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Surface active drugs significantly alter the drug output rate from medical nebulizers

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ABSTRACT

Background: Surface tension of aqueous solutions has a great impact on the resulting size of the produced aerosol droplets. Nevertheless, little attention has been drawn so far to the drug output of surface active substances during nebulization.
Methods: Six nebulizers with three different nebulization mechanisms were operated on the breath simulator and the dependence between the type of aerolisation mechanism, concentration of surface active substance and output rate was investigated.
Results: The results of this study confirm that surface active substances significantly affect nebulization. During ultrasonic nebulization of surface active substances a decrease of the residual drug concentration occurs. Simultaneously, higher amounts of aerosolized substance were observed in *in vitro* studies. These findings were dependent on the saturation of the liquid–air interface with molecules of a surface active substance. During jet nebulization, nevertheless, the process of an incorporation of substance molecules into aerosol droplets is associated with a strong solvent evaporation from the reservoir. In this case the resulting residual concentration and the aerosolized substance amount are dependent on the outbalancing of either an up-concentration of the surface active substance in the reservoir or rate of solvent evaporation. Vibrating membrane mechanism to nebulize solutions were observed to have minimal effects on the aerosolized substance quantity and residual concentration during nebulization.
Conclusions: Following conclusions for an efficient nebulization delivery of drugs with surface activity with respect to optimal delivery device can be made. Generally, ultrasonic devices produce higher drug output rates within shorter periods of time as compared to jet and vibrating membrane nebulizers. Accordingly, prescription instructions have to be adjusted to these findings. The study also emphasizes that aqueous nebulizer solutions should preferably be prescribed in conjunction with a specific nebulizer which has been tested *in vitro* and been used in *in vivo* studies, rather than to let patients choose their delivery device themselves.

1. Introduction

Inhalation of aerosols is an attractive route of therapeutic drug delivery. The delivery of substances directly to the lung enables the use of lower doses compared to other routes of administration (*i.e.* oral, buccal, rectal delivery) with an equivalent therapeutic response and lower systemic exposure. Therapeutics can be used for the topical application for treatment of respiratory diseases e.g. chronic lung diseases, acute or severe asthma, cystic fibrosis, chronic obstructive pulmonary disease, airway infections, etc. For the treatment of respiratory diseases rapid relief of symptoms, a reduction of side effects and good tolerance of the treatment can be emphasized as advantages of pulmonary drug delivery (Byat and Cook, 2004). Due to the vast, well-perfused absorptive surface and thin alveolar–capillary barrier the lung also offers a possibility for systemic drug application. These conditions allow fast absorption of medications into the bloodstream and rapid onset of action after inhalation (Price et al., 2002; Groneberg et al., 2003).

Inhaled therapy via nebulization has a well-documented history in the treatment of pulmonary diseases. Nebulizers are principally recommended for children and adults who have difficulties in coordinating inspiration and aerosol actuation and in the emergency

Abbreviations: ADA_G, aerosolized drug amount, determined by mass-balance method, μg; ADA_{HPLC}, aerosolized drug amount, determined by high performance liquid chromatography, μg; AO, aerosol output, determined by mass-balance method, g; BAC, benzalkonium chloride; C_0, initial drug concentration, μg/mL; C_1, residual drug concentration, μg/mL; m_0, initial nebulizer weight, g; m_2, nebulizer weight at the end of nebulization, g; NT, nebulization time, min; R, system recovery, %; RDA_G, residual drug amount, determined by mass-balance method, μg; RDA_{HPLC}, residual drug amount, determined by high performance liquid chromatography, μg; RV, residual volume, determined by mass-balance method, g; SBS, salbutamol sulfate; T_0, initial temperature of the nebulized solution, °C; T_1, residual temperature of the nebulized solution, °C.

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room for acute severe episodes of bronchospasm. Nebulization therapy is also indicated when large doses of inhaled drugs are required or when drugs are only available for nebulization (Esmond, 1998). There are a lot of factors which influence the efficiency of nebulization therapy. First of all, the individual lung anatomy can influence the aerosol deposition. Children, for example, can loose aerosol both during inspiration and expiration as their tidal volumes can be lower than aerosol output (Newman, 2000). Nose breathing, talking or drinking during treatment as well as disease related symptoms, such as cough, also decrease the aerosol deposition (O'Callaghan and Barry, 1997). Furthermore, the ability and motivation of a patient to perform nebulization treatment in the proper way has a strong influence on the lung deposition of the aerosol. Accordingly, nebulizers are designed with respect to size, portability, appearance, color, material, decoration, fashion and noise to improve patient compliance (Barry, 2002). The choice of a particular nebulizer construction influences the nebulizer output. During jet nebulization, for example, the rate of gas flow from the compressor influences the size of the produced droplets. At higher flow rates the particle size decreases and polydispersity of aerosol increases (Clay et al., 1983). During ultrasonic nebulization the size of primary aerosol droplets depends on the piezoelectric crystal frequency. Higher frequencies lead to smaller aerosol droplets. The diameter of the secondary generated droplets and, therefore, aerosol size distribution is critically dependent on the design of the nebulizer impaction system. The filling volume of the device both during jet and ultrasonic nebulization influences aerosol output and residual volume of the medication (Hess et al., 1996). It is, therefore, not very surprisingly that some authors showed that the delivered dose of the same medication from different types of nebulizers can differ significantly not only among brands but also among nebulizers within one brand (Hess et al., 1996; Barry and O'Callaghan, 1999).

The parameters to evaluate nebulization efficiency *in vitro* are aerosol output, fine particle fraction in the aerosol output, particle size distribution and residual drug amount. Some studies were carried out to measure the aerosol output from nebulizers gravimetrically as the difference of the nebulizer weight before and after nebulization (Loffert et al., 1994). However, since the aerosol output from the nebulizer consists of drug output fraction and solvent output fraction, caused by evaporation, this method was shown to be inefficient (Dennis et al., 1990; Allen and Langford, 1993). The gravimetrical method can overestimate the drug output from a nebulizer by up to 50%. The direct methods such as collecting and analyzing the drug on filters or the tracer technique are more accurate in measuring aerosol output *in vitro* (Barry and O'Callaghan, 1999; Gatnash et al., 1998; Dahlback, 1994). Determination of aerosol output based on measuring the residual volume after nebulization was also proposed (Eklund et al., 2000). Nevertheless, this method underestimates the amount of the drug left in the nebulizer chamber as it does not consider the evaporation of the solvent and concentration effect within the solution in the nebulizer reservoir. An acceptable method to measure residual drug amount is to assay the active drug from the reservoir using any suitable analytical method.

Physico-chemical characteristics of the nebulized medication have an influence on the nebulizer output as well. Liquids for inhalation, intended to be converted into aerosols by nebulizers, are solutions or suspensions. As long as a drug that is supposed to be delivered per nebulization must be solved or dispersed in a liquid medium, the addition of excipients cannot be avoided in some cases. Sometimes, to increase the drug solubility, the addition of co-solvents (ethanol or propylene glycol), solubilisation agents or pH adjustment is necessary. Preservatives could, for example, also be added to inhibit a microbial contamination. These compounds can lead to a change of the aerosol output and nebulization time due to changes in the physico-chemical properties of the solution or suspension. There are studies, showing the influence of factors such as surface tension, viscosity, density and temperature of the medication on the size of the produced droplets. It has been for example shown by Steckel and Eskandar (2003) that the surface tension of the solution influences the size of the produced droplets and, therefore, aerosol deposition. With decrease of surface tension, a decrease of the droplet size was shown. Viscosity of the solution also has an influence on aerosol output, aerosol particle size and nebulization time. The droplet size of aerosols produced by jet nebulization was found to be inversely proportional to the solution viscosity. Beyond the critical value of 6 cP the droplet size was found to increase with the increase of viscosity. Nebulization time was found to increase with the increase of the viscosity. Other authors report the ultrasonic nebulization of very viscous liquids to be inefficient (McCallion et al., 1996). Nevertheless, little attention has been drawn so far to investigate how the surface tension of the nebulized solution can alter the drug output from the nebulizer. A study of MacNeish et al. (1997) showed a difference in albuterol output between albuterol formulations with and without preservative (benzalkonium chloride) from different jet nebulizers . This fact was explained by the surface activity of benzalkonium chloride. The surface active substance causes the formation of foam continuously running back into the reservoir, but not adhering to the walls of the nebulizer and, therefore, allows more solution to be nebulized within the same time and decreases the device residual volume.

As a hypothesis of this study, it is supposed that the surface activity of a nebulized drug has an effect on the nebulizer output. The surface activity leads to a reduction of the surface tension of the nebulized solution which has an effect on the incorporation of drug molecules into the aerosol droplets. The aerolisation mechanism also influences this process. The aim of this study was, therefore, to investigate the aerolisation of liquids with reduced surface tension in comparison to water which will be a consequence of dissolving a surface active drug for nebulization therapy. This also refers to the effectiveness of the nebulization therapy, since, as a matter of fact, there are drugs used for this purpose which more or less feature surface activity. Characteristics of the nebulization efficiency such as aerosolized drug amount and residual drug amount can, therefore, be changed through this property.

Moreover, the aim of these investigations was to make some suggestions based on the received data for the efficient nebulization delivery for drugs showing surface activity with respect to the choice of an optimal delivery device.

2. Materials and methods

2.1. Model substances

Salbutamol sulfate (SBS, Astra Zeneca GmbH, Wedel, Germany) and benzalkonium chloride (BAC, Fluka Chemie GmbH, Buchs, Switzerland) were selected as model substances without and with surface activity. SBS was chosen as a drug with little to no effect on the surface tension of an aqueous solution. A 0.6 mg/mL solution of SBS in water was used for the experiments. Benzalkonium chloride in this study simulates a drug with properties of surface activity. A 0.1% (w/v) solution of BAC in water was used.

2.2. Nebulizers used in the study

Six nebulizers with three different nebulization mechanisms were involved in the study (Table 1). Multisonic® nebulizer is an ultrasonic nebulizer with continuous aerosol generation. The piezoelectric crystal fitted into the hand piece is in direct contact

Table 1
Nebulizers in the study.

Device name	Manufacturer	Type of nebulizer	Serial number
Multisonic®	Otto Schill GmbH, Probstzella, Germany	Ultrasonic	811110590
Optineb®	Nebu-Tec GmbH, Elsenfeld, Germany	Ultrasonic	20020752
Pari LC Star®	Pari GmbH, Munich, Germany	Jet	LOTCBQ6DA
Halolite®	Medic-Aid Limited, Bognor Regis, UK	Jet	2001AD700854
Prodose®	Profile Pharma, Chichester, UK	Jet	2003PD200114
Aeroneb® Pro	Aerogen Ltd., Galway, Ireland	VM	020430-042

with the drug solution in the reservoir. The crystal vibrates at a frequency of 2.4 MHz detaching droplets from the inhalation solution. The primarily generated droplets encounter the baffle that regulates the size of the particles by causing larger particles to drop back into the reservoir and to be re-cycled. The secondly generated droplets leave the device, carried out by the bypass air entering the nebulizer through the inhalation valve. Optineb® nebulizer accomplishes continuous aerosol production by using ultrasound as well. The vibration of the piezoelectric crystal is transmitted through a coupling liquid (water) to a nebulizer cup, detaching droplets from the solution for nebulization. The duration of treatment can be preset manually and the size of aerosol particles can be regulated using different baffle plates.

Pari LC Star® is a conventional, breath-enhanced, open vent nebulizer with constant aerosol generation. This nebulizer was used with the Pari TurboBoy® compressor (serial number CBW7PA0650, Pari GmbH, Munich, Germany) working at a maximum flow rate of 10.9 ± 1 L/min (at 23 °C and 952 hPa) with a corresponding maximum static pressure of 3.8 bar. Prodose® and Halolite® adaptive aerosol delivery (AAD) nebulizers are portable pneumatic aerosolization systems powered by a portable compressor. The Halolite® AAD has a manufacturer programmed preset dose. In the Prodose AAD® system the delivered dose is controlled by a Prodose Disk™ system. AAD system analyses the breathing patterns of the patient by measuring the flow-relevant pressure changes of the first three breaths to establish the shape of the breathing pattern and to measure the inspiration time for the determination of the aerosol pulse time. Both nebulizers generate aerosol only during patients' inspiration and deliver it during the first half of inspiration time which leads to a reduction of aerosol losses in the system. The AAD system continues to monitor the breathing pattern throughout the treatment for necessary adjustments. The Aeroneb® Professional Nebulizer System represents a vibrating membrane nebulizer. This nebulizer incorporates a membrane perforated with microholes and a vibration element working at near ultrasonic frequencies. The pressure generated from the vibration motion of the membrane draws the liquid through its holes where surface tension and hydrodynamic effects break it into a stream of precisely controlled droplets.

Nebulizers' reservoirs were cleaned with water, rinsed with demineralised water and dried prior each test. All nebulizers were used without any additional accessories.

2.3. Breathing patterns

The simulation was performed with a Pari Compas Breath Simulator (Pari GmbH, Munich, Germany). Regular sinusoidal human breathing patterns with tidal volumes of 500 mL were selected. A peak inspiratory flow rate of 20 L/min and a duration of one breathing cycle of 4 s (1:1 inhalation:exhalation ratio) were used in this study.

2.4. Determination of nebulization time

For the devices which do not control the nebulization time (NT), NT was determined while operating the nebulizer on the breath simulator. The tests were carried out in triplicate. The Multisonic® and Aeroneb® Pro nebulizers give an acoustic signal and switch off automatically when a certain residual volume is reached. The Optineb® device stops nebulization automatically when the preset time is over. For Pari LC Star® nebulizer the end of nebulization was defined as the sputtering point plus one additional minute. The sputtering point was defined as a time-point at which the first audible onset of discontinuous nebulization was recognized and the aerosol cloud visually became irregular. The Halolite® and Prodose® devices use the AAD system to determine the necessary inhalation time for a defined dose.

2.5. Quantitative assay

BAC and SBS were analytically assayed by high performance liquid chromatography (HPLC, Kontron Data System, Milan, Italy). The assay of both substances was performed on a reverse phase column (stationary phase LiChrospher 100 CN (5 μm) for BAC and LiChroChart 100 RP-18 (5 μm) for SBS, Merck KGaA, Darmstadt, Germany). The mobile phase for BAC was composed of 60% acetonitrile (C. Roth GmbH CoKG, Karlsruhe, Germany) and 40% acetate buffer (pH 5.8). 0.12% of 1-octanesulfonic acid sodium salt (Sigma–Aldrich, St. Louis, MO, USA) was added to the mobile phase. The flow rate was set at 2.0 mL/min. The detection was performed at 262 nm. The mobile phase for SBS was composed of 40% methanol (C. Roth GmbH CoKG, Karlsruhe, Germany) and 60% phosphate buffer (pH 3.0). 0.11% of heptanesulfonic acid sodium salt (Sigma–Aldrich, St. Louis, MO, USA) was added to the mobile phase. A flow rate of 1.2 mL/min was used. The detection was performed at 238 nm. For both substances each sample was analyzed twice (injected volume 80 μL). Calibration was done by an external standard method.

2.6. Experimental set-up (using the example of Pari LC Star® nebulizer)

The nebulizers were operated on the breath simulator. If not otherwise indicated each combination of nebulizer and formulation was analyzed in triplicate. The nebulizer was connected to the breath simulator via a Y-piece (Pari GmbH, Munich, Germany). All the connections were strengthened additionally with elastomeric film (Pechiney Plastic Packaging, Chicago, Il., USA) to avoid aerosol losses and aerosol exposure to the environment. The aerosol was collected on polypropylene inhalation and exhalation filters (3 M, Neuss, Germany). The filter pads were placed in the filter casings (Pari GmbH, Munich, Germany). Polypropylene filter pads were used to minimize the increase of filter resistance while the drug was depositing on the filter during the inhalation process. For the same reason filter pads were changed every 2 min. The test set-up for different nebulizers could differ from the standard test set-up (Fig. 1) with respect to the number of additional exhalation filters or connectors. Independent from the nebulizer type, it was insured that aerosol losses into the environment were avoided and the complete aerosol was collected and assayed.

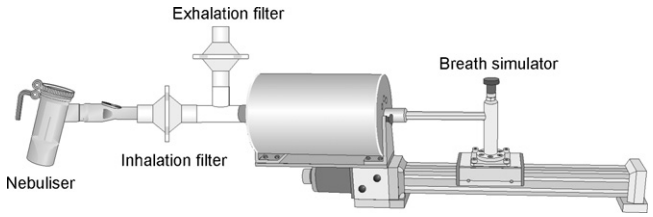


Fig. 1. Experimental set-up on the example of Pari LC Plus® nebulizer.

2.7. Determination of aerosol output and concentration effect

The empty nebulizer or nebulizer chamber was weighed on an analytical balance (Sartorius AG, Goettingen, Germany) and the initial weight (m_0, mg) was determined. 2 mL of the solution was transferred into a nebulizer, the weight m_1 was recorded. The concentration of the solution was assayed by HPLC and recorded as initial drug concentration (C_0, μg/mL). Breath simulator, compressor and nebulizer were turned on. After 1 min of the experiment, the nebulization was stopped by turning off the breath simulator, compressor or nebulizer and timer (Hanhart, Berlin, Germany). The inspiratory filter was changed including the bottom part of the filter casing. The filter was placed into the 200 mL glass beaker (Schott AG, Mainz, Germany), whereas the bottom part of the filter casing was used as a funnel. 15 mL of water was dispensed into the beaker to rinse the filter casing carefully. The measurements were restarted with the new filter. Each time increment of 2 min the inhalation filter, including the filter casing, were changed in the same way as described above. Nebulization was performed until the end of nebulization time. At the end of nebulization the weight of the nebulizer or nebulizer chamber (m_2, mg) was detected. The aerosol output (AO, g) was calculated as the difference between m_1 and m_2, assuming a liquid density of 1.0 g/cm^3 (mass-balance method). The residual volume (RV, g) was determined gravimetrically as the difference between m_2 and m_0. At the end of nebulization, samples were taken from the nebulizer reservoir and the drug concentration was assayed and defined as the residual drug concentration (C_1, μg/mL). The residual drug concentration was compared against the initial drug concentration.

2.8. Determination of aerosolized and residual drug amount

The inhalation and exhalation filter casings were resembled; pads were placed into a 200 mL glass beaker and extracted with a solvent. Covered glass beakers were placed on a shaker (Gesellschaft fuer Labortechnik GmbH, Burgwedel, Germany) for 30 min. Y-piece, mouthpiece and all the parts of the experimental set-up which have been in contact with the aerosol were resembled, separately placed into glass beakers and rinsed with solvent. The residual drug amount was recovered from the nebulizer reservoir. The samples were taken and the amount of drug was determined by HPLC. The aerosolized drug amount (ADA_{HPLC}, μg) was calculated as cumulative drug amount found in the parts of the experimental set-up, which had contact with the produced aerosol. The residual drug amount (RDA_{HPLC}, μg) was determined as a residual amount of drug left in the nebulizer reservoir after nebulization. The aerosolized and the residual drug amounts were also estimated gravimetrically (ADA_G, RDA_G, μg). The amount of drug determined by HPLC was compared with that estimated gravimetrically. The parameter of the system recovery (R, %) was calculated as cumulative drug amount assayed within the whole test set-up divided by the filled amount of the drug, reported in % of the filled amount. This parameter estimates the aerosol losses into the environment. The recovery was found to be in the range of $90\% < R < 100\%$ in all cases.

2.9. Determination of concentration effect and aerosolized amount of BAC by Pari LC Star® nebulizer at different flow rates of driving gas

Pari LC Star® nebulizer was investigated additionally at different driving gas pressures. For this experiment, the original compressor unit was replaced by a gas pressure cylinder with nitrogen. The operating pressure and, therefore, the gas flow rate could in this case be precisely regulated by means of a reducing valve. The pressure was set to 1–3 bar causing different flow rates of the driving gas. The rest of the set-up was the same as described above. After the end of the nebulization time, the concentration effect and the amount of substance deposited only on inhalation filters were assayed by HPLC.

2.10. Determination of temperature changes in the residual solution after nebulization

To evaluate the temperature changes during nebulization 2 mL of BAC solution was placed into the nebulizer reservoir. The initial temperature of the solution (T_0, °C) was measured with an electric thermometer (Conrad Electronic GmbH, Hirschau, Germany). The nebulizer was operated on the breath simulator till the end of nebulization time as described above. The reservoir temperature of the residual volume of the drug solution was recorded as T_1. All tests were carried out in triplicate for each nebulizer.

2.11. Determination of the surface tension of BAC solution before and after nebulization

The determination of surface tension and critical micelle concentration of BAC solutions was carried out with a Processor tensiometer K12 (KRÜSS GmbH, Hamburg, Germany). The tensiometer was connected with a thermostat bath (Haake, Berlin, Germany) to control the temperature. The plate method was used. Before each measurement the plate was rinsed with demineralised water, annealed to red-hot with a Bunsen burner and cooled to room temperature. The sample vessel was rinsed several times with demineralised water and the sample solution. The tensiometer was calibrated with demineralised water. For the determination of critical micelle concentration a series of solutions of different concentrations (%, w/v) were prepared. The surface tension of 0.1% BAC was measured before and after nebulization. Two nebulizers of

Table 2
Nebulization times detected during nebulization of BAC and SBS solution.

Device name	Time until sputtering (min), mean ± SD		Resulting time (min), mean ± SD		Comments
	BAC	SBS	BAC	SBS	
Multisonic®			7.0 ± 0.6	4.3 ± 0.7	
Optineb®			5.0	5.0	Preset
Pari LC Star®	3.4 ± 0.0	2.4 ± 0.0	4.4 ± 0.0	3.4 ± 0.0	
Halolite®			4.5 ± 0.5	4.7 ± 0.3	AAD
Prodose® 500 μg disk			11.2 ± 1.9	9.2 ± 0.2	AAD
Aeroneb® Pro			5.0	5.0	Preset

two different nebulization mechanisms were chosen for this test: Pari LC Star® nebulizer and Multisonic® nebulizer. The nebulizers were operated on the breath simulator until the end of nebulization time as described above. The tests were carried out in triplicate.

3. Results and discussions

3.1. Nebulization of BAC

Measured nebulization times for 2 mL filling volume are presented in Table 2. The Prodose® nebulizer with 500 μg disk showed the longest nebulization time, whereas the Pari LC Star® and Halolite® nebulizers showed the shortest nebulization times for the defined filling volume. Since the time of the treatment is among the factors influencing the patients' compliance, shorter nebulization times are preferred.

The aerosol output and residual volumes based on a mass-balance are presented in Table 3. The data obtained for Multisonic®, Optineb®, Pari LC Star® and Prodose® nebulizers were comparable. Nebulization with the Prodose® device resulted in an aerosol output of approximately 0.8 g and the longest nebulization time, whereas 1.1 g of medication was delivered by the Pari LC Star® nebulizer only within 4.4 min. Nebulization with Halolite® nebulizer resulted in the lowest aerosol output and the highest residual volume. Nebulization of 2 mL solution with Aeroneb® Pro nebulizer led to complete nebulization after 5 min. This is certainly an advantage leading to saving of medicament and therapy costs. But, since we were interested in the residual concentration of the nebulized solution, the filling volume was increased to 3 mL to be able to measure the concentration after 5 min of aerosol delivery.

Table 3
Aerosol output and residual volumes based on a mass-balance after nebulization of BAC solution.

Device name	AO (g), mean ± SD		RV (g), mean ± SD	
	BAC	SBS	BAC	SBS
Multisonic®	1.2 ± 0.0	0.9 ± 0.2	0.8 ± 0.0	1.0 ± 0.2
Optineb®	1.0 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
Pari LC Star®	1.1 ± 0.1	0.9 ± 0.0	0.9 ± 0.1	1.1 ± 0.0
Halolite®	0.2 ± 0.1	0.2 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
Prodose® 500 μg disk	0.8 ± 0.0	0.7 ± 0.1	1.2 ± 0.0	1.3 ± 0.1
Aeroneb® Pro[a]	1.6 ± 0.2	1.9 ± 0.0	1.4 ± 0.2	0.1 ± 0.0

[a] Was used with a filling volume of 3 mL.

For BAC solution a decrease of the residual solution concentration was observed during ultrasonic nebulization: by 21.0 ± 0.1% (Multisonic®) and 11.0 ± 0.1% (Optineb®) of initial concentration, respectively. Ultrasonic nebulization (Multisonic®) also led to an increase by 1.74 ± 0.04 mN/m of the surface tension of the BAC solution. For this experiment the 0.01% BAC solution (below the CMC) was used. Since the residual substance concentration is decreased during ultrasonic nebulization, an increase of the surface tension took place.

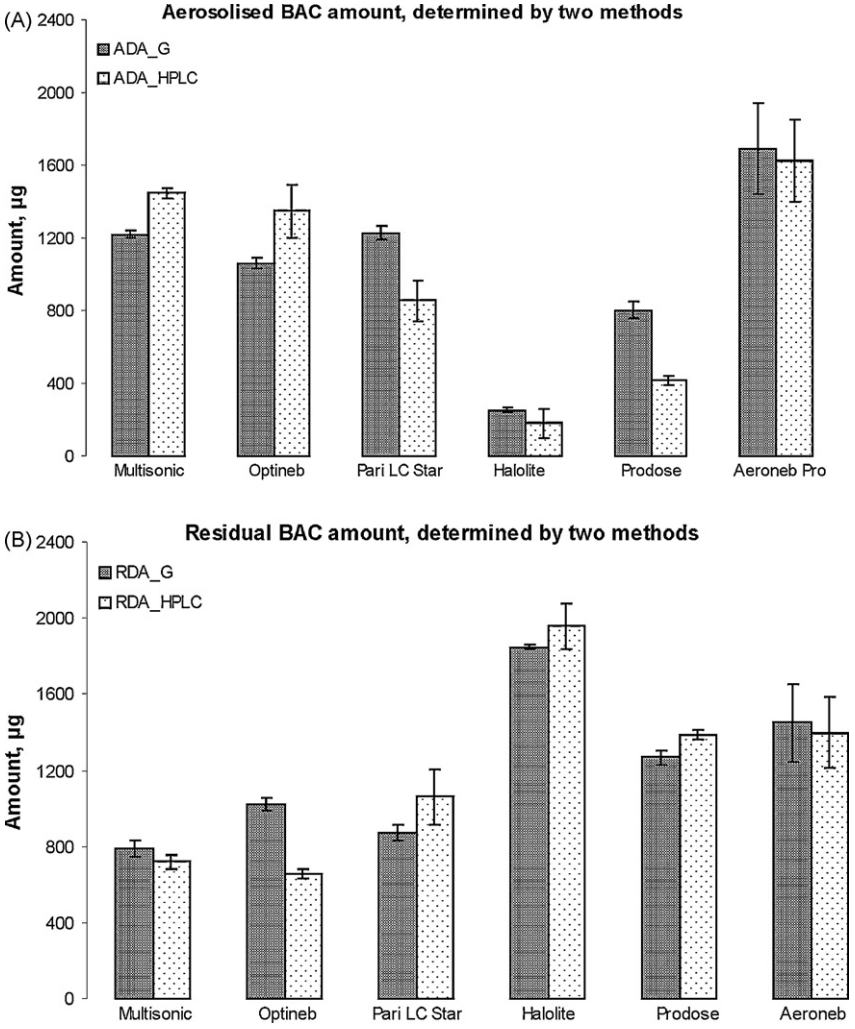


Fig. 2. (a) Aerosolized BAC amount. (b) Residual BAC amount.

Table 4
Temperature changes after nebulization of BAC solution.

Device name	Temperature change (°C),mean ± SD
Multisonic®	+10.1 ± 1.1
Optineb®	+8.9 ± 2.9
Pari LC Star®	−7.9 ± 0.4
Halolite®	−4.2 ± 0.6
Prodose® 500 μg disk	−4.2 ± 0.6
Aeroneb® Pro	+3.2 ± 0.3

The reduction of the residual drug concentration was associated with increased aerosolized drug amounts and decreased residual drug amounts yielded by the HPLC method compared to the mass-balance method for BAC solution (Fig. 2a and b). These results were surprising, since the gravimetrical analysis was reported to overestimate aerosolized drug amounts (Gatnash et al., 1998).

At the end of ultrasonic nebulization the temperature increase in the reservoir was determined (Table 4). Nebulization with Optineb® device in spite of the presence of coupling liquid also resulted in an increase of the reservoir temperature. The increase of the solution temperature during ultrasonic nebulization happens as a result of the heat transmission from the vibrating piezoelectric crystal.

For the jet nebulizers decreased aerosolized drug amounts and increased residual drug amounts were measured with HPLC assay as compared to the mass-balance method (Fig. 2a and b). The concentration of the solution in the reservoir increased by 11.0 ± 0.1% during nebulization with the Pari LC Star® device. Jet nebulization (Pari LC Star®) also caused a decrease of the surface tension of the nebulized solution by 1.12 ± 0.02 mN/m (0.01% BAC solution was nebulized) which is in accordance with the increase of the substance concentration in the reservoir.

During the nebulization with the AAD systems the results were dependent on the nebulization time. So during long nebulization times an increase of the residual substance concentration was observed: by 6.0 ± 0.5% (Prodose®, NT 11 min). During short nebulization times the residual concentration was found to decrease: by 3.0 ± 0.2% (Prodose®, nebulization was interrupted after 5 min) and by 11.0 ± 0.1% (Halolite®, NT 4.5 min). The temperature of the solution during jet nebulization is decreased (Table 4). The cooling effect in the case of jet nebulizers is a result of solvent evaporation which is caused by dry air entering the nebulizer from a compressor. The decrease of the temperature in the reservoir was smaller during nebulization with the Halolite® and Prodose® nebulizers, since these devices deliver the aerosol only during inhalation, decreasing in this way the time, when the air from the compressor has a contact with the solution in the reservoir.

A dependency between aerosolized drug amount and different nebulization mechanisms was observed. The mechanisms of droplet formation during jet and ultrasonic nebulization are principally different and are the cause for the differences in the observations between ultrasonic and jet nebulization considering the aerosolized and residual drug amounts and concentration effects within the nebulizer chamber.

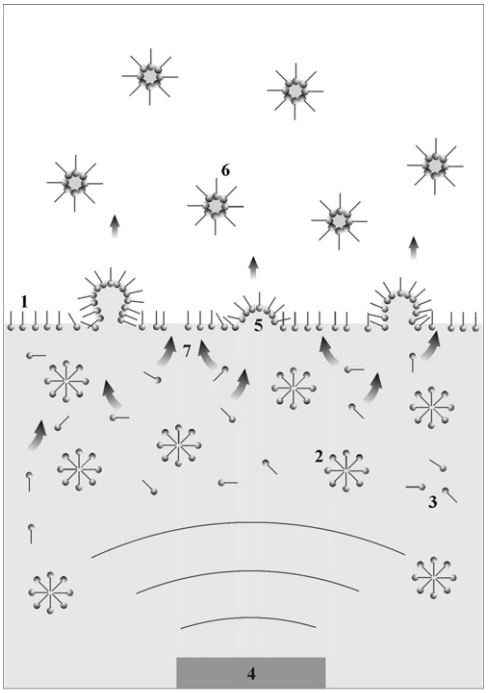
Droplet formation by ultrasonic nebulizers occurs by capillary wave formation and cavitation. Formation of cavitation bubbles occurs at low frequencies whereas capillary wave formation takes place at high frequencies. Cavitation theory proposes that a piezoelectric crystal, operating at low frequencies, vibrates the bulk liquid causing the formation of cavitation bubbles. The bubbles are formed due to the negative pressure of the sound waves which causes some vapor in the liquid to come out of the solution in the form of bubbles (Flament et al., 2001). As the air bubbles, which have a short life span, rise towards the air–liquid interface, the internal pressure of the cavitation bubbles equilibrates with the atmosphere, causing the bubbles to implode. As the bubble collapses at the liquid surface, parts of the liquid break free and form droplets. The capillary wave theory states that high frequency crystal vibrations cause formation of small waves, so called capillary waves. The capillary waves interfere to form peaks at the liquid surface which become unstable under the influence of continuous vibrations. Parts of the peak break free and form droplets. Large droplets either impact on baffles or return to the reservoir under the influence of gravity and are re-circulated (Hickey, 1996).

Molecules of a surface active substance in the solution in nebulizer reservoir orientate themselves at the liquid–air interface until this interface is saturated (below the critical micelle concentration). Above the critical micelle concentration the molecules of the surface active substances form micelles in the bulk of the solution. Critical micelle concentration for BAC was determined to be 0.05% (w/v). Therefore, in a 0.1% solution which was used in all the experiments, the liquid–air interface is completely saturated with BAC molecules. Surfactant molecules in free form in the bulk solution are in equilibrium with molecules in aggregated form (micelles) and the molecules at the air–liquid interface. Considering that during ultrasonic nebulization the initialisation of capillary wave formation and destruction of cavitation bubbles caused by vibrating of piezoelectric crystal are the main events which lead to the droplet formation taking place primarily at the surface of the solution in the nebulizer reservoir, this would lead to a preferred incorporation of the surface active drug molecules into aerosol droplets and, therefore, increase the amount of aerosolized drug molecules (Fig. 3). This effect predominates over the effect of solvent evaporation due to the temperature increase which is caused by the heat transmission from piezoelectric crystal.

Once the molecules of the surface active substance are deprived of the liquid–air interface, molecules from the bulk solution would replace them immediately so that a new equilibrium state will be reached. An increase of the aerosolized drug amount during ultrasonic nebulization results simultaneously in the observed decrease of the BAC content in the residual volume.

Molecules of a surface active substance in an aqueous aerosol droplet will orientate themselves to the surface of the droplet. The volume of the nebulized solution consists of small droplets while the residual volume is formed by bulk solution and larger droplets which are baffled and recirculate within the nebulizer reservoir. Since the surface/volume ratio for the smaller droplets is higher than that of the larger ones, the volume of the solution which is aerosolized contains more drug molecules than the residual volume. This leads to an increased aerosolized drug amount and a decreased residual drug amount associated with a decrease in the residual drug concentration. The decrease of residual drug concentration leads to an increase of surface tension of the solution in the reservoir. Differences observed for the Multisonic® and Optineb® nebulizer may be attributed to the presence of a coupling liquid in the Optineb® device, leading to the indirect energy transmission.

Jet nebulization is based on the Bernoulli principle. During aerosolization two processes take place simultaneously. Firstly, liquid is sucked through capillaries to the jet and aerosolized. Secondly, assuming preferred accumulation of the molecules of the surface active substance into the aerosol droplets, an increase of the aerosolized substance amount and a decrease of the residual concentration of the substance in the nebulizer reservoir would be observed, since the molecules of the surface active substance are orientated at the air–liquid interface (Fig. 4). In the case of BAC nebulization with the Pari LC Star® nebulizer, the effect of solvent evaporation predominates leading to an increase of the residual solution concentration, in spite of relatively short nebulization time (4.4 min), because of the continuous aerosol generation and high air flow rate through the nebulizer. Nebulization with the Prodose® nebulizer also led to the increase of the residual solution concen-



1 - surface saturation with the molecules of the surface active drug

2 - micelles in the bulk solution

3 - molecules of the surface active drug not associated in micelles

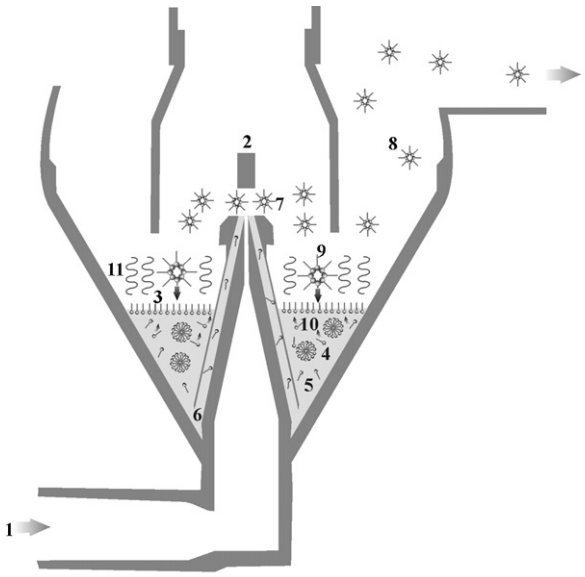
4 - piezoelectric crystal

5 - droplet formation at the surface, preferential inclusion and accumulation of the molecules of the surface active drug

6 - aerosol droplet

7 - immediate reorganisation and surface saturation with the molecules of the surface active drug

Fig. 3. Mechanism of ultrasonic nebulization of the surface active drugs.



1 - air from the compressor

2 - baffle

3 - surface saturation with the molecules of the surface active drug

4 - micelles in the bulk solution

5 - molecules of the surface active drug not associated in micelles

6 - feed tube of the Venturi system

7 - primary droplet formation around the nozzle of the Venturi system

8 - secondly generated droplets leaving the nebuliser

9 - recirculation of the aerosol droplets through impaction on a baffle

10 - immediate reorganisation and surface saturation with the molecules of the surface active drug

11 - solution evaporation from the nebuliser reservoir

Fig. 4. Mechanism of jet nebulization of the surface active drugs.

tration because of the long nebulization time (NT 11 min). During shorter nebulization times and non-continuous aerosol generation (Halolite®, NT 4.5 min and Prodose®, NT 5 min) the air flow through the nebulizer is not continuous and, therefore, evaporation of the solvent is not strong. In this case the reduction of the residual solution concentration was observed.

It was observed that during jet nebulization an increase of residual concentration happens if the nebulization is carried out at higher flow rates of driving gas through the device. The evaporation of the solvent is highly dependent on the air flow through the jet nebulizer chamber created by a compressor. At higher flow rates the evaporation effect will be more pronounced than at lower flow rates. Accordingly, the air flow rate used to operate the different nebulizers affects the evaporation of pure solvent and determines, whether a reduction of residual concentration (preferred aerosolization at low flow rates) or an up-concentration (evaporation pre-domination) is taking place. This suggestion could be confirmed when operating the Pari LC Star® nebulizer at different flow rates, generated by different gas pressures, namely, at 1–3 bar. At 1 bar, the residual concentration of BAC in the reservoir decreased by $9.1 \pm 4.9\%$, whereas at 2 bar the residual concentration has increased by $9.5 \pm 0.5\%$ and at 3 bar by $22.2 \pm 2.8\%$. These results support the assumption that the observed nebulization properties can be attributed to the surface activity of the substance. At low flow rates the effect of solvent evaporation is not strong; smaller droplets, leaving the reservoir, incorporate more molecules of the surface active substance, since they are orientated at the liquid surface. At higher flow rates the effect of solvent evaporation is stronger. This results in an increase of residual concentration of BAC solution. The increased flow rates also resulted in an increased amount of BAC deposited on inhalation filters. Greater flow rate going through the nebulizer reservoir causes an increase of aerosol amount generated per time and, therefore, results in an increased BAC amount deposited on inhalation filters. At 1 bar, 105.7 ± 5.7 μg of BAC was recovered from the inhalation filter. At 2 and 3 bar, the inhalation filter deposition was 347.5 ± 14.0 μg and 560.4 ± 13.1 μg, respectively.

For vibrating membrane nebulizers both methods showed no significant differences for the aerosolized and residual substance amounts. Also, no changes in the residual drug concentration were observed. The temperature within the nebulizer reservoir slightly increased as a result of energy transmission.

Due to the specific mechanism of nebulization, the effect of solvent evaporation is avoided. The surface activity of the drug did not influence the amount of the surface active substance molecules incorporated into aerosol droplets or left in the nebulizer reservoir.

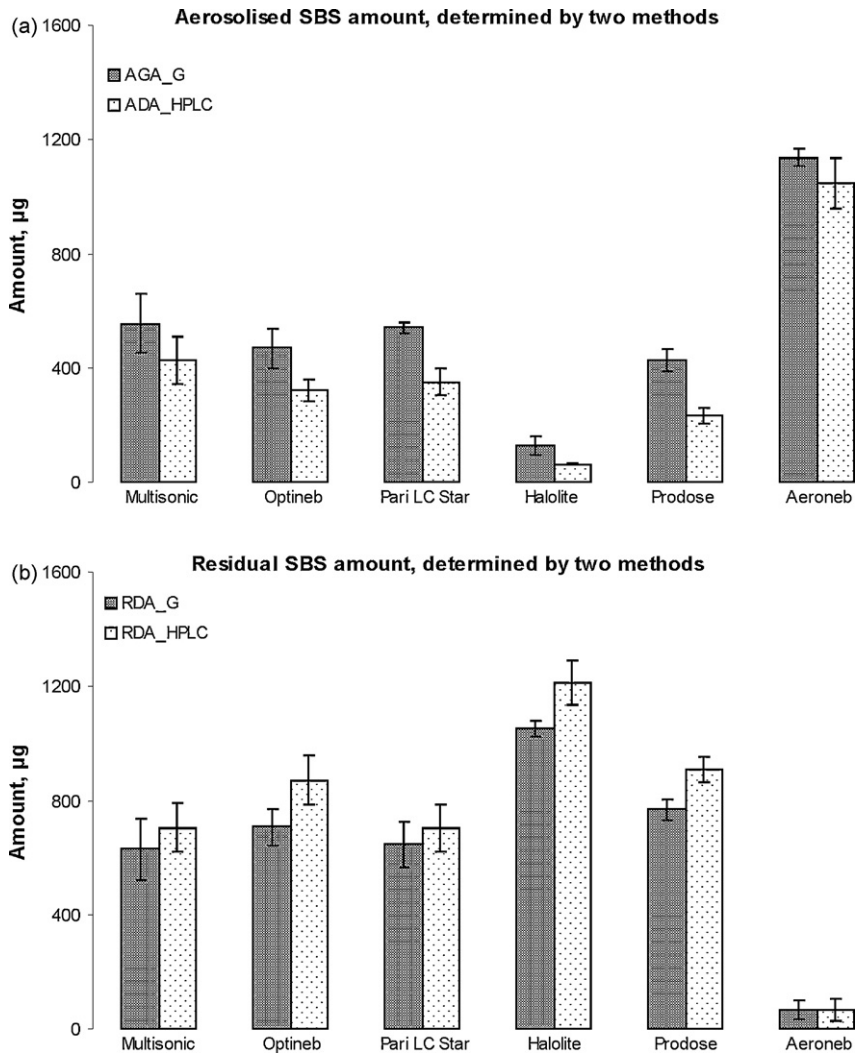


Fig. 5. (a) Aerosolized SBS amount. (b) Residual SBS amount.

3.2. Nebulization of SBS

Table 2 shows the measured nebulization times for SBS solution. The nebulization of SBS with the Multisonic® nebulizer reduced the nebulization time by approximately 3 min, compared to the nebulization duration of BAC. This could be explained by a reduction of the solution surface tension during nebulization of BAC, resulting in longer times for cavitation bubbles' and capillary waves' formation. All other nebulization times were found to be similar to that of BAC solution. The aerosol output and residual volume, which were measured with a mass-balance method, are presented in Table 3. The nebulization of SBS solution led to similar values for the aerosol output and residual volumes as during nebulization of BAC solution. Approximately the same aerosol output and residual volume were, nevertheless, reached within shorter nebulization time during nebulization with Multisonic® device.

For ultrasonic, jet and AAD jet nebulizers the HPLC method yielded lower aerosolized drug amounts and higher residual drug amounts than the mass-balance method (Fig. 5a and b). These data were different to those, obtained during nebulization of BAC solution. These observations were explained by the absence of the surface activity properties of SBS. So, we observed the normal up-concentration, caused by the solvent evaporation from the reservoir. The heat generated by a vibrating piezoelectric crystal leads to an increase of the temperature in the nebulizer reservoir during ultrasonic nebulization. The temperature increase causes the solvent in the reservoir to evaporate. During jet nebulization the nebulizer is driven by compressed air with low humidity and temperature. When getting into contact with compressed air, the water in the nebulizer reservoir starts to evaporate. The effect of solvent evaporation from nebulizer reservoir results in a second fraction of nebulizer output: the solvent output. Besides aerosolized droplets containing drug, a solvent vapor fraction is produced. Since the mass-balance method is based on measuring the mass losses of a nebulizer after nebulization, it does not consider the formation of a solvent vapor fraction, leading to the overestimation of aerosolized drug amount and was also observed during the nebulization of SBS. The evaporation of solvent from the nebulizer chamber leads to the up-concentration of the nebulized solution with all types of nebulizers. The concentration of SBS solution in the reservoir increased by approximately 10.0% ± 0.2% of the initial concentration (Multisonic®), 9.0% ± 0.6% (Optineb®), 9.0% ± 0.3% (Pari LC Star®), 5.0% ± 0.2% (Halolite®) and 2.0% ± 0.3% (Prodose®), respectively. The effect of solvent evaporation is stronger during conventional jet nebulization than during ultrasonic or jet–AAD nebulization and a stronger up-concentration effect was shown within the shortest nebulization time using Pari LC Plus® nebulizer. The longest nebulization time of ca. 9 min with Prodose® nebulizer still resulted only in a small increase of the residual concentration of the nebulized solution. This can be explained by the

application of the AAD system in the construction of this nebulizer, which generates an aerosol only during inhalation, leading in this way to the reduction of the evaporation effect. After nebulization with the Aeroneb® Pro device no or very little changes in the residual concentration were observed as the effect of solvent evaporation seems to be avoided. For vibrating membrane nebulizer HPLC and mass-balance method also showed no difference in the determination of aerosolized and residual drug amounts.

4. Conclusion

Parameters of aerosolized and residual drug amount as well as concentration effects in the nebulizer reservoir during nebulization of two substances with and without properties of surface activity were investigated in this study. An opposite behavior of these substances was observed. The obtained results support the hypothesis, that the molecules of surface active drugs and drugs without properties of surface activity are differently engaged in the process of aerosol droplet generation. These observations are crucial, since there are drugs used in the nebulization therapy which have surface active properties. Nebulization of the surface active drugs with ultrasonic nebulizers leads to higher aerosolized drug amounts as could be expected by mass-balance measurements which in the case of highly active drugs can lead to the development of side effects as well as to the reduction of treatment tolerability. During jet nebulization of surface active drugs, the solvent evaporation rate from the nebulizer reservoir was a principal effect influencing the nebulizers' efficiency.

The vibrating membrane nebulizer was found to be an optimal device to deliver a substance with surface activity. The residual volume in this device is reported to be minimal. This fact plays a role in the pulmonary delivery of highly cost-intensive drug products. The study also showed that no significant increase in reservoir temperature happens during operation of this type of nebulizers.

References

Allen, M., Langford, S., 1993. Variability in jet output. Chest 103, 1922–1923.
Barry, P.W., 2002. The future of nebulization. Respir. Care 47, 1459–1470.
Barry, P.W., O'Callaghan, C., 1999. An *in vitro* analysis of the output of salbutamol from different nebulizers. Eur. Respir. J. 13, 1164–1169.
Byat, M., Cook, A.M., 2004. Intrapulmonary administration of medications. J. Neurosci. Nurs. 36, 231–235.
Clay, M.M., Pavia, D., Newman, S.P., Clarke, S.W., 1983. Factors influencing the size distribution of aerosols from jet nebulizers. Thorax 38, 755–759.
Dahlback, M., 1994. Behavior of nebulizing solutions and suspensions. J. Aerosol Med. 7, 13–18.
Dennis, J.H., Stenton, S.C., Beach, J.R., Avenry, A.J., Walters, E.H., Hendrick, D.J., 1990. Jet and ultrasonic nebulizer output: use of a new method for direct measurement of aerosol output. Thorax 45, 728–732.
Eklund, L., Sundblad, B.M., Malmberg, P., Larsson, K., 2000. The salt output of a nebulizer – a comparison between two types. Respir. Med. 94, 139–144.
Esmond, G., 1998. Nebulizer therapy. Prof. Nurs. 14, 39–43.
Flament, M.P., Leterme, P., Gayot, A., 2001. Study of the technological parameters of ultrasonic nebulization. Drug Dev. Ind. Pharm. 27, 643–649.
Gatnash, A.A., Chandler, S.T., Connolly, C.K., 1998. A new method for measuring aerosol output using radioactive tracers. Eur. Respir. J. 12, 467–471.
Groneberg, D.A., Witt, C., Wagner, U., Chung, K.F., Fischer, A., 2003. Fundamentals of pulmonary drug delivery. Respir. Med. 97, 382–387.
Hess, D., Fisher, D., Williams, P., Pooler, S., Kacmarek, R.M., 1996. Medication performance. Effects of diluent volume, flow, and brand. Chest 110, 498–505.
Hickey, A., 1996. Inhalation Aerosols: Physical and Biological Basis for Therapy. Marcel Dekker Inc., New York, pp. 313–324.
Loffert, D.T., Ikle, D., Nelson, H.S., 1994. A comparison of commercial jet nebulizers. Chest 106, 1788–1792.
MacNeish, C.F., Meisner, D., Thibert, R., Kelemen, S., Vadas, E.B., Coates, A.L., 1997. A comparison of pulmonary availability between Ventolin (albuterol) nebules and Ventolin (albuterol) respiratory solution. Chest 11, 204–208.
McCallion, O.N.M., Taylor, K.M.G., Bridges, P.A., Thomas, M., Taylor, A.J., 1996. Jet nebulizers for pulmonary drug delivery. Int. J. Pharm. 130, 1–12.
Newman, S.P., 2000. Lung deposition from nebulizers. Eur. Respir. Rev. 10, 224–227.
O'Callaghan, C., Barry, P.W., 1997. The science of nebulised drug delivery. Thorax 52, 31–44.
Price, R., Young, P.M., Edge, S., Staniforth, J.N., 2002. The influence of relative humidity on particulate interactions in carrier-based dry powder inhaler formulations. Int. J. Pharm. 246, 47–59.
Steckel, H., Eskandar, F., 2003. Factors affecting aerosol performance during nebulization with jet and ultrasonic nebulizers. Eur. J. Pharm. Sci. 19, 443–455.