Influence of particle diameter on aerosolization performance and release of budesonide loaded mesoporous silica particles

Irès van der Zwaan a, Georgia A. Pilkington b,c, Göran Frenning a,*, Mikael Ekström d, Sabrina Valetti a,†, Gary R. Pitcairn b, Adam Feiler b,c

a Department of Pharmaceutical Biosciences and the Swedish Drug Delivery Center (SweDeliver), Uppsala University, P.O. Box 580, 751 23 Uppsala, Sweden
b Nanologica, Forskargatan 20 G, SE-151 36 Södertälje, Sweden
c Surface and Corrosion Science, KTH Royal Institute of Technology, SE-100 44 Stockholm, Sweden
d Iconovo AB, Ideongatan 3A-B, SE-223 70 Lund, Sweden

* Corresponding author.
E-mail address: goran.frenning@uu.se (G. Frenning).

Contents lists available at ScienceDirect
European Journal of Pharmaceutical Sciences
journal homepage: www.elsevier.com/locate/ejps

ARTICLE INFO

Keywords:
- Pulmonary drug delivery
- Mesoporous silica
- Controlled release
- Particle diameter
- Fine particle fraction

ABSTRACT

The potential of micron-sized amorphous mesoporous silica particles as a novel controlled release drug delivery system for pulmonary administration has been investigated. Mesoporous silica formulations were demonstrated to provide a narrower particle size distribution and (spherical) shape uniformity compared to commercial micronized formulations, which is critical for repeatable and targeted aerosol delivery to the lungs. The release profiles of a well-known pulmonary drug loaded into mesoporous particles of different mean particle diameters (2.4, 3.9 and 6.3 µm) were analysed after aerosolization in a modified Andersen Cascade Impactor. Systematic control of the release rate of drug loaded into the particles was demonstrated in simulated lung fluid by variation of the mean particle diameter, as well as an enhanced release compared to a commercial micronized formulation. The mesoporous silica formulations all demonstrated an increased release rate of the loaded drug and moreover, under aerosolization from a commercial, low-cost dry powder inhaler (DPI) device, the formulations showed excellent performance, with low retainment and commercially viable fine particle fractions (FPFs). In addition, the measured median mass aerodynamic diameter (MMAD) of the different formulations (2.8, 4.1 and 6.2 µm) was shown to be tuneable with particle size, which can be helpful for targeting different regions in the lung. Together these results demonstrate that mesoporous silica formulations offer a promising novel alternative to current dry powder formulations for pulmonary drug delivery.

1. Introduction

In recent decades, orally inhaled drugs have helped several million patients suffering from diseases, such as asthma and chronic obstructive pulmonary disease (COPD), by successfully reducing their symptoms (Anderson et al., 2022; Forbes et al., 2011). Besides providing local delivery to the lungs, the pulmonary route is also suitable for systemic delivery, with many known advantages in comparison to using the oral route (Islam and Gladki, 2008; Pilcer and Amighi, 2010). For example, inside the lungs there is a large available surface area, with a thin blood-alveolar barrier, that can facilitate the rapid onset of action and avoidance of the first pass metabolism (Islam and Ferro, 2016; Lorienta-Pastoriza et al., 2014). Many commonly used inhaled products utilise dry powder inhalers (DPIs), which deliver drugs to the lung in a solid form. The popularity of DPIs is largely due to them being considered more user-friendly, compared to pressurised Metered Dose Inhalers (pMDI), their high drug load capacity and the generally higher stability of drugs when formulated in a solid form (de Boer et al., 2017; De Boer and Thalberg, 2021; Velaga et al., 2018).

To successfully deliver to the lungs, the aerodynamic particle size of the powdered drugs is critical. It is known that, for drug delivery to the lungs, the powder particles should have an aerodynamic particle size between 1 and 5 µm; this has been shown to be the optimal size range, as particles above 5 µm will directly impact in the mouth and throat area (Islam and Gladki, 2008). Current DPI formulations are generally produced by milling or spray drying. These strategies can produce particles

* Corresponding author.
E-mail address: goran.frenning@uu.se (G. Frenning).

https://doi.org/10.1016/j.ejps.2024.106828
Received 5 March 2024; Received in revised form 24 May 2024; Accepted 8 June 2024
Available online 9 June 2024
0928-0987/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
in the correct size range, as well as offer some degree of control over the particle size and morphology. However, possible strategies towards tailoring the release profiles of loaded drug from the particles are quite limited (Chow et al., 2007; Rasenack and Müller, 2004; Shetty et al., 2020).

The ability to control the rate at which drugs are released into the airway surface liquid is anticipated to offer potential therapeutic benefits as this could influence the rate of absorption of the drug (Gerde et al., 2017; Patton et al., 2010; Riley et al., 2012). One delivery strategy that could provide both control over the particle size, as well as the possibility to control drug release profiles is the use of mesoporous particles made from amorphous silica. The ability to systematically tune the particle-size distribution could help targeting different regions in the lung.

Mesoporous silica particles have been extensively studied as a pharmaceutical delivery system since 2001, when the first pharmaceutical formulation incorporating mesoporous particles was shown (Vallet-Regi et al., 2001). While considered safe for use as food additives or in vitamin supplements (Barbé et al., 2004) mesoporous silica is not approved for pulmonary administration. However, early in vivo studies indicate that amorphous silica, as studied here, can be cleared from the lungs with no persistent or progressive toxicological effects, as is known to be the case for crystalline silica (Arts et al., 2007; Fruijtier-Polloth, 2012). In a more recent study, in vivo lung clearance of different amorphous silica particles was successfully demonstrated with a half-clearance time shorter than 6 h (Pilkington et al., 2023). In addition, Campus Pacheco et al. demonstrated no measurable toxicity for amorphous silica particles similar to those used in the present study (Campos Pacheco et al., 2024). Overall, these studies show the potential of using amorphous silica particles for inhalation.

The pore size of mesoporous particles can be carefully controlled, as can the size of the particles. In particular, it has been demonstrated that the highly tuneable pore size (2–50 nm) of porous silica particles can facilitate the loading of a wide variety of drugs and larger biological molecules, while their inherently high surface area can allow for high drug loading amounts (Bharti et al., 2015; Valetti et al., 2021, 2017a). Moreover, encapsulation within the silica matrix has been shown as an effective method for enhancing the release of poorly soluble drugs in

### Table 1

Characteristics of the Budesonide mesoporous silica formulations.

<table>
<thead>
<tr>
<th>Particle</th>
<th>Drug</th>
<th>Mean Particle Diameter (µm)</th>
<th>Average Pore Size (nm)</th>
<th>Specific Surface area (m²/g)</th>
<th>Drug loading (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Budesonide</td>
<td>2.4</td>
<td>11.6</td>
<td>312</td>
<td>16.8</td>
</tr>
<tr>
<td>B</td>
<td>3.9</td>
<td>11.1</td>
<td>298</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6.3</td>
<td>11.3</td>
<td>305</td>
<td>16.7</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2

Aerodynamic Particle Size Distribution (APSD) results for the mesoporous silica formulations from a ICOone inhaler at 64 L/min.

<table>
<thead>
<tr>
<th>Particle</th>
<th>Drug</th>
<th>Mean Particle Diameter (µm)</th>
<th>MMAD (µm)</th>
<th>FPF (%) of DD</th>
<th>DD (%) of MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Budesonide</td>
<td>2.4</td>
<td>2.8</td>
<td>70</td>
<td>80.1</td>
</tr>
<tr>
<td>B</td>
<td>3.9</td>
<td>4.1</td>
<td>40</td>
<td>91.2</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>6.3</td>
<td>6.2</td>
<td>15</td>
<td>96.3</td>
<td></td>
</tr>
</tbody>
</table>

*a* Median Mass Aerodynamic Diameter.  
*b* FPF - Fine Particle Fraction.  
*c* Delivered Dose.  
*d* Metered Dose.

Fig. 1. Schematic diagram of dose collection using the mACI and subsequent release experiments using a Transwell setup.

Fig. 2. Symbols represent MMAD values of encapsulated budesonide loaded into mesoporous particles of different diameters actuated from ICOone at 64 L/min. The error bars indicate the standard deviation of two replicates. Error bars are present in all points, however for the 3.9 (Particle B) and 6.3 µm (Particle C) the error bars are too small to be visible in the graph.
different budesonide loaded mesoporous formulations studied, which had an McGowan molecular volume. Budesonide is used as a lung-relevant 2021; Gulin-Sarfraz et al., 2019).

Within parentheses, the respective NGI cut-off diameters for each stage are

2. Materials and methods

2.1. Materials

The mesoporous particles used in this study were supplied by Nanologica AB. The particles are formed from pure amorphous silica (SiO₂; cf. Supplementary Information (SI); Fig. S1 and description below) and hydrophilic in nature due to the surface silanol groups. The hydrophilic nature of the silica is essential for dissolution and clearance of the particles in the lungs. The single point adsorption total pore volume of the particles and BET surface area were measured using a Tristar II 3020 analyzer (Micromeritics) and were between 0.8 – 0.9 cm³/g and 305 – 312 m²/g, respectively, for particles A, B and C. The average pore size was inferred from the single-point BET data as described earlier (Campos Pacheco et al., 2024). All particles had a comparable polydispersity with a d90/d10 between 1.3 – 1.7, determined by the Elzone II 5390 Particle size analyser (Micromeritics) that infers particle size from the volume of electrolyte that is displaced by a particle as it flows through the electrical sensing zone. X-Ray Powder Diffraction (XRD) analysis was performed to verify the absence of crystalline content in the mesoporous particles using a PANalytical X’Pert PRO diffractometer. The XRD samples were smeared onto a silicon zero background holder and analysed at room temperature between 2 – 40° in 2-theta, the sample was spun during the analysis to increase randomness (cf. SI; Fig. S1).

Particles of different mean diameters (2.4, 3.9 and 6.3 µm, particle A, B and C, respectively; cf. Table 1), and average pore sizes (11.1 – 11.6 nm) were loaded with budesonide (Zhou et al., 2018). All measurements were performed on single batches (1.5 g) of the loaded particles. All formulations were prepared using a proprietary method (Nanologica AB, Sweden). Briefly this involves suspending budesonide in a selected solvent, allowing or driving the adsorption inside the pores, whilst or before removing the solvent.

Validation that particles contained the loaded drugs in an amorphous form was provided by differential scanning calorimetry (DSC823e, Mettler Toledo AB) measurements (cf. SI; Fig. S2). In comparison, the micronized Turbuhaler formulation showed clear crystalline melting peaks within the same temperature range (cf. SI; Fig. S2).

The mass of drug contained with the particle matrix (drug loading) was between 16 and 17 wt% for all particles (cf. Table 1), determined by Thermal Gravimetric Analysis (TGA/DSC 3+ STARé System, Mettler Toledo AB). The drug load was determined from the weight loss upon heating, using unloaded mesoporous particles as reference (Campos Pacheco et al., 2024). Considering safety and patient convenience issues, 5–20 mg of mesoporous silica particles is considered suitable for a single delivery, which, for the given drug load, would correspond to a maximal dose of about 1–5 mg. Additionally, a commercial product containing budesonide (Pulmicort Turbuhaler®; 400 µg/dose) was purchased from Distansapoteket (Stockholm, Sweden) and the corresponding unformulated budesonide (Pharmaceutical Secondary Standard) was purchased from Sigma-Aldrich (Germany).

The drug release profiles were measured in simulated lung fluid (SLF, Gamble solution). For the SLF solution, sodium chloride, magnesium chloride, potassium chloride, disodium hydrogen phosphate, sodium

Fig. 3. NGI stage data presented as a percentage of delivered dose (DD) for the different budesonide loaded mesoporous formulations studied, which had an average diameter of 2.4, 3.9 and 6.3 µm (Particle A, B and C, respectively). Within parentheses, the respective NGI cut-off diameters for each stage are stated. The error bars indicate the standard deviation of two replicates.

Fig. 4. Influence of flow rate on the FPF (a) and the DD (b) of budesonide loaded 2.4 µm particles (Particle A) for two different DPI devices, the ICOone® and ICOcap®. The error bars indicate the standard deviation of at least two replicates, except for ICOcap® 45 L/min which only represents n = 1.
sulphate, calcium chloride dihydrate, sodium acetate, sodium hydrogen carbonate, sodium citrate dihydrate were purchased from Sigma-Aldrich (Germany). All organic solvents used (at least HPLC grade) were obtained from VWR (France). Ultrapure water from PURELAB flex (18.2 M\(\Omega\) cm, TOC \(\leq\) 4 ppb) was used for all experiments. Trifluoroacetic acid (TFA; at least 99 % pure) used during quantification was purchased from Sigma-Aldrich (Germany).

2.2. Particle morphology

Scanning electron microscopy (SEM) was employed to visualize the aerosolization of the formulations using the modified Andersen Cascade Impactor (mACI, see Section 2.4.2). For each formulation, a small amount of powder was deposited on top of a metal SEM holder coated with adhesive carbon tape. SEM images were taken using a Leo/Zeiss 1550 microscope (Jena, Germany); the SEM holders were coated with a thin layer of Au/Pd under argon using a sputter coater (Polaron, Quorum Technologies Ltd., Newhaven, UK). An in-lens detector with a magnification of 3000x, an acceleration voltage of 2.0 kV and a working distance between 2.4–2.5 mm was used for all formulations.

2.3. Aerosolization

The Aerodynamic Particle Size Distribution (APSD) of the formulations was assessed using a next generation impactor (NGI, Apparatus E, Copley Scientific Limited, Nottingham, UK) (EDQM, 2017) together with a device specific mouthpiece adapter for fixation of the inhaler to the USP inlet. Two commercially available devices obtained from Iconovo AB (Sweden) were studied. ICOOne is a single-dose, medium resistance device (64 L/min at 4 kPa) (Lastow and Arvidsson, 2021).
ICOcap is a capsule device loaded with size 3 capsules, with a low resistance. Flow rates of 45, 64 and 90 L/min were used for both devices. The time of aspiration was adjusted to obtain 4 L passing through the impactor according to Ph. Eur. (EDQM, 2017). Each inhaler was filled with approximately 10 mg of the powder. One actuation was performed per NGI repeat measurement. The results presented are the average of two repeated NGI measurements. A coating solution of about 15% w/v Brij® in Ethanol mixed with 4 g glycerol per ml was used in each of the NGI stage cups to minimise particle bounce during the aerosolization. 15 ml of an internal standard (IS; Propyl-4-hydroxybenzoate dissolved in 50% Ethanol) solution was added to inhaler, inlet, pre-separator, adapter, cups, Stage 1-Micro-Ori- fice Collector (MOC) and filter to dissolve the drug. The tray with the cups was shaken for 15 min. Stoppers were placed in the openings of the pre-separator (PS). The PS was then shaken vigorously for 15 min to dissolve the drug present in the PS. The inlet, adapter and device were repeatedly washed with the dispensed IS to collect the drug. Approximately 0.5–1 ml of sample solution was then taken from each sample.

Fig. 6. SEM images of budesonide particle formulations dispersed on filters: (a) Particle A (2.4 µm), (b) Particle B (3.9 µm), (c) Particle C (6.3 µm) and (d) the Pulmicort® Turbuhaler® formulation. Images (a)-(c) are at a magnification of 1000×. Image (d) was taken at a magnification of 5000× to visualize in more detail the morphology of the micronized drug aggregates.

Fig. 7. (a) Release profiles of loaded Budesonide particles with a mean particle diameter of 2.4 µm (Particle A), a mean particle diameter of 3.9 µm (Particle B) and a mean particle diameter of 6.3 µm (Particle C) and the Pulmicort® Turbuhaler® formulation in SLF. The release profiles were obtained from aerosolised fractions with an aerodynamic diameter below 4.4 µm. The symbols in (a) show the fraction of drug that has permeated the membrane, the error bars indicate the standard deviation of three replicates and the lines are fits of the transformed Weibull distribution functions. (b) Symbols represent t_{50} values comparing the release rate of encapsulated budesonide loaded into mesoporous particles of different diameters in SLF. The error bars indicate the standard deviation of three replicates. The dashed line represents a linear fit to the data to illustrate the trend that the rate of drug release increases as size of the particles decreases.
were interpolated from a logarithmic fit to the corrected mass distribution after actuation. The fine particle fraction (FPF), mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were interpolated from a logarithmic fit to the corrected mass of budesonide obtained from the inhalers and added to separate vials for HPLC analysis (see Section 2.5).

The total emitted dose was determined from the corrected total mass of budesonide deposited in the NGI. The inhaler retention was determined from the corrected mass of budesonide obtained from the inhalers after actuation. The fine particle fraction (FPF), mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were interpolated from a logarithmic fit to the corrected mass distribution as a function of the known cut-off diameters for a flow rate of 60 L/min (Table S1). The FPF is defined as the mass of particles < 5 μm in size, divided by the total emitted dose.

2.4. Drug release

2.4.1. Preparation of release media

Simulated lung fluid (Gamble’s solution; pH 7.4) was prepared according to the method reported by Marques et al. (Marques et al., 2011) containing 0.095 g/L magnesium chloride, 6.019 g/L sodium chloride, 0.298 g/L potassium chloride, 0.126 g/L disodium hydrogen phosphate, 0.063 g/L sodium sulphate, 0.368 g/L calcium chloride dehydrate, 0.574 g/L sodium acetate, 2.604 g/L sodium hydrogen carbonate and 0.097 g/L sodium citrate dehydrate. All buffers were filtered with a 0.2 µm syringe filter with a polyethersulfone membrane (Filtropur S 0.2, Sarstedt, Germany) before use.

2.4.2. Dose collection

Dose collection of the formulations was performed using a modified Andersen Cascade Impactor (mACI; cf. Fig. 1). The mACI is comparable to a standard ACI until Stage 1. However, after Stage 1, the normal stages are replaced with five hollow stages. Having these five hollow stages in the mACI allowed for particle sedimentation to occur at the final Filter Stage. With a flow rate of 60 L/min, the cut-off diameter is 4.4 μm after Stage 1, meaning that the fraction collected at the Filter Stage has an aerodynamic diameter below 4.4 μm. The flow rate in the mACI was 60 L/min, with a suction time of 0.3 s for all formulations. The sedimentation time was 20 min, after which the filters were collected for the release experiment. The dose collection method was based on an earlier work by Franek et al., to which the reader is referred for further details (Franek et al., 2018).

All formulations were individually weighed into a Screenhaler (Thalberg et al., 2016), an experimental set-up suitable for this type of investigations, coupled with a Turbuhaler® mouthpiece, which was then connected to the throat of the mACI. The amount of formulation weighed into the device was determined by the final amount of drug in the release experiment, this amount was fixed to be approximately 10 μg of drug. This resulted in a total amount of powder weighed in of roughly 5, 8, and 35 mg for particles A, B, and C, respectively.

A Turbuhaler® formulation was selected as a comparison to the budesonide mesoporous particle formulations as it is the current clinical standard on the market. The Turbuhaler® formulation consisted of micronized crystalline particles of budesonide (cf. SI; Fig. S2) in contrast to the amorphous budesonide in the mesoporous silica particles (cf. SI; Fig S2). Five doses of the Turbuhaler® were dispersed into the mACI to obtain a similar (10 μg) amount of drug on the filter, in the Filter Stage, as the mesoporous particle formulations.

To visualize the aerosolised particles deposited within the mACI SEM images were taken of the Filter Stage. To obtain the SEM pictures, an SEM stub was placed in the Filter Stage of the mACI and the mACI deposition conducted, as described above, before imaging.

2.4.3. In vitro drug release

After deposition of the particles in the Filter Stage of the mACI, the filters were transferred to the inserts of a Corning Transwell system (24 mm inserts, polycarbonate membranes, pore size 8 μm; Sigma-Aldrich, Germany) to determine the release profiles of the formulations (cf. Fig. 1). The release experiments as described in the following were based on previously published studies (Franek et al., 2018; van der Zwaan et al., 2022). The wells were prefilled with 2.3 mL of medium (Gamble solution) prior to placing the inserts in the well. After, an additional 0.7 mL of the same medium was added on top of the filters and the Transwell plate was placed on a shaking table (Heidolph Unimax 1010), which was set to a speed of 150 rpm. This step was considered as the start of the experiment, i.e., t = 0. Samples of 200 μL were taken at different time intervals; the sample volumes taken were replaced with fresh buffer to retain the same volume over time. After the final time point, 3 mL of methanol was added for an additional 30 min to determine the total amount of drug in the experiment. All release experiments were performed at room temperature. All experiments were performed under sink conditions. The solubility of budesonide in SLF has been previously reported to be 15 μg/mL (Al ayoub et al., 2022). To ensure sink conditions, a total dissolution volume of 3 mL and an average drug amount of 10 μg was used for all measurements.

Quantification of the samples was achieved using UPLC-UV (see Section 2.5). All measurements were performed in triplicate.

2.5. Quantitative analysis

The quantitation of the NGI experiments was performed by HPLC, using a reverse-phase system with a Symmetry XBridge RP 18 column (3.5 μm, 3.0 × 50 mm) and isotropic elution with an acetonitrile/phosphate buffer/sodium octanesulfonic acid mobile phase 25/75 (v/v).

Table 3

Table 3 Release/dissolution parameters obtained from fitting the solution of Eq. (1) to the experimental permeation data: characteristic time t_{63} and exponent n (mean values of three replicates with standard deviation in parenthesis).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>t_{63} (min)</th>
<th>n (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>129 (26)</td>
<td>0.44 (0.03)</td>
</tr>
<tr>
<td>B</td>
<td>152 (13)</td>
<td>0.51 (0.03)</td>
</tr>
<tr>
<td>C</td>
<td>197 (18)</td>
<td>0.65 (0.06)</td>
</tr>
<tr>
<td>Pulmicort® Turbuhaler®</td>
<td>224 (36)</td>
<td>0.91 (0.12)</td>
</tr>
</tbody>
</table>

Fig. 8. In (a) and (b), the original Weibull distribution functions, representing the fraction of released or dissolved drug, are shown vs. (a) time and (b) square root of time.
Detection was performed using UV-absorption at 214 nm and calibration and quantitation by internal standard methodology using 1 point standard calibration curve including the origin.

Quantification of the release samples was conducted using a Waters Acquity UPLC-UV I-Class system with a BEH C18 column (2.1 × 50 mm) with 1.7 µm particle size. The method used for quantification of the budesonide loaded particles was a gradient method using 65 % of mobile phase A and 35 % mobile phase B to a 20:80 ratio (A:B) in 1.33 min and back to 65:35 (A:B) in a total run time of 1.8 min. Mobile phase A and B were 0.03 % TFA in water and acetonitrile, respectively. The flow rate was 0.6 mL/min and the wavelength was 254 nm. The temperature of the column was set to 40°C. The temperature of the samples was set to 18°C and an injection volume of 2 µL was used for all samples. Quantification of the samples was achieved by using a standard curve with an external standard. The method was validated with an inter- and intraday variation with standard curve samples. The limit of detection (LOD) and limit of quantification (LOQ) for budesonide was 0.4 µg/mL and 1.2 µg/mL, respectively.

2.6. Data analysis

A procedure based on a previously developed mechanistic model (Frenning et al., 2020; van der Zwaan et al., 2022; van der Zwaan and Frenning, 2023) was used to reduce the effect of the Transwell membrane on the dissolution profiles. When drug is passively transported across the membrane and sink conditions prevail in the receptor compartment, the fraction of dissolved/released drug 1–s from the particles in the donor compartment is related to the fraction of permeated drug u into the acceptor compartment by an ordinary differential equation of the form

\[ \frac{du}{dt} + s = 1 - s \]  

Here, \( t_{perm} \) is a characteristic time for permeation that was determined as 9.1 ± 0.9 min (mean ± standard deviation) utilizing the fact that the fraction permeated drug increases as \( 1 - e^{-t/\tau_{perm}} \) when a drug solution is applied on top of the membrane. Since the time course of the fraction of drug remaining in solid form was unknown, a Weibull distribution function of the form \( 1 - e^{-t/\tau_{diss}} \) was substituted for \( 1 - s \) in Eq. (1). The unknown parameters \( t_{perm} \) and \( n \) were determined by fitting the u to the experimental permeation data using MATLAB (R2022b).

3. Results & discussions

3.1. Aerosolization performance

3.1.1. MMAD and FPF

It is of high importance for pulmonary administration to assess the aerosolization performance of a formulation. The fine particle fraction (FPF) and the median mass aerodynamic diameter (MMAD) of all three budesonide formulations, of different mean particle size, were determined using the NFI. The FPF and MMAD for each formulation when aerosolised from a single dose, low cost ICOOne® inhaler at flow rate of 64 L/min, are summarised in Table 2. The ratio between the delivered dose (DD) and the metered dose (MD) is also provided. In Fig. 2 the MMAD is compared to the mean particle diameter and it can be concluded that the MMAD is similar to the mean particle diameter of the formulations.

The results follow the expected trend, with increasing FPD and decreasing MMAD for decreasing mean particle diameter. The clinical standard formulation to deliver budesonide, the Pulmicort® Turbohaler® formulation, has a FPF around 50 % (Yoshida et al., 2017). Particles A and B with FPF values of 40–70 %, respectively, are broadly comparable to the commercial DPI and would be suitable for further development and clinical evaluation. Particle C, however, with a MMAD of 6.2 µm and a low FPF is not viable option for delivering drugs to the lungs. An FPF value of 15 % is too low for a DPI formulation to be considered viable as an inhaled product. Regardless of the delivered dose data, the majority of the dose would not reach the lungs and simply be deposited in the oropharynx (Borgstrom et al., 2006).

In Fig. 3, the percentage of the delivered dose of drug is shown for the different stages in the NFI. It can be seen that, for both the particles with an average diameter of 3.9 and 6.3 µm, the percentage of delivered dose was the highest (except for the inlet) in Stage 2 (around 30 %), with a cut-off diameter of 4.46 µm (Table S1). The formulation with an average particle diameter of 2.4 µm had the highest percentage of delivered dose in Stage 3, with a cut-off diameter of 2.82 µm. These results thus demonstrate that the aerodynamic distribution can be tuned according to the particle size, which can be helpful for targeting different regions in the lung.

3.1.2. Influence of flow rate on FPF and DD in two different DPI devices

The particle with smallest particle diameter of 2.4 µm was used to determine the influence of flow rate on the FPF and the DD for two different DPI devices, the ICOOne® and the ICOcap® (see Section 2.3). The FPF was measured for a flow rate of 45, 64 and 90 L/min for each device. In Fig. 4, it can be seen that the FPF was similar for the two devices and had a low flow rate dependency. For all three flow rates in both devices, the FPF was determined to be in a range of 60–80 %. For the ICOOne®, a weak increase (from 20 to 30 %) in the DD was observed upon increasing the flow rate. A similar trend between the DD and flow rate can also be observed for the ICOcap®, when comparing the flow rates of 45 L/min and 90 L/min. Some deviation in the DD was observed for 64 L/min, however, the standard deviation of this data point is significantly higher, therefore it would be inappropriate to draw any conclusions from this.

3.2. Dose collection of particles using mACI

SEM images were obtained to visualize the formulations before and after aerosolization using a modified ACI (mACI), prior to the dissolution studies (see Section 3.3). The mACI was used for dose collection as it allows the particles to sediment on the Filter Stage and, by using several smaller filters, for collection of several samples of the complete FPF. Fig. 5 shows the formulations before aerosolization. For all mesoporous silica formulations, individual spherical particles were present, with no large agglomerates. In comparison, particles from the Pulmicort® Turbuhaler®, appeared as multi-particle agglomerates made up of micronized budesonide particles (cf. Fig. 5d).

Fig. 6 shows SEM images of the formulations after aerosolization by the mACI, collected from the Filter Stage. All the mesoporous particle formulations (cf. Fig. 6a–c) maintained mechanical robustness after dispersion, with no apparent fracturing or breakage, and exhibited good dispersion on the filter. In comparison, the Pulmicort® Turbuhaler® formulation, showed smaller dispersed agglomerates after aerosolization (cf. Fig. 6d).

3.3. In vitro drug release

To compare the release profiles of the budesonide formulations to the current clinical standard, the release profiles of the silica formulations and budesonide collected from the Pulmicort® Turbuhaler® was determined in simulated lung fluid (SLF; pH 7.4). The Pulmicort® Turbuhaler® formulation showed the slowest release profile. In comparison, the release profiles of the budesonide formulations in SLF all exhibited notably faster release (cf. Figs. 6 and 7a). This difference can most likely be due to the different state of the drug in the different formulations. In the Pulmicort® Turbuhaler® formulation the drug is in a crystalline form, whereas the budesonide that is present in the mesoporous particles in an amorphous state (cf. SI; Fig. S2). By confining the drug and inducing an amorphous state, it has been similarly shown that
the solubility and the release rate of different drugs can be enhanced (Alonzo et al., 2010). In addition, it has previously been shown that dissolution enhancement of poorly soluble drugs can be influenced by the porosity of the particles, in particular the pore size (Valetti et al., 2017b). However, influence of the diameter of the silica particles on the dissolution behaviour has not yet been determined.

Here, effect of particle diameter on the release profile of budesonide, denoted also observed to increase with decreasing particle size. In all cases, the budesonide release profiles exhibited by the particle formulations were comparable pore size of approximately 11 nm (cf. Table 1). In all cases, the budesonide release profiles exhibited by the particle formulations were faster than that of the Pulmicort formulation. This enhanced release was also observed to increase with decreasing particle size.

The time required for 50% of the drug to reach the acceptor compartment, denoted t50, was extracted from the data in Fig. 7a. When comparing the t50 values of the three mesoporous silica budesonide formulations in SLF, a linear trend can be seen with particle diameter (cf. Fig. 7b), whereby the t50 increased with the diameter of the mesoporous particle. Regardless of the partly overlapping error bars in Fig. 7b, the linear trend can be confirmed by complete release profiles, used to determine the t50 value, shown in Fig. 7a.

As shown in Table 1, all parameters, including the specific surface area, are comparable for the three particles, except for the particle diameter, meaning that it is the only possible parameter that this effect could be attributed to. Such behaviour is likely to be correlated to changes in the diffusional path length, which increases with particle diameter. Increasing the particle diameter from 2.4 to 6.3 μm more than doubles the particle diameter, therefore the effective diffusion path summed over all the pores increases significantly. As the diffusion length increases, the number of collisions of the drug molecules with the silica surface and hence the rate of release from the particles decreases with particle diameter. In addition, the outer (enveloped) surface area is expected to be inversely proportional to the particle size (for spherical particles, the volume-specific outer surface area could be estimated as 6 /d where d is particle diameter). Hence, smaller particles have larger volume-specific enveloped surface area, something that could further contribute to the observed increase in dissolution rate for small particles.

It is known that the membrane present in the Transwell setup could affect the release profiles of rapidly dissolving drugs (Rohrschneider et al., 2015). Therefore, a mechanistic model (see Section 2.6) was used to reduce the effect of the Transwell membrane on the release profiles. It is seen that the transformed Weibull distribution functions, obtained as solutions of Eq. (1), fit the data well (solid lines in Fig. 7a). The extracted parameters are summarized in Table 3. The corresponding release/dissolution profiles are displayed in Fig. 8a. Hence, Fig. 8a displays the fraction of drug that has been released from the mesoporous silica particles into the liquid in the donor compartment, as obtained from the mechanistic model. This shall be contrasted with Fig. 7a that displays the fraction of drug that has permeated the Transwell membrane and thus reached the acceptor compartment.

Although the rank order between the permeation and release/dissolution profiles remains the same, it is evident that the permeation profiles do not properly reflect the fraction of released/dissolved drug, especially for short times. It is interesting to note that all mesoporous silica formulations exhibit an initial square-root-of-time kinetics, i.e., the fraction released increases linearly with square root of time, as can be seen in Fig. 5b. This result strongly points towards a diffusion-controlled kinetics, with negligible burst release, suggesting a nearly complete incorporation of budesonide in the pores of the mesoporous silica particles. On the contrary, the initial dissolution of Pulmicort® Turbuhaler® appears to depend nearly linearly on time. Such a kinetics would be obtained for dissolution of finite particles, and is thus consistent with the delivery of bare budesonide particles. The same conclusions can be drawn from the release/dissolution exponents n, which are about 0.5 for the mesoporous silica formulations and close to 1 for Pulmicort® Turbuhaler®.

4. Conclusions

In this study, the use of mesoporous silica particles as a novel controlled drug delivery system for dry powder pulmonary drug delivery was investigated. Formulations of budesonide were prepared with mesoporous silica powders of three different mean particle sizes. The formulations were then assessed through determination of their aerodynamic performance, physical robustness to aerosolization and release characteristics in simulated lung fluid in comparison to the clinical standard. To assess the aerodynamic performance of the budesonide-mesoporous particle formulations two commercially available DPI devices were employed, without any further optimisation.

Overall, all the mesoporous silica formulations exhibited excellent physical robustness after aerosolization. Compared to the clinical standard, two of the formulations showed a similar or enhanced FPF indicating a promising aerosol performance. Moreover, the deposition profiles of the three formulations demonstrated that the aerodynamic distribution could be systematically tuned according to the particle size, which could be helpful for targeting different regions in the lung. Such size control during the synthesis process of mesoporous silica particles also offers huge potential cost saving benefits for manufacturing, i.e., by minimising waste and losses due to fractionation.

During release studies, amorphization of the drug in the mesoporous network led to enhanced release overall compared to the clinical standard formulation in simulated lung fluid. Moreover, the mean particle size of the mesoporous particles was demonstrated to facilitate control over rate of release, which increased with decreasing particle diameter.

Although further work needs to be done to correlate these results to in vivo and the safety profile of using mesoporous amorphous silica particles for pulmonary administration needs to be fully addressed, the current study demonstrates that there is a possibility to manufacture mesoporous silica particles to narrow particle size ranges, optimal for dry powder inhalation and through careful choice of the particle diameters, deliver and release a drug with a high degree of control. Such tuneability and control could highly benefit the development of drug formulations for controlled delivery via the lungs and allow for tailoring to different therapeutic needs.
Delivery Center (SweDeliver) and financial support from Vinnova (Dnr 2019-00048) is gratefully acknowledged. SV and ME also acknowledge Knowledge foundation (KK-stiftelsen) grant 20190101. The authors would like to acknowledge Lucia Lazarova for her help with obtaining the SEM images.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2024.106828.

References


