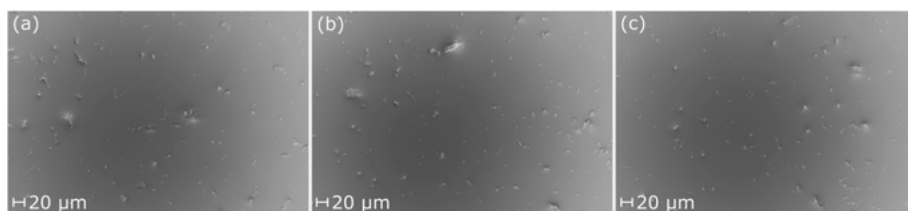


Figure 9. Scanning-electron micrographs of deposition of (a) Budesonide (Bud), (b) Beclomethasone dipropionate (BDP) and (c) Fluticasone propionate (FP).

Input parameters needed to analyse the dissolution profiles were c_s , t_{abs} , and σ . c_s was determined as described before, t_{diff} was determined by using the

experimentally obtained diffusion profiles and σ was obtained from the particle size distribution profiles for each drug individually. By using these input parameters the dissolution time, t_{diss} , could be determined. Besides this also the t_{63} was determined by using the Weibull fit. It could be concluded that the values extracted from the different approaches showed the same ranking order in dissolution time for the three different drugs (Fig. 10). Budesonide and Beclomethasone dipropionate had a t_{63} of around 13 min and Fluticasone propionate had significantly higher t_{63} of around 21 min. The extracted t_{diss} from the mechanistic model was around 3 min for Budesonide and Beclomethasone dipropionate and around 6 min for Fluticasone propionate. Even though the rank order of the three different compounds was the same for both approaches, the dissolution time differed. The mechanistic model indicated faster dissolution times than the Weibull fit.

When comparing the ranking order of the drugs with the solubility values of each drug, having the same dissolution time and profiles for Budesonide and Beclomethasone dipropionate was not as expected, as Beclomethasone dipropionate has a significantly lower solubility compared to Budesonide. However, previously performed absorption studies in human show a similar absorption time of 0.6 h for both Budesonide and Beclomethasone dipropionate, which shows good in vivo correlation of the dissolution profiles obtained in the in vitro system.^{74,75}

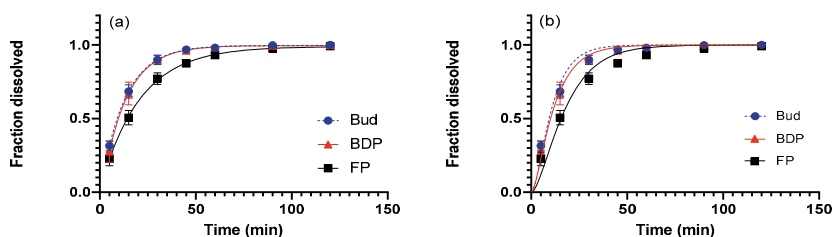


Figure 10. Dissolution profiles of Budesonide (Bud), Beclomethasone dipropionate (BDP) and Fluticasone propionate (FP) in PBS with 0.5% SDS. The solid lines in (a) represent fits of the semi-empirical model ($R^2 = 0.991, 0.977$ and 0.987) and in (b) of the mechanistic model ($R^2 = 0.988, 0.996$ and 0.980 for BUD, BDP and FP). The error bars indicate the standard deviation of six replicates.

By correcting the dissolution profiles for the effects of the diffusion of the drug across the membrane a clear vision could be given on how much the membrane actually effected the dissolution profiles (Fig. 11). It could be clearly seen that the membrane had a significant effect on the profiles, as the

fraction dissolved and the fraction released into the acceptor compartment was different. It could also be seen that for the more soluble drugs, Budesonide and Beclomethasone dipropionate, there was no difference between the current conditions and the sink conditions meaning that the dissolution profiles of these drugs were measured in sink conditions. However, a difference could be seen for Fluticasone propionate meaning that sink conditions were probably absent during the experimental determination of the dissolution profiles.

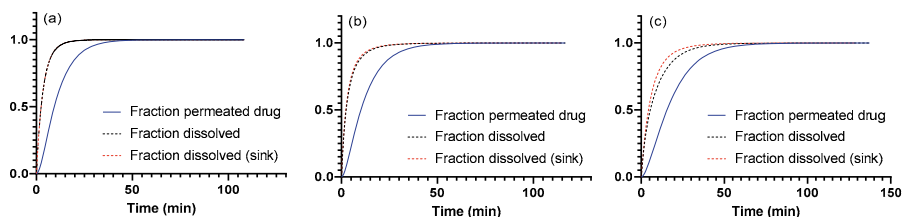


Figure 11. Fraction of permeated, fraction of dissolved drug and fraction of dissolved drug that would have been obtained under sink conditions for (a) Budesonide (Bud), (b) Beclomethasone dipropionate (BDP) and (c) Fluticasone propionate (FP).

Both models predicted the same ranking order but different dissolution times. The Weibull fit only uses the experimentally determined dissolution profiles as input parameters. The t_{63} values that are obtained from the Weibull fit are not only influenced by the dissolution but also by the effect of the membrane. The mechanistic model uses besides the experimentally determined profile, also the solubility of the drugs, the particle size distribution profiles of each drug and the effects of the diffusion across the membrane. Therefore, the mechanistic model gives more insight in the actual dissolution time, and the influences of the different parameters on the dissolution profiles. Using the mechanistic model is more cumbersome but it gives more insight in what influences the dissolution profiles and it will give more detailed understanding of the dissolution process and the dissolution setup that is used. When setting up dissolution experiments knowing how your system behaves could be highly valuable.

Characterisation of novel formulations

The dissolution profiles of clinically available drug products obtained from the Transwell system could be successfully characterised and understood in more detail by using the mechanistic model. However, as developing novel

formulations is of high importance, the ability to characterise novel formulations should be investigated. Therefore the potential of two novel drug delivery systems for pulmonary drug delivery was determined.

Amorphous mesoporous silica particles

The potential of micron-sized amorphous mesoporous silica particles as a novel modified release drug delivery system for pulmonary administration was investigated. The release profiles of two different pulmonary drugs loaded into mesoporous particles with different structural characteristics were analysed using a Transwell system after aerosolization with a mACI.

Deposition of particles

Prior to the dissolution experiments the particles were dispersed using a mACI. To confirm a successful dispersion of the particles SEM images were taken of the collection filters. Figure 12 shows that all the mesoporous silica formulations were well dispersed and maintained mechanical robustness after dispersion, with no apparent fracturing or breakage. In comparison, a standard clinical product, the Budesonide Turbuhaler® formulation, showed smaller dispersed agglomerates after dispersion (Fig. 12d).

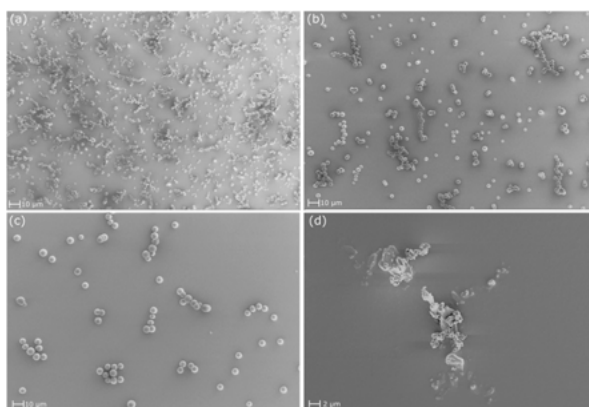


Figure 12. SEM images of Budesonide particle formulations dispersed on filters with an average particle diameter of (a) 2.4 μm , (b) 3.9 μm , (c) 6.3 μm and (d) the Budesonide Turbuhaler® formulation. Images (a)-(c) are at a magnification of 1000 \times . Image (d) was taken at a magnification of 5000 \times to visualize in more detail the morphology of the micronized drug aggregates.

Effect of particle diameter on in vitro drug release

The release profiles of three different mesoporous silica formulations loaded with Budesonide, with different average particle diameter, were compared to the current clinical standard, the Budesonide Turbuhaler® formulation (Fig. 13). What can be seen is that the Turbuhaler formulation showed the slowest

release with an average t_{63} value of 290 min, and that all three silica formulations had a significantly faster release. This difference is likely caused by the state of the drug in the different formulations. The Budesonide that is present in the mesoporous silica formulations is in an amorphous state, whereas the Budesonide present in the Turbuhaler formulation is in a crystalline form. It has been previously shown that the solubility and the release rate of different drugs can be enhanced by confining the drug in an amorphous state.⁸³

The three mesoporous particles, with average particle diameters of 2.4, 3.9, and 6.4 μm , all showed different release profiles. With increasing particle size, the release rate was decreasing, with a t_{63} of 140, 180, and 220 min for the formulations with an average size of 2.4, 3.9, and 6.4 μm , respectively. A linear trend can be seen with particle diameter (Fig. 13b). Irrespective of the partially overlapping error bars in Figure 13b the linear trend can be confirmed by the complete release profiles, used to determine the t_{63} value, shown in Figure 13a.

For all three formulations the particle diameter was the only parameter that was varying and could thus cause a difference in release between the different formulations, all other parameters, such as the specific surface, were comparable for the three formulations. This difference in release could be correlated to the changes in the diffusional path length, which changes with different particle diameters. Increasing the particle diameter from 2.4 to 6.3 μm more than doubles the particle diameter, therefore the effective diffusion path summed over all the pores increases significantly. With increased diffusional path, the number of collisions of the drug molecules with the silica increases and therefore the release rate of the formulations decreases.

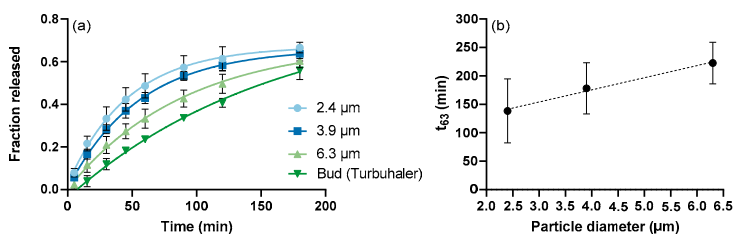


Figure 13. (a) Release profiles of loaded Budesonide particles with a mean particle diameter of 2.4 μm , 3.9 μm , and 6.3 μm , and the Budesonide Turbuhaler® formulation in SLF. The error bars indicate the standard deviation from the mean of three replicates. (b) Symbols represent t_{63} values comparing the release rate of encapsulated budesonide loaded into mesoporous particles of different diameters in SLF. The error bars indicate the standard deviation from the mean of three replicates. The dashed line represents a linear fit to the data to illustrate the trend that the rate of drug release increases as size of the particles decreases.

Effect of pore size on in vitro drug release

The release profiles of three different mesoporous silica particles, loaded with CMPD-X, were determined. The particle diameter of the formulations were similar, in contrast to the Budesonide loaded particles, however the pore sizes were different for all three formulations to determine the effect of pore size. As for the Budesonide loaded formulations, the release profile of the pure CMPD-X was determined (data not shown), and showed a significantly slower release rate compared to the silica formulations. As mentioned before, this is likely due to the drugs being encapsulated in the amorphous form in the silica particles, compared to the crystalline form for the pure drug.

It can be clearly seen that by increasing the pore size of the particles the release rate was also increased (Fig. 14). The formulation with a pore size of 2.3 nm had a t_{63} value of 53 min, the formulation with a pore size of 7.1 nm had a t_{63} value of 20 min, and the formulation with the biggest pore size of 11.6 nm had a t_{63} value of 15.2 min. A non-linear trend can be observed with pore size (Fig. 14b). What can be observed is that the formulation with the smallest pore size showed a significantly slower release profile compared to the other two formulations. It is likely that the more confined environment inside the pores of the formulation with the smallest pore size results in the diffusion of the surrounding medium being more limited, making it difficult for the drug molecules to be released.

For the CMPD-X loaded particles, it should also be noted that the loading amount varied between the different particles. It is therefore possible that the different loading amounts could have played a role in the differences in release profiles. More specifically, the formulation which had the smallest pore size of 2.3 nm has a lower loading percentage (13%) compared with that of the formulation with pore sizes of 7.1 nm (21%), whereas the loading amounts for the formulations with a pore size of 11.6 and 7.1 nm are more comparable (18.6 and 21%, respectively). However, if the loading amount were to play a dominant role in the release profiles, this would have resulted in formulation with pore size 7.1 nm exhibiting the fastest release, which was not observed. For this reason, it can be concluded that the observed variations in the release profiles of the particle formulations can only be attributed to the systematic modification of the particle pore sizes.

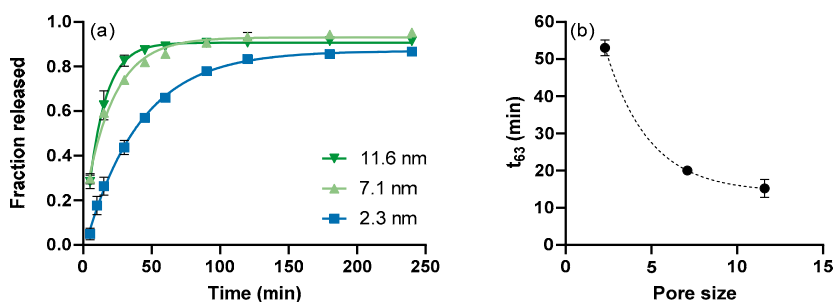


Figure 14. (a) Release profile of loaded CMPD-X particles with a pore size of 11.6 nm, pore size of 7.1 nm, and 2.3 nm in PBS with 0.5% SDS. The release profiles were determined with the fraction of particles with an aerodynamic diameter below 4.4 μm . The error bars indicate the standard deviation from the mean of three replicates. (b) The symbols represent t_{63} values comparing the release rate of encapsulated CMPD-X loaded into mesoporous particles with different pore sizes in PBS with 0.5% SDS. The dashed line represents an exponential curve with plateau as a guide for the eye. The error bars indicate the standard deviation from the mean of three replicates.

Hyaluronic hydrogels

The potential of hyaluronic acid hydrogel microparticles, loaded with Salbutamol, as a novel controlled release drug delivery system for pulmonary administration has been investigated. The FPF and the release profiles of the Salbutamol loaded microparticles with different compositions (20, 33, and 50 % of Salbutamol) were determined.

Fine particle fraction

To determine the aerosol performance of the developed formulations, the FPF of all formulations was determined using the FSI. The FPF represents the dose reaching the lungs with an aerodynamic size cut-off of 5 μm . All three drug-loaded formulations (HASS) exhibited a similar FPF between 48-56% (Fig. 15) that was significantly larger than that of the empty formulation (HAGA). This difference can most likely be attributed to the corrugated surface of the drug-loaded formulations compared to the smooth surface of the empty ones. No uniform spherical geometry was observed for the drug-loaded formulations, folded and fractured particles were obtained after spray drying of the formulations. The FPF of the formulations showed promising aerosol performance compared to clinical formulations of Salbutamol which showed an FPF of around 20%.⁸⁴

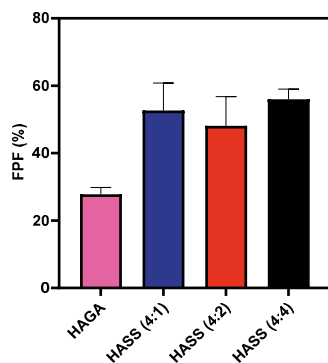


Figure 15. Fine particle fraction (FPF) of the hyaluronic hydrogels loaded with Salbutamol.

In vitro release

In vitro drug release from the drug-containing (HASS) formulations was determined using the Transwell setup and compared to that of pure Salbutamol. Due to the extremely high solubility of Salbutamol, water was used as a dissolution medium. By not using a more biorelevant dissolution medium, such as SLF, the dissolution profiles could possibly correlate less to the in vivo situation. However, as the focus of this study was on the development of a controlled release formulation, it was considered acceptable. It was more important to be able to obtain a full dissolution profile of all formulations, including the pure Salbutamol, to verify if there was a sustained release created by the formulations. In contrast to the previously obtained dissolution profiles, the Salbutamol particles were not dispersed by the mACI prior to the dissolution experiment. This was due to the limited amount of formulation available, and therefore the formulations as well as the pure drug were directly manually deposited on the filter. Since both the formulations and the pure drug were treated identical, regarding amount and way of depositing the drug on the filter, this should not have any significant influence on the differences observed in the dissolution profiles.

It can be observed from the effective dissolution profiles (Fig. 16) that the dissolution of the pure Salbutamol was the fastest overall, with a t_{63} of 8.4 min. This value was not significantly different from the t_{abs} (8.2 min), indicating that transport across the Transwell membrane (absorption) was the rate-limiting process for pure Salbutamol. All formulations showed a significantly slower effective dissolution compared to pure Salbutamol. Moreover, all three formulations showed a different dissolution profile and dissolution rate compared to each other. The slowest effective dissolution (t_{63} of 35.8 min) was observed for HASS (4:1) with 20% drug loading. A significantly faster effective dissolution (t_{63} of 22.5 min) was seen for HASS (4:2), with 33% loading.

The fastest effective dissolution (t_{63} of 16.6 min), which still was significantly slower than that of pure Salbutamol, was found for HASS (4:4), with 50% drug loading. Hence, all hydrogel formulations resulted in retarded release and the extent of retardation increased with decreasing drug content. It is plausible that a less dense network will be formed in the hydrogel when the amount of hyaluronic acid in the formulation is decreased, resulting in less interaction between the drug and gel. The swelling time may also be affected.

Determination of the total amount of drug in the experiment was done by adding methanol to the Transwell after the final time point. This normalization of the dissolution profiles apparently resulted in an additional amount of Salbutamol being measured, this was the case for both the drug-loaded formulations as well as for the pure Salbutamol. However, the extracted dissolution rates were independent of the normalization used.

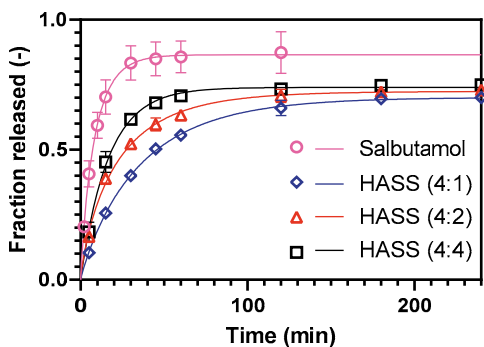


Figure 16. Release profiles obtained for Salbutamol and drug-containing microparticles (HASS). The solid lines represent fits of the Weibull distribution function.

Corrected Transwell dissolution profiles were obtained, using the previously developed mechanistic model, to minimize the effect of the Transwell membrane on the dissolution profiles (Fig. 17). As can clearly be seen, the dissolution of pure Salbutamol can be considered to be instantaneous. On the contrary, the release of Salbutamol from the formulations is retarded, and the extent of retardation decreases with the increasing amount of Salbutamol in the formulation, as would be anticipated from the uncorrected profiles discussed above. As clearly seen in Fig. 18, drug release is retarded for all formulations, with corrected t_{63} ranging from about 5 min for HASS (4:4), with 50% drug, to about 30 min for HASS (1:4), with 20% drug compared less than 1 min for pure Salbutamol. Moreover, a comparison between the corrected and effective t_{63} indicated that the effect of the membrane decreased with decreasing dissolution rate, as anticipated.

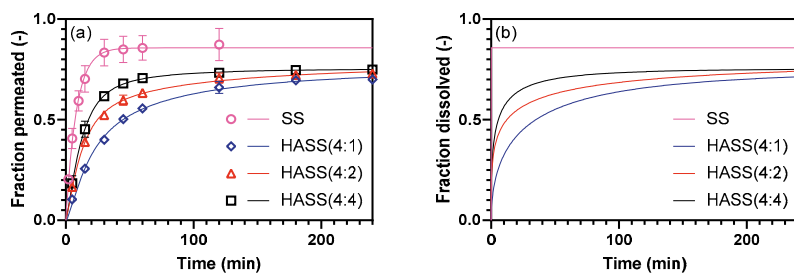


Figure 17. Determination of the corrected dissolution profiles: (a) Fit of the transformed Weibull distribution function to the experimental release data and (b) fraction dissolved Salbutamol.

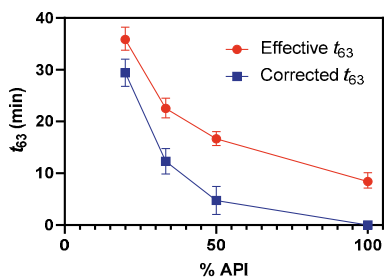


Figure 18. Comparison between effective (accounting for dissolution *and* absorption) and corrected (dissolution only) t_{63} .

Potential toxicity issues of investigated formulations

An extremely important factor in the development of novel pulmonary drug delivery systems is the safety of the novel formulations. Mesoporous amorphous silica particles are considered safe for the use of food additives or in vitamin supplements, in addition it has been previously reported that synthetic amorphous silica is cleared from the lungs without any progressive toxicological effects.^{85–87} Nevertheless, standard preclinical safety studies need to be conducted prior to any clinical evaluation of mesoporous silica particles as a potential pulmonary delivery system. Also for the hydrogel system safety studies need to be done before any form of clinical use. It has been shown that the concentration of crosslinking agent glutaraldehyde influences the cytotoxicity, therefore the amount of unlinked glutaraldehyde should be kept low.⁸⁸ However, the used hydrogel particles served as a model system and can be further optimised.

PBBM

Permeability input

The lung permeability (P_{eff}) used in the PBBM tools for Bud and FP were previously obtained by Eriksson et al.⁸⁹ However, not all input parameters were available for BDP in the literature and therefore building the PBBM tool for BDP was more challenging giving rise to more uncertainties. As mentioned earlier BDP is a prodrug which is directly metabolised in the lung to the highly active B-17-MP. It has been argued by Shao et al. that the lack of permeability data could be due to the fact that BDP metabolizes in the lung which could be challenging to determine these parameters with the presence of esterases.⁹⁰ As no previously reported human permeability value was known, the permeability value used in this study was a similar value as was used for Bud and FP. However, due to the lack of experimental data the permeability value should be seen as an approximation of the permeability of BDP.

Solubility input

The water solubility of the drug is often used as an input parameter in PBBM. However, in this study the solubility in PBS with 0.03% SDS was used, since the surfactant aided the experimental solubility measurement but is not expected to increase solubility since the SDS concentration is below the critical micelle concentration.⁹¹ As it is not clear yet which dissolution medium is the best to use, it is challenging to determine a standard solubility value to use in PBBM. The solubility values that have been used in this study were therefore the solubility value obtained in PBS with 0.03% SDS, the solubility value in 0.2% SDS and the solubility value obtained in 0.5% SDS.

The PK profiles obtained using different solubility input values can be seen in Figure 19. For Bud, regardless of the solubility value used, a good agreement between the observed PK profile and the predicted PK profiles was obtained. For both BDP and FP it can be observed that when the low solubility value was used (0.03% SDS) the model was underpredicting compared to the observed data. However, when the other two solubility values were used for the PBBM the agreement between observed and predicted was significantly improved.

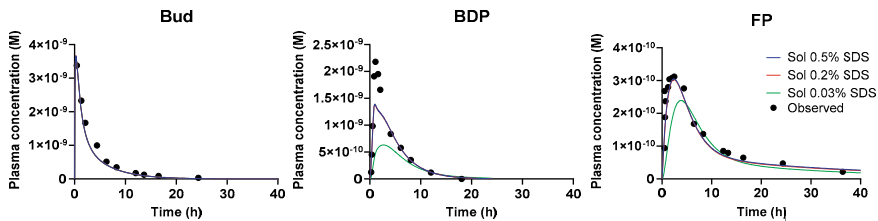


Figure 19. PK profiles of Bud, BDP and FP with observed PK profiles and simulated profiles with three different solubility (sol) values used to build the model.

Diffusivity input

It was observed in Figure 19 that adjusting the solubility value in the PBBM to a higher solubility significantly improved the fit between the observed and the predicted PK profiles. However, as mentioned before, it is not clear yet which dissolution medium and therefore solubility value should be used as a standard when simulating lung absorption. Selecting the right solubility value in PBBM would then be done based on the agreement between the observed and predicted data. To improve the PK fit between the observed and predicted data more mechanistically, different diffusivity values were calculated based on the presence of surfactant (Table 1). The diffusivity of all three drugs was determined in 0.5% SDS.

To validate the calculated diffusivity values, theoretical dissolution profiles simulated using the calculated diffusivity values of 0.5% SDS were compared with the corrected Transwell dissolution profiles, as these profiles were obtained in the in vitro setup using PBS with 0.5% SDS (Fig. 20). It can be observed that the theoretical profiles and the corrected Transwell profiles have good agreement for both BDP and FP. The theoretical dissolution profile for Bud showed a too fast dissolution compared to the corrected Transwell dissolution profile. However, as the dissolution of Bud occurs extremely fast this could be caused by practical limitations of the in vitro setup.

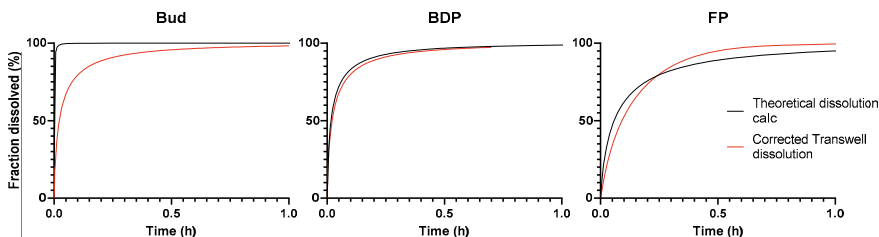


Figure 20. Theoretical dissolution profiles obtained using calculated diffusivity values compared to the corrected Transwell dissolution profiles.

Figure 21 compares the predicted PK profiles obtained for the standard water diffusivity and the calculated diffusivity in 0.5% SDS with the observed PK profiles. It can be seen that for Bud there is no difference between the predictions with the different diffusivity values. Both diffusivity values showed a good agreement with the observed PK profile. For BDP and FP it can be observed that changing the diffusivity value in the model, the agreement between the predicted and observed was improved. The water solubility, as mentioned above, underpredicts compared to the observed data.

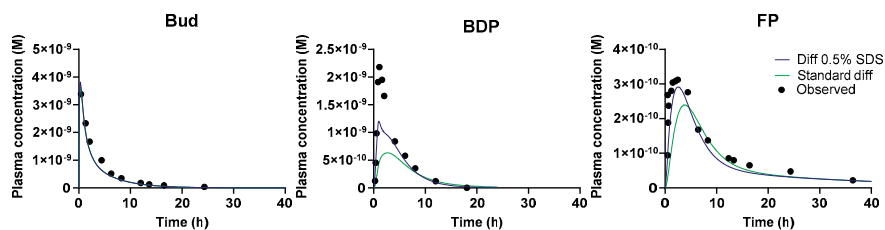


Figure 21. PK profiles of Bud, BDP and FP with observed PK profiles and simulated profiles with three different solubility (sol) values used to build the model.

Changing solubility or diffusivity input parameter

It can be observed that the agreement between the observed PK profile and the predicted profile can be improved by adjusting the solubility value as well as the diffusivity value. As mentioned earlier there is no standard dissolution medium yet available, and therefore improving the model with different solubility values is done based on the fit of the model.

However, calculated diffusivity values could also be used to improve the agreement between predicted and observed PK profiles. Using the calculated diffusivity a more mechanistically sound approach was used to obtain a good agreement with the observed data. The calculated diffusivity values take in to account the micellar fraction in the dissolution medium. It can be seen that with the same solubility but increasing amount of surfactant the diffusivity increases. This could explain that there is an effect of micelles on the dissolution profiles. The dissolution rate of the drug is defined by the solubility in the aqueous phase, for example in PBS, meaning that the dissolution rate of the drug should be similar when media with added surfactant are used.⁹² However, different dissolution profiles can be observed when using these different media. An explanation for this could be that in the presence of surfactant, the micelles speed up the dissolution by helping move particles from the surface in to the bulk.

The size and amount of micelles present in the media was determined based on SDS. The calculated diffusivity values are therefore not directly related to the in vivo situation as different types of surfactant are present in the lung lining fluid. Using the SDS a better understanding can be obtained on the effect of micelles on the in vitro dissolution profiles, however, further research needs to be done to better understand the effect of micelles on the in vivo situation.

Conclusions

In this thesis, an in vitro dissolution method was evaluated and characterised for assessment of novel pulmonary drug delivery systems, with a focus on future controlled release systems. This included determination of important characteristics of the in vitro method using a mechanistic model, evaluation of novel formulations in the characterised method and to establish PBBM tools using the micellar contribution. The main findings of the thesis are:

- Analysing experimental dissolution data using the developed mechanistic model showed good agreement between the model and the experimentally obtained data (**Paper I and II**)
- The developed mechanistic model could predict which process was the rate limiting step in the obtained dissolution profiles (**Paper I**)
- The developed mechanistic model provided the same rank order as the Weibull fit, however the model provided additional detailed understanding of the dissolution process and the dissolution setup used (**Paper II**)
- Mesoporous silica particles showed promising aerosolization and control over the release profiles by tuning different particle characteristics, making them interesting for further investigation as potential novel pulmonary formulations for controlled release (**Paper III**)
- Hyaluronic based hydrogels showed potential as a controlled release formulation for the lungs with a promising FPF and in vitro drug release that was retarded compared to the pure drug (**Paper IV**)
- The Transwell method can be used to characterise and analyse clinical available formulations, and can be used to determine the potential of novel pulmonary formulations (**Paper III and IV**)
- The micellar contribution to diffusion of poorly soluble inhaled drugs during in vitro dissolution was defined and validated using the corrected Transwell dissolution profiles (**Paper V**)

- PBBM tools were successfully established for Bud, BDP and FP using the diffusivity values taking into account the micellar contribution of the surfactant (**Paper V**)

Future perspectives

To summarise, an *in vitro* dissolution method has been characterised and PBBM tools were established for three orally inhaled drugs. Two novel potential controlled release drug delivery systems have been successfully analysed using this *in vitro* dissolution system. Ultimately, it would be highly beneficial if the corrected Transwell dissolution profiles and PBBM can be used to predict the lung absorption of novel pulmonary drug delivery systems. This would be extremely helpful in the early stage of pulmonary drug development, when selecting potential drugs or formulations to take further in to the development process. However, to further establish an *in vivo* relevant dissolution system to predict the performance of controlled release formulations a biorelevant dissolution medium has to be defined. In addition to that, the used PBBM tool has to be validated more, with a wider range of compounds, including different types of formulations.

Popular science summary

Delivering drugs through the lungs has been done for years in order to treat diseases like asthma and COPD (chronic obstructive pulmonary disease). Using the lungs for treating diseases is highly effective, as the lungs are extremely good at taking up drugs for local treatment, such as asthma, or systemic delivery to the blood. Nowadays, drugs that are delivered via the lungs act directly and have a short effect. However, to increase the duration of the effect of the drug, new products need to be developed that can release the drug over a longer period of time, and these are called controlled release systems.

One way of doing this is by slowing down the dissolution of the drug. For a drug to be taken up and have an effect, the drug particles need to dissolve and be in solution. By slowing down the dissolution, the drug will be taken up slower and the effect of the drug will be longer. To determine and predict how long the dissolution of a drug takes in the lung, different methods are developed. However, there are no standard methods yet for inhaled drugs and therefore several different methods are used. One of these methods is a membrane-type method consisting of two compartments. First the drug particles, in powder form, need to dissolve in the donor compartment, then the drug, in solution, needs to transport over the membrane to the acceptor compartment, where the drug concentration over time is determined. These dissolution profiles represent the dissolution of the drug and transport over the membrane, meaning that these profiles need to be well understood to determine what the dissolution profiles of the drug really is. To better analyse and understand these profiles, a mechanistic model can be used, which is built up out of several different mathematical equations.

The aim of this thesis was to evaluate and characterise a membrane-type dissolution method for assessment of new pulmonary drug delivery systems, with a focus on future controlled release systems. The mechanistic model that was used to analyse the dissolution profiles, could predict which process was the rate limiting step, the dissolution of the drug or the transport over the membrane, and the model could provide rank order of the analysed drugs that was similar to the rank order of the drugs in humans. Two new pulmonary formulations were successfully analysed using the membrane-type dissolution method and showed modified release and potential to be successfully inhaled.

Finally, an additional computational model was established to successfully predict the lung absorption in human for three clinically available drug products. This was done by determining how fast molecules can move in different dissolution liquids, and incorporating this in the computational model.

Populairwetenschappelijke samenvatting

Het toedienen van medicatie via de longen is een langgebruikte manier om bijvoorbeeld astma en COPD (chronisch obstructieve longziekte) te behandelen. Het gebruik van de longen voor de behandeling van ziekten is zeer effectief omdat de longen buitengewoon goed zijn in het opnemen van medicijnen voor lokale behandeling, zoals astma, of voor transportatie van medicijnen naar het bloed. Bestaande inhalatie medicijnen worden direct opgenomen en hebben een snel maar kort effect. Om de werkingsduur van het medicijn te verlengen en daarmee de doseringsfrequentie te verlagen, moeten er echter nieuwe producten worden ontwikkeld die het medicijn over een langere periode kunnen afgeven; de zogenaamde gecontroleerde afgifte medicijnen.

Om een medicijn op te nemen in het lichaam en een effect te hebben, moeten de medicijndeeltjes oplossen in het lichaamsvocht (in de longen, longvocht). Door het oplossen van de medicijndeeltjes in het lichaamsvocht te vertragen, kan de werkingsduur van het medicijn worden verlengd. Om te bepalen en te voorspellen hoe lang het oplossen van een medicijn in de longen duurt, kunnen verschillende methoden worden gebruikt. In tegenstelling tot medicijnvormen zoals pillen en capsules is er nog geen standaardmethode voor inhalatiegeneesmiddelen om dit te bepalen. Daarom wordt er wereldwijd onderzoek gedaan naar verschillende methoden.

Praktisch is het (nog) niet mogelijk om direct in mensen te meten hoe snel een bepaald medicijn in het longvocht oplost, daarom wordt de snelheid waarmee medicijnen effect hebben gebruikt om te vergelijken met in het lab verkregen data. Sommige medicijnen werken sneller dan andere en dus kan er een rangorde worden bepaald. De membraan methode is een veel onderzochte methode om oplosprofielen te verkrijgen in het laboratorium en waarmee het mogelijk is om deze rangorde in snelheid van het oplossen van medicijnen te relateren aan de rangorde in effect bij mensen. Deze membraan methode is gebruikt in dit proefschrift en bestaat uit twee compartimenten: het donor- en acceptorcompartiment. Bij deze methode lossen de medicijndeeltjes (in poedervorm) eerst op in het donorcompartiment, vervolgens wordt het medicijn (in oplossing) over het membraan naar het acceptorcompartiment getransporteerd. In dit compartiment wordt de medicijnconcentratie over tijd bepaald, ook wel

oplosprofielen genoemd. Deze oplosprofielen vertegenwoordigen het oplossen van het medicijn, maar daarnaast ook het transport over het membraan. Een zorgvuldige analyse van deze profielen is noodzakelijk om te bepalen wat de exacte oplosprofielen van het medicijn zijn. Voor deze analyse kan een mechanistisch model worden gebruikt dat is opgebouwd uit verschillende wetenschappelijke vergelijkingen.

Het doel van dit proefschrift was het evalueren en karakteriseren van deze membraan oplossingsmethode voor de beoordeling van nieuwe medicijnen voor inhalatie, met een focus op nieuwe toekomstige medicijnen met gecontroleerde afgifte.

Het mechanistische model dat werd gebruikt om de oplosprofielen te analyseren, kon voorspellen welk proces de snelheidsbeperkende stap was: het oplossen van het medicijn of het transport over het membraan. Het model kon ook de rangorde van de geanalyseerde medicijnen correleren met de rangorde in effect bij mensen. Met behulp van de membraan oplossingsmethode werden twee nieuwe toekomstige medicijnen voor inhalatie met succes geanalyseerd. De medicijnen toonden een gecontroleerde afgifte en potentie om met succes te worden geïnhaald. Ten slotte werd er een computer model/programma gebruikt om met succes de longabsorptie bij de mens te voorspellen voor drie medicijnen. Dit is gedaan door te bepalen hoe snel moleculen in verschillende oplosvloeistoffen kunnen bewegen en dit in het computer model op te nemen.

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Irès

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