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(54) **Title**: INHALABLE COMPOSITIONS

(57) **Abstract**: There is described a composition for nebulisation comprising a non-ionic hyperosmolar agent and an electrolyte, wherein the electrolyte can include a sodium or potassium salt, and the electrolyte can include xylitol, mannitol, sorbitol or saccharin. Also described is a method of forming a nebulised composition and its use in the treatment of respiratory conditions such as cystic fibrosis, bronchiectasis or asthma.

"Inhalable compositions"

Cross-Reference to Related Applications

The present application claims priority from AU 2010904885 filed 2 November 2010 the content of which is incorporated herein by reference.

Field of the invention

The present invention relates to inhalable compositions and their use as a medicament. In particular the invention relates to inhalable compositions containing non-ionic hyperosmolar agents.

Background

Mucocilliary clearance is the central innate defence mechanism of the airways against various foreign pathogens. The airway surface liquid (ASL) lining the respiratory epithelium, consists separately of the viscous mucus overlying a watery periciliary liquid (PCL) layer. The correct viscoelastic consistency of mucus is essential for its unique ability to remain provisionally attached to the epithelium whilst being mobile enough to be cleared by ciliary beating. In healthy airways, fluid exchange with the luminal epithelium promotes optimal hydration of the ASL for mucociliary function. However, in certain respiratory diseases including asthma, bronchiectasis and cystic fibrosis (CF), the ASL becomes dehydrated as a result of inflammation-stimulated mucus hypersecretion or ion transport defect. The cilia cannot fully extend due to the depleted volume of PCL. Cilia mobility and mucus clearance is further suppressed by a tightly adhering, overly viscous mucus layer. This is problematic for patients as it promotes formation of mucus plugs and subsequent infection and inflammation, particularly in the small airways, whilst impairing the penetration and activity of other aerosolised therapy into smaller airways and the lung.

Hyperosmolar agents aim to preclude development of these secondary complications by correcting the underlying hydration defect. Deposition of the medication on the airway epithelial surface establishes an osmotic gradient to induce transepithelial rehydration of the ASL, thus restoring the volume of the PCL and optimising the viscoelastic properties of mucus necessary for healthy mucociliary function. Ciliary beating is further stimulated by the release of factors such as adenosine phosphate and the induction of cough, which is necessary for clearance of sputum - the expectorated combination of inflammatory mediators, cellular components and foreign particles in mucus.

These agents are used primarily for their potential to be an economical replacement or adjunct for conventional sputum clearance therapy, typically expensive nebulised recombinant human deoxyribonuclease (rhDNase).

One hyperosmolar agent is nebulised hypertonic saline, which has been shown to improve mucociliary function. However, hypertonic saline does not have an extended therapeutic effect due to rapid absorption through airway epithelial sodium channels. In addition, the nebuliser itself has traditionally been cumbersome, which leads to difficulties with administration that overall reduces patient quality of life.

More recently, advances have been made with nebulisers with the advent of vibrating mesh nebulisers, which may in time supersede the conventional jet or ultrasonic nebuliser, as they significantly improving nebuliser performance. Aerosol generation is by an oscillating metal mesh that alternately loads the micron-sized holes with drug fluid and releases aerosol for patient inhalation. Primary droplets are optimally sized and require no recycling structures, translating into shorter treatment times and reduced size of the nebuliser unit. Device portability is further enhanced by its ability to function silently. The low velocity aerosol cloud minimises throat impaction and environmental wastage during exhalation, thereby enhancing lung deposition. Dose delivery is further augmented by the lack of drug loss as reservoir residual volume - typically in the range of 0.5-1.0mL for conventional nebulisers – and is an important advantage for cost-intensive medications. The considerable improvements in treatment time, dose delivery and device portability have the potential to enhance patient adherence and tolerability for nebulised therapeutics.

A second hyperosmolar agent is dry powder mannitol (a sugar alcohol), which has demonstrated similar therapeutic efficacy to nebulised hypertonic saline with potential for long-term improvements to lung function, and which avoids any difficulties associated with nebulisers. Mannitol overcomes the short duration of action of saline due to a lack of active transport for the sugar alcohol. The limited airway absorption necessitates clearance via the slower paracellular mechanism thereby theoretically prolonging its therapeutic activity. The use of a dry powder inhaler significantly improves the portability of the treatment as compared against a traditional nebuliser.

Nevertheless, there is a continuing need for improvements in therapeutic options to treat respiratory diseases, for example asthma, bronchiectasis and cystic fibrosis (CF).

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Summary

In work leading to the present invention, the inventors investigated the nebulistaion of hyperosmolar agents.

Surprisingly, the inventors have found that non-ionic hyperosmolar agents can be nebulised in an effective manner provided they are co-formulated with an electrolyte.

Further, the inventors have surprisingly found that certain non-ionic hyperosmolar agents, specifically higher solubility polyols, when co-formulated with an electrolyte and nebulised provide an enhanced rate of osmolar delivery.

According to one aspect of the invention, there is provided a nebulised composition comprising a non-ionic hyperosmolar agent and an electrolyte.

In another aspect there is provided a composition for nebulisation comprising a non-ionic hyperosmolar agent and an electrolyte.

In yet a further aspect, there is provided a composition for nebulisation comprising a non-ionic hyperosmolar agent and an electrolyte for use as a medicament.

In yet a further aspect, there is provided a composition comprising a non-ionic hyperosmolar agent and an electrolyte for use as a nebulised medicament.

In another aspect there is provided the use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte as a medicament.

In a further aspect there is provided the use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte as a nebulised medicament.

In yet a further aspect there is provided the use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte with a nebuliser, preferably a mesh nebuliser.

In another aspect there is provided a composition comprising a non-ionic hyperosmolar agent and an electrolyte for use in the treatment of a respiratory disease.

In a further aspect there is provided a nebulised composition comprising a non-ionic hyperosmolar agent and an electrolyte for use in the treatment of a respiratory disease.

In a further aspect there is provided a composition comprising a non-ionic hyperosmolar agent and an electrolyte, wherein the composition is nebulised by a nebuliser, preferably a mesh nebuliser, for use in the treatment of a respiratory disease.

In another aspect there is provided a method of treating a respiratory disease comprising administering to a patient in need thereof a therapeutically effective amount of a nebulised composition comprising a non-ionic hyperosmolar agent and an electrolyte.

In a further aspect there is provided a method of treating a respiratory disease comprising administering to a patient in need thereof a therapeutically effective amount of a nebulised composition via a nebuliser, preferably a mesh nebuliser, the composition comprising a non-ionic hyperosmolar agent and an electrolyte.

In another aspect there is provide the use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte for the manufacture of a medicament for the treatment of a respiratory disease.

In a further aspect there is provide the use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte for the manufacture of a nebulised medicament for the treatment of a respiratory disease.

In another aspect there is provided the use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte in the treatment of a respiratory disease.

In a further aspect there is provided the use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte in the preparation of a nebulised medicament.

In another aspect there is provided the use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte as a nebulised medicament in the treatment of a respiratory disease.

In yet a further aspect there is provided the use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte with a nebuliser, preferably a mesh nebuliser, in the treatment of a respiratory disease.

In another aspect there is provided a kit containing a nebuliser together with a composition comprising a non-ionic hyperosmolar agent and an electrolyte. Specifically, there is provided a kit containing a nebuliser together with a composition comprising a non-ionic hyperosmolar agent and an electrolyte wherein when the composition is nebulised with the nebuliser, a nebulised composition is formed.

In a further aspect there is provided a kit containing a mesh nebuliser together with a composition comprising a non-ionic hyperosmolar agent and an electrolyte. Specifically, there is provided a kit containing a mesh nebuliser together with a composition comprising a non-ionic hyperosmolar agent and an electrolyte, wherein when the composition is nebulised with the nebuliser, a nebulised composition is formed.

In another aspect there is provided a nebuliser containing a composition comprising a non-ionic hyperosmolar agent and an electrolyte. Specifically, there is provided a nebuliser containing a composition comprising a non-ionic hyperosmolar agent and an electrolyte, wherein when the composition is nebulised with the nebuliser, a nebulised composition is formed.

In a further aspect there is provided a mesh nebuliser containing a composition comprising a non-ionic hyperosmolar agent and an electrolyte. Specifically, there is provided a mesh nebuliser together with a composition comprising a non-ionic hyperosmolar agent and an electrolyte, wherein when the composition is nebulised with the nebuliser, a nebulised composition is formed.

In the above four aspects, when the composition is nebulised with the nebuliser, a nebulised composition is formed. Said another way, upon nebulisation with the nebuliser the composition forms a nebulised composition.

In yet a further aspect there is provided a method of forming a nebulised composition the method comprising nebulising a composition comprising a non-ionic hyperosmolar agent and an electrolyte in a nebuliser.

For the first time, the present invention has demonstrated that a non-ionic hyperosmolar agent may be delivered via a nebuliser in a form suitable for administration to a patient in need thereof and this has been achieved by the combination of the non-ionic hyperosmolar agent with an electrolyte in the composition. When a drug is solubilised or suspended in a fluid the physicochemical properties of the consequent formulation have a significant influence on aerosol generation, especially for mesh nebulisers. The present invention has identified that the addition of an electrolyte changes the physicochemical properties of the composition to facilitate aerosol production.

In an embodiment, the composition is a solution containing the non-ionic hyperosmolar agent and electrolyte dissolved therein.

In a further embodiment, the composition is a liquid containing the electrolyte and at least some of the non-ionic hyperosmolar dissolved therein but wherein at least some of the non-ionic hyperosmolar agent is suspended in the solution. Thus, the composition is a solution containing the non-ionic hyperosmolar agent and electrolyte dissolved therein which further contains at least some of the non-ionic hyperosmolar agent suspended in the solution.

The non-ionic hyperosmolar agent may be any pharmaceutically acceptable non-ionic agent which alters the osmolarity of the airways. In an embodiment, the non-ionic hyperosmolar agent may be selected from sugars and sugar alcohols.

Sugars and sugar alcohols are useful because they are osmotic agents and have also been shown to be non-toxic. Furthermore, they have a tolerable taste for patients during administration which may improve patient compliance. In a further embodiment, the non-ionic hyperosmolar agent may be selected from mannitol, sorbitol, and xylitol. In a particular embodiment, the non-ionic hyperosmolar agent is mannitol. In another particular embodiment, the non-ionic hyperosmolar agent is sorbitol. In another particular embodiment, the non-ionic hyperosmolar agent is xylitol.

In a particular embodiment, the composition contains more than one non-ionic hyperosmolar agent, which may be selected from one or more sugars or sugar alcohols or a combination of sugars and sugar alcohols. Accordingly, the one non-ionic hyperosmolar agents may be selected from one or more of mannitol, sorbitol or xylitol or mixtures thereof. In one embodiment the non-ionic hyperosmolar agent is mannitol. In another embodiment the non-ionic hyperosmolar agent is sorbitol. . In yet another embodiment the non-ionic hyperosmolar agent is xylitol. Particular preferred combinations include, but are not limited to: mannitol and sorbitol, mannitol and xylitol, sorbitol and xylitol.

Mannitol is a sugar alcohol that can be used to achieve long term improvement in lung function. There is a lack of active transport in the lungs for mannitol which necessitates clearance via the slower paracellular mechanism. Mannitol also has an excellent safety profile and is tolerable for patients due to its sweet taste.

Sorbitol is a diasteromer of mannitol. It shares a molecular weight, and gives similar aerosol characteristics and effects on airway function. However, its solubility is higher which gives it a higher potential fluid concentration. This can lead to either a decreased total fluid volume to be nebulised, thereby reducing treatment time; or can increase the amount of non-ionic hyperosmolar agent delivered to the patient in a given volume of liquid.

Xylitol, has a slightly higher osmotic value than mannitol or sorbitol and retains the low transepithelial permeability important for prolonged therapeutic effect.

The non-ionic hyperosmolar agent will be present in the composition in an amount sufficient to achieve a therapeutic effect but it is generally desirable to have a higher concentration so that a lower volume of liquid is needed to get the necessary dose. At the same time, too high a concentration may lead to problems delivering the nebulised composition. This can be due to the non-ionic hyperosmolar agent precipitating out of composition either in storage or during administration, which can lead to uneven dosing and can affect the performance of the nebuliser. Too high a concentration can also increase the viscosity to an extent that it decreases overall performance or even prevents the nebuliser from working. The actual concentration chosen will therefore depend on a number of factors, including the solubility of the agent.

In an embodiment, the composition contains the maximum solubilising amount of non-ionic hyperosmolar agent for the aqueous system in which it is solubilised. Said another way, the non-ionic hyperosmolar agent is present in a concentration at or just below its saturation point. In a further embodiment, the composition contains less than the maximum solubilising amount of non-ionic hyperosmolar agent for the aqueous system in which it is solubilised. In a particular embodiment, the composition contains 100% the amount of non-ionic hyperosmolar agent which can be solubilised. In a further embodiment the composition contains 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% or 10% of the amount of non-ionic hyperosmolar agent which can be solubilised.

As an example, if up to 180mg/ml of a given non-ionic hyperosmolar agent can be solubilised it may be desirable to prepare a composition containing 80% of the maximum amount, that is ~150mg/ml non-ionic hyperosmolar agent. At this level, the concentration is relatively high which minimises the total volume of composition that needs to be nebulised which reduces treatment time. However, the concentration is safely below the threshold where unwanted precipitation may occur.

Particular concentrations will depend on the non-ionic hyperosmolar agent but may be in the range of about 200-10mg/ml. The concentration may be selected from about: 200mg/ml, 190mg/ml, 180mg/ml, 170mg/ml, 160mg/ml, 150mg/ml, 140mg/ml, 130mg/ml, 120mg/ml, 110mg/ml, 100mg/ml, 90mg/ml, 80mg/ml, 70mg/ml, 60mg/ml, 50mg/ml, 40mg/ml, 30mg/ml, 20mg/ml, or 10mg/ml.

In a particular embodiment, if mannitol is the non-ionic hyperosmolar agent, the concentration may be in the range of about 200-70mg/ml, preferably about 200-100mg/ml, more preferably, about 170-140mg/ml. The concentration may be selected from about: 200mg/ml, 190mg/ml, 180mg/ml, 170mg/ml, 160mg/ml, 150mg/ml, 140mg/ml, 130mg/ml, 120mg/ml, 110mg/ml, 100mg/ml or 75mg/ml. Further particular concentrations are about: 170mg/ml, 160mg/ml, 150mg/ml or 140mg/ml. In one embodiment, the concentration is about 150mg/ml.

In a further embodiment, if sorbitol is the non-ionic hyperosmolar agent, the concentration may be in the range of about 1000-70mg/ml, preferably about 400-200mg/ml, more preferably about 400-300mg/ml, even more preferably about 300-350mg/ml. The concentration may be selected from about: 1000mg/ml, 900mg/ml, 800mg/ml, 700mg/ml, 600mg/ml, 500mg/ml, 400mg/ml, 350mg/ml, 300mg/ml, 250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml or 75mg/ml. Further particular concentrations are about: 400mg/ml, 380mg/ml, 360mg/ml, 320mg/ml, 300mg/ml, 280mg/ml, 260mg/ml, 240mg/ml, 220mg/ml or 200mg/ml.

In a further embodiment, if xylitol is the non-ionic hyperosmolar agent, the concentration may be in the range of about 640-60mg/ml, preferably about 300-400mg/ml, more preferably about 300-350mg/ml. The concentration may be selected from about: 640mg/ml, 620mg/ml, 600mg/ml, 580mg/ml, 560mg/ml, 540mg/ml, 520mg/ml, 500mg/ml, 480mg/ml, 460mg/ml, 440mg/ml, 420mg/ml, 400mg/ml, 380mg/ml, 360mg/ml, 350mg/ml, 340mg/ml, 334mg/ml, 330mg/ml, 320mg/ml, 300mg/ml, 280mg/ml, 260mg/ml, 250mg/ml, 240mg/ml, 220mg/ml, 200mg/ml, 180mg/ml, 160mg/ml, 140mg/ml, 125mg/ml, 120mg/ml, 100mg/ml or 63mg/ml.

There is an interaction between the choice of non-ionic hyperosmolar agent with its resultant solubility and the choice of concentration that is used. Higher concentrations are generally preferred since this leads to lower total volumes and therefore lower treatment times for the patient. However, there are two main limiting factors which can affect the maximum concentration that can be used. For lower solubility hyperosmolar agents the limiting factor may be precipitation and a concentration sufficiently below the saturation point may be needed to ensure that the agent remains in composition. For high solubility hyperosmolar agents the saturation point is less likely to be an issue but viscosity of the composition will become relevant. As the concentration is

increased the viscosity also increases. While this beneficially lowers droplet size, it also lengthens treatment time. Also, above a certain threshold, too high a viscosity can adversely affect performance or even prevent the nebuliser from working.

Thus, the viscosity of the composition affects a number of properties of the nebuliser. Increasing the viscosity lowers the average droplet size which leads to smaller droplets and lower lung deposition due to an inverse relationship between viscosity and droplet size. However, nebulisation becomes intermittent or ceases completely with too high viscosities. Furthermore, increasing the viscosity increases the treatment time since it takes longer to nebulise the composition. Thus, a balance should be struck with viscosity to achieve the optimum balance between total composition volume, droplet size and treatment time. The maximum usable viscosity will depend on the type of nebuliser used. In an embodiment, the viscosity of the composition is in the range of about: 1- 10cP, 1-4cP, 1-2.7cP, 1-3.8cP, 1-1.9cP. It may be <10cP (centipoise). In a further embodiment, the viscosity of the composition is <9cP, <8cP, <7cP, <6cP, <5cP, <4cP, <3.8cP, <3cP, <2cP, <2.7cP, <2.5cP, <2.4cP, <2.3cP, <2.2cP, <2.1cP, <2cP, <1.9cP, <1.8cP, <1.7cP, <1.6cP, <1.5cP, <1.4cP, <1.3cP, <1.2cP, <1.1cP, or <1cP. In a particular embodiment, the viscosity is less than about 1.9cP. In another embodiment the viscosity is less than about 2.7cP. In yet another embodiment the viscosity is less than about 3.8cP.

Overall, the specific concentration used will depend on the choice of non-ionic hyperosmolar agent.

With reference to mannitol, sorbitol and xylitol, in embodiments the solute concentrations of sorbitol and xylitol may be considerably below their saturation point. In contrast, the solute concentration of mannitol may be much closer to its saturation point. Thus whereas for mannitol the limiting aspect may be aqueous solubility, viscosity may be the ultimate factor determining maximal feasible fluid concentration for sorbitol and xylitol.

The total amount of non-ionic hyperosmolar agent delivered to the patient will depend on the efficacy of the active among other factors. In an embodiment, the total amount of non-ionic hyperosmolar agent delivered to the patient in a single therapeutic dose in the range of about 900-100mg, preferably 500-300mg. It may be selected from about: 900mg, 800mg, 700mg, 600mg, 500mg, 400mg, 300mg, 200mg, or 100mg although

the actual value could be higher or lower depending on the specific active agent. In a particular embodiment, if the non-ionic hyperosmolar agent is mannitol, an appropriate dose may be about: 500mg, 400mg or 300mg.

The compositions of the present invention also contain one or more electrolytes. It has been found that adding an electrolyte to the non-ionic hyperosmolar agent containing composition enhances performance and allows nebulisation to occur in a therapeutically useful way. On its own, mannitol has been found to be poorly nebulised and aerosol output was characterised by frequent periods of intermittent nebulisation that increased the treatment time. It has surprisingly been found that the addition of an electrolyte to the composition improves nebulisation performance by producing respirable droplets and reducing treatment time.

The electrolyte has been found to improve the physicochemical properties of the composition for nebulisation, which positively affects aerosol generation particularly for vibrating mesh type nebulisers. Without wishing to be bound by theory, one explanation for this may be that the electrolyte suppresses electrostatic charges present in the aqueous composition which improves the composition's flow through, and detachment from, the mesh of the nebuliser.

In an embodiment, the electrolyte may be selected from salts including sodium salts, potassium salts or calcium salts. The electrolyte may be selected from the group consisting of: sodium chloride, potassium chloride, sodium citrate dihydrate, potassium citrate, sodium bicarbonate, sodium carbonate, citric acid monohydrate, saccharin sodium, glycine, calcium acetate, calcium hydroxide, phosphoric acid, acetic acid, sodium acetate, or monosodium glutamate. The term 'electrolyte' is intended to encompass any molecule which contains physiologically acceptable ions, which dissociate into their respective cations and anions in composition. The present invention encompasses any pharmacologically acceptable ion or salt.

The present invention also includes the possibility that the non-ionic hyperosmolar agent is present in a salt form which provides the electrolyte. As an example, the non-ionic hyperosmolar agent may be saccharin sodium (i.e. a salt form of saccharin). The saccharin is present as the non-ionic hyperosmolar agent and the sodium forms the electrolyte.

Therefore, in embodiments of the invention the non-ionic hyperosmolar agent is added to the liquid in its salt form and this provides both the non-ionic hyperosmolar agent and the electrolyte.

In a particular embodiment, the electrolyte is selected from salts of sodium or potassium. In a further embodiment, the composition contains NaCl.

The use of NaCl has a further advantage in that it too independently may contribute to the osmotic effect of the nebulised composition and has already been found to be safe in nebulised therapies.

In an embodiment the electrolyte may be present in a concentration of about: 0.1% - 20%, 0.1-10%, 0.1-7%, 0.2-7%, 0.1-5%, 0.5-5%, 0.5-2%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10% w/v.

Increasing electrolyte concentration may decrease treatment times whilst simultaneously boosting the osmotic effect of the formulation. However, increasing the electrolyte concentration may adversely affect the taste of the plume as it becomes increasingly noticeable by the patient. Thus, an appropriate balance between efficacy and taste should be struck. The particular concentration of about 1% w/v has been found to strike this balance but it could optionally be increased to achieve shorter treatment times whilst boosting osmotic delivery.

The non-ionic hyperosmolar agent and electrolyte may be solubilised in any physiologically acceptable aqueous medium to allow for nebulisation. One such medium is water. Further compositions include buffer compositions or other physiologically acceptable mediums. Such mediums may contain electrolytes and if so, they may optionally be supplemented with additional electrolyte to assist with the overall nebulisation performance of the composition. In a particular embodiment, the aqueous composition is water.

The total amount of composition to be nebulised in a given treatment will depend on a number of factors including the solubility of the non-ionic hyperosmolar agent, with lower solubilities requiring greater total composition volumes. The total volume will also depend on the nature of the therapy with certain treatments requiring a higher or

lower dose. However, an important determining factor is the nebuliser itself since they typically have defined maximum reservoir volumes. Thus, in an embodiment, the amount of composition nebulised and delivered to the patient may be about: 0.1-10ml, 0.1-5ml, 0.5-1ml, 1ml, 2ml, 2.5ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml or 10ml.

The compositions of the present invention are nebulised before being inhaled by the patient. The process of nebulisation creates a fine mist of small droplets of composition that are of respirable size. Droplets are considered respirable if they can be inhaled and deposited onto one or more of the oropharynx and upper airways (including the trachea), lower airways (including the bronchus and bronchioles) or deposited in the alveoli. In particular embodiments the respirable droplets can be deposited onto the lower airways. The size of the droplet can be adjusted to target delivery to certain parts of the respiratory system, for example using smaller droplets to target the lower airways and larger droplets to target the upper airways. For example, if it is desired to be able to deliver agents to bronchus and bronchioles, then appropriate droplet size is needed to allow the particles to be carried to the target area and not deposit in the upper airways. Equally, in certain circumstances, it may be desirable to specifically target the upper airways and again, appropriate droplet size is needed.

The nebuliser produces large numbers of droplets and it is generally the case that within this large number it is expected that there will be a distribution of individual droplet sizes, ranging from very small through to large. There may be a normal distribution of droplet sizes with a percentage of the droplets within a given size range. Thus, when considering the droplets of the present invention the droplet size may be thought of as the average droplet size. Thus, for example, if the droplets are said to be about 1-3 microns in size, this represents an average droplet size with some droplets being smaller or larger than the average droplet size. In some embodiments, this average size may be the mean. In further embodiments, the size may be the median.

The droplets may be measured in terms of their aerodynamic diameter. Aerodynamic diameter provides a useful measurement of inhalable particles and takes into account factors that affect their aerodynamic properties. Aerodynamic diameter can be used to compare droplets of differing physical size and takes into account the density of the particle as well as its size. The aerodynamic diameter can be approximated by considering the following formulae: aerodynamic diameter (d_{ae}) = $\rho^{0.5} d_p$, where ρ is the density of the particle and d_p the physical diameter of the particle. The advantage of

considering an aerodynamic diameter is that it differentiates between otherwise similar physically sized particles that have different aerodynamic properties. As an example, a hollow sphere will have a lower density than an equally sized droplet. The aerodynamic diameter of the two spheres will be different with the hollow sphere being lower, even though the physical diameter is the same. Equally, seemingly differently shaped or sized particles or droplets may have the same aerodynamic diameter.

An advantage of considering the aerodynamic diameter of a collection of particles or droplets is that it can be measured empirically in an impactor and it is not necessary to look at each droplet individually to measure its physical size. The inhalation properties of the droplets are assessed and the average aerodynamic properties of the droplets determined to arrive at the average aerodynamic diameter. A typical measurement will identify what percentage of the total droplets are within various aerodynamic diameter size ranges.

In one embodiment the average aerodynamic diameter of the droplets is between from about 0.01 to about 20 microns. In a further embodiment, the average particle size is between from about 0.1 to about 20 microns. In a yet further embodiment, the average particle size is between from about 0.1 to about 15 microns, about 0.1 to about 10 microns, about 0.1 to about 7 microns, about 0.1 to about 6 microns, about 0.5 to about 10 microns, about 0.8 to about 10 microns, about 1 to about 10 microns, about 1 to about 9 microns, about 1 to about 8 microns, about 1 to about 7 microns, about 1 to about 6 microns, about 1 to about 5 microns, about 1 to about 4 microns, about 1 to about 3 microns, about 1 to about 2 microns, about 2 to about 3 microns, about 3 to about 4 microns, from about 3 micron to about 7 microns, about 4 to about 5 microns, about 4 micron to about 6 microns, about 5 to about 6 microns, or about 6 to about 7 microns.

Particular average aerodynamic diameter ranges are between from about 3 micron to about 7 microns, between from about 4 micron to about 6 microns, and between from about 5 micron to about 6 microns.

In a yet further embodiment, the average aerodynamic diameter is $\leq$ about 10 microns, $\leq$ about 9 microns, $\leq$ about 8 microns, $\leq$ about 7 microns, $\leq$ about 6 microns, $\leq$ about 5 microns, $\leq$ about 4 microns, $\leq$ about 3 microns, $\leq$ about 2 microns, $\leq$ about 1 micron.

In a yet further embodiment, the average aerodynamic diameter is about 1 micron, about 2 microns, about 3 microns, about 4 microns, about 5 microns, about 6 microns, about 7 microns, or about 8 microns.

The average size of the droplets are affected by a number of factors including the viscosity of the composition, the surface tension of the composition and also the type of nebuliser used. If necessary, these factors can be adjusted to achieve the desired droplet size.

The treatment time is also affected by a number of factors including the viscosity of the composition, the volume of composition and the type of nebuliser used. A lower treatment time is advantageous because it makes administration quicker and easier. In an embodiment, the average administration time for 2.5ml is <420 seconds, <360 seconds, <300 seconds, <240 seconds, <180 seconds, or <120 seconds. In particular embodiments, the total treatment time is less than 300 seconds or less than 250 seconds. Treatment times will vary correspondingly if the total volume of composition is changed.

In particular embodiments, the composition may contain mannitol in an amount of 150mg/ml. In a further embodiment, the composition may contain about 1% saline. In yet a further embodiment, the composition contains a saline-mannitol combination consisting of about 150mg/mL mannitol in about 1% saline. This provides an appropriate balance in that it maximises mannitol delivery within a reasonable time without adversely affecting taste.

In further embodiments, the composition may contain sorbitol in an amount of about 300-400mg/ml. Particular embodiments include about: 300mg/ml, 330mg/ml or 350 mg/ml. Again, in embodiments, the composition may contain about 1% saline. In yet a further embodiment, the composition contains a saline-sorbitol combination consisting of from about 300mg/mL to less than 400mg/ml sorbitol in about 1% saline, or preferably from about 300mg/mL to about 350mg/ml sorbitol in about 1% saline.

In further embodiments, , the composition may contain xylitol in an amount of about 300-400mg/ml. Particular embodiments include about 300mg/ml, 330mg/ml or 350 mg/ml. Again, in embodiments, the composition may contain about 1% saline. In yet a further embodiment, the composition contains a saline-xylitol combination consisting

of from about 300mg/mL to less than 400mg/ml sorbitol in about 1% saline, or preferably from about 300mg/mL to about 350mg/ml sorbitol in about 1% saline.

The nebuliser for use with the present invention may be a mesh nebuliser. Mesh nebulisers contain a micro-perforated metal plate that plays a central role in aerosol production. In an embodiment, the nebuliser is a static (or passive) mesh nebuliser. In an alternative embodiment, the nebuliser is a vibrating mesh nebuliser.

However, in an alternative embodiment, traditional nebulisers may also be used, including jet or ultrasonic nebulisers. Whilst it is believed that these will be equally effective, they may moderately increase the treatment time.

The compositions of the present invention may further include one or more additional therapeutic agents.

The compositions of the present invention may be useful in the treatment of respiratory or non-respiratory diseases. In a particular embodiment, the compositions of the present invention are useful in the treatment of respiratory diseases. In an embodiment, the respiratory disease(s) may be selected from asthma, bronchiectasis, emphysema, COPD and cystic fibrosis (CF). The term treatment should be broadly interpreted as covering both therapeutically treating a condition or disease, and also mitigating or targeting the effects of the diseases, without treating the underlying conditions themselves.

Diseases or conditions for which the present invention may apply include respiratory or non-respiratory conditions. Respiratory conditions include: bronchiectasis, COPD, bronchitis, allergy, rhinitis, emphysema, cystic fibrosis, pulmonary infection, tuberculosis, influenza, other lung infections, lung cancer and asthma. Non-respiratory conditions include diabetes, hypertension, hypercholesterolaemia, gout, infections (bacterial or viral), fever, pain (neurological or muscular).

In one embodiment, the respiratory condition is COPD. In a further embodiment, the respiratory condition is bronchitis. In yet a further embodiment, the respiratory condition is allergy. In yet a further embodiment, the respiratory condition is rhinitis. In yet a further embodiment, the respiratory condition is bronchiectasis. In yet a further embodiment, the respiratory condition is emphysema. In yet a further embodiment, the

respiratory condition is cystic fibrosis. In yet a further embodiment, the respiratory condition is pulmonary infection. In yet a further embodiment, the respiratory condition is asthma.

Particular conditions are cystic fibrosis (CF), bronchiectasis, emphysema or COPD. In a particular embodiment, the condition is cystic fibrosis.

Brief Description of the Figures

Figure 1 shows an Aeroneb®Go Vibrating Mesh Nebuliser.

Figure 2 shows the mass distribution of sodium chloride and mannitol in the Next Generation Impactor after nebulisation of mannitol at 150mg/mL in 1% NaCl (n=3, error bars not visible as negligible error between replicates).

Figure 3 shows the mass distribution for sobitol 300mg/mL in 1% saline (error not shown because negligible).

Figure 4 shows mass distribution for xylitol 334mg/mL in 1% saline (error not shown because negligible)..

Figure 5 shows respirable aerosol output and viscosity (at 20°C) for nebulised polyol fluids (error is not charted as errors bars are too small to be visible).

The invention will now be described with reference to the following examples although they should not be taken as limiting the invention.

Detailed Description

By way of example only, preferred embodiments of the invention are now described with reference to Figures 1-5.

In embodiments of the present invention, the nebuliser used may be a mesh nebuliser, including a static (passive) mesh nebuliser or a vibrating mesh nebuliser.

Static (passive) mesh nebulisers include the Omron Micro*Air*® NE-U22V nebuliser which was the first interpretation of the mesh nebuliser. In a similar fashion to

ultrasonic nebulisers, a transducer vibrating at 180MHz, generates pressure waves through the drug fluid. However, droplet formation is indirect, instead utilising the pressure waves to force the aqueous drug through the stationary mesh. Approximately 90% of the drug loaded into the reservoir is nebulised. The NE-U22V has a metal alloy mesh consisting of 6000 tapered holes with a diameter of ~3μm formed by electroplating.

Newer generations, termed vibrating mesh nebulisers, utilise vibration of the mesh itself. The mesh consists of ceramic piezo elements surrounding a domed aperture mesh plate. The mesh holes are conical, with the larger cross section in contact with the drug reservoir, and located at the centre of the plate which has the highest amplitude. Operation of this configuration has been likened to a "micropump", whereby each inward oscillation into the drug reservoir loads the individual micron sized mesh holes with aqueous drug, whilst the subsequent opposing oscillation ejects the drug as uniformly sized droplets. Depending on the nebuliser design, close to 100% of the loaded drug can be nebulised. Vibrating mesh technology is currently commercialised as either ODEM Touchspray or Aerogen OnQ, which are compared in Table A below.

Table A: *Mesh Nebuliser Technology*

Technology	**Aerogen OnQ**	**ODEM Touchspray**
Nebuliser	**Aeroneb® Go**	**PARI eFlow® Rapid**
Mesh	Nickel-Palladium Alloy; 1000 Electroformed Holes	Stainless Steel; 4000 laser drilled holes
Frequency	100kHz	116kHz
Treatment Time (relative)	2.5 (Aeroneb® Go); Aerosol output rate of 0.3-0.6mL/min	1 (eFlow® Rapid); Aerosol output rate of up to 1mL/min
Aerosol Velocity	Vertical; Standing Cloud	Horizontal; Low Speed
Mass Median Aerodynamic Diameter	3.6μm	3-4μm
Maximum Fill Volume	6mL	6mL

Other Comments	i) Aeroneb® Go and PARI eFlow® Rapid are the most popular nebulisers of the respective technologies. The Aeroneb® Pro is mechanically ventilated nebuliser for clinical settings. The PARI eFlow® SCF (sometimes known as the Trio) is essentially the same as the eFlow® Rapid but without the structurally integrated 1.2mL residual volume. ii) Stainless steel and nickel palladium alloy both provide corrosion resistance against the saline vehicles typically employed for nebule formulations. iii) The PARI eFlow® Rapid is not available off the shelf due to significantly higher drug delivery efficacy compared to conventional jet nebulisers. Instead, it is directly licenced to pharmaceutical companies for use with specific drug formulations.

The following experiments demonstrate that compositions containing a non-ionic hyperosmolar agent and an electrolyte can be nebulised to produce aerosolised mists containing respirable droplets which can be useful in respiratory treatment. The experiments are carried out using a variety of non-ionic hyperosmolar agents: mannitol, sorbitol and xylitol together with NaCl as the electrolyte.

Experiment 1: Mannitol

1. Methodology

1.1. Nebuliser

An Aerogen Aeroneb® Go mesh nebuliser (Lot 9136100319; Aerogen, Dangan, Galway, Ireland) (Figure 1) was used for nebulisation. The nebuliser was thoroughly cleaned between nebulisations, with consideration of the manufacturer's instructions. This involved rinsing with cold tap water, followed by soaking in hot tap water for five minutes, then rinsing with deionised water (ambient temperature). No detergent was used to minimise potential contaminant substances deposition on nebuliser components (e.g. fragrance). The nebuliser was oven dried (Model TED-66F; Serial 29890; Thermoline Scientific, Smithfield, NSW, Australia) at 70°C for 10 minutes then cooled

to ambient temperature by deionising fan (Serial 25724; Ion Systems, Berkeley, California, USA) before use.

1.2. Nebuliser Fluids

Fresh deionised water (MilliQ, Molsheim, France), sodium chloride (Batch 232931, LabServ™, Clayton, Victoria, Australia) and mannitol (Lot Batch E236A, Roquette Frères, Lestrem, France), were used as supplied, to prepare the following fluids for nebulisation: 1) Deionised Water as a baseline for comparison; 2) Mannitol 150mg/mL in deionised water to determine its nebulisation viability as a lone ingredient; 3) Saline alone at various concentrations (0.2%, 1%, 3%, 5%, 7% w/v) to determine the effect of concentration on treatment time and aerosol characteristics; 4) Mannitol 150mg/mL in Saline (0.2%, 1%, 3%, 7% w/v) to enhance aerosol output and respirable size fraction. The mannitol concentration of 150mg/mL was chosen to maximise delivery whilst considering its solubility at ambient temperatures (13% w/v at 14°C, 18% w/v at 25°C). All solutions were stored at room temperature (20±1°C) and used within 48 hours of manufacture.

1.3. Particle Size Characterisation and Instrument Selection

Characterisation of nebulised aerosols is commonly performed with the Next Generation Impactor (NGI), in accordance with General Chapter 2.9.44 (Preparations for Nebulisation: Characterisation) of the European Pharmacopeia, that recommends cascade impaction calibrated to a sampling flow rate of 15L min^{-1}. However, sizing by this method is notoriously difficult due to post-aerosolisation evaporation leading to undersizing of nebulised aerosol. This phenomenon is observed for both conventional jet nebulisers and the more recent vibrating mesh nebulisers, and is a result of heat and mass transfers as the aerosol travels through the NGI. The latter devices are primarily affected by entrainment of unsaturated ambient air to match the sampling airflow of impactor. Traditionally, this evaporative effect has been minimised by humidification of entrained air, to attain an internal NGI environment close to 100% relative humidity. More recently, there has been renewed interest in achieving this by lowering the temperature to 5°C through NGI refrigeration. Regardless of method, this is an additional requirement to an already tedious and time-consuming process.

Laser diffraction is a quicker and well-substantiated alternative for size characterisation of aqueous aerosols. The short distance between nebuliser mouthpiece and measurement zone, means that droplet evaporation in laser diffractometry is negligible,

permitting use at ambient conditions. The effectiveness of laser diffraction as a sizing option has further been established for vibrating mesh-nebulised aerosol. Although this technique suffers from multiple scatter, vignetting and beam steering, these are adjusted for in the current study. The geometric and aerodynamic diameter obtained from laser diffraction and cascade impaction (corrected for evaporation), respectively, exhibit a high level of correlation – a characteristic attributed to nebulised droplets resembling unit density spheres.

Laser diffractometry with the Spraytec (Malvern, UK) was used to determine aerosol distribution and total nebulisation time. An open bench configuration was chosen to minimise the distance, and hence evaporation, between nebuliser mouthpiece and laser measurement area. Air extraction through a SureGard® 1420 nebuliser filter (Lot L69470; BIRD Healthcare Pty Ltd, Melbourne, Australia) was implemented to maintain laser obscuration at 20 ± 2% and to prevent aerosol re-entry into the measurement zone. A new respiratory filter was used for each nebulisation. In accordance with standards for particle sizing with laser diffractometry set by the European Committee for Standardization (CEN), a 10mm gap was maintained between mouthpiece and measurement zone.

Detectors 1-5 were excluded from data analysis to account for beam steering. An algorithm to correct for multiple scatter embedded in the Malvern software was activated. It is noted that no significant multiple scattering was expected as light transmission remained above 70% for all measurements. No evidence of significant vignetting was observed.

All experiments were conducted within a climate controlled room with temperature maintained at 20±1°C and relative humidity at 30±1%. The reservoir of the nebuliser was filled with 2.5mL or 5mL of the respective nebuliser fluid prior to nebulisation. The filled nebuliser was weighed, then again post-nebulisation, to determine gravimetric output and residual volume. Particle measurement and extraction was initiated 5 seconds prior to nebulisation and ceased 10 seconds after no aerosol was detectable and reservoir confirmed to be empty. The prepared nebuliser fluids were each analysed in triplicates.

Treatment time was defined as the first to the last instance of detectable aerosol. All data points excluding the initial and last 5 seconds of nebulisation were then averaged for each measurement.

1.4. Aerosol performance by Cascade Impaction

A series of nebulisations (1% saline with 150mg/mL Mannitol) was performed on the Next Generation Impactor (NGI) to assess droplet content uniformity and the level of correlation with laser diffractometry. To limit evaporation of nebulised aerosol, the nebuliser and NGI were housed in a polycarbonate box with a controlled temperature (20±1°C) and relative humidity (>95%). The NGI sampling airflow was activated for 2 minutes prior to attachment of the nebuliser to allow for environmental equilibration between the NGI and experimental housing.

A rubber adaptor completed a sealed attachment of the nebuliser to the USP throat of the impactor. Impactor plates were not coated as it is not required for collection of aqueous aerosol and a hydrophobic layer may cause unintended post-deposition movement of the aqueous drug.

The NGI was operated with an extraction flow rate of 15L min^{-1} to mimic the midpoint of adult tidal breathing used with nebulisers and with consideration of expected revisions of the European Pharmacopeia standards for nebuliser-related testing.

1.5. Mass Assay

Before use, the nebuliser and NGI plates were oven dried at 70°C to evaporate any aqueous phase/the deposited aqueous drug aerosol then cooled to ambient temperature by deionising fan. The nebuliser, adaptor, USP throat and impactor plates were then individually rinsed with 5mL (15mL for nebuliser due to high solute concentration) of mobile phase (50mg/mL calcium disodium EDTA-Sigma-Aldrich Chemie B.V., Zwijndrecht, Netherlands- in deionised water). Mass assay of these samples were performed using High Performance Liquid Chromatography (HPLC).

Methodology for chromatographic separation of sodium chloride and mannitol was adapted from Hughes & Lindsay (1985). The Shimadzu HPLC system comprised of a CBM-20A controller, LC-20AT pump, SPD-20A RID-10A refractive index detector, SIL-20A HT Autosampler and column oven with LCSolution software (all Shimadzu Corporation, Japan). It was coupled with a dual column configuration, consisting of a

Sugar-Pak® 300mm x 6.5mm (i.d.) column (Waters, Milford, MA, USA) maintained at 77 ±1°C by a column heater (Waters, Milford, MA, USA), joined to a Resolve® C-18 column 150mm×3.9mm (Waters, Milford, MA, USA). A mobile phase consisting of 50mg/mL calcium disodium EDTA (Sigma-Aldrich Chemie B.V., Zwijndrecht, Netherlands) in deionised water was pumped through the system at a flow rate of 0.5mL min^{-1}, with a sample injection volume of 100µL.

Sodium chloride (Batch 232931, LabServ™, Clayton, Victoria, Australia) and mannitol (Lot Batch E236A, Roquette Frères, Lestrem, France) were separately dissolved in deionised water at sequential concentrations to obtain calibration curves (0.01 to 1% w/v (R^2=1.00) for sodium chloride; 0.015 to 15mg/mL (R^2=1.00) for mannitol) for result analysis.

1.6 Statistical Analysis

One-way ANOVA was used to identify any statistically significant differences between the various fluid formulations ($p<0.05$). Significant differences were further analysed with Tukey's post hoc test to identify the specific formulations involved. A p-value of <0.05 was considered statistically significant.

Unpaired two-sample t-tests for a 99% confidence limit were used to ascertain any significant differences in particle sizes *between* the three formulation groups – 2.5mL saline only, and 2.5mL and 5mL saline with mannitol, and treatment times. A p-value of >0.01 was considered statistically significant.

Results of Experiment 1

1.7 Single nebulisation of Water and solubilised Mannitol

Water (2.5mL) and mannitol (2.5mL) were observed to exit the nebuliser at irregular, intermittent intervals. The median droplet size and treatment times obtained were 13.79µm in 425 seconds and 11.27µm in 477 seconds, respectively. Median droplets of this size are considered too large for respiratory use.

1.8 Nebulisation of various concentration of Sodium Chloride alone and in combination with Mannitol

The data is separated into three groups: 2.5mL saline at various concentrations, 2.5mL and 5mL of 150mg/mL mannitol in saline of various concentrations.

1.8.1 Particle Size analysis of the nebulised formulations

In Table 1 the median droplet sizes obtained within each of the three manufactured groups is presented: 2.5mL of mannitol 150mg/mL in saline, 5mL mannitol 150mg/mL in saline and 2.5mL saline only (no mannitol), respectively. There was no significant difference in droplet sizes (p-value > 0.05) between the groups. Samples of different volume (2.5mL and 5mL) containing mannitol exhibited a similar particle size (p-value > 0.01). These were significantly different from the larger droplets generated by the 2.5mL saline only samples (p-value <0.01). The mean droplet sizes for the three formulations were 6.20±0.09, 5.75±0.07 and 5.74±0.09μm, respectively.

Geometric standard deviation (GSD) increased slightly with increasing saline concentration. However, there was no significant difference except between 0.2% saline and the higher concentrations. A significant, albeit small increase in GSD was observed when sodium chloride was combined with mannitol.

Table 1: *Median droplet volumetric diameter ± standard deviation (and geometric standard deviation in parentheses) for various hyperosmotic fluids*

saline concentration (%)	0.2	1	3	5	7	Mean (μm)	P-value
Mannitol 150mg/mL 2.5mL	5.70±0.01 (1.84)	5.76±0.04 (1.83)	5.81±0.07 (1.88)	-	5.70±0.07 (1.88)	5.75±0.07	0.157
Mannitol 150mg/mL 5.0mL	5.73±0.04 (1.83)	5.75±0.06 (1.86)	5.77±0.05 (1.89)	-	5.68±0.02 (1.88)	5.74±0.09	0.294
No Mannitol 2.5mL	6.28±0.05 (1.88)	6.20±0.10 (1.91)	6.23±0.10 (1.91)	6.08±0.08 (1.93)	6.18±0.04 (1.94)	6.20±0.09	0.148

1.8.2 Nebulisation Treatment Times

The mean treatment times with standard deviation for the three aforementioned formulations are summarised in Table 2.

For all formulations, treatment time is inversely related to saline concentration. In the saline only group, there was significant difference *between* (p<0.05) but not within the following two sets of treatment times: a) 0.2 and 1%; b) 3, 5 and 7% saline. Addition of mannitol increased treatment times within each saline concentration (p<0.01) for all three formulations. The formulation containing 5mL saline and mannitol exhibited significant differences in treatment times for all saline concentrations. A similar

difference was observed for the 2.5mL group except for the treatment times between 3 and 7%. The difference in time between the three formulations at each respective saline concentration was statistically significant ($p<0.01$).

Table 2: *Treatment times ± standard deviation for various hyperosmotic formulations*

Saline Concentration (%)	0.2	1	3	5	7	P-value
Mannitol 150mg/mL 2.5mL	298.0±4.24	261.0±3.46	245.3±4.93	-	243.7±2.52	0.00
Mannitol 150mg/mL 5.0mL	556.0±7.6	532.7±5.9	505.7±6.8	-	472.0±15.6	0.00
Saline Only 2.5mL	241.3±1.2	239.3±4.5	219.3±3.2	213.5±5.0	211.7±1.2	0.00

1.9 Gravimetric Output

Differences between pre- and post-nebulisation weights are shown in Table 3. There was no nebuliser fluid visible in the drug reservoir after each complete nebulisation. No significant difference (<0.05) was observed within each of the three groups. However, the addition of mannitol to saline increased gravimentric output in a statistically significant manner.

Table 3: *Gravimetric Output ± standard deviation for various hyperosmotic formulations*

Saline Concentration (%)	0.2	1	3	5	7	P-value
Mannitol 150mg/mL 2.5mL	1.85±0.05	1.69±0.04	1.60±0.00	-	1.57±0.04	0
Mannitol 150mg/mL 5.0mL	3.51±0.01	3.45±0.08	3.19±0.08	-	2.98±0.08	0
No Mannitol 2.5mL	1.60±0.00	1.633±0.06	1.51±0.09	1.56±0.04	1.48±0.05	0.037

1.10 Aerosol Characterisation by Cascade Impaction

Results from cascade impaction and mass assay from nebulisation of 2.5mL of aqueous solution (150mg/mL mannitol with 1% sodium chloride) are shown in Table 4, and compared to similar values obtained by laser diffraction.

The median volumetric diameter and median aerodynamic diameter obtained from laser diffraction and cascade impaction, respectively, were not significantly different. However, sizing by laser diffraction produced a notably lesser geometric standard deviation. The nebulised masses were only slightly different.

Figure 2 shows the mean proportions of saline and mannitol (i.e. concentration) recovered by mass assay were extensively similar. Error bars not included as standard error between samples was negligible.

Table 4: *Comparison between values for laser diffraction and aerosol cascade impaction for nebulisation of 2.5mL of 150mg/mL Mannitol in 1% NaCl aqueous solution.*

Saline Concentration (%)	Median Diameter (μm)	GSD	Nebulised Mass (grams)
Laser Diffraction	5.76 (0.04)	1.86 (0.01)	1.69 (0.04)
Cascade Impaction	5.69 (0.02)	2.24 (0.01)	1.54 (0.04)
P-Value	0.061	0.00	0.045

Discussion of Experiment 1

As can be seen from the result, deionised water alone and mannitol in aqueous solution were poorly nebulised. Aerosol output was characterised by frequent periods of intermittent nebulisation that contributed to a lengthy treatment. Median droplet size decreased from 13.79μm to 11.27μm with the addition of 150mg/mL mannitol to deionised water, but was still clearly outside a preferred respirable range (< 5-6μm). The median droplet size for saline was significantly smaller at 6.20 μm. Combination of the two solutes further decreased aerosol size to 5.75μm. Thus, the present invention has demonstrated that the addition of an electrolyte to a solution containing a non-ionic hyperosmolar agent reduces the droplet size to within a respirable range.

The current results show droplet size for all samples were unaffected by increasing sodium chloride concentration. Furthermore, there is a dramatic disparity between aerosol sizes obtained from mannitol and sodium chloride aqueous solutions (11.27μm vs. 6.20 μm, respectively). This suggests that the presence of an electrolyte is important for regular aerosol output and maintaining a respirable droplet size. However, beyond a certain minimum concentration required to suppress electrostatic contribution, ionic concentration is irrelevant unless it drastically affects other fluid physicochemical properties such as surface tension or viscosity.

The treatment times for 2.5mL of deionised water and aqueous solution of mannitol were 425 and 477 seconds, respectively, times more attributable to slower jet nebulisation therapy. The high treatment times are in large part attributed to the frequent unpredictable stoppages in nebulisation. The current results show inclusion of just 0.2% sodium chloride to the pure mannitol solution caused a dramatic drop in treatment time from 477 to 298 seconds, with consistent aerosol generation. On the other hand, the treatment time for mannitol-saline combination is lengthened compared to a similar concentration of saline alone, presumably due to the additive increase in viscosity. Overall, by increasing saline concentration, treatment time can be shortened.

A known issue with the Aeroneb Go™ nebuliser is aerosol deposition at the base of the nebuliser interior, as aerosol generation is directed downwards. The aerosol loss by this mechanism was significant for the various nebuliser fluids in the current study and reflected in the results for gravimetric output. The deposition amount however, did not appear dependent on the output rate and only slightly on the formulation.

Cascade impaction confirmed the recognized correlation with laser diffraction for nebuliser aerosol sizing. No significance between median diameters was observed provided evaporation is reconciled for during cascade impaction. Unlike jet nebulisers, vibrating mesh nebulisers do not suffer from a gradual increase in reservoir solute concentration from evaporation. Thus mesh nebulisers are expected to deliver a uniform dose over the course of nebulisation. Results from cascade impaction support this assumption with a consistent proportion of sodium chloride and mannitol across all assay samples and the original stock solution. Should solute concentration increase, mannitol – already close to saturation – was expected to precipitate out resulting in a lowered concentration relative to sodium chloride, which has a much higher solubility. Furthermore, it demonstrated that the presence of sodium chloride serves to enhance aerosol output without adversely affecting mannitol solubility and deposition. The disparity in geometric standard deviation may be a consequence of detector exclusion in laser diffraction to account for beam steering, loss of aerosol from the micro-orifice collector (stage 8 in the NGI) when using a low flow rate (15L min^{-1}), or a combination of both these factors.

Sodium chloride alone is capable of enhancing mucociliary clearance increasing hyperosmolarity and also increases aerosol output rate. It is clear that the addition of

sodium chloride – essentially an electrolyte - to an aqueous mannitol solution is necessary to impart a reasonable respirable fraction and treatment time. Mannitol solubility was not affected by the presence of sodium chloride even at concentrations up to 7%. Thus a combination of these hyperosmolar agents at high concentrations can be used to deliver a therapeutic osmotic effect in a much quicker time than by either agent alone.

A combination of 150mg/mL mannitol in 1% saline may be a suitable option for therapy, providing a balance between acceptable treatment time and taste tolerability.

Experiment 2: Sorbitol and Xylitol

The experiments were also carried out using the sugar alcohols sorbitol and xylitol with 1% NaCl as electrolyte. The use of higher solubility hyperosmolar agents was investigated to identify whether they overcome any limitations associated with the relatively low aqueous solubility of mannitol.

2. Methodology

The methodology was similar to that described in Experiment 1 with variations as indicated below.

2.1 Nebuliser

Two commercially available vibrating mesh nebulisers – an Aeroneb® Go provided by Aerogen (Lot 9136100319; Aerogen, Dangan, Galway, Ireland) and a PARI eFlow Rapid (Serial Number 9V11E08456; PARI GmbH, Starberg, Germany) – were used for nebulisation. The nebulisers were thoroughly cleaned between nebulisations, with consideration of the manufacturers' instructions. This involved rinsing with cold tap water, followed by soaking in hot tap water for ten minutes, then rinsing with deionised water. No detergent was used to minimise potential foreign substance deposition on nebuliser components (e.g. fragrance). The nebuliser was dried using compressed air then equilibrated to ambient temperature by a deionising fan (Serial 25724; Ion Systems, Berkeley, California, USA) before use. For the eFlow Rapid, the mesh was additionally reverse-nebulised using the provided Easycare cleaning aid (PARI GmbH, Starberg, Germany) every six nebulisations to prevent mesh clogging.

The relatively small number of nebulisations performed combined with adequate cleaning meant mesh clogging was not expected to severely affect performance of the nebulisers. Both nebulisers were retested following completion of the main experiment to ensure consistency of results that indicated minimal mesh clogging.

2.2 Nebuliser Fluids

The experiments were carried out using the following hyperosmolar agents: mannitol (Lot Batch E236A, Roquette Frères, Lestrem, France), sorbitol (Batch 603650; Unilab, Ajax Chemicals, Auburn, Australia) and xylitol (Batch 744229, Roquette Frères, Lestrem, France). The agents were used as supplied, to prepare the following fluids (in 1% w/v saline) for nebulisation: a) Mannitol (75mg/mL and 150mg/mL) for comparison with Experiment 1 above; b) Sorbitol (75, 150, 300 and 400mg/mL); c) Xylitol at osmotic concentrations equivalent to those for sorbitol (63, 125, 251 and 334mg/mL, respectively). The osmotic equivalence between the three polyols is presented in Table 5. One percent (1%) saline was chosen as the vehicle to give an appropriate balance between treatment time and taste tolerability. All solutions were stored at room temperature (20±1°C) and used within 48 hours of manufacture.

Table 5: *Osmole equivalents for the various concentrations of polyol fluids.*

	Polyol Fluid Concentration (mg/mL)		
Osmoles ($x10^{-3}$) per 2.5mL	**Mannitol**	**Sorbitol**	**Xylitol**
1.03	75	75	63
2.06	150	150	125
4.12	-	300	251
5.49	-	400	334

2.3 Particle Size Characterisation and Instrument Selection

Particle size characterisation and instrument selection was as described in Experiment 1 with the variations as indicated below.

In relation to laser diffraction and in contrast to Experiment 1, detectors 1-6 were excluded from data analysis to account for beam steering.

As with Experiment 1, all experiments were conducted within a climate controlled room with temperature maintained at 20±1°C and relative humidity at 30±1%. The reservoir of the Aeroneb® Go was filled with 2.5mL and the eFlow Rapid with 3.5mL of the respective nebuliser fluid prior to nebulisation – the higher volume in the eFlow to compensate for an approximate 1mL residual volume incorporated in its medication reservoir design. The filled nebuliser was weighed, then again post-nebulisation, to determine gravimetric output and residual volume. Particle measurement and extraction was initiated 5 seconds prior to nebulisation and ceased 10 seconds after no aerosol was detectable (detection limit at 10% obscuration) and reservoir confirmed to be empty (Aeroneb® Go) or the polyol fluid fell below the level of the vibrating mesh plate (eFlow Rapid).

Treatment time was again defined as the first to the last instance of detectable aerosol. All data points excluding the initial and last 5 seconds of nebulisation were then averaged for each measurement. The prepared nebuliser fluids (excepting 400mg/mL sorbitol) were each analysed in triplicates.

2.4 Aerosol performance by Cascade Impaction

A series of nebulisations of two promising formulation candidates (300mg/mL sorbitol and 400mg/mL Xylitol, in 1% w/v saline) was also performed on the Next Generation Impactor (NGI) using the Aeroneb® Go to assess droplet content uniformity and the correlation with laser diffractometry.

The methodology was as for Experiment 1 but the airflow was activated for 1 minute prior to attachment of the nebuliser to allow for environmental equilibration between the NGI and experimental housing.

2.5 Mass Assay

Following aerosoal deposition, the nebuliser and NGI plates were oven dried at 70°C to evaporate any aqueous phase. The solid deposition was then cooled to ambient temperature by deionising fan (Serial 25724; Ion Systems, Berkeley, California, USA). The nebuliser, adaptor, USP throat and impactor plates were then individually rinsed respectively with 20mL, 5mL, 5mL and 10mL of mobile phase (50mg/mL calcium disodium EDTA (Sigma-Aldrich Chemie B.V., Zwijndrecht, Netherlands) in deionised water). The various rinsing volumes reflect the expected solute mass for the sample and

an appropriate dilution for HPLC detection. Mass assay of these samples were performed using High Performance Liquid Chromatography (HPLC).

Methodology for chromatographic separation of sodium chloride and mannitol was as for Experiment 1. Sodium chloride, sorbitol and xylitol were separately dissolved in deionised water at sequential concentrations to obtain calibration curves (0.01 to 1% w/v (R^2=1.00) for sodium chloride; 0.015 to 15mg/mL (R^2=1.00) for sorbitol and xylitol) for result analysis.

2.6 Statistical analysis

Statistical analysis was as for Experiment 1.

Results of Experiment 2

2.7 Laser Diffraction

The results from laser diffraction are presented in Table 6 (Aeroneb® Go) and Table 7 (eFlowRapid). For the Aeroneb® Go, median particle size (5.7-6.9 μm) mainly decreases with polyol concentration then increases again at high concentrations (>300mg/mLfor Sorbitol and >251mg/mL for Xylitol). The respirable fraction (40-43%) is directly related to particle size and is defined as the percentage of aerosol <5μm. However, whilst the size differences between concentrations are statistically significant (*p*-value <0.05), they are clinically negligible. In contrast, nebulisation on the eFlow Rapid imparted considerable changes to particle size (3.6-4.7μm) and respirable fraction (53-80%) related to polyol concentrations. For both nebulisers, treatment t times are directly related to polyol concentration, with sorbitol at 400mg/mL exhibiting a disproportionately high treatment time compared to concentrations at 300mg/mL and below.

There is also a slight increase in mass output with polyol concentration for the Aeroneb® Go For this nebuliser, it is important to note that all charged fluid (2.5m L, weighing approximately 2,5g) is nebulised. Loss of mass measured post-aerosolisation, represents significant droplet impaction and deposition at the base of the nebuliser chamber. Conversely, the eFlow Rapid nebulises until reaching an in-built residual volume of approximately 1mL and there is minimal loss by deposition within the device.

Table 6: *Median Volumetric Particle Size (μm) ± Standard Deviation (GSD in parentheses), Treatment Time (Seconds) ± Standard Deviation, Aerosol Mass Output (gravimetric difference between nebuliser pre- and post-nebulisation weight) (grams) ± Standard Deviation and Respirable Fraction ± Standard Deviation for the Aernoeb®Go, assuming the initial 2.5mL fluid charge was completely nebulised for all of the various hyperosmotic polyol fluids.*

Polyol Fluid	Median Particle Size (μm)	Treatment Time (seconds)	Aerosol Mass Output (grams)	Respirable Fraction (%)
Mannitol 75mg/mL	6.0 ± 0.04 (1.9)	225 ± 1	1.6± 0.01	41 ± 1.1
Mannitol 150mg/mL	5.8 ± 0.01 (1.9)	245 ± 1	1.7± 0.01	42 ± 0.3
Sorbitol 75mg/mL	5.9 ± 0.05 (1.9)	220 ± 1	1.5± 0.02	41 ± 0.4
Sorbitol 150mg/mL	5.8 ± 0.00 (1.9)	230 ± 1	1.6± 0.01	43 ± 0.6
Sorbitol 300mg/mL	5.7 ± 0.01 (1.8)	310 ± 3	1.8± 0.01	43 ± 0.1
Sorbitol 400mg/mL	6.00 (1.8)	780	2.1	40
Xylitol 62.6mg/mL	5.9 ± 0.03 (1.9)	215 ± 3	1.5± 0.03	41 ± 0.2
Xylitol 125.3mg/mL	5.7 ± 0.03 (1.9))	230± 3	1.6± 0.01	43 ± 0.3
Xylitol 250.6mg/mL	5.7± 0.02 (1.9)	250 ± 2	1.7± 0.01	43 ± 0.2
Xylitol 334.1mg/mL	5.9 ± 0.02 (1.9)	320 ± 1	1.8± 0.01	42 ± 0.2

Table 7: *Median Volumetric Particle Size (μm) ± SD (GSD), Treatment Time ± Standard Deviation, Aerosol Mass Output (gravimetric difference between nebuliser pre- and post-nebulisation weight) ± Standard Deviation and Respirable Fraction ± Standard Deviation for the eFlow Rapid assuming the initial 3.5mL fluid charge (minus 1mL residual volume) was completely nebulised.*

Polyol Fluid	**Median Particle Size** (μm)	**Treatment Time** (seconds)	**Mass Output** (grams)	**Respirable Fraction** (%)
Mannitol 75mg/mL	4.6 ± 0.05 (1.6)	104 ± 1	2.4 ± 0.03	56 ± 0.3
Mannitol 150mg/mL	4.1 ± 0.09 (1.9)	130 ± 1	2.5 ± 0.03	64 ± 1.9
Sorbitol 75mg/mL	4.5 ± 0.03 (1.6)	160 ± 1	2.4 ± 0.03	58 ± 1.2
Sorbitol 150mg/mL	4.1 ± 0.09 (1.6)	150 ± 1	2.5 ± 0.06	65 ± 1.6
Sorbitol 300mg/mL	3.6 ± 0.09 (1.4)	230 ± 3	2.5 ± 0.05	77 ± 2.4
Sorbitol 400mg/mL	-	-	-	-
Xylitol 62.6mg/mL	4.7 ± 0.02 (1.7)	155 ± 3	2.4 ± 0.03	53 ± 2.1
Xylitol 125.3mg/mL	4.2 ± 0.07 (1.6)	180 ± 3	2.4 ± 0.04	63 ± 1.6
Xylitol 250.6mg/mL	3.9 ± 0.06 (1.5)	230 ± 2	2.5 ± 0.07	70 ± 1.1
Xylitol 334.1mg/mL	3.6 ± 0.08 (1.4)	280 ± 1	2.5 ± 0.05	80 ± 1.2

Figure 5 shows the osmolar output rate of respirable sized aerosol for various polyol formulations nebulised from both the eFlow Rapid and Aernoeb® Go. Again this

shows comparable output between polyol formulations at the lower two concentrations (75mg and 150 mg/mL mannitol and sorbitol; 63 and 125 mg/mL xylitol). For both nebulisers, maximum output rate was achieved at a concentration of 300 mg/mL for sorbitol and 334 mg/mL for xylitol, the latter formulation approximately doubling the output relative to 150 mg/mL mannitol.

2.8 Cascade Impaction

The comparison of important values between cascade impaction and laser diffraction for 300mg/mL sorbitol and 334mg/mL xylitol, in 1% w/v saline is illustrated in Table 8. The median particle size for the xylitol formulation showed a clear correlation between the two sizing methods. There was a statistically significant difference (*p*-value <0.05) between the methods for the sorbitol fluid but it is not practically significant. The geometric standard deviation and aerosolised mass also demonstrated slight differences.

Sorbitol 400 mg/mL in 1% w/v saline was assessed once only for both nebulisers as the rate of aerosolisation dropped far too significantly (no detection at all was observed for the eFlow Rapid) to be considered as a potential candidate for future study.

Table 8: *Comparison of median particle size, geometric standard deviation and aerosol mass between cascade impaction and laser diffraction for 300mg/mL sorbitol and 334mg/mL xylitol, in 1% saline.*

Sorbitol 300mg/mL	**Median Particle Size** (μm)	**Geometric Standard Deviation**	**Aerosol Mass Output** (grams)
Laser Diffraction	5.7 ± 0.01	1.8± 0.0	1.8± 0.01
Cascade Impaction	5.9± 0.08	2.1± 0.1	1.75± 0.0
Xylitol 334.1mg/mL			
Laser Diffraction	5.9 ± 0.02	1.9± 0.01	1.8± 0.01
Cascade Impaction	5.9± 0.05	2.2± 0.01	1.76± 0.0

Figures 3 and 4 depict the mean aerosol mass distribution for the two aforementioned formulations recovered by mass assay. In both cases, no sizeable differences was observed between the respective proportions of polyol and sodium chloride.

2.9 Fluid Viscosity

The viscosity of the various polyol fluids is presented in Table 9. Viscosity increases with polyol concentration for all fluids and is inversely related to temperature. At both room and refrigerated temperatures, sorbitol solutions shares similar viscosity values with the mannitol formulations. Both these polyols exhibit slightly higher viscosity at all equivalent osmolar concentrations relative to the xylitol solutions.

Table 9: *Viscosity of polyols of various concentrations at room and refrigerated temperatures.*

Polyol Fluid	**Viscosity at 20°C** (cPt)	**Viscosity at 5°C** (cP)
Mannitol 75mg/mL	1.2 ± 0.01	1.9 ± 0.01
Mannitol 150mg/mL	1.5 ± 0.01	**2.5** ± 0.01
Sorbitol 75mg/mL	1.3 ± 0.01	1.8 ± 0.01
Sorbitol 150mg/mL	1.6 ± 0.01	2.4 ± 0.01
Sorbitol 300mg/mL	2.7 ± 0.01	3.9 ± 0.01
Sorbitol 400mg/mL	3.8 ± 0.01	6.4 ± 0.01
Xylitol 62.6mg/mL	1.2 ± 0.01	1.7 ± 0.01
Xylitol 125.3mg/mL	1.4 ± 0.01	2.1 ± 0.01
Xylitol 250.6mg/mL	2.1 ± 0.01	3.1 ± 0.01
Xylitol 334.1mg/mL	2.7 ± 0.01	4.4 ± 0.01

Discussion of Experiment 2

The physicochemical properties of fluids for nebulisation affect aerosol generation, particularly for vibrating mesh type nebulisers. Of primary importance is the high electrostatic charge present in aqueous solutions that work to inhibit the flow and detachment of fluid through the mesh. Introduction of an electrolyte suppresses this charge, considerably improving particle size and treatment times. In this experiment,

this was accounted for by the obligatory 1% w/v saline component in the various polyol formulations.

Experiment 2 assesses how the viscosity of the solutions, which increases with increasing polyol concentration in aqueous solutions, affects nebuliser performance. It is believed that increasing viscosity decreases droplet size, which leads to prolonged treatment time as seen for both nebulisers in Table 6 and 7. Further, nebulisation has been found to slow down or completely halt at a critical viscosity value dependent on the mesh. This relationship was observed for the highest sorbitol concentration. A marked drop in output (Aernoeb® Go) or a non-detectable level of aerosolisation (eFlow Rapid) is eventually observed at 400mg/mL (Figure 5), the fluid viscosity (3.8±0.01cP) presumably having exceeded the aforementioned critical value. For both nebulisers, xylitol output plateaus at the equivalent of 400mg/mL sorbitol (334mg/m xylitol) without reaching the same precipitous decline in output seen for sorbitol. Without being bound by theory, the inventors believe this may be attributed to its lower relative molecular mass permitting greater osmolar equivalence with a lower solute mass and hence lesser contribution to viscosity (2.7 ± 0.01 cP for 334 mg/mL xylitol). Furthermore, the rate of viscosity increase is slower for the equivalent xylitol concentrations (Figure 5). The critical viscosity for the Aeroneb® Go and eFlow Rapid is thus inferred to be at a point between 2.7 and 3.8 cP. It is further noted that the solute concentrations of the sorbitol and xylitol sample fluids were considerably below their saturation point. Thus, whereas for mannitol the limiting aspect was aqueous solubility, viscosity is the ultimate factor determining maximal feasible fluid concentration for sorbitol and xylitol.

Both xylitol and sorbitol achieved superior osmolar output compared to solubilised mannitol. These higher outputs were observed at concentrations beyond the osmolar equivalent of 150mg/mL mannitol, confirming the importance of high polyol solubility for realising enhanced dosing capabilities. At 334mg/mL xylitol the osmolar output is double that achieved with 150mg/mL mannitol (Table 5). However, both the nebulisers have clear differences in performance. The shorter treatment time of the eFlow Rapid may be explained by its significantly greater number of mesh holes (four times that of the Aeroneb® Go) which directly relates to the number of aerosol droplets generated per time and hence output rate (Vecellio, 2006). Further, the smaller droplet sizes compared to the Aeroneb® Go are presumeably due to smaller mesh hole sizes that also confers significantly greater susceptibility to viscosity changes (Tables 6 and 7). These

characteristics are observed in the high respirable fractions for the eFlow Rapid that ranges between 53 and 80%. Differences in median particle sizes and geometric standard deviation between the various formulations may be statistically different, but the minor size variations are unlikely to be of clinical significance. Thus Xylitol is an alternative to nebulised mannitol because it may substantially improve the efficiency of hyperosmolar delivery without appreciably altering aerosol properties.

Further, the results of cascade impaction for the Aeroneb® Go demonstrate good overall correlation with laser diffraction. Slight discrepancies in median particle size for sorbitol could possibly be attributed to inaccuracies in sampling flow rate adjustments. The differences in geometric deviation are expected due to detector exclusion in laser diffraction to account for beam steering. More importantly, mass assay did not show any significant separation between sorbitol and sodium chloride (Figure 3) or xylitol and sodium chloride (Figure 4). This suggests the combination of the solutes did not adversely affect the capability to deliver a uniform dose throughout the course of nebulisation.

A recurring issue is the visible deposition, and hence loss, of aerosol within the chamber of the Aeroneb® Go as a consequence of downward aerosol velocity (Table 6). This may function analogous to the in-built 1mL residual volume of the eFlow Rapid that limits total aerosol delivery to a jet nebuliser. Nonetheless, the Aeroneb® Go has been employed as it demonstrates consistent performance in aerosol characteristics and output rate that are useful for *in vitro* reproducibility and comparisons.
Experiment 2 demonstrates that xylitol has the greatest potential amongst the sampled polyols for nebulised delivery using a mesh device. Experiment 2 clearly demonstrates that utilising higher solubility polyols, especially xylitol, is a viable method of enhancing the rate of therapeutic osmolar delivery by mesh nebulisation. 334mg/mL of xylitol was found to double the output achieved by near-saturated mannitol solution (150mg/mL). Increasing viscosity of polyol aqueous solutions with polyol concentration was the ultimate limiting factor, causing an abrupt drop in nebuliser performance beyond a critical viscosity as demonstrated by sorbitol 400mg/mL.

Although the formulations are expected to be stable and unlikely to require refrigeration, colder storage conditions, if used, would increase viscosity (Table 9) which could potentially limit viability of the higher polyol concentrations. It is anticipated that the current co-formulated electrolyte concentration (1% sodium

chloride) may be increased in future studies to shorten treatment times whilst boosting osmotic delivery.

Overall, mucociliary clearance is a crucial innate airway defence mechanism. Its function is compromised in respiratory disease states such as cystic fibrosis, bronchiectasis and asthma, due to dehydration of the airway surface liquid. The present invention has identified new avenues for the therapy of respirable diseases. It has identified that mannitol in deionised aqueous solution alone is unsatisfactorily aerosolised by vibrating mesh nebulisers, producing excessively high treatment times and low respirable aerosol fraction. The addition of an electrolyte, such as sodium chloride, which coincidently is itself a hyperosmolar agent, dramatically improves nebuliser output rate and produces a significantly higher aerosol respirable fraction. Moreover, treatment time is inversely related to electrolyte concentration.

The present invention has demonstrated that a number of non-ionic hyperosmolar agents, mannitol, sorbitol and xylitol may be used at various concentrations and with various electrolyte concentrations. The compositions of the present invention provide a pleasant-tasting alternative mucoactive agent to hypertonic saline, with longer therapeutic action, or prescribed for patients who have a poor response to rhDNase therapy.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:

1 A composition comprising a non-ionic hyperosmolar agent and an electrolyte for use in the treatment of a respiratory disease.

2 A composition for nebulisation comprising a non-ionic hyperosmolar agent and an electrolyte.

3 A composition comprising a non-ionic hyperosmolar agent and an electrolyte, wherein the composition is nebulised by a nebuliser, for use in the treatment of a respiratory disease.

4 A composition for nebulisation comprising a non-ionic hyperosmolar agent and an electrolyte for use as a medicament.

5 A composition comprising a non-ionic hyperosmolar agent and an electrolyte for use as a nebulised medicament.

6 Use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte as a medicament.

7 Use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte as a nebulised medicament.

8 Use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte in the preparation of a nebulised medicament.

9 Use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte with a mesh nebuliser.

10 Use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte for the manufacture of a medicament for the treatment of a respiratory disease.

11 Use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte for the manufacture of a nebulised medicament for the treatment of a respiratory disease.

12 Use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte in the treatment of a respiratory disease.

13 Use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte as a nebulised medicament in the treatment of a respiratory disease.

14 Use of a composition comprising a non-ionic hyperosmolar agent and an electrolyte with a nebuliser in the treatment of a respiratory disease

15 A method of forming a nebulised composition the method comprising nebulising a composition comprising a non-ionic hyperosmolar agent and an electrolyte in a nebuliser.

16 A kit containing a nebuliser together with a composition comprising a non-ionic hyperosmolar agent and an electrolyte, wherein when the composition is nebulised with the nebuliser, a nebulised composition is formed..

17 A kit containing a mesh nebuliser together with a composition comprising a non-ionic hyperosmolar agent and an electrolyte, wherein when the composition is nebulised with the nebuliser, a nebulised composition is formed.

18 A nebuliser containing a composition comprising a non-ionic hyperosmolar agent and an electrolyte, wherein when the composition is nebulised with the nebuliser, a nebulised composition is formed.

19 A mesh nebuliser containing a composition comprising a non-ionic hyperosmolar agent and an electrolyte, wherein when the composition is nebulised with the nebuliser, a nebulised composition is formed.

20 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17 or the nebuliser according to any one of claims 18-19, wherein the composition is a solution containing the non-ionic hyperosmolar agent and electrolyte dissolved therein.

21 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17 or the nebuliser according to any one of claims 18-19, wherein the composition is a solution containing the non-ionic hyperosmolar agent and electrolyte dissolved therein and at least some of the non-ionic hyperosmolar agent suspended in the solution.

22 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17 or the nebuliser according to any one of claims 18-19, wherein the composition has a viscosity in the range of about 1-10cP, about 1-4cP, about 1-2.7, about 1-3.8cP or about 1-1.9cP; or <10 cP, <9cP, <8cP, <7cP, <6cP, <5cP, <4cP, <3.8cP, <3cP, <2.7cP, <2.5cP, <2.4cP, <2.3cP, <2.2cP, <2.1cP, <2cP, <1.9cP, <1.8cP, <1.7cP, <1.6cP, <1.5cP, <1.4cP, <1.3cP, <1.2cP, <1.1cP, or <1cP.

23 The composition, the use, the method, the kit and the nebuliser according to claim 22, wherein the composition has a viscosity of <3.8cP.

24 The composition, the use, the method, the kit and the nebuliser according to claim 22, wherein the composition has a viscosity of <2.7cP.

25 The composition, the use, the method, the kit and the nebuliser according to claim 22, wherein the composition has a viscosity of <1.9cP.

26 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17 or the nebuliser according to any one of claims 18-19, wherein the non-ionic hyperosmolar agent and electrolyte are solubilised in an aqueous medium, preferably water.

27 A nebulised composition comprising a non-ionic hyperosmolar agent and an electrolyte.

28 A nebulised composition comprising a non-ionic hyperosmolar agent and an electrolyte for use in the treatment of a respiratory disease.

29 A method of treating a respiratory disease comprising administering to a patient in need thereof a therapeutically effective amount of a nebulised composition comprising a non-ionic hyperosmolar agent and an electrolyte.

30 A method of treating a respiratory disease comprising administering to a patient in need thereof a therapeutically effective amount of a nebulised composition via a nebuliser, the composition comprising a non-ionic hyperosmolar agent and an electrolyte.

31 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17, the nebuliser according to any one of claims 18-19, the nebulised composition according to any one of claims 27-28, or the method according to any one of claims 29-30,

wherein the non-ionic hyperosmolar agent is present in a concentration at or just below its saturation point.

32 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17, the nebuliser according to any one of claims 18-19, the nebulised composition according to any one of claims 27-28 or the method according to any one of claims 29-30,

wherein the non-ionic hyperosmolar is present in the composition in a therapeutically effective amount.

33 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17, the nebuliser according to any one of claims 18-19, the nebulised composition according to any one of claims 27-28, the method according to claim 29-30; or the composition, the use, the method, the kit, the nebuliser, the nebulised composition or the method according to any one of claims 31-32,

wherein the non-ionic hyperosmolar agent is selected from one or more sugars or sugar alcohols or combinations thereof.

34 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17, the nebuliser according to any one of claims 18-19, the nebulised composition according to any one of claims 27-28, the method according to any one of claims 29-30 or the composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to any one of claims 32-33,

wherein the non-ionic hyperosmolar agent is selected from one or more of mannitol, sorbitol, and xylitol.

35 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 34,

wherein the non-ionic hyperosmolar agent is mannitol.

36 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 34,

wherein the non-ionic hyperosmolar agent is sorbitol.

37 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 34,

wherein the non-ionic hyperosmolar agent is xylitol.

38 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 34,

wherein the non-ionic hyperosmolar agent is mannitol and sorbitol.

39 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 34,

wherein the non-ionic hyperosmolar agent is mannitol and xylitol.

40 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 34,

wherein the non-ionic hyperosmolar agent is sorbitol and xylitol.

41 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17, the nebuliser according to any one of claims 18-19, the nebulised composition according to any one of claims 27-28, the method according to any one of claims 29-30 or the composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to any one of claims 32-40,

wherein the composition contains 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% or 10% of the amount of non-ionic hyperosmolar agent which can be solubilised.

42 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17, the nebuliser according to any one of claims 18-19, the nebulised composition according to any one of claims 27-28, the method according to any one of claims 29-30; or the composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to any one of claims 32-41,

wherein the concentration of the non-ionic hyperosmolar agent is in the range of about: 200-10mg/ml; or is about: 200mg/ml, 190mg/ml, 180mg/ml, 170mg/ml, 160mg/ml, 150mg/ml, 140mg/ml, 130mg/ml, 120mg/ml, 110mg/ml, 100mg/ml, 90mg/ml, 80mg/ml, 70mg/ml, 60mg/ml, 50mg/ml, 40mg/ml, 30mg/ml, 20mg/ml, or about 10mg/ml.

43 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17, the nebuliser according to any one of claims 18-19, the nebulised composition according to any one of claims 27-28, the method according to any one of claims 29-30; or the composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to any one of claims 32-34,

wherein the non-ionic hyperosmolar agent is mannitol having a concentration in the range of about: 200-70mg/ml, preferably about 200-100mg/ml, more preferably about 170-140 mg/ml; or is selected from about: 200mg/ml, 190mg/ml, 180mg/ml, 170mg/ml, 160mg/ml, 150mg/ml, 140mg/ml, 130mg/ml, 120mg/ml, 110mg/ml, 100mg/ml or about 75 mg/ml, preferably about 170mg/ml, 160mg/ml, 150mg/ml or about 140mg/ml, more preferably about 150mg/mL.

44 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17, the nebuliser according to any one of claims 18-19, the nebulised composition according to any one of claims 27-28, the method according to any one of claims 29-30; or the composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to any one of claims 32-34,

wherein the non-ionic hyperosmolar agent is sorbitol having a concentration in the range of about: 1000- 70mg/ml, preferably 400 -200mg/ml, more preferably 400-300mg/ml, even more preferably 300-350 mg/ml; or is selected from about: 1000mg/ml, 900mg/ml, 800mg/ml, 700mg/ml, 600mg/ml, 500mg/ml, 400mg/ml,

350mg/ml, 330 mg/ml, 300mg/ml, 250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml, or about 75mg/ml, 380mg/ml, 360mg/ml, 320mg/ml, 280mg/ml, 260mg/ml, 240mg/ml, or about 220mg/ml.

45 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17, the nebuliser according to any one of claims 18-19, the nebulised composition according to any one of claims 27-28, the method according to any one of claims 29-30; or the composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to any one of claims 32-34,

wherein the non-ionic hyperosmolar agent is xylitol having a concentration in the range of: about 640-60mg/ml, preferably about 300-400mg/ml, more preferably about 300-350mg/ml; or is selected from about: 640mg/ml, 620mg/ml, 600mg/ml, 580mg/ml, 560mg/ml, 540mg/ml, 520mg/ml, 500mg/ml, 480mg/ml, 460mg/ml, 440mg/ml, 420mg/ml, 400mg/ml, 380mg/ml, 360mg/ml, 350 mg/ml, 340mg/ml, 334mg/ml, 330mg/ml, 320mg/ml, 300mg/ml, 280mg/ml, 260mg/ml, 250 mg/ml, 240mg/ml, 220mg/ml, 200mg/ml, 180mg/ml, 160mg/ml, 140mg/ml, 125mg/ml, 120mg/ml, 100mg/ml or 63mg/ml.

46 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17, the nebuliser according to any one of claims 18-19, the nebulised composition according to any one of claims 27-28, the method according to any one of claims 29-30; or the composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to any one of claims 31-45,

wherein the composition contains one or more electrolytes.

47 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 46,

wherein the electrolyte is selected from salts including sodium salts, potassium salts or calcium salts.

48 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 46,

wherein the electrolyte is selected from the group consisting of : sodium chloride, potassium chloride, sodium citrate dihydrate, potassium citrate, sodium bicarbonate, sodium carbonate, citric acid monohydrate, saccharin sodium, glycine, calcium acetate, calcium hydroxide, phosphoric acid, acetic acid, sodium acetate, or monosodium glutamate.

49 The composition according to any one of claims 1-5, the use according to any one of claims 6-14, the method according to claims 15, the kit according to any one of claim 16-17, the nebuliser according to any one of claims 18-19, the nebulised composition according to any one of claims 27-28, the method according to any one of claims 29-30 or the composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to any one of claims 31-48,

wherein the electrolyte is present in a concentration of about 0.1% - 20%, 0.1-10%, 0.1-7%, 0.2-7%, 0.1-5%, 0.5-5%, 0.5-2%, about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or about 10% w/v.

50 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 49,

wherein the electrolyte is present in a concentration of about 1% w/v.

51 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 49, wherein the composition comprises a saline-mannitol combination consisting of 150mg/mL mannitol in about 1% saline.

52 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 49, wherein the composition comprises a saline-sorbitol combination consisting of from 300mg/mL to less than 400mg/ml sorbitol in about 1% saline, preferably from about 300mg/mL to about 350mg/ml sorbitol in about 1% saline.

53 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 49, wherein the composition comprises a saline-xylitol combination consisting of from 300mg/mL to less than 400mg/ml sorbitol in about 1% saline, preferably from about 300mg/mL to about 350mg/ml sorbitol in about 1% saline.

54 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 49,

wherein the compositions includes a buffer.

55 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 49, wherein the compositions further includes one or more additional therapeutic agents.

56 The composition, the use, the method, the kit and the nebuliser, the nebulised composition or the method according to claim 49 wherein the compositions are useful in the treatment of respiratory diseases or conditions including those selected from asthma, bronchiectasis, emphysema, COPD, cystic fibrosis (CF), bronchitis, allergy,

rhinitis, emphysema, , pulmonary infection, tuberculosis, influenza, other lung infections, lung cancer and asthma.

57 The method according to claim 29 or 30 wherein the total amount of non-ionic hyperosmolar agent delivered to the patient in a single therapeutic dose is in the range of about 900-100mg, preferably about 500-300mg; or is selected from about 900mg, 800mg, 700mg, 600mg, 500mg, 400mg, 300mg, 200mg, or about 100mg.

58 The method of claim 57 wherein the non-ionic hyperosmolar agent is mannitol, delivered to the patient in a dose of about 500mg, 400mg or about 300mg.

59 The method of claim 57 or 58, wherein the amount of composition nebulised and delivered to the patient is about: 0.1-10ml, 0.1-5ml, 0.5-1ml, 1ml, 2ml, 2.5ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml or 10ml.

60 The method according to any one of claims 57-59, wherein the composition is nebulised into droplets having an average aerodynamic diameter in the range of about: 0.01 to 20, 0.1 to 20 microns, 0.1 to 15 microns, 0.1 to 10 microns, 0.1 to 7 microns, 0.1 to 6 microns, 0.5 to 10 microns, 0.8 to 10 microns, 1 to 10 microns, 1 to 9 microns, 1 to 8 microns, 1 to 7 microns, 1 to 6 microns, 1 to 5 microns, 1 to 4 microns, 1 to 3 microns, 1 to 2 microns, 2 to 3 microns, 3 to 4 microns, 3 to 7 microns, 4 to 5 microns, 4 to 6 microns, 5 to 6 microns, or 6 to 7 microns, or $\leq$ about 10 microns, $\leq$ about 9 microns, $\leq$ about 8 microns, $\leq$ about 7 microns, $\leq$ about 6 microns, $\leq$ about 5 microns, $\leq$ about 4 microns, $\leq$ about 3 microns, $\leq$ about 2 microns, $\leq$ about 1 micron, about 1 micron, about 2 microns, about 3 microns, about 4 microns, about 5 microns, about 6 microns, about 7 microns, or about 8 microns.

61 The method according to any one of claims 57-60, wherein the administration time for a 2.5ml dose of the composition is about: <420 seconds, <360 seconds, <300 seconds, <240 seconds, <180 seconds, or <120 seconds, preferably < 300 seconds or < 250 seconds.

62 The kit according to any one of claims 16-17 or the method according to any one of claims 29-30 or 57-61, wherein the nebuliser is selected from the group consisting of mesh nebulisers, jet nebulisers and ultrasonic nebulisers.

63 The kit or method according to claim 62, wherein the mesh nebuliser is a static mesh nebuliser or a vibrating mesh nebuliser.

64 A composition substantially as herein before described with reference to any one of the foregoing Figures and/or Examples, excluding comparative Figures and/or Examples.

65 Use of a composition substantially as herein before described with reference to any one of the foregoing Figures and/or Examples, excluding comparative Figures and/or Examples.

66 A method substantially as herein before described with reference to any one of the foregoing Figures and/or Examples, excluding comparative Figures and/or Examples.

67 A kit substantially as herein before described with reference to any one of the foregoing Figures and/or Examples, excluding comparative Figures and/or Examples.

68 A nebuliser containing a composition substantially as herein before described with reference to any one of the foregoing Figures and/or Examples, excluding comparative Figures and/or Examples.

69 A nebulised composition substantially as herein before described with reference to any one of the foregoing Figures and/or Examples, excluding comparative Figures and/or Examples.

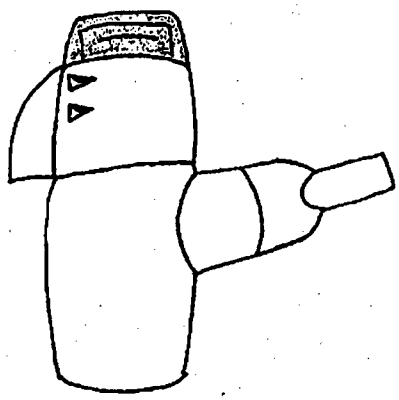

Figure 1

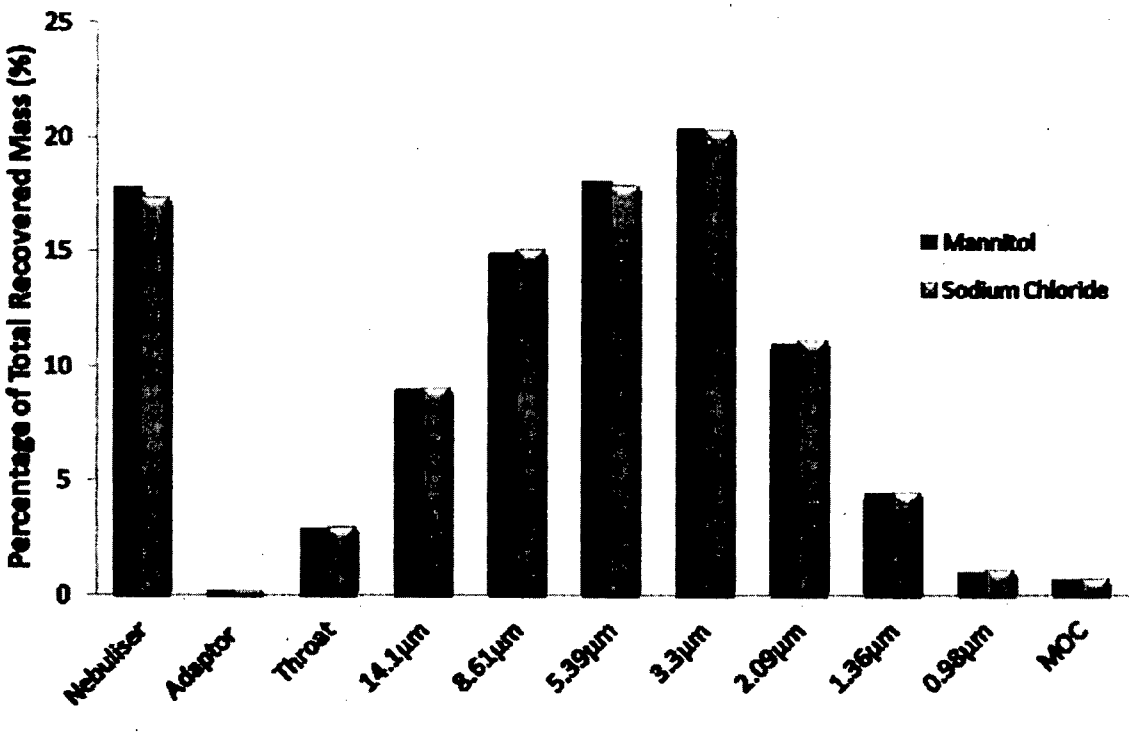
Percentage of Total Recovered Mass (%)
25
20
15
10
5
0
Mannitol
Sodium Chloride
Nebuliser
Adaptor
Throat
14.1µm
8.61µm
5.39µm
3.3µm
2.09µm
1.36µm
0.98µm
MOC

Figure 2

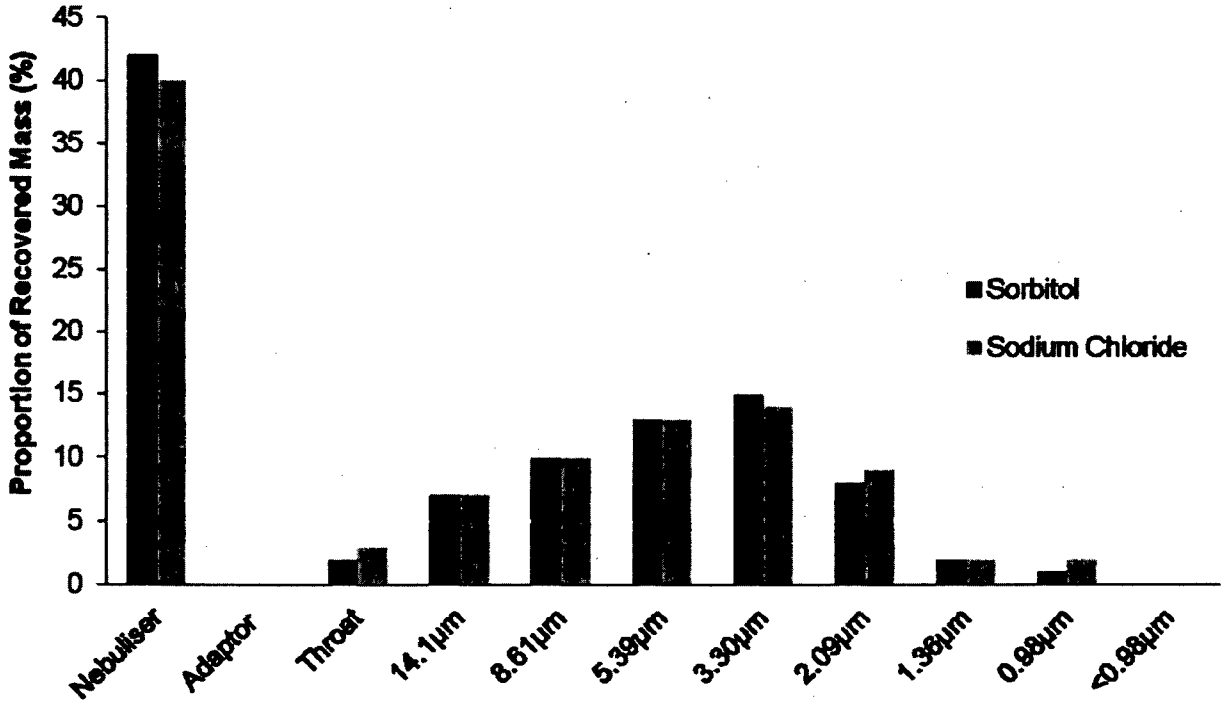
Proportion of Recovered Mass (%)
45
40
35
30
25
20
15
10
5
0
Sorbitol
Sodium Chloride
Nebuliser
Adaptor
Throat
14.1µm
8.61µm
5.39µm
3.30µm
2.09µm
1.36µm
0.98µm
<0.98µm

Figure 3

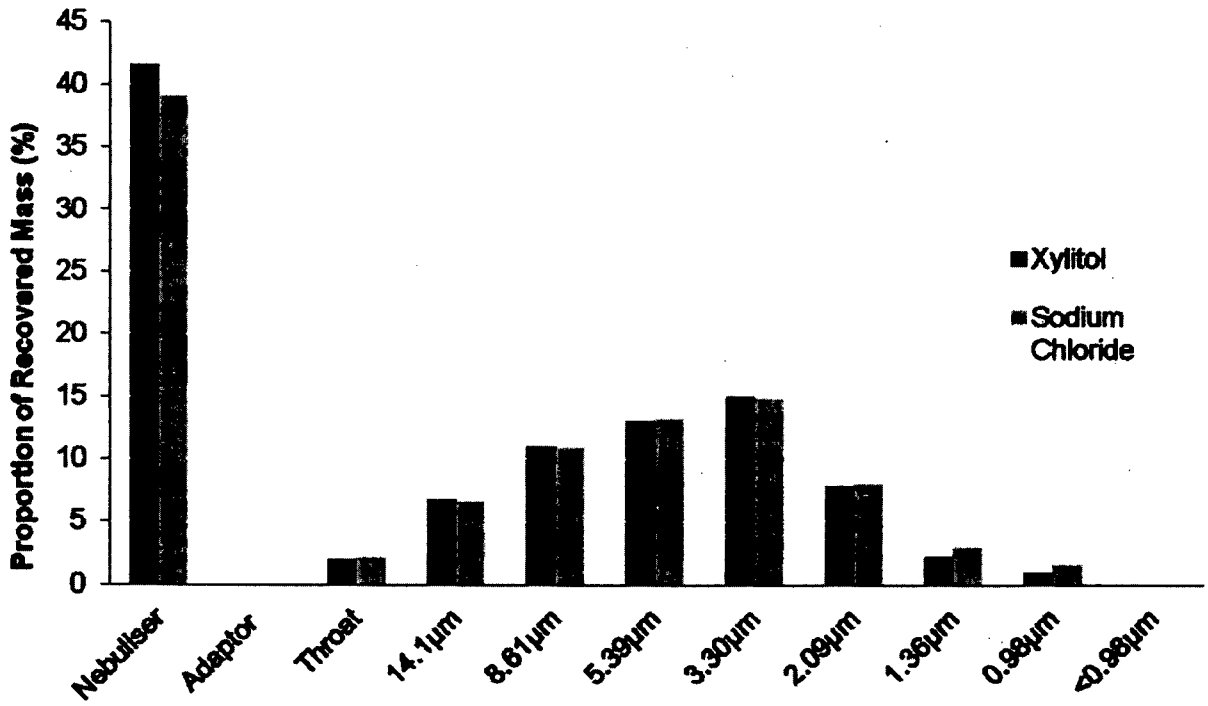
Proportion of Recovered Mass (%)
45
40
35
30
25
20
15
10
5
0
Xylitol
Sodium Chloride
Nebuliser
Adaptor
Throat
14.1µm
8.61µm
5.39µm
3.30µm
2.09µm
1.36µm
0.98µm
<0.98µm

Figure 4

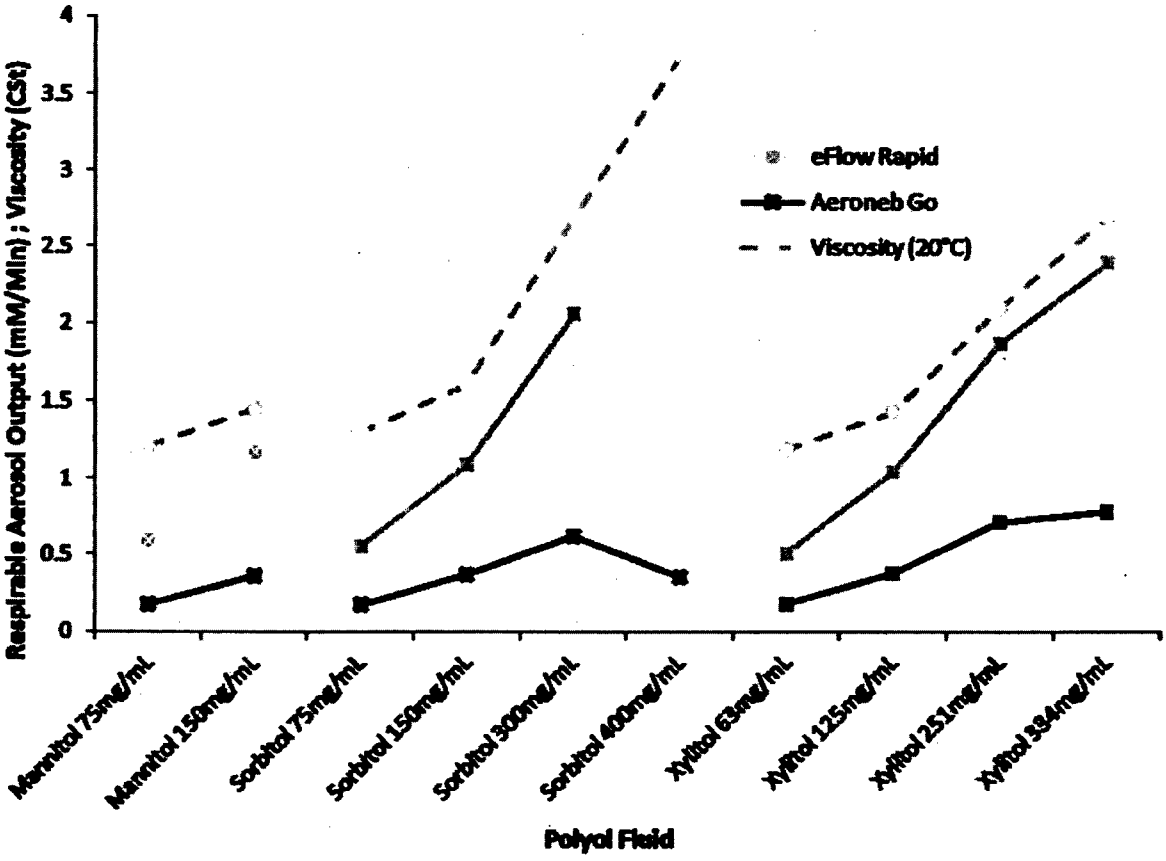
Respirable Aerosol Output (mM/Min) ; Viscosity (Cst)
4
3.5
3
2.5
2
1.5
1
0.5
0
eFlow Rapid
Aeroneb Go
Viscosity (20°C)
Mannitol 75mg/mL
Mannitol 150mg/mL
Sorbitol 75mg/mL
Sorbitol 150mg/mL
Sorbitol 300mg/mL
Sorbitol 400mg/mL
Xylitol 63mg/mL
Xylitol 125mg/mL
Xylitol 251mg/mL
Xylitol 334mg/mL
Polyol Fluid

Figure 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2011/001406

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 31/047 (2006.01) ***A61K 33/14*** (2006.01) ***A61P 11/06*** (2006.01)
A61K 31/425 (2006.01) ***A61P 11/00*** (2006.01) ***A61P 11/08*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Epodoc, WPI, Medline, Biosis, Chem Abs, Google (hyperosmolar, mannitol, xylitol, sorbitol, saccharin, Benzoic sulfimide, Ortho Sulphobenzamide, potassium, kcl, Sylvite, sodium, saline, nacl, Asthma, bronchi, emphysema, COPD, cystic fibrosis, allergy, rhinitis, pulmonary infection, tuberculosis, influenza, lung, respirat+, nebuli+, aerosol, inhal+, aspirat+, atomi[z,s]+)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/111680 A2 (PULMATRIX, INC) 30 September 2010 Paragraph 351, 354, 360 and Table 21	1, 2, 4-6, 10, 12, 32-35, 41 and 46-48
X	CLARK, A et al., "A Comparison of the Pulmonary Bioavailability of Powder and Liquid Aerosol Formulations of Salmon Calcitonin", Pharm Res. 2008, Vol. 25, No. 7, pages 1583-90 Abstract and page 1584	1-8, 15, 16, 18, 20, 26-28, 32-35, 41, 42, 46-49, 54, 55, 56 and 62

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 February 2012	10 February 2012
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer **SARAH HENDERSON** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 3 9935 9655

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/0193577 A1 (KELLER) 23 August 2007 Paragraphs 14-16, 18 , 124, Table 5	1-20, 26-30, 32-37, 41, 46-50, 54-56, 62 and 63
X	WO 2009/086470 A2 (AIRES PHARMACEUTICALS, INC) 9 July 2009 Pages 16 and 60	1-20, 26-30, 32, 33, 41, 46, 47, 49, 54, 56, 62, 63
X	WO 2002/011711 A2 (LONGWOOD PHARMACEUTICAL RESEARCH, INC) 14 February 2002 page 5, 16 and 20 and Example 11	1-8, 10-16, 18, 21, 27-30, 32-34, 36, 41, 46-49, 54-56
X	WO 2006/017726 A2 (IVAX CORPORATION ET AL.) 16 February 2006 Paragraph 22, 40 , 68 and 69	1-8, 10-16, 18, 20, 26-30, 32-35, 41, 46, 47
X	WO 2001/095885 A1 (GLAXO GROUP LIMITED) 20 December 2001 pages 1, 2 and claims	1, 2, 4-6, 10, 12, 32-37, 46, 47
X	WO 2007/134180 A2 (LACLEDE, INC) 22 November 2007 Paragraphs 4, 34, 38, 41, 82, 85, Examples 4 and 12	1-8, 10-16, 18, 21, 27-30, 32-34, 36, 44, 46, 47, 49, 55, 56, 62

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	METERSKY, M.L "New treatment options for bronchiectasis" Therapeutic Advances in Respiratory Disease, 2010, Vol.4, No.2, pages 93-99 Abstract, pages 95 and 96	1-63
X	WILLS, P.J., "Inhaled mannitol in cystic fibrosis", Expert Opin. Investig. Drugs, 2007, Vol.16, No.7, pages 1121-1126 abstract and Item 2.3	1-63
X	DURAIRAJ, L et al., "Safety assessment of inhaled xylitol in subjects with cystic fibrosis", Journal of Cystic Fibrosis, 2007, Vol.6, pages 31-34 Abstract	1-63
P,X	GAR YAN CHAN, J. et al., "Mannitol Delivery by Vibrating Mesh Nebulisation for Enhancing Mucociliary Clearance", Journal of Pharmaceutical Sciences, published online 31 January 2011, Vol. 100, No.7, pages 2693–2702	1-63

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2011/001406

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: **64-69**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The claims do not comply with Rule 6.2(a) because they rely on references to the description and/or drawings.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See Supplemental Box I

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-63 (in part, as they relate to Inventions 1 and 2)

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

Supplemental Box 1
(To be used when the space in any of Boxes I to IV is not sufficient)

Continuation of Box No: III

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

Invention 1: Claims 1-63 (in part). The feature of a composition of mannitol, xylitol, sorbitol or saccharin as the hyperosmolar agent and a sodium salt as the electrolyte is specific to this group of claims. The composition may be used as a medicament for treating conditions such as respiratory disorders.

Invention 2: Claims 1-63 (in part). The feature of mannitol, xylitol, sorbitol or saccharin as the hyperosmolar agent and a potassium salt as the electrolyte for use in the treatment and prevention of respiratory conditions is specific to this group of claims.

Invention 3: Claims 1-63 (in part). The feature of mannitol, xylitol, sorbitol or saccharin as the hyperosmolar agent and a calcium salt as the electrolyte is specific to this group of claims. The composition may be used as a medicament for treating conditions such as respiratory disorders.

Invention 4: Claims 1-46, 48-63 (in part). The feature of mannitol, xylitol, sorbitol or saccharin as the hyperosmolar agent and phosphoric acid as the electrolyte is specific to this group of claims. The composition may be used as a medicament for treating conditions such as respiratory disorders.

Invention 5: Claims 1-46, 48-63 (in part) The feature of mannitol, xylitol, sorbitol or saccharin as the hyperosmolar agent and acetic acid as the electrolyte is specific to this group of claims. The composition may be used as a medicament for treating conditions such as respiratory disorders.

Invention 6: Claims 1-46, 48-63 (in part). The feature of mannitol, xylitol, sorbitol or saccharin as the hyperosmolar agent and citric acid as the electrolyte is specific to this group of claims. The composition may be used as a medicament for treating conditions such as respiratory disorders.

Invention 7: Claims 1-46, 48-63 (in part). The feature of mannitol, xylitol, sorbitol or saccharin as the hyperosmolar agent and glycine acid as the electrolyte is specific to this group of claims. The composition may be used as a medicament for treating conditions such as respiratory disorders.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is the combination of an electrolyte and a hyperosmolar agent that is suitable for use as a medicament. However this feature does not make a contribution over the prior art because it is disclosed in:

D1 WO 2010/111680 A2 (PULMATRIX, INC) 30 September 2010

D2 CLARK, A et al., "A Comparison of the Pulmonary Bioavailability of Powder and Liquid Aerosol Formulations of Salmon Calcitonin", Pharm Res. 2008, Vol. 25, No. 7, pages 1583-90

[continued in Supplemental Box 2]

Supplemental Box 2
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Supplemental Box No 1

D3 US 2007/0193577 A1 (KELLER) 23 August 2007

D4 WO 2009/086470 A2 (AIRES PHARMACEUTICALS, INC) 9 July 2009

D5 WO 2002/011711 A2 (LONGWOOD PHARMACEUTICAL RESEARCH, INC) 14 February 2002

D6 WO 2006/017726 A2 (IVAX CORPORATION ET AL.) 16 February 2006

D7 WO 2001/095885 A1 (GLAXO GROUP LIMITED) 20 December 2001

D8 WO 2007/134180 A2 (LACLEDE, INC) 22 November 2007

Therefore in the light of these documents this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori.*

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU2011/001406

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2010111680	AU	2010229668	AU	2010229721	AU	2010229724
		AU	2010229730	CA	2754670	CA	2754677
		CA	2754680	CA	2754684	CA	2754691
		EP	2315580	WO	2010111640	WO	2010111641
		WO	2010111644	WO	2010111650		
US	2007193577	NONE					
WO	2009086470	AU	2008345034	CA	2710349	EP	2237788
		JP	2011507968	US	2009196930		
WO	0211711	AU	78115/01	CA	2417973	EP	1311294
		US	2002076382	US	2004198708		
WO	2006017726	AU	2005271349	BR	PI0513095	CA	2576131
		CN	101014607	EP	1778709	HK	1098758
		JP	2008509156	KR	20070042188	MX	2007001444
		NO	20070987	NZ	553292	RU	2007107935
		US	2007281893	US	7902158	US	2011118198
		ZA	200700758				
WO	0195885	AU	74236/01	EP	1292286	JP	2004503492
		US	2004037784				
WO	2007134180	AU	2007249348	CA	2687128	EP	2016187
		JP	2009539764	US	2010150897		

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX