Nitazoxanide-Loaded PLGA Nanoparticles for Therapeutic Intervention in Visceral Leishmaniasis

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Purpose
To develop and characterize PLGA nanoparticles loaded with Nitazoxanide (NTX) for use against visceral leishmaniasis.

Methods
NTX-loaded nanoparticles were prepared by emulsification-solvent diffusion employing Poloxamer 188 as a stabilizer. Taguchi orthogonal array (OA) experimental design was employed to evaluate process variables and optimize the nanoformulation using Design-Expert® software (Version 8.02). The average particle size (nm), zeta potential ($\zeta$) and polydispersity index (PDI) were evaluated using a Zetasizer Nano-ZS (Malvern). Size and morphology were further evaluated using transmission electron microscopy (TEM; Jeol, JEM-1400) and atomic force microscopy (AFM; A.P.E. Research). NTX content and entrapment efficiency (EE) were determined by a validated HPLC method. Uptake of nanoparticles in vitro was assessed in THP-1 derived macrophages, using flow cytometry (FACS Aria, BD Biosciences).

Results
Monodisperse (PDI=0.09±0.03) nanoparticles of size 154.2±5.7 nm and a negative $\zeta$ of -10.3±0.84 mV were reproducibly prepared. EE was 94.6±0.43% at 5% w/w drug loading. Formulations were size-stable on storage at 4°C over the last 120 days, and the study is continuing. Taguchi OA revealed that the mean diameter of particles increased with increase in the proportion of the polymer, but decreased with increase in the volume of organic phase. The in vitro drug release study by membrane dialysis demonstrated that NTX release was retarded when the polymer concentration was increased. After 24h 83.1% drug was released when drug: polymer ratio was 1:5 but only 46.5% and 39.1% drug was released when it was 1:10 and 1:20 respectively. The formulation exhibited significantly less haemolytic activity compared to plain drug at 200 $\mu$g/ml (P<0.05) upon incubation with rat RBCs. Flow cytometry results indicate targeting of nanoparticles to macrophages.

Conclusion
The nanoparticles meet pre-defined desirability criteria in terms of size, encapsulation efficiency and storage stability. Uptake of nanoparticles by cultured macrophages suggests that the formulation is suitable for efficient delivery of NTX to the cytosol of the Leishmania-infected macrophage. Preclinical development of the proposed formulation is required to investigate efficacy and toxicity in vivo.