Chapter 1 - CONCERNS ON NEBULIZATIONS

A. Introduction

The use of nebulizations for delivery of topically active drugs is becoming increasingly frequent as the first line treatment of acute or chronic lung diseases. Moreover, it is also an attractive way to deliver systemic drugs due to the large surface area of the alveolar epithelium and small airways. This large surface of diffusion allows absorption of some drugs into the blood circulation. This modality of administration has both clinical and diagnostic indications.

To maximize efficacy of nebulization, it is mandatory as a first step to provide to patients devices of quality. Various techniques and devices are available and must be selected to ensure patient compliance. Since 2001, guidelines to a better control on quality and efficiency of existing devices have been elaborated by an European norm (EN-FR13544-1)[1]. Obtaining therapeutic effect requires optimal conditions of nebulization. These conditions are influenced by devices' characteristics and technical (driving gas, flow) or individual variables. The latter are related to respiratory pattern, lung disease, anatomy, age and cognitive ability of subjects to use the selected device as well as laboratory data available.

Several *in vitro* and *in vivo* methods for evaluating nebulizers are now available. All of these methods may provide helpful indications to therapists to make the appropriate choice of device depending on the target and objectives. Understanding the needs to improve clinical practice for the use of nebulized therapy, the European Respiratory Society (ERS) has published, after reviewing related scientific and clinical principles, users' guidelines of nebulized treatments[2].

The aims of this thesis concerns 1) evaluation of a new modality of nebulization : the intrapulmonary percussive ventilation[3] and 2) comparisons of this modality with a classical well-studied jet nebulizer in various *in vitro* and *in vivo* conditions. Aerodynamic properties and deposition obtained by intyrapulmonary percussive ventilation will be measured. These measurements will allow a comparison to a well-studied device. Cascade impaction and laser diffraction will be used to obtain *in vitro*

parameters and lung deposition will be analysis by imaging and pharmacokinetic techniques.

B. Historical aspects of nebulization

Nebulizations were used before the first written records of medicine. In the Ayurvedic medicine whose origins date back 4,000 years, a traditional place was given to inhalation therapy by smoking preparations from herbs[4]. Dioscorides (first century), the Greek physician called "the Father of Pharmacy", recommended inhaled sulphur vapours. Patients were also encouraged by Galien to breathe sulphuric vapours from the Vesuvius[5]. Until the 19th century, vapours were known to be the only means for inhalation therapy. Medications were added to boiling water to allow inhalation and asthma cigarettes were documented as "fuming asthma remedies"[6].

The principle of liquid nebulization is to convert a solution into a suspension of a particle size that can be inhaled into the lower respiratory tract[7]. The first apparatus producing liquid nebulization was constructed in 1829 and the word "nebulizer" appeared in the Oxford Dictionary in 1872[8]. Before the appearance of the word "aerosol", other words were used (fog, micro mist…). In the middle of the 19th century, inhaling thermal waters were in fashion (Figure 1)[6] before falling out of use due to the potential risk of the substances associated to these waters. During the First World War, nebulizations made a comeback with the discovery of the value of inhaled adrenaline and ephedrine and they have never disappeared from the medical area[5].

Jet nebulization is the oldest of the currently used kind of aerosol delivery and first appeared before the Second World War[7]. The first real precursor to the modern T-piece plastic hand-held pneumatic nebulizer was the 1950's Wright nebulizer using a combination of gas flow, precise venturi orifices, and baffles. This pioneer apparatus was able to produce aerosol particles in the fine size range of 1–5 microns[4]. Ultrasonic nebulizers, introduced in the 60's[8], propagate high-frequency sound waves by a quartz and aerosolize a liquid by cavitation. Devices evolved over the last decades (Figure 1) to meet the patients' needs even if none of these systems meets all the requirements but rather they reach compromises between all the necessary criteria[9].

Figure 1: Illustrations of historical and recent methods of inhalation. Top : at the left, inhalator of Dr Renault[5], and at the right, asthma cigarettes[4]. Middle : at the left, Inhaler of infusion (Courtesy of Mark Sanders, Inhalatorium.com.), and at the right, asthma cigarettes[4]. Bottom : at the left, Inhalator thermal of water[5], and at the right, a modern nebulizer.

The use of nebulized drugs for the treatment of pulmonary diseases has advantages over oral, subcutaneous, intramuscular and intravenous routes of delivery. This modality allows selective treatment of the lungs by directly delivering high drug concentrations in the airways while reducing systemic adverse effects comparatively with oral route of administration. The onset of action is quicker with nebulized route of administration than with oral delivery and some drugs are only available for nebulized use.

Conversly there are also some identified disadvantages associated with aerosol drug therapy. An optimal technique of inhalation is necessary to increase drug delivery and potentially maximize efficacy. The proliferation of various devices may produce confusion for clinicians and patients. Nebulizations are less convenient than oral drug administration insofar as the time required for drug administration may be longer and some patients may find the device less portable. This last point is particularly true for conventional jet nebulizers.

Each type of aerosol device has its own advantages and disadvantages, and the clinician must have a good knowledge of all principal devices to provide optimal care for individual patients. Even though there are few therapeutic indications of nebulization, it is mandatory to provide the most appropriate device, that meets clinical and user requirements. Ease of use, nebulization time, drug output, particles size, amount of drug delivered and the portability are essential elements to determine the quality of a device.

C. Jet nebulizers and their design principles

Different manufacturers provide distinct devices. As comparison between two different devices – a classical well-studied jet nebulizer to a jet nebulizer using intrapulmonary percussive ventilation – is a major objective of this work, it is important to make a detailed description of general properties of both apparatus.

A pressurized gas supply is required as driving force for liquid nebulization. The basic principle of this kind of nebulization is based on the Bernouilli's principle, which states that within a tube, as the speed of a moving fluid increases, the pressure within the fluid decreases. A compressed gas directed through one or more narrow orifices entrains the solution. Bernouilli's equations states:

$$\frac{1}{2} m.v^2 + P = \text{constant}$$

where the first term of the sum represents kinetic energy and the second one, pressure.

A decrease of lateral pressure occurs when gas velocity increases. Then an increase in kinetic energy is coupled to a decrease in pressure. In practice, the fall in pressure where the power source enters in the nebulizer entrains the fluid contained in the collector (Figure 2). This meeting between gas and liquid produces primary generation of droplets with typical sizes between 40 and 60 μm. A baffle impacts these particles and a second generation of droplets is produced. Their size is ranged between 1 and 5 μm for the majority of particles. As it will explained further (See *Aerodynamic properties* in *Assessing nebulizer performance*), the particles diameters must be optimal for effective pulmonary drug delivery. The size of the particles allows the transport of a significant amount of drug without impacting the posterior pharyngeal wall. The aerosol is delivered into the inspiratory gas stream of the patient. Larger particles return to the liquid reservoir. Smye *et al.*[10] have shown that more than 99% of the particles may be returned to the liquid reservoir.

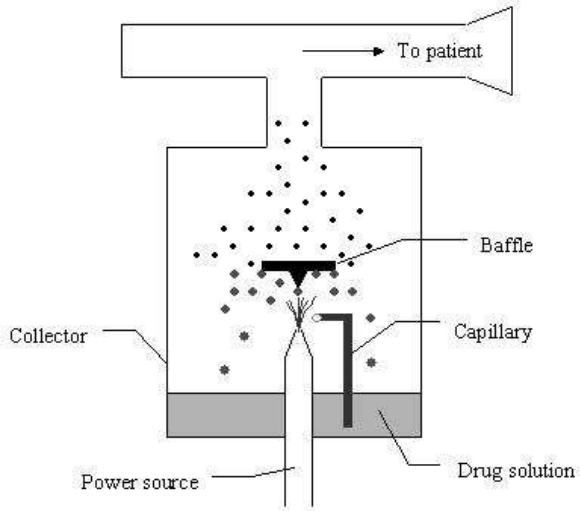

Figure 2: Classical design of a jet nebulizer

Nebulizer designs are of two types (Figure 3): the internal mixing design where gas flow interacts with the solution before leaving by the exit port, and the external mixing design where gas and solution interact after leaving the nozzle. There is no superiority of one approach over the other.

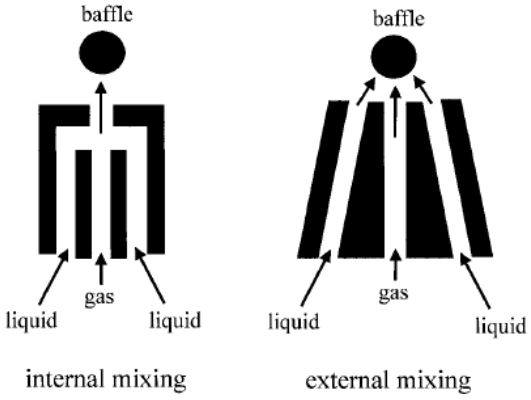

Figure 3 : The two kinds of nebulizers design[11]

In a constant-output nebulizer, particles are produced and released constantly during the entire respiratory pattern, so aerosol can be lost in the environment during exhalation. Constant-output nebulizers have been criticized as unreliable and inefficient, because only a low percentage of the nebulized dose reaches the patient[12]. To limit exhaled loss due to traditional jet nebulizer design, a new generation of

nebulizers have been developed. These have been called “valved open-vent design” or “breath-enhanced” and incorporate a mainstream design combined sometimes to additionnal valves. During inspiration, nebulizer output is increased by adding the inspiratory flow to the compressed air flow. During expiratory phase, a one-way valve – when existing – directs patient’s flow away from the nebulizer chamber and the particles, although continuously generated, remain in the collector (Figure 4). The drug output delivered by breath-enhanced nebulizers is higher than that delivered by constant-output nebulizer[13].

In vitro assessment of the rate of drug delivery and of the inhaled mass is more complex with these nebulizers[14], requiring a simulation of the human breathing patterns (peak inspiratory flow rates, tidal volumes, respiratory frequency and inspiratory duty cycles (Ti/Ttot)). Considering this limitation, with a filter placed at the inhalation port of the nebulizer, both the rate of delivery and the inhaled mass can be estimated *in vitro*[15]. In a breath-enhanced nebulizer, the rate of delivery is dependent upon the pattern of breathing. When air entrainment occurs, the inspiratory flow rate exceeds the compressor flow rate and the rate of delivery is mainly a function of the duty cycle (ratio of inspiratory time to total time)[15].

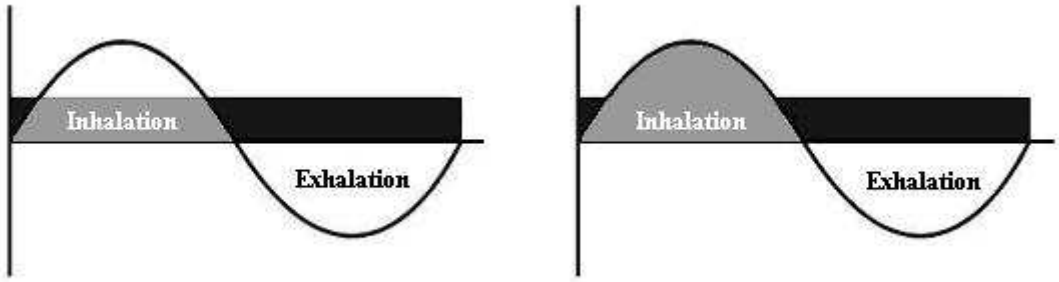

Figure 4: Representation of air entrainment for continuous (left) and breath-enhanced nebulizers (right) during a respiratory cycle

Several studies have evaluated the effects of this modification and reported a higher pulmonary deposition with this design than with a conventional nebulizer[13,16,17]. Increase in inspiratory flow with an open-vent nebulizer design improves nebulizer output and then decreases nebulization time which is finally related to compliance[18].

Some properties of nebulizers such as shape and size of tube and baffle, as well as some characteristics of solution such as density and viscosity, and driving gas influence aerosol droplets size, which is an essential factor favouring lung deposition and therapeutic effect.

1. Sidestream

The Sidestream model (Medic-Aid®, Brussels, Belgium) is a well-known nebulizer, operating as an open-vent jet nebulizer (Figure 5). Inspiratory flow is added to the driving pressure flow to generate aerosol. Aerodynamic and other physical properties of the Sidestream model have been described by several studies[19-21] and they will allow to check and confirm our results.

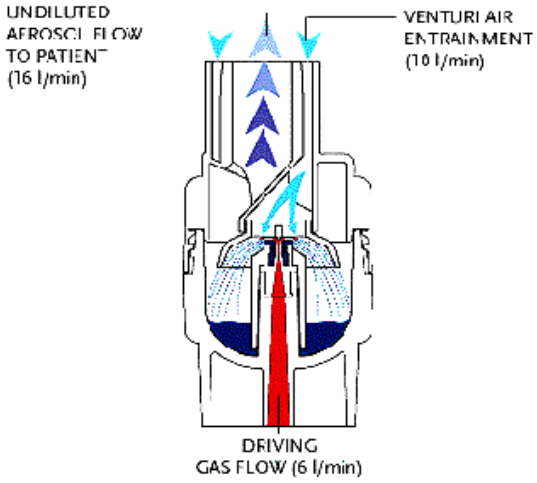

Figure 5: Scheme of Sidestream model

2. Intrapulmonary percussive ventilation

The intrapulmonary percussive ventilation has been elaborated by FM Bird in 1979[3]. This mode of ventilation is based on delivery of a small air volume (smaller than tidal volume) by percussions at high frequency. It is an adaptation of a pneumatic high frequency ventilatory mode in which a high flow of gas is delivered to the airways. Its theoretical interest is to combine ventilation and drainage with a nebulizer. Potential

effects claimed by the manufacturer are increase of mucociliary clearance, improvement of gas exchange and lung mechanics, stabilization of airway patency and humidification of airway.

Percussionaire (Percussionaire, Brussels, Belgium) is composed of two pieces: aerosol generator and phasitron. The first component is similar to that found in a classic jet nebulizer and the second, a dynamic venturi. Air volume under tidal volume is administrated via the phasitron where percussions are generated.

As illustrated in Figure 6 and 7, pulsed gas drives the dynamic venturi by successive round trips at a frequency chosen by the therapist. A diaphragm is bent by the increase in pressure. This deformation mobilizes the venturi towards the mouthpiece with a resulting effect of compression on spring. Inspiratory gate is then opened and expiratory gate is closed. Air entrainment at inspiratory gate and aerosol are generated by a venturi effect due to a decrease in pressure and an increase in gas flow.

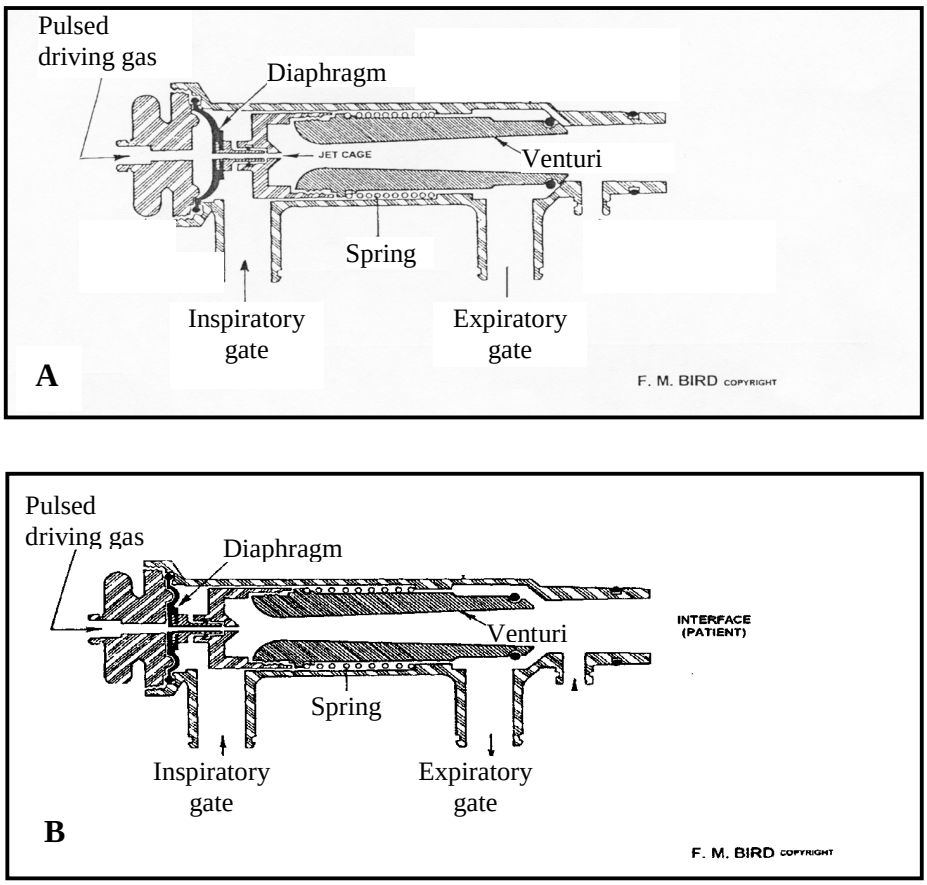

Figure 6: Phasitron of Percussionaire in inspiratory (A) and expiratory (B) position

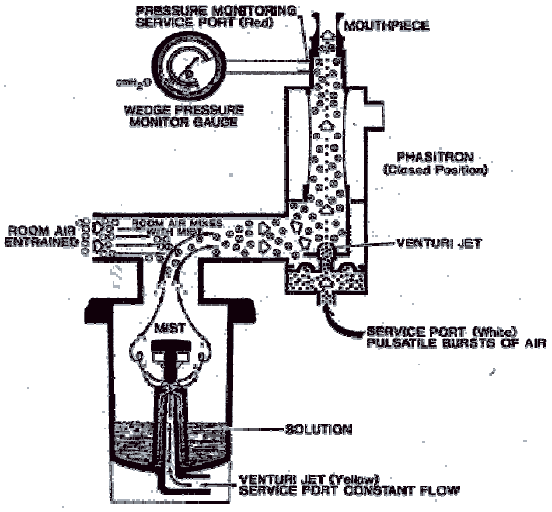

Figure 7: Nebulizer of Percussionaire

D. Assessing performance of nebulizers

1. Doses: concerns and definitions

In the context of nebulization, concerns on dose are particularly complex for the practitionner[22]. The term "dose" encloses in fact many different meanings. Different related issues are summarized in Figure 8.

Initial dose, called "*nominal dose*", represents the total prescribed dose and is theoretically device-specific. In practice, this issue is rarely considered. In this framework, nominal dose means the amount of drug initially placed in the collector of the nebulizer.

The "*emitted dose*" corresponds to the mass leaving the collector. It differs from the "*inhaled or delivered dose*" because a part of the emitted dose is either lost in the dead space of the device or by the open expiratory valve, whether existing.

The delivered dose is the amount of drug reaching the mouthpiece or leaving the face mask[23]. From this dose, the "*mouth dose*", the "*exhaled dose*" and the "*patient dose*" should be distinguished. The exhaled dose results from either an inadequate breathing

pattern (low tidal volume and high respiratory frequency) or unfavourable aerodynamic properties. The patient dose is that finally deposited into the patient (minus that impacting into the mouth). The mass of drug deposited into the lung, called "*lung dose*", represents the effective therapeutic component of the nominal dose. Usually a given therapeutic effect should be produced by a given lung dose irrespective of the delivery device. Lung dose can be divided into *central* and *peripheral lung doses*. They are generally expressed in percentage of various doses (nominal, delivered or patient dose) which can be confusing when interpreting data from different studies. "*Extrathoracic dose*" is responsible for adverse effects and corresponds to the dose of drug reaching the digestive tract.

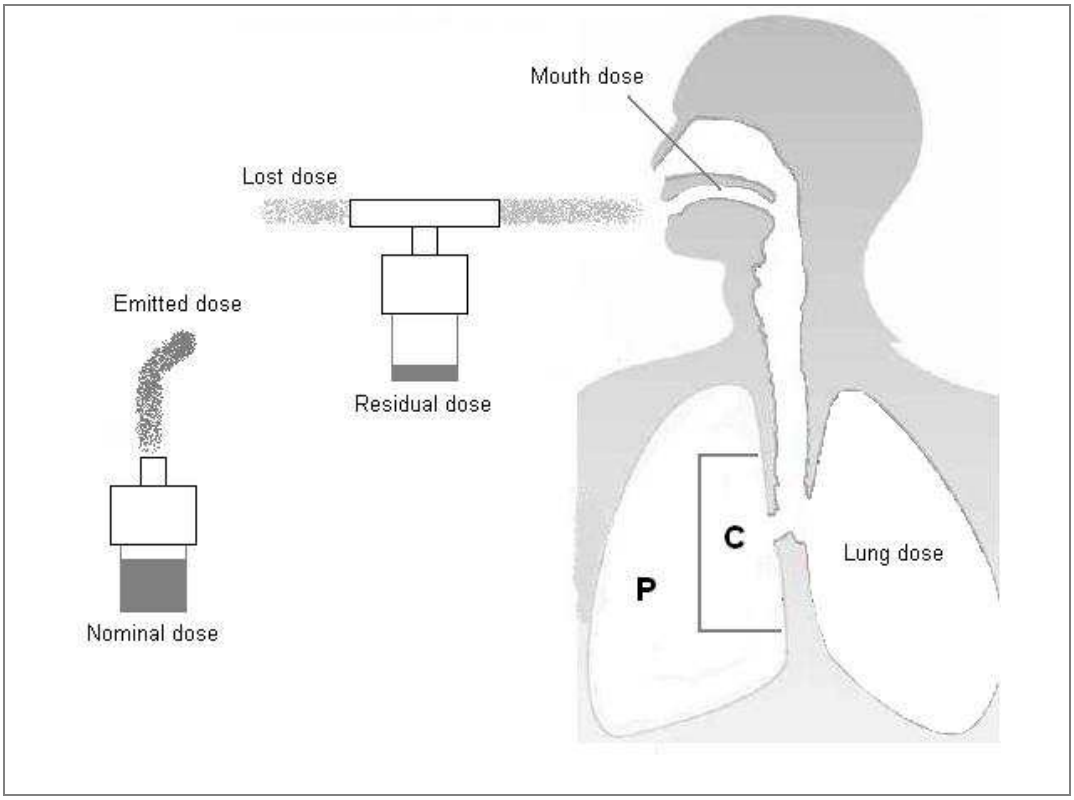

Figure 8: Different doses from nominal dose to lung dose.

2. Residual volume: concerns and definition

Residual (or dead) volume is the sum of volume remaining in the collector at the end of nebulization and volume corresponding to droplets fixed on the walls of the nebulizer. This endpoint has been discussed in a large number of publications. In 1993, Malone

demonstrated that continuing nebulization following the onset of sputtering point (time when nebulization becomes inconsistent) is not productive[24].

Various factors influence residual volume. Due to evaporative water loss, residual volume becomes increasingly more concentrated during the nebulization time[24-27]. This increase in concentration ranges from 5 to 37 percent to the initial drug concentration. Residual volume can be estimated by gravimetric method. Even though this method has the great advantage of allowing comparisons between nebulizers, it is not a reliable method for determining mass output of drugs because it overestimates nebulizer output[28-30]. Indeed, accurate and precise measuring of nebulized drug output requires considering both residual volume and concentration of the drug. Optimal gravimetric analysis should be coupled with techniques quantifying drug concentration.

Fall in temperature of nebulization solution occurs due to the evaporative cooling process[25] and due to the decrease of gas pressure (*perfect gas law*). Solution temperature directly affects nebulizer output[31] and droplet size produced by the nebulizer also varies directly with temperature[25].

Configuration of the nebulizer[32] and surface tension of nebulized solution[32,33] modify residual volume. A lower surface tension reduces residual volume by limiting tendency of droplets to remain on the walls of the nebulizer.

3. Lung deposition

Aerosolized medications can be administered by either nasal or oral route. The nasal vestibule is surrounded primarily by cartilage, unlike the more distal main nasal chamber that is surrounded by bony structures, the turbinate. During nasal delivery, due to the presence of cilia and turbinate, nebulized particles come into contact with the mucus trapping them. Administration by oral inhalation avoids the nasal filtering process.

Particle size plays here a major role depending on the anatomy of the upper airway. Posterior pharynx and the vocal cords present curvatures which larger particles (>5 µm) are often unable to negotiate and deposit there by impaction.

Three mechanisms govern particle transport in the human respiratory tract. Impaction referring to inertial force (Figure 9) corresponds to the process whereby a particle moving in a gas stream deviates from its path by hitting an obstacle or a target. Impaction of particles on epithelium of the respiratory tree or an obstacle promotes their local deposition[34,35]. This obstacle can be an airway bifurcation or an intrinsic or extrinsic obstruction (secretions, bronchial tumour). The effect of inspiratory flow and particle density is implicit in this definition of inertial impaction. The probability of impaction increases with increasing air velocity, breathing frequency, and particle size[34]. It means that effective drug delivery by the aerosol route must avoid the mechanisms designed to protect the lungs from particulate matter.

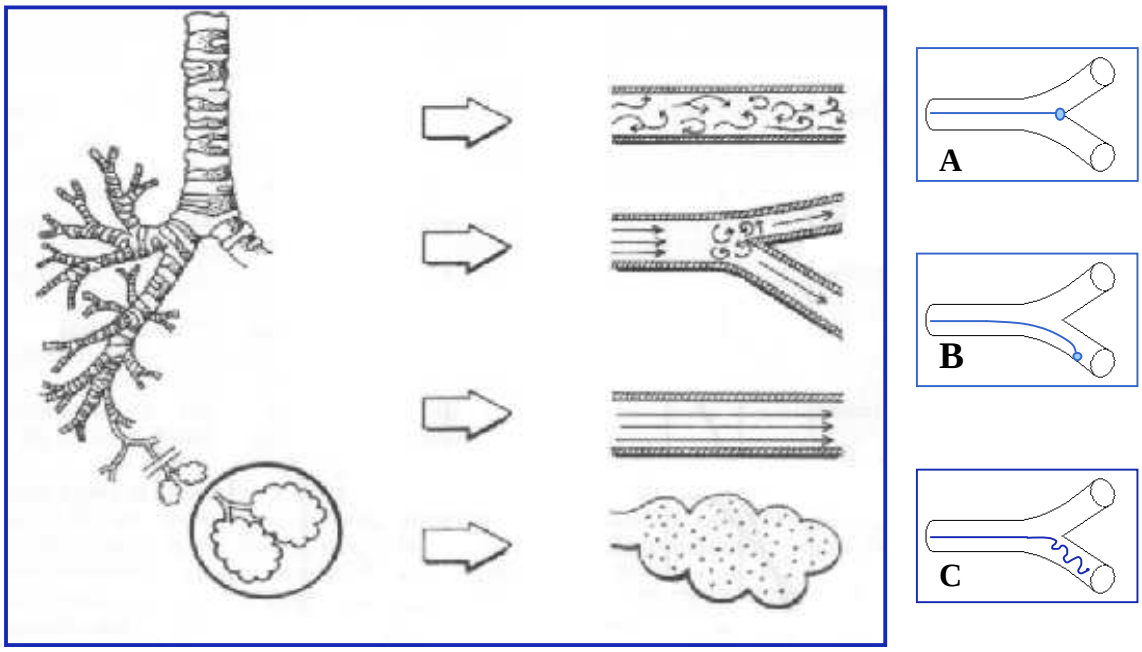

Figure 9: Three particle modes of deposition related to the respiratory tract (A: impaction; B: sedimentation; C: diffusion)

In the lower airway, two other mechanisms of deposition become effective: sedimentation and diffusion. Sedimentation is the process whereby the particles deposit through gravity which causes the particles to fall to the surface. Sedimentation arises whenever the gravitational force acting on a particle overcomes the total force

maintaining it into suspension[34]. Inhaled particles will then fall out of gas stream (Figure 9). This is an important mechanism in small airways because the gas velocity dramatically decreases at this level. The probability of sedimentation is proportional to both residence time of the particle in the airways and particle size.

Smallest particles move at random through the gas (Brownian movement). Ultrafine particles (with a diameter smaller than 0.1 μm) deposit into the respiratory tract only by diffusion (Figure 9). This mechanism is slow. Therefore, these particles may be exhaled before they impinge respiratory epithelium. Effectiveness of Brownian motion on particles deposition is inversely proportional to particle diameter smaller than 0.5 μm. This mechanism is important in bronchioles, alveoli, and at bronchial airway bifurcations[34].

Particle transport depends on some parameters related to particles such as size and density, and on airflow in the airway. The obvious corollary is that administration of aerosols by mouthpiece is much more favourable than by facemask. Other mechanisms are likely to be significant contributors to deposition. The physical motion of the heart generates an oscillating motion of air in the lungs, which results in an enhanced gas mixing in the airways[36,37].

Therapeutic effect depends directly on site of deposition. Optimal localization varies with drugs and their pharmacological site of action. It seems evident that the optimal site of action for bronchodilators is located in proximal airways and for antibiotics, the alveoli must be targeted. For glucocorticosteroids, site of action remains uncertain.

Nebulization is also responsible for systemic and local side effects. Extrathoracic deposition contributes to systemic side effects. After absorption by the digestive tract and after first-pass hepathic metabolisation, swallowed active drugs can reach the systemic circulation and may provoke adverse effects. Bennett *et al.*[38] highlighted that following administration of inhaled salmeterol and salbutamol, heart rate increases while plasma potassium concentration and diastolic blood pressure decrease, in a dose dependent manner. Burggraaf *et al.*[39] showed that inhaled beta-2 agonists have serious systemic vascular side effects including sudden death in hypoxic asthmatic patients. A substantial vasodilatation and possibly pulmonary shunting were suspected as a

possible underlying mechanism for explaining the association between sudden death and aerosolized treatment. Inhaled corticosteroids absorbed into the systemic circulation could also cause adverse effects. These systemic side effects, including calcium and phosphate metabolism dysregulation with subsequent risk of osteoporosis, adrenocortical suppression, bruising and skin thinning, posterior subcapsular cataracts and glaucoma are dose-dependent and obvious differences exist between molecules. Studies suggest that steroid-induced osteoporosis is clinically important since a large proportion of patients receiving long-term oral steroids experience rib or vertebral fractures[40]. If local side effects associated with different drugs like dysphonia, oropharyngeal candidiasis, hoarseness and pharyngeal discomfort, thirst, cough and tongue hypertrophy are common problems in children[41] and adult patients[42], they are considered to constitute infrequent and minor problems[42]. However, they can contribute to a poor compliance with therapy.

4. Aerodynamic properties

The amount of drug reaching the lower airways and the pattern of deposition are essential for pharmacological effects. When approaching concepts of doses in nebulization and their methods of measurements by *in vitro* techniques, it is important to note that only the emitted dose can be accurately measured. By contrast, precise determination of the site of deposition as well as the quantification of deposited drug can be only measured by *in vivo* procedures. Deposition depends directly on multiple factors such as the geometry of airway (anatomical factor), the pattern of inhalation (individual factor) and the aerodynamic properties of particles (technical factor). The latter can be assessed by *in vitro* techniques but the results obtained from this analysis must be integrated with the other factors.

Pulmonary deposition depends partly on aerodynamic properties of emitted particles. As shown in Figure 10, any given particle will not be deposited exclusively in a single area of the respiratory tract, however the particle aerodynamic properties will partly determine a preferential area of deposition[43].

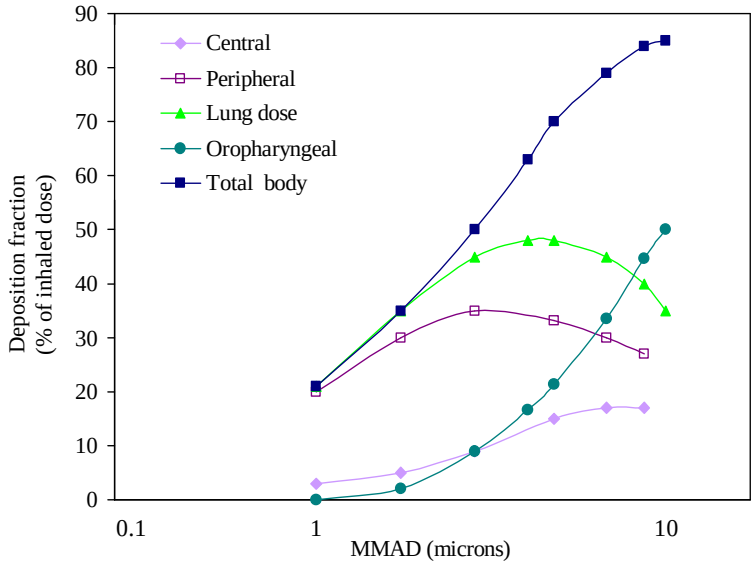

Figure 10: Relationship between mass median aerodynamic diameters (MMAD) (see further for explanation) measured by laser diffraction and deposition fraction in the healthy adult lung (based on *in vitro* models)[43]

Indeed, size of particles affects their mode of transport and their site of deposition. The importance to assess aerodynamic properties of inhaled particles is obvious from a clinical point of view. This allows predicting pulmonary deposition of a drug (Figure 11) even though the relationship between *in vitro* measurements and the clinical reality may be complex. This issue was extensively reviewed by Newman[44].

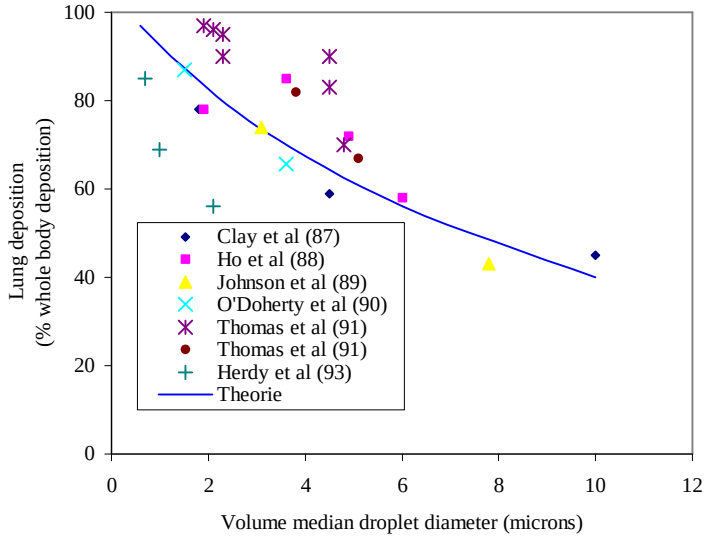

Figure 11: Correlation between the volume median diameters of an aerosol measured by laser diffraction and lung deposition (modified from[43])

Defining the size of particles inevitably raises the problem of specifically geometrical properties such as shape and dimensions. Should one consider the largest dimension or a sphere of an equivalent volume? When the particles are not spherical, the number of geometrical parameters defining them is increased and the concept of size becomes ambiguous. The selected parameter depends on the field of investigated particles. When deposition is evaluated, behaviour of the particle in contact with the airflow will be a key factor to determine the site and timing of particle deposition. For this reason, when considering nebulization, knowledge of the aerodynamic properties is required and a sphere of an equivalent volume is chosen.

The aerodynamic properties of particles are as follows:

- aerodynamic diameter (d_{ae}) meaning the diameter of a unit sphere of a given density which possesses the same speed as that of the measured particle. It takes into account the influences of both particle density and shape, and is characterized by the following equation[45]:

$$d_{ae} = d_{ps}\sqrt{\rho}$$

where d_{ps} = physical diameter of the particle

ρ = density of the particle

- distribution of sizes of particles meaning the distribution of particles represented by a histogram where frequency of appearance of particles is expressed for each diameter (Figure 12). Logarithmic transformation curve yields a normal or Gaussian distribution (Figure 13).

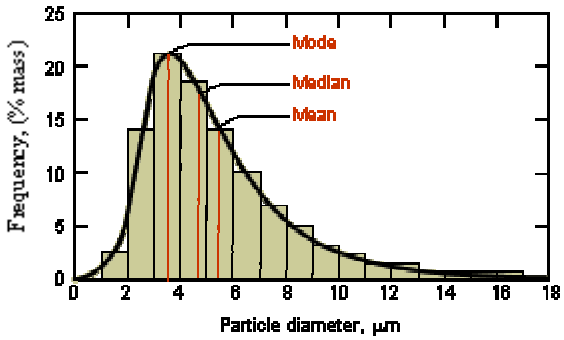

Figure 12: Histogram of a particle size distribution

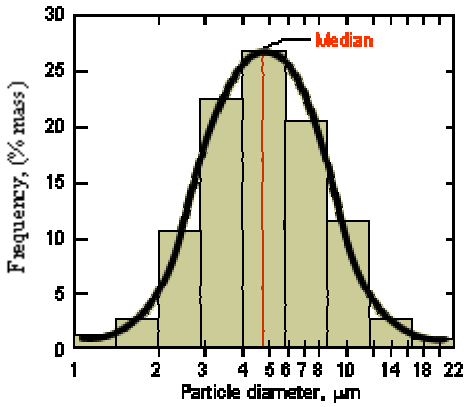

Figure 13: Histogram of a lognormal particle size distribution

- median aerodynamic diameter corresponding to the median of particle diameter on histograms of a lognormal particle size distribution (Figure 13).

- mass median aerodynamic diameter (MMAD) corresponds to the median diameter of particles when the latter are expressed as mass (i.e. 50% of the mass of particles have a diameter lower than, and 50% higher than, the MMAD).

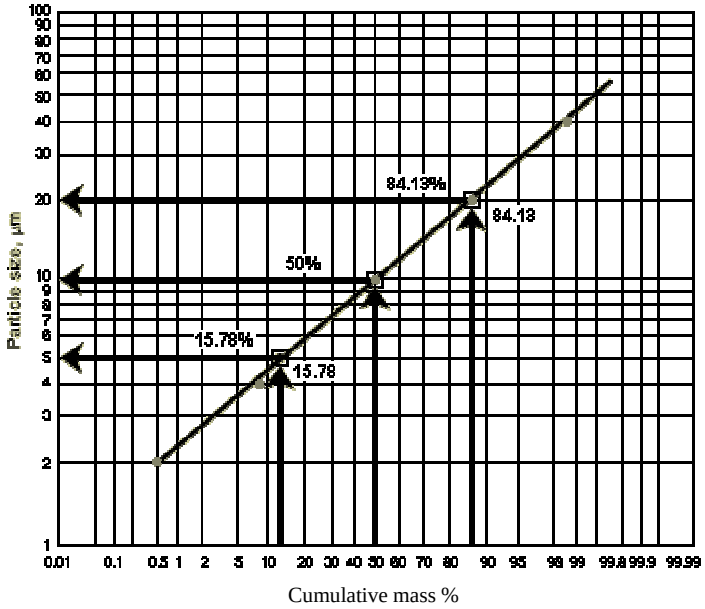

Figure 14: Graphical relationship between particle size and cumulative mass allowing to calculate mass median aerodynamic diameter (MMAD = 10 microns in this example) and geometrical standard deviation (σ = 2 in this example)

In the example shown in Figure 14, the MMAD is 10 microns. In the description of size distributions of pharmaceutical aerosols, referring to MMAD is preferred to median aerodynamic diameter because the mass reaching the lung is the most important concept. MMAD is in fact a key factor determining the site of aerosol deposition[46]. A MMAD of approximately 3 microns appears to be the optimal particle size for bronchodilation[47]. Whether greater MMAD is obtained, a large number of particles above the respirable size range will be left[48].

- geometrical standard deviation (σ) characterizes the dispersion of the distribution (narrow distribution (<1, but pratically impossible with nebulizers) or more generally large distribution in the case of pharmacological aerosols (>1.2)) and is calculated by the equation:

$$\sigma = d_{84\%}/d_{50\%} = (d_{84\%}/d_{15\%})^{1/2}$$

where $d_{x\%}$ represents particle diameter corresponding to x percentage of cumulative mass. In the example given in Figure 14, the σ is equal to 2 ($\sigma = 20/10 = (20/5)^{1/2}$).

- fine particles fraction (FPF) represents the percentage of particles, which potentially can penetrate and settle on in the airway. The preferred size range for inhaled drugs depends on the targeted area and is still controversial for some drugs[49]. Conceptually, when referring to an intermediate and peripheral pulmonary deposition, selected range of particles sizes extends from 1 to 5 microns. The concept of fine particles dose, corresponding to the mass of drug transported by particles contained in fine particles fraction, can also be used.

- emitted dose (ED) and fine particle dose (FPD) : emitted dose is often used in the presentation of *in vitro* performance data and corresponds to an amount of drug leaving the collector of the nebulizer.The fine particle dose can be calculated from the obtained data in accordance with the equation :

$$FPD = FPF \times ED$$

where FPD = Fine particle dose
FPF = Fine particle fraction
ED = Emitted dose

Aerodynamic properties of aerosols depend on the apparatus itself and on the solution tested. These properties can be measured mainly by two methods: cascade impaction and laser diffraction. Hence, we must be aware of different notions. A different particle variable (diameter, volume, surface…) is measured by a precise and particular technique.

a. Cascade impaction

As stated previously, one of the mechanisms of deposition of particles in the respiratory tract is the impaction. This phenomenon is assessed by cascade impaction. All devices recognized by both US and European Pharmacopoeia for this kind of measurements are based on this principle[45]. Standard testing procedure to characterize nebulizers established under the aegis of the European Committee for Normalization (CEN) proposed the cascade impaction as the gold standard method to measure particle size distribution.

Cascade impactors are the most used devices for *in vitro* measurement of particle size. They are the most accurate device to separate particles in the range of 0.3–12 μm and are well suited for characterizing almost all medical nebulizers. Fundamentally, they measure particle aerodynamic mass-weighted size distributions from which several parameters (MMAD, FPF, σ) can be derived in order to quantify nebulizer performance. In practice, the upper limit for fine particle fraction (FPF) is set at either

5.8 or 4.7 µm. This corresponds to plates with hole diameter of 5.8 and respectively 4.7 µm (see further). The FPF corresponds to the mass collected bellow these plates at a flow of 28.3 $Lmin^{-1}$. Only the upper limit is defined and therefore an underlying assumption is made that the fine particle fraction contains the entire amount of the dose collected in the cascade impactor smaller than this size. This hypothesis is appropriate for drugs which emitted dose is in the size range from 1 to 5 µm. However, this is not always true for some dry powder inhaler[45].

Andersen's cascade impactor is a model of this kind of device (Figure 15). It is conceived for simulating tracheobronchial tree as a collector of particles and is composed of:

- a bent tube or inlet aiming at mimicking to a great or less extent depending on its design, the human oropharyngeal region.
- a preseparator (PS), filtering the particles > 10 microns, which avoids their bounce on the first level and errors related to their reentrainment in the circuit.
- 8 stages, each one composed of an aluminium plate with 400 holes which diameter decrease on each floor. This diameter is called "*cut-off diameter*".
- a filter located downstream from the 8 levels to collect the particles of very small size.

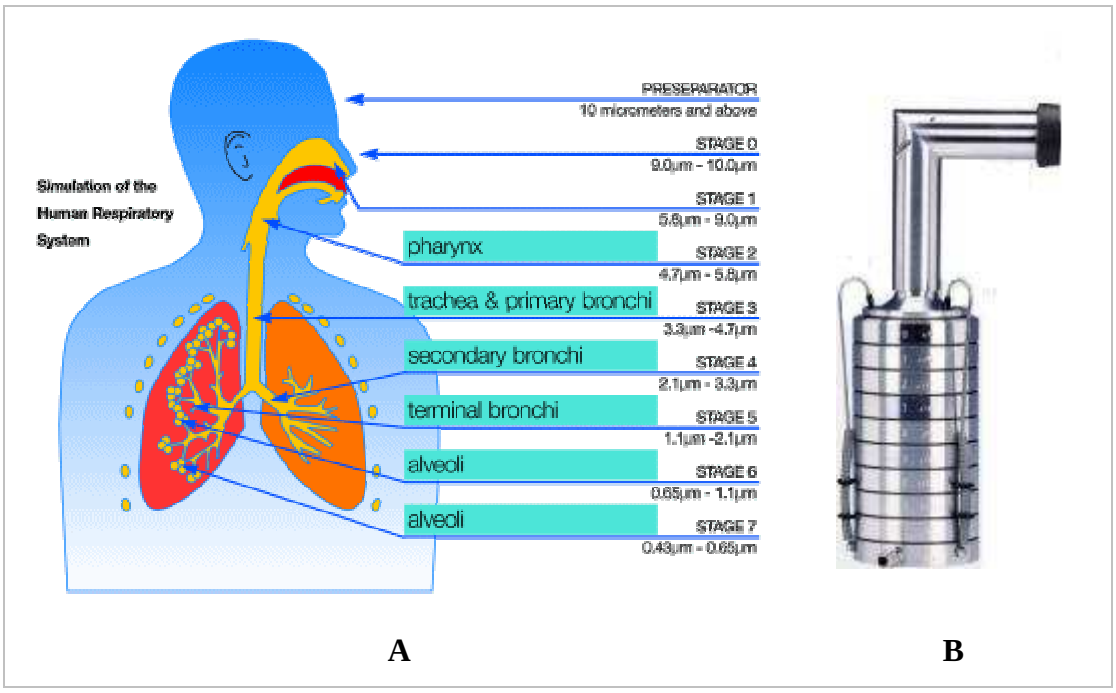

Figure 15: Pulmonary representation of stages of an impactor (A) and illustration of an Andersen cascade impactor (B)

The test consists in nebulizing a chosen substance through the impactor (Figure 16). The proportion of the drug collected on various stages is then measured.

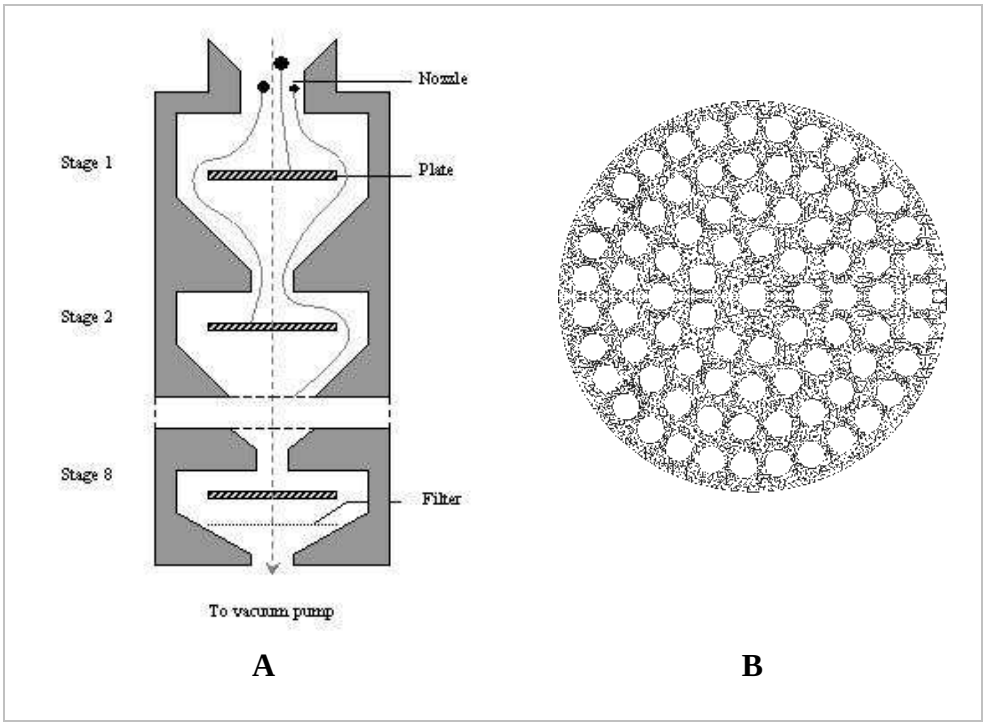

Figure 16: Cascade impactor (A) and a view of one of its plates (B)

The Andersen cascade impactor is connected to a pump producing a suction flow simulating the inspiratory flow of a patient. The nebulizer to be tested is connected tightly to the impactor through an inlet simulating the oropharynx. The design of this inlet varies according the different models.

The behaviour of the emitted particles within the impactor varies according to their size. If a particle has a diameter higher than the cut-off diameter of the stage, it will impinge on the plate by inertia. On the other hand, smaller particles will continue their progression (Figure 17). This process is repeated on every level. The speed of the particles will increase as the diameter of the holes decrease, and more and more small particles will deposit by impaction.

By measuring the amount of drug deposited on each plate, and adding them together, the total amount of deposited drug can be calculated. From this value, the proportion of drug deposition on each stage can be computed.

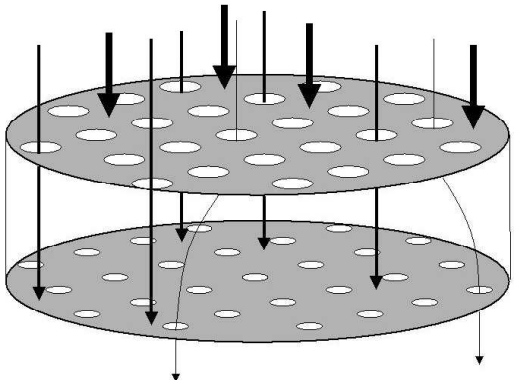

Figure 17: Behaviour of particles in relation with plates of the impactor

Effectiveness of the impactor is determined by the d_{50}, which is the cut-off diameter for which probability of capture of the particles is 50%. This depends on the hole diameter of each plate which varies from model to model. This diameter is influenced by a dimensionless number known as the Stokes number[45] :

$$K_I = \frac{C_c\, d_{ps}^2\, v\, \rho_p}{18 \mu\, D_c}$$

where

C_c= Cunningham's slip correction factor

d_{ps} = particle diameter (μm)

v = speed (cm/s)

ρ = particle density (g/cm^3)

μ = viscosity of the gas (g/cm . s)

D_c = cutoff diameter (μm)

The cut-off diameter depends inversely on the square root of flow. Impactors can operate with various flows. This is important particularly during evaluation of dry powder inhalers or metered-dose inhalers where inspiratory flow of the patient is higher than during spontaneous breathing used in nebulization. Once separation is carried out on the different stages, it remains to quantify, by chemical analysis, the collected solution for each fraction of size in order to obtain the distribution.

A disadvantage of cascade impactor is that it favours evaporation. Indeed, the flow from the pump is drier than the humidified air of nebulizer and contributes to evaporation of the particles[45].

b. Laser diffraction

This method is because the angle of diffraction of light, passing through a particle containing solution, is inversely related to particle size. Equipment is composed by a laser (Helium-Neon) with a fixed wavelength, and a detector, which is a photosensitive silicone cell with a variable number of detectors. Measurements can then be performed directly (without chemical analysis), quickly and simply by nebulizing a solution through the laser ray. Nebulized solution is collected either by an impactor or by an air extractor (Figure 18).

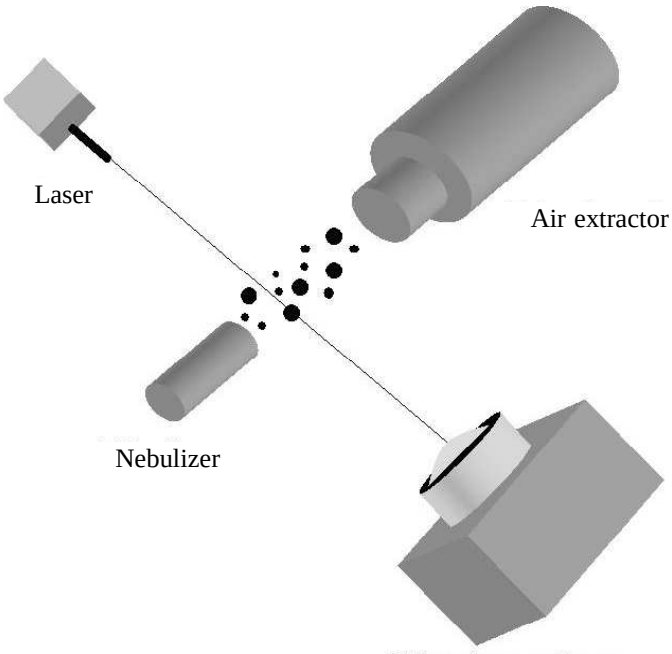

Figure 18: Experimental configuration of a laser diffractor

Two light scattering theories exist, that of Fraunhofer and that of Mie. The Fraunhofer's theory is applicable to spherical, nonporous and opaque particles of diameter higher than the wavelength and producing random movements (diffusion and absorption not taken into account). For smaller particles – such as in drug nebulization –, it is advisable to apply Mie's theory which takes into account not only the

phenomena of diffraction but also diffusion of particles in their medium. This increases the precision of measurement.

When light strikes an interface between two substances with different index of refraction - a complex number defined as the ratio of the speed of light in a vacuum to that in the material - two phenomena occur. An incident ray of light striking the interface at a given angle, measured between a line perpendicular to the interface and the propagation direction of the incident ray, will be reflected off the interface at the same given angle. In other words, the angle of reflection is equal to the angle of incidence. If the second substance is transparent to light, then a ray of light will enter the substance with different refractive index, and will be refracted, or bent, at an angle r, the angle of refraction. The angle of refraction is dependent on the angle of incidence and the index of refraction of the materials on either side of the interface.

In the laser diffraction method, the laser beam crossing the cell of analysis is propagated without deflection until it meets a particle. Index of refraction of a particle is different from index of the continuous phase. This change of index will create a refraction of laser beam. The laser beam reading a particle is diffracted while entering into the particle and when leaving this latter. Light finally will arrive on the detector after undergoing several variations of its axis of propagation (Figure 19).

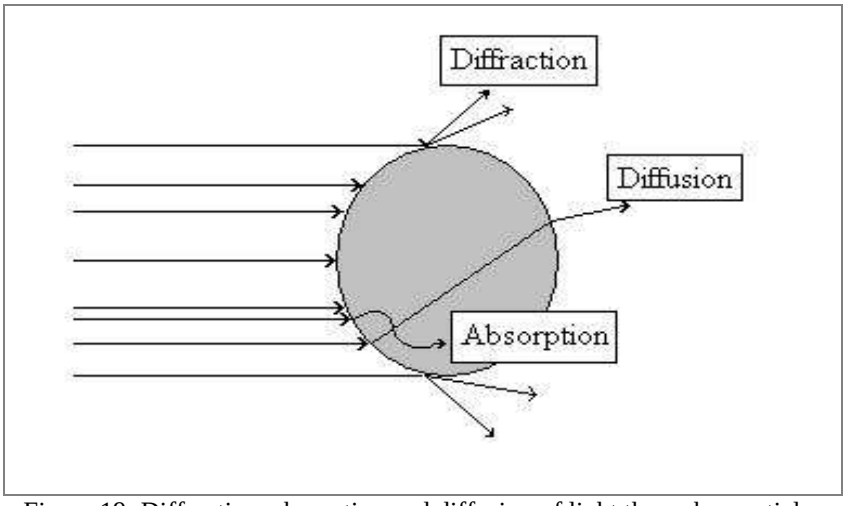

Figure 19: Diffraction, absorption and diffusion of light through a particle

E. Methods of evaluation of lung deposition

The relationship between *in vitro* particle size and lung deposition is complex. Prediction of lung deposition based on *in vitro* results is relatively difficult due to various influences although some studies shown a correlation between some parameters such as fine particle fraction or mass median aerodynamic diameter and mass of drug reaching the lung[50].

The aim of nebulization is deposition of the highest dose of drug into the lung[51]. Several validated methods allow *in vivo* measurements of deposition into the lungs but there is no optimal technique providing all necessary information.

1. Imaging techniques

Radionuclide scanning techniques show the distribution of particles within the lung. Planar gamma scintigraphy, where inhaled formulations are labelled with an emitting isotope (e.g., Technetium 99m-DTPA), is the most widely used method for assessing the deposition obtained from different nebulizers[52-55]. It is used routinely by a number of commercial and academic study centres for assessing inhaled drug delivery[56]. Using a gamma camera (Figure 20) and data processing system, this quick method provides 2-D pictures of anterior and posterior projections of the lung.

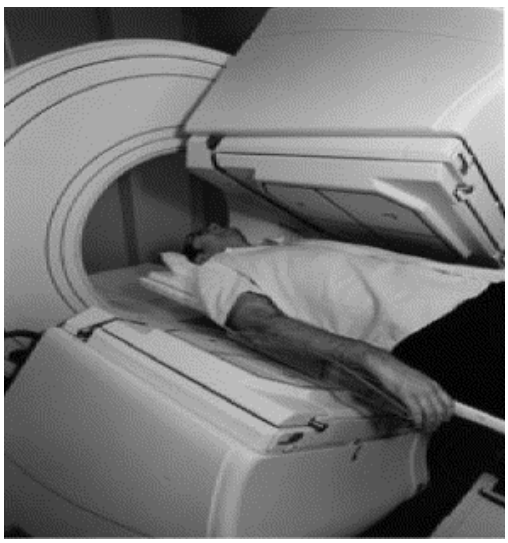

Figure 20: Gamma camera

Over the years, 2-D lung imaging has provided useful information regarding the deposition of inhaled particles and drugs (Figure 21).

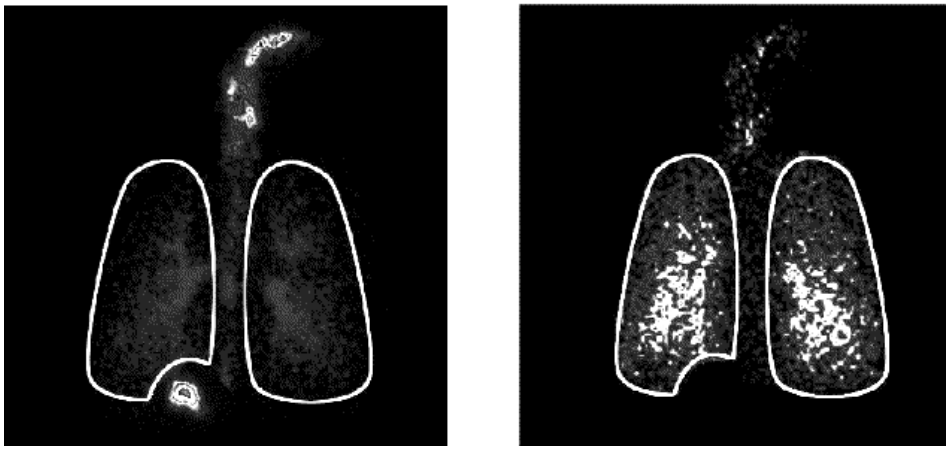

Figure 21: Scintigraphy resulting for a nebulization with a dry powder inhaler (at the left) and a nebulizer (at the right)

Planar gamma scintigraphy enables lung deposition to be quantified accurately for any inhaled drug, and provides data on regional deposition by dividing the lungs into a series of zones (Figure 22).

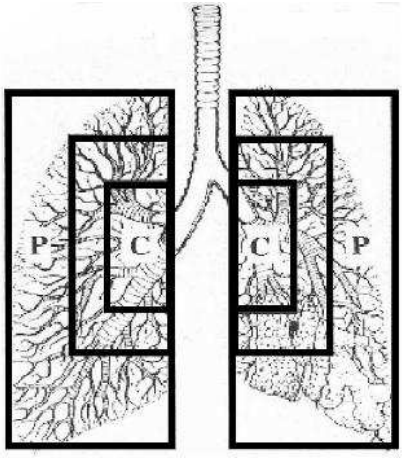

Figure 22: Algorythm for assessing regional lung deposition in gamma scintigraphy.
Three box-shaped region (central, intermediate, peripheral)

While extremely useful, scintigraphy suffers from limited resolution[57]. The radionucleotide 99mTc (half-life 6 h) is generally used to radiolabel the drug formulation.

For a 3-D picture, we need to use tomographic radionuclide imaging techniques. Two types exist: the single photon emission computed tomography (SPECT) and the positron emission tomography (PET). The first equipment produces images from a gamma-emitting radionuclide (Figure 23), and the second maps the distribution of a positron emitter[58]. SPECT use generally the same gamma camera as that used for planar imaging and PET detects gamma ray pairs emitted at 180 degrees from each other allowing the calculation of the original position of the positrons. The 3-D picture is obtained using a similar reconstruction technique for SPECT and for PET. The radionucleotide used for SPECT is 99mTc, whereas, for PET, both fluorine-18 and carbon-11 have been applied. The use of PET is more recent for measurement of aerosol deposition and more informative than SPECT[58]. The label can be incorporated directly into the drug molecule due to the availability of positron imaging radionuclides of physiologically compatible elements. SPECT relies principally on radiolabeling with Tc-99m, which is less easily attached to inhaled drugs. This is a particular problem for metered-dose inhaler and dry powder inhaler, as current techniques do not provide sufficiently robust labelling of the particles[58].

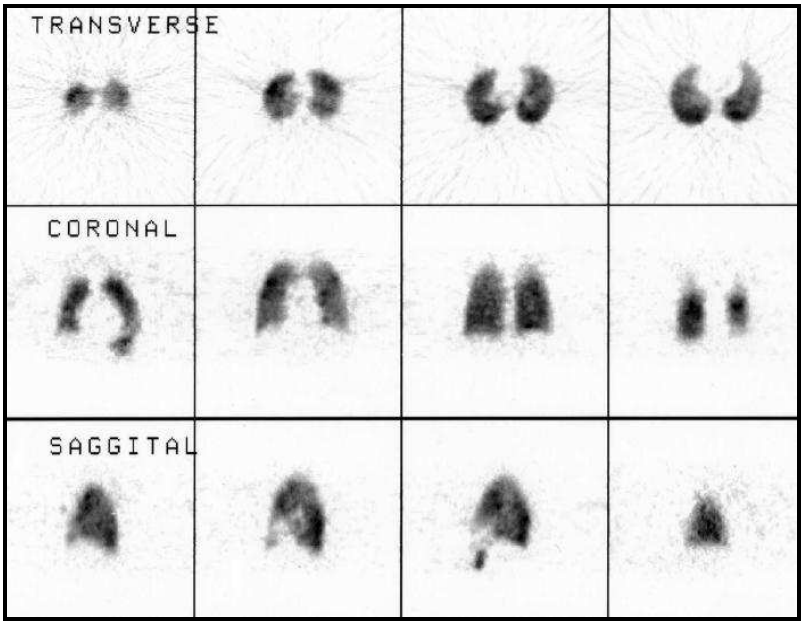

Figure 23 : Deposition distribution of an aerosol imaged using SPECT composed by views from the feet (transverse), from the front (coronal) and from the left side of the subject (sagittal)[58].

Three dimensional lung imaging improves accuracy of assessment of the regional distribution of deposited particles[53,57] but requires more time than scintigraphy. Phipps *et al.*[53] showed a statistically significant difference between penetration index following deposition studies in subjects measured with planar and tomographic gamma scintigraphy.

Another relevant interest of imaging techniques in this particular context is to provide precise information on localization of deposition of drugs in the lungs. However, dosage of radionucleotides can be difficult and radioelements necessitate cautious manipulations. Ethical aspects of using radiolabelling in aerosol research must be taken into consideration. The risk associated with radiolabelled studies can and must be quantified and minimised by careful design. Everard[59] discussed the use of radiolabelled aerosols and gamma scintigraphy in children and concluded that such studies can be undertaken only if the question cannot be answered otherwise. Using very low doses, these studies carried apparently a very low risk. For obvious reasons, this approach is however not recommended in clinical routine.

2. Pharmacokinetic

Pharmacokinetic methods have also been applied to assess the lung dose after nebulization in both healthy subjects and patients with pulmonary diseases using various protocols[60-69]. Blood or urinary sampling were used with success to compare efficacy of drug delivery between two or more nebulizers[17,70].

Quantification of drug concentrations in blood or in urine first provides information about systemic exposure. Blood samples give very low plasma concentration of drug but sampling collection is more convenient and less time consuming for blood than for urine. Urine collection is generally performed over a prolonged period and usually at fixed times which is restricting and often only acceptable for investigative studies. For routine use, it is inconvenient. Dequin *et al.* showed a good correlation between monitoring of urinary excretion of amikacin and lung deposition in cystic fibrosis (CF) patients[71] and Faurisson *et al.* demonstrated that a single-point urinary assay, applied to an overnight urine sampling (inhalation after the last micturition and just before

bedtime), provides an accurate estimate of the inhaled dose of an aminoglycoside. This methodology allows to monitor the dose administrated, but not the appropriate adjusted dose[72].

An inherent problem of pharmacokinetic approaches for assessing drug delivery to the lungs is that systemic drug levels not only result from drug absorbed through the lungs, but also from that deposited in oropharynx and then absorbed via the gastrointestinal tract[47]. Two possibilities can be considered to limit this inconvenient: nebulizing drugs with a negligible gastrointestinal absorption rate (i.e. aminoglycosides, fluticasone proprionate) or concomitantly with nebulization, intaking orally an activated charcoal for blocking oral absorption of the drug.

These methods can be used to determine whole lung deposition of drugs with negligible hepatic metabolism or, negligible or accurately known oral bioavailability, or for those which gastrointestinal absorption can be blocked by co-administration of charcoal. Antibiotics[73-77], bronchodilators[17,33,67,78] and corticosteroids[61,79-81] has been preferentially used as a marker to evaluate efficacy of different nebulizers or nebulizing conditions.

The disadvantage of pharmacokinetic methods is the lack of precision of site of deposition. Drugs tested must be standardized for allowing comparisons between studies because it has been reported that solutes penetrate the alveolar wall at increasingly different rates depending on the lipid solubility of the compound[82,83].

In vivo methods provide important information concerning deposition of a drug in the lung. However, none is considered able to substitute to all others for clinical assessment of inhaled drugs. Their real indication is elsewhere. As Newman *et al.* stated[84] : “Lung deposition data can act as a bridge to help cross the wide gulf that exists between the *in vitro* testing and the clinical trials programme, enabling more informed decisions to be made about the conduct of clinical studies than would otherwise be the case, and allowing the clinical trials programme to proceed with greater confidence of a successful outcome”.

3. Residual gravimetric technique

Gravimetric measurements, consisting in weighting the nebulizer before and after the nebulization, were initially used to assess nebulizer output. Unfortunately, the difference in nebulizer weights may not only result from the drug output but also from evaporation of the solution occurring during the nebulization. This basic method has been criticized[85] even though a good correlation between drug output and changes in weight has been reported for a large number of nebulizers and investigative conditions[29]. Replacing measuring of weight changes by determining drug concentrations before and after nebulization was not sufficient[86] and both approaches still overestimate drug output because they not take into account expiratory losses[87]. Then filtering techniques were introduced to measure drug output during the inspiratory time.

This technique is the simplest way to measure performance of a nebulizer. In a circuit composed of a nebulizer and either an artificial lung or a pump, a filter is placed just after the nebulizer to collect the delivered dose. Different experimental setups have been elaborated[16,88-92] but all use a simple process. The filter is assayed after the nebulization and, if a radionucleotide is administered, the radioactivity on the filter is measured[93]. This technique is specifically used to determine drug output of nebulizer and allows comparing different devices. Veccelio *et al.* adapted this time-consuming technique which may require a sophisticated equipment, such as a high performance liquid chromatograph, for analytical quantification of drugs used during nebulization[94]. When validated for a particular pharmacological agent, the residual gravimetric measurement is an accurate mean to determine the nebulizer output in comparison with the previous filtering technique[94].

F. Relationship between *in vitro* and *in vivo* data

The relationships between *in vitro* data (particle size, FPF…) and *in vivo* (scintigraphic or pharmacokinetic) data may be complex. Newman compared *in vitro* data and *in vivo* lung deposition data from 18 inhaled drugs[44]. Lung deposition was assessed by gamma scintigraphy and a four-stage liquid impinger measured particle size. The higher the

fine particle fraction (FPF), the higher the whole body deposition. As illustrated in Figure 24, results revealed a positive correlation between FPF and whole body deposition and, that FPF measurement systematically overestimated whole lung deposition (Figure 24).

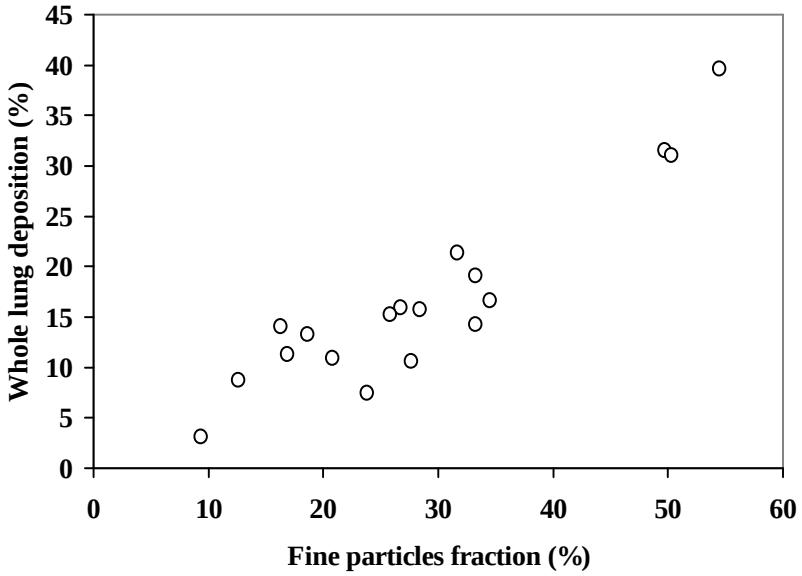

Figure 24 : Relationship between the lung deposition and fine particle fraction for 18 nebulizers[44]

This can be explained by complexity of human upper airway. The inlet throat used with impactor does not exactly represent the upper airways. Hence, FPF does not predict accurately human lung deposition mainly due to inlet throat. Olsson *et al.*[95] showed that when a conventional inlet was used, *in vitro* data failed to predict the relative lung deposition. Interestingly, when these results are obtained from metered-dose inhaler and dry powder inhaler, a better agreement was obtained using an anatomical model of the human upper airway as an inlet to the impactor.

Correlation found between FPF and lung deposition for dry powder inhaler by Olsson et al[95] has not been confirmed by other studies. Silkstone *et al.* investigated recently the correlation between *in vitro* and *in vivo* evaluation of inhaled salbutamol with different nebulizers. Significant correlations were reported (1) between *in vitro* respirable dose (proportion of particles under 5 microns multiplied by the mass of drug available for inhalation (measured by the filter technique)) and the amount of salbutamol excreted in the urine 30 min after the start of nebulization and, (2) between *in vitro* respirable dose and the total amount of salbutamol and its metabolites excreted over the 24 h following

the nebulization. The results demonstrated that *in vivo* / *in vitro* correlations do indeed exist[67].

Several studies assessed the relationship between lung deposition and therapeutic effect[51,96-100]. Generally, bronchodilators have been studied due to the difficulty to assess glucocorticosteroids response. A relationship between MMAD and bronchodilatation was shown[101]. Different nebulizers producing different MMAD were studied and the pulmonary function was measured after terbutaline administration. Respiratory parameters were variably modified depending on the nebulizer and on the MMAD. A lower MMAD gave a better pharmacological response. These results were confirmed by another radiolabel study of this group where lung deposition was evaluated for the same devices[102]. Similar results were obtained in a study evaluating response to dornase alpha in cystic fibrosis patients[21].

Drug deposition assessed from scintigraphic or pharmacokinetic studies allows a good predictor of the therapeutic response to inhaled drugs. Interpretation of lung deposition data can be difficult because if drug is delivered to the part of respiratory tract that is unsuitable for the desired effect (i.e. antibiotics to the central airways or bronchodilators to alveoli), its therapeutic effect will be reduced. Even though the predictive nature of *in vitro* particle size data is matter of debate, data obtained from this approach are still helpful to compare devices. Pritchard introduced the concept of the optimal lung to systemic drug concentration ratio. He stated that it is suitable that the quantity of drug delivered to the lung should be sealed to the total amount reaching the systemic circulation[103]. Once reaching the plateau of the dose-response curve, delivering a larger dose brings no additional clinical benefit and may rather produce adverse side effects[104]. These findings illustrated the necessity to determine the optimal dose to be administered.

G. Influencing parameters on nebulization

Several parameters or elements can influence lung deposition and then therapeutically effect of nebulized drug.

1. Interface

Pritchard wrote in an editorial of the Journal of Aerosol Medicine that interfaces are an essential factor for good compliance[105]. It is also an essential element of the nebulizer. In classical conditions, nebulizations can be administered using a mouthpiece or a face mask[106-108]. In particular situations, especially in small children, other interfaces can be used (Figure 25).

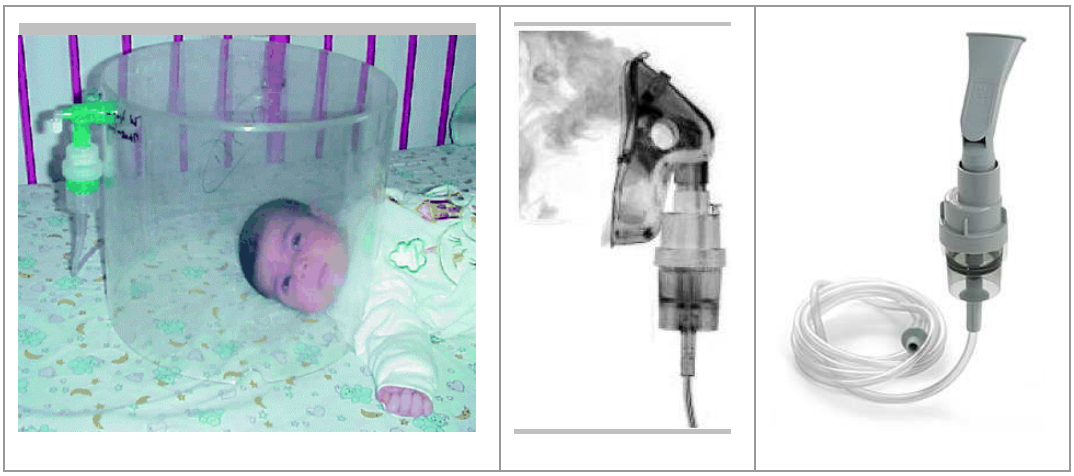

Figure 25: Various interfaces used for nebulization: hood, face mask and mouthpiece

Adult interfaces are often not well adapted to children. Infants and toddlers are often disturbed by the mask and thus reject them. It has been shown that when using a face mask, a very small space (1 cm) between the face of the child and the mask reduces the dose delivered by 50%[90]. Using alternative interfaces which do not touch the infant's face must be encouraged to facilitate acceptance[109]. A transparent hood or a tent covering the child's head were used to facilitate the nebulization. Hood was demonstrated to be more efficient than the face mask and more acceptable to the parents. Parents' preference are markedly and significantly in favour of the hood treatments[110] Moreover, hoods are inexpensive and can minimise the environmental contamination related to some drugs.

When comparing mouthpieces and facemasks, similar therapeutic effects occur with these both interfaces. As a result of nasal passage, droplets delivered from the nebulizer are filtered. A nearly 50% reduction of deposition in the lungs of healthy subjects was reported when comparing nasal to oral inhalation masks [108]. The nebulization with a

face mask can result in deposition on the face and in the eyes and the differences in deposition pattern on the face are depending on the localization of the insertion point of the nebulizer in the mask[111].

Use of a mouthpiece and certainly oral breathing should be encouraged even in children[112]. Kairaitis[113] highlighted two interesting points. First, most asthmatic patients spontaneously breathe simultaneously by the nose and by the mouth during bronchospasms while healthy subjects usually breathe exclusively via the nasal route, both with and without a facemask. Second, even when not acutely bronchoconstricted, these patients switch to oronasal breathing when wearing a facemask. Concerning mouthpiece, a study showed an influence of its size on deposition depending on particle size[114].

Data from Roth *et al.*[115] revealed that breathing through a nebulizer caused statistically significant changes in measured human breathing patterns when compared to normal breathing. An increase in tidal volume (34 %) and a decreased in respiratory frequency (39 %) was observed. Moreover, average duty cycle, i.e. the fraction of the breathing cycle during which inspiration takes place (Ti/Ttot), shifted 12 % towards a more symmetrical breath due to the unequal increase in the inhalation and exhalation times (55 % and 28 %, respectively). Askanazi *et al.* have previously reported an increase of 32.5 and 15.5% in tidal volume, respectively when using a mask or a mouthpiece accompanied with a nose clip, whereas respiratory frequency and inspiratory and expiratory time remained unchanged[116]. These observations have been later confirmed[117,118]. Absence of change in inspiratory time implies that increase in tidal volume is caused entirely by increased inspiratory flow.

Noseclip could potentially influence nebulization by modifying the route of breathing. It has been shown that when a nose clip is used during a nebulization, amount of drug delivered to the mouth is doubled[119]. However, wearing a nose clip does not seem to modify the lung dose[120]. It is documented that wearing a nose clip influences essentially the delivered dose without any effect on the lung dose, representing the therapeutical dose.

Some studies have investigated the impact of the diameter of the mouthpiece on deposition[114,121] and have shown that it is variable and depends on particle size[114]. Air velocity being slower with a larger mouth opening for a given inspiratory flow rate, a reduction in inertia of particles will result, decreasing the impaction of particles in the posterior oropharynx where the direction of the air stream changes abruptly[114].

2. Breathing Patterns

Breathing pattern during nebulization influences its efficacy. Tidal volume and frequency determine flow rates in different region of the respiratory airway, which influence the effectiveness of each deposition mechanism. Effect of change in breathing pattern has been previously studied[122-124]. Rapid respiratory frequency is often associated with increased deposition of larger particles in the upper respiratory tract. Conversely, calm and steady inhalation increases the number of particles that penetrate into the peripheral parts of the lungs, maximalizing deposition by sedimentation. Intersubject variability of deposition and differences in deposition between healthy subjects and patients were smaller for slow breathing pattern[125]. If an end-inspiratory breath-hold is regularly recommended, Zainudin *et al.* showed that an inhalation during tidal breathing is as effective as a combination of tidal breathing and deep breaths with or without breath hold[126].

Drug output of a nebulizer is influenced by inspiratory flow of the subject[28,127,128]. For breath enhanced nebulizers, it is influenced by air entrainment[13] which occurred when inspiratory flow exceeded nebuliser flow. In children for example, as a result of a low peak inspiratory flow, pure aerosol not diluted by entraining flow of air may be inhaled (Figure 26).

Once inspiratory flow is higher than nebulizer flow, the amount of drug delivered to the patient is diluted. A significant difference in the amount of drug produced by various nebulizers was demonstrated depending on breathing pattern[87]. Breathing pattern of a child or an adult also influences drug output. Drug delivery was increased with an adult breathing pattern[129].

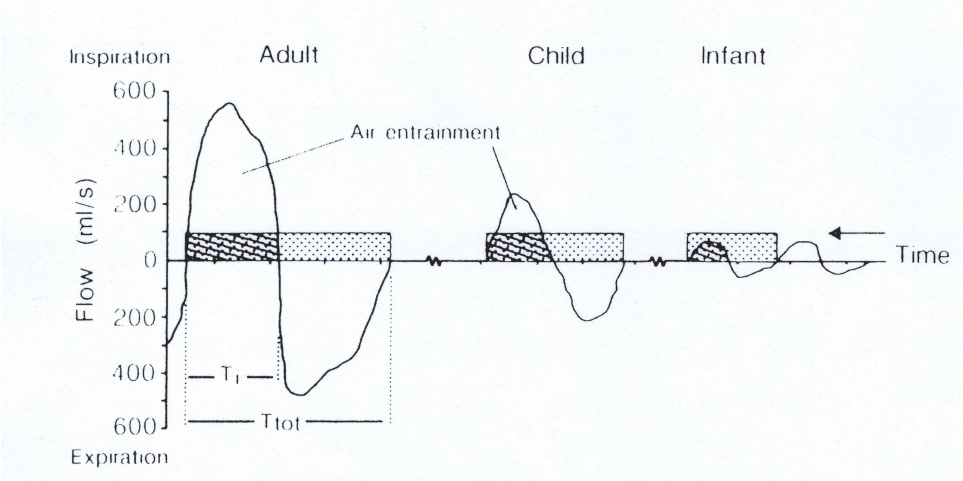

Figure 26 : Representation of air entrainment with typical flow-time curve from an adult, a child and a newborn[130]

3. Duty cycle

The duty cycle or the ratio of inspiratory to total respiratory time influences drug delivery to the lungs. Most of the drug reaching the lungs is delivered during inspiration.

Consequently, any reduction in duty cycle decreases lung deposition. Such a reduction in duty cycle occurs when the subject presents an airway obstruction. Crying also reduces dramatically duty cycle and hence deposition[131,132].

4. Age and sex

Age influences drug deposition when using a nebulizer. As mentioned previously in this chapter under “Inspiratory flow”, in very young children, drug delivery is enhanced due to pure aerosol inhalation. In older subjects, aerosol is diluted by entrainment of air which does not contain droplets of drug[130].

A small body size is related to smaller airways and results in an increased resistance of airways due to inversely relation of resistance to the fourth power of airway radius. This increase favours central airway deposition[133].

Small body area and lack of air entrainment compensate partially poor drug deposition measured in children. As a consequence, the delivered dose corrected for patient weight may be similar in infants and adults[130]. The small influence of patient height on deposition could be explained by predominant role played by the duty cycle, which is independent of height, and a less significant role played by minute ventilation, which is related to height[134]. In a radiolabelled study realized with cystic fibrosis patients, lung deposition was quantified and it was shown to be lower in infants than in older children[135]. Other available results[136] show that lung dose is age dependent, and for a fixed inhaled dose, the systemic exposure of an inhaled aerosol is independent of age. That means that the systemic dose increased proportionally with age. This suggests that from a safety perspective similar nominal doses can be prescribed irrespective of age. Studies in children often demonstrate preferentially high levels of oropharyngeal and laryngeal deposition[137]. In children, tidal volume increases and respiratory frequency decreases with increasing age[138].

Influence of sex on lung deposition can be explained by several elements. Gender differences of lung anatomy should be first pointed out[139]. Variations in the dynamic behaviour of larynx and vocal cords are another element[139]. This is particularly true in upper airways and large conducting airways[140]. Lung deposition was initially considered to be similar in both genders but it has been demonstrated that it is more proximal in women[139,141]. Pattern of breathing is also gender-related. Indeed, women breathe with a smaller tidal volume[142].

5. Disease

The structure of the bronchial and alveolar regions in normal lungs is considered a minor influence in standard healthy conditions. However, this is not the case for diseased lungs[143]. Obstructive diseases decrease the amount of drug reaching the lungs by increasing airway resistance. Alveolar ventilation can be considerably modified in lung diseases, particularly those associated with variations in resistance and compliance of some unit. Unit of lungs with an increased resistance tend to fill slowly thereby causing inequalities in ventilation distribution due to differences in time constants.

Distribution of aerosol is influenced by ventilation distribution[144] and depends on duration of inspiration, compliance and resistance. Obstructive patients present an heterogeneous deposition pattern marked by a pronounced deposition in the central airways[145] and various focal "hot" spots in different regions of the lung[146]. Bronchoconstriction increases central deposition and reduces peripheral deposition of RhDNAse in adults CF patients[147]. Using radiolabeled studies, Smaldone and Messina demonstrated that flow-limiting segments can be a major determinant of deposition in central airways[148,149]. Moreover, the pattern of breathing is modified in the majority of lung diseases[150] and, as seen above, has an influence on deposition.

6. Diluent (or fill) volume, flow and pressure of driving gas

Increasing diluent volume led to significantly increased nebulization time and greater delivery of the drug initially placed in the nebulizer by reducing trapped drug in residual volume. Hess *et al.*[28] and Malone *et al.*[24] have shown in similar studies that an increase in diluent volume results in a significant decrease of residual volume and in an increased amount of albuterol delivered to the mouth. Studies based on gravimetric measurement of the residual volume revealed comparable results.

An increased flow has an identical but smaller effect on drug output as an increased diluent volume[28,32,151,152] and produces also a decrease in MMAD and an increase of respirable output (mass of drug within the optimal range)[31]. Moreover, it decreases nebulization time, which is a positive factor-enhancing patient's compliance to the inhaled treatment. It was demonstrated that a longer nebulization time is judged less acceptable by the patients[18].

The driving pressure influences also directly the output of the nebulizer. The higher the driving pressure, the higher the output[153] and the shorter the time until dry running[154].

7. Hygroscopy and temperature

The lung is a humid environment where inhaled hygroscopic particles will gain or lose water until they become isotonic with the fluid lining the airways. The tonicity of the particle materials, the water content of the particles, the temperature and the humidity are other important factors for hygroscopic particle growth and deposition. After aerosol production, the droplets will partly evaporate to humidify the air[143].

Normally, the temperature of the airway increases from about 23 to 37°C and the humidity from about 8 to 44 $\mu g \cdot cm^{-3}$ as the airflow proceeds along the nasal, pharyngeal, laryngeal and tracheal airway. As inspiratory airflow rate increases, there is a large decrease in the air temperature of the upper airways. Then, many aerosols change their aerodynamic properties in real time after being generated.

The difference of pattern of deposition between solutions of various tonicities was evaluated. In healthy subjects, hypotonic solutions had an increased index of penetration and, in asthmatic patients; this influence was dependent on the sate of the airways. Authors concluded that tonicity of aerosols affect their deposition both through physical and physiological mechanisms[155,156].

8. Impact of these influences on studies

It seems extremely important to limit and control the impact of these influences on the results of a study-evaluating efficacy of nebulizers or drugs. The best way to establish lung deposition obtained by different devices is to measure the chosen variables depending on pharmacokinetic or imaging techniques simultaneously in the same conditions. Study on healthy subjects has the advantages to limit variations due to anatomical or mechanical modifications related to disease. Nevertheless, extrapolation to patients should be done with caution. Studies in healthy subjects are then mainly used to compare efficacy of devices or systemic activity of various drugs. Clinical effect of a drug must be evaluated on patients with lung disease.