A Method for Evaluation of Aerodynamic Particle Size Distribution of Novel Inhalable Light Emitting Particles for Photodynamic Therapy – Light4Lungs

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Summary

Light4Lungs is a Horizon 2020 project to investigate a novel therapeutic approach for treatment of antimicrobial resistant (**AMR**) chronic lung infections. Inhalable light emitting particles (**LEPs**) that excite endogenous bacterial photosensitisers by photodynamic effect and thereby eliminate pathogenic bacteria were formulated for aerosol delivery. LEPs are composed of a metal oxide core, a transition metal dopant, and a surface coating. Excitation spectrum is in the UV range with the emission peak in the blue spectrum. For pharmaceutical formulation development a novel quantification method for LEPs, independent of light emitting properties, was developed using a turbidimeter. Turbidity measurements were used to investigate LEP suspension stability and to establish the relationship between LEP concentration and turbidity readings. Standardised suspension preparation methods involving sonication and the addition of surfactant were established in order to prepare stable suspensions of LEPs for aerosolisation using an air-jet nebuliser. The physico-chemical properties of LEPs, in particular the tendency for surface adhesion, resulted in significant retention of material within the nebuliser. Furthermore, accurate and reproducible recovery of LEPs from a standard Next Generation Impactor, was not possible. The use of a Viable Andersen Cascade Impactor resulted in efficient and accurate recovery and facilitated determination of aerosol characteristics. The fine particle fraction (% <5µm) was >65% with a mass median aerodynamic diameter of approximately 1µm. Work is ongoing to investigate suitability of alternative nebuliser technology e.g. vibrating mesh, in order to optimise delivery of LEPs.

Key Message

Turbidity measurements were successfully used to assess LEPs suspension properties and also to enable quantification of LEPs. Initial aerosol characterisation studies indicated significant LEPs retention within the nebuliser reservoir and on inertial impactor surfaces. Use of a viable impactor resolved the latter issue while alternative nebuliser systems will be evaluated.

Introduction

In 2019 according to the World Health Organisation [1] lower respiratory tract infections remained the world's deadliest communicable diseases and ranked the fourth leading cause of death, giving rise to 2.6 million fatalities per year. Antimicrobial resistance (AMR) among patients with pulmonary infections is a high-risk factor.

A consortium of eight partners are participating in a Horizon 2020 Future and Emerging Technologies project entitled Inhalable Aerosol Light Source for Controlling Drug-Resistance Bacterial Lung Infections (acronym Light4Lungs) [2]. The project will evaluate a novel approach to eliminating pathogenic bacteria by photodynamic therapy as an alternative to antibiotics. The concept is summarised in **Figure 1**. It is envisaged that particle activation will be achieved by the excitation of LEPs via a light emitting diode with a maximum emission wavelength of 285 – 340 nm. Excitation of LEPs will occur following aerosol generation either into a holding chamber or directly prior to delivery to in-vitro/in-vivo models. The excitation process will be evaluated and refined to optimise the delivered dose of phosphorescent photons from the LEPs.

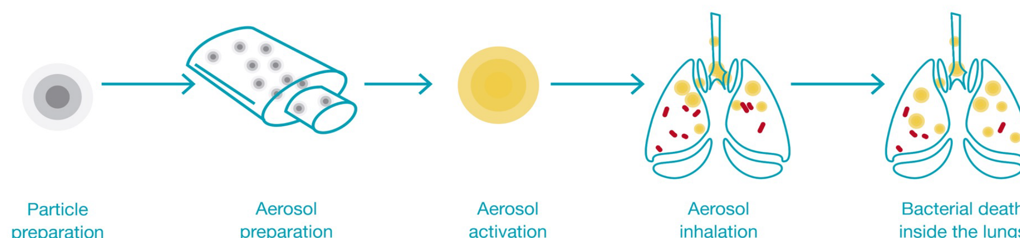

Figure 1. The Light4Lungs 5 step concept.

The LEP consist of a nano-sized (< 1 µm) metal oxide core, doped with a transition metal and surface coated to promote dispersibility. The duration of light emission from the excited particles is currently short i.e. approximately 1 minute, although research efforts are being made to extend this. The insoluble, biologically and chemically inert LEP will be suspended in aqueous media to allow aerosolization, prior to activation of the particles using an LED light source. Assessment of bactericidal efficacy will initially use appropriate bacterial cell cultures and biofilm models.

For pharmaceutical formulation development a novel method to assess LEPs suspension

quality and also to enable quantification of the LEP independently of their light emitting properties was required.

In this study we present a method for preparation of a stable suspension of the LEPs and a novel method of quantification, correlating sample turbidity with the mass of suspended particles. Using this quantitation method, we performed preliminary aerodynamic particle size distribution (APSD) evaluation of aerosols generated using an air-jet nebuliser. APSD measurements were performed by two different cascade impaction methods.

Experimental Methods

Primary LEP size evaluation using environmental scanning electron microscope and transmission election microscope was conducted by other consortium partners.

LEP suspension properties and quantification measurements were performed using a turbidimeter (TU5200, Hach UK) that collected scattered light at 90° to the incident light, turbidity was reported in terms of Formazin Nephelometric Units (FNU). The instrument used a monochromatic light source at 850 nm, thus avoiding interference due to absorption or emission from the LEP (maximum absorption and emission wavelengths of 280 nm and 480 nm respectively).

Initial LEP suspension stability experiments (0.5 mg/mL), were performed in water (HPLC grade, Fisher Scientific Ltd, UK) with a target of pH 7.0, subsequently the inclusion of surfactant i.e. 0.02 mg/mL polysorbate 80 (Fisher Scientific Ltd, UK) was evaluated. Samples were sonicated (Ultrasonic bath, S-series, Fisher Scientific Ltd, UK) for 40 minutes prior to cooling to ambient temperature to ensure temperature change did not influence sedimentation of LEPs. After cooling, sequential sampling of suspensions was performed over a 240 min period and turbidity readings were determined as a means of assessing suspension stability.

Validation of the quantification method was performed to establish linearity of response, limits of quantitation and detection, precision, accuracy and robustness. Linearity over the range 0.1 – 25 µg/mL was investigated by dilution of a stock suspension of LEPs (50 µg/mL) prepared as described above.

Having established suitable methods for preparation of stable suspensions and for quantitation of LEP, experiments were performed to investigate LEPs recovery from inertial impactor components. Subsequently, aerosol testing was conducted using a 6-stage viable sampler (Viable Andersen Cascade Impactor, vACI) operated at a flow rate of 28.3 L/min. The key feature of this impactor is that sample deposition was achieved by collection on stages comprised of glass Petri dishes containing 27 mL of polysorbate 80 solution (0.02 mg/mL) to optimise LEPs recovery. An in-line filter was connected downstream of the vACI, and testing was conducted in a glove box using filtered air (0.2 µm) to reduce the possibility of particulate contamination. Following aerosol deposition, the nebuliser, Inlet Port, Petri dishes and final filter were placed into separate sampling bags (Nasco Whirl-Pak, Fisher Scientific, UK) with appropriate volumes of recovery solution (0.02 mg/mL polysorbate 80), mixed by inversion and sonicated for approximately 10 min prior to sampling for turbidity analysis. Reporting of APSD was performed with Copley Inhaler Testing Data Analysis Software (CITDAS, Copley Scientific Ltd, UK).

Preliminary assessment of aerosol properties was conducted using an air-jet nebuliser. The Micro CirrusTM (Intersurgical Ltd, UK) was selected since this device can generate aerosols with mass median aerodynamic diameter (MMAD) of approximately 1.2 µm [3], suitable for delivery of LEPs to the periphery of the lung. The nebuliser was loaded with 5 mL of a 0.5 mg/mL LEP suspension (containing 0.02 mg/mL polysorbate 80) and aerosol output was collected for 18 minutes.

Measurements of LEPs conducted by consortium partners using environmental scanning electron microscopy and transmission electron microscopy, indicated that the average primary particle size was approximately 200 nm. Consortium partners also reported that effective dispersion of the LEPs was difficult and prolonged sonication was required, furthermore there was a tendency for the particles to adhere to the surfaces of equipment used during sample preparation and testing.

A stable aqueous colloidal suspension of LEPs (0.5 mg/mL) in 0.02 mg/mL polysorbate 80 (pH 7.0) was prepared by sonicating for 40 min. The suspension was subsequently sampled over a 240 min period to determine turbidity. Measurements indicated it was stable up to 60 min, beyond which appreciable changes in turbidity were measured (**Figure 2**) indicating sedimentation of the LEPs. It was considered that suspensions stabilised with polysorbate 80 would be suitable for future bactericidal efficacy experiments since this surfactant is used in commercial nebuliser formulations.

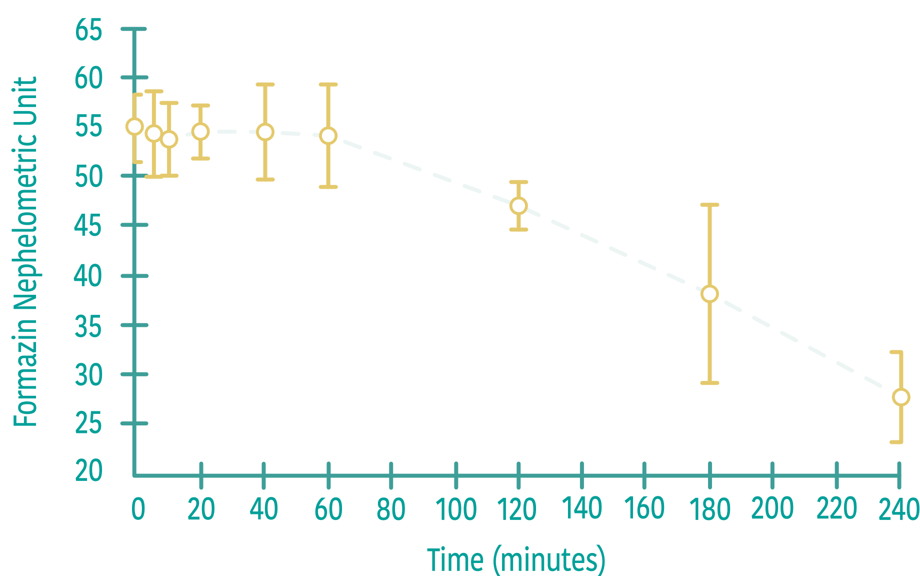

Figure 2. Suspension stability of LEPs (0.5 mg/mL) in aqueous solution following sonication for 40 min (mean n=3 ± SD)

For quantitation, a linear relationship between LEPs concentration (0 – 25 µg/mL) and turbidity was established (R2 value of 0.999), as shown in **Figure 3**. Calculated quantitation and detection limits were 0.67 µg/mL and 0.22 µg/mL respectively. Further assay characteristics such as accuracy, precision and robustness demonstrated that the method was fit for purpose.

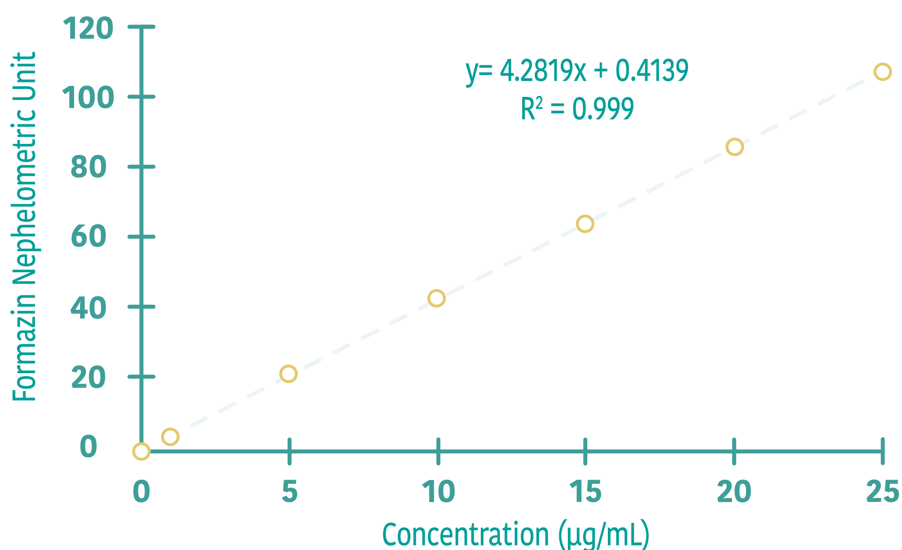

Figure 3. LEPs linearity concentration curve.

Method development work included assessments of LEPs recovery from components of the Next Generation Impactor (NGI) and the vACI. It was found that LEPs recovery from the vACI was more efficient and reproducible than the NGI. Despite surface coating of the NGI collection cups, LEPs were found to adhere strongly. The vACI is usually operated with glass Petri dishes containing 27 mL of agar, in order to enable culture of biological aerosols. However, we found that substituting agar with an equal volume of aqueous polysorbate 80 solution enabled effective, reproducible and rapid recovery of deposited nanoparticles. Prior to particle size analysis measurements, experiments were conducted to confirm that the liquid collection medium in the Petri dishes was not displaced and did not appreciably evaporate during aerosol collection tests (18 min operation). Use of liquid as a collection medium in the vACI has been reported previously [4].

Investigations of LEP suspensions aerosolised using the Micro-Cirrus jet nebuliser revealed significant hold-up in the nebuliser reservoir. This observation was consistent with findings of other consortium partners who reported surface adhesion

of the LEPs. However, of the emitted dose i.e. material deposited in the vACI, 65 % was within the fine particle fraction (FPF) i.e. % of emitted particles with an aerodynamic size of less than 5 μm, with a mass median aerodynamic diameter (MMAD) of 1.18 μm (**Table 1**). The distribution pattern of recovered nanoparticles in the vACI is shown in **Figure 4**.

Cascade impactor	FPF (%<5 μm)	MMAD (μm)	GSD
vACI	65.4 ± 2.64	1.18 ± 0.19	4.36 ± 1.29

Table 1. Summary LEPs aerosol characterisation from APSD evaluation with the viable Andersen Cascade Impactor (mean n=3, ± SD).

The key finding of aerosol generation studies was that retention of LEPs in the nebuliser reservoir was unacceptably high and an alternative nebuliser system will be evaluated. One such strategy would be to use of a vibrating mesh nebuliser, which would avoid recirculation of LEPs in the device reservoir and so help reduce particle adhesion.

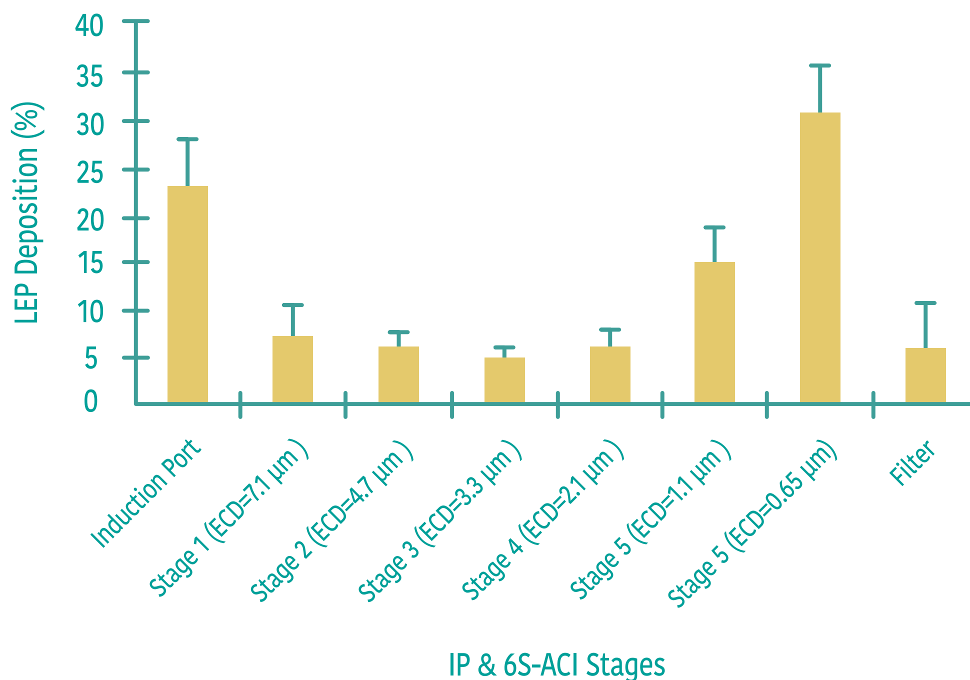

Figure 4. Recovery of LEPs (%Emitted Dose) from the vACI following delivery via the Micro-Cirrus jet nebuliser (mean n = 3, ± SD).

Conclusion

A novel application of turbidity measurement to determine suspension stability and enable quantification of LEPs, independent of their light emitting properties, is reported. Aqueous suspensions of particles, stabilised with polysorbate 80, were aerosolised using an air-jet nebuliser and characterised using a vACI. The aerodynamic properties of emitted LEPs were suitable for progression into studies to investigate excitation of aerosolised LEPs, however a significant fraction of the material was retained within the jet nebuliser. Alternative nebuliser technologies will be evaluated in future studies.

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Aknowledgement

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