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Research Paper

Pulmonary Spray Dried Powders of Tobramycin Containing Sodium Stearate to Improve Aerosolization Efficiency

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Purpose. Tobramycin microparticulate powders containing the hydrophobic adjunct sodium stearate were studied for their use as pulmonary formulations in dry powder inhalers.
Methods. Spray-dried powders were characterized in terms of particle size distribution, morphology, crystallinity, drug dissolution rate, toxicity on epithelial lung cells and aerosol efficiency.
Results. The presence of the sodium stearate had a direct influence on the aerosol performance of tobramycin spray-dried powders. Powders containing 1% *w/w* sodium stearate had fine particle fraction FPF of 84.3±2.0% compared to 27.1±1.9% for powders containing no adjunct. This was attributed to the accumulation of sodium stearate at the particle surface. Powders with higher sodium stearate concentrations (2% *w/w*) showed significantly lower FPF (66.4±0.9%) and less accumulation of sodium stearate at the particle surface. This was attributed to the formation of adjunct micelles, which remained internalised in the particle structure due to their reduced tropism toward the drying drop surface and molecular mobility. Preliminary analysis of the toxicity effect of sodium stearate on A549 cell lines showed that the adjunct, in the concentration used, had no effect on cell viability over a 24-h period compared to particles of pure tobramycin.
Conclusions. Tobramycin pulmonary powders with low level of sodium stearate, presenting high respiration performances and no overt toxicity on lung cells, could be used to improve therapeutic outcomes of patient with Cystic Fibrosis (CF).

KEY WORDS: antibiotic; dry powder inhaler; spray drying; pulmonary delivery; NGI; tobramycin.

INTRODUCTION

The administration of antibiotics to the respiratory tract of patients with lung infections is a promising therapeutic technique for the stabilisation and restoration of lung function (1). While parenteral antibacterial regimens are commonly used to treat acute infection in disease states such as cystic fibrosis (CF), bronchiectasis, pneumonia and chronic obstructive pulmonary disease (COPD) (2), the use of aerosolized antibiotics has been proven to ameliorate lung function, reduce systemic long-term toxicity as well as decrease hospitalization (3–5). Furthermore, when considering drugs such as aminoglycosides antibiotics administered parenterally, high doses (10 mg Kg^{-1}) (6) are required to overcome poor lung distribution (7), due to their high polarity and poor drug penetration into the endobronchial space (8). Consequently, these classes of antibiotics exhibit a narrow safety margin (9) since they may cause severe ototoxicity and nephrotoxicity in long-term therapy.

The administration of aminoglycosides by inhalation offers an attractive alternative due to the delivery of lower amount of antibiotic directly to the site of infection (TOBI®; 600 mg per 5 ml), while minimizing bioavailability (10) and reducing systemic side effects. Tobramycin solution for inhalation is the only antibiotic product approved by the Food and Drug Administration for respiratory delivery (11). Although the administration of tobramycin by nebulisation has many advantages for the treatment of lung infections, the formulation via nebulisation requires an extended administration time (approx 20 min) (12). Furthermore, the nebulisation approach has limited versatility and low efficiency, poor reproducibility and potential risk of bacterial contamination (13).

An alternative approach for delivering drugs to the lung is the use of dry powder inhalers (DPI) activated and driven by the patient's inspiratory flow (14). DPI formulations incorporate a powder containing the drug as micron-sized particles (aerodynamic diameter less then <5 μm) which, upon inhalation are aerosolised from the device to deposit in the respiratory tract (15). Dry powder inhalers have many advantages over liquid nebuliser systems; for example, DPIs are breath-actuated, require minimal patient coordination, are propellant free and have a short treatment time.

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Furthermore, recent studies in cystic fibrosis patients, have shown tobramycin dry powder inhalation resulted in a more efficient and rapid delivery of tobramycin than an equivalent tobramycin inhalation solution (16).

The major obstacle in DPI technology is the production of a drug powder with favourable aerodynamic properties. The attainment of a respirable powder of tobramycin is a difficult task due to the unsuitable size and shape of the original drug particles and, after processing, the hygroscopicity of the micronized powder (17). Spray drying provides a potential method of manufacturing particles suitable for inhalation, since it is possible to control, in one step, the characteristics of the particle output i.e., size, shape and density (18,19). However, spray drying still does not overcome the hydroscopic and thus cohesive nature of the final powder. Previous attempts to overcome this cohesive nature have included the incorporation of large amounts of lipophilic adjuncts (such as mixture of cholesterol and phospholipids), or the use of high-pressure homogenization and spray drying techniques to develop a formulation composed of a mixture of micro and nanoparticles (20–22).

The focus of this study was to prepare a series of tobramycin microparticles, with a high aerosolisation efficiency and resistance to environmental humidity, using small amounts of lipophilic excipients (≤2% *w*/*w*). These systems were evaluated in terms of the aerosol performance, morphology and structure.

MATERIALS AND METHODS

Materials

Tobramycin base (*T*) was supplied by Lisapharma (Erba, Co, Italy). Sodium stearate (NaSt) was purchased from Carlo Erba (Milan, Italy). Water was purified by reverse osmosis (MilliQ, Millipore, France). All solvents and chemicals were obtained from Biolab (Clayon, Victoria, Australia) and were of analytical grade.

Preparation of Micro-particulate Powders

Tobramycin solutions to be spray-dried were prepared by mixing a 30°C aqueous drug solution (1.5%) with an 30°C alcoholic solution of sodium stearate (0.07% *w*/*v*). Maintaining the solid content at 1% *w*/*v*, mixtures containing 30 parts of alcoholic and 70 parts of water solution were prepared by diluting stock solutions to the required drug:adjunct ratio. The following solutions, containing increasing sodium stearate percentages (calculated on tobramycin) were prepared: 0% (pure tobramycin), 0.25%, 0.5%, 1%, 1.5% and 2% *w*/*w* sodium stearate, respectively.

The 30°C solution was spray-dried using a Buchi B-192 (Buchi, Flawil, Switzerland) spray-drier using the following conditions: feed rate 3 ml min^{-1}, aspiration rate 100%; air flow rate 600 l h^{-1}, inlet and outlet temperatures 125°C and 75–78°C, respectively. Three batches for each concentration of sodium stearate were prepared.

Scanning Electron Microscopy

The morphology of each micro-particle formulation was investigated using Scanning Electron Microscopy (SEM) equipped with high resolution field emission microscope (0.6 nm at 30 kV) and Windoless EDS microanalysis (JSM 6000F JEOL, Japan). Samples were mounted on adhesive black carbon tabs (pre-mounted on aluminium stubs) and sputter-coated with platinum (Sputter coater S150B, Edwards High Vacuum, Sussex, UK) at 40 nm thickness prior to analysis. Samples of tobramycin spray-dried microparticles and tobramycin raw material were studied with high magnification (×20,000) EDS spot analysis. Specifically, EDS was used to analyse sodium counts in the samples since it is an element characteristic of the sodium stearate and not of the tobramycin structure (23). Nine particles in each sample were analysed to detect the presence of sodium on the surface.

Particle Size Distribution

Particle size distributions of the spray-dried powders were determined by laser scattering (Mastersizer 2000, Malvern, UK). Approximately 5–10 mg of each sample were dispersed in chloroform, sonicated for 10 min in a water bath (FXP12M Unisonics, Australia) and added to a small volume sample dispersion unit for analysis. Particle size distribution was measured in triplicate with an obscuration threshold of 15%. Data was expressed in terms of volume median diameter (VMD).

Microparticles X-ray Powder Diffraction

The crystallinity of all formulations was characterised using X-ray powder diffraction (XRD- S6000 Shimadzu Corporation, Japan) with the following settings: 5–45° 2θ, step time 2° min^{-1}, 25°C.

Tobramycin Chemical Analysis

The Tobramycin assay method used is described in the USP 31. For this specific analysis, an HPLC (Waters77+with auto sampler, 600 pump, 486 variable wavelength UV detector and a 600 controller with Millennium V.32 software; all Waters Ltd., Sydney, Australia) was used. Tobramycin was analysed at a detector wavelength of 365 nm, using a 3.9 mm×30 cm stainless steel (5 μm particle size) reversed-phase C18 column (Waters Ltd., Milford, MA, USA). The flow rate was 1.2 ml min^{-1}. Mobile phase was prepared by dissolving 2 g Tris- (hydroxymethyl)-aminomethane in 800 mL water, adding 20 mL of H_2SO_4 1 N and diluting with acetonitrile to 2 L. System suitability was also determined: peak symmetry factor 1.18, number of theoretical plates 707, peak resolution 5.11, precision 0.6%.

In Vitro Cell Toxicity of Tobramycin-Sodium Stearate Microparticles

The human alveolar basal epithelium A549 cell line, from the American Type Culture Collection (Manassas, VA, USA) was cultured in Dulbecco's Modified Eagles' Medium (DMEM) supplemented with 10% heat-inactivated foetal

bovine serum, 2 mM L-glutamine (ThermoTrace, Melbourne, VIC, Australia), 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B (Invitrogen, Carlsbad, CA). Cell cultures were grown in 10 cm plates in an atmosphere of 5% CO_2 in air in a humidified incubator at 37°C. The medium was exchanged every 2–3 days and cells were sub-cultured twice weekly. The toxicity of the microparticles on cells was evaluated using the MTT test. Cell respiration as indicator of cell viability was determined by the mitochondrial-dependent reduction of 3-(3,4-dimethylthiazol-2yl)-5-diphenyl tetrazolium bromide (MTT) (Sigma-Aldrich MO, USA) to formazan (24). The A549 cells were seeded in 96-well plates at a density of 3,200 cells/well. Each wells contained 100 μl of the same medium used for culturing the cells. Cells were grown at 37°C in a 5% CO_2 atmosphere for 24 h before use in viability assays.

Cytotoxicity of each batch of tobramycin-sodium stearate microparticle powders were investigated by replacing the cell culture medium with a suspension of the test powder in 100 μl of pre-warmed cell culture medium. Toxicity was determined after 24 h exposure by measuring cell respiration. In addition, a positive control test formulation was prepared by dissolving Triton X-100 in PBS to create a 0.5 mg ml^{-1} solution. Likewise, a negative control test formulation was also used in the form of the same culture medium. Only the 60 internal wells were used for result collection purposes. The remaining 36 wells contained only culture medium. After 24 h of cell incubation, 50 μl of the MTT solution (0.5 mg ml^{-1} in PBS) was added to each well. After 4 h the medium was removed and any formazan crystals generated were solubilised with 100 μl DMSO. The absorbance of each well was measured by spectrophotometry (SpectraMax 190, Molecular Devices, USA) at 550 nm after complete solubilisation of the crystals (25). The relative cell viability (%) was calculated as follows:

$$\text{Viability}(\%) = (A/C) \times 100$$

where A is the absorbance obtained for each of the concentrations of the test substance, and C is the average absorbance obtained for each of the negative control. Prior to analysis microparticle powders were subjected to gamma irradiation to ensure that bacterial contamination of powder did not influence toxicity. Briefly, cobalt 60 was used for irradiation (25 kGy) (Steritech, Sydney, Australia) and radiated samples were confirmed via the use of a gamma indicator dot placed on the outer of sterilisation bag. Exposure time was calculated according to the density of the samples and the received dose monitored via a dosimeter.

In Vitro Release Profile of the Tobramycin Microparticles

Since lipophilic water-insoluble adjunct was incorporated into the formulations, particle dissolution and drug release profiles were investigated. Briefly, Type-2 Franz cells (internal volume 20 ml, with heated water jackets, PermeGear, Inc. Bethlehem, PA, USA) were mounted in a six-station stirrer (V6B, PermeGear Inc. Bethlehem, PA, USA). Each Franz cell had a medium reservoir containing 0.05 M Phosphate Buffer (pH=7.4) at 37±0.5°C. The medium was circulated in the Franz cell reservoir using a peristaltic pump (Carter-Manostat) at flow rate of 5 ml min^{-1} (with two channels used for each cell). A 0.45 μm nitrocellulose membrane (MF™ Membrane Filters, Millipore, Bedford, MA, USA), previously soaked in the buffer, was mounted between donor and receptor compartments. The membrane diameter available for diffusion was 2.5 cm. The system was equilibrated approximately for 1 h; 50 mg of powder was evenly spread on the wet membrane on the Franz cell donor side. Samples of dissolution medium were taken from the reservoir at predetermined time and assayed using the HPLC method previously described. All samples were filtered through a 0.2 μm PTFE disposable filter (Whatman® plc, Kent, UK) and analysed in triplicate.

In Vitro Aerodynamic Assessment

The aerodynamic assessment of tobramycin powder was investigated using the Next Generation Impactor (NGI), as per Appendix XXI F of the British Pharmacopeia, without pre-separator. The NGI, at flow rate of 60 l min^{-1}, separates particles by aerodynamic size with stages 1–7 cut-off diameters of 8.06 (S1), 4.46 (S2), 2.82 (S3), 1.66 (S4), 0.94 (S5), 0.55 (S6) and 0.34 μm (S7), respectively. In addition a Micro Orifice Collector (MOC) below stage 7 ensures collection of particles less than 0.34 μm. The NGI was set at 60 l min^{-1}, using a calibrated flowmeter (TSI 3063, TSI instruments Ltd., Buckinghamshire, UK) equipped with a rotary vein pump and solenoid valve timer (Erweka GmbH, Germany). Approximately 20±2 mg of powder was manually weighed into a size 3, hard hydroxyl-propyl methyl cellulose capsule (Capsugel®, NSW, Australia). The capsule was then placed into the sample compartment of Aerolizer® DPI device, pierced and actuated for 4 s. The device, capsule, throat and all sample stages were then washed into suitable volumetric flasks before derivatization for the HPLC analysis. Each solution was tested in triplicate.

Analysis of drug deposition in the device/capsule, throat and NGI stages allows measurement of different deposition parameters. Specifically, the delivered doses (DD, the sum of drug collected from all stages, MOC and throat), the Fine Particle Mass (FPM, mg of drug lower than 5 μm aerodynamic diameter) and the Fine Particle Fraction (FPF: the ratio between FPM and DD) were calculated. The Mass Median Aerodynamic Diameter (MMAD) was determined by the % cumulative undersize on probability scale *versus* logarithmic aerodynamic diameter data.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA). Significant differences between formulations were analysed using post-hoc multiple comparisons, where p values of <0.05 (Fisher Pair wise) were considered to be significant. Unless otherwise stated data is represented in terms of mean and standard deviation.

RESULTS

Six batches of tobramycin powders containing different percentages of sodium stearate were manufactured by spray drying. All powder batches prepared presented a yield ranging between 64±2.2% and 70±5.7%. Unless otherwise

stated, each formulation will be referred as the concentration of sodium stearate in the solid (*w*/*w*). In order to understand the influence of hydrophobic adjunct on the aerosol performance of the microparticles, each formulation was evaluated in terms of physico-chemical properties.

Scanning Electron Microscopy and X-ray Dispersive Analysis

Representative scanning electron micrographs of tobramycin microparticles containing 0% and 1% *w*/*w* sodium stearate are shown in Fig. 1A, B, respectively. In general, the particles were spherical with smooth surfaces, indicative of an amorphous spray dried material. No substantial differences were observed between the two preparations. Furthermore, micrographs of samples containing different concentrations of sodium stearate (not shown) confirmed that adjunct concentration did not affect particle geometry or morphology.

X-ray dispersive analysis of the sodium presence on particle surface showed an increase in sodium stearate concentration between 0–1% *w*/*w*, resulting in a linear increase in sodium counts from 7.24±0.45 to 33.47±1.97 (R^2 value of 0.976). The value of 7.24 obtained for pure tobramycin is equivalent to the 'blank' constituted by the carbon tab coated with platinum used for mounting the sample. It represented the background reading and it was not subtracted from the samples analysed containing tobramycin. However, an increase in sodium stearate concentration over 1% resulted in a decrease in sodium counts to 13.64±0.55 for the particles containing 1.5% *w*/*w* of adjunct and 11.54±0.27 for particles at 2% *w*/*w*. The relationship over the total range of concentrations between the sodium stearate concentration and the sodium count on the microparticles surface is presented in Fig. 7B.

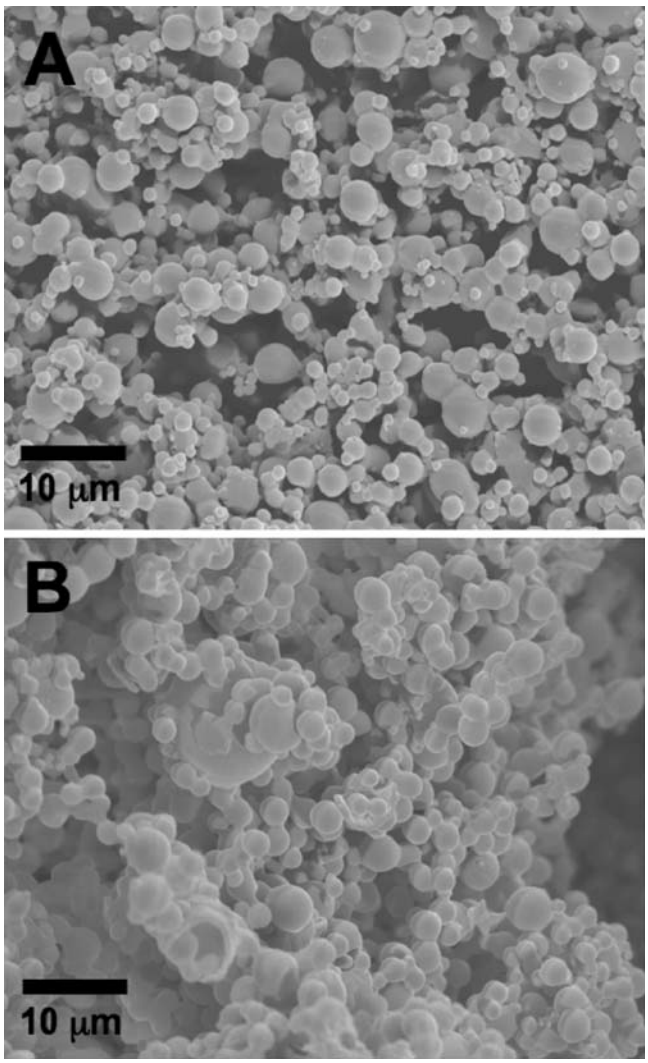

Fig. 1. Representative scanning electron micrographs of spray dried tobramycin microparticles containing (**A**) 0% and (**B**) 1% *w*/*w* sodium stearate.

Particle Size Analysis

The particle size distributions of each formulation is shown in Fig. 2. Analysis of the particle size indicated that the powders had similar mono-modal size distribution within a size range useful for respiratory delivery. However, variations in size were observed, and their significance can be evaluated from the mean values and standard deviations as reported. For example, the 50th percentile undersize ($d_{0.5}$) varied with respect to sodium stearate concentration. For example, $d_{0.5}$ values of 2.25±0.03, 2.28±0.16, 1.66±0.002, 1.49±0.007, 1.13±0.001 and 1.48±0.002 μm were observed for particles containing 0%, 0.25%, 0.5%, 1%, 1.5% and 2% *w*/*w* sodium stearate, respectively. Similarly, the 90th percentile undersize ($d_{0.9}$) values varied showing $d_{0.90}$ values of 8.19±3.01, 9.23±0.62, 4.24±0.02, 4.04±0.02, 3.94±0.001 and 4.23±0.04 μm for particles containing 0%, 0.25%, 0.5%, 1%, 1.5% and 2% *w*/*w* sodium stearate, respectively. However, regression analysis of $d_{0.5}$ or $d_{0.9}$ as a function of sodium stearate concentration indicated no linear relationship, since R^2 values of ≤0.680 were observed.

X-ray Powder Diffraction

X-Ray powder diffraction analysis of the spray dried samples (Fig. 3) presented a broad diffuse peak indicative of an amorphous material. Such observations are consistent with spray drying of many organic materials, specifically those which are composed of binary components (26). In addition, they are in good agreement with the SEM images (Fig. 1), which showed particles with smooth spherical structure.

In Vitro Cell Toxicity

To the author's knowledge, there have been limited studies into the relative toxicity of hydrophobic adjuncts delivered to the respiratory tract. Although more soluble than the acidic form (stearic acid), sodium stearate has relatively low aqueous solubility and thus potential toxicity during residence in the lung. Subsequently, the A549 lung epithelial cell viability after exposure to varied concentrations of different formulations of tobramycin-sodium stearate and of the stearate alone was investigated (Fig. 4). In general, analysis of the data indicated that there was no significant relationship between either sodium stearate or total microparticle concentration and cell viability. Minimum cell viabil-

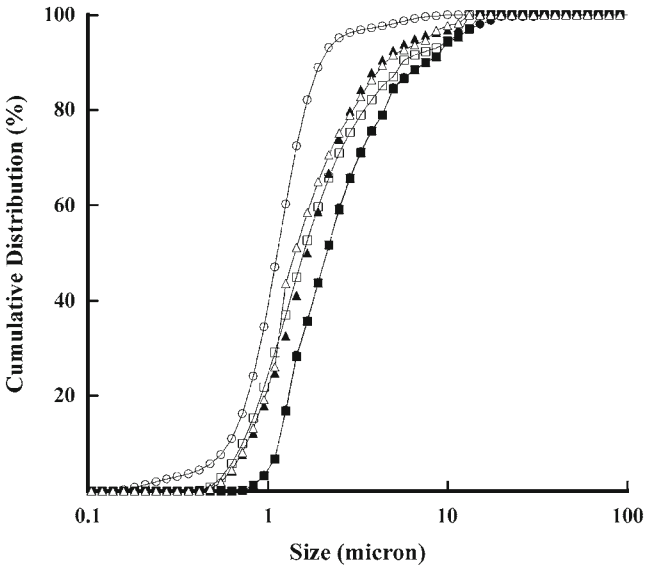

Fig. 2. Particle size distributions of spray dried tobramycin microparticles containing different concentrations of sodium stearate adjunct (T0 T0.25 T0.5 T1 T1.5 T2).

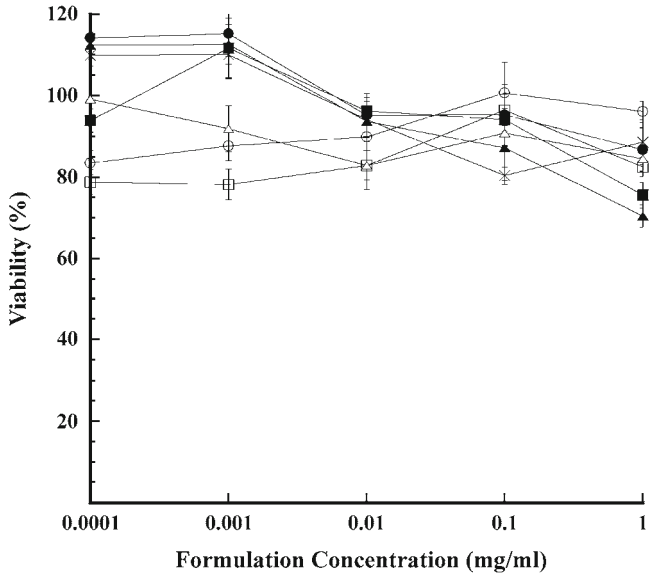

Fig. 4. A549 epithelial cell viability measured by MTT cytotoxicity assay after 24 h exposure to different concentrations of formulation (n=5; mean ± standard deviation). (NaSt T0 T0.25 T0.5 T1 T1.5 T2).

ity was exhibited at 70% by the formulation containing 0.5% of sodium stearate, a value non-significantly different from the one of pure tobramycin viability. Furthermore, the inhibitory cell concentration (IC) indicated all samples were viable with no $IC_{50\%}$ value. Consequently, it may be concluded that tobramycin-sodium stearate powders were not toxic under the conditions or timescale studied.

In Vitro Release of Tobramycin Microparticles

Currently, there is no pharmacopoeia method suitable for *in vitro* release studies of drugs for pulmonary administration as dry powders (27). Furthermore, traditional dissolution methodologies may not be suitable as they are not representative of the lung environment; where the lower bronchial tree has approximately 1 ml of fluid as a thin multilayer (28). In addition, it is likely that the release mechanism of drug from the microparticles upon impaction in the lung will be determined by a complex wetting, dissolution and diffusion mechanism (29). A recent study by Salama *et al.* (30) has shown that for inhalation powders, the determination of drug transported in a modified Franz cell through a wet filter

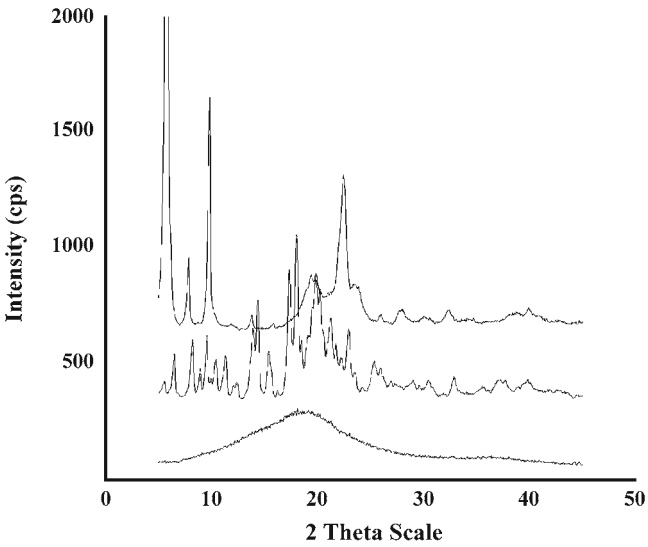

Fig. 3. X-Ray powder diffraction patterns of tobramycin, sodium stearate raw material and T1 formulation.

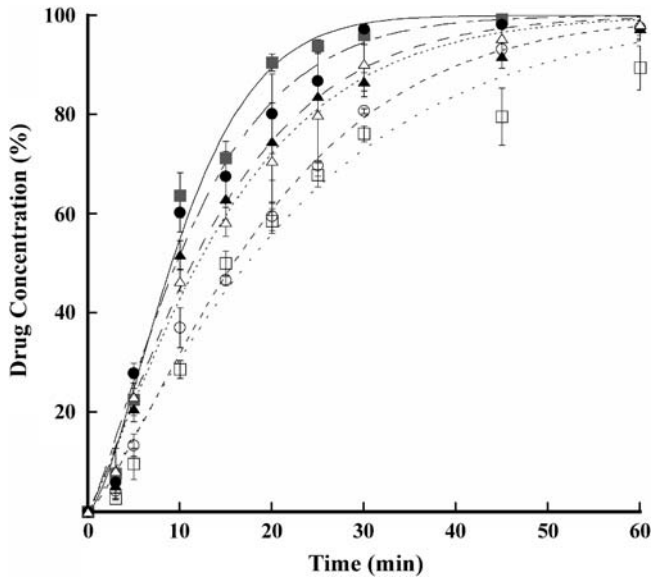

Fig. 5. *In vitro* transport profile of tobramycin from microparticles containing different concentrations of sodium stearate fitted with Weibull distribution equation. (n=6; mean and standard deviation). (T0, T0.25, T0.5, T1, T1.5, T2).

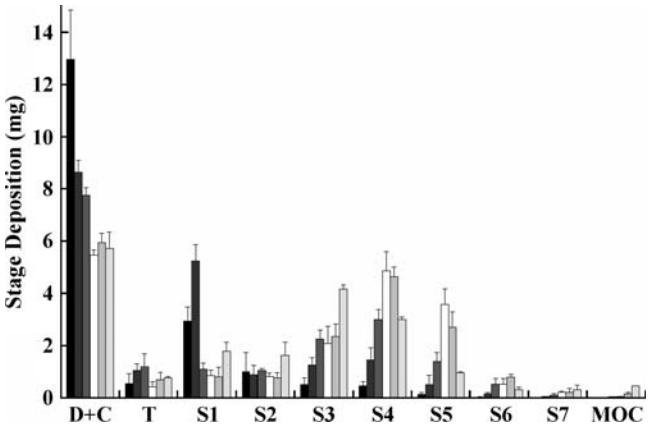

Fig. 6. Next Generation Impactor stage deposition of tobramycin microparticles containing different concentrations of sodium stearate. (n=3; mean ± standard deviation) (■ T0, ■ T0.25, ■ T0.5, □ T1, ■ T1.5, ■ T2). (D+C = Device+capsule).

membrane, on which the microparticles have been deposited, was suitable for investigating variations in release profiles of microparticulate systems. Therefore, to study the drug release from these particulates containing small amounts of adjunct, this previously reported method was used.

The drug release/diffusion profiles of tobramycin microparticles containing different concentrations of sodium stearate were presented as percentages of transported drug plotted as function of time (Fig. 5). The data was analyzed using the Weibull distribution equation and the relationship between sodium stearate concentration and time for 63.2% of tobramycin to be dissolved was analysed (Fig. 7C). The time for 63.2% of drug transported followed a parabolic profile with an increase observed with respect to sodium stearate concentration until 1% *w/w*. Further increase from 1% to 2% *w/w* resulted in an increase in transport rate and thus a decrease of the time parameter. Such results may be related to the decrease in sodium counts on the surface of the microparticles when lipophilic adjunct was increased beyond 1% *w/w*.

In Vitro Aerodynamic Assessment

The deposition (device/capsule, throat and NGI stages) of the microparticles after aerosolization is shown in Fig. 6. The *in vitro* respirability parameters are reported in Table I. Mass median aerodynamic diameter values, compared with the volume diameter, indicates a possible agglomeration effect for tobramycin microparticles without and with 0.25% NaSt; the difference between the two equivalent diameters was significantly reduced by increasing the content of sodium stearate, with a minimum for the powders containing the adjunct between 1% and 1.5%. Analysis of drug deposition on each stage suggested sodium stearate concentration had a significant effect on the aerosolization performance. The aerosolization and deposition performance of tobramycin microparticles without lipophilic adjunct presented the poorest performance, since a FPF of 27.1±1.9% was observed. This particle system also had the highest device and capsule recovery (12.98±1.88 mg), indicating a highly cohesive powder. The addition of a small quantity of adjunct (0.25% *w/w*) resulted in a significant decrease in capsule and device retention (8.65±0.45 mg), suggesting that the addition of adjunct aided powder flow by reducing interfacial interaction. Further addition of adjunct resulted in a small but significant decrease in device retention, considered that 2% *w/w* NaSt particles had 5.74±0.62 mg deposited in the device and capsules. In comparison, analysis of stage-3 through to the micro-orifice collection plate (respirable size ranges) indicated that the addition of small quantities of NaSt between 0.25% and 1% *w/w* resulted in high drug powder deposition. Furthermore, higher concentrations of sodium stearate between 1.5% and 2% *w/w* resulted in a decrease of deposition on stages 4–6. The values of FPF were in good correlation with these findings, since FPF increased from 27.1±1.9% with no adjunct, to a maximum of 84.3±2.0% at 1% *w/w* adjunct, followed by a decrease to 66.4±0.9% at 2% *w/w* when 2% *w/w* adjunct was added.

DISCUSSION

Tobramycin dry powders formulations for inhalation with high delivery efficiency (>80% FPF) have been characterised in terms of cell toxicity, release rate, and physico-chemical parameters. In addition the use of adjunct has also been investigated.

Previous studies have shown that addition of small amount of various compounds such as trileucine, chitosan, leucine, phenylalanine, or cyclodextrin can improve particles dispersibility (31–34). Cholesterol and phospholipids (>5% *w/w*) have also been employed in tobramycin particle formations by spray drying (20). In this study, sodium stearate was used as lipophilic adjunct to promote microparticle aerosolization and protect them from environmental humidity.

The relationship between sodium stearate concentration, aerosolization and deposition performance was not linear over the adjunct concentration range studied. A parabolic dependence was observed with a peak around the 1% content of sodium stearate. In order to further understand the effect

Table I. Deposition Parameter (n=3; Mean ± Standard Deviation) of the Different Formulations Measured by NGI

#	Metered dose (mg)	Delivered dose (mg)	FPM (mg)	FPF (%)	MMAD (μm)
T_0	18.67±0.95	5.69±1.01	1.55±0.39	27.18±1.9	7.49±1.19
$T_{0.25}$	19.39±1.31	10.74±1.25	3.52±0.69	32.80±1.49	7.63±1.02
$T_{0.5}$	18.52±0.19	10.74±0.97	7.32±0.39	68.17±1.65	2.79±0.10
T_1	18.99±0.19	13.52±1.37	11.41±1.64	84.35±2.0	2.29±0.14
$T_{1.5}$	19.18±0.41	13.25±1.00	10.77±0.57	81.26±1.70	2.17±0.25
T_2	19.28±0.33	13.54±0.59	9.00±0.45	66.45±0.9	2.95±0.14

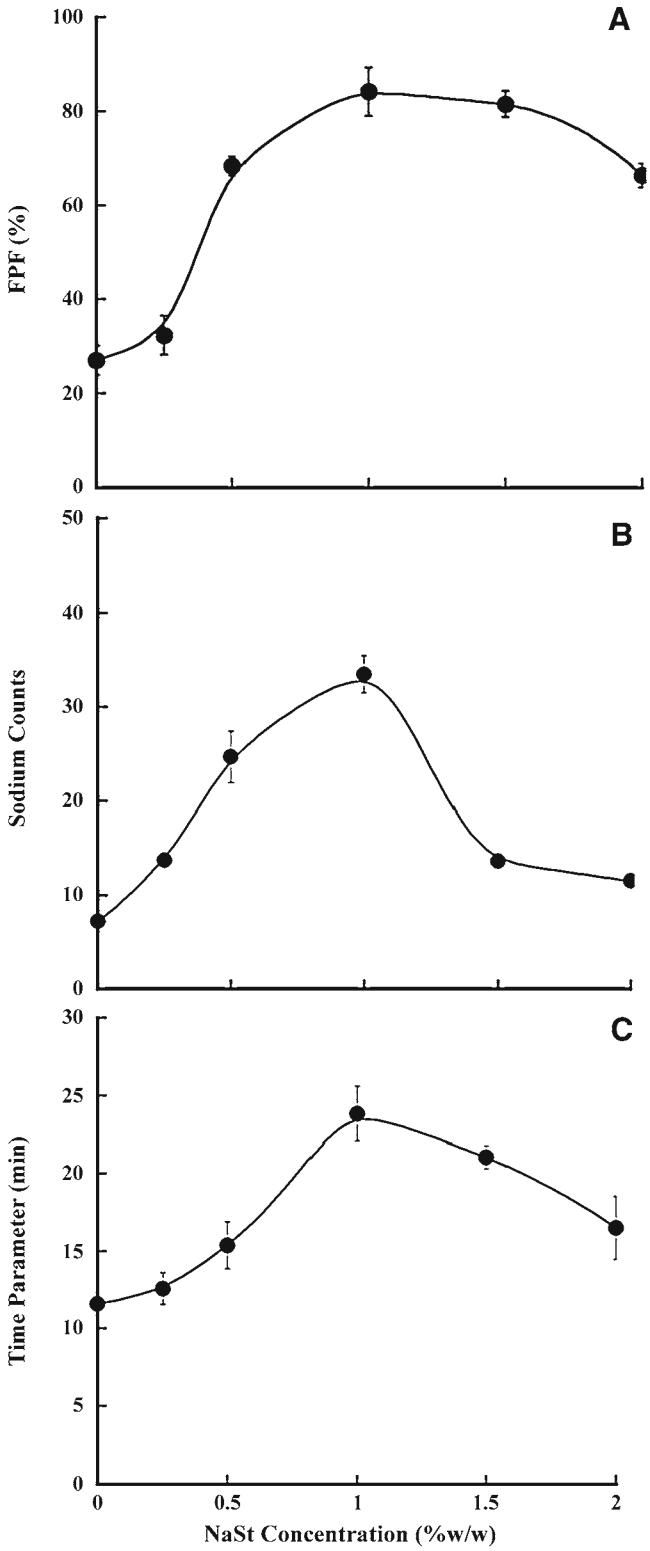

Fig. 7. Relationship between initial sodium stearate concentration and **A** fine particle fraction, **B** sodium counts and **C** time parameter, time necessary for the 63.2% of drug dissolved.

of this adjunct concentration on aerosol performance, the relationships between fine particle fraction, particle surface sodium count, release rate and sodium stearate concentration were collectively analysed and shown in Fig. 7A–C, respectively. Fig. 7A, B, shown a positive relationship between the presence of adjunct on the particle surface, as measured with sodium count, and aerosol performance.

During the spray drying process, the solutes in the sprayed droplets will have a radial distribution as the droplet evaporates. In a binary system, the final distribution of each component in the dry solid will be due to the molecular structure of the individual components and their relative molecular mass. The molecular mass of tobramycin and sodium stearate is 467.5 and 306.46 g/mol, respectively, suggesting that they would have similar transport rate in a rapidly drying droplet and thus be evenly distributed throughout the solid. However, sodium stearate has a much lower aqueous solubility than tobramycin (35,36) and is a surface-active agent. It may be assumed therefore, that the hydrophobic adjunct would accumulate at the droplet liquid–air interface during the drying process and deposit on the dried particles surface. This surface accumulation would result in the increase in relative sodium counts and large reduction in interfacial tension between the contiguous microparticles. This would results in a significant improvement of the aerosolization efficiency. For sodium stearate concentrations over 1% *w*/*w*, a significant decrease in sodium counts (Fig. 7B) was observed, suggesting a different mechanism of molecular re-distribution of the components during the drying process and internalisation of the lipophilic adjunct in the microparticles. Furthermore, comparing FPF and sodium counts for the 1.5% *w*/*w* adjunct microparticles, it is evident that the decrease in sodium count number was not immediately reflected by a lower respiratory performance. It is possible that the relative decrease in particle size between 1% and 1.5% *w*/*w* microparticles distribution, could have affected these results.

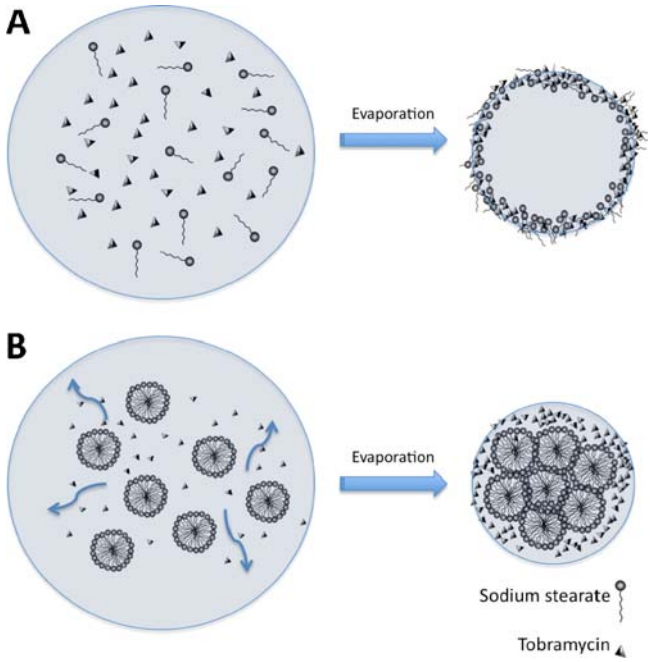

Fig. 8. Schematic of the proposed particle formation process for binary mixtures of tobramycin and sodium stearate during spray drying where **A** low sodium stearate concentrations and **B** high sodium stearate concentrations.

The *in vitro* drug transport (Fig. 7C), the sodium counts (Fig. 7B) and the *in vitro* deposition (Fig. 7A) correlated with the NaSt content following a parabolic behaviour with the maximum values around 1% *w*/*w* of adjunct. No relationship was found between release rate and size distribution, suggesting that the decrease in dissolution/transport rate of powders containing sodium stearate between 0% and 1% *w*/*w* was due to the increased concentration of the hydrophobic adjunct on the particle surface, affecting wetting and dissolution. A further increase from 1% to 2% *w*/*w* of NaSt resulted in a faster dissolution/transport rate suggesting, as confirmed with EDS analysis, the internalisation of the adjunct in the solid particulate.

The internalisation of the adjunct may be explained considering overall molecular structure of the components at different concentrations. The critical micelle concentration (CMC) for sodium stearate in aqueous solution has been reported between 4.0×10^{-4} and 5.6×10^{-4} m, based on experimental and theoretical calculations (37–39). Considering the molecular mass of 306.46 g/mol, the relative molar concentrations of sodium stearate in the spray dry solutions would be 3.3×10^{-4} m at a concentration of 0.01% *w*/*v* and 6.5×10^{-4} m at 0.02% *w*/*v*. These values correspond to final amount of 1% and 2% *w*/*w* sodium stearate within the microparticle formulation, which can be found at either side of the previously reported CMC range. Furthermore, the micelle structure in aqueous solution has been reported as containing 78 monomer groups (38). Assuming, the CMC and micelle structure in this complex mixture would be in a similar range to that of a pure aqueous solution, the micelle mass would be approximately 23,904 g/mol compared with 306 g/mol for the individual stearate molecule. Essentially, in a micellular form the sodium stearate component would have more than fifty times the molecular mass of tobramycin and would have reduced molecular mobility during the drying stage. However, this system is dominated by the relative surface activities and ionic nature of the materials used. Sodium stearate is a surface-active agent and, at low concentrations (less than 1%), the hydrophobic chains will preferentially distribute at the air/water inter-phase, pointing away from the microparticle core. At high concentrations (more than 1%) a micellular system is formed, where the hydrophobic tails are shielded, and the ionic (and thus hydrophilic) nature of the sodium stearate micelles will result in higher internalization, promoting tobramycin partition at the inter-phase.

A schematic of this theoretical re-distribution of sodium stearate in the micro-droplets during the spray drying process is shown in Fig. 8. This theoretical explanation took into account that during the evaporation process the concentration of sodium stearate will increase, suggesting that CMC will be reached during drying for all solutions. However, the drying process is very rapid and self-assembly of free sodium stearate will be hindered by the concomitant presence of other molecules in the droplet and the rapid nature of this process. Indeed, spray drying is used to circumvent the self-assembling of molecules in producing amorphous over crystalline forms.

CONCLUSION

Tobramycin was spray-dried in presence of varying amount of sodium stearate used as adjunct to control the aerosolization efficiency in a dry powder inhaler. The aerosol performance of the spray-dried powders was related to the percentage of adjunct in the microparticles in the range between 0.25% to 2.0% *w*/*w*. Adjunct between 1% and 1.5% *w*/*w* showed a positive linear increase of aerosol performance, followed by a significant decrease at 2%. In general, particles containing a final adjunct concentration of 1% *w*/*w* sodium stearate, where sodium stearate was concentrated for the majority on the surface of microparticles, provided the greatest aerosol performance (>80% FPF). The molecular form of stearate in the spray drying solutions and its redistribution in the microparticle growing during spray drying, was suggested as the determinant for the presence of sodium stearate on the surface of the microparticles. Preliminary cell toxicity studies have showed that the use of this hydrophilic adjunct at the concentrations shown before has no effect on cell viability over a 24 h period. The minimum value of cell viability was higher and non-significantly different from the value exhibited by tobramycin alone (approx 70%). In conclusion, this approach provides a feasible and attractive alternative to intravenous or nebulisation of antibiotics to treat respiratory infection. It is envisaged that this novel tobramycin pulmonary powder could ultimately be used to improve therapeutic outcomes for patients suffering from debilitating respiratory diseases such as Cystic Fibrosis, bronchiectasis and COPD.

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