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RESEARCH PAPER

Dissolution Techniques for *In Vitro* Testing of Dry Powders for Inhalation

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ABSTRACT

Purpose To evaluate different dissolution testing methods and subsequently develop a simple to perform but reproducible and discriminating dissolution technique for inhalative powders.

Methods From a dry powder a fraction of aerosolized particles with an aerodynamic particle size below 5 μm was collected on regenerated cellulose membranes using an abbreviated Andersen cascade impactor. The membrane was then transferred to the respective dissolution set up either paddle apparatus with membrane holder, flow through cell or Franz diffusion cell.

Results All tested dissolution techniques could discriminate between good and poorly soluble substances, but only the paddle apparatus differentiated between small variations of solubility. We showed that membrane coverage and particle diameter play an important role for the dissolution rate. The profiles were fitted with mathematical models (e.g., Weibull, first order) choosing the best fit for determination of the mean dissolution time. Furthermore, a correlation between the dissolution profiles obtained with Franz cell compared to paddle apparatus could be shown.

Conclusion The paddle apparatus with membrane holder has the best discrimination power with optimal reproducibility.

KEY WORDS diffusion · flow through cell · Franz diffusion cell · paddle apparatus · aerosol for inhalation

ABBREVIATIONS

ACI	Andersen cascade impactor
DPI	dry powder inhaler
HPLC	high performance liquid chromatography
MDT	mean dissolution time
PE	polyethylen
PEEK	polyetherketone
RC	regenerated cellulose
SEM	scanning electron microscopy
SLF	simulated lung fluid

INTRODUCTION

Dissolution is defined as the process by which solid substances enter into a solvent to yield a solution. The process is controlled by the affinity between the solid substance and the solvent (1). Dissolution testing of solid and semi-solid dosage forms is a standardized test method in all pharmacopeias but there is no standardized method to measure the dissolution behavior of inhaled powders. Dissolution testing for oral dosage forms was introduced to differentiate several formulation types and should allow an estimate of dissolution behavior *in vivo*. It is routinely used in quality control during development, manufacturing and storage (2). In the future, dissolution testing of inhaled powders

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might be a meaningful tool for the development of formulations containing higher amounts of actives and/or for formulations containing actives with slow dissolution kinetics (3).

There are several hurdles to develop a dissolution test for inhaled formulations. A) The respiratory tract has a large surface area (>100 m^2) but only a small amount of aqueous fluid (approximately 10–20 ml/100 m^2) (2,4). B) Different clearance mechanisms in the lower and upper lung. In the upper respiratory tract a thick mucus film acts as a physical and chemical barrier to drug and particle diffusion. Particles which are trapped in the mucus are rapidly cleared by mucociliary clearance. In the lower airways and alveoli the lining fluid is thin (1 μm or less) and drug particles might be cleared through phagocytosis by macrophages (5).

Despite these obstacles several *in vitro* approaches of dissolution testing of aerosols have been set up in the past.

Besides different techniques e.g., modified twin stage impinger, flow through cell, horizontal diffusion cell, Franz cell, USP apparatus 4 (flow through cell) and USP apparatus 2 (paddle apparatus) with or without membrane containing cassette or membrane holder several substances and dissolution mediums could be found in literature (1,6–11).

Davies and Feddah tested the fine particle dose of three inhalative glucocorticoids in a flow through cell utilizing water, simulated lung fluid (SLF) and modified SLF with L-α-phosphatidylcholine (1). Salama *et al.* studied the drug release from controlled release microparticles for inhalation. They showed that only the Franz cell could differentiate between several formulations with different Polyvinyl alcohol percentages (8). Bur *et al.* (12) tested several commercially available inhalers after size fractionating of powders with a multi-stage liquid impinger with Calu 3 cells. Finally there were several other approaches of dispersing dry powders directly in commercial available dissolution systems (13,14).

The aim of this study was to compare different dissolution testing methods and also to study their ability to differentiate between different formulations. Furthermore, the development of a robust and reliable testing procedure for inhalative powders was the objective. The methods studied are a modified USP apparatus 2 (paddle apparatus), a modified USP apparatus 4 (flow through cell) and as a diffusion model, a modified Franz cell. The Franz cell was chosen to mimic the diffusion controlled air-liquid interface of the lung. In contrast, the paddle apparatus and flow through cell have no air liquid interface. Paddle Apparatus with membrane holder is a dissolution and diffusion controlled test system, but the membrane is completely immersed. Compared to Franz cell and paddle apparatus flow through cell is not diffusion controlled, but flow rate controlled. Hence, approaches based on different principles were evaluated for comparison.

MATERIALS AND METHODS

Materials

Budesonide was purchased from Cipla (India). Fenoterol HBr and Substance A (crystalline base, amorphous base (DSC data in Supplementary Material Fig. S1) and bromide) were obtained from Boehringer Ingelheim (Ingelheim, Germany). Substances were micronized via jet milling. Only the amorphous base was produced by spray drying (Table I).

PBS buffer tablets for dissolution medium and Acetonitrile (HPLC grade) was purchased from Sigma Aldrich Chemicals (Steinheim, Germany). Regenerated cellulose membrane filters (25 mm and 100 mm diameter) of 0.45 μm pore size were obtained by Whatman (Dassel, Germany) and Spartan filters for solubility measurement by Schleicher und Schuell (Dassel, Germany). KH_2PO_4 for eluent buffer pH 3 was purchased from Merck (Darmstadt, Germany). HPLC vials were obtained by Waters (Milford, USA) and Macherey Nagel (Düren, Germany), inserts for vials by Wicom (Heppenheim, Germany), respectively.

Water was purified by the use of a Milli Q® System (Millipore, Molsheim, France).

Physical and Chemical Characterization

Scanning Electron Microscopy

Micronized Budesonide particle deposition patterns and behavior on membranes were studied by imaging membranes after particle deposition using scanning electron microscopy (Leo, Gemini Supra 55vP with SE detector) with an acceleration voltage of 1 kV. For samples, polyethylen (PE) capsules were filled with 10 mg and 1 mg respectively. The powders were dispersed by use of a HandiHaler® (Boehringer Ingelheim, Ingelheim, Germany) and the aerosolized particles were collected as described in part *Dose collection*. Micronized Budesonide and membranes without powder, respectively act as references.

Table I Molecular Weight and Log D of Budesonide, Fenoterol HBr and Substance A Free Base

	Budesonide	Fenoterol HBr	Substance A free base
log D	3.2 (15)	−0.9 (16)	2.4
Molecular Weight	430.5 (17)	384.3 (17)	683.8

Geometric Particle Sizing

Geometrical diameter of micronized drug powder was studied using laser diffraction (Helos, Sympatec GmbH, Clausthal-Zellerfeld, Germany). Samples were dispersed with a dry powder unit (Rodos, Sympatec GmbH, Clausthal-Zellerfeld, Germany) at a pressure of 3 bar. Samples were analyzed in triplicate.

Aerodynamic Particle Size Determination

For classification of aerodynamic particle size distribution the Andersen cascade impactor (ACI) was used.

Substances were filled into PE capsules (1 mg each Budesonide, Substance A amorphous base, crystalline base, dibromide, and 10 mg Fenoterol) and dispersed with a HandiHaler® at standard USP conditions (4 kPa, 4 l).

Afterwards each collection plate, preseperator, High Top, SIP, Adapter, HandiHaler® and capsule were rinsed with solvent and the concentration was determined via HPLC. Particle sizing was done in triplicate.

Solubility Measurement

Saturated solutions of Budesonide, Fenoterol and modifications of Substance A were prepared by adding powder to PBS buffer pH 7.4. Flasks were covered with aluminum foil and stored in an overhead shaker at 22 °C, 50 % rH in a climate cabinet. After 24 h the suspensions were filtered using a Spartan filter (13/0.45 RC). Adsorption of drugs onto filter and syringe was evaluated before performing solubility measurements. Fenoterol HBr was diluted three times. Concentrations were determined with HPLC. Solubility measurements were performed in triplicate.

High Performance Liquid Chromatography (HPLC)

All quantification measurements of Substance A, Budesonide and Fenoterol HBr were determined by HPLC. Analysis was performed using an Alliance system with UV–VIS detector (operating at 240 nm for Budesonide, 225 nm for Substances A and 280 nm for Fenoterol). Reverse phase chromatography was performed using a LiChrosphor 60 RP select B, 60×4 mm column, purchased from MZ Analysentechnik (Mainz, Germany). The column temperature was set to 40 °C. The injected volume of each sample was 10 µl. The mobile phase consisted of buffer pH 3 and Acetonitrile and was eluted at a flow rate of 1.7 ml min^{-1} for Budesonide and Substance A and 1 ml min^{-1} for Fenoterol.

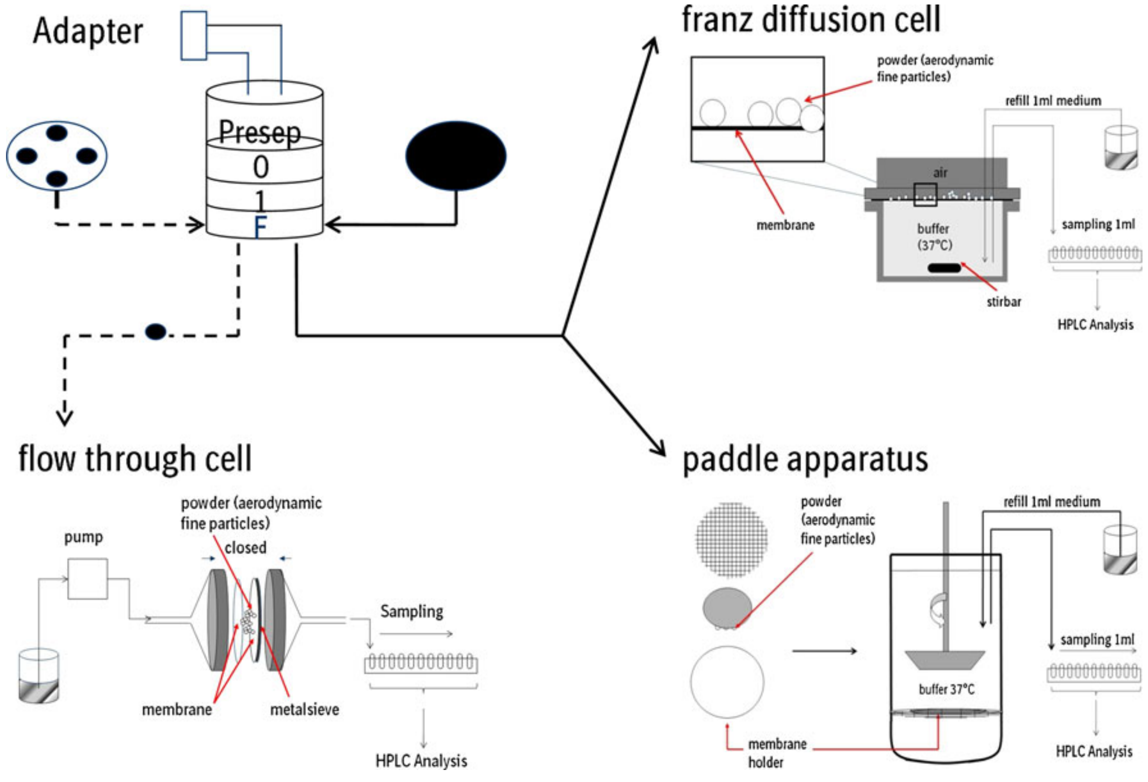

Fig. 1 Schematic diagram of abbreviated ACI and dissolution techniques. Powder was classified by the use of an abbreviated ACI. Particles less than 5 μm on 80 mm membranes were tested either in Franz diffusion cell or in paddle apparatus with membrane holder (particles faced down to the watch glass). Dissolution of particles smaller than 5 μm on 25 mm membranes were analysed in flow through cell. Figure modified from (3).

Table II Time Schedule for Dissolution Set-ups

Flow through cell		Franz cell		Paddle apparatus	
Sample 1–9	0.25 min	sample 1–21	3 min	sample 1–20	3 min
Sample 10–13	0. 5 min	sample 22–33	5 min	sample 21–31	5 min
Sample 14	1 min	sample 34–44	15 min	sample 32–48	15 min
Sample 15–19	5 min	sample 50–68	60 min	sample 49–65	60 min
Sample 20, 21	15 min	For Fenoterol first 20 samples differ, after see above (sample 21)			
		sample 1–21	1.5 min	sample 1–20	1 min

Dose Collection

For dose collection an abbreviated Andersen cascade impactor was used. Substances were filled into PE capsules (1 mg Budesonide, modifications of Substances A; 10 mg Budesonide, Fenoterol) and dispersed with a HandiHaler® at standard USP conditions (4 kPa, 4 l). Particles $d_{\text{aerodynamic}} < 5\ \mu\text{m}$ were collected on the filter membrane consisting of regenerated cellulose. For flow through cell experiments 4 filters (diameter 25 mm) were placed into a template. The template consists of PEEK and has four voids for the filters. The template placed into the filter stage of an ACI ensures an air stream only through the membranes. For Franz diffusion cell and paddle apparatus experiments a membrane with a diameter of 80 mm were used.

After drug deposition the filter membranes were removed from the ACI and placed into the corresponding dissolution test system.

Dissolution Apparatus

Release profiles of aerodynamically classified particles of Substance A in its different forms, Budesonide, and Fenoterol were investigated using three different methods: USP apparatus 2 (paddle apparatus), USP apparatus 4 (flow through cell) and modified Franz diffusion cell.

All experiments were done in triplicate and concentrations were determined with HPLC.

Dissolution tests were performed at 37 °C using degassed PBS buffer pH 7.4 as dissolution medium. The total amount of drug initially loaded on the membranes was measured using the maximum of the cumulatively released amounts plus the remaining quantity of particles on the membrane (determined at the end of each experiment). Percentages of drug released were calculated by dividing the amount of drug released by the drug mass loaded on the membrane after application with the ACI.

Flow Through Cell

An advanced flow through cell design (Fig. 1) (1) was modified by Boehringer Ingelheim to ensure uniform flow through the membrane which was obtained by a special insert. After dose collection the drug loaded membrane was covered with a second membrane and placed into the flow through cell. The dissolution medium was pumped through the apparatus using a HPLC pump in an open method set up. Flow rate was constant and set to 1 ml min^{-1}. According to a defined time schedule probe sampling was automated by a Gilson sample injector (Gilson, Middleton, UK) (Table II).

At the end of the experimental procedure all parts of the flow through cell were washed with a defined amount of solvent according to the used substance to determine the total recovery. As solvent were used Acetonitrile for Budesonide, Substance A amorphous base and Substance A crystalline base, purified water for Fenoterol and PBS buffer pH 7.4 for Substance A dibromide.

Franz Diffusion Cell

The Franz diffusion cell is based on a modified set up described by Franz (11) and Salama (8). The modified Franz cell consists of a dissolution medium reservoir filled with 1 l PBS buffer pH 7.4 and a heated membrane holder to

Table III Dissolution Models

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

Table IV Best Fits Describing Dissolution Curves from Which Respective MDT Was Determined

Dissolution technique	Substances	Capsule weight [mg]	Fit
Flow through cell	Budesonide	10	Weibull
	Budesonide	1	Weibull
	Substance A base crystalline	1	Weibull
	Substance A base amorphous	1	Weibull
	Substance A Br	1	Weibull
	Fenoterol	10	Weibull
Franz cell	Budesonide	10	First order
	Budesonide	1	First order
	Substance A base crystalline	1	First order
	Substance A base amorphous	1	First order
	Substance A Br	1	First order
	Fenoterol	10	First order
Paddle apparatus	Budesonide 50 rpm	10	Weibull
	Budesonide 100 rpm	10	First order
	Budesonide 140 rpm	10	First order
	Budesonide	1	Logistic
	Substance A base crystalline	1	Weibull
	Substance A base amorphous	1	First order
	Fenoterol	10	First order

avoid condensation. The membrane is placed into the holder, particles facing up, and brought in contact to the dissolution medium, barely touching it, ensuring that the formulation is at the air–liquid interface. Stirring speed was set to 100 rpm. Sampling was automated according to a defined time schedule (Table II). The solvent removed during sampling was refilled with fresh pre-warmed PBS buffer (37 °C) to maintain a constant dissolution medium volume. After finishing sampling the filter was removed and rinsed with 50 ml solvent for dissolving the remaining particles. The same solvents as described above were used.

Paddle Apparatus with Membrane Holder

The paddle apparatus with membrane holder was developed by Son *et al.* (10). The apparatus consists of a vessel with 1 l dissolution medium which is immersed in a water bath (Erweka DT70, Erweka, Heusenstamm, Germany). The membrane filter was placed with the particles towards the watch glass into the membrane holder (Copley Scientific, Nottingham, UK) and then placed into temperature-controlled paddle apparatus, release surface sided up. Distance between bottom edge of the paddle and surface of the membrane holder maintained 25±2 mm as requested for distance between paddle and bottom of the vessel in standard USP apparatus (14). At the beginning stirring speeds of 50 rpm, 100 rpm and 140 rpm were tested. All following experiments were performed with a stirring speed of 140 rpm. Automated sampling took place at a defined time schedule (Table II). Solvent removed during sampling was refilled with fresh pre-warmed PBS buffer (37 °C) to maintain the volume of the dissolution medium constant. After dissolution testing residual amounts of drug in and on

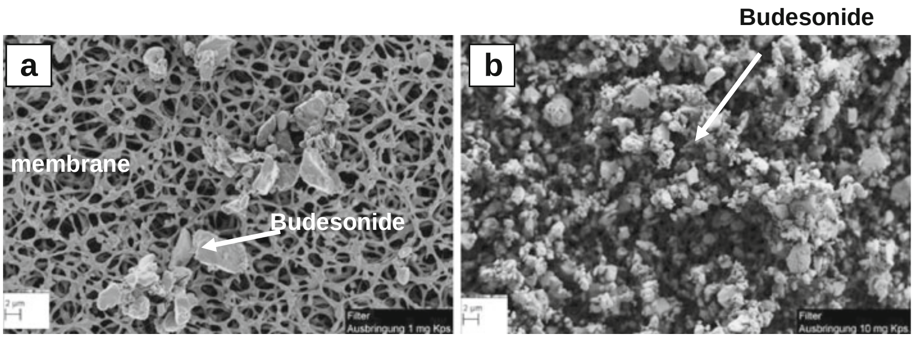

Fig. 2 SEM pictures of (**a**) membrane with Budesonide after dispersion of 1 mg capsule, (**b**) membrane with Budesonide after dispersion of 10 mg capsule via short stack ACI. Scale bar represents 2 μm.

Table V Geometric Particle Size (Mean ± Standard Deviation)

	Budesonide	Fenoterol	Substance A		
			Cryst. Base	Amorp. base	Dibromide
x10 [μm]	0.54 ± 0.01	0.69 ± 0.01	0.71 ± 0.02	0.52 ± 0.02	0.62 ± 0.01
x50 [μm]	1.53 ± 0.02	1.90 ± 0.01	1.64 ± 0.00	1.24 ± 0.03	1.67 ± 0.07
x90 [μm]	3.69 ± 0.03	4.03 ± 0.01	3.54 ± 0.08	3.07 ± 0.10	6.65 ± 0.63
Q5 [Vol%]	96.52 ± 0.18	95.83 ± 0.07	97.68 ± 0.48	97.45 ± 0.32	84.02 ± 2.24

x10, x50, x90=10 %, 50 %, 90 % of particle diameter in μm

Q5 = Vol% of particles with a diameter ≤ 5 μm

the membrane and watch glass were determined by washing both parts with each 50 ml solvent. Solvents were described above.

Data Treatment

From the experiments a mean dissolution curve with standard deviations were calculated and used for further analysis.

The resulting release curves were fitted with different mathematical models (Table III) and best fits were taken (Table IV) for calculating mean dissolution time (MDT) (Eq. 1) (18). Comparison of the different techniques and substances was done with MDT ± standard deviation.

$$MDT = \frac{\sum_{i=1}^{n} \bar{t}_i + \Delta M_i}{\sum_{i=1}^{n} \Delta M_i} \quad (1)$$

RESULTS

Physical and Chemical Characterization

Scanning Electron Microscopy

Particles collected on the filter are shown in Fig. 2. Images A and B show the differences between dispersion of a 1 and a 10 mg capsule. As apparent in A objects were widespread on the filter in contrast to B where particles are covering the complete surface forming heaps and agglomerates.

Geometric Particle Sizing

Size distribution of Budesonide, Fenoterol HBr and forms of substance A particles measured by the laser diffraction technique is shown in Table V. All substances except of substance A dibromide showed a high amount of particles less than 5 μm.

Table VI Particle Size Distribution, Mean ± Standard Deviation

	Budesonide [%]	Fenoterol [%]	Substance A		
			Dibromide [%]	Crystalline base [%]	Amorphous base [%]
HH + capsule	24.0 ± 1.8	11.2 ± 1.8	8.9 ± 2.6	27.7 ± 3.0	10.6 ± 3.4
Adapter	20.5 ± 7.2	20.8 ± 7.2	12.0 ± 7.5	9.6 ± 1.1	24.9 ± 5.0
SIP					
High top					
Presep	25.6 ± 3.8	48.7 ± 13.2	46.3 ± 8.2	8.1 ± 1.9	41.3 ± 4.3
Stage 0	2.2 ± 0.1	1.2 ± 0.2	1.1 ± 0.2	0.1 ± 0.2	0.4 ± 0.1
Stage 1	4.0 ± 0.2	2.0 ± 0.2	1.2 ± 0.2	2.2 ± 0.3	0.8 ± 0.1
Stage 2	4.7 ± 0.3	3.3 ± 0.2	0.9 ± 0.1	3.9 ± 0.5	1.3 ± 0.4
Stage 3	7.5 ± 0.6	4.6 ± 0.5	1.5 ± 0.5	9.3 ± 1.1	2.2 ± 0.6
Stage 4	6.9 ± 0.8	3.3 ± 0.7	1.7 ± 0.5	13.9 ± 1.9	2.3 ± 0.9
Stage 5	4.6 ± 0.4	1.3 ± 0.5	2.0 ± 0.5	8.1 ± 1.4	1.9 ± 0.7
Stage 6	0.8 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	1.4 ± 0.2	0.4 ± 0.2
Stage 7	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.4 ± 0.1	0.2 ± 0.2
Filter	0.4 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.2 ± 0.1
FPF (%) ACI	25.2 ± 1.9	12.9 ± 2.4	6.5 ± 1.8	37.4 ± 4.6	8.5 ± 2.8
FPF (%) abbreviated ACI	27.8 ± 2.7	17.6 ± 1.7	6.5 ± 4.0	45.5 ± 2.7	17.8 ± 3.7

Aerodynamic Particle Size Determination

Substance A, Budesonide and Fenoterol HBr particles were collected using ACI impaction plates. Results are shown in Table VI. Percentages were calculated based on the capsule load.

As shown in Table VI particle size distribution of Substance A dibromide and amorphous base is not significantly different to each other except stage 5. Furthermore, the amount of smallest particles (stage 6—filter) of Substance A is increased *versus* Budesonide. Table VI also shows only small difference between FPF calculated of data from particle size measurement of the whole ACI and directly determination of the FPF at the abbreviated ACI.

Solubility Measurement

The solubility of Budesonide in PBS buffer pH 7.4 was 16.68±0.21 μg ml^{-1} (literature solubility 23 μg/ml (1)). Fenoterol is classified as good soluble substance. We could measure a solubility of 42.86±0.48 mg ml^{-1}.

Solubility of Substance A crystalline base was 7 μg ml^{-1}, of amorphous base 210 μg ml^{-1} and for crystalline dibromide 265 μg ml^{-1}. Hence, the solubility could be ranked from Fenoterol > Substance A dibromide > Substance A amorphous base > Budesonide > Substance A crystalline base.

Dissolution Apparatus

The measured dissolution rates in dissolution apparatus are shown in Fig. 3. As expected from the solubility data, Fenoterol is fastest dissolved under all experimental conditions whereas the crystalline base of Substance A is always slowest. Furthermore, a higher amount of Budesonide deposited on the membrane resulted in a slower dissolution than a smaller amount.

Flow Through Cell

In flow through cell, dissolution profiles of Substance A dibromide and amorphous base were similar in the first 20 min. Hereafter the dissolution profile of the amorphous base (100 % dissolved amount after 30 min) increased slower than the one of dibromide (100 % dissolved amount after 30 min). Interestingly, Budesonide (1 mg) is faster dissolved despite its lower solubility than the dibromide and the amorphous base of Substances A. Except for Substance A dibromide and the amorphous base (9.06±2.13 min *vs.* 9.40±0.66 min) MDT of all tested substances is significantly different from each other (Fig. 4 groups a–e).

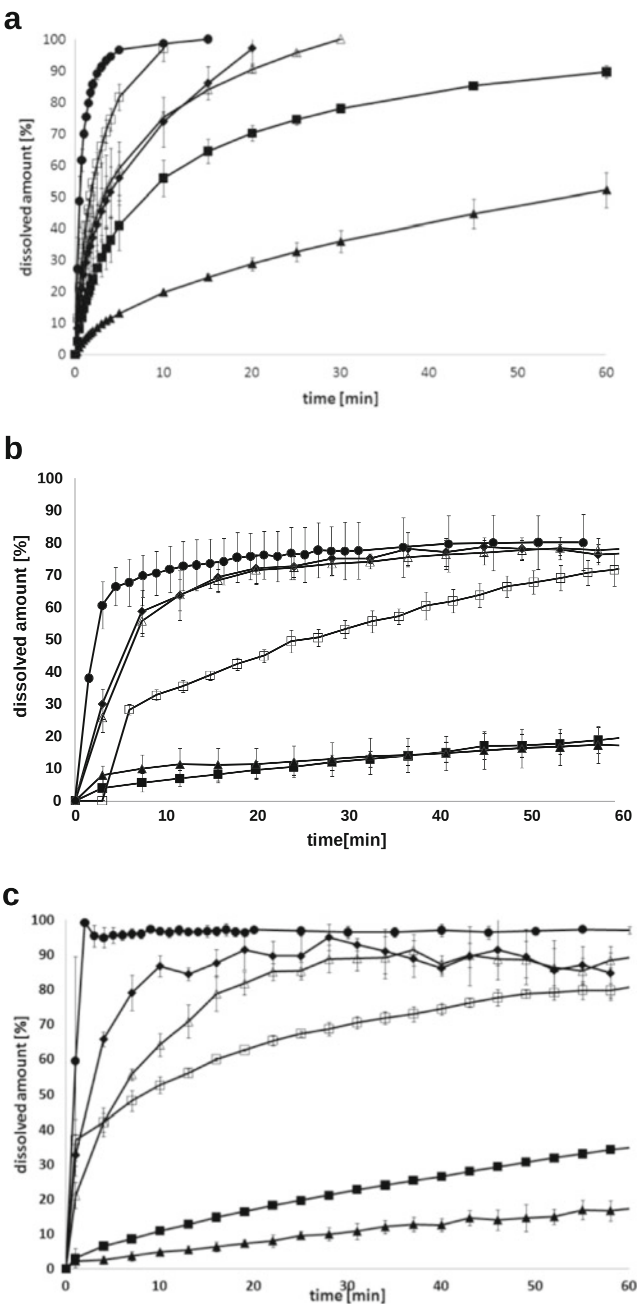

Fig. 3 Dissolution profiles in (**a**) flow through cell, (**b**) Franz cell, (**c**) paddle apparatus Budesonide 1 mg—□; Budesonide 10 mg—■; Fenoterol—●; Substance A dibromide—♦; Substance A crystalline—▲; Substance A amorphous—▵. *n*=3, mean ± SD for (**a**) crystalline base, Fenoterol, Budesonide 1 mg: *n*=2, mean ± SD.

Franz Diffusion Cell

In the first 60 min dissolution profile of Budesonide 10 mg *vs.* crystalline base of Substance A and profiles of Substance A dibromide *vs.* amorphous base over hole time, respectively are not significantly different from each other.

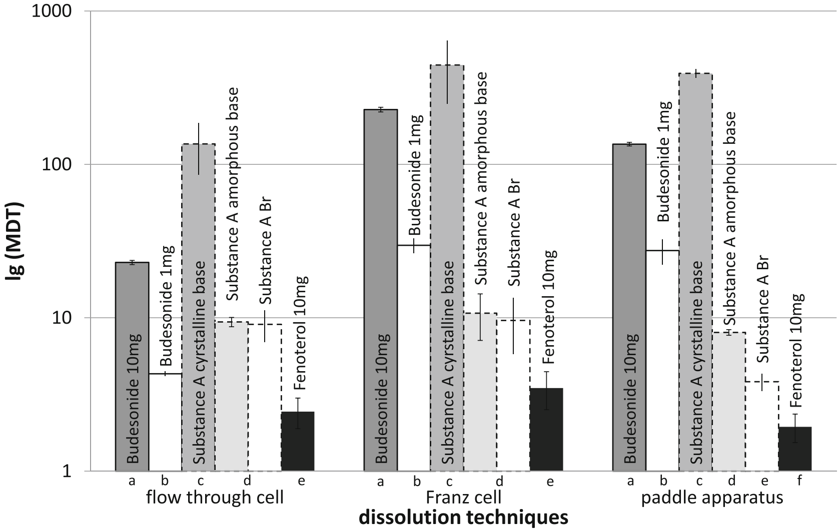

Fig. 4 MDT of Budesonide, Fenoterol and Substance A as determined with different dissolution techniques (n=3, mean ± 95 % confidence interval).

In Franz cell the dissolved mass never exceeded 90 %. Furthermore a wide range in standard deviation is noticeable. MDTs were significantly different except Substance A dibromide and amorphous base (8.25±3.84 min *vs.* 9.57±3.60 min) compared to each other (Fig. 4 groups a–e).

Paddle Apparatus with Membrane Holder

Figure 5a shows the dissolved amount of Budesonide in paddle apparatus with membrane holder at different stirring speed. MDT is shown in Fig. 5b. As already displayed in Fig. 5a 100 rpm and 140 rpm were not significantly different but at 140 rpm smaller errors were found. Therefore, 140 rpm was chosen for all other experiments.

In the paddle apparatus ranking of dissolution profiles corresponded to the solubility of substances. Additionally, discrimination between profiles of amorphous base and dibromide of Substance A could be achieved (Fig. 3c).

As shown in Fig. 4 groups a–f MDTs for all substances are significantly different to each other considering their 95 % confidence intervals.

Corresponding results for Budesonide were also found for two commercial DPIs: Budes®Easyhaler® 0.2 mg (Hexal, Holzkirchen, Germany) and Budecort®200 Novolizer® (Astellas Pharma, München, Germany).

DISCUSSION

The aim of this study was to compare three different dissolution techniques and the evaluation of the dissolution behavior of aerodynamically deposited fine particles.

As described above in the flow through cell set up Substance A dibromide and amorphous base showed a similar dissolution profile in the first 20 min and later the profiles separated. Furthermore, the dissolution of both after 3 min is significantly slower, even though having a higher solubility, in comparison to Budesonide (Fig. 3a).

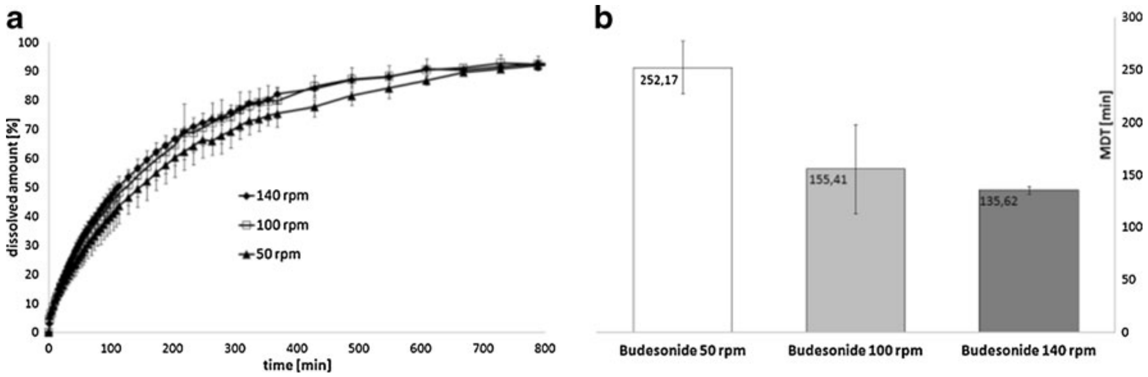

Fig. 5 (**a**) Release profiles of Budesonide at different stirring speed in paddle apparatus with membrane holder for Budesonide. Error bars indicate the standard deviation of three tests. n=3, mean ± SD. (**b**) Comparison of MDT for several stirring speeds in paddle apparatus with membrane holder for Budesonide. Error bars indicate the standard deviation of three tests. n=3, mean ± SD.

Under the assumption that particle density of all substances (used in this study) is the same, particles are spherical and geometric and aerodynamic diameter are similar, the reason might be found in particle size distribution of particles less than 5 µm. Especially for the smallest particles the surface increases dramatically with decreasing diameter. Amorphous base and dibromide show similar particle size while Budesonide particle size at most stages is significantly smaller (Table VI). From Noyes Whitney equation (18) the opposite would have been expected hence the reason could be found in flow through cell method set up.

At Franz diffusion cell, dissolution profiles were in expected order apart from the amorphous base and the dibromide of Substance A. A possible explanation could be particle size distribution for those two substances under the assumption described above. In this case ranking of substance is consistent with Noyes Whitney in combination with particle size distribution of ACI. In contrast, to the flow through cell, the dissolution profile of Budesonide was slower than those of Substance A amorphous base and dibromide, as expected. All Franz cell attempts could be fitted with first order model (Table IV). First order processes depend only on the concentration of the dissolving substance. So, substances with higher solubility are faster dissolved and can diffuse through the membrane in contrast to those with lower solubility. As shown in Fig. 3b Franz cell dissolution profiles reached never more than 90 % of recovery rate and showed a wide range in standard deviation. Contact area to dissolution medium under membrane might not be homogenous due to small air bubbles or wrinkles in membrane therefore wetting of particle on membrane is not consistent or even particles might not be wetted.

As described at paddle apparatus three different stirring speeds were tested. With increasing stirring speed, error bars decreased (Fig. 5 a, b) due to better circulation in the vessel. A stirring speed of more than 140 rpm was, however, not possible because the membrane holder was irregularly moving otherwise. Son *et al.* mentioned already a dead volume between membrane holder and bottom of the vessel and that circulation of dissolution medium is hindered despite medium circulates between the holder and the vessel wall around the holder (10). Hence stirring of dead volumes is only assured at higher stirrings speeds.

Paddle apparatus could discriminate between all substances tested and ranking of dissolution profiles was as expected.

A final comparison over all test apparatus is difficult to draw but there are some similarities and differences. Due to dissolution profiles all techniques could differentiate between good and poorly soluble substances. Furthermore, deposited mass on membrane plays an important role (Budesonide 1 mg *vs.* 10 mg, respectively) for dissolution as shown with dissolution profiles (Fig. 3) and SEM pictures (Fig. 2). A higher membrane loading lead to a decreasing dissolution rate. SEM picture after dispersing a 10 mg capsule showed complete coverage of the surface with heaps and agglomerates, having, compared to single particles, a smaller surface resulting in a slower solubility rate and might have wetting problems. Additionally, flow through cell results of Substance A differed from those published by Jensen *et al.* (3). They reported that the flow through cell could discriminate between different forms of Substance A. However, the capsule weight of Jensen was much higher than the 1 mg capsule weight used in the study described in this paper leading to high membrane coverage and hence modified dissolution.

For Budesonide and Substance A crystalline base flow through cell is the significantly fastest technique. Except for the dibromide of Substance A, results after dispersing a 1 mg capsule across all substances showed no significant differences between Franz cell and paddle apparatus. MDTs of substances in paddle apparatus present smallest error bars compared with Franz cell and flow through cell (Fig. 4). Consequently paddle apparatus is the most suitable technique.

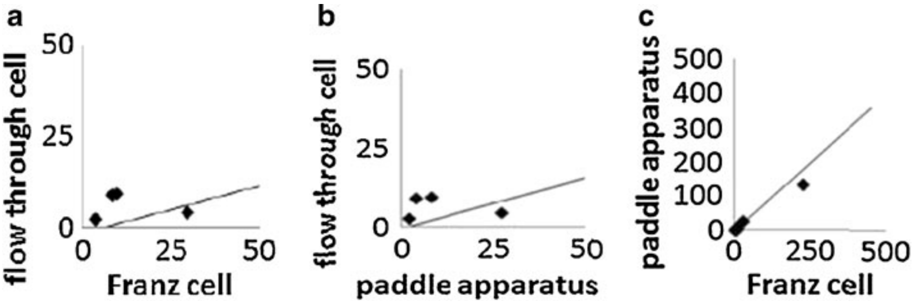

Fig. 6 Correlation between MDTs in minutes. (**a**) Flow through cell and Franz cell (R^2:0.8659). (**b**) Flow through cell and paddle apparatus (R^2: 0.9521). (**c**) Paddle apparatus and Franz cell (R^2: 0.9714) For better visibility in (**a**) and (**b**) axis are downscaled to 50 min. As a consequence the results for Budesonide 10 mg and substance A crystalline are not visible but included in the fitting.

The three dissolution methods differ in discrimination of Substance A amorphous base and dibromide. Flow through cell and Franz cell compared with paddle apparatus were not able to differentiate between the two compounds. Mechanistically Franz cell is similar to paddle apparatus but both are different from the flow through cell. Dissolution profiles of Franz cell and for almost all cases at paddle apparatus in contrast to flow through cell can be described with mathematic first order model (Table III). Moreover a good correlation of MDT between paddle apparatus and Franz cell could be shown in Fig. 6c, although paddle apparatus is the better discriminating and reproducible technique. For flow through cell no correlation of MDT with any other test set up could be found (Fig. 6a, b).

In contrast to the results of Salama *et al.* (8) in this study the Franz diffusion cell was not able to differentiate between substances if their solubility was very close. A possible reason might the different method set up. Salama *et al.* worked with controlled released drug polymer particles, a different membrane (nitrocellulose filter) and a much higher membrane load (20 mg) which showed here to influence the dissolution profile (8).

Paddle apparatus was superior to Franz cell and flow through cell because it was the only technique which could differentiate between different solubilities. Furthermore, paddle apparatus showed the best reproducibility with smallest standard deviation.

CONCLUSION

In this study we tested three dissolution techniques for inhaled powders. In order of solubility data we estimated with higher solubility a faster dissolution rate. We showed that all apparatus could discriminate between poor and good soluble substances but only paddle apparatus was able to discriminate materials with small differences in solubility while showing also good reproducibility. Furthermore we demonstrated that membrane loading has a large influence on dissolution and has to be strongly investigated in the future. Although Franz cell system due to air liquid interface is closer to lung, paddle apparatus is the best discriminating technique.

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