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Original Article

ZAP-AIR™MICRONIZER: IMPACT ON NEBULIZED SALBUTAMOL SULPHATE AND IPRATROPIUM BROMIDE PARTICLE SIZE

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ABSTRACT

Objective: This study aimed to investigate the effect of the patented Zap-Air™ Micronizer on the particle size reduction of salbutamol sulphate (Ventolin), ipratropium bromide (Atrovent), and their combination (Combivent) nebulizing solutions.

Methods: The study used an Omron Compressor Nebulizer NE-C28 and compressed oxygen at flow rates of 3L and 6L per minute. Particle size analysis was conducted using Malvern Spraytec Laser Diffraction System to measure DV4 (4% of particles in the sample), DV50 (50% of particles in the sample), DV90 (90% of particles in the sample). Drug deposition analysis was performed using the Next Generation Pharmaceutical Impactor (NGI) with High-Performance Liquid Chromatography (HPLC) to analyze the content at each stage.

Results: The Zap-Air™ Micronizer effectively reduced the particle size of salbutamol sulphate and ipratropium bromide to less than 2 µm. Both Unit 1 and Unit 2 micronizers showed significant particle size reduction, with the average size of 50% of particles (DV50) being below 1 µm. Drug release intensity was significantly reduced when using the micronizers compared to the nebulizer alone.

Conclusion: The Zap-Air™ Micronizer has the potential to enhance drug delivery and therapeutic effects in the treatment of Asthma and Chronic Obstructive Pulmonary Disease (COPD) by improving the deposition of salbutamol sulphate and ipratropium bromide into the smaller airways of the lungs.

Keywords: Particle size' 'COPD' 'Salbutamol sulphate', Ipratropium bromide

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INTRODUCTION

Pulmonary drug delivery is a form of drug targeting the lung, whether for topical or systemic action. For the former, the advantages of this delivery system include the possibility to use a relatively low dose, a low incidence of systemic side effects and for some drugs, a rapid onset of action [1, 2]. The lungs consist of a complex network of branching airways that looks like a tree. When a particle is aimed at penetrating the alveolar region and gaining access to the large epithelial target site, it must pass through the numerous airway bifurcations where it could potentially be deposited. The required aerosol size to deliver drugs to the whole lung involves an aerodynamic diameter of less than 5 µm [3]. For delivery to the alveolar epithelium, particles with an even smaller size, usually an aerodynamic diameter less than 3 µm, are required [4].

Medical nebulizers are widely used in respiratory treatments. Nebulisers generate medicine-containing droplets by atomization, delivering the medication to the patients through inhalation gas flow [5]. The size of the nebulized particles is one of the most important factors which determine the performance of a medical nebulizer, although other parameters, such as the breathing rate and movement, lung dimension and structure, also significantly affect the particle deposition in the lungs. The Mass Median Aerodynamic Diameter (MMAD) is often used to evaluate medical nebulizers. It is defined as the particle diameter separating the higher half of the particle mass from the lower half. Particle deposition characteristics are closely related to particle size; therefore, MMAD determines where, in the respiratory tract, and in what proportion, most of the particles are deposited after they are inhaled [6].

Inhaled Corticosteroids (ICSs) are widely used for the treatment of Chronic Obstructive Pulmonary Disease (COPD). They are often used in combination with bronchodilators. ICS particle size is measured in MMAD, which is in the range of 1 to 5µm for commonly used ICS devices [7]. Particle size has been proven to play an important role in the distribution of drugs within the lung [8]. Particles have been acknowledged to deposit in the respiratory tract by inertial impaction (3-6 µm), sedimentation (1-3 µm), and diffusion (<1 µm). To reach the lower respiratory tract past the carina, the MMAD of inhaled particles should be less than 5 µm in diameter; specifically, the particle size with the most efficient deposition in the small airways, the so-called extra-fine particle fraction, is said to be less than 2 µm [9]. It has been shown that smaller particles of inhaled salbutamol achieve greater overall lung deposition, as well as greater peripheral lung distribution [10]. In addition, smaller particles of long-acting beta-agonists have been associated with improved small airway responses measured by impulse oscillometry [11]. Additionally, studies suggest that decreasing the mass of large droplets in inhaled aerosols could lead to reduced local (oropharyngeal) side effects, thereby potentially improving patient tolerance and adherence to treatment regimens. Extrafine particles, having an MMAD of ≤2 µm, have also been shown to reach more peripheral parts of the airways to a greater extent [12, 13]. It has been reported that extra-fine particles increase drug uptake due to their increased surface area [14]. Extrafine particle size may result in a larger dispersion area, which reduces the local concentration. In addition, extra-fine particles could facilitate faster absorption and elimination, potentially lowering the risk of diseases such as pneumonia. Hence, altering the aerosol size within the narrow range of 1–5 µm affects the deposition within the lungs and could potentially alter both the efficacy and side effects of the drugs.

Heerfordt, C. K., *et al.* (2023) investigated the use of extrafine-particles of Beclometasone Dipropionate Hydrofluoroalkane Inhaler (HFA) and extrafine ciclesonide in patients with =COPD. A total of 35,691 patients were included, of whom 1,471 received extra-fine particle ICS. Among these patients, 4,657 were hospitalised due to pneumonia. They found that patients with COPD receiving extra-fine-particle ICS had a lower risk of hospitalisation due to pneumonia compared with patients receiving standard particle-size

ICS. Therefore, they concluded that the use of extra-fine-particle ICS in the treatment was associated with a lower risk of pneumonia hospitalisation in patients with COPD compared with those who received standard-size treatment [15]. Another study investigated the effectiveness of initiating extra-fine-particle versus fine-particle inhaled corticosteroids as asthma therapy in the Netherlands. Patients were matched (1:1) on relevant demographic and clinical characteristics over a 1-year baseline period. Their results concluded that extra-fine-particle ICS was associated with better asthma control than fine-particle ICS in patients prescribed their first ICS therapy, and this was reached at a significantly lower prescribed dose [16]. Mohamed Faisal Abdul Hamid *et al.* (2023) studied the effect of fine-particle size and extra-fine-particle size ICS in reducing airway resistance in asthmatic patients. Thirty-four subjects were recruited with a median asthma duration of 20 years. Subjects were grouped based on pre-existing inhalers; Extra fine and Fine-Inhaled Corticosteroid (ICS)/Long Acting Beta Agonist (LABA). Parameters assessed were the Asthma Control Test (ACT) score, Forced Expiratory Volume in 1 second (FEV1) and Impulse Oscillometry (IOS) inhaler technique periodically. They found that there was an improvement in FEV1 and ACT scores with extra fine ICS/lABA but no benefit in airway resistance [17]. Another related study investigated the effectiveness of changing from dual therapies or triple therapies (multiple inhalers) to Extrafine Single-Inhaler Triple Therapy (efSITT), which consists of the ICS beclomethasone, the Long-Acting β_2-Agonist (LABA) formoterol and the Long-Acting Muscarinic Antagonist (LAMA) glycopyrronium, in patients with moderate-to-severe COPD. Patients were recruited at 148 sites in Germany and assessed on Health-Related Quality of Life (HRQoL: measured by the COPD Assessment Test [CAT]), lung function and adherence (measured by the Test of Adherence to Inhalers [TAI]) at baseline and after six months. The treatment switch to efSITT resulted in the improvement of HRQoL, COPD-specific symptoms, lung function parameters and adherence under real-world conditions [18].

Therefore, this study aimed to investigate the effect of the patented micronisers on the size reduction of salbutamol sulphate (Ventolin), ipratropium bromide (Atrovent) and a combination of salbutamol sulphate and ipratropium bromide (Combivent) nebulising solutions using Omron Compressor Nebuliser NE-C28, and compressed oxygen at 3L and 6L per minute flow rates.

MATERIALS AND METHODS

Salbutamol sulphate standard, iptratropium bromide standard, phosphoric acid, methanol and acetonitrile HPLC grade were obtained from Sigma Aldrich. Atrovent, Ventolin and Combivent nebulising solutions were obtained locally. Oxygen was purchased from Warisan Alam Trading Sdn. Bhd.

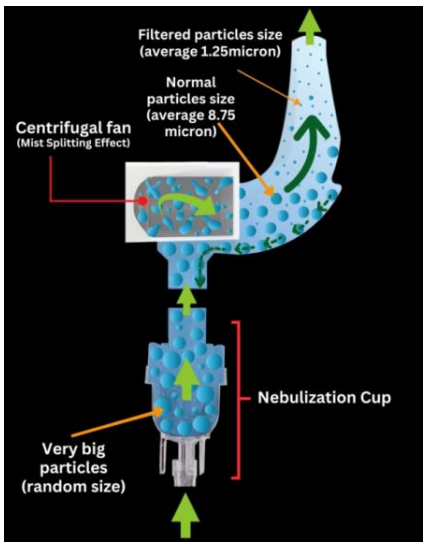

Fig. 1: Zap-Air™Micronizer mode of action

Microniser design

Zap-Air™Micronizer is a simple device that is made up of a mini air blower, housed inside a curved nozzle polymer casing with a battery. It transforms conventional mist into highly functional mist through a five-step process, which involves sucking, splitting, separating, filtrating, and blowing out, facilitated by a simple curved nozzle fan technology (fig. 1). By effectively filtering out the larger particles, the microniser ensures that the resulting mist predominantly consists of submicron-sized particles.

Selection of micronisers

Two different types of micronisers were investigated. The first microniser, referred to as Unit 1, operates at 6000 rpm and has an adjustable voltage range of 3.5, 4, 5, 6, 7, 8, and 9V. The second microniser, known as Unit 2, operates at a lower speed of 4200 rpm with a fixed voltage of 6V.

Selection of nebulisers

Two different types of nebulisers were investigated. The first type was the Omron Compressor Nebuliser NE-C28. The second type involves the use of compressed oxygen, with flow rates set at either 3L per minute or 6L per minute.

Selection of drugs

Three different types of drugs were investigated. The first drug was salbutamol sulphate, marketed as Ventolin, available in a nebulising solution with a concentration of 2.5 mg/2.5 ml. The second drug was ipratropium bromide, sold under the brand name Atrovent, in a nebulising solution with a concentration of 0.025% in a 20 ml solution. The third drug was a combination of salbutamol sulphate and ipratropium bromide, available as Combivent, containing 0.5 mg of ipratropium bromide and 3.01 mg of salbutamol sulphate per dose.

Particle size analysis

2.5 ml of each drug was nebulised using the stated nebulisers for 60 seconds, respectively, with and without the use of the micronisers using the Malvern Spraytec Laser Diffraction System. The flow rate was set to 28.3 l/min. The experiment was performed in triplicate and the average size was calculated. The size, DV4, DV50 (MMAD), DV 90 and the intensity of the drug released were calculated using Malvern Spraytec 3.20 software and compared.

Particle deposition analysis

The Next Generation Pharmaceutical Impactor (NGI) was used to investigate the deposition of drugs at the different levels of NGI. Before the experiment, the NGI apparatus was cooled at 3°C-4°C for 90 min. The NGI method followed that described by Abdelrahim and Chrystyn [19] with a standard T-mouthpiece, routinely used with each nebulized system, fitted tightly to the induction port of the NGI using a standard rubber mouthpiece adapter (fig. 2). Before another experiment was performed, the apparatus was cooled for 30 min before usage. The flow rate was set to 15 l/min. All the parts of the NGI were washed and allowed to dry. The collection plates were not sprayed with silicone fluid as it is recommended by the European Pharmaceutical Aerosol Group (EPAG) that the NGI collection cups do not require coating for nebulizer aerosol assessments. The NGI was assembled with a final filter after the Micro Orifice Collector (MOC) [20]. In this experiment, Unit 2 was selected from the results obtained in the particle size analysis experiment. In brief, 2.5 ml of salbutamol sulphate (SS) solution was added to the nebuliser compartment. The experiment was started (with and without microniser, respectively) for 60 seconds and stopped. 10 ml of HPLC solvent was used to rinse and collect the deposited drug in each of the NGI stages. The experiment was then continued until the whole solution was finished. Again, 10 ml of HPLC solvent was used to rinse and collect the deposited drug in each of the NGI stages. The collected samples were then sent for HPLC analysis. The experiment was repeated for ipratropium bromide (IPB).

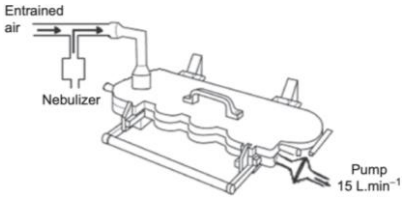

Fig. 2: Schematic design of the NGI methodology to measure the aerosol droplet size from a nebuliser [19]

HPLC analysis

The HPLC method was adapted from Sheena M. Raj *et al.* (2020) with some modifications [21]. A Luna C18 (2) column (150×4.6 mm i. d., 5-µm particle size; Phenomenex, CA, USA) fitted with a security guard column (C18 ODS; 4 × 3.0 mm i. d.; Phenomenex, CA, USA) was utilised for the chromatographic separation of SS and IPB. The equipment used was an HPLC analytical model, the Agilent 1200. The mobile phase was injected at a flow rate of 1 ml/minute. The mobile phase was made by combining acetonitrile with phosphate buffer (pH adjusted to 3.0±0.5 with o-phosphoric acid) in a 30:70 v/v ratio. The column temperature was set to 30 °C and the detection wavelength was 212 nm. The mobile phase was filtered and degassed prior to use. The chromatographic run was performed by injecting a 10 µl volume of sample and stopping at the end of 5 min. Before the injection of standards and samples, the column was equilibrated with the mobile phase for 60 min. Stock solutions of 1,000 µg/ml of SS and IPB were prepared using the mobile phase and necessary dilutions were made to acquire concentrations of 2–12 µg/ml.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Version 26.0 SPSS Inc., Chicago, IL, USA). Paired sample t-tests were conducted to detect significant differences between the paired groups. Additionally, one-way analysis of variance (ANOVA) followed by Tukey post-hoc test was employed to assess differences among multiple groups, with a confidence level of 95%.

RESULTS

Particle size analysis using omron compressor nebuliser NE-C28

Fig. 3 and 4 depict the particle size (DV4, DV50 and DV90) of the drugs using Unit 1 and Unit 2 micronisers, respectively. From the results obtained, with the various changes in voltage of the unit 1 microniser, the particle size was reduced to below 2 µm for all drugs. Similarly, for unit 2 microniser, the particle size was also significantly reduced to below 2 µm for all drugs. In general, it can be seen that the average particle size of 50% of particles is smaller, which is 1 µm or below.

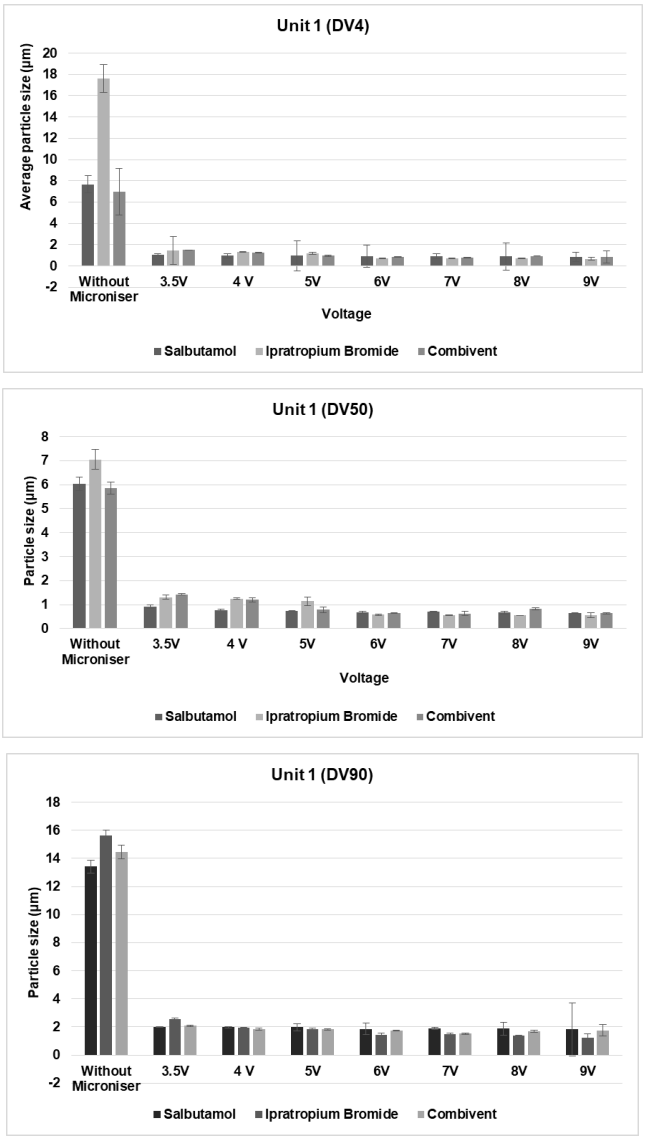

Fig. 3: Particle size of SS, IPB and combivent using unit 1 microniser (n = 3). Error bars indicated SD values

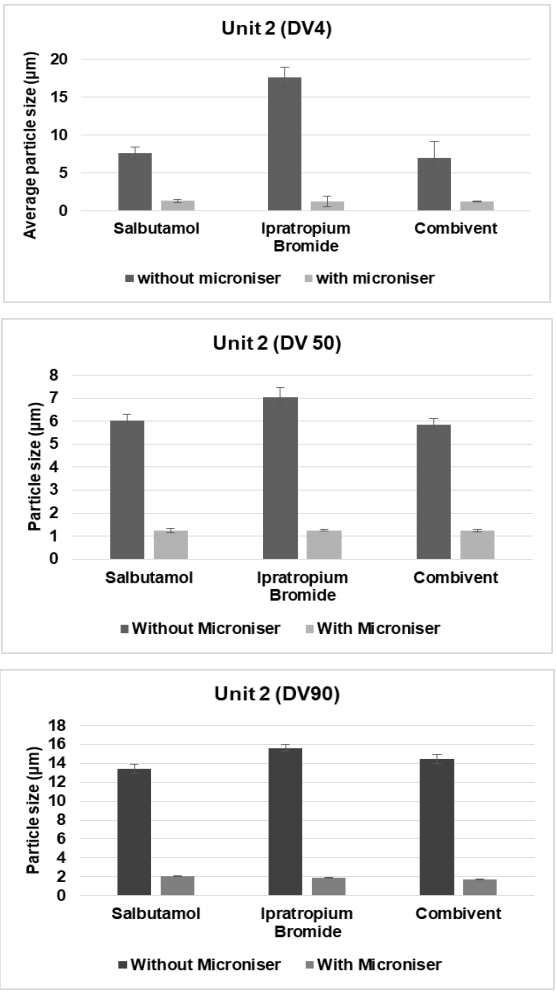

Fig. 4: Particle size of SS, IPB and combivent using unit 2 microniser (n = 3). Error bars indicated SD values

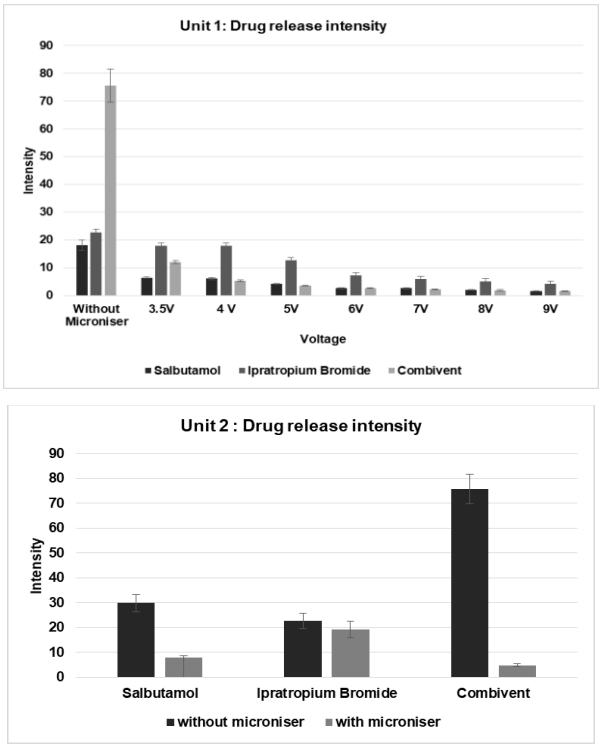

Fig. 5: Drug release intensity from unit 1 and unit 2 micronisers (n = 3). Error bars indicated SD values

Fig. 5 illustrates the release of drugs, both with and without the use of micronisers. It can be noted that SS and combivent drug release were significantly reduced by both micronisers compared to using just the nebuliser alone. However, the release of IPB is similar to that of using the nebuliser alone.

Particle size analysis using oxygen 3 L/min

Fig. 6 and 7 depict the particle sizes (DV4, DV50 and DV90) of the drugs using unit 1 and unit 2 micronisers, respectively. Significant size reductions to below 2 µm was achieved for all drugs when the nebuliser was used with the micronisers. However, for unit 1, as the voltage was increased to above 5V, the particle size measurement of combivent from the nebulising system was not able to be obtained.

Fig. 8 shows the drug release intensity from both Unit 1 and 2 micronisers. It can be seen that the amount or intensity of the drug being released is significantly reduced. It should also be noted that for unit 1 micronisers, when the voltage was increased to above 5 volts, no drug release from combivent was detected.

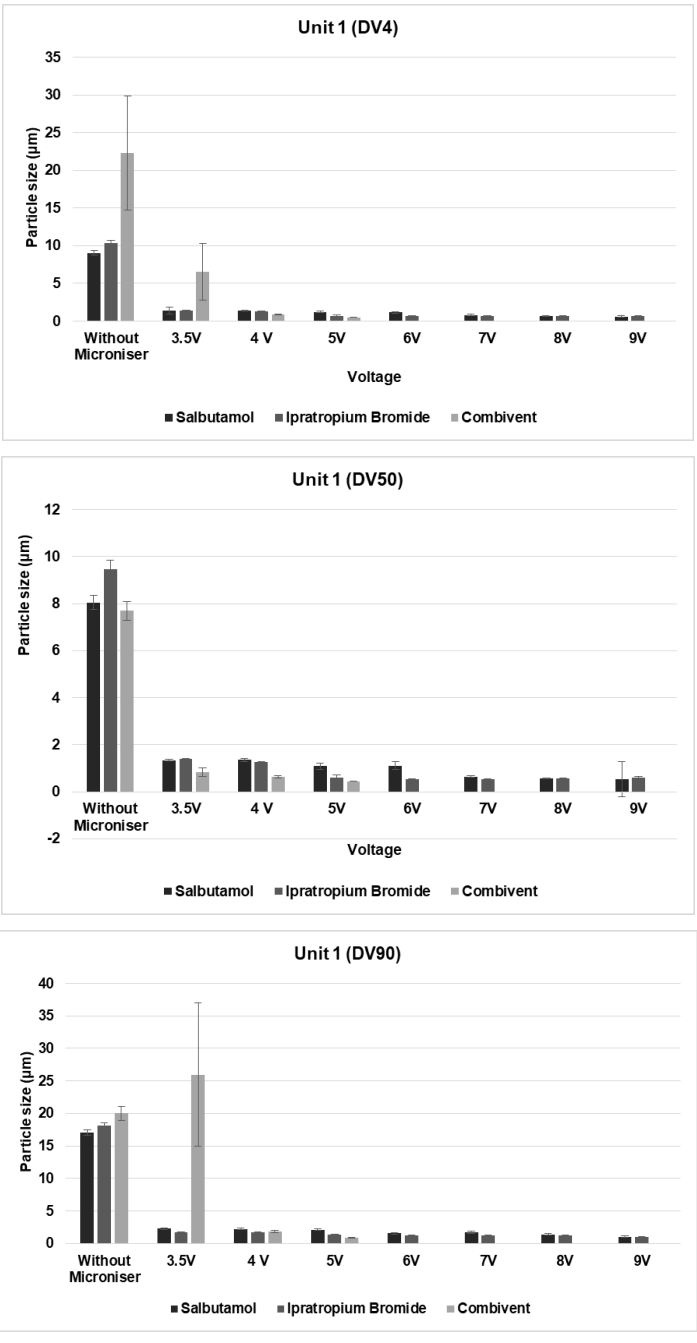

Fig. 6: Particle size of SS, IPB and combivent using unit 1 microniser (n = 3). Error bars indicated SD values

Particle size analysis using oxygen 6 l/min

Fig. 9 and 10 depict the particle sizes (DV4, DV50 and DV90) of the drugs using unit 1 and unit 2 micronisers, respectively. Again, the particle size of all drugs was significantly reduced when the micronisers were used with the nebulising system. The MMAD (DV50) of below 1µm was achieved for Unit 1 when the voltage was increased to 5 volts and above. As for unit 2, a particle size below 1 µm was achieved for salbutamol sulphate and Combivent. However, the use of both micronisers managed to reduce the particle size of all drugs to below 2 µm.

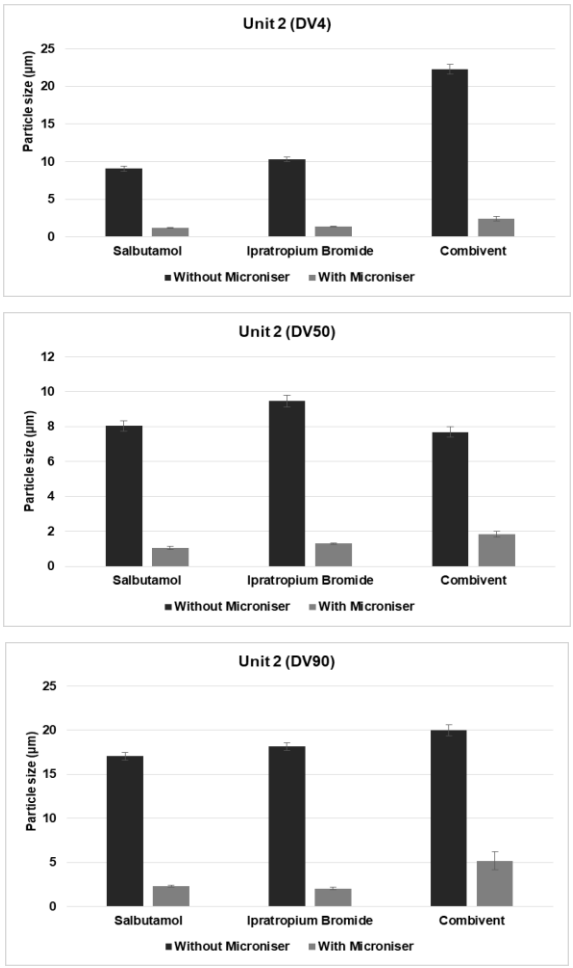

Fig. 7: Particle size of SS, IPB and combivent using unit 2 microniser (n = 3). Error bars indicated SD values

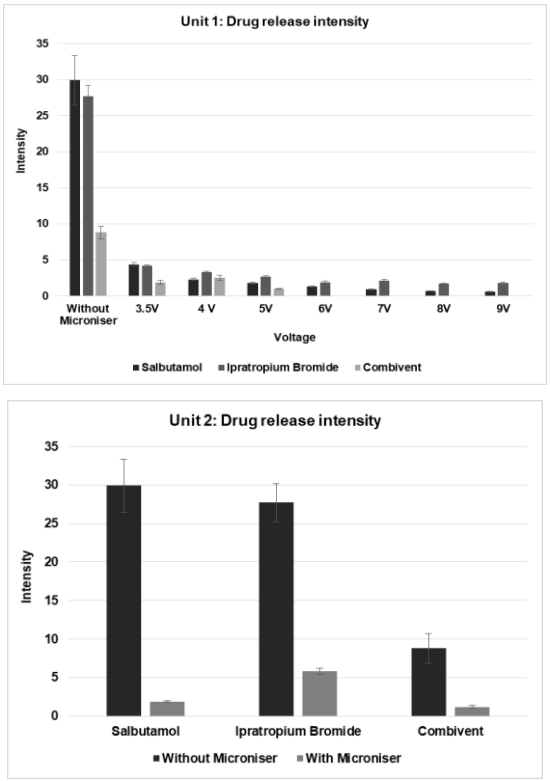

Fig. 8: Drug release intensity from unit 1 and unit 2 micronisers (n = 3). Error bars indicated SD values

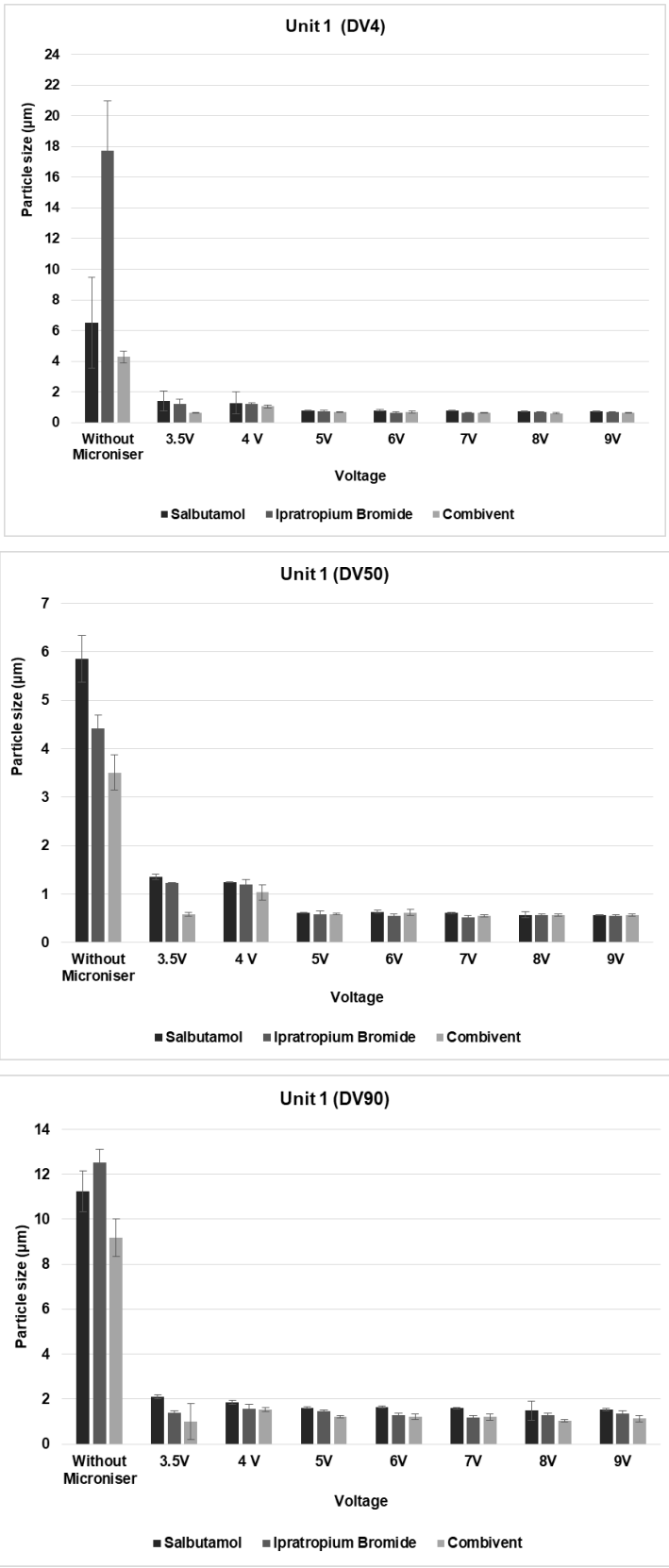

Fig. 9: Particle size of SS, IPB and combivent using unit 1 microniser (n = 3). Error bars indicated SD values

Fig. 11 shows the drug release intensity from both unit 1 and 2 micronisers. For unit 1, as the voltage increased, the intensity of drug released was reduced. As for unit 2, although the intensity of release was reduced, the rate at which the drug was released was still high as compared to the previous two nebuliser studies.

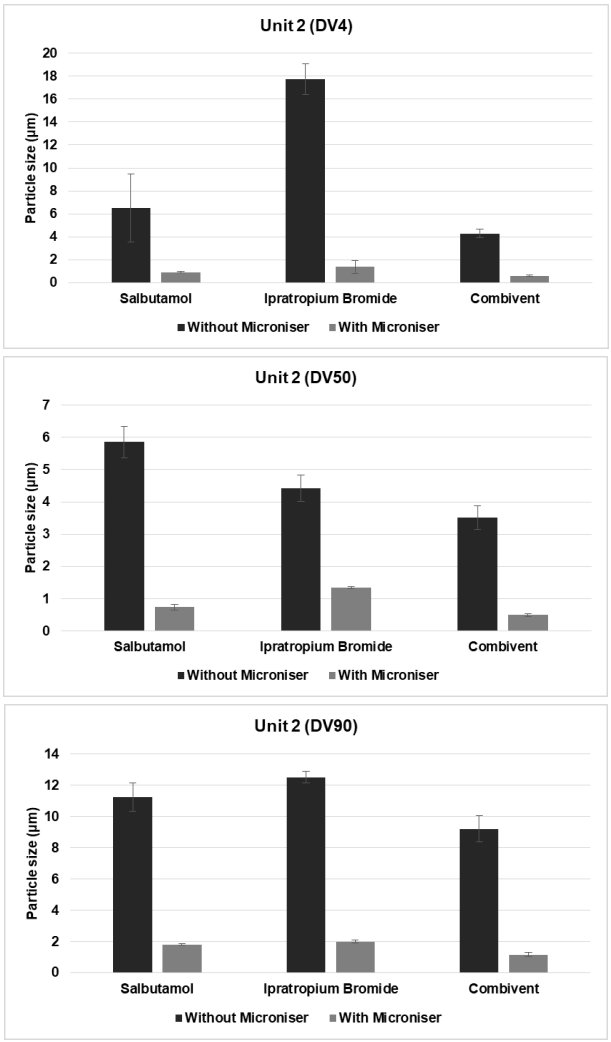

Fig. 10: Particle size of SS, IPB and combivent using unit 2 microniser (n = 3). Error bars indicated SD values

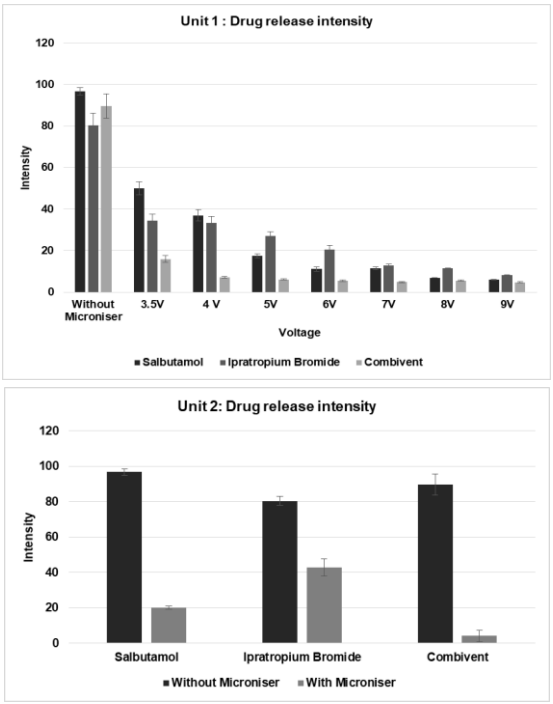

Fig. 11: Drug release intensity from unit 1 and unit 2 micronisers (n = 3). Error bars indicated SD values

Particle deposition analysis

Fig. 12 depicts SS and IPB drug deposition for the NGI stages. Drug deposition for SS is significantly higher at stages 7 and 8 of the NGI when the microniser is used. While for IPB, the deposition of drugs is higher at stages 5 and above, highest at stage 6 of the NGI when the microniser is used. For both drugs, their deposition is highest at stage 1 when the nebuliser is used alone.

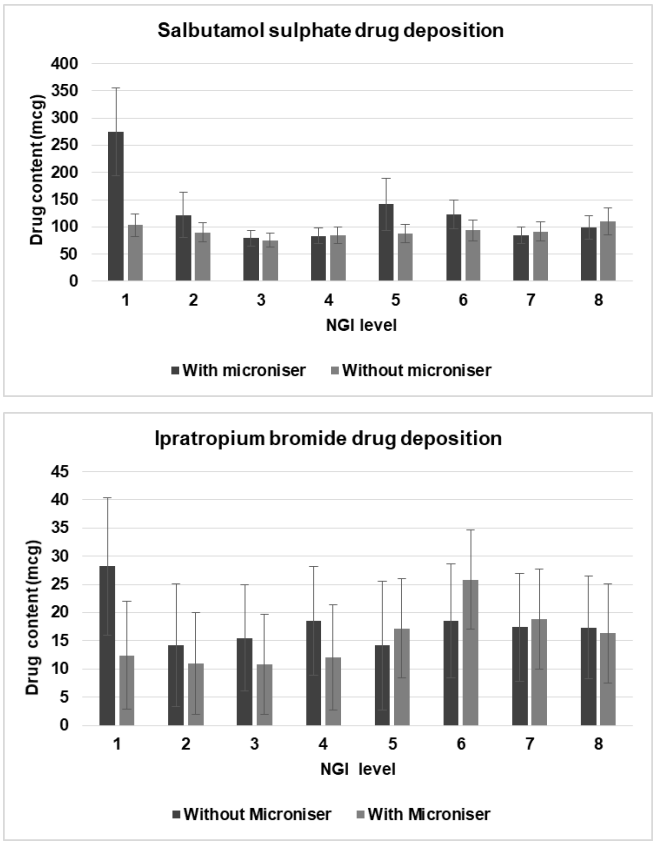

Fig. 12: SS and IPB deposition for the NGI stages (n = 3). Error bars indicated SD values

DISCUSSION

From our studies, when the micronisers were used together with the nebulisers, the particle size of SS, IPB and Combivent was significantly decreased to less than 2µm. However, the drug particle size reduction achieved by the microniser differed based on the drug and voltage used. As the voltage increased, the particle size decreased significantly. Similarly, Morteza Abdeyeh (2017) studied the effect of applied voltage and concentration on the size of nanoparticles produced by electrospray. Their findings showed that particle size decreases with an increase in applied voltage [22]. In contrast, Ravendran and Chou (2021) investigated the effect of voltage and anodizing time on nanocolloidal silver and their results showed that the particle size increases in diameter when there is an increase in voltage from 7.5 V to 12.5 V [23]. Studies conducted by Javad *et al.* [24] and Ahmet *et al.* [25] have also resulted in a similar outcome where the particle size increases with the increase of voltage. However, they reported that the particle size increases with anodizing time until a certain point before decreasing [26]. Weng Xinzen *et al.* investigated the trend of the average diameter of paclitaxel loaded nanoparticles in the voltage range of 18-26 kV. They reported that the average diameter of nanoparticles decreased first and then increased as the applied voltage increased. A minimum particle size was observed at 24 kV [27]. In our current study, we also found that the drug release for combivent from unit 1 is very low, and as the voltage was increased to 6V and above, no more drug release was detected when using omron compressor nebuliser NE-C28 and 3 l oxygen/min. However, when 6L oxygen/min was used, the drug released was observed throughout the different voltages. It was also noted that when using unit 2 microniser with omron compressor nebuliser NE-C28, there was no significant difference in drug release when comparing the use of or without the microniser.

In vitro determination of the particle size distribution, the fine particle dose and the emitted particles by nebulizers are important parameters that have the potential to indicate differences in the clinical response and side effects. The characterization of the aerosols emitted from nebulizers can be determined using the Next Generation Impactor (NGI). Previously, the Andersen Cascade Impactor (ACI) was used, but the limitation of high flow rate used decreased its use [28]. In the drug deposition study, unit 2 microniser was chosen as the drug intensity released was not significantly different from the nebulising system using Omron Compressor Nebuliser NE-C28. In general, when Omron Compressor Nebuliser NE-C28 was used alone, the drug deposition was higher in the lower stages of the NGI, where a larger particle size for both SS and IPB was indicated. When using the nebulising system with the microniser, it was observed that for SS and IPB, the drug deposition is higher at stages 7 and 5 of the NGI, respectively, indicating smaller particle size. The average cut-off size for stages 5, 6 and 7 of the NGI at 15L/min flow rate is 2.08,1.36 and 0.98µm, respectively. Analysis was also done to ascertain the amount of drugs deposited inside the induction port (the L-shape connector between the mouthpiece and the NGI), and it was found that a high amount of both SS and IPB was obtained when the Omron Compressor Nebuliser NE-C28 was used alone, indicating a higher concentration of drug particles which did not enter the NGI. Particle size analysis was performed to ascertain the size of SS particles in that compartment, and an average size of 224µm was obtained. The average cut off size for stage 1 of the NGI is 14µm at 15L/min flow rate. This supports the high amount of drug present in the induction port, as the particle size was too large to enter stage 1. Finally, for SS, it was calculated that a higher amount of drug was released when the miconiser was used compared to Omron Compressor Nebuliser NE-C28 alone.

There is an increasing awareness of the importance of small airways in asthma and COPD [29, 30] and the presence of numerous clinical phenotypes related to small airway involvement [31]. The small airways are less than 2 mm in diameter, and consist of the ducts between generation 8 and the alveoli. It has been claimed that finer aerosols than those delivered by most currently available inhalers may be needed to target these small airways more effectively in order to achieve better drug distribution over the whole bronchial tree [32]. A fixed-dose combination of the ICS Beclometasone Dipropionate (BDP) and LABA Formoterol Fumarate (FF) has been used for the treatment of COPD. This fixed-dose combination is made of an extrafine (i. e MMAD<2.0µm) formulation of both active components of BDP 100 µg and FF 6 µg per actuation [33]. The extrafine formulation provides efficient lung deposition, resulting in a reduction to about half the equivalent dose of a conventional BDP aerosol, which reduces systemic exposure. Small airway inflammation and narrowing are prime characteristics of COPD [34, 35], and the ability of this extrafine formulation to reach the small airways is therefore therapeutically important.

CONCLUSION

Zap-Air™Micronizer showed the capability of reducing the particle size of both SS and IPB to less than 2 µm. Therefore, it has the potential to deliver the drugs into the smaller airways of the lung, thus improving the delivery and therapeutic effects of SS and IPB in the treatment of asthma and COPD.

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AUTHORS CONTRIBUTIONS

Shariza Sahudin and Muhammad Izzuddin Zamery contributed significantly to the experimental work, data analysis, and manuscript writing. Shaharizuan Abdul Rahman participated in the experimental work with a primary focus on instrument setup and troubleshooting. Albert Kow was responsible for the design and development of the Zap-Air™ Micronizer.

CONFLICTS OF INTERESTS

All authors have none to declare

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