Correlation of ICS *in vitro* dissolution and pulmonary absorption

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TABLE OF CONTENTS

Contents page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure page

LIST OF ABBREVIATIONS

ACI	Anderson cascade impactor
ACN	Acetonitrile
AUC	Area under the curve
AUMC	Area under the first moment curve
BDP	Beclomethasone dipropionate
BUD	Budesonide
CFC	Chlorofluorocarbon
CIC	Ciclesonide
COPD	Chronic obstructive pulmonary disease
DDW	Double distilled water
DD	Delivered dose
Des-CIC	Desisobutyryl-Ciclesonide
DPI	Dry powder inhaler
DPPC	L-α phosphatidylcholine
EMA	European Medicines Agency
EtOH	Ethanol
FDA	Food and Drug Administration
FP	Fluticasone propionate
FPD	Fine Particle Dose

FPF	Fine Particle Fraction
GI	gastrointestinal
HFA	Hydrofluroalkane
HPLC	High performance liquid chromatography
ICS	Inhaled corticosteroid(s)
IPAC-RS	International Pharmaceutical Aerosol Consortium on Regulation and Science
IVIVC	*in vitro-/in vivo* correlation
LLOQ	Lowest Level of Quantification
MAT	Mean absorption time
MAIC	Major Analytical Instrument Center
MD	Metered dose
MDI	Metered dose inhaler
MDT	Mean dissolution time
MRT	Mean residence time
MRT_{inf}	Mean residence time of an infusion
$MRT_{i.v.}$	Mean residence time of an intravenous bolus injection
MeOH	Methanol
ml	Milliliter
mPBS	modified PBS

MW	Molecular weight
NGI	Next Generation Impactor
PBS	Phosphate buffered saline
PERC	Particle Engineering Research Center
(p)MDI	(Pressurized) metered dose inhaler
QbD	Quality by Design
SD	Standard deviation
SDS	Sodium Dodecyl Sulfate
SEM	Scanning electron microscope
SIP	Sample Induction Port
SLF	Simulated Lung Fluid
TEM	Transmission electron microscope
tPBS	PBS with polysorbate 80
(% v/v)	% volume in volume
(% w/v)	% weight in volume
µg	microgram

ABSTRACT

On the one hand dissolution testing and *in vitro/in vivo* correlations are regularly used or required by regulatory authorities for purposes such as quality testing or bioequivalence studies in the field of solid orally administered drugs. On the other hand dissolution testing for inhaled products regardless of bioequivalence testing or quality assessment purposes is relatively new and a standardized and validated method or guidance for regulatory use, as provided for solid oral formulations, is not available.

Working groups from the pharmaceutical industry such as e.g. the dissolution working group of the International Pharmaceutical Aerosol Consortium on Regulation and Science, the IPAC-RS, are dedicating their inter-company collaboration to provide a better understanding of the current scientific knowledge and by that support the discussion about the value and status of dissolution testing for inhaled products for all parties involved.

The scientific interest in characterizing inhalation formulations through dissolution testing is reflected by a growing number of publications which focus on the development of different dissolution methods and techniques. These methods additionally promote the scientific discussion and thus advance the understanding in this field besides the IPAC-RS working group dedicated to the topic.

In the case of inhaled corticosteroids, a specific type of inhalation drugs, recent publications focused on the dissolution behavior of this class of drugs when administered by different delivery devices. Most published methods had the long-term goal to develop a quality assessment tool for e.g. evaluating batch to batch variations after production. These methods have employed different dissolution media and set-ups with the attempt to develop standardized procedures and as a consequence the employed approaches differ widely (from flow through to limited fluid capacity arrangements),

Most approaches have in common that a suitable membrane or barrier has to be identified which provides a sufficient separation between a donor compartment, containing non-dissolved drug particles, and an acceptor (sampling) compartment which only contains dissolved drug particles.

In this dissertation it is shown that the adaption of a dissolution method based on a limited fluid capacity arrangement in Transwell® wells is suitable for testing three different Inhaled Corticosteroids (ICS) and their respective dissolution when previously being delivered from metered dose inhalers into an Andersen Cascade Impactor (ACI). The ACI arrangement itself was controlled for humidity, flow rate and temperature and it was shown that the final dissolution method works under sink conditions and is not affected by membranes, that in other published methods represented the rate limiting step (diffusion across commercially membranes can represent rate limiting step).

This dissertation focuses on the inhaled corticosteroid Ciclesonide (CIC) for which on the one hand little data exists regarding its dissolution behavior and on the other hand *in vitro/in vivo* relationships to CIC's pharmacokinetic behavior are limited. It was also of interest to compare CIC's behavior to those of two other steroids, namely Budesonide and Fluticasone propionate.

The development of the temperature and humidity controlled dissolution test procedure for the three compounds included (1) the selection of the dissolution apparatus including a suitable barrier and (2) the identification of a dissolution medium (Sodium dodecyl sulfate, SDS). Results generated with the optimized procedure, suggested that water solubility and dissolution testing without surfactant is not a good predictor for the dissolution rate of the drug in lung surfactant and also indicated that the dissolution medium needs to mimic the lung surfactant.

Ciclesonide which is one of the least water soluble drugs among the ICS was found to exhibit a rather rapid dissolution profile once the surfactant in this case SDS in the chosen concentration was added. Thus, the rank order obtained for water solubility (Ciclesonide less than Fluticasone propionate less than Budesonide) contrasts with the dissolution rate and solubility in the surfactant SDS (Ciclesonide about as soluble and fast as Budesonide and both much more soluble and faster than Fluticasone propionate).

However, the dissolution rates *in vitro* were shown to be a good indicator for differences in the absorption rate after inhalation as the rate of absorption (mean absorption time) obtained from pharmacokinetic studies agreed well with the *in vitro* data when surfactant was used within the dissolution media. Thus, the developed

dissolution method in surfactant containing medium is a good predictor for *in vivo* absorption rates of these ICS, assuming that the dissolution rate is the rate-limiting step. It also suggests that the selected surfactant and the pulmonary surfactant present in the lining fluid behave similarly regarding the dissolution of these ICS generated aerosols. The data further suggest that an agreement of *in vitro* and *in vivo* absorption rates is a strong indicator that the absorption for the investigated drugs is controlled by the dissolution of the drugs.

ZUSAMMENFASSUNG

Im Rahmen dieser Dissertation wurde gezeigt, dass die hier entwickelte und adaptierte Methode, basierend auf Transwell®-Wells mit kleinen Volumina, geeignet ist 3 inhalierbare Kortikosteroide (ICS), Budesonid, Fluticasonpropionat und Ciclesonid, und ihre Dissolutionseigenschaften nach Ausbringung aus Inhalatoren in einen Anderson Kaskaden-Impaktor (ACI) zu vermessen. Die Ausbringung der drei verschiedenen marktüblichen Dosieraerosole erfolgte in einen Luftstrom, der einen Volumenstrom, eine Luftfeuchtigkeit und Temperatur aufwies, wie es auch im Menschen zu erwarten ist. Es wurde gezeigt, dass die adaptierte Dissolution-Methode unter Sink-Bedingungen arbeitet und weiterhin, im Gegensatz zur literatur-bekannten Methode, unbeeinflusst vom handelsüblichen Membranmaterial ist.

Neben Budesonid und Fluticasonpropionat fokussierte sich die Arbeit auf Ciclesonid (CIC), zu welchem einerseits wenig öffentlich-zugängliche Daten existieren bezüglich des Auflösungsverhaltens und andererseits wenig bekannt ist über die Verknüpfung von *in vitro/in vivo* Daten zum pharmakokinetischen Verhalten von Ciclesonid.

Die Entwicklung der bevorzugten Apparatur für die drei Wirkstoffe beinhaltete (1) die Auswahl und Anpassung des Aufbaus inklusive der geeigneten Barriere zwischen Wirkstoff- und Medienseite und (2) die Festlegung eines geeigneten Tensids (Natriumdodecylsulfat, SDS) und seiner Konzentration für das Lösungsmedium. Die Ergebnisse zeigen, dass die reine Wasserlöslichkeit, d.h. ohne oberflächenaktive Stoffe, keine gute Vorhersage für das Auflösungsverhalten von Ciclesonid in der Lunge, d.h. in Anwesenheit von oberflächenaktiven Stoffen liefert, und solche somit notwendig sind, um das Lungenfluid und das Auflösungsgeschehen in der Lunge simulieren zu können.

Es konnte weiterhin gezeigt werden, dass die Dissolutions-Raten *in vitro* mit oberflächenaktiven Stoffen gut mit den pharmakokinetischen mittleren Resorptionszeiten (MAT) *in vivo* korrelieren, und es konnte gut zwischen den verschiedenen ICS diskriminiert werden. Die Daten legen die Vermutung nah, dass die Auflösung der zeitbestimmende Schritt bei der Resorption der Wirkstoffe in der Lunge ist.

GENERAL INTRODUCTION, AIM OF THE STUDY AND INTRODUCTION TO ICS DISSOLUTION TESTING: A LITERATURE REVIEW

Inhaled Corticosteroids in Asthma and COPD: An Overview

Inhaled corticosteroids (ICS) are the main anti-inflammatory therapy in persistent asthma worldwide [1]. They are also used to treat other inflammatory based respiratory diseases such as chronic obstructive pulmonary disease (COPD) [2]. Whereas Asthma is characterized by a reversible complex inflammation of the lung involving e.g. episodes of wheezing coughing and acute shortness of breath based on an allergic reaction to an allergen [3], COPD is mainly characterized by its irreversible inflammatory steadily progressing worsening of lung function manifested by more and more airflow limitation [4]. In the case of COPD **Figure 1** illustrates the illness in comparison to the normal lung physiology in that region [4].

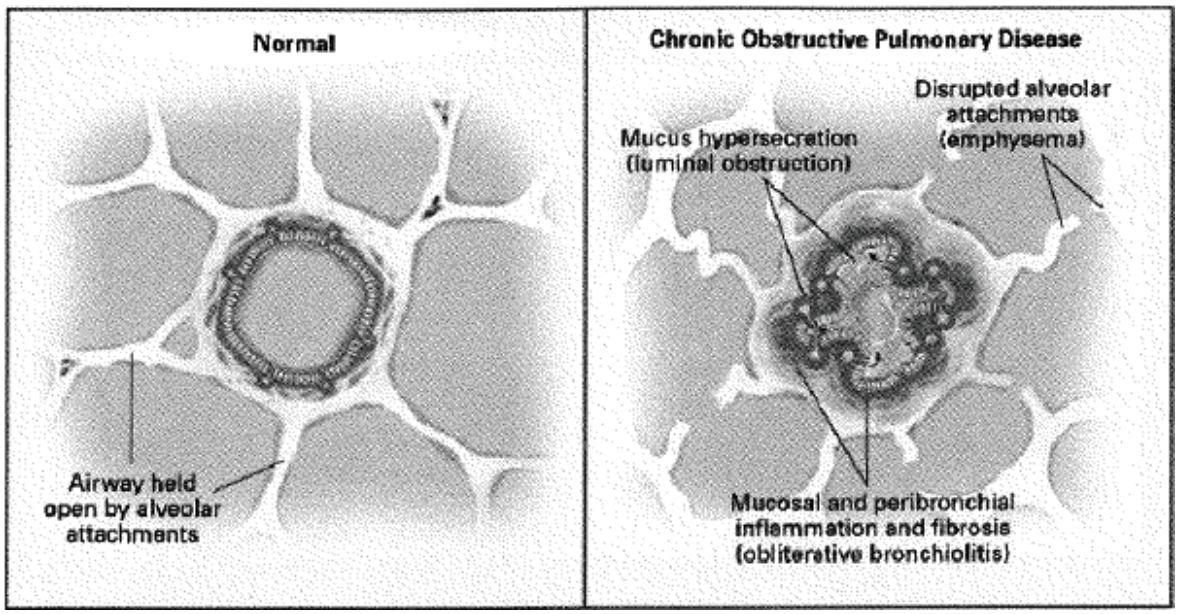


Figure 1: Comparison of normal and COPD affected airways [4]

The region which is portrait in **Figure 1** is the peripheral or deeper region of the airway and it can be seen that there is a limitation in opening as well as loss of alveolar attachment. In the normal lung there is a wide opening of the airway and the location of

this type of alveolar region can be seen in the magnification on the bottom of **Figure 2** [5].

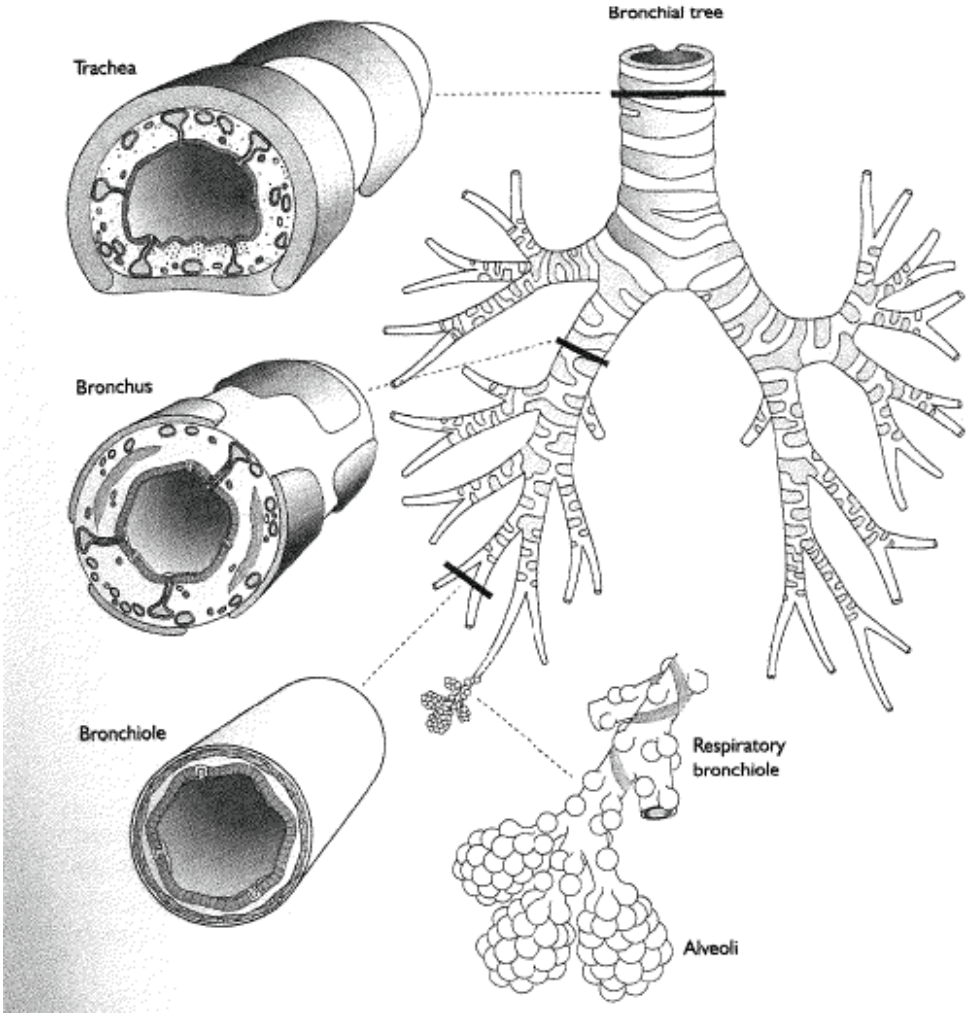


Figure 2: Illustration of different lung regions from bronchial tree to the alveoli [5]

Regarding medication for mild to moderate persistent asthma a low- to medium-dosed ICS therapy is typically chosen as it is characterized by a positive benefit to risk ratio in regard to side effects such as reduction in growth, glaucoma, cataracts, osteoporosis or even fractures [2]. Currently there are a number of different ICS in different devices and/or formulations together with different dose strengths on the market [6]. In this respect there are two main categories of devices which are used to deliver the aerosols to the desired site of action in the lung. One type of devices are

Dry Powder Inhalers (DPI) which contain the active drug as a powder formulation alone or are blended with carrier exipients such as lactose monohydrate or compounds like e.g. Mannitol [7]. The other main type of pulmonary drug delivery devices are Metered Dose Inhalers (MDI) which are powered by propellants such as Hydrofluroalkane (HFA) which is the newer type of propellants or the older ones which are intended to be phased out in the future (Chlorofluorocarbon (CFC) liquefied propellants) [7-9]. The formulations within the MDI's can be micronized or micro-crystallized powders or e.g. ethanol based solution formulations [10-12]. **Table 1** provides an exemplified overview of the performance of such devices and formulations regarding their lung deposition.

Table 1: Performance of selected ICS inhaler devices and formulations

Drug, Propellant, Device	% deposited in the lung of delivered dose	% deposited in the lung of metered dose
Ciclesonide; HFA, MDI [10]	52	
Budesonide CFC, MDI [13]	16	15
Budesonide, Powder, Turbohaler [13]	---	32
Fluticasone propionate, CFC, MDI [14]	---	25
Fluticasone propionate, HFA, MDI [14]	---	28
Fluticasone propionate, Powder, Diskus® [14]	---	16

Apart from the mentioned MDI and DPI there are also nebulizers and the spring driven, propellant free, soft mist inhaler Respimat®, which is a unique device in regard to the underlying technology and performance [15, 16]; but Respimat® is not available containing an ICS formulation.

Today the mode of action responsible for the anti-inflammatory effects of ICS in asthma and COPD is very much understood [17, 18]. The ICS promoted by their high

lipophilicity can cross the pulmonary cell membranes at the site of action very effectively. It binds to the cytosolic glucocorticoid receptor. The activated ligand-glucocorticoid complex can promote the modification of certain genes which leads to the production of anti-inflammatory proteins. These can then provide the desired effect against the inflammation itself.

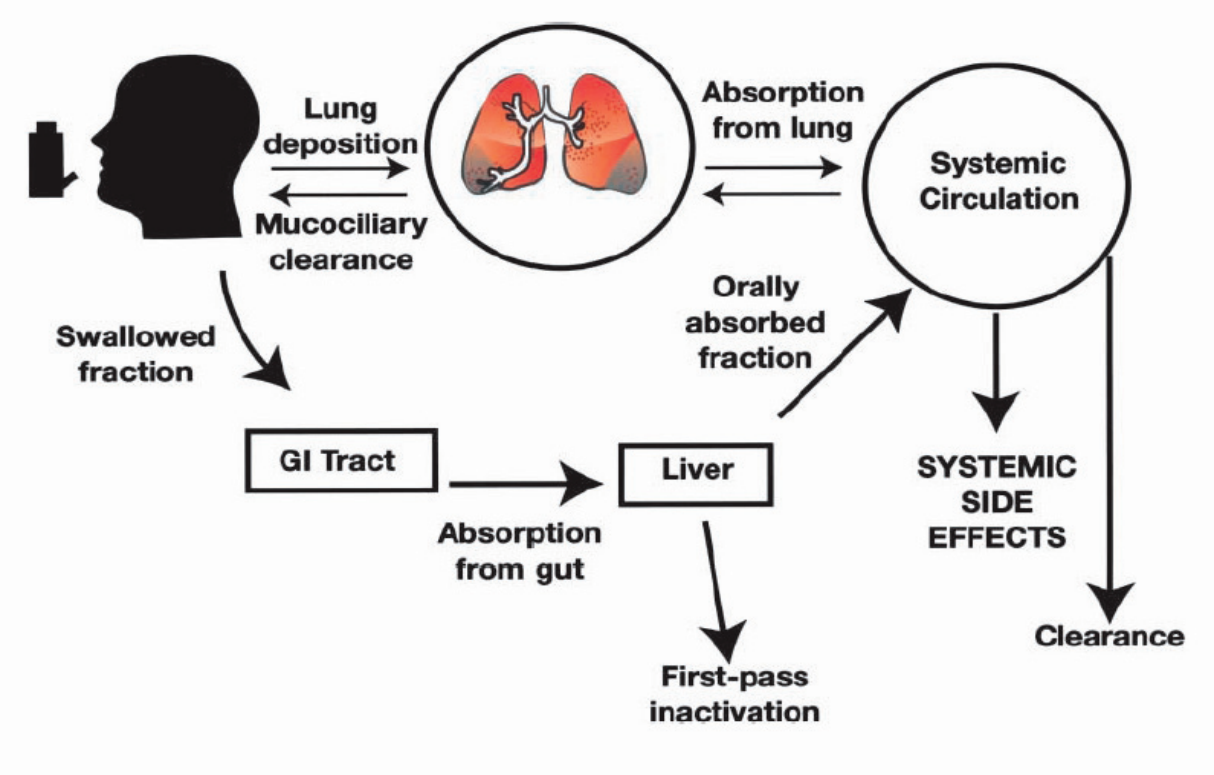


Figure 3: General overview of the fate of ICS drug deposition [19]

The fate of an ICS drug particle after inhalation is depicted in **Figure 3** [19]. Drug is either deposited in the lung or swallowed after it was deposited in the oropharyngeal region. If the drug is dissolving slowly at the site of action, a fraction of the deposited mass in the upper part of the lung might be cleared by mucociliary clearance and then swallowed [19].

Induction of the desired effects in the lung can only occur after dissolution. It is important to realize that all pulmonary available drug (only drug that dissolved) will be absorbed into the systemic circulation.

The non-inhaled fraction is swallowed after the inhalation maneuver. This fraction then enters gastrointestinal (GI) tract, is absorbed from the gut and can be inactivated through first pass metabolism in the liver or GI membranes or enter the systemic circulation. Unchanged absorbed drug can lead to possible side effects. This indicates that systemic side effects can be induced from drug having been absorbed from the lung and the GI tract. Both fractions will be eliminated through systemic clearance. The more efficient this clearance the smaller will be the systemic side effects [19].

The ICS themselves might be prodrugs that need to be metabolized to the active drug at the site of action (e.g. Ciclesonide – Desisobutyryl Ciclesonide (Des-CIC)), but represent generally active drug species that do not depend on metabolic activation (e.g. Fluticasone propionate). The ICS are additionally often divided into first generation and second generation corticosteroids whereas the newer inhaled corticosteroids such as Fluticasone propionate, Mometasone furoate and Ciclesonide are described to possess more favorable pharmacokinetic properties than the old ones (e.g. lower oral bioavailability) (see **Table 2**) [6, 20].

Table 2: General overview of pharmacokinetic properties and their interdependence for supporting efficacy, safety and airway selectivity of ICS [20]

Pharmacokinetic properties promoting safety and efficacy of inhaled corticosteroids. ↑, ↓=corresponds to improved, reduced or no influence, respectively.

Parameter	Efficacy	Safety	Airway selectivity
Formulation	Greater lung deposition with optimal particle size	Reduced incidence of local adverse events when lung deposition is maximized	↑ with optimal particle size
Prodrug structure	Increased efficacy if pulmonary retention is improved. Reduced efficacy if intact prodrug is systemically absorbed.	If prodrug completely activated, safety will not be affected. Local safety will be improved for prodrugs which are not activated in oropharyngeal area.	↑ if pulmonary retention of active metabolite is improved and/or oropharyngeal activation is reduced ↓ for prodrugs which are systemically absorbed
Pulmonary availability	High availability results in increased lung exposure	High availability increase systemic exposure but reduce oral waist	= for a drug with no or little oral availability ↑ for an orally available drug molecule
Receptor affinity	Increased anti-inflammatory activity with strong receptor binding	Reduced receptor binding results in lowered incidence of systemic adverse effects	=
Local esterification	Longer pulmonary retention prolongs efficacy	Little influence if limited peripheral esterification	↑ if greater local than systemic esterification
Oral availability	The orally available fraction will not contribute to efficacy	If oral availability is significant, systemic exposure and incidence of adverse effects will increase	↓
Lipophilicity	Increase lung retention and duration of action but also mucociliary clearance	Increased systemic retention might increase the risk of adverse events	= or ↓depending on relative degree of lung and systemic retention
Protein binding	Conceptually only free drug is associated with effect: therefore efficacy should decrease with increased protein binding	(systemic effects associated with free drug only)	= (in the linear part of the dose-response curve for efficacy and safety)
Systemic clearance	No effect on pulmonary efficacy of rapid clearance	Reduced potential for adverse effects with rapid clearance	↑ with increased clearance
Systemic half-life	Prolongation of systemic half-life unlikely to affect efficacy in the lung	Lower systemic risk with reduced elimination half-life	↑ with shorter half-life

Strategies during recent drug development of new or improved ICS can be summarized according to the outline below [19, 21, 22]:

- the optimization of targeting the site of action more efficiently to produce the required pulmonary anti-inflammatory effects without causing unwanted side effects
- the delivery of a high fraction of small particles in the inhalable range from the inhaler device
- delivery as a prodrug with pulmonary on-site activation of the ICS to the active drug

- a high affinity of the active drug to the glucocorticoid receptors in the lung
- a long pulmonary residence time together with reversible esterfication (see **Figure 4**)
- a low oral bioavailability to reduce the systemic side effects
- a high systemic clearance of both drug and if applicable the prodrug.

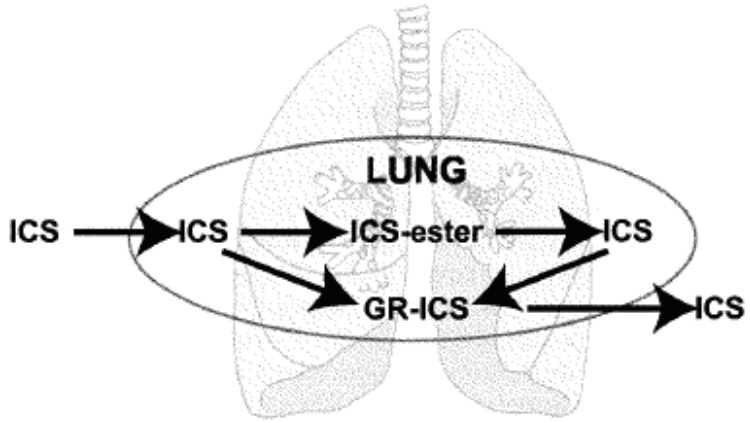
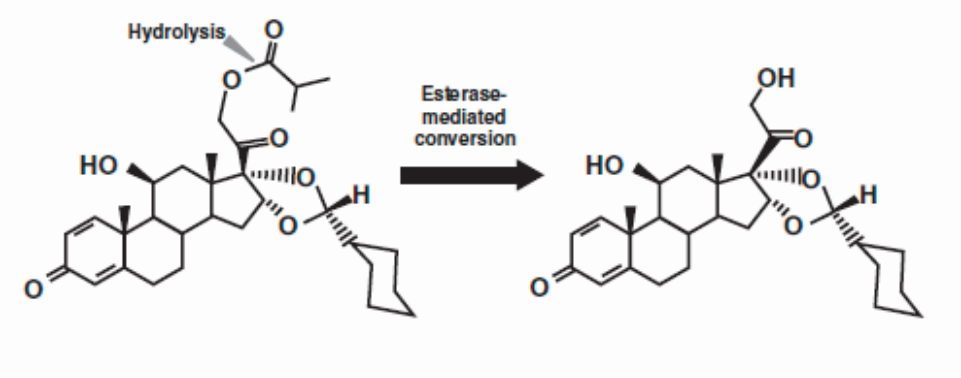


Figure 4: ICS reversible esterfication process [19]

Although all marketed ICS are regarded as relatively safe and efficacious in low- to medium-dosed applications especially Ciclesonide can be regarded as a corticosteroid with many characteristics an “ideal ICS” should possess [2, 23-27].

In order to achieve these characteristics the Alvesco® HFA-MDI which contains Ciclesonide produces a high fine particle fraction of this prodrug resulting in a lung deposition by the inhaler of approximately 50% or more [10, 28]. The prodrug which is deposited in the lung is mostly activated within the lung to the active metabolite Des-Ciclesonide (see **Figure 5**).



Ciclesonide Des-Ciclesonide

Figure 5: Ciclesonide activation to active metabolite Des-Ciclesonide with minor adaptation from [29, 30]

After activation it exhibits a high affinity to the glucocorticoid receptor and is described to have a long pulmonary residence time attributed to a reversible esterfication trapped with fatty acid conjugates resulting in a possible depot-like effect of the active drug Des-Ciclesonide at the site of action [26]. The initial drug's, Ciclesonide, low oral bioavailability coupled with a very high protein binding in plasma (only free drug is able to induce effects) leads to a reduced risk of side effects for this ICS [31, 32]. In addition Ciclesonide and Des-Ciclesonide, which enters the systemic circulation, is rapidly cleared from the body [31].

Although different, Budesonide and Fluticasone propionate both also possess beneficial pharmacokinetic properties in order to achieve a relatively safe risk to benefit ratio. The two ICS are characterized by a low oral bioavailability and in connection with improved delivery devices with higher inhalable fractions can further reduce the likelihood of adverse events. This is due to the fact that even less of the drug is being swallowed by the patient once the inhaler provides a more suitable pulmonary deposition [33, 34].

Budesonide similar to Ciclesonide undergoes reversible esterfication within the target organ resulting in the formation of intra-pulmonary depots [35]. This feature represents one of the possible mechanisms to extend the residence time within the lung and thus providing a prolongation for the desired anti-inflammatory effects of the inhaled ICS at the site of action [35].

In general the insight and scientific knowledge of the physiochemical properties, pharmacokinetics, pharmacodynamics, delivery technologies and formulations of marketed ICS is relatively comprehensive [1, 6, 24]. Critical issues related to the efficacy and safety of ICS, such as the dose strengths and dosing regimens (once daily vs. twice daily) for an optimal effect to side effect ratio can be found to be discussed by different authors [1, 6, 24, 36].

One focus which has recently been addressed is the discussion of the implications of the *in vivo* dissolution rate of ICS for pulmonary targeting [37-39]. This is additionally characterized by the emergence of various proposals regarding *in vitro* dissolution testing methods for this class of Asthma and COPD medications [40-43].

Aim of the Study

Recent discussions in the pharmaceutical industry, regulatory authorities and academia regarding the bioequivalence of inhaled dosage forms includes the discussion about the effect of the dissolution rate of drug particles at the site of action within the lung. In addition to other well established *in vitro* aerosol test methods such as particle size characterization by cascade impactors and impingers or delivered dose (DD) measurements by the help of sampling tubes, it is currently of interest to present and/or develop a standardized dissolution test method for orally inhaled products.

In the light of the growing discussion of dissolution testing it is the aim of this study to review and assess published dissolution test methods and to provide an improved and cost effective alternative dissolution test which consequently is applied to a selection of inhaled anti-inflammatory drugs namely Inhaled Corticosteroids. The proposed research, therefore, includes the optimization of a dissolution test method.

As one important challenge within such *in vitro* approaches is the fact that the rate limiting step is often not the dissolution process, but the diffusion of drug across membranes or barriers that divide donor and receptor (sampling compartment) the separation element used between donor and acceptor side and the choice of the dissolution medium itself needed attention. It was also important to challenge the developed dissolution model by testing its *in vitro/in vivo* predictability when applied to pharmacokinetic studies and to relate all results to the physicochemical properties of the selected ICS. Hopefully, this work will further increase our scientific understanding of the fate of ICS at the site of action.

Introduction to ICS Dissolution Testing: A Literature Review

The process of dissolution is characterized by a solid particle entering a solvent in liquid phase. The kinetics of such a process is often described by a mass transfer equation by Noyes-Whitney or Nernst-Brunner (**Equation 1**).

$$\frac{dm}{dt} = \frac{DS}{h}(\boldsymbol{Cs} - Cb) \qquad \textbf{Equation 1}$$

In this equation dm/dt represents the change or in other words the dissolution rate in mass per time. Cb is the concentration of the dissolved solid in the bulk phase at the respective time t. Cs resembles the saturation solubility of the solid within the bulk phase of the solid particle. h is the thickness of the diffusion layer around the particles. S represents the surface area which is exposed to the dissolution media and D is the diffusion coefficient of the drug in the unstirred layer surrounding the particles.

Dissolution profiles are often used to visualize the kinetic process of dissolution and also to compare different profiles. These profiles are obtained by sampling of bulk phase from a dissolution system at respective time points which are usually analyzed by HPLC. For solid oral dosage forms the apparatus in which such dissolution testing takes place, for e.g. quality reasons, in the pharmaceutical industry is a paddle apparatus (USP 2). For inhalation dosage forms there are a variety of adaptations of dissolution test methods or new proposals which will be discussed further in this chapter.

Over the last few years dissolution testing for inhaled substances has gained interest within the respiratory research, development and regulatory science communities. Different groups and teams are proposing dissolution test methods for inhaled products [40, 41, 44]. It is has been stressed that these tests may facilitate the general understanding of the processes occurring in the target organ and thus support drug development and scientific understanding [40, 42, 45]. However, a standardized

method which is accepted by all regulatory agencies worldwide and with which dissolution kinetics can easily be assessed *in vitro* is still missing [41].

The regulatory authorities themselves are not in agreement when it comes to dissolution testing of orally inhaled products. Agencies have not yet accepted or provided any guidance on dissolution testing for inhaled products while information on dose content uniformity testing, particle size distribution measurements up to pharmacokinetic and pharmacodynamic studies are available and regulated [46-50]. Only acceptance criteria for dissolution testing for solid oral dosage forms for quality testing or *in vitro/in vivo* correlations to achieve bioequivalence for two products are regulated [51, 52].

The discussion in the pharmaceutical industry, academia and international pharmaceutical working groups has gained considerable interest. This includes discussions on the relevance of dissolution rate testing for Quality by Design (QbD) considerations [53]. This must be interpreted in the light of the fact that QbD itself is an initiative which was originally started by the authorities themselves as early as 2006 [54].

However, it is of scientific interest to provide more insight into the pulmonary dissolution process as it is a physiologically influenced process that determines the pulmonary absorption and residence time in the lung and as such affects the degree of pulmonary targeting [19, 37]. A thorough understanding and assessment of this process is important for the evaluation of generic and new inhalation products with respect to its pharmacological profiles. A visualization of the processes in question can be found in **Figure 6**.

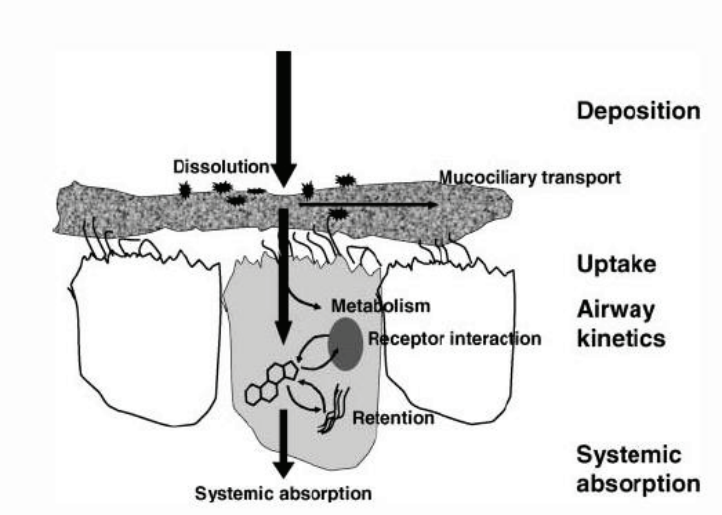


Figure 6: Schematic visualization of the fate of drug particles from deposition up to systemic absorption [20]

For a brief description of the fate of an inhaled drug particle at the site of action it can be said that the deposited particles (which might be generated from a solution based MDI through evaporation of the propellant) have to dissolve prior to their uptake into the pulmonary lining fluid (estimated 10-30 ml in humans) [20]. Only after dissolution of solid particles into the lining fluid the particles can cross the membrane of the lung epithelium to then exhibit the desired pharmacological effect (**Figure 6**).

This assumes that the dissolution process is fast enough before so called mucociliary clearance in the upper airways can remove non-dissolved particles from the target organ [55]. The process of mucociliary clearance is achieved by the mucociliary transport of particles out of the lung in **Figure 6.** Once dissolved the particles can enter the cell, be activated if necessary, enter the ester depot or directly induce the desired effect after which the drug will be absorbed into the systemic circulation [20].

This short description from particle deposition to systemic uptake explains that although the majority of processes prior to the deposition as well as the processes after crossing the membrane of the lung epithelium are relatively well understood either by aerosol generation technologies or e.g. pharmacokinetics, comparative data on the dissolution of ICSs into the lining fluid plays an important role and should be thus supported by *in vitro* assessments.

Work has been published using a variety of different approaches and dissolution media [40, 41, 43, 44]. A comprehensive overview and discussion of these approaches has recently been provided by the IPAC-RS Dissolution Working Group [56]. They concluded that dissolution testing is a valuable technique in the development of inhaled dosage forms, but that a standardized method has not been proposed which can finally serve as a standard for the industry [56]. In the interest of this dissertation a selection of these dissolution publications will be discussed to provide a broader understanding of the topic.

In 2001 *Pham and Wiedman* described the importance and relevance of surfactants for dissolution processes within the lung by comparing Budesonide in Survanta®, a bovine derived lung surfactant, with results obtained in saline and low concentration sodium dodecyl sulfate (SDS) solution media [44]. They came to the conclusion that surfactants regardless of their origin can enhance the rate of the dissolution process and hence help to mimic the *in vivo* dissolution process more realistically [44]. This finding is very important for all dissolution test methods which are intended to be used to correlate results to the *in vivo* dissolution of drug particles at the site of action as the lung itself provides surfactants within the lining fluid which significantly influence the dissolution process [57].

In 2003 *Davies and Feddah* proposed a method for assessing the dissolution of aerosolized Fluticasone propionate, Budesonide and Triamcinolone acetonide powder formulations in a flow through cell after sampling the drug particles in an Andersen Mark II Cascade Impactor (ACI) (**Figure 7**) and different dissolution media [41].

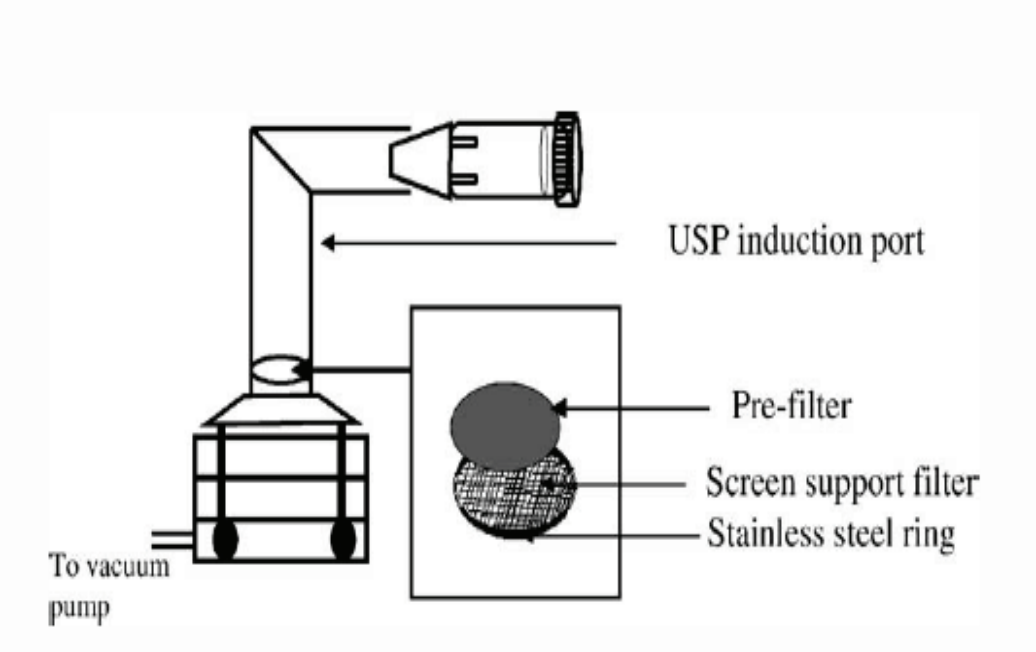


Figure 7: Particle collection within USP induction port in Andersen Mark II Impactor onto fiber glass filter [41]

Besides water simulated lung fluid (SLF) and modified SLF with L-α phosphatidylcholine (DPPC) was used as the dissolution media [41]. The composition of these two artificial and the real life lung fluid can be found in **Table 3** [41, 58, 59].

Table 3: Composition of actual and simulated lung fluids a [59] b [58] c [41]

Ion	Actual[a]	Simulated lung fluid[b]	Modified simulated lung fluid with 0.02% DPPC[c]
Calcium, Ca^{2+}	5.0	5.0	5.0
Magnesium, Mg^{2+}	2.0	2.0	2.0
Potassium, K^{+}	4.0	4.0	4.0
Sodium, Na^{+}	145.0	145.0	145.0
Total cations	156.0	156.0	156.0
Bicarbonate, HCO_3^{-}	31.0	31.0	31.0
Chloride, Cl^{-}	114.0	114.0	114.0
Citrate, $H_5C_6O_7^{3-}$	–	1.0	1.0
Acetate, $H_3C_2O_2^{-}$	7.0	7.0	7.0
Phosphate, HPO_4^{2-}	2.0	2.0	2.0
Sulphate, SO_4^{2-}	1.0	1.0	1.0
Protein	1.0	–	–
L-α-Phosphatidylcholine	–	–	200 mg
Total anions	156.0	156.0	156.0
pH	7.3–7.4	7.3–7.4	7.3–7.4

DPPC: L-α-phosphatidylcholine.

The flow through cell set-up which was kept at 37 °C allowed for dissolution testing under sink conditions and also enabled the user to influence the dissolution parameters online (e.g. flow rate, pH, temperature and the media itself) (**Figure 8**).

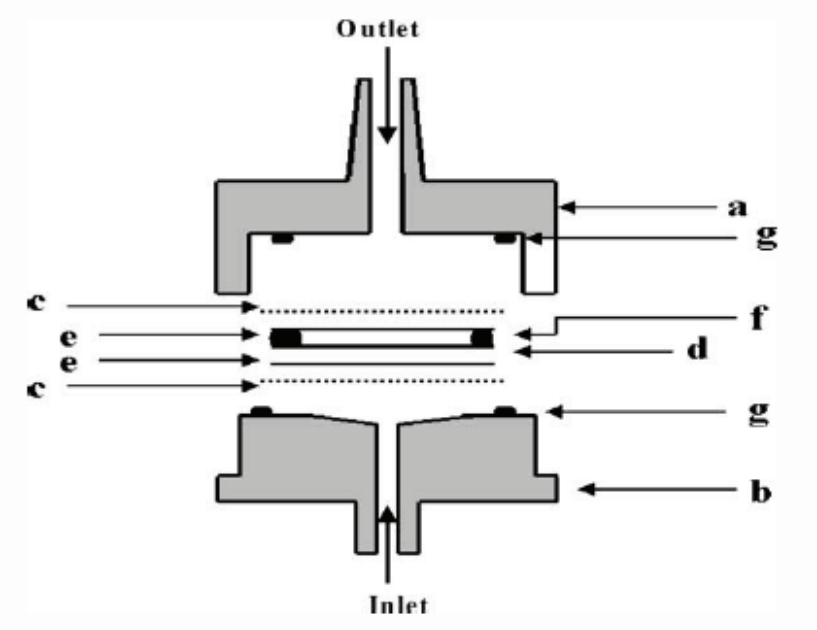


Figure 8: Schematic cross-section view of flow through cell set-up [41]

The above cross section of the device (**Figure 8**) explains the general set-up of the flow through cell in detail. The upper (a) and lower (b) parts of the holder provide the inlet and outlet of the complete device while holding the stainless steel screen support filters (c) with the inner glass fiber which contains the drug particles (d) together with 0.45 µm pore size cellulose acetate membrane filters that are separated by a 1mm Teflon ring (f) and an additional sealing ring element (g) [41].

Sink conditions are sometimes problematic to achieve due to the very low water solubility of the ICS which are tested, but this challenge is eliminated by this specific set-up due to the possibility to influence the flow properties through the cell online and thus providing the necessary flow through volume for sink conditions. Sink conditions are generally defined in way that the volume of the dissolution medium is at least three times greater than the maximum volume which would be needed to saturate said medium with the drug (up to 10 times for very poorly soluble drugs, e.g. ICS). In a flow through arrangement this condition can easily be achieved due to the fact that there is always fresh dissolution medium available for the dissolution process and thus saturation of the medium is avoided per se.

In regard to the dissolution kinetics of the ICS, *Davies and Feddah* conclude that Budesonide and Triamcinolone acetonide follow the Nernst-Brunner equation

(**Equation 1**) in the different dissolution media [41]. For Fluticasone propionate, but not for Budesonide and Triamcinolone acetonide, the authors postulated that there is a similarity in the slow *in vitro* dissolution rate to the longer residence time of the drug in the lung as it had been already described by other authors [60]. An overall evaluation of the proposed dissolution test leads one to the conclusion that besides a relatively complex and possible cost intensive set-up due to the necessity of customized parts (USP sample induction port (SIP) modification) the method can be used for the assessment of the dissolution of ICS. This might include batch release tests that probe for differences in the dissolution behavior. However, it would be important to establish *in vitro/in vivo* correlations for compounds covering a wide range of dissolution behavior and pulmonary dissolution/absorption characteristics.

A recently proposed dissolution method which according to the authors could potentially be also be used for quality control purposes for inhalation products was published by *Son, Horng, Copley and McConville* in 2010 [43]. The authors chose a set-up in which they used a customized membrane holder which had previously been placed into a Next Generation Impactor (NGI) cup before being exposed to various dissolution media within a standard paddle USP 2 dissolution apparatus [43].

The tested drug substances were Budesonide delivered from the HFA-Ventolin® pMDI and Albuterol sulfate from the Pulmicort Flexhaler® DPI [43]. The dissolution media in this study ranged from simulated lung fluid (SLF), 0.2 M phosphate buffer at pH 7.4 to phosphate-buffered saline (PBS), modified PBS (mPBS) which contained dipalmitoylphosphatidylcholine (DPPC) up to PBS with polysorbate 80 (tPBS).

Single to multiple doses emitted from delivery devices were deposited in order to assess drug loading and particle size effects in the above mentioned media [43]. The membrane holder was placed into the cup or stage of the NGI which was previously found to achieve a reliable amount of drug after the smallest number of device actuations [43]. This is based on the fact that the minimum number of doses had to provide enough deposited mass for an analytical examination of the complete dissolution process by HPLC above the lowest level of quantification (LLOQ) (**Figure 9**).

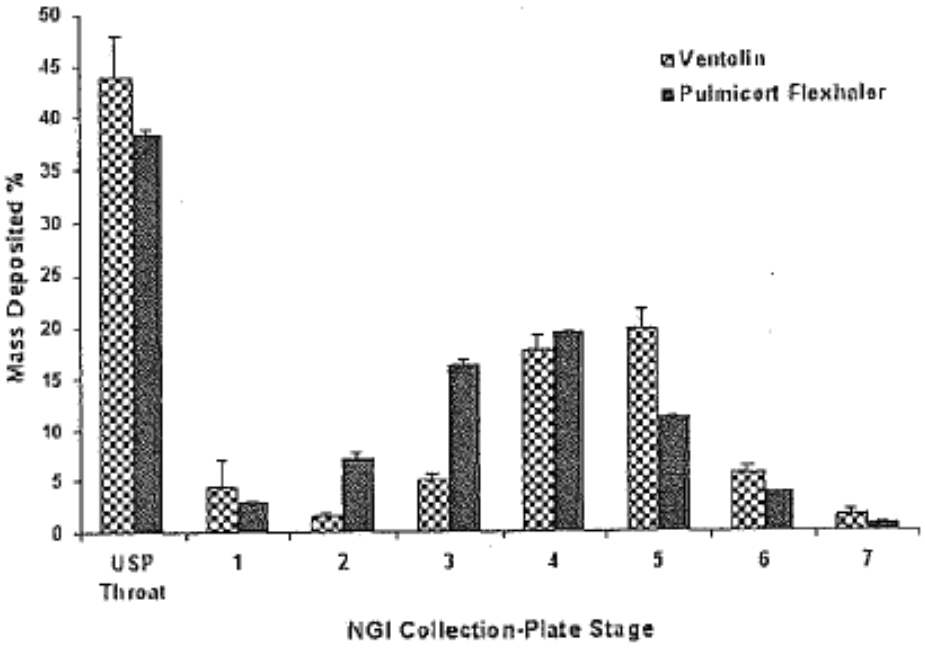


Figure 9: Particle size distribution on NGI stages/cups of Ventolin® HFA MDI (at 30 L/min) and Pulmicort Flexhaler® (at 60 L/min) after 5 actuations [43]

The authors reported that they observed differences in the dissolution behavior of Budesonide in the different media [43]. They concluded the best suitable dissolution media for Budesonide was PBS containing 0.2% Polysorbate 80 when comparing drug loading and device actuation [43].

In connection to a drug loading dependence it was surprisingly found in the case of Budesonide that the hydrophobic drug does not exhibit the fastest dissolution profile in SLF as one would expect. Budesonide dissolved the fastest in the surfactant Polysorbate 80 based media [43].

For the other compound Albuterol sulfate the authors explain that due to the high solubility of the drug in water it was found that the respective dissolution profiles seem more independent of the dissolution media and or drug loading [43].

In summary it can be concluded that the discussed dissolution test method requires the alteration and customization of standard aerosol characterization and dissolution equipment in connection with an identification of a suitable media including possible concentration evaluations of surfactants for poorly soluble compounds.

Whether such efforts can justify the claim that this method can be used as a general quality control procedure appears to be open to further discussion.

Another dissolution test method proposes the use of semi-permeable Transwell® diffusion cell inserts which were used in Transwell® permeable supports to examine the dissolution of five different ICS previously delivered from seven MDI or dry powder inhalers (DPI) [40]. The drug particles were caught on filter membranes which were then placed within an ACI onto a stage on which respirable particles deposit upon impaction (**Figure 10**).

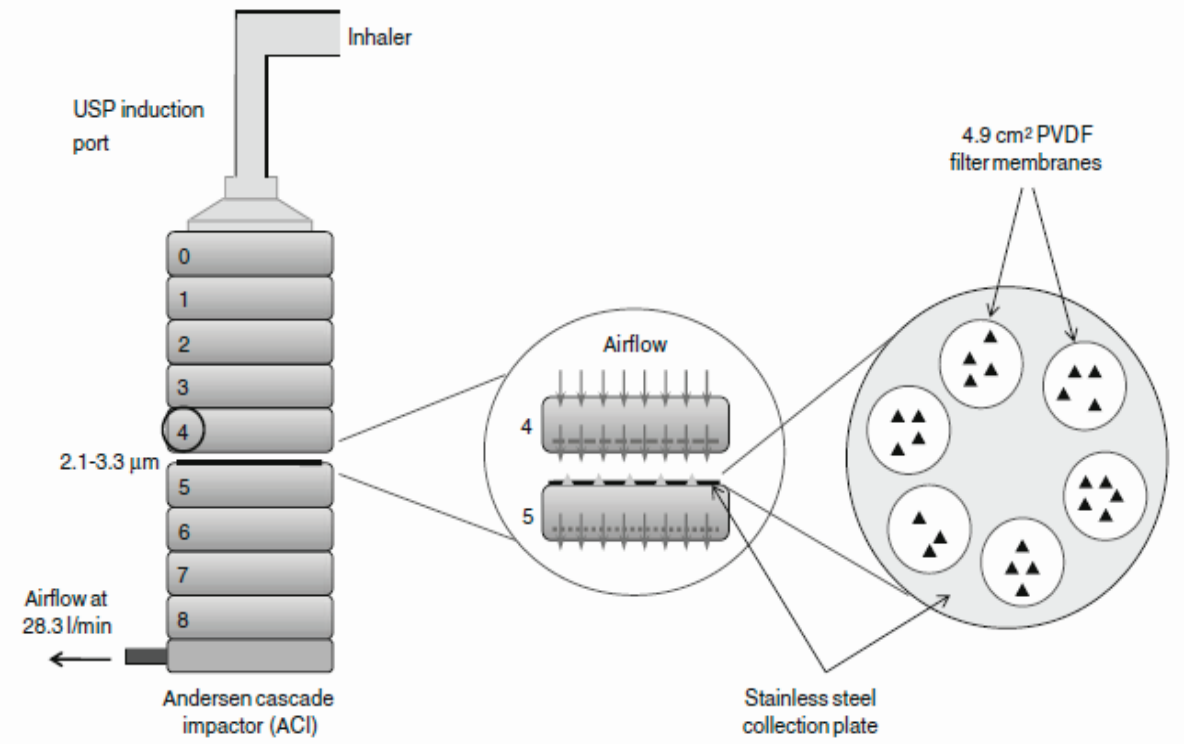


Figure 10: Respirable particle collection for dissolution in Transwell® wells [40]

The dissolution itself was then performed by transferring the PVDF filter membranes drug facing downwards into the Transwell® inserts and starting the dissolution process by adding 0.04 ml of either PBS or DDW as the dissolution media itself (**Figure 11**). The Transwell® support wells were filled with 1.4 ml of the respective dissolution fluid and kept at 37 °C and near 100% humidity during the dissolution experiment [40].

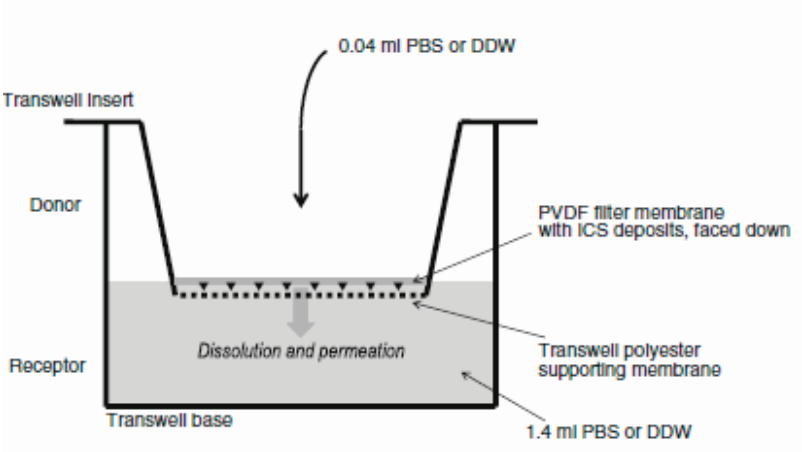


Figure 11: Schematic illustration of dissolution test set-up in Transwell® [40]

Due to the sampling intervals and withdrawal of aliquots (0.5 ml) from the receptor side in the well at the sampling points it was shown that there is a significant turnover of fresh dissolution media over the time course of a single experiment [40].

In some cases this could contribute to sink conditions or near sink conditions depending on the solubility of each of the individual ICS in PBS or double distilled water (DDW).

However, results with Flunisolide in solution suggested that the membrane seems to represent a significant diffusion barrier, which might mask faster dissolution processes as the solution diffusion and the dissolution of the drug lead to the same profile. In general it was found that the dissolution profiles within this system conformed with the aqueous solubility of the tested ICS [40]. However, sink conditions were clearly often not reached. The most striking example for non-sink conditions was observed for Fluticasone propionate. Especially in this case there was no difference in dissolution kinetics when Fluticasone propionate was previously delivered from two different inhalers [40]. In one case the Diskus® DPI which is formulated with lactose as the carrier of the drug substance was used and in the other case the drug was delivered from the Flovent® HFA-MDI [40]. Under these conditions the dissolution profiles of the two Fluticasone propionate formulations (Flovent® HFA MDI vs Diskus® DPI) were identical with only 2% of the drug being dissolved after 5 hours [40]. These results do not correlate in any way with the *in vivo* dissolution or absorption kinetics of Fluticasone propionate.

Literature data reports a dissolution time for Fluticasone propionate of 8h in bronchial fluid [61]. Additionally, there is also publically available data which shows that for Fluticasone propionate depending on the delivery device and also subjects (healthy vs. asthmatics) the mean absorption time (MAT) in the lung can differ [34]. The MAT here is the mean time it takes for a drug to enter the systemic circulation which implies that something which enters the systemic circulation needs to dissolve at the site of action first. The suggested dissolution approach was unable to detect differences in the absorption profiles observed after inhalation of the two formulations *in vivo* [34]. Hence a surfactant based dissolution test method would be more suitable to identify possible differences of the dissolution behavior of Fluticasone propionate as *in vivo* findings show that more than 2% of deposited drug dissolve and are absorbed in e.g. 5 hours. In summary all this argues for a lack of *in vitro/in vivo* correlation. The *in vivo* dissolution process for Fluticasone propionate is slow [40, 60, 62, 63] and is the rate limiting step for the absorption into the systemic circulation.

In the case of the other compounds tested the authors observed significant differences in dissolution profiles when the same drugs are delivered from different devices [40]. In addition to testing the different inhalers and the corresponding ICS the dissolution study also investigated drug loading and particle size effects on the dissolution profiles [40]. The drug loading effects were depending on the aqueous solubility of the substance in question [40]. Thus the dissolution and permeation into the receptor well decreased the more ICS was deposited onto the filter membrane and the effect was more prominent depending on the actual aqueous solubility of the ICS [40].

Regarding particle size it was found that the dissolution increased with decreasing particle sizes [40]. The particles themselves were captured on the stages of the ACI on which according to the theory particles deposit in the inhalable range due to their size [40].

In conclusion the authors present results of an *in vitro* dissolution method which tested the respirable particles of 5 ICS from 7 different inhalers [40]. Apart from the Fluticasone propionate example clear differences were shown for the dissolution

behavior of ICS depending on the device from which the particles were generated from [40].

It was shown that in PBS or DDW respectively that the kinetic dissolution profiles of the ICS not only depend on their solubility in the said media, but additionally also on formulation, deposited mass, and particle size [40]. The need for relatively small quantities of dissolution media (1.4 ml) combined with the fact that the sampling itself provides some turnover within the Transwell® receptor well, makes this method easily adaptable to other inhaled substances. The method provides ease in handling and requires limited resources to test aerosols. This makes it attractive as a basis for further investigations in the field of ICS dissolution behavior.

Due to the possibility to use the Transwell® wells to grow a monolayer of Callu-3 cells on the insert membrane the set-up was also used test the effect of different ICS when the respirable drug particles were deposited onto the said membrane including cells [45]. The authors come to the conclusion that there are differences in the expected dissolution *in vivo* in the lung compared to *in vitro* water based dissolution testing which can from their perspective be explained by a so-called post-dissolution "escape" which is believed to promote sink conditions in the case of readily metabolized prodrugs such as Beclomethasone dipropionate (BDP) and Ciclesonide [45]. This thought that the hydrolysis of the two prodrugs can be at least part of the reason for the fast dissolution on the luminal side and therefore explain the rapid absorption into the cells was earlier discussed by *Edsbäcker et al.* on the basis of work by *Rohdewald et al.* [20, 64].

Overall, while the dissolution approach has some important advantages, review of the results indicates that the membrane which is incorporated into the Transwell® system can be the rate limiting step at least for faster dissolving compounds such as Flunisolide (no difference between dry powder particles and Flunisolide solution) [40]. A membrane can thus also be influencing the dissolution rate of Ciclesonide and possibly Beclomethasone dipropionate which are both very insoluble in water. *In vivo* the dissolution rate of the drugs seems to be affected by metabolism and its effect on sink conditions as described e.g. in the second study [45].

In addition, it needs further investigations whether approaches without the use of surfactant in general, are suitable to describe the conditions present in the lung. It was therefore one of the goals of this work, to evaluate effects of membrane and dissolution media on the results of dissolution tests for commercially available drugs such as Ciclesonide, Budesonide and Fluticasone propionate.

MATERIALS, METHODS AND DATA TREATMENT

Ciclesonide materials and dissolution standard set-up A and adapted set-up B including visualization and characterization of Ciclesonide HFA-MDI generated particles

Materials: 24mm Transwell® with 0.4µm Pore Polyester Membrane Inserts with 6 well plates (Corning, Inc., Acton, MA) were purchased together with the Fisherbrand® Filter Papers Q8 Cat. No.: 09-790C and a Space Saver acrylic desiccator through Fisher Scientific (Pittsburgh, PA, USA). Acetonitrile (HPLC Grade), Ethanol (HPLC Grade), Potassium Sulfate (Fisher Chemical, Fisher Scientific), Sodium Dodecyl Sulfate (Fisher Chemical Fisher Scientific) and Phosphate Buffered Saline Solution (Fisher BioReagents, Fisher Scientific) were also purchased from Fisher Scientific (Pittsburgh, PA, USA).

A table top temperature control only oven; Fisher ISOTHEMP® 100 Series Model 126 G, was provided by the Department of Pharmaceutics of the College of Pharmacy of the University of Florida together with the HPLC equipment consisting of an HP 1050 Series HPLC pump, an HP 1100 autosampler and an HP 1050 UV-detector using a Waters C8; 5 µm; 4.6 x 150 mm column.

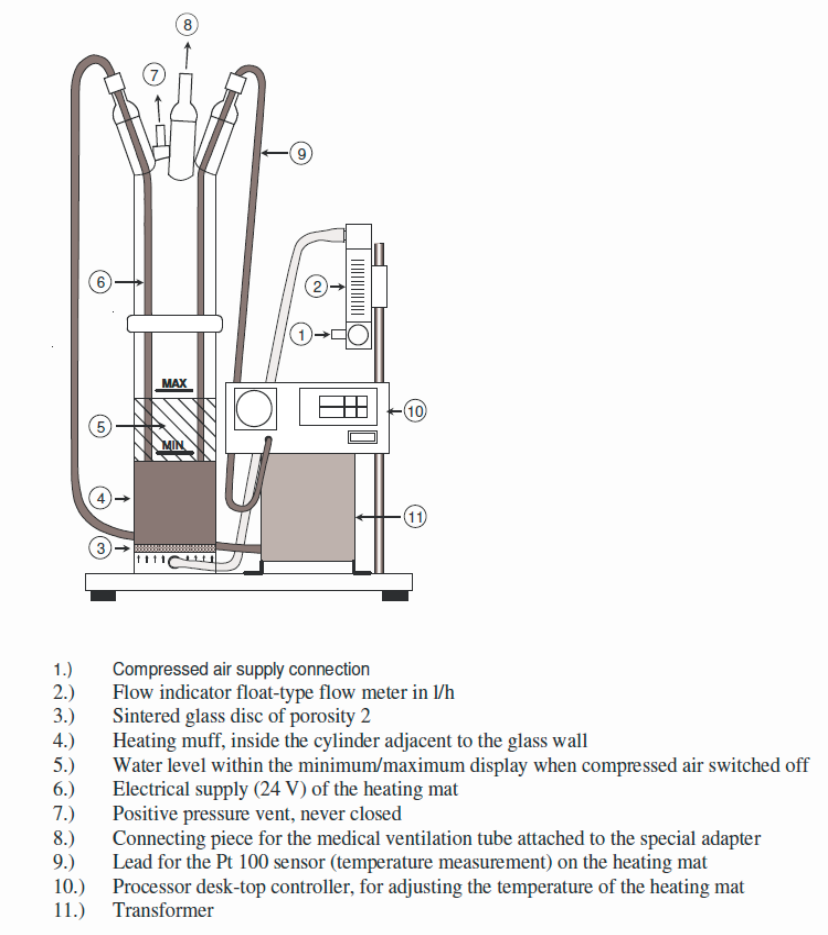
Table 4 provides a short overview of the quantitative analytical method for Ciclesonide. For the solubility testing of the Ciclesonide 25mm syringe filter, 0.45 µm MCE, sterile ,Fisherbrand, Fisher Scientific, Made in Ireland and BD 10ml syringe, Luer-Lock Tip, Becton and Dickinson, Franklin Lakes, NJ were purchased from Fisher Scientific (Pittsburgh, PA, USA).

A NuAire IR Autoflow CO2 water jacketed incubator (model no.: NU-2600) in which the sample was kept and stirred for the solubility testing was provided by the Department of Pharmaceutics of the College of Pharmacy of the University of Florida.

Table 4: Summary of Ciclesonide quantitative analytical method

Drug	Analysis	Column	Mobile phase	Flow rate	Detection	LOQ
Ciclesonide	HPLC-UV	Waters C8 4.6 x 150mm , 5 µm	Acetonitrile/DDW 80/20 (v/v)	1.2 ml/min	242 nm	0.25 [µg/ml]

A humidifier set-up generally consisting of an air flow indicator to measure air input (l/h) into a glass water container with inner heating mat and a processor desk-top controller for adjusting the temperature of the heating mat was donated by Boehringer Ingelheim GmbH & Co. KG (**Figure 12**) for all experimental work.



1.) Compressed air supply connection
2.) Flow indicator float-type flow meter in l/h
3.) Sintered glass disc of porosity 2
4.) Heating muff, inside the cylinder adjacent to the glass wall
5.) Water level within the minimum/maximum display when compressed air switched off
6.) Electrical supply (24 V) of the heating mat
7.) Positive pressure vent, never closed
8.) Connecting piece for the medical ventilation tube attached to the special adapter
9.) Lead for the Pt 100 sensor (temperature measurement) on the heating mat
10.) Processor desk-top controller, for adjusting the temperature of the heating mat
11.) Transformer

Figure 12: Humidifier for producing close to 100% r.h.

A connecting humidifying housing for the MDI was designed from a commercially available spacer by inverting the MDI adapter of the spacer on the ACI side and providing a hole for a laboratory glove finger within the housing body in order to be able to actuate the MDI within the humidified and temperature controlled air (**Figure 13**).

This set-up was connected to the humidifier on the air entrance side via the humidifier connection (**Figure 13**). All open areas of the design were additionally sealed with laboratory or duct tape for leakage prevention of the humidified air.

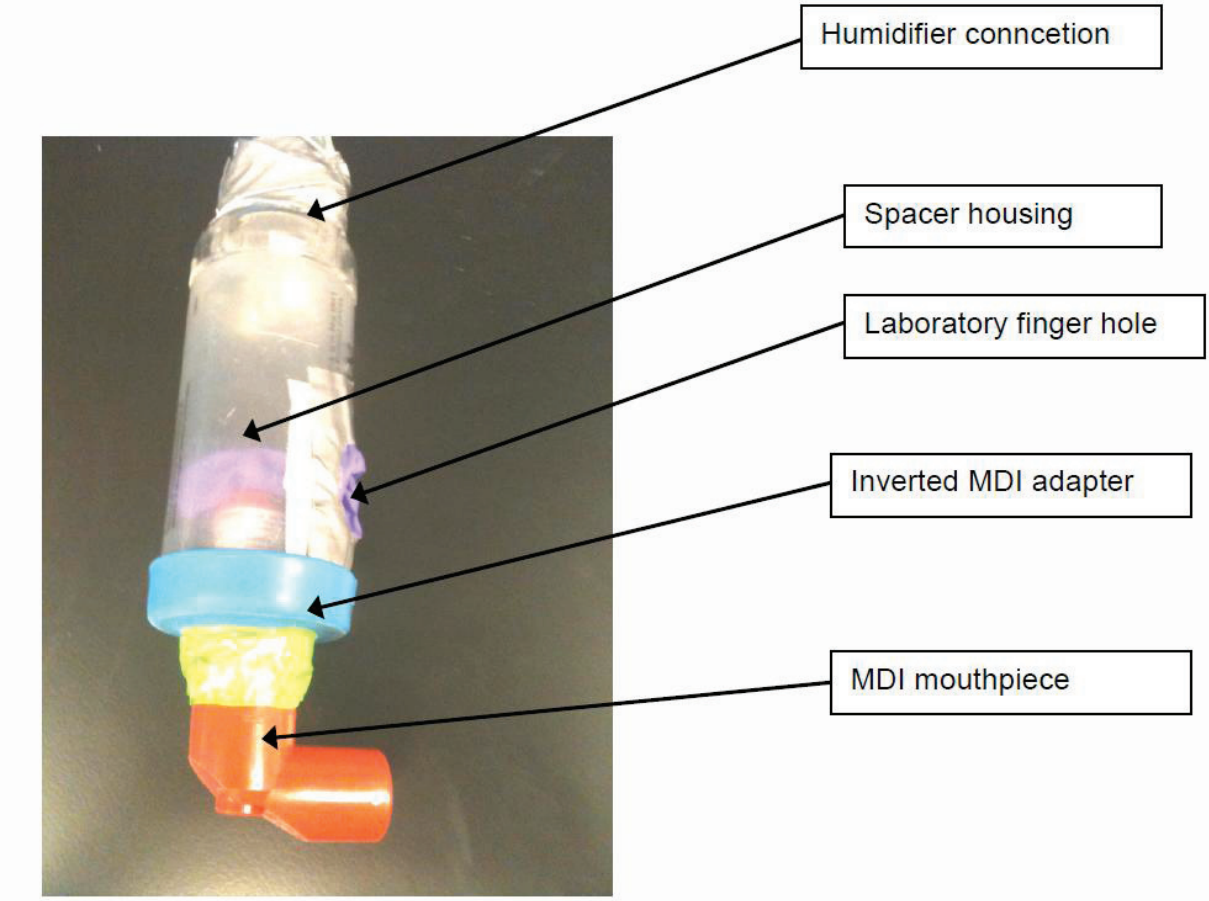


Figure 13: Image of self designed MDI humidity housing

An 8-stage stainless steel Andersen Cascade Impactor (Copley Scientific, UK) was provided by Boehringer Ingelheim GmbH & Co. KG (**Figure 14**) together with a flow control and a measuring unit including a pump (Andersen Samplers Inc., Atlanta, Georgia provided by the Department of Pharmaceutics of the College of Pharmacy of the University of Florida). Schott Duran glass dishes to rinse the stages of the ACI after the cascade measurement and prior to the HPLC analysis were provided by Boehringer Ingelheim GmbH & Co. KG.

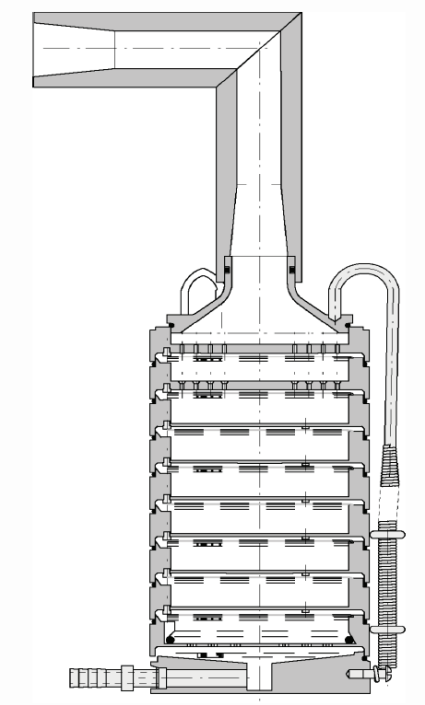


Figure 14: Cross section of an ACI assembled including Sample Induction Port (SIP)

A humidity and temperature control unit (Rotronic Hygropalm 2) was provided by Boehringer Ingelheim GmbH & Co. KG in order to be able to measure the humidity and temperature leaving the ACI together with a valve arrangement for controlling the air flow time for sampling.

An Alvesco® (160 mcg Ciclesonide) HFA-MDI (Sepracor Inc. Marlborough, MA) and Ciclesonide reference standards were provided by the Department of Pharmaceutics of the College of Pharmacy of the University of Florida for the study.

For the visualization and characterization of the Ciclesonide particles, solid metal blocks and carbon tape were provided by the Department of Pharmaceutics of the College of Pharmacy of the University of Florida together with the access to a Scanning Electron Microscope (SEM) JEOL JSM-6335F within the Particle Engineering Research Center (PERC) of the Major Analytical Instrument Center (MAIC).

Copper mesh grids together with the access to a JEOL 2010F Scanning Transmission Electron Microscope (TEM) of the Major Analytical Instrument Center

(MAIC) of the University of Florida were also provided and organised by the Department of Pharmaceutics of the College of Pharmacy of the University of Florida.

Sample Collection of Ciclesonide for dissolution standard set-up A and adapted set-up B including sample collection for SEM and TEM particle analysis

Using the hardware described above, the experimental set-up utilized a humidified and temperature controlled air flow which was generated by a pump at 28.3 L/min and entered the inhaler within the specially designed humidifier inhaler housing (spacer based **Figure 13**) in order to mimic *in vivo* like administration of the aerosol. A heating mat within the humidifier controlled the water temperature in the humidifier while the humidity was maintained at close to 100% r.h. The air temperature and humidity were measured after the pump and the ACI by a hygrometer together with the air flow.

In all experiments the inhaler was connected to an 8-stage stainless steel ACI via a tailored mouthpiece adapter. When dissolution testing was intended to be the successive experiment, 24 mm diameter filter papers were placed on stage 4 of the ACI (**Figure 15**). For preliminary experiments which focused on drug deposition on the stages of the ACI under ambient conditions the humidifier was not used.

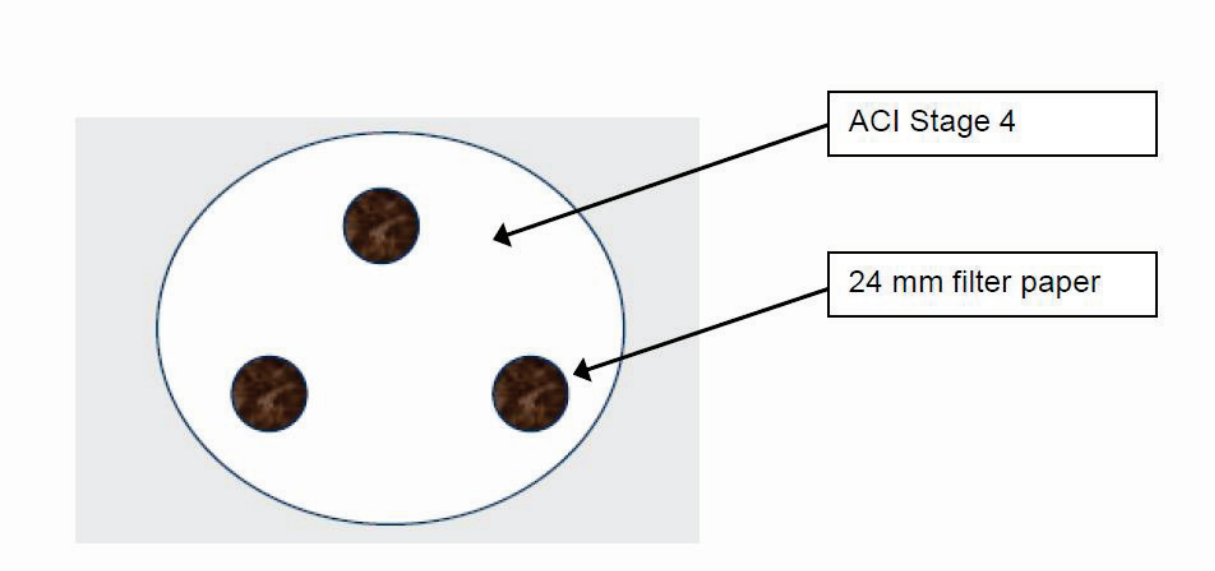


Figure 15: Position of 24 mm filter papers on stage 4 of the ACI

The inhaler was actuated 5 times employing the following procedure. After the first actuation, the air flow was stopped for 8 - 10 seconds by switching the pump off, followed by delivering the next dose after restart of the pump. This was repeated until 5 actuations were delivered. For the dissolution tests the filter papers were immediately transferred to the dissolution set-up orientated according to set-up A or B and initiated upon completion of the 5 actuations with 0.04 ml of 0.5% SDS (% w/v) solution.

Figure 16 illustrates the set-up of the aerosol particle collection for the dissolution testing as described above. The adapter, the stages of the ACI, and the filter were rinsed with 5 ml of ethanol/ DDW 80/20 (% v/v) to analyze the delivered dose and the particle size distribution from the inhaler within the ACI experiment. The mouthpiece was also rinsed using 10 ml of ethanol/ DDW 80/20 (% v/v).

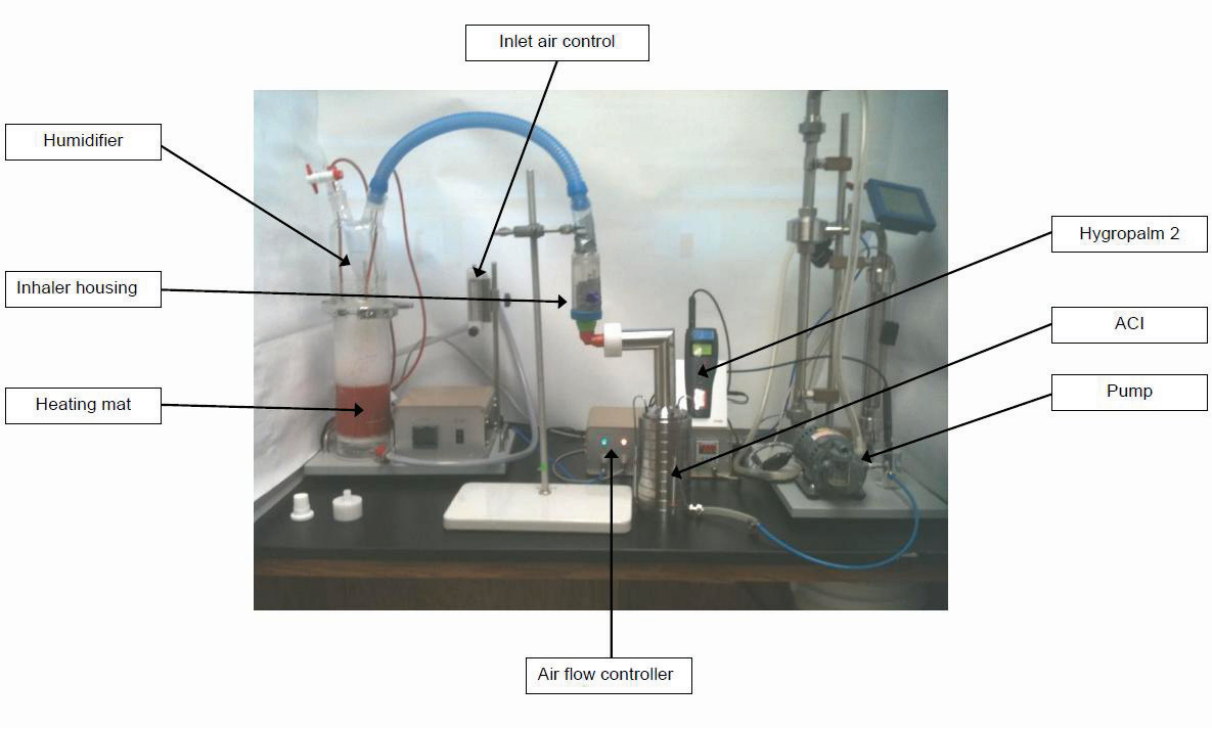


Figure 16: Aerosol particle sample collection set-up for dissolution test

When particles were intended to be collected for further characterization with SEM or TEM either carbon tape was attached to stage 4 of the ACI for the SEM analysis (operated at 5 kV) or the copper mesh grids were placed onto stages or areas of interest for the TEM analysis. All samples were immediately analyzed with the exception of one run for which the copper mesh grid sample of ACI stage 5 which was left at ambient room conditions for another TEM analysis 14 days after being produced. For the SEM 3D visualization images were taken from -5, 0, +5 degree angles while focus remained on the same spot of the sample and 3D images were electronically assembled using Alicona's MeX® software.

Dissolution Set-up A

Dissolution set up A was similar to that described by *Aora et al*. [40]. It was established to allow comparison of the optimized set-up B with results from literature. Thus, set-up A used an unchanged Transwell® plate with inserts, which contained the commercially available membrane. Within this set-up, either Ciclesonide solution or an MDI generated respirable ACI particle fraction were compared with respect to the rate

the drug enters the receptor compartment. Ciclesonide was either dissolved in SDS solution (0.5% SDS (% w/v) in Phosphate Buffered Saline (PBS) pH 7.4) leading to a drug concentration of 10 µg per 0.04 ml of this SDS solution or a respirable MDI fraction deposited on the filter paper (24 mm diameter) was transferred onto the membrane of the Transwell® system with the aerosol particles facing the membrane of the inserts and finally initiated with 0.04 ml 0.5% SDS (% w/v) (**Figure 17**). The filter paper was also loaded with the drug solution within the Transwell® insert for Ciclesonide SDS solution diffusion tests (**Figure 17)**.

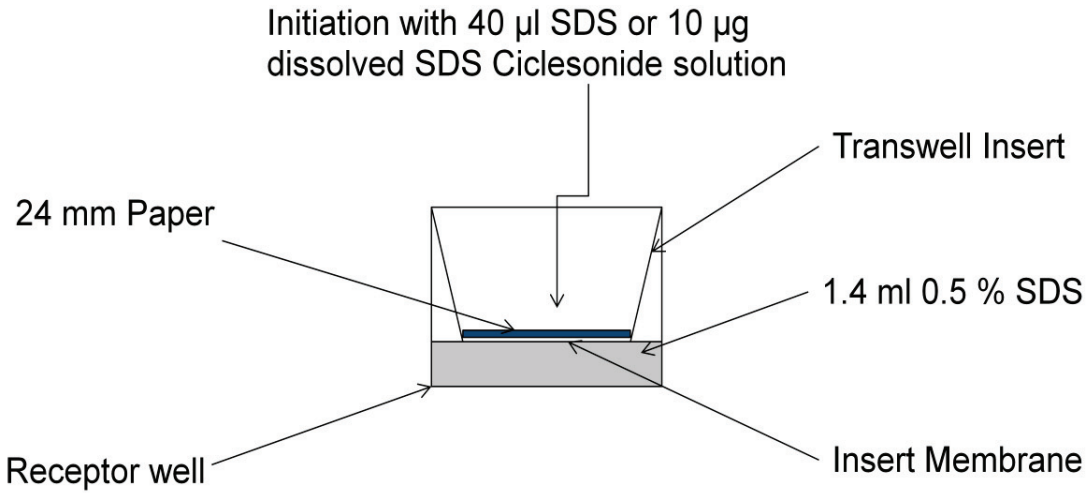


Figure 17: Dissolution and diffusion initiation and sampling set-up A based on [40]

Sampling of 0.5 ml of the medium from the receptor well and immediate replenishing of fresh medium was performed in the Transwell® wells while the set-up was kept under the controlled environmental conditions (37 °C and ~100% rel. humidity) in the Fisher temperature control only oven.

Samples of 0.5 ml were taken at 0, 10, 20, 30, 45, 60, 90, 120, 180, 240 and 300, 360, 420, 480, 1440 min and then analyzed by HPLC. After the final sample the filter papers were removed from the Transwell® well and also analyzed for remaining drug.

The Transwell® plates consisting of 6 wells were placed into a desiccator on top of a 200 ml saturated potassium sulfate water solution which provided the required relative humidity of near 100% within the desiccator. The kinetics of the ICS's dissolution rate were analyzed.

Dissolution Set-up B

The above described method was adapted in a further experimental set-up on the basis of the initial results. The adaptation and redesign included (a) removal of the Transwell® standard membrane, (b) a technical alteration (thermo-formed notches, see below) of the Transwell® system, (c) changing the filter paper orientation (drug facing up) of the deposited drug within the insert and (d) increasing the dissolution media volume within the Transwell® receptor compartment (**Figure 18**).

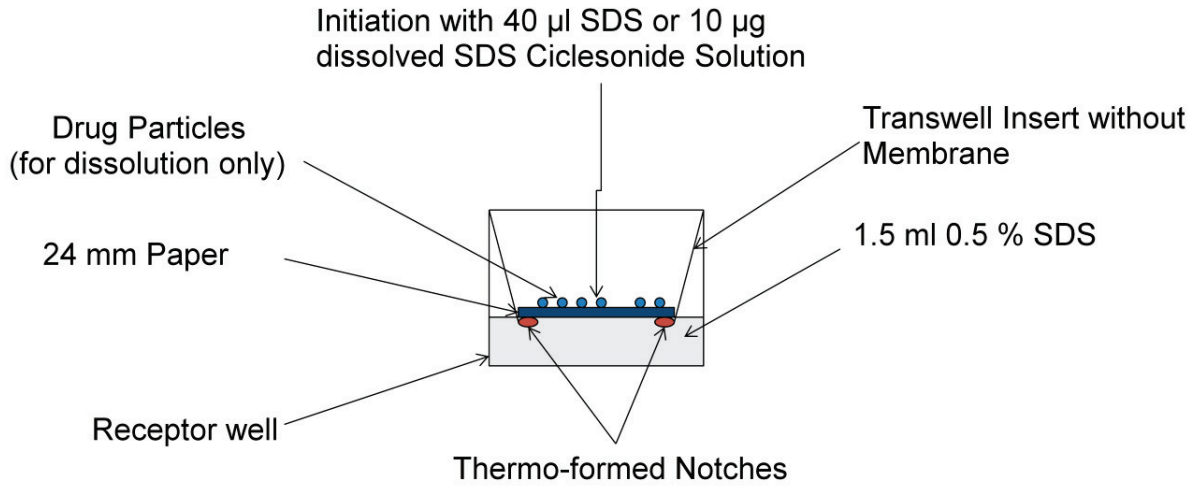


Figure 18: Dissolution and diffusion initiation and sampling set-up B (diffusion for Ciclesonide only)

For the first step of the adaptation process (a) the membrane was mechanically removed from the bottom of the insert. The device was cleaned from all remaining parts of the membrane by using a sharp instrument. Next (b), the insert was exposed to a heating plate until the material of the insert transformed into a softer state. A metal plate was then used to deform the insert by placing pressure on its top in an evenly manner until the deformation of the said insert reached the mark that originates from the injection tool at the bottom part of the insert. In doing so it was possible to achieve a controlled thermo-forming process which provided consistent and comparable remodeled inserts. The thermo-forming process provided notches on the inside of the inserts which can then function as holders for the 24 mm filter papers.

In contrast to the dissolution set-up A and the previously discussed literature method [40], the filter paper was now (c) transferred into the inserts with the drug particles facing upwards. (d) Due to the fact that some material of the inserts needed to be used to form the notches making them smaller and thus the bottom of the inserts did not reach as deep into the wells as before, the volume of dissolution media in the receptor well was adapted to the new situation by adding 0.1 ml of the 0.5% SDS (% w/v) dissolution medium solution. This resulted in a total starting volume within the wells of 1.5 ml.

Because of the missing membrane, transfer of the drug into the receptor compartment was faster. Sampling times were adjusted to taking 0.5 ml for the HPLC analysis from the receptor well at 0, 10, 20, 30, 45, 60, 90, 120, 180, 240 and 300, 360 min due to the finding that the dissolution and diffusion processes reached 100 % into the receptor well significantly faster than compared to the earlier set-up.

Materials for Fluticasone propionate and Budesonide dissolution testing

Materials: 24mm Transwell® with 0.4µm Pore Polyester Membrane Inserts with 6 well plates (Corning, Inc., Acton, MA) were purchased together with the Fisherbrand® Filter Papers Q8 Cat. No.: 09-790C and a Space Saver acrylic desiccator through Fisher Scientific (Pittsburgh, PA, USA), Acetonitrile (HPLC Grade), Methanol and Ethanol (HPLC Grade), Potassium Sulfate (Fisher Chemical, Fisher Scientific), Sodium Dodecyl Sulfate (Fisher Chemical Fisher Scientific) and Phosphate Buffered Saline Solution (Fisher BioReagents, Fisher Scientific) were purchased from Fisher Scientific (Pittsburgh, PA, USA).

A table top temperature control only oven; Fisher ISOTHEMP® 100 Series Model 126 G, was provided by the Department of Pharmaceutics of the College of Pharmacy of the University of Florida together with the HPLC equipment consisting of an HP 1050 Series HPLC pump, an HP 1100 autosampler and an HP 1050 UV-detector. **Table 5** provides a short overview of the quantitative analytical method for Budesonide and Fluticasone propionate.

For the solubility testing of the Fluticasone propionate and Budesonide 25 mm syringe filter, 0.45 µm MCE, sterile ,Fisherbrand, Fisher Scientific, Made in Ireland and

BD 10ml syringe, Luer-Lock Tip, Becton and Dickinson, Franklin Lakes, NJ were purchased from Fisher Scientific (Pittsburgh, PA, USA). A NuAire IR Autoflow CO2 water jacketed incubator (model no.: NU-2600) in which the samples for the solubility testing were kept and stirred was provided by the Department of Pharmaceutics of the College of Pharmacy of the University of Florida

Table 5: Summary of Budesonide and Fluticasone propionate quantitative analytical methods

Drug	Analysis	Column	Mobile phase	Flow rate	Detection	LOQ
Fluticasone	HPLC-UV	Waters C8 3.9 x 150mm , 5 µm	Acetonitrile/DDW 70/30 (v/v)	1.2 ml/min	239 nm	0.25 [µg/ml]
Budesonide	HPLC-UV	Waters C8 4.6 x 150mm , 5 µm	Acetonitrile/DDW 50/50 (v/v)	1.5 ml/min	242 nm	0.25 [µg/ml]

An 8-stage stainless steel ACI (Copley Scientific, UK) with tailored mouthpiece adapters for the inhalers were provided by Boehringer Ingelheim GmbH & Co. KG (Figure 14) together with a flow control and a measuring unit including a pump (Andersen Samplers Inc., Atlanta, Georgia) which was provided by the Department of Pharmaceutics of the College of Pharmacy of the University of Florida.

Flovent® (Fluticasone Propionate 44 µg/actuation) and Symbicort® (Budesonide 80 µg/actuation and Formoterol 4.5 µg/actuation) MDI inhalers were also provided by the Department of Pharmaceutics of the University of Florida. Schott Duran glass dishes to rinse the stages of the ACI after the cascade measurement and prior to an HPLC analysis were provided by Boehringer Ingelheim GmbH & Co. KG.

Methods for Fluticasone propionate and Budesonide dissolution testing

Sample Collection of Fluticasone propionate and Budesonide

The experimental design was similar to that for Ciclesonide in set-up B. The experimental set-up included the control of the air flow generated by the pump at 28.3 L/min into the inhalers which were connected to an 8-stage stainless steel ACI via the tailored mouthpiece adapters. For dissolution testing 24 mm diameter filter papers were placed on stage 4 of the ACI (**Figure 15**). The inhalers were actuated 10 times in the case of the Flovent® MDI and 5 actuations for the Symbicort® MDI. After every actuation, the air flow was upheld for 12 seconds followed by turning the pump off and restarting it before the next actuation.

The drug Formoterol in the Symbicort® MDI was not analyzed by HPLC. Previous literature research revealed that Budesonide and Formoterol are delivered independently from one another by the MDI and thus not being chemically or physically connected [65]. The filter papers were immediately transferred to the dissolution set-up and initiated with 0.04 ml of the 0.5% SDS (% w/v) solution. The adapter, the stages of the ACI, and the filter were rinsed with 5 ml of methanol/ DDW 70/30 (% v/v) to analyze the delivered dose from the inhalers within the ACI experiment. The mouthpiece was also rinsed using 10 ml of methanol/DDW 70/30 (% v/v).

Dissolution Set-up B for Fluticasone propionate and Budesonide

Within this set-up, Fluticasone propionate and Budesonide MDI generated respirable ACI particle fractions were compared with respect to the rate of drug that enters the receptor compartment.

Therefore, the aerosol cloud of the MDIs was emitted into the humidified and temperature controlled air-flow, as described above. This was done in order to mimic a more *in vivo* like administration with respect to temperature and humidity. The respirable MDI fraction deposited onto the filter paper of 24 mm diameter within stage 4

of the ACI was transferred into the Transwell® system drug side facing upwards. They were afterwards initiated with 0.04 ml of the 0.5% SDS (% w/v) dissolution medium which was formulated in Phosphate Buffered Saline (PBS) pH 7. 4 (**Figure 19**).

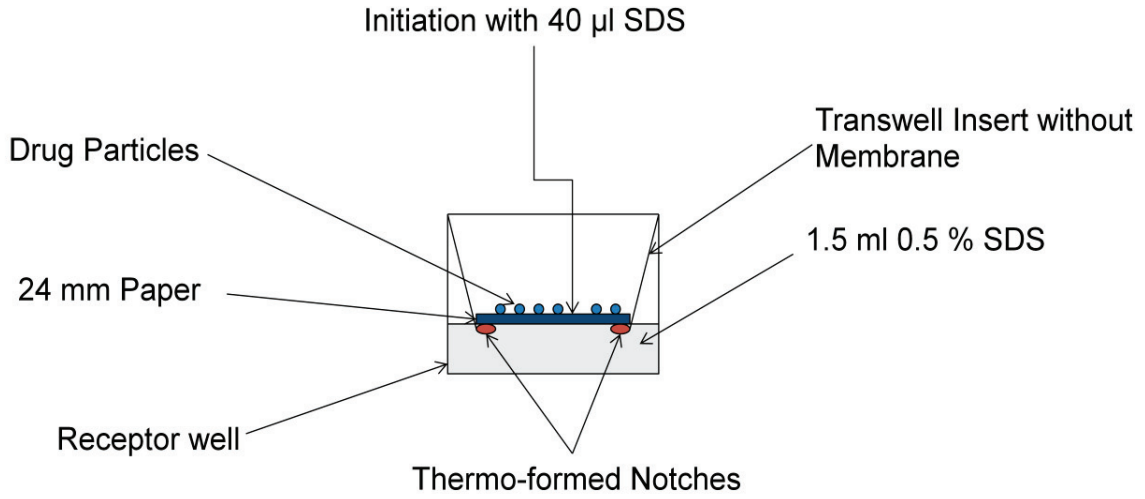


Figure 19: Dissolution initiation and sampling set-up for Fluticasone propionate and Budesonide

Sampling of 0.5 ml of the dissolution medium from the receptor well and immediate replenishing of fresh medium was performed in the Transwell® wells while the set-up was kept under the controlled environmental conditions (37 °C and ~100% rel. humidity) in the Fisher temperature control only oven. For Fluticasone propionate and Budesonide samples of 0.5 ml were taken from the receptor side of the well at 0, 10, 20, 30, 45, 60, 90, 120, 180, 240 and 300, 360, 420, 480, 1440 min and then analyzed by HPLC according to the methods described in **Table 5**.

After the final sampling the filter paper was removed from the Transwell® well and analyzed for remaining drug. The samples of Budesonide were diluted 50% (v/v) with Ethanol/DDW 80/20 (% v/v) prior to the HPLC analysis. The Transwell® plates consisting of 6 wells were placed into a desiccator on top of a 200 ml saturated potassium sulfate water solution which provided the required relative humidity of near 100% within the desiccator. The kinetics of the ICS dissolution rates were analyzed.

Solubility testing of Fluticasone propionate, Budesonide and Ciclesonide in SDS solution

Approximately 5 mg of Fluticasone propionate, Budesonide and Ciclesonide drug substance were weight into a 200 mL glass cup and the glass cup was afterwards filled with 100 ml of the 0.5% SDS solution. The glass vial was then placed into the Nuaire, IR AutoFlow CO_2 water-jacketed incubator and was kept at around 37° C.

The samples were constantly stirred by a magnetic stirrer inside the glass vial and samples were taken at 30 min, 60 min, 120 min, 180 min and 24h filtered through a 0.45 µm filter tip of a 10 ml syringe to be analyzed by HPLC according to the methods described in **Table 4** and **Table 5.** The samples of Budesonide were diluted 50% (v/v) with Ethanol/DDW 80/20 (% v/v) prior to the HPLC analysis.

Data Treatment and Profile Analysis

Data obtained by HPLC were analyzed in the following way. Concentrations present in the receptor well at a given sampling time point were transformed into amounts present in the receptor compartment and consequently adjusted by the dissolution that occurred during previous sample removals (0.5 ml per sampling time point). The dissolution and diffusion experiments were analyzed based on these adjusted amount of dissolved drug at each time point within the receptor well of the Transwell® plates.
The amount of drug reaching the receptor well during one sampling period was calculated by subtracting from the cumulative amount that entered the receptor well up to the sampling time of interest, the amount which entered the compartment up to the previous sampling time point.

The overall amount of drug over the course of the entire experiment was determined by testing the 24 mm filter paper for remaining drug after the last measured time point. The resulting total amount was then set at 100% cumulative drug released to express both dissolved and diffused measurements. Afterwards the results were plotted versus time.

The resulting plots were visually and statistically compared to assess similarity or dissimilarity. The statistical comparison was done by employing a one-way analysis of variance (ANOVA), where $p<0.05$ was considered to be significant. The dissolution and in the case diffusion profiles were additionally mathematically fitted by different models (**Table 6**) using Least-Square-Mean-Error estimators and best fits were used to calculate mean dissolution and diffusion times for all profiles. All calculations were performed using the statistical software GNU R Version 2.14.

Table 6: Overview of the mathematical models and equations

First order	First order mass balance	$r(t) = a(1-\exp(-bt))$
Hixson Crowell	erosion release mechanism	$r(t) = a - (1-b*t)^3$
Higuchi	Fickian diffusion	$r(t) = at^{1/2}$
Korsmeyer-Peppas	diffusion mechanism	$r(t) = at^b$
Weibull	life time distribution	$r(t) = 1-\exp(-at^b)$
Logistic	population-dynamics	$r(t) = a/(1+\exp(-b*time-c))$

The profiles were also tested with a model independent method which uses a comparison based on difference (f_1) (**Equation 2**) and similarity (f_2) (**Equation 3**) factors.

$$f_1 = \frac{\sum_{j=1}^{n} \left| R_j - T_j \right|}{\sum_{j=1}^{n} R_j} \times 100$$

Equation 2

$$f_2 = 50 \times \log\left\{\left[1 + (1/n)\sum_{j=1}^{n}\left|R_j - T_j\right|^2\right]^{-0.5} \times 100\right\}$$

Equation 3

The difference test is provided by a percent error over all measured time points and then expressed in the f_1-value which determines significant difference between (T) and (R) profiles. The f_2-value examination is based on mean squared differences to test the profiles in question for similarity. The similarity test is referred to by EMA and FDA for solid oral dosage forms in an equal manner [66-68]. The values for (f_1) and (f_2) in order to show difference or as it can be found in the guidelines for similarity only are:

f_1: values lower than 15 (0-15) indicate no difference between the two tested profiles

f_2: values higher than 50 (50-100) indicate similarity between two profiles

According to the European Medicines Agency (EMA) the examination for similarity for solid oral dosage forms should be based on at least 12 single dosage units at a minimum of 3 time points [68]. Targeting the same category of products in the form of solid oral dosage forms the FDA requires justification for the use of an appropriate statistical method [66, 67].

The dissolution and diffusion data used in this study do not fully fulfill these requirements, but the tests were employed to add to the other modes of profile interpretation and thus are not seen in isolation or the sole basis for similarity or difference assessments of the profiles.

The mean dissolution time (MDT) of all profiles was calculated on both the actual data and also the fitted data according to **Equation 4**.

$$MDT = \frac{\sum_{i=1}^{n} \bar{t}_i \cdot \Delta M_i}{\sum_{i=1}^{n} \Delta M_i}$$

Equation 4

For ease of comparison and clarification the MDT is based on the complete data sets as the visualized profiles were limited to certain time spans for comparative reasons.

Fluticasone propionate is an example of such a case where the profiles are only visualized up to 5 hours in this dissertation for comparative reasons to e.g. Budesonide. The actual sampling of Fluticasone propionate exceeded the visualization. The reason for this is that Budesonide dissolves much faster reaching 100% dissolved drug within the 300 min displayed [cf. **Figure 33**]. Budesonide could thus not be measured experimentally for the same time span as Fluticasone propionate.

RESULTS AND DISCUSSION

Ciclesonide ACI deposition evaluation; visualization of particles; dissolution and diffusion testing in set-up A and B

The literature suggested several methods for dissolution testing of inhaled medications [40-43]. In the case of ICS the *in vitro* dissolution behavior itself is highly influenced by the water solubility of these compounds themselves [40]. Some of the ICS have relatively high water solubilities and thus should exhibit a rather fast absorption *in vivo*. The relative high water solubility of these compounds may nevertheless not be reflected in prolonged absorption times *in vivo* if other mechanisms are responsible for a prolonged pulmonary residence time. There are drugs e.g. Budesonide which possess a very fast dissolution and absorption into the cells of the lung [34]. whose prolonged residence time, at least for a small portion of the delivered dose, is due to formation of lipophilic Budesonide esters in pulmonary cells, that are captured in these cells and only leave the lung after cleavage of the ester bond (reactivation to Budesonide) [69]. On the other hand, fast absorption of rather lipophilic drugs have been reported, that cannot be explained by the poor water solubility [45]. An example is Beclomethasone dipropionate , which is also characterized by a short MAT although its solubility in water is low [45].

In the case of Ciclesonide which exhibits one of the lowest water solubility values among marketed ICS [70] performing dissolution tests in water-based medium (no organic solvent, no surfactant) is challenging or often impossible because of the low concentrations observed in such systems. It therefore makes sense to try to find more realistic solvent based systems that e.g. contain surfactants similar to conditions in the lung; especially as this might increase solubility, will facilitate sink conditions and consequently influence the dissolution rates. Adding a surfactant to the dissolution medium is thus reasonable as this mimics components in the lung lining fluid which contains surfactant like substances such as phospholipids (90%) and proteins (10%) which are constantly synthesized and excreted by epithelial cells type II in order to reduce surface tension [71-73].

The dissolution test method described by *Arora et al* used Transwell® wells within a temperature and humidity controlled environment and represents a feasible approach from a technical point of view [40]. However, it did not incorporate surfactants [40].

The fact that only PBS and water were used as the dissolution media in this original set-up would not allow for Ciclesonide dissolution testing. This, in addition with experience in the laboratory of Prof. Dr. Hochhaus at the University of Florida, Department of Pharmaceutics, with using surfactants as a necessary component within dissolution and drug release studies argued for the use of such surfactants in this study [74].

The following chapters will thus focus on an adequate adaptation of the existing method [40] for testing the dissolution of Ciclesonide when introducing a surfactant to the dissolution medium.

The surfactant SDS and its concentration were chosen on the basis of findings from previous authors [74], to provide sink conditions for the ICS and the recommendation given by e.g. the FDA when dissolution testing for poorly water soluble drugs is intended where explicitly SDS is mentioned as such an option as a surfactant [51].

In addition, it was of interest to evaluate whether the chosen method is fast, accurate and cost effective. Due to the fact that Transwell® wells and inserts in combination with all other necessary materials can be purchased “off the shelf” the above mentioned dissolution test method is an ideal starting point for developing a dissolution test for aerosolized Ciclesonide drug particles.

Ciclesonide ACI Preliminary Depositing Stage Determination for Dissolution Studies

A preliminary experiment was conducted in order to define the stage of the ACI which provides the highest chance to retrieve a suitable amount of representative drug particles under humidified conditions. The reason for selecting these conditions was to mimic *in vivo* like drug deposition on the filter papers before transferring them to the dissolution set-ups. The stages were assessed with regard to mass of Ciclesonide deposited after 5 actuations of the Alvesco® HFA-MDI.

A smaller set of ACI experiments (n=3) revealed that when ambient laboratory conditions were used instead of the humidified and temperature controlled conditions the inhalable fraction of the emitted dose from the inhaler shifted to lower stages of the ACI. The overall fine particle fraction of both environmental conditions remained nearly the same around 45-50 % of the delivered dose of 160 µg Ciclesonide per actuation (**Figure 20**).

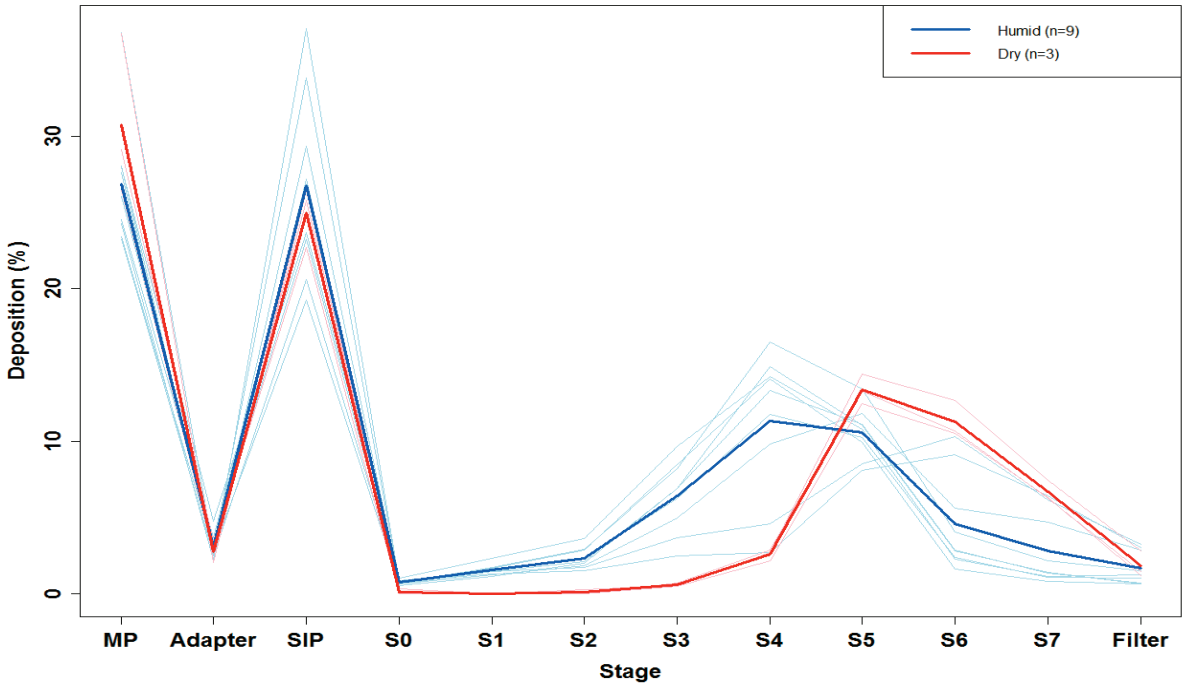


Figure 20: Particle size distribution of Ciclesonide when delivered by an HFA-MDI under two different environmental conditions

The results are confirmed by published data for the HFA-MDI which reported around 50% lung deposition when deposition is determined by scintigraphy examination [28]. Interestingly the two profiles in this preliminary study are similar in regard to losses in higher, non-respirable stages of the ACI set-up (MP= mouthpiece; Adapter; SIP; S0-S1 = Stage 0-1) whereas the inhalable stages (S2-Filter) seem to be less affected by the already mentioned shift of particle size distribution towards lower stages. Thus, differences in environmental conditions (temperature, humidity) do not contribute to a significant reduction of the overall inhalable fraction based on the delivered dose, but the inhalable particles are generally smaller under ambient conditions.

Additionally, there is also a tendency that, although the humidified environmental conditions were well controlled, the variation in deposition on the stages 2-7 were more pronounced under controlled than under ambient conditions (cf. **Figure 20**).

In summary this preliminary experiment revealed that the 24 mm filter paper on stage 4 of the ACI captured the highest amount of Ciclesonide within the inhalable range under humidified conditions.

Ciclesoinde Particle visualization and examination by SEM and TEM

For addressing the question what the particle characteristics and properties are that are generated during deposition of the solution based MDI of Ciclesonide, particles emitted from the Alvesco® inhaler were collected on stage 4 of the ACI on carbon tape or copper mesh grids on which deposited particles were analyzed by a Scanning Electron Microscope (SEM) and a Transmission Electron Microscope. The TEM was also used to characterize single particles with respect to their crystalline or amorphous state. The image produced by the SEM analysis revealed that deposited particles were forming piles or agglomerates (**Figure 21**).

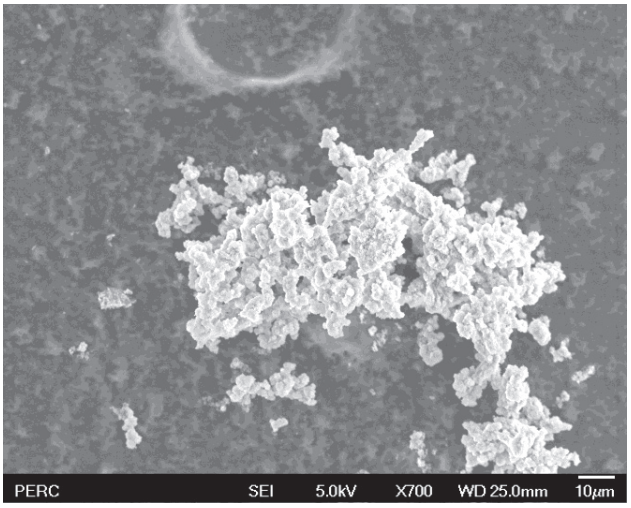


Figure 21: Ciclesonide ACI stage 4 SEM

It should be stressed that this way of deposition will differ from the *in vivo* situation as piles are likely due to the ordered geometry of openings within the ACI *in vitro* while a diffuse particle distribution will occur in the inhalation airstream in vivo. Thus, these piles or agglomerations are caused by the holes above the stages in the ring elements of an ACI. The particles have to follow through these holes in the

airstream and upon deposition are centralized at certain points on the stage below. The piles of the particles become even more visible by a 3D visualization of such agglomerations (**Figure 22**).

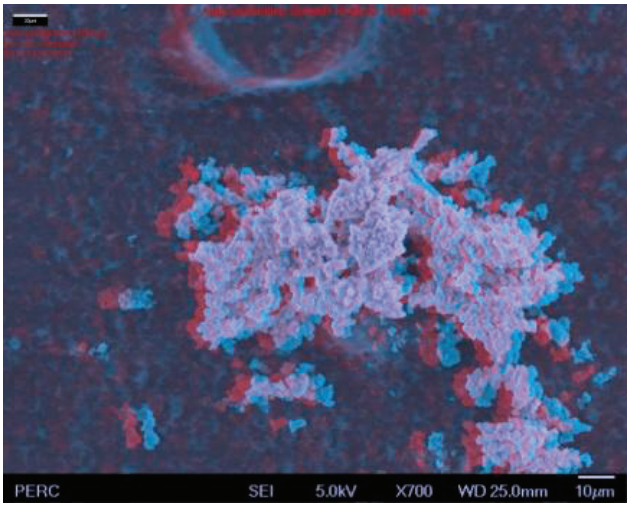


Figure 22: 3D SEM image of Ciclesonide particles deposited onto ACI stage4

In all upcoming dissolution trials these or similar structures have to be envisioned as the dissolving depot of the drug substance. In fact all ACI based dissolution models will be measuring piles and not single particles. A detailed discussion of this finding is not the focus of this dissertation as they cannot be avoided in this arrangement, but it could surely be an additional area of research.

Unlike the SEM pictures the TEM images represent the visualization of single particles. The copper mesh grids are smaller than the pieces of carbon tape which were used and especially on stage 4 of the ACI the number of particles was smaller than the focused piles and agglomerates of the SEM (**Figure 23**)

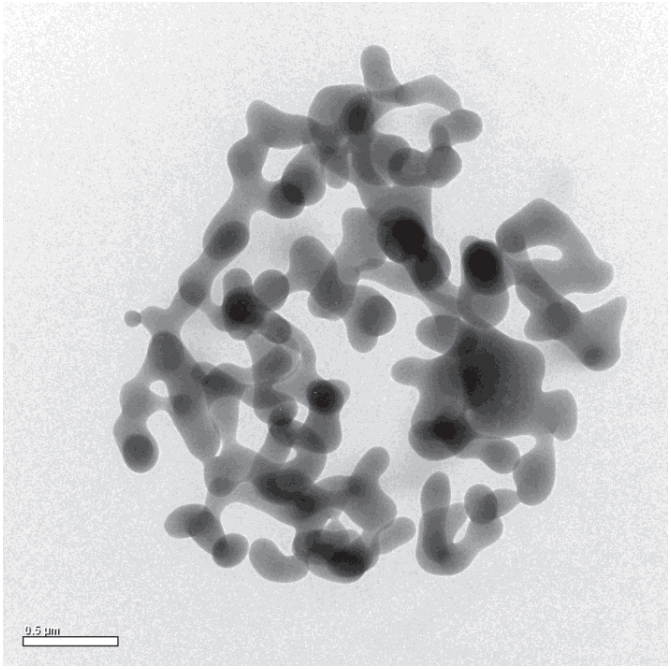


Figure 23: TEM image of an ACI stage 4 captured Ciclesonide particle

The individual structure of the particle is also different when compared to the SEM image of particles. The image in **Figure 23** can be regarded as characteristic for the Ciclesonide particles as additional samples which are not shown appear in the same manner. The thermodynamical state analysis done on this particle by electron diffraction in the TEM reveals that the particle is amorphous (**Figure 24**).

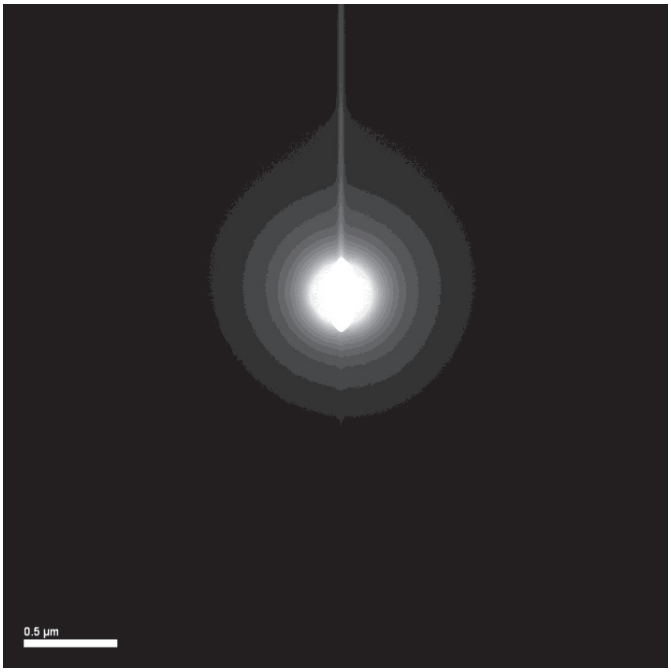


Figure 24: Amorphous electron diffraction pattern of Ciclesonide particle (cf. **Figure 23**)

After analyzing various single particles (not all shown) by the help of this technology it can be said that all particles which were found were deemed to be amorphous by the corresponding electron diffraction pattern. **Figure 25** represents such an example.

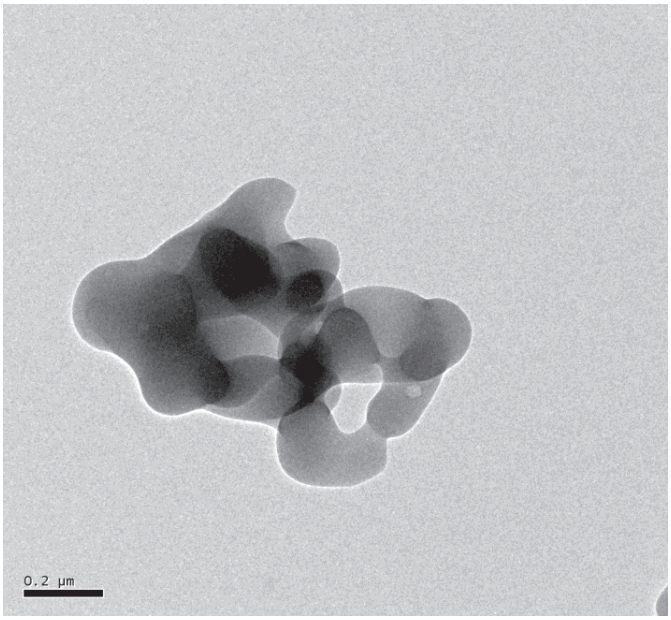


Figure 25: Ciclesonide Stage 5 particle 14 days after being captured in ACI and stored at ambient temperature

It is of interest to note that the particles in **Figure 25** were still amorphous after 14 days of being kept at ambient laboratory conditions (**Figure 26**).

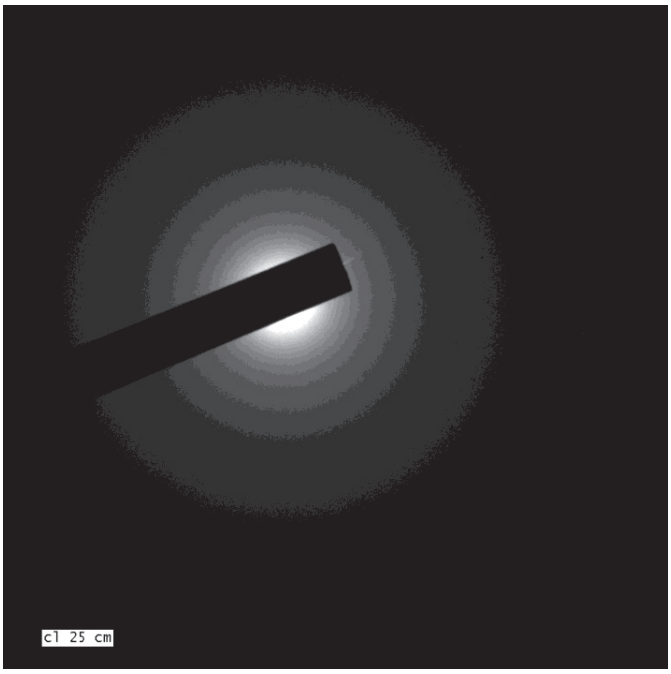


Figure 26: Amorphous electron diffraction pattern of Ciclesonide particle from **Figure 25**

The amorphous state of Ciclesonide has been found stable over longer periods of time if kept below 60 °C [70]. This explains the stability of the amorphous content of the aerosol particles as the particles emitted from the inhaler are definitely colder, especially if one considers the evaporation effect of the HFA/EtOH mixture when released at room temperature.

Ciclesonide dissolution and diffusion testing in set-up A and B

First experiments compared the transfer rate of Ciclesonide from solution and particles obtained from stage 4 when dissolution set-up A (membrane present) was employed. All experiments were performed with 0.5 % SDS (% w/v) containing medium. A comparison of media with and without SDS was not possible because of the very low concentrations of Ciclesonide which can be achieved in medium not containing SDS.

To test the diffusion of dissolved material across the membrane preliminary experiments were performed with Ciclesonide solution in SDS containing medium. The concentration and amount of the Ciclesonide/SDS solution to be loaded onto the filter paper was chosen in such a way that the amount of drug (10 µg) was equivalent to the amounts generally obtained on stage 4 of the ACI experiments when delivered from the MDI after five actuations. This amount was dissolved in 40 µl of SDS solution which was identical to the volume used to initiate the dissolution of drug particles.

The dissolution profiles of the first set of ACI deposited drug particles were found to be visually indistinguishable from profiles obtained for Ciclesonide solution (**Figure 27**).

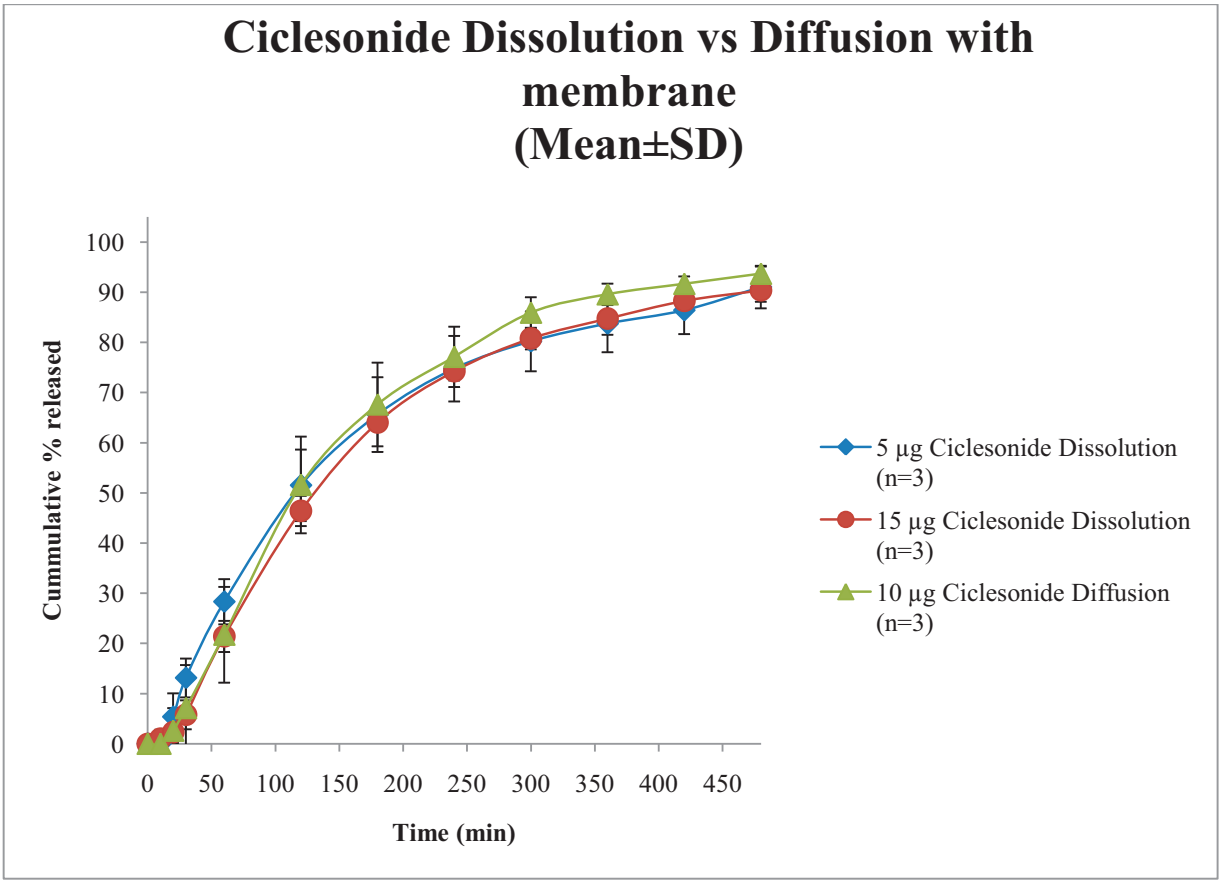


Figure 27: Ciclesonide Diffusion vs. Dissolution with insert membrane (lines between data points for visualization only)

Not only are most time points individually indistinguishable regarding their respective mean values, the error bars of these profiles are additionally also overlapping. The described observations were not significantly influenced by the drug loading on the filter papers as can be seen for the dissolution profiles for 5 µg and 15 µg deposited per paper and the diffusion driven result which contained 10 µg of Ciclesonide (**Figure 27**). All three profiles overlap completely or at least their respective error bars. Thus the statistical and mathematical examination does also reveal that the profiles do not differ or can be regarded as similar when all three were tested against each other by employing **Equation 2** and **Equation 3**. The respective values are shown in **Table 7** for the comparison of the profiles:

Table 7: f_1 and f_2 calculation of Ciclesonide diffusion and dissolution with membrane

Profile vs. Profile	15 µg dissolution vs. 10 µg diffusion	15 µg dissolution vs. 5 µg dissolution	5µg dissolution vs. 10 µg diffusion
f_1	7.17	3.46	6.37
f_2	68.32	81.66	66

The profiles in direct comparison reveal in addition to the visually observable similarity that all f_1 values are below 15 and thus cannot be regarded as significantly different from one another. The same is true for the f_2 calculation where all profiles lead to values higher than 50 and thus reflect similarity. The one-way ANOVA calculation is another evidence of similarity of the three profiles (calculation not shown). The values for the MDT, expressed as mean diffusion and dissolution times, are also very similar (**Table 8**).

Table 8: Overview of MDT of Ciclesonide in set-up with membrane

Profile	Dissolution 15 µg	Diffusion 10 µg	Dissolution 5 µg
MDT (min)	215.58	181.51	229.04

The rather long transfer rates for drug in solution suggest that the membrane between donor and receptor compartment must represent a significant barrier. The similarity of solution and particle profiles indicates that crossing the membrane represents the rate limiting step for entering the receptor compartment, even if drug particles are assessed and that the dissolution process is much faster. Experiments using this set-up are therefore not suitable to assess the dissolution process of Ciclesonide aersosol drug particles.

With the goal of optimizing the system, it was decided to remove this membrane and identify an alternative separator between donor and receptor compartment. It was evaluated whether systems whose barrier between the initial drug loaded side and the

receptor well which was based only on filter paper itself are more suitable for dissolution testing (**Figure 18**).

A first set of experiments revealed that once the membrane was removed the diffusion of Ciclesonide from solution into the receptor compartment was faster compared to the previously conducted diffusion trials using the 10 µg Ciclesonide and 0.5 % SDS (% w/v) solution (**Figure 28**).

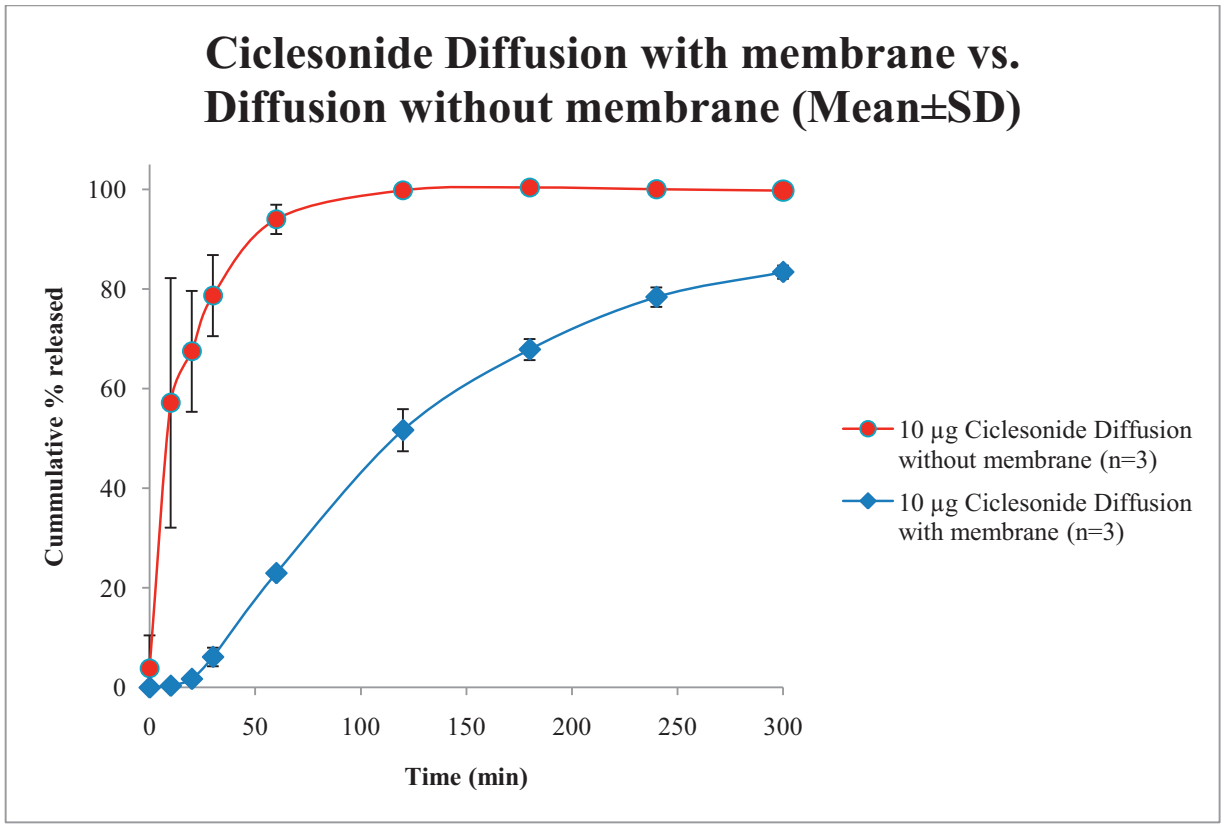


Figure 28: Diffusion of Ciclesonide/SDS solution (10 µg) into receptor well with and without membrane (lines between data points for visualization only)

It was also observed that the initial shape of the curve changed in comparison to the previous experiment. There was a slower initial rise in the profile when the membrane was involved which was followed by a steady and continuous faster phase which leads to a biphasic sigmoidal-like profile in this case. An initial hydration process of the membrane might be responsible for the delayed transfer in the presence of the membrane.

When the membrane was no longer present the diffusion profile exhibited an early and fast rise reaching a plateau and a complete transfer of Ciclesonide into the receptor well within roughly 60 minutes. The time necessary for a complete diffusion across the membrane into the receptor well exceeded 300 min in the first set of experiments. The mathematical and statistical evaluation and comparison of the two profiles add to the visual observation. The f1 and f2 test of the two profiles also underlines the visual observation as can be seen in **Table 9**.

Table 9: f_1 and f_2 calculation of Ciclesonide diffusion with and without membrane

Profile vs. Profile	10 µg Ciclesonide diffusion with membrane vs. without membrane
f_1	55.43
f_2	15.29

In this case the f_1 value clearly indicates that the two profiles are different by definition. Additionally, the f_2 value does not allow assuming similarity as the value is well below the minimum target value for similarity which is 50. With respect to the one-way ANOVA analysis it can be said that the calculation also shows that the two profiles are different. The MDT for the membrane diffusion which was used for this set of comparative experiments is 179.73 min and without the membrane it drops to 19.29 min (**Table 10**).

Table 10: MDT diffusion with vs. without membrane

Profile	Diffusion with membrane	Diffusion without membrane
MDT (min)	179.73	19.29

Overall these experiments show that the membrane strongly influenced the diffusion of Ciclesonide from the donor side with the filter paper to the receptor well and thus are masking the monitoring of the dissolution process for Ciclesonide. In order to be able to fully evaluate the effect of the removal of the membrane on monitoring the dissolution process, the dissolution of ACI captured particles of Ciclesonide was evaluated with the new set-up. In this case the obtained dissolution profile of the drug particles which were deposited onto the filter papers was compared to the diffusion profile of Ciclesonide in solution within the same set-up without the membrane (**Figure 29**).

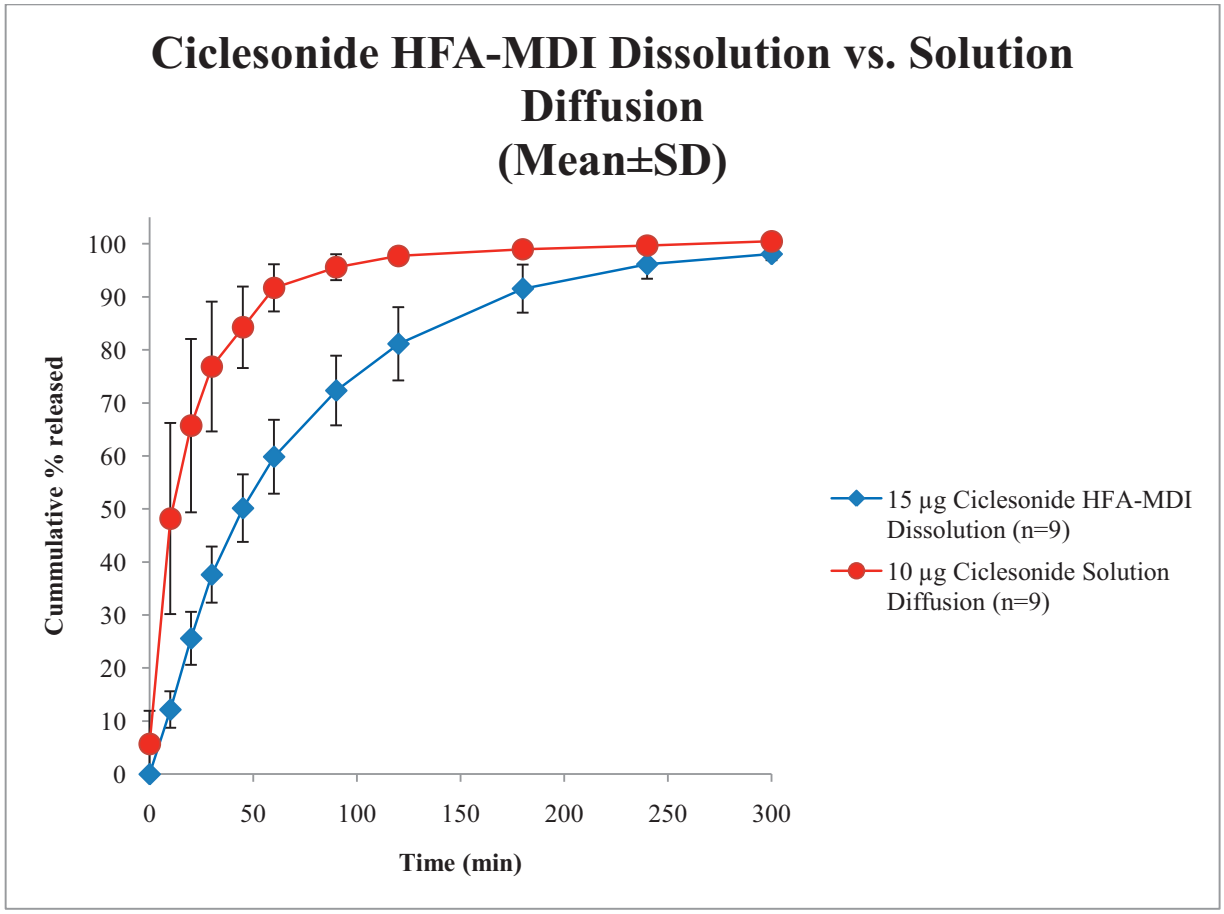


Figure 29: Ciclesonide Diffusion vs. Dissolution without insert membrane (lines between data points for visualization only)

It is visually apparent that the two profiles differ and that the dissolution set-up without the membrane is more suitable to investigate the dissolution behavior of Ciclesonide when administered by the HFA-MDI. Due to the challenges to deposit exactly 10 µg of Ciclesonide on the filter papers the mean amount of Ciclesonide for the dissolution experiments (n=9) was 15 µg. Single experiments with 10 µg drug deposited do not differ significantly (data not shown), but were not reproducible enough.

Because of the faster transfer rates, the sampling times were also adapted to the new set-up including more time points in the first two hours. Due to the increase in transfer rate within the new set-up, the addition of the sampling points helped to visualize the difference within the first two hours. The mathematical and statistical analysis of the two profiles supports the visually apparent observation. The f_1 and f_2 values indicate that the profiles differ and thus are therefore not similar (**Table 11**).

Table 11: f_1 and f_2 calculation of Ciclesonide diffusion vs. dissolution

Profile vs. Profile	Ciclesonide diffusion vs. dissolution
f_1	36.94
f_2	29.7

The one-way ANOVA calculation also indicates a significant difference of the two profiles. The MDT of the two profiles are 26 min for the diffusion and 66 min for the dissolution (**Table 12**).

Table 12: MDT Ciclesonide dissolution and diffusion without membrane

Profile	Ciclesonide Dissolution	Ciclesonide Diffusion
MDT (min)	65.7	26.09

The MDT of the dissolution when the membrane was present for 15 µg of Ciclesonide was 216 minutes as indicated in **Table 8**. This value dropped to 66 min

(**Table 12**) for the same drug amount when the membrane was removed (filter paper served as barrier between donor and acceptor compartment). Although the two profiles differ in the number of experiments and the sampling time points, the visualization in **Figure 30** clearly shows the difference between the two methods.

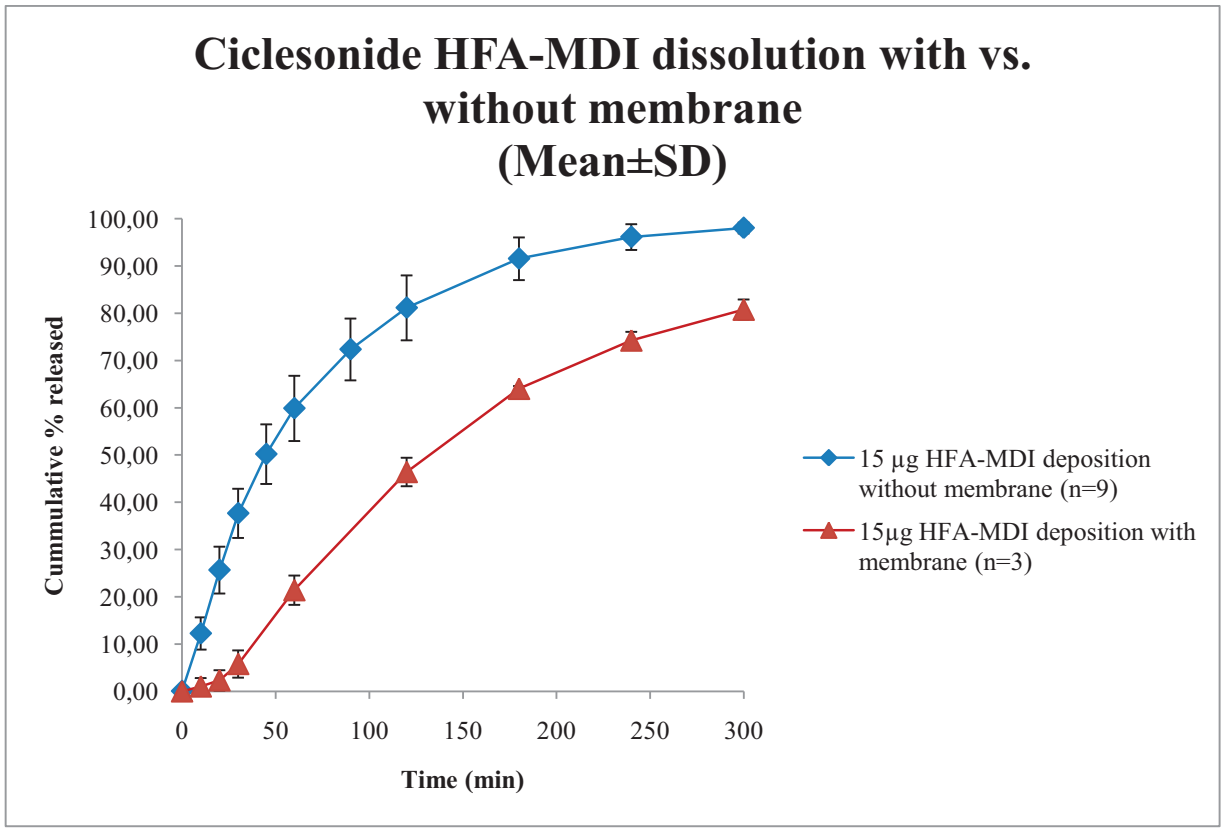


Figure 30: Dissolution of 15 µg Ciclesonide in set-up A vs. B (lines between data points for visualization only)

Whereas in the dissolution test without the membrane 100 % of the deposited Ciclesonide dissolved and entered the receptor compartment within 5 hours, the method with the membrane in place only reaches 80 % after 300 min. The greatest difference can e.g. be seen after the first hour of sampling where the dissolution testing without the membrane rises up to 60 % in comparison to 20 % with the membrane. Thus, the membrane is the rate limiting step for the transfer of already dissolved Ciclesonide into the receptor compartment and not the process of dissolution of dissolving particles, as it should be.

Fluticasone propionate and Budesonide dissolution testing in set-up A

The two ICS Budesonide and Fluticasone are generally prescribed in order to treat asthma and COPD. Both drugs are characterized by a prolonged residence time in the target organ, due to reversible esterfication in the case of Budesonide and a slow dissolution of the deposited drug particles within the lung in the case of Fluticasone [20, 23, 35, 63, 75, 76]. The inhalers which were used for the dissolution experiments of this work are both suspension based metered dose inhalers which contain micronized Budesonide and microcrystallized Fluticasone propionate respectively [77, 78]. The prolonged residence time at the site of action has been discussed to support the efficacy of the drugs against lung inflammation [62, 75, 76].

Pharmacokinetic studies suggested that the absorption of Budesonide from the target organ is rapid [34]. This is only possible if the dissolution process is also fast. For Fluticasone propionate on the other hand depending on the evaluated delivery system and formulation absorption proceeds for up to 7 hours or more [20]. Most authors explain differences in the absorption rate with differences in the physicochemical properties of the two drugs and their effect on the dissolution rate [57].

Thus the rapid dissolution in the lung fluid of Budesonide, Flunisolide and Triamcinolone acetonide to name two additional ICS with similar properties is explained by the much higher water solubility of these compounds. The clear differences in absorption rates of Budesonide and Fluticasone propionate *in vivo* make them highly suitable to validate the *in vitro* dissolution test set-up, as one would expect the same differences in the dissolution rates *in vitro*, if the test system reflects the situation *in vivo* with respect to sink conditions and dissolution medium, and if the rate limiting step in the *in vitro* system truly is the dissolution step. Often dissolution test methods described in the literature, so far, might not fulfill these demands [40].

It was the aim of this part of the project to compare the two ICS with the optimized dissolution test method (set-up B). It should be re-emphasized that the dissolution medium contained surfactant as being the case in the lung, and that drug particles were within the respirable fraction. In order to further mimic the in vivo situation the dissolution test itself was performed at 37 °C and almost 100 % relative humidity.

As mentioned before, filter paper was used as the barrier between donor and acceptor compartment to achieve a faster transfer of dissolved drug between the compartments. The five actuations of the MDI for Budesonide led to a deposition of around 20 µg per filter paper. In the case of Fluticasone propionate the deposition which was achieved by 10 actuations of the inhaler was 10 µg. It was not possible to achieve the exact same amount of deposition for both drugs. The conditions however make it more challenging to achieve a positive outcome of the experiment (hypothesis was that Budesonide should dissolve faster than Fluticasone propionate).

The dissolution of Budesonide and the transfer into the receptor compartment was fast. There was a complete dissolution of the deposited 20 µg of Budesonide in less than 120 min (**Figure 31**).

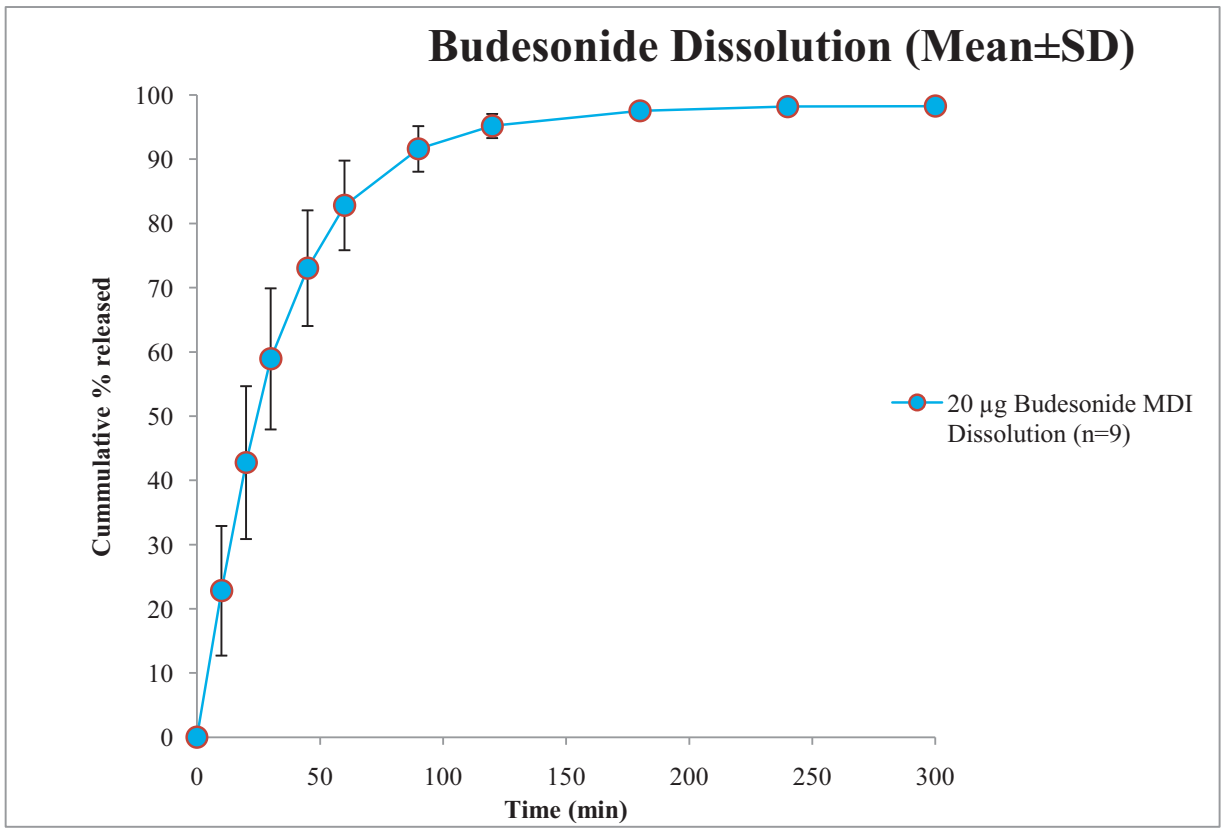


Figure 31: Budesonide MDI dissolution (lines between data points for visualization only)

This result mirrors the expected dissolution behavior of the drug. In contrast to the dissolution behavior found for Budesonide in this study, *Arora et al.* when depositing even smaller doses of Budesonide with the Transwell® system (including the original

membrane, no surfactant) did not reach a complete dissolution even after 5 hours of sampling [40]. This additionally argues for the use of the proposed dissolution system.

Fluticasone propionate was deposited in the described system onto the filter papers in the 10 µg range. The dissolution profile visually differs significantly from that of Budesonide (**Figure 31**).

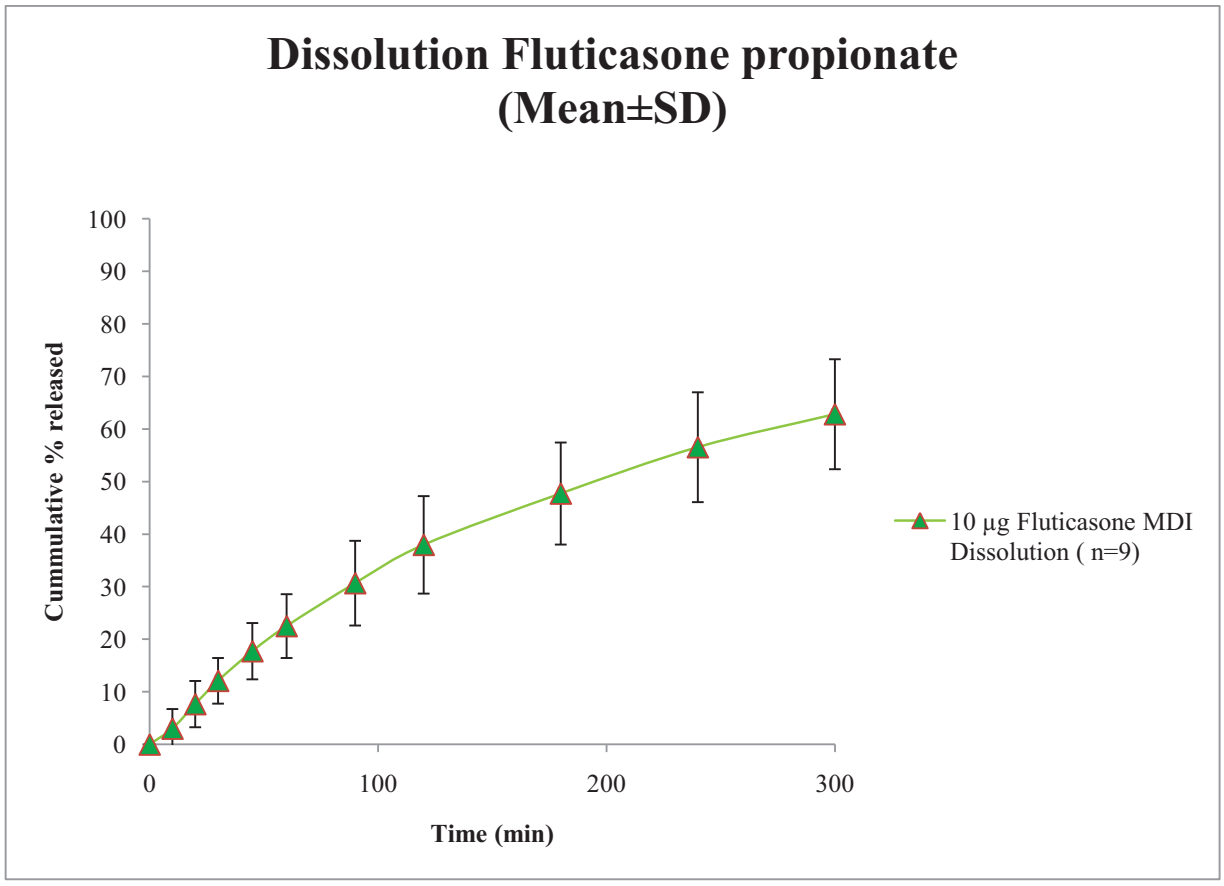


Figure 32: Fluticasone propionate MDI dissolution (lines between data points for visualization only)

The time for a full dissolution of the deposited amount exceeded the sampling time of 8 hours (**Figure 32**). There was a complete dissolution of Fluticasone propionate at some time point after 8 hours because there were only small amounts left when the filter was analyzed after 24 hours (results not shown). For ease of comparison the profile for Fluticasone propionate is only shown up to 300 min like the Budesonide example (**Figure 31**). A direct comparison of the two profiles shows that

the dissolution of Budesonide drug particles is much faster compared to that of Fluticasone propionate (**Figure 33**).

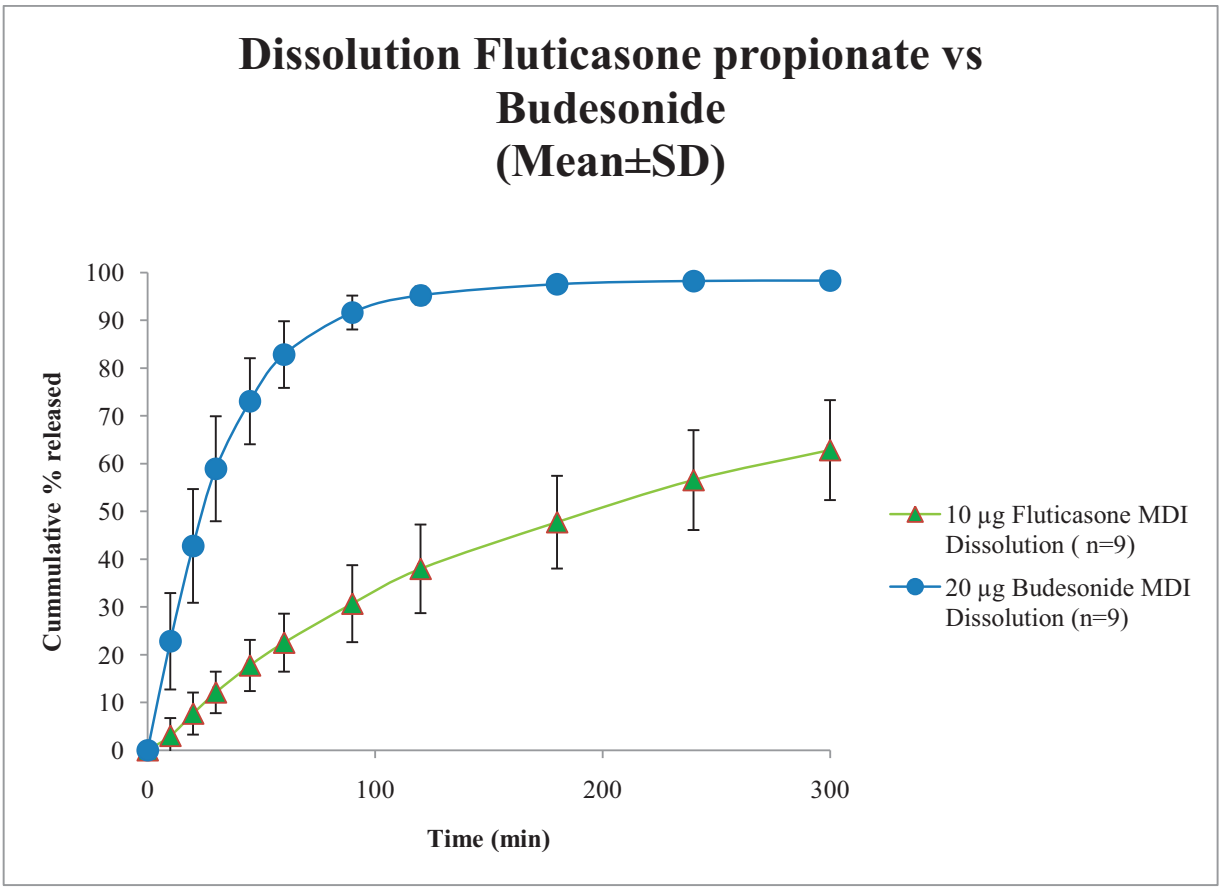


Figure 33: Budesonide vs. Fluticasone MDI Dissolution (lines between data points for visualization only)

The mathematical and statistical evaluation and comparison of the two profiles support the visual observation. The f1 and f2 test of the two profiles as previously done for Ciclesonide also underlines the visual observation as can be seen in **Table 13**.

Table 13: f_1 and f_2 calculation of Budesonide and Fluticasone propionate dissolution

Profile vs. Profile	Budesonide vs. Fluticasone propionate
f_1	60.73
f_2	17

In this case the f_1 value indicates that the two profiles are different. Additionally, the f_2 value does not allow assuming similarity as the value is well below the minimum target value for similarity which is 50. With respect to the one-way ANOVA analysis it can be said that the calculation also shows that the two profiles are different. The MDT for Budesonide was 35 min and for Fluticasone propionate 301 min (**Table 14**).

Table 14: MDT 20 µg Budesonide vs. 10 µg Fluticasone propionate

Profile	Budesonide	Fluticasone propionate
MDT (min)	34.67	301.11

All data sets were fitted using a range of models. It can generally be said that the first-order and Weibull model seem to express the closest fit to all the measurements with the Weibull model mirroring the data even better in some of the cases. **Appendix 1** gives an overview of all profiles and fits.

In summary one can conclude that the dissolution set-up B was able to provide a good model of the dissolution of the two ICS when compared to their respective *in vivo* situation. Budesonide dissolves and is then absorbed very fast within the lung [79]. The *in vitro* tested aerosolized particles of the drug also dissolved in a similar time span in the dissolution test. The same can be said for Fluticasone propionate pointing to the fact that the absorption in the lung takes much longer *in vivo* [34] than that of Budesonide and that when the dissolution set-up B was used also provided a longer dissolution time of the Fluticasone propionate drug particles.

The similarity of dissolution profiles generated by the proposed method and *in vivo* absorption profiles [34, 79] argue for a closer examination. The findings must be seen in relationship to the other ICS, Ciclesonide, and in the light of the physicochemical properties of the three ICS as a whole.

While the solubility in water increases in the order of CIC<FP<BUD [20, 70], this order changes in the presence of SDS. Here Fluticasone propionate becomes the least soluble ICS whereas Ciclesonide and Budesonide are much more affected by the presence of the surfactant in regard to solubility (**Table 15**).

Table 15: Water solubility [20, 70] vs. SDS solubility of ICS

Drug	Water solubility	0.5 % SDS solubility
Ciclesonide	> 0.1µg/ml	300 µg/ml*
Fluticasone propionate	0.14 µg/ml	150 µg/ml*
Budesonide	14 µg/ml	470 µg/ml*

*solubility of respective ICS in SDS after 24 h

Generally the rate of dissolution in the SDS solution agreed with the solubility differences of the compounds in the medium itself.

Budesonide vs. Fluticasone propionate and Ciclesonide dissolution, lung absorption and pharmacokinetics

Theoretically, all processes involved in the pulmonary absorption of drug into the blood stream can determine pulmonary absorption rate. These processes include time to dissolve, time to diffuse to pulmonary cells, time to enter cells by crossing cell membranes, time spent within the cells (prolonged e.g. through formation of intracellular esters), time to cross membranes and enter the blood stream [19]. While prolonged intracellular residence time through esterification processes have been described for a small percentage of the overall doses of Ciclesonide and Budesonide [26, 69, 80], one has speculated, that the rate limiting process for some of the inhaled glucocorticoids represents the dissolution process [20, 37, 57]. If this hypothesis is true for ICS, a good agreement between *in vitro* dissolution and pulmonary absorption kinetics have to be

observed. It was therefore of interest to compare in *vitro* dissolution behavior with pharmacokinetic data of fast and slowly absorbed ICS and correlate these results to their physicochemical properties (lipophilicity, solubility).

The bridging of the dissolution results to *in vivo* data can be achieved by including the evaluation of relevant parameters from pharmacokinetic studies. In order to do so the mean absorption times (MAT, the average time an average molecule will need to enter the blood stream) in the lung can be calculated by the direct comparison of intravenous pharmacokinetic data with those after inhalation. The necessary parameters and equations all evolve around the plasma concentration time profiles of these different routes of administration of the drugs in question [81]. The foundation is laid by calculating the respective area under the curve (AUC) from the plasma concentration versus time plots. In addition, the area under the first moment curve (AUMC) needs to be calculated, which can be derived from plots that show the product of concentration and time (Concentration*t; y-axis) as a function of time (t; x-axis). The mean residence time (MRT; the average time a drug molecule stays in the body) after inhalation (MRT_{inh}) and after intravenous (iv) administration (MRT_{iv}) can then be easily calculated from the ratio of AUC and AUMC observed either after inhalation or iv administration. The MAT (average time a drug particle spends in the lung) is then the difference of MRT after inhalation and MRT after iv administration [81].

Knowing the dissolution time of an inhaled ICS is important because of other events that occur in the upper part of the lung. The upper part of the lung contains a clearance mechanism for inhaled “dust” particles, the mucociliary clearance [79]. Small “hairs” swipe particles out of the lung. As the process takes time, this clearance mechanism is only relevant for drug particles that do not dissolve immediately (e.g. Fluticasone propionate). The bigger respirable drug particles which deposit in the ciliated central regions of the lung, the better they can be cleared by mucociliary clearance provided that the substance which was deposited does not dissolve rapidly and hence escapes the clearance mechanism by dissolution (**Figure 34**) [19, 79]. The pulmonary available dose is consequently smaller for such drugs than those that dissolve fast and can escape the clearance mechanism. Knowing the dissolution rate *in vivo* is therefore of relevance for the drug development process.

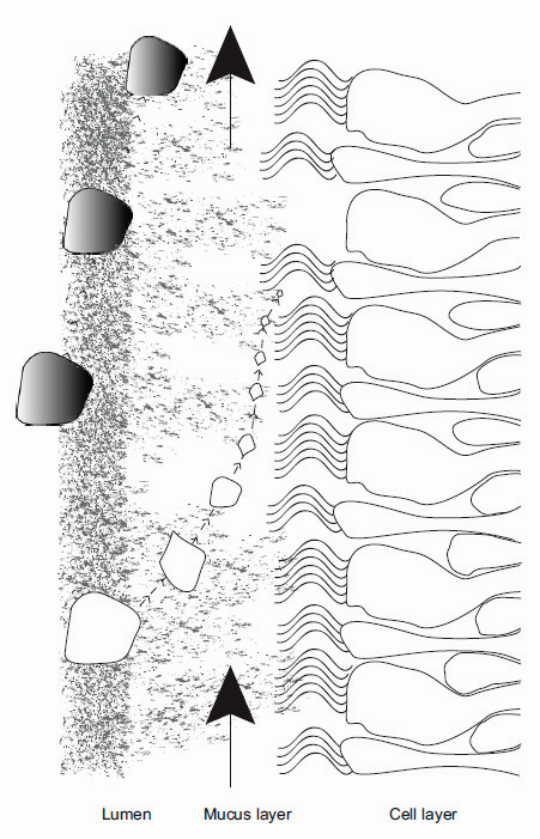


Figure 34: Mucociliary clearance (dark particles) in contrast to a rapidly dissolving particle (light particles) [79]

The dark particle as shown in **Figure 34** which does not dissolve rapidly enough can be cleared in an upward direction by the lung to be swallowed whereas the light particle in Figure 34 does dissolve rapidly and can then enter into the cells of the lung [79]. Thus in the case of the deposition of a slowly dissolving ICS the systemic exposure will be reflected in differences in the pharmacokinetic data; by e.g. lower AUC values for data which was obtained from asthmatic patients as the drug is no longer available for systemic exposure due to the connected limited oral bioavailability in this class of compounds. In the case of a rapidly dissolving ICS like Budesonide the mucociliary clearance does not have the time to exhibit such effects as the drug dissolves before the mucociliary escalator can remove drug particles out of the lung [79, 82].

Regarding the peripheral or deeper deposition it can be said that the much smaller respirable particles which deposit within this non ciliated region are not prone to

such a clearance mechanism and therefore slowly dissolving drugs like e.g. Fluticasone propionate have enough time to dissolve at these sites which is then reflected in longer MAT values [79, 82-85].

The importance of the dissolution behavior for the pulmonary fate of ICS has been discussed so far only on a qualitative level for Budesonide and Fluticasone propionate [34, 79, 82], as hard dissolution rate data were not available for this discussion. This is now possible with the availability of data for Budesonide and Fluticasone propionate and Ciclesonide in the developed dissolution test (set-up B) including Ciclesonide's surprising behavior.

Budesonide vs. Fluticasone dissolution and lung absorption

In vitro dissolution data for Budesonide and Fluticasone propionate are in agreement with the physiochemical properties (lipophilicity, water solubility) of the two compounds [40, 41]. For Budesonide and Fluticasone propionate their differences in lipophilicity and water solubility predict their behavior in dissolution tests, independent of the choice of a surfactant containing or surfactant free dissolution medium. Budesonide exhibits a much higher water solubility of about 14-21 µg/ml compared to that of Fluticasone propionate that has a solubility of water of 0.14 µg/ml [20]. Differences are also reflected in differences in the octanol/water partition coefficient, which is a good indicator of the smaller lipophilicty of Budesonide [79, 86]. In addition, a clinically relevant dose of Budesonide would only require 1-15 ml for dissolution, while a clinically equivalent amount Fluticasone propionate would require 2 l of water to dissolve [79].

In agreement with these physicochemical properties of the two drugs, Budesonide dissolves much faster than Fluticasone propionate in water [40] and surfactant containing medium (**Figure 33** and reference [41]). The calculation of the measured dissolution times (MDT, calculated in accordance with the MRT) in this dissertation allows for testing a correlation between dissolution rate and the *in vivo* MAT for the two compounds (see **Table 16**).

Table 16: MAT and MDT in set-up B for Fluticasone propionate and Budesonide

ICS	MAT (h)	MDT in set-up B (h)
Fluticasone propionate	5-7 [34]	5
Budesonide	< 1 (0.6 – 0.9) [34]	< 1 (0.5)

Water based dissolution approaches (no surfactant) do not show a good correlation to the *in vivo* results [40] as dissolution times in such a systems take much longer than the *in vivo* absorption process. By way of example only about 2% of Fluticasone and just 80% of Budesonide dissolve in a water based, non surfactant containing Transwell® arrangement within a 5 hour period [40] whereas 100 % of Budesonide and about 50% of Fluticasone propionate dissolve when applying the surfactant based dissolution method in adapted Transwell® set-ups of this dissertation.

The availability of a method that allows to predict the dissolution behavior *in vivo* is important, e.g. for projecting whether the pulmonary fate of the drug will be affected by mucociliary clearance in the central portions of the lung. As an example, **Figure 35** indicates that a more central deposition generally only observed in asthmatic patients will affect the pulmonary available dose for Fluticasone propionate (the slow dissolution will allow the mucociliary clearance to remove particles), while for Budesonide a more central deposition, as seen for asthmatics will not reduce the pulmonary available dose, as the fast dissolution will prevent removal of solid Budesonide particles [82].

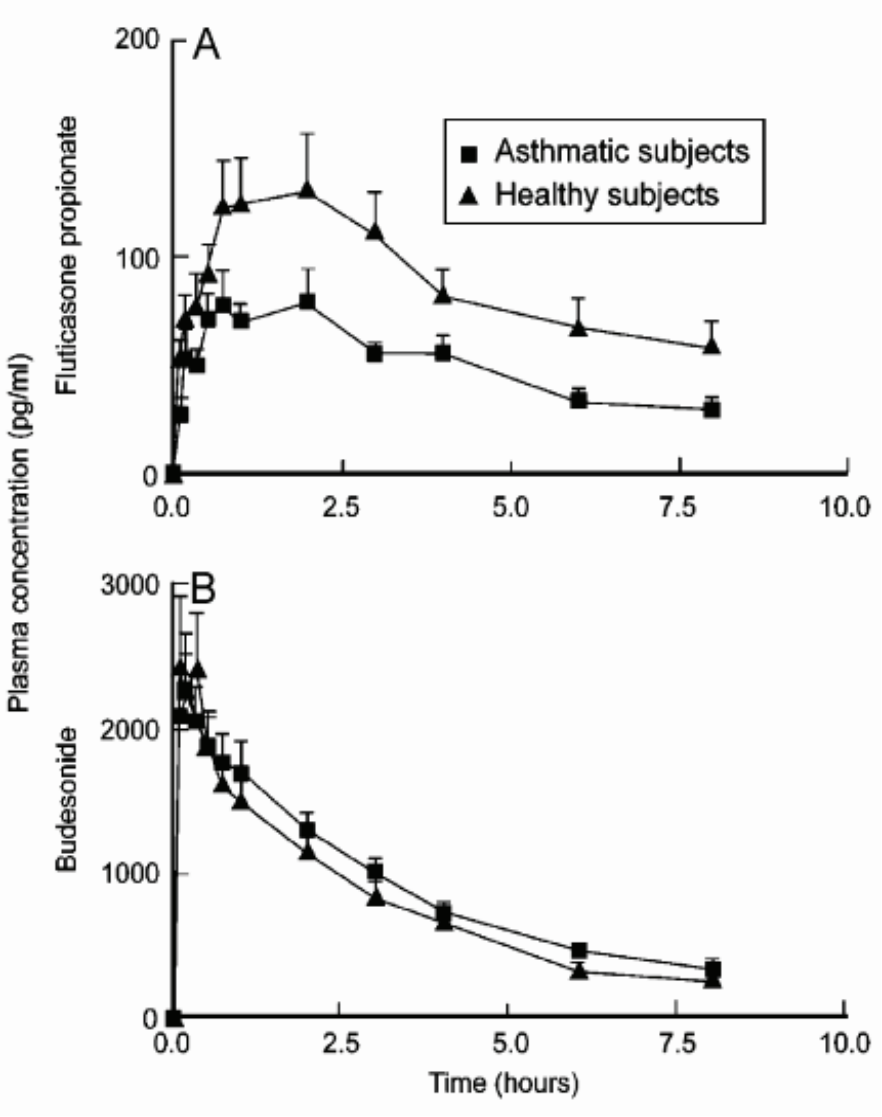


Figure 35: Plasma Concentration time profiles of Fluticasone propionate and Budesonide in asthmatic and healthy subjects [82]

Similar projections can be made for formulations that deposit more centrally (larger particles) or more peripherally (smaller particles). Differences in formulation dependent c/p ratios will not affect the pulmonary available dose for Budesonide, but it will be the case for Fluticasone propionate.

In summary, the good correlation between *in vitro* (MDT) and *in vivo* data (MAT) for the two ICS that differ in their physicochemical properties seems to suggest that the developed system is of relevance for the drug development process. It should be

stressed again that the use of surfactant and of a non-membrane based separators between donor and acceptor compartments is vital.

Ciclesonide lung dissolution and adsorption at the site of action

Ciclesonide is an ICS which is very insoluble in water. In fact less than 1 µg/ml have been found to dissolve in water [20, 70]. As mentioned earlier Budesonide dissolves much better in water by providing values between 14 – 21 µg/ml and Fluticasone propionate reaching values more in the range of Ciclesonide with 0.14 µg/ml [20, 70, 79]. Interestingly, Ciclesonide does not behave like Fluticasone propionate in the set-up B of this dissertation with the addition of the surfactant SDS and the technical alterations. In fact Ciclesonide dissolves very fast when the surfactant SDS is added (see **Table 17**).

Table 17: MDT in set-up B for Ciclesonide vs Fluticasone propionate

ICS	MDT in set-up B (h)
Ciclesonide	1
Fluticasone propionate	5

As the MDT in the surfactant based dissolution set-up B differs significantly from the one of Fluticasone propionate, despite their similar water solubility, it is of interest to discuss the MAT of Ciclesonide in the lung and finally compare this value to the *in vitro* MDT for the two drugs as illustrated in **Table 17**.

Regarding the lipophilicity of the three ICS in question which is also connected to drug absortion in the lung it can be said that Budesonide is the least lipophilic followed by Fluticasone and then Ciclesonide [86]. In a relative rank order of the respective lipophilicity Budesonide can be assigned a (1), Fluticasone propionate a (3.2) and Ciclesonide a (4) [86]. Lipophilicity is an important factor for membrane permeability in the lung and thus the more lipophilic a compound is, the faster it will enter the cells after dissolution. Although Ciclesonide is more lipophilic, it has the lowest water solubility of the three compounds.

Therefore, based on the low water solubility one would expect Ciclesonide to show a slow dissolution, similar to that of Fluticasone propionate. However, much shorter MDT were derived in the proposed *in vitro* system.

Interestingly, clinical studies with Ciclesonide showed that the pulmonary available dose does not depend on the central to peripheral deposition ratio, as similar pharmacokinetic results were obtained for healthy volunteers and asthmatics [87, 88]. This can be regarded as another hint that Ciclesonide is rapidly absorbed in the lung.

Additionally, the clinical pharmacology review package of Ciclesonide provides the necessary data package in order to be able to discuss the mechanism and rate of Ciclesonide lung absorption when delivered by an inhaler [89]. The aim of the study was to investigate the systemic availability of the active metabolite Des-Ciclesonide of the prodrug Ciclesonide when administered by an HFA-MDI and intravenously.

This review includes a pharmacokinetic evaluation after different routes of administration [89]. **Table 18** shows the overview for the intravenous and the HFA-MDI data for Ciclesonide, including the non-compartmental analysis.

Table 18: Ciclesonide i.v. versus inhaled selected pharmacokinetic parameters [89]

Parameter	MDI published	i.v. published
$AUC_{t-\infty}$ (ng*h/ml)	1.92	5.41
C_{max} (ng/ml)	4.89	21.91
T_{max} (h)	0.17	0.18

Unfortunately, the authors did not present the MRT estimate for Ciclesonide for both routes of administration. However, a graphical representation compared the concentration time profiles after intravenous administration and inhalation of a solution based MDI. Using the details of **Figure 36** (estimates of the mean concentrations for

any given time point) [89], the missing values were consequently self-calculated. From the graphical representation of the results (**Figure 36**) mean concentrations for the time points were taken [89].

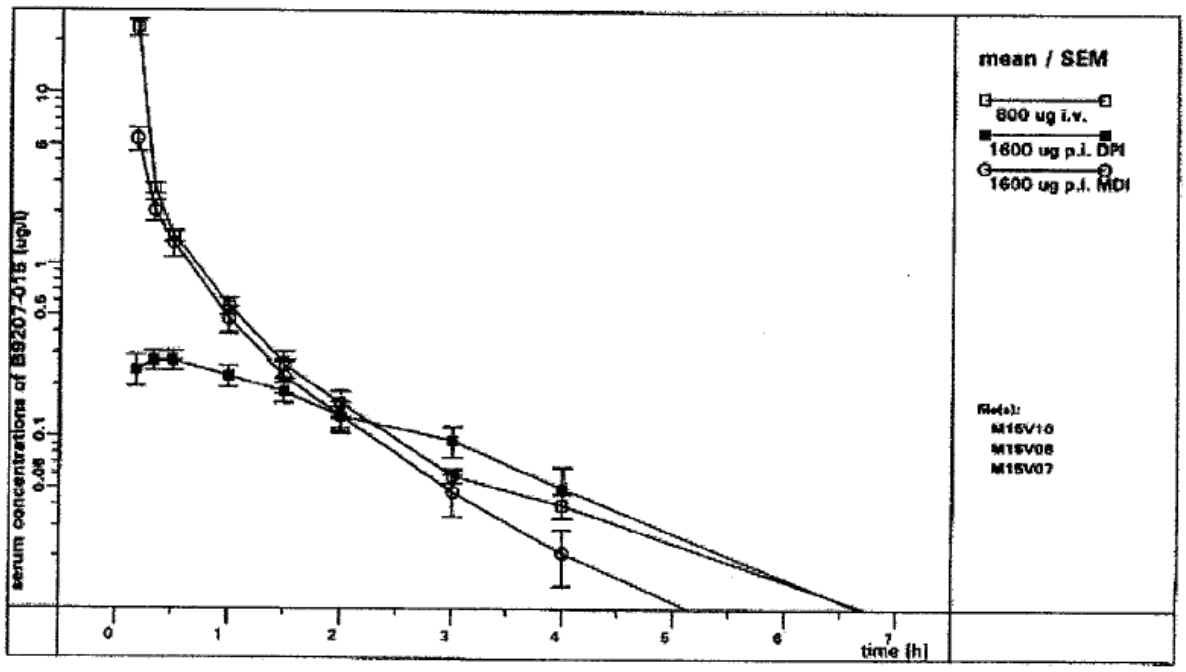


Figure 36: Ciclesonide i.v. and HFA-MDI pharmacokinetic plasma concentration time profiles [89]

This allowed calculation of the mean AUMC estimates (**Table 20**). The knowledge of the corresponding AUC estimates (calculated accordingly, **Table 19** and verified by the published AUC [89], **Table 18**) allowed further calculation of the MRT estimates of these non-compartmental parameters which are based on the statistical moment theory [81, 90, 91].

Table 19: Comparison of estimated AUC and published AUC for Ciclesonide when administered by different routes

Parameter	MDI published	MDI estimated	i.v. published	i.v. estimated
$AUC_{t-\infty}$ (ng*h/ml)	1.92	1.92	5.41	6.16

Table 19 provides a direct comparison of the estimated AUC. On the basis of the individual concentration values for every single time point the AUMC which is shown in **Table 20** was estimated.

Table 20: Estimated AUMC for Ciclesonide when administered by different routes

Parameter	MDI estimated	i.v. estimated
$AUMC_{t-\infty}$ (ng*h*h/ml)	1.29	2.03

The MRT for a compound in the body can be calculated according to **Equation 5** using the obtained AUMC and AUC values.

$$MRT = \frac{AUMC}{AUC} \qquad \textbf{Equation 5}$$

In order to estimate the MRT for the intravenous route ($MRT_{i.v.}$) it is important to note that in the case of Ciclesonide the administration was achieved by a short time infusion of T=10 minutes [90], which needed to be considered by **Equation 6**.

$$MRT_{i.v.} = MRT_{inf} - \frac{T}{2} \qquad \textbf{Equation 6}$$

The $MRT_{i.v.}$ for the intravenous bolus injection is thus 0.24 h when using the estimated AUC (i.v.) and AUMC (i.v.) in **Table 19** and **Table 20** respectively and applying it to **Equation 6**. The MRT for the MDI can be estimated in a similar way using **Equation 5** and the estimates of **Table 19** and **Table 20**. A complete overview of the results for both MRT obtained by the estimations are presented in **Table 21.**

Table 21: Overview of estimated MRT for i.v. and MDI administration of Ciclesonide

Parameter	MDI estimated	i.v. estimated
MRT (h)	0.67	0.24

The MRT of the different routes of administration can now be used for the estimation of the MAT in the lung for the MDI by applying **Equation 7**.

$$MAT_{lung} = MRT_{lung} - MRT_{i.v.} \qquad \textbf{Equation 7}$$

According to these relationships, the MAT for the MDI is 26 min or 0.43 h as listed in **Table 22** below. This result is in line with the findings of other authors [45].

Table 22: MAT for Ciclesonide MDI

Parameter	MDI estimated
MAT (h)	0.43

This indicates that the absorption of Ciclesonide at the site of action delivered by the MDI is much faster than the reported MAT for Fluticasone propionate [34].

In summary, the Ciclesonide MDI delivered particles are being absorbed with a rate similar to that of Budesonide for which an MAT of less than 1 hour as illustrated in **Table 23** was reported [34]. Thus, despite Ciclesonide's high lipophilicity and low water solubility values that are more in agreement with those of Fluticasone propionate, the uptake at the site of action and consequently the dissolution in the lung agrees more with that of Budesonide.

A complete overview of the *in vitro* MDT and the MAT correlation of the three ICS can be found in **Table 23**.

Table 23: MAT and MDT in set-up B for Fluticasone propionate, Budesonide and Ciclesonide

ICS	MAT (h)	MDT in set-up B (h)
Fluticasone propionate	5-7 [34]	5
Budesonide	< 1 (0.6 – 0.9) [34]	< 1 (0.5)
Ciclesonide	< 1 (0.43)	1

Here it becomes clear that the dissolution set-up B in connection with the addition of a surfactant is able to show good correlation to the *in vivo* absorption kinetics not only for Budesonide and Fluticasone propionate, but more importantly also for Ciclesonide delivered by the HFA-MDI.

GENERAL CONCLUSION AND SUMMARY

This work was conducted as limited or no data are available for the *in vitro* dissolution behavior of aerosolized Ciclesonide and its potential relevance for predicting the *in vivo* pulmonary absorption rate.

To achieve this goal, representative particles formed by the marketed HFA-Alvesco®-MDI under humidity and temperature controlled conditions were characterized and visualized by scanning electron microscopy and diffraction patterns obtained from transmission electron microscopy. The dissolution behavior of Ciclesonide was then assessed based on a published (set-up A) and a new and improved method (set-up B) under temperature and humidity controlled conditions. Both methods used a commercially available Transwell® system for separating undissolved and dissolved drug and used in this work, contrary to published work, surfactant based dissolution media. Set-up A used the commercially available Transwell® system without change (including the use of the standard membrane to separate donor and acceptor compartment) while set-up B used a new membrane-free system [40]. The membrane was not only removed in order to be able to capture the dissolution process, but the Transwell® well itself had to be technically adapted for the purpose of the study. This was achieved by thermoforming a groove or notch on the inside of the well which was then used to place the filter papers onto. This filter paper as the new diffusion barrier was then used for dissolution testing. In a further development step the volume of SDS solution, the surfactant which was used, was adapted to a level of liquid that better facilitated the new set-up in regard to wetting the lower surface of the filter paper. Drug was previously deposited in an ACI onto upper side for dissolution testing.

The following results were obtained. Stage 4 of an Andersen Cascade Impactor was identified in preliminary experiments as the stage on which most inhalable particles of Ciclesonide deposited. Thus, dissolution tests were performed with this fraction. When Ciclesonide collected on stage 4 and Ciclesonide in solution were compared within set-up A indistinguishable profiles were observed. The visual, mathematical and statistical evaluation of the profiles led to the conclusion that membrane diffusion was rate limiting and not the dissolution of Ciclesonide solid particles. Consequently this

work was able to show that only after the removal of the membrane from the Transwell® arrangement and the exchange for filter paper as the diffusion barrier, dissolution testing of Ciclesonide becomes meaningful.

The initial tests in the newly developed set-up B were performed by comparing the diffusion of already dissolved Ciclesonide in the SDS solution without the membrane to the results with the membrane from before. Ciclesonide entered the receptor compartment much faster.

These results confirmed that the diffusion across the original Transwell® membrane was rate limiting and thus made monitoring of the dissolution process impossible. Only after its removal it was possible, as shown in this dissertation, to distinguish between the dissolution of deposited Ciclesonide particles and the diffusion of Ciclesonide in solution.

Surprisingly, the dissolution rate of the deposited Ciclesonide was faster than expected for drugs of such high lipophilicity and low water solubility, especially as ICS with similar physicochemical properties were dissolving more slowly in another dissolution test method [40]. However, the rather fast dissolution of Ciclesonide in the developed system agreed with its fast absorption *in vivo*. To further validate the proposed method (no membrane, use of surfactant), the in vitro dissolution behavior of Budesonide, an ICS with fast absorption and dissolution kinetics and that of Fluticasone propionate, a drug with slow pulmonary absorption and dissolution was evaluated [40, 41, 44, 45, 53]. Results indicated that Budesonide and Fluticasone propionate exhibit their expected dissolution behavior in the newly developed set-up B. These results allowed putting the finding that Ciclesonide dissolves rapidly once a suitable amount of a surfactant is added into context. Future studies should investigate the detailed mechanism behind this finding including this and other surfactants and concentrations.

In addition solubility studies in surfactant based dissolution media already indicated that the solubility of Ciclesonide increased over-proportionally in surfactant when these data were compared to surfactant induced solubility increases observed for Budesonide and Fluticasone propionate. Thus, the rapid dissolution rates of Ciclesonide were not only due to the possible amorphous nature of the particles

generated from the HFA-Alvesco®-MDI as shown by the help of a TEM analysis, but also due to the very pronounced enhancement in solubility in SDS containing media.

The developed dissolution set-up B was able to mimic the *in vivo* dissolution of the three ICS as mean absorption times of Ciclesonide, Budesonide and Fluticasone propionate also agreed with the mean dissolution times determined *in vitro*. This good *in vitro/in vivo* correlation indicates the importance of the dissolution process for the pulmonary absorption kinetics of Ciclesonide and other inhaled corticosteroids, in general. Overall, the good *in vitro/in vivo* correlation of the proposed method, that could not be observed for other methods, suggested that surfactant containing dissolution media together with optimized separation of donor and acceptor compartments are important for mirroring the dissolution in the lung.

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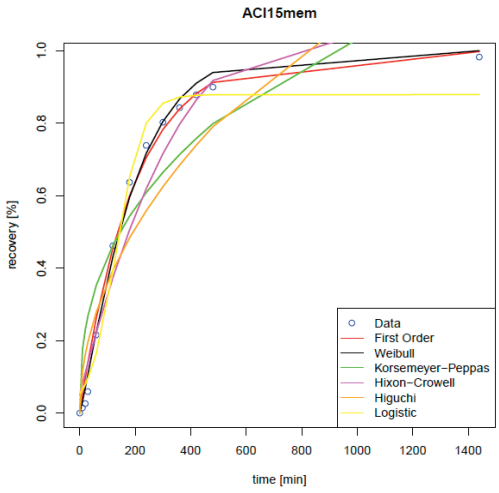
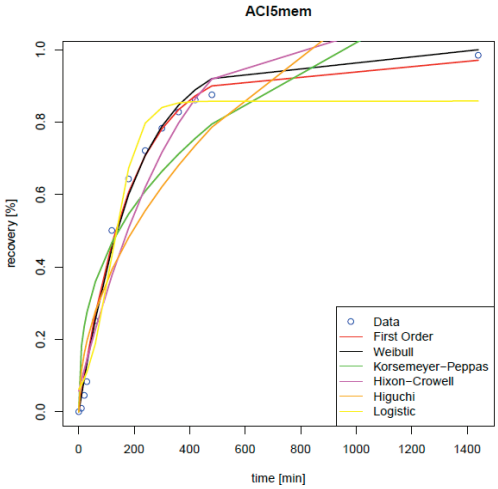
APPENDIX

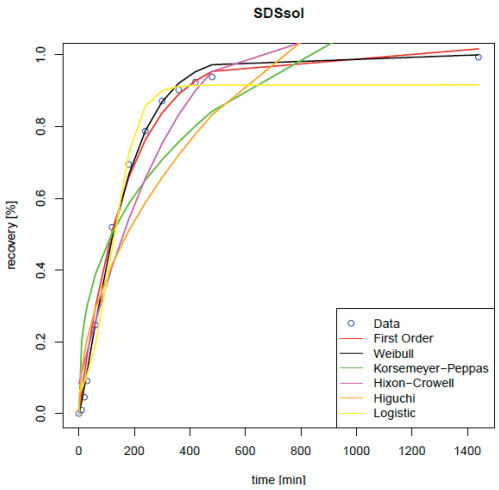
Appendix 1

1. Ciclesonide with membrane dissolution and diffusion plots and data fits

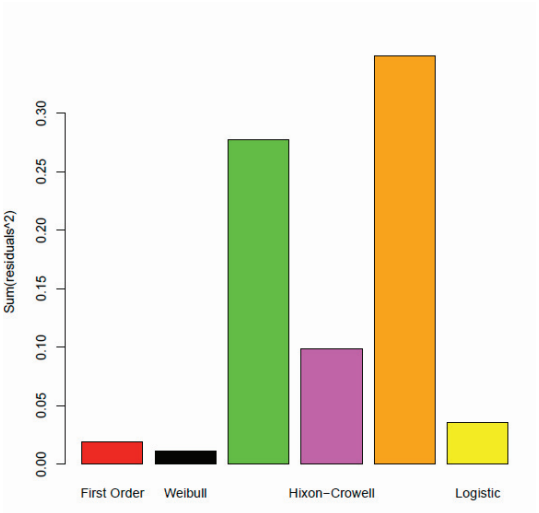
   a) 5 µg and 15µg dissolution and 10 µg diffusion fitted plots

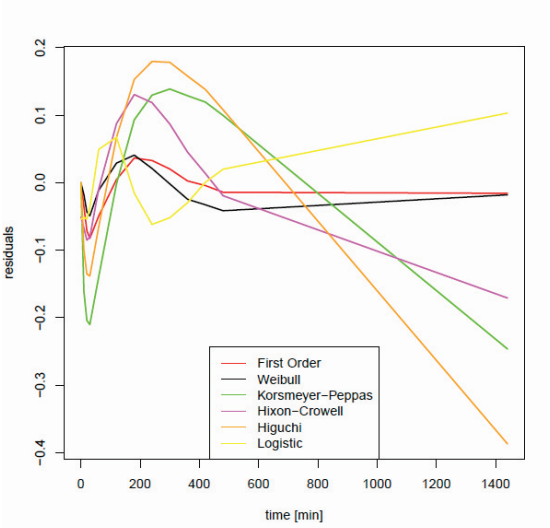




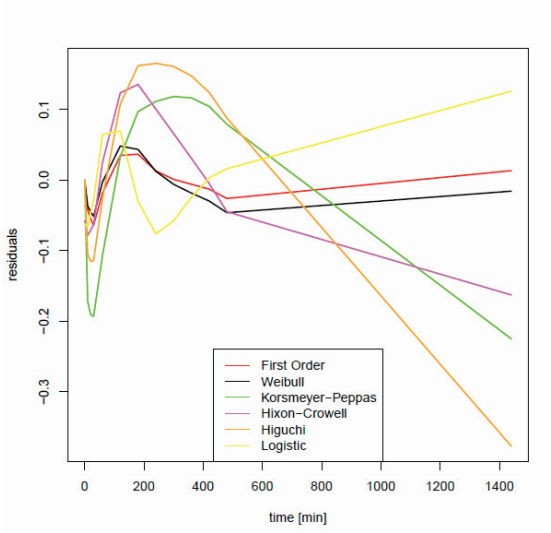


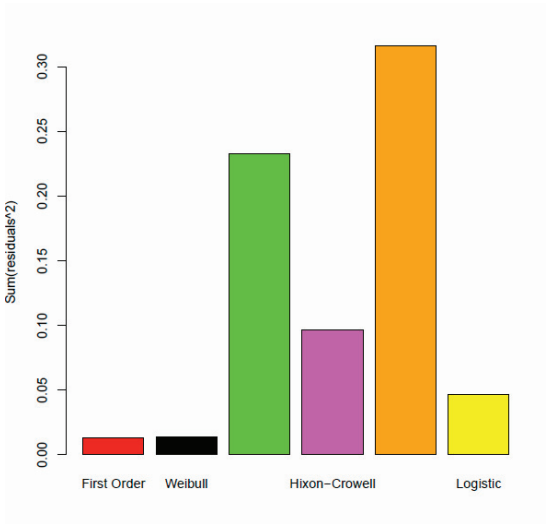
b) 15 µg Ciclesonide dissolution data fits



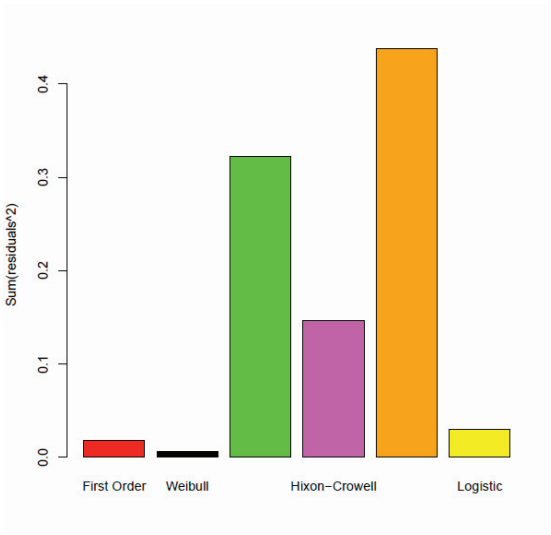


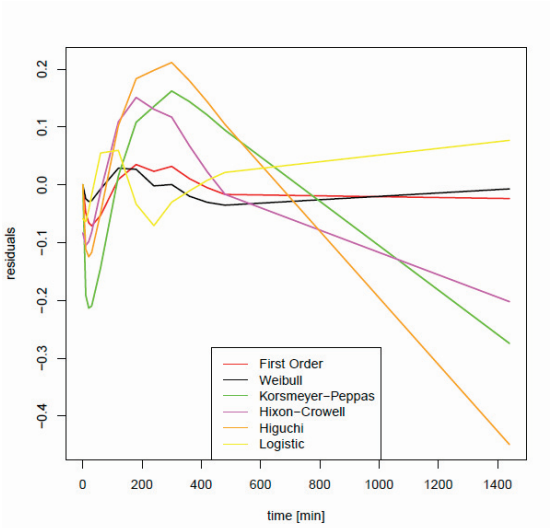
c) 5 µg Ciclesonide dissolution data fits





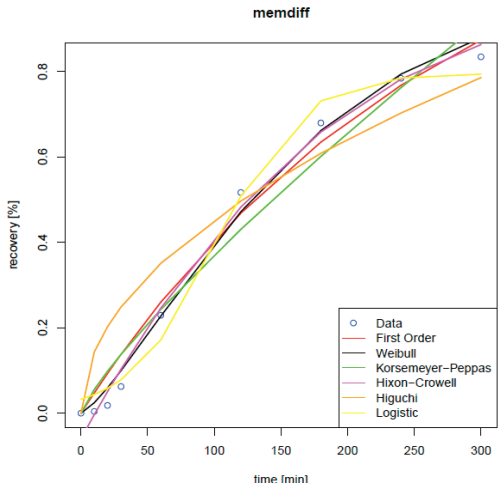
d) 10 µg Ciclesonide diffusion data fits

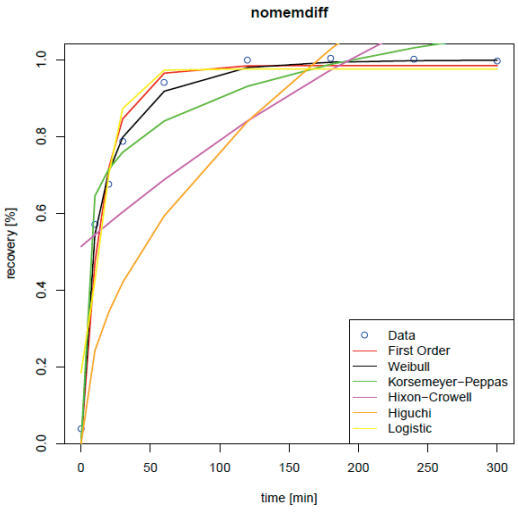




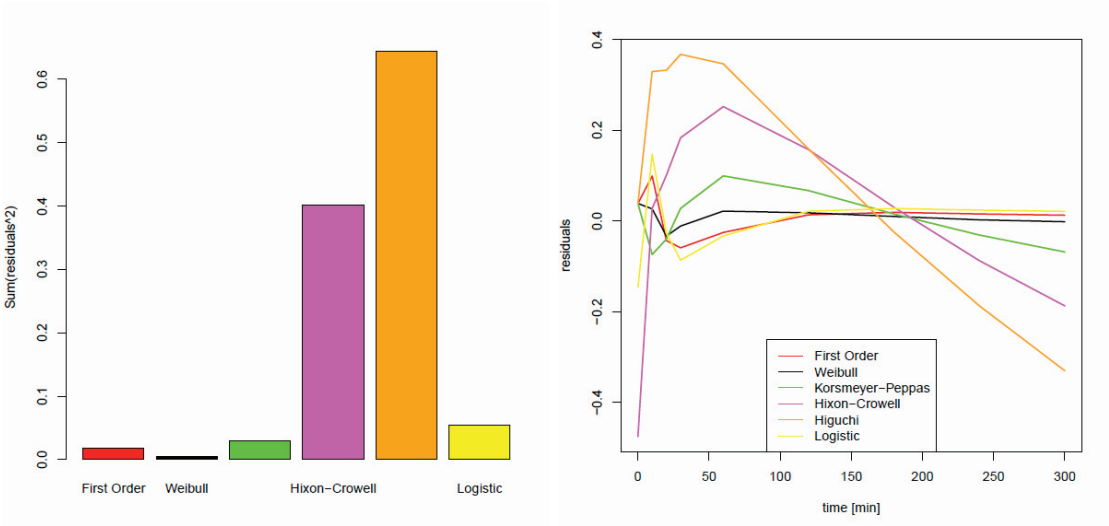
2. Ciclesonide diffusion with and without membrane plots and data fits

a) Ciclesonide diffusion with and without membrane fitted plots

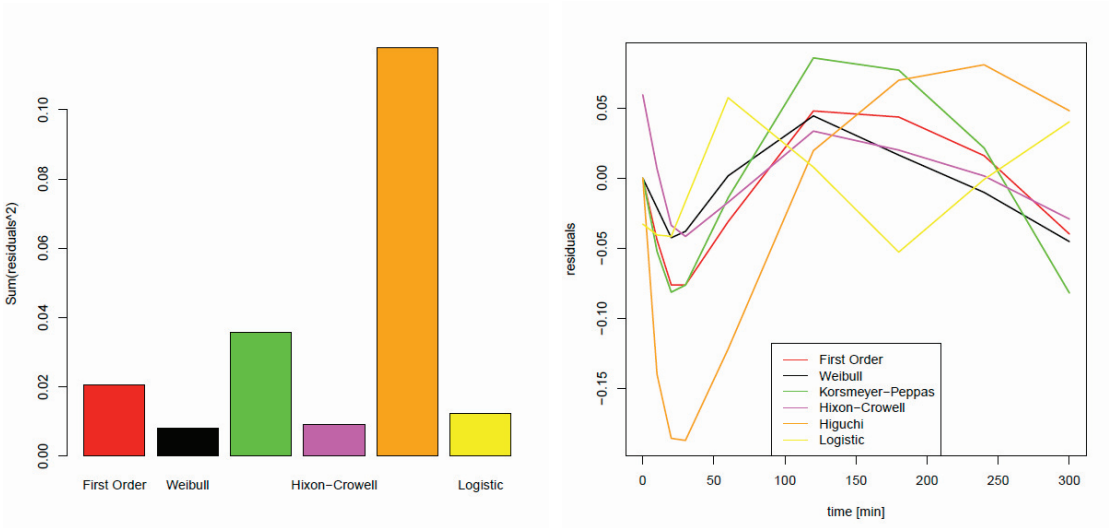




b) Ciclesonide diffusion without membrane data fits

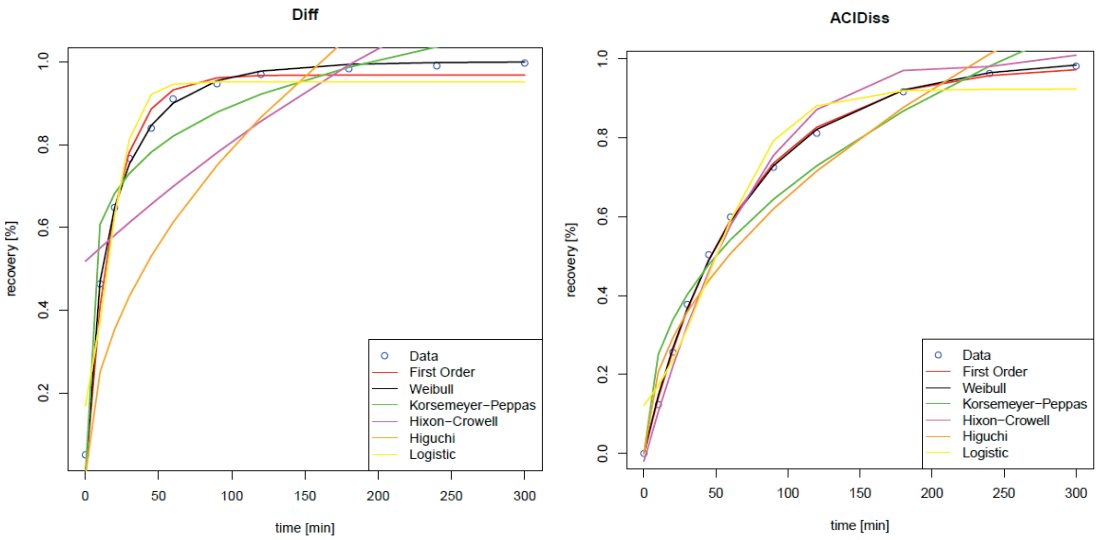


c) Ciclesonide diffusion with membrane data fits

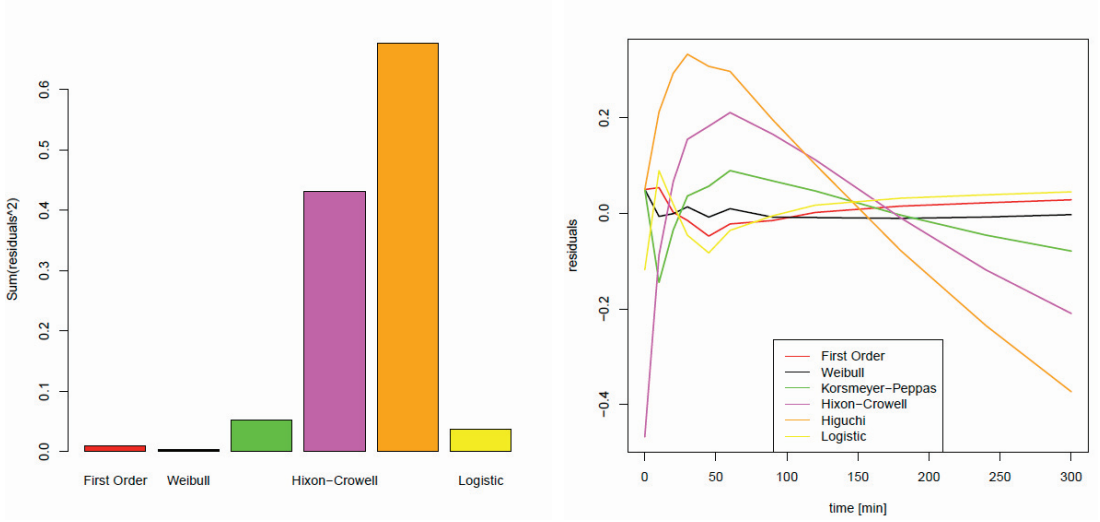


3. Ciclesonide dissolution and diffusion no-membrane plots and data fits

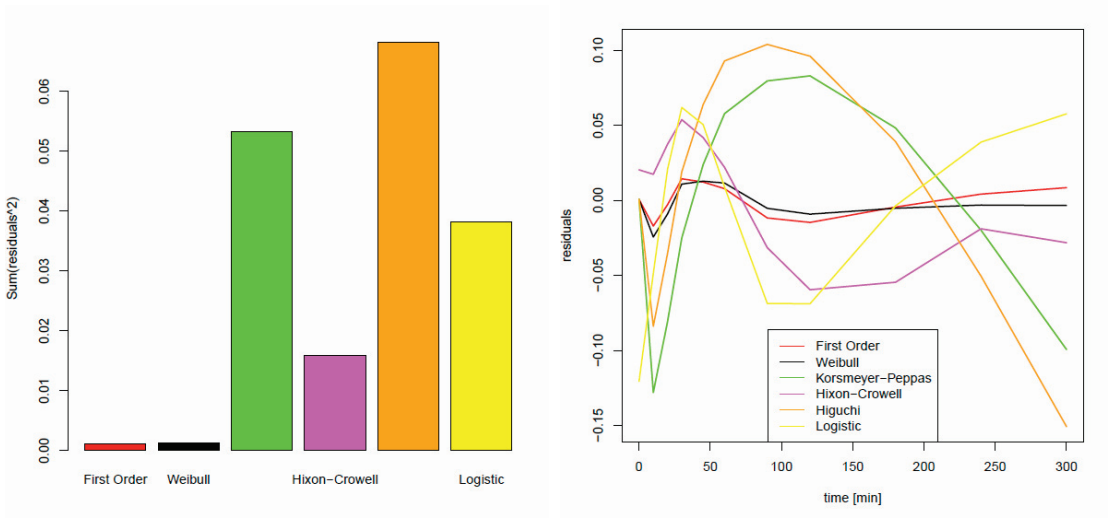
a) Ciclesonide dissolution and diffusion no-membrane fitted plots



b) Ciclesonide without membrane diffusion data fits

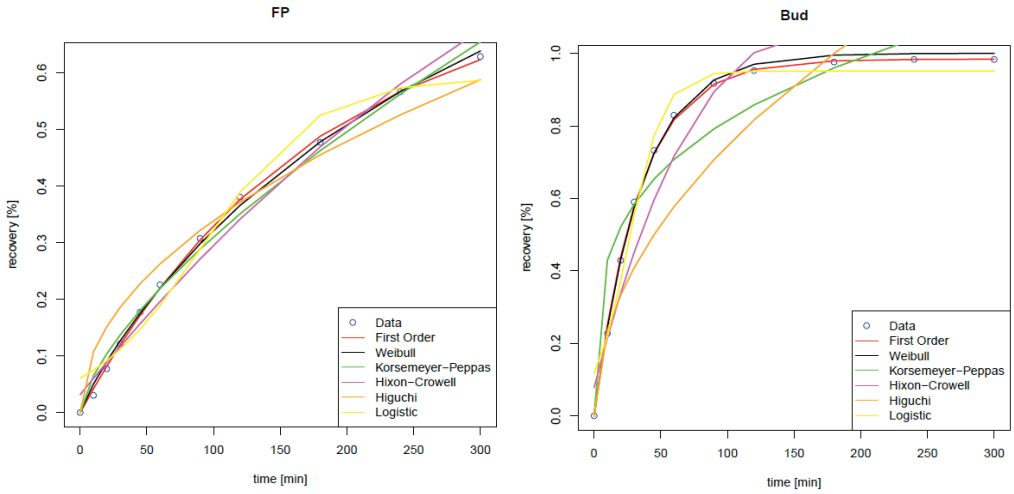


c) Ciclesonide without membrane dissolution data fits

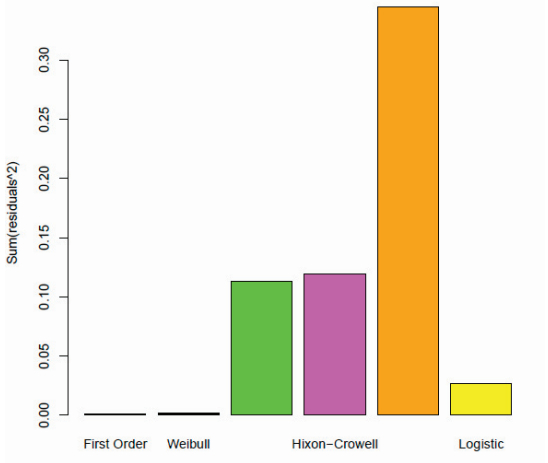


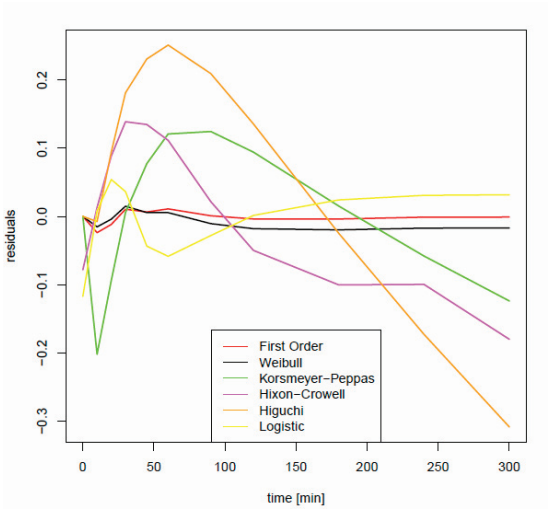
4. Budesonide vs. Fluticasone propionate plots and data fits

a) Budesonide (Bud) and Fluticasone propionate (FP) fitted plots



b) Budesonide dissolution data fits





c) Fluticasone propionate dissolution data fits