



Inhaled product dissolution as a predictive tool of drug bioavailability: a p38 MAPK inhibitor case study using Respicell® dissolution apparatus

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ABSTRACT

The inhaled p38 α/β mitogen-activated protein kinase inhibitor PF-03715455 is an example of “inhalation by design” pharmacologically active substance which maximize pulmonary residence and local efficacy, demonstrating a slow absorption rate and a prolonged pulmonary retention. However, for dry powder inhalers dissolution in the pulmonary lining fluid can become the rate-limiting step for drug absorption and bioavailability. The present study investigated the relationship between *in vitro* dissolution and *in vivo* pulmonary pharmacokinetics of two micronized PF-03715455 powders with different solid state, amorphous vs pseudo-crystalline. Powders were characterized by scanning electron microscopy and aerosol performance analysis using the PreciseInhale® coupled with cascade impactor. Dissolution studies were performed with RespiCell® apparatus directly on the respirable fraction obtained from PreciseInhale® aerosolization. Pulmonary pharmacokinetics were then evaluated following intratracheal administration in rats.

Both formulations exhibited comparable aerodynamic properties. However, marked differences were observed in dissolution behavior. After 8 h, only 3% of the amorphous powder and 0.4% of the pseudo-crystalline powder dissolved, confirming the extremely low aqueous solubility of the compound. These differences strongly influenced *in vivo* performance. The amorphous formulation showed enhanced systemic exposure and faster decline of lung concentrations, whereas the pseudo-crystalline powder exhibited prolonged pulmonary retention with minimal plasma exposure. The results demonstrated a clear correlation between dissolution rate and pulmonary absorption kinetics.

Overall, the study highlights the critical role of dissolution testing in predicting the *in vivo* behavior of poorly soluble inhaled drugs and supports the use of biologically relevant *in vitro* dissolution methods during early-stage formulation development.

1. Introduction

Recent research has advanced our understanding of actual lung drug concentrations and strategies to maximize local exposure, enabling the desired pharmacological response at doses suitable for the development of inhaled products (Cooper et al., 2012). The concept of “inhalation by design” has thus emerged, emphasizing the rational design of inhaled drugs (Glossop et al., 2010; Mazzucato et al., 2023; Yeadon, 2011) and formulations (Alhajj et al., 2023; Buttini et al., 2018) to maximize

pulmonary residence and local efficacy while minimizing systemic exposure. A representative example of this approach is PF-03715455, reported as a potential inhaled p38 α/β mitogen-activated protein kinase (p38 MAPK) inhibitor developed for the treatment of COPD and severe asthma (Millan et al., 2011; Norman, 2015). The p38 isoforms have been shown to play a key role in inflammatory responses, and their inhibition has demonstrated therapeutic potential in COPD, particularly when combined with other anti-inflammatory steroids (Ahmadi et al., 2023).

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In a previous publication, we reported the *in vivo* pharmacokinetic profile of the drug solution that demonstrated a slow absorption rate and a prolonged pulmonary retention: a desirable property for pulmonary administration (Marenghi et al., 2020). For dry powder inhalers (DPI) formulations, however, the dissolution process may significantly influence the drug's bioavailability following pulmonary delivery (Somby et al., 2023; Velaga et al., 2018). Indeed, the dissolution test has emerged as a valuable tool for *in vitro* characterization of inhaled drug products (Buttini et al., 2014; Lewis et al., 2014). More importantly, the dissolution rate can represent a critical factor influencing absorption kinetics and, consequently, therapeutic efficacy for poorly soluble, high dosage and/or biologic inhaled drugs (Nokhodchi et al., 2023; Radivojev et al., 2021).

As the pharmaceutical fields continue to advance toward more complex and targeted pulmonary therapies, the development and standardization of robust *in vitro* dissolution methods are becoming a critical issue. There is, therefore, an urgent need to establish standardized *in vitro* dissolution apparatuses able to reflect the *in vivo* fate of inhaled drugs *in vivo*, enabling accurate prediction of their pharmacokinetic and pharmacodynamic performance (Ayoub et al., 2022; Nokhodchi et al., 2023). Such systems would improve the mechanistic understanding of drug dissolution, absorption, and clearance within the lungs, thereby facilitating rational formulation design and optimization (Radivojev et al., 2019).

Efforts in this direction are expected to bridge the existing gap between *in vitro* and *in vivo* performance, ultimately enhancing the safety, efficacy, and reliability of inhaled medications. RespiCell® apparatus, for example, provides an air-liquid interface (ALI) configuration that better reproduces the physiological environment of the respiratory tract. Unlike the conventional Franz diffusion cells, which is primarily intended for membrane permeation studies, the RespiCell® apparatus is specifically designed for dissolution testing of inhaled formulations and can accommodate the aerosolized particle collection filters containing the respirable fraction of the formulation obtained from cascade impactors. This enables dissolution testing to be performed directly on the deposited inhalable dose under physiologically relevant conditions. This system has shown potential for establishing *in vitro-in vivo* correlations (IVIVCs) by linking dissolution kinetics of poorly soluble aerosolized drugs deposited in the airways to their pharmacokinetic and pharmacodynamic profiles (Patterlini et al., 2024; Sonvico et al., 2021).

The present research evaluated the *in vivo* bioavailability of the dry powder formulation of PF-03715455 administered to rodents using the PreciseInhale® system, and to correlate these findings with *in vitro* dissolution data. The dissolution test was carried out on the respirable fraction collected after aerosolization with the PreciseInhale®, in order to maximize its predictive relevance to *in vivo* performance. This research provides an important contribution to the field by emphasizing the role of predictive *in vitro* dissolution testing for inhaled drugs with poor aqueous solubility. In particular, it proposes a preliminary dissolution testing approach capable of characterizing and differentiating the dissolution profiles and behaviors of dry powder inhaler (DPI) formulations following pulmonary administration. Such an approach has the potential in the early discovery stage to reduce or even replace *in vivo* studies by enabling the prediction of *in vivo* bioavailability based on *in vitro* dissolution outcomes, potentially in the framework of the principles of the recently proposed Inhalation-based Biopharmaceutics Classification System (iBCS) (Bäckman et al., 2022; Forbes et al., 2025; Hastedt et al., 2024, 2022).

2. Materials and methods

2.1. Materials

The p38 MAPK inhibitor PF-03715455 was supplied by Chiesi Farmaceutici S.p.A. (Parma, Italy) as a micronized crystalline and amorphous powder with a particle size distribution in the range of 2–3

µm. The compound was used as received, without further processing. Other materials employed for analytical purposes were acetonitrile (ACN) HPLC grade, formic acid, ammonium formate (NH₄COOH), and dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Darmstadt, Germany). The salts used for the preparation of Gamble's solution, such as magnesium chloride, sodium chloride, potassium chloride, calcium chloride, sodium dihydrogen phosphate, sodium hydrogen carbonate, were also obtained from Sigma Aldrich (Milan, Italy). Ultrapure water (>18 MΩ·cm) was obtained using Reagent Grade Milli-QA10 Advantage water purification system (Milli-Q, MilliporeSigma, Merck, Darmstadt, Germany).

2.2. Methods

2.2.1. Aerosol generation

Aerosol of the PF-03715455 dry powder formulations were generated using the PreciseInhale® system (Inhalation Sciences Sweden AB, Stockholm, Sweden). This system (Fig. 1) consists of a custom-made exposure manifold and an aerosol generator comprising a variable-volume pressure chamber and a fixed-volume powder chamber connected via a main valve to a holding chamber, where aerosolization takes place. The system is fully automated and computer-controlled, allowing precise regulation of aerosol generation and exposure parameters (Fioni et al., 2018; Selg et al., 2013). Particle size distribution (PSD) of the aerosol was assessed by coupling the PreciseInhale system with an 8-stage Marple cascade impactor (MSP Corp., Shoreview, MN, USA). Filters from each stage were analyzed gravimetrically to determine the mass of powder deposited, which was then used to calculate the Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD). The PSD data served as input for estimating the lung-deposited fraction of the aerosol using the Multiple-Path Particle Dosimetry (MPPD) model (ARA Inc., Albuquerque, NM, USA), configured for a breathing rate of 135 bpm and a tidal volume of 1.3 mL. Aerosol concentration was monitored in real time using a Casella Microdust Pro light-scattering photometer (Casella CEL Inc., Buffalo, NY, USA), calibrated against the inhaled mass using an *in vitro* filter-based method. The aerosol delivery setup was configured to ensure accurate dose control, with airflow parameters continuously monitored and adjusted via the system's dedicated software (Fig. 1).

2.3. Scanning electron microscopy (SEM)

The morphology, *i.e.* size, shape, and surface features, of the powders under study were examined using a Field-Emission Scanning Electron Microscope (Zeiss Auriga Compact, Carl Zeiss, Oberkochen, Germany). Powders were dispersed on carbon-tape-covered aluminum stubs, and any surplus particles were eliminated using a mild nitrogen flow. In order to image micrometric-sized insulating particles without the necessity for metallization, the acceleration voltage of the primary electron beam was reduced at 1.0 kV. Magnifications 1,000 ×, 5,000 × and 10,000 × were used to capture the images.

2.4. Powder X-ray diffraction analysis

The data were collected on a Smartlab XE diffractometer (Rigaku Europe, Neu-Isenburg, Germany) in θ – θ Bragg–Brentano geometry with Cu K α radiation. The samples were placed on zero-background Si sample holder and exposed to radiation ($1.5^\circ \leq 2\theta \leq 40^\circ$) at a scan rate of $1^\circ/\text{min}$. The diffracted beam was collected on a two-dimensional (2D) HyPix 3000 solid-state detector; 5° radiant Soller slits were used as a compromise for high flux and moderate peak asymmetry at low angles. A beam stopper and antiscatterer air component were used to mitigate the scattering at a low angle.

2.5. Dissolution studies

In vitro dissolution tests were conducted using the RespiCell®

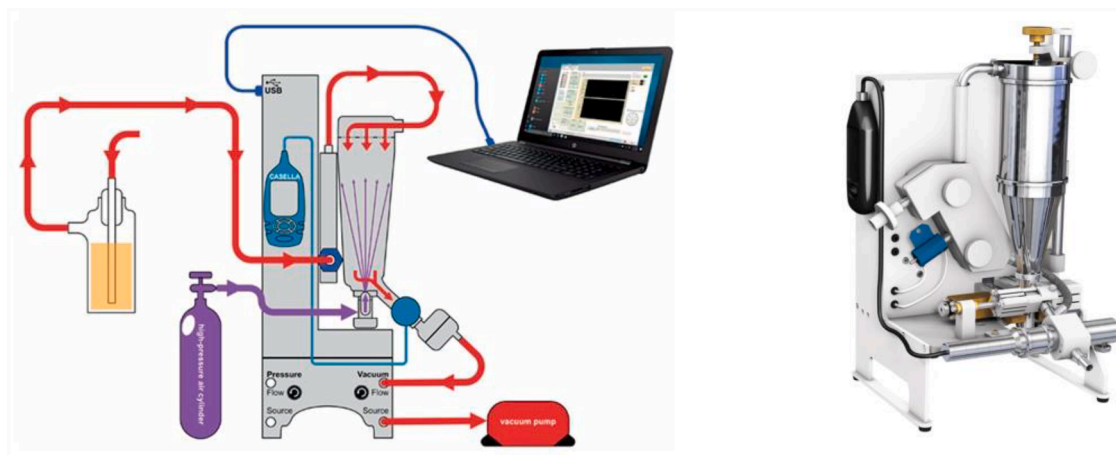


Fig. 1. Schematic representation of the aerosolization system. Before aerosol exposures, the upper part of the tracheal catheter or the restrainer tube is connected to the PreciseInhale platform, thus creating a closed system. The aerosol generation and delivery procedure are executed by the exposure control program using an active dose-control system.

dissolution apparatus (EU Design Registration No 006649570–0001; DISA S.p.A., Milan, Italy) (Fig. 2) (Sonvico et al., 2021). This vertical, two-compartment system consists of a donor and an acceptor chamber. The acceptor chamber was filled with 180 mL of dissolution medium and equipped with a 10 cm side arm for sampling. The two chambers were separated by a polycarbonate membrane (Nucleopore™, 0.4 μm pore size, 90 mm diameter; Whatman, Little Chalfont, UK), allowing direct contact with the dissolution medium.

The apparatus was jacketed and connected to a thermostatic water bath (Eco Silver E4, Lauda, Assago, Italy) maintained at 37 ± 0.5 °C. The dissolution medium, used to solubilize the poorly water-soluble drug, was Gamble's solution, consisting of MgCl_2 (1 mM), NaCl (103 mM), KCl (4 mM), CaCl_2 (2.5 mM), NaH_2PO_4 (1 mM), and NaHCO_3 (31 mM) (Marques and Loebenberg, 2011).

At predetermined time points (30 min, 1 h, 2 h, 4 h, 6 h, and 8 h), 1 mL samples were withdrawn from the side arm and analyzed by LC–MS according to a validated method published previously (Marengi et al., 2020). The chromatographic analyses were performed on an Agilent 1260/1200 UHPLC system (Agilent Technologies, Santa Clara, CA, USA). Separation was achieved using a Phenomenex Kinetex UHPLC column (EVO C18, 5 μm , 100 Å, 50×2.1 mm; Phenomenex, Torrance, CA, USA). The column temperature was maintained at 40 °C, and the injection volume was set to 5 μL . The aqueous mobile phase (eluent A) consisted of 10 mM ammonium formate, while the organic mobile phase (eluent B) was acetonitrile containing 0.1% formic acid. Separation of PF-03715455 from the biological matrix was achieved using a gradient elution ranging from 10% to 95% eluent B. Detection was performed using a triple quadrupole mass spectrometer (QTRAP® 4000 System, AB SCIEX, Concord, ON, Canada) equipped with an electrospray ionization (ESI) source operating in positive ion mode. Under these optimized conditions, the total run time was 7.5 min, and PF-03715455

eluted at 3.76 min.

After each sampling, the withdrawn volume was replaced with 1 mL of preheated fresh dissolution medium.

At the end of the experiment, the membrane filter was removed and washed with 15 mL of a 1:1 (v/v) acetonitrile–water mixture to quantify the undissolved drug fraction. Each test was performed in triplicate for each formulation. The total drug amount was calculated as the sum of the dissolved fraction and the drug recovered from the filter. In all experiments, the total drug recovery exceeded 80% of the nominal deposited dose.

The PF-03715455 drug powders with different solid state used for the dissolution test corresponded to the respirable fraction collected with the PreciseInhale® impactor. To collect this fraction, only the first five stages of the impactor were employed. Each stage was equipped with a 25 mm glass fiber filter (Whatman GF/A, Cytiva, UK). The filter from the fifth stage, on which the respirable particles were deposited (cut-off < 4.75 μm), was selected for the dissolution test. For the amorphous powder the fraction collected was 605 ± 34.65 μg ; while for the pseudo-crystalline was 501 ± 57.53 μg .

The filter was carefully removed and positioned upside down at the center of the polycarbonate membrane of the RespiCell® apparatus, ensuring that the deposited powder was in direct contact with the membrane surface (Fig. 2). Under these conditions, the dissolution process occurred exclusively through the polycarbonate membrane, allowing the assessment of the dissolution behavior of the same respirable fraction used for *in vivo* administration.

2.6. Animal studies

The experimental procedures involving animals, carried out at Chiesi Farmaceutici S.p.A., (Parma, Italy), were approved by the Italian

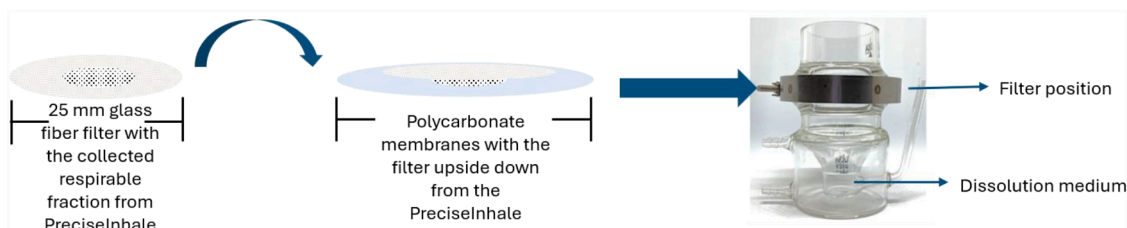


Fig. 2. Schematic representation of the preparation for the dissolution of the respirable fraction collected using a fast screening impactor. The filter of the PreciseInhale with the respirable fraction is then combined with the polycarbonate membrane upside down and place on the RespiCell apparatus for the dissolution process.

Ministry of Health (Aut. N. 1104/2020-PR) and complied with the European and Italian regulations for animal care and use of laboratory animals. Animal studies were performed in accordance with the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), for which the accreditation was granted in June 2024, and with efforts to minimize the number of animals used and their suffering.

Thirty male CD rats, weighing 0.302 to 0.346 kg, were used for intratracheal administration of PF-03715455 amorphous and pseudo-crystalline powders with the PreciseInhale® system.

For each administration, 1 mg ($\pm 10\%$) of PF-03715455 powder was loaded into the PreciseInhale® system. Animals were anesthetized with sevoflurane (Abbott, Maidenhead, UK) and positioned supine. The trachea was visualized using a laryngoscope (Welch Allyn Inc., Skaneateles Falls, NY, USA), and a steel catheter (2.02 mm OD, 1.67 mm ID, 6 cm length) was inserted to the tracheal bifurcation. The animal was then connected to the exposure block, and stable breathing was confirmed before exposure. The target deposited lung dose of the aerosolized powder was 10 $\mu\text{g}/\text{lung}$. The inhaled dose required to reach this target was calculated and entered into the system software, which automatically terminated the exposure upon reaching the desired dose. Animals were then returned to their cages and monitored until full recovery. Blood samples (1 mL) were collected via aortic puncture at 0.083, 1, 2, 4, 7, 24, 48, 96, and 168 h post-dosing. Three rats were sacrificed for each time point. Blood samples were immediately transferred into heparinized polystyrene tubes. After sample collection, animals were sacrificed by total bleeding through aortic cutting off. Plasma was separated by centrifugation ($1,500 \times g$, 10 min, 4°C) and stored at -80°C . Lungs and trachea were excised, rinsed with cold saline, weighed, and stored at -80°C . Before analysis, tissues were homogenized in saline/acetonitrile (50:50, v/v; 3 mL/g tissue) using a Precellys Evolution homogenizer with Cryolys cooling (Bertin Technologies, Montigny-le Bretonneux, France). Lung homogenization was performed at 7800 rpm (3×12 s, 40 s pause), and trachea at 8,000 rpm under the same conditions, maintaining temperatures below 10°C . Plasma and lung homogenized were analysed by mass spectrometry following the method published and validated previously (Marenghi et al., 2020).

2.7. Pharmacokinetics parameters and statistics

All results are reported as mean value and standard deviation of at least 3 replicates, if not stated otherwise. All statistical analyses were performed using Prism Software Version 10.6.1 (Prism, Version 10.6.1, GraphPad Software Inc., La Jolla, CA, USA). The aerosol parameters Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) were calculated using Excel Office Software.

The following pharmacokinetic parameters were calculated by non-compartmental analysis using the mean plasma and lung concentration values: λ_z the apparent terminal elimination rate constant, calculated from the slope of the linear regression curve obtained by fitting the natural logarithms of at least three time points plasma concentration values vs time over the terminal phase; $t_{1/2}$: the apparent terminal elimination half-life, calculated by the equation: $t_{1/2} = \ln 2 / \lambda_z$; C_{last} the concentration value at the last sampling time (T_{last}); T_{last} the last sampling time at which a measurable concentration was found; AUC_{last} : the area under the plasma concentration/time curve up to the last sampling time (T_{last}); AUC_{inf} the area under the plasma concentrations vs time curve up to infinity, calculated by the equation: $\text{AUC}_{\text{inf}} = \text{AUC}_{\text{last}} + (C_{\text{last}}/\lambda_z)$; MRT_{last} the mean residence time from the time of dosing to the last measurable concentration, calculated as follows: $\text{MRT}_{\text{last}} = \text{AUMC}_{\text{last}}/\text{AUC}_{\text{last}}$.

3. Results

3.1. Morphology of p38 MAPK inhibitor powders

To elucidate the morphology of the powders under investigation, SEM was performed. Representative images acquired at different magnifications of are presented in Fig. 3.

The amorphous powder (left side) consists of collapsed and fragmented spherical particles, a morphology typical of spray-dried materials. The rather heterogeneous powder is characterized by hollow shells, ruptured porous microspheres, and agglomerated fine particulates, as evident in the upper left panel of Fig. 3.

In contrast, the crystalline powder (Fig. 3 right side panels) exhibits flaky plate-like (lamellar) particles with flat facets and sharp edges, even if the crystals shape is not always regular with sections of the particles appearing spongy, possibly as a result of the drying or micronization of the solid. These plates show a pronounced tendency to stack and agglomerate into dense clusters, with smaller particulates adhering to their surfaces.

In terms of particle size, the amorphous powder appears to have a roughly bimodal particle distribution with larger particles and fragments in the order of several tens of microns and a population of regular spherical microparticles as small as 1 μm . The crystalline powder appears to form clusters relatively regular in size with dimension in the 1–10 μm .

3.2. Powder X-ray diffraction analysis

X-ray diffraction (XRD) analysis was carried out to verify the structural differences between the two powders (Fig. 4). The diffraction pattern of the nominally crystalline sample was clearly distinct from that of the amorphous sample, confirming a different structural arrangement. Nevertheless, the absence of sharp and well-defined Bragg peaks indicates that the material cannot be considered highly crystalline. Conversely, its diffraction profile also differs significantly from that of a fully amorphous material. Therefore, the sample is more appropriately described as possessing a low degree of crystallinity, characterized by limited long-range structural order, and in consequence will be referred to as pseudo-crystalline.

3.3. Aerosol performance

Both pseudo-crystalline and amorphous powders were efficiently aerosolized using the PreciseInhale® system, with aerosol yields well above 10%. The particle size distributions were consistent with respirable aerosols, with MMAD values of 3.38 μm and 3.51 μm , and GSD values of 1.71 and 1.82 for the crystalline and the amorphous powders, respectively (Table 1). These parameters confirmed the suitability of the formulation for pulmonary delivery.

3.4. Dissolution studies with the Respicell apparatus

The dissolution study of the p38 MAPK inhibitor was performed using Respicell, an apparatus designed for the dissolution of inhalation products that allows the product wetting and dissolution process to happen at the wet surface of a polymeric membrane used to separate the donor from the acceptor compartments. The dissolution was performed using a simulated lung fluid (Gamble's solution, pH 7.4) (Radivojevic et al., 2019) in non-sink conditions since the respiratory tract contains only a very limited volume of epithelial lining fluid and reproducing these non-sink conditions is considered an important aspect for achieving physiological relevance during *in vitro* dissolution experiments for inhaled products, as reported also by other groups (Eedara et al., 2022). The profiles obtained are outlined in Fig. 5. It is important to point out that the dissolution was performed on the respirable fraction obtained directly from the PreciseInhale and data were

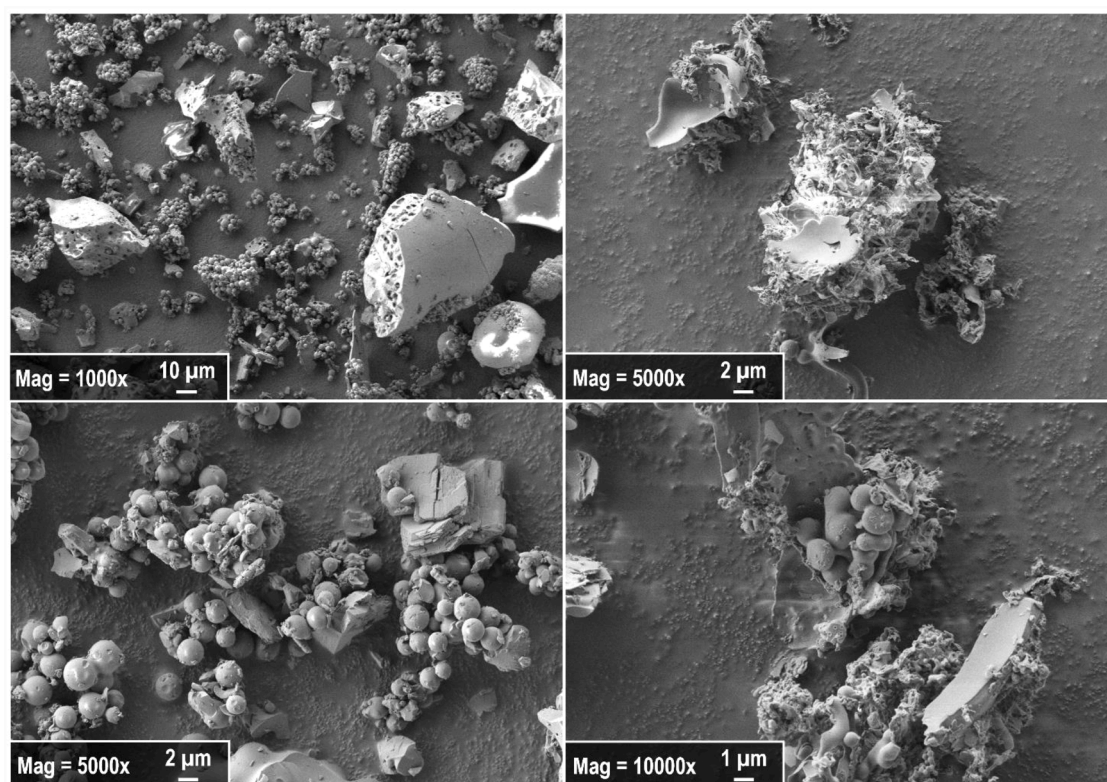


Fig. 3. SEM images of amorphous (left side) and crystalline (right side) powders of PF-03,715,455.

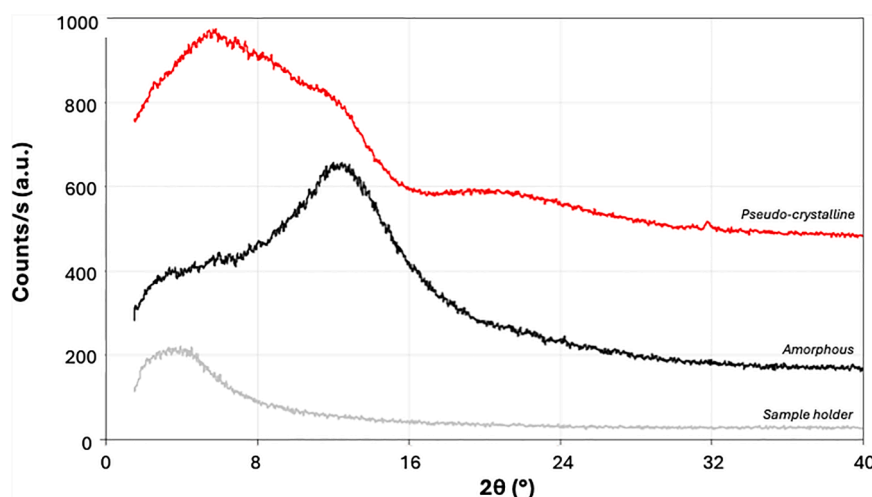


Fig. 4. Staked plots of PXRD patterns collected from the pseudo-crystalline sample (red line) and the amorphous sample (black line). Sample holder trace, not subtracted, is also reported (grey line) for the sake of clarity. Very broad Bragg peaks support the low degree of crystallinity of the pseudo-crystalline sample.

Table 1

PF-03715455 Micronized amorphous and pseudo-crystalline powders aerosol parameters.

PARAMETERS	POWDER	
	Amorphous	Pseudo-crystalline
Yield of Aerosolization (%)	33.9	34.8
MMAD (μm)	3.51	3.38
GSD	1.82	1.71

expressed as percentage of the total respirable fraction. The dissolution profiles highlighted that after 8 h, just 3% of the amorphous powder and

0.4% of the pseudo-crystalline powders were dissolved, indicating an extremely slow dissolution process potentially a combination of low aqueous solubility and possibly poor wettability. Statistically significant differences were observed at 6 and 8 h ($p < 0.05$), confirming the superior dissolution behavior of the amorphous formulation compared with the pseudo-crystalline formulation.

3.5. Pharmacokinetics of the p38 MAPK inhibitor amorphous powder

Fig. 6 depicts the pharmacokinetic profiles of the amorphous powder in both plasma and lung tissues after intratracheal administration of the aerosolized powders in rats using the PreciseInhale apparatus. The

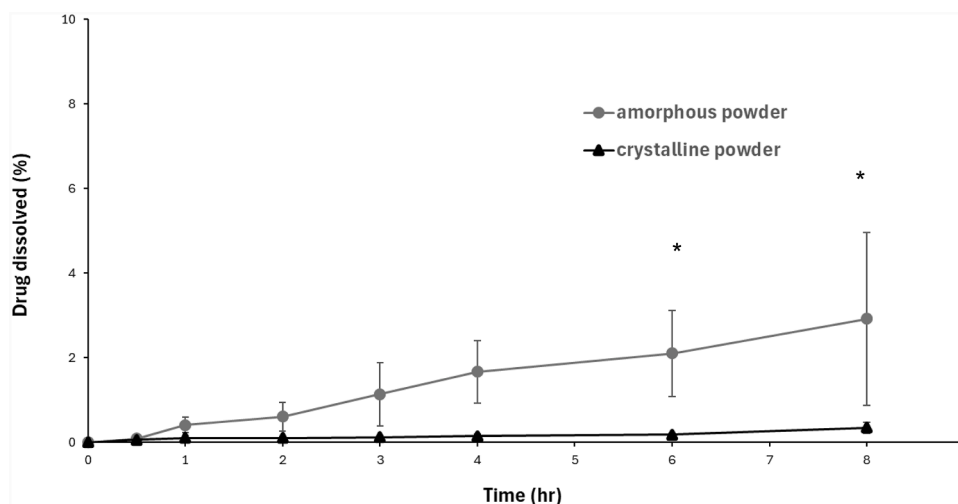


Fig. 5. Dissolution profiles of the p38 MAPK inhibitor pseudo-crystalline and amorphous powders. Statistically significant differences were observed at 6 and 8 h (two-way ANOVA followed by Bonferroni's multiple comparisons test, $p < 0.05$).

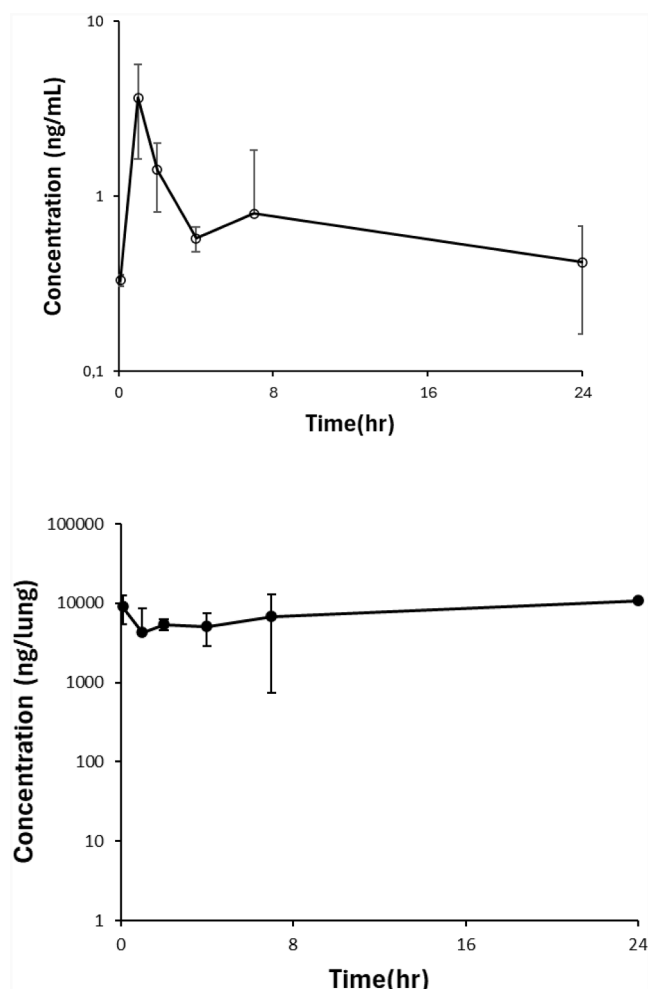


Fig. 6. Mean PF-03715455 plasma (upper panel) and lung concentrations (lower panel) after intratracheally administration of micronized amorphous powder.

deposited dose was 10 µg/lung per rat. Prolonged retention in the lung was observed, with quantifiable concentrations detected up to 168 h post-dosing (Fig. 8). This was reflected by pharmacokinetic parameters

summarized in Table 2 demonstrating a prolonged systemic absorption, as evidenced by the elevated AUC_{last} and MRT_{last} values. The high MRT_{last} further suggested a persistence of the compound in the systemic circulation, likely resulting from continuous absorption through the lung.

3.6. Pharmacokinetics of the p38 MAPK inhibitor pseudo-crystalline powder

Fig. 7 presents the pharmacokinetic profiles of the pseudo-crystalline powder in plasma and lung tissue. The deposited dose was 10 µg/lung per rat. Prolonged retention in the lung was observed, with quantifiable concentrations detected in the lungs up to 168 h post-dose (Fig. 8). In contrast, plasma concentrations were detectable only up to 4 h, with a mean residence time (MRT_{last}) of 1.36 h. The pharmacokinetic parameters reported in Table 2 indicate limited systemic absorption, likely attributable to poor dissolution, limited absorption rates and comparatively rapid systemic clearance. Indeed, Fig. 8, which reports lung concentrations at 96 and 168 h, highlights that drug levels remained very high, approximately one order of magnitude greater than those of the amorphous formulation, in agreement with the dissolution results.

4. Discussion

The *in vivo* and *in vitro* results obtained for the dry-powder formulations of the p38 MAPK inhibitor highlighted the pivotal role of the dissolution process in determining pulmonary drug availability. Although only the dissolved fraction is able to permeate the pulmonary epithelium and interact with its pharmacological target, the undissolved deposited particles act as a local drug reservoir, gradually reintegrating the dissolved fraction. Consequently, for poorly water-soluble inhaled compounds, the dissolution rate can become the rate-limiting step governing both local drug availability and systemic absorption.

Table 2

Pharmacokinetic parameters in rat plasma after amorphous and pseudo-crystalline powder aerosol after intratracheal administration.

PK PARAMETERS	POWDER	
	Amorphous	Pseudo-crystalline
AUC_{last} (ng·hr·mL ⁻¹)	40.94	0.79
AUC_{inf} (ng·hr·mL ⁻¹)	43.48	0.99
$t_{1/2}$ (hr)	60.20	1.62
MRT_{last} (hr)	33.39	1.36

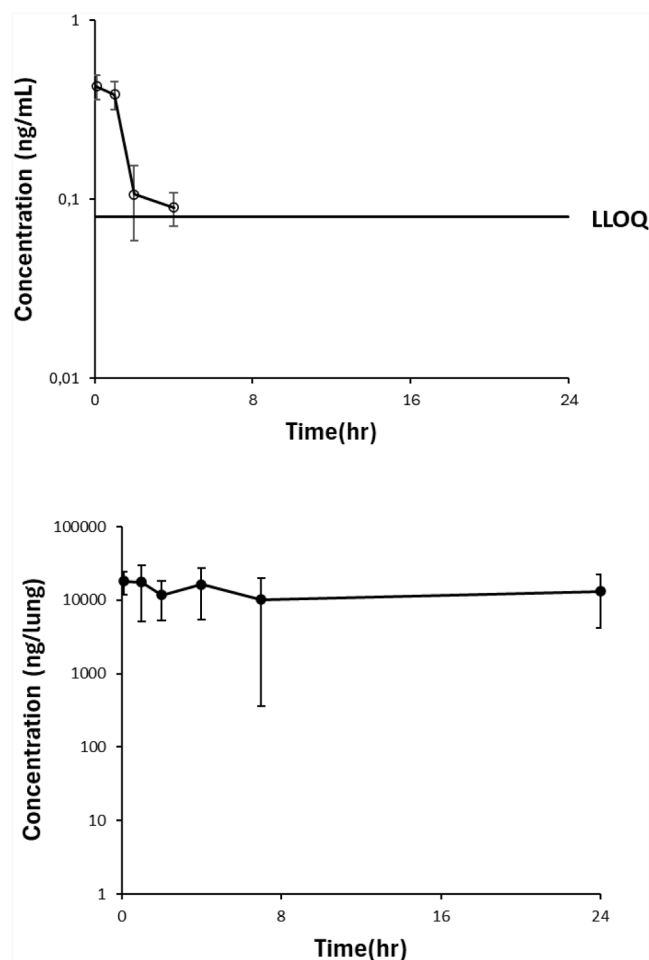


Fig. 7. Mean PF-03715455 plasma (upper panel) and lung concentrations (lower panel) after IT administration of micronized pseudo-crystalline powder.

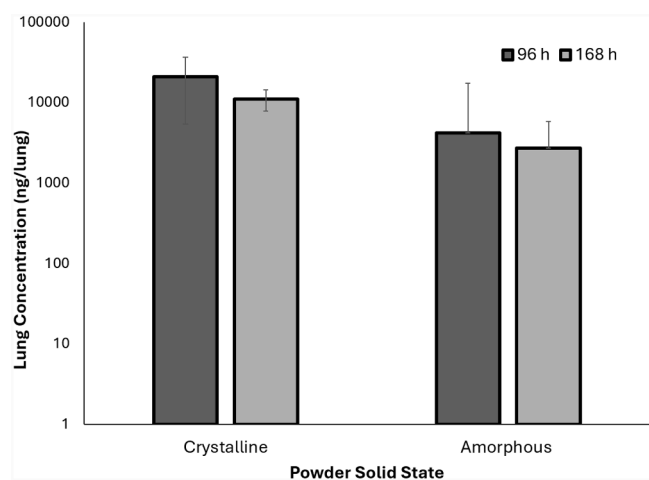


Fig. 8. Histogram representation of PF-03715455 lung concentrations at 96 h and 168 h following intratracheal administration of the micronized pseudo-crystalline powder.

Interestingly, the two powders investigated, despite exhibiting different solid-state characteristics and markedly different morphologies (*i.e.*, spongy large clusters for the crystalline powder and small microparticles for the amorphous formulation), displayed similar aerodynamic properties in terms of aerosolization yield and MMAD. Possibly this is due to

the lower density of the porous clusters evidenced for the pseudo-crystalline powder, a feature often exploited to improve the aerodynamic characteristics of inhalation products (Buttini et al., 2012; Scherließ et al., 2022; Sou and Bergström, 2021). In any case, the similar aerodynamic behavior allowed a fair comparison of the two powders tested in terms of dissolution properties and *in vivo* pharmacokinetics after pulmonary administration in rodents.

The p38 MAPK inhibitor compound selected was initially designed, in agreement with the “inhalation by design” strategy, in order to extend its half-life and residence in the lung, but this strategy apparently resulted in a significant downside, *i.e.* poor aqueous solubility (0.67 µg/mL, *in house* data). This physico-chemical property of PF-03715455 deeply impacted a feature often overlooked in the development of inhalation products: dissolution rate. Even if Gamble’s solution is one of the simplest simulated lung fluids and it is mainly a surrogate of lung interstitial fluid, it is often used as medium for dissolution experiments of inhalation products (Marques and Loebenberg, 2011).

The dissolution test showed that after 8 h, the pseudo-crystalline powder remained essentially undissolved. Even if the solid state had an impact on the dissolution, with an improved dissolution rate for the amorphous powder and a 10-fold improvement in the dissolved fraction, the overall value did not reach 5%. It is true that *in vivo*, especially at alveolar level a contribution to the dissolution from the physiologic alveolar surfactant is expected to promote hydrophobic, poorly soluble drug wetting and dissolution (Das and Stewart, 2016; Wiedmann et al., 2000), however, the dissolution test performed with Respicell allow to evaluate the powders behavior in conditions closely related to those found *in vivo*. In fact, the inhaled powder is not dissolving in a large volume of bulk solution as in the case of the GI tract, but it is deposited onto as superficial film of liquid. Similarly, in the Respicell apparatus the dissolution process occurs at the medium interface and in semi-stagnant hydrodynamic conditions avoiding the effect of stirring or medium flow conditions typical of paddle and flow-through apparatuses (Sonvico et al., 2021). In these conditions, it was possible to measure the very low dissolution rate for PF-03715455 in the two powders tested, but also to highlight the advantage of the amorphous solid state in promoting the dissolution.

These differences were reflected in the animal experiments in which the powders were administered intratracheally. Indeed, lung retention was prolonged while the plasma concentration remained at a very low level, highlighting an incomplete dissolution. In terms of comparison between the two powders tested, the amorphous formulation resulted in a markedly different pharmacokinetic profile. Lung concentrations were initially high but declined more rapidly than the pseudo-crystalline form. Plasma exposure was significantly increased, with AUC_{last} approximately 50-fold higher than that of the pseudo-crystalline powder. This discrepancy was predicted by the dissolution experiment, which showed that the amorphous powder dissolved substantially faster than the pseudo-crystalline form. In contrast, the pseudo-crystalline material exhibited extremely limited dissolution, with only 0.4% of the dose dissolving over 8 h. Interestingly, the plasma concentrations after the pulmonary administration of the pseudo-crystalline powder were detectable only up to 4 h, since the values afterwards declined below the lower limit of detection of the method. It appears clear that in these conditions, despite poor permeability of the compound, the elimination rate of the drug exceeds the dissolution rate leading to significantly lower plasma half-life ($t_{1/2}$) and MRT values for the pseudo-crystalline powder in comparison to the amorphous one. On the contrary for this latter solid form, dissolution rate was *in vivo* sufficient to maintain plasma concentrations at least for 24 h.

These results are likely a reflection of local conditions in the lung, with active drug concentrations not efficiently sustained in the case of the extremely low dissolution rate of the pseudo-crystalline drug.

This difference is consistent with the distinct solid-state properties of the two forms. The highly ordered and compact structure of the pseudo-crystalline state confers greater thermodynamic stability and reduces

molecular mobility, thereby limiting solvent penetration and slowing dissolution. Conversely, the amorphous form, characterized by a disordered molecular arrangement and higher free energy, facilitates solvent interaction and enhances the dissolution rate (Adhikari et al., 2022; Kawabata et al., 2011). These findings support the emerging paradigm that, especially for inhaled powders with low aqueous solubility, dissolution in the lung epithelial lining fluid can become the rate-limiting step for absorption into the systemic circulation. As noted by Somby et al., slow dissolution can serve as the absorption-rate-limiting mechanism, thereby prolonging lung exposure but reducing systemic uptake (Somby et al., 2023). A review by Eedara et al. reported that the dissolution from the solid state to the lining fluid is often the primary barrier for the inhaled particles, before the trans-epithelial transport occurs (Eedara et al., 2022). In a pharmacokinetic modelling context, the models for characterizing the pharmacokinetics of orally inhaled drugs also identify deposition, solid-state dissolution, and absorption as the critical triad governing systemic exposure (Borghardt et al., 2015).

In the research presented, the very low fraction dissolved *in vitro* and the limited plasma exposure suggested that the powders, despite their intended high lung residence, on one hand improved the local retention but on the other side limited the systemic (and possibly local) availability in terms of actual dissolved drug.

Recently, an evidence-based inhalation Biopharmaceutics Classification System (iBCS) has been proposed as a framework to provide a qualitative assessment of the technical and clinical risk related to the development of a new inhalation drug (Hastedt et al., 2022). In order to classify a drug in one of the four iBCS classes, several attributes and characteristics of the drug substance, e.g. solubility and permeability, and of the drug product, e.g. dose and dissolution rate, need to be taken into account. In particular, the boundaries of the grid separating the four iBCS classes (Fig. 9) are based on two fundamental parameters represented by the effective permeability and the Dose Number (Do), calculated according to the Eq. (1), knowing the lung dose (Mi), the volume of solution available for dissolution (Vi, that in the iBCS model is estimated in 10 mL of lung fluids), and the solubility of the drug (Csi) (Bäckman et al., 2022).

$$Do = \frac{(M_i/V_i)}{C_{si}} \quad (1)$$

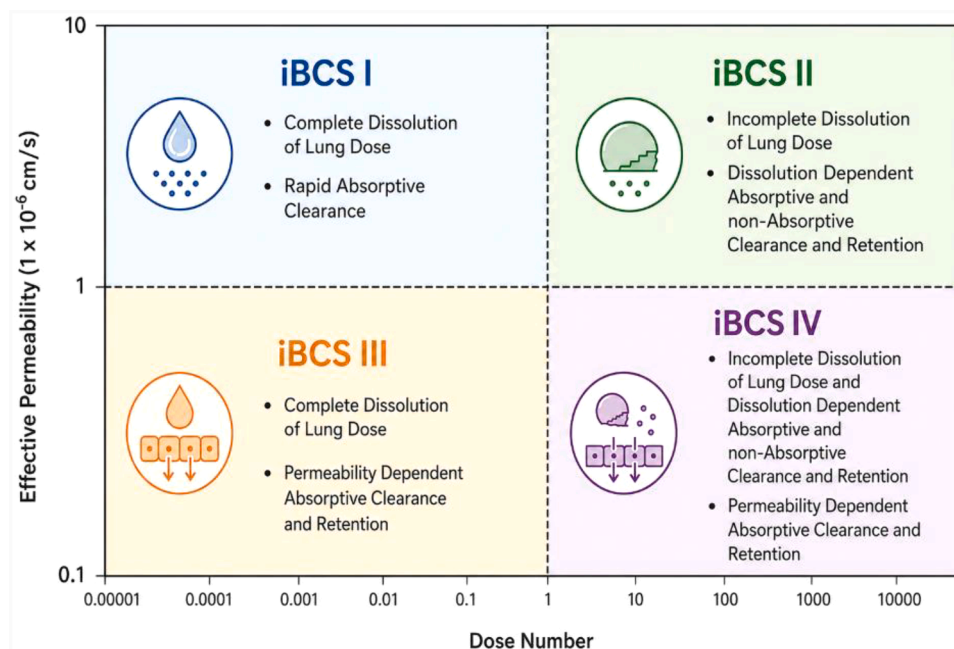


Fig. 9. An iBCS classification scheme depicting permeability and dose number categories (high vs. low), along with the dissolution and absorption characteristics associated with drugs in iBCS Classes I–IV (Hastedt et al., 2022).

Now PF-03715455 permeability obtained in CaCo-2 monolayers was $0.3 \cdot 10^{-6}$ cm/s (in house data) and its dose number can be calculated considering the dosing of 680 µg BID used in clinical trials for moderate to severe uncontrolled asthma (ClinicalTrials.gov ID NCT02366637) and severe chronic obstructive pulmonary disease (ClinicalTrials.gov ID NCT02219048). Taking in consideration, the compound solubility value, the administered dose and a conservative assumption of only around 20% lung deposition (i.e. subtracting from the dose the fraction not reaching the lung and the one eliminated via mucociliary clearance) the calculated Do value for PF-03715455 is around 19. As a result, PF-03715455, with a dose number above 1 and a permeability below $1 \cdot 10^{-6}$ cm/s, can be classified as an iBCS class IV drug, characterized by poor solubility and permeability and consequently expected to show incomplete dissolution of the lung dose and incomplete absorption, due to a dissolution-limited absorptive clearance in the non-sink *in vivo* conditions (Hastedt et al., 2024). All these iBCS predictions are duly confirmed by the *in vitro* and *in vivo* results presented in this research work.

From a formulation point of view, this implies that “inhalation by design” compounds selected to have low solubility for enhanced lung residence but developed without balanced dissolution kinetics may miss the therapeutic objective. This highlights the need to optimize not only aerodynamic and deposition characteristics, but also the solid-state, formulation and dissolution behavior of inhaled drug compounds falling in iBCS classes II and IV. Furthermore, the results presented evidence the importance of the dissolution testing for inhaled drugs: a formulation designed for prolonged lung residence must still ensure sufficient dissolution to enable absorption and effect. In fact, *in vitro* dissolution testing of the respirable fraction can provide predictive insight into *in vivo* bioavailability for inhaled poorly soluble drugs, potentially avoiding sometimes costly *in vivo* studies of formulations that are unlikely to achieve systemic exposure despite adequate deposition.

5. Conclusion

The present study demonstrated that the solid-state properties of PF-03715455 markedly influenced its dissolution behavior and, consequently, its pulmonary pharmacokinetic profile following inhalation. Although the amorphous and pseudo-crystalline powders exhibited

comparable aerodynamic performance, the amorphous formulation showed a significantly faster dissolution rate, resulting in greater systemic exposure and lower lung retention. In contrast, the pseudo-crystalline formulation dissolved only to a very limited extent, leading to prolonged pulmonary retention and minimal plasma exposure. These findings confirm a clear relationship between *in vitro* dissolution behavior and *in vivo* pulmonary bioavailability for this poorly soluble iBCS Class IV compound.

The results further indicate that the dissolution rate of poorly soluble inhaled drugs is a decisive factor in determining both lung retention and systemic exposure following pulmonary administration. Therefore, optimizing only the aerodynamic properties of dry powder formulations is not sufficient; careful attention must also be paid to dissolution behavior to achieve the desired therapeutic profile. The use of predictive *in vitro* dissolution testing, as demonstrated with the RespiCell® apparatus, represents a valuable tool for early-stage formulation screening and may contribute to reducing the reliance on animal studies. Overall, these findings support the integration of biorelevant dissolution testing into the development workflow of inhaled drug products and encourage further studies aimed at establishing robust *in vitro*–*in vivo* correlations.

CRediT authorship contribution statement

Virginia Patterlini: Writing – original draft, Investigation. **Giulia Marengi:** Writing – original draft, Investigation. **Alessandro Fioni:** Writing – review & editing, Funding acquisition, Conceptualization. **Giovanna Trevisi:** Methodology. **Paolo Pio Mazzeo:** Methodology. **Eride Quarta:** Validation, Resources. **Francesca Buttini:** Validation, Supervision. **Fabio Sonvico:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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