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Next Generation Nebuliser Formulations

Background

The use of aerosols in inhaled delivery remains one of the oldest forms of drug therapy in history. It was employed as far back ancient Egyptian times to deliver the vapour of black henbane to provide relief to the breathless patient [1]. The delivery of drug containing aerosols directly to the respiratory system has significant advantages over drug delivery using other routes. Not least of which is avoidance of the first pass metabolism of the hepatic system and assurance of local action with regards to respiratory conditions like asthma and COPD. Thus, favouring enhanced disease targeting and reduced occurrence of adverse effects which result from systemic exposure to drug from other routes.

As a PhD student, my focus has been on the effects of pMDI (pressurised metered dose inhaler) excipients on the disposition of inhaled corticosteroids in lung tissue in the treatment of asthma. Corticosteroids remain the mainstay of treatment in asthma to control inflammation and prevent exacerbations and as such the need for a thorough understanding of the delivery mechanisms of these medicines cannot be over-emphasised. I hope to gain greater insight into the effects of glycerol which is a bulking agent used in the formulation of beclomethasone dipropionate for delivery via pMDI on formulation characteristics, aerosol particle properties and lung permeability.

Following on from the work I carried out for the final year of my MPharm degree which involved exploring inhaled drug delivery using nebulisers, I am continuing to address the need for alternative formulations, more specifically the potential of microemulsions for the delivery of poorly aqueous soluble drugs using nebulisers.

Introduction and Experimental Aims

Nebulisers are niche drug delivery devices which are employed in primary and secondary care settings. They are used to deliver much higher drug doses over a longer time period than conventional inhaler devices. Another advantage of using a nebuliser to deliver drug containing aerosol is that it does not require the complex breathing/actuation co-ordination that the use of other aerosol devices like pMDIs or DPIs (Dry powder inhalers) would entail. This makes nebulisers an excellent choice for administering inhaled therapy in the very young and the elderly populations [2]. They also have the advantage of being able to produce small droplets in the nanometre range that penetrate the lungs more deeply. Thus ensuring that a therapeutic dose is achieved with minimal physiological effort on the user's part.

Glucocorticoids are a class of anti-inflammatory drugs that are used in the treatment of a range of pulmonary disorders. These drugs have poor aqueous solubility; a property that facilitates their formulation as suspensions when they are to be delivered using nebulisers. The glucocorticoid investigated in this instance is fluticasone propionate (FP) and it is practically insoluble in water. These steroid suspensions, however, have been shown to be less than optimal in their production and

delivery of drug during nebulisation [3]. Some of the shortcomings include: need for preservatives (which may be irritant to the respiratory tract) [4] and time-consuming aseptic preparation techniques to ensure the minimum sterility assurance level for FP and/or excipients as filtration sterilization would lead to significant loss of drug from the suspension formulation; excessive impaction on the upper airways and subsequent ciliary clearance leading to loss of delivered dose [5]due to the production of larger aerosol droplet sizes from suspensions and finally inconsistency in aerosol droplet drug concentration [6,7].

Microemulsions are thermodynamically stable, lipid-based systems that have proven to be useful drug delivery systems [8]. They are usually isotropic mixtures of water, oil, surfactant and co-surfactant (and drug) that greatly enhance the solubility of poorly water-soluble drugs by incorporating the drug molecule in the oil phase within the surfactant micelles. Although microemulsions have been formulated for drug delivery via a range of administration routes, not many of these have been designed for the respiratory route. This may be due to the relatively small number of pharmaceutically acceptable excipients approved for inhaled drug delivery [9] which represents a considerable barrier to the development of novel formulations for this route. The formulation of microemulsions of the pulmonary route is also limited by the sensitivity of the respiratory tract to some of the surfactants and co-surfactants typically used to stabilise the microemulsions. For example, substances like phenol and ethylenediaminetetraacetic acid (EDTA) which are used in formulations for their preservative and chemical stabilising properties respectively have been known to cause bronchoconstriction [10].

Microemulsions have however been hypothesized to be better formulations for drug delivery because they behave, for the most part, like solutions. Thus, microemulsions allow for sterilisation via filtration, [11] more uniform distribution of aerosol droplet size and greater reproducibility in terms of droplet drug content.

There is thus an opportunity to apply these systems to the field of inhaled drug delivery. Microemulsions represent a new formulation concept for inhalation with real advantages over the relatively more established products.

The aim here was to develop fluticasone propionate microemulsions for pulmonary delivery by nebulisation. Specific objectives are to (i) design prototype formulations, (ii) describe the formulations in terms of their physicochemical characteristics, and (iii) evaluate their suitability as nebuliser formulations by assessing product performance in terms of fine particle dose, inhaled dose, and aerodynamic size profile. Finally, the aerosol output and aerodynamic performance of a licensed FP suspension, Flixotide® nebules, will be measured to enable comparison with the novel microemulsions.

Preliminary Data.

Methodology and results

Table I: Microemulsions and their components

***ME1** [4] **(Adapted budesonide nebuliser formulation)**	**ME2** [11] **(Adapted ocular dexamethasone formulation)**	**ME3** [12] **(Adapted Topical bifonazole Hydrogel)**
Fluticasone Propionate 0.25mg/g	Fluticasone Propionate 0.25mg/g	Fluticasone Propionate 0.25mg/g
Tween 80 10% w/w	Kolliphor EL 15.0% w/w	Tween 80 37.5% w/w
Ethanol 1.0% w/w	Propylene Glycol 15.0% w/w	Isopropyl Alcohol 12.5% w/w
Isopropyl myristate ***(Crodamol in original formulation)** 1.0% w/w	Isopropyl myristate 5.0% w/w	Oleic acid 10 % w/w
Normal saline (0.9%w/v) To required weight	Water To required weight	Water To required weight

The microemulsions were formulated for use with a jet nebuliser, the Pari LC Sprint. The jet nebuliser uses high velocity gas from a compressed gas source shot through the drug containing reservoir to produce an aerosol.

The formulations in this study; ME1, ME2 and ME3, were prepared using excipients and methods reported to produce drug microemulsions with good biocompatibility [4,11-12]. With the notable exception of ME1 where isopropyl myristate was used as oil phase in place of the medium chain triglyceride, crodamol GTCC **(Table I)**. A variety of microemulsions investigated for different routes of drug administration were selected to allow investigation of a broad range of microemulsion characteristics that could affect their performance as drug delivery vehicles for nebulisation. The FP concentration of the microemulsions were identical in every instance; 0.25 mg of active ingredient per 1g of formulation.

The solubilisation capacity of the micromemulsions formed which is a function of the properties and concentration of excipients was also evaluated. Samples of each microemulsion were filtered through a 0.22µm pore-diameter membrane filter. The original samples and the filtrates were then diluted and prepared for assay using HPLC. The assumption that formed the basis for this test was that any FP molecule(s) contained in a droplet of disperse phase with size less than or equal 0.2µm was considered

as solubilized or encapsulated in the microemulsion. Conversely, particles greater than 0.2μm would be retained by the filter and thus considered to be insoluble residue.

Viscosity of the test formulations was measured using a Brookfield LVDV device at room temperature. Of the microemulsions produced, ME3 could not be nebulised and so testing beyond viscosity was not carried out.
Testing of the delivery rate and delivered dose was carried out as per European Pharmacopoeia specification in triplicates. The breath simulator was set to run at 15 breaths/minute to mimic the normal human adult breathing frequency. The nebuliser was connected to the breath simulators via filters that were positioned to collect nebulised FP. A sputtering sound was taken to signal the point when the formulation had been nebulised to dryness. The FP content on the filters were determined using HPLC.

Table II: Viscosity; Fluticasone Propionate solubilisation capacity; Delivery rate and aerosol output. Comparison of delivery rate and delivered dose of ME1, ME2 and Flixotide® suspension. Data represents mean ± Standard Deviation, n = 3 Jet (Pari LC Sprint) nebuliser was used.

Formulations	Solubilising capacity (%)	Viscosity (cP)	Delivery rate (mg/min)	Emitted Dose (%)
ME1	49.62 ± 1.22	2.64	0.04 ± 0.002	27.01 ± 0.004
ME2	84.74 ± 1.26	7.74	0.02 ± 0.001	20.70 ± 0.010
ME3	97.22 ± 0.06	107.00	**FAILED TO NEBULISE**	
Flixotide® Nebules	**NOT MEASURED**		0.04 ± 0.020	52.48 ± 0.203

To determine the aerodynamic size profile produced by the formulations, the next generation impactor was used to fractionate the aerosol droplets according to their aerodynamic size. Each formulation, including Flixotide®, was delivered using the Pari LC Sprint jet nebuliser. The FP content of each stage of the NGI was assayed using HPLC.

Statistical analysis performed using ANOVA. P values < 0.05 were taken to be statistically significant.

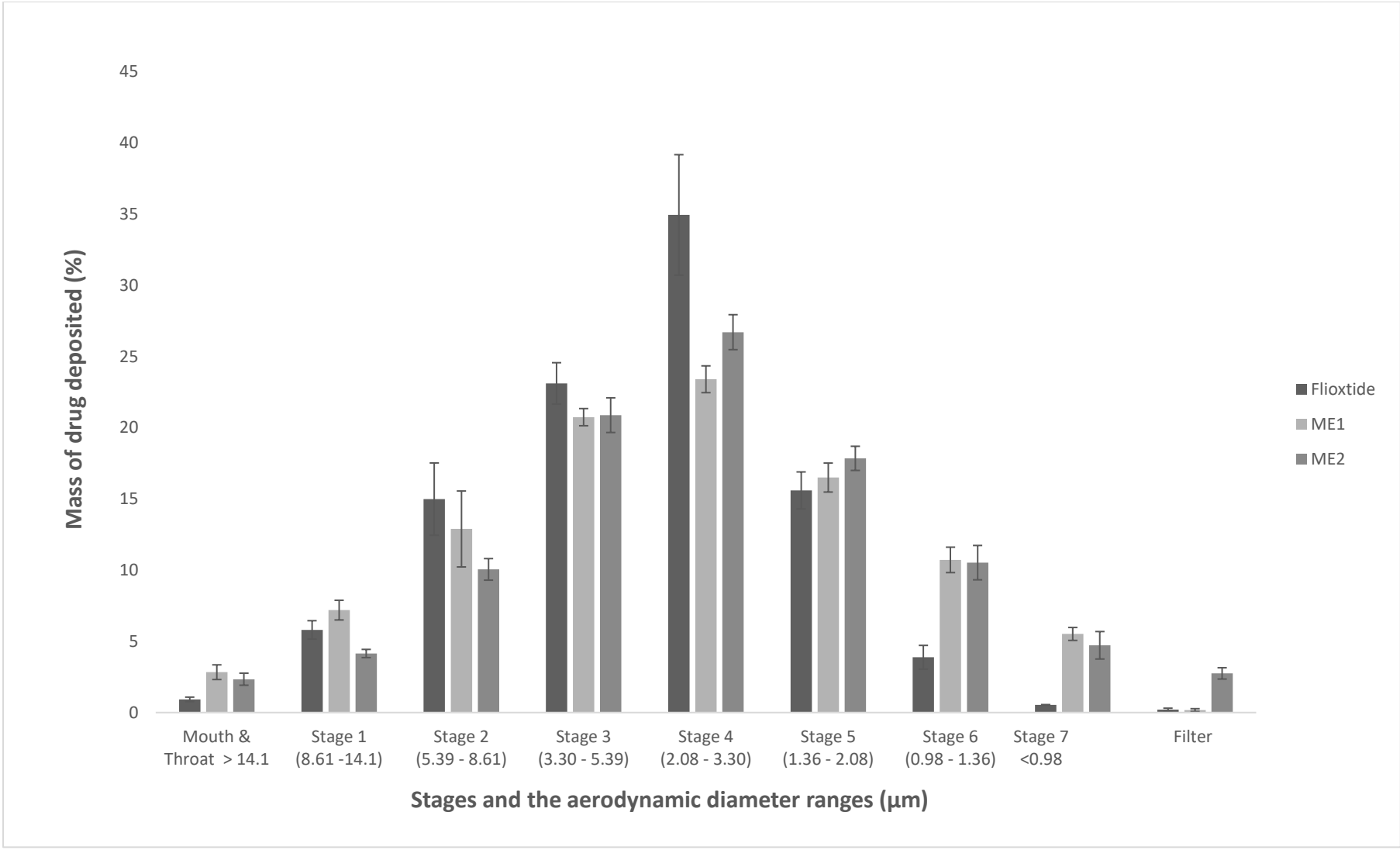

Figure 1: ***In vitro*** **aerodynamic deposition profile for the different stages specific to Next generation pharmaceutical impactor, NGI (Copley Scientific, Nottingham, UK) at flow rate 15L/min. Mass of drug in each stage is calculated as a percentage of total deposition in NGI body.**

Table III: Fine particle dose (FPD) of microemulsion (ME1 and ME2) and suspension (Flixotide®) formulations of FP. FPD (Fine particle dose) = percentage of droplets that are <5µm in size. Data represents mean ± standard deviation, n = 3.

Formulation	Fine Particle dose (%) FPD	Mass Median Aerodynamic Diameter (µm)
ME1	73.23 ± 3.70	4.60 ± 0.26
ME2	79.55 ± 1.24	4.18 ± 0.20
Flixotide®	73.96 ± 2.87	4.95 ± 0.24

Discussion

ME1 had the least FP solubilisation with 49.6% efficiency **(Table II)**. The highest solubilisation of the test formulations was ME3 with 97.2% efficiency. This could be due to increasing surfactant concentration of which ME3 had the most and ME1, the least. Whilst this presents as an avenue for increasing solubilisation in future studies, the limitation of increased risk of toxicity of these irritant chemicals to the cells and tissues of respiratory tract [10,13] must be considered. Although ME3 had near total encapsulation, the inability of the nebuliser to generate aerosols from the formulation made it unsuitable for inhaled delivery. This finding was attributed to the very high viscosity of the formulation [14].

In a clinical setting, how quickly an inhaled dose is administered has major effects on patient adherence. In the context of aerosol output of an inhaled product, the optimal formulation would be one that delivers the maximum amount of drug in the shortest possible time. Overall there was no major improvement over Flixotide® for the delivery rate or inhaled dose in the test formulations **(Table II)**. This was perhaps due also to the viscosity of the microemulsions which would have slowed the rate at which the formulations could go through the nozzle of the jet nebuliser. The inefficiency observed with the inhaled dose of all the formulations could be due to evaporative losses and drug wastage which are common issues associated with continuously operated jet nebulisers [15].

The aerodynamic profiles show that all three formulations including Flixotide® deposited the most FP on Stage 4 (cut-off value > 3.30 µm). However, their deposition patterns varied across all stages **(Figure 2)**. In the pathology of asthma, inflammation occurs throughout the respiratory tract but more predominantly in the lower airways. Particles within the range of 1 – 5 µm usually deposit in the lower airways [16]. Thus, the optimal formulation would be one that produces a high proportion of droplets in that size range. This property has been quantified in this study as the fine particle dose (FPD). There was no significant difference between the FPD values ($p > 0.05$) for ME1 and Flixotide® which indicates similar deposition patterns for both formulations **(Table III)**. This finding is in line with the results from solubilisation capacity studies where ME1 was seen to exhibit some properties characteristic of a suspension (lowest capacity). ME2 was shown to produce the most droplets that are likely to deposit in the lower airways as it had the most favourable FPD and MMAD (Mass median aerodynamic diameter). This advantage over Flixotide® could be due to the presence of surfactants in the microemulsion that reduce interfacial tension between the phases and facilitate the formation of smaller droplets [17].

According to the British Pharmacopoeia, the pore size of filter used for sterility testing should be no greater than 0.45 µm. The pore size used in this study was smaller and yet allowed for up to 85% drug recovery from ME2 suggesting that perhaps with manipulation of the surfactant concentration to increase encapsulation/solubilisation in either formulation, filtration can be employed as a means of terminal sterilisation of this formulation.

As was alluded to previously, the desired outcome would be to produce an FP microemulsion that exhibits improvements over the commercial formulation and attempts to circumvent some of the issues plaguing aerosol drug delivery from suspension formulations like inability to filter-sterilise and increased aerosol droplet size. The ability of the prototype formulation to permit sterilisation via filtration can be considered a significant advantage as it reduces or possibly eliminates the need to use other means of sterilisation which are substantially more expensive and time-consuming. The use of microemulsions in inhaled drug delivery constitutes a perhaps under-investigated body of work. Consequently, this study represents an exciting opportunity to explore the possibilities of the applications of these systems.

Future work

The results of this study indicate that the formulation of FP microemulsions for nebuliser devices is a realisable outcome. However, the next steps are to optimise the microemulsion formulations with the view to improving the physicochemical and aerodynamic properties. Some of the approaches include:

- Reformulating ME1 with MCT which is the oil phase in the formulation upon which it was based could lead to some improvement in solubilizing capacity and aerodynamic performance.
- Manipulating the surfactant concentration of ME1 to maximize solubilisation of FP. Also, decreasing the surfactant concentration in ME2 as this might reduce the viscosity and increase the delivery rate.
- Measuring the effect of dissolution of solubilized drug versus suspended drug using *in vitro* dissolution assays. Modelling the effect of dissolution on the concentration-time profiles of FP in the lungs and evaluating the results as a means of optimizing aerosol therapy.
- Testing the physical and chemical stability of the microemulsions as per the relevant specification
- Investigating the safety of the microemulsions especially when exposed to the lung tissue to ascertain any toxicity.

About the applicant

Before becoming a qualified pharmacist in August 2016, I undertook my pre-registration training year at King's College Hospital NHS Foundation Trust. During this time, I was involved in asthma and COPD clinics where I was fortunate to understand patients' experience of their inhaled medicines. Being in a position to appreciate the role of aerosol engineering in drug therapy for pulmonary disease was one of the main inspirations for my current research career.

Extracurricular activities that I have engaged in over the years have furnished me with skills that I have subsequently found to be indispensable to my progress so far. I understand the importance of team work and of fostering team spirit in situations where the people involved have very diverse roles and cultures. For the final two years of my undergraduate degree, I was the secretary of WWF (World Wildlife Fund) society at my university. As a member of the executive team, I was involved in the planning and execution of events to raise funds and awareness for and of our cause. This required me, on numerous occasions, to work with people who were different from myself and ensure that everyone still had a voice. The interpersonal skills, leadership and organisational skills I obtained during these times have helped me integrate into the teams that I encounter in my research career.

Given my previous experiences in various fields of work, I am confident that I possess the necessary skills to undertake my PhD with all that it entails and emerge with success. I hope to be considered favourably for the C.N. Davies Award.

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