Gaining an insight into the importance of each inhalation manoeuvre parameter using altered patients' inhalation profiles

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Abbreviations: IP, inhalation profile; ACI, Andersen Cascade Impactor; DPIs, dry powder inhalers; FPD, fine particle dose; FPF, fine particle fraction; HPLC, high performance liquid chromatography; PIF, Peak inhalation flow; IT, inhalation time; Vin, inhaled volume; LOD, limit of detection; LOQ, limit of quantification; MMAD, mass median aerodynamic diameter; PIL, patient information leaflets; RA, residual amount; DD, delivered dose; TRD, total recovered dose

32 **ABSTRACT**

33 The aerodynamic particle size distribution (APSD) and dose emission characteristics of
34 indacaterol Breezhaler have been measured using ex-vivo methodology and altering original
35 COPD inhalation manoeuvre profiles to investigate the influence of inspiratory parameters
36 namely, the peak inhalation flow (PIF), inhalation acceleration rate (ACIM) and inhaled volume
37 (Vin) by keeping two parameters fixed and varying the other one.

38 All inhalation parameters had an impact on the dose emission, dose emptying from the
39 capsule/device in the order of PIF>Vin>ACIM. ACIM and Vin have almost an equal effect on the
40 delivered dose (DD) and the fine particle dose (FPD) but the impact of the PIF was the most
41 pronounced. PIF also had the greatest impact on the mass median aerodynamic diameter
42 (MMAD). Producing an optimal inhalation manoeuvre combining the highest PIF, ACIM and Vin
43 in one single inhalation would improve both the quantity and the quality of the aerosol. Making
44 two separate inhalation from each prepared dose would also be useful. This ex-vivo methodology
45 replaying altered COPD profiles with the breath simulator allows an insight on the importance of
46 each inhalation manoeuvre parameter which would have not been possible using conventional
47 in-vitro Pharmacopoeial methodology.

48 **Keywords:**

49 Andersen cascade impactor (ACI), Peak inhalation flow (PIF), Inhalation volume (Vin),
50 Acceleration rate (ACIM), Breath simulator (BRS) and Onbrez Breezhaler®

1. Introduction

The drug delivery to the local site of action in the lungs and reducing the systemic side effect is paramount in the treatment and management of respiratory disorders such as asthma and COPD[1,2]. Thus, the pulmonary route is the important route of drug administration in the treatment of respiratory diseases. In order to deliver the medication via the inhalation route, different inhaler devices are available nebulisers, pressurised metered dose inhalers (pMDIs) and dry powder inhalers (DPIs). The latter is becoming the most widely used device. The dose emitted from a DPI operates upon the inhalation manoeuvre generated by the patient, which means no co-ordination is required between dose actuation and the inhalation manoeuvre, as the case with most pMDIs [1,3].

The amount and the quality of the dose emitted from a DPI is dependent on its formulation. The performance of a DPI formulation depends on detachment of drug particles from the carrier surface and deaggregation of drug agglomerates during aerosolization, any factor that affects the drug-carrier interaction may also affect drug delivery and deposition. Several parameters have been identified including carrier size and size distribution, shape and surface roughness. The latter has been identified to be closely related to surface energy and the adhesion force between drug and carrier[4].The design and resistance of the device and inhalation profiles (IPs) characteristics of the inhalation manoeuvre made by a patient are equally important in enhancing aerosol performance from DPIs [5–7].

The patient's IP is characterised by three factors namely the peak inhalation flow (PIF), the inhaled volume (V_{in}) and initial acceleration at the start of the inhalation manoeuvre (ACIM)[8,9].There is a large inter-individual variability of IPs made by patients with asthma and

73 chronic obstructive pulmonary disease (COPD) when they inhale through DPIs [10,11] and
74 therefore prescribing the right inhaler to the right patient together with training them how to
75 use the device are crucial [7,12,13].

76 Dose de-aggregation and the clinical efficacy of DPIs is governed by the characteristics of the IP
77 generated by the patient [3,14]. In-vitro assessment of DPIs using compendial methodologies
78 (USP, 2014) to probe the total emitted dose (TED) and aerodynamic particle size distribution
79 (APSD) of the inhaled products are quality control tests that are not reflective of real life patient
80 use [8,15] . Nevertheless, since the systemic drug delivery is a function of the total emitted dose
81 (TED), which acts as an indicator of systemic safety, while lung deposition is a function of the fine
82 particle dose (FPD), a marker of dose efficacy [16,17] then these in-vitro methodologies do
83 provide some useful insights related to patient use. To compliment this, the European Medicines
84 Agency (EMA) have recommended that these should be measured using a range of inhalation
85 PIFs achieved by the patients (EMA, 2006). However, these methods use a vacuum pump to
86 generate an inhalation profile which is not achievable by a human [8,9,15] .

87 We have adapted the compendial in-vitro methodologies, based on using the Andersen Cascade
88 Impactor (ACI), by using the inhalation profiles of patients instead of the traditional vacuum
89 pump [9]. Using this ex-vivo methodology, inhalation manoeuvre parameters such as PIF, Vin and
90 ACIM have been shown to affect the dose delivery from DPIs[9,15]. During routine real life use
91 the contribution of each of the inhalation parameters on dose emission from a DPI is not known.
92 Our previous study in-vitro using Dose unit sampling apparatus (DUSA) and Andersen Cascade
93 impactor (ACI)[18] allowed us to investigate the effects of the PIFs and Vins separately by fixing
94 one parameter at a time to study the impact of each parameter on the dose emission and

aerodynamic characteristics of inhaled aerosol. Our study in-vitro showed that inhaled volume becomes more important in improving dose emission and drug deposition, especially at low PIF. Due to the square wave inhalation profile of this in-vitro method dictated by the vacuum pump, the PIF is reached instantly upon actuation of the dose contrary to the gradual increase in ACIM for a bell-shaped inhalation profile of a patient making correlations difficult and the results from the in-vitro study are overestimated in comparison of what is happening in-vivo .Using our ex-vivo method a patient profile can be altered by varying one inhalation manoeuvre parameter at a time whilst fixing the two other parameters to help understand the impact of each inhalation parameter and its contribution to overall drug emission and drug deposition. Therefore, this study focuses on the use of COPD patients' altered IPs to investigate the effect of each inspiratory parameter (PIF, V_{in} and ACIM) on the overall dose emission and APSD of indacaterol from an Onbrez Breezhaler®.

2. Materials and methods

2.1. Inhaler device

Onbrez Breezhaler® (Novartis Pharmaceuticals Ltd, CH) used in the study. Each capsule contains indacaterol maleate equivalent to 150 µg of indacaterol.

2.2. Altered inhalation profiles

The IPs of 37 patients with different COPD severity aged 55-79 with a mean age of 66, when they inhaled through a Breezhaler® loaded with an empty capsule were measured using an inhalation profile recorder [11] . The Breezhaler® is a low resistance device with an intrinsic resistance of 0.0177 (kPa)^{0.5}(min L⁻¹) [19]. The patients were given the patient information leaflet (PIL) to read and trained to inhale as fast as they can from the start of the inhalation manoeuvre and continue

for as long as possible. The inspiratory parameters (e.g., PIF, ACIM and Vin) were obtained from each profile [10]

The IPs with a PIF of low (40 L/min) medium (65 L/min) and high (85 L/min) flows were chosen.

The ACIM and Vin of these profiles were altered to obtain Vin values of 1, 2 and 3 L and ACIM values of 2, 4 and 8 L/s², the total number of altered IPs in the present study were 27 IPs. The inhalation volume was altered by increasing or decreasing the inhalation time (Ti) after the PIF of the profile. The ACIM was altered by increasing or decreasing the time to PIF.

27 profiles were used (9 profiles varying the PIF from low (40 L/min) to medium (65 L/min) to high flow rate (85 L/min) but fixing the ACIM and Vin, 9 profiles varying the ACIM from 2 to 4 to 8 L/s² but with fixed PIF and Vin and finally 9 profiles with varying the Vin from low (1L) to medium (2 L) to high (3 L) but with fixed PIF and ACIM).

2.3. Experimental set-up

(a) Aerodynamic particle size distribution (APSD)

Figure 1, previously described by [8,9] provides a schematic design of the method used to identify the APSD. The Andersen Cascade Impactor (ACI; Copley Scientific Ltd, UK) was set up at either 60 or 90 L/min using the appropriate stages and pre-separator with the required flow rate drawn through the ACI using a dry powder flow controller (TPK2100; Copley Scientific Ltd, UK) and vacuum pump (HCP5; Copley Scientific Ltd, UK). A mixing inlet was placed between the adult version of the Alberta's Idealised Throat (AIT; Copley Scientific Ltd, UK) and the pre-separator of the ACI. Supplementary air from a compressed air source was introduced into the ACI, through the side arm of the mixing inlet, at the same flow rate that was drawn through the ACI, so that the airflow at the mouthpiece of the AIT was zero (0 L/min). During the set-up a flow meter was

attached to the AIT to ensure zero flow at the mouthpiece when adjusting the supplementary flow to equal the air drawn through the ACI. A breath simulator (BRS 3000, Copley Scientific Ltd), was programmed with the inhalation flow against time data of each IP. It was positioned between the supplementary air and the mixing inlet. When the programmed IP was drawn from the supplementary air, the IP was replayed through the DPI in situ at the mouthpiece of the AIT because of the constant flow drawn through the ACI via the dry powder controller unit. Thus, the IPs facilitated dose de-aggregation and emission of indacaterol from the capsule loaded in the Breezhaler®, into the ACI. For each determination, three separate capsules were aerosolised into the ACI by replaying the IP for each capsule and three separate dose emission determinations were made (n=3) for each IP.

(b) Delivered dose and residual amounts

The delivered dose (DD) was determined using a dose unit sampling unit (DUSA; Copley Scientific Ltd, UK). The method is similar to that of the pharmacopoeias (USP, 2014) except that the vacuum pump was replaced by inhalation profiles drawn through the DUSA using the breath simulator (BRS3000; Copley Scientific Ltd, UK). A 150µg indacaterol capsule was loaded into the Breezhaler®, according to the instructions for use, and the inhaler was inserted into the mouthpiece of the DUSA. An inhalation profile was then replayed, and the emitted dose was captured in the DUSA. Each determination was carried out three times for each IP. The delivered dose (DD) was determined after recovering the dose emitted into the DUSA and the residual amount from that left in the capsule and device

2.4. HPLC assay

The amount of indacaterol deposited on each stage of the ACI and the final filter, the pre-separator, AIT, and the mixing inlet were recovered by appropriate rinsing followed by measurement using a validated HPLC method.

The HPLC system used for samples quantification was Prominence liquid chromatography LC-20 AT (Shimadzu, UK). The specifications of the system component are as follow

- Pump: LC-20AT prominence pump. Oven: column oven (CTO-10AS VP)
- Autosampler: Prominence Auto Sampler (SIL-20A HT) fitted with a 200 µL loop (Shimadzu Ltd, UK)
- Degasser: Prominence degasser (DGU-20A5) (Shimadzu Ltd, UK)
- Detector: SPD-20A prominence UV-vis detector (Shimadzu Ltd, UK)

The HPLC method used was validated using Dexamethasone as an internal standard. The limit of quantification and limit of detection for indacaterol were 0.17 and 0.07 µg/mL respectively. A summary of the HPLC method is shown in Table 1 [8,20]

2.5. Data Analysis

The Copley Inhaler Testing Data Analysis Software (CITDAS version 2.0, Copley Scientific Ltd, UK) was used to calculate the aerodynamic dose emission parameters.

The total emitted dose (TED) was obtained from the cumulative amounts of indacaterol deposited in the Alberta's throat (IAT), the pre-separator, mixing inlet and all the stages of the ACI. The FPD was the mass associated with particles <5 µm and the extra fine particle dose (EFPD) was the mass of powder associated with particles < 2 µm [21] . The fine particle dose was

expressed as a % of the label claim and the fine particle fraction (FPF) was the FPD divided by the TED. The MMAD was the size corresponding to the 50th percentile of the cumulative mass-weighted distribution of the amount deposited in the ACI. The geometric standard deviation (GSD) indicates the variability in the aerosol particle size. The GSD is representing the dispersion of the inhaled particles whereas particles with ≤ 1.2 are monodisperse and particles with GSD of ≥ 1.2 are polydisperse. Large particle mass (LPM) was calculated as mass of the particles $> 5\mu\text{m}$ deposited in the upper part of the ACI.

The delivered dose (DD) was the total amount deposited in the DUSA, the residual amount (RA) was the amount left in the device and capsule after drawn a inhalation profile and the total recovered dose (TRD) was the sum of DD and RA.

SPSS version 16.0 software (SPSS Inc., Chicago, USA) was used for the statistical analysis. A two-way analysis of variance (ANOVA) was used to determine any significant differences between the DD, FPD, %FPF, MMAD between different inhalation profiles.

3. Results

To study the effect of the PIF, we maintained the ACIM and V_{in} fixed. To study the impact of the ACIM, we maintained the PIF and V_{in} fixed and finally to investigate the impact of the V_{in} , we maintained the PIF and ACIM fixed and the results are summarised in Tables (2, 3 and 4). Dose delivery uniformity and dose emission from an Onbrez Breezhaler® was also conducted using DUSA for the same profiles (Table 5). At any fixed ACIM and V_{in} , increasing the PIF from 40 to 85 L/min resulted in a significant increase ($p < 0.05$) in the DD, FPD, EFPD and a significant reduction ($p < 0.05$) in the RA and MMAD. The effect of the PIF on the aerodynamic characteristics of the

emitted dose was even more pronounced when the PIF was combined with the highest ACIM (8 L/s²) and the highest Vin (3 L) Tables (2, 3 and 4).

At any fixed PIF and Vin, the FPD and DD increased significantly ($p < 0.05$) with increasing the ACIM (Figure 2, Table 5). RA showed also some marginal decrease but still significant ($p < 0.05$) (Table 5). Whereas, the MMAD was not affected by the ACIM (Figure 3). At any fixed PIF and ACIM, the DD (Table 5), and FPD (Figure 2) increased significantly with increasing the Vin, whereas, RA (Table 5) showed a significant decrease ($p < 0.05$) but MMAD (Figure 3) was not affected with the Vin. Most changes in the aerodynamic dose emission characteristics of indacaterol were observed by combining all optimal inhalation manoeuvre parameters (highest PIF, highest ACIM and highest Vin) (Tables 2,3 and 4). About 50% of the indacaterol 150 µg nominal dose was deposited in the impactor as large particle mass (LPM) (Tables 2,3 and 4) irrespective of the PIF, ACIM and Vin. GSD values are > 1.2 and showed no significant variation with the PIF, ACIM and Vin (Tables, 2,3 and 4). Dose delivery study with the DUSA corroborated with the impactor study with regards to the DD and RA (Table 5).

4 Discussion

Onbrez Breezhaler® dose emission and aerodynamic characteristics were studied previously [18,22–24]. This device was chosen because patients were able to generate a wide range of PIFs, Vins and ACIMs. Previous In-vivo studies showed that not all the patients were able to generate 4kPa pressure drop across the inhaler with 4 L inhalation volume as recommended by the pharmacopoeia to assess the in-vitro dose emission from DPIs [10,11]. Patients using Breezhaler® were able to inhale with least inspiratory effort and generate a high PIF when compared to Elipta®

and Handihaler® [22,24,25]. Breezhaler® requires a minimum PIF of 30 L/min and optimal PIF of 60 L/min when compared to Genuair® which requires an optimal flow > 45 L/min [26]

Using patient IPs, the major influence on dose emission and its characteristics was found to be the PIF. This flow dependent dose emission of indacaterol from the Breezhaler® is consistent with that reported by others [22,23]. Also, the results are consistent with the dose emission characteristics of glycopyrronium from the Breezhaler® [24]. The largest difference was found to be between 40 and 65 L/min suggesting that 65 L/min could be regarded as the cut-off inhalation flow. This is faster than that previously reported [27]. Using inhalation profiles amongst the 37 COPD patients we studied 9 inhaled profiles < 65 L/min. Previous studies have shown that out of 26 patients only one patient achieving a PIF of < 52 L/min [22], while Colthorpe et al., (2013) reported that only 2 patients achieving a PIF < 65 L/min[24]. In our previous inhalation profiles performance study, patients inhaling through Breezhaler® were able to generate an average V_{in} and ACIM of 2.1 L and 4.2 L/s² [8].

The ex-vivo methodology was used to identify the impact of each of the inhalation manoeuvre parameter (PIF, V_{in} and ACIM) on the aerodynamic and dose emission characteristics of indacaterol 150 µg capsules from a Breezhaler®. It has been reported that these parameters play an important role in both dose emission and drug deposition into the lungs [8,9,15] but it's very difficult to differentiate which one of these parameters has the most dominant effect. The information provided in the patient information leaflets (PIL) when inhaling through DPIs is vague, such as inhale as 'inhale forcefully'. The meaning of this phrase is clear to the aerosol formulation scientists (i.e., to achieve an optimal PIF, ACIM and V_{in}) but not for all patients. This will result in the misuse of the inhaler device by the patient, resulting in the patient getting a sub-

245 optimal dose. Knowing which parameter of the inhalation manoeuvre is the most important will
246 ease patient's training and education by the inhalation health care practitioners to help the
247 patient manage their own disease. Unfortunately, no data is available in the literature regarding
248 the influence of each inhalation parameter. Compendial methods use a vacuum pump that
249 provides an unachievable fast acceleration and square wave inhalation profile that is not possible
250 for any human to replicate. Also, recommendations to use an inhalation flow corresponding to a
251 pressure drop of 4kPa and an inhaled volume of 4 L is only achieved by very small numbers of
252 patients [10,11] .The compendial methodologies provide dose emission data that uses optimal
253 inhalations and so can only be regarded as quality control data and not reflective of patient use.
254 Most commercially available DPIs are a breath actuated devices, and the dose emission from the
255 inhaler relies on the above mentioned inspiratory parameters to generate particles within the
256 respirable range ($\leq 5 \mu\text{m}$) with the greatest tendency for lung deposition [28,29]. The intrinsic
257 device resistance is an important factor that determines the extent of the patient's ability to
258 generate the required pressure drop inside the device for effective dose de-aggregation and
259 dispersion [30,31]. Indacaterol Breezhaler® belongs to low resistance devices category with a PIF
260 of 100 L/min and above to generate 4kPa pressure drop [22] . To attain a pressure, drop of 4kPa
261 in the Breezhaler® requires an inhalation flow of 107 L/min. This is above the recommended flow
262 to use cascade impactors, so it is useful to study dose emissions using inhalation profiles.
263 However, using the ACI and the Next Generation Impactor (NGI) using flows below 90 and 100
264 L/min, respectively, is not recommended. Nevertheless although dose emission from the Onbrez
265 Breezhaler® is dependent on the inhalation flow rate [18,22,23] knowledge of the dose emission
266 above these maximum flow is not important because dose de-aggregation and empty of the dose

from the capsule and device should approach the nominal dose and, therefore, not clinically significant. However, if a patient uses the Breezhaler® with an inhalation flow above 100 L/min oropharyngeal deposition will start to increase [7,32]

The dose emission study from various profiles was carried out using the DUSA (Table 5). To study the impact of the PIF on the indacaterol dose emission, we fixed the values of both the V_{in} and ACIM. DD increased significantly ($p < 0.05$) with the PIF at any fixed V_{in} and ACIM suggesting dose emission flow rate dependency of the Onbrez Breezhaler® (Figure 4). The increase in the dose emission was associated with a significant increase ($p < 0.05$) in the FPD (Figure 2), extra fine particle dose (EFPD) (Tables 2,3 and 4) and a reduction in the MMAD (Figure 3). For examples, at the weakest V_{in} (1 L) and ACIM (2 L/s^2), DD increased from 116,80 (1.25) μg at 40 L/min to 125.41 (0.73) at 85 L/min (Table 5) and the corresponding FPD increased from 29.4 (0.7) μg to 34.7 (0.7) μg (Figure 2), whereas the EFPD increased significantly ($p < 0.05$) from 19.7 (1.1) μg to 25.2 (0.3) μg . The fine particles dose obtained for indacaterol was in line with clinical study conducted on glycopyrronium bromide using the same inhaler device Breezhaler®. FPF of approximately 36% of the total emitted dose was generated in both studies suggesting the reliability of Breezhaler® in delivering the same FPD irrespective of the drug used [33].

The residual amount (RA) left in the capsule and device also showed a substantial decrease with increasing the PIF from 40 L/min to 85 L/min at a fixed ACIM and V_{in} (Figure 4). In the DUSA study for example at the above mentioned weakest V_{in} (1 L) and ACIM (2 L/s^2), the RA decreased from 33.51 (1.00) μg at 40 L/min to 22.76 (0.54) μg at 85 L/min (Table 5). The impact of the PIF on indacaterol dose emission, dispersion shown from the increase in the $\text{EFPD} \leq 2 \mu\text{m}$, increasing the amount of respirable particles reaching the distal stages of the impactor (FPD) and

contributing to dose emptying is obvious. But the impact of the PIF on reducing the large particle mass (LPM) is not substantial as approximately 50% of the nominal dose was deposited and lost in the upper part of the impactor as LPM irrespective of the flow rate used.

The effect of the PIF on dose emission characteristics and dose retention is further enhanced by increasing the inhaled volume (V_{in}) and the acceleration of inhalation at the start of the inhalation manoeuvre (ACIM). For example, DD increased from 116.80 (1.25) μg at PIF (40L/min), ACIM (2 L/s²), V_{in} (1 L) (Table 5) to 134.83 (1.58) μg at PIF (85L/min), ACIM (8 L/s²), V_{in} (3 L) (Table 5). Similarly, the FPD increased from 29.4 (0.7) μg to 49.8 (1.0) μg (Figure 2), EFPD increased by two fold from 19.7 (1.1) μg to 39.16 (0.2) μg , MMAD was reduced from 3.4 (0.1) to 2.6 (0.0) μm (Figure 3) and dose retention in device and capsule was reduced by 50% from 33.51 (1.00) μg to 14.58 (2.44) μg (Table 5). Thus, by combining all optimal inhalation parameters, i.e., highest PIF, highest ACIM and the highest V_{in} in one single inhalation, the quantity and the quality of the inhaled indacaterol is improved to maximise drug delivery to the lungs, whilst providing more particles in the extrafine range ($\leq 2 \mu\text{m}$) with high tendency for peripheral deposition.

The effect of ACIM could not be investigated using traditional pharmacopoeial methods, due to the square wave generated by the vacuum pump resulting in a rapid acceleration through the DPI, beyond what typical patient can generate during routine use [10]. However, this was made possible with the breath simulator. The impact of the ACIM alone was also investigated by fixing the PIF and the V_{in} . DD increased significantly ($p < 0.05$) with the ACIM at any fixed PIF and V_{in} (Table 5). The increase in the dose emission was accompanied with a significant ($p < 0.05$) increase in the FPD (Figure 2), effective extra fine dose (EFPD) showed a marginal increase, almost no significant effect on the MMAD (Figure 3). The impact of the PIF on the DD, FPD, EFPD and

MMAD was more significant ($p < 0.05$) than the ACIM. Since the acceleration rate is involved at the start of the inhalation manoeuvre and from these results it seems that the ACIM has more effect on dose emission and detachment of the indacaterol from the surface of the carrier to maximise the amount of aerosol exiting the device increasing DD (Figure 4) and FPD (Figure 2), but the effect of ACIM on drug dispersion is limited as shown by EFPD (Tables 2,3 and 4) and MMAD (Figure 3) results which were marginally affected by the ACIM. For examples, at the weakest PIF (40 L/min) and V_{in} (1 L), DD increased from 116.80 (1.25) μg at 2 L/s^2 to 121.31 (0.16) μg at 8 L/s^2 (Table 5) and FPD increased from 29.4 (0.7) μg to 34.7 (0.7) μg (Figure 2), whereas the EFPD increased from 19.7 (1.1) μg to 25.2 (0.3) μg (Tables 2). The residual amount (RA) left in the capsule and device showed a slight decrease with increasing the ACIM from 2 L/s^2 to 8 L/s^2 (Figure 4). MMAD showed almost no significant variation with the ACIM (Figure 3).

This detailed study on the effect of the initial acceleration of the inhalation manoeuvre (ACIM) showed its importance in dose emission of the inhaled indacaterol which is in accordance with other authors [9,14,15]. Patients may achieve the same PIF and V_{in} but with different accelerations. The one inhaling fast through Onbrez Breezhaler® can benefit more from the inhaled dose.

Effect of the inhaled volume (V_{in})

Inhalation volume (V_{in}) is another inhalation manoeuvre parameter to be considered when evaluating the performance of DPIs. The Pharmacopoeial DPIs testing in-vitro suggested that a V_{in} of 4 L should be drawn through the inhaler. However, most of the patients with asthma and COPD were unable to achieve this value, and only a few patients were able to exceed 2 L inhalation volume with a mean value $\geq 2 \text{ L}$ [10,11,34]. The inhaled volumes used to quantify the

aerodynamic particle size distribution of inhaled indacaterol exceeds the internal volume of the ACI (1.155 L) [35] to allow sufficient sampling time for the aerosol bolus transfer from the inhaler device to distal stages of the ACI [36,37].

The impact of the V_{in} at a fixed PIFs and ACIMs was investigated. For example, at the lowest PIF (40 L/min) and at any ACIM, increasing the V_{in} from 1 L to 3 L increased significantly ($p < 0.05$) the DD from 116.80 (1.25) μg to 123.98 (0.44) μg at ACIM of 2 L/s², from 117.96 (2.08) μg to 124.61 (2.65) μg at ACIM of 4 L/s² and from 121.31 (0.16) μg to 127.30(1.35) μg at 8 L/s² (Table 5). It is interesting to note that the impact of the V_{in} on the DD is less pronounced at high ACIM (8L/s² and High PIF (85 L/min))(Figure 4). For example, at the highest PIF (85 L/min) and the highest ACIM 8 L/s² (Table 5), the DD showed only slight but significant increase ($p < 0.05$) from 129.40 (1.68) μg to 134.83 (1.58) μg with increasing the V_{in} from 1 L to 3 L respectively. When the PIF and ACIM are low (e.g., PIF (40 L/min) and ACIM (2 L/s²) increasing the inhaled volume can be used to promote drug emission with a subsequent increase in the FPD (Figure 2). The importance of V_{in} was in agreement with previous studies carried out by Abadelah et al., (2017) Easyhaler® [38] Breezhaler® [18]. The impact of the V_{in} on the FPD is significant ($p < 0.05$) and this is obvious from Figure 2. The increase in the V_{in} was associated with a significant ($p < 0.05$) increase in the DD (Figure 4) and FPD (Figure 2) irrespective of the PIF and ACIM. This shows the importance of the V_{in} in promoting drug delivery to the lungs from capsule based DPIs such as Onbrez Breezhaler®. The EFPD is marginally increased with the V_{in} , whereas MMAD (Figure 3) showed almost no change with the V_{in} suggesting that the V_{in} is involved more in mass transfer rather than aerosol dispersion to cause a further decrease in the MMAD as shown from the present results (Figure 3). The V_{in} has a significant effect ($p < 0.05$) on the RA left in the

capsule and device as shown from DUSA study (Figure 4). For example, at the weakest PIF (40 L/min) and ACIM (2 L/s²), the RA significantly reduced ($p < 0.05$) from 33.51 (1.00) μg to 24.05 (1.01) μg with increasing the V_{in} from 1 to 3 L (Table 5) and on the other hand at the same PIF (40 L/min) and V_{in} 1 L, increasing the ACIM from 2 L/s² to 8 L/s² has promoted capsule and device emptying but significantly ($p < 0.05$) to a lesser extent when compared to the V_{in} (Table 5). For example, at a fixed PIF (40 L/min) and 1 L V_{in} , increasing the ACIM from 2 to 8 L/s² has reduced the RA from 33.51 (1.00) μg to 27.98 (2.77) μg , this reduction in the RA was smaller compared to the V_{in} (Table 5). The most efficient inhalation manoeuvre parameter with regards to capsule and device emptying was $\text{PIF} > V_{in} > \text{ACIM}$. The residual amount was reduced by 50 % by combining all the inhalation manoeuvre parameters to their optimal values (i.e., increasing the PIF from 40 L/min to 85 L/min; ACIM from 2 L/s² to 8 L/s²; V_{in} from 1 L to 3 L). It is interesting to note that complete emptying of the capsule and device is still a challenge irrespective of the inhalation manoeuvre. Thus, more work on the inhaler device is needed to improve dose emptying from capsule based devices such as Onbrez Breezhaler®.

Effect of inhalation manoeuvre parameters on MMAD and EFPD

The β -receptors are abundant in the alveolar wall a region where no smooth muscle exists and whose functional configuration is unknown. The location of β -receptors in the lungs suggests that drug targeting is important for maximum therapeutic effect. For example, salbutamol requires a more peripheral delivery to the medium and small airways to produce a therapeutic effect [16]. Thus, particle size affects the lung deposition of an aerosol and influences the clinical effectiveness. Zanen et al., (1996) reported that “in patients with mild to moderate asthma, particles with an MMAD of approximately 3 μm were more effective than larger particle size

aerosol in producing bronchodilation since it has the best penetration and retention in the lungs in the presence of airways narrowing” [39]. Breezhaler® was effective in producing particles with an MMAD of around 3 µm irrespective of the PIF, ACIM and Vin (Figure 3). ACIM and Vin have almost a negligible effect on the MMAD whereas the indacaterol dispersion is more caused by the PIF especially when combined with a fast and prolonged inhalation to increase the amount of indacaterol reaching the distal stages of the impactor with more particles in the extrafine range ($\leq 2 \mu\text{m}$) with high tendency for peripheral deposition as shown at high PIF (85 L/min), ACIM (8 L/s²) and Vin (3 L) (Table 4). Particles with an MMAD < 3µm are important in targeting smaller airways, and they found to be useful in the management of respiratory diseases [40,41]

4. Conclusion

The Ex-vivo methodology on dose emission characteristics of indacaterol from Onbrez Breezhaler using altered COPD patients’ IPs provides an insight on how each inhalation manoeuvre parameter contributes to the overall dose emission and its characteristics. PIF was the most important parameter of the inhalation manoeuvre affecting the DD, FPD, EFPD, MMAD and RA. Thus, dose de-aggregation, aerosol dispersion and drug retention in the device and capsule are affected by the PIF. The ACIM affected mainly the DD and FPD, whereas the Vin has an effect on the DD, FPD and RA. The ACIM and Vin have almost equally the same impact on the DD and FPD. ACIM and Vin are involved more in mass transfer rather than aerosol dispersion. Combining all optimal parameters of the inhalation manoeuvre in a single inhalation improves both the quantity and the quality of the emitted dose. Thus, achieving a high peak flow faster with a

prolonged inhalation would not only promote the highest emission of the dose, but also increases the amount of the fine and extra fine drug particles being emitted.

Conflict of interest

The authors declare no conflicts of interest in this work.

Henry Chrystyn has no shares in any pharmaceutical companies. He has received sponsorship to carry out studies, together with Board Membership, consultant agreements and honoraria for presentation, from several pharmaceutical companies that market inhaled products. These include Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Innovata Biomed, Meda, Napp.

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Figure 1: Schematic diagram of the experimental set-up of the Andersen Cascade Impactor (ACI) with Breath simulator (BRS).

Figure 2: Mean (SD) Fine particle dose (FPD) represented as percentage of the nominal dose μg for each inhalation profile (n=3)

588 Figure 3: Mean (SD) Mass median aerodynamic diameter (MMAD) for each
589 inhalation profile

Figure 4: Delivered dose (DD) and Residual amount (RA) of indacaterol using DUSA for all the inhalation profiles, represented as percentage of the nominal dose (150µg). (A; Low PIF 40 L/min, B; Medium PIF 65 L/min, C; High PIF 85 L/min)

Table 1: HPLC methodology parameters

Column	Phenomenex RP-Kinetex 18 5µm, 250mm 4.6 mm
Column temperature	40 °C
Mobile phase	Methanol / 25 mM Potassium hydrogen orthophosphate, adjusted to pH 2.5 using orthophosphoric acid 60:40 % v/v
Flow rate	1 mL/min
Injection volume	20 µL
Detection ,UV	259 nm
Run Time	6 min
Internal standard	Dexamethasone 1µg/mL

Table 2: Mean (SD) indacaterol APSD for low PIF (40 L/min) profiles (n= 3) using 3 doses for each determination [150µg nominal dose].

	Vin ~ 1L		
	ACIM ~ 2.0 L/s²	ACIM ~ 4.0 L/s²	ACIM ~ 8.0 L/s²
FPD (µg)	29.4 (0.7)	31.6 (0.2)	34.7 (0.7)
%FPF	25.9 (3.2)	27.6 (1.9)	28.8 (2.3)
LPM (µg)	70.2 (1.6)	68.3 (1.2)	72.8 (1.4)
EFPD (µg)	19.7 (1.1)	23.8(1.0)	25.2 (0.3)
MMAD (µm)	3.4 (0.1)	3.2 (0.1)	3.1 (0.0)
GSD	1.8 (0.0)	1.8 (0.0)	1.9 (0.0)
	Vin ~ 2L		
	2.0 L/s²	4.0 L/s²	8.0 L/s²
FPD (µg)	33.3 (1.8)	35.8 (1.9)	38.3 (0.5)
%FPF	28.3 (0.8)	30.3 (3.0)	31.4 (1.2)
LPM (µg)	70.4 (1.9)	69.7 (2.8)	70.5 (2.5)
EFPD (µg)	21.1(0.1)	21.8 (0.9)	23.6 (0.4)
MMAD (µm)	3.3 (0.1)	3.3 (0.1)	3.4 (0.1)
GSD	1.8 (0.1)	1.8 (0.1)	1.8 (0.0)
	Vin ~ 3L		
	2.0 L/s²	4.0 L/s²	8.0 L/s²
FPD (µg)	36.5 (0.7)	38.8 (1.1)	40.3 (0.7)
%FPF	30.3 (1.2)	31.9 (3.0)	32.7 (0.9)
LPM (µg)	70.7 (2.8)	71.4 (2.5)	71.8 (3.2)
EFPD (µg)	25.7 (0.2)	26.6 (0.3)	26.8 (0.5)
MMAD (µm)	3.1 (0.0)	3.1 (0.1)	3.2 (0.1)
GSD	1.9 (0.0)	2.0 (0.0)	1.9 (0.1)

FPD, Fine particle dose; FPF, Fine particle fraction; LPM, Large particle mass; EFPD, extra fine particle dose; MMAD, Mass median aerodynamic diameter; GSD, Geometric standard deviation

Table 3: Mean (SD) indacaterol APSD for medium PIF (65 L/min) profiles (n= 3) using 3 doses for each determination [150 µg nominal dose].

	Vin ~ 1L		
	ACIM ~ 2.0 L/s²	ACIM ~ 4.0 L/s²	ACIM ~ 8.0 L/s²
FPD (µg)	37.9 (1.3)	38.1 (0.6)	40.5 (0.4)
%FPF	32.7 (3.0)	32.9 (1.2)	32.9 (3.2)
LPM (µg)	67.9 (4.4)	66.8 (0.9)	69.4 (2.2)
EFPD (µg)	31.04 (0.8)	30.40 (0.2)	32.89(0.7)
MMAD (µm)	2.9 (0.1)	2.9 (0.1)	2.8 (0.0)
GSD	2.0 (0.0)	1.9 (0.1)	2.1 (0.0)
	Vin ~ 2L		
	2.0 L/s²	4.0 L/s²	8.0 L/s²
FPD (µg)	39.0 (0.4)	39.9 (0.5)	42.5 (1.6)
%FPF	33.0 (2.2)	33.5 (0.8)	34.2 (1.7)
LPM (µg)	73.6 (0.9)	68.7 (1.6)	70.7 (2.3)
EFPD (µg)	27.85 (1.3)	32.11 (0.6)	34.85 (1.0)
MMAD (µm)	3.0 (0.1)	2.9 (0.1)	2.9 (0.1)
GSD	2.2 (0.1)	2.0 (0.0)	2.0 (0.0)
	Vin ~ 3L		
	2.0 L/s²	4.0 L/s²	8.0 L/s²
FPD (µg)	40.3 (0.3)	41.7 (0.8)	43 (0.3)
%FPF	33.2 (2.3)	34.0 (1.9)	34.2 (3.2)
LPM (µg)	66.1 (1.2)	70.6 (2.8)	72.4 (2.2)
EFPD (µg)	32.98 (0.9)	33.88 (0.6)	36.09 (0.9)
MMAD (µm)	2.9 (0.0)	2.8 (0.1)	2.7 (0.0)
GSD	2.0 (0.0)	1.9 (0.0)	2.1 (0.1)

FPD, Fine particle dose; FPF, Fine particle fraction; LPM, Large particle mass; EFPD, extra fine particle dose;

MMAD, Mass median aerodynamic diameter; GSD, Geometric standard deviation

Table 4: Mean (SD) indacaterol APSD for high PIF (85 L/min) profiles (n= 3) using 3 doses for each determination [150 µg nominal dose].

	Vin ~ 1L		
	ACIM ~ 2.0 L/s²	ACIM ~ 4.0 L/s²	ACIM ~ 8.0 L/s²
FPD (µg)	40.7 (1.1)	42.0 (1.0)	43.7 (0.2)
%FPF	34.3 (1.1)	35.4 (0.6)	35.8 (1.9)
LPM (µg)	67.9 (2.7)	71.0 (1.9)	73.1 (2.9)
EFPD (µg)	33.26(0.5)	30.85 (0.3)	32.91 (1.2)
MMAD (µm)	3.0 (0.1)	2.9 (0.0)	2.8 (0.1)
GSD	2.0 (0.1)	2.0 (0.0)	2.1 (0.1)
	Vin ~ 2L		
	2.0 L/s²	4.0 L/s²	8.0 L/s²
FPD (µg)	41.8 (0.2)	43.4 (0.3)	44.8 (1.5)
%FPF	34.7 (0.5)	35.8 (1.8)	36.2 (0.5)
LPM (µg)	72.4 (1.6)	72.6 (1.0)	74.1 (3.5)
EFPD (µg)	30.35 (0.8)	32.32 (0.4)	34.44 (0.2)
MMAD (µm)	2.9 (0.0)	2.8 (0.0)	2.7 (0.0)
GSD	2.1 (0.0)	2.0 (0.0)	2.0 (0.0)
	Vin ~ 3L		
	2.0 L/s²	4.0 L/s²	8.0 L/s²
FPD (µg)	44.1 (1.0)	46.8 (0.5)	49.8 (1.0)
%FPF	35.9 (1.7)	37.8 (1.3)	38.7 (2.0)
LPM (µg)	73.5 (2.4)	71.8 (3.0)	73.0 (2.6)
EFPD (µg)	32.34 (0.8)	35.03 (0.2)	39.16 (0.2)
MMAD (µm)	2.9 (0.0)	2.8 (0.1)	2.6 (0.0)
GSD	2.0 (0.0)	2.0 (0.0)	2.1 (0.0)

FPD, Fine particle dose; FPF, Fine particle fraction; LPM, Large particle mass; EFPD, extra fine particle dose; MMAD, Mass median aerodynamic diameter; GSD, Geometric standard deviation

Table 5: Mean (SD) indacaterol delivered dose and residual amounts (in µg) for all profiles using 1 dose for each determination (n=3 separate determinations). [150µg nominal dose].

	40 L/min	65 L/min	85 L/min
1 L -2 L/s²			
DD (µg)	116.80 (1.25)	121.84 (0.11)	125.41 (0.73)
RA(µg)	33.51 (1.00)	25.36 (1.04)	22.76 (0.54)
TRD(µg)	150.32 (0.25)	147.21 (0.93)	148.17 (0.19)
1L -4 L/s²			
DD (µg)	117.96 (2.08)	122.99 (3.50)	126.27 (3.50)
RA(µg)	30.58 (0.73)	24.92 (3.18)	22.32 (3.71)
TRD(µg)	148.54 (1.35)	147.92 (0.32)	148.59 (0.21)
1L-8 L/s²			
DD (µg)	121.31 (0.16)	125.39 (1.06)	129.40 (1.68)
RA(µg)	27.98 (2.77)	21.19 (1.49)	19.70 (0.62)
TRD(µg)	149.29 (2.93)	146.58 (0.43)	149.11 (1.06)
2L-2 L/s²			
DD (µg)	118.32 (1.26)	120.00 (0.05)	129.37 (2.99)
RA(µg)	29.38 (4.48)	21.84 (1.90)	20.24 (1.74)
TRD(µg)	147.70 (1.22)	147.34 (1.85)	149.61(1.25)
2L-4 L/s²			
DD (µg)	119.48 (0.28)	126.19 (2.38)	130.06 (1.12)
RA(µg)	27.74 (0.53)	21.16 (0.35)	19.88 (0.85)
TRD(µg)	147.22 (0.81)	147.35 (2.73)	151.22 (1.47)
2L-8 L/s²			
DD (µg)	122.81 (0.79)	128.26 (1.11)	131.83 (1.95)
RA(µg)	24.75 (1.39)	20.42 (1.19)	19.72 (0.98)
TRD(µg)	147.56 (0.61)	148.68 (0.08)	151.55 (0.76)
3L-2 L/s²			
DD (µg)	123.98 (0.44)	126.83 (1.47)	131.29 (0.64)
RA(µg)	24.05 (1.01)	19.31 (0.77)	18.20 (0.80)
TRD(µg)	148.03 (0.57)	146.14 (0.69)	149.49 (0.17)
3L-4 L/s²			
DD (µg)	124.61 (2.65)	128.73 (1.36)	132.30 (1.78)
RA(µg)	23.53 (2.00)	19.49 (0.55)	16.51 (1.56)
TRD(µg)	148.14 (0.65)	148.22 (1.91)	148.82 (0.22)
3L-8 L/s²			
DD (µg)	127.30 (1.35)	129.47 (0.31)	134.83 (1.58)
RA(µg)	20.43 (1.42)	17.93 (0.20)	14.58 (2.44)
TRD(µg)	147.73 (4.77)	147.40 (0.51)	149.41 (0.86)

DD, Delivered dose; RA, Residual amount; TRD, Total recovered dose