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Review

# The function and performance of aqueous aerosol devices for inhalation therapy

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**Keywords**
nebulizer function; lung targeting; aerosol suspension; aerosol solution; protein therapeutics

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## Abstract

**Objectives** In this review paper, we explore !!!the interaction between the functioning mechanism of different nebulizers and the physicochemical properties of the formulations!!! for several types of devices, namely jet, ultrasonic and vibrating-mesh nebulizers; colliding and extruded jets; electrohydrodynamic mechanism; surface acoustic wave microfluidic atomization; and capillary aerosol generation.

**Key findings** Nebulization is the transformation of bulk liquids into droplets. For inhalation therapy, nebulizers are widely used to aerosolize aqueous systems, such as solutions and suspensions. The interaction between the functioning mechanism of different nebulizers and the physicochemical properties of the formulations plays a significant role in the performance of aerosol generation appropriate for pulmonary delivery. Certain types of nebulizers have consistently presented temperature increase during the nebulization event. Therefore, careful consideration should be given when evaluating thermo-labile drugs, such as protein therapeutics. We also present the general approaches for characterization of nebulizer formulations.

**Summary** In conclusion, the interplay between the dosage form (i.e. aqueous systems) and the specific type of device for aerosol generation determines the effectiveness of drug delivery in nebulization therapies, thus requiring extensive understanding and characterization.

## Introduction

### The evolution of nebulization technologies

Commercially available technologies to transform a liquid dosage form into an aerosol for medical inhalation purposes have evolved significantly over the last century. Fundamentally, aerosol generation in the form of droplets has evolved from using human-powered techniques (manually compressed hand bulbs), followed by the advent of gas-powered devices (the air-jet stream principle) and to electronic powered systems (using the ultrasound effect, including recent adaptations to create vibrating-mesh micropumps). More recently, mechanical and electromechanical systems have been applied to develop novel aerosol production technologies (i.e. soft mist inhalers). The emerging technologies still include new nebulizing concepts, involving mechanisms such as electrohydrodynamic atomization and surface acoustic wave microfluidic atomization as well as capillary aerosol generators (CAG). Nebulizers are usually selected over other medical inhalers (e.g. pressurized metered-dose inhaler (pMDI), or dry powder inhaler (DPI)) either due to the high drug deposition potential, or the negation of required patient training of complex inhalation manoeuvres. Additionally, nebulizers have an innate capacity to aerosolize special formulations (e.g. recombinant human deoxyribonuclease (rhDNAse) or antibiotics not available as other inhalation dosage forms).[1]

In addition to the progress of the basic principles of nebulization, the innovation has advanced further to encompass the so-called 'smart' technologies, with the objective to increase drug deposition to the lungs. Breath-enhanced nebulizer systems, such as the Pari LC Star® (PARI Respira-

tory Equipment, Inc., Midlothian, VA) and the AeroEclipse® (Trudell Medical International, London, ON) devices, have an inspiratory flow rate to match that of the patient, increasing delivery of droplets, while returning the flow rate to baseline during exhalation.[2] In addition, breath-actuated devices, such as the AeroEclipse® and Halolite®, deliver aerosols after preprofiling a patient's breathing pattern. The I-neb Adaptive Aerosol Delivery (by Respironics®) delivers aerosol only during the initial phase of inhalation.[3–5] The Aer$_X$™ insulin Diabetes Management System (iDMS®; developed by Aradigm [Aradigm Corporation, Hayward, CA] and Novo Nordisk (Novo Nordisk A/S, Bagsværd, Denmark) is a breath-activated inhalation system that also allows for patient monitoring in order to ensure compliance to an adequate inhalation technique at optimal breathing conditions.[6,7] Other technologies are available to monitor adherence of patients to MDIs, such as the SmartMist®, the Doser CT® and the MDILog®.[8]

Nebulization is the process to convert a liquid dosage form into fine droplets using a particular device. Therefore, specific properties of bulk formulations in conjunction with the functional mechanism of a specific inhaler can dramatically influence the droplet characteristics and overall aerosol production. These droplet characteristics, together with patient dependent factors, in turn determine the quality and extent of drug deposition to the lungs (Figure 1).

## Particle deposition and related characterization methods for pulmonary delivery

Deposition throughout the respiratory airways of particles with different sizes is governed by different forces. Larger particles are highly affected by velocity, due to their relatively high mass, and therefore deposit by inertial impaction. Alternatively, sedimentation generally occurs to particles when gravitational forces are significant. Overall, larger particles are more likely to deposit in the upper airways while smaller sized particles tend to reach the deep lungs via sedimentation. At the smallest end of the scale, particles moving by Brownian motion are prone to be exhaled. More often than not, the droplets formed in most nebulizer systems present somewhat a heterogeneous size distribution. Thus, the dispersity of the size distribution is also an important parameter to be considered in deposition. Overall, it is generally accepted that particles with aerodynamic sizes between 1 and 5 μm may be deposited in the deep lungs.[9]

Essentially, two methods have become prominent in analysing droplet sizes generated by nebulizers; these are inertial impaction and laser diffraction (LD). The first one relates to the drug concentration and is correlated to the hydrodynamic airflow and inertial impaction of droplets with specific sizes; the distribution of droplets is evaluated gravimetrically to determine a mass median aerodynamic diameter (MMAD). This parameter is the equivalent droplet size in which half (50%) of the droplets are smaller and 50% are larger than the specified diameter. The MMAD is calculated by following the evaluation of drug amount deposited in different stages of a cascade impactor apparatus. Commonly, the geometric standard deviation (GSD) is reported to indicate the dispersity droplet size distribution around the MMAD. Laser diffraction is only applied to solution systems, as it is derived from a volume-based measurement and is supported by the principle of homogeneous drug concentration of these aqueous dosage forms. For this technique, the MMAD is usually interchangeably referred to as volume mean diameter (VMD) and the dispersity is sometimes given as span (10% percentile subtracted from 90% percentile and divided by VMD).

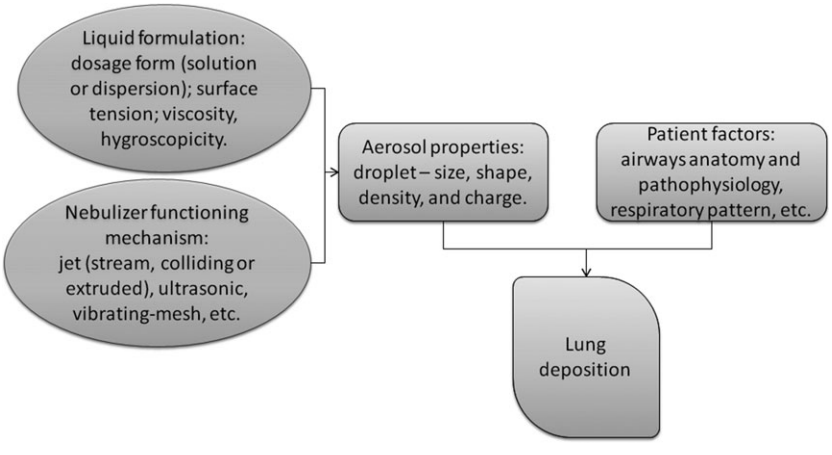


**Figure 1** Factors influencing lung deposition from nebulizer formulations.

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A reduced nebulization time is always desired in order to boost patient compliance to treatment, and nebulizer systems capable of delivering relatively high amounts of drug are generally preferred. Therefore, measurement of aerosol output (amount and rate) is essential to establish nebulization performance. This analysis has been traditionally performed either on weight basis (gravimetrically, by simply weighing a nebulizer reservoir before and after nebulization) or on drug amount basis. However, care should be practiced when relying on the gravimetric method. For instance, weight loss analysis can overestimate drug output due to evaporative effects of jet nebulizers,[10,11] or due to a heterogeneous nebulization of drug containing droplets (i.e. the generation of droplets that contain varying amounts of drug) during aerosolization. These effects could potentially be exacerbated during the nebulization of dispersed systems, such as suspensions or liposomes.[12]

### Context

We present the different mechanisms of aerosol generation, herein defined as nebulization by the transformation of bulk liquids into droplets. From this perspective, we included 'soft mist inhalers' (SMI) considering their functioning mechanism, which is based on aerosol emission of small volumes/doses at slow velocity. We have subdivided the devices presented here into three categories: established, emerging and investigational nebulizers. The established nebulizers are those that have been extensively investigated and are commercially well-established, namely jet, ultrasonic and vibrating-mesh nebulizers. The emerging nebulizers are aerosol generators with mechanisms more recently developed: colliding jets; extruded jets; and electrohydrodynamic principle. The technologies may still be in evaluation on clinical trials. Commonly, there may be not more than a couple of them currently being marketed so their application, adoption and investigation have been limited thus far compared to established nebulizers. Finally, the surface acoustic wave microfluidic atomizer and the capillary aerosol generators comprise the investigational nebulizers and are yet to have clinical data presented or a commercial medical device to be launched, although there have been several exploratory studies carried out.

We explore the nebulization performance of different methods of aerosol generation for solution and dispersed systems based on the bulk characteristics of liquids, with emphasis on the influence of changes in surface tension and viscosity to aerosol production. We recognize the importance of density, but, because the vast majority of the nebulizing formulations are based on aqueous systems, overall changes might be small and its influence may be limited. Importantly, the nebulizer system comprises all components attached to the aerosol generation device, as nebulizer performance varies with respect to other factors beyond just droplet formation, such as flow characteristics and airway connection tubing properties.[13] Ultimately, our intention is to lay out the importance of the interplay between inhalation device design and formulation.

## Established Nebulizers

A summary of the technologies, their functioning mechanisms and selected examples of commercially available devices are presented in Table 1.

### Jet nebulizers

The basic functioning principle of jet nebulization is that a compressed gas (i.e. air) is forced through a tubing system, which is in turn connected to a nozzle. As the air velocity increases with the decrease in the tubing cross-sectional area, a zone of low pressure is created around the nozzle (Venturi effect). As the high-velocity jet passes tangentially or coaxially through the Venturi nozzle, the pressure drop created causes the liquid formulation to rise up on a feed tube from the liquid reservoir (Bernoulli effect). A primary droplet is then formed as an aerosol; a large droplet may subsequently impact on baffles or onto the nebulizer walls, recycling into the reservoir. Droplets small enough circumvent these barriers (secondary droplets) and form the respirable aerosol generated from jet nebulizers.[14] Therefore, nebulizer design and dimensions greatly influence the characteristics of the secondary aerosol formation. This reason reinforces that nebulizers should be evaluated as a multicomponent system for the respirable aerosol generation, as opposed to characterization of the inhalation formulations based on isolating the single mechanism of aerosol production itself (primary aerosol generation). Although the influence of surface tension and viscosity on the size of primary droplets is well described, the secondary aerosol characteristic is a complex function of jet nebulizer systems.[14,15] Figure 2 illustrates the functioning mechanism of jet nebulizers.

The performance of these nebulizer systems (compressor/nebulizer combinations) to produce water droplets has been compared extensively, with MMAD values measured using laser diffraction varying from 2.6 to 10.2 μm.[16] Treatment time reduction with these systems can be achieved by increasing airflow rate and using a small initial fill volume, although these measures can slightly change the aerosol characteristics.[14,16] Overall, decrease in droplet size (with increased aerosol polydispersity) and increase in aerosol output can be expected for higher airflow rates and

**Table 1** Established nebulizers, their functioning mechanisms and examples of commercially available devices

| Technology | Functioning mechanism | Examples of commercially available devices |
|---|---|---|
| Jet nebulizers | Air is forced through a tubing system connected to a nozzle. The air velocity increases (due to decrease in tubing cross-sectional area) creating a low-pressure zone around the nozzle (Venturi effect). As the high-velocity jet passes tangentially or coaxially through the Venturi nozzle, the pressure drop created causes the formulation to rise up on a feed tube from the liquid reservoir (Bernoulli effect). A primary droplet is formed as an aerosol; a large droplet may subsequently impact on baffles or onto the nebulizer walls, recycling into the reservoir. Droplets small enough circumvent these barriers (secondary droplets) and form the respirable aerosol. | AeroEclipse II; Assister KN-180; Genki; Hudson T Up-draft; Marquest Acorn II; Medel Clenny; Medel SkyNeb; Mefar 2000; Micromist; Millicon S; Nesco; Nissho; Omron NE; Pari LC Jet Plus; Pari LC Sprint; Pari LC Star; Pari LL; Sidestream; Sidestream Plus; Ventstream. |
| Ultrasonic nebulizers | Acoustic waves are generated by a piezoelectric transducer that converts electrical signal into oscillatory mechanical movement. This mechanism creates oscillatory pressure disturbances that travel through a bulk liquid to be aerosolized. Two widely discussed possible mechanisms for wave destabilization at the liquid surface are responsible for producing droplets: cavitation and capillary. | Beurer IH50; Devilbiss Ultraneb; DP 10; Easimist; Euroneb; Liberty; Medix Electronic; Multisonic; NE320; Omron U1; Optineb; Polygreen KN-9210; SAM LS; Spira Ultra; Sonix 2000; Systam. |
| Vibrating-mesh nebulizers | Micropump systems: energy forcing liquid to flow through small apertures of a plate or membrane.<br>*Passive*: a piezoelectric crystal generates vibration from electrical force to a transducer horn that is in contact with the formulation. The vibration creates waves in the nebulizer reservoir that travel towards a perforated plate positioned in front of the transducer horn. Consequently, aerosols are created once the fluid flowing through the membrane is enough to cause drop detachment.<br>*Active*: a dome-shaped membrane is directly connected to a vibrating piezoelectric element. Following the application of electric current, the membrane moves up and down causing the liquid formulation to be rapidly extruded through the mesh. | *Passive*: Omron MicroAir;<br>*Active*: Aeroneb Pro and Pari eFlow. |

higher initial fill volumes.[17,18] Irrespective of initial fill volume, a study with water clearly showed that output rate was not constant over time for the twenty-three jet nebulizer systems investigated, varying anywhere from 0.05 to 0.29 mL/min at different time points within the same aerosolization event.[16] This was an important study comparing the capacity of different nebulizer/compressor combinations to aerosolize a reference liquid (water). However, the fact that these systems promoted a device-specific variable decrease in temperature (4 to 8 °C)[19] does not allow us to evaluate the effect of the important temperature-dependent properties (i.e. surface tension and viscosity) and their effect on nebulization performance.

Nebulizer systems are capable of delivering high amounts of drug, and nebulizer formulations are primarily comprised of aqueous systems that can avoid damage to lung physiology. However, the presence of different excipients will almost certainly alter the physicochemical properties of liquids, even given the limited options for inhalation delivery due to potential toxicological effects of certain inactive ingredients.[20] For this reason, the characterization of the liquid formulation in conjunction with nebulization performance has been investigated in a number of recent studies. Very small droplet sizes (MMAD between 0.5 and 1 μm as measured by a 6-stage cascade impactor) were generated from jet nebulization of a simple hydroalcoholic solution (4% v/v ethanol in water) of Prostaglandin $E_1$, aiming to treat neonatal hypoxemic respiratory failure.[21] The small ethanolic content was of a sufficient amount to decrease surface tension and viscosity values to approximately 61 mN/m and 0.982 cP, respectively. Cyclodextrin complexation of poorly water-soluble formoterol has provided solutions with surface tension and viscosity values of 54–56 mN/m and 1.16–1.18 cP, respectively.[11] Jet nebulization of these solutions has shown VMD values varying between 3 to 5 μm, with drug output rates of approximately 30–60 μg/min for four different nebulizer systems. Interestingly, the authors report that rate of formulation output is greater than rate of the formoterol emitted, indicative of the formation of aerosol droplets with varying drug concentration.

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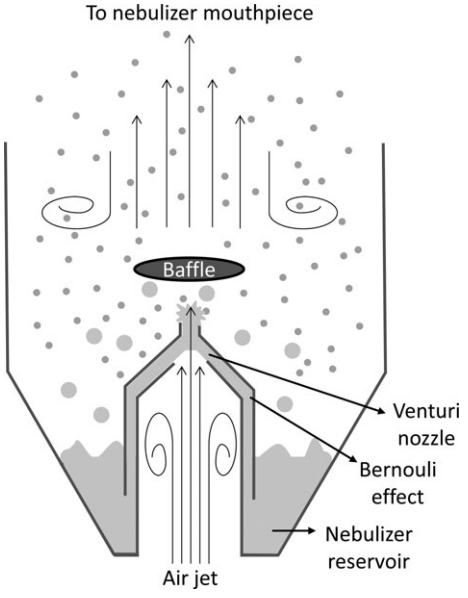


**Figure 2** Schematic diagram showing the functioning mechanism of jet nebulizers.

The effect of surface tension on jet nebulization output of solutions was clearly illustrated when Ventolin® (albuterol sulfate; Glaxo Canada, Inc., Montreal, QC) was added to a tobramycin intravenous solution supplied by Eli Lilly Canada.[18] The presence of benzalkonium chloride as preservative in the Ventolin® formulation caused the surface tension of the final mixture to change from 66 mN/m (tobramycin IV solution diluted with saline) to 31 mN/m (tobramycin IV solution containing 0.5 mL of Ventolin® formulation and diluted with saline, with a final benzalkonium chloride concentration of 0.00125% w/v). As a result, an increased drug output (10–50%) was observed for the lower surface tension solution. Similar results have been reported in another investigation that used three different jet nebulizer systems.[19] Importantly, authors from both studies highlighted the magnitude of increased drug output further related to differences in jet nebulizer systems and parameters (i.e. airflow rate) studied. Conversely, studies on solutions with increasing concentrations of heparin have shown a concomitant increase in kinematic viscosity, but no change in surface tension.[17] This increase in viscosity is in general translated into increased output rate and decreased droplet sizes when solutions of calcium, sodium and low molecular weight heparin are jet nebulized. Interestingly, analysis of droplet size over time within a 15-min nebulization run using a highly concentrated sodium heparin solution (19 900 IU/mL) displayed a decrease in MMAD from 2.5 to 1.9 μm, with no change in GSD.

A drop in temperature caused by the latent heat of evaporation of the nebulizer solution is only one formulation attribute changing over time during jet nebulization.[22] According to Steckel and Eskandar, while studying the changes occurring within a 10-min nebulization period, an increased drug concentration can also be expected as the water evaporates. This can be attenuated by the presence of buffer in saline solution, which causes a drop in the saturated vapour pressure. Moreover, the investigators found that while viscosity increases due to temperature drop of the nebulizer solution, surface tension decreases due to the increased nebulizer solution concentration. Most importantly, the authors explain that, within a 10-min nebulization period, as jet nebulization occurs and water starts to evaporate, the temperature drop promotes an increase in viscosity and a reduction in saturated vapour pressure. Consequently, an initial increase in droplet size is observed. As the process continues and the nebulizer solution concentrates, the reduction in surface tension provides droplets with smaller VMD values.

As indicated, it is very valuable to have knowledge during formulation development with respect to an understanding of the influence of physicochemical properties of liquids (i.e. surface tension and viscosity) on aerosol droplet size and output for these inhaler devices.[23] The addition of surface active agents to water changes the secondary aerosol properties in a device-specific manner, with an overall inverse relationship relative to aerosol output.[24] However, a more intricate relationship between surface tension and droplet size can be expected. In some cases, this relationship between surface tension and droplet size may be inversely related, and in other cases, it may reach a peak value. Irrespective of the observed relationship, the size of the emitted droplets appears to be independent of the critical micelle concentration, and respirable output results overall agree with total output trends.[24] Viscosity effects are clearer, with jet nebulization being more efficient in terms of respirable output with liquids of low viscosity (1–6 cP). Thereafter and up to ceasing nebulization, increased viscosity increases MMAD as well as aerosol output, also in a device-specific manner.[23,25,26]

Jet nebulizers have been shown to be capable of aerosolizing protein solutions. Recombinant human deoxyribonuclease I (rhDNase I, also known as dornase alfa) has been tested and successfully delivered to the cystic fibrosis patient airways using jet nebulizer systems, to alleviate excessive mucus accumulation. [27,28] In fact, there are only three different jet nebulizer systems approved for delivery of dornase alfa to treat patients with cystic fibrosis and these are (nebulizer/compressor system) as follows: the Marquest Acorn II/DeVilbiss Pulmo-Aide; the Hudson T Up-draft/DeVilbiss Pulmo-Aide; and the Pari LC Jet Plus/Pari Inhaler Boy.[29,30] Limited methionine oxidation and no aggregation of insulin-like growth factor-1 (IGF-1) were observed upon nebulization with jet and vibrating-mesh nebulizers.[31] However, studies to evaluate jet nebulization

on protein degradation must always be considered. It is apparent that through different mechanisms, the micellar properties of Tween-80 and the hydrodynamic size as well as the influence of polyethylene glycol (PEG) on the conformational structure of protein in the air–water interface have shown to aid in protein stabilization during air-jet nebulization.[32] Chitosan provides an additional protective effect, possibly via ionic interactions between its positive charge and the negatively charged enzyme.[33] Moreover, protein solutions are commonly freeze-dried to provide greater physicochemical stability.[34] When sodium polyphosphate, calcium chloride or magnesium sulfate are used as cryoprotectants in a protein formulation (aviscumine), decreased surface tension and increased viscosity are seen.[35] The droplet size of jet nebulized formulations was observed to be slightly decreased when containing these excipients as compared to normal saline. Meanwhile, these components also provide protection to aviscumine destabilization caused by the air-jet process. For the treatment of emphysema, and potentially cystic fibrosis, the addition of antifoams, such as span 65, or a mixture of cetyl alcohol and tyloxapol, to protein solutions of $\alpha_1$-protease inhibitor also decreased surface tension without altering viscosity.[36] An overall increased amount of jet nebulized protein was observed, while the cetyl alcohol/tyloxapol antifoam mixture provided an improved respirable fraction.

Dispersed dosage forms can also be delivered to the lungs using jet nebulizers.[37,38] The aerosolization efficiency is highly device-dependent.[39,40] For instance, thirty different jet nebulizer systems show respirable fractions of Pulmicort Respules® (budesonide suspensions) ranging from 15% to 50% but with very different output rates.[41] Reportedly, these suspensions present drug particle sizes of 2–3 μm.[42] Suspensions of non-deformable-shaped (latex) microspheres of 1 to 10 μm were also consistently nebulized with a Pari® air-jet nebulizer.[43] Nanoemulsions of budesonide (10.9 nm) prepared using ultrasonication presented improved aerosol characteristics for pulmonary delivery following jet nebulization. MMAD values were around 5.0–5.5 μm for the nanoemulsion compared to 7.0–8.0 μm for the standard suspension. Additionally, the nanoemulsion had better aerosol output, thus allowing for a much improved respirable fraction.[44] Lipid nanoemulsions of amphotericin B were prepared with commercially available products for total parenteral nutrition (Intralipid® and Clinoleic®; Baxter International, Inc., Deerfield, IL) and also successfully aerosolized using jet nebulizers.[45] Also, chitosan has been used as a nanocarrier to formulate poorly water-soluble compounds for air-jet nebulization.[46,47] Jet nebulization of nanoparticle dispersions of deoxyribonuclease I (DNase I) showed similar results, while greater than 50% activity of the protein is maintained.[48] Importantly, the nebulization performance of suspensions using jet nebulizers is also dependent on formulation properties, with different excipients and methods of preparation providing rather variable drug deposition patterns.[49] In addition, drug nanoparticle aggregation may also occur during jet nebulization.[50]

Many liposomal formulations have been aerosolized with this method. Liposome components included soya (SPC) or egg phosphatidylcholine (EPC), cholesterol, and a variety of synthetic phospholipids, such as 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC).[51] With increased phospholipid concentration (1,2-dilauroyl-sn-glycero-3-phosphocholine – DLPC), an increase in the Non-Newtonian apparent viscosity of budesonide and ciclosporin liposomes has been observed, promoting reduction in drug mass output rate following jet nebulization.[52] Nevertheless, the differences in nebulizer design as well as lipid concentration (and therefore viscosity) are factors influencing secondary droplet sizes.[53] Ultimately, the type of phospholipid influences the jet nebulization performance differently for particular compounds.[54] For instance, in delivering plasmid DNA complexed with a cationic liposome, the tested formulation with viscosity of 6 cP and surface tension of 42 nM/m was best nebulized using an AeroEclipse II jet nebulizer when compared to several commercially available jet, ultrasonic and vibrating-mesh nebulizers.[55]

Powders of phospholipid-coated particles (proliposomes) are ready for hydration to form liposomes and can be directly dispersed within the jet nebulizer reservoir for efficient aerosolization.[12] However, the shear effect of air-jet aerosolization can be expected to affect the physical stability of multilamellar vesicles (MLV).[56] A slightly higher physical stability of liposomes to jet nebulization can be achieved when MLVs are extruded through 1-μm polycarbonate filters.[57] Further reduction in particle size of MLVs by extrusion through 0.4-μm filters did not improve physical stability, in terms of retained entrapped drug following jet nebulization, but did provide an improved drug-to-aerosol mass output. *In vitro* studies suggest that liposomal drug encapsulation with DPPC is beneficial for deposition to the deep lungs with air-jet nebulization when compared to free drug, mainly for poorly water-soluble compounds.[58] Supposedly, the decrease in surface tension caused by this phospholipid can bring advantages to the adsorption kinetics of the liposomes to lung surfactants. [59]

## Ultrasonic nebulizers

During the function of an ultrasonic nebulizer, acoustic waves are generated by a piezoelectric transducer that converts electrical signal into oscillatory mechanical move-

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ment. With frequencies of approximately 20 KHz, this mechanism creates oscillatory pressure disturbances that travel through a bulk liquid which is to be aerosolized. Cavitation occurs when pressure disturbances propagating through the liquid cause zones of low pressure, this creates vapour bubbles. At the collapse of these bubbles, shock waves close to the air–liquid interfacial region lead to surface destabilization creating the droplets. Alternatively, liquid excitation by ultrasonication causes capillary waves going outwards from the surface region up to a collapsing point in which droplets are generated. These two widely discussed possible mechanisms for wave destabilization at the liquid surface are responsible for producing droplets, namely cavitation and capillary.[60,61]

Conversely to air-jet systems, ultrasonic nebulizers promote an increase in solution temperatures to as much as 10 °C above the starting temperature after a 5- to 10-min aerosolization period.[19,62] This phenomenon of increasing temperature is caused by the high energy input of the piezoelectric crystal. Additionally, a higher magnitude of increase in drug concentration within a 10-min nebulization period is observed compared to jet nebulizers.[22,62] On the other hand, the addition of buffer salts or saline solution has also a relatively greater effect in decreasing drug concentration differences as well. Nonetheless, ultrasonic nebulizers are capable of maintaining a more constant VMD over time during the same nebulization event, while causing viscosity as well as saturated vapour pressure at the air–water interface to drop during aerosolization.[22] Increased concentration of buffer solution promotes increase in VMD caused by increase in viscosity, decrease in saturated vapour pressure or a surface tension drop.

Considering the functioning mechanisms of aerosol generation, it is extremely important to independently evaluate formulations by comparison of different devices. For instance, for formulations of heparin with increased concentration (and therefore increased kinematic viscosity, but no variation in surface tension), aerosol characteristics were unsatisfactory for ultrasonic nebulizers, presenting variable MMADs from 5.5 to 7 μm. This variation was not observed for air-jet nebulizers, as previously discussed.[17] The ultrasonic aerosolization of solutions containing macromolecules is another concern. For instance, activity of the protein aviscumine is highly affected by ultrasonic nebulizers compared to air-jet systems.[35] Notably, a device in which water was used as medium to propagate the ultrasonic waves presented less accentuated aviscumine degradation than when the protein solution was used as transducer medium. Nevertheless, the investigation also showed that salts used as cryoprotectants decreased surface tension and increased viscosity, but did not alter droplet size significantly. In addition, the salts were not as capable of providing protection to the protein solution during ultrasonic aerosolization as they were for jet nebulization, and could not be ruled out as a possible contributor to extensive protein instability. On the other hand, aerosolization of a protein solution of α1 protease inhibitor (viscosity of 1.25 mPa.s and surface tension of 53 mN/m) using a variable frequency ultrasonic nebulizer (up to 2.4 MHz) provides adequate VMDs of approximately 1.6 μm at different vibration levels of the piezoelectric crystal.[63] More importantly, the protein molecular weight and anti-elastase activity are maintained despite the stress caused by the ultrasonic nebulization. As the protein is a thermolabile compound, this stabilization is related to the heat absorption of a coupling liquid that is designed with the ultrasonic nebulizer to act as a buffer, avoiding excessive temperature increases in the formulation to be aerosolized. Therefore, it appears that the thermal and mechanical stresses caused by ultrasonic nebulization are potential reasons for the unsuitability of these devices to aerosolize large molecules. However, when studying nebulization of lactate dehydrogenase solutions, no simplistic evaluation could be inferred for the capability of different types of nebulizers (jet and ultrasonic) to effectively aerosolize this protein solution, as enzyme activity was maintained across the board.[64] This reinforces the need to specifically determine the effectiveness of a device to aerosolize protein solutions.

Overall, ultrasonic nebulizers are incapable of generating aerosols from high viscosity liquids (i.e. greater than 6 cP).[23,25,55,65] For less viscous liquids, an inverse relationship to the respirable output occurs. And comparing liquids with decreasing surface tension, peak values for VMDs outbalance the trough values of total output resulting in an optimal respirable output from ultrasonic nebulizers concurring with droplet size patterns generated.[23,24] In general, ultrasonic nebulizers present a less heterodisperse aerosol than jet nebulizer systems.[23]

Ultrasonic devices are well known for not being appropriate to deliver microparticulate dispersed dosage forms, such as budesonide suspensions, and MLV liposomes.[12,51,66,67] An ultrasonic nebulizer has shown to selectively aerosolize the continuous aqueous phase of a latex microsphere suspension while the microspheres were left in the reservoir.[43] Radiolabelled solid lipid nanoparticles, however, have been effectively delivered to the lungs using this aerosol generation mechanism to study lymphatic uptake.[68] Furthermore, recent studies show that ultrasonication does not rupture nor does it cause aggregation or agglomeration of drug particle size encapsulated in lipid nanocarriers.[69] Further investigations are warranted to determine nebulization performance of these formulations as well as whether this resistance of solid lipid nanoparticles to nebulization is related to particle composition or structure and size.

## Vibrating-mesh nebulizers

Vibrating-mesh nebulizers can be classified as micropump systems because aerosol generation from this technology is a result of energy forcing liquid to flow through small apertures of a plate or membrane. There are two types of micropump nebulizers: passive or active vibrating-mesh systems. The passive vibrating-mesh nebulizer (i.e. Omron MicroAir®; Omron Healthcare, Inc., Lake Forest, IL) is composed of a piezoelectric crystal which generates vibration from electrical force to a transducer horn that is in contact with the liquid formulation.[70,71] The vibration then creates waves in the nebulizer reservoir that travel towards a perforated plate (with aperture diameter of approximately 3 μm) positioned in front of the transducer horn. Consequently, aerosol droplets are created once the fluid flowing through the membrane is enough to cause drop detachment. Alternatively, active vibrating-mesh nebulizers (i.e. Aerogen Aeroneb® [Aerogen, Inc., Galway, Ireland] and Pari eFlow® [PARI Respiratory Equipment, Inc., Midlothian, VA]) have a dome-shaped membrane (aperture sizes of approximately 4 μm and 20 μm, respectively) directly connected to a vibrating piezoelectric element.[56,72] Following application of electric current, the liquid formulation is rapidly extruded through the mesh as a consequence of the downward and upward movements of said membrane; this action generates the droplets.[73]

This class of nebulizers, particularly with the Aeroneb Pro® (Aerogen, Inc., Galway, Ireland), presents the lowest change in temperature of the nebulizer solution among the inhalers discussed so far, with a small increase of about 3 °C over a 5-min nebulization period,[19] although the temperature continues to grow linearly[62]. Nevertheless, the temperature increase is nebulizer-dependent as seen with the similarly active vibrating-mesh device, Pari eFlow®, and can also be influenced by the volume of formulation in the nebulizer reservoir.[74] Cooling strategies of the formulation in the nebulizer reservoir have shown promise in circumventing this challenge, and it may be greatly necessary for thermally unstable drugs, such as protein therapeutics. In addition, changes in drug concentration over a 10-min nebulization event are negligible, differently from what is observed for jet and ultrasonic nebulizers.[62] This particular technological advance in functioning mechanism offers the benefit of promoting its selection for use in clinical trials of inhalation therapies.[75,76]

Both active and passive vibrating-mesh nebulizers are highly dependent on formulation characteristics. The influence of bulk liquid characteristics on aerosol generation of solutions has been systematically evaluated.[77,78] Both systems have been demonstrated to ineffectively produce aerosols from solutions that have viscosities higher than 2 cP, with their total aerosol output being independent of physicochemical properties of liquids.[55,77,79] The passive mesh technology yields slightly larger droplets than the active mesh system, but compensates to provide a similar respirable output by having a higher total aerosol output. An increased viscosity provides a decrease in droplet size, and a consequently higher respirable output from both mesh systems, but the overall output rate is compromised for passive mesh nebulizers. The influence of surface tension on aerosol properties is less clear, but it is known that fluids with low viscosity and low surface tension seem more desirable for greater nebulization performance.[77] With the Pari eFlow® nebulizer, increase in solution viscosity showed decrease both in aerodynamic diameter and output rate while increase in electrolyte concentration showed increase in output rate and decrease in aerodynamic diameter.[80,81] Therefore, the proportion of respirable droplets generated is dependent on the interplay between output rate and aerodynamic diameter, which in turn are each highly driven by the physicochemical properties of the formulation. A low ion concentration is crucial for providing less variable aerosol generation with vibrating-mesh nebulizers.[77,78,82] Investigations using several sodium halides showed that solutions containing ions with greater polarizing abilities (i.e. NaI) presented superior aerosol performance due to their greater presence at the air–water interface.[83] Aerosol charge distribution from active vibrating-mesh nebulizers has recently been investigated with (TDMA). The results showed that the levels of negatively charged droplets from nebulization of normal saline are superior to that of positively charged particles and that the fraction of charged particles is greater for those below 200 nm in size.[84] Although charge distributions can greatly differ with different nebulizer configurations and formulation compositions, TDMA was successfully used to determine submicron particle charge, which can influence patterns of drug deposition in the lungs.

Not all available apertures produce droplets all of the time though; this is highly dependent on the interactions between the bulk liquid formulation and the vibrating membrane.[78] Importantly, the orifices of a mesh can get clogged over time, despite emphasizing cleaning instructions to patients that aerosolize solutions.[85] As a result of clogging, dramatic variations in output rate and subsequent delivered dose can be problematic. In extreme clogging situations, the device may even be caused to switch-off automatically. For these reasons, thorough cleaning of the vibrating-mesh must be conducted and the membrane should be periodically evaluated for clogging. In a clinical setting, timely replacement of the membrane as well as dedication of device to specific formulations should be considered to avoid cross contamination.

In general, active vibrating-mesh nebulizers more efficiently deliver solutions of low viscosity than jet nebulizers,

while passive devices present comparable performance,[86–89] although this may differ in specific populations.[90] On the other hand, passive vibrating-mesh nebulizers more efficiently deliver protein solutions than the air-jet systems.[4,28,91] For the last two decades, only jet nebulizers have been approved to deliver dornase alfa, but recently, the Pari eFlow® (active vibrating-mesh) nebulizer has shown promise in a technical feasibility study.[92] Sparing-material and high-throughput approaches to screen formulations are often sought to expedite the drug development process. Hertel and co-workers have developed a surrogate method using a Pari eFlow® to determine the feasibility in nebulizing biopharmaceutical products.[93] Using agitation at elevated temperatures to mimic nebulizer-related stresses, protein stability upon nebulization could be predicted and excipients could be screened. Vibrating-mesh nebulizers can also successfully deliver poorly water-soluble drugs to the lungs from dispersed systems, such as nanosuspensions.[38,94–99] The drug particle size of nanosuspensions can be maintained for this particular aerosolization, including nanoparticles prepared using freeze-drying with different lyoprotectants.[62,100,101] Active vibrating-mesh nebulizers have shown to present superior aerosolization performance compared to a passive vibrating-mesh system when tested with a suspension of nondeformable, latex microspheres, although incurring in particle fragmentation.[43] Despite being able to better aerosolize drug suspensions than jet nebulizers, the delivery of nanoemulsions of budesonide demonstrates an even more pronounced improvement, with better drug output and fine particle fraction.[44]

Active devices have been shown to be capable of delivering liposomal formulations of water-soluble drugs as well,[102] demonstrating a superior performance when compared to air-jet and ultrasonic systems (greater physical stability and output rate).[12,56,67] Nevertheless, comparable performance of these three types of nebulizers was demonstrated when nebulizing ultradeformable liposomes, which are stress-responsive vesicles containing Tween-80 and ethanol.[103] Attributed to the addition of these excipients, ultradeformable liposomes were found to be less stable to aerosolization than conventional liposomes regardless of type of nebulizer. Manufacturer customization of the active vibrating-mesh with larger aperture sizes (8 μm as opposed to the commonly available 4 μm) has been shown to provide a lower extent of MLV liposome disruption compared to air-jet nebulization, but no significant difference when compared to the normal aperture size vibrating-mesh.[56,57] Extrusion of MLV liposomes through 1-μm membrane filters improved drug output from large mesh aperture nebulizers, but further decrease in lamellarity (using a 0.4 μm filter) was not deemed beneficial.[57] Reconstitution of liposomal formulations with various hydration media provides differences in aerosolization performance of active vibrating-mesh nebulizers, based on the physicochemical properties of the medium.[104] Interestingly, the drug particle size increases have been observed in the nebulizer reservoir. This increase could indicate that aggregation or accumulation can occur due to a cut-off size of liposomes that may be extruded through the mesh during aerosolization. Utilization of different lipid mixtures may enable prolonged drug release upon pulmonary deposition, mainly for liposomes presenting higher phase transition temperatures.[105]

The vibrating-mesh nebulizers are even capable of appropriately aerosolizing more complex dosage forms. Cytosine–phosphate–guanine oligodeoxynucleotides incorporated into gelatin nanoparticles have been successfully aerosolized with both active and passive vibrating-mesh nebulizers while maintaining its *in vitro* immunomodulating effect in equine lung cells.[106] When dendrimers of polyamidoamine (PAMAM) were complexed with a poorly water-soluble compound, the active vibrating-mesh and the jet nebulizers presented comparable aerosolization performance and superior to that of passive vibrating-mesh nebulizers.[107] Together with jet nebulizers, vibrating-mesh nebulizers have also shown to be effective in nebulizing niosomes, an alternative to liposomes made of nonionic surfactant vesicles.[71]

Formulation properties of dispersed systems also highly influence the nebulization performance of these devices. In a recent study, our research group has demonstrated that rheological behaviour of aqueous dispersion is indicative of

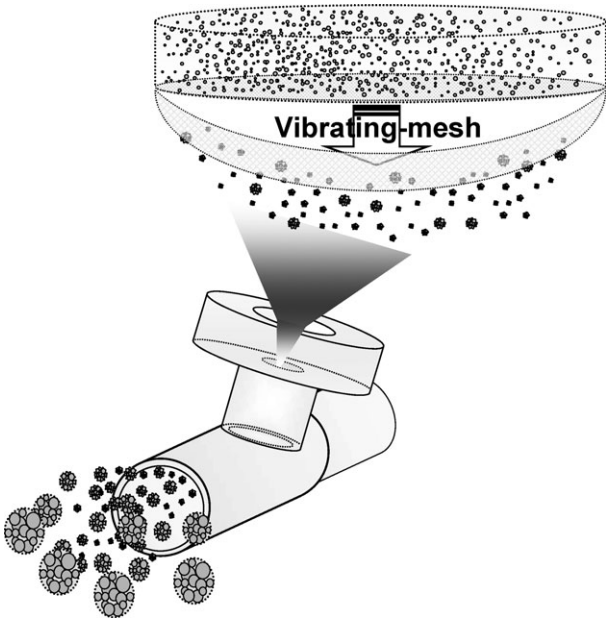


**Figure 3** Schematic diagram showing the functioning mechanism of a vibrating-mesh nebulizer aerosolizing a suspended dosage form. Reprint with permission from Taylor & Francis Ltd.

**Table 2** Emerging nebulizers, their functioning mechanisms and examples of commercially available devices

| Technology | Functioning mechanism | Examples of commercially available devices |
|---|---|---|
| Colliding jets | When a compressed coiled spring positioned in the bottom of a liquid reservoir is released, the formulation is pushed through two precisely engineered nozzles (uniblock) positioned in a specific preset angle that allows liquid jets to converge and collide against each other. The uniblock is comprised of finely engineered microchannels that filter the solution before jet formation in the outlet nozzle. As a result, aerosols are generated at a slow speed. | Respimat |
| Extruded jets | Three-layer laminate strip: the first layer contains a microvolume liquid reservoir blister that is heat sealed to the second (lid) layer. A nozzle array completes the third layer where micrometre holes are laser drilled. Index holes align the multilayer system, which is then connected to a handle to form a final assembled package (strip) fit to the device accordingly. During operation, a piston forces the first layer of the strip towards the nozzle array. As the liquid ruptures the lid layer and rapidly extrudes through the microholes, liquid break-up occurs. | $Aer_X^{TM}$ |
| | Rayleigh break-up theory using lithography (wafer stepper and etching techniques) to engineer different micron-sized spray nozzles. Following actuation, a loaded spring mechanically controls the release of the drug solution contained in a metering valve. As the liquid formulation is extruded through the spray nozzle, the patient's inspiratory flow pulls the formed droplets from a Venturi-like mouthpiece channel into the lungs. | Medspray |
| Electrohydrodynamic atomization | A liquid is slowly fed to a positive potential, electronically controlled capillary nozzle surrounded by a gas flow sheath. An electric field is then created between the nozzle and a counter-electrode; also positively charged, independently from the capillary nozzle. A Taylor cone-jet is formed between the capillary nozzle and the counter-electrode once the electrical stress outbalances the surface tension, generating charged droplets. A corona discharge then controls the droplet charge generating a monodisperse aerosol. | Mystic™ |

nebulization performance using vibrating-mesh nebulizers.[108] Therefore, it is imperative to evaluate many of the properties of the dispersion (e.g. drug particle size distribution, zeta potential, rheology, etc.) when considering aerosolization of this dosage form. Figure 3 shows an illustration of the operating principle of vibrating-mesh nebulizers to aerosolize suspended dosage forms.

## Emerging Nebulizers

A summary of the technologies, their functioning mechanisms and selected examples of commercially available devices are presented in Table 2.

### Colliding jets

In the Respimat® device (Boehringer Ingelheim GmbH, Ingelheim am Rhein, Germany) a compressed coiled spring positioned in the bottom of a liquid reservoir serves to store the energy necessary to operate this system. When the spring is released, the formulation is pushed through two precisely engineered nozzles (uniblock) positioned in a specific preset angle that allows liquid jets to converge and thus collide against each other. The uniblock is comprised of finely engineered microchannels that filter the solution before jet formation in the outlet nozzle. As a result, aerosols are generated at a slow speed. Hence, the name soft mist inhaler (SMI), which,

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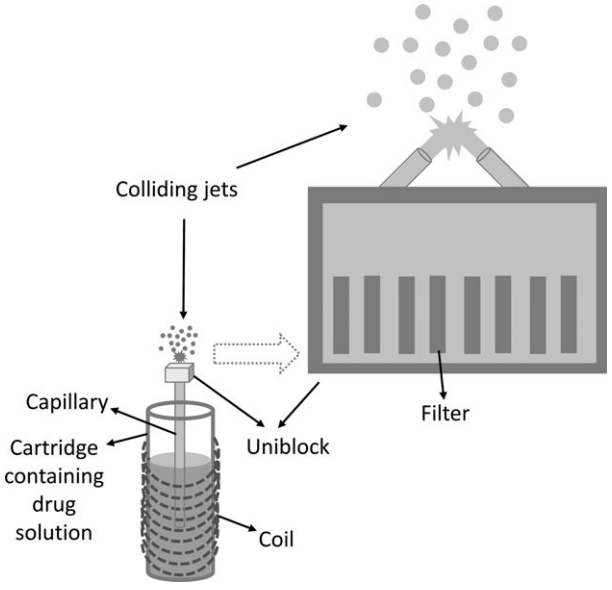


**Figure 4** Schematic diagram showing the functioning mechanism of colliding jet nebulizers.

based on the definition of conversion from liquid to aerosol droplet, can be considered in the context of this review a subcategory of nebulizers. Figure 4 presents a schematic diagram for the functioning mechanism of colliding jet. A high deposition of small particles in the mouthpiece was recently found to occur with the current Respimat® design, due to a zone of recirculation created around the nozzle outlet.[109,110]

The rationale for developing this system was to overcome the disadvantages of other inhalers. The aerosol cloud lasts longer and travels slower (10 m/s for aqueous drug solutions) than aerosols generated by pressurized metered-dose inhalers (pMDI) (50 m/s).[111] Other comparisons show that the mist generated from Respimat® can be up to ten times slower than pMDIs and last 1.2 to 1.6 seconds in the air.[112] The characteristic slow velocity mist avoids high drug deposition in the oropharynx and negates the need for patient synchronization as seen with all pMDI devices.[113] Mixing the concepts of the functional mechanism of nebulizers with the advantage of having a portable inhaler, Respimat® was first available for clinical use in Europe and was only recently approved in the United States.[114,115] It provides a multidose of 120 actuations that are precisely delivered [116] using this mechanical-powered platform. In addition to being independent on inspiratory effort (as observed in some dry powder inhaler systems), it is portable and user-friendly to patients.[117,118]

Respimat® is designed to deliver drug solutions, but not dispersed systems.[119] Successful clinical trials in asthma patients with an aqueous solution containing ipratropium bromide and fenoterol hydrobromide led to the approval of Berodual®.[120,121] Follow-up studies demonstrated its efficacy and safety, despite the presence of benzalkonium chloride and EDTA in the formulation.[122,123] In fact, Berodual® was shown to provide better efficiency in drug delivery to the lungs, and the nominal dose of the active ingredients could be decreased by two- to fourfold when using this device, compared to conventional DPI or pMDIs (with or without the use of spacers).[110,124,125] Similar results were found when treating patients with chronic obstructive pulmonary disease (COPD).[126–129] Inhalation solutions of tiotropium have been shown to be safe in asthma patients,[130] but an increased risk of mortality has been reported for patients with COPD using Respimat®.[131] The high efficiency of this device also allows for the delivery of acidic solutions with pH values as low as 2.7, as well as ethanolic solutions, to be safely delivered to asthma patients without causing adverse events.[132,133] The flexibility of this platform has allowed the use of a novel $\beta_2$-agonist solution (olodaterol) in a hydroalcoholic mixture to be evaluated for pulmonary delivery.[134] Due to its capacity to produce submicron droplets, Respimat® has been employed to investigate the concept of the enhanced excipient growth approach. In this concept, particles increase in size as they enter the airways, achieving appropriate size for pulmonary deposition.[135]

Although, to our knowledge, there is no report on a systematic investigation, surface tension and viscosity of liquids may also play a role in the performance of this device. Analysis of an ethanolic solution of the steroid flunisolide showed a higher fine particle fraction and slower aerosol cloud speed (7.5 m/s) compared to an aqueous solution of $\beta_2$-agonist fenoterol containing also benzalkonium chloride and ethylenediaminetetraacetic acid (EDTA).[111] The physicochemical properties that result from the components utilized in each of the aforementioned formulations are likely to have been responsible for the apparent differences in nebulization performance. Finally, device handling is considered safe, with unintentional misuse being likely to show no harmful or unwanted side effects due to facial or ocular deposition.[136]

## Extruded jets

A three-layer laminate strip assembled to form the unit dose package of the Aer$_x$™ device (Aradigm Corporation, Hayward, CA) for the pulmonary delivery of aqueous formulations. The first layer contains a microvolume liquid reservoir blister that is heat sealed to the second (lid) layer. A nozzle array completes the third layer where micrometre holes are laser drilled. Index holes align the multilayer system, which is then connected to a handle to form a final assembled package (strip) fit to the device accordingly. During operation, a piston forces the first layer of the strip towards the nozzle array. A minimum pressure, dependent on the surface tension, is needed to impart the necessary

velocity to the liquid jet stream. As the liquid ruptures the lid layer and rapidly extrudes through the microholes, liquid break-up occurs. This break-up is dependent on the liquid viscosity that is generating the aerosol droplets.[137] This functioning mechanism produces a slow velocity mist based on a mesh technology and is commercialized as Aer$_X$™.

This technology is capable of aerosolizing solution dosage forms, including testosterone[138] and opioids[139,140] (e.g. morphine[141–144] and fentanyl[145]). An ethanolic formulation containing a poorly water-soluble prodrug candidate for pulmonary delivery was also successfully delivered using this device.[146] The prodrug had an MMAD of 3 μm and a GSD of 1.3, with a pharmacokinetic study showing systemic absorption following pulmonary delivery comparable to that of intravenous administration. Patient posture and breathing manoeuvre were not shown to influence the diffuse pattern in lung distribution of aerosols generated using this technology.[147] Despite its usual small volume reservoir (i.e. 45 μL), Aer$_X$™ is capable of delivering high doses of therapeutic agents in solution. Two inhalations from this system were twice as effective in delivering an inhaled drug candidate to the lungs compared to up to 15 min of aerosolization using conventional air-jet nebulizers. The superior performance can be attributed to the improved aerosol output (higher respirable dose) that this soft mist inhaler provides.[148]

Furthermore, protein solutions can be aerosolized using the extruded jets mechanism. When an interleukin-4 receptor drug was aerosolized to the lungs, together with a radiolabeling compound in a saline solution, a higher peripheral deposition was found when compared to air-jet nebulization.[149] The higher peripheral deposition could be explained by differences in aerosol properties that showed MMAD values of 2.0 and 3.5 μm, and GSDs of 1.35 and 2.5, for the Aer$_X$™ and air-jet nebulizer, respectively. Importantly, Aer$_X$™ delivered five times faster, three to four times more drug (relatively to their initial protein charge) than the air-jet system. Similar results were found when compared to a pMDI device for the deposition profile of a radiolabeling solution.[150] Importantly, bolus inhalation of dornase alfa using this extruded jets mechanism to treat cystic fibrosis patients may in the future be a possible alternative to the currently approved jet nebulizer systems.[151] However, the possibility of macromolecule degradation must always be considered on a case-by-case basis. DNA-based drug products can be prone to degradation following extruded jet nebulization.[152] Plasmid DNA protected by encapsulation in cationic lipids (lipoplexes) can avoid such degradation when this nonviral gene therapy formulation is aerosolized to the lungs using Aer$_X$™. Ion concentration plays an important role in production of aerosols via this mechanism due to suppression of electrostatic charges.[153] And the addition of sodium chloride to lipoplex formulations has shown an improved emitted dose.[152]

The possibility of delivering insulin to diabetes patients via the lungs is a subject that has been widely investigated.[154] Insulin solutions have also been delivered with this technology.[140,155] In particular, this has been the only system used for inhaled insulin in liquid dosage form when most of the other attempts are with formulations in dry powder form.[6,156] Recently, a long-term study comparing prandial inhaled insulin compared to subcutaneous administration showed encouraging results.[157] In this insulin study using the iDMS technology, the authors concluded that, after one year, both routes of administration of insulin were comparably safe and efficacious, although further optimization was needed to avoid risk of nocturnal hypoglycaemia with the inhaled dosage form.

When drug particles are in the nanoscale size range, this technology can also produce aerosol from dispersed systems. Solid lipid nanosuspensions of ketoprofen and indomethacin were prepared via supercritical fluid extraction of emulsions. Aerosolization using Aer$_X$™ and Aer$_X$™ Essence (electronically and mechanically controlled) produced fine particle fractions of 60–80% and emitted doses of 50–60%, which resulted in fine particle doses of approximately 40%.[158,159] Importantly, suspensions of a few hundred nanometres were not as effectively delivered using micron-sized nozzle extruders as those suspensions with drug particle sizes below 100 nm.[158,160] Sub-micron-sized nozzle extruders are also being considered for development, in which viscosity and drug particle size of dispersions are expected to have a greater impact on the aerosolization profile.[159] In addition, a miniaturized version of Aer$_X$™ has been developed and is due to be used in large animals (i.e. dogs).[161] This system might bring great value to future proof-of-concept studies for safety and tolerability of drug candidates for inhalation therapy.

Medspray® (Medspray BV, Enschede, Netherlands) is a recently developed technology that applies the extruded jets principle from the Rayleigh break-up theory to produce aerosols.[162,163] It is a hand-held, liquid metered-dose inhaler in which lithography (wafer stepper and etching techniques) is used to engineer different micron-sized spray nozzles. Following actuation by the patient, a loaded spring mechanically controls the release of the drug solution contained in a metering valve. As the liquid formulation is extruded through the spray nozzle, the patient's inspiratory flow pulls the formed droplets from a Venturi-like mouthpiece channel into the lungs. The device therefore requires some synchronization, with the patient pushing the drug release button a few seconds after initializing the inspira-

tory manoeuvre. On the other hand, as the aerosol production rate is controlled by the device (spring), it avoids dose emission variability that could be caused by differences in pressure and speed of actuation by a patient. A slow mist (4 m/s) is created at an inspiratory flow of 30 L/min by a patient. Weber further considered the influence of liquid viscosity on Rayleigh's basic analysis of jet instability to describe a relationship between water aerosol droplets and nozzle diameters.[162,164] During the development phase of the Medspray® inhaler, nozzles of 1.5, 2.0 and 2.5 μm in diameter generated droplets with aerodynamic diameters of 4.0, 5.0, and 6.0 μm, respectively. Further studies showed that the larger droplets (6.0 μm) are more effective for improving the pulmonary function in asthmatic patients.[165]

### Electrohydrodynamic mechanism (Mystic™)

In the Mystic™ drug delivery platform (Battelle Memorial Institute, Inc., Columbus, OH) liquid is slowly fed to a positive potential, electronically controlled capillary nozzle surrounded by a gas flow sheath. An electric field is then created between the nozzle and a counter-electrode; also positively charged, independently from the capillary nozzle. A Taylor cone-jet is formed between the capillary nozzle and the counter-electrode once the electrical stress outbalances the surface tension, generating charged droplets. Subsequently, a corona discharge controls the droplet charge generating a monodisperse aerosol.[166] This functioning mechanism is called electrohydrodynamic atomization (EHDA) or electrospray and has been recently adapted for pulmonary delivery of drugs.[167] Under the trade name Mystic™, it is currently being developed by the Battelle Memorial Institute.[168] This technique is also widely used in pharmaceutical applications for ionization in mass spectroscopy,[169] thin film formation [170,171] and particle engineering.[172–175] Particularly, this technique can consistently produce highly monodisperse aerosols (with GSD values between 1.2 and 1.4).[176]

Control of certain variables during EHDA can greatly benefit the aerosol generation for inhalation purposes. Flow rate is directly related to droplet size while surface tension presents an inverse relationship.[166,176,177] The surrounding gas sheath influences the electric breakdown threshold, preventing corona discharge at the tip of the nozzle. Utilization of a small concentration of carbon dioxide (0.5%) in the gas sheath helps stabilize the electrospray in cases when fluids of high surface tension (i.e. pure water) require a voltage greater than the electric breakdown threshold. Ion concentration can also help stabilize the electrospray and produce smaller droplet sizes. When adding low concentrations of sodium chloride (0.005% w/w) to pure water, increased water conductivity can be achieved while not affecting surface tension. Thus, electrical current can flow more effectively, producing smaller particle sizes.[176] However, higher concentrations of NaCl can increase polydispersity, which can be a problem for pulmonary delivery of certain pharmaceutical preparations (i.e. isotonic solutions).[166] Viscosity also appears to influence aerosol generation with this mechanism, although systematic investigation is warranted.[176] Droplet charge control through the corona discharge system can avoid deposition in the oropharynx despite droplet size.[166] An increase in drug concentration can increase droplet size and polydispersity, but does not change MMAD and GSD values significantly over time for the same aerosolized system.[176]

Clinical trials using EHDA aerosol generation have shown the feasibility of delivering ethanolic solutions of beclomethasone dipropionate.[178] Interestingly, evaluation of monodisperse aerosols (GSD < 1.2) shows bioavailability of larger droplets (MMADs of 2.5 and 4.5 μm) to be greater when compared to small droplet aerosols (MMAD of 1.5 μm). Additionally, this technology can produce aerosols from dispersed dosage forms.[179] Electrospraying of negatively charged nanoliposomes of DPPC, 1,2-Dipalmitoyl-sn-Glycerol-3-[Phospho-rac-(1-glycerol)] sodium salt (DPPGNa) and cholesterol presented a bimodal size distribution (peaks at 35 and 100 nm) caused by different agglomeration patterns inside the capillary nozzle during aerosolization.[180] Head-to-tail and side-by-side juxtaposition were identified during aerosolization of suspensions with high lipid mass concentration. Notably, the physicochemical properties of the dispersed system (i.e. drug particle size of nanosuspension) can influence the jet break-up characteristics.[181] Nevertheless, EHDA is a gentle technique that can be successfully used in the ionization of macromolecules for analysis with mass spectroscopy.[182,183] Not surprisingly, large biomolecules are aerosolized with this mechanism without suffering thermal degradation, even at high concentrations of protein solutions.[184] Very importantly, this technology has shown to be more effective for aerosol delivery of gene therapy than jet, ultrasonic and vibrating-mesh nebulizers.[185] Recently, the electrohydrodynamic principle has been used to develop a novel nanoaerosol device.[186] However, further studies are warranted due to the challenges of pulmonary delivery of nanoaerosols, such as high proportion of exhaled nanodroplets and limited dose due to high rate of droplet coagulation at high aerosol concentration.

## Investigational Nebulizers

A summary of the technologies and their functioning mechanisms are presented in Table 3.

**Table 3** Investigational nebulizers and their functioning mechanisms

| Technology | Functioning mechanism |
|---|---|
| Surface acoustic wave microfluidic atomization | This technology uses propagating waves to generate aerosols similarly to traditional ultrasonic nebulizer. Instead of millimetre-order wavelengths propagating through the bulk liquid, the nanometre amplitude Raleigh waves travel on the surface of a piezoelectric substrate at a much higher frequency (10–20 MHz). With a microsyringe pump continuously delivering a solution on top of a lithium niobate ($LiNbO_3$) substrate, the x-propagating acoustic waves generate aerosol from the formed capillary waves. |
| Capillary aerosol generator | Liquid is pumped into one end of a heated microcapillary. Once inside the tube, the formulation vapourizes before it exits from the other end where it mixes with the cooler surrounding air. This cooling causes the vapour to supersaturate and therefore initiate nucleation. A subsequent increase in droplet size occurs due to condensation of the surrounding vapour onto the formed nuclei, generating the aerosol. Reservoir chambers are used to control droplet coagulation and, consequently, droplet size. |

## Surface acoustic wave microfluidic atomization

Much like the traditional ultrasonic nebulizers, this novel technology uses propagating waves to generate aerosols. However, it is designed in a way that, instead of millimetre-order wavelengths propagating through the bulk liquid, the nanometre amplitude Raleigh waves travel on the surface of a piezoelectric substrate at a much higher frequency (10–20 MHz).[187–189] The surface acoustic wave (SAW) is therefore a highly efficient method to drive fluid motion. With a microsyringe pump continuously delivering a solution on top of the lithium niobate ($LiNbO_3$) substrate, the x-propagating acoustic waves generate aerosol from the formed capillary waves.[187] With a significantly more efficient energy transfer, a considerably lower energy input is required (1–3 W). A lower energy input results in the feasibility of a portable hand-held device.[190]

The droplet diameter during SAW atomization is directly proportional to surface tension and inversely proportional to the viscosity of liquids.[191] Due to the higher surface tension and lower viscosity, water produces larger droplets when compared to fluids such as ethanol and octanol.[190] Ethanol and octanol have similar surface tensions (22–27 mN/m), but the latter presents a greater viscosity of 7.3 cP, compared to 1.1 cP for ethanol. Aerosolization of octanol using SAW results in smaller droplets than with ethanol. Further development of this system could therefore be an alternative to jet nebulizers for aerosolization of highly viscous fluids due to the limitations described above for other nebulizer types (i.e. ultrasonic and vibrating-mesh). Of equal importance, the aerosol output is directly related to the power input, but its increase compromises droplet size and dispersity.[192] In general, an optimal power input to produce aerosols for delivery to the deep lung at a reasonable rate has been shown to be around 1.5 watts.[190]

The delivery of large molecules is expected to be feasible as proteins have been shown to maintain their activity.[193,194] The pulmonary delivery of plasmid DNA using this technology has also shown to be feasible and insulin solutions have been successfully aerosolized.[192,195] Recent studies have shown that limiting the amplitude modulation to 1 KHz mitigates potential protein denaturation and plasmid DNA fragmentation without compromising nebulization performance; an important advance of this technology to enable gene and vaccine delivery.[196] The SAW microfluidic process may be unsuitable for atomization of dispersed systems (i.e. suspensions) due to concentration of particles via nucleation templating.[197,198] But this *a priori* disadvantage has further found an application in the production of pharmaceutical nanoparticles.[193,199,200]

## Capillary aerosol generator

In this aerosolization process, a liquid solution is pumped into one end of a heated microcapillary. Once inside the tube, the formulation vapourizes before it exits from the other end where it mixes with the cooler surrounding air. This cooling causes the vapour to supersaturate and therefore initiate nucleation. A subsequent increase in droplet size occurs due to condensation of the surrounding vapour onto the formed nuclei, generating the desired aerosol for pulmonary delivery.[201,202] The appropriate droplet size can be achieved by controlling droplet coagulation using reservoir chambers.[203]

The surface tension and the viscosity of liquids appear to greatly influence the production of aerosols from CAG. Using a variety of vehicles, the values found for MMAD varied greatly, up to ten times.[204] Furthermore, both concentration and the physicochemical characteristics of solutes influence the aerosol generation.[205] As seen with Respimat®, this type of nebulizer has been used to pro-

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duce submicron droplets to investigate the concept of enhance excipient growth.[135] Importantly, by dissolving benzyl in propylene glycol, it has been shown that both evaporate and condensate simultaneously.[203] The aerosol droplet is also dependent on energy input, with trough in MMAD at about 40 Joules.[206] It is not feasible to aerosolize thermolabile substances using CAG, as the vapour jet temperature reaches between 150 to 200 °C.[206] Studies with the antiemetic perphenazine dissolved in propylene glycol required a higher energy input (84–95 J), but still showed acceptable stability of this substance with the CAG aerosolization process.[207] So far, utilization of this technology intended for inhalation therapy has been limited to preclinical studies.[208]

## Characterization of nebulizer formulations

In spite of the great significance of nebulization therapy in clinical practice, very little has been done to standardize the characterization of nebulizer formulations. Assessment of the nebulizer device itself is available under an European Standard[209] and the European Respiratory Society presents guidelines on nebulization therapy.[1] Nebulizers were not covered in the United States Pharmacopoeia (USP) General Chapter *<601> Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers*. Only very recently, the first supplement of USP 34 – NF 29 brings the standardization of characterization tests for nebulizer products.

The General Chapter *<1601> Products for Nebulization – Characterization Test* of the USP now establishes, based on the dose delivered to a patient intrinsic to the formulation characteristics in conjunction with the device chosen (nebulizer system), two analyses for the assessment of nebulization performance:

- Drug Substance Delivery Rate and Total Drug Substance Delivered (TDD); and
- Aerodynamic Assessment of Nebulized Aerosols.

The first test determines the rate and total amount of drug delivered. A breathing simulator is recommended to be used at specific airflow rates, established depending on the targeted patient population (neonates, infants, children or adults).[210] Instead of continuous delivery, breathing patterns more appropriately measure drug mass output from nebulizers. In this analysis, a volume of formulation specified for therapy is filled to the nebulizer reservoir. The device, positioned as intended to use, is connected to a filter enclosed in a holder, which is then connected to the breathing simulator. The nebulization is started and, at regular intervals, the filter is substituted for a new one. The drug mass deposited in each filter is then suitably analysed and used to calculate the results as follows:

$$R_i = \frac{m_i}{t_i} \quad \text{TDD} = \sum_{i=1}^{n} m_i$$

Where $R_i$, $m_i$ and $t_i$ are the rate, the drug mass and the time interval used for collection at the ith interval, respectively, and $n$ is the total number of filters collected.

Among various cascade impactors, the next-generation impactor (NGI) is the apparatus recommended by the USP for the assessment of aerodynamic droplet sizes from nebulizer systems, because it is a direct measurement of drug mass deposited based on aerodynamic droplet sizes.[211–216] Alternatively, laser diffractometry is accepted for droplet size measurement specifically for homogeneous solutions, but not for dispersed systems or when significant droplet evaporation occurs.[217,218] The test should be performed at airflow rate of 15 L/min and with a cooled impactor to avoid droplet evaporation.[219–221] The seven stages of the NGI therefore present the following cut-off diameters: 0.98, 1.36, 2.08, 3.30, 5.39, 8.61, and 14.1 μm. Besides the micro-orifice collector (MOC) plate, an external filter is also recommended to collect very small droplets. Plate coating to avoid droplet bounce and re-entrainment, and the use of a preseparator are unnecessary. Impactor stage overloading should be avoided by adequately establishing a feasible time interval for drug deposition during the test, a balancing capability with that of sensitivity of the analytical method employed to determine drug mass.

If a normal distribution of the deposited drug is observed, the MMAD and GSD can be determined from the log cut-off size versus probability scale (probit) of cumulative mass, starting at the MOC/external filter. Intercept of this curve identifies MMAD, as probit of 50% is equal to zero. GSD can be determined from the slope of the linear portion of the curve or as follows:

$$\text{GSD} = \sqrt{\frac{\text{Size relative to } 84.13\% \text{ deposition}}{\text{Size relative to } 15.87\% \text{ deposition}}}$$

The mass fraction of drug deposited in each plate should also be presented, including the deposition in the induction port.

The characterization of the physicochemical properties of the formulations is very important to help determine the factors influencing droplet formation from nebulization systems with different functioning mechanisms. There are innumerous methods available to measure surface tension, including the Capillary Rise and the Du Noüy ring methods.[222,223] Recently, our group has developed a simple

and quick method using a Texture Analyser.[224] Likewise, viscosity can be measured using various techniques, such as: capillary (or Ostwald-Cannon-Fenske) viscometer, falling-sphere viscometer, and rotational (cup-and-bob, and cone-and-plate) viscometers.[225,226]

When dispersed systems (e.g. suspensions, liposomes, etc.) are to be nebulized, it is very important to characterize the drug particles in bulk liquid in order to better understand the nebulization performance based on the different mechanisms of aerosol generation from the appropriate devices.[227] Among the different methods, measurement of drug particle size and charge should be considered. Particle size and particle size distribution can be analysed via laser diffraction or dynamic light scattering.[228] Measurement of zeta potential based on the principle of dynamic electrophoretic mobility can inform the magnitude of attraction or repulsion between particles in suspension.[229,230] Very importantly, it should be considered that the rheology of dispersions (i.e. suspensions and emulsions) is much more complex than the simple measurement of viscosity for Newtonian fluids.[231,232] Non-Newtonian behaviour of fluids may be a factor influencing the nebulization performance of these systems depending on the type of nebulizer used. In addition, the aerosol output from these systems, based on gravimetrical analysis, may be misleading with respect to the real drug mass that is being aerosolized.

## Conclusions

The technology to produce aerosols from liquid formulations for inhalation therapy has greatly evolved in a continuous manner from the traditional jet and ultrasonic nebulizers to emerging technologies based on mechanisms such as surface acoustic waves, electrohydrodynamic atomization, and capillary aerosol generation. Smart technologies have further improved success through monitoring of patient adherence to therapy. The physicochemical properties of the formulations in conjunction with the nebulizer design and mechanism of function greatly determines the aerosolization performance. Increase in temperature in the nebulizing formulation has been reported for several types of devices and care should be taken when evaluating thermolabile drugs, such as protein therapeutics. Overwhelmingly, ion concentration, surface tension and viscosity can highly influence aerosol generation and a greater understanding of their role in nebulization performance is a large part of the puzzle towards improved nebulization therapies. The recent establishment of compendial characterization tests for nebulization products will greatly favour *in vitro* comparison of devices, which should ultimately translate into better *in vivo* efficiency.

## References


1. Boe J *et al.* European Respiratory Society Guidelines on the use of nebulizers. *Eur Respir J* 2001; 18: 228–242.
2. Leung K *et al.* Comparison of breath-enhanced to breath-actuated nebulizers for rate, consistency, and efficiency. *Chest* 2004; 126: 1619–1627.
3. Denyer J, Dyche T. The adaptive aerosol delivery (AAD) technology: past, present, and future. *J Aerosol Med Pulm Drug Deliv* 2010; 23: S1–S10.
4. Hardaker LEA, Hatley RHM. In vitro characterization of the I-neb adaptive aerosol delivery (AAD) system. *J Aerosol Med Pulm Drug Deliv* 2010; 23: S11–S20.
5. Geller DE. The science of aerosol delivery in cystic fibrosis. *Pediatr Pulmonol* 2008; 43: S5–S17.
6. Mudaliar S. Inhaled insulin using AERx (R) insulin diabetes management system (AERx (R) iDMS). *Expert Opin Investig Drugs* 2007; 16: 1673–1681.
7. Owens DR *et al.* Alternative routes of insulin delivery. *Diabet Med* 2003; 20: 886–898.
8. Julius SM *et al.* Accuracy of three electronic monitors for metered-dose inhalers. *Chest* 2002; 121: 871–876.
9. Carvalho TC *et al.* Influence of particle size on regional lung deposition – What evidence is there? *Int J Pharm* 2011; 406: 1–10.
10. Ocallaghan C *et al.* Inaccurate calculation of drug output from nebulizers. *Eur J Pediatr* 1989; 148: 473–474.
11. Thi THH *et al.* Characterization and in vitro evaluation of the formoterol/cyclodextrin complex for pulmonary administration by nebulization. *Eur J Pharm Biopharm* 2009; 72: 214–218.
12. Elhissi AMA, Taylor KMG. Delivery of liposomes generated from proliposomes using air-jet, ultrasonic, and vibrating-mesh nebulisers. *J Drug Deliv Sci Technol* 2005; 15: 261–265.
13. Gurses BK, Smaldone GC. Effect of tubing deposition, breathing pattern, and temperature on aerosol mass distribution measured by cascade impactor. *J Aerosol Med-Deposit Clearance Effects Lung* 2003; 16: 387–394.
14. McCallion ONM *et al.* Jet nebulisers for pulmonary drug delivery. *Int J Pharm* 1996; 130: 1–11.
15. Mercer TT. Production of therapeutic aerosols – principles and techniques. *Chest* 1981; 80: 813–818.
16. Smith EC *et al.* Comparison of 23 nebulizer compressor combinations for domiciliary use. *Eur Respir J* 1995; 8: 1214–1221.
17. Bendstrup KE *et al.* Characterization of heparin aerosols generated in jet and ultrasonic nebulizers. *J Aerosol Med-Deposit Clearance Effects Lung* 1999; 12: 17–25.
18. Coates AL *et al.* The choice of jet nebulizer, nebulizing flow, and addition of albuterol affects the output of

tobramycin aerosols. *Chest* 1997; 111: 1206–1212.
19. Arzhavitina A, Steckel H. Surface active drugs significantly alter the drug output rate from medical nebulizers. *Int J Pharm* 2010; 384: 128–136.
20. Pilcer G, Amighi K. Formulation strategy and use of excipients in pulmonary drug delivery. *Int J Pharm* 2010; 392: 1–19.
21. Sood BG *et al.* Jet nebulization of prostaglandin E-1 during neonatal mechanical ventilation: stability, emitted dose and aerosol particle size. *Pharmacol Res* 2007; 56: 531–541.
22. Steckel H, Eskandar F. Factors affecting aerosol performance during nebulization with jet and ultrasonic nebulizers. *Eur J Pharm Sci* 2003; 19: 443–455.
23. McCallion ONM *et al.* Nebulization of fluids of different physicochemical properties with air-jet and ultrasonic nebulizers. *Pharm Res* 1995; 12: 1682–1688.
24. McCallion ONM *et al.* The influence of surface tension on aerosols produced by medical nebulisers. *Int J Pharm* 1996; 129: 123–136.
25. McCallion ONM, Patel MJ. Viscosity effects on nebulisation of aqueous solutions. *Int J Pharm* 1996; 130: 245–249.
26. Broniarz-Press L *et al.* The effect of shear and extensional viscosity on atomization in medical inhaler. *Int J Pharm* 2014; 468: 199–206.
27. Diot P *et al.* RhDNase I aerosol deposition and related factors in cystic fibrosis. *Am J Respir Crit Care Med* 1997; 156: 1662–1668.
28. Johnson JC *et al.* Aerosol delivery of recombinant human DNase I. In vitro comparison of a vibrating-mesh nebulizer with a jet nebulizer. *Respiratory Care* 2008; 53: 1703–1708.
29. Fiel SB *et al.* Comparison of three jet nebulizer aerosol delivery systems used to administer recombinant human DNase-I to patients with cystic fibrosis. *Chest* 1995; 108: 153–156.
30. Geller DE *et al.* Effect of smaller droplet size of dornase alfa on lung function in mild cystic fibrosis. *Pediatr Pulmonol* 1998; 25: 83–87.
31. Germershaus O *et al.* Insulin-like growth factor-I aerosol formulations for pulmonary delivery. *Eur J Pharm Biopharm* 2013; 85: 61–68.
32. Niven RW *et al.* Protein nebulization.2. Stabilization of G-CSF to air-jet nebulization and the role of protectants. *Int J Pharm* 1996; 127: 191–201.
33. Albasarah YY *et al.* Stabilizing protein formulations during air-jet nebulization. *Int J Pharm* 2010; 402: 140–145.
34. Chang LQ, Pikal MJ. Mechanisms of protein stabilization in the solid state. *J Pharm Sci* 2009; 98: 2886–2908.
35. Steckel H *et al.* Effect of cryoprotectants on the stability and aerosol performance of nebulized aviscumine, a 57-kDa protein. *Eur J Pharm Biopharm* 2003; 56: 11–21.
36. Flament MP *et al.* Influence of formulation on jet nebulisation quality of alpha(1) protease inhibitor. *Int J Pharm* 1999; 178: 101–109.
37. Terzano C *et al.* Comparison of the efficacy of beclometasone dipropionate and fluticasone propionate suspensions for nebulization in adult patients with persistent asthma. *Respir Med* 2003; 97: S35–S40.
38. Britland S *et al.* Droplet aerodynamics, cellular uptake, and efficacy of a nebulizable corticosteroid nanosuspension are superior to a micronized dosage form. *Biotechnol Prog* 2012; 28: 1152–1159.
39. Barry PW, O'Callaghan C. An in vitro analysis of the output of budesonide from different nebulizers. *J Allergy Clin Immunol* 1999; 104: 1168–1173.
40. Dahlstrom K *et al.* Systemic availability and lung deposition of budesonide via three different nebulizers in adults. *Annals Allergy Asthma Immunol* 2003; 90: 226–232.
41. Berg EB, Picard RJ. In vitro delivery of budesonide from 30 jet nebulizer/compressor combinations using infant and child breathing patterns. *Respiratory Care* 2009; 54: 1671–1678.
42. Vaghi A *et al.* In vitro comparison of nebulised budesonide (Pulmicort Respuless (R)) and beclomethasone dipropionate (Clenil (R) per Aerosol). *Pulm Pharmacol Ther* 2005; 18: 151–153.
43. Najlah M *et al.* The effects of suspension particle size on the performance of air-jet, ultrasonic and vibrating-mesh nebulisers. *Int J Pharm* 2014; 461: 234–241.
44. Amani A *et al.* Evaluation of a nanoemulsion-based formulation for respiratory delivery of budesonide by nebulizers. *Aaps Pharmscitech* 2010; 11: 1147–1151.
45. Nasr M *et al.* Amphotericin B lipid nanoemulsion aerosols for targeting peripheral respiratory airways via nebulization. *Int J Pharm* 2012; 436: 611–616.
46. Moazeni E *et al.* Preparation and evaluation of inhalable itraconazole chitosan based polymeric micelles. *DARU J Pharmaceut Sci* 2012; 20: 85.
47. Gilani K *et al.* Development of respirable nanomicelle carriers for delivery of amphotericin B by jet nebulization. *J Pharm Sci* 2011; 100: 252–259.
48. Osman R *et al.* Enhanced properties of discrete pulmonary deoxyribonuclease I (DNaseI) loaded PLGA nanoparticles during encapsulation and activity determination. *Int J Pharm* 2011; 408: 257–265.
49. Tadros MI. The influence of sodium hyaluronate, L-leucine and sodium taurocholate on the nebulization of aqueous betamethasone-17-valerate suspensions. *Aaps Pharmscitech* 2008; 9: 243–249.
50. Dailey LA *et al.* Nebulization of biodegradable nanoparticles: impact of nebulizer technology and nanoparticle characteristics on aerosol features. *J Controlled Release* 2003; 86: 131–144.
51. Gaspar MM *et al.* Inhaled liposomes-current strategies and future challenges. *J Biomed Nanotechnol* 2008; 4: 245–257.
52. Waldrep JC *et al.* High dose cyclosporin A and budesonide-liposome aerosols. *Int J Pharm* 1997; 152: 27–36.

53. Bridges PA, Taylor KMG. An investigation of some of the factors influencing the jet nebulisation of liposomes. *Int J Pharm* 2000; 204: 69–79.
54. Desai TR *et al.* A facile method of delivery of liposomes by nebulization. *J Controlled Release* 2002; 84: 69–78.
55. Davies LA *et al.* Aerosol delivery of DNA/Liposomes to the lung for cystic fibrosis gene therapy. *Human Gene Therapy Clin Develop* 2014; 25: 97–107.
56. Elhissi AMA *et al.* Formulations generated from ethanol-based proliposomes for delivery via medical nebulizers. *J Pharm Pharmacol* 2006; 58: 887–894.
57. Elhissi AMA *et al.* Physical stability and aerosol properties of liposomes delivered using an air-jet nebulizer and a novel micropump device with large mesh apertures. *Int J Pharm* 2007; 334: 62–70.
58. Chimote G, Banerjee R. In vitro evaluation of inhalable isoniazid-loaded surfactant liposomes as an adjunct therapy in pulmonary tuberculosis. *J Biomed Mat Res Part B-Appl Biomat* 2010; 94B: 1–10.
59. Chimote G, Banerjee R. Evaluation of antitubercular drug-loaded surfactants as inhalable drug-delivery systems for pulmonary tuberculosis. *J Biomed Mater Res, Part A* 2009; 89A: 281–292.
60. Yeo LY *et al.* Ultrasonic nebulization platforms for pulmonary drug delivery. *Expert Opin Drug Delivery* 2010; 7: 663–679.
61. Ramisetty KA *et al.* Investigations into ultrasound induced atomization. *Ultrason Sonochem* 2013; 20: 254–264.
62. Beck-Broichsitter M *et al.* Impact of lyoprotectants for the stabilization of biodegradable nanoparticles on the performance of air-jet, ultrasonic, and vibrating-mesh nebulizers. *Eur J Pharm Biopharm* 2012; 82: 272–280.
63. Flament MP *et al.* Influence of the technological parameters of ultrasonic nebulisation on the nebulisation quality of alpha 1 protease inhibitor (alpha 1I). *Int J Pharm* 1999; 189: 197–204.
64. Khatri L *et al.* An assessment of jet and ultrasonic nebulisers for the delivery of lactate dehydrogenase solutions. *Int J Pharm* 2001; 227: 121–131.
65. Broniarz-Press L *et al.* The effect of shear and extensional viscosities on atomization of Newtonian and non-Newtonian fluids in ultrasonic inhaler. *Int J Pharm* 2015; 485: 41–49.
66. Nikander K *et al.* The conventional ultrasonic nebulizer proved inefficient in nebulizing a suspension. *J Aerosol Med-Deposit Clearance Effects Lung* 1999; 12: 47–53.
67. Lehofer B *et al.* Impact of atomization technique on the stability and transport efficiency of nebulized liposomes harboring different surface characteristics. *Eur J Pharm Biopharm* 2014; 88: 1076–1085.
68. Videira MA *et al.* Lymphatic uptake of pulmonary delivered radiolabelled solid lipid nanoparticles. *J Drug Target* 2002; 10: 607–613.
69. Pardeike J *et al.* Development of an Itraconazole-loaded nanostructured lipid carrier (NLC) formulation for pulmonary application. *Int J Pharm* 2011; 419: 329–338.
70. Newman S, Gee-Turner A. The Omron MicroAir vibrating mesh technology nebuliser, a 21st century approach to inhalation therapy. *J Applied Therap Res* 2005; 5: 29–33.
71. Elhissi A *et al.* Air-jet and vibrating-mesh nebulization of niosomes generated using a particulate-based proniosome technology. *Int J Pharm* 2013; 444: 193–199.
72. Lass JS *et al.* New advances in aerosolised drug delivery: vibrating membrane nebuliser technology. *Exp Opin Drug Deliv* 2006; 3: 693–702.
73. Watts AB *et al.* Current therapies and technological advances in aqueous aerosol drug delivery. *Drug Dev Ind Pharm* 2008; 34: 913–922.
74. Hertel S *et al.* That's cool! – Nebulization of thermolabile proteins with a cooled vibrating mesh nebulizer. *Eur J Pharm Biopharm* 2014; 87: 357–365.
75. Coates AL *et al.* Higher Tobramycin concentration and vibrating mesh technology can shorten antibiotic treatment time in cystic fibrosis. *Pediatr Pulmonol* 2011; 46: 401–408.
76. Coates AL *et al.* Testing of nebulizers for delivering magnesium sulfate to pediatric asthma patients in the emergency department. *Respiratory Care* 2011; 56: 314–318.
77. Ghazanfari T *et al.* The influence of fluid physicochemical properties on vibrating-mesh nebulization. *Int J Pharm* 2007; 339: 103–111.
78. Zhang G *et al.* Performance of the vibrating membrane aerosol generation device: Aeroneb micropump nebulizer (TM). *J Aeros Med-Deposit Clear Effects Lung* 2007; 20: 408–416.
79. Chan JGY *et al.* Delivery of high solubility polyols by vibrating mesh nebulizer to enhance mucociliary clearance. *J Aerosol Med Pulm Drug Deliv* 2012; 25: 297–305.
80. Beck-Broichsitter M *et al.* On the correlation of output rate and aerodynamic characteristics in vibrating-mesh-based aqueous aerosol delivery. *Int J Pharm* 2014; 461: 34–37.
81. Beck-Broichsitter M *et al.* Nebulization of active pharmaceutical ingredients with the eFlow®rapid: impact of formulation variables on aerodynamic characteristics. *J Pharm Sci* 2014; 103: 2585–2589.
82. Chan JGY *et al.* Mannitol delivery by vibrating mesh nebulisation for enhancing mucociliary clearance. *J Pharm Sci* 2011; 100: 2693–2702.
83. Najlah M *et al.* A study of the effects of sodium halides on the performance of air-jet and vibrating-mesh nebulizers. *Int J Pharm* 2013; 456: 520–527.
84. Simones MP *et al.* Measurement of the size and charge distribution of sodium chloride particles generated by an Aeroneb Pro® pharmaceutical nebulizer. *Europ J Nanomed* 2014; 6: 29–36.
85. Rottier BL *et al.* Changes in performance of the Pari eFlow (R) rapid and Pari LC Plus (TM) during 6 months use by CF patients. *J Aerosol Med Pulm Drug Deliv* 2009; 22: 263–269.
86. Pitance L *et al.* Delivery efficacy of a vibrating mesh nebulizer and a jet

nebulizer under different configurations. *J Aerosol Med Pulm Drug Deliv* 2010; 23: 389–396.
87. Ari A *et al.* Influence of nebulizer type, position, and bias flow on aerosol drug delivery in simulated pediatric and adult lung models during mechanical ventilation. *Respiratory Care* 2010; 55: 845–851.
88. Skaria S, Smaldone GC. Omron NE U22: Comparison between vibrating mesh and jet nebulizer. *J Aerosol Med Pulm Drug Deliv* 2010; 23: 173–180.
89. Coates AL *et al.* A comparison of amount and speed of deposition between the PARI LC STAR® jet nebulizer and an investigational eFlow® nebulizer. *J Aerosol Med Pulm Drug Deliv* 2011; 24: 157–163.
90. Lenney W *et al.* Lung deposition of inhaled tobramycin with eFlow rapid/LC Plus jet nebuliser in healthy and cystic fibrosis subjects. *J Cyst Fibros* 2011; 10: 9–14.
91. Harris K. *et al.* In vitro effects of vibrating mesh nebulization and radiopharmaceuticals on interferon gamma. In: European Respiratory Society. 2007.
92. Scherer T *et al.* A technical feasibility study of dornase alfa delivery with eflow® vibrating membrane nebulizers: aerosol characteristics and physicochemical stability. *J Pharm Sci* 2011; 100: 98–109.
93. Hertel S *et al.* Prediction of protein degradation during vibrating mesh nebulization via a high throughput screening method. *Eur J Pharm Biopharm* 2014; 87: 386–394.
94. McConville JT *et al.* Targeted high lung concentrations of itraconazole using nebulized dispersions in a murine model. *Pharm Res* 2006; 23: 901–911.
95. Tam JM *et al.* Amorphous cyclosporin nanodispersions for enhanced pulmonary deposition and dissolution. *J Pharm Sci* 2008; 97: 4915–4933.
96. Vaughn JM *et al.* Single dose and multiple dose studies of itraconazole nanoparticles. *Eur J Pharm Biopharm* 2006; 63: 95–102.
97. Beck-Broichsitter M *et al.* Following the concentration of polymeric nanoparticles during nebulization. *Pharm Res* 2013; 30: 16–24.
98. Beck-Broichsitter M *et al.* Boosting the aerodynamic properties of vibrating-mesh nebulized polymeric nanosuspensions. *Int J Pharm* 2014; 459: 23–29.
99. Watts AB *et al.* Preclinical evaluation of tacrolimus colloidal dispersion for inhalation. *Eur J Pharm Biopharm* 2011; 77: 207–215.
100. Beck-Broichsitter M *et al.* Pulmonary drug delivery with aerosolizable nanoparticles in an ex vivo lung model. *Int J Pharm* 2009; 367: 169–178.
101. Packhaeuser CB *et al.* Stabilization of aerosolizable nano-carriers by freeze-drying. *Pharm Res* 2009; 26: 129–138.
102. Li ZL *et al.* Characterization of nebulized liposomal amikacin (Arikace (TM)) as a function of droplet size. *J Aerosol Med Pulm Drug Deliv* 2008; 21: 245–253.
103. Elhissi AMA *et al.* Nebulization of ultradeformable liposomes: the influence of aerosolization mechanism and formulation excipients. *Int J Pharm* 2012; 436: 519–526.
104. Elhissi A *et al.* Vibrating-mesh nebulization of liposomes generated using an ethanol-based proliposome technology. *J Liposome Res* 2011; 21: 173–180.
105. Beck-Broichsitter M *et al.* Correlation of drug release with pulmonary drug absorption profiles for nebulizable liposomal formulations. *Eur J Pharm Biopharm* 2013; 84: 106–114.
106. Fuchs S *et al.* Towards an inhalative in vivo application of immunomodulating gelatin nanoparticles in horse-related preformulation studies. *J Microencapsul* 2012; 29: 615–625.
107. Nasr M *et al.* PAMAM dendrimers as aerosol drug nanocarriers for pulmonary delivery via nebulization. *Int J Pharm* 2014; 461: 242–250.
108. Carvalho TC *et al.* Development and characterization of phospholipid-stabilized submicron aqueous dispersions of coenzyme Q10 presenting continuous vibrating-mesh nebulization performance. *J Liposome Res* 2013; 23: 276–290.
109. Longest PW, Hindle M. Evaluation of the respimat soft mist inhaler using a concurrent CFD and in vitro approach. *J Aerosol Med Pulm Drug Deliv* 2009; 22: 99–112.
110. Pitcairn G *et al.* Deposition of corticosteroid aerosol in the human lung by Respimat (R) Soft Mist (TM) Inhaler compared to deposition by metered dose inhaler or by Turbuhaler (R) dry powder inhaler. *J Aeros Med-Deposit Clear Effects Lung* 2005; 18: 264–272.
111. Zierenberg B. Optimizing the in vitro performance of Respimat. *J Aeros Med-Deposit Clear Effects Lung* 1999; 12: S19–S24.
112. Hochrainer D *et al.* Comparison of the aerosol velocity and spray duration of Respimat (R) Soft Mist (TM) Inhaler and pressurized metered dose inhalers. *J Aeros Med-Deposit Clearan Effects Lung* 2005; 18: 273–282.
113. Dalby R *et al.* A review of the development of Respimat((R)) Soft Mist (TM) Inhaler. *Int J Pharm* 2004; 283: 1–9.
114. Dolovich MB, Dhand R. Aerosol drug delivery: developments in device design and clinical use. *Lancet* 2011; 377: 1032–1045.
115. Keating G. Tiotropium Respimat® Soft Mist™ Inhaler: a review of its use in chronic obstructive pulmonary disease. *Drugs* 2014; 74: 1801–1816.
116. Spallek MW *et al.* Optimizing nozzles for soft mist inhalers. In: *Respiratory Drug Delivery VIII*. Tucson, AZ, USA: Virginia Commonwealth University, Richmond, VA, USA, 2002.
117. Son YJ, McConville JT. Advancements in dry powder delivery to the lung. *Drug Dev Ind Pharm* 2008; 34: 948–959.
118. Islam N, Gladki E. Dry powder inhalers (DPIs) – a review of device reliability and innovation. *Int J Pharm* 2008; 360: 1–11.

119. Kesser KC, Geller DE. New aerosol delivery devices for cystic fibrosis. *Respiratory Care* 2009; 54: 754–767.
120. Kunkel G *et al.* Respimat (R) (a new soft mist inhaler) delivering fenoterol plus ipratropium bromide provides equivalent bronchodilation at half the cumulative dose compared with a conventional metered dose inhaler in asthmatic patients. *Respiration* 2000; 67: 306–314.
121. Goldberg J *et al.* Improved delivery of fenoterol plus ipratropium bromide using Respimat (R) compared with a conventional metered dose inhaler. *Eur Respir J* 2001; 17: 225–232.
122. Hodder R *et al.* Low incidence of paradoxical bronchoconstriction in asthma and COPD patients during chronic use of Respimat (R) soft mist (TM) inhaler. *Respir Med* 2005; 99: 1087–1095.
123. Koehler D *et al.* Low incidence of paradoxical bronchoconstriction with bronchodilator drugs administered by Respimat (R) Soft Mist (TM) Inhaler: Results of phase II single-dose crossover studies. *Respiration* 2004; 71: 469–476.
124. von Berg A *et al.* Efficacy and safety of Ipratropium bromide plus fenoterol inhaled via Respimat (R) Soft Mist (TM) inhaler vs. a conventional metered dose inhaler plus Spacer in children with asthma. *Pediatr Pulmonol* 2004; 37: 264–272.
125. Vincken W *et al.* Long-term efficacy and safety of ipratropium bromide plus fenoterol via Respimat (R) Soft Mist (TM) inhaler (SMI) versus a pressurised metered-dose inhaler in asthma. *Clin Drug Invest* 2004; 24: 17–28.
126. Iacono P *et al.* Improved delivery of ipratropium bromide using Respimat (R) (a new soft mist inhaler) compared with a conventional metered dose inhaler: cumulative dose response study in patients with COPD. *Respir Med* 2000; 94: 490–495.
127. Kilfeather SA *et al.* Improved delivery of ipratropium bromide/fenoterol from Respimat (R) Soft Mist (TM) Inhaler in patients with COPD. *Respir Med* 2004; 98: 387–397.
128. Anderson P. Use of Respimat Soft Mist inhaler in COPD patients. *Int J Chron Obstruct Pulmon Dis* 2006; 1: 251–259.
129. ZuWallack R *et al.* Efficacy and safety of ipratropium bromide/albuterol delivered via Respimat (R) inhaler versus MDI. *Respir Med* 2010; 104: 1179–1188.
130. Kerstjens HAM *et al.* Tiotropium improves lung function in patients with severe uncontrolled asthma: a randomized controlled trial. *J Allergy Clin Immunol* 2011; 128: 308–314.
131. Singh S *et al.* Mortality associated with tiotropium mist inhaler in patients with chronic obstructive pulmonary disease: systematic review and meta-analysis of randomised controlled trials. *Br Med J* 2011; 342: d3215.
132. Leclerc V *et al.* Acute local tolerability of acidic aqueous vehicles delivered via Respimat (R) soft Mist(TM) inhaler in hyperreactive asthma patients. *Respiration* 2007; 74: 691–696.
133. Patel KR *et al.* Inhaled ethanolic and aqueous solutions via respimat soft mist inhaler are well-tolerated in asthma patients. *Respiration* 2006; 73: 434–440.
134. Bouyssou T *et al.* Pharmacological characterization of olodaterol, a novel inhaled beta(2)-Adrenoceptor agonist exerting a 24-hour-long duration of action in preclinical models. *J Pharmacol Exp Ther* 2010; 334: 53–62.
135. Hindle M, Longest PW. Condensational growth of combination drug-excipient submicrometer particles for targeted high-efficiency pulmonary delivery: evaluation of formulation and delivery device. *J Pharm Pharmacol* 2012; 64: 1254–1263.
136. Newman SP *et al.* An in vitro study to assess facial and ocular deposition from Respimat (R) Soft Mist (TM) inhaler. *J Aeros Med-Deposit Clear Effects Lung* 2007; 20: 7–12.
137. Schuster J *et al.* The AER(X)(TM) aerosol delivery system. *Pharm Res* 1997; 14: 354–357.
138. Davison S *et al.* Pharmacokinetics and acute safety of inhaled testosterone in postmenopausal women. *J Clin Pharmacol* 2005; 45: 177–184.
139. Farr SJ, Otulana BA. Pulmonary delivery of opioids as pain therapeutics. *Adv Drug Deliv Rev* 2006; 58: 1076–1088.
140. Gonda I *et al.* Inhalation delivery systems with compliance and disease management capabilities. *J Controlled Release* 1998; 53: 269–274.
141. Otulana B *et al.* Safety and pharmacokinetics of inhaled morphine delivered using the AERx System in patients with moderate-to-severe asthma. *Int J Clin Pharmacol Ther* 2004; 42: 456–462.
142. Thipphawong JB *et al.* Analgesic efficacy of inhaled morphine in patients after bunionectomy surgery. *Anesthesiology* 2003; 99: 693–700.
143. Dershwitz M *et al.* Pharmacokinetics and pharmacodynamics of inhaled versus intravenous morphine in healthy volunteers. *Anesthesiology* 2000; 93: 619–628.
144. Ward ME *et al.* Morphine pharmacokinetics after pulmonary administration from a novel aerosol delivery system. *Clin Pharmacol Ther* 1997; 62: 596–609.
145. Mather LE *et al.* Pharmacokinetics of orally inhaled fentanyl in healthy subjects. *J Clin Pharmacol* 2000; 40: 1060.
146. Okumu FW *et al.* Evaluation of the AERx pulmonary delivery system for systemic delivery of a poorly soluble selective D-1 agonist, ABT-431. *Pharm Res* 2002; 19: 1009–1012.
147. Chan HK *et al.* Deposition of aqueous aerosol of technetium-99 m diethylene triamine penta-acetic acid generated and delivered by a novel system (AER(x)) in healthy subjects. *Eur J Nucl Med* 1999; 26: 320–327.
148. Cipolla DC *et al.* Bolus Administration of Ixml: Studying the Feasibility of Delivering High Doses of Drugs Using the AERx Pulmonary Delivery System. In: *Respiratory Dru Delivery VII*. Tarpon Springs, FL, USA: Serentec Press, Raleigh, NC, USA, 2000.
149. Sangwan S *et al.* Aerosolized protein delivery in asthma: gamma camera

analysis of regional deposition and perfusion. *J Aeros Med-Deposit Clear Effects Lung* 2001; 14: 185–195.
150. Farr SJ *et al.* Comparison of in vitro and in vivo efficiencies of a novel unit-dose liquid aerosol generator and a pressurized metered dose inhaler. *Int J Pharm* 2000; 198: 63–70.
151. Geller D *et al.* Bolus inhalation of rhDNase with the AERx system in subjects with cystic fibrosis. *J Aeros Med-Deposit Clear Effects Lung* 2003; 16: 175–182.
152. Deshpande D *et al.* Aerosolization of lipoplexes using AERx (R) Pulmonary Delivery System. *Aaps Pharmsci* 2002; 4: 12–21.
153. Rosell J *et al.* Suppression of electrostatic changing of AERx aerosols. *J Aeros Med* 2001; 14: 405, P2–23.
154. Heinemann L. New ways of insulin delivery. *Int J Clin Pract* 2011; 65: 31–46.
155. Boyd B *et al.* Effect of gender and device mouthpiece shape on bolus insulin aerosol delivery using the AER(x) pulmonary delivery system. *Pharm Res* 2004; 21: 1776–1782.
156. Mastrandrea LD, Quattrin T. Clinical evaluation of inhaled insulin. *Adv Drug Deliv Rev* 2006; 58: 1061–1075.
157. Moses RG *et al.* Safety and efficacy of inhaled insulin (AERx((R)) iDMS (1)) compared with subcutaneous insulin therapy in patients with Type 1 diabetes: 1-year data from a randomized, parallel group trial. *Diabet Med* 2009; 26: 260–267.
158. Wilbanks TM, Schuster JA. Aerosol drug delivery to the lung periphery using nano-scale technologies, In: *2005 International Conference on MEMS, NANO and Smart Systems, Proceedings*. 2005: 127–128.
159. Chattopadhyay P *et al.* Production of solid lipid nanoparticle suspensions using supercritical fluid extraction of emulsions (SFEE) for pulmonary delivery using the AERx system. *Adv Drug Deliv Rev* 2007; 59: 444–453.
160. Yim D *et al.* Feasibility of pulmonary delivery of nano-suspension formulations using the AERx® System. *J Aeros Med* 2001; 18: 101–102.
161. Deshpande DS *et al.* Gamma scintigraphic evaluation of a miniaturized AERx (R) pulmonary delivery system for aerosol delivery to anesthetized animals using a positive pressure ventilation system. *J Aeros Med-Deposit Clear Effects Lung* 2005; 18: 34–44.
162. de Boer AH *et al.* In vitro performance testing of the novel medspray (R) wet aerosol inhaler based on the principle of Rayleigh break-up. *Pharm Res* 2008; 25: 1186–1192.
163. Rayleigh L. On the Instability of Jets. *Proceed London Mathemat Soc* 1878; 10: 4–13.
164. Weber C. Zum Zerfall eines Flussigkeitsstrahles. *J Appl Mathemat Mechanics/Zeitschrift fur Angewandte Mathematik und Mechanik* 1931; 11: 136–154.
165. Munnik P *et al.* In vivo performance testing of the novel medspray (R) wet aerosol inhaler. *J Aerosol Med Pulm Drug Deliv* 2009; 22: 317–321.
166. Tang K, Gomez A. Generation by electrospray of monodisperse water droplets for targeted drug-delivery by inhalation. *J Aerosol Sci* 1994; 25: 1237–1249.
167. Zimlich WC *et al. The Development of a Novel Electrohydrodynamic Pulmonary Drug Delivery Device. in Respiratory Dru Delivery VII. 2000*. Tarpon Springs, FL, USA; Raleigh, NC, USA: Serentec Press.
168. Clear N *et al.* Miller P, Shepherd M. Electrohydrodynamic aerosol drug delivery: experience in early clinical development. *J Aeros Med* 2004; 17: 95, 9.
169. Smyth WF, Rodriguez V. Recent studies of the electrospray ionisation behaviour of selected drugs and their application in capillary electrophoresis-mass spectrometry and liquid chromatography-mass spectrometry. *J Chromatogr A* 2007; 1159: 159–174.
170. Yurteri CU *et al.* Producing pharmaceutical particles via electrospraying with an emphasis on nano and nano structured particles – a review. *Kona Powder Particle J* 2010; 28: 91–115.
171. Kumbar SG *et al.* A preliminary report on a novel electrospray technique for nanoparticle based biomedical implants coating: precision electrospraying. *J Biomed Mat Res Part B-Appl Biomat* 2007; 81B: 91–103.
172. Xie J *et al.* Electrohydrodynamic atomization for biodegradable polymeric particle production. *J Colloid Interface Sci* 2006; 302: 103–112.
173. Ding L *et al.* Fabrication of monodispersed taxol-loaded particles using electrohydrodynamic atomization. *J Controlled Release* 2005; 102: 395–413.
174. Jung JH. Electrohydrodynamic nanospraying of ethanolic natural plant extracts. *J Aerosol Sci* 2011; 42: 725–736.
175. Jaworek A. Electrostatic micro- and nanoencapsulation and electroemulsification: a brief review. *J Microencapsul* 2008; 25: 443–468.
176. Ijsebaert JC *et al.* Electro-hydrodynamic atomization of drug solutions for inhalation purposes. *J Appl Physiol* 2001; 91: 2735–2741.
177. Singh S *et al.* Parameterization of an electro-hydrodynamic atomization based aerosol generator. *Part Sci Technol* 2013; 31: 494–500.
178. Esposito-Festen JE *et al.* Pharmacokinetics of inhaled monodisperse beclomethasone as a function of particle size. *Br J Clin Pharmacol* 2007; 64: 328–334.
179. Davies DN*et al.* Liquid formations for electrohydrodymanic spraying containing polymer and suspended particles, USPTO, Editor. 2011, Battelle Memorial Institute, Columbus, OH, USA. p. 25.
180. Chattopadhyay S *et al.* Size distribution and morphology of liposome aerosols generated by two methodologies. *Aerosol Sci Technol* 2010; 44: 972–982.
181. Jayasinghe SN, Edirisinghe MJ. Jet break-up in nano-suspensions during electrohydrodynamic atomization in the stable cone-jet mode. *J Nanosci Nanotechnol* 2005; 5: 923–926.
182. Charvat A *et al.* Time-resolved micro liquid desorption mass spectrometry: mechanism, features, and kinetic applications. *Aust J Chem* 2006; 59: 81–103.

183. Murariu M *et al.* Model peptide-based system used for the investigation of metal ions binding to histidine-containing polypeptides. *Biopolymers* 2010; 93: 497–508.
184. Pareta R *et al.* Electrohydrodynamic atomization of protein (bovine serum albumin). *J Mat Sci-Mat Med* 2005; 16: 919–925.
185. Lentz YK *et al.* Rationale for the selection of an aerosol delivery system for gene delivery. *J Aeros Med-Deposit Clearan Effects Lung* 2006; 19: 372–384.
186. Morozov VN *et al.* Generation and delivery of nanoaerosols from biological and biologically active substances. *J Aerosol Sci* 2014; 69: 48–61.
187. Yeo LY, Friend JR. Ultrafast microfluidics using surface acoustic waves. *Biomicrofluidics* 2009; 3: 012002.
188. Alvarez M *et al.* Surface vibration induced spatial ordering of periodic polymer patterns on a substrate. *Langmuir* 2008; 24: 10629–10632.
189. Friend J, Yeo LY. Microscale acoustofluidics: microfluidics driven via acoustics and ultrasonics. *Rev Mod Phys* 2011; 83: 647–704.
190. Qi AS *et al.* Miniature inhalation therapy platform using surface acoustic wave microfluidic atomization. *Lab Chip* 2009; 9: 2184–2193.
191. Qi A *et al.* Interfacial destabilization and atomization driven by surface acoustic waves. *Phys Fluids* 2008; 20: 074103.
192. Alvarez M *et al.* Rapid generation of protein aerosols and nanoparticles via surface acoustic wave atomization. *Nanotechnology* 2008; 19: 455103.
193. Alvarez M *et al.* Rapid production of protein-loaded biodegradable microparticles using surface acoustic waves. *Biomicrofluidics* 2009; 3: 014102.
194. Qi A *et al.* The extraction of liquid, protein molecules and yeast cells from paper through surface acoustic wave atomization. *Lab Chip* 2009; 10: 470–476.
195. Rajapaksa A *et al.* Effective pulmonary delivery of an aerosolized plasmid DNA vaccine via surface acoustic wave nebulization. *Respir Res* 2014; 15: 60.
196. Rajapaksa A *et al.* Enabling practical surface acoustic wave nebulizer drug delivery via amplitude modulation. *Lab Chip* 2014; 14: 1858–1865.
197. Li H *et al.* Surface acoustic wave concentration of particle and bioparticle suspensions. *Biomed Microdevices* 2007; 9: 647–656.
198. Tan MK *et al.* Microparticle collection and concentration via a miniature surface acoustic wave device. *Lab Chip* 2007; 7: 618–625.
199. Friend JR *et al.* Evaporative self-assembly assisted synthesis of polymeric nanoparticles by surface acoustic wave atomization. *Nanotechnology* 2008; 19: 145301.
200. Forde GM, *et al.* Straightforward biodegradable nanoparticle generation through megahertz-order ultrasonic atomization. *Appl Phys Lett* 2006; 89: 4105.
201. Hindle M, *et al.* High efficiency fine particle generation using novel condensation technology. In: *Respiratory Drug Delivery VI*. Hilton Head, SC, USA; Buffalo Grove, IL, USA: Interpharm Press, 1998.
202. Howell TM, Sweeney WR. Aerosol and a Method and Apparatus for Generating Aerosol, USPTO, Editor. 1998, Philip Morris Incorporated. New York, NY, USA. p. 14.
203. Hong JN, *et al.* Control of particle size by coagulation of novel condensation aerosols in reservoir chambers. *J Aeros Med-Deposit Clear Effects Lung* 2002; 15: 359–368.
204. Gupta R *et al.* Vehicle effects on the performance of a novel pharmaceutical condensation aerosol generator. *AAPS PharmSci*. 2001; 3: 3150 (suppl).
205. Gupta R *et al.* Solute and concentration effects in a novel pharmaceutical condensation aerosol generator. *AAPS PharmSci* 2001; 3: 3148(suppl).
206. Shen X *et al.* Effect of energy on propylene glycol aerosols using the capillary aerosol generator. *Int J Pharm* 2004; 275: 249–258.
207. Li XH *et al.* Stability and characterization of perphenazine aerosols generated using the capillary aerosol generator. *Int J Pharm* 2005; 303: 113–124.
208. Werley MS *et al.* Non-clinical safety and pharmacokinetic evaluations of propylene glycol aerosol in Sprague-Dawley rats and Beagle dogs. *Toxicology* 2011; 287: 76–90.
209. CEN, Respiratory therapy equipment – Part 1: Nebulizing systems and their components, E.C.f. Standardization, Editor. 2007, BSI.
210. Sangwan S *et al.* Lung deposition and respirable mass during wet nebulization. *J Aeros Med-Deposit Clear Effects Lung* 2003; 16: 379–386.
211. Marple VA *et al.* Next generation pharmaceutical impactor (A new impactor for pharmaceutical inhaler testing). Part I: Design. *J Aeros Med-Deposit Clear Effects Lung* 2003; 16: 283–299.
212. Mitchell JP, Nagel MW. Cascade impactors for the size characterization of aerosols from medical inhalers: their uses and limitations. *J Aeros Med-Deposit Clear Effects Lung* 2003; 16: 341-+.
213. Mitchell J *et al.* In vitro and in vivo aspects of cascade impactor tests and inhaler performance: a review. *Aaps Pharmscitech* 2007; 8: 237–248.
214. Waldrep JC *et al.* Comparative analysis of methods to measure aerosols generated by a vibrating mesh nebulizer. *J Aeros Med-Deposit Clear Effects Lung* 2007; 20: 310–319.
215. Mitchell J *et al.* Non-impactor-based methods for sizing of aerosols emitted from orally inhaled and nasal drug products (OINDPs). *AAPS PharmSciTech* 2011; 12: 965–988.
216. Mitchell JP, Nagel MW. Particle size analysis of aerosols from medicinal inhalers. *KONA Powder Particle* 2004; 22: 32–65.
217. de Boer AH *et al.* Characterization of inhalation aerosols: a critical evaluation of cascade impactor analysis and laser diffraction technique. *Int J Pharm* 2002; 249: 219–231.
218. Mitchell JP *et al.* Laser diffractometry as a technique for the rapid assessment of aerosol particle size

from inhalers. *J Aeros Med-Deposit Clear Effects Lung* 2006; 19: 409–433.
219. Marple VA *et al.* Next generation pharmaceutical impactor: A new impactor for pharmaceutical inhaler testing. Part III. Extension of archival calibration to 15 L/min. *J Aeros Med-Deposit Clear Effects Lung* 2004; 17: 335–343.
220. Berg E *et al.* Determination of nebulizer droplet size distribution: a method based on impactor refrigeration. *J Aeros Med-Deposit Clear Effects Lung* 2007; 20: 97–104.
221. Berlinski A, Hayden JB. Optimization of a procedure used to measure aerosol characteristics of nebulized solutions using a cooled next generation impactor. *J Aerosol Med Pulm Drug Deliv* 2010; 23: 397–404.
222. Bummer PM. Interfacial Phenomena. In: Hendrickson R, ed. *Remington: The Science and Practice of Pharmacy*. Baltimore, MD: Lippincott Williams & Wilkins, 2005: 280–292.
223. Minko T. Interfacial Phenomena. In: Sinko PJ, ed. *Martin's Physical Pharmacy and Pharmaceutical Sciences*. Baltimore, MD: Lippincott Williams & Wilkins, 2006: 437–467.
224. Carvalho TC, *et al.* Application of a Pull on a Disk Method to Measure Surface Tension of Liquids. *AAPS Pharm Sci Tech* 2012; 13: 305–312.
225. Longer M. Rheology. In: Sinko PJ, ed. *Martin's Physical Pharmacy and Pharmaceutical Sciences*. Baltimore, MD: Lippincott Williams & Wilkins, 2006: 561–583.
226. Schnaare RL *et al.* Rheology. In: Hendrickson R, ed. *Remington: The Science and Practice of Pharmacy*. Baltimore, MD: Lippincott Williams & Wilkins, 2005: 338–357.
227. Nagvekar AA *et al.*. Current analytical methods used in the in vitro evaluation of nano-drug delivery systems. *Curr Pharm Anal* 2009; 5: 358–366.
228. Shekunov BY *et al.* Particle size analysis in pharmaceutics: principles, methods and applications. *Pharm Res* 2007; 24: 203–227.
229. Lyklema J. Molecular interpretation of electrokinetic potentials. *Curr Opin Colloid Interface Sci* 2010; 15: 125–130.
230. Kallay N *et al.* Electrostatic potentials at solid/liquid interfaces. *Croat Chem Acta* 2010; 83: 357–370.
231. Barnes HA et al.. Rheology of Suspensions. In Walters K, ed. *An Introduction to Rheology*. Amsterdam, The Netherlands: Elsevier, 1989: 115–137.
232. Derkach SR. Rheology of emulsions. *Adv Colloid Interface Sci* 2009; 151: 1–23.