In vitro aerodynamic characterization of the dose emitted during nebulization of tobramycin high strength solution by novel and jet nebulizer delivery systems

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A B S T R A C T

Background: Chronic infections with *Pseudomonas aeruginosa* are a leading cause of morbidity in patients with cystic fibrosis (CF). The aim of tobramycin inhalation therapy in CF patients with chronic pulmonary infection is to deliver high amounts of drug directly to the site of infection. TOBI® is a tobramycin nebulizer solution (300 mg/5 ml) approved by FDA for maintenance therapy for patient with CF. The 20% tobramycin sulfate solution was reported as the optimal and maximal concentration.
Methods: Nebulization of high strength tobramycin solution (20% tobramycin sulfate) (HSTS) has been assessed in this study by using different selected high performance nebulizer delivery systems: two different designs of jet nebulizers, and three new nebulizers based on vibrating mesh technology. The aerosol particle size distribution and output characteristics were measured for in vitro performance assessment of the nebulizer systems. The methodology was adapted from the current European standard, EN 13544-1:2001E.
Results: The particle size distribution characteristic measurements showed that all tested nebulizers may be suitable for inhalation of HSTS. The mean (SD) of highest percentage of fine particles (<5 μm) was 77.64 (2.3) % for Sidestream®, at flow rate 16 L/min. The highest respirable inhaled mass was for Pari LC Plus® combined with PariBoy*N*® compressor, with mean (SD) 90.85 (8.6) mg. The mean (SD) of highest drug wastage percentage was 63.9 (3.9) % for Sidestream® jet nebulizer combined with compressed air cylinder at flow rate 16 L/min, while the lowest was 2.3 (0.26) % for NE-U22 Omron® (high frequency).
Conclusions: The HSTS can be nebulized by all tested nebulisers but the high frequency NE-U22 Omron® and Aeroneb Go® are more efficient. When the HSTS compared to TOBI®, the respirable inhaled dose was increased to more than 73%.

1. Introduction

Pulmonary infections are the primary cause of morbidity and mortality among cystic fibrosis (CF) patients. *Pseudomonas aeruginosa* is the most common bacterial pathogen associated with pulmonary exacerbations in CF [1], so antipseudomonal antibiotics combinations often include aminoglycosides [2,3] as part of the treatment regimen, due to their potent activity and low potential for development of resistance [4]. Antibiotics in CF are used on an intermittent basis to treat pulmonary exacerbations to lengthen the interval between them [5–9]. Systemic antibiotic therapy is limited by the poor penetration of most intravenously administered agents into bronchial secretions, and the impairment of their biological activity by purulent secretions. Moreover, repeated courses of intravenous antibiotics increase risk of bacterial antibiotic resistance and complications associated with prolonged use. The majority of clinical trials of inhaled antibiotics have investigated their efficacy as suppressive therapy in the period between acute exacerbations. Inhaled antibiotics can be used as maintenance treatment for patients chronically infected with *P. aeruginosa* to improve pulmonary function and reduce the frequency of hospital admission. Aminoglycoside antibiotics are among those commonly aerosolized because they are chemically stable, have a long post-

antibiotic effect, have a low background level of resistance, and have an acceptable taste. Tobramycin is one of the aminoglycosides with the lowest systemic toxicity.

Recently, the safety and efficacy of inhaled tobramycin solution 300 mg/5 ml (TOBI)® were approved by FDA for maintenance treatment of *Pseudomonas aeroginosa* lung infections in CF patients [10–12]. The approved tobramycin inhalation regimen therapy is 300 mg twice daily, during three courses of 28 days. Serum tobramycin concentration was 1 mg/L, measured 1 h after inhalation of 300 mg tobramycin with a Pari LC Plus® nebulizer on days 3 and 10, during a treatment period of 28 days [13]. In another study, the mean (SD) of tobramycin serum level was 1.27 mg/L (1.07) after inhalation of single dose 600 mg tobramycin with Wisto Senior® ultrasonic nebulizer [14]. Many assessed studies for tobramycin nebulization have been reported [15–20].

Compared to intravenous therapy, these values are low. Plasma tobramycin levels at least 8 mg/L are necessary to treat exacerbations with intravenous administration. Toxic serum level of tobramycin is > 12 mg/L, and this wide range between serum level of inhaled tobramycin and toxic serum level encourages trials of nebulized high strength dose of tobramycin to achieve the optimal inhaled dose.

An in vitro study by Le Brun et al. [21,22] assessed nebulization of several tobramycin sulfate solutions ranging from 5 to 30% (w/v), delivered by 14 commercially available jet and ultrasonic nebulizers. They reported that a 20% tobramycin sulfate solution was the optimal and maximal concentration to be aerosolized, and the jet nebulizer was most suitable. The delivery of a high amount of tobramycin into the lungs is largely dependent upon nebulizer performance, which is significantly affected by the physicochemical properties of drug solution such as osmolarity, pH, ionic strength, viscosity, density, and surface tension.

In this present study, in vitro aerodynamic characterization of the dose emitted during nebulization of high strength dose of tobramycin by novel and jet nebulizer delivery systems has been studied to determine optimum combinations for delivery of high dose of tobramycin.

2. Materials and methods

2.1. Method of study

Nebulization of tobramycin high strength solution (200 mg/ml) has been evaluated by using different selected high performance nebulizer delivery systems. The most important characteristic for nebulizer performance assessment is the aerosol particle size distribution and output that are produced by nebulizer device. The methodology applied for the determination of the aerosol particle size distribution and aerosol output was adapted from the current European standard [23]. The nebulization time, simplicity of use, cost, and cleaning and sterilization requirements are also important characteristics for nebulizer performance. All measurements were performed at ambient conditions.

2.2. Tobramycin solution

The aerosolized tobramycin formulation was tobramycin sulfate (USP XXIII quality, Dumex-Alpharma A/S, Denmark) 200 mg/ml in 0.18% sodium chloride (w/v). The osmolarity and pH values of tobramycin sulfate 20% are acceptable and tolerated for aerosolized solutions. The osmolarity was 335 mmol/kg measured by 5500 Vapour pressure osmometer (Wescor, Inc., Logan, Utah, USA). Sulfuric acid and sodium hydroxide were used to adjust the pH of the drug solution to 6, measured by Corning M240 pH meter (Ciba Corning Diagnostic, Surbury, Suffolk, UK). All physical measurements were performed at ambient temperature.

2.3. Nebulizer devices

Different nebulizer systems were used: two different designs of jet nebulizers and three new nebulizers based on vibrating mesh technology. The jet nebulizers were the breath-enhanced jet nebulizer Pari LC Plus® attached to a PariBoy*N*® compressor and the constant output jet nebulizer Sidestream® attached to Porta-Neb® compressor and compressed air cylinder at different gas flow rates: 12 and 16 L/min. The new nebulizers were the NE-U22 Omron® static mesh nebulizer (low and high frequencies), and the Aeroneb® Go vibrating mesh nebulizer. The nebulizer fill volumes were 5 ml and 2.5 ml for jet and new nebulizers, respectively.

The Sidestream® attached to Porta-Neb® compressor is fast, accurate drug delivery every time. It is a constant-output open-vent jet nebuliser. The open vent on the top of nebuliser chamber improves aerosol output by allowing the negative pressure to suck extra air into the chamber. This markedly increases the air flow out of the nebuliser and pushes out more small droplets to be inspired. The aerosol is produced or released constantly during inspiration and expiration. It is a reusable nebuliser and can be cleaned in the dishwasher, boiled or autoclaved.

The PARI LC Plus nebulizer is the “gold standard” for aerosolized medication delivery.

It is a breath-enhanced open-vent jet nebuliser that uses two valves to maximise aerosol delivery and minimise medication wastage. The inspiratory valve is opened during inspiration to allow extra air to be sucked through the nebuliser chamber and closed during exhalation to decrease air flow through the chamber. It is designed for children as well as adults and works with any compressor. It is a reusable nebuliser that is recommended for six months use and it can be cleaned in the dishwasher, boiled or autoclaved.

The MicroAir® NE-U22 nebuliser uses an ultrasonic transducer to generate vibration (180 kHz) of the drug solution and push the droplets through the static mesh which can then be inhaled directly by the patient. Unlike jet and ultrasonic nebulisers, the aerosol is not recycled in the mesh nebuliser. The mesh cannot be disinfected by an autoclave process and should be submerged in a 0.1% benzalkonium solution.

The Aeroneb® Go nebuliser has a novel design and generates aerosol using the OnQ™ electronic micropump. It consists of a domed aperture plate with precision-formed holes (1000 holes) and a vibrational element which vibrates at 100 kHz. This creates a micro-pumping action producing a fine particle, low velocity aerosol using no propellants or compressors. It cannot be disinfected by an autoclave process and there are no specific recommendations for disinfecting.

2.4. Aerosol particle size distribution

The Aerosol particle size is an important characteristic of nebulizer performance. Particles too large do not reach the lower respiratory tract, whereas particles too small are exhaled [24–26]. It has been shown that smaller particles are produced at higher nebulizer flow [27]. The Aerosol particle size should be 2–5 μm for air way deposition, and 1–2 μm for parenchymal deposition [28]. The Aerosol particle size produced by the nebulizer was determined by using the eight-stage Marple 298 cascade impactor (Copley Scientific Limited, UK).

The Aerosol particle size is often expressed in terms of mass median aerodynamic diameter (MMAD). The amount of tobramycin extracted from cascade filters was determined by HPLC.

Mass Median Aerodynamic Diameter (MMAD) is defined as the

Table 1
Summary of mean (SD, n = 10) aerosol particle size distribution characteristics during nebulization tobramycin high strength solution of by selected nebulizer delivery systems.

Nebulizer device	MMAD μm	GSD μm	Fine particles <5 μm (mg)	% FPF	VMD μm
Sidestream® Porta-Neb compressor	2.7 ± 0.3	2.5 ± 0.1	2.6 ± 0.2	47.6 ± 3.5	2.6 ± 0.2
Sidestream® (Flow rate 12 L/min)	1.5 ± 0.01	2.2 ± 0.5	8.2 ± 0.6	68.8 ± 2.5	3.3 ± 0.4
Sidestream® (Flow rate 16 L/min)	1.2 ± 0.1	2.5 ± 0.2	10.7 ± 1.6	77.6 ± 2.3	3.1 ± 0.2
Pari LC Plus® PariBoy compressor	1.9 ± 0.1	2.4 ± 0.1	7.4 ± 0.5	59.6 ± 0.8	3.3 ± 0.2
Aeroneb Go®	2.1 ± 0.1	2.1 ± 0.1	9.2 ± 1	59 ± 1.7	2.7 ± 0.2
NE-U22 Omron® (High frequency)	2.7 ± 0.3	2.3 ± 0.1	5.6 ± 0.4	47.3 ± 3.8	2.3 ± 0.3
NE-U22 Omron® (Low frequency)	2.3 ± 0.2	1.7 ± 0.1	2 ± 0.4	57 ± 4.4	2 ± 0.2

diameter at which 50% of the particles by mass are larger and 50% are smaller.

Geometric Standard Deviation (GSD) is a measure of the spread of an aerodynamic particle size distribution.

The size distribution was expressed in terms of the volume median diameter (VMD), which is the value of the particle size that divides the population into two equal halves. The VMD is directly related to the mass median diameter (MMD) by the density of the particles.

According to Clark and Borgstrom [29], the cumulative deposition data were plotted against stage cut-off diameter, and fitted with a logarithmic regression curve to determine the particle size at 50% of the accumulated deposition (Mass median aerodynamic diameter, MMAD), geometric standard division (GSD), and volume median diameter (VMD), and recorded as the mean of ten determinations.

2.5. Aerosol output

The performance of nebulizer efficiency was evaluated using high dose of aerosolized tobramycin (20%) by determination of the aerosol output characteristics that include respirable inhaled mass, aerosol output rate, deposited dose, nebulization time, and residual dose volume using adult breathing simulator modelling (Pari Respiratory Equipment, Inc). These parameters were recorded as the mean of ten determinations. The respirable inhaled mass was collected on a filter placed between the nebulizer and breathing simulator modelling under conditions of simulated normal adult breathing (tidal volume 500 ml, 15 breaths/min, and inspiratory:expiratory ratio 50%) then the extracted amount and the residual medication left in the device were determined by the developed HPLC method. The duration of nebulization (Treatment time) was measured one minute past sputter (jet nebulizers) using stopwatch or to the end of aerosol generation (ultrasonic nebulizers and Aeroneb Go). The aerosol particle size distribution and aerosol output were determined at ambient conditions (temperature 23 ± 2 °C, relative humidity 45% r.h to 75% r.h, and pressure from 86 kPa to 106 kPa).

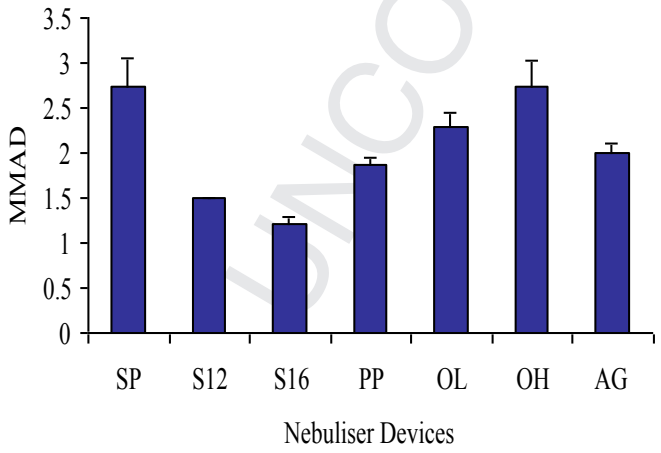

Fig. 1. Mean (SD, n = 10)) MMAD obtained by nebulizer systems during nebulization of high strength dose of tobramycin. Key: SP = Sidestream®/PotaNeb® compressor, S12 and S16 Sidestream®/air cylinder at flow rate 12 and 16 L/min, PP = Pari LC Plus®/PariBoy®, OL and OH = low and high frequency of NE-U22 Omron®, and AG = Aeroneb® Go.

2.6. Tobramycin assay

The tobramycin concentration was determined by using validated HPLC method following pre-column derivatization with fluorescein isothiocyanate (FITC) [30]. The Guideline on Bioanalytical Method Validation. EMEA/CHMP/EWP/192217/2009, 21 July 2011 [31] was used as a guide to validated the analytical method.

3. Results and discussion

Le Brun et al. [32] have reported that 20% tobramycin sulfate in normal saline (osmolarity 483 mmol/kg and pH 7.6) was the optimal and maximal concentration to be aerosolized. In this study, 0.18% NaCl was used with 20% tobramycin sulfate to achieve the osmolarity of drug solution to 335 mmol/kg. This value will be tolerated after inhalation, since an osmolarity of an antibiotic solution in the range of 150–550 mmol/kg is acceptable [33].

Sulfuric acid and sodium hydroxide were added to adjust the pH of drug solution to 6, which is an acceptable value for aerosolized solutions. After jet nebulization, the osmolarity was increased to 400 mmol/kg in Sidestream® and Pari LC Plus® jet nebulizers due to evaporation of the solvent, while the pH value was constant. Although the osmolarity of drug solution rose to 400 mmol/kg

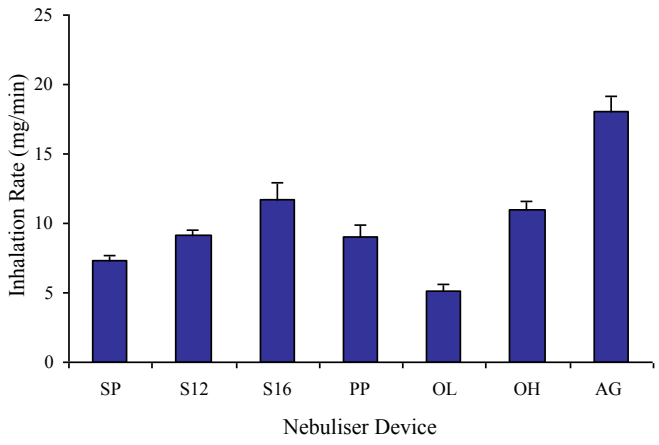

Fig. 2. Mean (SD, n = 10) inhalation rate of tobramycin produced by different nebulizer systems during nebulization of high strength dose of tobramycin.

Table 2
Summary of means (SD, n = 10) aerosol output characteristics during nebulization tobramycin high strength solution of by different nebulizer delivery systems.

Nebulizer device	Fill volume	Nebulization time (min)	Inhalation rate (mg/min)	Respirable inhaled Mass (mg)	Respirable inhaled mass %	Residual of dose volume %
Sidestream® Porta-Neb compressor	5 ml	18 ± 0.3	7.4 ± 0.3	62.9 ± 2.2	9.6 ± 0.3	51.3 ± 3.5
Sidestream® (Flow rate 12 L/min)	5 ml	11.5 ± 0.2	9.16 ± 0.4	72.57 ± 3.3	11 ± 0.5	55.5 ± 3.7
Sidestream® (Flow rate 16 L/min)	5 ml	7.5 ± 0.2	11.68 ± 1.3	68 ± 7.7	10.4 ± 1.2	63.9 ± 3.9
Pari LC Plus® PariBoy compressor	5 ml	17 ± 1.1	8.97 ± 0.9	90.9 ± 8.6	13.6 ± 1.3	46.9 ± 2.5
NE-U22 Omron® (Low frequency)	2.5 ml	28 ± 1.3	5.18 ± 0.5	82.6 ± 7.8	20.6 ± 1.1	7.8 ± 0.5
NE-U22 Omron® (High frequency)	2.5 ml	13 ± 0.5	11 ± 0.6	67.4 ± 3.8	25.2 ± 2.4	2.3 ± 0.3
Aeroneb Go®	2.5 ml	7.3 ± 0.2	18 ± 1.1	78.4 ± 5	23.9 ± 1.5	33.2 ± 2.2

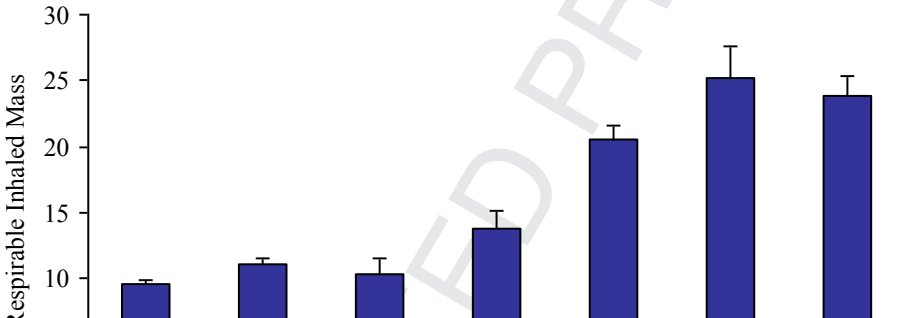

Fig. 3. Mean (SD, n = 10) respirable inhalation mass of tobramycin produced by different nebulizer systems during nebulization of high strength dose of tobramycin.

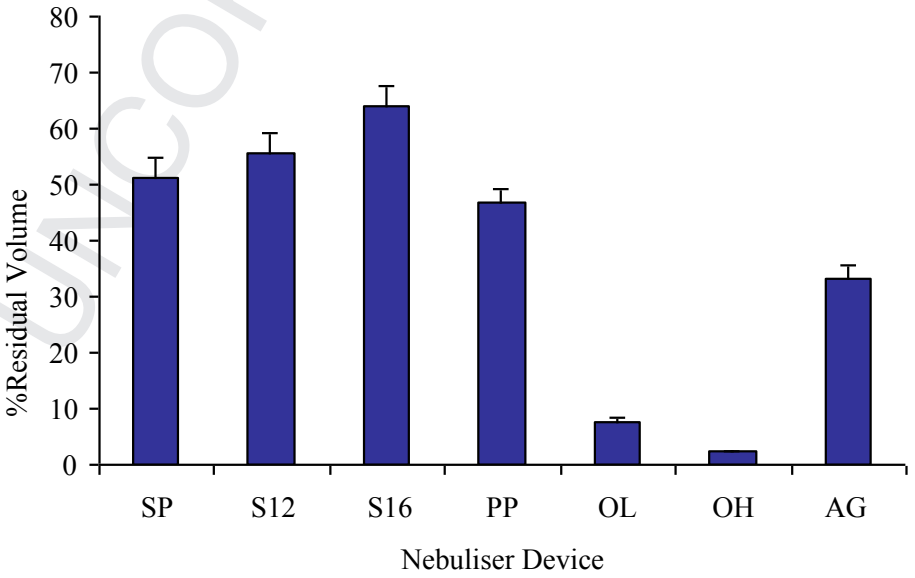

Fig. 4. Mean (SD, n = 10) residual volume percentage of tobramycin after nebulization of high strength dose of tobramycin by different nebulizer systems.

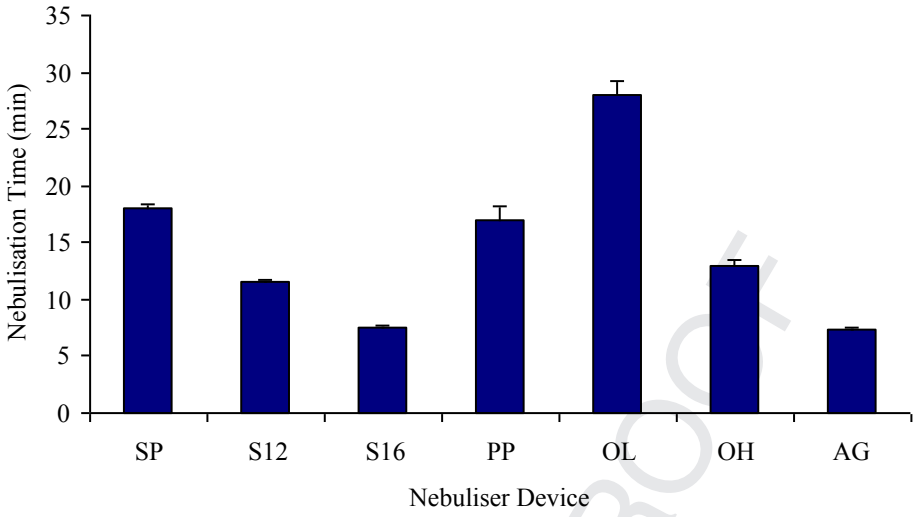

Fig. 5. Mean (SD, n = 10) nebulization time of nebulization of high strength dose of tobramycin by different nebulizer systems.

during nebulization, it was still within the acceptable range. The particle size distribution is dependent on the type of nebulizer or the combination of compressor and nebulizer [34–37] In the experiments, the Sidestream® was combined with PortaNeb® compressor (flow rate 6.8 L/min) and compressed air cylinder at flow rate 12 and 16 L/min. The Pari LC Plus® was combined with PariBoy*N*® compressor (flow rate 7 L/min). Although all tested nebulizers produced droplets within the respirable size, the smallest particle size was observed for Sidestream® at flow rate 16 L/min which can be explained by the higher flow rate. The low frequency of NE-U22 Omron® produced droplet particles smaller than high frequency. The heterodispersity of aerosol produced from vibrating mesh nebulizers was less than those produced from jet nebulizers. All the nebulized aerosols were polydispersed with span values ranging from 2.58 to 3.28 μm for jet nebulizers, and 1.57–2.65 μm for vibrating mesh nebulizers. The mean (SD) of highest percentage of fine particles (<5 μm) was 77.64 (2.3) % for Sidestream®, at flow rate 16 L/min. The particle size distribution characteristic measurements are summarized in Table 1. When comparing the nebulizers, the particle size distribution results showed that all may be suitable for inhalation of a 20% tobramycin sulfate solution, as shown in Fig. 1.

Increasing the driving gas flow through jet nebulizers will increase the drug output and decrease the nebulization time [38]. In tested jet nebulizers, the inhaled amount of tobramycin in the first minute was increased, while the total inhaled amount decreased with increasing flow rate (6.8–16 L/min). The PortaNeb® compressor was the optimum combination with the Sidestream® jet nebulizer. However, the highest respirable inhaled mass was for Pari LC Plus® combined with PariBoy*N*® compressor, with mean (SD) 90.85 (8.6) mg. The NE-U22 Omron® (low and high frequency) and Aeroneb Go® were showed a high percentage of respirable inhaled mass with fill volume less than the jet nebulizers. The drug wastage was increased with jet nebulizers due to evaporation of solvent during jet nebulization, leading to a gradual increase in the concentration of drug solution left behind.

The mean (SD) of highest drug wastage percentage was 63.9 (3.9) % for Sidestream® jet nebulizer combined with compressed air cylinder at flow rate 16 L/min, while the lowest was 2.3 (0.26) % for NE-U22 Omron® (high frequency). The aerosol output characteristics are summarized in Table 2. When the results data of the particle size distribution and output measurements are combined, the high frequency NE-U22 Omron® and Aeroneb Go® are preferred to nebulize 20% tobramycin sulfate. As shown in Figs. 3–5, the NE-U22 Omron® high frequency showed high inhalation rate, high respirable mass, less drug wastage, and reasonable nebulization time, while Aeroneb Go® showed the shortest nebulization time. When compared to TOBI®, the respirable inhaled mass (<5 μm) produced by nebulization of tobramycin sulfate 20% was increased to more than 40% (see Fig. 2).

4. Conclusion

In conclusion, the high strength tobramycin solution (20% tobramycin sulfate) can be nebulized by all tested nebulisers but the high frequency NE-U22 Omron® and Aeroneb Go® are more efficient. When the high strength tobramycin solution compared to TOBI®, the respirable inhaled dose was increased to more than 73%.

The particle size distribution characteristic measurements showed that all tested nebulizers may be suitable for inhalation of a 20% tobramycin sulfate solution while the aerosol output measurements showed that the NE-U22 Omron® high frequency and Aeroneb Go® are the best.

The NE-U22 Omron® high frequency showed high inhalation rate, high respirable mass, less drug wastage, and reasonable nebulization time, while Aeroneb Go® showed the shortest nebulization time.

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