

CHAPTER 2- Nanoparticulate drug delivery systems and applications in the treatment of tuberculosis

Abbreviations

CSIR - Council for Scientific and Industrial Research

DDS - drug delivery systems

ELPs - elastin-like polypeptides

EMA - European Agency for the evaluation of Medicinal products

FDA - Food and Drug Administration

IM - intramuscular

LCST - lower critical solution temperature

PBCA - poly-n-butylcyanoacrylate

PIBCA - polyisobutylcyanoacrylate

PEO - polyethylene oxide

PEG - polyethylene glycol

PLA - polylactic acid

PLGA - polylactic-co-glycolic acid

PMDA - Pharmaceutical and Medical Device Agency

PNIPAAm - poly-(N-isopropylacrylamide)

PPO - polypropylene oxide

PPS - poly(propylene sulfide)

RES - reticuloendothelial system

2.1 Introduction

The promise of nanotechnology based drug delivery systems in addressing the challenges of TB chemotherapy discussed in Chapter 1 is being explored. It is hypothesised that the size and physicochemical properties of nanoparticles may improve bioavailability of the drugs by improving drug absorption (Ahmad *et al.* 2006:415), facilitate transport through biological barriers (Lockman *et al.* 2002:1) as well as enhance uptake of poorly soluble drugs (Kipp, 2004:109). In addition, the ability to chemically modify the surface of the particles has been reported to allow targeting to diseased sites (Torchilin, 2006:136). Polymeric drug delivery systems have become much more sophisticated over recent years. A system can be designed to respond to changes in the biological environment by delivering or ceasing to deliver based on these changes (Brannon-Peppas, 1997:35). Various research groups in the United States of America, India, China and South Africa are extensively exploring the potential of nanoparticles as a drug delivery vehicle for anti-tuberculosis drugs (du Toit *et al.* 2008; Dutt & Khuller 2001; Pandey *et al.* 2003b; Pandey *et al.* 2005; Swai *et al.* 2008). Furthermore, Ahmad *et al.* (2007:414) reported that sustained drug release can be achieved by nanoencapsulation of the anti-tuberculosis drugs thus introducing the possibility of reducing the duration of therapy as well as the dosing frequency (Ahmad *et al.* 2007).

The term ‘drug delivery systems’ (DDS) is an all-encompassing term describing the design of the drug, the vehicle employed for the delivery of the drug and the route of administration. DDS usually consists of a polymeric or lipid carrier system that is designed to transport drugs to their target sites. The ideal system design would be to reach its target site in such a manner that maximum therapeutic activity is provided, minimum degradation or inactivation occurs until the target site is reached and adverse reactions are minimised (Mahato, 2007:3). These factors need to be considered when designing any drug delivery system.

Classification of DDS can be done by dividing it in a broad spectrum of two groups, namely macromolecular drug carrier systems and particulate carrier systems, that includes microspheres, nanospheres and liposomes (Mahato, 2007:4). Although these are ideal objectives to strive towards, they are not easily achieved in practice since pharmacokinetic and pharmacodynamic factors of the drug (discussed in chapter 1) and the physicochemical properties of the carrier system greatly influence the success of a drug delivery system. Therefore, it is crucial to design a DDS in such a way as to control these factors. Table 2.1

briefly describes how a DDS can control and correct poor drug properties to ensure maximal targeting and efficacy. Various DDS will be described in further detail with specific focus on nanoparticulate systems since nanocarriers have revolutionized research in DDS optimisation.

Table 2.1 PK and PD parameters that can be averted through drug delivery systems. Adapted from (Allen & Cullis 2004:1820).

Limitation	Implication	Effect of DDS
Poor solubility limiting effective formulation	Since hydrophobic drugs may precipitate in aqueous media, a suitable pharmaceutical design can be difficult to achieve. Furthermore, there are toxicities associated with the use of excipients such as Cremphor	DDS such as liposomes or lipid micelles (discussed in section 2.5 and 2.6, respectively) provide both hydrophilic and hydrophobic environments, thereby enhancing drug solubility
Tissue damage on extravasation	Instant or delayed drug release of certain drugs, such as the cytotoxic drug doxorubicin can lead to tissue damage such as tissue necrosis	When drug release is regulated as is possible with DDS instant or delayed extravasation of drugs can be controlled thus reducing or eliminating tissue damage
Rapid degradation of the drug <i>in vivo</i>	Drugs such as the camptothecins lose activity at physiological pH following administration	DDS limit premature degradation and lower doses of the drug is required since the concentration that will reach the tissues will still be within effective concentrations
Unfavourable pharmacokinetics	Rapid clearance or metabolism of drugs by the kidneys as a function of poor kinetics occurs, affecting therapeutic efficacy thus requiring the use of higher doses or continuous infusion	The design of DDS can alter PK of the drug and reduce clearance
Poor biodistribution	Drugs that are well distributed in the body can affect healthy tissue, resulting in dose-limiting side effects	The particulate nature of a certain DDS design can decrease the volume of distribution thereby reducing side effects in healthy tissue

2.2 Polymeric drug delivery

Polymers are formed when smaller molecules, or monomers, are chemically linked. Both natural and synthetic polymers are used in the field of drug delivery. Synthetic polymers used to deliver drugs are lactic acid based such as polylactic acid (PLA) and poly(lactic)-co-glycolic acid (PLGA). The fact that these polymers are biodegradable contributes to their wide use in drug delivery since they don't have to be surgically removed from the body, following biodistribution. Biodegradation of polymers can be through various mechanisms, eventually leading to clearance of the monomers or metabolites via various biological pathways. Chitosan, a natural polymer derived from chitin, have also shown great success. Chitosan is of particular interest in oral and nasal drug delivery due to its mucoadhesive properties. Most natural polymers used in DDS contain reactive sites that may be

manipulated or modified for a variety of clinical applications (Dang & Leong 2006:490). These polymers can deliver a wide range of drugs to diseased tissues for prolonged periods and can avoid chronic inflammation and long term implications (Mahato, 2007:34).

The use of polymers in drug delivery is due to their versatile nature. They can be designed to control the release of the drug. Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or active agent in such a way that the active agent is released from the material in a predesigned manner (Brannon-Peppas, 1997). The release may be constant over a long period, it may be cyclic over a long period, or be triggered by the environment or other external stimuli. It is thus important to have a thorough understanding of the physical, chemical and biological properties of a polymer before formulating a controlled drug delivery device. These polymers can be used in drug targeting, by designing it in such a manner so that targeting ligands can be attached to assist in delivering a drug to a specific target site (Manolova *et al.* 2008:1411).

The focus of the research presented in this thesis is on PLGA nanoparticles formulated with chitosan coated with and without PEG. These polymers will now be discussed in further detail.

2.2.1 PLGA

Due to the many disadvantages associated with natural polymers (e.g. variations in composition and therefore properties), synthetic biodegradable polymers such as PLGA have received increased attention in the field of drug delivery due, not only to their excellent biodegradability, but also to the high degree of biocompatibility (Jain, 2000:2475).

PLGA is one of the most extensively used synthetic polymers for drug delivery applications. Examples of its wide application include tissue engineering (Wang *et al.* 2010), controlled release DDS (Oh & Lee 2007) and vaccines (Jiang *et al.* 2005), to name a few. The synthesis of PLGA involves the random ring opening polymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. Successive monomeric units of lactic and glycolic acid are linked together to form PLGA via ester linkages during the polymerization process and a resultant linear aliphatic polyester product as illustrated in Figure 2.1 is yielded (Astete, 2006:247). Lactic acid has been observed to be more hydrophobic than glycolic acid; therefore lactic-rich PLGA copolymers are less

hydrophilic, absorb less water and degrade more slowly (Jain, 2000:2476). Thus, based on the parameters required for the drug delivery system, various monomer ratios of PLGA can be applied. Furthermore, the biodegradation of the PLGA polymer is an important parameter that directly influences properties such as mechanical strength, swelling, hydrolysis and subsequent biodegradation of the polymer (Wu & Wang 2001:25). The crystallinity of the copolymer is dependent on the type and molar ratio of the monomer components in the copolymer chain (Figure 2.1) (Jalil & Nixon 1990:297).

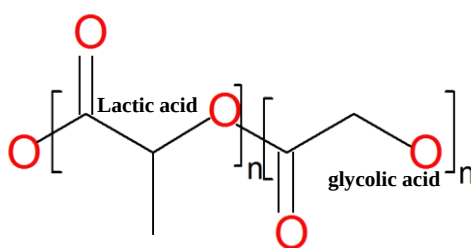


Figure 2.1 Chemical structure of PLGA indicating the two monomers, lactic acid and glycolic acid. The “n” indicates the number of times units are repeated (Jalil & Nixon 1990:297).

The nanoparticles in this study are composed of PLGA polymers with 50:50 ratios of glycolic and lactic acids, which mean they are hydrolysed much faster than those containing higher proportions of either of the two monomers (Kitchell & Wise 1985:436). PLGA copolymer undergoes bio- and hydrolytic degradation in aqueous environment (Wu & Wang 2001; Jalil & Nixon 1990; Kitchell & Wise 1985). Raghuvanshi *et al* (1993) proposed a three-phase biodegradation mechanism for PLGA; (1) random chain scission process where the molecular weight of the polymer substantially decreases, but significant weight loss and no soluble monomers are formed; (2) In the middle phase a decrease in molecular weight accompanied by rapid loss of mass and soluble oligomeric and monomer products are formed; and (3) Soluble monomer products formed from soluble oligomeric fragments. This phase is that of complete polymer solubilisation (Raghuvanshi *et al.* 1993). PLGA has also been documented as demonstrating stimuli-responsive properties. Yoo *et al* (2010:11206) reported a shape-shift of PLGA from an elliptical disk to a sphere in response to pH, temperature and chemical stimulus (Figure 2.2) (Yoo & Mitragotri 2012).

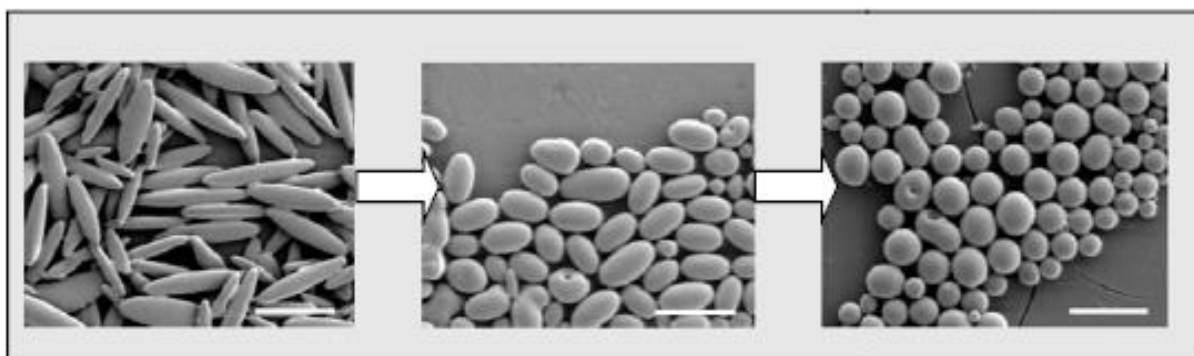


Figure 2.2 Shape switching of PLGA particles switched in deionized water at 37 °C. (Left: 0 min; Centre: after 2 min; Right: after 5 min). (Scale bar: 5μm.) Taken from (Yoo & Mitragotri 2012) with permission.

In addition to the examples listed where PLGA has been applied, it has also been extensively applied in TB drug delivery systems. Dutt and Khuller (2001:831) formulated entrapped INH and RIF in PLGA microparticles for the purpose of sustained drug delivery for subcutaneous administration. *In vivo* studies revealed sustained release of 6-7 weeks in mice, whereas a release of only 24 hours was observed for free drug. Their results possibly offer an improvement for tuberculosis chemotherapy. However, subcutaneous administration is not the ideal form of drug delivery and thus patient discomfort becomes an issue (Dut & Khuller 2001). PLGA nanoparticles have been documented to demonstrate similar controlled release results (Ahmad *et al.* 2008:145). Therefore, it could be assumed that PLGA nanoparticles are suitable candidates for drug delivery by improving the biodistribution of the drug.

2.2.2 PEG

PEG is an oligomer or polymer of ethylene oxide, which is chemically synonymous to PEO but tends to be a shorter polymer. PEG is available in different molecular weights (Mw 1000-35000). PEG can be attached various molecules such as proteins and can thus protect them from rapid hydrolysis or degradation within the body, resulting in increased blood circulation time of the bound molecules and lower immunogenicity of proteins. The process of covalently coupling PEG to other molecules is referred to as PEGylation (Li *et al.* 2001:203). PEGylation technology is now widely used in drug delivery and among the advantages mentioned above are optimized pharmacokinetics and decreased frequency of administration as a function of the improved bioavailability and blood circulation time (NOF Corporation, 2012). PEG is also helpful in increasing drug solubility (Mahato, 2007:18) and can prevent a process called opsonisation and thus increases the systemic circulation time of a particle coated with PEG. This process is described in further detail in chapter 4.

2.2.3 Chitosan as polymeric carrier

Chitosan is a natural polymer derived from the chitin of crustacean shells. Unlike other natural polymers, chitosan is positively charged and mucoadhesive which provides an explanation as to its increased interest for drug delivery. It also possesses favourable biocompatibility and does not induce an immune response. Chitosan has been used to enhance the preparation of micro and nanoparticles (Agnihotri *et al.* 2004:8) and has been investigated for its potential use in anti-tuberculosis drug delivery (Pandey & Khuller 2004). Drug release through chitosan occurs through the polymer matrix, either by diffusion of the drug through the matrix or by erosion of the polymer surface. Various pharmaceutical applications for chitosan have been reported such as colonic-targeted delivery, mucosal delivery, cancer therapy, gene delivery, topical delivery and ocular delivery (Agnihotri *et al.* 2004:18). Santhosh *et al.* (2007:72) documented the hepatoprotective property of chitosan by demonstrating in their study that chitosan co-administration normalized the INH-RIF-induced protein metabolism and hepatic antioxidant defence system. Their paper also classified chitosan as an antihepatotoxic agent (Santhosh *et al.* 2007). Coating drug-loaded particles or spheres with chitosan has the functionality of a reinforcing polymer, which retards the degradation of the polymeric shell, resulting in slow drug release kinetics. When used in conjunction with another natural polymer such as alginate, it is able to sustain the drug levels in plasma of anti-tuberculosis drugs for up to seven days.

2.3 Nanoparticle drug delivery

To date, numerous reviews on nanotechnology-based drug delivery systems have been published (Imani, 2008; Kreuter, 2007; Mohanraj & Chen 2006; Pandey & Khuller 2006; Gelperina *et al.* 2005; Sung *et al.* 2007; Medina *et al.* 2007; Brigger *et al.* 2002), including studies in the field of cancer chemotherapy, treatment of infectious diseases as well as gene and vaccine delivery. Paul Erlich first proposed the concept of nanotechnology application to drug delivery in 1891. The application was a new paradigm in pharmacotherapy for cell-targeted drug delivery. He hypothesised the treatment of pathogens in the human body with a chemical entity, which has a high affinity for the specific presenting pathogen by his so-called “magic bullets” (Couvreur & Vauthier 2006; Kayser *et al.* 2005; Winau *et al.* 2004). This theory is supported by the assumption that drug delivery to a specific target site in the human body is largely dependent on particle size, physicochemical properties, drug stability (Park, 2007) and unique recognition of the site by the particle’s modified surface.

There are already commercialized applications of this technology in the field of drug delivery (Table 2.3).

Table 2.3 Nanotechnology applications in drug delivery approved by the Food and Drug Administration (FDA) since 2000 (Gerencher, 2006; Ledet & Mandal 2012:9).

Pharmaceutical Company	Drug	Description	Application	Year approved
Abraxis Bioscience	Abraxane®	Albumin bound paclitaxel. Albumin serves as a drug carrier	Cancer drug designed as the next generation, nanoversion of Taxol®	2005
Wyeth Pharmaceuticals	Rapamune®	Nano-crystallization of the drug sirolimus	Prevents organ rejection in kidney transplant patients	2000
Merck	Emend®	Nano-crystallization of the drug aprepitant	Prevents chemotherapy-induced nausea and vomiting	2003
Novavax	Estrasorb®	Nanoemulsion of the hormone estradiol	Hormone-replacement therapy prepared as a nanoemulsion for topical administration	2003
Smith & Nephew and Nucryst Pharmaceuticals	Acticoat®	Nanosilver formulated antimicrobial	Wound dressing	2005
Elan Nanocrystal® Technology	Megace® ES	Nano-crystallization of the drug megestrol acetate	A drug designed to stimulate appetite	2004
Élan Nanocrystal® Technology	Tricor®	Nano-crystallization of the drug fenofibrate	A cholesterol-lowering drug	2004
ALZA Corporation STEALTH® Technology	Doxil®	Liposomes encapsulating doxorubicin	Anti-cancer drug	2005
UCB	Cimzia®	PEGylated fragment of a humanized anti-TNF- α antibody	Crohn's disease and rheumatoid arthritis	2008
Atrix Laboratories	Eligard®	Leuprolide acetate and PLGH-polymer formulation	Advanced prostate cancer	2002
Pfizer/Eyetch Pharmaceuticals	Macugen®	PEG-anti-VEGF aptamer	Neovascular age-related macular degeneration	2004
Roche	Mircera®	Chemically synthesized ESA, methoxy PEG-epoetin beta	Symptomatic anaemia associated with chronic kidney disease	2007
Royalty Pharma	Neulasta®	Conjugate of PEG and filgrastim	Chemotherapy-induced neutropenia	2002
Roche	Pegasys®	PEGylated interferon-alpha-2a	Hepatitis C	2002
Schering-Plough	Pegintron®	PEGylated interferon-alpha-2b	Hepatitis C	2001
Shire	Renagel®	Polyamine (polymer loaded with amine groups)	Chronic kidney disease	2000
Pfizer	Somavert®	PEGylated human growth hormone receptor antagonist	Acromegaly	2003

The physico-chemical properties of nanoparticles which are discussed in detail below have led to various research groups exploring the application of nanoparticulate drug delivery to other complex diseases, such as TB, fungal and parasitic infections, viral infections including HIV and metabolic diseases such as diabetes as well as osteoporosis (Couvreur & Vauthier 2006).

Various nanoparticulate systems have to date been engineered in the field of nanomedicine, such as ceramic and metal nanoparticles e.g., gold nanoparticle and quantum dots. Polymeric micelles, liposomes, dendrimers and nanoparticles have also been explored. All of these nanoparticles can be formulated to carry therapeutic drugs, peptide drugs, DNA and low/high molecular weight compounds (Yih & Al-Fandi 2006:1186). However, this literature review will only address polymeric biodegradable and biocompatible nanoparticles.

2.3.1 Preparation of nanoparticles

The two of the primary methods of preparing nanoparticles for drug delivery are freeze-drying of the emulsion and spray-drying of the emulsion. Nanoparticles are essentially colloidal polymeric systems and depending on the process used for preparation, either nanospheres or nanocapsules are formed. The difference between the two is in the conformation of the drug within the nanoparticle. Nanocapsules are vesicular systems where the drug is localised within the nanoparticles surrounded by a polymeric membrane. For nanospheres, a matrix system is produced where the drug is essentially distributed throughout the polymer core (Abdelwahed, 2006:1689).

2.3.1.1 Preparation via freeze-drying

Besides the preparation of nanoparticles, liposomes and nanoemulsion, freeze-drying has been used in the preparation of vaccines, viruses and proteins. The major disadvantage of the freeze-drying cycle (freezing-primary drying-secondary drying) is that it is a time consuming and expensive process. The freezing (solidification) step is where the liquid suspension/emulsion cooled to the point where the liquid becomes so viscous that it solidifies. This results in an amorphous crystalline phase (Abdelwahed *et al.* 2006:1692). During the primary drying step (sublimation step), sublimation of the ice from the frozen product occurs and a porous plug is formed (Abdelwahed *et al.* 2006:1693). Secondary drying is the final step where water absorbed to the product is removed. According to

Abdelwahed *et al* (2006:1693) freeze-dried nanoparticles should have three main characteristics. Firstly, the primary chemical and physical characteristic of the product which includes an elegant cake appearance, a short reconstitution time, low/unmodified particle size distribution within the suspension and unaffected activity of the encapsulated drug. Secondly, the nanoparticles produced should have an acceptable relative humidity and thirdly, display long-term stability. Examples of successfully freeze-dried nanoparticle methods include nanoprecipitation (Saez *et al.* 2000:379; de Chasteigner *et al.* 1996:116), salting out (De Jaeghere *et al.* 1999:859), double emulsion (Zambaux *et al.* 1999:109), micro-emulsion method (Cui, 2003:689), emulsion-evaporation (Lemarchand *et al.* 2003:1284), polymerization (Roy *et al.* 1997:165) and melt homogenization method (Lim, 2002:135). Despite these successes, there remains the disadvantage of freeze-drying in which the encapsulated drug influences the freeze-drying process and the lyophilisation process could cause the encapsulated drug to leak out or degrade as is the case with proteins (Abdelwahed *et al.* 2006:1699).

2.3.1.2 Preparation via the spray-drying route

Spray-drying is a form of particle engineering to obtain particles of a certain size and shape for use in various applications among which pharmaceutical drug delivery (Vehring, 2008:999). Particles formed by means of spray-drying are spherical in shape and this shape can be described by their geometric diameter (Vehring, 2008:1000). Spray-drying is similar to spray-pyrolysis, which is a liquid-to-particle conversion (Okuyama & Longgoro 2003:538). The only difference between the two is the precursors used. The method involves droplets atomized from the starting solution introduced to heat. The final product is formed by evaporation of the solvent, diffusion of solute, drying, precipitation, reaction between precursor and surrounding gas. Furthermore, pyrolysis, or sintering may occur (Okuyama & Longgoro 2003:538). Precursors typically used in spray-drying are colloidal particles or solutions. If the suspension used consists of colloidal nanoparticles as primary particles, the resultant product will also comprise of nanoparticles (Okuyama & Longgoro 2003:538). Therefore spray-drying is a suitable method of nanoparticle preparation, the basic principle being that one droplet forms one particle. This method is both cost and time effective. Nanoparticles have been successfully formulated by double emulsion solvent evaporation (Lamprecht *et al.* 1999:97; Kalombo, 2008:1), nanoprecipitation (Raffin-Pohlmann *et al.* 2002:305; Stanisçuaski-Guterres *et al.* 2000:195), salting out (McCarron *et al.* 2006:480) and polymerization (Kriwet *et al.* 1998:149).

2.3.2 Properties of nanoparticles

Ultrafine particles or nanoparticles are in the size range of 10-100nm (Okuyama & Longgoro 2003:537). However, in pharmaceutical applications, nanoparticles are defined as particulate dispersions or solid particles ranging from 10-500nm in size (Pison *et al.* 2006:343) or even 10-1000nm as literature have shown (Ledet & Mandal 2012:7). Materials formulated or existing at a nanometre scale exploits novel physical, chemical and biological properties and these affects pharmaceutical factors (Pison *et al.* 2006:342). Among the methods currently used to formulate and manufacture nanoparticles are emulsion solvent evaporation techniques and/or solvent exchange, salting out and supercritical technology as previously described (section 2.3.1). The challenges in the application of these techniques include aspects such as low drug loading capacity and encapsulation efficiency as well as poor control of the uniformity of particle size distribution and the use of solvents (Park, 2007), which greatly affect pharmaceutical parameters of the encapsulated drugs.

The encapsulation of a drug within a biodegradable polymer has been reported to minimize first pass metabolism via protection of the drug in the core of the polymeric shell (Couvreur & Vauthier 2006). The nano-size range of particles provides improved drug absorption due to the intracellular delivery of the drug and passive targeting of specific tissues or cells that macromolecules or drugs generally do not reach. It is also possible to facilitate active targeting to a specific site. This is accomplished by functionalizing the nanoparticle surface with specific molecules or ligands such as monoclonal antibodies, RNA/DNA aptamers or peptides to enhance binding and interactions with specific receptors which are expressed by the cell populations at the diseased site (Kingsley *et al.* 2006:344).

Near zero-order release kinetics, modification and improvement of pharmacokinetic parameters such as tissue distribution profile and the capacity to apply a variety of architectures during formulation are also achieved through nanoparticulate drug delivery (Couvreur & Vauthier 2006). It has also been reported that nanoparticles improve bioavailability by eliminating food effects on absorption (Wu *et al.* 2004:135).

Most chemotherapeutic agents such anti-tuberculosis and anti-cancer agents cause dose-dependent side effects. Nano-based drug delivery systems present the capacity to be formulated for the purpose of controlled release (Pandey & Khuller 2005:230), therefore

posing the possibility to reduce dose frequency and subsequent dose-related side effects (Medina *et al.* 2007:553). In addition, nanoparticles present pharmaceutical improvement as drug carrier systems in that they can improve drug stability (long shelf-life), have a high carrying capacity (ability to encapsulate large quantities of drug molecules), incorporate hydrophilic and hydrophobic substances and also tailored variable routes of administration (Gelperina *et al.* 2005:1487). Properties of nanoparticles that may affect PK and pharmaceutic parameters of drugs include molecular weight, crystallinity, porosity, particle size, surface properties, zeta potential and controlled release. These properties are all interlinked and collectively affect PK in formulations.

2.3.2.1 Molecular weight

The analysis of polymer molecular weight distribution has been used to elucidate the possible transport and clearance mechanism of the nanoparticles, the interaction between the drug and polymer as well as the mechanism of degradation of the nanoparticles. Molecular weight also has an effect on the glass transition temperature and the crystallinity of the particles and hence will affect the degradation profiles of the particles (O'Donnell & McGinity 1997:25). Low molecular weight particles have been reported to exhibit an increase in degradation products within a short period of incubation and the oligomers making up the particles decrease in molecular weight. However, with particles made up of high molecular weight polymers, the molecular weight remains constant for longer and degradation occurs at a slower rate when compared to low molecular weight polymers.

Degradation has been observed to be the main factor for drug release in low molecular weight polymers after the initial burst stage. On the other hand, high molecular weight polymers follow a slow drug release profile as a function of diffusion followed by the main drug release in the inner matrix due to polymer degradation. Various authors have explored the possibility of using low molecular weight polymers in combination with high molecular weight polymers to obtain a desired two phase release profile (O'Donnell & McGinity 1997).

It has been documented that polymeric nanoparticles made up of numerous small oligomers with a molecular weight of between 600-3000 Dalton (Da) are more easily eliminated and also results in a reduced overload of the RES (Couvreur *et al.* 1986:147) when these particles are administered intravenously. Therefore, molecular weight analysis is vital in predicting the drug release kinetics and drug dissolution, which are important parameters in dosage form

design (Vila *et al.* 2004:124). These pharmacokinetic factors can be tailored to obtain a longer lifespan in the blood circulation by varying the molecular weight of the polymers.

2.3.2.2 Crystallinity

Crystallinity in nanoparticle formulation is an important consideration during the development process that greatly affects the solubility and dissolution characteristics of the drug. Amorphous or non-crystalline forms (Mahato, 2007:21) of the particles may present faster dissolution rates compared to the crystalline forms. Therefore, it is vital that the crystallinity of the polymer be determined prior to dosage form design and the crystallinity of the specific formulation after manufacture. Also, in some cases amorphous particles may result in increased bioavailability compared to crystalline forms since the amorphous form of a compound is always more soluble and shows a higher dissolution rate than the corresponding crystal form (Mahato, 2007:21; York, 2002).

When considering the crystallinity of polymeric nanoparticles it is suggested that degradation occurs first in the amorphous regions of the particle, followed by a slower degradation in the crystalline regions. This observation thus suggests that the crystallinity of the polymer affects the degradation rate and drug release kinetics. Izumikawa *et al.* (1991:137) observed that at low drug loading, the polymer dominated the crystalline properties of the particles and no crystallinity was observed for the drug. At high drug loading capacity, crystallinity was dependent on the organic solvent removal process, i.e. at slow solvent removal rates particle crystallinity was observed for both the drug and the polymer, but faster removal resulted in amorphous spheres (Izumikawa *et al.* 1991). Another consideration is the fact that a particle can exist in more than one form depending on the micro environment (York, 2002).

In addition, during the preparation and storage of encapsulated drug particles, drug polymorphs could be present, resulting in varied release kinetics and glass transition temperatures (Yu *et al.* 1998:118). The importance of different polymorphic forms is that they are capable of changing from a metastable to a stable polymorphic form with consequent changes in parameters such as melting and boiling point, as well as impacting on release kinetics and bioavailability. These are very important factors in dosage form design because the different crystalline states may exhibit different activities of the encapsulated drugs (Mahato, 2007:22).

2.3.1.4 Porosity

In general, nanoparticles are prepared by an oil-in-water (o/w) or oil-in-water-in-oil (o/w/o) emulsion–solvent evaporation method. During this process, when the solvent is removed via evaporation, it results in pores within the particles. This can significantly affect the dissolution and diffusion of the encapsulated drug. It has been suggested that during the release of a drug that is in close proximity to the pores of the particles, the drug would diffuse out of the pores. However, for drug molecules located within the network of pores, they would have to first diffuse to the closest pores, and then be released to the outside (Lemaire *et al.* 2003:100). The longer diffusion path of these drug molecules would then affect the effective restricted diffusion coefficient of the drug, which is a function of the diffusivity of the drug, the porosity (ϵ) and the tortuosity (τ) of the matrix (Veith *et al.* 2004:220). All of these factors play a major role in predicting the release kinetics of the drug, which is a vital parameter to elucidate primarily in controlled release systems such as nano-based delivery systems.

Sant *et al.* (2005:203) conducted a porosity study involving nanoparticles encapsulating propafenone hydrochloride, a drug used in cardiac arrhythmia. These formulations contained varying amounts of triethylamine as well as a control where no triethylamine was incorporated. The studies showed that the porosity of the different formulations varied as a function of percentage drug loading, with a higher drug loading showing a higher volume contribution of smaller pores. The pore structure was complex revealing a slow drug release during *in vitro* studies. Furthermore, the porosity of particles also affects the extent of lung deposition of drugs in pulmonary drug delivery as was observed by Edwards *et al.* (1997:1870) where their study on porous microparticles, illustrated that the porous nature of the microparticles resulted in increased systemic bioavailability of the inhaled drug, due to increased aerosolization efficiency when compared to non-porous particles of similar aerodynamic diameter (Edwards *et al.* 1997).

2.3.1.5 Surface area

Nano-sized particles have a larger surface-to-volume ratio than microparticles. With nanoparticles, during formulation some of the drug adsorbs onto the surface of the nanoparticles, particularly in emulsion based techniques of preparation, this results in an initial burst release due to the large surface area, thus affecting the drug release kinetics (Dorozhkin, 2009:1994).

Kondo *et al* (1993:798) documented an increase in bioavailability because of a 10-fold reduction in particle size. This occurs due to an increase in surface area and consequently dissolution rate. However, if the particles agglomerate e.g. in the GI tract, the surface area would decrease with a subsequent decrease in bioavailability (Kondo *et al.* 1993). Thus, it is imperative that the surface charge of the particles is maintained, or steric hindrance be introduced, to avoid agglomeration, thereby greatly increasing surface area for dissolution. Therefore, one would expect that when the bioavailability is dissolution rate limited, a 10-fold decrease in particle size would be necessary to increase surface area with a consequent increase in dissolution rate as well as bioavailability (Liversidge & Cundy 1995:95).

2.3.1.6 Particle size

The sub-micron size of nanoparticles offers a number of distinct advantages over microparticles in drug delivery due to the fact that these particles are in the size region of macromolecules. This physical characteristic, particularly for particles less than 100 nm in size, allows these particles to reach virtually all tissues in the body (McNeil, 2005:586). Nanoparticles have in general, relatively higher intracellular uptake compared to microparticles. This was demonstrated by Desai *et al* (1997:1570) whereby 100 nm size nanoparticles showed 2.5 fold greater uptake compared to 1 μ m and six-fold higher uptake compared to 10 μ m microparticles in Caco-2 cell line (Desai *et al.* 1997). Furthermore, these particles can cross barriers that in general make it difficult for conventional therapeutic compounds to reach targeted site. Reports on nanoparticles crossing the blood brain barrier, the stomach epithelial and even the skin have been presented (Koziara *et al.* 2003:1772).

When conventional drugs are administered orally, they are absorbed into the systematic circulation via the portal blood, and undergo first pass metabolism, which thus leads to poor oral bioavailability. However when particles less than 500 nm in size are orally administered, they are capable of crossing the M cells in the Peyer's patches and the mesentery on the surface of the gastrointestinal mucosa, entering the lymphatic transport system and thus avoiding pre-systemic hepatic metabolism. This therefore results in increased absorption and thus also increased bioavailability of the encapsulated drug (Jain, 2008, Brannon-Peppas, 1997). Desai *et al* (1996:1841) demonstrated a significantly higher nanoparticle uptake in the Peyer's patches in the small intestine at 100 nm compared to higher size ranges following

oral gavage. Histological evaluation showed these nanoparticles to be diffused throughout the submucosal layer and also on the serosal side of the Peyer's patch (Desai *et al.* 1996).

The definitive characteristic of any nanoparticulate system is clearly its nanometre size since the dissolution rate of a drug is a function of intrinsic solubility and particle size of the carrier (Liversidge & Cundy 1995). Studies have shown that by reducing the particle size, the rate of dissolution may be increased, resulting in a higher oral bioavailability (Liversidge & Cundy 1995). Therefore, it is possible to manipulate size to control release kinetics.

2.3.1.7 Surface hydrophobicity

In drug delivery, the nanoparticles surface hydrophobicity determines the extent of adsorption of blood components, mainly opsonins, onto the surface of the particles. Opsonins are proteins that promote the activation of the complement system and assist in phagocytic uptake by macrophages (Kingsley *et al.* 2006:344). Binding of these opsonins onto the surfaces of nanoparticles act as a bridge between nanoparticles and phagocytic cells. For the purpose of prolonged circulation, prevention of opsonin adsorption can be achieved by (a) surface coating of nanoparticles with hydrophilic polymers/ surfactants; (b) formulation of nanoparticles with biodegradable copolymers with hydrophilic segments such as PEG, PEO, poloxamers, poloxamines and polysorbate 80 (Mohanraj & Chen 2006:564).

2.3.1.8 Zeta potential

Particles in a medium carry a thin layer of ions and solvent around them (called the electrical double layer), which results in an interaction of the nanoparticle with the biological environment. The surface separating the stationary medium from the moving particles and its bound ions is called the slipping plane. The potential at this surface is called the zeta potential, which is dependent on the electrolyte concentration and the pH of the suspending particle medium (Labhasetwar *et al.* 1998:1231). Zeta potential in nanoparticles reflects the electrical potential of particles and is influenced by the chemical composition of the particle and the medium in which it is dispersed. Nanoparticles with a high positive or negative zeta potential have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles (Müller *et al.* 2000:168). The zeta potential can also be used to determine whether a charged active material is encapsulated within the centre of the nanoparticle or adsorbed onto the surface of the particles (Mohanraj & Chen 2006:564).

Opsonisation is also influenced by zeta potential. This effect is a vital parameter to consider when designing drug-loaded nanoparticles particularly for intravenous administration. Plasma proteins make the particle more susceptible to phagocytosis and thus leading to their clearance from the body. High negative zeta potential values in serum are indicative of particles that have become highly opsonised, and may also indicate poor formulations (Freiberg & Zhu 2004:1). A positive surface charge of the Nanoparticles enhances its attachment to the negatively charged cellular membrane, thus improving intracellular uptake, in turn improving the bioavailability of the drugs.

2.3.1.9 Controlled release

Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or active agent in such a way that the active agent is released from the material in a predesigned manner. The release may be constant over a long period, it may be cyclic over a long period, or it may be triggered by the environment or other external events. In addition, the type and molecular weight of polymer that the particles are composed of greatly influences the release kinetics (Brannon-Peppas, 1995:1) as previously discussed. Different triggers known to induce controlled release are pH, temperature, magnetism and redox potential.

pH response

Drug release can be controlled by triggering a pH dependent release mechanism. Incorporation of pH-sensitive groups (e.g. poly(methacrylic-g-ethylene glycol) onto the surface of the polymeric particles can allow targeting and controlled-release of the drug at specific tissues of which the pH differs from other tissues (Dai *et al.* 2004:229).

Temperature response

Temperature is another parameter that is explored to attain controlled-release. For this purpose, when the polymer is below its LCST the polymer acquires a hydrophilic state due to hydrogen bonding with water molecules, e.g. PNIPAAm. Above the LCST, the polymer becomes hydrophobic due to the disruption of the hydrogen bonds. In the hydrophilic state of the polymer, the drug release rate is higher than when the polymer is in the hydrophobic state (Heath *et al.* 2007:236). This parameter has been applied in cases where in a diseased state the temperature in that region varies to that of the normal state (Heath *et al.* 2007:236). Thus,

it is possible to further enhance the properties of nanoparticles by manipulating the polymer type and content.

Magnetic response

Drug release can also be controlled by formulating magnetic particles dispersed in polymeric microspheres. The magnetic response is then activated by applying a magnetic field, which causes a change in the pores of the particle. A resultant change in swelling then occurs thereby releasing the drug (Brannon-Peppas, 1997). Furthermore, magnetic responsive polymers have also been used in site-specific targeting where the functionalized drug-loaded particles are transported by magnetic forces to the targeted cell population (Schütt *et al.* 1999:99). The efficiency of this mechanism has been reported in site-specific targeting to cancer cells (Lübbe *et al.* 2001:203). An example of a magnetic-responsive polymer is the formulation of the thermo-responsive polymer PNIPAAm as a hydrogel with incorporated magnetic particles (Zrinyi, 2000:98).

Oxidative response

Another mechanism in which drug release can be controlled is by the use of oxidative-responsive polymers. An example of such a polymer is poly-(propylene sulphide) (PPS) formulated as a copolymer with PEG with a disulphide bond (Rehor *et al.* 2008:1960). Under oxidative conditions, PPS is oxidised and loses its strong hydrophobic character. The reduction in hydrophobicity is due to the conversion of the disulphide bond into sulfoxides and eventually sulfones. Therefore, when a hydrophobic drug is encapsulated via hydrophobic interactions with PPS in these particles, upon exposure to oxidative conditions as in a diseased state, the drug would be released in a more accelerated release profile compared to normal physiological conditions (Rehor *et al.* 2008).

2.4 Toxicological concerns

Although the advantages for nano-drug delivery systems are extensive, the potential side effects derived from the use of particles at a nano-level have become an important consideration as well as a rising cause for debate amongst researchers. These effects have been grouped and have emerged as a sub-discipline of nanotechnology now termed nanotoxicology (Fischer & Chan 2007:565). The main cause for concern is based on the already considered advantageous property of the mobility of nanoparticles due to their nano-size range. This characteristic of nanoparticles poses the potential disadvantage that entrance

into the skin, lungs and intestinal tract with resulting deposition in various organs, may possibly affect cellular integrity and vital pathways in tissues (Medina *et al.* 2007:552). *In situ* studies have demonstrated that nanoparticles of less than 100 nm cross the blood-brain barrier. However, the mechanism of transport and possible toxicity has not been fully elucidated (Koziara *et al.* 2003:1772).

There is insufficient data to support toxicity of nanomaterials utilised in drug delivery *in vivo*. Fischer and Chan (2007) documented the need for detailed *in vivo* studies that will address the toxicity concerns. However, the review shows that although the areas of metabolism, immunotoxicity and complement activation may be of interest, any cause for concern in the field of drug delivery of biodegradable and biocompatible polymer-based nanoparticles is still unknown (Fischer & Chan 2007). However, it is postulated that since the polymers are biocompatible, biodegradable and FDA approved, no toxic effect should be observed.

Much work in elucidating the possible adverse effects of nanomaterials has been conducted on metal nanoparticles. This is primarily due to the rapid increase in the production of these materials, fuelled by the growing need for some properties that these materials provide (Nel *et al.* 2006:622). Merget *et al* (2002:625) reported an increased risk to lung diseases such as chronic obstructive pulmonary disease (COPD), chronic bronchitis, lung cancer and TB due to exposure to crystalline silica dust (Merget *et al.* 2002). There is an additional risk of cytotoxicity caused by tissue damage or cellular polymer overloading if the nanoparticles do not undergo biodegradation (Magenheim & Benita 1991:231). Kagan *et al* (2005:312) reported an inflammatory response to single-walled carbon nanotubes in the lungs of mice, which progressed from an acute inflammatory phase to fibrogenic events. These factors are vital considerations when utilising nanotechnology. Thus, effort is being put into determining the ecotoxicological as well as the biological risks associated with these materials (Gwinn & Vallyathana 2006:1818).

2.5 Regulatory considerations

The area of nanotechnology has had a positive impact on treating medical conditions with prolonged treatment regimens. However, there are obstacles hindering the development of these systems. The general scepticism of the average person relating to new technology such as nano based technologies needs to be addressed (Bennet & Sarowitz 2005).

Approval authorities such as the FDA in USA, the European Agency for the evaluation of Medicinal products (EAM) in Europe and the Pharmaceutical and Medical Device Agency, KIKO (PMDA, KIKO) in Japan all have the final say in the registration of new drug entities, new dosage forms and new drug delivery devices (Cone & Walker 2004).

The general concerns of the FDA, which is the largest regulatory body, about nanotechnology are about safety, quality and characterization of material and environmental impact (Couvreur & Vauthier 2006). The FDA, however, concluded that the current requirements for safety testing of medicinal products is sufficiently rigorous and are currently believed to be adequate. Thus, there are currently no specific testing requirements specifically for nanotechnology products (MHRA, 2006, Couvreur & Vauthier 2006). However, since the knowledge of nanoscale materials are always increasing, the FDA Nanotechnology Task Force recommended in their 2007 report that such applications should be taken into account, on a case-by-case basis, whether an FDA-regulated product containing nanoscale materials qualifies for an existing categorical exclusion or whether extraordinary circumstances exist (Von Eschenbach, 2007).

2.6 Developments in nanotechnology-based drug delivery systems

Developments in nanotechnology-based therapeutic applications have yielded success in the field of cancer therapy, such as nanoparticles of paclitaxel stabilized by albumin which is approved by the FDA for use in the chemotherapy of refractory metastatic breast cancer. In diabetes mellitus, polyethylcyanoacrylate nanospheres as biodegradable polymeric carriers have been found useful in oral insulin delivery in streptozotocin-induced diabetic rat model.

Nanotechnology has already addressed many therapeutic challenges with regards to improving treatment of complex diseases. Drug-loaded nanoparticles have demonstrated increased efficacy and reduced toxicity by improved intracellular penetration in cancer chemotherapy when formulated for site-specific targeting (Craig, 1998). For example, the originally approved formulation of paclitaxel was a parenterally administered formulation consisting of the drug, 527 mg/ml of polyoxyethylated castor oil (Cremophor EL) and 49.7% v/v of absolute ethanol. The unfortunate side effects of the inclusion of cremophor in this formulation included severe cases of hypotension, urticaria, angioedema and potentially fatal anaphylactic reactions (Maitra *et al.* 2002). They then designed a nanoparticulate formulation in which the ethanol concentration was greatly reduced and cremophor was completely

excluded. These were nanoparticles of polymeric micelles that entrap/solubilize paclitaxel without affecting its cytotoxic properties, thus increasing efficacy with less side effects (Maitra *et al.* 2002).

As mentioned earlier and the core focus of this thesis is the use of nanoparticle-based drug delivery in tuberculosis chemotherapy. In the field of ocular disorders, dendrimers have been shown to have potential. VivaGel[®] which is still undergoing trials, is an anti-HIV drug based on dendrimer technology (Ahmad, 2007). In the treatment of various bacterial infections, nanoparticles have demonstrated increased efficacy and reduced toxicity by controlling biodistribution, improving intracellular penetration (in macrophages, cell-presenting antigens and dendritic cells) and facilitating mucosal absorption. The same effects were observed for metabolic disease, autoimmune diseases, pain treatment and gene therapy. An overall increase in bioavailability as well as improving protection against degradation has also been seen in these treatments (Couvreur & Vauthier 2006).

Site-specific targeting of drug-loaded nanoparticles would be the most effective way to treat *M.tb* concentrated in macrophages, in various organs and tissues as well as latent or semi-dormant *M.tb*. Optimal design of such a system will ensure a decrease in dose frequency, minimize side effects and increase efficacy with consequent increase in patient compliance.

2.7 Potential use of nanoparticles as drug delivery systems for anti-tuberculosis drugs

Extensive studies have been conducted to validate the hypothesis that nanoparticle-based drug delivery has enormous potential for improving current TB chemotherapy. Of these, the largest knowledge contribution towards understanding the potential of nanoparticle-based drug delivery systems for the purpose of TB chemotherapy, has been the research group of Prof. G.K. Khuller (Dutt & Khuller 2001; Pandey *et al.* 2003a; Pandey & Khuller 2004; Sharma *et al.* 2004, Sharma *et al.* 2004; Pandey *et al.* 2005; Pandey & Khuller 2005; Ahmad *et al.* 2005; Ahmad *et al.* 2006; Ahmad *et al.* 2007; Pandey & Khuller 2007; Ahmad *et al.* 2008). These are summarised in chapter 5.

2.7.1 Current research approaches

Ahmad *et al* (2006:414) conducted a study that demonstrated the potential for the use of nanoparticle drug delivery in providing a sustained release profile for anti-tuberculosis drugs. For INH encapsulated in nanoparticles, therapeutic plasma levels were maintained above the MIC for as long as 11 days versus 12 hours for free INH. This validates the potential for reducing the dose frequency of TB drugs and thus the possibility to address the challenge of poor patient compliance (Ahmad *et al.* 2006).

Kisich *et al* (2007:158) evaluated the *in vitro* efficacy of a PBCA nano-encapsulated MXF versus free-MXF. An MIC of 0.1 µg/ml was observed for nanoencapsulated MXF as opposed to 1 µg/ml for free MXF in macrophage cells infected with H₃₇R_v. Encapsulated MXF demonstrated three times more efficient accumulation in macrophages than free MXF. Free MXF reached a maximum concentration of ~125 µg/ml in 5 minutes and the encapsulated MXF continued to accumulate within the macrophages for up to one hour to a concentration of ~325 µg/ml.

Pandey *et al* (2003:984) administered RIF, INH and PZA encapsulated in PLGA-nanoparticles once over 10 days via oral gavage to guinea pig TB models. The results were comparable with the efficacy of 46 conventional daily doses. Also, three divided doses administered every 10th day via the pulmonary route in guinea pigs demonstrated the same comparable efficacy to 46 conventional daily doses i.e. no tubercle bacilli was detected in the lung as assessed on the basis of colony forming units (cfu) (Pandey *et al.* 2003a). Pandey and Khuller (2006:1149) conducted a study postulating the potential efficacy of nanoencapsulated TB drugs in a cerebral TB model, which was confirmed by bacilli in meningeal smears. PLGA-nanoparticles encapsulating RIF, INH, PZA and ETB were orally administered in a murine model. RIF, INH and PZA were detected for up to nine days in the brain, except for ETB, which was only detected until day six. Five oral doses of the same formulation to *M.tb* infected mice administered every 10th day showed undetectable bacilli in the meninges. These results illustrated the potential for the use of nanoparticles in extra-pulmonary TB (Pandey & Khuller 2006).

Other novel applications were also documented by Sharma *et al* (2004:761), which included lectin-conjugated nanoparticles that resulted in the absence of necrosis in mice treated via the

pulmonary route compared to untreated infected mice (Sharma *et al.* 2004). The application of eight oral nano-encapsulated streptomycin doses illustrated the possible replacement of 24 intramuscular (IM) doses. In a similar study, Ahmad *et al.* (2008:147) demonstrated the potential efficacy of nanoencapsulated econazole, which could synergistically enhance the activity of other drugs such as MXF. In addition to the above research, there are various reports on nanoparticulate drug delivery in TB chemotherapy such as an *in vitro* controlled-release INH-loaded nanosystem and self-assembled nanostructures from lipid derivatives of INH (du Toit *et al.* 2008; Jin *et al.* 2008).

The nanoparticle formulation challenges have been addressed by a novel reduced-step double emulsion solvent-evaporation spray-drying process developed by the Council for Scientific and Industrial Research (CSIR) TB nano-drug delivery group (Kalombo, 2008).

Despite the fact that controversy exists regarding the possible toxicity of nanoparticles, these studies also indicate no detectable biochemical hepatotoxicity. Since the polymers used are biocompatible and biodegradable and these nanoparticles are eventually cleared from all organs and tissues. These studies validate the promise that nanoparticulate drug delivery systems hold for tuberculosis chemotherapy. To date, no clinical data has been reported for this application, but enough pre-clinical evidence as presented above, exists to postulate the eventual turning point to successful tuberculosis chemotherapy.

2.7.2 Prospects in nanoparticle drug delivery

Nanoparticle drug delivery of anti-tuberculosis drugs has demonstrated promising data in animal studies. Table 2.4 illustrates the improved PK parameters because of drugs encapsulated in nanoparticulate drug delivery systems.

Table 2.4 Comparison of PK parameters for conventional versus nanoencapsulated anti-tuberculosis drugs

PK parameters	First-line TB drugs				Nanoencapsulated drugs				Reference
	Conventional				Nanoencapsulated				
	<i>RIF</i>	<i>INH</i>	<i>PZA</i>	<i>ETB</i>	<i>RIF</i>	<i>INH</i>	<i>PZA</i>	<i>ETB</i>	
t½ (h)	2.3-6	3-4	8-11	3-4	41.50	30.70	47.30	27.70	(Gurumurthy <i>et al.</i> 1999)
t _{max} (h)	3	1-2	3.11±1.18	2-4	24	24	24	24	(Gurumurthy <i>et al.</i> 1999, Katzung 2000, Ahmad <i>et al.</i> 2006)
C _{max} (µg/ml)	11	3-7	38.7	2-5	1.07	13.51	50.2	22	(Ahmad <i>et al.</i> 2006, Gurumurthy <i>et al.</i> 1999)
AUC _{0-∞} (µg/ml)	53.47	76.95	340.7	6.9-54	164.24	81.784	3432	298.22	(Ahmad <i>et al.</i> 2006, McIlleron <i>et al.</i> 2006)
MIC (µg/ml)	0.25	0.2-0.5	8-20	≤2	0.25	0.2-0.5	8-20	≤2	(Franzblau <i>et al.</i> 1998, Pillai <i>et al.</i> 1999, Katzung 2000)

Values are not absolute and are based on approximate values according to literature.

These values indicate the improved PK parameters of TB drugs as a result of the application of nanoencapsulation. Nanoparticulate drug delivery provides a longer circulation time and thus a longer half-life due to the slow release profile. It also has the capability to maintain plasma levels above the MIC with an extended time to reach maximum plasma concentrations (t_{max}). These properties along with higher C_{max} values results in an increased area under the curve, thus facilitating an improved PK profile.

Various methods of employing the nanoparticulate technique for delivery of tuberculosis drugs have been reported. Pandey *et al* (2003:376) encapsulated RIF, INH and PZA in PLGA nanoparticles for oral delivery by the multiple emulsion technique. The nanoparticles were administered at every 10th day and after five oral doses of treatment, no tubercle bacilli could be detected in the tissues. This formulation achieved an encapsulation efficiency of 56.9% for RIF, 66.3% for INH and 68% for PZA (Pandey *et al.* 2003a). The encapsulation efficiency is a measurement of the amount of drug encapsulated within the nanoparticle during formulation.

In a similar study, Zahoor *et al* (2005:300) formulated alginate-based nanoparticles for inhalation. Two formulations were prepared by the cation-induced gelification technique with INH, RIF and PZA in one formulation and INH, RIF, PZA and ETB in the other. The encapsulation was successful in obtaining above 80% encapsulation efficiency for all four drugs. Above 80.5% of particles were within respirable range and doses 15 days apart were

comparable with 45 daily doses of conventional drugs. They thus concluded that there is definite potential for the use of this formulation in TB therapy. However, more investigations are needed before attempting human clinical trials (Zahoor *et al.* 2005).

An investigation was conducted with solid lipid nanoparticles intended for broncho-alveolar drug delivery, where the delivery system was formulated by incorporating INH, RIF and PZA into nanocrystalline suspensions. A single nebulization in guinea pigs resulted in therapeutic drug concentrations being maintained in the plasma for five days (Pandey & Khuller 2005). Research is on-going on a global scale in the optimisation of nanoencapsulation of TB drugs and with the promising results already documented.

2.8 Drug targeting in TB

Macrophages are vital cells for the survival of *M.tb* within the host. These cells originate from monocytes. Macrophages are phagocytes, acting in both non-specific and specific defence of vertebrate animals. Their role is to phagocytose (engulf and digest) cellular debris and pathogens either as stationary or mobile cells, and to stimulate lymphocytes and other immune cells to respond to the pathogen (Banchereau & Steinmann 1998:245). When a macrophage ingests a pathogen, the pathogen becomes trapped in a food vacuole, which then fuses with a lysosome. Within the lysosome, enzymes and toxic peroxides digest the invader. However, *M.tb* has become resistant to these methods of digestion. Thus, although this mycobacterium is taken up by the alveolar macrophages, it is able to survive and multiply within the macrophages (Maartens & Wilkinson 2007:2030). For this reason, activated macrophages have become an essential focus point in drug targeting for tuberculosis therapy.

Ahsan *et al* (2002:30) described the mechanism of uptake of liposomes by macrophages. This was described in different steps (1) stable adsorption to the cell surface; (2) cellular uptake of intact vesicles by an energy-dependant mechanism and (3) lysosomal degradation of the liposomes and their content thus releasing the drug for action on engulfed mycobacteria. At the time of their review in 2002, liposomes were the most widely studied and most advanced carrier systems for targeted delivery to macrophages (Ahsan *et al.* 2002).

Anisimova *et al* (2000:165), the first to report nanoparticles encapsulating anti-TB drugs, conducted a study in which they infected macrophages to evaluate the antimicrobial activity of RIF, INH and streptomycin against intracellular *M.tb* persisting in human monocyte-

derived macrophages. These drugs were encapsulated in nanoparticles with mean size of approximately 250 nm. The particles were prepared by an emulsion polymerization technique using poly-n-butylcyanoacrylate (PBCA) and polyisobutylcyanoacrylate (PIBCA). In this study, evaluation of PBCA-PIBCA nano-encapsulated INH and SM revealed a decrease in the MIC *in vitro*. The nanoparticles containing Tween 80 as stabilizer had an 8-fold accumulation of INH in human monocytes and exposure to infected macrophages resulted in complete inhibition of bacterial activity for free INH with an MIC of 0.12 µg/ml and 0.03 µg/ml for encapsulated INH. Streptomycin had 7-fold higher intracellular accumulation compared to extracellular with an MIC of 1.0µg/ml for encapsulated SM and 4.0 µg/ml for free SM. There was no significant difference between MIC (reported at approximately 0.25 µg/ml) for the free and encapsulated RIF. Therefore, drug targeting to macrophages for TB therapy is possible and an avenue well worth exploring (Anisimova *et al.* 2000).

2.9 Conclusion

The impact and advantages of nanoparticulate drug delivery systems have been presented in this chapter. The potential to revolutionize TB chemotherapy in patients is becoming increasingly evident. Nanotechnology provides a critical platform for the development of novel drug delivery systems aimed at new drug delivery techniques for effectively targeting drugs to the diseased site, in order to increase patient compliance and reduce healthcare costs (Pison *et al.* 2006). As previously mentioned (Chapter 1), one of the major obstacles of tuberculosis chemotherapy is patient non-compliance mainly due to dose frequency and duration of therapy. The on-going interest in the field of drug delivery for these drugs focuses on the optimisation or improvement of these two factors. The fact that nanoparticles can be manipulated in many ways to suit the drug gives great potential for its use in TB drug delivery. Among these advantages, high stability, high carrier capacity, its ability to incorporate hydrophilic and hydrophobic substances, its variable routes of administration and its ability to be designed to enable controlled drug delivery are particularly noteworthy (Gelperina *et al.* 2005). Pharmaceutical companies and other institutions across the globe are making a concerted effort to develop nanotechnology in drug delivery and this is validated by a very real need to enhance current drug delivery approaches (Emerich, 2006).

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