



Original article

The effect of inspiratory parameters after two separate inhalations on the dose emission of theophylline from low and high resistance dry powder inhalers

Mohamad Abadelah^{a,b}, Jumannah Al-Assadi^a, James Rooney^a, Hassane Larhrib^{a,*}^aDepartment of Pharmacy and Pharmaceutical Sciences, University of Huddersfield, Huddersfield HD1 3DH, United Kingdom^bDepartment of Pharmacy and Pharmaceutical Sciences, University of Tobruk, Libya

ARTICLE INFO

Article history:

Received 20 March 2019

Accepted 2 November 2019

Available online 13 November 2019

Keywords:

Peak inhalation flow

Inhalation volume

Theophylline

Handihaler[®]Onbrez Breezhaler[®]

ABSTRACT

The dose emission from DPIs can be affected by the inspiratory parameters achieved by the patient as well as the device in-use. Conventional in-vitro dose emission methodology was used, but instead of using inhalation volume (Vin) of 2 or 4 L and peak inhalation flow (PIF) corresponding to 4 kPa, a range of PIFs (28.3, 60, 90 and 120 L min⁻¹) and Vins (0.5, 0.75, 1, 1.5, 2, and 4 L) were used. The formulation was composed of spray dried Theophylline as a model drug with Lactohale[®] α lactose monohydrate carrier. The formulation was aerosolised using two DPIs; a low resistance Breezhaler[®] and high resistance Handihaler[®]. The formulation showed a consistent dose content uniformity with a Coefficient of Variation (CV) of 1.70%. The drug distribution on the surface of the carrier was obvious from the SE micrographs with some drug particles lodged into lactose crevices. The dose emission after the first inhalation (ED1) and total emitted dose (TED) of theophylline increased with PIF and Vin, irrespective of the inhaler device. However, the dose delivered was superior for the Handihaler[®] compared to Breezhaler[®]. Drug retention in the capsule and device was high at low PIFs and Vins and reduced after the second inhalation. Therefore, our study supports the recommendations for patients who cannot achieve sufficient PIF and Vin to inhale twice for each dose to ensure the better clinical outcome.

© 2019 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Most of the dry powder inhalers (DPIs) are breath actuated devices relying on the inspiratory effort by patients during the

Abbreviations: DPIs, Dry powder inhalers; ED, Emitted dose; ED1, Dose emitted after first inhalation; ED2, Dose emitted after second inhalation; DUSA, Dose unit sampling apparatus; TED, Total Emitted Dose; TRD, Total recovered dose; TRA, Total residual amount; PIF, Peak inhalation flow; Vin, inhalation volume; PIL, Patient information leaflets; TRA, Total Residual amount; SEM, Scanning electron microscope; API, Active pharmaceutical ingredient.

* Corresponding author at: Department of Pharmacy and Pharmaceutical Sciences, University of Huddersfield, Queensgate, Huddersfield HD1 3DH, United Kingdom.

E-mail addresses: J.Al-Assadi@hud.ac.uk (J. Al-Assadi), jj.rooney@hud.ac.uk (J. Rooney), e.larhrib@hud.ac.uk (H. Larhrib).

Peer review under responsibility of King Saud University.



inhalation manoeuvre to successfully de-aggregate and disperse the powder formulation into fine particles with the capabilities of lung deposition (Chrystyn, 2003; Haidl et al., 2016; Laube et al., 2011). The design of the device inhalation channel, metering cup and the air inlet determine the intrinsic resistance of the inhaler device (DeBoer et al., 1997). The DPIs are classified into three main groups depending on their intrinsic resistance; Low, Medium and High resistance device. This classification is usually determined by the peak inhalation flow (PIF) generating a 4 kPa across the inhaler device (Clark and Hollingworth, 1993; Dal Negro, 2015). Patients do not inhale in the same manner through DPIs; patients usually generate a fast inhalation through low resistance devices when compared to slow flow through high resistance devices (Al-Showair et al., 2007; Azouz and Chrystyn, 2012; Laube et al., 2011). The inhalation volume (Vin) is another important parameter for successful dose emission and dose emptying, especially from capsule based DPIs. For this reason, the manufacturer patient information leaflet (PIL) instructs the patients to inhale twice for each capsule dose to empty the dose efficiently (Abadelah et al., 2017b; Haughney et al., 2010). Not all patients

can achieve the required PIF and Vin for each DPI. Patients with limited lung capacity have difficulties achieving sufficient inhalation manoeuvre resulting in low dose delivered to the lungs (Azouz et al., 2015; Haidl et al., 2016). For in-vitro DPIs dose emission testing, the pharmacopoeia compendial method recommends the use of a PIF corresponding to 4 kPa pressure drop with a Vin of 2 or 4 L (FDA, 2018; USP, 2014). Several studies have used the pharmacopoeia recommended PIFs and Vins when testing DPIs, however, the results showed that increasing the volume showed no significant improvement when using Vin above 2L (Abdelrahim, 2010; Alaboud, 2011; Yakubu et al., 2013). The patients who are suffering from COPD or asthma were unable to achieve neither 4 kPa pressure drop nor 4 L Vin and some patients were unable to achieve a Vin of 2 L. In the present study, the pharmacopoeial method for testing DPIs was used, however, instead of PIF corresponding to 4 kPa and a Vin of 4L, a range of PIFs (28.3, 60, 90 and 120 L min⁻¹) and Vins (0.5, 0.75, 1, 1.5, 2, and 4 L) were used. The study was designed to assess the effect of inhaler's design (intrinsic resistance), PIF and Vin on dose emission after the first (ED1) and second inhalation (ED2) using a model drug theophylline aerosolised from two different inhaler devices Onbrez Breezhaler[®] and Handihaler[®].

2. Materials and methods

2.1. Inhaler device

The inhaler devices used in the present research were low resistance (Breezhaler[®] Novartis, United Kingdom) and High resistance (Handihaler[®] Boehringer Ingelheim, Germany).

2.2. Chemicals and solvents

Ultra-purified water (Barnstead; Thermo Fisher Scientific, UK). Lactose (Lactohale[®]; DEF Pharma, Germany) was used as a carrier for dry powder formulation. Theophylline (Sigma Aldrich, UK) was chosen as the model drug to investigate dose emission after aerosolisation from two capsule based devices Breezhaler[®] and Handihaler[®].

2.3. Determination of inhalers airflow resistance

The intrinsic resistance of the Onbrez Breezhaler[®] and Handihaler[®] devices were measured to determine the threshold inhalation flow required to generate enough turbulent energy in the device to de-aggregate the powder formulation (Fig. 1). The speci-

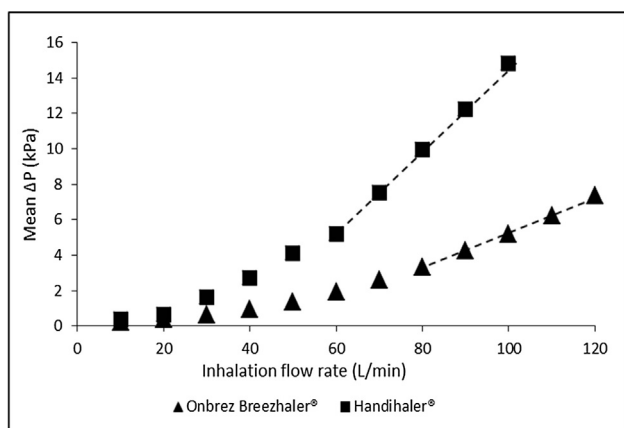


Fig. 1. Relationship between pressure drop inside the inhaler and the inhalation flow rate.

fic resistance to airflow (R) of each device was calculated from the linear relationships between the square root of pressure drop (ΔP) against the volumetric flow (Q) between 28.3 and 120 L min⁻¹. $\sqrt{\Delta P} = R \times Q$ (Clark and Hollingworth, 1993).

2.4. Formulation procedure of theophylline with the carrier

The formulation consisted of two components namely Lactohale[®] α -lactose monohydrate (carrier) and theophylline (API). Lactohale[®] was sieved manually so as to avoid particle abrasion using a stack of sieves composed of 63 and 90 μ m. The supplied theophylline was spray dried to provide the desired size (1 to 5 μ m) for inhalation.

The theophylline solution was prepared by dissolving 3 g of commercial theophylline in 500 mL ultra-purified water. The aqueous solution was spray dried using the LabPlant spray dryer (LabPlant, UK) using the following settings: feed rate (20 mL min⁻¹), 3 ms⁻¹ fan setting, 120 °C inlet temperature and 72 °C outlet temperature. The sprayed drug droplets were dried instantaneously to produce uniform particles with a smooth surface and a size range of 1 to 5 μ m, making them suitable for pulmonary delivery.

The powder was formulated by using 1:67.5 w/w drug to carrier ratio and an order mix was carried out according to Larhrib (1999) (Larhrib et al., 1999). The formulation was then filled manually into size 3 hard gelatine capsules; each capsule contained approximately 27.4 \pm 0.2 mg of the formulation with a nominal dose of 400 \pm 3 μ g theophylline. An exact amount of drug and carrier equivalent to the production of 100 capsules was weighed and mixed manually in a 40 mL glass vial using an order mix followed by mixing in a Turbula mixer (Glen Mills Inc., US) for 30 min at a speed of 72 rotations per minute. Lactose sieved powder (63–90 μ m), spray dried Theophylline powder, dry powder inhalation formulation, empty capsules and filled capsules with the powder formulation were each contained in a 40 mL amber glass bottle and stored over silica gel at room temperature (23 °C) until required for further investigation.

2.5. Characterisation of the theophylline powder formulation by Scanning electron microscope (SEM)

SEM investigation of the shape, particle size and surface texture of Lactose and Theophylline (Fig. 2) formulation were carried out using a Jeol 6060LV SEM (Jeol Ltd., UK). A sample of the powder formulation was scattered onto the aluminium stage stub, by a gentle tapping motion, to provide a thin layer that was suitable to view the particles. The movement of the sample was restricted by adhering the sample to a double-sided conductive carbon adhesive tape (Agar Scientific, UK). As the sample is non-conductive, the sample was coated with a thin layer of gold (15 to 20 nm) (Quorum Technologies Ltd., UK) using a Quorum SC7620 Sputter Coater (Quorum Technologies Ltd., UK).

2.6. Characterisation of the powder formulation by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

DSC experiments were conducted using a DSC 822^e instrument (Mettler-Toledo, UK), with a refrigerated cooling system (RCS). Nitrogen was used as the purge gas, flowing at 50 mL min⁻¹ through the DSC cell and at 150 mL min⁻¹ through the RCS units. Aluminium non-hermetic DSC pans were used throughout the study. The mass of each empty sample pan was matched with the mass of the empty reference pan to \pm 0.1 mg. The instrument was calibrated using indium and zinc standards and approximately 2.5 \pm 0.2 mg of sample (Lactose monohydrate and Theophylline) was used for each run. After sealing the pans, the pans were placed

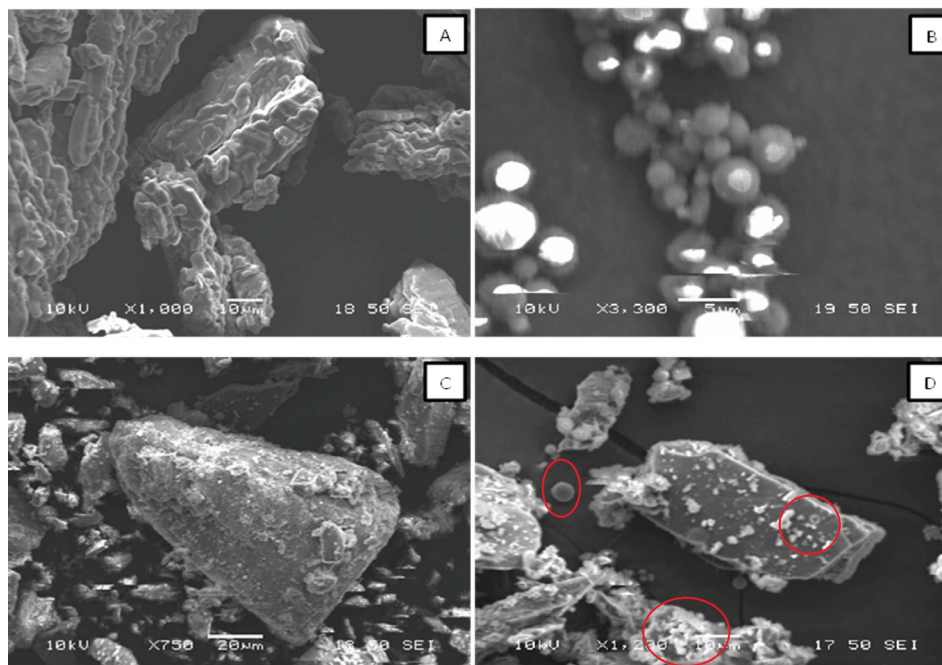


Fig. 2. SEM images of Theophylline formulation; a). Commercial Theophylline, b). Spray dried Theophylline, c). α -lactose monohydrate, d). Lactose and Theophylline in the formulation.

in the DSC furnace which had been pre-equilibrated at 25 °C. Before each measurement, the samples were allowed to equilibrate for 5 min at 25 °C and were then heated to 250 °C at a heating rate of 10 °C min⁻¹. Each sample was analysed in duplicate. The DSC results were analysed using the STAR[®] SW 9.01 version (Mettler-Toledo, Leicester, UK). Theophylline, lactose and the powder formulation samples were analysed using DSC and TGA.

2.7. Determination of Beer-Lambert's law range and plotting of calibration curve of theophylline

A working stock solution of theophylline of 100 $\mu\text{g mL}^{-1}$ was prepared, from which different aliquots of 0.2 mL to 1.6 mL (0.2 mL intervals) were taken in a series of 10 mL volumetric flasks. The volume was made up with ultra-purified water to obtain a series of working standard solutions of 2 to 16 $\mu\text{g mL}^{-1}$ (2 $\mu\text{g mL}^{-1}$ interval), respectively. The absorbance of each theophylline concentration was obtained spectrophotometrically using the Jenway 7200 COLE-PARMER Ltd against the ultra-purified water (blank) in triplicates at a wavelength of 272 nm and a calibration curve was constructed. The standard Concentrations ranged from 2 to 16 $\mu\text{g mL}^{-1}$ as follow 2, 4, 6, 8, 10, 12, 14, 16 $\mu\text{g mL}^{-1}$. The drug follows linearity in the concentration range 2 $\mu\text{g mL}^{-1}$ to 16 $\mu\text{g mL}^{-1}$ with a correlation coefficient value of 0.999 and linearity equation $y = 0.0642 \times -0.052$. The accuracy of the method was validated by adding a known amount of the stock solutions were added to theophylline sample solutions at three different levels 80%, 100%, and 120%. The solutions were reanalyzed by the proposed method. The method was accurate and showed that the % amount found was between 98.54% and 99.98% with % RSD < 2.

2.8. Theophylline content uniformity

Drug content uniformity was carried out by taking 10 random samples, each weighing 27.4 ± 0.02 mg so that each aliquot contains approximately 400 ± 3 μg theophylline. Three aliquots were taken from the top, 3 from the middle, and 3 from the bottom of

the vial containing the powder formulation and the last 10th aliquot was taken randomly from the bulk powder contained in a glass vial. Each aliquot was dissolved in ultra-purified water and made up to 100 mL volume. The absorbance was measured using UV-Spectrophotometer at a wavelength of 272 nm. The absorbance readings were converted into a mass of theophylline using the calibration curve whilst taking the dilution factor into consideration. The coefficient of variation (%CV) was used to assess the homogeneity of the powder blend.

2.9. Dose emission, residual amount and recovered dose from Breezhaler[®] and Handihaler[®]

The total emitted dose (TED) after two separate inhalations of theophylline emitted from Breezhaler[®] and Handihaler[®] was determined using a DPI dose unit sampling apparatus (DUSA) (Copley Scientific, UK). The dose emission methodology used was similar to that reported in the pharmacopoeia (US, 2014) expect that a range of PIF and Vin values were used instead of the flow corresponding to a 4 kPa pressure drop and an inhaled volume of 4 L (Fig. 3). For each experiment, two DUSAs were used to collect dose emitted after first (ED1) and second (ED2) inhalation, the TED was the sum of those inhalations. The emitted dose of theophylline was measured by collecting one dose at different PIFs and Vins. For each determination, one capsule containing 400 μg theophylline was inserted into the inhaler device (Breezhaler[®] and Handihaler[®]) according to the manufacturer's instructions in the PIL. The determination was replicated three times for each set of PIF and Vin. The dose recovery procedure was carried out after the second inhalation by recovering the theophylline dose emitted into DUSA 1, DUSA 2 using ultra-purified water. The filter (Whatman[®], Fisher Scientific Ltd, UK) of DUSA was immersed into an appropriate volume of water and sonicated for 10 min to detach and dissolve all particles entrained and captured by the filter. The device and capsule were washed separately to determine the total residual amount (TRA). The total recovered dose (TRD) was calculated as the sum of the TED and TRA.

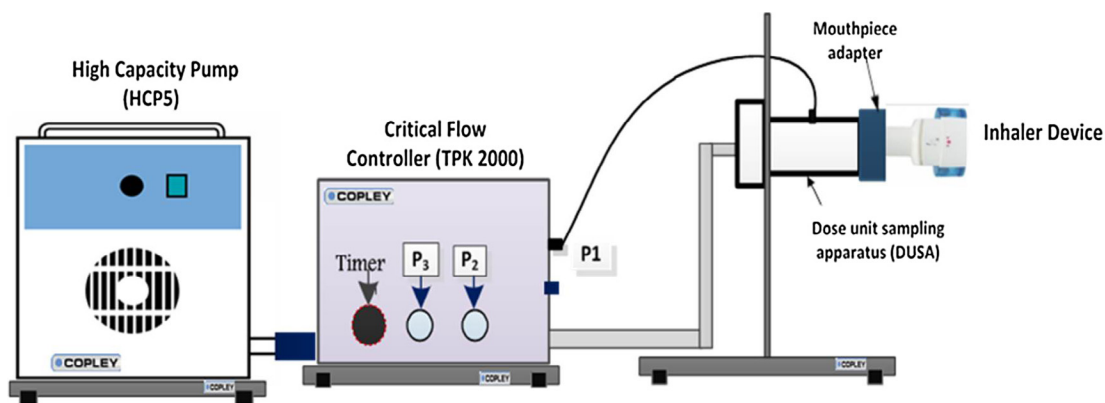


Fig. 3. A schematic diagram of DPIs dose emission methodology (Abadelah, 2018).

2.10. Data analysis

The statistical analysis comprised of a three-way factorial analysis of variance (ANOVA) which was carried out using the statistical analysis software, SPSS Statistics (SPSS Inc., Chicago, USA). The ANOVA was used to determine the significant effect of PIF, V_{in} and inhaler resistance on dose emission parameters: the TED, TRA, ED1 and ED2.

3. Results and discussion

3.1. Device resistance to airflow

The relationship between the pressure drop and the PIF was not linear for both devices as seen in Fig. 1, however, there is important information that can be extracted from the graph such as the threshold flow required to de-aggregate the formulation. The threshold PIF is the onset of when a small change in the airflow causes a large change in the pressure drop (Dal Negro, 2015). The estimated threshold PIF for the Onbrez Breezhaler[®] device was 90 L min^{-1} and for the Handihaler[®] device was 60 L min^{-1} as this is where the slope becomes linear as shown in Fig. 1; the devices would not operate efficiently below these flow (Laube et al., 2011). The threshold PIF for the Handihaler[®] was lower than that of the Onbrez Breezhaler[®]. The curve for the Handihaler[®] device for the pressure drop test is steeper and more curved than that of the Onbrez Breezhaler[®]; the same PIF for Handihaler[®] produces a larger pressure drop than the one observed in the Onbrez Breezhaler[®] in accordance with previous studies (Azouz et al., 2015; Dal Negro, 2015). Fig. 1 shows that the Onbrez Breezhaler[®] exhibits a lower intrinsic resistance in comparison to the Handihaler[®] device.

3.2. Content uniformity

The content uniformity test of the formulation was carried out by randomly choosing 10 aliquots from different locations of the vial containing the powder formulation. Fig. 4 shows that the drug content in the formulation was uniform with a % CV of 1.70%. In each sample, the nominal dose of $400 \mu\text{g}$ of theophylline was recovered with a mean (Standard Deviation) of 10 samples was $402.73 \pm 6.85 \mu\text{g}$ suggesting that formulating procedure, sampling and analysing were accurate and reproducible.

3.3. Formulation characterisation

The DSC and TGA graphs for the spray dried theophylline showed no event throughout the heating of the sample. However, the sample was anhydrous and only heated up to $250 \text{ }^\circ\text{C}$. Theo-

phylline has a higher melting point of approximately $275 \text{ }^\circ\text{C}$ which can be seen by the grey heating line (Szterner et al., 2010). The spray drying formed spherically shaped particles with a smooth surface and of suitable size for inhalation.

The SEM images of lactose showed a tomahawk shape with a rough surface, as seen in Fig. 2. The TGA (Figs. A.1–A.3) and DSC graphs (Figs. A.4–A.6) for lactose corroborate with each other in terms of transition events. For both, dehydration occurs just before $150 \text{ }^\circ\text{C}$, melting at approximately $219 \text{ }^\circ\text{C}$ and degradation occurring just after melting. This pattern is also consistent with the DSC and TGA graphs for the formulation, as α -lactose monohydrate was the most dominant component in the weight of the powder formulation. The effects of temperature on theophylline may have been observed if the TGA and DSC analyses were to continue to higher temperatures but the heating was limited to $250 \text{ }^\circ\text{C}$ due to lactose degradation above $220 \text{ }^\circ\text{C}$. TGA for both commercial and spray dried theophylline showed no loss of water of dehydration suggesting the anhydrous form of theophylline.

Fig. 2(d) has three components which are indicated by red rings. One of the components observed was that the theophylline adhered to the lactose (the right most ring) whilst the other is free theophylline (the left most ring). The component in the bottom most rings shows multiple theophylline particles lodged in a crevasse of lactose, referred to as hot spots or active sites by inhalation formulation scientists. The active sites are defined as areas on the surface of the carrier particle which are more adhesive than others (Peng et al., 2016). This may impede drug detachment during inhalation manoeuvre as the drug is held in the active site and, therefore, requires a higher inhalation force to be dislodged, thus causing a decrease in the emitted dose and fine particle dose.

3.4. Dose emission

3.4.1. Emitted dose 1 (ED1)

The dose emission after the first inhalation was affected by the inspiratory parameters, PIF and V_{in} , as well as the intrinsic resistance of the inhaler device (Figs. 5 and 6). The ED1 emitted from Handihaler[®] was higher than Breezhaler[®] suggesting that the turbulence energy generated inside the Handihaler was higher when compared to Breezhaler (Fig. 1). The ED1 increased significantly ($p < 0.05$) with increasing the PIF and V_{in} for both devices, however, the higher the inhalation flow was, the smaller the difference between the two inhalers. For example, at PIF of 28.3 L min^{-1} and V_{in} of 500 mL inhalation volume, the ED1 for Handihaler[®] was $271.98 \pm 5.0 \mu\text{g}$ and for Onbrez Breezhaler[®] was $231.37 \pm 0.80 \mu\text{g}$ with a difference of $40.61 \mu\text{g}$. However, when the PIF was 120 L min^{-1} and the volume was 500 mL , the dose emitted from Handihaler[®] was $294.46 \pm 0.22 \mu\text{g}$ and from Onbrez Breezhaler[®] was $281.37 \pm 7.31 \mu\text{g}$ with a smaller difference of $13.09 \mu\text{g}$.

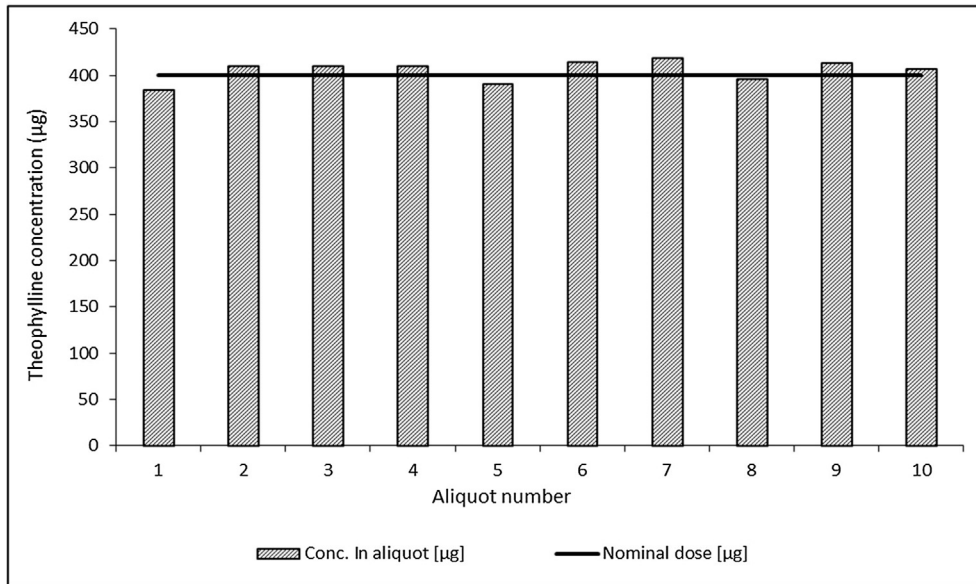


Fig. 4. Dose content uniformity of Theophylline formulation.

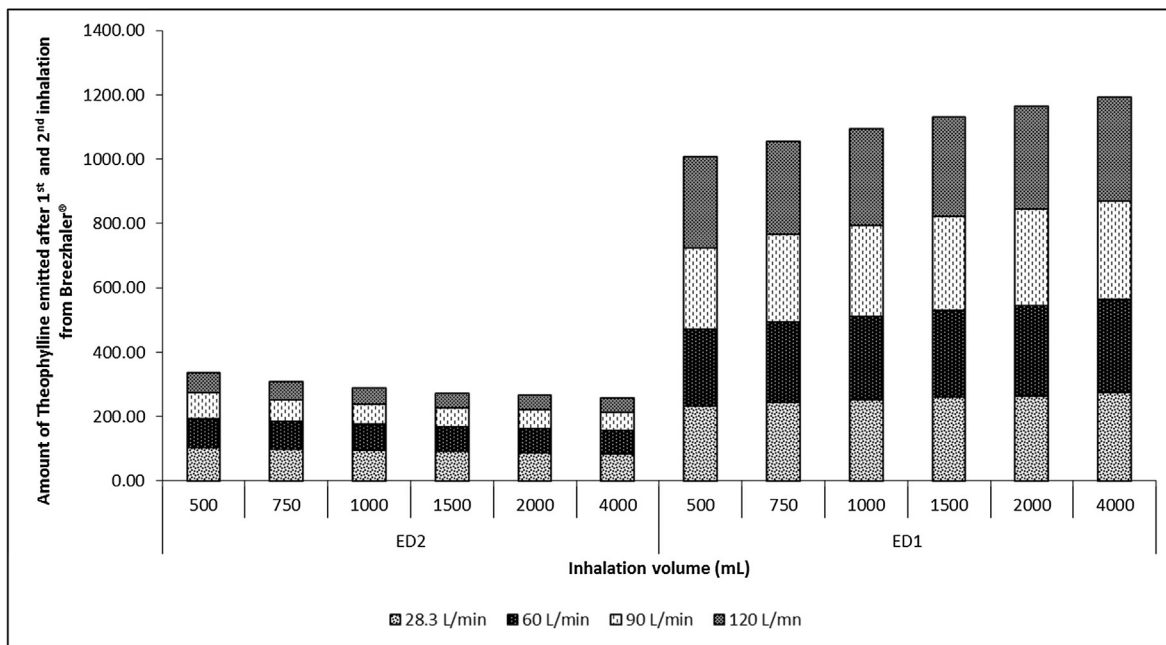


Fig. 5. The amount of Theophylline emitted after first and second inhalation from Onbrez Breezhaler® at different inspiratory parameters of PIF and VIN.

The PIF had a more significant effect on Onbrez Breezhaler® as any change in flow rate would cause a large change in ED1. For example, at Vin of 4000 mL, when the PIF was 28.3 L min⁻¹, the emitted dose from Onbrez Breezhaler® was 274.86 ± 9.70 µg and at 120 L min⁻¹ was 322.26 ± 0.93 µg with a difference of 47.402 µg. As for the Handihaler®, when the PIF was 28.3 L min⁻¹, the emitted dose was 293.36 ± 9.48 µg and at 120 L min⁻¹ was 318.47 ± 5.76 µg with a smaller difference of 25.12 µg. There is a larger difference in Onbrez Breezhaler® than Handihaler® (Tables 1 and 2).

The Vin also showed a significant effect on the ED1 ($p < 0.05$). Increasing the Vin resulted in more dose emitted from the device after the first inhalation suggesting that more time was required to empty the capsule. For example, ED1 from Breezhaler® was 231.37 ± 0.80 µg at PIF of 28.3 L min⁻¹ and a Vin of 500 mL, but

when the Vin increased to 4000 mL the ED1 increased to 274.87 ± 9.70 µg.

The PIF and Vin showed an impact on theophylline dose emission after first inhalation suggesting that higher PIF and longer inhalation time are required to enhance the dose de-aggregation and dose emission. The results of the present study are in line with previous studies (Abadela et al., 2017a; Abdelrahim, 2010; Alaboud, 2011; Colthorpe et al., 2013; Kamin et al., 2002; Pavkov et al., 2010). The ED1 showed this trend due to the difference in the intrinsic resistance between Handihaler® and Breezhaler®, where Handihaler has higher intrinsic resistance and therefore higher turbulent energy generated inside the device during the inhalation manoeuvre. Furthermore, the internal resistance of the device partly depends on the inhalation channel (Abadela et al., 2017b; Coates et al., 2004). The Onbrez Breezhaler® has a longer

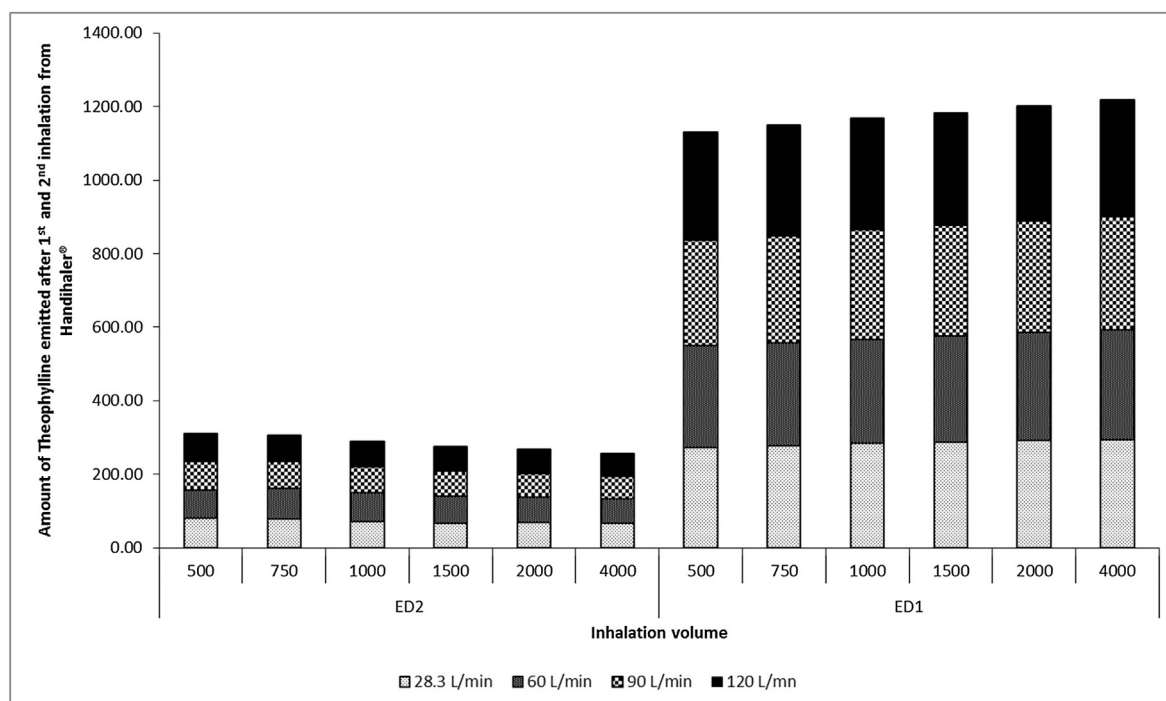


Fig. 6. The amount of Theophylline emitted after first and second inhalation from Handihaler® at different inspiratory parameters of PIF and VIN.

Table 1

Mean (SD) of Theophylline dose emission results using Onbrez Breezhaler® at different PIFs and Vins [n = 3].

Vin (mL)	ED1 (µg)	ED2 (µg)	TED (µg)	TRA (µg)	TRD (µg)
PIF = 28.3 L/min					
500	231.37 (0.80)	102.69 (10.98)	334.06 (10.18)	66.25 (1.24)	400.31 (8.94)
750	242.42 (0.49)	96.52 (4.36)	338.94 (12.85)	59.67 (0.80)	398.62 (23.64)
1000	251.25 (1.02)	94.58 (4.65)	345.83 (5.67)	46.21 (0.00)	392.04 (5.67)
1500	259.73 (0.35)	90.64 (12.27)	350.37 (12.62)	44.49 (4.92)	394.86 (2.30)
2000	263.21 (0.40)	86.38 (0.22)	349.59 (6.18)	40.01 (2.04)	389.60 (2.21)
4000	274.86 (9.70)	83.75 (0.04)	358.61 (10.75)	37.07 (1.24)	395.68 (8.51)
PIF = 60 L/min					
500	239.79 (13.15)	89.20 (3.94)	328.99 (9.21)	71.51 (0.53)	400.50 (9.74)
750	250.03 (8.68)	86.91 (3.45)	336.94 (12.13)	62.24 (7.08)	399.18 (19.22)
1000	258.70 (6.51)	82.75 (13.68)	341.45 (10.19)	49.09 (16.12)	390.54 (4.07)
1500	269.38 (1.68)	78.37 (8.19)	347.74 (9.87)	45.71 (5.67)	393.45 (15.54)
2000	280.96 (9.12)	75.42 (8.81)	356.38 (0.31)	34.63 (1.33)	391.01 (1.64)
4000	289.51 (2.88)	72.42 (6.96)	361.93 (8.09)	33.00 (4.16)	394.92 (9.92)
PIF = 90 L/min					
500	253.47 (13.19)	82.65 (2.18)	336.13 (11.01)	58.42 (0.80)	394.55 (10.21)
750	272.60 (15.13)	69.89 (2.13)	341.98 (17.25)	62.30 (4.09)	404.29 (13.17)
1000	285.03 (18.07)	61.68 (3.10)	346.71 (13.16)	50.72 (7.89)	397.43 (3.28)
1500	292.08 (19.79)	56.61 (3.01)	348.68 (16.78)	39.76 (1.86)	388.44 (14.92)
2000	299.75 (3.36)	58.01 (3.41)	357.76 (0.04)	38.07 (2.83)	395.83 (2.88)
4000	305.10 (4.91)	57.58 (7.31)	362.68 (12.22)	28.68 (3.37)	391.36 (10.59)
PIF = 120 L/min					
500	281.37 (7.31)	60.43 (3.54)	341.79 (10.85)	75.77 (2.66)	417.56 (8.19)
750	290.42 (4.69)	54.04 (8.15)	344.46 (3.45)	52.79 (0.44)	397.24 (3.01)
1000	298.87 (11.33)	50.66 (3.54)	349.53 (14.88)	38.82 (8.15)	388.35 (6.73)
1500	309.26 (2.04)	46.78 (2.39)	356.04 (4.43)	47.71 (0.89)	403.75 (3.54)
2000	319.16 (4.87)	45.12 (3.14)	364.27 (8.02)	27.05 (5.14)	391.32 (2.88)
4000	322.26 (0.93)	43.08 (4.34)	365.34 (3.41)	25.74 (3.45)	391.07 (0.04)

inhalation channel than the Handihaler®. The particles travelling through the Onbrez Breezhaler® must travel a further distance than the Handihaler® before reaching the exit mouth piece of the device. This means that a higher air flow or volume was required to emit the drug from the Onbrez Breezhaler® than the Handihaler® due to the distance of travel. The results show that the Onbrez Breezhaler® required higher flow to emit similar doses to the Handihaler®; in line with previous studies (Abadelah et al., 2017a; Alaboud, 2011; Laube et al., 2011).

3.4.2. Emitted dose 2 (ED2)

The dose emission after the second inhalation was measured to assess the dose that the patient might leave in the device if insufficient inhalation parameters were achieved by a patient from the first inhalation. Figs. 5 and 6 clearly show that the ED2 decreased with increasing the PIF and Vin, the impact of PIF and Vin was significant ($p < 0.05$), however, the impact of inhaler type (intrinsic resistance) was less pronounced on ED2 ($p > 0.05$). This happened because most of the dose was emitted after ED1 and the amount

Table 2
Mean (SD) of Theophylline dose emission results using Handihaler® at different PIFs and Vins [n = 3].

Vin (mL)	ED1 (µg)	ED2 (µg)	TED (µg)	TRA (µg)	TRD (µg)
PIF = 28.3 L/min					
500	271.98(5.00)	80.12(6.60)	352.09(1.59)	45.15(2.21)	397.24(3.81)
750	276.11(1.90)	78.18(5.11)	354.29(13.20)	38.64(1.86)	392.92(11.34)
1000	283.87(6.95)	71.63(4.07)	355.51(11.03)	29.56(0.18)	385.06(10.85)
1500	287.79(5.14)	67.09(3.23)	354.88(1.90)	51.03(4.87)	405.91(2.97)
2000	290.76(13.15)	67.85(1.39)	358.61(14.54)	20.60(2.57)	379.21(21.97)
4000	293.36(9.48)	66.41(3.23)	359.77(6.24)	20.79(0.00)	380.55(6.24)
PIF = 60 L/min					
500	277.55(5.09)	76.96(2.57)	354.51(7.66)	38.82(0.18)	393.33(7.84)
750	280.68(3.85)	83.59(6.47)	364.27(2.61)	55.10(4.60)	419.38(7.22)
1000	282.50(2.97)	78.19(12.82)	360.69(0.16)	36.26(14.43)	396.94(14.59)
1500	288.13(1.13)	73.76(1.59)	361.89(1.73)	33.81(2.30)	395.71(0.58)
2000	294.14(6.51)	69.94(15.41)	364.09(8.90)	28.49(1.15)	392.58(10.05)
4000	298.34(7.22)	65.94(3.63)	364.27(10.85)	24.73(3.63)	389.01(7.22)
PIF = 90 L/min					
500	286.35(8.06)	77.05(4.38)	363.40(12.44)	34.00(2.57)	397.40(9.87)
750	291.76(4.11)	73.64(10.90)	365.40(15.01)	31.31(1.42)	396.71(13.60)
1000	297.34(0.93)	69.51(1.86)	366.84(0.93)	24.86(1.68)	391.70(0.75)
1500	300.44(0.35)	66.97(2.61)	367.40(2.26)	21.04(2.17)	388.44(4.43)
2000	304.44(7.53)	64.53(4.29)	368.97(13.24)	43.96(6.10)	412.93(7.14)
4000	308.55(3.76)	62.46(3.50)	371.01(7.26)	20.73(5.05)	391.73(12.31)
PIF = 120 L/min					
500	294.46(0.22)	75.67(7.84)	370.13(7.62)	30.43(2.13)	400.56(9.74)
750	300.34(4.56)	71.23(7.39)	371.57(2.83)	21.92(1.42)	393.48(4.25)
1000	304.35(0.84)	69.88(10.05)	374.23(14.21)	20.35(0.62)	394.58(14.83)
1500	306.70(4.60)	68.19(4.87)	374.89(9.47)	19.60(1.33)	394.49(10.80)
2000	311.43(3.30)	64.31(13.85)	375.73(9.15)	19.29(2.30)	395.02(16.85)
4000	318.47(5.76)	60.39(3.29)	378.86(11.04)	16.59(1.15)	395.46(11.89)

left in the device was small for the second inhalation, especially at high PIF and Vin.

The results showed that the higher the PIF, the smaller the ED2 became. This was because, as the PIF increased, ED1 increased, leaving less dose to be emitted for ED2. At the lowest and intermediate PIF values (28.3 and 60 L min⁻¹), the Onbrez Breezhaler® had a generally higher ED2 than Handihaler®; for example, at the PIF of 28.3 L min⁻¹ and inhalation volume of 500 mL, Onbrez Breezhaler® had an ED2 of 102.69 µg ± 10.98 and the Handihaler® had an ED2 of 80.12 ± 6.60 µg. This was because of the ED1 of the Onbrez Breezhaler® was lower than the Handihaler® and so there was more dose to be emitted for the second inhalation in comparison to the Handihaler®. However, at higher PIFs (90 and 120 L min⁻¹), the Handihaler® generally had a higher ED2 than the Onbrez Breezhaler®; for example, at PIF of 120 L min⁻¹ and inhalation volume of 4000 mL, Handihaler® emitted 60.39 ± 3.29 µg and Onbrez Breezhaler® emitted 43.08 ± 4.34 µg. This change occurred due to the change in the intrinsic resistance between the devices; Handihaler® has a higher intrinsic resistance than Onbrez Breezhaler®. There was more resistance-induced turbulence in the Handihaler® combined with flow induced allowing more dose to be emitted in comparison to that from Onbrez Breezhaler®.

Furthermore, the Vin also showed a significant impact on the ED2; the higher the Vin, the lower was the ED 2 emitted. For example, at the flow of 28.3 L min⁻¹, the Onbrez Breezhaler® has an ED2 of 96.52 ± 4.36 µg at the volume of 750 mL and an ED2 of 86.38 ± 0.22 µg at the inhaled volume of 2000 mL (Table 1). The results showed more dose was emitted after the first inhalation resulting in less dose available for the second inhalation with increasing inhaled volume.

3.4.3. Total emitted dose (TED)

The TED was calculated as the sum of dose emitted after first and second inhalation (TED = ED1 + ED2). The results showed that, by increasing the PIF and Vin, significantly increased the TED (p < 0.05) for both devices Handihaler® and Breezhaler®. The higher the PIF and Vin, the higher was the TED, however, when PIF and Vin increased, the difference in the TED between the two

inhalers was reduced. For example, at PIF of 28.3 L min⁻¹ and Vin of 500 mL, the Onbrez Breezhaler® had a TED of 334.06 ± 10.18 µg and Handihaler® had a TED of 352.09 ± 1.59 µg, with a difference in TED of 18.03 µg. When the inhalation volume was increased to 4000 mL, the TED for the Onbrez Breezhaler® was 358.61 ± 10.75 µg and for Handihaler® was 359.77 ± 6.24 µg with a smaller difference of 1.16 µg. The difference was not very drastic in the case where the PIF was increased, for example, at Vin of 500 mL and PIF of 28.3 L min⁻¹ Onbrez Breezhaler® TED was 334.06 ± 10.18 µg and 336.13 ± 11.01 µg at 90 L min⁻¹, with a difference of only 2.067 µg. For the Handihaler® TED of 352.09 ± 1.59 µg and 370.13 ± 7.62 µg, respectively, when the PIF was increased from 28.3 to 120 L min⁻¹ at a Vin of 500 mL (Figs. 7 and 8).

The results of the present study are in accordance with the task force recommendation for the use of DPIs (Laube et al., 2011). The increase in the ED1, TED with the increase of the PIF is not always advantageous, because the oropharyngeal deposition also increased when fast inhalation manoeuvre is used (Haughney et al., 2010). If the amount of drug impacting in the oropharyngeal region is high, the clinical effect is compromised reducing the effectiveness of the drug. Inhaler devices with higher intrinsic resistance (Handihaler®) do not require a very high inhalation PIF when compared to low resistance devices (Breezhaler®) so the drug leaving the inhaler device will not be travelling at a very high velocity; allowing for a lower chance of oropharyngeal deposition by impaction. DPIs with higher intrinsic resistances have greater lung deposition than devices with lower resistance due to reduced oropharyngeal drug deposition (Chrystyn and Price, 2009; Laube et al., 2011).

The Vin is representing the duration of the inhalation patient can achieve during the inhalation manoeuvre. It can be seen from the results that higher Vin improves the TED for both devices, supporting the study carried out by Azouz and Chrystyn in 2012, however, not all patients can achieve a high Vin. Patients with asthma and COPD can neither achieve a high Vin nor high PIF, which is even worse in exacerbation (Al-Showair et al., 2007; Laube et al., 2011). In practice, patients with reduced lung capacity and who have difficulties achieving high PIF should be advised to prolong

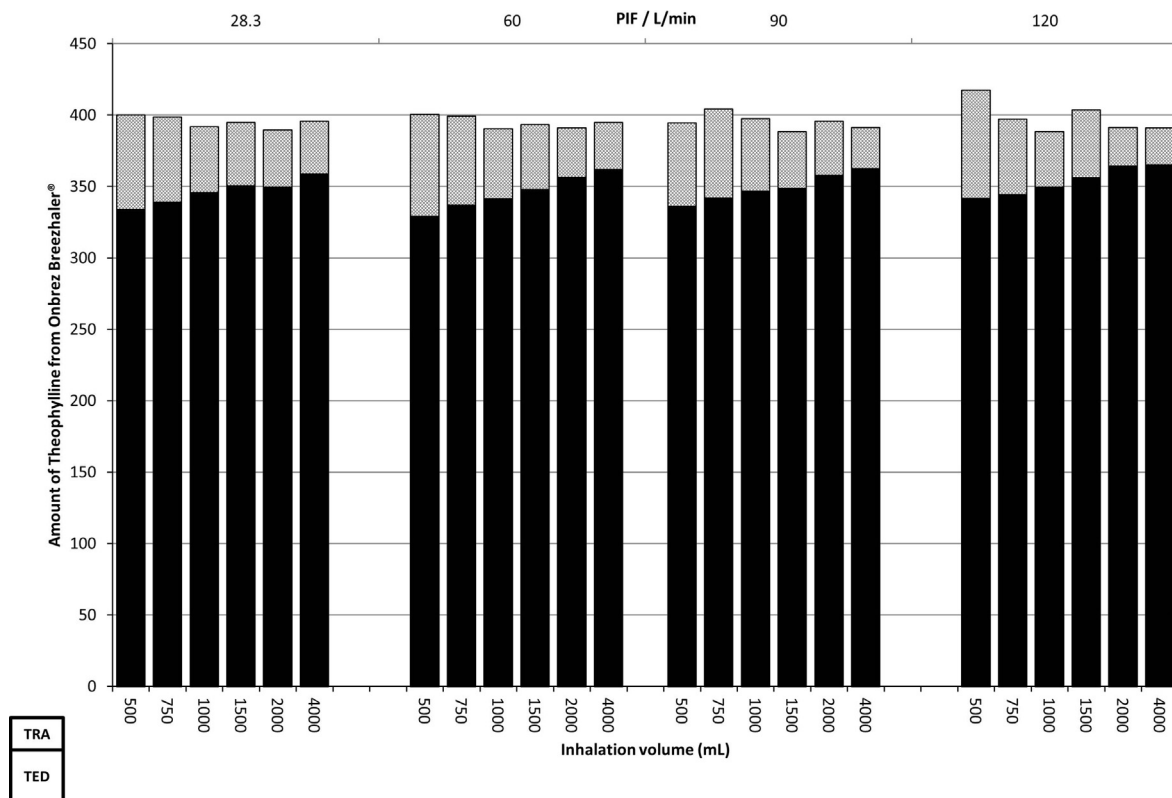


Fig. 7. The amount of total emitted dose (TED) and total residual amount (TRA) of Theophylline using Onbrez Breezhaler® [TED (Solid Column) TRA (Pattern Column)].

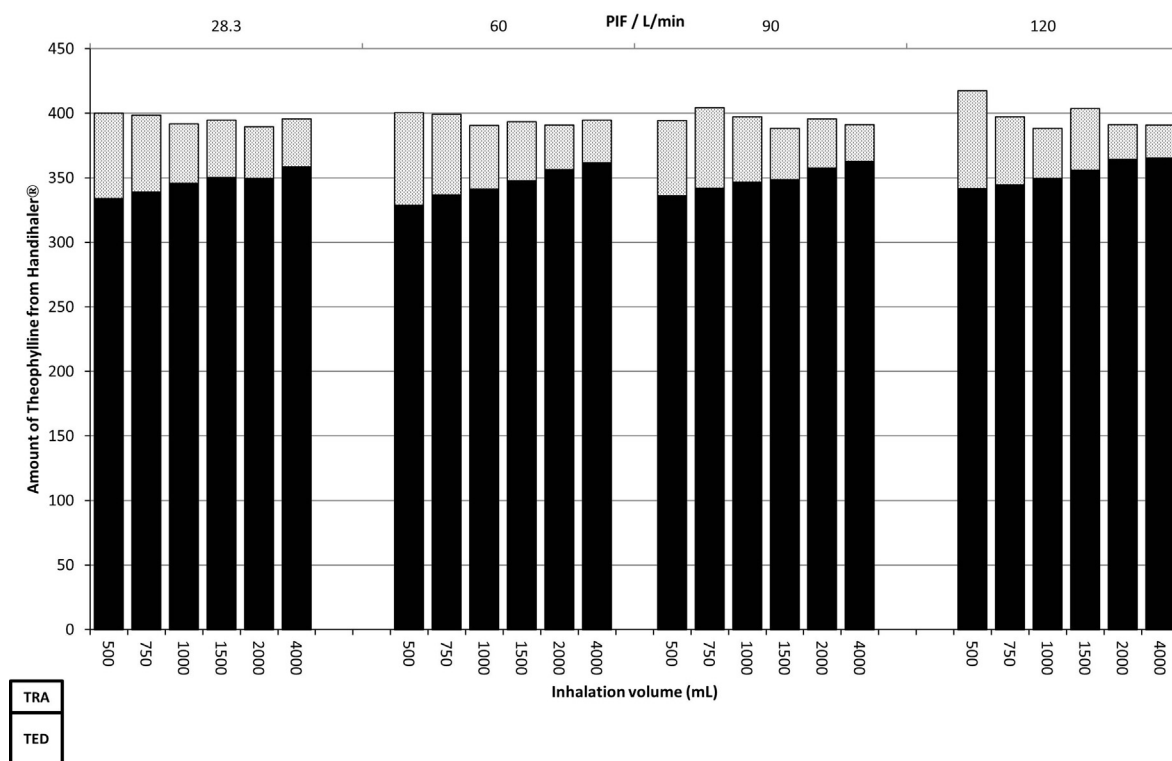


Fig. 8. The amount of total emitted dose (TED) and total residual amount (TRA) of Theophylline using Handihaler® [TED (Solid Column) TRA (Pattern Column)].

their inhalation as the later would increase the inhaled volume and, thereby the TED.

It is interesting to note that after the second inhalation (ED2), most of the drug was released from the capsule resulting in less dif-

ference between TED at different PIF and Vin. Thus, patients would need to inhale twice in order to benefit from each dose. This explains manufacturers PIL recommending that two inhalations should be taken by users for effective dose emission and dose emptying.

Abdelrahim (2010) used dose unit sampling apparatus (DUSA) to investigate the effect of the inhalation flow varying from 10 to 60 L min⁻¹ at 2 inhalation volumes (2 L and 4 L) on the emitted dose of terbutaline from Bricanyl Turbuhaler[®] after one and two inhalations (Abdelrahim, 2010). The results obtained in the current work are in line with the finding from Abdelrahim (2010) despite the difference in the inhalation condition in terms of range of inhalation flow, volume and the inhaler device. The emitted dose after one inhalation (ED1) and two inhalations (TED) increased with the increase of PIF and Vin. Abdelrahim et al., (2013) used a mixing inlet attached to an ACI to investigate the impact of low inhalation flows (10 to 60 L min⁻¹) on total emitted dose (TED) and fine particle dose (%FPF) at 4 L inhaled volume for Terbutaline from a Bricanyl Turbuhaler[®]. Their results showed that both TED and FPD increased significantly with inhalation flow. However, the impact of the inhalation flow was more pronounced on the FPD than TED. In the current work, a wider range of PIF and Vin was used and our results showed that emitted dose after first inhalation and TED were dependent on both PIF and Vin irrespective of the inhaler device either Breezhaler[®] or Handihaler[®]. The present study concluded the same conclusion to that reported by Abdelrahim (2010) that it is essential for a patient to inhale twice and as deep and hard as possible from each dose to benefit from the inhaled medication especially for patients with low lung capacity.

Ali and Abdelrahim (2014) used data mining technology based on artificial neural networks and genetic algorithms to model and optimise Terbutaline Emitted from a DPI. The optimised models demonstrated that an optimum emitted dose >76% could be obtained if the dose was withdrawn as two inhalations with an inhaled volume of 4 L and inhalation flow of 60 L min⁻¹. Our data showed that after two inhalations, the theophylline dose emitted was 90% and 91% from Breezhaler[®] and Handihaler[®] respectively, this suggests that our data was in line with the optimised models described by Ali and Abdelrahim. Boshra et al., (2018) compared the performance of two inhaler devices namely Diskus[®] and Aeroliser[®] using two separate inhalations. Their results showed that at an inhalation flow ≤ 30 L min⁻¹ two inhalations resulted in higher TED than one inhalation. Our results showed that second inhalation is more important especially when a low inhalation flow is used. For those patients who cannot generate a high inhalation flow rate due to low lung capacity, it is recommended to inhale twice from the same dose. The in-vitro results from the present research are in accordance with the in-vivo study carried out by Boshra et al., (2019) that it is essential to inhale twice and as hard and deep as possible from each dose when using DPI especially with COPD-patients having poor inspiratory efforts such as elderly patients and children.

3.4.4. Total residual amount (TRA)

The dose emptying from the capsule and device is demonstrated by the total residual amount (TRA), which represents the amount of drug retained in the device and capsule after the two inhalations. The inspiratory parameters PIF and Vin significantly ($p < 0.05$) affect the dose retained in the capsule and device, where increasing PIF and Vin resulted in a decrease in TRA, for both devices.

The Onbrez Breezhaler[®] clearly had a higher TRA as shown in Fig. 7. This means that Handihaler[®] has a better dose delivery performance when compared to Breezhaler[®]. For example, at the PIF of 28.3 L min⁻¹, the TRA of the Onbrez Breezhaler[®] was 66.25 \pm 1.24 μ g and the TRA of the Handihaler[®] was 45.15 \pm 2.21 μ g. The Handihaler[®] emitted 21.10 μ g more than the Onbrez Breezhaler[®].

Drug retention inside the inhaler continues to be a factor, plaguing the performance of novel inhalers (Tajber et al., 2009). Drug

retention varies between inhalers in that some studies have reported between 30 and 50% of the nominal dose is retained within the device (Heng et al., 2013). The difference in the inhaled volume between DPIs was attributed to their internal design and the intrinsic resistance, for example a significant increase in the fine particle fraction was shown with the Aerolizer[®] when the air inlet size was reduced and the results were explained by the increase in the air velocity and turbulent inside the inhaler device (Zhou et al., 2013; Abadelah, 2018). The difference in the Vin between the Diskus[®] and Turbuhaler[®] was attributed to the length of the inhalation channel. The inhalation channel in the Turbuhaler[®] is relatively long and includes a cyclone, whereas the inhalation channels of the Diskus[®] is very short. The low resistance devices have also been reported to require larger inhaled volume than higher resistance devices (Azouz and Chrystyn, 2012). The Easyhaler[®] design, i.e., short inhalation channel together with a high intrinsic resistance may have both accounted for only a small inhaled volume been required by this device, thus allowing the dose to leave the inhaler.

It is important that the complete dose is released from the inhaler so as to maximise the therapeutic effect, minimising drug wastage and avoiding potential dosage errors during the next inhalation. Some drug particles remained in the device irrespective of the PIF and Vin, as shown in this study.

From the results of this current study, it is evident that patients would benefit from two inhalations for each dose enabling the patient to receive most of the dose from the capsule. One inhalation may not be sufficient to emit the complete dose as seen in the ED1. By employing the second inhalation, the total emitted dose was much higher. Both devices are breath actuated and the PIF and Vin showed an impact on the total emitted dose as it is the case of most breath actuated DPIs.

Prescribing the right inhaler to the right patient is always important to ensure a better clinical outcome. Previous studies have shown that patients normally get a better clinical outcome from inhalers with a higher resistance (Chrystyn and Price, 2009). The present study showed that both devices were consistent in terms of dose delivery, but with more dose was delivered from Handihaler[®], however, taking into account the effort required to achieve certain PIF differ between low and high resistance device, patients with limited lung capacity or who cannot achieve higher PIF and Vin should inhale twice from capsule based devices to ensure better dose emptying and dose emission.

4. Conclusion

The inspiratory parameters PIF and Vin affected the dose emission of theophylline formulation from Handihaler[®] and Breezhaler[®]. ED1 and TED increased with increasing the PIF and Vin. Handihaler[®] delivered more dose of theophylline than Breezhaler[®]. None of these devices were able to empty the full dose from the capsule, irrespective of the PIF and Vin used, and some effort still has to be made by DPI manufacturers to improve the design of their inhaler devices. Our study supports the recommendations for patients who cannot achieve sufficient PIF and Vin to inhale twice for each dose to ensure the better clinical outcome.

Declaration of Competing Interest

All authors declare no conflicts of interest in this work.

Appendix A

(See Figs. A.1–A.6).

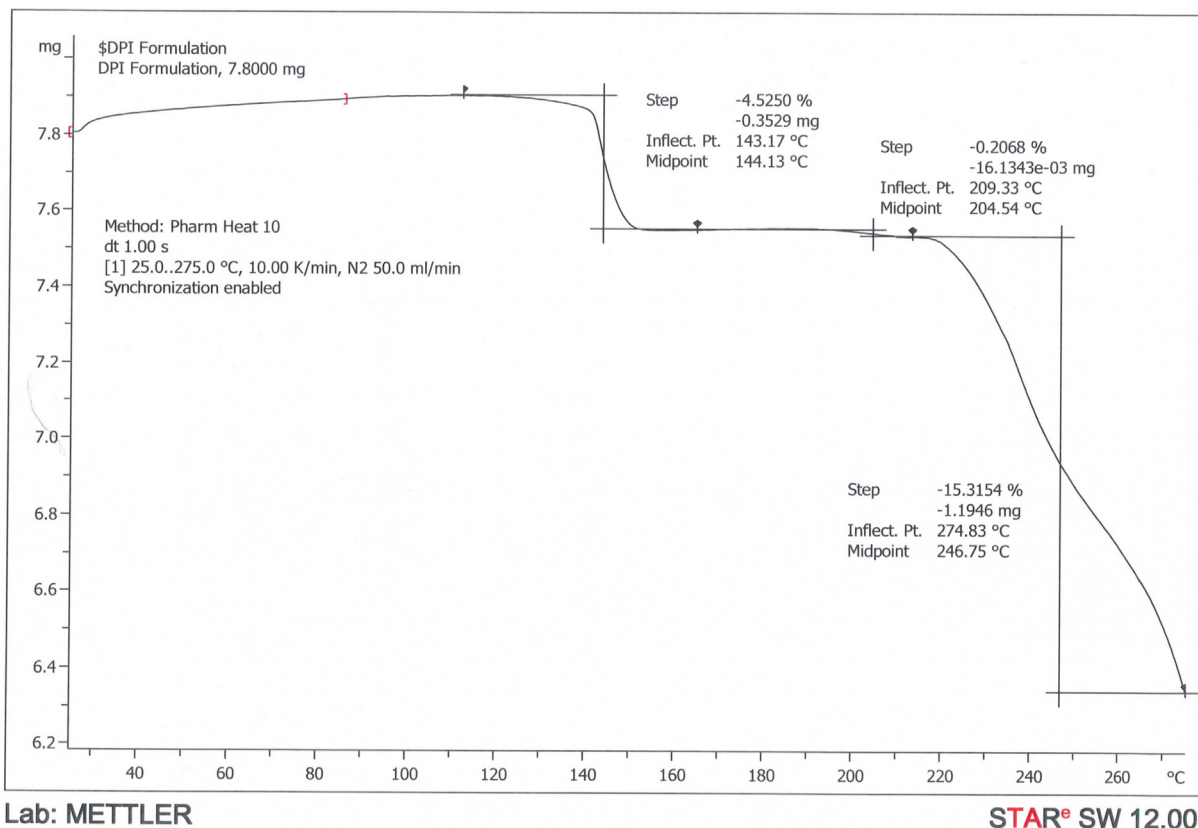


Fig. A.1. TGA graph of the DPI formulation; Spray dried Theophylline and Lactohale[®].

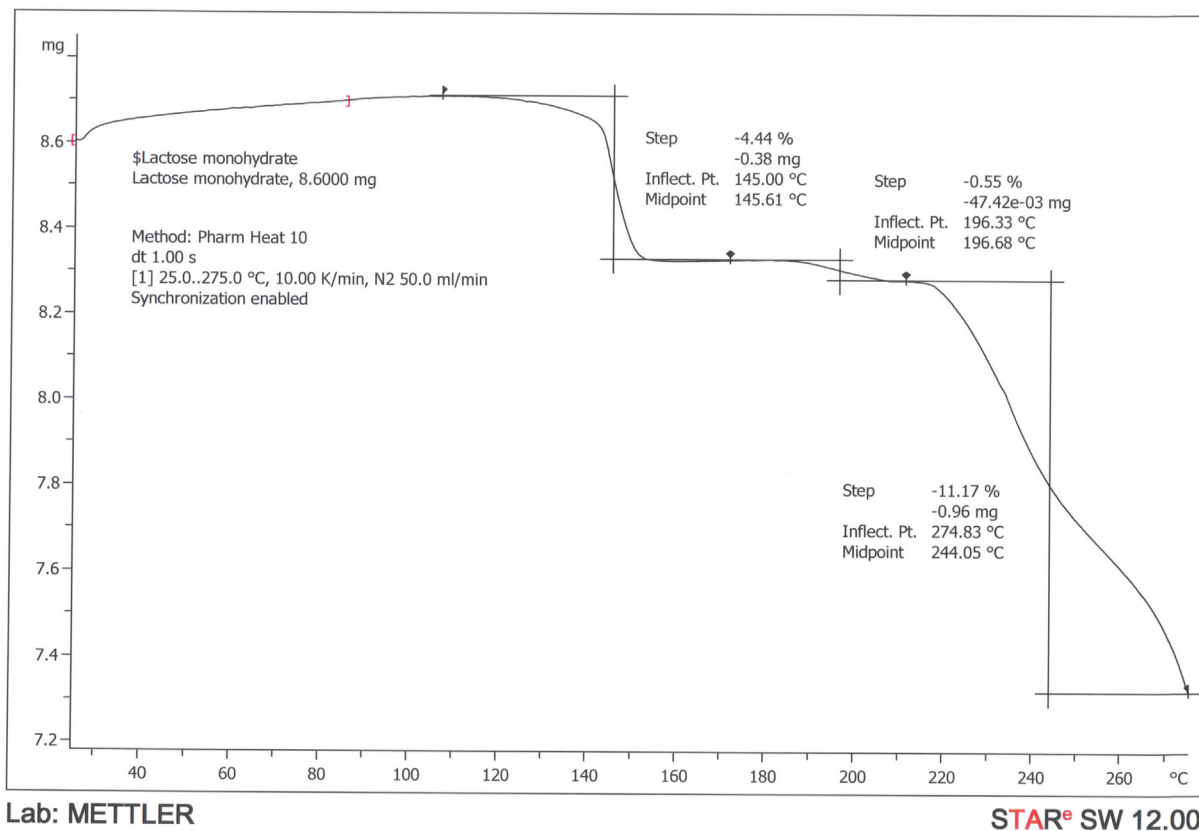


Fig. A.2. TGA graph of Lactohale[®] (63–90 μm).

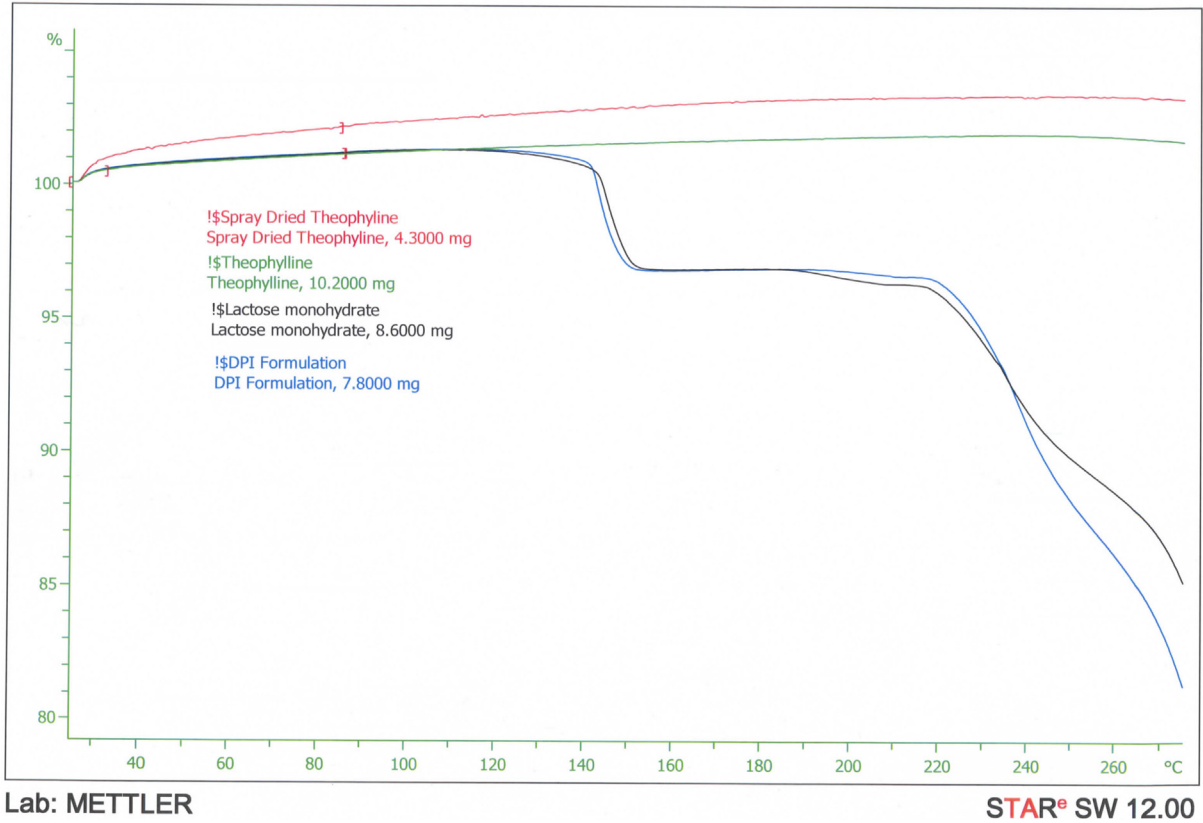


Fig. A.3. TGA graph of spray dried theophylline, commercial theophylline, and Lactohale® and DPI formulation.

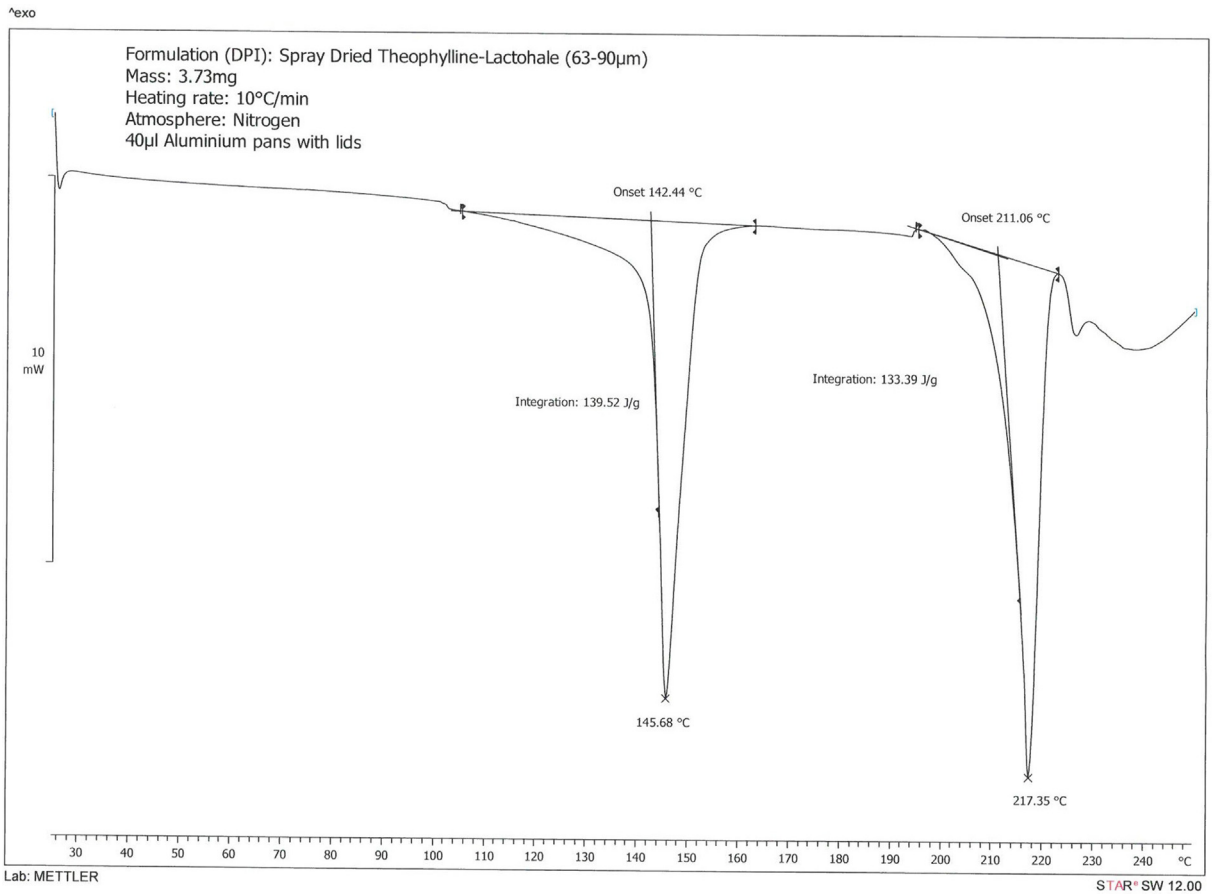


Fig. A.4. DSC graph of DPI formulation; Spray dried Theophylline and Lactohale®.

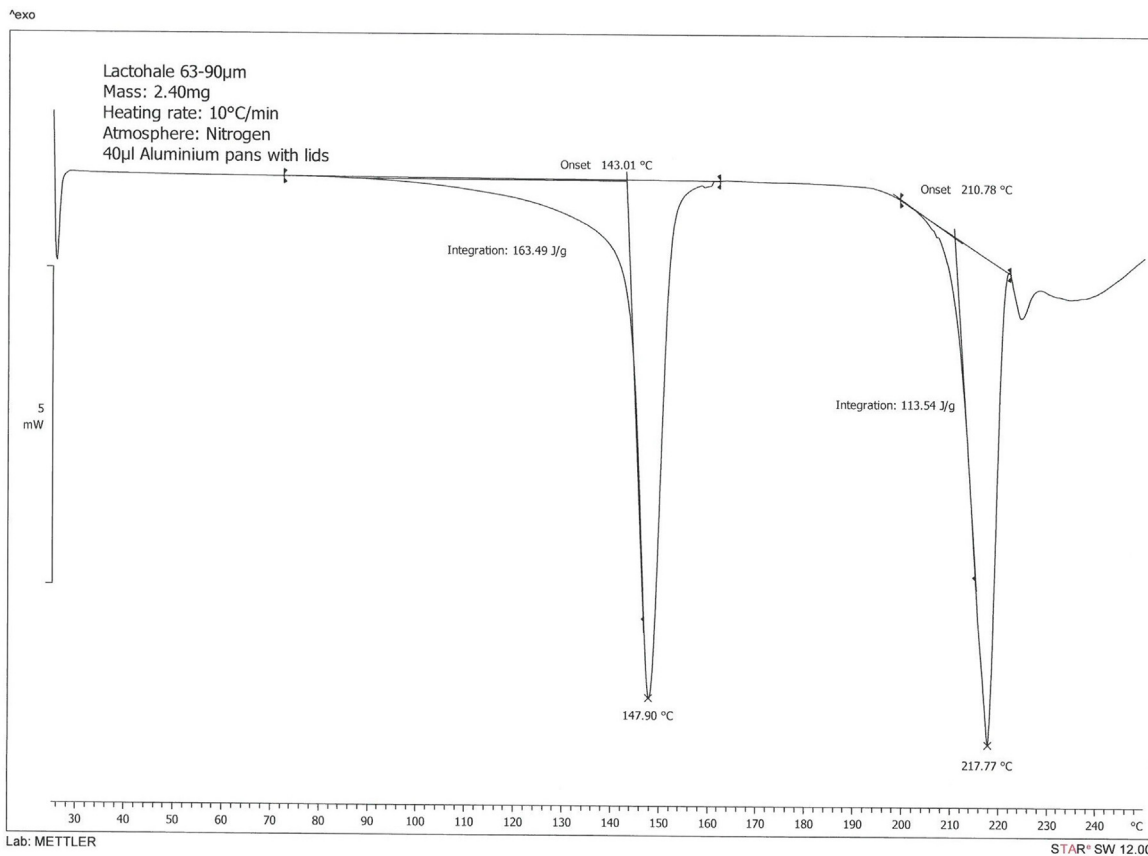


Fig. A.5. DSC graph of Lactohale[®] (63–90 μ m).

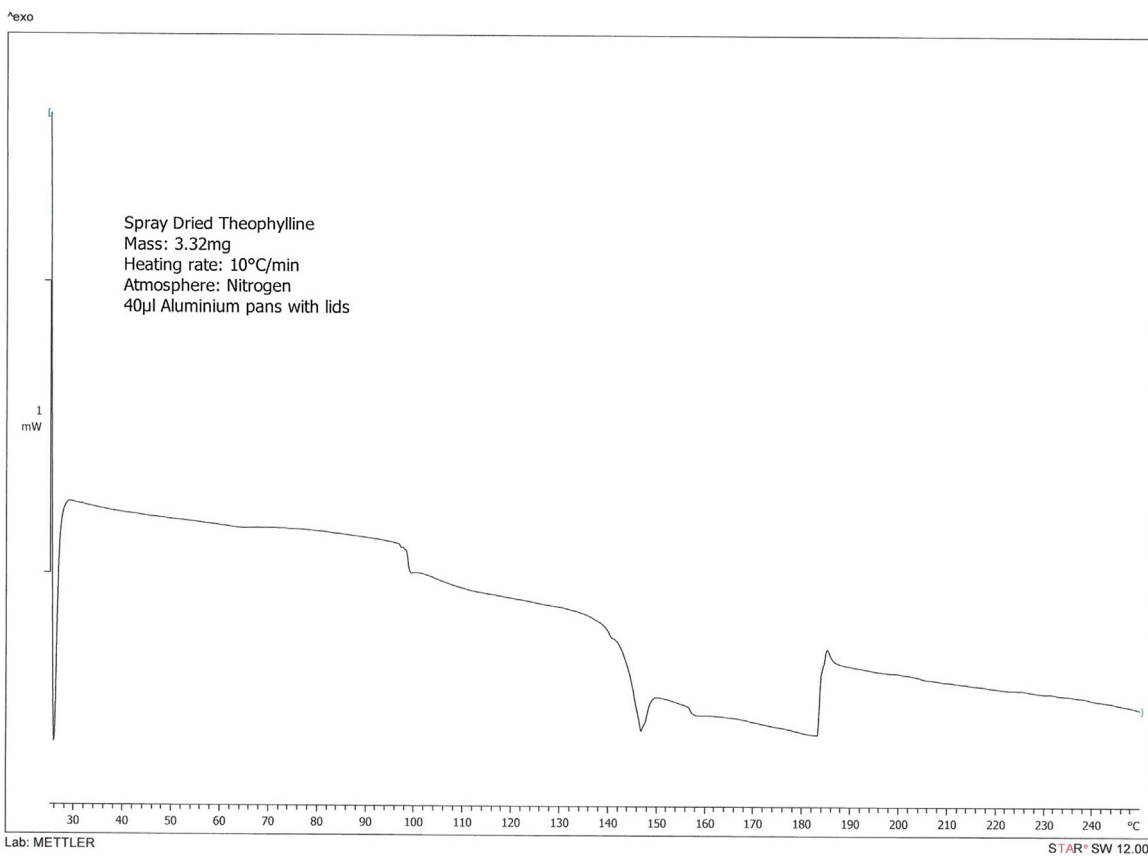


Fig. A.6. DSC graph of Spray dried theophylline.

References

- Abadelah, M., 2018. Effect of Inhalation Manoeuvre Parameters In-Vitro and Ex-Vivo on the Dose Emission and the Aerodynamic Characteristics of Formoterol and Indacaterol from Marketed Dry Powder Inhalers (Doctoral dissertation, University of Huddersfield).
- Abadelah, M., Chrystyn, H., Bagherisadeghi, G., Abdalla, G., Larhrib, H., 2017a. Study of the emitted dose after two separate inhalations at different inhalation flow rates and volumes and an assessment of aerodynamic characteristics of indacaterol Onbrez Breezhaler® 150 and 300 µg. *AAPS PharmSciTech*. <https://doi.org/10.1208/s12249-017-0841-y>.
- Abadelah, M., Hazim, F., Chrystyn, H., Bagherisadeghi, G., Rahmoune, H., Larhrib, H., 2017b. Effect of maximum inhalation flow and inhaled volume on formoterol drug deposition in-vitro from an Easyhaler® dry powder inhaler. *Eur. J. Pharm. Sci.* 104. <https://doi.org/10.1016/j.ejps.2017.03.035>.
- Abdelrahim, M.E., 2010. Emitted dose and lung deposition of inhaled terbutaline from Turbuhaler at different conditions. *Respir. Med.* 104, 682–689.
- Abdelrahim, M., Assi, K., Chrystyn, H., 2013. Dose emission and aerodynamic characterization of the terbutaline sulphate dose emitted from a Turbuhaler at low inhalation flow. *Pharm Dev Technol.* 18 (4), 944–949.
- Al-Showair, R.A.M., Tarsin, W.Y., Assi, K.H., Pearson, S.B., Chrystyn, H., 2007. Can all patients with COPD use the correct inhalation flow with all inhalers and does training help?. *Respir. Med.* 101, 2395–2401.
- Alaboud, S., 2011. In-vitro inhalation performance for formoterol dry powder and metred dose inhalers. In-vitro characteristics of the emitted dose from the formoterol dry powder and metred dose inhalers to identify the influence of inhalation flow, inhalation volume and the number of inhalation per dose.
- Ali, A., Abdelrahim, M., 2014. Modeling and optimization of terbutaline emitted from a dry powder inhaler and influence on systemic bioavailability using data mining technology. *J. Pharm. Innov.* 9 (1), 38–47.
- Azouz, W., Chetcuti, P., Hosker, H.S.R., Saralaya, D., Stephenson, J., Chrystyn, H., 2015. The inhalation characteristics of patients when they use different dry powder inhalers. *J. Aerosol Med. Pulm. Drug Deliv.* 28, 35–42.
- Azouz, W., Chrystyn, H., 2012. Clarifying the dilemmas about inhalation techniques for dry powder inhalers: integrating science with clinical practice. *Prim Care Respir J* 21, 208–213. <https://doi.org/10.4104/pcrj.2012.00010>.
- De Boer, A., Bolhuis, G., Gjaltema, D., 1997. Inhalation characteristics and their effects on in vitro drug delivery from dry powder inhalers: Part 3: The effect of flow increase rate (FIR) on the in vitro drug release. *Int. J.*
- Boshra, Marian S., Almeldien, Ahmed G., Eldin, Randa Salah, Elberry, Ahmed A., Abdelwahab, Nada Sayed, Salem, Mohamed Nabil, Rabea, Hoda, Abdelrahim, Mohamed E.A., 2018. Total emitted dose of salbutamol sulphate at different inhalation flows and inhalation volumes through different types of dry powder inhalers. *Exp Lung Res.* 44 (4–5), 211–216.
- Boshra, Marian S., Almeldien, Ahmed G., Eldin, Randa Salah, Elberry, Ahmed A., Abdelwahab, Nada Sayed, Salem, Mohamed Nabil, Rabea, Hoda, Abdelrahim, Mohamed E.A., 2019. Inhaled salbutamol from Aerolizer and Diskus at different inhalation flows, Inhalation volume and number of inhalations in both healthy subjects and COPD patients. *Exp Lung Res.* 1–8.
- Chrystyn, H., 2003. Is inhalation rate important for a dry powder inhaler? Using the In-Check Dial to identify these rates. *Respir. Med.* 97, 181–187.
- Chrystyn, H., Price, D., 2009. Not all asthma inhalers are the same: factors to consider when prescribing an inhaler. *Prim Care Respir J.* 18, 243–249.
- Clark, A.R., Hollingsworth, A.M., 1993. The relationship between powder inhaler resistance and peak inspiratory conditions in healthy volunteers—implications for in vitro testing. *J. Aerosol. Med.* 6, 99–110.
- Coates, M.S., Fletcher, D.F., Chan, H.-K., Raper, J.A., 2004. Effect of design on the performance of a dry powder inhaler using computational fluid dynamics. Part 1: Grid structure and mouthpiece length. *J. Pharm. Sci.* 93, 2863–2876. <https://doi.org/10.1002/JPS.20201>.
- Colthorpe, P., Voshaar, T., Kieckbusch, T., 2013. Delivery characteristics of a low-resistance dry-powder inhaler used to deliver the long-acting muscarinic antagonist glycopyrronium. *J. Drug.*
- Dal Negro, R.W., 2015. Dry powder inhalers and the right things to remember: a concept review. *Multidiscip. Respir. Med.* 10, 1.
- FDA, 1998. Food and Drug Administration; Guidance for Industry Metered Dose Inhaler (MDI) and Dry Powder Inhaler (DPI) Drug Products [Online]. Available: https://www.fda.gov/ohrms/dockets/ac/00/backgrd/3634b1c_sectiond.pdf [Accessed 5th September 2018].
- Haidl, P., Heindl, S., Siemon, K., Bernacka, M., Cloes, R.M., 2016. Inhalation device requirements for patients' inhalation maneuvers. *Respir. Med.* <https://doi.org/10.1016/j.rmed.2016.07.013>.
- Haughney, J., Price, D., Barnes, N., Virchow, J., 2010. Choosing inhaler devices for people with asthma: current knowledge and outstanding research needs. *Respir. Med.*
- Heng, D., Lee, S., Ng, W., Chan, H., Kwek, J., 2013. Novel alternatives to reduce powder retention in the dry powder inhaler during aerosolization. *Int. J.*
- Kamin, W.E.S., Genz, T., Roeder, S., Scheuch, G., Trammer, T., Juenemann, R., Cloes, R. M., 2002. Mass output and particle size distribution of glucocorticosteroids emitted from different inhalation devices depending on various inspiratory parameters. *J. aerosol Med.* 15, 65–73.
- Larhrib, H., Zeng, X., Martin, G., Marriott, C., 1999. The use of different grades of lactose as a carrier for aerosolised salbutamol sulphate. *Int. J.*
- Laube, B.L., Janssens, H.M., de Jongh, F.H.C., Devadason, S.G., Dhand, R., Diot, P., Everard, M.L., Horvath, I., Navalesi, P., Voshaar, T., 2011. What the pulmonary specialist should know about the new inhalation therapies. *Eur. Respir. J.* 37, 1308–1417.
- Pavkov, R., Mueller, S., Fiebich, K., Singh, D., 2010. Characteristics of a capsule based dry powder inhaler for the delivery of indacaterol. *Med. Res.*
- Peng, T., Lin, S., Niu, B., Wang, X., Huang, Y., Zhang, X., Li, G., Pan, X., Wu, C., 2016. Influence of physical properties of carrier on the performance of dry powder inhalers. *Acta Pharm. Sin. B* 6, 308–318. <https://doi.org/10.1016/j.APSB.2016.03.011>.
- Szterner, P., Legendre, B., Sghaier, M., 2010. Thermodynamic properties of polymorphic forms of theophylline. Part I: DSC, TG, X-ray study. *J. Therm. Anal. Calorim.* 99, 325–335. <https://doi.org/10.1007/s10973-009-0186-1>.
- Tajber, L., Corrigan, O.I., Healy, A.M., 2009. Spray drying of budesonide, formoterol fumarate and their composites—II. Statistical factorial design and in vitro deposition properties. *Int. J. Pharm.* 367, 86–96.
- USP, 2014. United States Pharmacopeia, USP 38-NF 33.
- Yakubu, S.I., Assi, K.H., Chrystyn, H., 2013. Aerodynamic dose emission characteristics of dry powder inhalers using an Andersen Cascade Impactor with a mixing inlet: The influence of flow and volume. *Int. J. Pharm.* 455, 213–218. <https://doi.org/10.1016/j.IJPHARM.2013.07.036>.
- Zhou, Q.T., Tong, Z., Tang, P., Citterio, M., Yang, R., Chan, H.-K., 2013. Effect of device design on the aerosolization of a carrier-based dry powder inhaler—a case study on Aerolizer® Foradile®. *AAPS J.* 15, 511–522.