DEVELOPMENT OF NANOCOMPOSITE CORTICOSTEROID PARTICLES FOR USE IN ASTHMA

By

GINA PATEL

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This work is dedicated to my Mum, grandparents, husband and family, for their love and support.

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ACI	Anderson cascade impactor
ACN	Acetonitrile
AUC	Area under the curve
AUC_{brain}	Area under the receptor occupancy time profile of the brain
AUC_{liver}	under the receptor occupancy time profile of the liver
AUC_{lung}	under the receptor occupancy time profile of the lung
BDP	Beclomethasone dipropionate
BMP	Beclomethasone monopropionate
BUD	Budesonide
CFC	Chlorofluorcarbon
CH-MF	Chitosan coated mometasone furoate poly(lactic acid) nanoparticles
CIC	Ciclesonide
CIC-AM	Ciclesonide active metabolite
CL	Clearance
COPD	Chronic obstructive pulmonary disease
DCM	Dichloromethane
DDW	Double distilled water
DD	Delivered dose
Des-CIC	Desisobutyryl ciclesonide
DPI	Dry powder inhaler
DPPC	1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine
EE	Encapsulation efficiency
F	Bioavailability
FDA	Federal drug administration

FLU	Flutamide
F_{oral}	Oral bioavailability
FP	Fluticasone propionate
FPF	Fine particle fraction
f_u	Fraction of drug unbound
g	Gram
GINA	Global initiative for asthma
HFA	Hydrofluroalkane
HPLC	High performance liquid chromatography (Ultraviolet detection)
ICS	Inhaled corticosteroid
kV	Kilo volt
MAIC	Major Analytical Instrument Center
MD	Metered dose
MDI	Metered dose inhaler
MeOH	Methanol
MF	Mometasone furoate
MF-PLA	Mometasone furoate and poly(lactic acid) nanoparticles
mL	Milliliter
ms	Milisecond
MW	Molecular weight
nm	Nanometer
ODS	Octadecyl silane
PBS	Phosphate buffered saline
PLA	Poly(lactic acid)
PLD	Pulsed laser deposition

PLGA	Poly(lactic-co-glycolic acid)
pMDI	Pressurized metered dose inhaler
PT	Pulmonary targeting
PVA	Polyvinyl alcohol
RRA	Relative receptor affinity
RPM	Rotations per minute
SD	Standard deviation
SEM	Scanning electron microscope
SMI	Soft Mist Inhaler
TA	Triamcinolone acetonide
TAP	Triamcinolone acetonide phosphate
TA-PLGA	Triamcinolone acetonide and poly(lactic-co-glycolic acid) nanoparticles
TAP-lip	Triamcinolone acetonide phosphate liposomes
TAP-sol	Triamcinolone acetonide phosphate solution
Tg	Glass transition temperature
Vdss	Volume of distribution at steady state
W	Watt
% v/v	% volume in volume
% w/v	% weight in volume
% w/w	% weight in weight

Abstract of Dissertation Presented to the Graduate School
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Gina Patel

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Asthma is a chronic inflammatory condition of the airways resulting in episodes wheezing and breathlessness. Inhaled Corticosteroids (ICS) are used to prevent the occurrence of asthma attacks. The clinical effect of ICSs depends on the time the drug resides in the lung – therefore increasing the drug residence time in the lung improves asthma therapy. It has been proposed that nanoparticles could escape clearance mechanisms in the lung and adhere strongly to the lung surface, leading to increased residence time. There are two main barriers to this approach, firstly, nanoparticles cannot deposit in the lung, and instead they are exhaled. Secondly, the particles must be formulated to release drug slowly in order to take advantage of the increased residence time.

In order to further improve lung targeting of corticosteroids, poly(lactic-co-glycolic acid) PLGA and poly(lactic acid) PLA were used to produce polymeric nanoparticles of Triamcinolone Acetonide (TA) and Mometasone Furoate (MF) using the solvent evaporation technique. TA was used as a model corticosteroid to allow optimization of various production parameters to produce PLGA nanoparticles. The solvent evaporation technique was used to produce polymeric nanoparticles. A number of

process parameters may be varied to alter nanoparticle size and drug encapsulation efficiency. Polyvinyl alcohol (PVA) surfactant concentration and PLGA content were varied to determine their influence on particle size, drug encapsulation efficiency, particle morphology and *in vitro* release characteristics of TA nanoparticles. Increasing PLGA content resulted in a trend of increasing particle size and drug encapsulation. As PVA concentration was increased particles tended to reduce in size and drug loading. Nanoparticles produced ranged in particle size between 156-209nm. In addition when a low concentration of 1% or 2% w/v PVA was used to produce nanoparticles combined with the use of only 200 mg PLGA, TA crystals were observed by scanning electron microscopy. *In vitro* release studies revealed TA-PLGA nanoparticles released drug at a similar rate to micronized TA, with 50% drug release being observed within 15 minutes. In order to produce nanoparticles which can deliver drug at a slower rate compared to micron sized particles, a number of changes can be made to nanoparticle production; a more lipophilic drug MF in combination with a more hydrophobic polymer, PLA can be used to further slow down drug release. Subsequently MF nanoparticles (MF-PLA) were using 10 mg MF, 400 mg PLA and 1% w/v PVA, these particles showed slow release compared to MF contained in the Asmanex® Twisthaler™. To further reduce MF release rate, nanoparticles were coated with chitosan. *In vitro* release studies showed that chitosan coated MF-PLA nanoparticles (CH-MF) showed significantly slower release compared to both uncoated nanoparticles and MF contained within the Asmanex® Twisthaler™. *In vitro* release studies determined 100% MF occurred after 1 hour for the Asmanex® formulation, in comparison at this time only 50% and 24% MF was released from MF-PLA and CH-MF respectively.

A novel spray dryer was designed so that operating conditions of this device allowed outlet temperatures to remain below the glass transition temperature of PLGA and PLA, thus making this system suitable to spray dry polymeric nanoparticles. Incorporation of these nanoparticles into lactose based microspheres by spray drying resulted in spherical nanocomposite microspheres. Analysis of these microspheres showed complete incorporation of nanoparticles into the formulation. Optimal conditions for incorporation of nanoparticles into microspheres were using a composition of 75% nanoparticles and 25% lactose. The fine particle fraction of the microspheres was comparable to that of MF from the Asmanex® Twisthaler™. A biphasic release of MF was observed from the microspheres, with a significantly slower release compared to Asmanex®. The spray drying process did not seem to alter the release properties of chitosan coated nanoparticles.

CHAPTER 1
INTRODUCTION

Asthma

In the United States (US) asthma affects 16.4 million adults and 7 million children and is increasing in prevalence [1-3]. Asthma can be defined as a complex inflammatory disease of the airways involving many inflammatory mediators. Asthma is a chronic reversible inflammatory disease of the airways that is characterized by episodes of wheezing and breathlessness [4]. A component of asthma involves chronic inflammation, if left untreated this can progress to airway remodeling, resulting in irreversible airway narrowing [5].

Asthma Treatment

The Global Initiative for Asthma (GINA) was established in 1993 in order to focus on reducing asthma prevalence, morbidity and mortality throughout the world. Published guidelines for the treatment and prevention of asthma were produced by GINA. Current therapy includes Inhaled Corticosteroids (ICS), long-acting β_2 agonists, leukotriene modifiers, theophylline and short acting β_2 agonists for immediate relief [4]. ICS therapy targets the underlying inflammation present in asthma, to reduce long term consequences such as progression of asthma to reversible obstruction in the airways. ICS therapy is therefore the cornerstone for asthma treatment and is recommended for all severity levels of persistent asthma by GINA [4, 6].

Adverse Effects of ICS

Pulmonary drug delivery has many attractive prospects for local and systemic action. Selectively targeting drug delivery to the lung allows lower doses to be

administered providing a reduction in systemic side effects whilst maintaining clinical efficacy. Although ICS are considered the “gold standard” in asthma management they are responsible for many local and systemic adverse effects. Local side effects include oropharyngeal candidiasis, dysphonia, perioral dermatitis (which may occur with the use of spacer devices or nebulizers attached to face masks), cough, thirst and in rare cases tongue hypertrophy. Additionally systemic side effects include adrenal insufficiency, cataracts, glaucoma, growth retardation in children, osteoporosis, increased bone fractures, skin thinning and skin bruising [7-11]. New developments in synthetic corticosteroids have lead to improvements in pulmonary targeting and reductions in adverse effects [12].

Fate of ICS

In order to produce further improvements in the area of ICS therapy, it is necessary to be fully aware of the fate of corticosteroids following inhalation, as described in Figure 1-1. Following inhalation a fraction of drug is deposited in the lungs, a certain portion of the dose can be deposited in the oropharynx, and if this is not rinsed may be swallowed. Subsequently the swallowed portion is available for oral absorption; if the drug has significant oral bioavailability, it enters the systemic circulation to produce unwanted adverse effects. Drug may enter the systemic circulation as a result of absorption from the lung and gastrointestinal tract, both of which contribute towards systemic availability of the ICS [12-14]. Newer ICS such as fluticasone propionate (FP) and mometasone furoate (MF) have negligible oral bioavailability (F_{oral}), therefore this route does not significantly contribute to the systemically available dose [14]. A fraction is deposited in the lungs where it must first undergo dissolution to exert its desired

pharmacologic effect; dissolved drug will be absorbed into the systemic circulation, particulate matter deposited in the conducting airways however, will be subject to clearance by the mucociliary escalator. In order to achieve high pulmonary targeting drug dissolution should be slow, allowing pulmonary glucocorticoid receptors to be occupied for a longer period of time. On the other hand if particles dissolve much slower than the rate at which they are cleared from the lung, receptor occupancy over a given time period will be reduced. In order to achieve pulmonary targeting, corticosteroids should provide drug release at a rate comparable to their clearance from the lung [15].

Pulmonary Targeting of ICS

Receptor occupancy-time profiles may serve as a surrogate marker for pulmonary and systemic effects; cumulative receptor occupancy allows direct comparisons to be made between local efficacy and systemic adverse effects [16]. For a given dose increased cumulative receptor occupancy in the lungs compared to the systemic circulation results in improved pulmonary targeting; providing better efficacy and reduced systemic adverse effects [12, 14, 15]. Various factors are important for pulmonary targeting of ICS, these are based on pulmonary or systemic factors. Pulmonary pharmacokinetic factors include deposition efficiency and region of deposition in the lung of the ICS, also pulmonary residence time and lung tissue binding are important. Pulmonary pharmacodynamic factors include receptor binding affinity and selectivity. Systemic factors include oral bioavailability, clearance, volume of distribution, protein binding, tissue binding and affinity to transporters [15]. To

summarize, pulmonary targeting for inhaled drugs is influenced by both the inhaler device and formulation and are discussed below [17].

Ideal Corticosteroid

An ideal ICS should have high pulmonary targeting, defined as the difference between cumulative lung and liver receptor occupancies. High pulmonary versus oropharnygeal deposition is desired, however as a significant fraction of inhaled drug is deposited in the oropharynx from which it is subsequently swallowed, it follows that an ideal ICS should posses negligible oral bioavailability. This is the case for newer corticosteroids such as fluticasone propionate, mometasone furoate, ciclesonide and des-ciclesonide, which have less than 1% oral bioavailability [18, 19]. Systemic bioavailability is the sum of pulmonary and oral bioavailability, since oral bioavailability is negligible for the newer corticosteroids, pulmonary absorption is responsible for the significant portion of drug available to the systemic circulation. An ideal corticosteroid should be removed rapidly from the systemic circulation to reduce systemic adverse effects. The maximal clearance rate from the liver is approximately 90 L/min, which is equal to the liver blood flow, most corticosteroids possess systemic clearance rates that are similar to blood flow. ICS have high hepatic extraction, resulting in low systemic exposure and enhanced pulmonary targeting, described in Table 1-1. Further improvements in this area will be difficult as clearance of ICS are already close to that of liver blood flow, improvements may be achieved through the possibility of increased extra hepatic metabolism. Increased pulmonary residence prolongs drug action in the lungs, thereby improving efficacy. Utilization of slow release inhaled formulations will increase pulmonary residence time and targeting. In the case of slowly dissolving

drugs, the effect of mucociliary clearance predominates; as a result drug particles are removed from the lungs before they are able to completely dissolve, thus reducing pulmonary targeting [15]. Inhaled formulations capable of providing slow release combined with reduced clearance from the lung are able to improve pulmonary targeting; this approach increases efficacy allowing lower doses to be administered. As a result fewer systemic side effects will be experienced.

Inhaler Device

Commonly used inhaler devices include Dry Powder Inhalers (DPIs) and pressurized Metered Dose Inhalers (pMDIs) and to a lesser extent nebulizers, in addition a new category of inhalers termed “soft” mist inhalers (SMI) has been developed recently. pMDI devices utilize propellants to aerosolize a solution or suspension, however they require the patient to co-ordinate both device actuation and inspiration; resulting in poor inhaler technique, especially in young children and the elderly. In addition to this a blast of high velocity cold propellant can impact the oropharynx, in a small number of patients this will induce a gag reflex [20]. DPIs avoid problems with inhaler technique as they are usually driven by the patient’s own breath. This itself however creates difficulty in many asthmatic and COPD patients who do not have the necessary lung function [12].

The inhalation device plays a vital role in delivering drug to the lung. In the early years of ICS therapy doses were delivered with low pulmonary deposition efficiencies of approximately 10% or less [12]. In recent times however, new inhaler devices capable of delivering >30% of the dose to the lung have been developed [13, 21, 22]. An example of which comes from the re-development of the Qvar containing beclomethasone dipropionate (BDP) from a CFC (CFC-BDP) to a HFA (HFA-BDP)

propellant, as a result pulmonary deposition increased from <10% to 50-60%. In addition HFA-BDP deposited less drug on the oropharynx and resulted in a more even distribution throughout the lung [21]. Modulite® technology was developed to allow HFA propellants to replace CFCs in pMDIs. The formoterol Modulite® HFA inhaler provides a respirable fraction of 35% and 32.5% respectively. Slower plume velocity reduces impaction of the aerosol in the throat and therefore leads to greater lung deposition. In addition slower plume velocities over a longer time period reduce problems associated with poor hand-breath co-ordination; the inhaled dose is delivered over a longer time period, therefore despite poor timing of the inhaler device with the patient's inspiration, majority of the dose will still be inhaled. High speed photography of the aerosol cloud produced following actuation of the Modulite® device shows a greater plume length, reduced velocity and extended spray duration of up to 220 ms with formoterol Modulite® compared to CFC propelled salmeterol. This approach leads to reduced deposition in the oropharynx and subsequently improved pulmonary targeting [23]. The Respimat® belongs to a new class of inhaler devices termed "soft" mist inhalers (SMIs). It does not contain any propellant, instead it utilizes the mechanical energy from a spring to aerosolize droplets through a two channel nozzle, resulting in the production of an aerosol as a result of impaction of two converging jets of liquid at a carefully controlled angle. The uniblock is the key element of the Respimat®, constructed with a silicon wafer bonded to a small (2 mm x 2.5 mm) borosilicate glass plate. The spray is generated over approximately 1.5 seconds; this results in a gentle mist of respirable particles and allows more time for the patient to coordinate device actuation with inspiration. As the Respimat® produces a gentle mist which emerges at

a slower velocity compared to the aerosol cloud from pMDIS, less drug is deposited in the oropharynx [22, 24, 25]. The Respimat® delivers a greater fraction to the lung, it is possible to reduce the Metered Dose (MD) and still maintain the same clinical efficacy. The dose of ipratropium/fenoterol hydrobromide delivered using the Respimat® may be reduced by 50% of the dose administered using a pMDI with a spacer device, while producing the same clinical effect [25]. Improvements in pulmonary deposition may increase pulmonary selectivity, especially for corticosteroids with high oral bioavailability. Improvements in lung deposition are not as significant for newer ICS with low oral bioavailability [12].

Influence of Mass Median Aerodynamic Diameter

Deposition of particles in the lung is governed by its mass median aerodynamic diameter (MMAD). Respirable particles are in the range 1-5µm and will deposit effectively in the lung. Very small particles (<1µm) will not be effectively deposited and are exhaled. Large particles (>10µm) will be deposited in the tracheobronchial region and then swallowed [26]. Depending on the oral bioavailability of the compound this may contribute to therapeutic efficacy and adverse effects. For example many of the more recently developed inhaled corticosteroids such as fluticasone propionate, mometasone furoate, ciclesonide and des-ciclesonide have minimal oral bioavailability thus this route does not contribute significantly towards systemic drug exposure [12].

Controlled Release Inhaled Formulations

A number of methods to prolong pulmonary residence time of inhaled formulations have been employed. For example budesonide forms reversible fatty esters within cells, this forms a depot of inactive drug until the ester is broken down to the active form. This fatty acid esterification of budesonide, prolongs its action within the lungs

allowing once daily dosing and reduced systemic adverse effects [27-31]. In order for a corticosteroid to undergo fatty acid esterification it must possess a steric-hindrance-free hydroxyl group at the carbon 21 position [31]. Liposomal formulations have also been investigated to provide slow release following inhalation, however, liposomes have problems with stability due to leakage of drug during storage or jet-milling of lyophilized formulations [16, 32]. Solid lipid nanoparticles and microparticles provide slow drug release and increased stability in comparison to liposomes [33-35]. Biodegradable polymers such as PLGA and PLA are used widely in the drug delivery due to their biocompatible nature and approval for use as excipients [36]. PLGA microspheres have been formulated to release dexamethasone continuously over one month [37]. Drug particles may also be coated with nano thin layers of polymer to produce slow release formulations, pulsed laser deposition of PLA onto glucocorticoids resulted in slower release budesonide [38]. Large porous low density microparticles containing albuterol demonstrated sustained bronchodilation over at least 16 hours compared to 5 hours provided by non-porous particles of similar MMAD [39]. PLGA and PLA nanoparticles have received a great deal of attention, a number of studies have described their use for providing slow release. Usually a biphasic release profile was observed in most cases, initially a burst release is observed, followed by a slow release profile [40-46]. Slow release formulation of nanoparticles may be problematic, due to their large surface area over which diffusion out of the polymer matrix occurs many studies observed very fast drug release [47-49]. Mucoadhesive properties of chitosan allow nanoparticle retention time to be further increased, a study by Yamamoto et al showed surface modified PLGA

nanoparticles, modified with chitosan had prolonged effects compared with unmodified particles and also had reduced clearance[50].

Polymeric Nanoparticles

Polymer nanoparticles of PLGA or PLA are increasingly becoming the focus of attention as they are biocompatible and biodegradable [36, 51]. Degradation products are glycolic and lactic acid, natural bi-products of the Krebs cycle, readily eliminated from the body by further breakdown to carbon dioxide and water [36, 51]. PLGA degradation occurs by hydrolysis, and is dependent on molecular weight, conformation and polymer composition [52, 53]. Rates of polymer degradation are fastest when the composition is 50% lactic acid and 50% glycolic acid, thus we will use this for nanoparticle production [53]. PLGA degradation however, may also be dependent on the type of drug encapsulated [54].

Polymeric corticosteroid nanoparticles have been developed for a number of medical applications, for example, cancer, arthritis, choroidal neovascularization and neural drug delivery to name a few [40, 42, 43, 55-59]. Techniques for production of polymeric nanoparticles include solvent evaporation, nanoprecipitation, supercritical fluid precipitation, wet milling and high pressure homogenization [60-64].

The solvent evaporation technique is used commonly, it involves the production of a microemulsion in which both drug and polymer are dissolved in the organic phase, Figure 2-1 shows the scheme of production of nanoparticles using the solvent evaporation technique. The organic phase is usually a volatile compound such as dichloromethane or acetone, this will diffuse into the aqueous phase and evaporate, leaving behind a nanoparticle suspension. Budhian et al explored the influence of various production parameters on particle size and drug encapsulation efficiency [65].

Increased polymer lead to improved drug encapsulation, with a gradual increase in particle size. PVA surfactant concentration less than 0.5% w/v resulted in a bimodal particle size distribution due to insufficient stability of the microemulsion.

Solid Lipid Nanoparticles (SLN) and Microparticles

Solid lipid nanoparticles (SLN) have been studied for their potential in providing sustained drug delivery [34]. Dexamethasone SLN particles have been developed using soybean lecithin and glycerol tristearate. A biphasic *in vitro* release was observed for these particles with an initial burst of approximately 70% followed by slower release [35]. The lipid matrix of these SLN dispersion is more mobile in comparison to PLGA nanoparticles, thus slow release drug formulations may be more challenging [34]. Slow release salbutamol acetonide lipid microparticles have also been developed by Jaspart et al. Encapsulation efficiency of the formulations developed was greater than 87%, however high drug loading of around 25% w/w resulted in crystallization of drug particles outside of the microparticles. In vitro release studies determined microparticles formulated with lower drug loading produced a slower drug release, however all solid lipid microparticles released at a slower rate compared to pure salbutamol acetonide [33].

Low Density Microspheres

Large porous particles are more efficiently aerosolized as they produce fewer aggregates and are easier to re-disperse within an air stream. It is also possible that these large porous microparticles are too large to be engulfed by macrophages [66]. Large porous microparticles can be produced by spray drying, this results in particles with a similar MMAD to smaller non-porous particles. Large porous estradiol particles were aerosolized into the lungs of rats using an endotracheal tube. These particles

were shown to release over a longer time period of approximately 5 days compared to only release over 1 day with non-porous particles of similar MMAD [67].

Pulsed Laser Deposition (PLD)

A nano-thin coating of a polymer such as PLA or PLGA may be applied with the use of Pulsed Laser Deposition (PLD). High energy pulses of ultraviolet light are directed onto a polymer disc to create a plume of nanoparticles which are subsequently deposited onto a dry powder, thereby producing a nano-thin coating as shown in Figure 1-2 [68].

PLD can be used to produce sustained drug release, mean dissolution time for budesonide particles coated was 4.7 ± 0.1 hours compared to only 1.2 ± 0.5 hours for uncoated budesonide. Improved pulmonary targeting of PLA coated budesonide was demonstrated in neonatal rats. Table 1-2 shows AUC of receptor occupancy-time profiles of budesonide in neonatal rats in the lung and liver. It was seen that following intratracheal administration of uncoated budesonide, AUC for the lung and liver were indistinguishable from one another, whereas following administration of PLA coated budesonide a higher AUC_{lung} was seen compared to AUC_{liver}; indicating improvement in pulmonary targeting with PLA coated budesonide [69].

Physicochemical differences between drugs also play an important role in influencing improvements in pulmonary targeting following polymer coating. Triamcinolone Acetonide (TA) and budesonide were coated with PLA, no difference between dissolution profiles was observed between coated and uncoated TA, however the more lipophilic corticosteroid, budesonide clearly showed slower drug release after coating with PLA [70].

Oligolactic Acid (OLA)

Oligolactic acids (OLAs) are short chain versions of polylactides, which are biocompatible and approved by the FDA for use with implantable devices. OLAs are formulated as excipients in MDI inhalers by 3M, they have been used as solubilizers, suspending agents and produce sustained release within the lung. Sustained release is achieved by the formation of OLA-drug matrix (solution formulations) or OLA-coated drug particle (suspension formulations) [71, 72].

Pro-drugs

Many ICS, such as BUD or FP are administered in their pharmacologically active form; others may be inhaled as pro-drugs that must first undergo conversion to their active form. This approach can be utilized to improve pulmonary by achieving therapeutic drug concentrations at the target site whilst minimizing unwanted side effects at other sites. Deposition of ICS in the mouth and oropharynx can give rise to adverse effects such as oral candidiasis and dysphonia. Two currently available ICS include beclomethasone dipropionate and ciclesonide are converted to their active metabolites by esterase enzymes within the pulmonary epithelium [73-75]. It has been shown that bioactivation of ciclesonide is very low in the oropharynx, therefore less active drug is present in comparison administration of budesonide or FP [14]. Ciclesonide, a newer inhaled corticosteroid is converted to its active metabolite by ester cleavage at the C21 position, resulting in the formation of desisobutyryl-ciclesonide with 100-fold higher potency. Combined with high systemic clearance of both the parent compound and the active metabolite, local and systemic adverse effects are reduced. In addition the active metabolite of ciclesonide undergoes fatty acid esterification within the lung, to prolong retention within the lung, described in further detail later [74-77].

Ester Formation

Pulmonary retention of inhaled corticosteroids may be enhanced due to reversible fatty acid esterification. Conjugation has only been reported for budesonide, triamcinolone acetonide and des-ciclesonide. This process occurs within cells in the lung and forms a reservoir of lipid conjugated drug. Conjugated corticosteroid is slowly hydrolyzed by enzymes to release free drug. In order for this fatty acid conjugation to take place the ICS must possess a steric-hindrance-free hydroxyl group at the carbon 21 position. Reversible fatty acid conjugation provides increased anti-inflammatory action at the target site resulting in improved pulmonary selectivity [27-31, 74, 75, 78-80]. In general, rapid ester formation and slow ester hydrolysis leads to improved pulmonary targeting; this is further improved in combination with a high systemic clearance.

The corticosteroid budesonide is moderately lipophilic with a relatively fast dissolution, followed by a rapid absorption from the lung. Budesonide would not be expected to have a prolonged duration of action in the lung when compared to fluticasone propionate, a corticosteroid with both higher lipophilicity and relative receptor affinity [80]. Formation of the fatty acid ester, budesonide oleate however, prolongs retention in the airways and allows for once daily dosing [29].

Ciclesonide is a corticosteroid which first undergoes metabolism to the active form, des-ciclesonide followed by reversible fatty acid ester formation. This ester formation was confirmed to occur in the human lung in a single dose, open-label, non-randomized study in 20 patients. The metabolites des-ciclesonide, des-ciclesonide-oleate and des-ciclesonide-palmitate were detected in the central and peripheral lung tissue [73].

Budesonide, however was shown to be esterified more rapidly and to a greater extent in comparison to ciclesonide in rat tracheal tissue [79].

Future developments in corticosteroids may involve selection of compounds which undergo fatty acid esterification [81].

Liposomes

Liposomes have been extensively studied due to their ability to incorporate both hydrophilic and hydrophobic drugs, as well as ability to produce a variety of particle sizes and have been used as drug carriers since the late 1960s [82]. Liposomes are composed of phospholipids which are endogenous in the lung, improving compatibility. Nebulized BDP-DLPC liposomes administered to healthy volunteers are well tolerated in doses equivalent to those currently used for treatment in asthma [83]. Modified liposomes may be used to target delivery to different cells, liposomes prepared with mannosylated cholesterol derivatives can enhance uptake into macrophage cells [84]. Liposomal formulations have also been investigated to provide slow release following inhalation, however, liposomes have problems with stability due to leakage of drug during storage or jet-milling of lyophilized formulations [16, 32]. Solid lipid nanoparticles and microparticles provide slow drug release and increased stability in comparison to liposomes [33-35]. It has been demonstrated that slow drug release from liposomes is able to improve pulmonary selectivity in the rat model. Triamcinolone Acetonide Phosphate (TAP) liposomes sized 200nm and 800nm were shown to produce biphasic drug release *in vitro*, with the later resulting in slower release). In vivo studies in male F-344 rats were performed in order to compare pulmonary selectivity of these liposomes with TA solution and liposomes. TA liposomes release drug rapidly under sink conditions, and thus both the TA solution and liposome formulation would not be

expected to produce pulmonary targeting, Table 1-3 show 800nm TAP liposomes produced the greatest pulmonary targeting; pulmonary targeting is defined as the area under the curve of the receptor occupancy time profile of the lung compared to the liver (Pulmonary targeting (%*hr) = AUC_{lung} - AUC_{liver}). Slow drug release from liposomes allows reduction in dose and dosing frequency, thereby reducing systemic adverse effects observed whilst maintaining clinical effects in the lung [16]. It has been shown that budesonide encapsulated into stealth liposomes, delivered once a week was able to provide an equivalent anti-inflammatory effect as once daily administration of budesonide [85].

Enhancing Mucoadhesive Properties

Chitosan is a polysaccharide containing an amino group which may be positively charged, particles composed or coated with chitosan increase interaction with negatively charged lung epithelial cells. In addition chitosan may also influence release characteristics of drug particles. Gelatin has also been utilized to produce particles. Rifampicin liposomes coated with chitosan had greater mucoadhesive properties and lower toxicity towards A549 epithelial cells compared to uncoated liposomes. Table 1-4 demonstrates the relationship between zeta potential and mucoadhesive properties of rifampicin liposomes. Uncoated negatively charged liposomes showed the lowest amount of adhesion, followed by uncharged liposomes, with positively charged chitosan coated liposomes showing the most mucoadhesion [86].

Objectives

Increased pulmonary retention has been observed following inhalation of nanometer sized particles in comparison to micronized particles [87, 88]. In addition polymers such as PLGA and PLA are able to reduce drug release from nanoparticles.

We hypothesize that nanoparticles may be spray dried with lactose to produce nanocomposite microspheres; these microspheres will be capable of delivery to the lung following inhalation. The above hypotheses will be tested by the following specific aims;

- Preparation and characterization of slow release polymeric corticosteroid nanoparticles.
- *In vitro* drug release testing to determine slow release characteristics of nanoparticles.
- Design of a spray dryer able to enable nanoparticles to be spray dried at temperatures lower than operating temperatures of commercially available spray dryers.
- Preparation of spray dried nanoparticles to form nanocomposite microspheres with MMAD in the respirable range.
- Determine *in vitro* release characteristics of spray dried formulations to investigate the effect of spray drying on release rate.

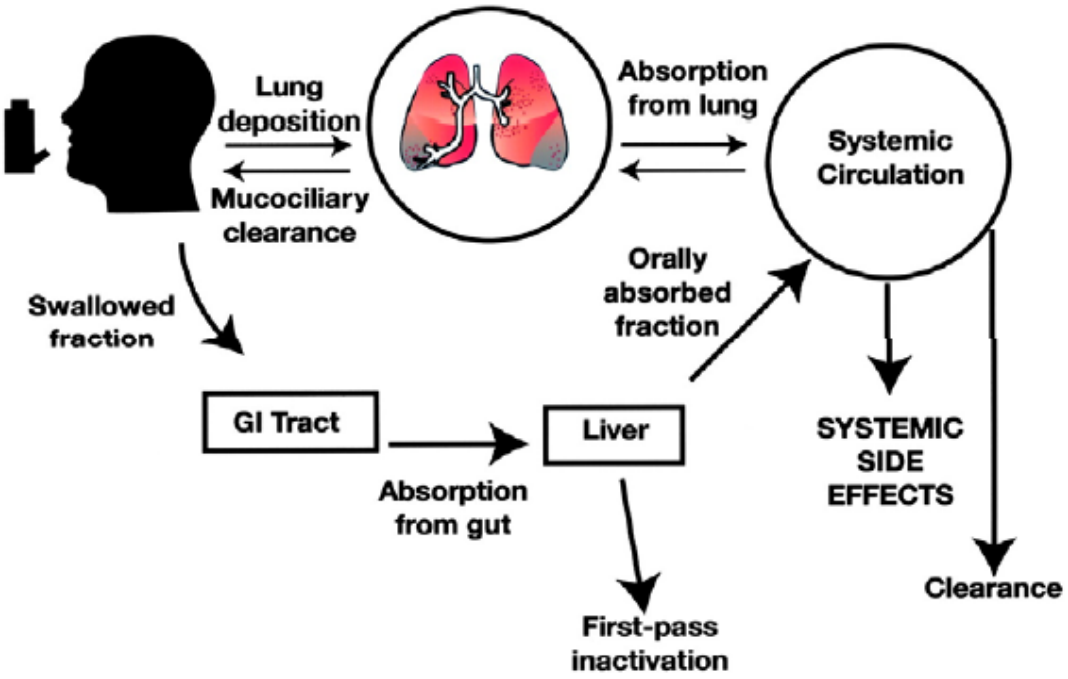


Figure 1-1. Fate of Inhaled Corticosteroids [12]

Table 1-1. PKPD properties of inhaled corticosteroids [15]

ICS	RRA	CL (L/hr)	Vdss (L)	F_{oral} (%)	f_u (%)
BDP	53	150	20	15-20	13
BMP	1022	120*	424	26	-
FLU	190	57	96	20	20
TA	233	37	103	23	29
BUD	935	84	18,311	12	
FP	1800	69	318	<1	10
MF	2900	54	-	<1	1-2
CIC	12	140	207	<1	1
Des-CIC	1200	228*	897	<1	<1

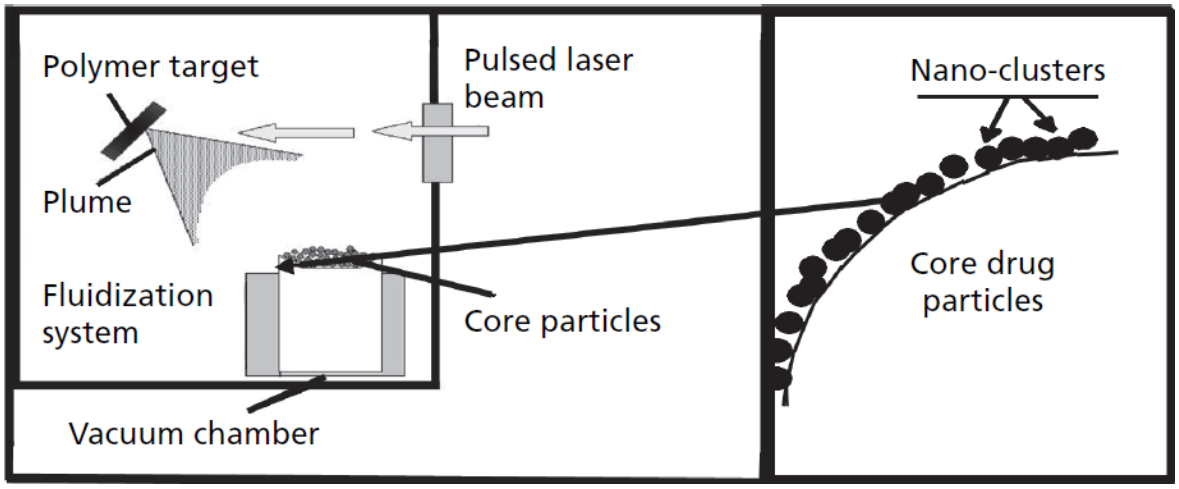


Figure 1-2. Pulsed laser deposition (PLD) - Nanoclusters of polymer from the target are deposited on larger micronized drug particles as continuous coatings that sustain the release rate of drug in solution [38]

Table 1-2. Average AUCs (n=3) in the lung, liver and brain and pulmonary targeting (PT) in neonatal rats after intratracheal administration (50 µg/kg) of uncoated budesonide and PLA coated budesonide [69]

Formulation	Dose (µg/kg)	AUC_{lung}	AUC_{liver}	AUC_{brain}	PT (AUC_{lung}/AUC_{liver})
Uncoated budesonide	50	58.4 ± 12.9	56.4 ± 6.8	38.3 ± 6.7	1.03 ± 0.13
PLA coated budesonide	50	75.8 ± 3.7	46.6 ± 14.5	29 ± 7	1.72 ± 0.46

Table 1-3. Cumulative Receptor Occupancy (AUC), Pulmonary Targeting and Mean Pulmonary Effect Times (MET) After Intratracheal Administration of Escalating Doses of TAP in 800 nm Liposomes [16]

	TAP-sol	TA-lip 200 nm	TAP-lip 200 nm	TAP-lip 800 nm
Lung	370 ± 50	320 ± 85	770 ± 120	1070 ± 70
Liver	340 ± 40	380 ± 10	620 ± 150	700 ± 140
Pulmonary Targeting (%*hr)(AUC_{lung}-AUC_{liver})	30 ± 10	-60 ± 80	150 ± 60	370 ± 70
Pulmonary Targeting (%*hr)(AUC_{lung}/AUC_{liver})	1.0	0.85	1.2	1.5
Mean Pulmonary Effect Time (hr)	3	2.4	5.7	>6.2

Table 1-4. Influence of liposome composition on mucoadhesion and zeta potential [86]

Liposome composition	Mucin adsorbed on liposomes (%) Mean value (SD)	Zeta-Potential (mV)
PC/Chol	17.0 ± 8.3	+0.09 ± 0.54
PC/PG/Chol	7.4 ± 4.4	-22.9 ± 2.1
$[PC/Chol]_{CHT}$	47.1 ± 1.2	+4.4 ± 1.9
$[PC/PG/Chol]_{CHT}$	90.9 ± 7.6	+24.98 ± 0.91
DSPC/Chol	46.2 ± 4.1	+0.93 ± 0.77
DSPC/PG/Chol (9:1:5)	25.1 ± 8.1	-19.9 ± 2.3
$[DSPC/Chol]_{CHT}$	66.6 ± 2.2	+5.4 ± 2.7
$[DSCP/PG/Chol]_{CHT}$	93.1 ± 4.1	24.43 ± 0.62

CHAPTER 2
DEVELOPMENT AND CHARACTERIZATION OF SLOW RELEASE POLYMERIC CORTICOSTEROID NANOPARTICLES

Introduction

Nanoparticles may have the possibility of providing increased retention in the airways compared to micron sized particles [87]. Nanoparticles have been extensively studied in the field of toxicology. A number of studies have been conducted in order to determine deposition, retention and translocation of ultrafine particles in the lung. Technetium Tc 99m (^{99m}Tc)-radiolabeled 100nm carbon particles were administered to healthy subjects and COPD patients by nebulizer. The central/peripheral (C/P) distribution was controlled by administering either a shallow or deep aerosol bolus. A shallow aerosol bolus is used to deliver either more centrally compared to a deep aerosol bolus inhalation. 48 hours following a shallow aerosol bolus, 70% and 82% of particles were retained within the airways of healthy non-smokers and COPD patients respectively. It has been shown that nanoparticles are retained in the lung for a longer time period compared with micron sized particles; 24 hours following inhalation >70% of nanoparticles are retained in the airways, in comparison only 10% of particles greater than 6μm are retained [87, 88]. It has been proposed that increased retention on nanoparticles in the lung is as a result of greater displacement of nanoparticles into the aqueous surfactant film compared to micron sized particles [87, 89, 90].

In recent times nanotechnology has received much attention, potential applications include imaging and diagnosis, targeted drug delivery and controlled drug release [91, 92]. Nanotechnology itself is not a new concept, liposomes were first used as drug carriers in the late 1960s [82]. Polymer nanoparticles of PLGA or PLA are increasingly becoming the focus of attention as they are biocompatible, biodegradable and have

been approved by the FDA for use in implantable devices [36, 51]. Degradation products are glycolic and lactic acid, natural bi-products of the Krebs cycle, readily eliminated from the body by further breakdown to carbon dioxide and water [36, 51]. PLGA degradation occurs by hydrolysis, and is dependent on molecular weight, conformation and polymer composition [52, 53]. Rates of polymer degradation are fastest when the composition is 50% lactic acid and 50% glycolic acid, thus this was used for production of nanoparticles [53]. PLGA degradation however, may also be dependent on the type of drug encapsulated [54]. Drug release from particles is also influenced by polymer composition. Increased lactide:glycolide results in slower release, possibly due to increased hydrophobicity of the polymer as well as increased solid state solubility of hydrophobic drugs in the polymer matrix. It has also been determined that PLGA with an ester terminated end group released hydrophobic drugs at a slower release rate compared with a carboxylic acid end group [93].

Polymeric corticosteroid nanoparticles have been developed for a number of medical applications, for example, cancer, arthritis, choroidal neovascularization and neural drug delivery to name a few [40, 42, 43, 55-59]. Techniques for production of polymeric nanoparticles include solvent evaporation, nanoprecipitation, supercritical fluid precipitation, wet milling and high pressure homogenization [60-64]. Advantages and disadvantages of the various methods for nanoparticle production are discussed in Table 2-1.

The solvent evaporation technique is used in the production of monodispersed spherical polymeric nanoparticles. This technique is used commonly, it involves the production of a microemulsion in which both drug and polymer are dissolved in the

organic phase, Figure 2-1 shows the scheme of production of nanoparticles using the solvent evaporation technique. The organic phase is usually a volatile compound such as dichloromethane or acetone, this will diffuse into the aqueous phase and evaporate, leaving behind a nanoparticle suspension. Nanoparticles may be collected and washed by centrifugation, followed by lyophilization to allow storage as a dry powder. A number of process parameters may be manipulated to influence particle size and drug loading. Budhian et al explored the influence of various production parameters on particle size and drug encapsulation efficiency [65]. Increased polymer content lead to improved drug encapsulation, with a gradual increase in particle size. Increased polymer content allows more drug to be dispersed within the polymer matrix, thus resulting in higher encapsulation efficiency. As the polymer contained in the organic phase increases however, viscosity of this phase is also increased, thus resulting in formation of larger o/w microemulsion droplets and larger nanoparticle size. PVA surfactant concentration less than 0.5% w/v resulted in a bimodal particle size distribution due to insufficient surfactant concentration for production of a stable microemulsion.

The solvent evaporation method has been used to produce slow release nanoparticles of a number of different compounds including paclitaxel, etanidazole, bezopsoralen, flurbiprofen and dexamethasone; a biphasic *in vitro* release was observed in most cases [40-46]. It is difficult to compare results from different studies however, as drug release rates depend on release conditions such as presence of sink conditions, release media and sample separation methods (filtration, centrifugation or dialysis). Though each method has its advantages it is possible that certain artifacts may result due to the separation method used. For example retardation of drug release

from dialysis bags may cause release profiles to reflect diffusion through the dialysis membrane rather than from the formulation [34, 94]. In these situations, it is possible for the drug to interact with the dialysis membrane, slow release observed is due to an interaction with the membrane rather than the actual release rate from the nanoparticle formulation; therefore it is necessary to ensure that the chosen dialysis membrane does not interact with the drug of interest, as well as inclusion of adequate controls in the experiment. In addition, release rate can further be reduced by coating with another polymer such as chitosan. Chitosan is polysaccharide derived from the deacetylation of chitin, commonly obtained from the shells of crustaceans. It is biocompatible and can be degraded by lysozymes present in all mammalian cells [95]. Cationic chitosan is able to coat nanoparticles due to an electrostatic interaction with the negatively charged carboxylic acid end group present on the polymer PLGA or PLA [96]. Chitosan has been used to coat PLGA nanoparticles as well as liposomes [86, 96-99]. PLGA nanoparticles surface modified with chitosan also had increased retention in the lung compared to PLGA nanoparticles due to improved mucoadhesive properties of chitosan [50].

Hypothesis

We hypothesize that polymeric nanoparticles with a monodispersed particle size distribution can be formed using the solvent evaporation method. Various parameters during this process will be manipulated to influence particle size and drug encapsulation. We hypothesize that increasing polymer content will increase particle size and drug encapsulation efficiency. Also increasing PVA concentration will allow smaller oil-in-water microemulsion droplets to be formed, resulting in smaller particles with lower drug encapsulation. Larger particles may be formed with reducing PVA

concentration, however a minimum content must be achieved in order to sufficiently stabilize the microemulsion and result in a monodispersed particle size distribution.

It is hypothesized that chitosan coated nanoparticles will be able to release at a slower rate in comparison with uncoated particles. Electrostatic interaction between the positively charged chitosan and negatively charged polymer allows chitosan to coat polymeric nanoparticles. It is important that the polymer chain is terminated with a carboxylic acid end group and is not ester terminated, to ensure interaction with the chitosan. Methods to produce chitosan coated particles will be investigated and optimized.

In vitro release testing will be performed by the batch/filter method [100]. This method reduces artifacts which may be observed by using the dialysis method for separation of particles from free drug. It is hypothesized that polymeric nanoparticles will release drug at a slower rate compared to the control micronized drug.

Materials and Methods

Chemicals

Micronized TA was purchased from PCCA Inc. (Houston, TX, USA). MF was donated by Ipca laboratories ltd (Mumbai, India). PLGA and PLA were purchased from Lactel Absorbable Polymers (Pelham, AL, USA). DCM and ACN were purchased from Fisher Scientific (Pittsburgh, PA, USA). PVA and medium molecular weight chitosan were obtained from Sigma Chemical Co. (St. Louis, MO).

Nanoparticle Preparation

Polymeric nanoparticles of either TA or MF were prepared using the solvent evaporation technique [47]. TA will be used as a model drug to allow investigation of

process parameters on particle size, drug encapsulation efficiency and *in vitro* release profiles. The polymer used was either DL-PLGA (50:50 inherent (0.55-0.75 dL/g inherent viscosity) or DL-PLA (0.54 dL/g inherent viscosity). In brief, 10 mg of drug and 200-400 mg polymer were dissolved in 5 mL DCM, this was pre-emulsified with 5 mL of the aqueous PVA phase by vortexing for 30 seconds in a 20 mL glass scintillation vial using the Fisher vortex genie 2 at speed 9. The pre-emulsion was added to the remaining 45 mL PVA solution and sonicated on ice (Sonics Vibra Cell Ultrasonicator Newtown, CT) at 60 W for 5 minutes to form an oil-in-water microemulsion. PVA concentration was varied between 1 and 3% w/v for each PLGA level, (n=3). DCM was allowed to evaporate under gentle stirring on the magnetic stirrer for 4 hours to produce a nanoparticle suspension which was collected by centrifugation at 20,000 rpm for 40 minutes and washed Beckman J2-21 (Beckman Coulter, Inc.Fullerton, CA) using the JA-20 rotor. Nanoparticles were lypophilized using a Labconco freeze dryer (Labconco Corporation, Kansas City, MO). Dried nanoparticles were stored in amber glass vials at 4°C in a dessicator.

Two different methods to prepare chitosan coated PLA nanoparticles were studied. Figure 2-2 shows details of these two methods, the incubation method involves dispersion of PLA nanoparticles in either 0.1% or 1% w/v chitosan solution. In situ coating allows the chitosan coating to be applied in the same step as formation of the nanoparticles. Nanoparticles are prepared using the solvent evaporation technique, however chitosan is already dissolved in the aqueous PVA phase of the microemulsion. During formation of the nanoparticle suspension, the positively charged chitosan is coated onto the negative PLA particles by an electrostatic interaction. The optimal

method was chosen based on the ability to form unimodal nanoparticles. The aqueous phase of the oil-in-water microemulsion was produced using PVA and chitosan in 1% v/v acetic acid in DDW. 400 mg PLA and 10 mg MF were dissolved into 5 mL DCM, this was pre-emulsified with 5 mL of the aqueous phase by vortexing for 30 seconds. This was combined with the remaining 45 mL of the aqueous phase and sonicated on ice for 5 minutes at 60 W to produce an oil-in-water microemulsion. The emulsion was gently stirred for a further 4 hours to allow the DCM to evaporate and leave behind a chitosan coated nanoparticle suspension. The resultant suspension was centrifuged and washed using a 1% v/v acetic acid solution. Chitosan coated nanoparticles were dispersed in a small volume of double distilled water followed by lyophilization and stored in amber glass vials in a desiccator at 4°C.

Drug loading and Encapsulation Efficiency

In order to determine the drug content of the lyophilized nanoparticles approximately 5 mg of the dry powder was weighed. The particles were then dissolved using 2 mL DCM and placed on an orbital shaker (Bellco biotechnology, Vineland, NJ) overnight to ensure disintegration of the nanoparticles. The DCM was evaporated off using a Jouan RC10.10 vacuum centrifuge (Thermal Fisher, Asheville, NC), the dried residue was dissolved in 1 mL mobile phase (ACN:DDW, 60:40) and analyzed by HPLC-UV (Hewlett Packard Series 1050) using a Phenomenex Ultracarb 30 4.6x150 mm ODS column using mobile phase at a flow rate of 1.2 mL/min sample peaks were quantified at 254 nm wavelength. Concentrations for the caliberation curve were 10, 20, 40, 60, 80 and 100 μg/mL with an R squared value of at least 0.997. Figure 2-3 describes the method by which EE and drug loading of nanoparticles was determined.

Drug loading and encapsulation efficiency were determined by the equations below. Theoretical drug loading (% w/w) was calculated by calculating how much drug would be theoretically present in 100 g of the lyophilized powder provided that no drug or polymer is lost during the nanoparticle production process, the equation for which is described below.

$$Actual\ Drug\ loading\ (\%\ w/w) = \frac{Amount\ of\ drug\ contained\ in\ NP}{Total\ weight\ of\ NPs\ weighed} \times 100$$

$$Theoretical\ drug\ loading\ (\%\ w/w) = \frac{Drug\ weighed}{Drug + Polymer\ weighed} \times 100$$

$$Encapsulation\ Efficiency\ (\%\ w/w) = \frac{Actual\ drug\ loading\ (\%\ w/w)}{Theoretical\ drug\ loading\ (\%\ w/w)} \times 100$$

Particle Size, Zeta Potential and Morphology

Particle size and zeta potential were determined using the Nanotrac (Microtrac) and Brookhaven ZetaPlus. SEM was performed to observe particle morphology using scanning electron microscope (SEM) JEOL JSM-6335F instrument (Major Analytical Instrument Center (MAIC), UF, Gainesville, FL). Briefly, formulations were placed on carbon stubs which were coated with carbon using a vacuum evaporator. SEM was conducted using 2 kV.

***In vitro* Drug Release Study**

In vitro release was performed in 100 mL 1% v/v tween 80 in PBS, shaken at 30 rpm in a hot shaker (Bellco biotechnology, Vineland, NJ) at 37°C over 24 hours under constant sink conditions. Tween 80 was used to increase the saturation concentration of TA and MF in the dissolution media to 83 µg/ml and 25 µg/ml with addition of 1% v/v tween 80 at 37°C. In order to maintain sink conditions maximal concentrations obtained did not exceed 10% of the saturation concentration. At specific time points (0, 15, 30,

90, 120, 180, 240, 360, 600 and 1440 minutes), 1 mL was removed using a pipette and subsequently filtered using a 0.02 μm filter (Whatman Anotop plus filter), 1 mL fresh buffer was replaced into the dissolution media. The maximum concentration obtained was determined based on analysis of remaining undissolved drug at the end of the dissolution testing. After the last time point, the dissolution media was centrifuged at 20,000 rpm for 40 mins using the Beckman J2-21 (Beckman Coulter, Inc.Fullerton, CA) using the JA-20 rotor. The pellet was dissolved in 2 mL DCM and placed on an orbital shaker overnight (Bellco biotechnology, Vineland, NJ) to ensure disintegration of the particles. HPLC was used to quantify released drug concentrations using a caliberation curve ranging from 0.5-10 ug/mL. Controls used were micronized TA and MF contained in the Asmanex® formulation from the reservoir based inhaler device, Twisthaler™, Schering-Plough.

Results and Discussion

Influence of PLGA and PVA on Encapsulation Efficiency and Particle Size of Nanoparticles

A factorial design was implemented to determine influence of PLGA and PVA concentration on particle size and encapsulation of TA within nanoparticles. Encapsulation efficiency of TA nanoparticles decreased with increasing PVA concentration, shown in Figure 2-4 as expected. Increased PVA surfactant concentration in the microemulsion aqueous phase results in an increased solubility of TA in the aqueous phase. A consequence of this is increased loss of drug during nanoparticle production is reduced encapsulation efficiency and drug loading. Increasing PLGA content did not have any significant effect on encapsulation efficiency. Increasing PVA concentrations resulted in a trend of decreased particle size, as shown

in Figure 2-5. Increased surfactant concentration in the oil-in-water microemulsion leads to the formation of smaller oil phase droplets; as a result particle size of nanoparticles decreases with increased PVA concentration. Increasing PLGA content results in the formation of larger nanoparticles, this is as a result of increased viscosity of the oil phase of the microemulsion; larger organic phase droplets are formed leading to an increase in particle size. This is in agreement with studies performed by Budhian et al; increasing PVA concentration results in reduced drug loading initially, between 1-2% w/v PVA then plateau. Particle size also decreased with increasing PVA concentration in their study, however with the use of >5% w/v PVA particle size increased. This is due to competing effects of increased stabilization of the microemulsion with increasing surfactant, which reduces particle size. High PVA concentration increases viscosity of the aqueous phase, leading to reduced net shear stress for droplet breakdown during formation of the microemulsion, therefore resulting in larger particles [65]. The PVA concentration range we investigated was 1-3% w/v, thus it was determined increasing PVA concentration reduced encapsulation efficiency and a trend of reduced particle size was observed. Budhian et al also investigated the influence of PLGA content on particle size and drug loading. Increasing PLGA content lead to a gradual increase in particle size and drug loading, as was observed in our experiments producing TA-PLGA nanoparticles.

Influence of PLGA and PVA on Morphology of Nanoparticles

TA crystals were observed for two formulations of TA-PLGA nanoparticles, these were both formulated using 200mg PLGA using 1% and 2% w/v PVA, SEM of these formulations are shown in Figure 2.6 and Figure 2.7. These TA crystals were only

observed when particles were formulated with both low polymer contents and surfactant concentrations. There must be sufficient polymer for the drug to disperse within in the solid state; drug not present in the polymer matrix must then be washed off [93]. When only 200 mg PLGA is used, there is insufficient polymer for the TA to disperse within, coupled with low PVA surfactant, unencapsulated drug is not washed away leaving behind free TA crystals. Although it appears that encapsulation efficiency for these two samples is greater than 50%, this is not due to TA trapped in PLGA nanoparticle matrix. Figure 2.8 shows spherical TA-PLGA nanoparticles produced with 400 mg PLGA and 1% w/v PVA, no TA crystals were observed in this formulation. Formulations produced using 300-400 mg PLGA did not contain any unencapsulated drug crystals, as determined by SEM.

In Vitro Release Study of TA-PLGA Nanoparticles

Various methods to determine *in vitro* drug release of nanoparticles have been reported in the literature. Regenerated cellulose ester membranes and dialysis bags have been used to physically separate nanoparticles from the dissolution media. Preliminary studies performed however, indicated interaction of corticosteroids with the membrane and delayed release of TA from within dialysis bags. If this method is used, it is essential to find dialysis membranes which do not interact with the drug being studied, as well as use of adequate controls [94]. Separation by filtration was used to determine *in vitro* release to avoid problems which occurred with the use of regenerated cellulose dialysis membranes.

In vitro release studies were performed on TA-PLGA nanoparticles formulated with either 300 mg or 400 mg PLGA using both 1% and 2% w/v PVA. These formulations had good drug encapsulation efficiency as well as a unimodal particle size.

In comparison to the control of micronized TA, nanoparticle formulations made with only 300mg PLGA released at a slightly faster rate, as shown in Figure 2-9. TA-PLGA nanoparticles formulated with 400mg PLGA were shown to release at the same rate as micronized TA, refer to Figure 2-10. All formulations tested release drug at a very similar rate to the control micronized TA. The surface area to mass ratio of nanoparticles is very high compared with micron sized particles, thus if no modification is made to the formulation, nanoparticles would release drug at a much faster rate compared to their micron sized counterpart. TA-PLGA nanoparticles posses some slow release characteristics as they are able to release drug at a similar rate to micronized TA.

Chitosan Coated and Uncoated PLA MF Nanoparticles

Two methods for production of chitosan coated polymeric nanoparticles were investigated. The chitosan coating procedure was optimized to produce particles coated particles with a unimodal particle size distribution. Lyophilized MF-PLA nanoparticles that were incubated with either 0.1% or 1% w/v chitosan solutions resulted in nanoparticles that had multimodal particle size distributions. A possible explanation for this is due to incomplete dispersion of nanoparticles in the viscous chitosan solution, leading to coating of agglomerated nanoparticles. SEM of MF-PLA nanoparticles incubated with a chitosan solution showed a bimodal particle size distribution (Figure 2-11). In situ chitosan coating involves coating during the formation of nanoparticles. Chitosan is present in the aqueous phase of the microemulsion, positively charged chitosan adheres onto the negatively charged PLA nanoparticles. Addition of 1% w/v chitosan to the aqueous phase results in bimodal particle size distribution. High viscosity of 1% w/v chitosan solution resists droplet breakdown during

sonication, producing some larger particles. In addition, particles produced from 1% w/v chitosan solutions are more difficult to collect by centrifugation, due to the high viscosity of the solution. The use of 0.1% w/v chitosan within the situ coating procedure resulted in a unimodal particle distribution and can be seen by SEM (Figure 2-13) and can be compared to uncoated MF-PLA nanoparticles (Figure 2-12). Particle size of chitosan coated particles was 226 nm compared to 212 nm for uncoated particles. Although particle size does not significantly increase following coating with chitosan, the presence of chitosan is confirmed by the reversal of zeta potential from negative charge on uncoated nanoparticles to positive charge on coated particles, as shown in Table 2-3. In order for chitosan to coat particles, the PLA polymer must terminate with a carboxylic acid group and the amine group on chitosan must be protonated, with 1% v/v acetic acid.

In vitro release of chitosan coated particles was compared to uncoated MF-PLA nanoparticles and Asmanex® as a control are shown in Figure 2-14. A biphasic release was observed for both the chitosan coated and uncoated nanoparticles. Initially the burst release of MF-PLA nanoparticles is similar to the Asmanex® formulation, followed by slow release. Chitosan coated MF-PLA nanoparticles (CH-MF) release approximately 20% by 30 minutes, followed by a much slower release rate compared to MF or Asmanex®. After 24 hours of *in vitro* release, CH-MF nanoparticles only released 43% MF; in comparison, all MF from the Asmanex® formulation was released in 1 hour. *In vitro* release data show that chitosan coating allows much slower drug release from nanoparticles. Drug release occurs by diffusion out of the nanoparticles as well as due to degradation of chitosan and PLA. In the short term, diffusion processes

determine drug release, however over longer time periods degradation of chitosan and PLA play a more dominant role. Similar observations were made from testosterone contained in a PLGA film, initial release was as a result of diffusion from the film, followed by a slower release due to the hydrolytic degradation of PLGA [101]. Both chitosan coated and uncoated nanoparticles formulated with MF were able to release at a slower rate compared to the formulation contained within the commercially available Asmanex® Twistnaler™, currently used by oral inhalation for long-term asthma management. PLGA microspheres of rifampicin coated with chitosan were shown to produce a smaller burst effect compared to uncoated particles by Manca et al [97], these results are in agreement with our observations from chitosan coated and uncoated PLA nanoparticles.

Conclusion

- Increased PLGA content resulted in a general increase in particles size with significant increase in drug encapsulation efficiency and loading.
- Increased PVA concentration resulted in reduced nanoparticle size, encapsulation efficiency and drug loading.
- Chitosan coated nanoparticles were not significantly larger than uncoated particles, suggesting only a thin coating layer.
- Reversal of zeta potential from negative for uncoated particles to positive for chitosan coated particles confirmed presence of chitosan coating.
- *In vitro* release studies showed that TA-PLGA nanoparticles released at a similar rate to micronized drug particles.
- *In vitro* release of MF-PLA and CH-MF produced a biphasic drug release profile.
- *In vitro* release determined that CH-MF formulation released at the slowest rate, with only 43% drug release over 24 hours.
- Both chitosan coated (CH-MF) and uncoated MF-PLA nanoparticles released drug at a slower rate compared to MF contained in the Asmanex® Twisthaler™.

- Chitosan coated particles, however, released at a much slower rate, only 20% burst release was observed, followed by slow drug release.

- Chitosan coated and uncoated nanoparticles prepared with the corticosteroid MF were able to release drug at a slower rate compared to MF contained in the Asmanex® Twisthaler™.

- Chitosan coated MF-PLA nanoparticles were chosen for further development into nanocomposite microspheres due to slow release rates observed.

Table 2-1. Advantages and disadvantages of various methods for production of nanoparticles

	Advantages	Disadvantages
Wet Milling	Crystalline nanoparticles	Contamination with grinding material Batch-to-batch variation Microbial growth @30ºC
High pressure homogenization	40-500nm Small or large scale	Aggregation and coalescence of nanosuspensions Changes in crystallinity
Supercritical fluid process	No organic solvent	Depends on efficiency of atomization Hydrophobic:hydrophilic particles cannot be produced – inadequate solvent systems Poorly water soluble drugs have poor CO_2 solubility → low particle production Resolved by high temperatures High pressures
Solvent evaporation	Spherical nanoparticles Monodispersed nanoparticles Ease of production Suitable for hydrophobic and hydrophilic drugs	Use of organic solvent Not suitable for industrial use

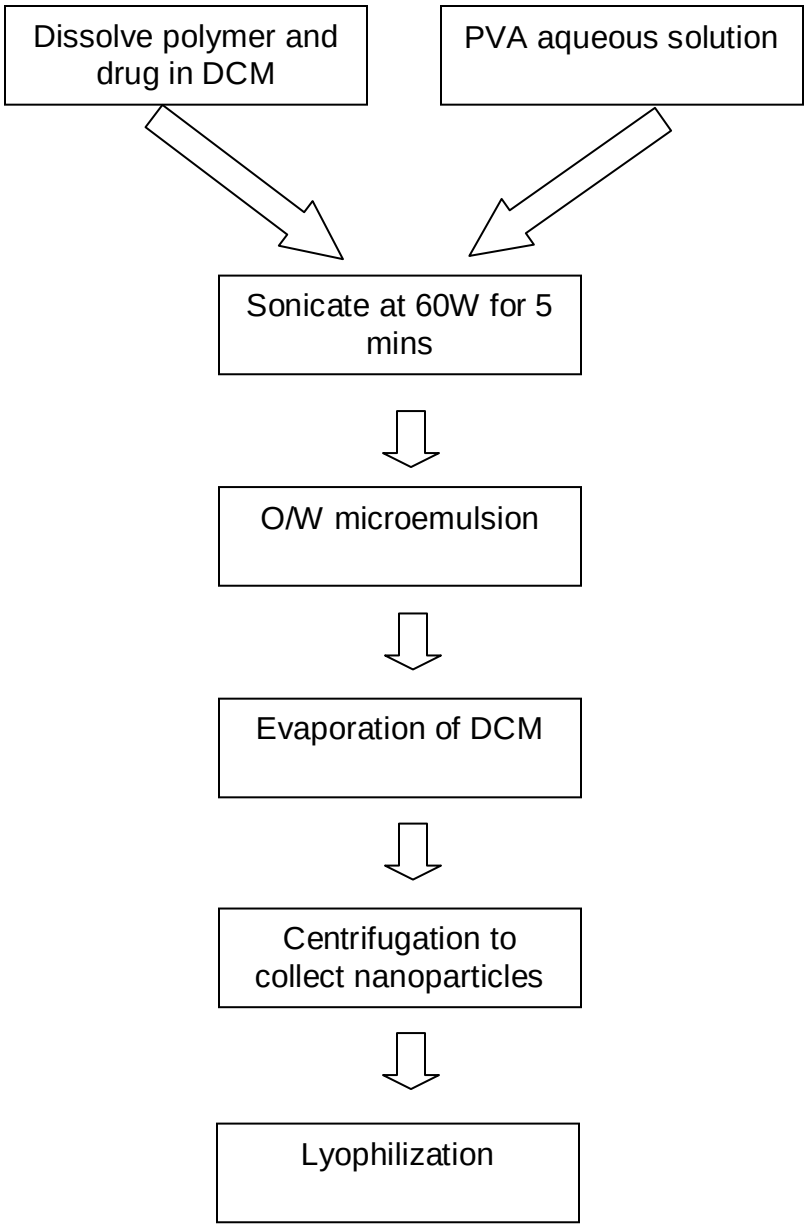


Figure 2-1. Solvent evaporation technique to produce nanoparticles

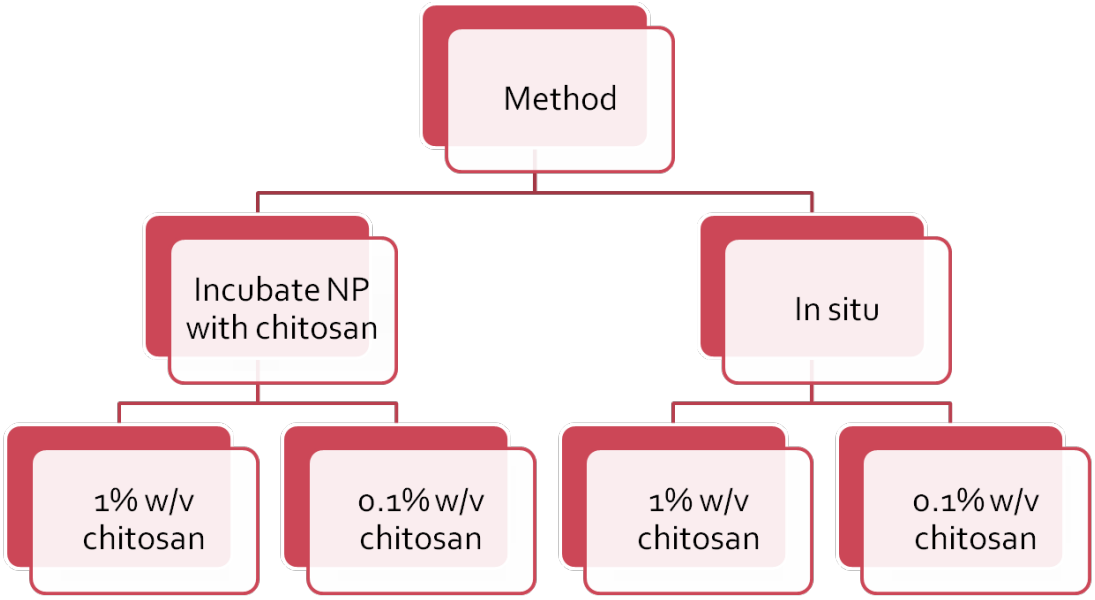


Figure 2-2. Schematic detailing methods to coat nanoparticles with chitosan

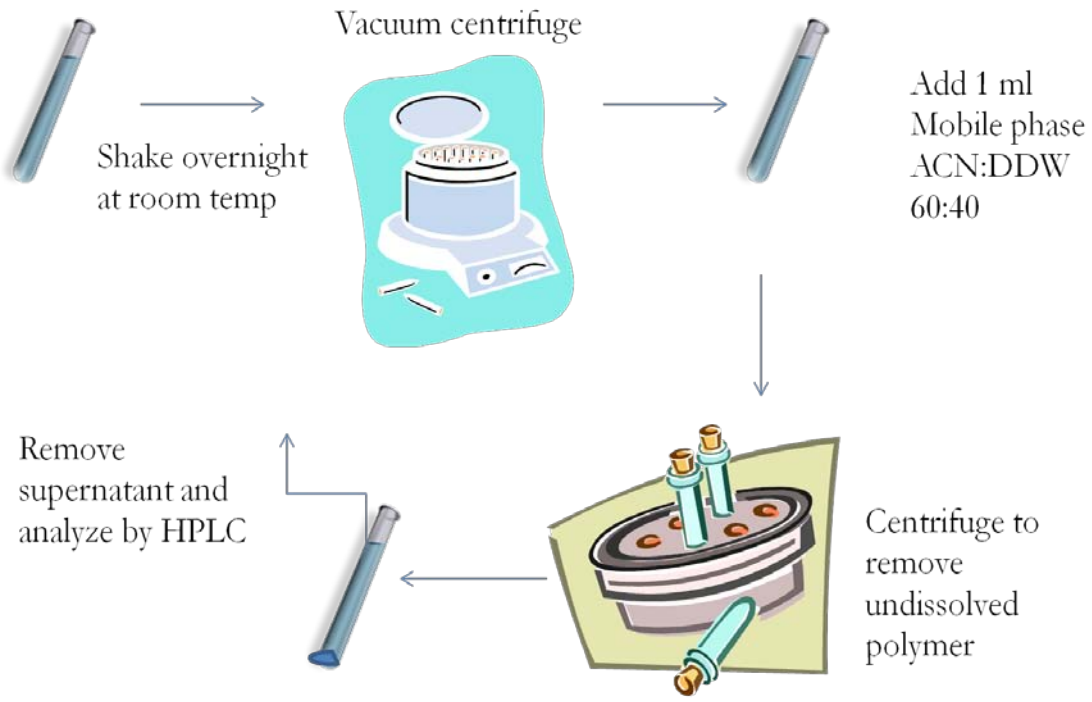


Figure 2-3. Determine encapsulation efficiency and drug loading of nanoparticles

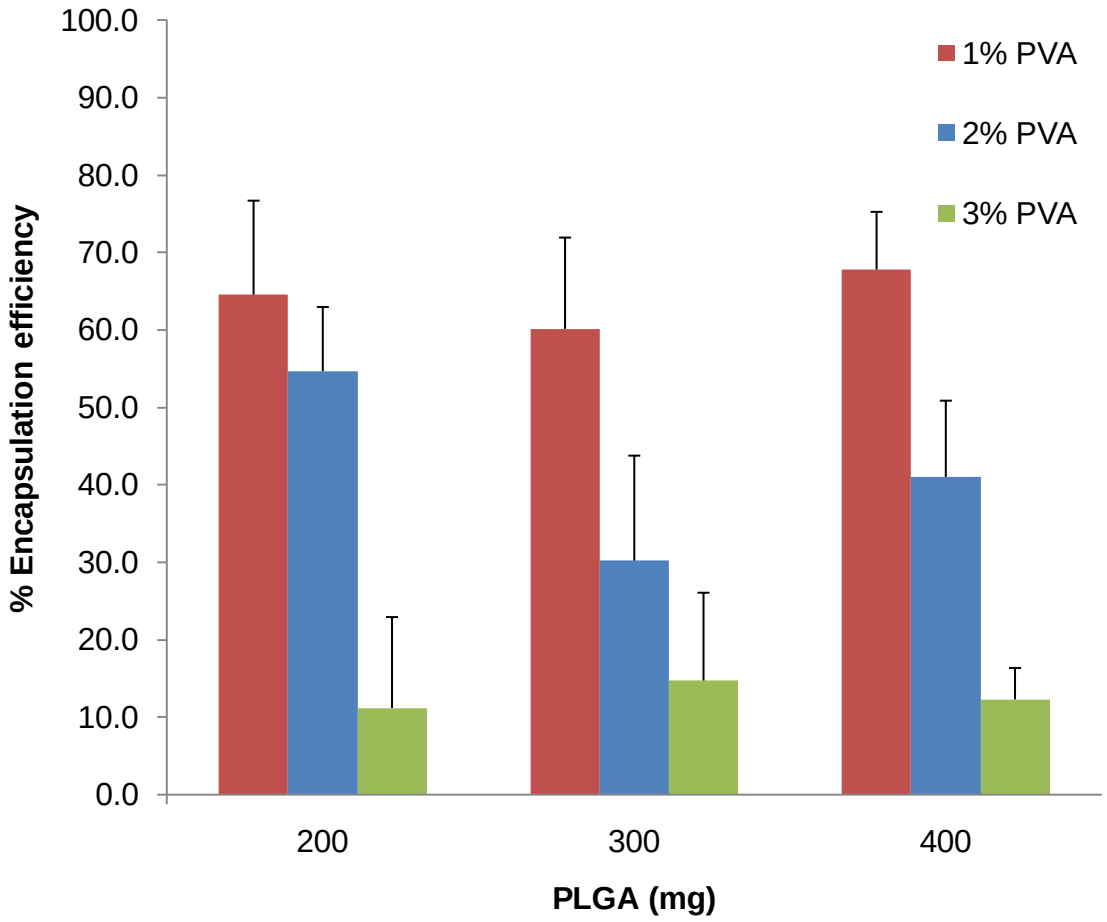


Figure 2-4. Influence of PLGA and [PVA] on encapsulation efficiency of TA into nanoparticles

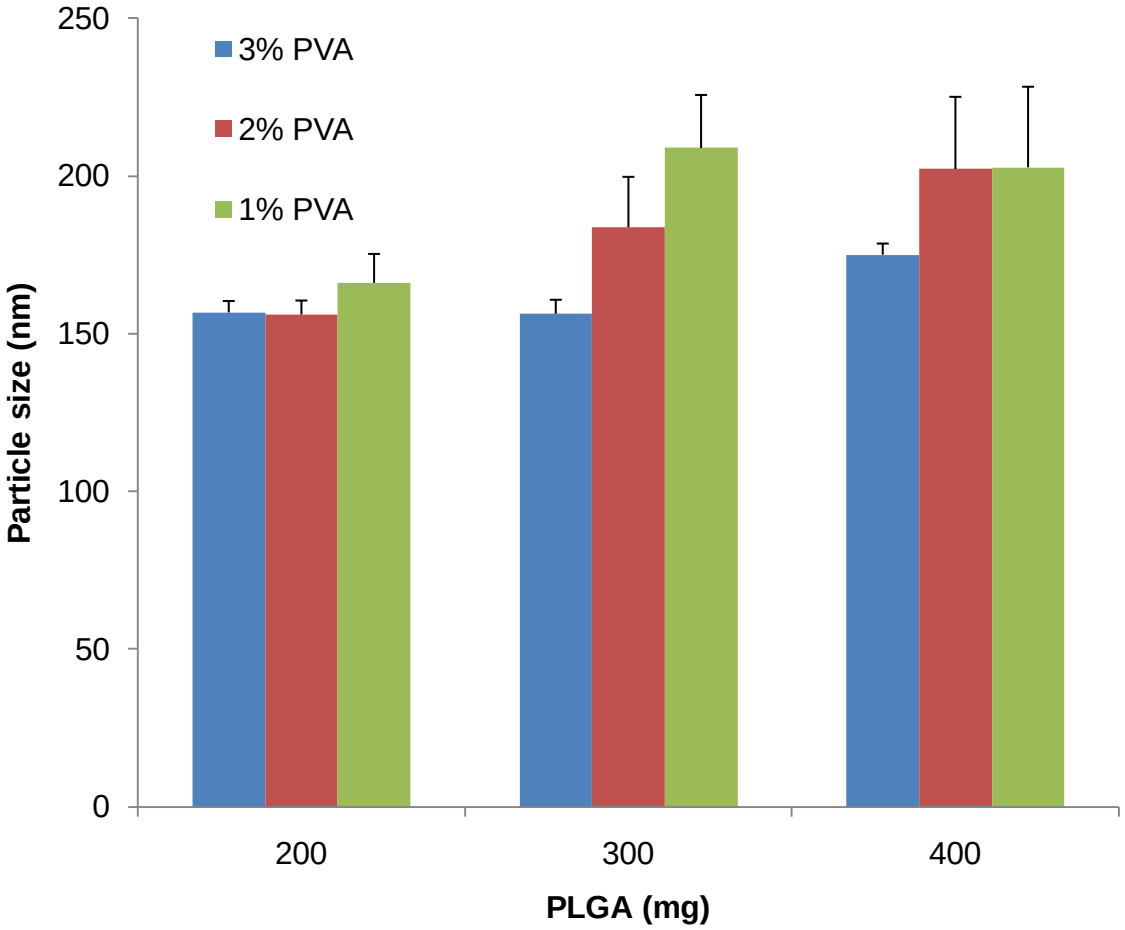


Figure 2-5. Influence of PLGA and [PVA] on particle size of TA nanoparticles

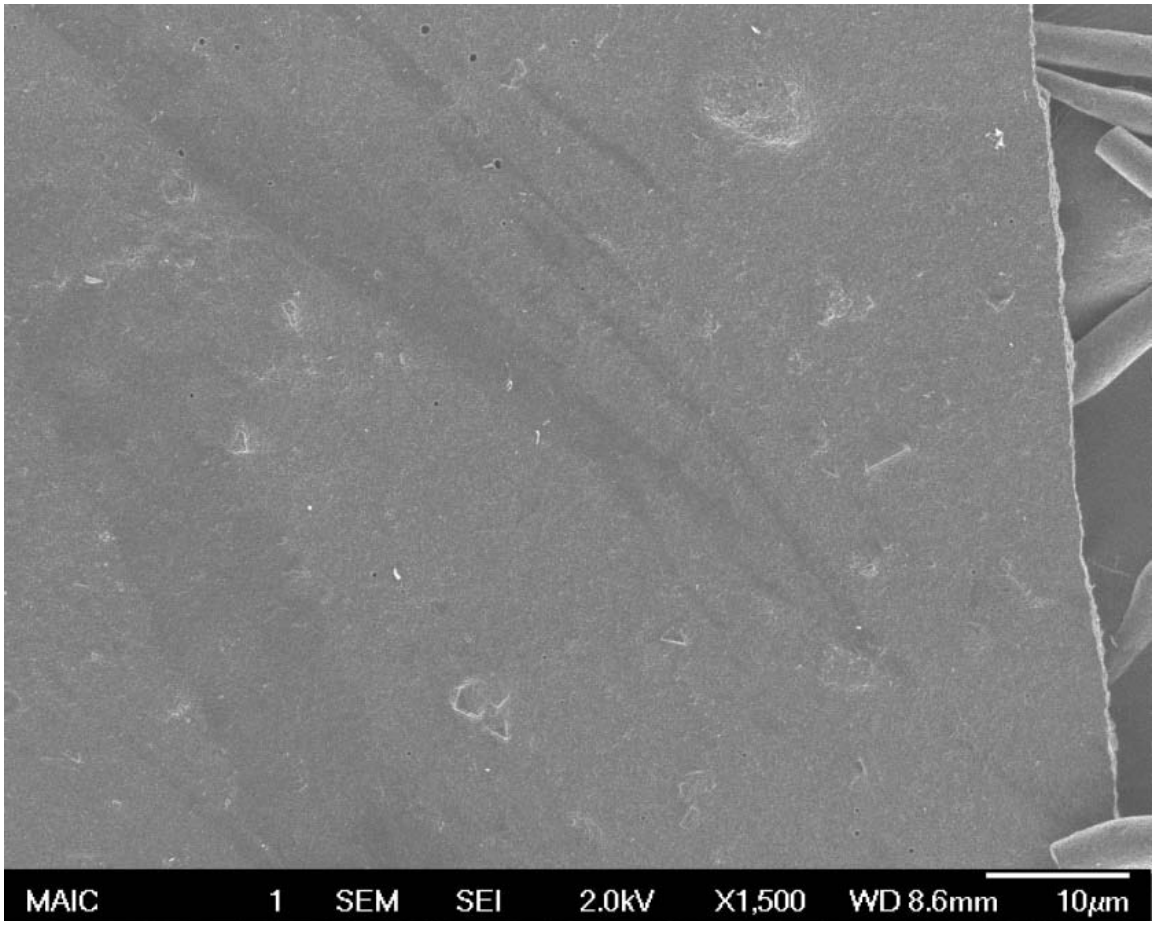


Figure 2-6. SEM of TA-PLGA formulated with 200mg PLGA and 1% w/v PVA

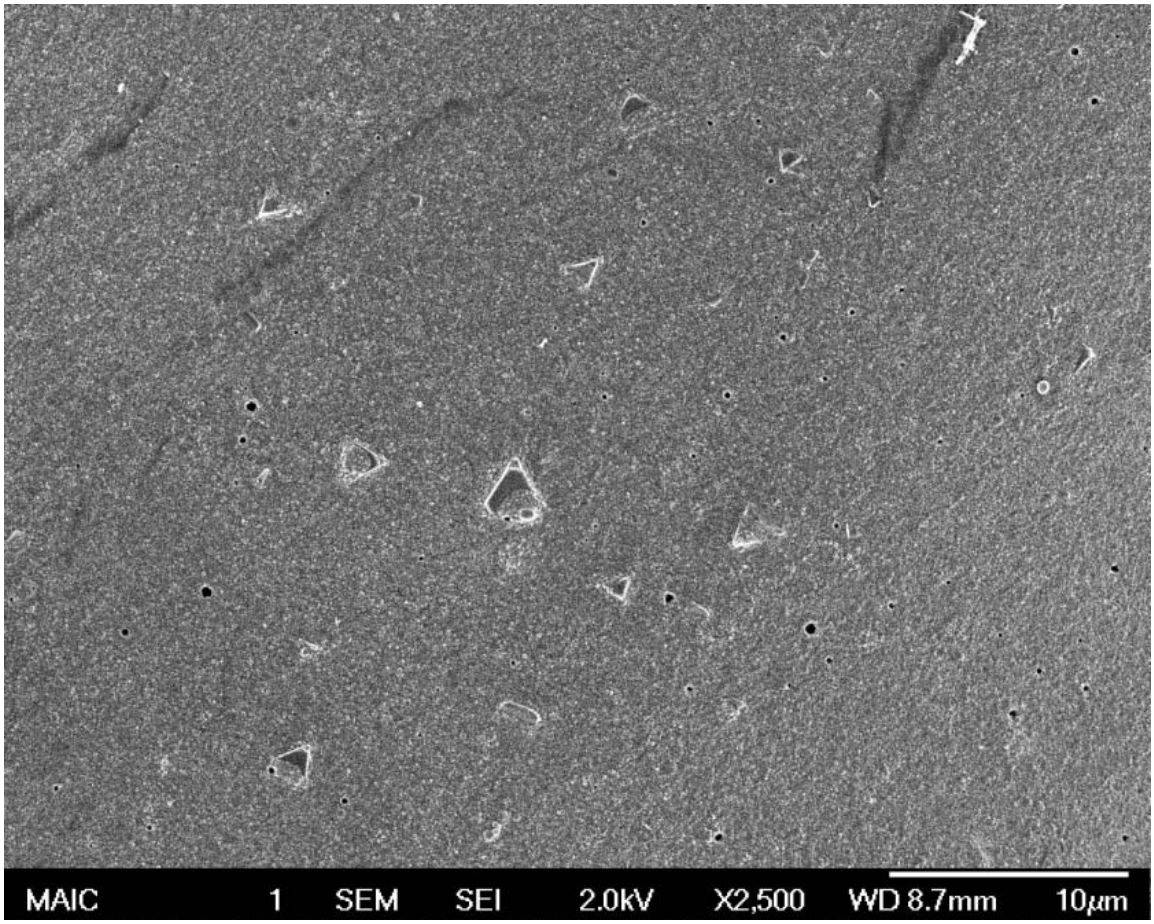


Figure 2-7. SEM of TA-PLGA formulated with 200mg PLGA and 2% w/v

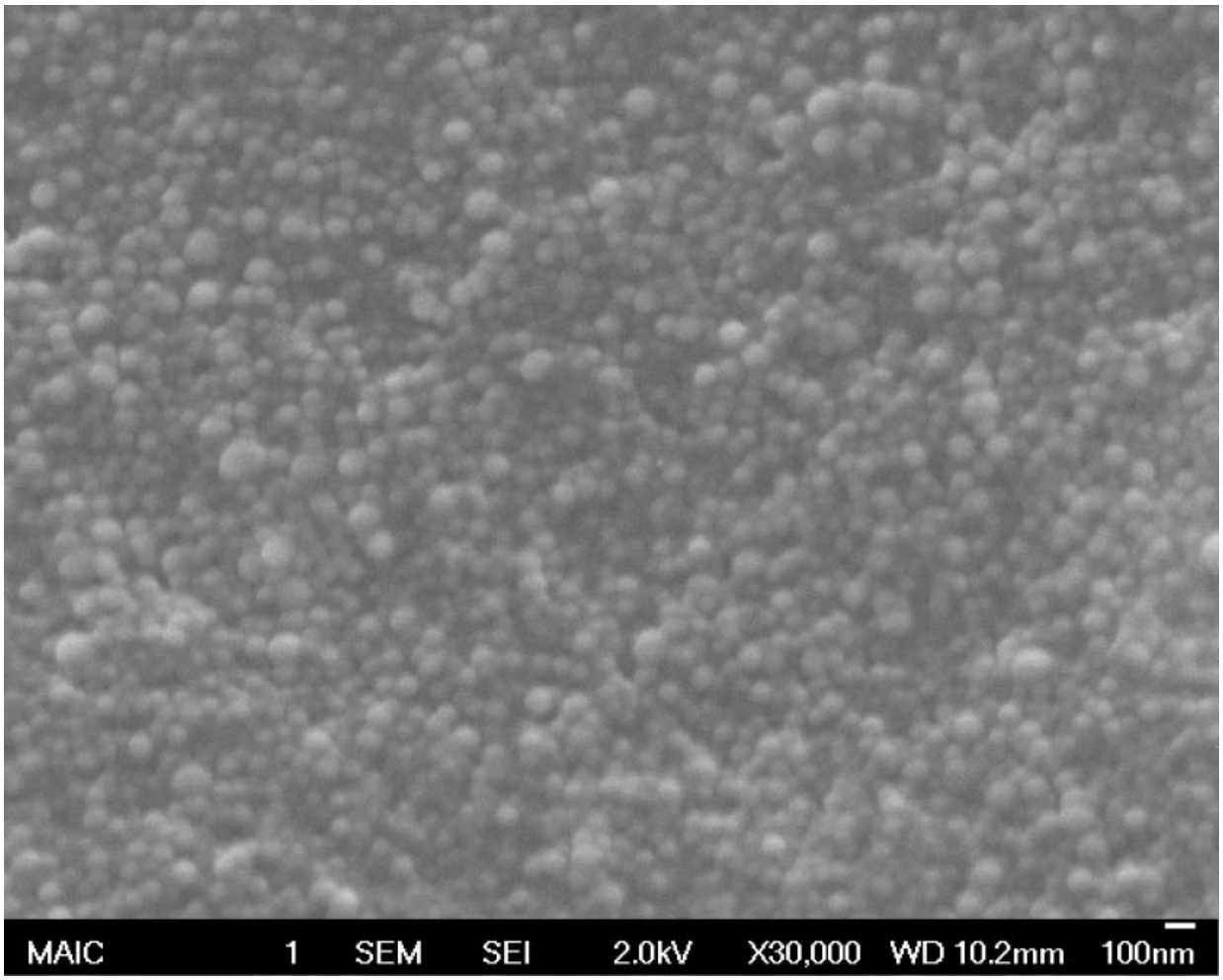


Figure 2-8. SEM of TA-PLGA formulated with 400mg PLGA and 1% w/v PVA

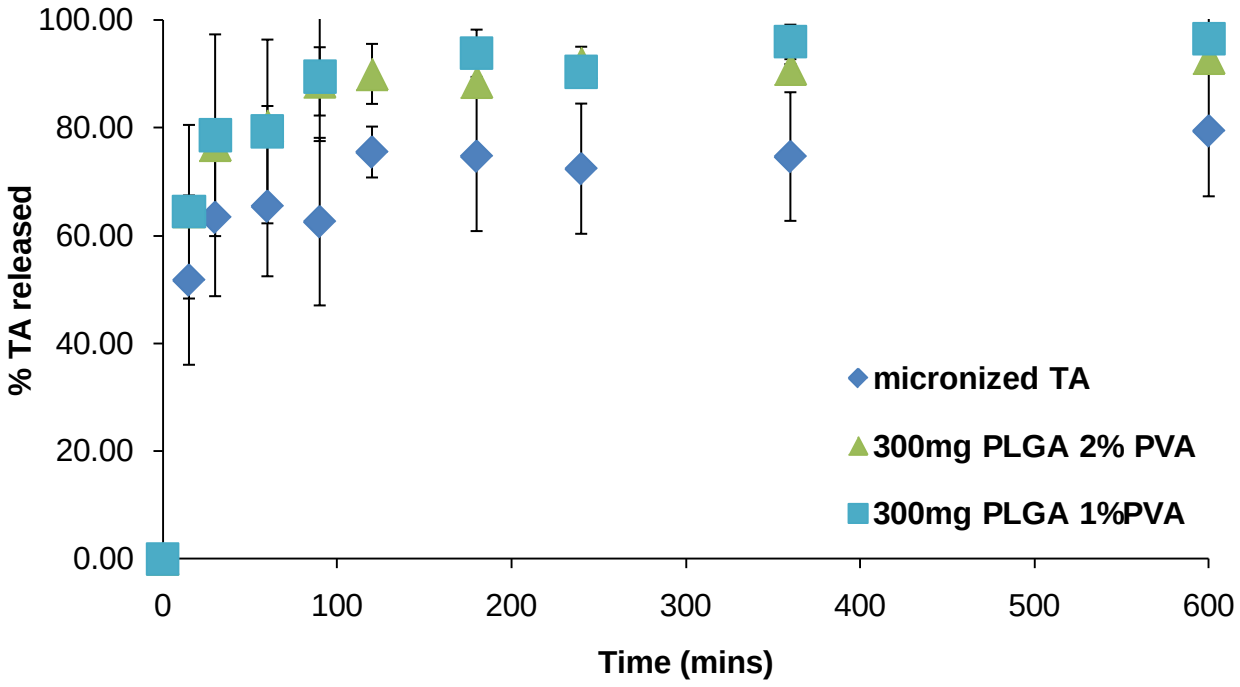


Figure 2-9. *In vitro* release TA-PLGA nanoparticles formulated with 300mg PLGA using 1% or 2% w/v PVA

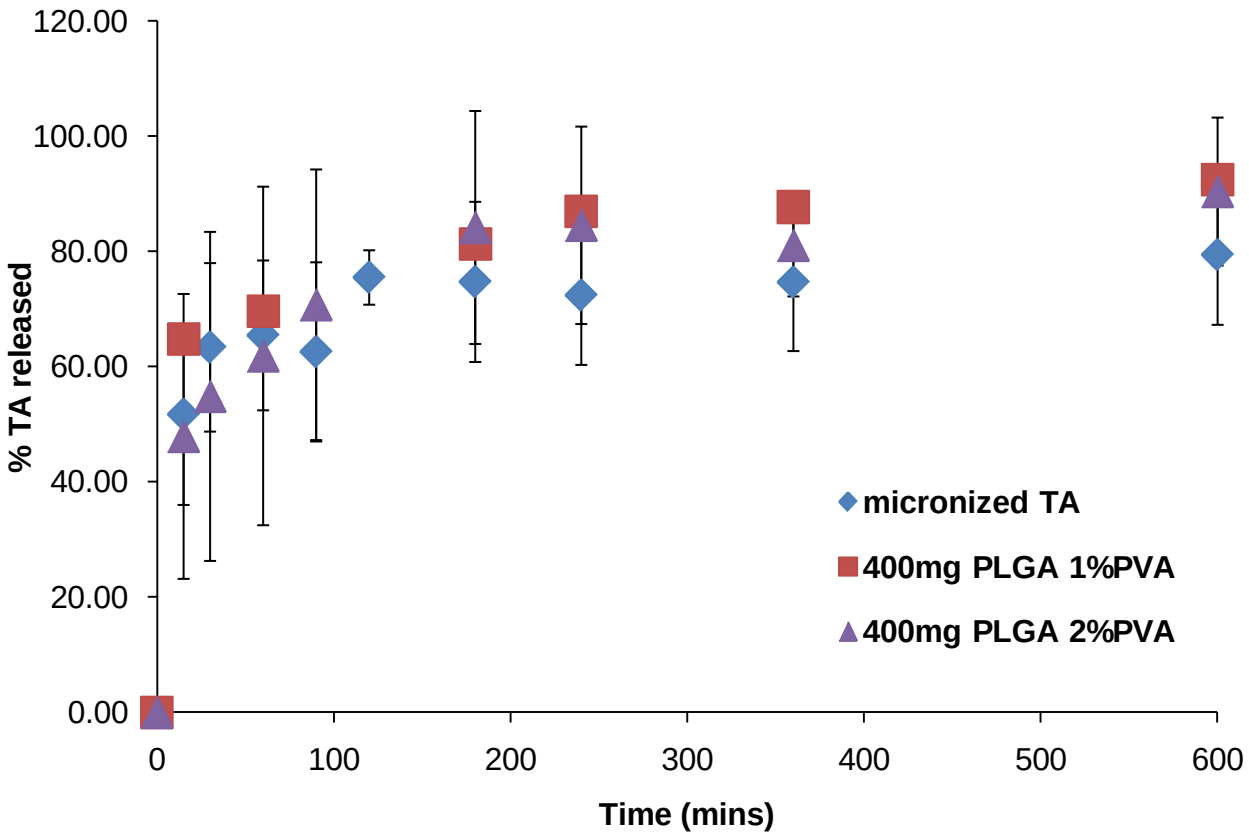


Figure 2-10. *In vitro* release of TA-PLGA nanoparticles formulated with 400mg PLGA and 1% or 2% w/v PVA

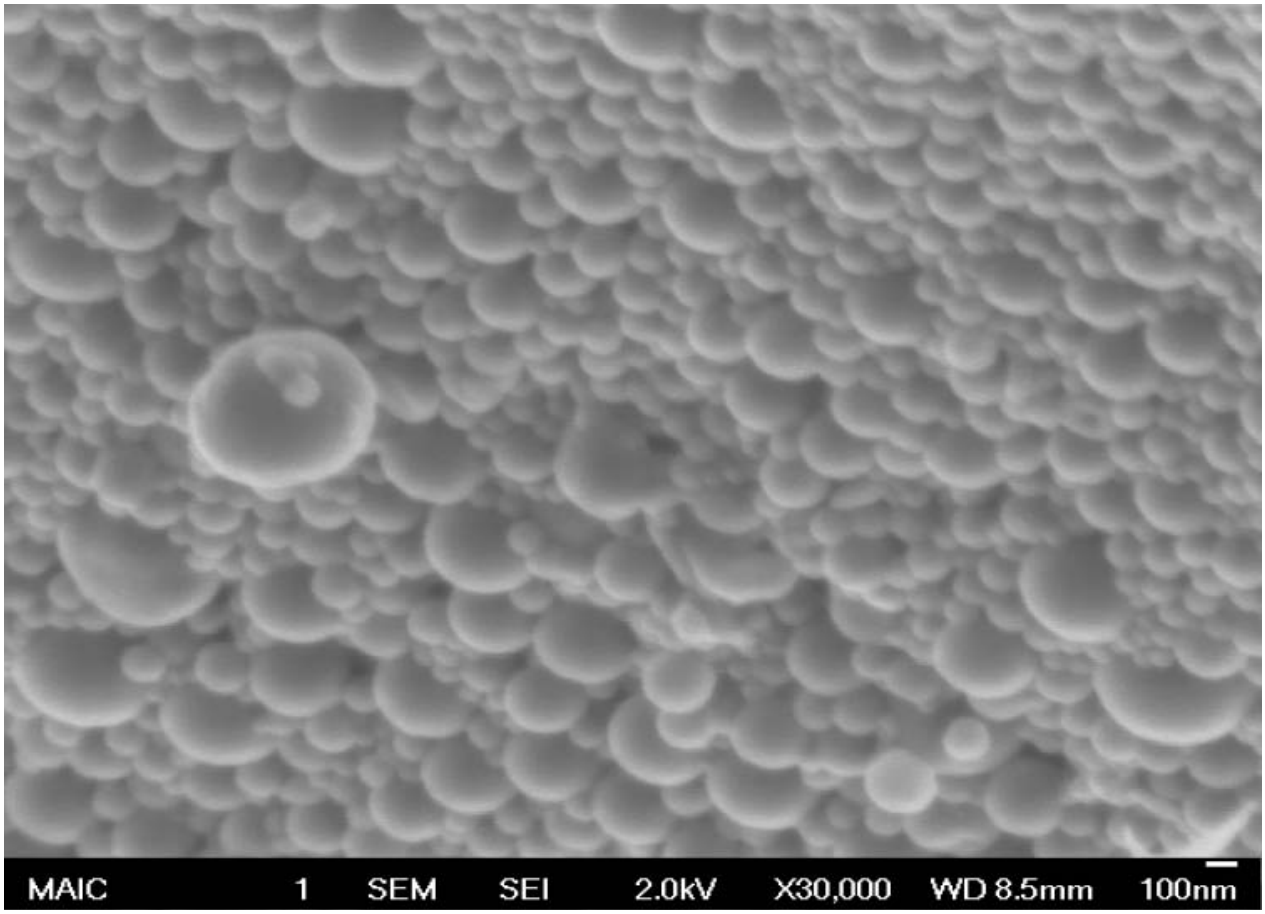


Figure 2-11. SEM of MF-PLA nanoparticles incubated with chitosan 1% w/v

Table 2-2. Particle size distribution for chitosan coated MF-PLA nanoparticles prepared with 0.1% or 1% w/v chitosan by either incubation or in situ coating with chitosan

Method	Particle size (nm)	SD (nm)
Incubation - 0.1% w/v chitosan	Bimodal	-
Incubation - 1% w/v chitosan	Bimodal	-
In situ - 0.1% w/v chitosan	226	10
In situ - 1% w/v chitosan	Bimodal	-

Table 2-3. Influence of chitosan coating to MF-PLA nanoparticles on encapsulation efficiency, drug loading, particle size and zeta potential

Formulation	Drug loading (%w/w)	SD (%w/w)	Particle size (nm)	SD (nm)	Zeta potential (mV)
PLA nanoparticles	1.4	0.1	212	14	-36.0
Chitosan coated PLA nanoparticles	2.0	0.1	226	10	13.7

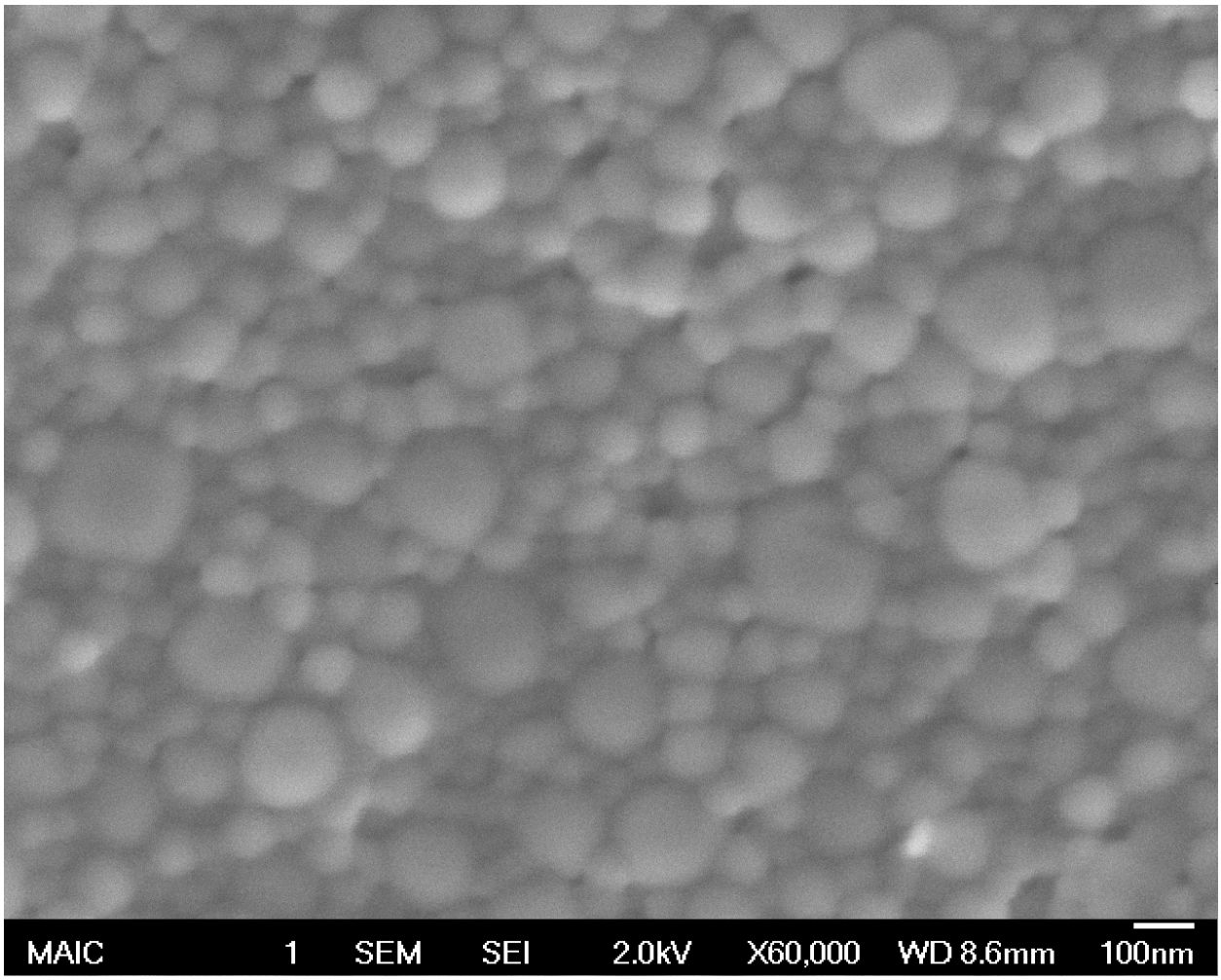


Figure 2-12. SEM of uncoated MF-PLA nanoparticles formulated with 400mg PLA, 1% w/v PVA

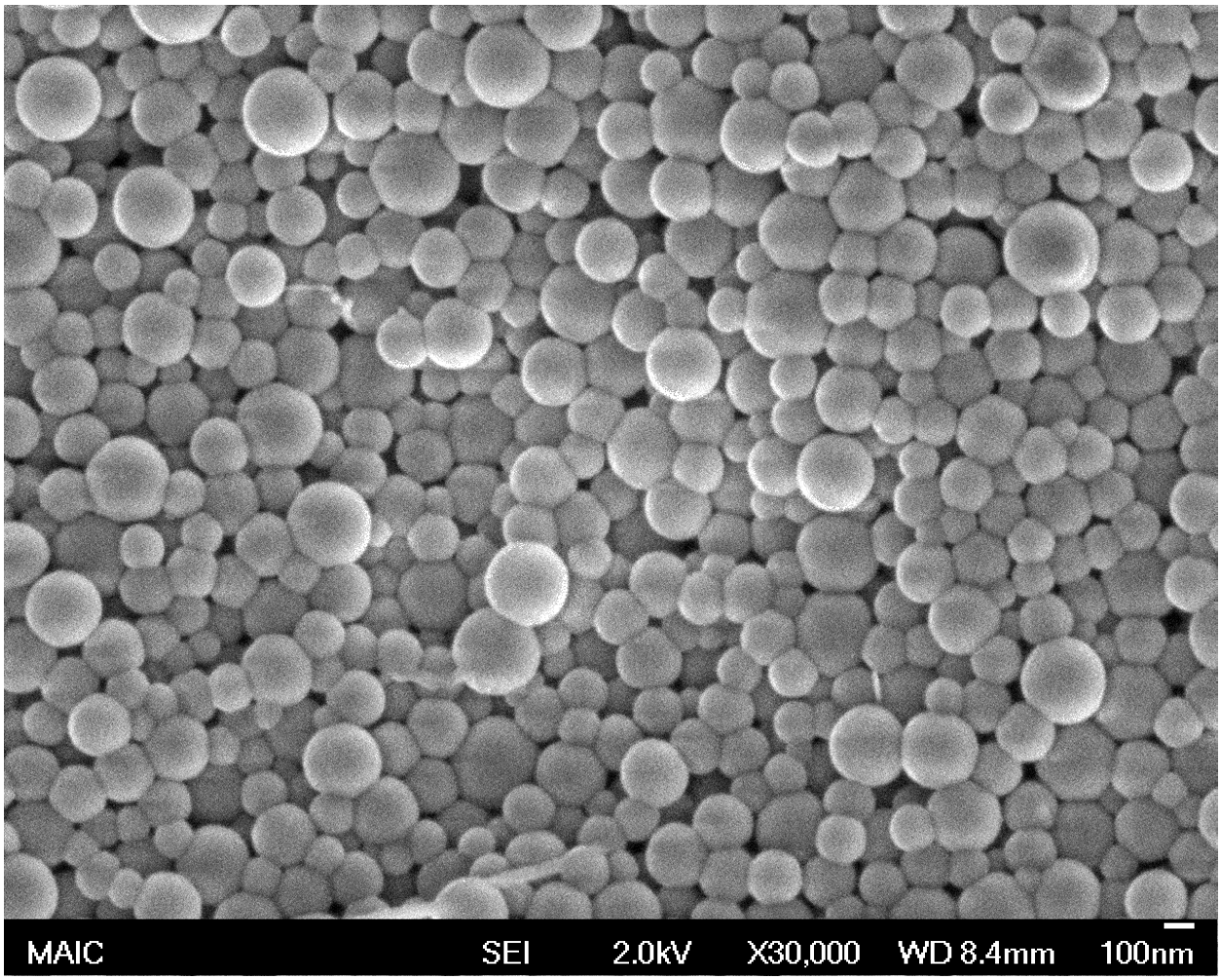


Figure 2-13. SEM of chitosan coated MF-PLA nanoparticles formulated with 400mg PLA, 1% w/v PVA and 0.1% w/v chitosan

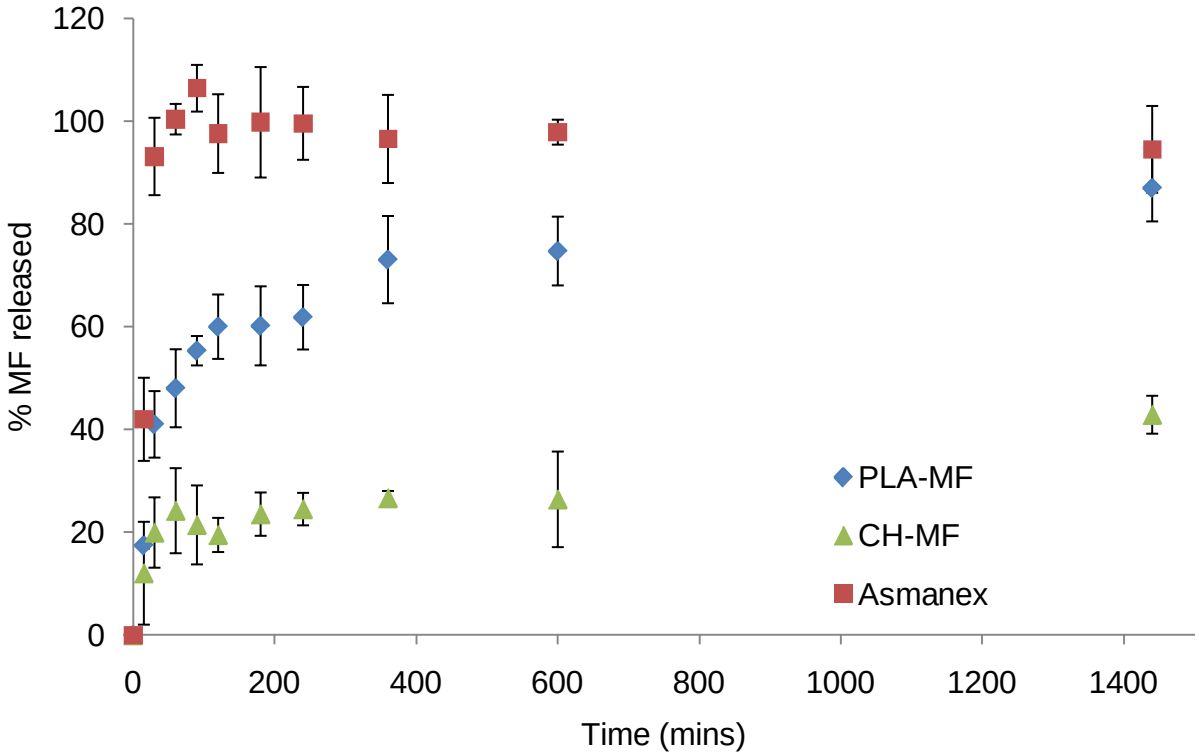


Figure 2-14. *In vitro* release of MF-PLA, chitosan coated MF-PLA (CH-MF) and micronized MF

CHAPTER 3
DEVELOPMENT OF NANOCOMPOSITE MICROSPHERES

Introduction

Although nanoparticles may be capable of providing increased pulmonary retention and slow drug release, they are not efficiently deposited within the airways. In order to deposit in the airways particles must have an MMAD in the respirable range of 1-5μm [26]. Nanoparticles must first be incorporated into micron sized carrier particles, upon exposure to the aqueous environment of the lung, these particles are released, and deposit nanoparticles onto the lung epithelium [102]. Nanoparticles may be spray dried with an excipient such as lactose to form nanocomposite microspheres with good flow properties [103-105]. Ely et al developed effervescent spray dried nanoparticles, these contain an active method to breakdown microspheres once deposited in the lung; citric acid and carbonate react when delivered to the humid airways for form bubbles of carbon dioxide and disperse nanoparticles [106]. Hadinoto et al investigated the influence of nanoparticle size, chemical nature and feed concentration of polystyrene and colloidal silica nanoparticles to be spray dried on the ability to form hollow nanocomposite particles and found that a particular nanoparticle concentration threshold must be reached in order to produce hollow microspheres composed of nanoparticles alone [107]. Addition of phospholipids also reduced phagocytic uptake, nanocomposite particles have been formulated by spray drying nanoparticles with phospholipids, however these particles resulted in slower drug release after spray drying [108].

Spray dried gelatin nanoparticles were found to be significantly larger following spray drying under conventional conditions in a commercially available instrument,

however no size change was seen for polybutylcyanoacrylate particles [105, 109]. Spray dried PLGA dexamethasone nanoparticles were spray dried with 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC) and hyaluronic acid. The resultant microspheres produced released dexamethasone at a slower rate compared to nanoparticles alone [103]. Aspirin nanoparticles spray dried with increasing concentrations of phospholipids initially had similar burst release rates to nanoparticles alone, however, following the initial burst nanocomposite particles with higher phospholipid contents released at a slower rate [107]. Similar findings have also been noted in other studies [103]. Large porous lactose particles have been developed by the Edwards group, the advantage of these are reduced aggregation on storage compared to smaller non-porous particles with an equivalent MMAD [102].

Lactose is a commonly used excipient for inhaled formulations, a number of studies have used lactose to form nanocomposite microspheres [105, 106, 109]. PLGA nanoparticles spray dried with trehalose or lactose are able to release nanoparticles on contact with lung lining fluid. If these nanocomposite microspheres are produced by spray drying using inlet temperatures of 100°C or higher, it is no longer possible for nanoparticles to be released from the formulation [110]. Commercially available spray drying systems operate with inlet temperatures ranging from 100-220ºC, however, this may be problematic for use with polymeric nanoparticles. PLGA and PLA which are used to form nanoparticles have glass transition temperatures of 45-50°C and 50-60°C respectively, thus spray drying at higher temperatures will result in changes in particle shape and possibly agglomeration of nanoparticles [111].

Hypothesis

We hypothesize that a spray dryer capable of operating under lower temperatures than commercially available will allow nanoparticles to be incorporated into microspheres without changes in particle morphology, size or *in vitro* release characteristics. During the drying of aerosol droplets within the spray dyer, nanoparticles are protected from excessive heat due to evaporation of water. Once the aerosol is dried, nanoparticles may be exposed to high temperatures resulting in agglomeration of particles as well as changes in drug release rates, thus it is especially important that the outlet temperature of the spray dryer should be lower than the Tg of the polymer nanoparticles. Lactose will be used as an excipient; we hypothesize that nanoparticles will be freely re-dispersed from nanocomposite microspheres composed of lactose.

Materials and Methods

Chemicals

MF was donated by Ipca laboratories ltd (Mumbai, India). PLGA and PLA were purchased from Lactel Absorbable Polymers (Pelham, AL, USA). Extra-fine lactose was donated by EM industry (Hawthorne, NY, USA). DCM and ACN were purchased from Fisher Scientific (Pittsburgh, PA, USA). PVA and medium molecular weight chitosan were obtained from Sigma Chemical Co. (St. Louis, MO).

Development of Spray Dryer and Optimization of Operating Conditions

DL-PLA has a glass transition temperature (Tg) of 50-60°C, a novel spray dryer capable of operating at temperatures lower than the Tg of the polymers was developed (Figure 3-1); this allowed nanoparticles to be spray dried without causing particles to aggregate during the process. Operating conditions of the spray dryer will be optimized to allow

dried microspheres to be collected using an Anderson cascade impactor (Copley Scientific, UK). Initially, PLA-TA nanoparticles were spray dried with lactose to determine the lowest temperatures and additional parameters under which dried microspheres may be produced. Parameters such as sweep air flow, starting temperature of the drying chamber, nebulizer air flow, nebulizer flow rate were varied. Particle morphology was observed by SEM to determine particle morphology and incorporation of nanoparticles into microspheres.

During method development, in order to determine operating parameters for spray drying, total collection of TA-PLA nanoparticles on the ACI was evaluated by weight of particles deposited on each stage. In addition, each stage was rinsed with 5 mL MeOH and total TA content will be evaluated following dilution with DDW to a produce 50:50 MeOH:DDW solution. Total mass of nanoparticles collected on each stage was determined. This allowed a fast screening method to determine the composition of nanocomposite microspheres produced as well as an approximation of the MMAD of the formulation.

The spray dryer design is shown in Figure 3-1. Warm dry air is directed into the heating chamber from the bottom of the instrument. The port placed at the bottom of the heating chamber is angled to allow the warmed dry sweep air to produce a vortex through the heating chamber; preventing nebulized aerosol droplets impinging the spray dryer walls. This results in reduced loss of formulation within the spray dryer, to improve the final yield of spray dried nanoparticles. In addition to this, it increases the time the aerosol droplets reside in the heating chamber, allowing a more gentle heat to be applied. In addition width and height of the aerosol generated using the nebulizer

was measured, as a result the diameter of the drying chamber was designed to be wider than the aerosol plume created by the glass nebulizer to reduce impaction and loss to the chamber walls.

Inlet temperature was maintained in the range 75-80°C while outlet temperature was less than 35°C. Parameters such as temperature, nebulizer air flow, flow rate of nanoparticle suspension to be aerosolized and air flow through the spray drying device were optimized in order to allow complete drying of the microspheres without subjecting nanoparticles to temperatures above Tg. Optimal conditions were found to be with inlet airflow of 15 L/min, 0.5 ml/min flow of nebulizer suspension with the whole system under slightly negative pressure. Inlet temperatures less than 75°C resulted in incomplete drying of the microspheres, which could be observed by the collection of wet particles in the ACI. Higher flow rates of the nebulized suspension also resulted in wet particles being produced. The nanoparticle/lactose suspension is sprayed into a heated drying chamber using a type A concentric circle glass nebulizer (Meinhard, Golden, CO) at a rate of 0.5 mL/min under 20 psi with a corresponding air flow of 0.8 L/min, shown in Figure 3-2. The nebulizer is able to operate at a maximal pressure of 30 psi, with a linear correlation between air flow rate and pressure between 15 and 80 psi, (Air flow (L/min) = 0.0328xPressure(psi) + 0.1401 with $R^2 = 0.9997$). The heated chamber was maintained under a slightly negative pressure in order to improve aerosol drying. Warm dry sweep air at a flow rate of 15 L/min was used to create a vortex within the drying chamber to prevent aerosol droplets from impinging onto the chamber walls. Inlet and outlet temperatures were approximately 78°C and <35°C respectively; outlet temperatures should be maintained below the Tg of the polymer in order to prevent

nanoparticle agglomeration. Typically commercial spray dryers operate at much higher temperature, for example lactose is spray dried at an inlet temperature of 160°C and an outlet temperature of 105°C, this method would be unsuitable for use with polymeric nanoparticles [112]. An Anderson cascade impactor was set up in-line with the spray dryer as a method of both collecting the spray dried formulation and also to selectively collect the respirable fraction. Air flow through the impactor was set to 20 L/min, following spray drying, the formulation was retrieved from stages 2-7. Particles collected are expected to have a MMAD below 6.9µm.

Spray Dried Chitosan Coated MF-PLA Nanoparticles

Optimal spray dryer conditions determined were used for further development of spray dried nanocomposite microspheres. In Chapter 2, chitosan coated PLA nanoparticles were shown to produce the slowest drug release compared to uncoated nanoparticles or MF contained in the Asmanex® formulation (Figure 2-14), as a result were spray dried with lactose. Chitosan coated nanoparticles (CH-MF) were suspended in a lactose solution, then spray dried. Total solid feed content to be nebulized was initially set to 5% w/v while the CH-MF:lactose composition was varied (10:90, 25:75, 50:50, 75:25 and 90:10). MMAD, particle morphology, nanoparticle entrapment efficiency and *in vitro* release characteristics of spray dried CH-MF nanoparticles were determined as described below.

Nanoparticle Entrapment Efficiency and Loading into Nanocomposite Microspheres

In order to determine the percentage nanoparticle content of the nanocomposite microspheres as well as the nanoparticle encapsulation efficiency 5 mg of the nanocomposite microspheres were weighed and dissolved in DCM on the orbital shaker

for overnight. DCM was placed in the vacuum centrifuge (Jouan RC10.10) to dryness, the dried residue was dissolved in 1 mL ACN:DDW 60:40 and analyzed by HPLC (Hewlett Packard Series 1050). Drug loading and encapsulation efficiency were determined by the equations below.

$$Drug\ loading\ (\%\ w/w) = \frac{Actual\ MF\ (ug)}{Weight\ of\ Formulation\ (ug)} \times 100$$

$$Encapsulation\ Efficiency = \frac{Actual\ drug\ loading\ (\%\ w/w)}{Theoretical\ drug\ loading\ (\%w/w)} \times 100$$

Morphology of Nanocomposite Microparticles

SEM was performed to observe particle morphology using Scanning Electron Microscope (SEM) JEOL JSM-6335F instrument (Major Analytical Instrument Center (MAIC), UF, Gainesville, FL). The spray dried formulation was placed on a carbon stub which was coated with carbon using a vacuum evaporator, SEM was performed at 2 kV. Lactose alone was spray dried under the same conditions to allow comparison of particle morphology of spray dried particles which do not contain any nanoparticles.

Determine MMAD of Nanocomposite Microparticles

Particle MMAD of spray dried hybrid particles was determined using an 8-stage, non-viable Anderson cascade impactor (Copley Scientific), stainless steel collection plates were used, the inhaler device was coupled to the impactor using a tailor made adapter. The operational airflow used was 39 L/min for 6 seconds per actuation. Surfaces of the particle collection sites on the ACI were coated with an ethanolic solution of brij 35 in glycerol to avoid bias caused by particle bounce [113]. Each of the plates was coated with 50 μL brij 35 solution, with the pre-seperator being coated with 100 μL to reduce the bounce effect, ethanol will evaporate before HPLC analysis. The sample induction

port (SIP), high top, pre-separator and plates from each stage were washed with 5 mL acetic acid buffer pH 4, to remove the chitosan coating, this was followed by incubation with 5 mL acetonitrile to release MF from within the PLA matrix. The samples were centrifuged then analyzed by HPLC as described above. Two capsules were half filled with 10 mg of the spray dried formulation; the capsule was placed in the center chamber of the device with the overlapping end pointing upwards and delivered via the HandiHaler® (Figure 3-4). Capsules were pieced in the HandiHaler®, however the size of the holes produced was designed to be smaller diameter than that used in the commercial device, in order to prolong the residence/delivery time of the formulation within the capsules and thereby allowing for a longer de-agglomeration process of the formulation particles. The respirable fraction of the spray dried nanoparticles formulation was compared with that of the commercially available MF formulation delivered from the Asmanex inhaler device (Figure 3-5). The impactor will not give a continuous distribution of particles, but will categorize particle size into certain size ranges [114]. The respirable dose was calculated based on particles with MMAD <4.9 µm by ACI analysis, based on MF collection from stage 2 and below, based on the particle size cutoff shown in Table 3-1. The Metered Dose (MD) was based on the MF content using the actual amount of formulation weighed into the capsules for inhalation. The Delivered Dose (DD) was determined based on the total recovered MF from all stages of the ACI, pre-separator, Sample Induction Port (SIP), high top and adapter. Drug remaining in the device and capsules were not included within the DD. Fine particle dose (FPD) was determined by the summation of all MF collected from stage 2 and below and was compared to DD to determine FPF.

$$Fine\ Particle\ Fraction = \frac{Fine\ Particle\ Dose\ (ug)}{Delivered\ Dose\ (ug)} \times 100$$

In vitro drug Release Study

In vitro release was performed in 100 mL 1% v/v tween 80 in PBS, shaken at 30 rpm in a hot shaker (Bellco biotechnology, Vineland, NJ) at 37°C over 24 hours under constant sink conditions, as previously described in Chapter 2. Tween 80 increased the saturation concentration of MF in the dissolution media to 25 µg/ml at 37°C. In order to maintain sink conditions maximal concentrations obtained did not exceed 10% of the saturation concentration. At specific time points (0, 15, 30, 90, 120, 180, 240, 360, 600 and 1440 minutes), 1 mL was removed using a pipette and subsequently filtered using a 0.02 µm filter (Whatman Anotop plus filter), 1 mL fresh buffer was replaced into the dissolution media. HPLC was used to quantify released drug concentrations using a calibration curve ranging from 0.5-10 ug/mL with an R^2>0.996. Spray dried CH-MF formulations were compared to MF from the Asmanex® Twisthaler™.

Results and Discussion

Development of Spray Dryer and Optimization of Operating Conditions

A spray dryer capable of operating with inlet temperatures less than the Tg of PLGA or PLA was designed. Additionally it was able to spray dry formulations using mg quantities of materials. Initially PLA-TA nanoparticles were used to optimize the spray dryer operational parameters, SEM of this formulation can be seen in Figure 3-9. Figure 3-6 shows MMAD of particles collected directly from the ACI, a large fraction of the total spray dried microspheres obtained are less than 5.8 µm, Figure 3-7 also shows this as the total mass collected at each stage. Figure 3-8 shows that in general the total nanoparticle:lactose content did not vary between the collection stages, with an

exception of a much lower incorporation of nanoparticles into microspheres <0.65 µm. This may be due to exclusion of nanoparticles from microspheres that are very small, as the nanoparticles are also sized at least 200 nm. Initially mass of formulation deposited on the collection surfaces were determined by gravimetric analysis as a convenient method to quantify larger amounts of collected formulation. However, for smaller masses, it becomes more accurate to perform analysis by HPLC as gravimetric analysis is not able to differentiate between formulation weight and water weight, therefore spray dried CH-MF nanoparticles were further analyzed by HPLC [115].

Spray Dried Chitosan Coated MF-PLA Nanoparticles - Influence of CH-MF:lactose Ratio on Morphology of Spray Dried Microspheres

Chitosan coated MF-PLA (CH-MF) nanoparticles were used to optimize composition of nanocomposite microspheres. The effect of increasing nanoparticle:lactose ratio on particle morphology was investigated. CH-MF nanoparticle content was varied at 10%, 25%, 50% and 75% of the total feed of 5% w/v being spray dried. Higher nanoparticle content was not possible as a result of blockage of the nebulizer nozzle with the use of 90% nanoparticle composition. At low composition of 10% nanoparticles, microspheres formed were spherical as shown in Figure 3-10, however, as nanoparticle content increased, particles appeared more deflated and hollow as shown in Figures 3-11, 3-12 and 3-13. Advantages of spray dried hybrid particles containing higher nanoparticle content include a lower mass to be inhaled as well as production of hollow particles which in turn will have lower MMAD as in comparison to solid particles with similar particle size. Hollow particles tend to have larger particle sizes compared to non-porous particles of the same MMAD. This property allows deposition of these particles in the same regions of the airways,

however less aggregation should be expected on storage. As a result of this, chitosan coated nanoparticles were developed using a high nanoparticle:lactose ratio of 75:25. The high solid feed content of 5% w/v tended to lead to clogging of the nebulizer nozzle, therefore particles were spray dried with a solid feed concentration of 1.25% w/v. Lactose alone was spray dried at a concentration of 1.25% w/v to determine surface morphology of these microspheres in the absence of incorporated nanoparticles. Figure 3-14 shows the SEM of spray dried lactose alone, microspheres have a smooth surface in comparison to lactose spray dried with nanoparticles. The resultant microspheres were still produced using a ratio 75% CH-MF nanoparticles and 25% lactose, therefore the final formulation will have the same nanoparticle and drug loading.

Spray Dried Chitosan Coated MF-PLA Nanoparticles – Nanoparticle Incorporation

Chitosan coated nanoparticles were spray dried with lactose 75% and 25% respectively, with a solid feed concentration of 1.25% w/v. Solid feed content was reduced from 5% w/v from previous studies with uncoated MF-PLA nanoparticles to 1.25% w/v as chitosan coated nanoparticles tended to clog the nebulizer at high concentrations. A relatively high nanoparticle:lactose ratio was chosen in order to produce a formulation which contained a clinically appropriate dose and could be administered by one or two inhalations after filling capsules for use in the HandiHaler®. Theoretical MF loading into the spray dried CH-MF formulation is 1.5% w/w, actual MF loading was very similar at 1.43% w/w (SD=0.08% w/w). It was determined that 96% (SD=6%) of the total nanoparticles used were incorporated into the final spray dried formulation. SEM of the formulation contained within the Asmanex® Twisthaler™ is shown in Figure 3-15, this shows a blend of lactose with MF.

Spray Dried Chitosan Coated MF-PLA Nanoparticles - MMAD

MMAD of spray dried CH-MF nanoparticles were characterized by ACI to determine FPF of the delivered dose from the HandiHaler® at 39 L/min for 6 seconds. The United States Pharmacopeia recommends that DPIs should be tested at a flow rate which corresponds to a pressure drop across the inhaler of 4 kPa [116]. As the HandiHaler® produces a pressure drop of 4 kPa at 39 L/min, this was the air flow tested. On inspiration the capsule vibrates inside the center chamber, this results in mechanical agitation of powder aggregates contained and results in their break up [117]. Increasing retention of the formulation within the capsule before delivery to the lung allows increased time over which de-aggregation may occur. In order to increase the time that the spray dried formulation is mechanically agitated within the device; holes used to pierce the capsule were customized to be smaller than those usually made with the HandiHaler®, while allowing the capsule to empty with one inhalation. FPF of MF contained in Asmanex® Twisthaler™ was used as a control for the spray dried CH-MF nanoparticles. The Metered Dose (MD) and Delivered Dose (DD) of MF delivered from the Asmanex® Twisthaler™ produced by Schering-Plough is available as 220 µg or 440 µg and 200 µg or 400 µg respectively [118].

The HandiHaler® is a high efficiency inhaler and was used as it is capable of delivering large doses up to 50 mg by inhalation [119]. This allows a clinical dose of MF from the spray dried chitosan coated nanoparticle formulation to be administered with one or two inhalations. In addition, prepared spray dried formulations can be packaged into capsules with relative ease in comparison to other DPI devices such as the Flixotide™ Diskhaler™ which would require special blister packing equipment to be used [120].

FPF on DD from the spray dried chitosan coated nanoparticle formulation was 14.6% (SD=4.3%), whereas the Asmanex® Twisthaler™ had 20.0% (SD=4.7%). Three batches of spray dried formulation were compared in order to determine batch to batch variability of the spray drying process (n=3 per batch), shown in Figure 3-16. MMAD of MF from the Twisthaler™ was evaluated in order to make comparisons with the FPF on DD of the spray dried nanoparticle formulation with a known commercial formulation. Literature values for FPF of the Asmanex® TwisthalerTM are reported to be 39.9% (SD=2.5) and 35.6% (SD=3.4) at 200 µg and 400 µg DD with an airflow of 60 L/min. The fine particle dose was however defined as particle with MMAD <6.5 µm [121]. Differences between ACI conditions will account for the lower FPF observed with the Asmanex® compared to the literature reported values. To summarize, FPF of the DD from the spray dried CH-MF formulation is similar to commercially available MF from Schering-Plough's Asmanex® Twisthaler™.

Spray Dried Chitosan Coated MF-PLA Nanoparticles – *In vitro* release

MF blended with lactose was removed from the Asmanex® Twisthaler™, Figure 3-15. At 1 hour 100% of MF from the Asmanex® is released, in comparison, spray dried CH-MF exhibits an initial burst, followed by slower release. Spray dried CH-MF shows a slightly greater burst effect compared to CH-MF nanoparticles alone of 30% and 20% respectively. At 24 hours approximately 70% of the total MF contained within the spray dried formulation is released, however, CH-MF nanoparticles alone only released 43% of the total MF content at this time point (Figure 3-17). Lyophilized nanoparticles must be re-dispersed in a lactose solution before they can be spray dried. It is possible that during this process, MF is able to disperse to the surface of the CH-MF nanoparticles, leading to a greater burst release. Following the initial phase, release rate from CH-MF

nanoparticles and spray dried formulation were similar, indicating that nanoparticles did not aggregate during the spray drying process. *In vitro* release of CH-MF nanoparticles did not change following spray drying. Additionally, the spray dried formulation released MF at a much slower rate compared to the commercial MF prepared in the Asmanex® Twisthaler™. Studies conducted by other groups observed slower drug release from PLGA nanoparticles spray dried with lipids, however it is unclear if this is a result of high temperatures used or a property of the lipids used [102, 107].

Conclusion

- The spray dryer was designed to operate at outlet temperatures below the Tg of PLA or PLGA.
- Spray dried particle morphology was influenced by nanoparticle:lactose composition used.
- Spherical microspheres with MMAD in the respirable range were produced containing nanoparticles.
- Increase in nanoparticle:lactose ratio resulted in changes in microsphere morphology.
- Lower nanoparticle:lactose ratios produced spherical microspheres whereas higher nanoparticle concentrations produced hollow particles as shown by SEM.
- Spray dried chitosan coated MF-PLA nanoparticles composed of 75% nanoparticles and 25% lactose are able to provide a clinically appropriate dose in approximately two inhalations using 20 mg of the formulation from the HandiHaler®.
- FPF on DD of the spray dried chitosan coated MF-PLA nanoparticles was 14.6% (SD=4.3%), whereas the Asmanex® Twisthaler™ was 20.0% (SD=4.7%).
- *In vitro* release of MF from the spray dried chitosan coated MF-PLA nanoparticles was much slower than from the formulation contained within the Asmanex® Twisthaler™.
- A biphasic release profile was observed for spray dried CH-MF hybrid particles

- After CH-MF nanoparticles were spray dried, initial burst release observed was faster than for CH-MF nanoparticles alone. Following the initial burst however, the release rates were found to be similar.

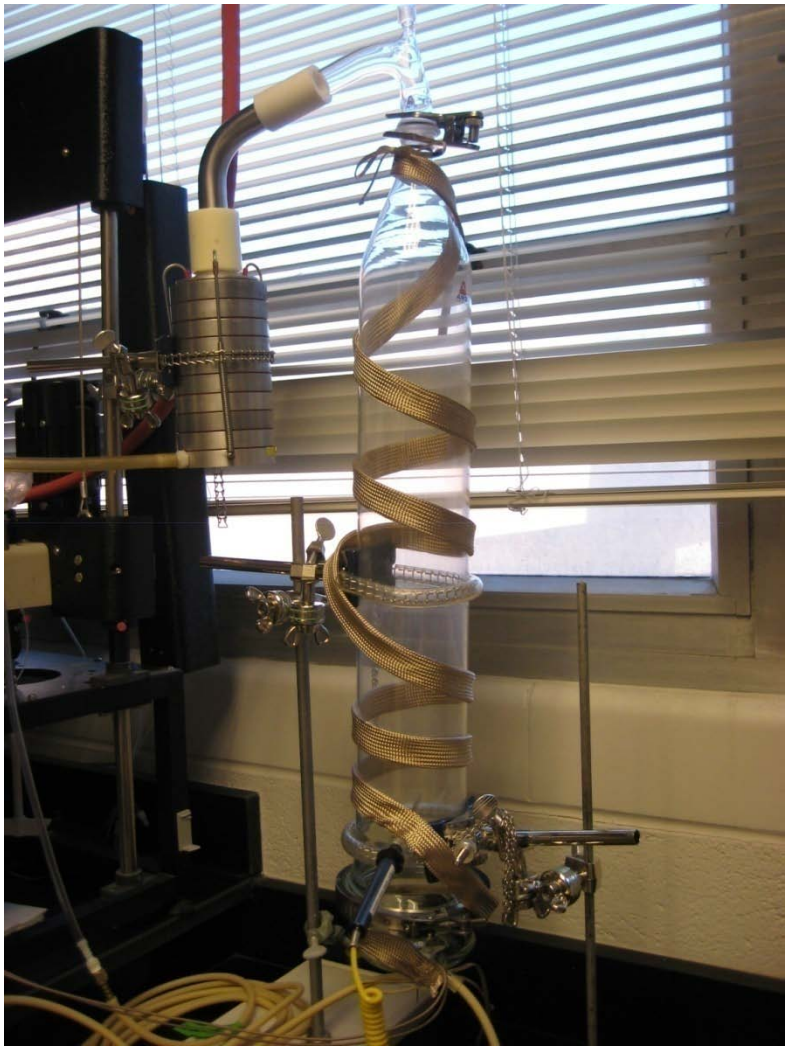
- 

Figure 3-1. Spray dryer, designed to operate under lower temperatures than commercially available instruments

Figure 3-2. TR-30-A3 nebulizer, type A flush capillary lapped nozzle 30 psi, 3 mL/min [122]

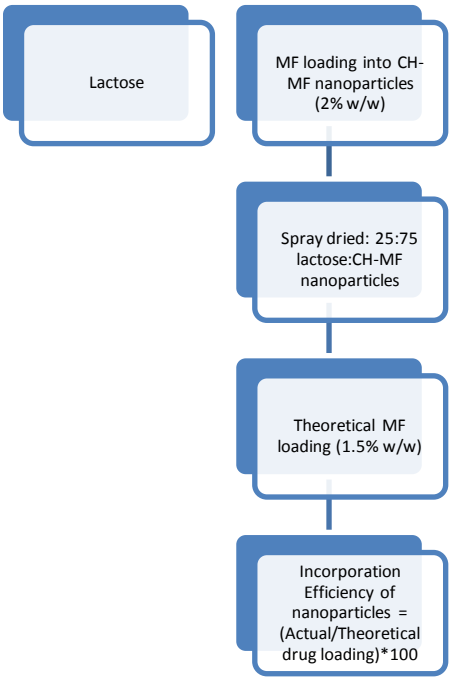


Figure 3-3. Schematic to determine incorporation efficiency of nanoparticles into spray dried formulation

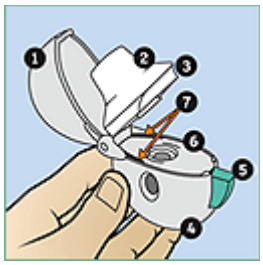


Figure 3-4. HandiHaler® (1) dust cap, (2) mouthpiece, (3) mouthpiece ridge, (4) base, (5) green piercing button, (6) center chamber, (7) air intake vents [123]

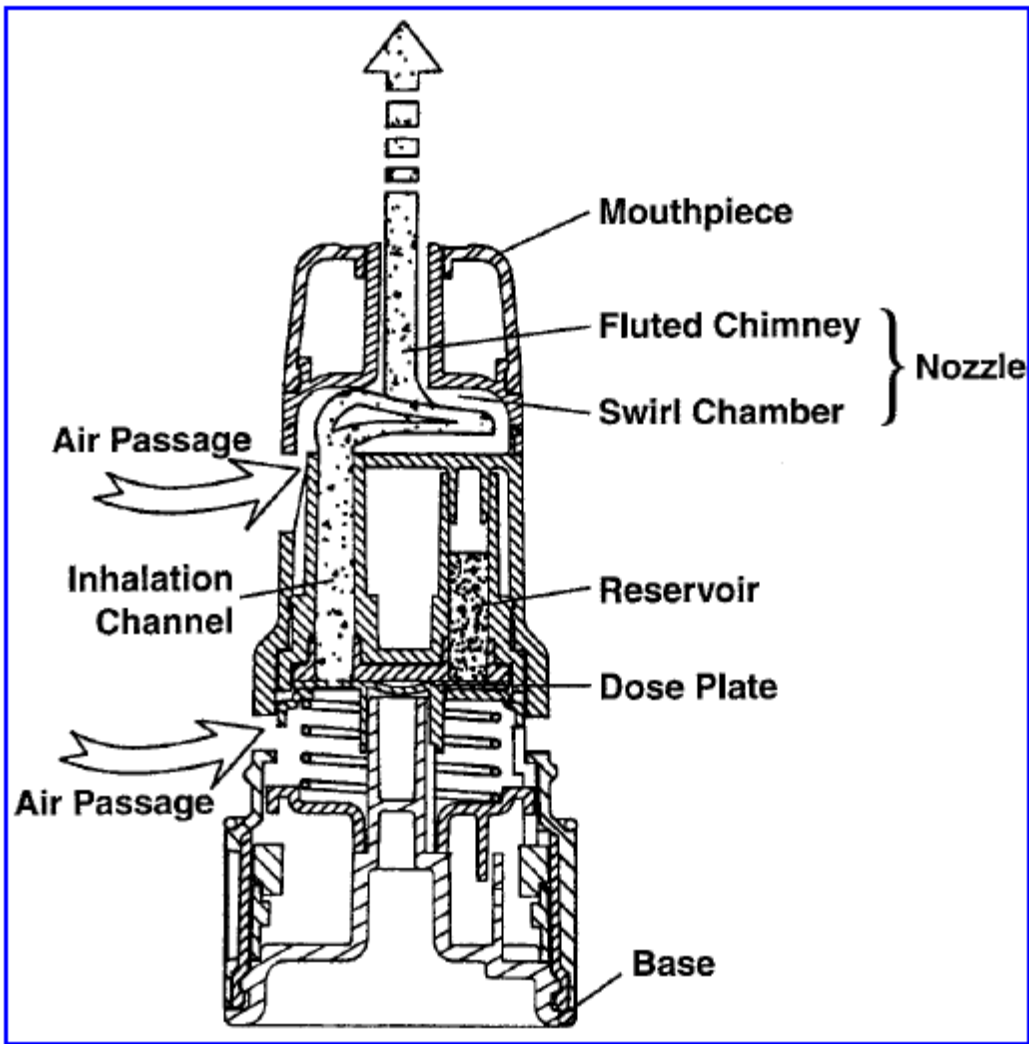


Figure 3-5. Cross section of Asmanex® Twisthaler™ [121]

Table 3-1. MMAD cutoff for ACI analysis based on air flow rate (L/min)

L/min	20.0	22.5	28.3	30.0	39.0	45.4	60.0
Stage							
0	17.8-10.7	16.8-10.1	15.0-9.0	14.6-8.7	12.8-7.7	11.8-7.1	10.3-6
1	10.7-6.9	10.1-6.5	9.0-5.8	8.7-5.6	7.7-4.9	7.1-4.6	6.2-4.0
2	6.9-5.6	6.5-5.3	5.8-4.7	5.6-4.6	4.9-4.0	4.6-3.7	4.0-3.0
3	5.6-3.9	5.3-3.7	4.7-3.3	4.6-3.2	4.0-2.8	3.7-2.6	3.0-2.2
4	3.9-2.5	3.7-2.4	3.3-2.1	3.2-2.0	2.8-1.8	2.6-1.7	2.3-1.0
5	2.5-1.3	2.4-1.2	2.1-1.1	2.0-1.1	1.8-0.9	1.7-0.9	1.4-0
6	1.3-0.8	1.2-0.8	1.1-0.7	1.1-0.7	0.9-0.6	0.9-0.6	0.8-0
7	0.8-0.5	0.8-0.4	0.7-0.4	0.7-0.4	0.6-0.3	0.6-0.3	0.5-0
Filter	<0.5	<0.4	<0.4	<0.4	<0.3	<0.3	<0.3

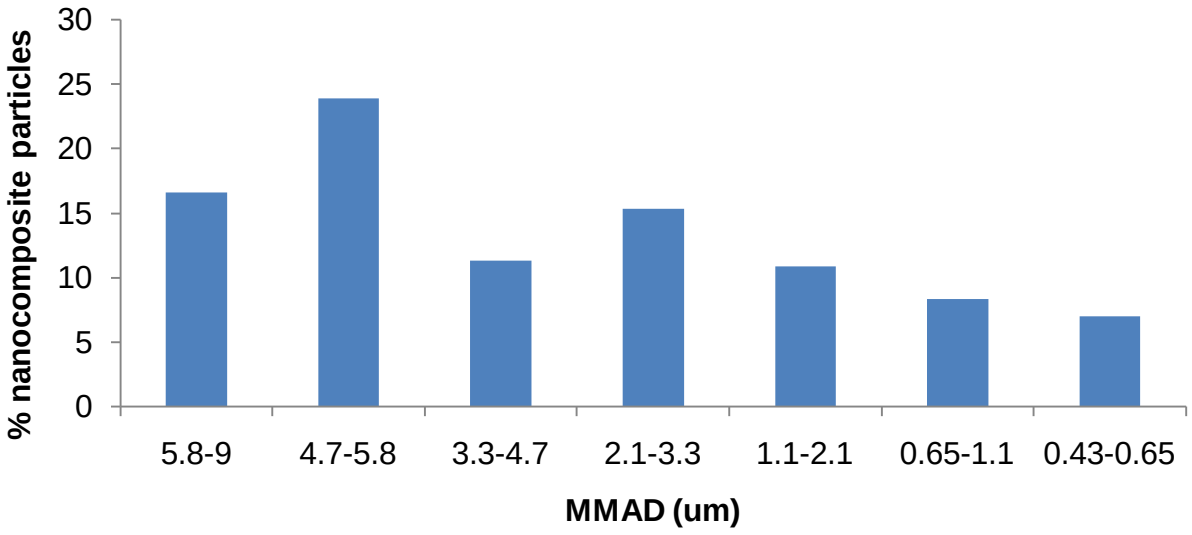


Figure 3-6. MMAD of nanocomposite PLA-TA particles

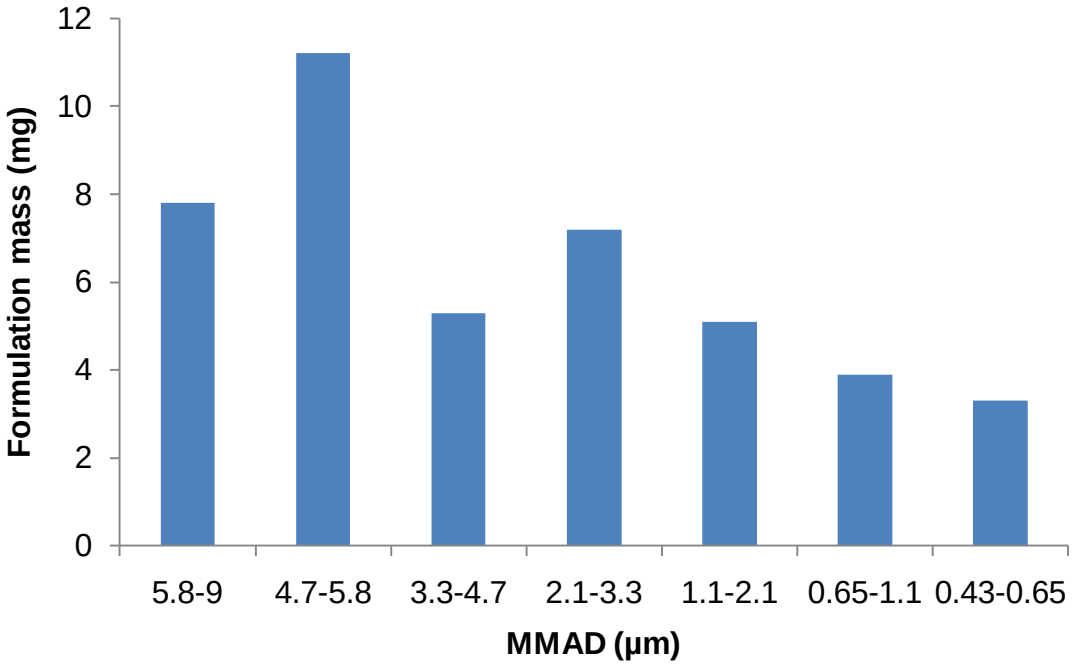


Figure 3-7. Total PLA-TA nanoparticles for each MMAD range

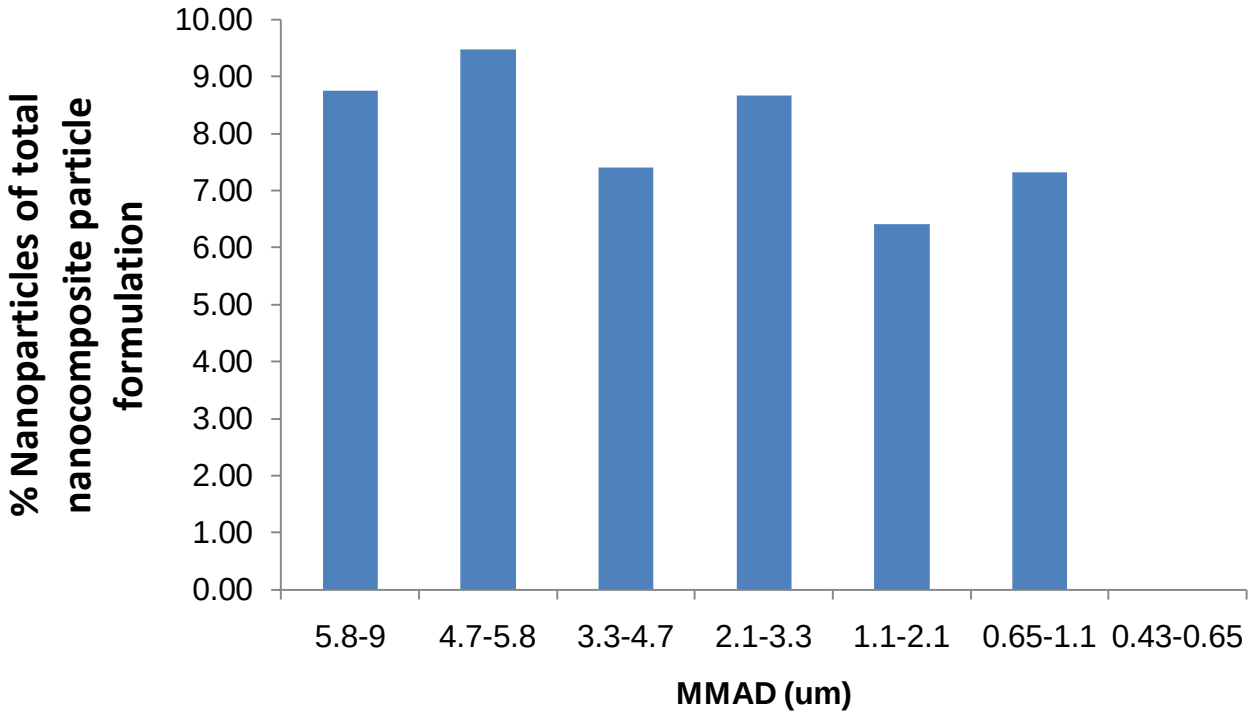


Figure 3-8. Percentage of PLA-TA nanoparticles at each particle cutoff compared with total weight

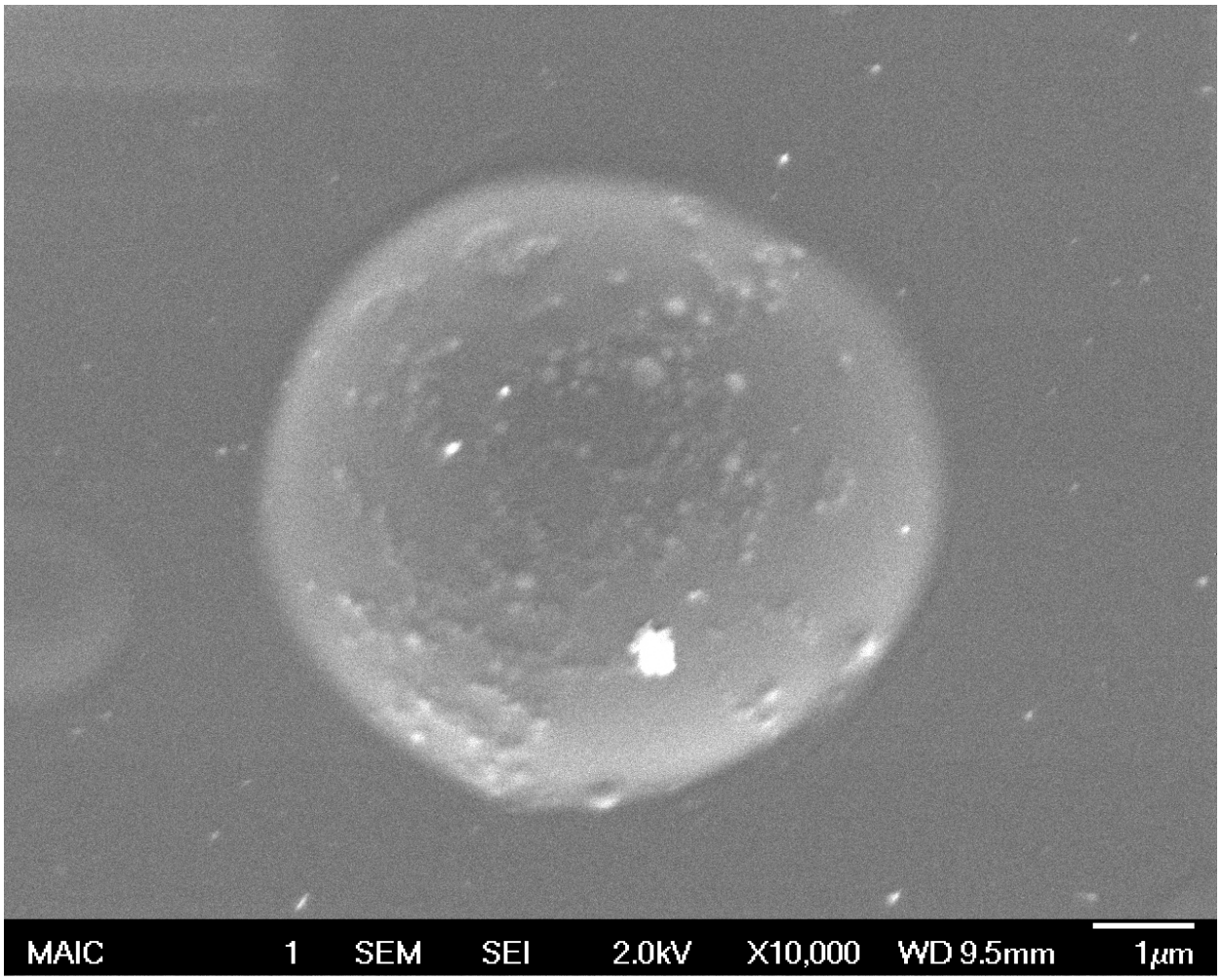


Figure 3-9. SEM of PLA-TA nanoparticles spray dried with lactose

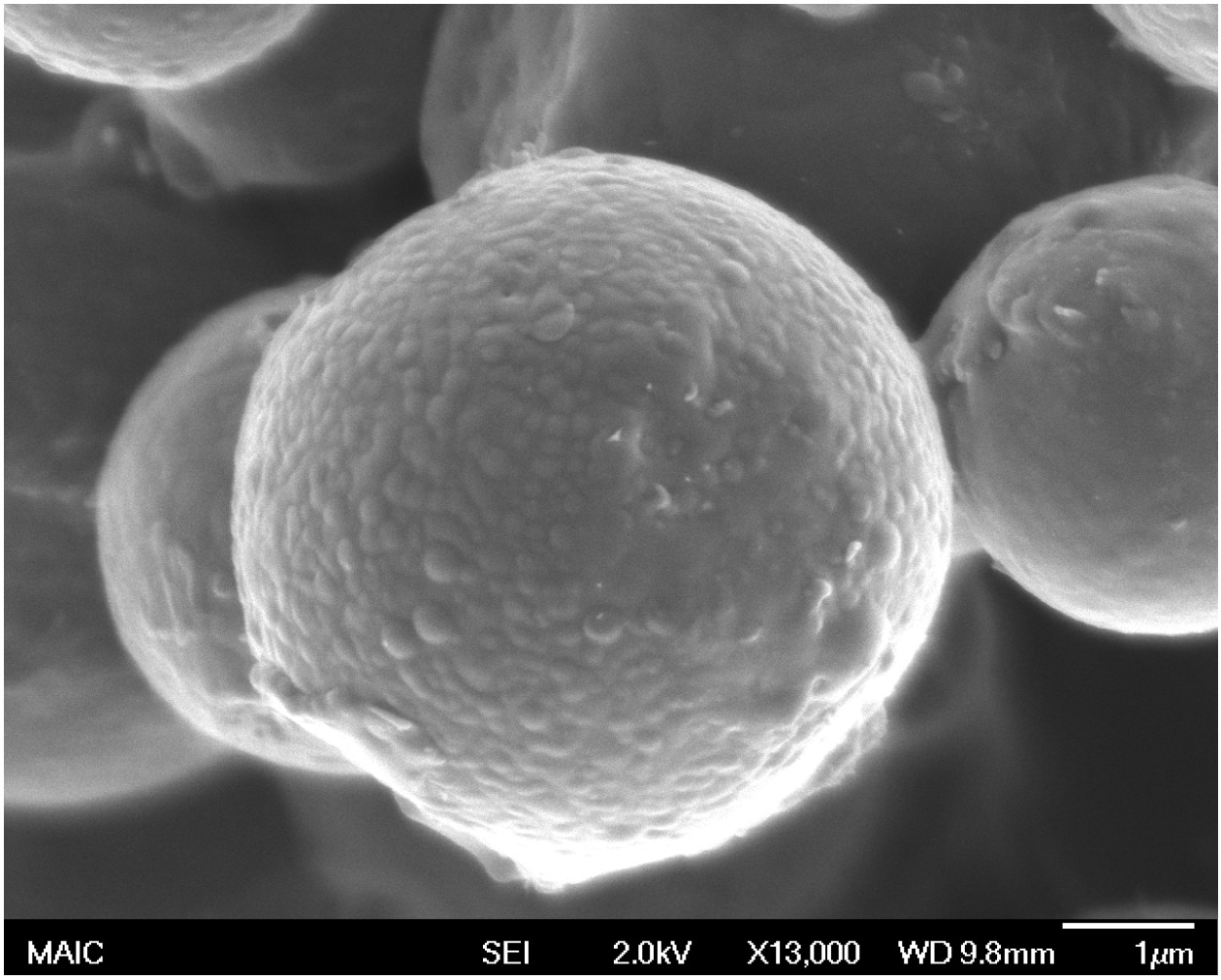


Figure 3-10. Spray dried chitosan coated MF-PLA nanoparticles containing 10% of total solid feed as nanoparticles

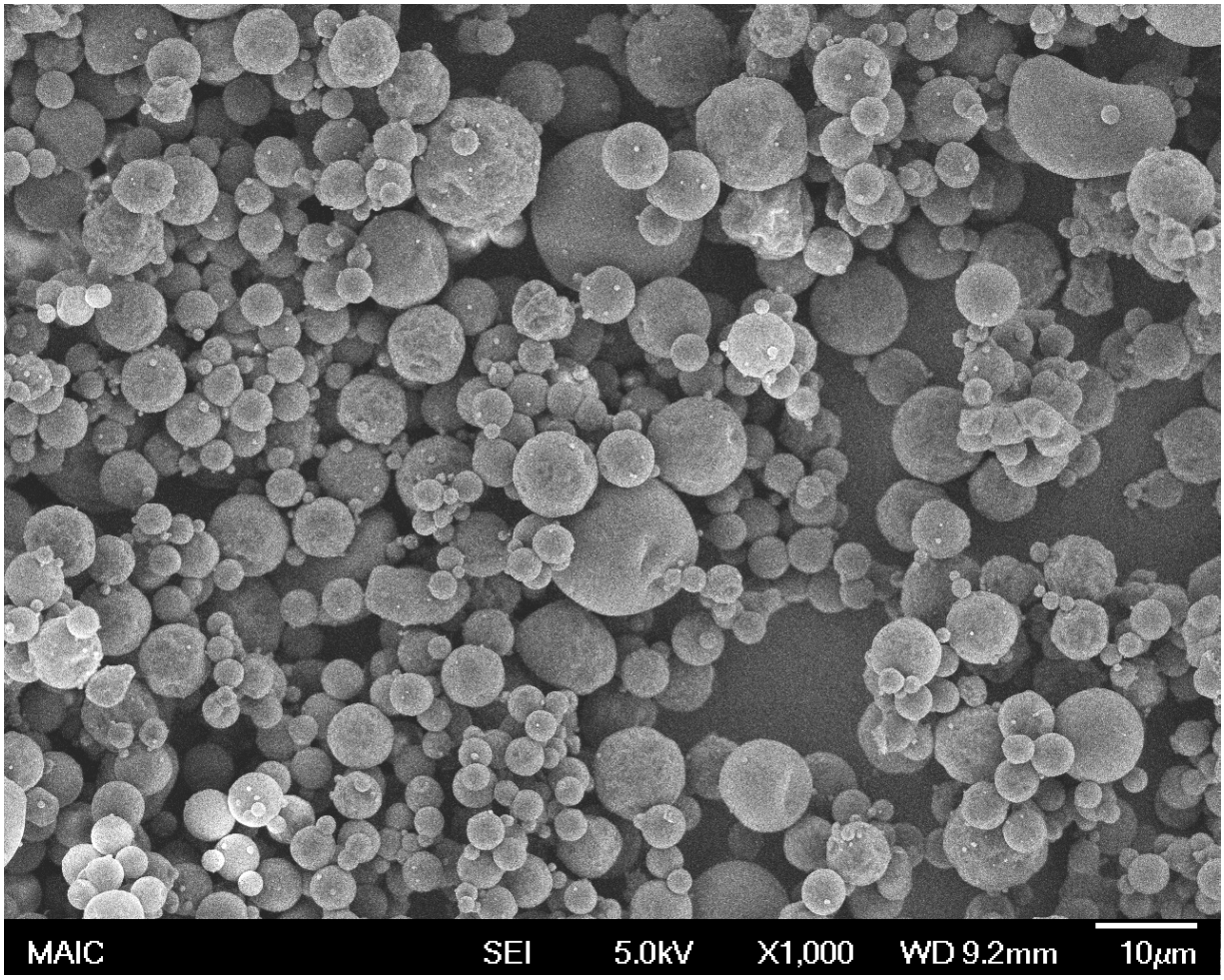


Figure 3-11. Spray dried chitosan coated MF-PLA nanoparticles containing 25% of total solid feed as nanoparticles

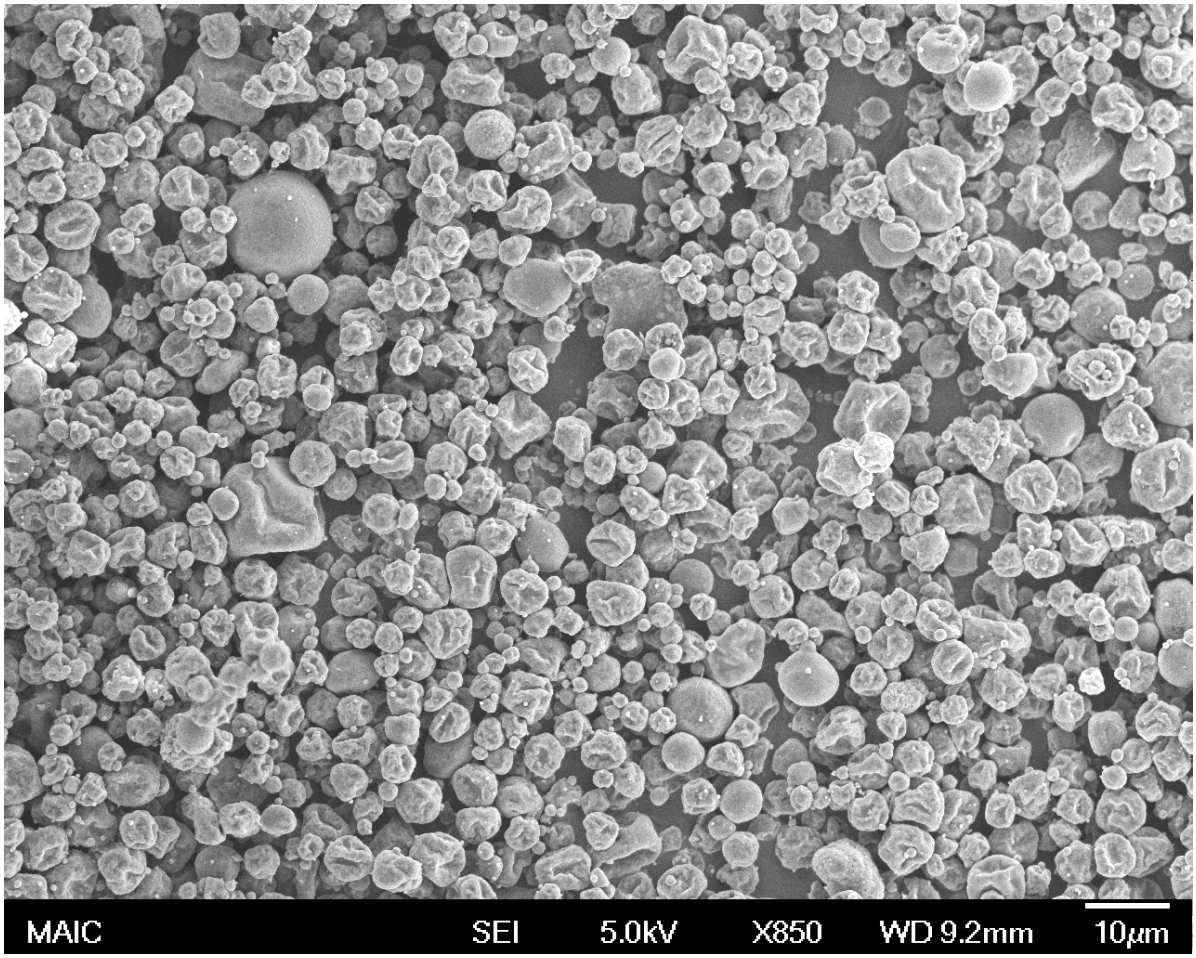


Figure 3-12. Spray dried chitosan coated MF-PLA nanoparticles containing 50% of total solid feed as nanoparticles

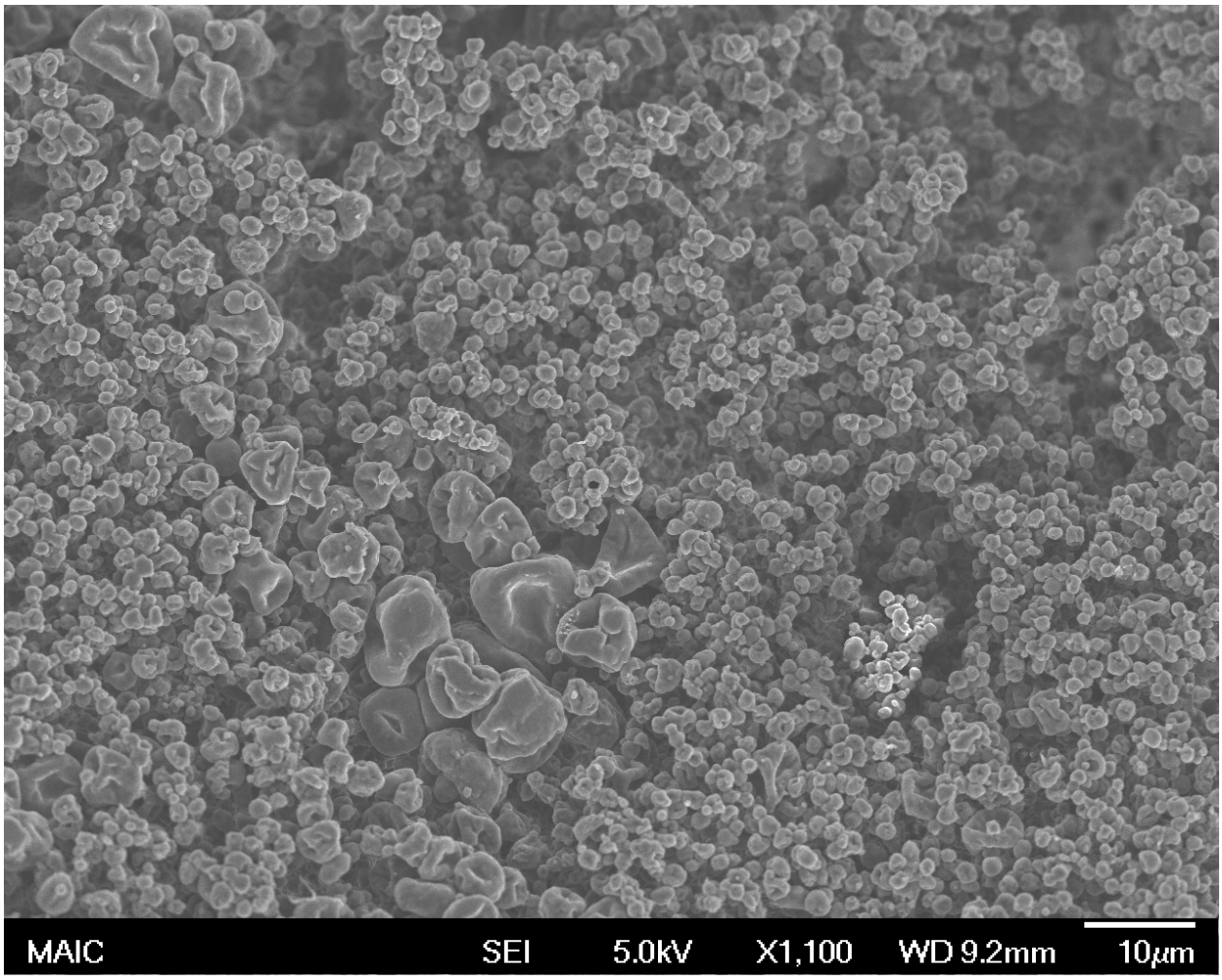


Figure 3-13. Spray dried chitosan coated MF-PLA nanoparticles containing 75% of total solid feed as nanoparticles

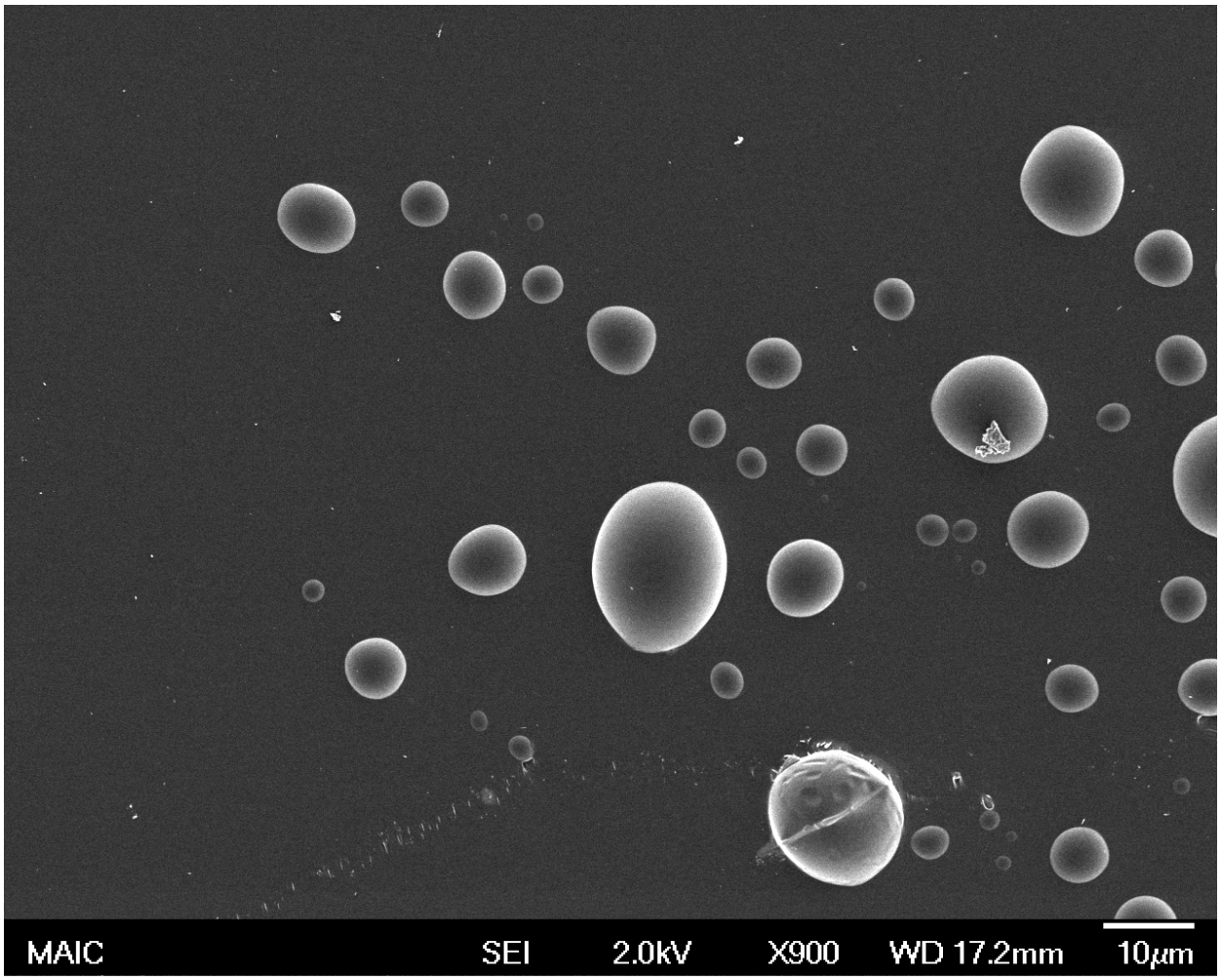


Figure 3-14. Spray dried lactose, 1.25% w/v solid feed content

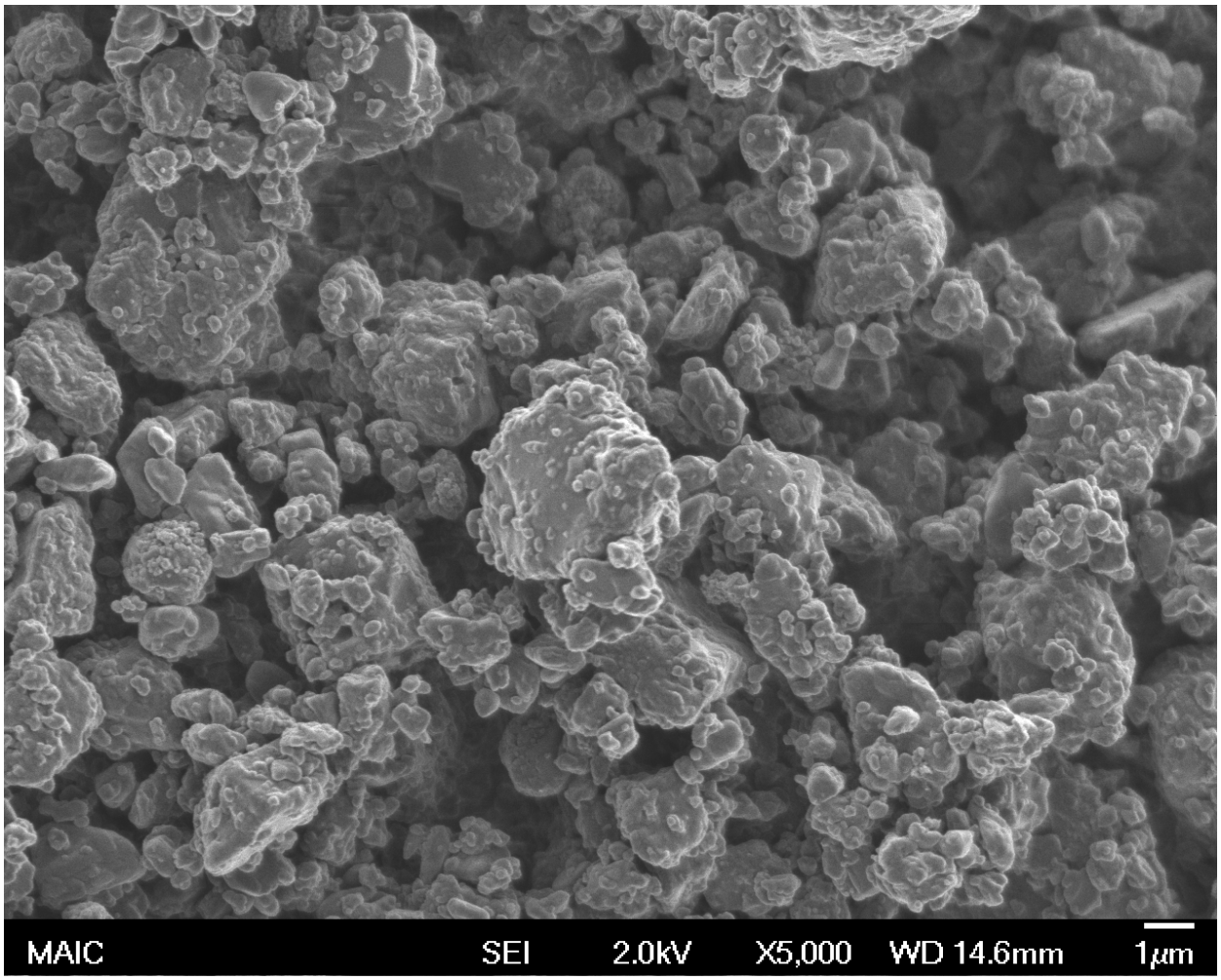


Figure 3-15. SEM of formulation contained in Asmanex® Twisthaler™

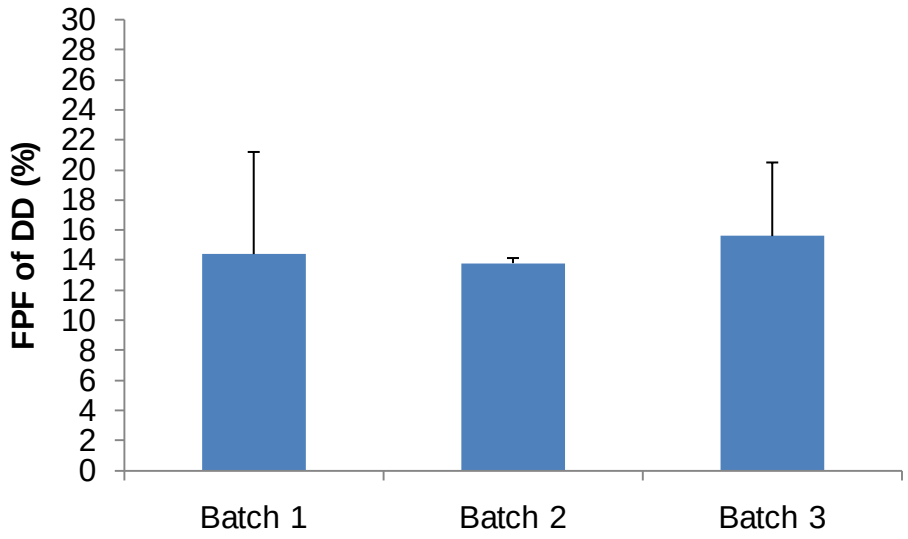


Figure 3-16. FPF of DD (%) of spray dried chitosan coated nanoparticles, to compare batch to batch variability (n=3)

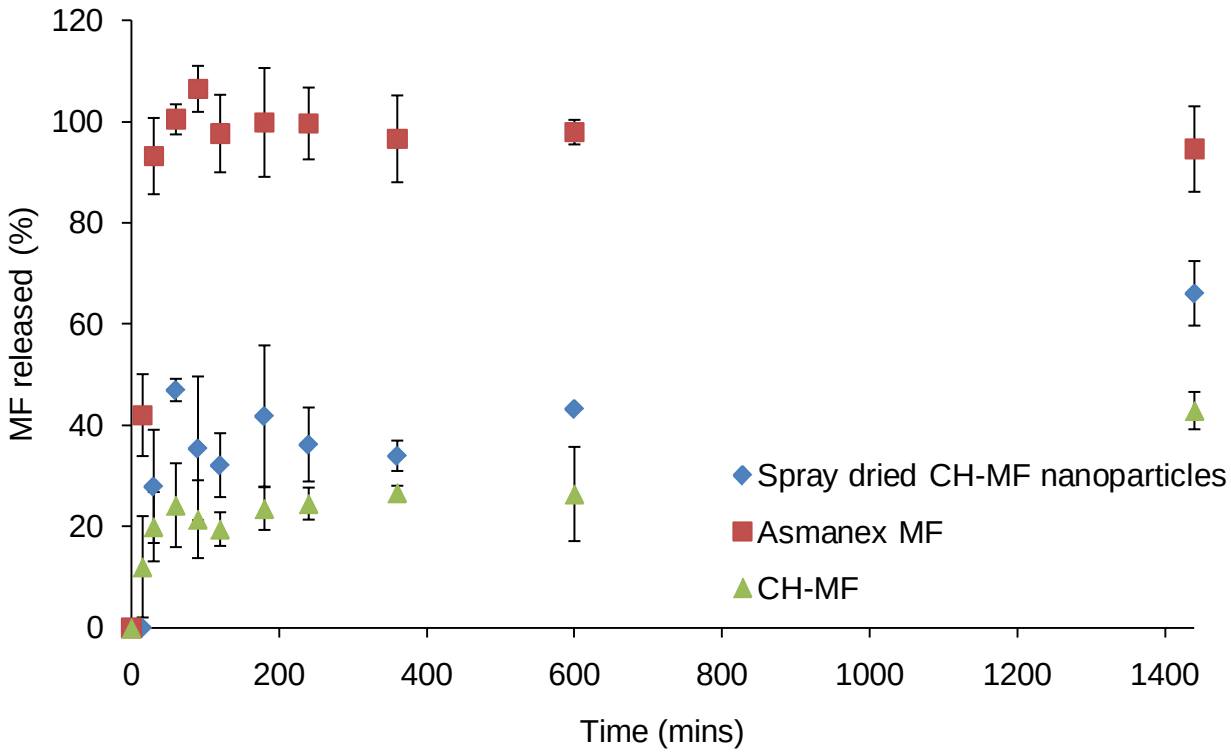


Figure 3-17. *In vitro* release of MF from spray dried CH-MF nanoparticles and Asmanex®

CHAPTER 4
SUMMARY

Polymeric nanoparticles containing PLGA and the model drug TA were developed using the solvent evaporation. Spherical unimodal TA-PLGA nanoparticles were produced using this technique. It was shown that various process parameters could influence particle size, drug encapsulation and drug release. Increasing polymer content was shown to increase encapsulation of TA in TA-PLGA nanoparticles and result in a trend of increasing particle size. As PVA surfactant concentration was increased, encapsulation efficiency decreased and smaller nanoparticles were formed. These TA-PLGA nanoparticles were able to release at a similar rate as micronized TA. Further developments were made using PLA to form nanoparticles. Mometasone furoate was used as this is a more lipophilic drug and would show slower drug release from polymer nanoparticles. Drug release was further reduced by coating MF-PLA nanoparticles with chitosan. MF released at a substantially slower rate from chitosan coated nanoparticles compared with MF-PLA nanoparticles and micronized MF that was used as a control.

Nanocomposite particles were developed using a spray dryer that was developed; this spray dryer operated with outlet temperatures below the Tg of PLA or PLGA making it suitable for spray drying polymer nanoparticles. The spray dryer was able to produce particles with MMAD in the respirable range. Nanocomposite microspheres were able to deliver a clinically relevant dose with two SPIRIVA® capsules filled with 10 mg of the formulation using the HandiHaler® device. SPIRIVA® capsules were placed in the center chamber of the HandiHaler® and pierced by pressing the button to a pre-determined level. Holes size produced in the capsule was customized to increase

retention within the device for a longer time period and therefore increase the time over which de-aggregation may occur. As a result of both the formulation and optimizing the inhaler device for use with this formulation, the FPF was similar to that from the Asmanex® Twisthaler™.

MF release from the spray dried CH-MF was compared to the nanoparticles before spray drying and also MF formulation contained within the Asmanex® Twisthaler™. MF was release rapidly from the Asmanex® with 100% release by 1 hour. In comparison both CH-MF and the spray dried formulation exhibited a biphasic release profile. CH-MF nanoparticles showed a burst release of approximately 20% compared to 30% from the spray dried formulation. A possible explanation for this may be as a result of having to re-disperse lyophilized nanoparticles in a lactose solution before spray drying. This could cause some of the MF to diffuse to closer to the surface of the nanoparticles and result in a greater burst effect observed with spray dried nanoparticles. Following the initial burst, both the CH-MF nanoparticles and spray dried formulation release with a similar rate. *In vitro* release from nanoparticles was not altered by the spray drying process.

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BIOGRAPHICAL SKETCH

Gina Patel was born in 1981, London, England. She graduated from the University of Bath, Bath, UK in 2003 as a Master of Pharmacy. During her studies she participated in an internship at the University of Florida under the supervision of Dr Hochhaus. After qualification as a pharmacist she worked as a hospital pharmacist at Musgrove Park Hospital, Taunton, UK before joining the Department of Pharmaceutics, University of Florida.