FORMULATION AND EVALUATION OF LIQUID CRYSTALLINE NANOSTRUCTURES FOR THE PULMONARY DELIVERY OF DOCETAXEL

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UNIVERSITI SAINS MALAYSIA

2025

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by

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Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

May 2025

ACKNOWLEDGEMENT

In the name of Almighty Allah, the Most Gracious, the Most Merciful. All praise is due to Allah for granting me the strength, wisdom, and perseverance to complete this research and earn my Ph.D.

I extend my heartfelt gratitude to my mentor, Professor Dr. Habibah A. Wahab, for her exemplary leadership, continuous support, and invaluable guidance. Her encouragement and inspiring discussions were vital to this work.

I am also sincerely thankful to my co-supervisors, Associate Professor Dr. Yusrida Darwis, Dr. Thaigarajan Parumasivam, and Associate Professor Dr. Anan Yaghmur, for their support and trust in my capabilities. Special thanks to Dr. Noratiqah Binti Mohtar for generously allowing me to conduct part of my research in her lab.

My appreciation goes to the School of Pharmaceutical Sciences, led by Associate Professor Dr. Nurzalina Binti Abdul Karim Khan, for fostering professional growth through enriching programs. I also thank the Institute of Postgraduate Studies, Universiti Sains Malaysia, for the USM Fellowship, and the Ministry of Higher Education Malaysia for supporting this work through FRGS (FRGS/1/2020/SKK0/USM/01/4).

Finally, my deepest love and thanks to my family especially my beloved parents, wife Dr. Reem Abou Assi, siblings for their unwavering support and encouragement throughout this journey.

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%	Percent
ACI	Andersen Cascade Impactor
ANOVA	Analysis of Variance
A549	Adenocarcinomic human alveolar basal epithelial cells
°C	Celsius
cm	Centimetre
cm^2	Squared centimetre
CC	Formulation with Glyceryl Monooleate and Poloxamer 407®
CCP	Formulation with Glyceryl Monooleate, Poloxamer 407® and Polyvinyl Alcohol
DTX	Docetaxel
GMO	Glyceryl Monooleate
GCit	Formulation with Glyceryl Monooleate and Citrem
HLB	Hydrophilic Lipophilic Balance
HPLC	High-Performance Liquid Chromatography
hr	Hour
LOD	Limit of Detection
LOQ	Limit of Quantification
LCN	Liquid Crystal Nanocarriers
µl	Microliter
mg/ml	Milligram per millilitre
min	Minute
ml/min	Millilitre per minute
mM	Millimole
mV	Millivolt

N	Theoretical plate number
NGI	Next Generation Impactor
nm	Nanometre
PDI	Polydispersity index
PL90G®	Phospholipid 90G®
PVA	Polyvinyl Alcohol
PTFE	Polytetrafluoroethylene
RH	Relative Humidity
rpm	Revolutions per minute
RSD	Relative Standard Deviation
SAXS	Small-angle X-ray scattering
SCit	Formulation with Phospholipid 90G® and Citrem
SD	Standard deviation
SEM	Standard Error of the Mean
TEM	Transmission Electron Microscopy
UK	United Kingdom
USA	United States of America
USFDA	United States Food and Drug Administration
USP	United State Pharmacopoeia
UV	Ultraviolet
WHO	World Health Organisation
w/v	Weight per volume
w/w	Weight per weight
ZP	Zeta potential
ζ	Zeta potential

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FORMULASI DAN PENILAIAN STRUKTUR NANO KRISTAL CECAIR UNTUK PENGHANTARAN PULMONARI DOCETAXEL

ABSTRAK

Kanser paru-paru merupakan punca utama kematian berkaitan kanser di seluruh dunia. Docetaxel (DTX) ialah agen kemoterapi yang banyak digunakan; namun, keterlarutan yang rendah, bioavailabiliti yang terhad, dan ketoksikan sistemik yang tinggi mengehadkan keberkesanannya. Formulasi intravena konvensional menggunakan etanol dan surfaktan seperti polisorbat 80 (Tween 80®), yang menyebabkan kesan sampingan teruk, termasuk nefrotoksisiti dan hipersensitiviti. Situasi ini menekankan keperluan untuk penghantaran ubat yang disasarkan bagi meningkatkan keberkesanan DTX sambil mengurangkan ketoksikan sistemik. Penghantaran pulmonari melalui nanopartikel berasaskan lipid, khususnya struktur nano kristal cecair (LCNs), menawarkan pendekatan tidak invasif dan berkesan dengan pematuhan pesakit yang lebih baik. Kajian ini bertujuan untuk membangunkan dan mencirikan LCNs bermuatan DTX yang boleh diinhalkan bagi penghantaran pulmonari, dengan mengoptimumkan keberkesanan antikanser serta berpotensi mengurangkan kesan sampingan. LCNs disediakan melalui pengekstrusian, memastikan kehilangan fasa terdispersi yang minimum dan mengekalkan integriti struktur. Penyebaran sinar-X sudut kecil (SAXS) mengesahkan struktur nano dalaman kubik, membuktikan pengekstrusian sebagai kaedah fabrikasi yang boleh diskalakan. Satu siri empat eksperimen faktorial penuh menghasilkan 40 formulasi, di mana 11 memenuhi kriteria pemilihan berdasarkan kandungan lipid tertinggi, saiz zarah (PS) terkecil, dan indeks polidispersitas (PDI) < 0.25. DTX dimuatkan pada kepekatan 2, 5, dan 10 mg/mL, dengan 5 mg/mL dikenal pasti sebagai kepekatan optimum. Lima

formulasi (CC8, CCP15, GCit7, SCit7, dan SCit8) menunjukkan kecekapan penjerapan (EE%) > 65% serta PS yang sesuai (149.5–238.4 nm) dan PDI (< 0.2). Kajian pelepasan ubat secara *in vitro* menunjukkan pelepasan DTX yang berterusan selama 72 jam. Kajian aerosol menggunakan nebuliser jenis air-jet dan mesh mengesahkan pemendapan pulmonari yang berkesan. LCN menunjukkan nilai diameter aerodinamik median jisim (MMAD) antara 3.38 ± 0.25 μm hingga 4.63 μm (air-jet) dan 3.90 ± 0.11 μm hingga 4.63 ± 0.28 μm (mesh). Pecahan zarah halus (FPF%) berada dalam julat 67.40 ± 1.03% hingga 83.72 ± 1.78% (air-jet) dan 66.24 ± 0.70% hingga 76.84 ± 0.35% (mesh), menunjukkan pemendapan ubat pada peringkat 4 dan 5 dalam next generation impactor (NGI), mencadangkan penyasaran yang berkesan ke kawasan bronkial-alveolar. Ujian sitotoksisiti pada sel A549 menunjukkan nilai IC50 yang setanding dengan DTX bebas, mengekalkan aktiviti antikanser yang berpotensi. Kajian kestabilan mengesahkan bahawa penyimpanan dalam peti sejuk adalah optimum bagi memelihara integriti LCNs dalam jangka masa panjang. Penemuan ini menonjolkan potensi DTX-LCN boleh diinhalkan sebagai strategi baharu untuk terapi kanser paru-paru yang disasarkan, menawarkan keberkesanan terapeutik yang lebih baik sambil berpotensi mengurangkan ketoksikan sistemik dan kesan sampingan.

FORMULATION AND EVALUATION OF LIQUID CRYSTALLINE NANOSTRUCTURES FOR THE PULMONARY DELIVERY OF DOCETAXEL

ABSTRACT

Lung cancer is the leading cause of cancer-related deaths worldwide. Docetaxel (DTX) is a widely used chemotherapeutic agent; however, its poor solubility, low bioavailability, and high systemic toxicity limit its therapeutic efficacy. Conventional intravenous formulations utilise ethanol and surfactants such as polysorbate 80 (Tween 80®), which cause severe adverse effects, including nephrotoxicity and hypersensitivity. This underscores the need for targeted drug delivery to enhance DTX efficacy while minimising systemic toxicity. Pulmonary delivery via lipid-based nanoparticles, particularly liquid crystalline nanostructures (LCNs), presents a non-invasive and efficient approach with improved patient compliance. This study aimed to develop and characterise inhalable DTX-loaded LCNs for pulmonary delivery, optimising anticancer efficacy while potentially reducing adverse effects. LCNs were prepared via extrusion, ensuring minimal dispersed phase loss and structural integrity. Small-angle X-ray scattering (SAXS) confirmed cubic internal nanostructures, validating extrusion as a scalable fabrication method. A series of four full factorial experiments yielded 40 formulations, of which 11 met the selection criteria of highest lipid content, smallest particle size (PS), and a polydispersity index (PDI) < 0.25. DTX was loaded at 2, 5, and 10 mg/mL, with 5 mg/mL identified as optimal. Five formulations (CC8, CCP15, GCit7, SCit7, and SCit8) demonstrated entrapment efficiency (EE%) > 65% and exhibited favourable PS (149.5–238.4 nm) and PDI (< 0.2). *In vitro* drug release studies demonstrated a

sustained release of DTX over 72 hours. Aerosolisation studies using air-jet and mesh nebulisers confirmed effective pulmonary deposition. LCNs exhibited mass median aerodynamic diameter (MMAD) values of 3.38 ± 0.25 μm to 4.63 μm (air-jet) and 3.90 ± 0.11 μm to 4.63 ± 0.28 μm (mesh). Fine particle fraction (FPF%) ranged from 67.40 ± 1.03% to 83.72 ± 1.78% (air-jet) and 66.24 ± 0.70% to 76.84 ± 0.35% (mesh), demonstrating drug deposition at next generation impactor (NGI) stages 4 and 5, suggesting effective bronchial-alveolar targeting. Cytotoxicity assays on A549 cells revealed IC50 values comparable to free DTX, maintaining potent anticancer activity. Stability studies confirmed that refrigeration optimally preserved LCNs integrity. Stability studies confirmed that refrigeration was optimal for preserving LCNs integrity over time. These findings highlight the potential of inhalable DTX-LCNs as a promising strategy for targeted lung cancer therapy, offering enhanced therapeutic efficacy while potentially mitigating systemic toxicity and adverse effects.

CHAPTER 1

INTRODUCTION

1.1 Background

Lung cancer was first described by researchers in the mid-19th century and considered relatively rare at the time. However, the incidence of lung cancer began to rise dramatically in the early 20th century, reaching 10% of all cancers by 1918 and over 14% by 1927. This increase was largely attributed to the rise in cigarette smoking following World War I (Witschi, 2001). Today, lung cancer is the leading cause of cancer-related deaths worldwide, surpassing even breast cancer as the top cancer killer among women in developed countries (Siegel et al., 2024).

Early theories about lung cancer's causes in the first half of the 20th century focused on environmental factors. The 1918 influenza pandemic was thought to be linked to a rise in lung cancer cases during that time. Researchers also suspected that the Human Papillomavirus, which was found in patients with head and neck cancer, might cause bronchogenic cancer due to the proximity of these body organs. However, the evidence linking Human Papillomavirus to lung cancer is limited (Ruegg, 2015). Exposure to chemicals like arsenic, silicone, asbestos, radon, tar, and others was identified as a possible cause of bronchogenic cancer. For instance, arsenic exposure was common among miners, copper smelters, and farmers who inhaled soil and pesticide fumes (Fucic et al., 2010). Medical reports noted arsenical keratosis in patients treated with Fowler’s solution for psoriasis and rheumatic fever. Exposure to silicone dust was linked to lung cancer (Hinojosa et al., 2018). Asbestos, widely used in clothing and building materials, was also suspected as a cause of lung cancer

(Hubaux et al., 2012). The rise of automobiles and tar-related air pollution was also linked to lung cancer (Chen et al., 2015). From 1950 onwards, medical investigations continued into environmental causes of lung cancer, including bird dander, second-hand smoke, and familial links. Bird keepers were initially thought to have increased lung cancer (Parascandola, 2023). Familial links were also explored in the 1960s, with suggestions that second-hand smoke might be a contributing factor. Second-hand smoke exposure was confirmed as a significant risk for lung cancer (Ruegg, 2015).

Pathophysiologically, lung cancer is defined as a malignant tumour that begins in the cells of the lung tissues, commonly in the cells lining air passages. It can be broadly categorised into two main types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC, accounting for about 80-85% of cases, includes several subtypes such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma (Zhang et al., 2023). SCLC, comprising about 10-15% of cases, is known for its aggressive nature and tendency to spread rapidly. The types of cancer are distinguished by the appearance of the cancer cells under a microscope. Accurate staging of cancer, based on the size of the tumour and extent of spread, is crucial for guiding treatment decisions (Zhang et al., 2023).

Diagnosing lung cancer typically involves a combination of imaging tests (such as X-rays, CT scans, and PET scans), biopsies (where tissue samples are taken for examination), and other tests to determine the stage and type of cancer. Symptoms often include persistent coughing, chest pain, shortness of breath, coughing up blood, and unintentional weight loss. Early detection is critical for improving treatment outcomes (Ning et al., 2021), but unfortunately, the symptoms typically start to appear only at the advanced stages of the disease and sometimes they are masked by other concurrent respiratory conditions. Accordingly, the majority of patients are diagnosed

with lung cancer while the disease is at its advanced stages and turns out to be incurable by the currently available treatments (Postmus et al., 2017) with a very poor prognosis of a 5-year survival rate of only 21% (Huang et al., 2023).

The treatment strategy depends on the type, stage of lung cancer and the physical state of the patient. Conventional methods include surgery, high-dose intravenous chemotherapy, radiation therapy, targeted treatments, immunotherapy, and photodynamic or laser therapy. Surgery is typically reserved for early-stage lung cancer and is often paired with chemotherapy and/or radiation to eliminate cancer cells (Postmus et al., 2017). Chemotherapeutic agents like docetaxel (DTX), cisplatin, paclitaxel, and etoposide, either alone or in combination, are the standard treatment.

DTX is a second-generation taxane approved for the treatment of lung and breast cancers, hormone-refractive prostate cancer and advanced gastric cancer (Farha & Kasi, 2023). In lung cancer, DTX is often used as part of combination therapy or as a second-line treatment for advanced disease (Gubens & Wakelee, 2010). DTX is administered intravenously, which remains the only route of its administration. As with other anti-cancers, the therapeutic efficacy of DTX is limited due to severe adverse effects and systemic toxicity, poor selectivity, and the development of multiple drug-resistance (Nurgali et al., 2018; Zhao et al., 2018). Moreover, the drug has very low water solubility (6-7μg/mL) and it is a substrate for P-glycoprotein (Alshamrani et al., 2022). Besides, the DTX commercial preparations typically contain Polysorbate 80 (Tween 80®) as a solubilising agent which has side effects, including acute hypersensitivity reactions as well as accumulative fluid retention, resulting in weight gain, peripheral oedema and, occasionally, pleural or pericardial effusions (Schwartzberg & Navari, 2018). Therefore, the utilisation of novel drug delivery

systems such as nanoparticles for pulmonary drug delivery may overcome the problems with the current formulations of DTX.

Nanotechnology has become a significant approach for improving current therapies and creating innovative treatments and diagnostic techniques for lung cancer. Advances in this field have led to the development of various nanosystems, including lipid-based nanocarriers, which hold the potential to transform lung cancer treatment (Gonciar et al., 2019; National Cancer Institute, 2017). These nanosystems can encapsulate drugs with different physicochemical characteristics, enable combination therapies, and exploit the enhanced permeability and retention (EPR) effect for more effective passive targeting. Additionally, their surfaces can be functionalised with targeting molecules for active targeting, enabling precise delivery to cancer cells and tumours.

Pulmonary delivery of anticancer drugs via lipid-based nanoparticles for lung cancer treatment is a growing and expanding area of research. This route of drug administration is non-invasive (needle-free), provides better patient compliance, and can be self-administered. It can be used to overcome the drawbacks associated with the oral or intravenous routes that may include high levels of systemic toxicity, the poor aqueous solubility of the anticancer agents, low drug accumulation within the tumour, and high rates of tumour relapse (Andrade et al., 2011; Bailey & Berkland, 2009; Madumani Amararathna, 2019; Paranjpe & Muller-Goymann, 2014). However, successful pulmonary delivery systems and clinical responses are affected by many factors, including physiological and pathophysiological factors, delivery devices and the model drugs' pharmacokinetic/pharmacodynamics properties.

Liquid crystalline nanostructures (LCNs) are lipid-based drug delivery systems and are attracting growing research attention (Leu et al., 2023). These nanocarriers are often termed ‘mesophases’ to describe their status of being an intermediate state of matter between the isotropic liquid and the solid crystal. LCNs are thermodynamically stable and can be dispersed in the presence of a stabiliser into smaller particles such as cubosomes and hexosomes that preserve the complex and highly ordered internal nanostructure. Cubosomes and hexosomes have a high potential for use in drug delivery applications due to their unique structural and physicochemical properties (Azmi et al., 2015; Kim et al., 2015; Rajabalaya et al., 2017). They have been employed successfully to deliver therapeutic agents through different routes of administration. However, until now, their potential role in pulmonary drug delivery has yet to be well investigated. Since lung surfactants are capable of forming various endogenous LCNs, this intrinsic property supports the potential role of exogenously prepared, drug-loaded LCNs for pulmonary delivery. Inhaled LCNs may serve as localized drug depots within the lungs, enhancing the efficiency of anticancer pulmonary drug delivery. However, literature on this approach remains limited, with only one study investigating the pulmonary delivery of bedaquiline via cubosomes for the treatment of non-small cell lung cancer (NSCLC) (Patil et al., 2021).

1.2 Problem Statement

Lung cancer is the most common type of diagnosed cancer and the leading cause of cancer-related deaths (Bray et al., 2018; WHO, 2021a). More persons die from lung cancer annually outnumbering the death cases number from cancer of the prostate, breast and colon combined (American Cancer Society, 2019; National Cancer Institute, 2021,). Furthermore, the World Health Organisation (WHO) through the

Global Cancer Observatory estimates that the lung cancer incidence and mortality rates for both sexes and at all ages will increase by 72.5% and 76.3%, respectively (from 2018 to 2040) (WHO, 2021b, 2021c).

DTX remains a cornerstone in lung cancer treatment; however, its therapeutic efficacy is significantly hindered by its poor aqueous solubility (0.025 μg/mL) and limited membrane permeability (1×10^{-6} cm/s), attributed to its pK_a of 10.97 and log P of 4.1. These physicochemical properties classify DTX as a Biopharmaceutical Classification System (BCS) Class IV drug, indicating both low solubility and permeability (Kuppens et al., 2005; Sohail et al., 2018). As a result, DTX is administered intravenously; however, its commercial formulation, Taxotere®, necessitates the use of solubilising agents such as Tween 80®, which is associated with severe hypersensitivity reactions (5–40% incidence), renal and hepatic toxicity, and injection-site adverse events (Clarke & Rivory, 1999; Kenmotsu & Tanigawara, 2015; Sanofi, n.d.; Valicherla et al., 2016). Furthermore, the ethanol co-solvent in these formulations induces central nervous system effects, including dizziness, nausea, and facial flushing, affecting 44.3% of patients during or shortly after infusion (FDA, 2024; Won et al., 2023). Besides, DTX like other chemotherapeutic agents, exhibits poor tumour selectivity and is prone to multidrug resistance, resulting in diminished efficacy and severe systemic toxicity, including anaemia, nausea, vomiting, nephrotoxicity, and neurotoxicity (Khan et al., 2018; Nurgali et al., 2018; Zhao et al., 2018). These challenges highlight the urgent need for innovative targeted therapies and advanced drug delivery systems, such as nanoscale materials, that offer superior efficacy, enhanced tumour targeting, and improved safety profiles.

The pulmonary respiratory route can be used efficiently for both local and systemic drug delivery, and it offers many advantages over the other routes of

administration (Liang et al., 2015). The alveolar region of the lung contains a very low amount of aqueous fluid and a high amount of lung surfactants; thus, these surfactants can form different types of liquid crystalline phases such as the cubic, inverse cubic, inverse hexagonal and lamellar phases. However, the mechanisms by which these phases influence respiratory drug delivery remain relatively unexplored (Das & Stewart, 2016; Wauthoz & Amighi, 2014).

Lipid-based liquid crystalline nanostructures (LCNs), such as cubosomes and hexosomes, are capable of encapsulating therapeutic agents with varying degrees of lipophilicity and physicochemical properties, including chemotherapy drugs such as DTX. These nanocarriers have been effectively employed for the delivery of drugs and diagnostic agents via multiple routes, such as oral, intravenous, transdermal, ocular, and intranasal (Azmi et al., 2015; Rajabalaya et al., 2017). However, their potential application in pulmonary drug delivery remains largely underexplored, and the feasibility of aerosolising these nanocarriers to create inhalable anticancer drug-loaded LCNs has not been extensively studied.

On the other hand, while various methods exist for preparing LCNs with desirable physicochemical properties, achieving small particle size and low polydispersity index through simple and efficient techniques remains a challenge, the currently available methods for producing LCNs, such as top-down approaches (Iqbal et al., 2023) and microfluidics (Nath et al., 2024), often depend on high-energy techniques like sonication or high-pressure homogenisation or require specialised microfluidic systems. Moreover, the most widely adopted low-energy technique, mechanical stirring with an overhead stirrer, can also lead to a substantial loss of dispersed phase components. Reports indicate that approximately 28% ± 0.5% of the dispersed phase is lost, primarily accumulating on the paddles of the overhead stirrer

(Esposito et al., 2003). Therefore, there is a need for simpler, more efficient methods that can be applied effectively at both the research and industrial scales.

Extrusion, a well-established technique used to reduce particle size in other lipid-based systems like liposomes, provides a simpler, scalable, and reproducible alternative (Manca et al., 2024). Surprisingly, despite its potential, the use of extrusion in LCNs production has received limited attention. The first use of extrusion in 2013 effectively managed the size of radiolabelled cubosomes (Nilsson et al., 2013), while more recent studies from 2019 (Uyama et al., 2019) and 2021 (Malheiros et al., 2021) confirmed its success in producing phytantriol- and GMO-based cubosomes with small and consistent particle sizes. However, these studies did not sufficiently address issues such as the loss of lipids and stabilisers during extrusion, or the impact on the internal structure of LCNs.

This research proposes LCNs as alternative carriers for DTX to overcome the challenges associated with its administration. The study aims to provide insights into the formulation of DTX-loaded LCNs, identifying the key parameters that influence their development. It focuses on extrusion as a low-energy method for LCNs production, aiming to minimise disperse phase loss, optimise particle size and polydispersity index (PDI), and evaluate the structural integrity of the LCNs post-extrusion. Additionally, the research investigates the critical factors affecting the efficacy of these nanocarriers in pulmonary delivery, offering a potential solution to the limitations of current DTX formulations.

1.3 Research Questions

i) Is it possible to prepare LCNs using extrusion? Besides, what are the critical parameters that affect the formulation of different types of DTX-LCNs drug delivery systems?

ii) How do these key parameters affect the physicochemical characteristics, aerosolisation, drug release profile, stability, and safety of the DTX-LCNs?

iii) Can the formulated DTX-LCNs be effectively aerosolised through nebulisation using air-jet and mesh nebulisers? Additionally, what are their aerosolisation characteristics when evaluated with the Next Generation Impactor (NGI)?

iv) What is the cytotoxicity profile of the developed DTX-LCNs on the model human lung adenocarcinoma cell line (A549)?

v) What are the insights on the short-term stability of the developed DTX-LCNs?

1.4 Research Hypothesis

In this work, it is hypothesised that:

i) LCNs naturally exist in the lungs, formed by lung surfactants such as lamellar bodies and tubular myelin. The formulation of exogenous LCNs could replicate these endogenous structures, offering a promising approach for pulmonary drug delivery. Given their potential to serve as drug depots in the lungs, LCNs should be further investigated for their role in enhancing drug retention and localized therapeutic delivery, particularly in lung cancer treatment.

ii) LCNs are composed of compounds classified as Generally Recognized as Safe (GRAS), making them a biocompatible and safe option for pulmonary drug delivery.

iii) LCNs exhibit sufficient stability and drug-encapsulation capacity, enabling their use as effective nanocarriers for the pulmonary delivery of DTX and other anticancer drugs.

iv) DTX-LCNs can be further engineered into micron-scale structures with optimal aerodynamic properties for efficient pulmonary drug delivery via nebulization.

1.5 Research Objectives

i) To formulate well-characterised DTX-LCN dispersions using extrusion method for pulmonary delivery.

ii) To identify and evaluate the critical parameters that govern the constitution of stable and efficient DTX-LCNs for pulmonary drug delivery.

iii) To investigate the physicochemical properties, the aerodynamic characteristics, and the release profile of the prepared DTX-LCNs.

iv) To study the cytotoxicity of the developed DTX-LCN formulations.

v) To evaluate the short-term stability of the optimised DTX-LCN preparations.

CHAPTER 2

LITERATURE REVIEW

2.1 Lung Cancer

Lung cancer presents a significant global health challenge, ranking among the most frequently diagnosed cancers worldwide. It accounts for approximately 11.4% of all reported cancer cases and is the leading cause of cancer-related deaths, responsible for about 18% of all cancer mortalities across genders (Mehta et al., 2021). In the United States alone, the American Cancer Society projected around 235,760 new lung cancer cases in 2021, constituting 12.4% of all new cancer diagnoses, with an estimated 131,880 deaths, making up 21.7% of all cancer-related deaths (Fuentes et al., 2021). Annually, more individuals succumb to lung cancer than to prostate, breast, and colon cancers combined (Pakzad et al., 2015; Weaver, 2023). Moreover, according to the World Health Organisation (WHO) Global Cancer Observatory, between 2020 and 2040, both the incidence and mortality rates of lung cancer are projected to increase by 64.4% and 67.5%, respectively, across all ages and genders (Global Cancer Observatory, 2020). According to the Malaysian Study on Cancer Survival (MySCan), lung cancer in Malaysia has a 5-year relative survival rate of 11.0% and a median survival time of 6.8 years (Yap et al., 2024).

2.1.1 Lung Cancer Causes and Mechanisms of Development

Lung cancer can develop due to various environmental and genetic factors and their interactions. The primary cause remains tobacco smoking, with smokers having a significantly higher risk of developing lung cancer compared to non-smokers, ranging from 10- to 30-fold. Other important factors include exposure to second-hand

smoke, industrial and environmental hazards such as radon, asbestos, and metals like chromium, cadmium, and arsenic, as well as exposure to different organic chemicals and ionizing radiation. A positive history of respiratory illnesses like bronchitis, emphysema, and tuberculosis also contributes to lung cancer risk. In familial cases, first-degree relatives of individuals with lung cancer have a 2- to 3-fold increased risk of developing lung cancer and other malignancies, many of which are not related to smoking (Li, 2021).

The development of lung cancer involves a multistep process characterised by the accumulation of genetic and epigenetic alterations that activate growth-promoting pathways and inhibit tumour-suppressor pathways. Lung cancer develops through the activation of growth-promoting proteins like v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), epidermal growth factor receptor (EGFR), BRAF, MEK-1, HER2, MET, ALK, and rearranged during transfection (RET), as well as inactivation of tumour suppressor genes such as TP53, PTEN, RB1, LKB11, and p16/CDKN2A (Walter et al., 2015). These genetic alterations can occur through mechanisms like gene amplification, point mutations, and structural rearrangements. Lung cancer mutations have been identified in key oncogenes and pathways, providing potential therapeutic targets for treatment strategies. The molecular basis of lung cancer is complex and heterogeneous, with advancements in understanding molecular alterations playing a crucial role in improving lung cancer diagnosis, prognostication, and treatment outcomes (Cooper et al., 2013; Gou et al., 2024).

2.1.2 Lung Cancer Types and Classifications

As illustrated in Figure 2.1, lung cancer is broadly categorised into two main types based on histology and behaviour: small-cell lung cancer (SCLC) and non-small-

cell lung cancer (NSCLC). SCLC, which constitutes about 15% of all lung cancer cases, is characterised by rapidly growing tumours composed of small cells and tends to spread early to other parts of the body, such as the brain, liver, and bones. This type of lung cancer is strongly associated with cigarette smoking (Mohtar et al., 2021; Tlemsani et al., 2020).

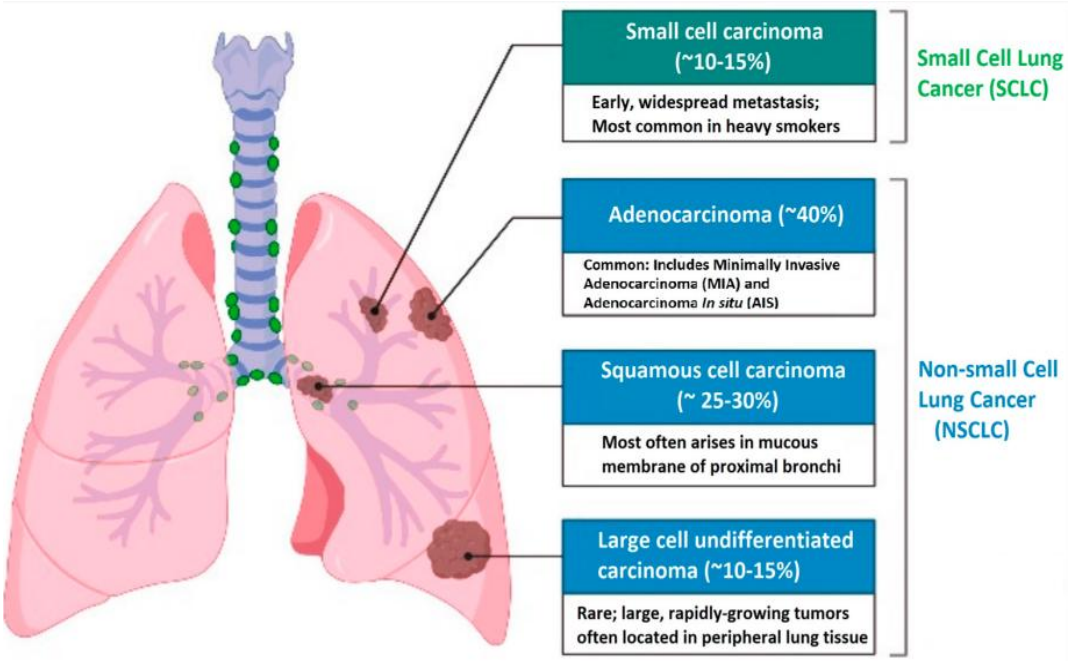

Figure 2.1 Histological classification of lung cancer. Adapted from (Restrepo et al., 2023)

On the other hand, NSCLC comprises approximately 85% of all lung cancer cases and is further subdivided into different histologic subtypes based on the appearance of tumour cells under the microscope and the type of cells from which they originate. The three main subtypes of NSCLC based on histology are adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma. Adenocarcinoma, the most common type of NSCLC accounting for about 40% of cases, usually develops in the outer regions of the lung and can occur in both smokers and non-smokers. Squamous cell carcinoma, accounting for about 25-30% of NSCLC cases, typically arises in the

central part of the lungs, near the bronchial tubes. Large-cell carcinoma, representing about 10-15% of NSCLC cases, can appear in any part of the lung and tends to grow and spread quickly (Dorantes-Heredia et al., 2016). Accurate histologic classification of lung cancer subtypes is essential for guiding treatment decisions and predicting patient outcomes.

2.1.3 Treatment of Lung Cancer

The choice of treatment strategy for lung cancer depends on factors such as the cancer type, stage, and the patient's physical condition. Traditional chemotherapy is a fundamental component of lung cancer treatment, often used in combination with other therapies like surgery, radiation, targeted treatments, and immunotherapy. While best supportive care focuses on the management of disease-related symptoms and the provision of physical, practical, emotional, and spiritual support to improve the quality of life for patients with cancer (Zhong et al., 2013).

Chemotherapy involves the use of drugs to destroy cancer cells by impeding their growth and division. It has been shown to improve both the length and quality of life for people with lung cancer of all stages. Chemotherapy regimens consist of specific cycles given over a set period, and the choice of drugs is influenced by the type of lung cancer. Common chemotherapy drugs for lung cancer include DTX, carboplatin, cisplatin, etoposide, gemcitabine, paclitaxel, pemetrexed, and vinorelbine (Lee, 2019; Li et al., 2023).

Before the chemotherapy era, the median overall survival for metastatic lung cancer was only 3.9 months. Platinum-based combinations, particularly cisplatin and carboplatin, have been pivotal since the 1980s. The platinum-based chemotherapy provides a significant survival advantage compared to supportive care, with a one-year

overall survival rate of 15% versus 5%. This approach remains standard, prescribed to about 80% of lung cancer patients either alone or with radiotherapy. In the 2000s, pemetrexed-cisplatin doublets emerged as a notable contender. The phase 3 clinical trial demonstrated an increase in overall survival by 1.7 months in non-squamous NSCLC compared to gemcitabine-cisplatin doublets, emphasising the histology's relevance in treatment. Additionally, maintenance therapy with pemetrexed showed improved progression-free survival and overall survival in non-squamous NSCLC, with minimal adverse effects. Figure 2.2 illustrates chemotherapy's evolving clinical benefits, correlating precision with improved overall survival rates (Heuvers et al., 2012; Lee, 2019; Zhong et al., 2013).

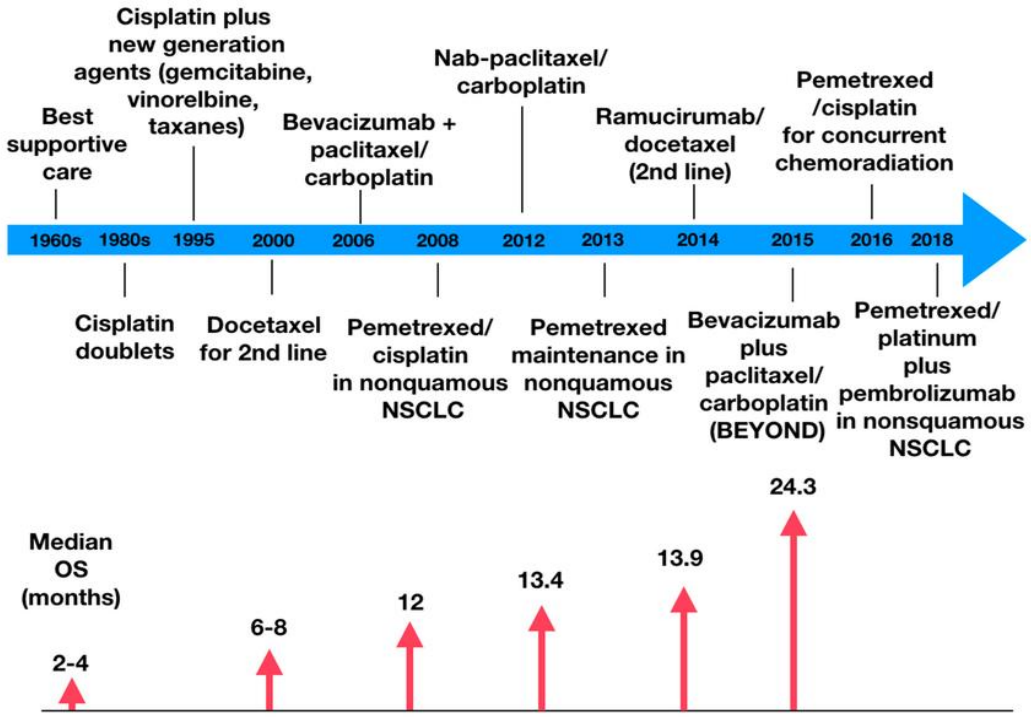

Figure 2.2 Evolution of chemotherapy treatment in NSCLC, highlighting some landmark drugs and the accompanying increase in median overall survival. NSCLC = non-small cell lung cancer. OS = overall survival. Adapted from (De Mello et al., 2020)

However, chemotherapy also affects healthy cells in the body, leading to side effects like fatigue, low blood cell counts, infection risk, nausea, and hair loss (Kaur et al., 2022).

Besides chemotherapy, other treatments and advanced therapy options for lung cancer may include:

i) Immunotherapy: has revolutionised lung cancer treatment over the past decade, changing the landscape for patients with lung cancer. Immunotherapy utilises the patient's immune system to fight cancer, with significant improvements in patient outcomes. It is now a standard part of treatment plans for patients with newly diagnosed metastatic disease, offering long-term cancer-free survival for approximately 20% of patients and changing the perception that lung cancer is universally fatal (Steven et al., 2016). Drugs like checkpoint inhibitors (e.g., pembrolizumab, nivolumab) have been shown effective against NSCLC lung cancer patients by enhancing the body's immune response against cancer cells (Cui et al., 2020).

ii) Targeted Therapy: Drugs targeting specific genetic mutations (e.g., EGFR, ALK) that support cancer growth and survival, leading to more efficient cancer management by blocking target genes related to tumour progression without causing significant damage to normal cells. Such approaches have revolutionised the treatment of NSCLC, offering more effective and less toxic alternatives to traditional chemotherapy (Li et al., 2023).

iii) Surgery: Such an approach includes three main surgical options lobectomy, segmentectomy and pneumonectomy. In lobectomy, the entire lobe of the lung containing the tumour will be removed. This is the most common surgery for non-small cell lung cancer (NSCLC). As for segmentectomy, it is also named (wedge resection) and involves the removal of the lobe part which is suitable for patients with limited lung capacity or small early-stage tumours. Pneumonectomy involves the removal of the entire lung, typically used when the tumour is centrally located or cannot be removed with other types of surgery (Hoy et al., 2019).

iv) Minimally Invasive Surgery: Techniques such as video-assisted thoracoscopic surgery (VATS) and robotic-assisted surgery enable precise tumour removal with reduced recovery times and fewer complications (Lampridis et al., 2023).

v) Radiation: Advanced techniques like stereotactic body radiation therapy (SBRT) deliver high doses of radiation precisely to tumours while sparing surrounding healthy tissue (Lo et al., 2009).

However, despite the advancements in targeted therapy and immunotherapy, traditional chemotherapy remains a crucial and expanding part of lung cancer management, demonstrating its efficacy across different stages, histologies, mutational subtypes, and immunologic statuses (Araghi et al., 2023).

2.1.4 Routes of Drug Administration for Lung Cancer Therapies

The choice of administration route for lung cancer treatment depends on the specific therapy, disease stage, and desired outcome. While more than 60% of anticancer medications are designed as oral treatments for clinical use, their effectiveness is often hindered by limited absorption in the body, leading to their underutilisation (Oliveira et al., 2021). The harsh environment of the gastrointestinal tract, including digestive enzymes, gastric acids, pH, and poor permeability of the intestinal epithelium, are major obstacles to minimising the therapeutic effects of drugs through oral administration (Hua, 2020). Besides, low bioavailability is another problem with orally administered drugs, especially with highly lipophilic anticancer drugs such as DTX and paclitaxel (Stuurman et al., 2013).

The intravenous (IV) infusion remains preferred for certain chemotherapeutic agents and immunotherapies to ensure direct delivery into the bloodstream without passing through the gastrointestinal tract. When combined with other treatment modalities like oral administration, and radiotherapy, IV administration has shown promising results in producing more effective outcomes than single-treatment methods. However, the efficacy of IV administered anticancer drugs remains limited due their poor selectivity, the development of multidrug resistance, in addition to the associated severe side effects which in turn limit their use and requiring close monitoring by healthcare professionals to ensure proper administration and minimise potential complications (Aryal et al., 2023).

Inhalation presents an intriguing and promising route for the administration of anticancer drugs in lung cancer treatment. Localised pulmonary drug delivery offers significant advantages, as it circumvents the limitations of other administration routes,

enabling the direct targeting of lung tumour cells with anticancer agents (Abdulbaqi et al., 2021). This approach and its potential benefits are further discussed in the following sections.

2.2 Inhalable Anticancer Therapy Via Lipid-based Nanocarriers: Main Advantages and Critical Challenges

Nanotechnology is representing one of the powerful tools in the hands of researchers today for enhancing the currently available classical therapies and developing new therapeutic strategies and diagnostic tools to combat lung cancer. The extensive research in this field yielded a wide range of nanosystems (including lipid-based nanocarriers) that have the potential to dramatically change how lung cancer is treated nowadays (Gonciar et al., 2019; National Cancer Institute, 2017; Zhao et al., 2018). This is attributed to their ability to entrap drugs with different physicochemical properties, suitability for combination therapy, and enhanced permeability and retention (EPR) effect which makes them highly effective in passive targeting. Besides, their surface could be functionalised with different targeting moieties for active targeting, so they can selectively target the cancerous cells and neoplasms.

Drug delivery for the treatment of lung cancer is achieved mainly via the intravenous and oral routes of administration. Local drug delivery methods at the tumour site are also considered for certain cases.

The use of inhalable lipid-based nanocarriers could provide many advantages over conventional routes for lung cancer treatment, especially for patients with surgically unresectable lung cancer. Pharmacokinetically, inhalation allows the delivery of chemotherapeutic agents to the target cancer cells and avoids hepatic metabolism; thus, rapid onset of action, lower dosing and fewer systemic distribution

and toxicities are expected (Dolovich & Dhand, 2011). Moreover, the alveolar region in the lungs has a large surface area of ~100 m^2, extensive vasculature, and limited drug-metabolizing enzymatic activity compared to other organs such as the liver and the gastrointestinal tract. In addition, the alveolar epithelium is extremely thin (0.1–0.2 μm), which is much thinner than that in the upper bronchial tree (50–60 μm). Thus, drug absorption and bioavailability in the targeted region may be further improved (Lee et al., 2015b; Patton & Byron, 2007). Additionally, phospholipids, which are major constituents of many lipid-based nanoparticles, especially liposomes and some types of LCNs, are present in the lungs and constitute almost 90% of lung surfactants (Garmany et al., 2006). This favours the design of a more biocompatible lipid-based formulation and enhances lung tolerability to the delivered anti-cancer agent. All these factors may significantly decrease treatment failures, development of drug resistance, and chemotherapy interruptions that are responsible for the repopulation of cancerous cells. Subsequently, tumours refractory to traditional systemic chemotherapy could also potentially respond to inhalational therapy.

Pulmonary drug delivery via lipid-based carriers allows anticancer drugs to target and reach various lung tumours via different pathways. After deposition in the respiratory tract, the inhaled drug can target lung tumours by directly penetrating the tumour via the achievement of elevated local concentrations and significantly high concentration gradients of therapeutic agents at the lung tumour site. Certain types of lung tumours such as squamous cell carcinomas or bronchioloalveolar cell carcinomas that are found next to or within the airways might take up the deposited drug by direct penetration. Furthermore, drugs delivered to the lung by inhalable lipid carriers can be absorbed into the local blood circulations. Due to the communication between the bronchial and pulmonary circulations, sufficient drug concentration could reach lung

tumours that lack a direct connection with the main airways depending on the tumour site. The bronchial vasculature nourishes lung tumours if they are located in the conducting region, while the pulmonary circulation feeds them if they are sited in the respiratory region (Kwok & Chan, 2016; Mangal et al., 2017; Williams et al., 2007). However, the absorption, lung clearance mechanisms, biodistribution, and tumour penetration of inhaled drug-loaded particles are subject to many factors such as the physicochemical properties of the drug/particles, the characteristics and composition of the used formulation, the site of dug deposition, the histological features of the respiratory system, and the associated pathological condition. In this regard, Haque et al. evaluated how inhaled liposomal formulation affects existing lung disease by comparing the pulmonary pharmacokinetic behaviour of drug-loaded ^{3}H-labelled PEGylated liposomes after intratracheal administration to healthy rats and rats with bleomycin-induced lung inflammation by following both ^{3}H label and drug. The results showed that liposomes were initially cleared more rapidly from inflamed lungs than from healthy ones but exhibited similar rates of lung clearance after three days. This was interesting given that mucociliary clearance was more efficient from healthy lungs, despite evidence of higher mucus retention in inflamed lungs and reduced association of the liposomes with lung tissue. The plasma pharmacokinetics of ^{3}H-phosphatidylcholine revealed higher liposomal bioavailability and more prolonged absorption from inflamed lungs. Concentrations of the pro-inflammatory cytokine IL-1β were increased in bronchoalveolar lavage fluid after a single pulmonary dose of liposomes to rats with inflamed lungs, but no other significant changes in inflammatory lung markers were identified in healthy or bleomycin-challenged rats (Haque et al., 2019). Moreover, inhaled drugs are also drained by the lymphatic system; they are commonly detected in the lungs' lymph nodes (Figure 2.3).

Consequently, these nodes are considered potential targets for inhaled drugs to suppress cancer metastasis to and from the lungs (Sharma et al., 2001; Tatsumura et al., 1993; Zarogoulidis et al., 2012).

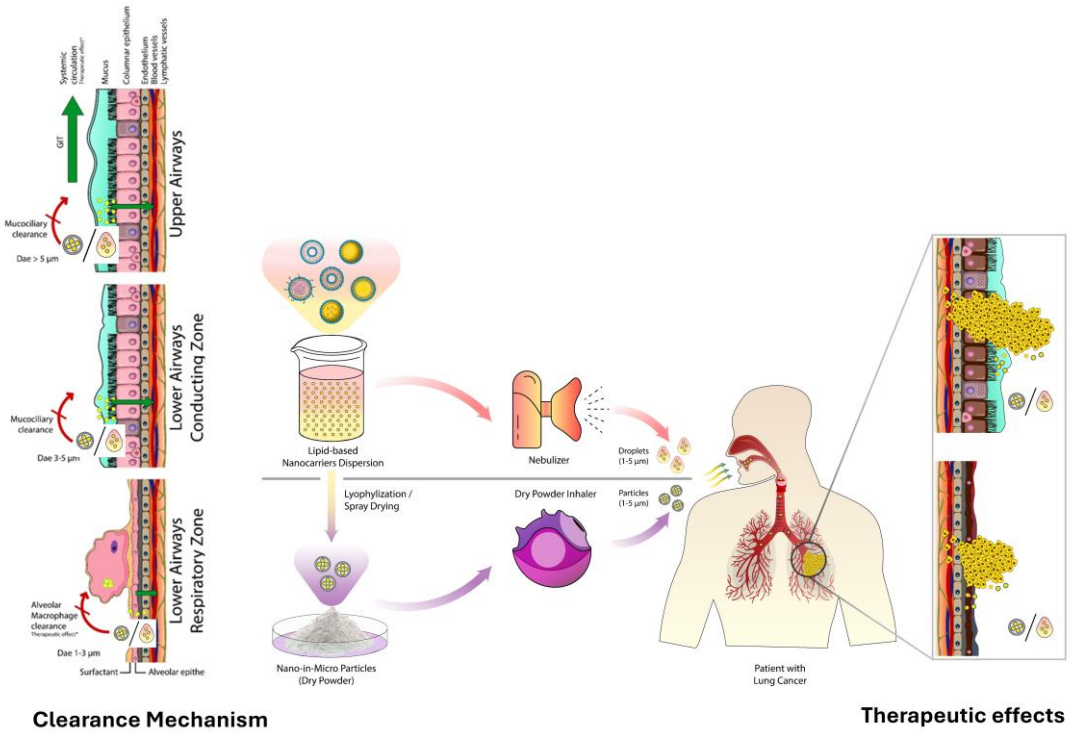

Figure 2.3 The involved clearance mechanisms and therapeutic effects of the inhaled anticancer agents loaded nanocarriers. D_{ae}, aerodynamic diameter; GIT, gastrointestinal tract; NPs, nanoparticles.

Videira et al. (2002) described the biodistribution of radiolabelled (99mTc) solid lipid nanoparticles (SLNs) following their pulmonary delivery to male Wistar rats. A 60 minute dynamic image acquisition was performed in a gamma camera, followed by static image collection at 30 minutes intervals up to 4 hours post inhalation. Radiation counting was performed in organ samples collected after the animals were sacrificed. The results revealed a significant uptake of the radiolabelled SLNs into the lymphatics after inhalation and a high distribution rate in peri-aortic, axillar, and inguinal lymph nodes (Videira et al., 2002). Due to all these remarkable advantages inhalation therapy has the potential to become an effective and safe delivery method for the treatment of lung cancer.

Despite the aforementioned advantages, the pulmonary drug delivery of anticancer drugs via lipid-based nanocarriers for lung cancer treatment is confronted by some challenges and limitations. One of the major concerns is lung tolerance and the potential risk of local pulmonary toxicity and adverse effects because of the cytotoxic activity of anticancer drugs themselves. Besides, the health of lung cancer patients is often impaired either due to lung cancer complications or because of the presence of other concomitant lung diseases such as asthma or chronic obstructive pulmonary diseases that can significantly affect patients' ability to tolerate the inhalable anticancer therapy.

Results from the so far conducted and published clinical trials (Section 2.7) of nebulised liposomal chemotherapeutics such as 9-nitrocamptothecin (9NC) (phase I) (Verschraegen et al., 2004), cisplatin (CIS) (phase I and Ib/IIa), (NCT00102531) (Chou et al., 2013; University of New Mexico, 2010a; Wittgen et al., 2007) for the treatment of LC revealed that they have relatively safe profiles. The most reported side effects or dose-limiting toxicities (DLT) were mainly related to the respiratory tract,

where grade 3 chemical pharyngitis and grade 3 bronchitis are reported as the most severe side effects (Verschraegen et al., 2004; Wittgen et al., 2007). To reduce these adverse effects, prophylactic doses of bronchodilators and/or corticosteroids before starting the anticancer inhalation therapy were used and/or recommended in clinical trials; they were found to help control these effects (Kosmidis et al., 2019; Lemarie et al., 2011; Verschraegen et al., 2004; Zarogoulidis et al., 2012).

Furthermore, the inhaled drug-loaded particles are faced with various lung clearance mechanisms depending on various factors (Figure 2.3). These mechanisms can clear these particles from the lungs before reaching their targeted sites or reduce their residence time before exerting their desired therapeutic effects. The mucociliary clearance is the predominant mechanism in the conducting zone; the inhaled particles will be carried from the bronchial region to the larynx and then transferred to the gastrointestinal tract by swallowing. Almost 80 to 90% of the inhaled particulates can be excreted from the central and upper airways by this mechanism within 24 hours. The lining mucus blanket (with a thickness of up to 30 μm) secreted by the goblet cells in this region represents another barrier. Additionally, particles on the alveolar epithelium (respiratory zone) may be phagocytosed by alveolar macrophages leading to lysosomal degradation, or they are taken to the upper respiratory tract by mucociliary escalator. The macrophages tend to engulf particles with a geometric size of 0.5 to 5 μm. The alveolar epithelium, on the other hand, is covered by lung surfactants which can aid in drug dissolution and diffusion. If drugs are dissolved, they are either absorbed by the blood or lymphatic circulations or subjected to enzymatic degradation (Dabbagh et al., 2018; Hidalgo et al., 2015; Lee et al., 2015a; Olsson et al., 2011; Ruge et al., 2013).