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A system for the production and delivery of monodisperse salbutamol aerosols to the lungs

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Abstract

An aerosol system is described for the generation and delivery of measured doses of monodisperse therapeutic drug particles to the human lungs. The system comprises a spinning top aerosol generator (STAG), aerosol chamber and inhalation control unit. Monodisperse aerosols allow drug particle size effects to be studied as the dose is within a narrow size distribution and when combined with controlled inhalation may lead to more precise targeting of therapeutic drug to the airways. Using the STAG, particles in the size range 1.5–12 μm were generated and their mass median aerodynamic diameter (MMAD) and concentration measured using an aerodynamic particle sizer (APS). The application and validation of the system with the bronchodilator drug salbutamol sulphate is described, and its potential use in the study of aerosol particle size effects is discussed.

Keywords: Monodisperse aerosols; Human; Lung; $Beta_2$-agonist; Spinning top aerosol generator

1. Introduction

Aerosol particle size and mode of inhalation are the two most important factors to consider for achieving optimal drug delivery to the lungs. The main physical processes governing the deposition of inhaled drug particles and droplets in the airways are inertial impaction, gravitational sedimentation and diffusion. The relative contribution of each of these to the total amount of drug deposited is primarily determined by the particle aerodynamic diameter. Most commercial inhalers produce polydisperse particles with a wide range of sizes, and it has been shown that approximately 20% of the dose from a correctly used metered dose inhaler (MDI) is delivered to the lungs (Melchor et al., 1993). The 'respirable fraction' is the aerosol dose contained within particles between 2 and 6 μm aerodynamic diameter, which is thought to be the ideal size range for inhalation throughout the airways. This has been predicted by mathematical and experimental modelling of deposition efficiency as a function of particle aerodynamic diameter (Köbrich et al., 1994; Heyder et al., 1986). These models also indicate that particles with aerodynamic diameters less than 2 μm will mainly deposit in the alveolar region or may be exhaled, whilst particles greater than 6 μm are likely to impact in the oropharynx. In order

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to study the particle size effects of drugs within the airways it is important to use aerosols of narrow size dispersity. Monodisperse aerosols are ideal for these studies as they have a geometric size distribution (GSD) of <1.22 (Morrow, 1981).

The STAG is one of the best-known instruments for generating particle aerosols of narrow size dispersity. It is capable of producing aerosol particles over a wide range of sizes. The principle of using a spinning disc to produce a spray of droplets of almost uniform size was first demonstrated in 1947 (Johnson and Walton, 1947). It was noted that the aerosol droplet size could be varied by changing the speed of the disc. A later design used compressed air to drive the disc (Walton and Prewett, 1949). May (1949) made further significant changes to the design to produce the forerunner of the present day device and later designed the first commercial version of the apparatus known as the STAG Mark I (May, 1966). The Mark II version, reviewed in 1991 (Melton et al., 1991), added further important improvements to the design enabling it to be used more easily and efficiently.

The STAG is considered an important research instrument for delivering inert monodisperse particles to the lung for the measurement of aerosol penetration and mucociliary clearance (Thomson and Short, 1969; Pavia et al., 1977; Pavia et al., 1980; Hasani et al., 1992). It has also been used to produce radiolabelled Teflon particles, which have been used as indirect markers of drug incorporated into an MDI in place of the drug (Newman et al., 1981) or in an MDI and a dry powder inhaler (DPI) in addition to the drug (Zainudin et al., 1990). More recently, the STAG has been used to generate unlabelled monodisperse drug particles (Zanen et al., 1996) in order to investigate the optimal particle size of β_2-agonist for delivery to the lungs of asthmatics.

The aim of this research was to develop a versatile system able to produce and deliver stable pharmacologically active monodisperse drug particles of various sizes. We describe the methods and validation of the production of monodisperse salbutamol sulphate aerosols using the STAG Mark II (Research Engineers Ltd., London, UK), and its use as a potentially valuable tool capable of delivering aerosols for inhalation and for the investigation of bronchodilator particle size effects within the human airways in vivo.

2. Materials and methods

2.1. Apparatus

A diagram of the aerosol generation and delivery apparatus is shown in Fig. 1. The STAG comprises an air driven steel disc (also known as a top), a stator and support assembly, a liquid feed needle assembly with remote height adjustment, a control unit housing the disc and exhauster air pressure regulators, and a digital disc speed indicator. It is housed in a purpose-built Perspex tank (aerosol chamber) with a volume of 150 l in which the aerosol particles accumulate. It is supported on a pedestal in the centre of the chamber. The source of the compressed air used to drive the disc and provide suction for the exhaust system, as well as inflate a balloon valve system is an air compressor (Motivair Ltd., Twickenham, UK). This provides clean, dry compressed air with a pressure of 5.5 bar at maximum demand.

Compressed air is delivered to the base of the STAG via pneumatic couplings through the wall of the aerosol chamber from the externally located disc and exhaust control unit. This unit contains regulators, which allow manual control of the air pressure applied to the disc and exhaust, and also contains a digital rotational speed indicator linked to a sensor located close to the STAG disc. This combination enables very accurate and reproducible control of the disc speed from outside of the chamber.

An outlet leads from the front of the aerosol chamber to a four-way inflatable balloon valve system (Hans Rudolph Inc., K.C., MO., USA) and a mouthpiece by which subjects may inhale the generated aerosol. An inlet at the rear allows air to be drawn into the chamber via a Fleisch flow transducer head. Alternatively, an APS (model 3310 with 3302 100:1 Diluter, TSI Inc., St. Paul, MN, USA) can be placed at the outlet in order to sample the aerosol concentration within the chamber. The Fleisch head is coupled to a pneumotachograph (CS5T Electrospirometer, GM Instruments Ltd., UK), which measures the flow and volume of inspired gases. This is electrically connected to the inhalation control unit, which has been designed in our workshop (Biomedical Engineering Department, Royal Brompton Hospital), to control the breathing manoeuvre. This unit controls the switching of compressed air to three balloon valves to inflate

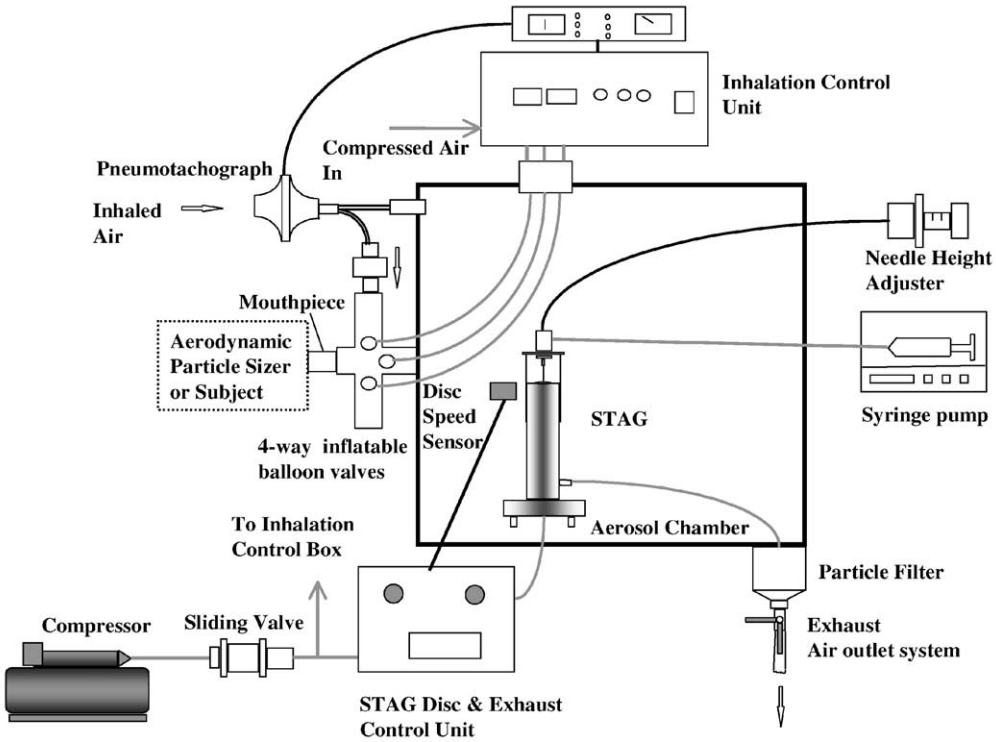

Fig. 1. Diagram of the aerosol generation and delivery system showing the spinning top aerosol generator, the aerosol chamber and the inhalation control unit. The aerodynamic particle sizer or subject can be positioned at the mouthpiece depending on whether the aerosol is being generated or delivered.

or deflate them. When operated in automatic mode the unit is triggered by the subject's first inspiration. The valves open and close in a designated sequence allowing the volume inhaled, breath-hold pause and number of breathes to be controlled. Exhaled breath can be diverted to a separate circuit where a filter can be used to collect particles.

The upper surface of the disc is flat and the conical lower surface is grooved and lies within the stator cup when at rest. The stator assembly is interfaced to the hollow steel body of the STAG by an elastomer damping ring to ensure stability of rotation. The disc is driven at high speed by the compressed air impinging on the grooves in the lower surface, which arrives through jets in the stator. Liquid to be aerosolised is fed by a syringe pump, via a needle, to the centre of the disc (Fig. 2). The liquid spreads to the edge of the disc where it accumulates until the centrifugal force acting upon it exceeds the retaining force of surface tension. The liquid is expelled tangentially, and forms rings of spherical droplets of two sizes known as primary and secondary droplets. The diameter of the primary droplet d is given by Eq. (1), where R is the disc radius, ω the angular velocity and T and ρ are the

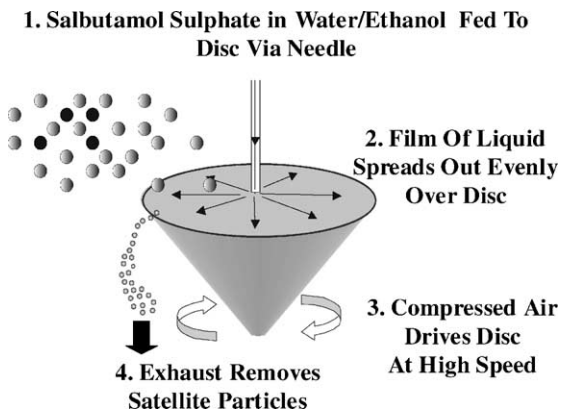

Fig. 2. Principle of operation of the STAG disc to produce monodisperse aerosols.

liquid surface tension and density.

$$d \propto \frac{1}{\omega}\sqrt{\frac{T}{R\rho}} \quad (1)$$

Therefore, droplet size decreases as the angular velocity of the disc increases. The secondary droplets are approximately 1/4 of the diameter of the primary droplets. These "satellite" droplets are removed by the suction produced by the exhaust system. The exhaust system can be varied to allow the airflow conditions around the disc periphery to be optimised so that all satellite droplets are removed whilst leaving the main droplet ring undisturbed. If the exhaust is set too high it will remove primary droplets as well. This function can also be useful for lowering the aerosol concentration around the STAG if necessary.

2.2. Technical considerations

In order to reproducibly generate monodisperse particles the apparatus must be carefully maintained and operated. The STAG needs to be completely level when operating otherwise the disc will not spin correctly. It requires frequent cleaning so that the exhaust system does not become blocked and the disc coated with drug. It is important for the needle to be centred and as close to the disc surface as possible, without actually touching, so that liquid flows out evenly without dripping. The needle height control of the STAG Mark II makes this much easier than its predecessor. Solution dripping or pulsing onto the disc surface rather than flowing leads to greater numbers of secondary particles being produced and wider aerosol dispersity. We found that drips could be eliminated by drilling a small hole in the centre of the disc to enable the needle to be lowered slightly below the surface of the disc. This also enables the exact centring of the needle relative to the disc, which is crucial for monodisperse aerosols to be produced. The surface wetting properties of the disc need to be good to enable the liquid to flow evenly over the surface. Regular cleaning of the disc helps to maintain this and we found that improved results were obtained when the surface was polished with fine abrasive paper to remove the original matt finish.

Table 1
Three concentrations of salbutamol sulphate, water and ethanol solutions used to generate monodisperse particles with the STAG

Material	Solution 1	Solution 2	Solution 3
Salbutamol sulphate (mg)	100	50	25
Sterile water (ml)	2	2	2
Ethanol (ml)	25	25	25

2.3. Experimental procedures

2.3.1. Salbutamol formulations for the generation of monodisperse aerosols

Initial experiments were aimed at generating monodisperse aerosols of salbutamol sulphate. The disc speed and drug concentrations were varied and the resulting particle distributions sampled with the APS. The MMAD and GSD of each distribution were recorded. Salbutamol sulphate was first dissolved in a small volume of water and then thoroughly mixed with a larger volume of ethanol (96% pharmaceutical grade, Martindale brand). Several concentrations of salbutamol sulphate, water and ethanol were prepared to identify which produced a good range of particle sizes with narrow dispersity and an acceptable aerosol concentration. The final three solution concentrations are given in Table 1.

2.3.2. Particle size and disc rotor speed relationship

A series of measurements were undertaken to calibrate the rotational speed of the disc with particle size generated using each of the three solutions described above. The particle MMAD and GSD were measured with the APS. Particles distributions were only considered acceptable if they had a GSD < 1.22. These measurements were made four times for each concentration with fresh solutions used every time (Fig. 3).

2.3.3. Validation of aerosol size and distribution

The aerosol particle size generated was sampled using an Andersen cascade impactor (Graseby-Andersen, Smyrna, GA, USA) and the particle distributions compared with those determined by the APS (Fig. 4). The Andersen cascade impactor plates were coated in a solution of 1% silicone oil in hexane and allowed to dry prior to assembling the impactor to prevent particle bounce. In order to make a comparison between

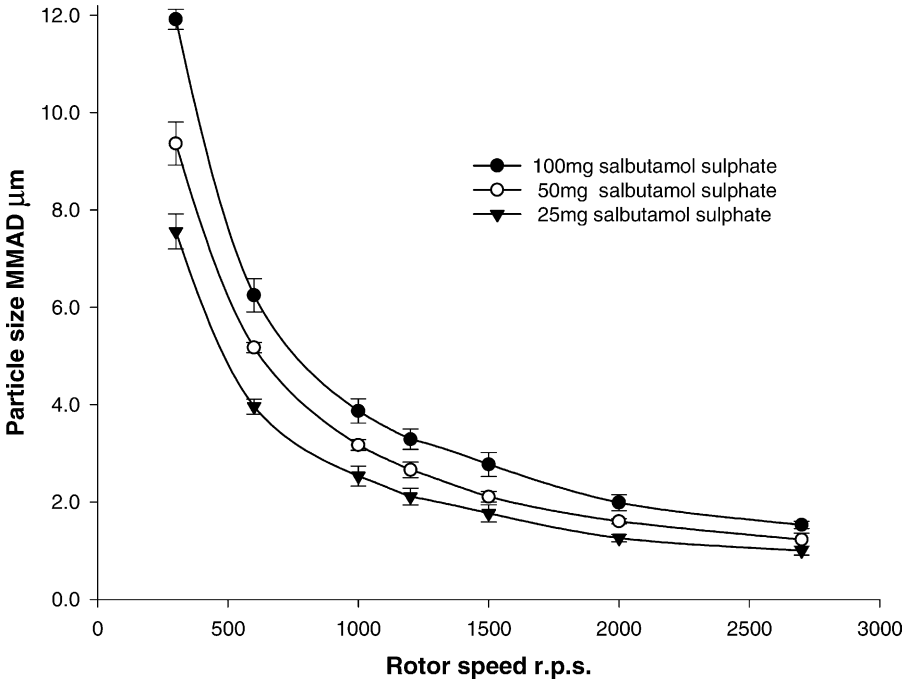

Fig. 3. Mean particle size (MMAD) vs. disc speed for three concentrations of salbutamol sulphate. Drug is dissolved in 2 ml sterile water and 25 ml ethanol. Each curve is the mean of four repeat measurements using fresh solution each time. Error bars indicate the S.D. of each data point.

the two instruments the aerosol was generated with the APS at the mouthpiece and the MMAD and GSD were measured. The APS was then replaced, by the Andersen cascade impactor and its pump turned on for 3 min at 28.3 l/min. The impactor was disassembled and the collection plates and backup filter removed and washed into separate volumetric flasks using methanol (HPLC grade)/water (70:30). The average aerosol concentration that we were sampling was 20 μg/l. We sampled over 3 min to allow sufficient drug to build up on the plates. Each plate was visually inspected prior to washing and white powder was visible on the impactor plates that had collected most drug.

The salbutamol concentrations in each flask were measured using an ultraviolet (UV) spectrophotometer (Kontron Instruments, Milton Keynes, UK) at a wavelength of 276 nm. To calibrate we produced a stock solution of 100 mg salbutamol sulphate in 100 ml methanol /water (70:30) and diluted it to produce a series of solution concentrations ranging from 10 mg in 50 ml (0.2 mg/ml) down to 25 μg in 50 ml (0.5 μg/ml). We measured the corresponding absorption values and plotted the drug concentrations against the absorption values on a graph and produced a linear regression line using Graphpad Prism. We found the limits of detection and quantification to be about 100 μg in 50 ml (2 μg/ml).

The aerosol MMAD and GSD were determined by calculating the cumulative percentage of drug less than the effective cutoff diameter (ECD) of each plate versus the effective cutoff diameter. The MMAD is the ECD at the cumulative 50% value and the GSD is the ratio of the diameter at the 84.1% cumulative percentage to the 50% value.

2.3.4. Stability of monodisperse aerosol distributions

During the generation process the ethanol and water rapidly evaporated. The stability of aerosols with MMADs 1.5, 3 and 6 μm was demonstrated by switching the syringe pump off so that no further aerosol was generated (Fig. 5). The aerosol was then monitored over several minutes using the APS to ascertain whether the MMAD of the particles changed due to

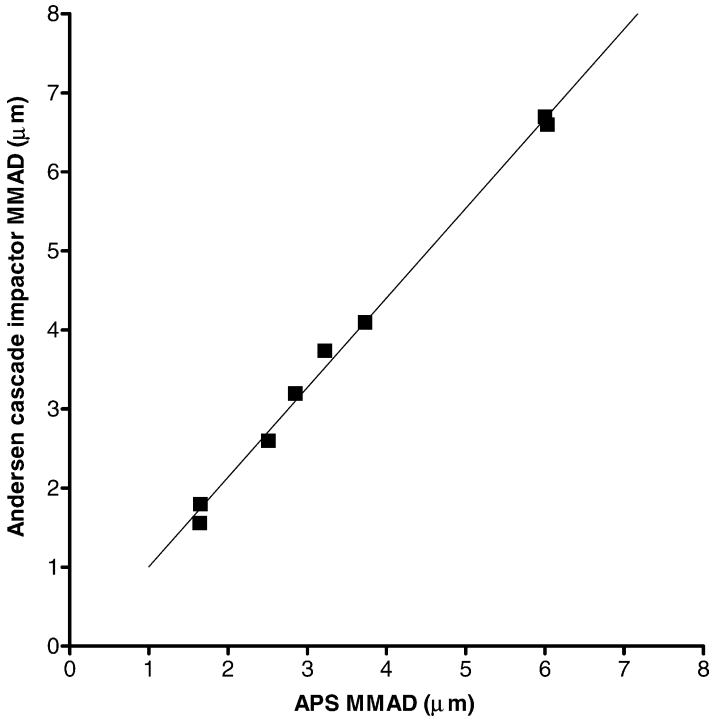

Fig. 4. Comparison of particle MMAD for the Andersen cascade impactor and the aerodynamic particle sizer. Equation of trend line $y = 1.133x - 0.112$ ($r^2 = 0.995$)

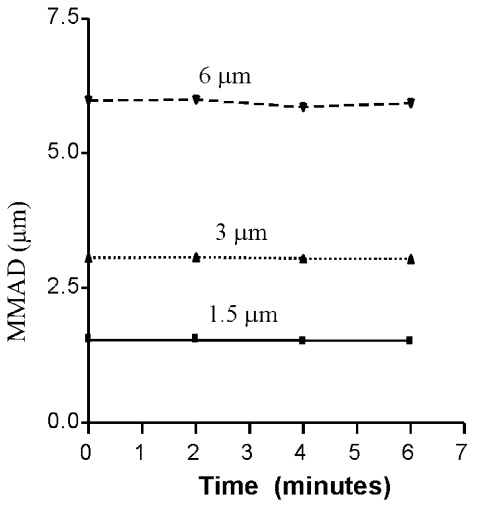

Fig. 5. Stability of particle MMAD in the aerosol chamber, for three particle diameters, at 0, 2, 4 and 6 min following the termination of the spinning top aerosol generator. The measurements were made by the APS.

further evaporation of ethanol or whether the particles were already dry. In another experiment glass slides were placed in the aerosol chamber for collecting particles and later inspection by a scanning electron microscope (SEM). SEM images of particles of size 1.5, 3 and 6 μm are shown in Fig. 6.

2.3.5. *Verification of the aerosol mass generated*

The dose of salbutamol aerosol generated for delivery, as measured by the APS, was confirmed using in vitro methods. The accuracy of the APS is crucial in ensuring delivery of the correct dose. In order to do this the drug concentrations at the mouthpiece were sampled and compared to the APS readings. Aerosol was allowed to build-up in the chamber and generation was then terminated, by turning off the syringe pump, slowing the disc down and allowing the aerosol concentration to decay whilst being sampled by the APS. The time at which the syringe pump was turned off was noted and referred to as time zero and the concentration at this point was taken as the start concentration. During the decay period two aerosol samples

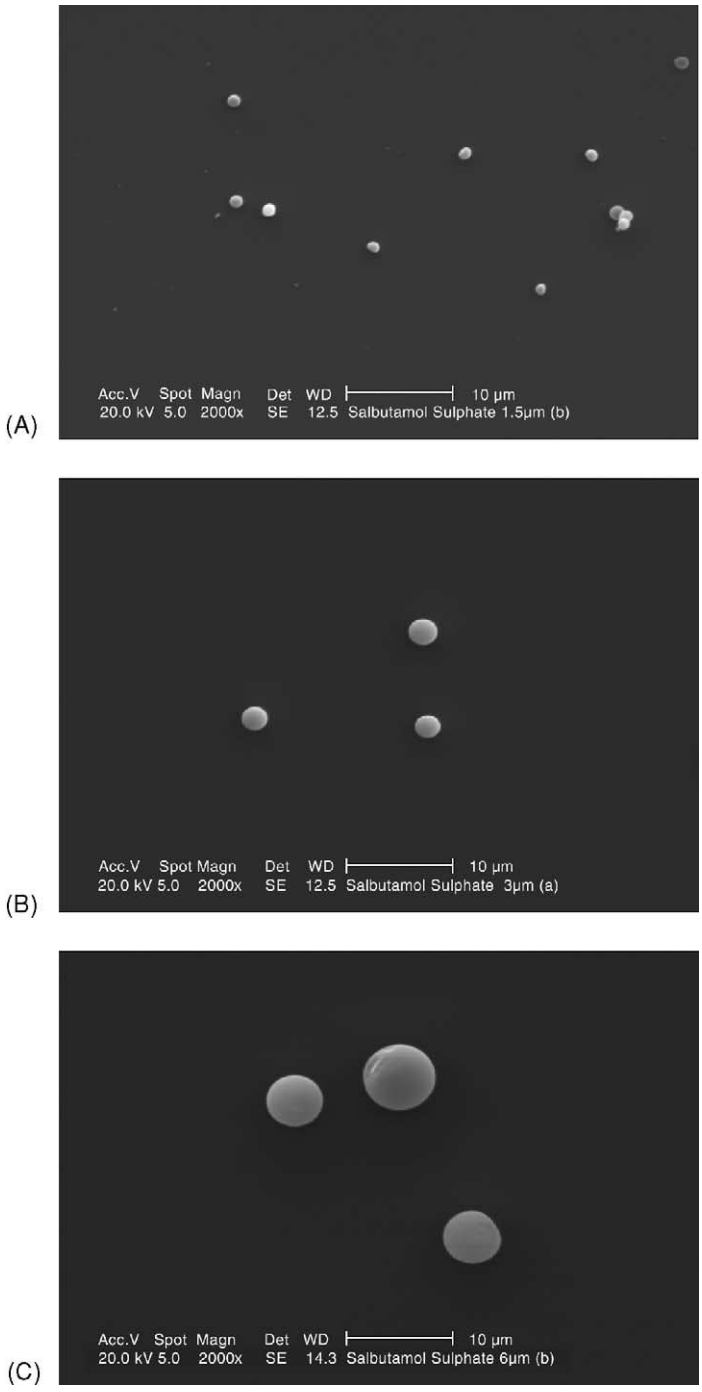

Fig. 6. Scanning electron microscope images of salbutamol sulphate particles of MMAD (A) 1.5 μm, (B) 3 μm and (C) 6 μm generated by the STAG.

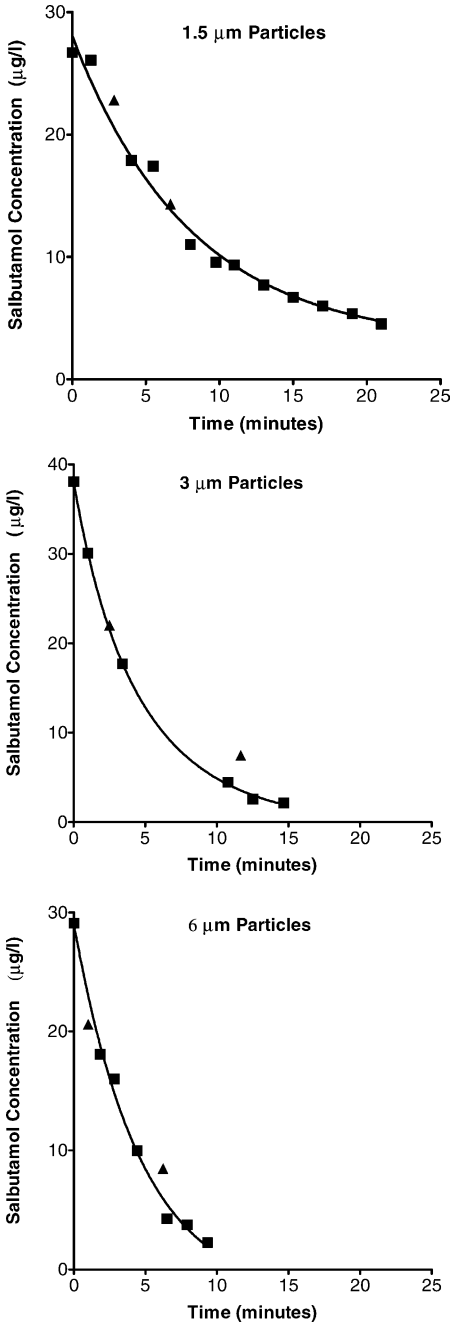

Fig. 7. Comparison of drug concentrations generated by the STAG and collected on filters (▲) with those measured by the APS (■) for three sizes of particles. Time is measured from the termination of aerosol generation at time 0.

were collected, by drawing air through filters (Respigard II), from the mouthpiece by a pump at 30 l/min for 3 min. The time of each sample was noted and followed by further APS measurements. Drug concentrations derived from the mass of particles trapped in the filters were later measured by removing the filter material and washing with a solution of methanol and water (70:30). The total mass of drug collected was determined using the UV spectrophotometer. This value was divided by the volume of air drawn through each filter to give the actual aerosol drug concentrations at the time of the filter sample. The concentrations measured for the two filters were then compared to those measured by the APS by plotting them on the same concentration–time graphs (Fig. 7). Aerosols of MMAD 1.5, 3 and 6 μm, representing particles within the respiratory range, were validated using these methods. These measurements were repeated several times for each particle size and the data plotted as salbutamol concentration (μg/l) determined by the APS versus salbutamol concentration (μg/l) collected on filters (Fig. 8).

3. Results

We have shown that it is possible to generate a range of salbutamol particle sizes using the STAG, by varying the disc speed and solution concentration. Fig. 3 shows the relationship between particle size and disc speed for the three different concentrations of salbutamol, water and ethanol. Mean values of four repeat measurements are shown with error bars indicating the S.D. of each value. As the particle diameter d is inversely proportional to the angular velocity ω Eq. (1), increasing the disc rotor speed leads to smaller particle sizes.

The APS measurements were compared to an Andersen cascade impactor (Fig. 4) yielding a regression line for the MMADs of $y = 1.133x - 0.112$ ($r^2 = 0.995$). These results indicate a good correlation between the particle size given by the APS and the Andersen cascade impactor.

Fig. 5 indicates that the monodisperse aerosols were stable, and that their particle size did not change over a period of 6 min following generation. It suggests that evaporation of ethanol and water was complete, and that the drug particles were dry. Fig. 6 shows SEM

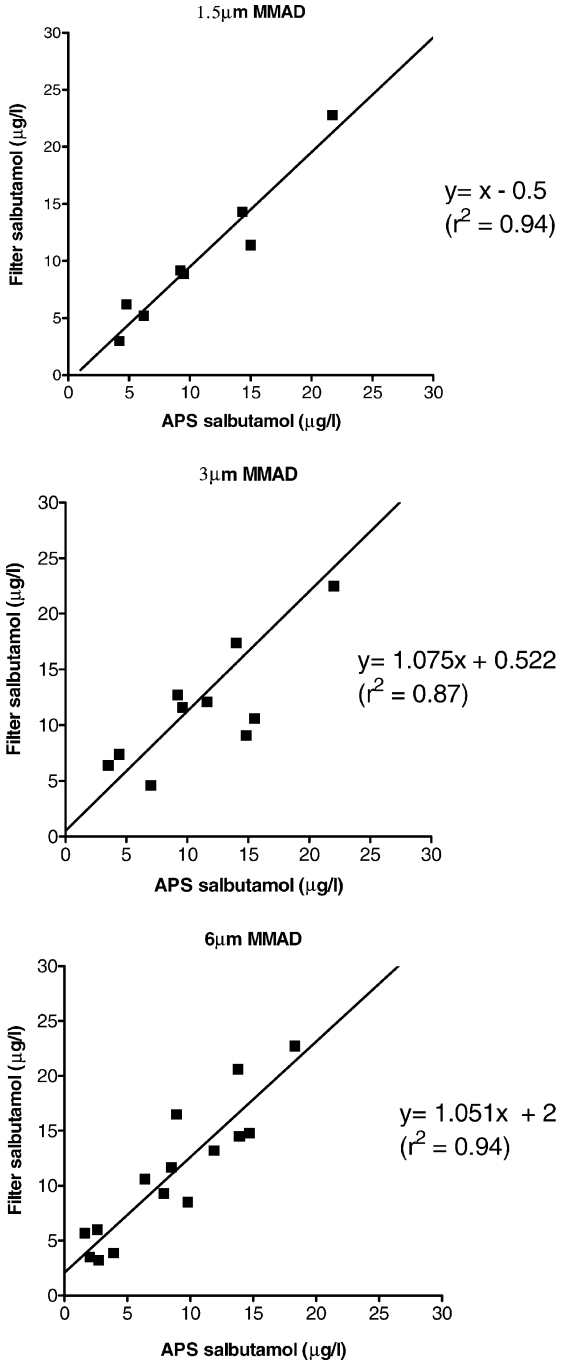

Fig. 8. Comparisons of drug concentrations collected in filters to those determined by the APS using the same methods to measure the data as that shown in Fig. 7 for particles of MMAD 1.5, 3 and 6 μm. Linear regression lines and their equations and r^2 values are shown.

images of particles generated by the STAG of MMAD 1.5, 3 and 6 μm. The SEM pictures show spherical dry particles of salbutamol sulphate and indicate a good correspondence between the physical diameters and the aerodynamic diameters as given by the MMADs of the aerosols. The concentration of aerosol produced by the STAG is low and given the volume of the aerosol chamber (150 l) the glass slides placed in the chamber were only able to collect a small portion of the particles. There is some variation in the physical particle size but this might be expected even from a monodisperse distribution unless its GSD was exactly 1. We measured both the MMAD and the GSD of the distribution at the same time as collecting the SEM slides and found the GSDs were all less than 1.2.

The drug concentrations measured by the APS were validated using concentrations derived from drug collected on filters, (Fig. 7). A measure of the accuracy of this relationship is shown by linear regression analysis of the repeated data (Fig. 8), and the trend lines and associated equations are given. They show a direct relationship between the two with small constant terms in each of the equations. The concentration decay curves of Fig. 7 also give an indication of the rate of settling of the particles and show that as expected the large particles settle much faster than the smaller particles.

4. Discussion

The aerosol generation and delivery system described is a versatile investigative tool for generating monodisperse aerosol distributions of therapeutic drug particles and allows them to be delivered to the airways in a controlled manner. The spinning top aerosol generator is capable of producing well-defined salbutamol sulphate particles of a wide range of sizes. We have validated the aerosol output characteristics for three particle sizes of monodisperse salbutamol (1.5, 3 and 6 μm) and showed that the APS can be used to give a direct measure of particle size and drug concentration. Measurements made by the APS and the Andersen cascade impactor on the same aerosol distributions gave comparable MMADs although the Andersen values were slightly higher. We also found that the GSDs measured by the Andersen were larger than 1.22. However, the Andersen is not ideal for

monodisperse aerosol distributions as it separates the sample into only 9 size intervals, with cutpoints ranging from 0.4 to 9 μm (Graseby-Andersen, 1985), compared to 52 size intervals for the APS, ranging from 0.5 to 20 μm. The Andersen size intervals are wider than those of the APS giving it less resolution to discriminate the spread of a narrow aerosol distribution.

It was necessary to validate the output from the APS by comparing it to an established chemical analysis method since it has serious limitations that can cause it to give erroneous output if these are not taken into account. The APS is a time of flight instrument and calculates each particle's aerodynamic diameter by measuring its time to pass between a laser beam, split into two beams, while being accelerated by an air flow, rather than using a direct drug assay. Its main advantage is that it can give an almost real-time measurement of aerosol MMAD and concentration but assumes the aerosol is composed of spherical particles of uniform density. It is unable to distinguish between drug and non-drug particles and may give incorrect readings due to particle coincidence if the incoming particle concentration is high (Mitchell and Nagel, 1999). These problems are important in wet aerosols and where large numbers of non-drug containing excipient particles are present such as those generated by pressurised MDIs and DPIs that use carrier particles. The aerosols generated by our system are homogeneous, dry and slow moving.

There are several benefits of this generator system over previous systems. Comparisons of the relative clinical effects of particles of different sizes can be made where the inhalation manoeuvre is consistent. Single or cumulative doses of drug can be delivered at various particle sizes and the response measured. Alternatively, the particle size and dose can be made constant and the inhalation manoeuvre varied. The versatility of the system stems from the facility to set the volume of air inhaled from the aerosol chamber and from the room and to include a breath-hold pause and exhalation breath in each automatic cycle. If this is combined with consistent lung inflation at the start of each breath and consistent inhalation flow rate, a reproducible breathing manoeuvre can be obtained removing one possible confounding factor in drug delivery. This is the most important improvement between this system and those of previous studies (Patel et al., 1990; Zanen et al., 1994) where subjects inhaled slow vital capacity breaths from the chamber until the correct dose was delivered. Our system also has a larger aerosol chamber, which aids aerosol mixing and allows evaporation of solvent prior to inhalation without the requirement of drying the particles separately.

The process by which drug is delivered to the lungs and the inhalation manoeuvre is similar to that used for an MDI with a spacer attachment rather than for an MDI alone or a DPI. The particles are inhaled from rest with slow steady breaths without the need for coordination. This is in contrast to that required for an MDI where the high discharge speed into the mouth and need for coordination leads to inevitable impaction losses in the oropharynx. It is also different to that required for many DPIs where a higher initial breath is necessary to get the drug particles airborne. However, the fundamental concepts of aerosol behaviour in the airways are still relevant. Thus, the effect of particle size on aerosol deposition patterns can be applied and the system provides an environment whereby these effects can be isolated from the other more patient related effects. The required particle size can be accurately obtained by adjusting the solution concentration and disc speed. The APS can then be used to verify the particle size and measure the concentration of aerosol in the chamber prior to inhalation. Monodisperse aerosols are achievable over a wide range of sizes and we found that a GSD as low as 1.0 can sometimes be obtained. The system has been tested on a number of asthmatic patients and after some coaching each has been able to adapt their breathing pattern to its requirements. Most patients found the procedures easy to learn and the instrument comfortable to use.

The validation data given in this paper is with the β_2-agonist salbutamol sulphate. However, there is plenty of scope for monodisperse aerosol studies using longer acting β_2-agonists or corticosteroids, provided the drug can be easily dissolved in a suitable solvent.

5. Conclusions

We have established an aerosol system capable of providing better understanding of important drug delivery concepts. It has the potential to deliver aerosols to patients in a controlled manner for investigating bronchodilator particle size effects in vivo.

Future clinical studies may yield information that can help in the development of more efficient and user-friendly inhaler systems. If indeed there is an optimum particle size or range of sizes that aerosols should be delivered at, then in order to maximise delivery potential, the ideal inhaler should be formulated to produce the dose at this size. However, the choice of particle size will depend on the distribution of the target receptors within the lung for the drug in question, device characteristics and the capability of particle engineering technology.

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