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OPTIMIZATION OF LOCAL TOBRAMYCIN DELIVERY INTO LUNGS BY CHANGING THE PHYSICOCHEMICAL PROPERTIES OF TOBRAMYCIN INHALED SOLUTION FOR IMPROVING NEBULIZER PERFORMANCE USING SELECTED HIGH-PERFORMANCE NEBULIZER DELIVERY SYSTEMS

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Abstract:

Chronic lung infection with Pseudomonas aeruginosa is a major cause of increased morbidity and mortality in cystic fibrosis (CF) patients. Lung function and overall quality of life have improved as a result of the tobramycin inhalation solution (TIS). For determining the optimum combinations to deliver a large amount of tobramycin at the site of infection in the lung, three sets of TIS formulations havebeen designed in this study by changing the physicochemical properties of the drug solution for improving nebulizer performance. The CEN methodology for the determination of particle size distribution and aerosol output characteristics has been used for in-vitro assessment of tobramycin-tested formulations. All measurements were performed at ambient conditions. Different selected high-performance nebulizer delivery systems were used: two different designs of jet nebulizers and two new nebulizers based on vibrating mesh technology. The two jet nebulizers were Pari LC Plus® and Sidestream® powered by PariBoyN® and Medix AC 2000 Hi Flow compressors, respectively, with a fill volume 5ml. The vibrating-mesh nebulizers

were high-frequency NE-U22 Omron® ultrasonic vibrating mesh and Aeroneb® Go electronic micropump nebulizers, with 2.5ml fill volume.

In conclusion, the particle size distribution and aerosol output data in this study showed that significantly affected by the physicochemical properties of the drugsolution such as osmolarity, pH, ionic strength, viscosity, density, and surface tension in conjunction with the nebulizer design on droplet size as well as aerosol output of TIS to maximize tobramycin delivery into the lungs with selected jet and ultrasonic vibrating mesh nebulizers. The probability of increasing tobramycin absorption in- vivo still required more investigations.

Keywords: *Cystic fibrosis; Tobramycin; TOBI®; nebulizer; jet nebulizers; ultrasonic vibrating mesh nebulizer; electronic micropump nebulizer; CEN method; surfacetension; ion concentration; viscosity.*

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1. INTRODUCTION:

Tobramycin inhalation therapy is used to provide large doses of the medication right to the site of infection in CF patients with persistent lung infection [1,2]. The U.S. Food and Drug Administration (FDA) subsequently approved inhaled tobramycin 300mg/5ml (TOBI®) in 1997. The efficacy and safety of inhaled tobramycin solution (300 mg twice daily for three cycles of 28 days is the recommended tobramycin inhalation regimen therapy) [3,4,5,6]. Therefore, in patients with cystic fibrosis (CF) who have moderate-to-severe lung disease and persistently positive airway cultures for P. aeruginosa, the US treatment guidelines strongly advise chronic use of inhaled tobramycin [7,8]. According to the recommendations of the European Consensus Guidelines, the treatment options for chronic P. aeruginosa infection in CF patients include either continuous inhalation of inhaled colistin or an intermittent (1-month on, 1-month off) regimen of inhaled aminoglycosides [9]. The US treatment guideline recommendations for the use of TIS in alternate months in CF patients aged 6 years were reinforced by the European Cystic Fibrosis Society Standards of Care best practice guidelines [10]. During a 28-day treatment period, the serum tobramycin concentration was 1 mg/L and was determined 1 hour after 300 mg of tobramycin was inhaled using a Pari LC Plus® nebulizer on days 3 and 10 [11,12]. A single dose of 600 mg of tobramycin was inhaled using a Wisto Senior® ultrasonic nebulizer in another trial, and the mean (SD) tobramycin serum level was 1.27 mg/L (1.07) [13]. These values fall short of intravenous (IV) therapy. Plasma tobramycin levels of at least 8mg/L are necessary to treat exacerbations with IV administration. Trials with nebulized high-strength doses of tobramycin are encouraged in order to find the ideal inhaled dose due to the large difference between the serum level of inhaled tobramycin and the toxic serum level of tobramycin, which is >12 mg/L. In an in- vitro study, Le Brun et al. (1999a, 1999b) assessed how well 14 commercially available jet and ultrasonic nebulizers nebulized different tobramycin sulphate solutions ranging from 5 to 30% (w/v). According to their findings, the jet nebulizer was best for producing an aerosol of tobramycin sulphate at a 20% concentration [14,15]. Trejo (2004) has compared the nebulization of several tobramycin solutions ranging from 100 to 300mg/ml to commercial tobramycin formulations 32 and 60 mg/ml delivered by MicroNeb® III jet nebulizer powered by Medix AC® 2000 compressor. She reported that the optimal inhaled output was with tobramycin solution 250mg/ml within a nebulization time of 11.2 min [16]. In children with CF, the nebulization of 75 mg TIS with the I-neb led to a systemic exposure similar to thatof 300 mg TIS with the PARI-LC Plus, with no obvious clinical effects after one month of inhalation [17]. The significant treatment burden in this patient population is made worse by prolonged administration and cleaning durations, high administration frequency, and difficult nebulizer delivery methods. Therefore recently, many study trials were done to improve delivery efficiency to the airways and substantially reduce the delivery time, tobramycin inhalation powder (TIP) has also been approved as a dry powder in Europe and the US under the brand name TOBI® Podhaler™ (Novartis Pharma AG, Basel, Switzerland) for the management of CF patients with *P. aeruginosa* infection [18]. A medical investigation by Hamed, K. Using data from US insurance claims, et al. (2017) compared adherence in 145 newly treated CF patients receiving TIP with those receiving the conventional tobramycin inhalation solution (TIS). They claimed that when compared to TIS, TIP is related with improved adherence. This might be a result of the same effectiveness but quicker administration time, simplicity of use, and general patient satisfaction with this delivery strategy. To completely explain the variations in adherence between TIP and TIS, they came to the conclusion that more research would be necessary [19]. Although the tobramycin inhalation powder (TIP) has also been approved in Europe and the US for the management of CF patients with *P.*

aeruginosa infection [20], post-inhalation cough is reported as a common side effect associated with both wet- and dry-powder inhalation in patients with CF in various clinical studies [21,22,23]. Furthermore, aerosolized TIS is recommended by the American Thoracic Society (ATS) in their CF Pulmonary Guidelines and is considered to be the standard of care as part of the chronic management of pulmonary disease associated with CF [24]. A recent study showed the vibrating-mesh nebulizer utilized without cleaning was consistent and dependable after repeated usage over 28 days [25], overcoming the drawback of nebulizer cleaning. Therefore, our studies will concentrate on aerosolized TIS. The respirable inhaled dose was increased to more than 73% in the previous study by Mashat et al. (2016), which contrasted the nebulization of high-strength tobramycin solution (20% tobramycin sulphate) to TOBI® [26]. On the other hand, nebulizer performance, which is greatly influenced by the physicochemical characteristics of the drug solution such as osmolarity, pH, ionic strength, viscosity, density, and surface tension, is crucial for the delivery of a high concentration of tobramycin into the lungs [27].So, the performance of selected nebulizer delivery systems by changing the physicochemical properties of TIS has been investigated and assessed in this study. Three sets of designed TIS formulations prepared by using some additives that lower surface tension and/or increase the viscosity of drug solution were developed to determine the optimum combinations for delivery of a high dose of tobramycin.

2 MATERIALS AND METHODS:

2.1 Method of Study

This study aims to establish the optimum combinations to deliver a large amount of tobramycin at the site of infection in the lung. Sets of designed TIS have been developed and evaluated by using different mechanisms of selected high-performance nebulizer delivery systems. The CEN methodology for the determination of particle size distribution and aerosol output characteristics has been used for in-vitro assessment of tobramycin-tested formulations cording to the CEN (Committee European de Normalization) method [28]. All measurements were performed at ambient conditions.

2.2 Tobramycin solution

2.2.1 Sets of Designed Tobramycin Formulations

The physicochemical properties of the TIS formulations in conjunction with the nebulizer design and mechanism of function greatly determine the aerosolization performance. The surface tension, ion concentration, and viscosity can highly influence aerosol generation and a greater understanding of their role in nebulization performance is a large part of the puzzle toward improved nebulization therapies [29]. In this study, three sets of tobramycin formulations have been developed by changing the physicochemical properties of TIS for improving nebulizer performance for large- amount delivery and/or improving pulmonary distribution and/ or absorption of tobramycin. The concentration of tobramycin was within the approved concentration (60mg/ml). The developed formulations of TIS were:

- Formulation I: Tobramycin-free base in Tween 80 surfactant.
- Formulation II: Tobramycin sulphate in Tween 80 surfactant.
- Formulation III: Tobramycin-free base in buffer with Tween 80 surfactant.

2.2.1.1 Formulation I

Physiologically, pulmonary surfactants are essential for life as it lines the alveoli to lower surface tension, which keeps the alveoli from collapsing after exhalation and makes breathing easy [30]. To mimic the action of these substances, the safe and common artificial surfactant has been added in TIS. The Tween 80 (polysorbate 80, polyoxyethylene sorbitan monooleate) is a non-ionic surfactant that is widely used as an emulsifier in pharmaceuticals and food products. It is approved by the US Food and Drug Administration for use in of 1% of selected foods [31].

Theoretically, the aerosol particle size should be proportional to the surface tension of the drug solution [32,33]. Thus, this study aimed to understand the influence of surfactant contributions on particle size distributions emitted from nebulizer systems by lowering the surface tension of drug solution.

In this study, Formulation-I was composed of tobramycin free base 60 mg/ml dissolved in 0.9% NaCl (w/v) prepared in Tween 80 surfactant solution 0.001% (w/v). Sulphuric acid and sodium hydroxide were added to adjust the pH of the drug solution to 6. The osmolarity of the tobramycin solution (Formulation I) was 361mmol/kg.

2.2.1.2 Formulation II

This developed formulation was designed as:

- Lower the viscosity of the drug solution was

achieved by decreasing of sodium chloride concentration from 0.9% to 0.18% (w/v).

- Tobramycin was used as sulphate salts to understand the influence of sulphate ions in lowering the surface tension of drug solution [34], and to achieve extra reduction of the surface tension with Tween 80 non-ionic surfactant.

Formulation-II was composed of tobramycin sulphate 91.44 mg/ml (equivalent to 60mg/ml tobramycin base) dissolved in 0.18% NaCl (w/v) prepared in Tween 80 surfactant solution 0.001% (w/v). Sulphuric acid and sodium hydroxide were added to adjust the pH of drug solution to 6. The osmolarity of tobramycin solution was 338mmol/kg.

2.2.1.3 Formulation III

Tobramycin free base in concentration of 60mg/ml was dissolved in 20mM phosphate buffer which prepared in Tween 80 surfactant solution 0.001% (w/v). Phosphoric acid and sodium hydroxide were added to adjust pH of solution to 6. The aim of using phosphate buffer was to increase the viscosity and lower surface tension of drug solution by increasing phosphate ions, and to find a general relationship between salt types and the changes in aerosol size and output characteristics. The osmolarity of drug solution was 565mOsmol/kg. Two different types of buffers (phosphate and tricine) in variable concentrations were examined to find a general relationship between the formulation component and the changes in the droplet size, and to choose a suitable buffer for tobramycin nebulizing solution. In literature data [34]. The effects of buffer type and concentrations on tobramycin particle size distribution were examined. Tricine buffer showed higher viscosity than phosphate buffer salts due to its amino acid nature. According to particle size distribution data, the phosphate buffer (20mM) was a suitable buffer for increasing viscosity of TIS.

2.3 Nebulizer devices

Different selected high performance nebulizer delivery systems were used: two different designs of jet nebulizers and two new nebulizers based on vibrating mesh technology. The two jet nebulizers were Pari LC Plus® and Sidestream® powered by PariBoy*N*® and Medix AC 2000 Hi Flow compressors, respectively, with fill volume 5ml. The new nebulizers were high frequency NE-U22 Omron® ultrasonic vibrating mesh and Aeroneb® Go electronic micropump nebulizers, with 2.5ml fill volume.

2.4 Aerosol particle size distribution

The performance of a nebulizer is significantly influenced by the aerosol particle size. While too-small particles are expelled, too-large particles prevent the lower respiratory tract from being reached [35,36,37]. It has been demonstrated that increased nebulizer flow results in the production of smaller particles [38]. For parenchymal deposition, the aerosol particle size should be 1-2 m and 2–5 m, respectively [39]. The eight-stage Marple 298 cascade impactor was used to measure the size of the aerosol particles the nebulizer produced (Copley Scientific Limited, UK). The mass median aerodynamic diameter is a common unit of measurement for the aerosol particle size (MMAD). This is the particle diameter about which 50% of the mass of the aerosol particles is distributed. The HPLC method was used to determine the amount of tobramycin extracted from cascade filters. 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), fine particle fraction (percentage of the aerosol between 1-5µm that deposits in the lung, FPF) and volume median diameter (VMD), and recorded as the mean of ten determinations, this according to Clark and Borgstrom [40]. Generally, to achieve a therapeutic effect, particles of nebulized drugs should have an MMAD of less than 5µm. Larger particles (5-100µm) are principally deposited in thenasopharynx, whilst particles of less than 5µm will be predominately deposited in the lungs, including alveolar deposition. Particles that are less than 0.5 µm will reach the alveolar region with around l5% of the drug delivered being deposited. This is because the technique of deposition in the lung is due to gravitational factors, and the small size of the particles means that the time necessary for the medication to deposit in any substantial quantity is actually longer than the breathing cycle, meaning thatthe bulk of the drug will be exhaled again [41].

Formulations I and II were based on lowering the surface tension of drug solution to improve the performance of jet and vibrating mesh nebulizers, while the formulation III was based on increasing the viscosity of drug solution, which improves the performance of jet nebulizers. The osmolarity of solution was measured by using a 5500-vapour pressure osmometer. The aerosol particle size distribution characteristics were measured by Marple

298 cascade impactor, while the aerosol output was measured by Pari breathing simulator under conditions of simulated normal adult breathing (tidal volume 500ml, 15 breaths/min, and inspiratory: expiratory ratio 50%),at ambient conditions.

2.5 Aerosol output

By using adult breathing simulator modelling (Pari Respiratory Equipment, Inc), The nebulizer efficiency was evaluated using developed TIS by determination of the aerosol output characteristics that include respirable inhaled mass (RIM), aerosol output rate, deposited dose, time of nebulization, and residual dose volume These parameters were reported as the mean of ten determinations. The respirable inhaled mass was collected on a filter placed between the nebulizer and breathing simulator modeling under conditions of simulated normal adult breathing (tidal volume 500ml, 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. Treatment time was measured one minute past sputter (jet nebulizers) or to the end of aerosol generation (ultrasonic and Aeroneb® Go nebulizers). The aerosol particle size distribution and aerosol emission were measured at ambient temperatures (23°C, 45% to 75% relative humidity, and pressures between 86 and 106 kPa).

2.6 Tobramycin Assay

By using the validated HPLC method following pre-column derivatization with fluorescein isothiocyanate (FITC), tobramycin concentration has been determined[42].

2.7 Data Analysis

The aerosol particle size distribution measurements, which include MMAD, VMD GSD, and FPF were calculated as the mean of three determinations. The aerosol output measurements were determined as the mean of five determinations. The statistical analysis was carried out using SPSS V15.0 (SPSS Inc., Chicago, USA) software program. The mean ratio (95% confidence interval) for all tested nebulizers was calculated. All developed formulations were compared to TOBI® by using a one- way ANOVA test. A mean difference (99.9% confidence interval) was calculated also, and the probability value of $p<0.001$ was considered to be significant.

3. RESULTS AND DISCUSSION:

The results of aerosol particle size distribution and aerosol output measurements during nebulization of TOBI® and developed TIS formulations by selected jet and vibrating mesh nebulizer delivery systems summarized in Tables 1,2,3. These data can be used for assessment the performance of nebulizer devices during nebulization of TOBI® and developed TIS formulations. In Formulation-I (lowering the surface tension of TIS), it can be concluded that the smallest particle sizes MMAD (2.26±0.06µm) and the largest FPF (53.96±1.1%) produced by the NE-U22 Omron® nebulizer. The aerosol output measurements were satisfactory results that respirable inhaled mass (RIM) and respirable inhaled mass percentage (RIM%) were increased that 41±2.2 mg and 27.7±1.4%, respectively as shown in Figures 3,5.

When reducing the viscosity of TIS in Formulation-II, the smallest particle sizes MMAD (1.38±0.1µm), the largest FPF (68.5±2.9%) and the largest RIM (63.9±2.2mg)produced by the Sidestream® powered by Medix AC 2000 Hi Flow compressor while, the largest RIM% (32.8±1%) generated by the NE-U22 Omron® nebulizer during nebulization of Formulation-II as shown in Figures1,2.

On the other hand, increasing the viscosity of TIS as designed in Formulation-III, the aerosols droplet size was decreased in both Sidestream® and Pari LC Plus® jet nebulizers and the NE-U22 Omron® nebulizer. The MMAD was 1.48±0.04, 1.39±0.01, and 1.87±0.05µm, respectively and largest FPF (86.5 ± 0.8%, 70.8± 0.28%, and 63.4±0.7%, respectively). Moreover, the aerosol output measurements were favourable outcome that RIM was 65.1± 1.9, 72.7 ± 1.3, and 48.63± 1.2 mg, respectively while, respirable inhaled RIM% was 21.7± 0.63%, 24.2± 0.45%, and 32.54± 0.8%, respectively.

The mean difference (99.9% CI) of in-vitro measurement data obtained by selected high-performance jet nebulizer systems during nebulization of designed TIS formulations compared to TOBI®, using one-way ANOVA test (Tukey HSD) illustrate in Tables 4,5.

The nebulizer's performance is significantly affected by the physicochemical properties of the drug solution, such as viscosity, density and surface tension. From the data available in the literature [43,44,45,46,47], it appears that there is an inverse relationship between the droplet size and surface tension of nebulizing solution in both jet and vibrating mesh nebulizers. Altering the surface tension of nebulizer fluids by surfactant addition should influence the size and output characteristics of theaerosol produced. McCallion et

al. (1996) have studied the effect of surfactant addition on the droplet size, and output characteristics emitted from both jet and ultrasonic nebulizers. They reported that the surface tension is inversely related to the droplet size and a higher release of aerosol with decreasing of surface tension.

In this study, Tween 80 non-ionic surfactant was added to tobramycin (60mg/ml) and the surface tension of the drug solution was measured in variable Tween 80 concentrations ranging from 0.0001 to 0.1% (w/v) to attain the critical micelles concentration (CMC). Four Tween 80 concentrations 0.0001, 0.001, 0.01, and 0.1% (w/v) were prepared for TIS (tobramycin 60mg/ml) and the surface tension values were measured as 55, 46, 45.5, and 45mN/m, respectively. These results indicated that the CMC was attained at a concentration of 0.001% (w/v) Tween 80. Water was used as a standard solution, while TOBI® inhaled solution was used as the reference solution. The surface tension values of water and TOBI® solution were 72.7 and 62mN/m, respectively. The advantage of choosing Tween 80 was that its CMC was attained at relatively low concentrations, which avoids alteration of tobramycin antibacterial activity. In Formulation I, decreasing in droplet MMAD and an increase in FPF by lowering the surface tension of TIS by the addition of 0.001% Tween 80 surfactant was observed with the NE-U22 Omron® nebulizer which produced the smallest particle sizes (MMAD 2.26±0.06µm) and largest FPF (53.96±1.1%) as shown in Figures 1,2. Furthermore, the aerosol output data showed that RIM and RIM% have increased 41±2.2 mg and 27.7±1.4%, respectively as shown in Figures 3,4. In Formulation II, the viscosity of the drug solution was reduced by decreasing sodium chloride concentration from 0.9% to 0.18% (w/v), and tobramycin was used as sulphate salts to keep the osmolarity the of drug solution within the ideal range, of 150-550 mmol/ kg, and tan o make extra reduction of surface tension of drug solution with 0.001% Tween 80 surfactant. The surface tension was 45.2mN/m and the osmolarity was 338mmol/kg. When comparing Formulation-II to TOBI® formulation, the MMAD value was decreased in the Sidestream® jet nebulizer and highly significantly decreased ($p<0.001$) in the NE-U22 Omron® nebulizer during nebulization of Formulation-II as shown in Figure1**.** Furthermore, the FPF, RIM, and RIM% were increased in the Sidestream® and highly significantly increased ($p<0.001$) in the NE-U22 Omron® nebulizer, as shown in Figures 2,3,4.

In Formulation III, Increasing the viscosity of TIS by the addition of phosphate buffer (20mM) has been studied. Two different types of buffers in variable concentrations were added to the tobramycin solution to study their effect on the aerosol droplet size. According to particle size distribution data, the phosphate buffer (20mM) was a suitable buffer for increasing the viscosity of TIS. aerosol droplet size was decreased by increasing the viscosity of TIS when phosphate buffer was added to TIS during the nebulization of Formulation-III in both Sidestream® and Pari LC Plus® jet nebulizers. A decrease in aerosols droplet MMAD and increases in FPF by lowering the surface tension of TIS was observed with Sidestream® and Pari LC Plus® jet nebulizers and the NE-U22 Omron® nebulizer which produced the smallest particle sizes (MMAD 1.48±0.04, 1.39±0.01, and 1.87±0.05µm, respectively) and largest FPF (86.5 ± 0.8%, 70.8± 0.28%, and 63.4±0.7%, respectively). The MMAD value was significantly decreased ($p<0.001$) in the Pari LC Plus® jet nebulizer while FPF was significantly increased ($p<0.001$) in Sidestream®.

The viscosity of the drug solution is particularly important: aerosol droplet size is inversely proportional to the viscosity of nebulizing solution in jet nebulizers. However, although high-viscosity fluids produce small droplets, they require a longer time to dry. On the other hand, the aerosol droplet size is proportional to the viscosity of nebulizing solution in ultrasonic nebulizers [48,49]. In this study, although the Sidestream® and Pari LC Plus® jet nebulizers produced the smallest particle sizes MMAD (1.48±0.04, 1.39±0.01µm, respectively) and the largest FPF (86.5 ± 0.8, 70.8±0.28%, respectively) during nebulization Formulation III (when the TIS viscosity increased) and they were significantly improved when compared with TOBI® as shown in Tables 5, the NE-U22 Omron® ultrasonic vibrating mesh was the optimum performance of nebulizer systems.

However, all developed TIS formulations showed maximum tobramycin delivered into the lungs, the Formulation III was the best outcome. Furthermore, the NE-U22 Omron® ultrasonic vibrating mesh nebulizer produced the optimum performance with all developed TIS formulations.

3.1 Limitation:

While Formulation III was the best outcome, the nebulization time was increased with the NE-U22 Omron® and significantly increased with the Pari LC Plus® jet nebulizer as shown in Figure 5.

4. CONCLUSIONS:

In conclusion, the aerosol particle size distribution and aerosol output data of all developed TIS formulations showed satisfactory outcomes to achieve optimum tobramycin delivery into lungs by changing the physicochemical properties of drug nebulizing solution using selected jet and vibrating mesh nebulizers when compared to the TOBI® formulation as shown in Tables 4,5 but the probability of increasing tobramycin absorption in-vivo still required to more investigations.

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Table 1 Summary of means (SD, n=3) aerosol particle size distribution measurements during nebulization of developed TIS formulations by jetnebulizer delivery systems.

Formulations	Sidestream®				Pari LC Plus®			
	MMAD µm	GSD µm	FPD	% FPF <5µm	MMAD µm	GSD µm	FPD	% FPF <5µm
TOBI®	1.96 ±0.03	2.7±0.07	1.47±0.16	64.6± 1.04	1.99±0.17	2.67±0.14	2.2±0.22	57.3 ±2.58
Formulation-I	2.1 ±0.01	1.8±0.01	6.39±0.37	60.7± 0.32	2.29±0.07	2.58± 0.03	3. 8±0.21	52.71 ±0.8
Formulation-II	1.38 ±0.1	2.8 ± 0.1	3.02±0.34	68.5± 2.9	2.24±0.1	2.7 ± 0.13	3.66±0.3	53.2 ± 1
Formulation-III	1.48±0.04	2.5±0.28	2.39±0.08	86.5 ± 0.8	1.39±0.01	2.5 ± 0.04	2.74±0.11	70.8± 0.28

Table 2 Summary of means (SD, n=3) aerosol particle size distribution measurements during nebulization of developed TIS formulations byselected vibrating mesh nebulizer delivery systems.

Formulations	Aeroneb® Go				NE-U22 Omron® (high frequency)			
	MMAD µm	GSD µm	FPD	% FPF <5µm	MMAD µm	GSD µm	FPD	% FPF <5µm
TOBI®	2.02±0.08	2.11±0.07	2.81±0.25	59.9± 1.4	2.63± 0.2	2.21±0.04	3.47±0.33	48.81±2.7
Formulation-I	2.83±0.1	2.28±0.01	2.39±0.08	46.2 ± 1.3	2.26±0.06	2.26±0.07	2.19±0.01	53.96±1.1
Formulation-II	2.32±0.1	2.55±0.21	4.22±0.08	52.8±1.75	1.6±0.01	2.29±0.26	4.47±0.14	64.6± 0.6
Formulation-III	2.46±0.02	2.41±0.02	2.39±0.08	51 ± 0.3	1.87±0.05	2.63±0.08	2.39±0.08	63.4±0.7

Table 3 Summary of means (SD n=5) aerosol output measurements during nebulization of developed TIS formulations by selected nebulizer deliverysystems.

Formulations	Sidestream®			Pari LC Plus®			Aeroneb® Go			NE-U22 Omron® (high frequency)		
	Neb. Time (min)	Respirable Inhaled Mass (mg)	% Res. Inhaled Mass	Neb. Time (min)	Respirable Inhaled Mass (mg)	% Res. Inhaled Mass	Neb. Time (min)	Respirable Inhaled Mass (mg)	% Res. Inhaled Mass	Neb. Time (min)	Respirable Inhaled Mass (mg)	% Res. Inhaled Mass
TOBI®	13.3 ± 0.5	56.8 ± 1.2	18.9 ± 0.4	13 ± 0.33	49.5± 2.58	16.5 ± 0.8	7 ± 0.09	42.1± 1.9	28.1± 1.3	8.2 ± 0.17	38.85 ± 1	25.9± 0.67
Formulation-I	13.4± 0.07	41.2± 3.29	13.7± 1.1	12.3± 0.12	41.2± 3.3	18.1± 0.6	5.4 ± 0.21	43 ± 1.8	28.67± 1.2	11± 0.14	41.6 ± 2.2	27.7 ± 1.4
Formulation-II	14 ± 0.42	63.9 ± 2.2	21.3± 0.74	11 ± 0.28	48.3 ± 1.4	16.1± 0.47	3 ± 0.17	24 ± 1.7	16.1± 1.18	12 ± 0.2	49.2± 1.5	32.8 ± 1
Formulation-III	13.6± 0.3	65.1± 1.9	21.7± 0.63	18 ± 0.37	72.7 ± 1.3	24.2± 0.45	3 ± 0.31	24 ± 0.5	16 ± 0.35	11.8± 0.35	48.63± 1.2	32.54± 0.8

Neb. Time= Nebulisation time.
% Res. Inhaled Mass= % Respirable inhaled mass.

Table 4 Mean difference (99.9% CI) of in vitro measurement data obtained by selected high performance jet nebulizer systems during nebulization ofdeveloped TIS formulations compared to TOBI® formulation, using one-way ANOVA test (Tukey HSD).

Tobramycin Formulation	Sidestream®									Pari LC Plus®								
	MMAD			% FPF			% Respirable Inhaled Mass			MMAD			% FPF			% Respirable Inhaled Mass		
	MD	99.9% CI		MD	99.9% CI		MD	99.9% CI		MD	99.9% CI		MD	99.9% CI		MD	99.9% CI	
		Low	UP		Low	UP		Low	UP		Low	UP		Low	UP		Low	UP
Formulation-I	-0.59 *	-0.78	-0.41	3.88	-1.63	9.40	5.19 *	2.99	7.40	-0.29	-0.9	0.27	4.61	-6.1	15.32	-1.57	-3.40	0.24
Formulation-II	0.15	-0.04	0.33	-3.88	-9.40	1.63	-2.37 *	-4.57	-0.18	-0.24	-0.8	0.32	4.08	-6.6	14.80	0.39	-1.43	2.21
Formulation-III	0.047	-0.14	0.23	-21.88 *	-27.4	-16.4	-2.76 *	-4.96	-0.56	0.61*	0.05	1.18	-13.5 *	-24.2	-2.78	-7.74*	-9.56	-5.92

99.9% CI=Confidence Interval.
Low=Lower Bound,
Up=Upper Bound.
MD=Mean Difference.
* Mean difference significant at 0.001 level ($p<0.001$).

Table 5 Mean difference (99.9% CI) of in vitro measurement data obtained by vibrating mesh nebulizers during nebulization of developed TIS formulations compared to TOBI® formulation using one-way ANOVA test (Tukey HSD).

Tobramycin Formulation	Aeroneb® Go									NE-U22 Omron® (high frequency)								
	MMAD			%FPF			%Respirable Inhaled Mass			MMAD			%FPF			%Respirable Inhaled Mass		
	MD	99.9% CI		MD	99.9% CI		MD	99.9% CI		MD	99.9% CI		MD	99.9% CI		MD	99.9% CI	
		Low	UP		Low	UP		Low	UP		Low	UP		Low	UP		Low	UP
Formulation-I	-0.81*	-1.36	-0.27	13.73 *	6.07	21.40	-0.59	-4.26	3.07	0.37	-0.39	1.14	-5.15	-15.6	5.26	-1.81	-4.19	0.57
Formulation-II	-0.29	-0.84	0.25	7.08	-0.58	14.74	12.0 *	8.33	15.6	1.03 *	0.37	1.70	-15.81*	-24.8	-6.79	-6.93*	-9.31	-4.55
Formulation-III	-0.44	-0.98	0.11	8.98 *	1.32	16.65	12.06*	8.39	15.7	-0.35	-0.76	0.21	9.56 *	1.87	15.25	11.46*	7.17	16.1

99.9%CI=Confidence Intervals.
Low=Lower Bound,
Up=Upper Bound.
MD=Mean Difference.
* The mean difference is significant at the 0.001 level ($p<0.001$).

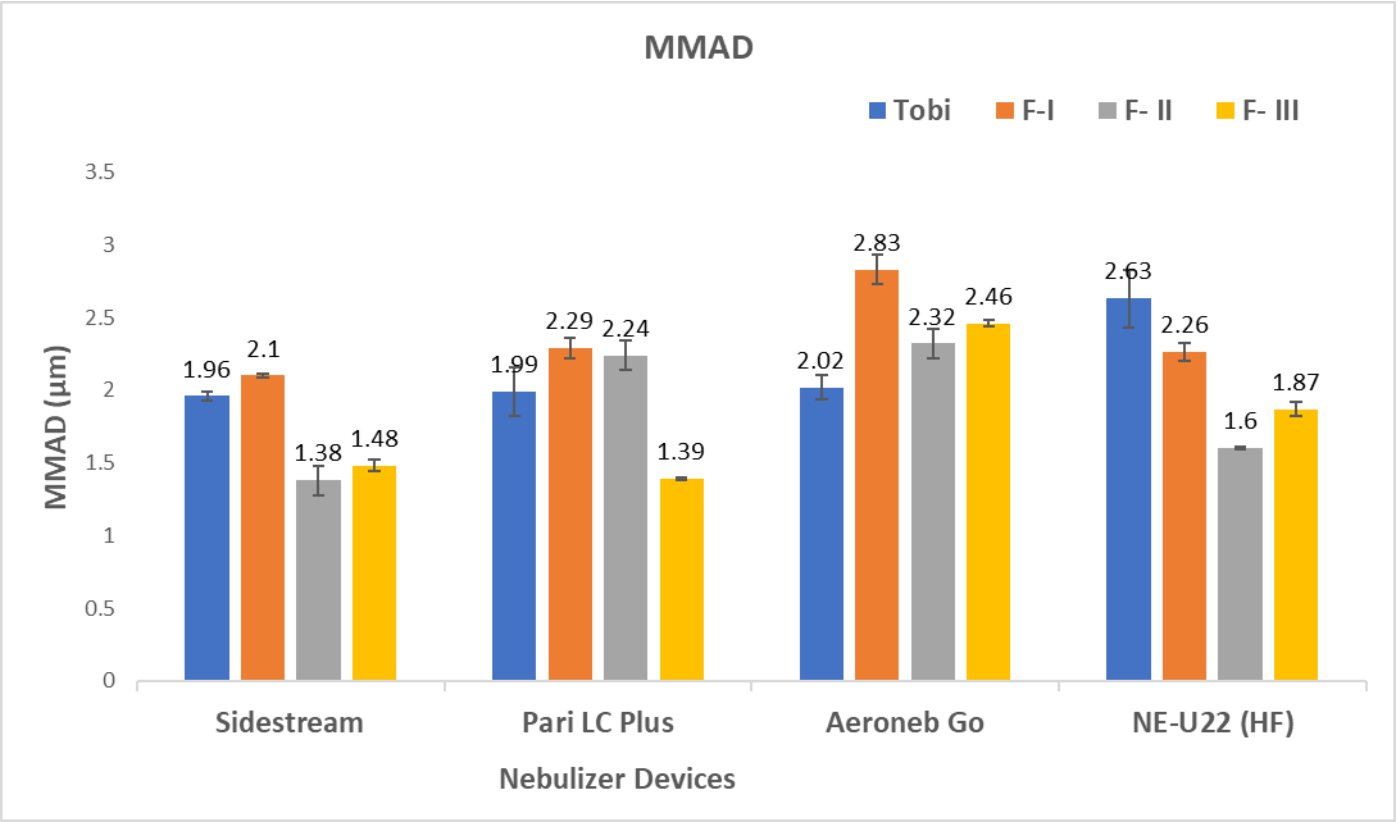

Figure 1 The mean (SD) MMAD of the emitted dose for the TOBI®, Formulation I, II, and III using different nebulizer delivery systems.

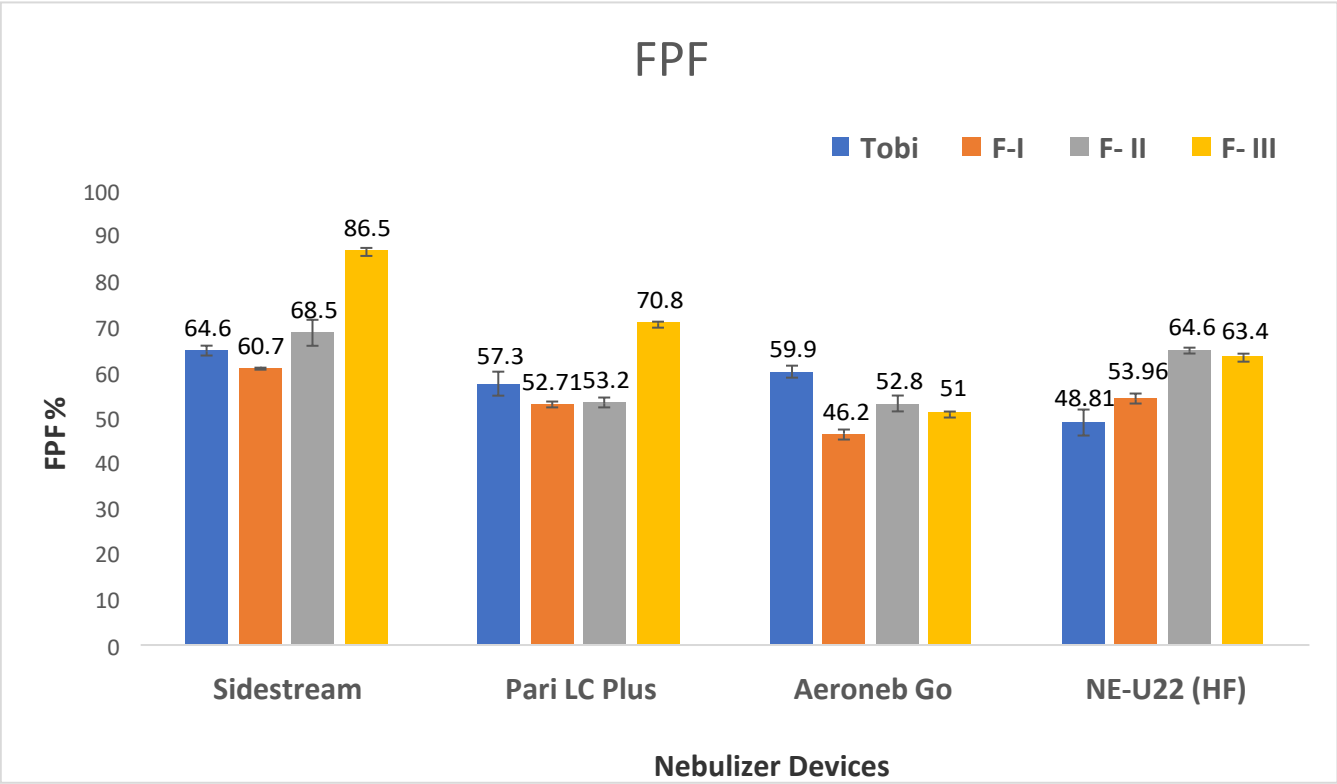

Figure 2 The mean (SD) FPF of the emitted dose for the TOBI®, Formulation I, II, and III using different nebulizer delivery systems.

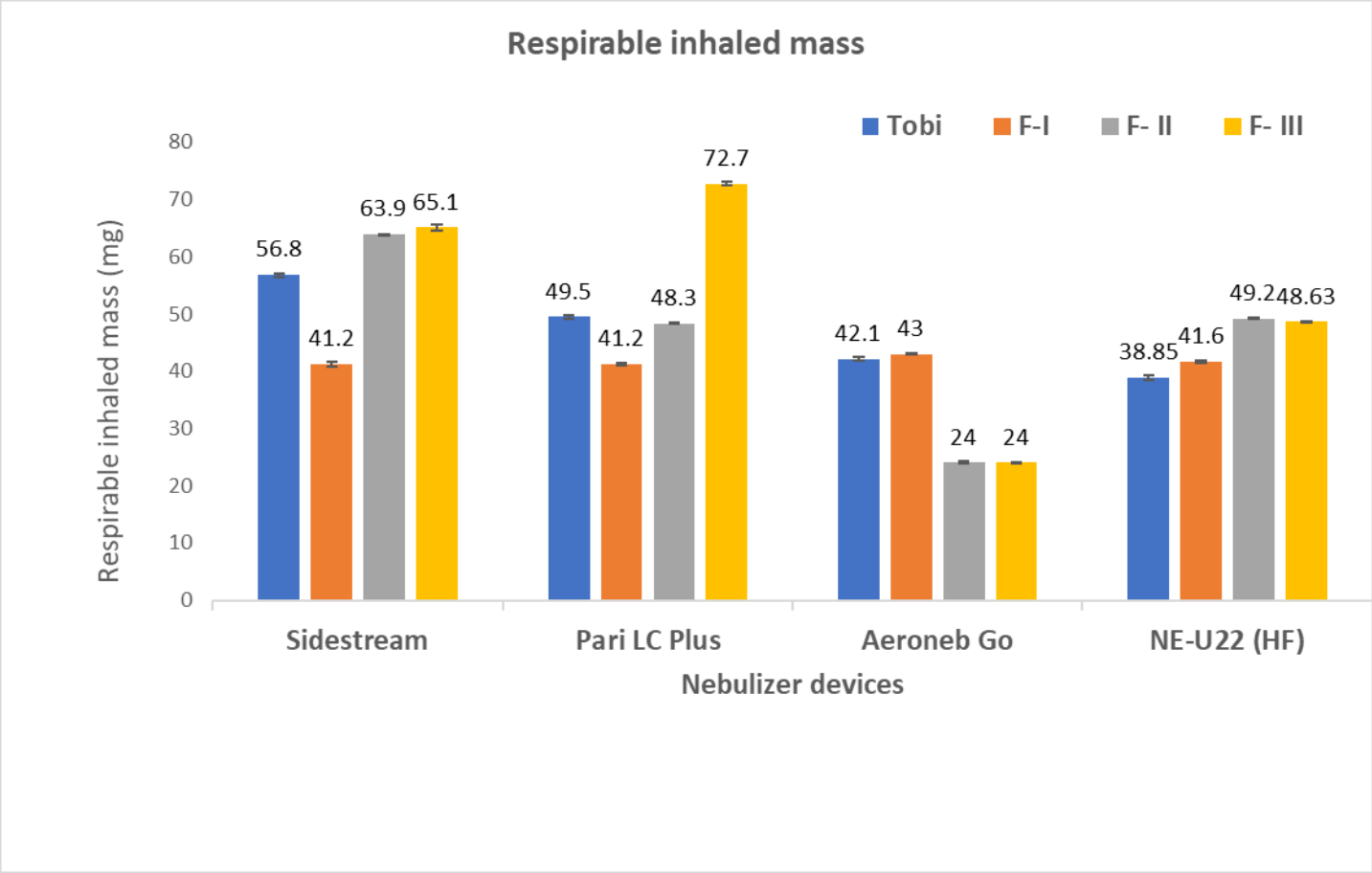

Figure 3 The mean (SD) respirable inhaled mass during nebulization of the TOBI®, Formulation I, II, and IIIusing different nebulizer delivery systems.

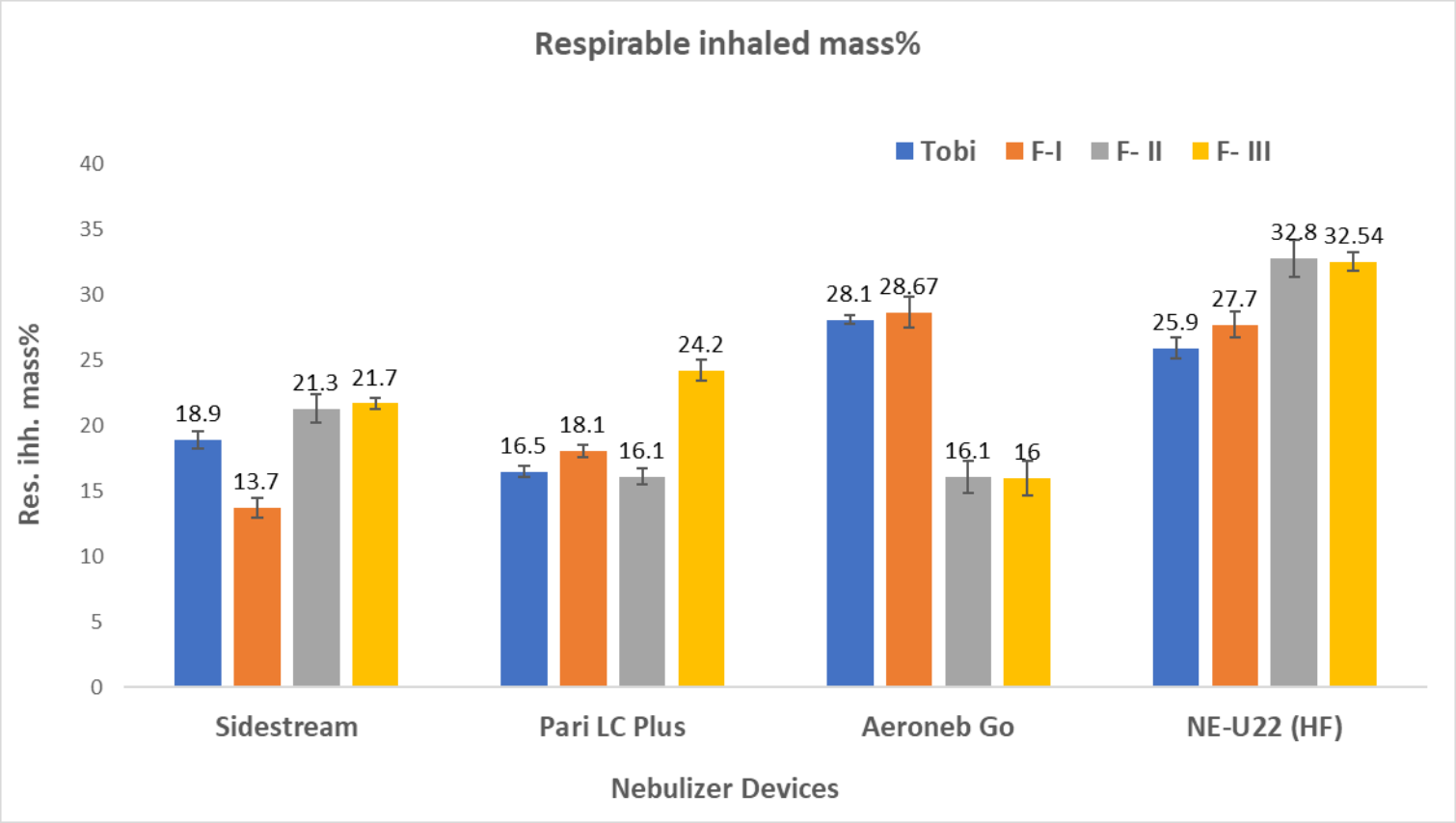

Figure 4 The mean (SD) respirable inhaled mass % during nebulization of the TOBI®, Formulation I, II, and IIIusing different nebulizer delivery systems.

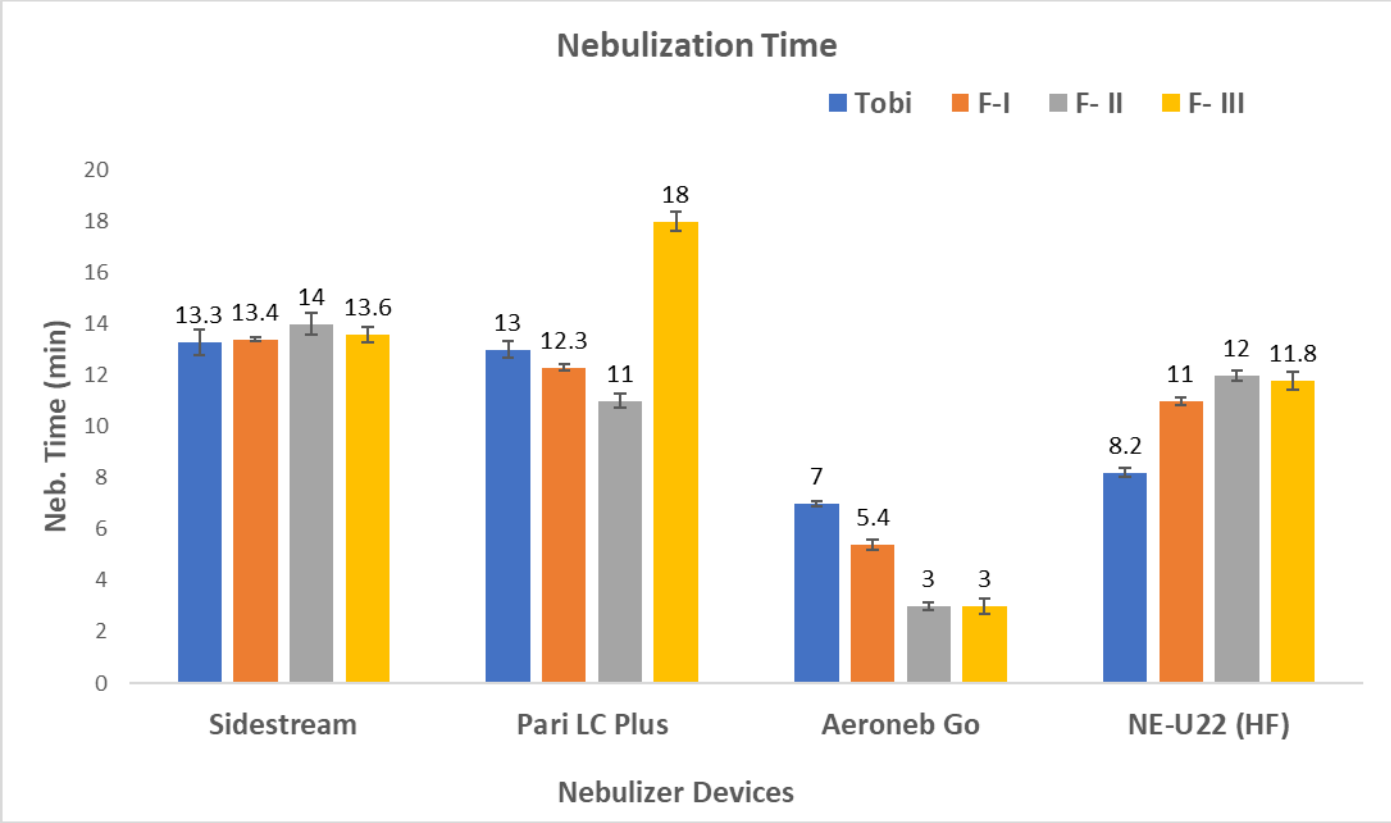

Figure 5 The mean (SD) nebulization time during nebulization of the TOBI®, Formulation I, II, and III using different nebulizer delivery systems.