# DEVELOPMENT OF DRUG-LOADED NANOPARTICLES SUITABLE FOR INHALATION AS TARGETED TREATMENTS FOR *MYCOBACTERIUM TUBERCULOSIS*

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**Background:** Tuberculosis (TB) is an infectious disease caused mainly by *Mycobacterium tuberculosis* (*Mtb*). It primarily affects the lungs but in some cases, other organs can be affected. According to the World Health Organization (WHO), millions of cases of TB are recorded yearly making it the one of the top causes of death worldwide. Nowadays, there is a rise of multi-drug resistant TB (MDR-TB), whereby patients become resistant to first-line therapy. MDR-TB cases are related to patient non-compliance to medications, due to lengthy dosage regimens and side effects. The ultimate goal of this study is to prepare Poly Lactic –*co*-Glycolic acid (PLGA) nanoparticles (NPs) suitable for delivery into the lungs via inhalation. Nanoparticles with a range of particle sizes were prepared and studied to improve targeting to the alveolar macrophages (AM), which are the host cells for *Mtb,* and enhance its biodistribution.

**Methods:** PLGA NPs loaded with a fluourescent dye (Rhodamine B) were formulated in three different sizes 200, 300 and 500 nm approximately by varying drug to polymer ratio 1:100, 1:10 and 1:50 (w/w%), the organic phase volume and stirring speed. The cellular uptake of the Rhodamine B PLGA NP was investigated using THP-1 derived macrophages, as an *in vitro* model of AMs and was confirmed by confocal microscopy using Hoechst 33342 and CellMask green dyes to stain nucleus and cytoplasm, respectively. Then, Rifampicin, as a model TB drug, was loaded into PLGA NPs using nanoprecipitation method.

**Results:** For Rhodamine B loaded PLGA NPs, a significant effect was seen on the particle size and PDI of the NPs when process parameters were altered including the organic phase volume, stirring speed and the dye content amount. Our results confirm the successful formulation of Rhodamine B-loaded PLGA NPs of sizes circa 200, 300 and 500nm for 1:100, 1:10 and 1:50 dye to polymer ratio (w/w%), respectively. Extensive cellular uptake of all Rhodamine B-loaded PLGA NPs was evident in THP-1 derived macrophages with significant distribution within the cytoplasm for all NPs. RIF loaded PLGA NPs with a particle size <200nm were then prepared. Polydispersity index (PDI) was consistent between batches and <0.2. Zeta potential was almost neutral for all formulations (-0.8 - -2.1 mV). The encapsulation efficiency (EE) of RIF, however, within the all prepared NPs was very low and current work seeks to enhance this using alternative manufacturing methods.

**Conclusions:** PLGA NPs within the size range 200-500nm demonstrated effective cellular uptake in AMs. Data obtained from confocal microcopy indicates no difference in the uptake among different sized particles. However, further quantitative analysis, using techniques such as high content cell analysis currently underway will better determine the correlation between the size of NPs and cellular uptake. Alternative manufacturing methods including double-emulsion solvent evaporation (DESE), microfluidics and supercritical fluid-based approaches are being explored to increase drug encapsulation efficiency and scale-up the process.