PEGYLATED PHOSPHOLIPID NANOMICELLES CONTAINING BUDESONIDE OR BECLOMETHASONE DIPROPIONATE FOR PULMONARY DELIVERY

MOHANAD NAJI SAHIB

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By

MOHANAD NAJI SAHIB

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LIST OF ABBREVIATIONS & SYMBOLS

$R_{adjusted}$	Adjusted coefficient of determination
AIC	Akaik Information Criterion
ANOVA	Analysis of variance
k_{BL}	Baker-Lansdale release constant
BDP	Beclomethasone Dipropionate
BALF	Bronchalveolar lavage fluid
BUD	Budesonide
CL	Clearance
cm	Centimetre
COPD	Chronic obstructive pulmonary disease
CMC	Critical micelle concentration
DSC	Deferential scanning calorimetry
ºC	Degree centigrade
DW	Distilled water
%DL	Drug loading percent
DPIs	Dry powder inhalers
DSPE	1,2-Distearoyl-sn-glycero-3-phosphoethanolamine
ECD	Effective cut-off diameter
ED	Emitted dose
%EE	Entrapment efficiency percent
Eq	Equation
FD	Freez drying
Fig	Figure

FPF	Fine particle fraction
k_1	First order release constant
FDA	Food and drug administration
FTIR	Fourier transform infrared spectroscopy
GSD	Geometric standard deviation
$t_{1/2}$	Half-life
HPLC	High performance liquid chromatography
HPH	High pressure homogenisation
ICH	International conference on harmonisation
k_H	Higuchi release constant
k_{HC}	Hixon-Crowell release constant
h	Hour
ICSs	Inhaled corticosteroids
ip	Intraperitoneal
kg	Kilogram
LOD	Limit of detection
LOQ	Limit of quantification
LC-MS-MS	Liquid chromatography-tandem mass spectrometry
L	Litre
MMAD	Mass median aerodynamic diameter
MDIs	Metered dose inhalers
µ	Micro
µg	Microgram
µL	Microlitre

µm	Micrometer
mg	Milligram
mg/ml	Milligram per millilitre
min	Minute
nm	Nanometer
NGI	Next generation impactor
OVA	Ovalbumin
%	Percent
%RSD	Percent of relative standard deviation
%RE	Percent relative error
PBS	Phosphate buffer saline
PCS	Photon correlation spectroscopy
PEG	Polyethylene glycol
PEG-PE	Polyethylene glycol- phosphatidyl ethanolamine
PI	Polydispersity index
PLGA	Poly(L-lactic-co-glycolic acid)
ppt	Precipitated
PASW	Predictive analytics software
PEG-DSPE	1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxy-poly(ethylene glycol)
RRA	Relative receptor affinity
REGWQ	Ryan-Einot-Gabriel-Welsch Q step-down procedure
SEM	Scanning electron microscopy

SD	Sprague-Dawley rat
sd	Standard deviation
S.E.	Standard error
SSMs	Sterically stabilised phospholipid nanomicelles
SSPs	Sterically stabilised particles
$T_{25\%}$	Time for 25% of drug release
$T_{50\%}$	Time for 50% of drug release
$T_{75\%}$	Time for 75% of drug release
$T_{80\%}$	Time for 80% of drug release
TEM	Transmission electron microscopy
Tukey HD	Tukey Honestly Significant Difference
UV	Ultraviolet
UK	United Kingdom
USA	United State of America
v/v	Volume by volume
w/w	Weight by weight
WHO	World Health Organization
%Y	Yield percent
k_o	Zero order release constant
Z-ave	Mean particle size

LIST OF PUBLICATIONS & COMMUNICATIONS

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National Journals

1. Sahib, M. N., Darwis, Y., Peh, K. K. and Tan, Y. T. F. (2011). Formulation and *in vitro* pulmonary deposition of budesonide nanomicelles using different nebulisers. *Malaysian journal of Pharmacy*. 1 (9), 362.
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Awards

1. Student Research Award (2011)

   Award 1st prize for outstanding work in the field of aerosol in medicine from the International Society for Aerosol in Medicine at 18th world congress of the International Society for Aerosol in Medicine, ISAM 2011 (June 18-22, 2011 Congress Center De Doelen, Rotterdam, the Netherlands).

2. USM Postgraduate Student Fellowship Award

   A fellowship for 3 years (2008-2010), awarded from Institute of Postgraduate Studies (IPS), Universiti Sains Malaysia (USM)

Conferences

International conferences

1. Sahib, M. N., Darwis, Y., Peh, K. K. and Tan, Y. T. F.. Formulation and *in vitro*, *in vivo* evaluation of self-associated beclomethasone dipropionate in PEGylated phospholipid nanomicelles for nebulisation. Drug Delivery to the Lungs 22- DDL22 (December 7-9, 2011 Edinburgh International Conference Center, Edinburgh, Scotland, United Kingdom).
2. Sahib, M. N., Darwis, Y., Peh, K. K. and Tan, Y. T. F.. Formulation and *in vitro* evaluation of sterically stabilised phospholipid nanomicelles loaded with beclomethasone dipropionate for nebulisation. 2nd Asian Symposium on Pharmaceutical Science and Technology (September 19-20, 2011 Xi'an, China).
3. Sahib, M. N., Darwis, Y., Peh, K. K. and Tan, Y. T. F.. Formulation and *in vitro*, *in vivo* evaluation of self-associated budesonide in phospholipid-based

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Seminar

1. Sahib, M. N., Darwis, Y., Khiang, P. K. and Tan, Y. T. F.. Preparation of Polymeric Micelles from PEGylated Phospholipids as Pulmonary Delivery

System. One Day Symposium in Pharmaceutical Technology (March 24, 2009 Meeting Room, School of Pharmaceutical Sciences, USM, Penang, Malaysia).

2. 2nd Seminar On The Use Of Animals In Science: Ethical & Practical Considerations (June 23-24, 2009, Lecture Hall X, School of Pharmaceutical Sciences, USM, Penang, Malaysia).
3. FAPA -AASP Workshop on Pharmacy Practice and Education: Pharmacy Education for Sustainable Pharmacy Practice (June 12, 2009 Meeting Room, School of Pharmaceutical Sciences, USM, Penang, Malaysia).
4. Viscometer Instrument Training Seminar.
5. HPLC Operating Machine Seminar.

NANOMISEL FOSFOLIPID TERPEGILAT YANG MENGANDUNGI BUDESONID ATAU BEKLOMETASON DIPROPIONAT UNTUK PENYAMPAIAN PULMONARI

ABSTRAK

Tujuan kajian ini adalah untuk merumuskan dan menilaikan nanomisel yang mengandungi kortikosteroid yang tak-terlarutkan air, budesonid (BUD) atau beklometason dipropionat (BDP) untuk penyampaian pulmonari dengan menggunakan polimer- polimer terPEGilat (PEG_{5000}-DSPE atau PEG_{2000}-DSPE).

Kesemua nanomisel fosfolipid yang terstabilkan secara sterik (SSMs) telah berjaya disediakan dengan menggunakan kaedah pemendakan mendakan bersama dan konstitusi semula. Rumusan SSM telah dicirikan dengan menggunakan kaedah fisikokimia yang berbeza. Terdapat perbezaan yang signifikan antara kecenderungan pemelarutan maksimum PEG_{5000}-DSPE dan PEG_{2000}-DSPE bagi BUD, iaitu lebih kurang 605.71±6.38 dan 646.27±4.93 μg/ml, masing-masing. Kecenderungan pemelarutan maksimum PEG_{5000}-DSPE dan PEG_{2000}-DSPE bagi BDP adalah lebih kurang 209.65±7.74 dan 210.01±5.28, masing-masing. Keputusan ini menunjukkan bahawa polimer terPEGilat mempunyai kecenderungan pemelarutan BUD yang lebih tinggi daripada BDP. Purata saiz partikel pada pemelarutan maksimum BUD:PEG_{2000}-DSPE (15.97±1.91 nm) dan BDP:PEG_{2000}-DSPE (15.44±1.66 nm) adalah lebih kecil daripada BUD:PEG_{5000}-DSPE (20.45±1.65 nm) dan BDP:PEG_{5000}-DSPE (19.99±0.98 nm). SSMs PEG_{5000}-DSPE tanpa drug telah berjaya diliofilkan pada kepekatan lebih kurang 5mM, manakala 10mM PEG_{2000}-DSPE diperlukan untuk liofilisasi. Terdapat perbezaan yang tidak signifikan dalam saiz

partikel, potensi zeta dan indeks polidispersiti di antara SSMs termuatkan drug dan SSM stanpa drug bagi polimer terPEGilat yang sama sebelum dan selepas proses liofilisasi. Peratusan hasil dan pemuatan drug bagi semua SSMs termuatkan drug adalah melebihi 95% dan 0.72%, masing-masing. Kedua-dua BUD dan BDP didapati berada dalam keadaan amorfus dengan DSC dan tidak bertindak balas secara kimia dengan polimer-polimer terPEGilat seperti yang ditunjukkan oleh spektrum FTIR. Pemeriksaan mikroskop elektron pemancaran (TEM) menunjukkan nanopartikel berbentuk sfera, sementara kajian mikroskop elektron imbasan (SEM) menunjukkan bentuk partikel BUD dan BDP yang berbeza berbanding dengan SSMs tanpa drug dan SSMs termuatkan drug yang terliofil. Kajian kestabilan jangka panjang dan jangka pendek menunjukkan bahawa SSMs termuatkan drug yang diliofilkan adalah stabil selama 1 tahun apabila disimpan pada suhu 4 ºC dan -20 ºC.

Dua kaedah HPLC yang ringkas dan sensitif telah dibangunkan untuk menganalisis kepekatan BUD dan BDP dalam rumusan yang berbeza. Keputusan pencirian aerodinamik menunjukkan SSMs termuatkan drug adalah lebih baik daripada rumusan dagangan mikroampaian Pulmicort Respules® dan Clenil®. Tambahan lagi, kajian pelarutan in *vitro* menunjukkan pelepasan drug daripada SSMs yang lebih berpanjangan daripada produk rujukan yang setara. Perbandingan bagi kesan farmakodinamik di antara SSM termuatkan drug dan produk rujukan menunjukkan kelebihan SSM dalam mengurangkan bilangan sel-sel keseluruhan dan diferensial, dan memperbaiki perencatan sel inflamatori. Keputusan kajian ini telah menunjukkan potensi besar sistem pembawa nano yang telah dibangunkan untuk penyampaian kortikosteroid kepada tapak sasaran dalam rawatan asma dan penyakit inflamatori saluran pernafasan yang lain.

PEGYLATED PHOSPHOLIPID NANOMICELLES CONTAINING BUDESONIDE OR BECLOMETHASONE DIPROPIONATE FOR PULMONARY DELIVERY

ABSTRACT

The aims of the present study were to formulate and evaluate nanomicelles containing poorly water soluble corticosteroids, budesonide (BUD) or beclomethasone dipropionate (BDP) for pulmonary delivery using PEGylated polymers (PEG_{5000}-DSPE and PEG_{2000}-DSPE).

All the sterically stabilized phospholipid nanomicelles (SSMs) were successfully prepared using a co-precipitation and reconstitution method. The SSMs were characterised by different physicochemical methods. There were significant differences between the maximum solubilisation tendencies of PEG_{5000}-DSPE and PEG_{2000}-DSPE for BUD, which were approximately 605.71±6.38 and 646.27±4.93 μg/ml, respectively. The maximum solubilisation tendencies of PEG_{5000}-DSPE and PEG_{2000}-DSPE for BDP were approximately 209.65±7.74 and 210.01±5.28, respectively. These results showed that PEGylated polymers had greater tendencies to solubilise BUD than BDP. The mean particle sizes at maximum solubilisation of BUD:PEG_{2000}-DSPE (15.97±1.91 nm) and BDP:PEG_{2000}-DSPE (15.44±1.66 nm) were smaller than BUD:PEG_{5000}-DSPE (20.45±1.65 nm) and BDP:PEG_{5000}-DSPE (19.99±0.98 nm). Blank SSMs of PEG_{5000}-DSPE were successfully lyophilised at a concentration of about 5 mM, while 10 mM of PEG_{2000}-DSPE was needed for lyophilisation. There were insignificant differences in the particle size, zeta potential and polydispersity index between drug-loaded SSMs and blank SSMs of the same

PEGylated polymer before and after lyophilisation. The yield and drug loading percentages of all the drug-loaded SSMs were more than 95% and 0.72%, respectively. Both BUD and BDP were found to be amorphous by differential scanning calorimeter (DSC) and did not interact chemically with the PEGylated polymers as shown by Fourier transform infrared spectroscopy (FTIR). The transmission electron microscope (TEM) examination showed spherical nanoparticles, while the scanning electron microscope (SEM) investigation indicated that the shapes of the BUD and BDP particles were very different from the lyophilised blank and drug-loaded SSMs. Short- and long-term stability studies showed that the lyophilised drug-loaded SSMs were stable for 1 year when stored at both 4ºC and -20ºC.

Two simple yet sensitive HPLC methods were developed in order to analyse the concentrations of BUD and BDP in different formulations. Aerodynamic characterisation of drug-loaded SSMs found that the SSMs were more superior than the marketed Pulmicort Respules® and Clenil® microsuspensions. In addition, the *in vitro* dissolution studies showed more prolonged drug release from the SSMs than their corresponding reference products. The pharmacodynamic study of drug-loaded SSMs showed the superiority of these formulations in reducing the total and differential cell counts and in enhancing the inhibition of inflammatory cells when compared with the reference products. The results of the present study indicated the great potential of the developed nanocarrier systems for the delivery of corticosteroids to the target sites for the treatment of asthma and other airway inflammatory diseases.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Asthma

Asthma is a chronic disorder of the conducting airway characterised by reversible narrowing of the airway due to inflammation and airflow obstruction that typically manifests itself as chest tightness, wheezing, cough and dyspnoea (Lemanske and Busse, 2003). The World Health Organization (WHO) estimates that about 150 million people worldwide are affected by asthma (Johansson and Haahtela, 2004). Asthma is an allergic process in origin as more than 50% of adults and at least 80% of children with the disorder suffer from an allergy (Lemanske and Busse, 2003; Johansson and Haahtela, 2004).

Inflammation in asthma involves many pathways that use various mechanisms or cells, cytokines and proinflammatory mediators. All these exert alterations in large and small airway structures, thickening the walls and producing excessive mucus and inflammatory exudates (Cerasoli, 2006). Different types of cells are involved in asthma pathogenesis, such as mast cells, eosinophils, basophils, neutrophils, macrophages, epithelial cells and lymphocytes, which contribute to ongoing airway inflammation through releasing a number of cytokines (Jarjour and Kelly, 2002). However, asthma is a heterogeneous disease with respect to immunopathology, clinical phenotypes, responses to therapies and natural history (Holgate, 2008).

Based on symptom frequency and severity, asthma can be classified into 4 categories: mild intermittent, mild persistent, moderate persistent and severe

persistent (Lenfant and Taggart, 1999). To control this disease, pharmacotherapy treatment is the standard management in most asthmatic patients (Eid, 2004). This includes short-acting (albuterol) and long-acting (salmeterol) beta-agonists, inhaled corticosteroids (ICSs) (beclomethasone dipropionate (BDP), budesonide (BUD) and fluticasone), leukotriene modifier (montelukast), chromones (cromolyn sodium and nedocromil sodium), methylxantines (theophylline), anticholynergic antimuscarinic drugs (ipratropium bromide) and anti-IgE (omalizumab). Treatment options depend on the symptom frequency and severity provided by the National Asthma Education and Prevention Program (NAEPP) (Lenfant and Taggart, 1999; Eid, 2004) and the Global Initiative for Asthma (GINA) (Bateman *et al.*, 2008).

Among these medications, ICSs are the most efficient for treating asthma due to their potent effects on inflammatory cells (diminish inflammatory cell function and activation), as well as altering chemotaxis (specifically neutrophils), impairing cytokine synthesis and release, reducing vascular leakage and mucus production, and increasing beta-adrenergic response (Szefler, 1991).

Understanding the pathophysiology of asthma has demonstrated the important role of ICSs in the first-line therapy for asthmatic patients for decreasing the risk of mortality (Suissa *et al.*, 2000). Different ICSs (alone or in combination with a beta-agonist) with different devices are available to treat asthma and are approved by the Food and Drug Administration (FDA) (Table 1.1).

Table 1.1 ICSs with different devices for treating asthma.

Model drug	Brand name®	Device
Beclomethasone Dipropionate	QVAR	MDI*
Budesonide	Pulmicort Respules	Microsuspension for nebulisation
Budesonide	Pulmicort Turbohaler	DPI**
Budesonide	Pulmicort Flexhaler	DPI
Budesonide and formoterol fumarate	Symbicort	MDI
Ciclesonide	Alvesco	MDI
Fluticasone propionate	Flovent HFA	MDI
Fluticasone propionate	Flovent Diskus	DPI
Mometasone	Asmanex	DPI
Fluticasone propionate and salmeterol xinafoate	Advair HFA	MDI
Fluticasone propionate and salmeterol xinafoate	Advair Diskus	DPI

* Metered Dose Inhaler; ** Dry Powder Inhaler

1.2 Inhaled Corticosteroids to Treat Asthma

Corticosteroids are not all equivalent due to the drug itself and/ or delivery device, which cause critical clinical differences in efficacy and safety (Allen *et al.*, 2003). Figure 1.1 gives a basic understanding of the fates of the ICSs in the human body.

Although ICSs are first line in treating persistent asthma, they lack a favourable reputation in terms of safety and tolerability due to local and systemic side effects (Table 1.2) (Hanania *et al.*, 1995; Lipworth, 1999; Kelly and Nelson, 2003). These

side effects lead to low compliance or adherence to medications and, as a consequence, poor asthma control, thus significantly increasing asthma morbidity and mortality risks (Rossi *et al.*, 2007). Therefore, different corticosteroids and devices have been developed to provide a higher therapeutic ratio with high potency, excellent efficacy, and optimum safety and tolerability, as shown in Table 1.1 (Cerasoli, 2006).

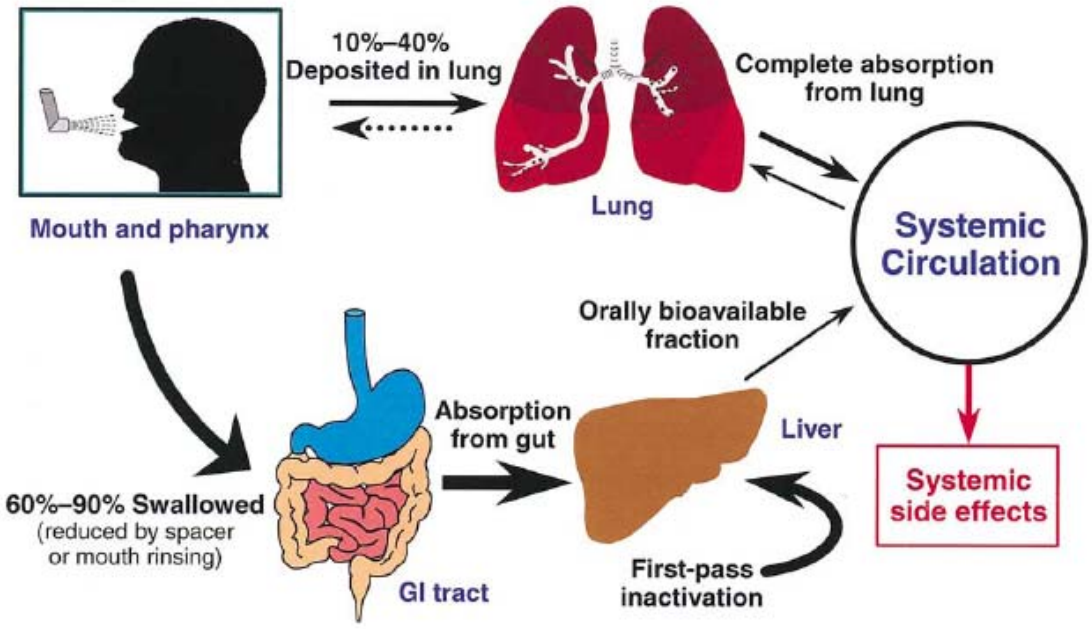

Figure 1.1 Fate of ICSs (adapted from (Allen *et al.*, 2003)).

1.3 Pulmonary Drug Delivery System

The local treatment of lung disorders such as asthma and chronic obstructive pulmonary disease (COPD) via pulmonary drug delivery offers many advantages

over oral or intravenous routes of administration as direct deposition of a drug at the diseased site increases local drug concentrations, improves the pulmonary receptor occupancy and reduces the overall dose required, therefore, reducing the side effects that result from high drug doses (Bailey and Berkland, 2009).

Table 1.2 Local and systemic side effects of ICSs (Adapted from (Dahl, 2006)).

Local side effects	Systemic side effects
Pharyngitis	HPA-axis suppression*
Dysphonia	Decrease in growth velocity and leg growth in children
Reflex cough	Decrease in bone mineral density
Bronchospasm	Bone fractures
Oropharyngeal candidiasis	Osteoporosis
	Skin thinning and bruising
	Cataracts and glaucoma

* HPA , hypothalamic, pituitary, adrenal

However, successful pulmonary delivery systems and clinical responses are affected by many factors including physiological and pathophysiological factors, delivery devices and corticosteroid pharmacokinetic/pharmacodynamic properties.

1.3.1 Physiological and Pathophysiological Factors

The respiratory system consists of the trachea, which divides into two bronchi. The bronchi branch into smaller bronchioles and finally the terminal bronchi, which end with the alveolar sac. Conducting airways are lined with ciliated epithelium and the lumen of the bronchiole is lined with serous fluid upon which floats a layer of

mucus. Cilia movement clears the mucous (mucociliary clearance) layer toward the proximal airways, where it is either swallowed or expectorated (Bailey and Berkland, 2009). The alveoli are composed of non-ciliated epithelium and an extremely thin barrier between the pulmonary lumen and the blood capillaries for efficient mass transfer (Brain, 2007).

Particle depositions in the lungs take place by inertial impaction, sedimentation or diffusion. Those with an aerodynamic diameter >10 μm are subjected to inertial impaction in the oropharyngeal region where they have little therapeutic effect, while particles with aerodynamic diameters of <1 μm mostly reach the alveolar region, but do not mostly deposit and are therefore exhaled. However, particles with aerodynamic diameters between 1 and 5 μm are efficiently deposited in the lung periphery to exert therapeutic effect (Musante *et al.*, 2002; Sung *et al.*, 2007).

Once drug molecules deposit in the lungs, they either penetrate the mucus and become absorbed or are subjected to mucociliary clearance (Bailey and Berkland, 2009). Mucociliary clearance in patients with acute asthma or COPD is markedly reduced and this clearance function is improved with the use of beta-agonists (Messina *et al.*, 1991; Lindberg *et al.*, 1995). The decrease in mucociliary clearance is compensated by cough clearance (Edsbäcker *et al.*, 2008).

Solubility of the inhaled drug particles and hence its pulmonary absorption may differ considerably between different drugs depending on their molecular weight, partition coefficient, hydrogen bond properties and polar surface (Tronde *et al.*, 2003a; Tronde *et al.*, 2003b). After particles deposit on the surface of the airways,

they are wetted and dissolve into the airway lining fluid. Inhaled drug particles with low solubility take a substantial period of time for solubilisation and partitioning between the phases of the airway lining, and are preferentially cleared from the airways by mucociliary transport and phagocytosis. Inhaled drug particles with high solubility enter into and dissolve in the airway lining fluid more rapidly, and are therefore less susceptible to mucociliary clearance (John *et al.*, 1994; Geiser *et al.*, 2000; Lay *et al.*, 2003).

In asthmatic patients, the systemic uptake of corticosteroid drugs also differs from that in healthy subjects, which depends on the severity of the disorder. For example, systemic uptake of fluticasone is lower in asthmatic patients than in healthy volunteers, while BUD systemic exposure has been shown to be higher than fluticasone in asthmatic patients and healthy volunteers (Harrison *et al.*, 2001; Harrison and Tattersfield, 2003). These results have been confirmed by pharmacokinetic results following intravenous administration of fluticasone and BUD in healthy and asthmatic patients, which are identical for both groups (Brutsche *et al.*, 2000; Thorsson *et al.*, 2001). In addition, regional distribution of the inhaled corticosteroid differs substantially between healthy subjects and asthmatic or COPD patients where the airways are smaller or obstructed, which may lead to reduced therapeutic effect, given that most beneficial effects of a corticosteroid occur when it is evenly distributed throughout the lungs since inflammatory cells are present throughout the airways and alveolar tissue in asthma (Kraft *et al.*, 1996; Carroll *et al.*, 1997). Uneven distribution tends to impact the inhaled corticosteroid in the proximal parts of the lung that are subject to mucociliary clearance (Saari *et al.*, 1998).

1.3.2 Delivery Device

There are three major types of inhalation devices used for pulmonary drug delivery: nebulisers, metered dose inhalers (MDIs) and dry powder inhalers (DPIs). From *in vitro* evaluation, each device has its own aerodynamic characteristics that may affect the clinical response (Sahib *et al.*, 2010). The advantages and disadvantages of each system are summarised in Table 1.3.

Table 1.3 Advantages and disadvantages of pulmonary delivery devices (adopted from (Labiris and Dolovich, 2003)).

Inhalation device	advantages	disadvantages
Nebulizers (jet, ultrasonic)	No specific inhalation technique or co-ordination required Aerosolizes most drug solutions Delivers large doses Suitable for infants and people too sick or physically unable to use other devices	Time consuming Bulky Nonportable Contents easily contaminated Relatively expensive Poor delivery efficiency Drug wastage Wide performance variation between different models and operating conditions
MDIs	Compact Portable Multidose (approximately 200 doses) Inexpensive Sealed environment (no degradation of drug) Reproducible dosing	Inhalation technique and patient co-ordination required High oral deposition Maximum dose of 5 mg Limited range of drugs available
DPIs	Compact Portable Breath actuated Easy to use No hand-mouth co-ordination required	Respirable dose dependent on inspiratory flow rate Humidity may cause powders to aggregate and capsules to soften Dose lost if patient inadvertently exhales into the DPI Most DPIs contain lactose

1.3.2.1 Nebulisers

The important advantage of using nebulisers is the delivery of the therapeutic agents to infants and young children or geriatric patients, who often lack the coordination and/or ability to cooperate actively and achieve optimal delivery with the pressurised metered-dose or powder inhalers, and allows for inhalation during tidal breathing (Berg and Picard, 2009). However, most prescribed drugs using nebuliser delivery devices never reach the lungs and the majority of the drug is either retained within the nebuliser or released into the environment during expiration with an average of 10% of the dose deposited in the lungs (O'Callaghan and Barry, 1997).

From a clinical point of view, although jet nebulisers have been used for aerosol delivery of water soluble compounds and micronised suspensions (like Pulmicort Respules®), their use with hydrophobic drugs has been inadequate (Waldrep *et al.*, 1997). Different nebulisers have different aerodynamic characteristics and therefore, give different therapeutic responses. Berg and Picard (2009) used thirty jet nebulisers to evaluate the aerodynamic characteristics of BUD and found different values. Moreover, Vaghi *et al.* (2005) showed the effect of formulation on the nebulisation characteristics of BUD (Pulmicort Respules®) and beclomethasone dipropionate (BDP) (Clenil® per Aerosol). In addition, suspension formulations are not nebulised as efficiently as solution formulations using ultrasound nebulisers (Nikander *et al.*, 1999). Recently, advances in nebuliser development have improved nebuliser efficiency, such as the Pari LC nebuliser, which has been shown to have more efficient output than the Omron nebuliser (Smaldone *et al.*, 1998; Berger, 2009). Furthermore, to overcome drug wastage during exhalation, the Akita device (Activaero, Gemunden, Germany) allows individualised controlled inhalations in

combination with either a Pari jet nebuliser or an eFlow vibrating mesh, thus ensuring that the aerosol is delivered to the patient during inspiration only (Kesser and Geller, 2009).

1.3.2.2 MDIs

MDIs are common delivery devices used for inhalation because they are portable and inexpensive. Even though the dosing with MDIs is more reproducible than that with DPIs, they are generally more difficult to use because they need coordination between actuation and inhalation to ensure optimal drug deposition in the lungs (Cochrane *et al.*, 2000). In addition, only 10 to 20% of the nominal dose of MDIs is deposited in the lungs due to large particle size and high speed spray that causes approximately 50 to 80% of the drug to be deposited in the oropharyngeal region (Newman *et al.*, 1981). Different spacer devices and breath-actuated MDIs have been developed to eliminate coordination requirements. Using a spacer produces finer particles, but does not change the distribution of the aerosol in patients with airway obstructions, only decreasing particle deposition in the oropharyngeal region and increasing the dose delivered to the lungs (Dolovich *et al.*, 1983; Newman and Newhouse, 1996; Kelly, 1998). Although breath-actuated MDIs increase the deposition of the therapeutic agent in the lungs, they do not decrease particle deposition in the oropharyngeal (Newman *et al.*, 1991; Chapman *et al.*, 1993; Cochrane *et al.*, 2000).

1.3.2.3 DPIs

These devices do not need coordination from the patients as they are breath-actuated and depend on the inspiratory flow rate, which can sometimes be difficult to replicate and tend to agglomerate due to electrostatic interactions and/or hygroscopic phenomena (Cochrane *et al.*, 2000; Khilnani and Banga, 2008). Lung deposition is approximately 12 to 40% of the emitted dose with 20 to 25% of the drug being retained within the device (Pedersen, 1996; Dolovich, 1999). Although DPIs depend on the inspiratory flow rate (i.e., it is affected by patient status), it has been found that patients admitted to the emergency room can sufficiently create a drug aerosol that results in a good clinical effect. However, a lung deposition study of budesonide showed that when the inhalation flow decreased from 58 L/min to 36 L/min, the lung deposition of BUD decreased from around 28% to around 15% (Borgstrom *et al.*, 1994).

1.3.3 Pharmacodynamic and Pharmacokinetic Properties of ICSs

1.3.3.1 Pharmacodynamic Properties of ICSs

The pharmacological effect of corticosteroids is mediated through the glucocorticoid receptor. Therefore, the receptor binding affinity determines the difference in potency of the different ICSs (Table 1.4), with a higher receptor affinity linked to a higher pharmacological response (Derendorf, 1997). The receptor binding affinities are measured relative to an affinity of 100 for the standard dexamethasone (Winkler *et al.*, 2004). The potency of ICSs ranked in descending order is as follows: mometasone fuorate > fluticasone propionate > beclomethasone-17-monopropionate

> des-ciclesonide > budesonide > beclomethasone > beclomethasone dipropionate > ciclesonide. From a clinical point of view, receptor binding affinity can be compensated by administering dose equivalents. Therefore, the pharmacokinetic properties of the ICSs are the most important factors for evaluating their safety and efficacy (Allen *et al.*, 2003).

Table 1.4 Pharmacokinetic and pharmacodynamic parameter of ICSs (adapted from (Winkler *et al.*, 2004)).

ICSs	RRA	F_{oral} (%)	Fu (%)	CL (L/h)	Vdss (L)	$t_{1/2}$ (h)
Mometasone fuorate	2300	<1	1-2	54	-	5.8
Fluticasone propionate	1800	<1	10	66-90	318-859	7-8
Beclomethasone dipropionate	53	15-20	13	150	20	0.5
Beclomethasone-17-monopropionate	1345	26	-	120	424	2.7
Beclomethasone	76	-	-	-	-	-
Ciclesonide	12	<1	<!	152	207	0.36
Des-ciclesonide	1200	<1	<1	228	897	3.4
Budesonide	935	11	12	84	183-301	2.8

CL, Clearance; F_{oral} , oral bioavailability; fu, fraction unbound; RRA, relative receptor affinity; $t_{1/2}$, half-life; Vdss, volume of distribution at steady state.

1.3.3.2 Pharmacokinetic Properties

Pharmacokinetics is a concentration-time relationship at the site of action. Pharmacokinetics properties of different ICSs are shown in Table 1.4. Some of the

ICSs are prodrugs, which are inactive compounds that are activated in the body after administration to exert their effects. Using prodrugs is beneficial due to reducing the risk of local as well as systemic side effects. For example, if an active corticosteroid is inhaled, some of the drug is deposited in the mouth and oropharynx, leading to side effects such as oral candidiasis, dysphonia and hoarseness. Inhaled prodrugs might reduce the incidence of local side effects in the mouth and oropharyngeal region due to the inactive drug form deposited there (Derendorf, 2007). Two ICSs are prodrugs, BDP and ciclesonide, which are activated to their active metabolite beclomethasone-17-monopropionate and des-ciclesonide, respectively (Freiwald *et al.*, 2005; Derendorf, 2007). A clinical trial of ciclesonide showed a lower incidence of oropharyngeal adverse effects than fluticasone propionate (Kaliner, 2006).

A large part of the inhaled drug (approximately 40 to 90%) is swallowed and available for systemic absorption. Oral bioavailability depends on the drug molecules as well as the delivery device. As only systemic absorption produces systemic side effects, it is desirable that oral bioavailability of ICSs be very low (Winkler *et al.*, 2004). For example, oral bioavailability of fluticasone propionate is less than 1% while that for 17-beclomethasone monopropionate is 26% (Thorsson *et al.*, 1997; Daley Yates *et al.*, 2001).

In addition to oral bioavailability, all the drug that is deposited in the lungs is absorbed systemically (Allen *et al.*, 2003). Delivery devices used for inhalation are an important factor for pulmonary bioavailability like the drug itself (Ben-Joseph, 2000). For example, absolute bioavailability of fluticasone propionate with DPI has

been shown to be approximately 17%, while its bioavailability is around 26 to 29% with MDI (Mackie *et al.*, 2000).

Inhaled corticosteroid should be eliminated from systemic circulation in order to reduce systemic adverse effects. All ICSs are eliminated in the liver with values close to the liver blood flow. Therefore, development of new corticosteroids with high intrinsic hepatic clearance is unnecessary, since such steroids are not cleared more efficiently (Winkler *et al.*, 2004). ICSs that are primarily present in tissues have large volumes of distribution, which suggests good penetration into the target tissues in the lungs and good pharmacodynamic activity (Allen *et al.*, 2003). The volumes of distribution are correlated with the lipophilicity of the ICSs. The more lipophilic a corticosteroid is, the more it binds to the tissue, i.e., the higher the volume of distribution it will have (Winkler *et al.*, 2004).

Protein binding is also an important parameter because only free corticosteroid molecules can interact with corticosteroid receptors. Most ICSs have same protein binding percentage (10%), except for ciclesonide (1%). This causes ciclesonide to elicit much less cortisol suppression than other inhaled corticosteroids (Lipworth *et al.*, 2005). The half-life parameter correlates to volume of distribution and clearance and is estimated after intravenous administration. However, the actual half-life of ICSs in the lungs depends on the pulmonary residence time (mean absorption time) and lipid conjugation. For example, the half-life of fluticasone propionate is between 7 and 8 hours after intravenous administration and around 14 hours after inhalation and this is due to its low water solubility, thus leading to low absorption time and high availability in the lungs (Thorsson *et al.*, 1997; Thorsson *et al.*, 2001). While

lipid conjugation is pronounced with BUD, it forms reversible esters with fatty acids in the lungs and could increase the pulmonary half-life (Miller-Larsson *et al.*, 1998).

1.4 Nanotechnology for Pulmonary Delivery

In pharmaceutical terms, a nanoparticle is defined as a particle with a size ranging from 1 or 10 to 1000 nm (Brigger *et al.*, 2002; Sung *et al.*, 2007; Gao *et al.*, 2008; Kaur *et al.*, 2008). However, particles larger than 200 nm are easily cleared from the circulation, given that spleen filtration captures particles exceeding 250 nm and liver filtration captures particles greater than 150 nm (Bawarski *et al.*, 2008). Furthermore, capillaries of a tumour rarely exceed 300 nm in diameter (Moghimi *et al.*, 2001) and those smaller than 260 nm can escape phagocytosis by macrophages (Yang *et al.*, 2008b). Therefore, current nanopharmaceutical formulations focus on particles smaller than 200 nm (Bawarski *et al.*, 2008). The National Nanotechnology Initiative (NNI) defines nanotechnology as the study and use of structures in the size ranging from 1 to 100 nm (Zullo *et al.*, 2002; Mishra *et al.*, 2010).

Nanocarriers have received a lot of attention in medical and drug formulations in recent years due to their advantages. They improve pharmacokinetics, minimise toxicity of therapeutic agents by their preferential accumulation at the target site (Alexis *et al.*, 2008), increase the solubility of hydrophobic compounds and increase their stability (Hayama *et al.*, 2008). They efficiently deliver therapeutic agents to the target organ due to their smaller size and higher barrier permeability (Wang *et al.*, 2002; Lukyanov *et al.*, 2003). They are formulated from biodegradable materials, which decrease the possibilities of hypersensitivity reactions and affords good tissue compatibility (Panyam and Labhasetwar, 2003).

Nanocarriers for the pulmonary system is becoming a popular method to deliver therapeutic or diagnostic agents for local or systemic effects (Table 1.5) (Ely *et al.*, 2007; Azarmi *et al.*, 2008). They offer many advantages that include:

1. Achievement of relatively uniform distribution of drug dose through the lungs (Sung *et al.*, 2007).
2. Increase in the solubility of hydrophobic drugs and deposition in the fluid lining the lungs, which protects them from mucociliary clearance until their dissolution (Sung *et al.*, 2007; Abdulla *et al.*, 2010).
3. Extended pharmacokinetic and pharmacodynamic of the inhaled drug in the airway system, which improve therapeutic management by reducing dose frequency and improving compliance, thus reducing side effects and cost (Saks and Gardner, 1997; Hardy and Chadwick, 2000).
4. Suitability to deliver macromolecules and protect them from degradation (Zhang *et al.*, 2001).
5. Nanoparticles are more superior than microparticles in penetrating the airway sputum, which can improve the therapeutic efficacy of anti-asthmatic drugs (Lai *et al.*, 2009; Suk *et al.*, 2009).

1.4.1 Nanocarrier Systems for Pulmonary Delivery

1.4.1.1 Liposomes

Liposomes are attractive candidates for pulmonary drug delivery vehicles, since they can be prepared from compounds endogenous to the lungs (such as lung surfactant) (Justo and Moraes, 2003). However, when liposomes are delivered to the lungs in the

liquid state, leakage of the encapsulated therapeutic agent occurs due to shearing forces (Leung *et al.*, 1996). Therefore, liposomes in dry powder form are used to deliver agents to the lungs (Lu and Hickey, 2005; Chougule *et al.*, 2007; Chougule *et al.*, 2008; Changsan *et al.*, 2009). Different therapeutic agents have been delivered to the lung using the liposomal formulation (Waldrep *et al.*, 1997; Waldrep *et al.*, 1998; Saari *et al.*, 1999; Huang and Anderson, 2002; Bhavane *et al.*, 2003; Wong *et al.*, 2003; Ohmori *et al.*, 2005; Gagnadoux *et al.*, 2008).

1.4.1.2 Polymeric Nanoparticles

The main advantages of polymeric nanoparticles in pulmonary drug delivery are in the control of drug release and protection of the therapeutic agent from degradation (Zhang *et al.*, 2001; Bivas-Benita *et al.*, 2004c; Zahoor *et al.*, 2005; Ohashi *et al.*, 2009). Many biodegradable or biocompatible materials are used to prepare polymeric nanoparticles, such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), alginic acid, gelatin and chitosan (Mansour *et al.*, 2009). However, long-term administration of these should be assessed as the degradation products of these polymers may affect the physiological function of respiratory surfactant in the alveoli, which in turn will affect pulmonary immunity control and adversely affect breathing. Toxicity and biodegradability of these polymers should also be closely examined for pulmonary delivery after repeat dosing (Mansour *et al.*, 2009). Many examples of these formulations with different therapeutic agents are found in the literature (Rudolph *et al.*, 2002; Pandey *et al.*, 2003; Zahoor *et al.*, 2005; Azarmi *et al.*, 2006; Li and Huang, 2006; Seong *et al.*, 2006; Yamamoto *et al.*, 2007; Chono *et al.*, 2008; Kimura *et al.*, 2009; Ko *et al.*, 2009).

Table 1.5 Nanocarrier formulations for the pulmonary system.

Therapeutic areas	Model drugs	References
Local	Sodium cromoglicate	(Nolan *et al.*, 2011)
Local	Beclomethasone Dipropionate	(Jaafar-Maalej *et al.*, 2011)
Local	Paclitaxel	(Gill *et al.*, 2011)
Local	Tacrolimus	(Watts *et al.*, 2011)
Local	Levofloxacin	(Cheow *et al.*, 2011)
Local	Doxorubicin	(Roa *et al.*, 2011)
Local	Interfering RNA (siRNA)	(Jensen *et al.*, 2010)
Local	Diatrizoic acid	(El-Gendy *et al.*, 2010)
Local	5-Fluorouracil	(Kalantarian *et al.*, 2010)
Systemic	Thymopentin (TP5)	(Li *et al.*, 2010)
Local	Rifampicin	(Abdulla *et al.*, 2010)
Local	Celecoxib	(Patlolla *et al.*, 2010)
Local/systemic	Itraconazole	(Yang *et al.*, 2010)
Local	Levofloxacin	(Kho *et al.*, 2010)
Local	Rifampicin	(Saraogi *et al.*, 2010)
Local	Cisplatin	(Tseng *et al.*, 2009)
Local	Paclitaxel	(Hureaux *et al.*, 2009)
Local	Paclitaxel	(El-Gendy and Berkland, 2009)
Local	Tobramycin	(Pilcer *et al.*, 2009)
Local/ systemic	Nifedipine	(Plumley *et al.*, 2009)
Local	Budesonide	(El Gendy *et al.*, 2009)
Local	Ciprofloxacin	(Zhao *et al.*, 2009)
Systemic	Insulin	(Liu *et al.*, 2008)
Systemic	Insulin	(Bailey *et al.*, 2008)
Local	Pranlukast hemihydrate	(Mizoe *et al.*, 2007)
Local	Itraconazole	(Alvarez *et al.*, 2007)
Local	Doxorubicin	(Azarmi *et al.*, 2006)
Systemic	Tetrahydrocannabinol	(Van Drooge *et al.*, 2005)
Local	Rifampicin; Isoniazid; Pyrazinamide	(Zahoor *et al.*, 2005)
Local	Rifampicin; Isoniazid; Pyrazinamide	(Pandey and Khuller, 2005)
Local	Beclomethasone Dipropionate	(Darwis and Kellaway, 2001)
Local	Ketotifen fumarate	(Joshi and Misra, 2001)

1.4.1.3 Lipid Nanocarriers

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) are a good example of nanocarrier systems for pulmonary delivery (Pandey and Khuller, 2005; Chattopadhyay *et al.*, 2007; Liu *et al.*, 2008; Patlolla *et al.*, 2010). SLN and NLC are made from solid lipids and solid lipids with spatially incompatible liquid lipids, respectively (Hu *et al.*, 2005). These formulations are produced as alternatives to polymeric nanoparticles as they have a faster *in vivo* degradation and higher tolerability in the lungs compared to polymeric nanoparticles (Müller *et al.*, 2000; Westesen, 2000; Liu *et al.*, 2008; Doktorovová *et al.*, 2010; Patlolla *et al.*, 2010). Lipid nanocarriers are fully appreciated as pulmonary delivery systems only when they are formulated from physiological lipids, which have little or no cytotoxicity compared to polymer-based systems (Müller *et al.*, 1997; Heydenreich *et al.*, 2003).

1.4.1.4 Submicron Emulsions and Suspension

Aerosolisation of submicron emulsions or suspension as a liquid or dry powder form is an interesting way to deliver therapeutic agents (Bivas-Benita *et al.*, 2004a; Shekunov *et al.*, 2006; El-Gendy and Berkland, 2009; Amani *et al.*, 2010; Cheow *et al.*, 2011). However, more studies are required to assess the adverse effects of formula surfactants and oils on lung alveolar function (adverse interactions with lung surfactant) (Mansour *et al.*, 2009).

1.5 Methods for Preparing Inhaled Formulations

Many methods are used to prepare nanocarrier systems depending on the material that is used. However, there are two main processes to produce different inhaled therapeutic nanocarriers (Bailey and Berkland, 2009):

1.5.1 Top-down Method

In this method, large solid particles are mechanically broken down into smaller sizes. The most common method is wet milling and high pressure homogenisation (HPH) (Van Eerdenbrugh *et al.*, 2008). Although they are scalable, reliable and capable of producing nanoparticles with narrow size distributions (Patravale *et al.*, 2004), they have some disadvantages due to the mechanical nature. They are time and energy consuming because they need up to a few days to produce nanoparticles (Wiedmann *et al.*, 1997; Ostrander *et al.*, 1999; Tam *et al.*, 2010). It is strongly suggested that these processes produce a high amorphous content of the model drug, which leads to long-term stability issues of the drug (Rabinow, 2004; Chow *et al.*, 2007). In addition, there is a high chance for contamination from milling media or the homogeniser chamber (Patravale *et al.*, 2004; Chow *et al.*, 2007). Many therapeutic agents are formulated as a nanosystem using these methods found in the literature (Wiedmann *et al.*, 1997; Ostrander *et al.*, 1999; Jacobs and Müller, 2002; Rabinow, 2004; James *et al.*, 2008; Chiang *et al.*, 2009; Shrewsbury *et al.*, 2009; Yang *et al.*, 2010).

1.5.2 Bottom-up Method

From the molecular level (dissolved drug), the nanoparticle can be formed with a better control of particle properties than the top-down method (Chow *et al.*, 2007).

1.5.2.1 Solvent Evaporation Method

1.5.2.1.1 Spray Drying

This method has been used efficiently to produce respirable colloidal systems (Freitas and Müller, 1998; Maa *et al.*, 1998; Duddu *et al.*, 2002; Stahl *et al.*, 2002; Purvis *et al.*, 2006; Mizoe *et al.*, 2007; Yang *et al.*, 2008e). It produces a dry powder in one step and allows the control of particle size and morphology by changing the experimental setup (Vehring, 2008). The solvent of the droplet is thermally removed and therefore cannot be used for heat sensitive drugs. In addition, it has an inefficient yield (Rogers *et al.*, 2002). It is also used in the production of SLN (Freitas and Müller, 1998).

1.5.2.1.2 Cryogenic Solvent Evaporation

To overcome the problem of using heat in removing solvent in spray drying, a cryogenic solvent evaporation process can be used. It has a high and efficient yield reaching 95% compared to spray drying (Barron *et al.*, 2003). It includes a droplet spraying into a cryogenic liquid and then lyophilising the frozen droplets. Different techniques use the principle of the cryogenic solvent evaporation process, which include spray freezing into liquid (SFL) (Rogers *et al.*, 2002; Hu *et al.*, 2004; Engstrom *et al.*, 2008; Yang *et al.*, 2008c), ultra rapid freezing (URF) (Sinswat *et al.*,

2008; Yang *et al.*, 2008c; Engstrom *et al.*, 2009) and thin film freezing (TFF) (Engstrom *et al.*, 2008; Engstrom *et al.*, 2009).

1.5.2.1.3 Evaporative Precipitation into Aqueous Solution (EPAS)

EPAS is another solvent evaporation method (Hoeben *et al.*, 2006; McConville *et al.*, 2006) where heated water is used instead of the cryogen as in SFL (Chen *et al.*, 2002).

1.5.2.1.4 Microemulsion

A nano- or microemulsion base can be used to trap the drug solution and subsequently remove the solvent under vacuum to produce a nanosuspension after rehydration or frozen and lyophilised or spray-freeze dried to produce dry nanoparticles. This method is commonly used to produce polymeric nanoparticles (Dailey *et al.*, 2003a; Pandey *et al.*, 2003; Bivas-Benita *et al.*, 2004b; Cook *et al.*, 2005; Dailey *et al.*, 2006; Shi *et al.*, 2007).

1.5.2.1.5 Condensation Aerosol Generation

This process includes the condensation of a liquid or solid that is previously vaporised using a heated capillary by ambient air to form particulates suitable for inhalation (Gupta *et al.*, 2003; Li *et al.*, 2005). A few examples of this method can be found in the literature (Hong *et al.*, 2002; Gupta *et al.*, 2003; Li *et al.*, 2005).

1.5.2.1.6 Rapid Expansion of Supercritical Solutions (RESS)

Pre-heated supercritical fluid solution composed from CO_2 and a model drug is ejected through a nozzle, resulting in solute precipitation due to the sudden drop in density and solubility power (Charpentier *et al.*, 2008; Martín and Cocero, 2008; Pasquali *et al.*, 2008). Carbon dioxide is usually used because it does not affect the environment when vented into the atmosphere (Snavely *et al.*, 2002).

1.5.2.2 Antisolvent Method

This method involves the production of supersaturated solution from mixing the solution with an antisolvent that induces nucleation and simultaneous growth of nanoparticles by condensation and coagulation (Mehnert and Mäder, 2001; Dalvi and Dave, 2009). This includes the supercritical fluids process (gas antisolvent (GAS) or supercritical fluid antisolvent (SAS)), supercritical fluid extraction of emulsions (SFEE)) and the liquid antisolvent process (uses high gravity and sonication) (Chattopadhyay *et al.*, 2006; Reverchon and Adami, 2006; Shekunov *et al.*, 2006; Zhang *et al.*, 2006b; Okamoto and Danjo, 2008; Pasquali and Bettini, 2008; Dhumal *et al.*, 2009; Zhao *et al.*, 2010).

1.6 Polymeric Micelles

In pharmaceutical technology, many drug-releasing and drug-targeting systems have been developed to reduce drug degradation and enhance the amount of drug in the target area (Jones and Leroux, 1999). In general, poor water-soluble agents are usually associated with poor absorption and bioavailability upon oral administration (Lipinski *et al.*, 2001). In addition, from a pharmacological point of view,

hydrophobicity is beneficial for the drug–tissue relationship but formulation, solubilisation and stabilisation of these agents are problems that should be investigated (Sezgin *et al.*, 2006). Accordingly, many pharmaceutical delivery systems from polymeric micelles have been introduced recently (Jones and Leroux, 1999) due to their solubilising potential of poorly soluble drugs and the formation of small particle sizes (less than 100 nm) by which they evade phagocytosis (Kwon and Okano, 1996).

Polymeric micelle so-called colloidal dispersions are self-assembled core-shell nanostructures formed in an aqueous solution consisting of hydrophobic fragments of amphiphilic molecules forming the core of a micelle, which is segregated from the environment by hydrophilic parts of the molecules that form the micelle corona (Gao *et al.*, 2002; Riess, 2003). Cargo space (core) formed from the hydrophobic segment solubilises a variety of poorly soluble therapeutic and diagnostic agents. This solubilisation increases the bioavailability and circulation time after parenteral administration, as well as modifying the pharmacokinetics and biodistribution of the therapeutic agents (Jones and Leroux, 1999; Torchilin, 2001). The small size of the micelles permits their extravasation and accumulation in a variety of pathological sites such as tumours (Sezgin *et al.*, 2006). Additionally, polymeric micelles are easily prepared on a large scale (Torchilin, 2001).

Polymeric micelles are more stable than micelles prepared from conventional detergents and some amphiphilic co-polymers have critical micelle concentration (CMC) values as low as 10^{-6} M (Sung Bum *et al.*, 1996; Kabanov *et al.*, 2002). This is especially important from a practical point of view, since upon dilution with a

large volume of biological fluid, micelles with a high CMC value may dissociate into monomers and their content may precipitate (Torchilin, 2004). Moreover, polymeric micelles have high kinetic stability due to the presence of multiple sites capable of mediating the hydrophobic interaction between each other and/or hydrophobic drugs (Yokoyama *et al.*, 1993).

The major driving force behind self-association of amphiphilic polymers is the decrease in free energy of the system due to the removal of a hydrophobic segment from the surrounding aqueous media with the formation of a micelle cargo space stabilised with hydrophilic blocks exposed to water (Kwon and Okano, 1999). The properties of the core and corona of the polymeric micelles give the characteristic behaviour in the biological system (Gref *et al.*, 1995; Cammas *et al.*, 1997; Kuntz and Mark Saltzman, 1997; Inoue *et al.*, 1998). Different polymeric micelle copolymers (but not restricted) have been investigated (Table 1.6) to solubilise and target many poorly soluble compounds. An important type of polymeric micelle is phospholipid micelles, which are sterically stabilised phospholipid nanomicelles (SSMs) composed of polyethylene glycol and phospholipids (PEGylated phospholipids).

1.7 PEGylated Phospholipid Micelles

PEGylated phospholipids are similar to the amphiphilic copolymers of the A-B type, except that the hydrophobic part is a lipid instead of a hydrophobic polymer block. Acyl chains of lipids form the cargo space of the polymeric micelles that encapsulate or solubilise a variety of poorly soluble therapeutic and diagnostic agents (Lukyanov and Torchilin, 2004). One of the most important PEGylated phospholipid is