

***In vitro* System to assess the size-dependent dissolution profile of inhalable aerosols**

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Summary

The assessment of the drug release profile from a dry powder formulation is a critical issue to evaluate bioavailability, lung clearance and toxicology of inhalative drugs. In the present work we developed a system that mimics the inhalation and deposition process of particles in the lung and subsequently, allows the online measurement of the released drug substance. A HandiHaler® device was attached to an Anderson Cascade Impactor containing a cellulose membrane. This allowed the immobilization of a powder fraction below a defined aerodynamic diameter. The dissolution profile was determined in a Franz diffusion cell used as an air-liquid interface model. Spray dried Budesonide and Salbutamol/polymer-particles were used as test formulations. The results showed a clear differentiation of particles according to their aerodynamic diameter. A faster drug release was observed with smaller particles.

Introduction

The bioavailability of inhalable drugs depends on the lung deposition efficiency, deposition site, lung clearance rate and the dissolution profile. Geometric size and aerodynamic behavior are thus considered as critical properties in the development of pulmonary formulations. Usually the lung deposition of a dry powder formulation requires deagglomeration of particles through an inhaler device with subsequent inhalation. Consequently, only a fraction of the powder aerosol with a particular morphology and size will effectively deposit in the lung and be therapeutically available. As the dissolution profile also depends on the particle size it is therefore crucial to analyse the particle fraction that would deposit in the lung in a human application. Standard dissolution tests are designed for oral applications in which the formulation is usually submerged in the dissolution media. Under these conditions micrometer-sized particles with a poor colloidal stability tend to aggregate, adding a significant artefactual diffusion barrier to the system. In other approaches a flow through dissolution cell is used in which the particles are subjected to a constant flow of the dissolution media. In vivo, particles deposited in the alveolar lung region are located in the epithelial lining fluid. Sink conditions are thus not necessarily present and morphological changes of the particles are likely to occur due to the high local concentration of the drug.

Our goal was to develop a dissolution assay that mimics the in vivo situation in the following regards:

- Deagglomeration of the powder particles using a human dry powder inhaler
- Investigation of the particle fraction that would deposit in the lung
- Immobilization of the particles to avoid aggregation in the dissolution media
- Use the air-liquid interface deposition model

In addition, we wanted to avoid time-consuming, HPLC-based analytics of the drug substance.

The strategy was to take advantage of the Anderson Cascade Impactor in order (i) to generate a dry powder aerosol using an inhaler device, (ii) to separate particles according to their aerodynamic properties and (iii) to deposit the particles onto a membrane that would impede further aggregation of the immobilized material. The air-liquid interface model was realized using a Franz diffusion cell.

Material and Methods

Spray drying was performed using a Büchi Mini Spray Dryer B-290. Budesonide was spray dried as a 10% solution in dichloromethane with an outlet temperature of 56 °C. The dry powder was collected through a high performance cyclone and showed a particle size of $X_{50} = 2 \mu\text{m}$ and $X_{90} = 4.6 \mu\text{m}$ as measured by a Sympatec Helos Laser Diffractometer equipped with a Rodos dispersing unit. For the Salbutamol/polymer-microparticles the Poly(lactic-co-glycolic acid) - poly(ethylene glycol)-block-co polymer LGPt8546 (Boehringer Ingelheim) was used. It has a triblock-co-polymer structure with a central 6 kDa PEG block. The polyester part contains 85 % L-lactide and 15 % glycolide. The molecular weight of the total molecule is approximately 150 kDa. Salbutamol sulphate/polymer-microparticles were spray dried as a w/o-emulsion using dichloromethane as the organic phase. 750 mg Salbutamol sulphate were dissolved in 100 ml water, added to 400 ml of 0.75 % polymer solution and subjected to ultrasounds in order to generate a stable emulsion. Spray drying was performed with an outlet temperature of 50 °C and generated particles with a size of $X_{50} = 3 \mu\text{m}$ and $X_{90} = 6.9 \mu\text{m}$.

The dissolution system is displayed in Figure 1. A cellulose filter membrane (RC 55, Whatman) was placed onto the Stage F (Filter) of an Anderson Cascade Impactor (ACI, Copley Scientific). A HandiHaler® (Boehringer Ingelheim) containing a PE-capsule filled with 10 mg dry powder was connected to an adaptor fitting to the ACI-stages. The ACI was run at 39 L/min during 6.15 sec corresponding to a human breath. To collect particles with an aerodynamic diameter (d_{ae}) < 5 μm the stages 0 and 1 were inserted above the filter stage. To collect particles with $d_{ae} < 2 \mu\text{m}$ stages 1-4 were used. The membrane was then placed into a Franz diffusion cell with an empty upper compartment mimicking the air compartment inside the lung. The lower compartment contained PBS buffer (Sigma). The temperature was maintained at 37 °C and the Franz diffusion cell was connected to a UV/Vis-

Spectrometer (Lambda 35, Perkin Elmer) to measure the Salbutamol concentration at 225 nm. For Budesonide a Franz diffusion cell with a basal compartment of 500 ml was used in order to maintain sink condition. Budesonide release was measured using a Waters 2487 UV-detector connected to a HPLC-pump. Absorption was measured at 240 nm. For longer measurements the baseline drift was compensated by subtracting the absorption at 350 nm.

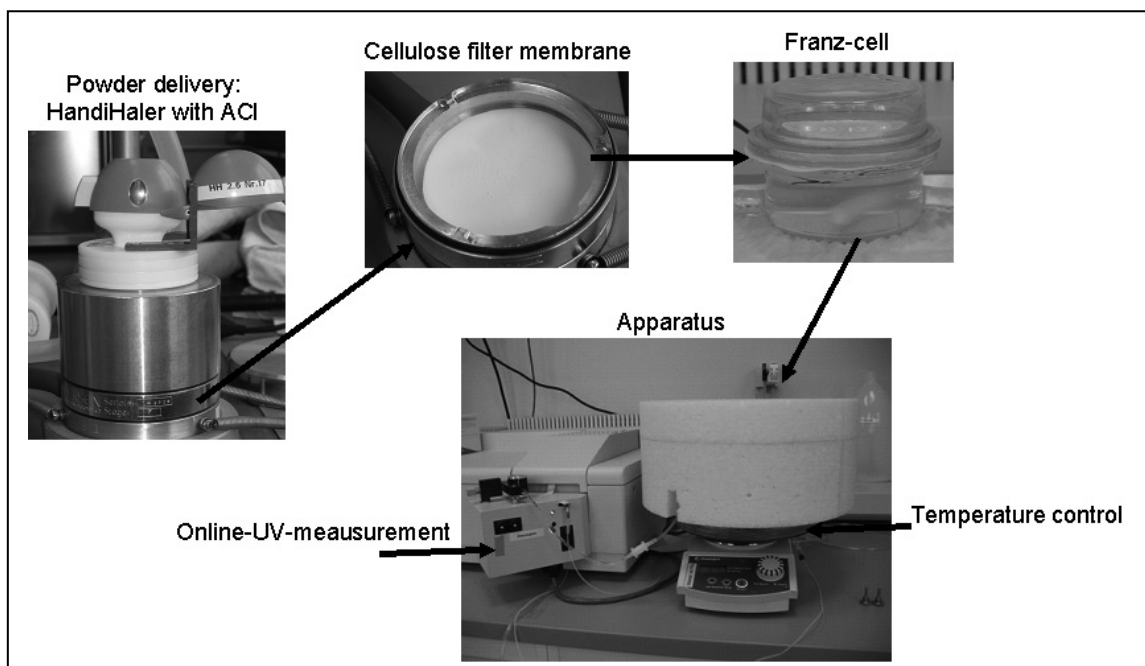


Figure 1. The experimental set up showing the HandiHaler® - Anderson Cascade Impactor unit containing the filter membrane and the Franz diffusion cell.

Results and discussion

The Franz diffusion cell was characterized using Salbutamol sulfate (Sbs) as a model drug. To assess the time necessary to display a concentration change inside the basal compartment Sbs solution was injected into the dissolution media prior to the measurement (Figure 2). The results showed that the maximum concentration was reached after 5 minutes. When Sbs-solution was brought onto the membrane a further delay of 5 minutes was observed that did not vary when Sbs dry powder was used. The results thus show that a concentration change at the membrane will be detected with a delay of 10 minutes which is negligible compared to the time span of a typical dissolution experiment.

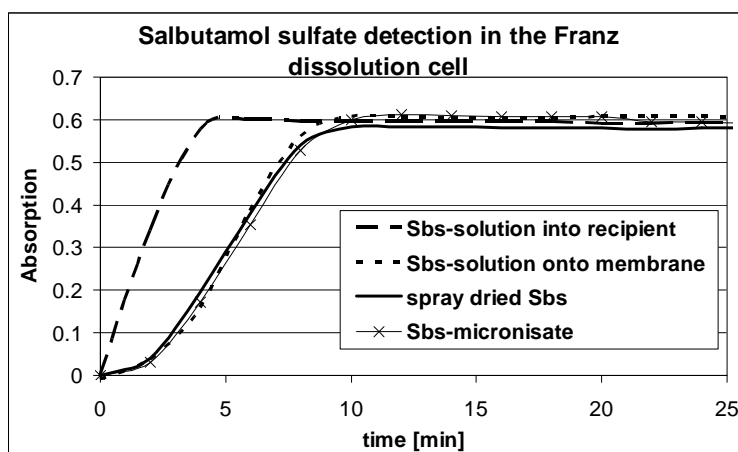


Figure 2. Characterization of the Franz diffusion cell containing the cellulose filter membrane

The drug release profile of Salbutamol/polymer-microparticles are shown in Figure 3. To investigate the total particle population (full fraction) no additional stage was placed above the filter in the ACI. In this case a burst release of 55 % was observed followed by a retarded release of the remaining drug over more than 24 h. To investigate the effect of the particle size particles with a d_{ae} smaller than 2 or 5 μm were deposited on the filter

membrane. An increasing burst release was observed with a decreasing particle size which can be attributed to a higher particle surface area. Since the particle fraction smaller than 1 μm represents less than 1 % of the total drug amount (not shown) it can be neglected in the present assay. The particles with a $d_{ae} < 5 \mu\text{m}$ can thus be attributed to the fine particle fraction. Hence, the system allows the differentiation between the total particle population generated during the spray drying process from the fraction that would deposit in the human lung.

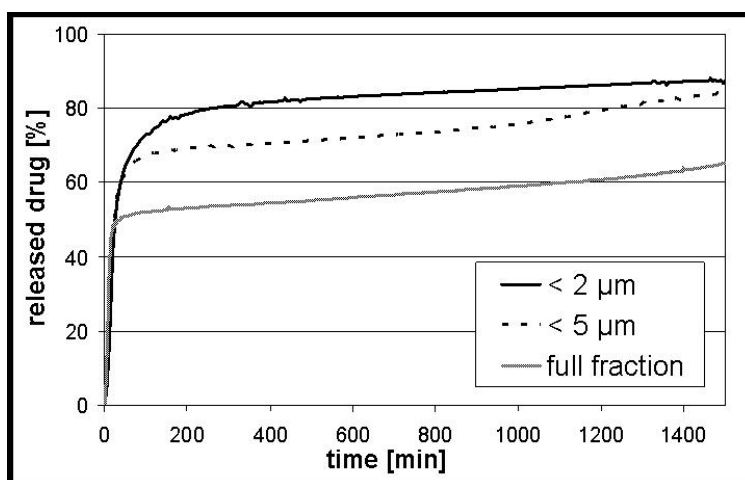


Figure 3. Dissolution profile of spray dried Salbutamol/polymer particles in relation to their aerodynamic diameter.

Budesonide is a hydrophobic substance with a water solubility of $\sim 10 \mu\text{g/ml}$ at 20 $^{\circ}\text{C}$. The dissolution profile of spray dried pure Budesonide particles is shown in Figure 4. Under the experimental condition a dissolution time of more than 24 h was observed with the whole particle mixture (full fraction). Similar to the Salbutamol-containing particles a size dependent dissolution profile was also observed with a decreasing particle size.

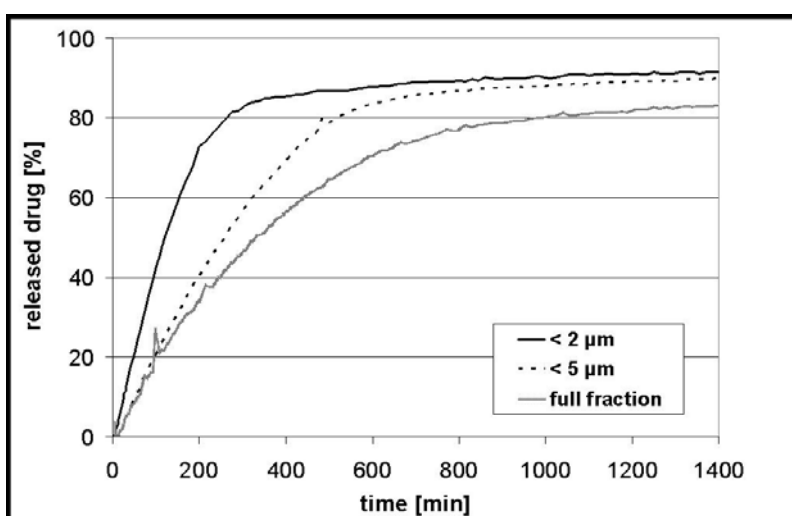


Figure 4. Dissolution profile of spray dried pure Budesonide particles in relation to their aerodynamic diameter.

Conclusions

Our aim was to analyse particles according to their aerodynamic properties and to take into account the particular air-liquid-interface present in the lung tissue where materials deposit. The drug release profiles of the tested formulations depended on their aerodynamic particle size. In particular, smaller particles showed a faster drug release as shown using Salbutamol sulphate as well as Budesonide as model drugs. Based on this system a rapid screening of formulations is possible revealing information on the actual particle fraction that would deposit in the lung. Furthermore, particles are located at an air-liquid interface and are dispersed onto a membrane that prevents aggregation during the dissolution process. We argue that compared to standard dissolution systems, e.g. flow cells, this set-up best mimics the conditions prevalent in the lung.