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Corresponding Author: Dr Boitumelo Semete, PhD (Biochemistry)

Corresponding Author's Institution: CSIR

First Author: Boitumelo Semete, Phd

Order of Authors: Boitumelo Semete, Phd; Lonji Kalombo, MSc; Lebogang Katata, PhD; Paul Chelule, PhD; Laetitia Booysen, MSc; Yolandy Lemmer, MSc; Hulda Swai, PhD

**Abstract:** Current therapeutic management of tuberculosis is inadequate due to non-compliance, lengthy course of treatment and drug-related side effects. In order to address these setbacks, we are developing a nanotechnology drug delivery system whereby anti-TB drugs can be administered in a single dose that maintains an active level of the drug for at least a week. Poly(lactic-co-glycolic acid) (PLGA) was used to prepare a multiple nano-emulsion which was subsequently spray dried or lyophilized via freeze drying. The resulting nanoparticles were characterized based on size, zeta potential, morphology and drug encapsulation efficiency. Particle uptake was analyzed in CaCo-2 cells via confocal microscopy, as well as in vivo, by analyzing macrophages of peritoneum cells of mice. Furthermore, the drug release profile was analyzed in vivo.

Nano-sized polymeric PLGA particles of size ranging from 200-300 nm (zeta potential ~16 mV) were synthesized, with a smooth spherical surface and a drug encapsulation efficiency of 50-65% for INH and RIF. It was observed from the in vitro release assays, that the drugs were released in a slow manner compared to free drugs. The particles were taken up in vitro by CaCo-2 cells as well as in vivo by macrophages of the peritoneum. The uptake of the particles by cells will enable improved bioavailability of the encapsulated drugs, in that intracellular drug release can be obtained.

## Potential of improving the treatment of Tuberculosis through Nanomedicine

Boitumelo Semete <sup>1</sup>, Lonji Kalombo <sup>1</sup>, Lebogang Katata <sup>1</sup>, Paul Chelule <sup>1</sup>, Laetitia Booysen <sup>1,2</sup>,  
Yolandy Lemmer <sup>1,3</sup>, Hulda. S Swai <sup>1</sup>

<sup>1</sup>Council for Scientific and Industrial Research (CSIR), Polymers and Bioceramics, Pretoria,  
0001, South Africa

<sup>2</sup>Department of Pharmaceutics, North-West University, Potchefstroom Campus, Potchefstroom,  
2520, South Africa

<sup>3</sup>Department of Biochemistry, University of Pretoria, Pretoria, 0001, South Africa

Corresponding Author  
Boitumelo Semete

### Physical address

CSIR  
Meiring Naude Road  
Building 14C  
Brummeria  
Pretoria  
0001

### Postal address

P O Box 395  
Pretoria  
0001  
South Africa

Tel: 27 12 841 2601

Fax 27 12 841 3553

Email: [bsemete@csir.co.za](mailto:bsemete@csir.co.za)

## Abstract

Current therapeutic management of tuberculosis is inadequate due to non-compliance, lengthy course of treatment and drug-related side effects. In order to address these setbacks, we are developing a nanotechnology drug delivery system whereby anti-TB drugs can be administered in a single dose that maintains an active level of the drug for at least a week. Poly(lactic-co-glycolic acid) (PLGA) was used to prepare a multiple nano-emulsion which was subsequently spray dried or lyophilized via freeze drying. The resulting nanoparticles were characterized based on size, zeta potential, morphology and drug encapsulation efficiency. Particle uptake was analyzed in CaCo-2 cells via confocal microscopy, as well as *in vivo*, by analyzing macrophages of peritoneum cells of mice. Furthermore, the drug release profile was analyzed *in vivo*.

Nano-sized polymeric PLGA particles of size ranging from 200-300 nm (zeta potential ~16 mV) were synthesized, with a smooth spherical surface and a drug encapsulation efficiency of 50-65% for INH and RIF. It was observed from the *in vitro* release assays, that the drugs were released in a slow manner compared to free drugs. The particles were taken up *in vitro* by CaCo-2 cells as well as *in vivo* by macrophages of the peritoneum. The uptake of the particles by cells will enable improved bioavailability of the encapsulated drugs, in that intracellular drug release can be obtained.

Key words: PLGA nanoparticles; Tuberculosis; Nanomedicine; Drug release

## **List of abbreviations**

ECRA: Ethics Committee for Research on Animals

EE: Encapsulation Efficiency

ETB: Ethambutol

FACS: Fluorescence activation cell sorting

FBS: Fetal bovine serum

FD: Freeze dry

HPLC: High Performance Liquid Chromatography

INH: Isoniazid

Ip: intraperitoneal

MDR-TB: Multidrug Resistant Tuberculosis

MIC: Minimum inhibitory concentration

M.tb: Mycobacterium tuberculosis

Mt: Total mass of drug

Mu: Mass of unencapsulated drug

MRC: Medical Research Council

PBS: Phosphate buffered saline

PECS: Peritoneal exudates cells

PEG: Poly(ethyleneglycol)

PYR: Pyrazinamide

PLGA: Poly(lactic-co-glycolic acid)

RIF: Rifampicin

SD: Spray dry

TB: Tuberculosis

TEER: Transepithelial electrical resistance

XDR-TB: Extremely Drug Resistant Tuberculosis

w/o/w: water - oil - water

## **Background**

Tuberculosis (TB) is currently one of the leading causes of death in South African adults. Annually, approximately 250 000 new cases of TB occur and it is ranked 5<sup>th</sup> amongst the top 22 high burden countries of the world, which collectively account for over 80% of all TB cases (WHO, 2009). A major reason for the escalation of TB is the evolution of the AIDS epidemic. It is estimated that approximately 70% of adult TB cases aged 15-49 years are HIV-infected and approximately 1.7% of new cases are multi-drug resistant (WHO, 2007). Although an effective therapeutic regimen is available, patient non-compliance resulting in treatment failure, as well as the emergence of multi-drug resistant (MDR) and extremely drug resistant (XDR) strains of TB still pose a challenge (Wright *et al.*, 2004). The first line drugs that are currently administered to treat TB include rifampicin (RIF), isoniazid (INH), pyrazinamide (PYR) and ethambutol (ETB). The current doses administered are high compared to the required minimum inhibitory concentration (MIC) of the drugs (Smith *et al.*, 1999). This is because the drugs have poor bioavailability and low permeability, which is a function of elimination or clearance of drugs before reaching their target site (Smith *et al.*, 1999). Thus the drawbacks of conventional chemotherapy necessitate the development of a carrier system which can release drugs slowly over prolonged time periods and maintain the MIC for a number of days.

The application of nanotechnology in the health care sector, also referred to as nanomedicine, has gained ground over the past 5 years. The field of drug development could potentially benefit greatly from nanomedicine in terms of addressing current shortfalls such as lack of specificity, toxicity of the therapeutic compounds, low solubility leading to lowered bioavailability and thus reduced efficacy (Langer, 2000). The nanometer size-ranges of delivery systems offer certain distinct advantages for drug delivery. Due to their sub-cellular and sub-micron size, nanoparticles can penetrate deep into tissues through fine capillaries, cross the fenestration present in the epithelial lining and are generally taken up efficiently by the cells. This allows efficient delivery of therapeutic agents to target sites in the body (Singh and Kim, 2000).

The small size also facilitates intracellular and para-cellular absorption or uptake of the particles. To date various hydrophilic surfactants such as polyethylene glycol (PEG) have been used to

enhance the bioavailability, by increasing the mean residence time of the encapsulated drug through minimizing opsonisation of the particles (Storm *et al.*, 1995). Poloxamers, poloxamines and other polymers such as chitosan can also be used for this purpose. Furthermore, the reported ability to functionalize these polymeric nanoparticles with ligands such as antibodies, peptides, carbohydrates and DNA has enabled researchers to target specific diseased cells within the body, thus further increasing the specificity and efficacy of the therapeutic drugs (Farokhzad and Langer, 2006; Torchilin, 2006).

Much of the advances in drug delivery have been primarily on cancer treatment, however various groups are working on anti-TB drug encapsulation. Dutt and Khuller (2001) formulated entrapped INH and RIF in PLGA microparticles for sustained drug delivery following subcutaneous administration. *In vivo* studies revealed sustained release of 6-7 days in mice, whereas a release of only 24hrs was observed for free drugs (Dutt and Khuller, 2001b). Pandey *et al* (2003) also encapsulated RIF, INH and PZA in PLGA nanoparticles for oral delivery by the multiple emulsion technique. The nanoparticles were administered at every 10<sup>th</sup> day and after 5 oral doses of treatment, no tubercle bacilli could be detected in the tissues (Dutt and Khuller, 2001b). This formulation achieved an encapsulation efficiency of 56.9% for RIF, 66.3% for INH and 68% for PZA (Pandey *et al.*, 2003). The group has conducted various studies in anti-TB drug delivery with very positive data. In addition, Kisich *et al.*, 2007 illustrated that encapsulated moxifloxacin accumulated in macrophages approximately three-fold times that of the free drug, and was detected in the cells for at least six times longer than free moxifloxacin at the same extracellular concentration. Inhibition of intracellular *Mycobacterium. Tuberculosis (M.tb)* growth with encapsulated moxifloxacin was achieved at the concentration of 0.1ug/ml, whereas the same effect with free moxifloxacin required a concentration of 1 ug/ml (Kisich *et al.*, 2007).

In this paper we report the encapsulation of anti-TB drugs using a novel spray drying technique as well as a freeze drying technology. The focus is specifically on RIF and INH encapsulation. We will illustrate how we have managed to modify physiochemical properties of the particles and attain sustained drug release over a period of days, both *in vitro* and *in vivo*. We further indicate

that our particles are taken up by cells and also that the activity of the drugs against *M.tb* is still maintained in the process of encapsulation.

## **Methods**

### **Preparation of PLGA nanoparticles via freeze drying**

Poly, DL, Lactic-co-Glycolic Acid (PLGA) 50:50 (Mw: 45000-75000) nanoparticles loaded with anti-tuberculosis drug were prepared using a modified multiple emulsion solvent evaporation technique (Lamprecht *et al.*, 1999) followed by freeze drying (FD). A solution of the hydrophilic drug (INH) was dissolved in 2 ml of an aqueous phosphate buffer solution (PBS) pH 7.4 was emulsified for a short period with a solution of 100mg PLGA dissolved in 8 ml of ethyl acetate (EA), by means of a high speed homogeniser (Silverson L4R) with a speed varying between 3000 and 5000rpm. The mass ratio of the drug to the polymer was 1:1. This water-in-oil (w/o) emulsion obtained was transferred into a specific volume of an aqueous solution of 1% w/v of the polyvinyl alcohol (PVA) (Mw: 13000-23000 and partially hydrolysed (87-89%)) as a stabiliser. The mixture was further emulsified for 5min by homogenisation at 5000 or 8000rpm. When hydrophobic drug (e.g. rifampicin) was used, it was dissolved together with PLGA in the organic solvent and the mass ratio polymer/drug was kept constant to 1:2. The water-in-oil-in water (w/o/w) double emulsion obtained was gently stirred overnight at room temperature to remove the organic solvent. Thereafter, drug-loaded PLGA nanoparticles were harvested by ultracentrifugation (37000g for 15min) followed by a series of wash steps with de-ionised water. The recovered pellet was re-dispersed in a small volume of de-ionised water to be lyophilized for 3 days.

### **Preparation of PLGA nanoparticles via spray drying**

An extensive investigation has been conducted on another method of preparing PLGA nanoparticles via spray drying (SD), where the double emulsion (w/o/w) obtained was directly fed into a bench top Buchi mini-spray dryer (Model B-290) and spray dried at a temperature ranging between 95 and 110°C, with an atomizing pressure varying between 6 and 7bars. PEG was used in the formulation as an excipient to increase the *in vivo* residence time of nanoparticles in the blood stream (Torchilin and Trubetskoy, 1995). In order to further increase the residence

time and enhance uptake of nanocarriers through the gastro-intestinal tract, nanoparticles were coated with a mucoadhesive and positively charged polysaccharide, i.e. chitosan as recommended in previous reports (Cui *et al.*, 2006;Takeuchi *et al.*, 2005). The summary of formulations investigated is presented in Table 1.

## **Particle characterization**

### **Particle Size and Zeta potential**

Particle size and distribution were measured by Dynamic Laser Scattering or Photon Correlation Spectroscopy using Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, UK). For each sample 1-3mg of nanoparticles were suspended in filtered water (0.2µm filter), then vortexed and/or sonicated for few minutes. Each sample was measured in triplicate. The zeta potential was also determined using the same instrument.

### **Determination of encapsulation and loading efficiency**

To determine drug concentration in the particles, i.e the encapsulation efficiency (EE), an indirect method was used. For that 10 mg of nanoparticles were suspended in a small volume of de-ionized water (10ml) and washed by centrifugation to remove the un-encapsulated drug. The supernatant was analyzed using a UV-spectrophotometer (UV-Vis, Thermo Spectronic Helios) set at a corresponding wavelength of the specific drug (e.g. 262 and 330nm for INH and RIF: respectively). Drug concentrations were determined using a standard calibration curve. The amount of drug incorporated into polymeric nanoparticles is then deduced from the difference between the initial amount put in the formulation and the amount found in the supernatant after washing of nanoparticles. Drug encapsulation and loading efficiency were calculated as follows:

$$EE = [(M_t - M_u) / M_t] \times 100$$

EE = Encapsulation efficiency in (%),  $M_t$  = Total mass of drug used in the formulation,  $M_u$  = Mass of un-encapsulated drug obtained from the supernatant.



$$\text{Drug loading (in \%)} = (\text{MD}/\text{MT}) \times 100$$

MD = Actual mass of drug encapsulated into nanoparticles and MT = Mass of nanoparticles

### **Analysis of surface morphology**

Surface morphology of anti-TB drugs-loaded PLGA nanoparticles was studied by scanning electron microscopy (LEO 1525 Field Emission SEM).

### ***In vitro* release studies**

*In vitro* drug release of INH and RIF was performed in phosphate buffered saline (0.1 M PBS, pH 7.4). In this method, three replicates of each sample containing nanoparticles a known concentration of RIF and INH were dispersed in 100 ml of PBS in a shaker incubated at 37°C. All the assays were performed in triplicate. At specified intervals, 500 µl of sample was sampled and replaced with the same volume of fresh PBS. The collected samples were analyzed by HPLC using the method adapted from Mohan *et al.* 2003 with minor modifications (Mohan *et al.*, 2003). The second method involved placing the suspended particles in a dialysis membrane with a molecular weight cut off of 10-12 kDa, and immersing the membrane into a PBS solution. For positive controls, 10mg of each of the drugs was dialysed into 100 ml of PBS. Sample collection and analysis was performed in a similar manner as that of PBS suspensions.

### **Particle uptake studies**

#### ***In vitro* particle uptake**

Particle uptake studies were performed in CaCo-2 cells, a colon carcinoma cell line purchased from Highveld Biologicals. The cells were cultured in 25 cm<sup>3</sup> flasks at 37°C in humidified atmosphere (90% humidity), 5% CO<sub>2</sub>, DMEM, 1% non-essential amino acid, 1% glutamine, 10% Fetal bovine serum (FBS). (Penicillin, 100U/ml and streptomycin, 100 µg/ml). Stocks of the cells were prepared in culture medium, containing 20% FBS, and 10% sterile glycerol. The cells were maintained according to routine cell culture procedures.

For the analysis of particle uptake, cells were seeded onto a polycarbonate filter (0.4  $\mu\text{m}$  pores, 4.71  $\text{cm}^2$ -Costar/Millicell (Millipore, Co.) at a density of  $1 \times 10^5$  cells/ $\text{cm}^2$  in the same conditions as above. The transepithelial electrical resistance (TEER) was measured with the Millicell-ERS (Millipore, Co.) The values measures were an indication of the confluency of the cells and how tight the junctions are between the cells. A TEER measurement  $>250 \Omega/\text{cm}^2$  indicated the formation of an intact monolayer with tight enough junctions to perform the drug transport studies (Artursson *et al.*, 1996). The cells were grown for approximately 25 days in order to get to a value close to  $200 \Omega/\text{cm}^2$ . Once this value was obtained the cells were analyzed by confocal microscopy. In order to visualize the lysosomes, the cells we stained with 1X lysotracker green, following the incubation with the nanoparticles. Particles that were labeled with Rhodamine 6G were used in this experiment. The incubations were performed over a 3-hr period.

### ***In vivo* particle uptake**

*In vivo* studies in Balb/c mice were approved by the Ethics Committee for Research on Animals (ECRA) of the South African Medical Research Council (MRC). The procedures followed in these studies were in accordance with institutional guidelines and mice weighing between 20-25 g were selected and housed under standard environment conditions at ambient temperature of  $25(^{\circ}\text{C})$ , and supplied with food and water *ad libitum*. Rhodamine 6G labeled PLGA nanoparticles were administered via the oral route once daily over five days and another group via the intraperitoneal (ip) route once to unchallenged Balb/C mice. Thioglycolate was administered via the oral and intraperitoneal routes respectively for the detection of activated macrophages. The cells were harvested after peritoneal lavage. The particle uptake by macrophages was determined via fluorescence activation cell sorting (FACS). Antibodies specific to macrophage and specifically phagocytotic macrophages, anti-MOMA-2 and anti-CD11c were utilized for the distinction of macrophage cells from the rest of the peritoneal exudate cell (PECS) population.

### **Determination *in vitro* efficacy**

#### ***Direct BACTEC 460***

BACTEC 12B vials (Becton Dickinson) were primed with 5%  $\text{CO}_2$  and 100  $\mu\text{l}$  the antimicrobial, which contains Polymyxin B, Amphotericin B, Naladixic acid, Trimethoprim, Azlocillin

(PANTA™ Becton Dickinson) in reconstitution fluid. 100ul of McFarland No.1 stock of the H<sub>37</sub>RV (1X10<sup>8</sup>/ml) was added to each vial and incubated at 37°C. Control vials were treated with reported MIC concentration of RIF and INH and the samples vials were treated with the same drug concentration however the drugs in this case were encapsulated in PLGA nanoparticles. Three vials were used for each group. Two controls were included, one a 10 X dilution and the second at 100 X dilution of the stock. Readings were taken daily until the bacterial growth index reached a maximum in the control samples.

### **Determination of *in vivo* release profile**

PLGA-drug loaded nanoparticles were administered via the oral gavage to unchallenged Laca mice on day 1 at a therapeutic dose of RIF 12 mg/kg, INH 10 mg/kg and PZA 25 mg/kg. Mice were divided into two groups. One group received free RIF, INH and PZA and that the other received PLGA-RIF, INH and PZA. Mice were bled via the retro-orbital plexus once each day for a period of 5 days. Drug concentrations were determined in plasma by LC-MS.

## **Results and Discussion**

### **Particle Characterization**

#### **Particle size, size distribution (SD) and zeta potentials (ZP),**

The size of nanoparticles was optimized against a benchmark of 500nm as it is reported that smaller particles (average size < 500nm) undergo passive diffusion through cells and different tissues in the body (Jani, 1990). Various parameters were optimized to obtain an average particle size ranging between 200 and 350 nm, with an average polydispersity index of 0.2. The increase of shear rate by increasing the speed of the homogenizer caused a decrease in particle size.

All samples made via freeze drying showed a negative zeta potential. The addition of chitosan to provide positive surface charge resulted in microparticles (data not shown). This problem has been alleviated by spray drying the double emulsion containing chitosan and PEG in the formulation (i.e. D and F in Table 2). Although RIF was loaded in the polymeric matrix of the carrier, there were no significant differences with INH-loaded nanocarriers in terms of all characteristics investigated, i.e size, size distribution and Zeta potential.

### **Encapsulation efficiency (EE)**

As indicated in Table 2, a considerable fraction of the active compound (i.e. INH) was incorporated into the nanoparticles. The encapsulation was improved by increasing the amount of PVA molecules for the same amount of particles. This might be attributed to a better coverage of the stabilizer onto the surface of particles that might limit the escape of drug molecules from the carrier during the preparation process.

Although RIF is highly hydrophobic, which should imply high encapsulation efficiency attributed to its low diffusion rate through the aqueous phase, its encapsulation is still relatively low when compared to INH samples. This might be attributed to the fact that it is located in the polymeric shell (as opposed to the inner aqueous core), which might make it susceptible to loss during washing. The current EE for freeze dried samples is at 60%, where for spray dried samples it is 70%. Further work in improving the EE is in progress.

### **Analysis of surface morphology**

As shown in Table 2, high volume of stabilizer i.e PVA, led to well distributed and uniform INH-loaded PLGA nanoparticles with an average size around 200nm, characterized by a very smooth surface as depicted by the SEM image in Figure 2, as opposed to particles made with low volume of stabilizer (see Figure 1).

It has been reported that small size (less than 500nm) and a spherical shape give rise to an enhanced efficiency of cell internalization. A spherical particle possesses the right curvature allowing its attachment onto the cell (Trewyn *et al.*, 2008). It is equally admitted that spherical particles offer maximum volume for drug incorporation. We also observed that the use of lactose in the spray dried formulation helped to decrease the dimpling effect that was previously observed on both INH and RIF-loaded nanoparticles (SEM results not shown).

### ***In vitro* drug release profile**

Minimal drug release was observed from nanoparticles suspended in PBS or in particles suspended in the dialysis membrane as depicted in Figure 3. Evidence of initial burst release can

be observed in the first 1-2 hours. The release lasted for approximately 24 hours for both drugs as depicted in Figure 3A, in contrast to free drugs whose release was complete in 6 hours (Figure 3B). Additionally, there was no significant difference in the amounts of drug released in the Freeze dried and Spray dried formulations. In the two methods, approximately 15-40% of surface-entrapped drugs were removed during the washing stage when the encapsulation efficiency was determined. Thus, the amount of drugs released from nanoparticles only accounted for less than 2% of encapsulated drugs. We postulated that the rest of the drugs (62-78%) are possibly still trapped in the nanoparticles.

Dutt and Khuller (2001) also had the same observation, where they observed that when pre-washed drug-loaded nanoparticles were suspended in PBS, there was negligible (initial 3–7% followed by <1%) *in vitro* drug release for 6 weeks (Dutt and Khuller, 2001a). Polymer biodegradation and adequate agitation usually play a crucial role in drug release from nanoparticle systems (Ahlin *et al.*, 2002). It is also hypothesized that drug release from NPs is through bioerosion of nanoparticles (Siepmann and Göpferich, 2001). Since hydrolytic enzymes were not included in PBS, the slow rate of nanoparticle degradation could be attributed to this factor. It is envisaged that faster release rate could be observed in the biological milieu with hydrolytic enzymes present.

## **Particle uptake studies**

### ***In vitro* particle uptake**

To determine particle uptake by CaCo-2 cells, cell were treated with 10 ug/ml PLGA at a density of  $1 \times 10^5$  cells/cm<sup>2</sup>. It was evident as indicated in Figure 4 that the particles of the specified size range were taken up within 30 min. However, after 60 min, a greater proportion of the particles were taken up. Based on this data, it can be suggested that the Rhodamine loaded nanoparticles co-localized with the lysosomes, as indicated by the orange colour in Figure 4.

Our results are in agreement with similar reports where it has been illustrated that nanoparticles are taken up by cells (Kisich *et al.*, 2007). This results support the data by Kisich *et al.* 2007, where it was illustrated that free drugs did not accumulate in the cells at the same concentration

as encapsulated drugs. Thus, nanoparticulate encapsulation of drugs may enhance drug uptake into the cells. The use of CaCo-2 cells in this specific study enabled the *in vitro* analysis of uptake in a cell model of the intestine. Thus, it can be suggested that the prepared particles should be taken up by cells of the intestine in patients.

### ***In vivo* particle uptake**

An *in vivo* method was followed as described in section 2.4.2 to confirm particle uptake. Initially macrophages were characterized from the subpopulation of PECS as indicated in Figure 5B, using thioglycolate induced macrophage proliferation a positive control and as well as anti-CD11c and MOMA-2 to characterize these cells. In general macrophages are large in size and also granular, thus they are detected in the higher ranges of the forward and side scatters.

The analysis of PECS subsequent to intraperitoneal and oral administration of Rhodamine labeled PLGA nanoparticles indicated that peritoneal macrophages did take up the particle as indicated in Figure 6. The method was slightly modified in order to detect for Rhodamine fluorescence.

From this data, it can be suggested that PLGA particles are taken up *in vivo* by macrophages. This is indicated by a population of macrophages that is positive for the Rhodamine particles. Although uptake of particles by macrophages has previously been reported (Ahsan *et al.*, 2002), much of the research was conducted *in vitro*. With this approach, we were able to illustrate that subsequent to intraperitoneal and oral administration, fluorescently labeled PLGA nanoparticles of the reported size range were taken up by the macrophages of the peritoneum. Further assays still needs to be conducted to illustrate whether the uptake of the particles elicits immune response or not by analyzing the cytokine profile in mice post administration of the particles.

### ***In vitro* efficacy**

MIC concentrations of INH and RIF were added into vials inoculated with H<sub>37</sub>R<sub>V</sub>. From the reading taken on the days indicated in Figure 7, it was evident that the encapsulated drugs still exhibited *in vitro* efficacy against H<sub>37</sub>R<sub>V</sub>, comparable to the free drugs. The BACTEC 460 assay is generally conducted to analyse the susceptibility of *M.tb* to the test drugs. Similar to data

presented by Kisich *et al.*, 2007, we were able to illustrate *in vitro* that the method of encapsulation, i.e multiple emulsion freeze drying or spray drying techniques do not have any effect on the potency of the drugs.

### ***In vivo* release profile**

As depicted in Figure 8, a sustained release profile of RIF, INH and PZA was observed over a period of at least 6 days when compared to free drugs which reached levels below the MIC within 16 hrs. With encapsulated drugs, drug concentration in plasma above the MIC level of RIF and INH were sustained for 5/6 days. Although PZA levels in plasma were below the MIC, a sustained release profile was observed over the same period of 4.5 days.

The difference between our results and those of Pandey *et al.*, (2003) is the use of a novel spray drying technology as well as the surface modified of the particles to increase the residence time in the circulation, via the attachment of PEG and chitosan. The scalability of our technology also makes it feasible for easier entry into the market.

### **Conclusions**

This study presents a novel technology that can be applied to encapsulated anti-TB drugs to improve the bioavailability of the anti-TB drugs. The ability to release the drugs at the MIC concentration over a period of days, presents the promise of reducing the doses currently administered, and also minimizing the dose frequency, from a daily intake of antibiotics to a postulated once a week intake. Furthermore, we have illustrated *in vivo* that macrophages which are the cells of targeted in TB chemotherapy do take up the particles post oral administration of the nanoparticles.

Nanomedicine, presents a fields that will greatly advanced the field of drug development and drug delivery. With nano-based drug delivery systems, it is envisaged that various drugs that could not enter the market due to adverse side effects etc, can be reformulated using nanotechnology for better efficacy and lesser adverse side effects.

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## Tables

**Table 1. Different formulations investigated**

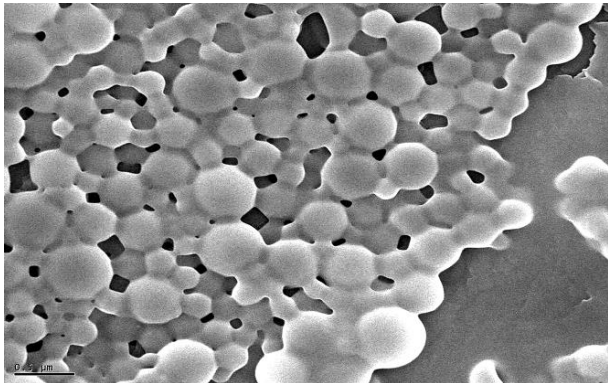
| <b>Formulation</b> | <b>1% PVA Vol (ml)</b> | <b>Speed of homogenisation (rpm)<sup>a</sup></b> | <b>Type of drying</b> | <b>Drug</b> |
|--------------------|------------------------|--|-----------------------|-------------|
| A                  | 20                     | 3000-5000  | FD                    | INH         |
| A1                 | 20                     | 5000-8000  | FD                    | INH         |
| B                  | 40                     | 3000-5000  | FD                    | INH         |
| B1                 | 40                     | 5000-8000  | FD                    | INH         |
| C                  | 40                     | 3000-5000  | FD                    | RIF         |
| C1                 | 40                     | 5000-8000  | FD                    | RIF         |
| D                  | 40                     | 5000-8000  | SD                    | INH         |
| F                  | 40                     | 5000-8000  | SD                    | INH         |

<sup>a</sup> = The first speed for first emulsion and the second refers to the speed of the second emulsion.

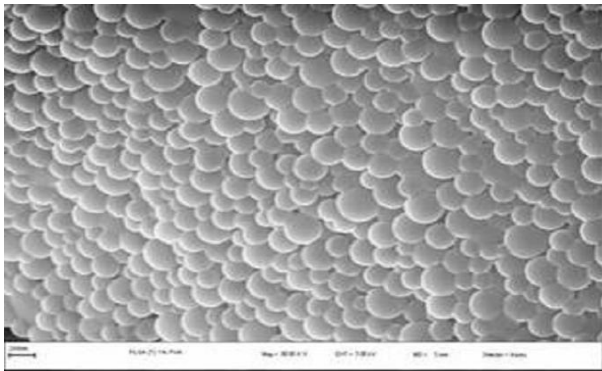
**Table 2. Characteristics of nanoparticles (n=3)**

| <b>Formulation</b> | <b>Size (nm)</b> | <b>SD</b>   | <b>ZP (mV)</b> | <b>EE</b> |
|--------------------|------------------|-------------|----------------|-----------|
| A                  | 486± 7           | 0.53 ± 0.11 | --18 ± 3       | 35 ± 4    |
| A1                 | 373± 23          | 0.27 ± 0.07 | -11 ± 3        | 51 ± 3    |
| B                  | 300 ± 9          | 0.24 ± 0.06 | -14 ± 3        | 53 ± 5    |
| B1                 | 210± 13          | 0.12 ± 0.03 | -14 ± 2        | 60 ± 3    |
| C                  | 328± 18          | 0.3 ± 0.09  | -15 ± 2        | 48 ± 3    |
| C1                 | 280± 23          | 0.21 ± 0.08 | -10 ± 4        | 58 ± 5    |
| D                  | 297± 22          | 0.15 ± 0.06 | +16 ± 2        | 65 ± 2    |
| F                  | 321± 33          | 0.21 ± 0.06 | +19 ± 1        | 70 ± 3    |

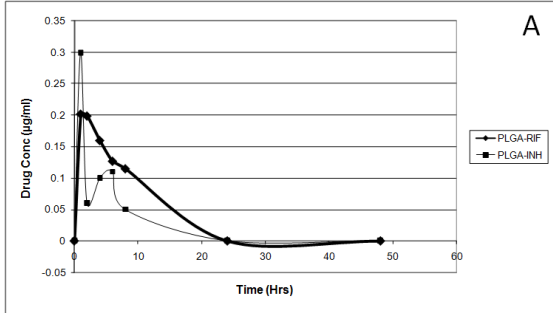
**Figures**



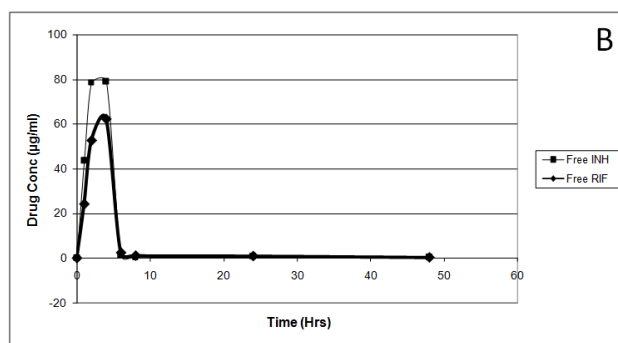
**Figure 1.** SEM picture of freeze dried formulation A1 (Prepared with 20ml PVA 1% w/w), The bar represents 200nm).



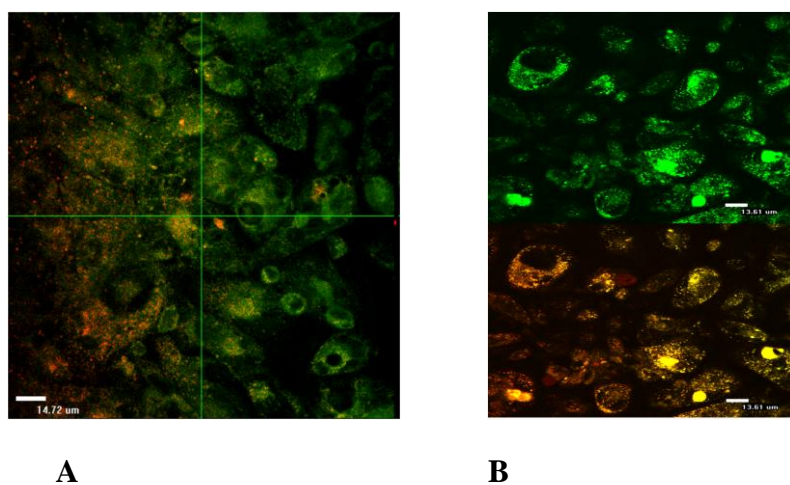
**Figure 2.** SEM picture of freeze dried formulation B1, (Prepared with 40ml 1% PVA). The bar represents 200nm.



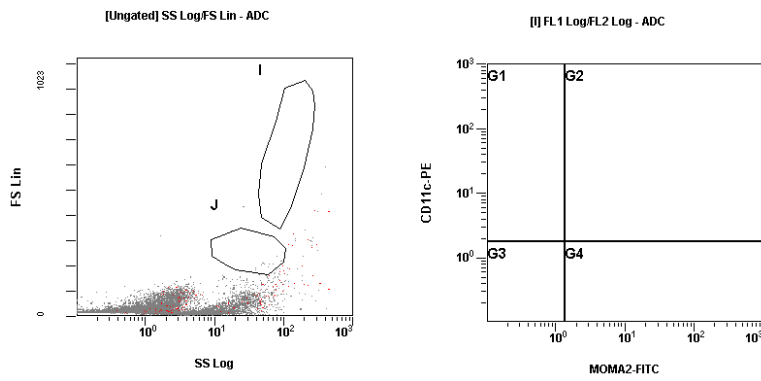
**Figure 3:A:** *In vitro* release of INH and RIF from PLGA nanoparticles suspended in 0.1M



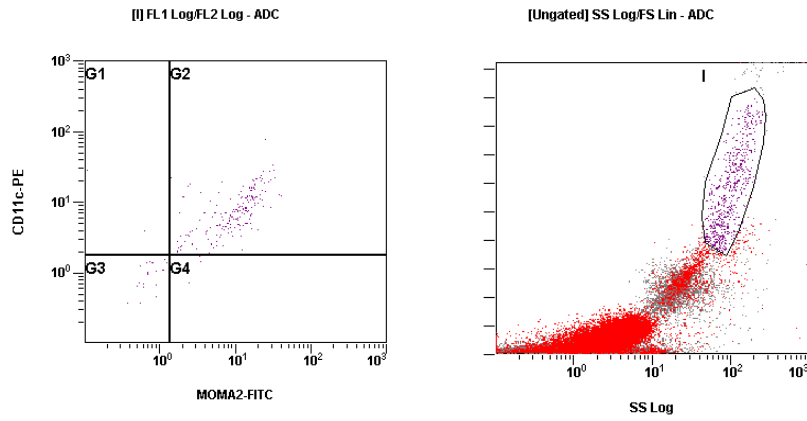
PBS pH 7.4. **B**: Positive control of free drugs following dialysis (10mg/100ml PBS) with stirring at 37 deg C.



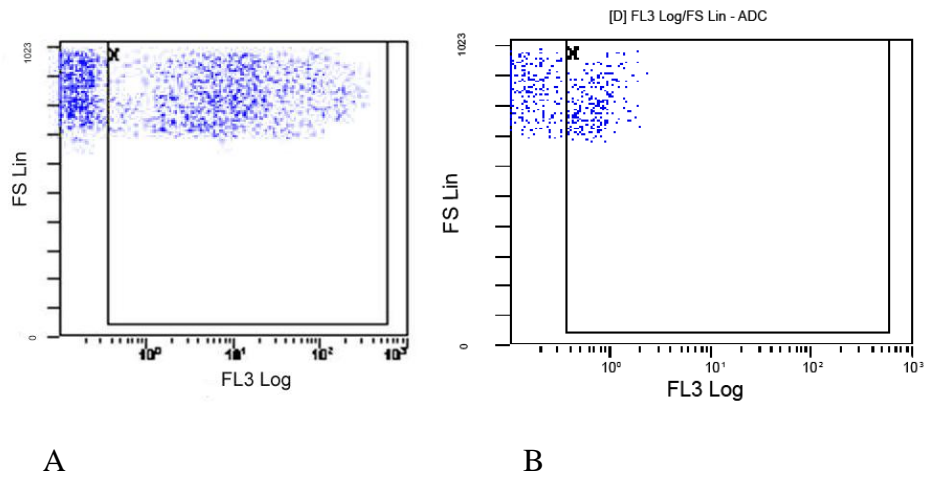
**Figure 4:** A indicates a Z-stack of 30 min incubation and B depicted a 60 min incubation period.



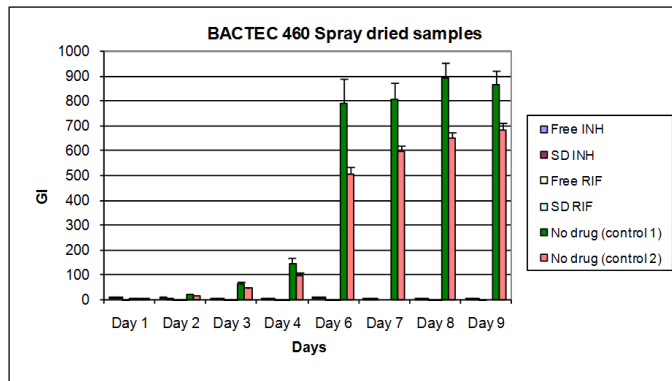
**Figure 5A:** FACS data of PECS subsequent to saline administration to mice.



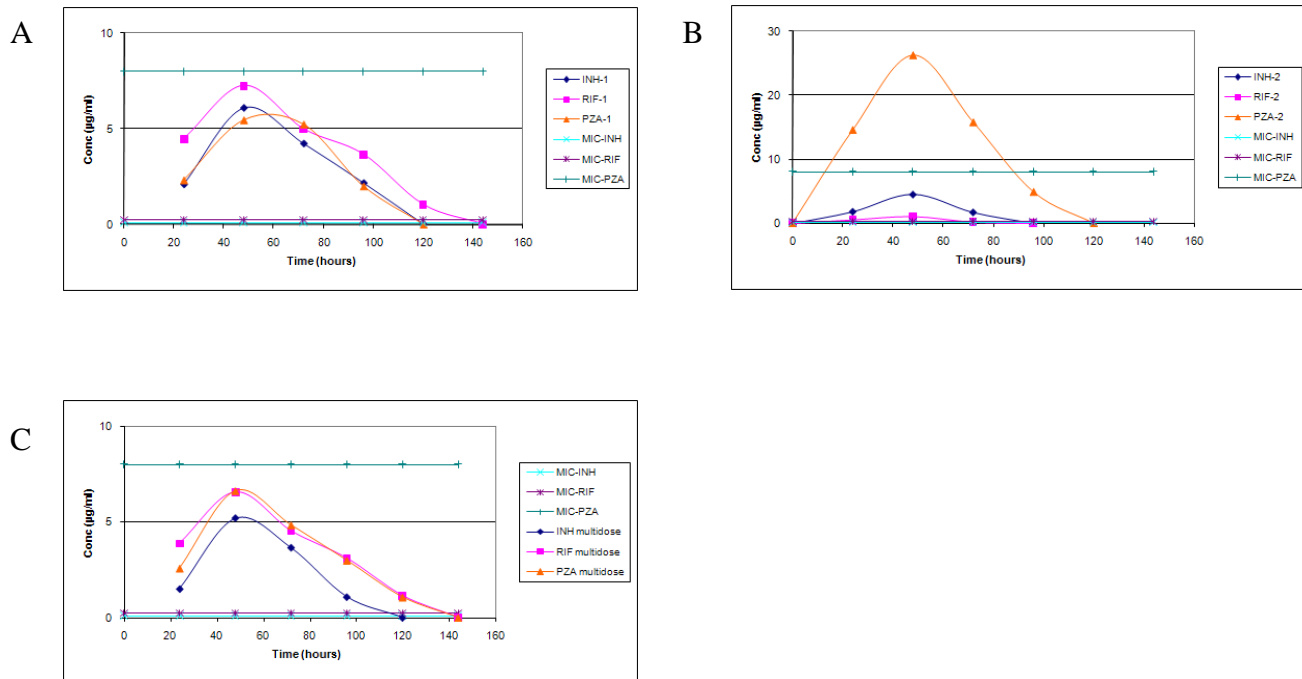
**Figure 5B:** FACS data of PECS subsequent to thioglycolate administration to mice.



**Figure 6:** A indicates macrophage cells subsequent to ip administration and B indicates oral administration of Rhodamine labeled particles



**Figure 7:** BACTEC 460 data of encapsulated RIF and INH



**Figure 8:** *in vivo* release of free drugs vs spray dried nanoparticles encapsulating RIF, INH and PZA. A. Samples with high concentration of lactose (5% w/v) and B. samples with low concentration of lactose (4% w/v). C. The multidose indicates a formulation where RIF, INH and PZA were encapsulated in combination, i.e all drug in one nanoparticle.