EFFERVESCENT PROLIPOSOMES FOR AEROSOL DELIVERY TO PARANASAL SINUSES

BY

OSHADIE KORALE

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Dedication

I dedicate this achievement to my parents, fiancé, brother, my grandmother and friends; without their encouragement and love this would not have been possible.
STUDENT DECLARATION FORM

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Doctor of Philosophy (PhD)

School

Pharmacy and Biomedical Science, University of Central Lancashire
Abstract

This study aims to design and develop effervescent proliposomes that could disintegrate in water and liberate liposomes, and to investigate the potential suitability of liposomes generated for aerosolization to target paranasal sinuses.

Novel effervescent proliposomes prepared with Soya phosphatidylcholine (SPC) and Dipalmitoylphosphatidylcholine (DPPC) successfully generated stable liposomes with an improved disintegration time of less than 5 min. Differences in lipid composition were found to influence liposome size and drug entrapment of the hydrophobic drug Beclometasone dipropionate (BDP). Mannitol-based formulations developed with DPPC:Chol (1:1) produced liposomes of 7.54±0.15 µm with a drug entrapment efficiency of 82.15±8.29%. Addition of the mucoadhesives alginic acid or chitosan to effervescent proliposomes made with SPC was found to hamper BDP entrapment in liposomes.

Effervescent proliposomes produced SPC:Chol liposomes that also proved beneficial for entrapment of the hydrophilic drug Xylometazoline hydrochloride (XH).

The Pari Sinus (pulsating aerosol technology) and Pari Sprint (non-pulsating technology) nebulizers were used for liposome delivery to a nasal cast. Choice of carrier did not affect the liposome’s ability to withstand shearing. A novel system of a Sar-Gel® (water indicating paste) coated clear nasal cast fixed to a two-stage impinger system was set up to analyze drug deposition within the nasal cast cavity. Sinus drug deposition with effervescent mannitol, DPPC:Chol formulation was observed to be highest at 48.45±2.75 cm² with pulsation compared to deposition of 35.52±11.11 cm² without pulsation. Drug distribution studies indicated that the Pari Sinus deposited 10.47±2.9% drug, while the Pari Sprint deposited only 4.6±1.4%. The degree of drug loss was higher with conventional liposomes in the Pari Sinus nebulizer, indicating that the degree of bilayers disruption depended on formulation.
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List of Abbreviations

Aeroneb Go (Mesh): Aeroneb Go vibrating-mesh nebulizer

Aeroneb Pro (Large mesh): A customized (8 μm-aperture) Aeroneb Pro

ANOVA: Analysis of variance

AUC: Area under the curve

BDP: Beclometasone dipropionate

Chol: Cholesterol

CT: computer tomography

D₂O: Deuterium oxide

Dryness: Status where no aerosols are seen for at least 30 s

DLPC: Dilauroylphosphatidylcholine

DLVO: Derjaguin-Landau-Verwey-Overbeek

DMPC: Dimyristoylphosphatidylcholine

DMPG: Dimyristoylphosphatidylglycerol

DPI: Dry powder inhaler

DPPC: Dipalmitoylphosphatidylcholine

DPPG: Dipalmitoylphosphatidylglycerol

DTPA: Diethylenetriaminepentaacetic acid

EE: Drug entrapment efficiency

EF: Effervescent

FPF: Fine particle fraction

GR: Glucocorticoid receptor

HPLC: High-performance liquid chromatography

HSPC: Hydrogenated Soya phosphatidylcholine

Impinger: Two-stage (twin) impinger

LUV: Large unilamellar vesicle
MRI: magnetic resonance imaging
MLV: Multilamellar vesicle
MVL: Multivesicular liposome
MS: Maxillary sinus
NAMCS: National ambulatory medical care survey
NO: Nitric oxide
NON-EF: Non-effervescent
NSAID: Non-steroidal anti-inflammatory drug
OLV: Oligolamellar vesicle
OMC: Ostiomeatal complex
Omron (Mesh): Omron Micro Air NE-U22 vibrating-mesh nebulizer
P: Probability value
Pari Sinus: Pari Sinus™ Pulsating Aerosol System
Pari Sprint: Pari LC® Sprint Nebulizer attached to a PARI Turboboy®SX compressor
PC: Phosphatidylcholine
RE: Retained entrapment
SD: Standard deviation
SEM: Scanning electron microscopy
Span: (90% undersize-10% undersize)/ 50% undersize
SPC: Soya phosphatidylcholine
SUV: Small unilamellar vesicle
TEM: Transmission electron microscopy
T_m: Main phase transition temperature
VMD: Volume median diameter (50% undersize)
XH: Xylometazoline hydrochloride
CHAPTER 1
INTRODUCTION
1.1. Nasal Drug Delivery

The nasal drug delivery route has been exploited for decades in the administration of systemic drugs and has been used as an alternative to oral drug delivery (Touitou and Illum, 2013). The nasal route of drug delivery was conventionally used for treatment of local diseases such as nasal infections, congestions and allergies. However, more recently the nasal route has shown promising results in drug delivery of small molecular weight, such as polar drugs, peptides, proteins, and other drugs (Illum, 2003). Nasal drug delivery is a significant mode of transport for drugs that need a rapid onset of action, such as in case of crisis treatment in pain management (Illum, 2002; Pires et al., 2009).

There are number of advantages for the use of nasal drug delivery, such as increased levels of drug absorption, fast therapeutic effect, non-invasiveness and the provision of a large surface area for drug absorption. Moreover, the permeable endothelial membranes in the nose and the rich blood flow within the nasal mucosa may provide a unique opportunity for a wide range of drugs to be absorbed and escape the first pass hepatic metabolism (Pires et al., 2009; Türker et al., 2004). High total blood flow per cm³, easy accessibility, and possibility of drugs to be delivered straight to the brain along olfactory nerves also make the nasal drug delivery an attractive substitute to the parenteral route (Illum, 2002; Ridley et al., 1995).

Other benefits, such as the need for lower doses than traditional oral formulations, help decrease the side effects caused by the delivery of the drugs directly into the blood stream. Drugs delivered via nasal administration can either act locally within the nose, such as nasal decongestants, or systemically, such as anti-migraine drugs, hormonal treatments, etc. (Jadhav et al., 2007). Nasal delivery is also a needle-free (i.e. non-invasive) approach for drug administration (Djupesland, 2013). Nasal delivery may improve patient compliance compared to the parenteral route of drug administration, offering avoidance of harsh conditions (e.g. unsuitable pH levels) such as that of the gastrointestinal tract.
Respiratory infections such as influenza (nasal influenza vaccine from Berna Biotech (CH)) are now being tackled through the use of nasal cavity vaccinations (Illum, 2002). Initiation of secondary immune responses at a distant mucosal site and direct delivery of vaccines to lymphatic tissue are also positives of the nasal drug delivery (Jadhav et al., 2007).

Despite the number of advantages there are a few limitations of nasal drug delivery, mainly the epithelium barrier, rapid mucociliary clearance, mucosal barrier, and enzymatic activity. Poor contact of formulations in nasal mucosa is one of the major drawbacks of nasal delivery. Nasal mucociliary clearance is principally responsible for the lack of absorption of certain drugs, and the resident time of the drugs are furthermore reduced due to mucociliary clearance (Marttin et al., 1998). Nasal drug delivery is also hampered during a diseased condition, resulting in impaired absorption (Jain, 2008). Diseased conditions in the nose could amplify or diminish mucociliary clearance or absorption of drugs. This could have significant consequences for drugs with narrow therapeutic index; therefore, lack of reproducibility can be observed in nasal delivery. Nasal drug delivery might be inconvenient, leading to nasal irritation (Rahisuddin et al., 2011). Poor delivery of the drug across the nasal epithelium is also one of the limitations of nasal drug delivery (Davis and Illum, 2003).

1.2. Anatomy of Nasal Cavity

The nostrils and the external nose consist of long dual chambers (one third of nasal cavity), which are 5 cm high and 10 cm long, while the total surface area of the nasal cavity is approximately 150 cm² with a total volume of 15 ml (Baroody, 1997). The primary function of the nasal cavity is to warm, filter air, and give moisture to the air entering the lungs, while filtering out the dust and small particles from entering the body by trapping it in hair and a mucus layer (Lang, 1989). Both nasal cavities have a septal wall and lateral wall; the vertical fin (nasal septum) divides the nasal cavity in two.
Temperature and humidity of the air is regulated by inferior, middle, and superior turbinates, which also dominates a major part of the nasal cavity (Figure 1-1).

The inferior, middle (and superior) meatus are located under and lateral to the turbinates; inferior and middle meatus function as the opening to the nasolacrimal duct and the paranasal sinuses (Mygind and Dahl, 1998). The small orifices called ostia connect the paranasal sinuses to the nasal cavity. The nasal cavity contains columnar epithelial cells, which are ciliated or non-ciliated (Figure 1-2). Columnar cells also have microvilli, which increases area of absorption. Cilia beats 700–1000 times per minute, transporting mucus containing trapped particles to the throat (Clarke and Pavia 1980).
Figure 1-2: Anatomy of nasal mucosa (source: Clarke and Pavia 1980).
1.3. Para Nasal Sinuses (Sinuses)

1.3.1. Sinuses and sinusitis

Sinuses are also referred to as paranasal sinuses, which are pockets or cavities of air present in the cranial bones. They connect the nose and the facial part of the skull, which helps air pass and mucus drain. Sinuses are lined with mucus; they filter and humidify the air and improve vocal resonance (Fagnan, 1998). The hollow pockets of sinus lighten the weight of the skull (the head). Vital functions of our heads are protected by the air pockets in sinuses in trauma situations by acting as crumple zones. The ostiomeatal complex acts as the common drainage pathway for frontal, maximal sinuses, and anterior ethmoid (Rao and El-Noueam, 1998).

Paranasal sinuses are of a complex nature due to their range of pneumatization and bony variants (Ogle et al., 2012). Inflammation of the paranasal sinuses is known as sinusitis. Sinusitis is caused by several reasons, such as allergies, infections (bacterial, viral, or fungal), or autoimmune diseases. Sinusitis can be divided into four groups according to the duration of the disease. These are acute (less than four weeks), sub-acute (four to eight weeks in duration), chronic sinusitis (symptoms lasting longer than eight weeks), and recurrent acute sinusitis (three or more episodes per year lasting more than two weeks) (Slavin et al., 2005). The infection may be caused by bacteria such as Streptococcus pneumoniae, Haemophilus influenza or Moraxella catarrhalis for acute sinusitis (Leung and Katial, 2008) while Staphylococcus aureus (Brook et al., 2008) with a combination of aerobic and anaerobic bacteria (Brook and Frazier, 2005) was detected in chronic sinusitis.

Breathing is vital for all animals, and the nasal pathway is the main airway. Air volume of 20-30 l/min is inhaled and exhaled during sleep, rest, and mild exercising (Cole, 1996). Paranasal cavities range from 5–30 ml (Tarhan et al., 2005). Ostia is the opening that connects the sinus to the nasal passage; it ranges from 1–3 mm in diameter and has 10–
15 mm length narrow ducts, that help the paranasal cavities transit the nasal passage (Tarhan et al., 2005)

1.3.2. Prevalence

Diagnosing sinusitis from common respiratory infections such as the common cold and influenza occurring from bacterial infections is challenging due to both conditions sharing similar symptoms. However, in 1990, antibiotics were prescribed for 92% of patients in the United Kingdom, and 85–98% of patients in the United States for upper respiratory and sinus infections (Ashworth et al., 2005; Hickner et al., 2001). In the United Kingdom, in 2002–2003, 73000 beds were taken by patients having chronic sinusitis).

A summary of health statistics of US adults reported in 2012 in their national health survey that 28.5 million (12.1%) of non-hospitalized adults were diagnosed with sinusitis (Vital and Health Statistics, series 10, number 260, 2012). In 2009, the National Ambulatory Medical Care Survey (NAMCS) reported 11.7 million patients being diagnosed with chronic sinusitis (Vital and Health Statistics, series 10, number 260, 2012).

1.3.3. Anatomy of sinus

Paranasal sinus development is closely linked to facial bone development. Initially, sinuses develop as evaginations of mucosa in the third and fourth months of foetal development (Anderhuber et al., 1992). By the age of 12, the child's sinuses are developed to adult size (Fujioka and Young, 1978). Rapid development in stages of the maxillary sinuses is observed between the ages of 2–3 years and then a slower development is observed until the age of 7–8 years (Eggesbø, 2006). Kaliner et. al., in 1997 stated that the paranasal sinuses’ final structure is as exclusive as a set of finger prints (Kaliner et al., 1997).
Sinuses could be divided into four pairs:

- **Frontal sinuses**, which are situated just above the eyes in the centre of the forehead on both right and left sides.
- **Maxillary sinuses**, which are positioned behind the cheekbones, near the upper jaws. Maxillary sinuses are considered the largest of the sinuses.
- **Sphenoid sinuses**, which are located in the sphenoid bone. Sphenoid sinuses are in close proximity to the optic nerve and pituitary glands.
- **Ethmoid sinuses**, which are a collection of 6–12 small cavities independent of the nasal cavity. These sinuses are not single sacs like the other sinuses.

![Image of healthy sinus and sinusitis](http://healthstalk.blogspot.co.uk/2012/01/sinusitis.html)

*Figure 1-3: Sinus and sinusitis.* (Source: [http://healthstalk.blogspot.co.uk/2012/01/sinusitis.html](http://healthstalk.blogspot.co.uk/2012/01/sinusitis.html))
1.3.4. Pathophysiology

Sinusitis (Figure 1-3) is a condition when sinus drainage is obstructed and normal mucus transport is reduced with diminished ventilation in the sinuses. Inflammation of the sinuses usually leads to obstruction of sinus ostium, resulting in reduced mucus drainage. Bacterial infections in the sinuses lead to a decrease in cilia beating down to 300 beats per min\(^1\); normal ciliary beat frequency is 1000–1500 beats per min. Ciliated columnar cells (30%) experience metaplastic changes to mucus secreting goblet cells during an inflammation. Sinus blockage results in an environment in which bacteria could thrive, due to reduced pH and diminished oxygen tensions (Loevner, 2008).

There are number of factors predisposing people to sinusitis, such as allergic and non-allergic rhinitis, anatomic variations similar to septal deviation, choanal atresia, dental infections, trauma, immunodeficiency (e.g. IgA deficiency), adenoid hypertrophy, hormonal conditions and factors, nasal dryness, upper respiratory infections, inhalation of irritants, and acquired immune deficiency (Eggesbø, 2006; Tomassen et al., 2011; Winstead, 2003). Acute sinusitis is the most common form of upper respiratory tract infections, which are usually viral in origin. Acute sinusitis can also be caused by a blockage of ostia. Maxillary and anterior sinuses in particular are the most common sinuses seen in both acute and chronic sinusitis (Hamilos, 2000). Inflammation of the sinuses leads to thickening of mucosal lining, hyperplasia, and oedema. Normal thickening of a healthy sinus is known to be around 4 mm. Mucosal thickening of maxillary sinuses is often observed with asymptomatic patients (Eggesbø et al., 1999; Zinreich et al., 1988).

Ciliated cells in sinuses move mucus against gravity due to lateral and inferior positioning of the sinuses. Cilia in each of the cells beat in different diffractions in each sinus, resulting in mucosa being moved from sinuses to the choanae, with a unique mucous flow pattern (Hilding, 1966). When different flow patterns of different sinuses
meet, stagnation occurs; long immobilization will also result in sinusitis, especially in ostiomeatal complex (OMC). Mechanical obstructions of OMC also interferes with mucociliary clearance (Eggesbø, 2006).

The recruitment of inflammatory mediators and production of mucosa is increased by nasal mucosa in response to viruses, which leads to future congestion and swelling. Interleukin-1 beta, Interleukin-6 and Interleukin-8 play a major role in acute sinusitis. Interleukin-3 supports inflammatory mediators in chronic sinusitis (Rudack et al., 1998). Nitric oxide (NO) is observed in high concentrations in the sinuses, produced by epithelial cells in the sinuses. NO functions by the sterilization of sinuses and improving ciliary motility. Decreases in nitric oxide amounts are observed in sinusitis (Jain et al., 1993).

Chronic sinusitis is triggered by mucosal swelling, loss of cilia, sinus obstruction, and bacterial and viral infections. In addition, cystic fibrosis, ciliary dyskinesia, and chronic conditions of sinusitis are due to impaired mucociliary clearance. Asthma, rhino-sinusitis, and other upper respiratory diseases may also lead to chronic sinusitis (Bachert et al., 2006).

1.3.5. Complications of sinusitis

Treatment is given to relieve the patient’s pain and pressure in the sinuses and to clear up the infection. Treatment helps to improve the discharge of mucus and decrease the sinus swelling. Preventing permanent damage to the tissue lining of the sinus and scar tissue formation is also one of the main objectives of sinusitis treatment. If not treated, acute sinusitis could cause toxic shock syndrome and acute local effect. Cellulitis, proptosis, chemosis, and ophthalmoplegia can also be caused by acute sinusitis. Furthermore, orbital cellulitis, subperiosteal abscess, and orbital abscess are also complications that can arise from acute sinusitis. Acute sinusitis often leads to flare-ups of asthma. Acute sinusitis sometimes occurs along with ear infections.
The main three areas that result in complications of the sinuses are in the orbital (60–75%), intracranial (15–20%), and bony area (5–10%) of the sinuses. The orbital area of the sinuses are the most commonly involved complication site (Bailey et al., 2006). Preseptal cellulitis, orbital cellulitis, subperiosteal abscess, orbital abscess, and cavernous sinus thrombosis all occur in the orbital site, sometimes even co-currently (Hassan and Ramadan, 2014) (Agayev and Yilmaz, 2008). The intracranial area of sinus complication is most commonly observed in male teenagers rather than children, due to their developed frontal and sphenoid sinuses. Meningitis, cavernous, sagittal, venous sinus thrombosis, or intracerebral, subdural, and epidural abscess are the major intracranial complications observed as a consequence of sinusitis (Achilles and Mösges, 2013; Bailey et al., 2006; Hicks et al., 2011; Ramachandran and Ramachandran, 2009). Bony sinus complications are relatively rare (Raja et al., 2007). There are reports of only 20–25 cases of bony sinus complications in the post-antibiotic era, with reports of 50 paediatric cases or less in 10 years of study (Blumfield and Misra, 2011). Furthermore, osteitis and osteomyelitis are also bony sinus complications that arise from dental issues. Pott’s puffy tumour also often results from inflammation of the frontal sinuses and is also a bony sinus complication.

Long-lasting sinusitis that persists for more than eight weeks can lead to chronic sinusitis. Superimposed acute sinusitis is the most common complication of chronic sinusitis. Nasopharynx pus causing adenoiditis is seen commonly in paediatric chronic sinusitis patients. A high percentage of paediatric patients suffer from pus in the nasopharynx, which can later develop into secondary serious or purulent otitis media. When acute sinusitis is not resolved, it can lead to mucosal hyperplasia and possible development of nasal polyps. Chronic sinusitis can also lead to meningitis, an infection that spreads to the lining of the brain causing vision problems through infection of the eye sockets that could lead to permanent blindness or reduced vision. In children, dacryocystitis and laryngitis are also common complications of chronic sinusitis. Untreated chronic sinusitis can even
lead to life-threatening conditions such as cystic fibrosis (Sharma et al., 1994). If not treated, sinusitis could cause the need for surgery to drain the mucus (Sharma et al., 1994). If the infection is not treated at an early stage, it could disseminate further to the bones and brain, leading to osteomyelitis, infection of the eye socket, blood clot, and brain infection (Rosenfeld et al., 2007).

1.3.6. Treatment for sinusitis

1.3.6.1. Topical and systemic therapy for sinusitis treatment

Topical decongestants, oral decongestants, intranasal corticosteroids, topical steroids, antibiotics, nasal saline, antihistamines, changes in diet, topical cromolyn, or mucolytics are used for relieving the symptoms of sinusitis. Nasal saline wash and steam inhalation are also used, along with other treatments to help humidify dry secretions, reducing inflammation of the mucosal lining and minimizing viscosity of the mucus. Physicians often suggest some of the above-mentioned non-drug therapies, along with antibiotic therapies for tackling sinusitis. These adjunctive therapies, even though never investigated for their effectiveness, are believed to help recover ciliary functions, decrease inflammations, and improve sinus draining (Mabry, 1993; Zeiger, 1992).

Decongestants to relieve nasal congestion are available in the form of tablets, nasal sprays, nasal drops, and liquids. Decongestants work by reducing the inflammation of the mucous membrane by constricting the blood vessel in the mucous membrane. The reduced blood supply results in a decrease of congestion and blockage. Nasal decongestants do not cure sinusitis but offer temporary relief of the symptoms (Lalwani, 2011). Nasal decongestants mainly affect the nasal blood supply, unlike oral decongestants, but a drawback of nasal decongestants is rebound congestion. Long-term use of nasal decongestants can result in swelling of the sinus membranes as the decongestants wear off, leading to additional congestion. Nasal and oral decongestants act on alpha1 and alpha2 adrenoceptors (Malm, 1994).
First-line treatment for acute sinusitis is often antibiotics, usually amoxicillin (for two weeks) or amoxicillin-clavulanate (Augmentin™). Antibiotics prescribed for the treatment of sinusitis typically cover *S. pneumoniae, H. influenzae*, and *M. catarrhalis*. Antibiotics targeting beta-lactamase inhibitors are also used for *H. influenzae* and *M. catarrhalis* (Sinus and Allergy Health Partnership, 2000). Patients who are allergic to Beta-lactams usually obtain prescriptions for trimethoprim-sulfamethoxazole (Bactrim™), clarithromycin (Biaxin™), or azithromycin (Zithromax™) as a substitute. When first-line treatment is not showing promising results, an alternative broad spectrum of antibiotics is prescribed. The third to fifth most common diagnosis with antibiotic prescriptions in Nordic countries was for sinusitis (André et al., 2002). It was estimated that 15–21% of antibiotic prescriptions in outpatient care were for sinusitis patients (The Cochrane Collaboration, 1996).

Chronic sinusitis treatment usually involves broad-spectrum antibiotics (four to six weeks), and topical intranasal steroids (Spector et al., 1998). Antibiotics used for the treatment of chronic sinusitis are amoxicillin-clavulanate, cefpodoxime proxetil, moxifloxacin, and levofloxacin, to cover organisms that are observed in acute sinusitis, but also cover *Staphylococcus* species and anaerobes. Short courses of oral steroids are also used for extreme cases of mucosal thickening and congestion treatment (Fagnan, 1998). However, according to Fagnan (1998), treatment with antibiotics for chronic sinusitis often leads to limited benefit.

1.3.6.2. **Surgical treatment for sinusitis**

Surgical treatment for sinusitis is considered when all other medical therapy fails. Surgical treatment is also carried out in the case of endocranial complications, septic complications, orbital complications, and malignant growth. (Bachert et al., 2003).

Sinus surgery is considered along with a nasal endoscopy, and ostiomeatal complex along with an endoscopically guided culture. Sinus surgery helps to clear the sinus of chronic
infections, edema, and polyps. Unlike older days, where open surgery was performed for chronic sinus diseases, now a much safer and simpler operation of endoscopic sinus surgery is performed with local anaesthesia (Slavin et al., 2005). Aspiration is also one of the simpler surgical treatments for sinusitis. Drainage is used for acute frontal sinusitis treatment, resorting to mucociliary clearance and improving ventilation of the sinuses (Stammberger, 1994). The most commonly practiced sinus surgery is functional endoscopic sinus surgery. Affected parts of mucosa are separated, which helps drainage and ventilation when the wounds are healed. Sense of smell, mucociliary clearance and nasal respiration, and quality of life overall are improved in the patient’s post-surgery. However, surgery does have drawbacks of potential injuries to orbits and dura (Cumberworth et al., 1994).

1.3.6.3. Difficulty in targeting paranasal sinuses

Current oral and intravenous antimicrobials and corticosteroids have significant side effects. A high number of sinus surgeries is an indication of need for better treatment methods and delivery methods of oral, topical, and systemic drugs (Schappert and Rechtsteiner, 2008). Therefore, direct delivery of drugs to the site of action has been considered. Topical drug delivery at the nasal cavity and sinuses has many advantages, namely acting straight on the site of inflammations, avoiding systemic side effects with high concentrations of drug being localized at the target area, and an increased rate of response to therapy.

Nasal irrigation, douches, neti pots, and different saline concentrations (isotonic and hypertonic) are often used for removal of sinusitis causing factors such as pollutants, irritants, inflammatory products, bacteria, mucus, and antigens (Achilles and Mösges, 2013). However, these procedures result in a high volume and high pressure (leading to shearing forces for removal of mucous and inflammatory products from the sinuses). This
mechanism is not appropriate for drug delivery where longer mucosal contact, local absorption, and minimal clearance is expected for better drug delivery.

Topical drug delivery to a sinus is difficult as paranasal sinuses are hollow cavities that are non-ventilated and perfused. Sinuses are also highly protected by a particular filtration process (Sahin-Yilmaz and Naclerio, 2011). In vivo and in vitro studies have shown that even though paranasal sinuses are poorly ventilated there is low deposition of the nebulized drug in the affected areas. Therefore, finding improved methods to increase the drug delivery to the sinuses is important for the treatment of chronic sinusitis (Moeller et al., 2009). Furthermore, flow and pressure fluctuation between nasal passage and the sinus cavity increases airflow, therefore aiding in ventilation of the sinuses (Krüner et al., 2013).
1.4. Nasal Drug Delivery Devices

Drug delivery to the sinuses through aerosols offers many advantages over the invasive means of drug delivery and oral drug delivery. Corticosteroids and antibiotics have been delivered to the nasal cavity through various methods, such as nasal drops, nasal sprays, nebulized aerosols, and irrigation, but not all of these methods are suitable for targeting the sinuses. Due to the location of the sinuses, nasal drops, nasal sprays, or irrigation are not necessarily suitable for drug delivery. However, nebulization has recently been exploited for drug delivery to the sinuses with results indicating possible success. Nasal irrigation is a suitable method for the removal of inflammatory cells, reducing inflammation, increasing mucociliary clearance, and even drug delivery post-sinus surgery. Irrigation as described previously is not suitable for drug delivery before surgery (Albu, 2012).

Nasal spray pumps generate large droplets of 50–100 µm in diameter with deposition of 70–150 µl per puff (Albu, 2012). Currently, different formulations of saline, decongestants, mucolytics, and steroids have been used along with nasal sprays. However, nasal sprays do not cause appreciable deposition of formulation on the sinuses. A major part of deposition upon using nasal sprays is the anterior part of the nose; these sprays are not suitable for sinus targeting (Möller et al., 2011).

Another nasal drug delivery device on the market is the breath-actuated bidirectional delivery devices (OptiMist™; OptiNose AS, Oslo, Norway). OptiMist™ has a breath-actuation mechanism along with a conventional spray pump that gives out droplets around the size of 43 µm. Comparison of OptiMist™ with nasal sprays has demonstrated that OptiMist™ causes a large deposition of the drug in the upper posterior sector (in the middle meatus and sinus ostia) and lower depositions in the anterior segments of the nose (Djupesland et al., 2006).
Medical nebulizers are inhalation devices that deliver aerosols from an aqueous formulation. Characteristics of aerosols generated are dependent on the operating principle and design of the nebulizer as well as the properties of the formulation. There are three main types of medical nebulizers: air jet, ultrasonic, and vibrating-mesh nebulizers. Small droplet sizes generated by nasal nebulizers have been observed to be superior for drug deposition when compared to spray pumps (Suman et al., 1999).

1.4.1. Aerosol drug delivery to sinus

For efficient aerosol transport to the sinus, it is required that aerosols are deposited in the posterior nasal cavity. Recently, few nasal devices have been developed to produce different size aerosols (in diameter) with different flow patterns, such as ‘pulsating’, ‘sonic’, ‘acoustic’, as well as utilizing pressure differences to target the sinuses. Aerosols that can penetrate to the posterior nasal cavity is of an aerodynamic diameter below 5 μm (ICRP Publication 66, 1994). In conditions such as sinusitis the normal deposition of drug within the nose is blocked. Therefore, the flow rate should be regulated at moderate level. New technologies such as ViaNase (Kurve Technology Inc., Lynnwood, WA, US) and the OptiNose (OptiNose AS, Oslo, Norway) do not use a pulsating drug delivery system, but may promote the awareness for the need of new aerosol delivery devices with more efficient mechanisms to maximize deposition in the nasal sinuses. Using conventional nasal devices, deposition in the nasal sinuses is unlikely due to the large size of the particles generated (>10 μm). This is further proven due to the lack of ventilation in the sinuses, and studies done with nasal casts using these devices (Möller et al., 2008; Sato et al., 1981).

Pulsating aerosol technology was introduced with the use of resonance conditions for gas exchange in secondary spaces and surrounding spaces; this was first suggested by Hermann Von Helmholtz (Keller et al., 2010). Guillerm and colleagues discovered the basics of pulsating aerosols in studies for sinus drug delivery (Guillerm et al., 1959). Kauf
studied model cavities and the aerosol’s ability to pass to secondary spaces such as sinuses cavities. Human cadavers and a nasal cast were used by Sato and co-workers, as well as Hyo and co-workers, for continuations of Kauf's studies, confirming deposition efficiencies to be between 1–4% (Keller et al., 2010). The first commercial pulsating aerosol technology produced was developed by La Diffusion Technique Francaise (Atomisor Automatic Manosonique Aerosol, DTF, Saint Etienne, France).

1.4.2. SinuNeb™ device

The SinuNeb™ (PARI Respiratory Equipment, Inc., Midlothian, VA) is a passive diffusion nebulizer producing smaller, particle-sized aerosols delivered in a constant direction with a slower velocity. Aerosols generated by SinuNeb™ are around 3 μm in diameter and are used for the delivery of antibiotics and anti-fungal formulations to the sinuses (Albu, 2012). Aerosols are transported to the sinuses through a hollow tube with two perforations at one end, through which the liquid is aspirated transnasally. In spite of that, a review by Aetna (2002, p. 593) suggests an insufficient amount of clinical studies are seen to support the claims of the manufacturer, which state that the drug can be nebulized directly into the lining of the sinuses to increase the rate of response, effectiveness, and reduction of infection. Manufacturers of SinuNeb™ also claim fewer side effects are observed by using SinuNeb™ when compared to side effects seen with oral delivery or intravenous administration.

A study published by Schuschnig et al. (2009) compares the nebulization efficiency of the AeroNeb Go (Aerogen, Galway, Ireland), the SinuNeb™ (Sinus Pharmacy, Carpinteria, CA), the Atomisor AMSA and Atomisor Sonique Box (both DTF, St. Etienne, France), and the VibrENT™ prototype (PARI Pharma, Munich, Germany) to the sinuses using a novel nasal cast model developed by PARI. The study concluded that only VibrENT™ (in pulsating mode) deposited a significant amount of drug (19% of the label claim = LC) to the sinuses. Standard nebulizers only managed to deliver less than 0.06%
of the drug to the artificial nasal cast model developed by PARI Researchers, this lower depositions explain that pulsation and pressure fluctuations constitute to the key principle behind drug deposition in the nasal sinuses (Schuschnig et al., 2009).

1.4.3. **RinoFlow™ nasal aerosol delivery device**

The RinoFlow™ nasal aerosol delivery device (Respironics, Inc., Cedar Grove, NJ) deposits aerosols in the size range of 20–30 μm in a controlled flow directly into the sinuses (Ranade et al., 2003). Volunteers were used in a study where technetium Tc99m was nasally administrated by the subjects using the Politzer Manoeuvre. Three out of the five subjects had inconsistent deposition in the frontal and maxillary sinuses. The sample size was too small to recognize the significance and the healthy volunteer group did not have blockages; therefore, future investigations should involve patients with sinusitis. However, Negley et al. (1999) have concluded that the results are promising (Negley et al., 1999). Another study has compared the distribution of nasal irrigation isotonic solution on eight volunteers using three irrigation techniques. The deposition was analyzed using computer tomography imaging. Three irrigation techniques, positive-pressure irrigation (Sinus Rinse™), negative-pressure irrigation (inhalation through sniffing), and passive diffusion (RinoFlow™) were tested. In that study, the RinoFlow nebulizer was found unsuccessful in ethmoid penetration. However, ethmoid penetration was observed in the other two cases (Albu, 2012).

1.4.4. **PARI VibrENT™ device**

The PARI VibrENT™ is a modified electronic nebulizer that generates aerosols through a perforated vibrating membrane. The nebulizer operates using the PARI eFlow technology with an amendable pulsation (flow pulsation at 25 Hz) to generate aerosols with a diameter of 3 μm and a flow rate of about 3 l/min (Keller et al., 2010). Three healthy male non-smoking volunteers were recruited for the study by Keller et al. (2010). ⁸¹ᵐKr-gas gamma camera imaging along with ⁹⁹ᵐTc-DTPA aerosols were delivered through a
Pari Vibrent nebulizer to analyze the deposition of the aerosol solution by pulsating air flow and nasal pump spray (Keller et al., 2010; Möller et al., 2010). Results indicated successful sinus deposition using the Pari Vibrent, while no deposition occurred into the sinuses using the nasal pump. Resident time of the drug was also observed to be threefold longer with the Pari Vibrent system, showing 71±17% total deposition in the nasal cavity and 6.5±2.5% deposition in the sinuses (Möller et al., 2010).

Another study investigates the deposition and nebulization effect using a novel nasal cast, developed by PARI GmbH (Munich, Germany). Budesonide solution was nebulized and deposition was measured by a high-performance liquid chromatography (HPLC). 15.9% was deposited in the paranasal cavity while 57.7% of the total volume was deposited in the nasal cavity. Deposition in each sinus cavity ranged from 0.1 to 7%. The highest deposition was observed in the ostium diameters from 1.5 to 3 mm (Schuschnig et al., 2008).

1.4.5. Pari Sinustar™ device
The Pari Sinustar™ is an FDA-approved device used for aerosol delivery to the upper respiratory airways and for the treatment of sinusitis. The Pari Sinustar™ produces aerosols with a size of 2.9 µm and total output of 180 ml/min. The total percentage of aerosols under the size of 5 µm are around 79% (Scheinberg and Otsuji, 2002). Pari Sinustar™ was compared to Ayr, Afrin, and Zicam nasal spray devices to investigate the deposition profile in sinuses (Kundoor and Dalby, 2010). The study indicated that the inhaled flow rate did not have a significant effect on the deposition pattern, while the Afrin nasal spray and Pari Sinustar™ nebulizer did have a significant difference in deposition when compared; the nebulizer covered a greater deposition area compared to the nasal spray. The Pari Sinustar™ nebulizer deposited aerosols on a greater surface compared to the spray pumps investigated in the study.
1.4.6. Pari Sinus™ device pulsating aerosol system

The Pari Sinus™ Pulsating Aerosol System (PARI GmbH, Starnberg, Germany) (Figure 1-4) was developed in Germany in 2003 by PARI GmbH. The Pari Sinus nebulizer is supplied with a pulsating aerosol system that provides a vibrating pulse. The aerodynamic size of the aerosol droplets generated by this nebulizer results in direct delivery to the sinuses. ICRP Publication 66 (1994) states that the aerodynamic diameter of aerosols should be below 3 µm with a moderate flow rate to reach the posterior nasal cavity. Pari Sinus is currently being marketed for the treatment of upper respiratory airway diseases such as chronic sinusitis, rhinitis, and nasal allergies.

A Pari Sinus consists of a Pari LC star jet nebulizer, with a pulsation of 44 Hz (Lass et al., 2006). This nebulizer has been reported to deposit a significant proportion of the aerosolized medication in the sinus cavities when the jet flow was set up at 6 l/min and the temperature of the surrounding environment was 23 °C. The ‘snake-like’ movement of the aerosols flow helps the aerosols reach the hidden pockets of the sinus cavities (PARI Respiratory Equipment, Inc., 2012) (Figure 1-5).
Figure 1-4: PARI Sinus™ Pulsating Aerosol System (PARI GmbH, Starnberg, Germany). (Source: http://www.medema.co.uk/pari_sinus_nebuliser__efficient_medication_deposition__in_the_nasal_cavities)
The Pari Sinus nebulizer is used for delivery by attaching the LC® Sprint Sinus Nebulizer with nasal adapter to one nostril at a time while keeping the other nostril closed with a nose plug. During the delivery, the soft palate should be kept closed. This directs the aerosols to the second output nostril from the delivery nostril. These protocols help aerosols to be deposited in the lung more efficiently. To ensure that optimal pressure transduction to the sinuses is achieved, it is vital that the output resistor and closure of the soft palate is done (Keller et al., 2010; Möller et al., 2011; 2010)

![Diagram of aerosol delivery](http://www.pari.com/products/sinus/product/detail/info/sinus_pulsating_aerosol_system.html)

**Figure 1-5:** Pari Sinus nebulizer aerosol delivery to sinus with snake-like aerosol movement. (Source: http://www.pari.com/products/sinus/product/detail/info/sinus_pulsating_aerosol_system.html)

The Pari Sinus nebulizer has been compared to the nasal spray in order to understand its capacity of aerosol deposition into the nasal sinus cavities (Schuschnig et. al, 2006). It was observed that significantly higher drug doses were deposited by the use of Pari Sinus compared to nasal sprays. It is expected that due to greater deposition, less medication would be required, possibly resulting in reduced side effects.
The efficiency of Pari Sinus was further explored by Valentine et al. (2008) where the sinonasal penetration of nasal douching and the Pari pulsed nebulizer were tested on a highly dissected cadaver model. Methylene blue stained solutions were used in the nebulizers and douching and sinuses deposition was observed. Significant increases in the intensity of the deposition pattern, percentage, and circumference of the stain were noted for the Pari Sinus nebulizer, compared to the nasal douching. Regular ethmoid sinus staining was observed by the Pari Sinus, while the other sinuses were variably reached when sinuses were analyzed individually for deposition. The frontal sinus had a deposition of 43%; maxillary sinus 46%, and sphenoid 54% (Valentine et al., 2008).

Five healthy volunteers were used to investigate sinus ventilation using $^{81m}$Kr-gas gamma camera imaging and $^{99m}$Tc-DTPA radiolabel aerosols in order to investigate retention time over a 24-hour period. Nasal pump sprays and pulsating aerosols were used. Results indicated no deposition when the nasal pump was sprayed in the sinuses. Deposition was around 6.5% for the pulsating airflow within the sinuses, with a slow reduced clearance of the deposited material. Residence time of the drug deposited via pulsating aerosols was also observed to have increased threefold compared to the nasal spray in the nasal cavity (Möller et al., 2010).

A study was conducted using nasal casts and healthy volunteers to recognize sinus ventilation and paranasal deposition using a pulsating airflow. The study demonstrated a promising result of an 8% increase in the nasal deposition of the drug within the sinuses using pulsating aerosols compared to nasal pump sprays. The study also demonstrated that the retention kinetics of the aerosols were longer in the nose when using the pulsating airflow (Möller et al., 2011).
1.5. Liposomes

1.5.1. An overview of liposomes and proliposomes

Liposomes are phospholipid bilayer vesicles used for the encapsulation of drugs and nutrients (Li et al., 2015). Phospholipids are naturally occurring, or synthetic amphipathic lipids (Figure 1-7). Liposomes are vesicles having self-closed structures of lipid bilayers due to their thermodynamic phase and self-assembling characteristics of the amphipathic molecules (Chrai et al., 2002) (Figure 1-6). Vesicles are shaped through self-assembly, a spontaneous process of phospholipids that change into ‘closed-up’ structures when dispersed in aqueous media. Phospholipid molecules arrange themselves into bilayer sheets that lower unfavourable interaction between the aqueous medium and the long hydrocarbon fatty acid chains. This state leads to low energy and maximum stability; bilayer sheets then start folding to form the sealed bilayer vesicles (Figure 1-6).

Figure 1-6: Mechanism of liposome formation. (Source: Sharma V. K et al., 2010)
Water-soluble drugs and lipid-soluble drugs can be effectively entrapped in liposomes. Lipophilic drugs are entrapped among the bilayers while hydrophilic drugs are entrapped within the aqueous spaces of the liposomes (Sharma, 2009) (Figure 1-7). Liposomes are also used as carriers of unstable compounds, such as antimicrobials, and antioxidants for applications in cosmetic, pharmaceutical, food, and farming industries. Liposomes protect the functionality of the unstable molecules until they reach the designated site by shielding them from decomposition (Akbarzadeh et al., 2013). Hydration temperature, choice of phospholipid, nature of membrane additives, size reduction technique, addition of kinetic energy, and the nature of the drug all can affect the physical nature of liposome formulation, and the stability and entrapment of the drug included (Surender Verma et al., 2010).

Figure 1-7: Structure of liposomes. (Source: http://www.medscape.com/viewarticle/734055_3)
1.5.2. Historical background of liposomes

Liposomes were first discovered in 1961 by Dr A. Bangham (Bangham and Horne, 1964). Bangham and co-workers discovered that phospholipids tend to curl and form unilamellar or multilamellar vesicles in the presence of appropriate solvents.

In the modern era from 1985 until today, liposomes have been used in various fields such as mathematics, chemistry, colloid science, biology, and other fields. The first liposomal product marketed for medicinal use was Ambisome™, a parenteral Amphotericin B formulation that is given intravenously for the treatment of systemic fungal infections. Doxorubicin, Daunorubicin, Amikacin, and Hepatitis A vaccine have all emerged as liposomal-based products in the market (Sharma Vijay et al., 2010).

1.5.3. Characterization of liposomes

Liposomes can be classified according to the number of lipid bilayers they have in multilamellar vesicles (MLV), small unilamellar vesicles (SUV), giant unilamellar vesicles (GUV), and large unilamellar vesicles (LUV) (Storm and Crommelin, 1998) (Figure 1-8). Liposomes can also be categorized according to their size (small, intermediate, or large) or based on their manufacturing methods such as reverse phase evaporation vesicles and thin film hydrated (hand-shaken) vesicles, etc. Unilamellar vesicles consist of a single lipid bilayer with a size range of 50–250 nm (Immordino et al., 2006). The size and surface properties of liposomes may affect their biological half-life following intravenous administration. The number of liposome bilayers could also be a deciding factor of the entrapment efficiency of drugs incorporated into liposome formulations. Unilamellar liposomes are mainly used for the delivery of water-soluble drugs due to their large aqueous cores. Multilamellar vesicles consist of many bilayers arranged in an onion-skin-like arrangement and are large in size (1–5 μm). Lipid-soluble drugs are usually entrapped for delivery in MLV liposomes due to their high lipid content (Sharma and Sharma, 1997).
Figure 1-8: Types of basic structures and liposome size.
(Source: http://www.hindawi.com/journals/jdd/2011/863734/fig1/)
1.6. Advantages and Disadvantages of Liposomes

1.6.1. General advantages of liposomes

There are many advantages for using liposomes. For example, liposomes provide a controlled and sustained drug delivery. They could be designed to be a targeted carrier. They are biodegradable, and may increase the therapeutic effect of the encapsulated drug. Moreover, they can carry both water-soluble and lipid-soluble drugs and protect them against instability caused by the surrounding in vivo environment (Goyal et al., 2005). Storm and Crommelin (1998) summarized the answer to the question of why to use liposomes, simply by stating ‘Direction, Duration, Protection, Internalization and Amplification’.

1.6.2. Liposomes as drug carriers

Entrapment of drugs in liposomes improves the solubility of lipophilic and amphiphilic drugs (Amphotericin B, some peptides, anthracyclines) and the passive targeting of the immune system cells, resulting in the potential for use as carriers of vaccines, immunomodulators, and antimonials (Akbarzadeh et al., 2013). Liposomes also help improve drugs by protecting them from the surrounding environment and allowing sustained release (systemically and locally). Biocompatibilities, biodegradability, non-toxicity, and the multidisciplinary nature of their use all have made liposomes highly suitable for drug delivery and targeting. The ‘milieu interne’ phenomenon (lipophilic and aqueous environment in one system) gives liposomes a unique advantage of being able to transport hydrophobic, amphipathic, and hydrophilic drugs. Liposomes have also been used to offer site-avoidance in cases such as Doxorubicin and Amphotericin B, which constitute the bases for drug targeting (Akbarzadeh et al., 2013).
Site-specific targeting of liposomes has been used with anti-inflammatory, anti-cancer, and anti-infection drugs to reduce side effects and improve the therapeutic outcome. Mucoadhesive properties of liposomes also help improve penetrations of the encapsulated drug molecules into the tissues (e.g. corticosteroids, anaesthetics, and insulin) (Akbarzadeh et al., 2013) (Figure 1-9). Liposomes also have the ability to be formulated in suspension such as an aerosol, gel, cream, or lotion (Sipai et al., 2012). Liposomes can be generated from dry powder precursors (i.e. proliposomes) for hydration prior to administration. Liposomes have been used for most routes of drug administration such as pulmonary, oral, intramuscular, nasal, topical, subcutaneous, and intravenous (Sipai et al., 2012).

![Acceptance of liposome into cell](source: sampathkumar et al., 2012)
The versatile nature of liposomes has allowed entrapment of small molecules and macromolecules (haemoglobin, interleukin-2) (Akbarzadeh et al., 2013; Kapoor et al., 2014; Surender Verma et al., 2010). Encapsulation of toxic drugs in liposomes may help reduce exposure of sensitive tissues to toxic drugs. Liposomes have also been flexible in coupling with site-specific ligands to improve targeting of the encapsulated drug in cases of anti-cancer and anti-microbial therapy (Sipai et al., 2012).

1.6.3. Drawbacks of liposomes

Drawbacks of liposomes include the liability of liposomal phospholipids to oxidation and hydrolysis, as well as the tendency of the liposome dispersions to microbial contamination during storage because phospholipids are natural food components (Omri and Ravaoarinaro, 1998). Other drawbacks of liposomes include the possibility of losing drug encapsulation due to chemical decomposition. Another disadvantage is that liposome production is expensive (Anwekar et al., 2011).

One major drawback of liposomes is their rapid elimination from the blood. Reticulo-endothelial system cells, mainly the liver, capture liposomes and clear them from the blood (Torchilin, 2005). This drawback has now been addressed by the development of long-circulating liposomes by changing lipid composition, size, and charge of the vesicle. The most significant changes in liposomes were achieved by coating the vesicles with polyethylene glycol polymer to improve blood circulation and reduce the uptake by the mononuclear phagocyte system (stealth liposomes). This modification has improved the ability of liposomes to encapsulate active molecules, and target high efficiency and activity (Immordino et al., 2006).

1.6.4. Proliposomes

Payne and co-workers (1986) designed an alternative to the direct formation of liposomes in order to offer an approach to overcoming the problems of conventional liposome instabilities. Liposomes can be made via passive loading and active loading methods.
Passive loading can be divided into three categories: mechanical dispersion method, solvent dispersion method, and detergent removal method (Huang et al., 2014). Proliposomes are stable, powdered phospholipid formulations that generate liposomes by the addition of an aqueous phase (Payne et al., 1986). Thus, a lot of liposome drawbacks could be overcome by the use of particulate-based proliposomes as an approach to generate liposomes.

The most common method of proliposomes manufacture includes film deposition on the carrier method, the spray drying method, the fluidized bed method, and supercritical anti-solvent method (Shaji and Bhatia, 2013). The film deposition on carrier method is done via deposition of a film of drugs and phospholipid onto water-soluble carrier material. Volatile organic solvent is introduced dropwise via a feed tube to the rotary evaporator flask on to the bed of carrier in the flask (Shaji and Bhatia, 2013). The solvent is then evaporated under a vacuum. The spray drying method involves a single step of both particle formation and drying. The spray drying method can be used on both aqueous and non-aqueous systems (Shaji and Bhatia, 2013). The fluidized bed method uses particle-coating technology and is used for large-scale production of proliposomes. The supercritical anti-solvent method uses apparatuses with a supercritical carbon dioxide (sCO₂) fluid state held at or above its critical temperature and pressure to prepare proliposomes (Shaji and Bhatia, 2013).

Liposomes are vesicles composed of phospholipid bilayers. Drawbacks of liposomes include their liability to oxidation and hydrolysis, and the tendency of the liposome dispersions to become microbially contaminated during storage because phospholipids are natural food components (Eichman and Robinson, 1998; Omri and Ravaoarinoro, 1998). There is also the possibility of losing drug encapsulation due to chemical decomposition of the lipids in the formulation. A lot of these drawbacks could be
overcome by the use of particulate-based proliposomes by being available in dry form, making it easy to store, distribute, transfer, and measure (Shaji and Bhatia, 2013).

1.6.5. Nasal delivery of liposomes

Sustained delivery of drugs to specific sites in the body achieved by carriers such as liposomes have attracted great interest. While the use of liposomes has been studied extensively in nasal delivery (Illum, 2003; Ravouru et al., 2013) liposomes in nasal drug delivery especially have proven advantages by causing decreased mucociliary clearance due to formulation viscosity. Efficient and increased drug absorption is noted through the opening of ‘new pores’ in the paracellular tight junctions in phospholipid membranes of nasal mucosa, which helps with liposome incorporation (Mainardes et al., 2006).

Nifedipine-bearing MLV liposomes given via nasal delivery have been successful in achieving a sustained plasma concentration and decreases in mucociliary clearance (Mainardes et al., 2006). A study done on a liposomal formulation of levonorgestrel, coupled with mucoadhesive polymers such as carbopol and chitosan for nasal delivery, has demonstrated increased drug bioavailability, increased contact time of the drug, and enhanced absorption through decreased mucociliary clearance (Shahiwala and Misra, 2004a). The study by Shahiwala and Misra (2004b) also states that using liposomes with colloidal carriers leads to decreased drug-dosing frequencies and decreased systemic side effects by maintaining blood concentrations from 6–60 hours (Shahiwala and Misra, 2004b). Many other studies support the use of liposomes in nasal delivery (Heurtault et al., 2010; Mainardes et al., 2006; Türker et al., 2004).

Permeability of liposomes with insulin entrapped and insulin solution (with and without treatment of sodium glycocholate) have been studied on the nasal mucosa of a rabbit. Results indicated that insulin loaded on to liposome solution has superior permeability to insulin solution (Maitani et al., 1992). Desmopressin-loaded liposomes were investigated on nasal mucosa. Researchers state positively charged liposomes had the most superior
nasal permeability, while negatively charged liposomes demonstrated less permeability compared to positive liposomes but superior permissibility compared to solution of desmopressin without liposomes (Law et al., 2001).

1.6.6. Nasal delivery of drugs through aerosolized proliposomes

Drug delivery to the pulmonary or nasal systems via nebulized aerosols may need a carrier system, especially when water solubility of the drug is poor. Liposomes may entrap drugs and when delivered intranasally they can enhance the uptake of the drug by the nasal mucosa. This approach has been exploited for nasal delivery of vaccines (Heurtault et al., 2010). However, the poor stability of liposomes is a serious obstacle since phospholipids are liable to oxidation and hydrolysis when present in aqueous formulations. Proliposomes are carbohydrate carrier particles coated with phospholipids using simple techniques, offering enhanced formulation stability. Nasal delivery is a needle-free approach, comprising a comfortable and painless mode of drug delivery for the treatment of local and systemic diseases.
1.7. Effervescent Formulations for Nasal Drug Delivery

1.7.1. Introduction to effervescent formulations

Effervescence is a reaction that happens in water and results in the liberation of carbon dioxide as a result of acid-base reactions. For nearly 200 years, effervescent formulations were mainly used in oral delivery (Eichman and Robinson, 1998) but only a limited number of studies have been published on the use of effervescent formulations in nasal and pulmonary delivery (Ely et al., 2007; Katare et al., 1995, 1990; Wei et al., 2013; Zhao et al., 2010). As a general context of preparation, effervescent formulations (containing carbonates, acid substance, or hydrogen carbonates) are made to permit the occurrence of an acid-base reaction in water, resulting in tablet/granule disintegration with subsequent liberation of the drug from the solid compact. The shaking provided via effervescence (because of the liberated carbon dioxide) helps to dissolve the drug that was originally included in the formulation. Effervescent tablets are also uncoated, so when water is present the reaction takes place immediately and carbon dioxide is released (Lindberg and Hansson, 2006). European Pharmacopeia 5 state, Effervescent granules should typically disintegrate or its medicinal ingredients dissolve in less than 5 min (Council of Europe, 2004, p. 606)

Effervescent formulations may offer a means of enhancing the dissolution of the incorporated drug, and hence its absorption through biological membranes can be improved (Coletta and Kennon, 1964). Effervescent granules also modify the releases and are designed to control the rate of release of the drug and delay the active component from releasing too quickly (Aulton and Taylor, 2013).

Effervescent formulations, apart from containing acids/acid salts, bicarbonates/carbonate salts, also contain fillers, binders, sweeteners, flavours, and lubricants. These products are then mixed in to effervescent formulations tableted by either wet granulations, fusion method, fluid-bed granulation, or direct compression (Aslani and Jahangiri, 2013). The
key benefit of effervescent solid formulations is its ability for the solid formulations to disperse in water and quickly be available in liquid formulation. This property of effervescent formulations has been used in many industries (dental hygiene, household cleaners, medicines, food supplements, detergents, etc.) with a market value thought to be in multibillions (Lindberg and Hansson, 2006).

Effervescent tablets, granules, and powders are mentioned in pharmacopeia and in medical products in the current market (Lindberg and Hansson, 2006). Effervescent formulations are often available in the market in tablet form. Currently, the most commonly used effervescent table is the aspirin tablet (Palanisamy et al., 2011). Effervescent oral tablets have many advantages over conventional solid dosage forms, namely, improved palatability, sparkling solution, portability, help for patients who cannot swallow tablets, and the stability of products that are unstable in liquid form, which are often stable as effervescent tablets. Effervescent liquid also address issues related to dissolution (absorption rate and extent of bioavailability). However, there are drawbacks, such as expensive manufacturing due to needing large amounts of excipients and special facilities to produce effervescent products. Effervescent products also need special packaging to minimize contact with moisture and air (Prabhakar and Krishna, 2011). Effervescent tablets are also bulky compared to conventional solid dosage form tablets. Effervescent formulations are sensitive to moisture and temperature; therefore, a relative humidity of 25% or less and a temperature of 25 °C are needed for manufacturing (Prabhakar and Krishna, 2011).

Drugs formulated as effervescent tablets are often drugs difficult to digest or that cause disruption in the stomach, pH-sensitive drugs (amino acids and antibiotics), and drugs requiring a large dose. The typical effervescent tablet is 1 in in diameter with a weight of 5 g in total. If the dose is larger, effervescent granules could be available in sachet form (Prabhakar and Krishna, 2011).
Laxative effervescent suppositories that release carbon dioxide have been researched and have been available on the Swedish market for many years (Hakata et al., 1993). Effervescent vaginal suppositories have also been studied (Kurobe et al., 1983). A pulsatile and gastric floating drug delivery system based on a reservoir system consisting of effervescent core and polymeric coating have been investigated (Krögel and Bodmeier, 1999).

1.7.2. **Effervescent liposomes**

Very limited studies have been done on effervescent formulations coupled with liposomes. Effervescent Ibuprofen proliposomes were first investigated for systemic administration by Katare and co-workers. Soya bean lecithin, Stearylamine, and cholesterol were incorporated into effervescent formulations, which produced regular- and uniform-sized (1–4 µm) liposomes with high drug encapsulation efficiency (Katare et al., 1990). Phospholipids in uniform liposomes were protected by an inert umbrella product that helped with the hydration of lipids by the high shear pressure given off by the effervescence reaction.

A study was carried out by the same research group in 1995 to explore the potential of indomethacin effervescent proliposomes. The liposomes were studied for their anti-inflammatory activity following systemic delivery using experimental rats, which confirmed superior anti-inflammatory activity of the liposomal formulation of the drug compared to a conventional drug preparation (Katare et al., 1995). More recent studies have demonstrated that effervescent proliposomes were used with docetaxel (composed of docetaxel/Tween-80/Phospholipon 90H/cholesterol/citric acid) for pulmonary drug delivery. The formulations were produced using a solid dispersion technique, which produced liposomes of 1 µm, negatively charged with a favourable lung-targeting effect. Following the addition of the aqueous phase, the formulation’s composition was docetaxel, Tween-80, Phospholipon 90H, cholesterol, and citric hydrated in
NaHCO$_3$ solution (Zhao et al., 2010). Wei Y et al. (2013) studied liposomes made with Phospholipon 90H and Tween-80 for delivery of paclitaxel to the lung in rabbits. Liposomes were 8.166±0.459 µm in size, negatively charged with high drug entrapment efficiency, indicating that effervescent proliposomes are a promising drug delivery system (Wei et al., 2013).

The paclitaxel liposomes drug delivery system was prepared by solid dispersion and effervescent techniques targeting the lung (Zhao et al., 2011). Liposomes contained between 80/HSPC/cholesterol (0.03 : 3.84 : 3.84, mol/mol), containing paclitaxel and lipids (1 : 40, mol/mol). Liposomes were found to have a mean size of 0.5±15.43 µm, a span of 0.28±0.02, zeta potential of −20.93±0.06 mV, and a drug entrapment of 95.17±0.32%. Liposomes were found to be stable for least three months at 6±2 °C. It was observed that paclitaxel liposomes had a drug concentration of 15 fold higher than of paclitaxel injection at 2h in the lung (Zhao et al., 2011). Research concluded that liposomes loaded with drug was an effective drug carrier system.

The lung-targeting injectable liposome formulation consisting of particles or powder loaded with medicine for solid phase and effervescent has been studied. In this study, proliposomes and effervescent were packed separately and mixed together in an effervescent dispersion technique before administration of the injection(Yu et al., 2010). Research on pulmonary delivery to the lung with the use of effervescent formulations was conducted by Ely et al., (2007) using formulations of dry effervescent powders for pulmonary delivery. Spray drying of effervescent drug powder was made by incorporating polybutylcyanoacrylate nanoparticles and ciprofloxacin. The effervescent powder was made with the aim of generating the effervescence within the pulmonary system. As yet, the delivery of effervescent proliposomal formulations via nebulizers to treat sinusitis has not been investigated.
1.8. Model Drugs for the Study of Couples with Effervescent Liposomes

1.8.1. Beclometasone dipropionate (hydrophobic)

Beclometasone dipropionate (BDP) (Figure 1-10) is a glucocorticoid steroid that is insoluble in water (i.e. hydrophobic) and has an anti-inflammatory effect, reducing the ability to produce immune reactions. BDP acts on the body by inhibiting inflammatory cells such as mast cells, eosinophils, basophils, lymphocytes, macrophages, and neutrophils. BDP also prevents the release of inflammatory mediators such as histamine, leukotrienes, and cytokines. Monoester 17 and monopropionate (17-BMP) are activated by hydrolysis in vivo by BDP demonstrating binding affinity for humans’ glucocorticoid receptor (GR), resulting in anti-inflammatory effects (Wang et al., 2011). All corticosteroids can cause side effects, associated with adrenaline inhibition and significant bone density decrease) (Wilson et al., 1997).

Figure 1-10: Chemical structure of beclometasone dipropionate (BDP).
(Source: http://upload.wikimedia.org/wikipedia/commons/2/21/Beclometasone_dipropionate.png)
Long-term use of this drug orally causes serious side effects, such as a cough, oral candidiasis, bad mouth odour, hoarseness, nasal congestion, pain, headache, and visual changes. Thus, the formulation of this drug as effervescent proliposomes will not only enhance its solubility but may also shorten the course of therapy by enhancing the drug absorption (Messerli et al., 1975; Mygind, 1973).

1.8.2. Xylometazoline hydrochloride (XH)

Xylometazoline hydrochloride (XH) (Figure 1-11) is a hydrophilic (i.e. water-soluble) drug, usually used as a topical nasal decongestant (Eccles et al., 2008). The typical concentration of the drug given to an adult is around 0.1% w/v XH and 0.05% for children under 12 years. XH works by constricting the blood vessels and increasing nasal airflow. Nasal congestion happens due to the inflammation of the large veins in the nose and/or infections or inflammation due to nasal allergy. XH also causes constriction of smaller arteries. Due to the constriction, nasal airflow increases and blockage of the nose is reduced, resulting in easier breathing for the patient (Castellano and Mautone, 2002).

XH mimics the molecular shape of adrenaline and is a derivative of imidazole which binds to alpha-adrenergic receptors in nasal mucosa. XH results in sympathomimetic effects and is not for patients with heart issues and high blood pressure. Long-term use of XH will decrease the effectiveness of the drug and result in an increased tolerance of the drug by decreasing in the number of drug receptors. Chronic congestion is also one of the side effects of XH, known as rebound congestion. Degeneration of the nasal mucosa membrane can also be a side effect of the long-term use of XH.
Figure 1-11: Chemical structure of xylometazoline hydrochloride (XH). (Source: http://upload.wikimedia.org/wikipedia/commons/8/8a/Xylometazoline_Structural_Formulae_V_2.png)
1.9.  Aim of This Thesis

The aim of this thesis is to design and develop an effervescent proliposomes formulation that could disintegrate in water and liberate liposomes in a matter of minutes by improving its disintegration time compared to conventional proliposomes. This will potentially help to improve dosing and produce liposomes available for immediate administration. The suitability of effervescent liposomes in terms of their characteristics, entrapment, and suitability for delivery through a nebulizer will be compared with the conventional liposomes. The hydrophobic model drug Beclometasone dipropionate (BDP), and also in some formulations the hydrophilic drug Xylometazoline hydrochloride, have been investigated in this study. The formulations of effervescent proliposomes were prepared using the slurry method. Mannitol was used as a sugar-based carrier and formulations were tested with or without cholesterol. Dipalmitoyle phosphatidylcholine (DPPC) and Soya phosphatidylcholine (SPC) lipids were chosen as the candidate lipids for this experiment to understand the impact of cholesterol in different types of lipids in the presence of effervescent ingredients. All formulations contained effervescent ingredients (bicarbonate, sodium benzoate, and citric acid). Formulations were also tested for their ability to produce stable liposomes in the presence of effervescent salts alone, without the sugar-based carrier (sugar-free). Mucoadhesive polymers, such as alginic acid or chitosan, were incorporated to improve the bioadhesive properties. The inclusion was then investigated for its suitability in effervescent liposomes. This study also aims to investigate the potential suitability of liposomes for aerosolization to target the paranasal sinuses using a nebulizer. The Pari Sinus nebulizer and the Pari Sprint nebulizer were then compared on the basis of their ability to deliver aerosols to the nasal cavity and sinuses using a unique system that was developed by incorporating a nasal cast (transparent nasal cast model) coated with Sar-Gel® (water indicating paste) to an impinger. The deposition patterns of nebulized formulations were
photographed and images were analyzed using Adobe® Photoshop, and pixels were counted and then converted into cubic centimetres. The ability of the Pari Sinus and Pari Sprint nebulizers to deposit aerosols into the full nasal cavity and sinuses alone were then compared. The final outcome of this thesis is to develop a liposomal effervescent formulation that is more efficient, with reduced disintegration time suitable for the delivery of drugs to the sinuses compared to conventional liposomes via a nebulizer.
Aim of thesis in schematic graph

1.10. Element of Originality:

Novelty aspects of the project are:

1) the design of Beclometasone dipropionate effervescent formulations for the treatment of sinusitis;

2) employment of proliposomes technology in designing nanotechnology systems to target the parasinuses;

3) understanding how the presence of cholesterol in effervescent liposomes affect different lipids used for the formulation;

4) the employment of the Pari Sinus nebulizer to deliver effervescent nanotechnology-based formulations;

5) the investigation of the suitability of effervescent liposomes in delivering hydrophilic drugs by loading Xylometazoline hydrochloride;

6) the development of a novel HPLC method to determine drug entrapment for Xylometazoline hydrochloride;

7) the development of a unique system with a twin impinger and a transparent nasal cast model coated with Sar-Gel® (water indicating paste) to study the deposition area of nebulized aerosols in the upper respiratory tract.

To my best knowledge, the potential of effervescent formulations of Beclometasone dipropionate has not yet been explored, and proliposome technology has not been investigated for targeting the parasinuses. Moreover, very little research has been conducted on the novel Pari Sinus nebulizer. So far, the sinuses have not been targeted with effervescent liposomal formulations. This project is specifically designed to develop effervescent formulations in the form of proliposome granules that could disintegrate in water and liberate liposomes with the model drugs for delivering aerosols that can target the parasinuses via the Pari Sinus nebulizer.
CHAPTER 2
METHOD AND MATERIALS
2.1. Materials

Table 2-1: Chemicals and Supplier

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Suppliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya Phosphatidylcholine (Lipoid S-100)</td>
<td>A gift from Lipoid, Switzerland</td>
</tr>
<tr>
<td>Dipalmitoyl Phosphatidylcholine (DPPC)</td>
<td>Lipoid, Switzerland</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>Sigma-Aldrich, UK</td>
</tr>
<tr>
<td>Cholesterol (Sigma grade, ≥99%),</td>
<td>Sigma-Aldrich, UK</td>
</tr>
<tr>
<td>Citric acid</td>
<td>Sigma-Aldrich, UK</td>
</tr>
<tr>
<td>Sodium benzoate</td>
<td>Sigma-Aldrich, UK</td>
</tr>
<tr>
<td>Xylometazoline hydrochloride (XH)</td>
<td>Sigma-Aldrich, UK</td>
</tr>
<tr>
<td>Deuterium oxide (For NMR, 99.8% atom %D)</td>
<td>Acros Organics, UK</td>
</tr>
<tr>
<td>Beclomethasone dipropionate</td>
<td>Acros Organics, UK</td>
</tr>
<tr>
<td>Deuterium oxide (For NMR, 99.8% atom %D)</td>
<td>Acros Organics, UK</td>
</tr>
<tr>
<td>HPLC water (HPLC gradient grade)</td>
<td>Fisher Scientific, UK</td>
</tr>
<tr>
<td>Methanol (HPLC grade)</td>
<td>Fisher Scientific, UK</td>
</tr>
<tr>
<td>Ethanol (99.8+% absolute duty free for HPLC certified HPLC)</td>
<td>Fisher Scientific, UK</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Fisher Scientific, UK</td>
</tr>
<tr>
<td>Sar-Gel®</td>
<td>Sartomer, US</td>
</tr>
</tbody>
</table>
2.2. Preparation of proliposomes

2.2.1. Preparation of particulate-based proliposomes

The following method was used for proliposome preparation. All proliposome formulations were made using the same method. The samples are as follows:

Table 2-2: Non-effervescent particulate-based formulations investigated

<table>
<thead>
<tr>
<th>Formulations (Lipid:Carrier)</th>
<th>Carrier</th>
<th>Lipid</th>
<th>Solvent</th>
<th>Drug (mol%)</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:5 w/w</td>
<td>Sucrose</td>
<td>SPC</td>
<td>Chloroform</td>
<td></td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:5 w/w</td>
<td>Sucrose</td>
<td>SPC</td>
<td>Chloroform</td>
<td>2.5 mol%</td>
<td>BDP</td>
</tr>
<tr>
<td>1:5 w/w</td>
<td>Mannitol</td>
<td>SPC</td>
<td>Chloroform</td>
<td>-</td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:5 w/w</td>
<td>Mannitol</td>
<td>SPC</td>
<td>Chloroform</td>
<td>2.5 mol%</td>
<td>BDP</td>
</tr>
<tr>
<td>1:5 w/w</td>
<td>Mannitol</td>
<td>SPC</td>
<td>Ethanol</td>
<td>-</td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:5 w/w</td>
<td>Mannitol</td>
<td>SPC</td>
<td>Ethanol</td>
<td>2.5 mol%</td>
<td>BDP</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol</td>
<td>SPC</td>
<td>Ethanol</td>
<td>-</td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol</td>
<td>SPC</td>
<td>Ethanol</td>
<td>2.5 mol%</td>
<td>BDP</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol</td>
<td>DPPC</td>
<td>Ethanol</td>
<td>5 mol %</td>
<td>BDP</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol</td>
<td>DPPC</td>
<td>Ethanol</td>
<td>-</td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol</td>
<td>SPC</td>
<td>Ethanol</td>
<td>5 mol %</td>
<td>BDP</td>
</tr>
</tbody>
</table>

2.2.2. Sucrose-based conventional liposomes (SPC-based) with hydrophobic drug

The following is a detailed explanation of the preparation of the first sample. The same method has been applied for all samples with changes of weights and ingredients as described in Table 2-1. For preparation of (Table 2-2) 1:5 w/w lipid to sucrose (carrier) ratio empty proliposomes, sucrose was ground in a ball mill rotated on a rotating roll mixer. The resulting powder was then sieved and the fraction of the sucrose particles between the sizes of 300–500 µm was collected for the manufacture of proliposomes with
a 1:5 w/w phospholipids to carrier ratio. Sucrose particles (1.25 g) were placed in a pear-shaped 100 ml flask and attached to a customized rotary evaporator (Büchi Rotavapor R-114, Büchi, Switzerland, Büchi, Switzerland) and the temperature of the water bath was set at 40°C. A chloroformic solution of Soya phosphatidylcholine (SPC, 60 mg/ml) and cholesterol were dissolved and poured on to the sucrose inside the round bottom flask. The round bottom flask was attached to the rotavapor and negative pressure continued for two hours to remove the solvent and generate dried proliposomes. The vacuum was released to collect the proliposomes and store them in a glass vial at -18°C.

For drug-loaded formulations (Table 2-2): This procedure was repeated by inclusion of 2.5 mol% Beclometasone dipropionate in the lipid phase for 1:5 w/w lipid to sucrose proliposomes with 2.5 mol% drug. Hydration of the proliposomes for non-effervescent liposomes, the samples was vortexed for 2 min (Stuart, SA8). The proliposomes were stored and annealing of the manufactured liposomes was carried out just prior to further testing.

The same procedure was repeated for all mannitol formulations with different ingredients as in Table 2-1. When preparing mannitol-based proliposomes, grinding was not necessary but the same procedure as the method as section 2.2.2 was repeated by substituting sucrose with mannitol. 1:5 w/w phospholipid to carrier ratio formulation with mannitol, 1.25 g of mannitol was placed in a 100 ml pear-shaped flask attached to a customized rotary evaporator (Büchi Rotavapor R-114, Büchi, Switzerland).

2.2.3. Mannitol-based conventional liposome (SPC-based) with hydrophobic drug

The following is a detailed explanation of the preparation of the first sample for mannitol non-effervescent formulations (Table 2-2). The same procedure was repeated for all mannitol formulations with different ingredients as in Table 2-1. When preparing mannitol-based proliposomes, grinding was not necessary but the same procedure as the method in section 2.2.2 was repeated by substituting sucrose with mannitol. 1:5 w/w
phospholipid to carrier ratio formulation with mannitol, 1.25 g of mannitol was placed in a 100 ml pear-shaped flask attached to a customized rotary evaporator (Büchi Rotavapor R-114, Büchi, Switzerland). Soya phosphatidylcholine (SPC, 60 mg/ml) and cholesterol dissolved in chloroforming solution was poured in to the pear-shaped flask. The round bottom flask was attached to the rotavapor and negative pressure continued for two hours to remove the solvent and generate dried proliposomes. The vacuum was released to collect the proliposomes and store them in a glass vial at -18 °C.

The same procedure was carried out for drug-loaded liposome formulations with the addition of 2.5 mol% drug BDP in lipid phases for 1:5 w/w lipid to mannitol proliposome ratio. For hydration, proliposomes were dissolved in water and vortexed for 2 min. Proliposomes were stored and annealing of formulations was done just prior to testing.

2.2.4. Mannitol-based conventional liposomes (DPPC-based) with hydrophobic drug

When preparing DPPC lipid-based samples (Table 2-2.), the method mentioned in section 2.2.3 was used to produce SPC-based liposomes was substituted with DPPC lipid. The round bottom flask in the rotary evaporator (Büchi Rotavapor R-114, Büchi, Switzerland) was used and the temperature of the water bath was set up at 55° C instead of 40° C.

2.2.5. Mannitol-based conventional liposomes (SPC-based) with hydrophilic drug

Xylometazoline hydrochloride, drug-loaded liposome formulations (Table 2-2) were made with SPC according to section 2.2.3 with the exception of drug loading at lipid phase. The hydrophilic drug was loaded to the liposomes at the hydration phase, instead of adding the drug in the lipid phase. The drug was added along with the proliposomes at the hydration phase. Proliposomes were stored at 18 °C, until annealing and drug were loaded at hydrating and vortexed for 2 min. Purification step of XH is mentioned in details at section 2.4, and section 2.42.
2.3. Characterization of Liposomes

2.3.1. Particle size analysis

Particles are passed through a beam of laser light and the scatters of the incident light are focused onto a Fourier lens. The Fourier lens in turn focuses the scattered light onto a detector array and the range of the particle sizes is calculated from the collected diffracted light data with the use of an inversion algorithm. Liposomes were placed in the dispersion unit of the Malvern Mastersizer 2000 (Malvern Instruments Ltd, UK) followed by measurement of the volume median diameter (VMD) and span to represent the size and size distribution respectively. The VMD represents the 50% undersize while span = (90% undersize – 10% undersize)/ VMD. The polydisperse mode was chosen with a stirring speed of 1360 rpm for analysis.

2.3.2. Zeta potential (surface charge) analysis

The zeta potential of liposomes may indicate how they behave in vivo and also help to identify any changes that would be made on the surface of the liposomes upon inclusion of certain materials. The zeta potential also provides information of stability of the particles in a suspension. Calculation of the zeta potential is automatic with the correlation of electrophoretic mobility when using the zeta sizer instrument (Malvern Instruments, UK). The zeta potential cell was thoroughly washed with deionized water to minimize possible cross-contamination. The cell was carefully loaded with the sample to avoid the creation of bubbles that may interfere with the measurements. The right software options were selected and measurement was performed.

2.3.3. Surface morphology using scanning electron microscopy (SEM)

A scanning electron microscope (SEM) (EMITECH, UK) is operated by generating an image of the specimen by scanning it with a beam of electrons. The scan is done in a raster pattern. When the electrons from the microscope interact with the atoms of the specimen they produce signals that give out information about the composition of the
specimen and its surface topography and also other characteristics such as the electrical conductivity of the specimen. The SEM is an instrument that can produce highly magnified images that can go down to the nanometre size range of particles.

Proliposome particles using a range of carrier size fractions, such as 300–500 μm using SPC or SPC:Chol (1:1), molar ratio were air-dried onto an SEM stub (TAAB Laboratories Equipment Ltd., UK). Samples were gold-coated for 5 min in a EMITECHK550 sputter coater (EMITECH, UK). The samples were then viewed, photographed, and video-printed using the Philips XK 20 SEM. The same procedure was repeated for all samples.
2.4. **Entrapment Studies**

2.4.1. **High-performance liquid chromatography (HPLC studies) for drug Beclometasone dipropionate (BDP)**

To investigate the HPLC entrapment of liposomes, the separation of liposomes was achieved by centrifugation. Liposomes and BDP crystals have a different density that helps with separation. Centrifugation results in sediments of suspended particles sinking, while liposomes tend to float. Batavia 2001 also investigated if the density difference of water and deuterium oxide help the separation of liposomes from the unincorporated BDP crystals.

2.4.2. **Separation of entrapped and unentrapped drug**

Prior to the HPLC, studies of the hydrophobic drug BDP were carried out for all proliposomes formulations. 30 mg/ml of each formulation was dissolved for separation. Each sample was centrifuged (bench centrifuge: Jencons-PLS, Spectrafuge 24D) at the speed of 13000 rpm (15300 relative centrifugal force) for 90 min and liposomes (in D2O the floating layer) were removed using a Pasteur pipette. Liposomes were dissolved by the addition of methanol in order to release the drug for subsequent analysis by HPLC. Methanol was also added to the BDP spot that contained the unentrapped drug to determine the unentrapped drug fraction. A mobile phase was added to dissolve the plasma membrane just before HPLC analysis. To analyze the separations of liposomes and crystals, the spot that appears in the centrifuge tube was investigated using a light microscope to establish they were BDP crystals.

The hydrophilic drug XH samples did not need suspension in D2O. 30 mg/ml of each formulation was dissolved for separation in HPLC gradient water. Each sample was centrifuged at the speed of 13000 rpm (15300 relative centrifugal force) for 90 min. The
liposomal layer deposited in the bottom was removed with a Pasteur pipette and a mobile phase was added to dissolve the plasma membrane just before HPLC analysis.

2.4.3. High-performance liquid chromatography (HPLC) analysis of Beclomethasone dipropionate (BDP)

An Agilent system, 1200 Serious USA, HPLC machine was used and HPLC analysis was conducted at room temperature using a high chrome ODS (4.6 x 250 mm) Eclipse XDB-C18, 4.6 x 150 mm, Agilent, UK column. The following method was adapted from Batavia and co-workers (2001). HPLC grades of methanol and water were used in a 3:1 ratio to constitute the mobile phase. The mobile phase was set to have a flow rate of 2 ml/min. The sample injection volume was set at 50 µl, and UV detection at 238 nm was employed. A calibration curve was constructed using ascending drug concentrations in methanol.

2.4.4. High–performance liquid chromatography (HPLC) analysis of Xylometazoline hydrochloride (XH)

Xylometazoline hydrochloride samples were analyzed by using HPLC Agilent Technologies. XH was assayed using a Synergie 4U MAX 250 x 4.60 mm column (Agilent, UK column). The mobile phase, consisting of acetonitrile and water was eluted at a flow rate of 1 ml/min with this specific gradient profile (Table 2-3)

<table>
<thead>
<tr>
<th>Step. No.</th>
<th>Time (min)</th>
<th>Water (%)</th>
<th>Acetonitrile (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>11.00</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>11.01</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>14.00</td>
<td>85</td>
<td>15</td>
</tr>
</tbody>
</table>

The injection volume was 50 µl and the fluorescence detector employed an absorbance wavelength of 225.4 nm signal and reference of 360,100. Stop time for each sample was 8 min. Under these conditions, the retention time for prednisolone was 10.3 min.
Specificity

The specificity of our method was proved by comparison of a blank sample consisting of mobile phase, acetonitrile, water, 1:1 (Figure 2-1) and prednisolone sample in that mobile phase (Figure 2-2).

![Figure 2-1: Blank sample assayed by the HPLC method.](image)

*Figure 2-1: Blank sample assayed by the HPLC method.*

![Figure 2-2: Xylometazoline hydrochloride sample assayed by the HPLC method.](image)

*Figure 2-2: Xylometazoline hydrochloride sample assayed by the HPLC method.*

Linearity

An acceptable linearity was established in the concentration range of 0 – 40 µg/ml ($R^2$=0.9945) (Figure 2-3).

![Figure 2-3: Calibration curve of prednisolone for the concentration 0–50 µ.](image)

*Figure 2-3: Calibration curve of prednisolone for the concentration 0–50 µ.*
Accuracy and Precision

The HPLC method was also tested for accuracy and precision. Table 2-4 shows accuracy evaluation for the concentration range of 5–40 ng/ml, and precision is shown in Table 2-5.

**Table 2-4: Accuracy of the HPLC method for the concentration range 5–40 ng/ml**

<table>
<thead>
<tr>
<th>Average actual concentration (µg/L)</th>
<th>Average calculated concentration (µg/L)</th>
<th>Average accuracy</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.45</td>
<td>5.24</td>
<td>99.14</td>
<td>0.17</td>
</tr>
<tr>
<td>10.12</td>
<td>10.01</td>
<td>100.29</td>
<td>5.41</td>
</tr>
<tr>
<td>15.94</td>
<td>14.99</td>
<td>98.14</td>
<td>2.67</td>
</tr>
<tr>
<td>20.99</td>
<td>20.98</td>
<td>100.07</td>
<td>1.05</td>
</tr>
<tr>
<td>25.31</td>
<td>24.45</td>
<td>99.76</td>
<td>3.71</td>
</tr>
<tr>
<td>30.40</td>
<td>29.93</td>
<td>96.99</td>
<td>6.40</td>
</tr>
<tr>
<td>35.21</td>
<td>34.94</td>
<td>100.50</td>
<td>2.98</td>
</tr>
<tr>
<td>40.05</td>
<td>39.24</td>
<td>101.94</td>
<td>2.18</td>
</tr>
</tbody>
</table>

**Table 2-5: Precision of the HPLC method after 10 measurements of XH sample**

<table>
<thead>
<tr>
<th>Concentration of XH (µg/l)</th>
<th>Average AUC of 10 measurements</th>
<th>SD</th>
<th>SD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.02</td>
<td>2278.03</td>
<td>0.63</td>
<td>0.19</td>
</tr>
</tbody>
</table>
2.5. Effervescent Proliposome Formulations

2.5.1. Preparation of effervescent formulations

Granules were prepared within the rotary evaporator. Both SPC and DPPC lipids were used for formulations and mannitol added or exempted to understand how it impacted on liposomes.

Cholesterol was also included or exempted to understand the interaction with different careers and lipids

*Table 2-6: Effervescent ingredients used for preparation of effervescent proliposomes*

<table>
<thead>
<tr>
<th>Name</th>
<th>Effervescent content</th>
<th>Amounts (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beclomethasone dipropionate</td>
<td>Drug</td>
<td>5.75 mg</td>
</tr>
<tr>
<td>Mannitol</td>
<td>Carrier</td>
<td>1250 mg</td>
</tr>
<tr>
<td>SPC</td>
<td>Phospholipid</td>
<td>83.33 mg</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Cholesterol</td>
<td>41.6 mg</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>Carbonate source</td>
<td>1705 mg</td>
</tr>
<tr>
<td>Citric acid anhydrous</td>
<td>Acid Source</td>
<td>1375 mg</td>
</tr>
<tr>
<td>Sodium benzoate</td>
<td>Lubricant</td>
<td>110 mg</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Binder</td>
<td>25 ml</td>
</tr>
</tbody>
</table>
Table 2-7: Effervescent proliposome formulations tested for drug delivery to sinuses

<table>
<thead>
<tr>
<th>Lipid: Carrier ratio</th>
<th>Carrier</th>
<th>Cholesterol</th>
<th>Lipid</th>
<th>Solvent</th>
<th>Drug (mol%)</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10 w/w</td>
<td>Mannitol with effervescent salts</td>
<td>Yes</td>
<td>SPC</td>
<td>Ethanol</td>
<td>-</td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol with effervescent salts</td>
<td>Yes</td>
<td>SPC</td>
<td>Ethanol</td>
<td>5 mol%</td>
<td>BDP</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Effervescent salts alone</td>
<td>Yes</td>
<td>SPC</td>
<td>Ethanol</td>
<td>-</td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Effervescent salts alone</td>
<td>Yes</td>
<td>SPC</td>
<td>Ethanol</td>
<td>5 mol%</td>
<td>BDP</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol with effervescent salts</td>
<td>No</td>
<td>SPC</td>
<td>Ethanol</td>
<td>-</td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol with effervescent salts</td>
<td>No</td>
<td>SPC</td>
<td>Ethanol</td>
<td>5 mol%</td>
<td>BDP</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Effervescent salts alone</td>
<td>No</td>
<td>SPC</td>
<td>Ethanol</td>
<td>-</td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Effervescent salts alone</td>
<td>No</td>
<td>SPC</td>
<td>Ethanol</td>
<td>5 mol%</td>
<td>BDP</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol with effervescent salts</td>
<td>Yes</td>
<td>SPC</td>
<td>Ethanol</td>
<td>5 mol%</td>
<td>XH</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Effervescent salts alone</td>
<td>Yes</td>
<td>SPC</td>
<td>Ethanol</td>
<td>5 mol%</td>
<td>XH</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol with effervescent salts</td>
<td>No</td>
<td>SPC</td>
<td>Ethanol</td>
<td>5 mol%</td>
<td>XH</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Effervescent salts alone</td>
<td>No</td>
<td>SPC</td>
<td>Ethanol</td>
<td>5 mol%</td>
<td>XH</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol with effervescent salts</td>
<td>Yes</td>
<td>DPPC</td>
<td>Ethanol</td>
<td>-</td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol with effervescent salts</td>
<td>Yes</td>
<td>DPPC</td>
<td>Ethanol</td>
<td>5 mol%</td>
<td>BDP</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Effervescent salts alone</td>
<td>Yes</td>
<td>DPPC</td>
<td>Ethanol</td>
<td>-</td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Effervescent salts alone</td>
<td>Yes</td>
<td>DPPC</td>
<td>Ethanol</td>
<td>5 mol%</td>
<td>BDP</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol with effervescent salts</td>
<td>No</td>
<td>DPPC</td>
<td>Ethanol</td>
<td>-</td>
<td>Drug-free</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Mannitol with effervescent salts</td>
<td>No</td>
<td>DPPC</td>
<td>Ethanol</td>
<td>5 mol%</td>
<td>BDP</td>
</tr>
<tr>
<td>1:10 w/w</td>
<td>Effervescent salts alone</td>
<td>No</td>
<td>SPC</td>
<td>Ethanol</td>
<td>-</td>
<td>Drug-free</td>
</tr>
</tbody>
</table>
2.5.2. **Effervescent proliposome formulation (mannitol-based and salt-based)**

The following method was used to manufacture 1:10 w/w lipid to carrier (mannitol or salt) ratio with 10 mol% BDP (5.75 mg) loaded with effervescent proliposome granules with ethanol as a solvent. 1:5 w/w phospholipid (SPC or DPPC) to carrier ratio (mannitol:1250 mg or effervescent salts), sodium bicarbonate (1705 mg), citric acid (1375 mg), and sodium benzoate (110 mg) were weighed and placed in a pear-shaped 250 ml round bottom flask. Phospholipid (83.33 mg) and cholesterol (41.6 mg) (2:1 ratio) were dissolved in 25 ml of ethanol and added to the ingredients. The flask was then attached to the rotary evaporator (Büchi Rotavapor R-114, Büchi, Switzerland) with water adjusted to 35 °C for SPC formulations or 50 °C for DPPC formulations (Figure 2-4). The sample was then left under vacuum for two hours until the solvent evaporated and dried proliposome granules were obtained. The proliposome granules were scraped from the bottom and stored in glass vials at -18 °C. Hydration of the proliposomes and annealing of the manufactured liposomes were carried out prior to further testing by adding 30 mg of effervescent proliposomes in 1 ml of water. Slat-based effervescent samples were made in the same manner omitting mannitol the sugar-based carrier. For samples without cholesterol, the same procedure was continued omitting cholesterol. Liposomes were characterized according to the methods described in sections 2.2 and 2.3 after incorporating the effervescent constituents to the samples. Characterization of proliposomes with and without the effervescent property was compared.

*Figure 2-4: Effervescent BDP-loaded proliposome production.*
2.5.2.1. **Effervescent liposomes coated with mucoadhesive**

Effervescent liposomes were made as described in section 2.5.2. Effervescent proliposomes (150 mg) were hydrated in 5 ml alginic solution or chitosan solutions. Both mucoadhesive alginic acid and chitosan solutions 0.2 W/V % and 1 W/V% were used. Mucoadhesive solutions (5 ml) were used for the hydration of 150 mg of effervescent proliposomes.

2.5.2.2. **Effervescent liposomes with hydrophilic drug XH**

Effervescent liposomes were made as described in section 2.5.2, however, drug loading was done at the hydration phase for the hydrophilic drug by dissolving the drug alone in water and hydrating the effervescent granules (150 mg in 5 ml). Samples were then characterized before and after sonication according to sections 2.2 and 2.3. Sonicated samples were done for 20 sec to downsize the liposome size. Each sample was then centrifuged at a speed of 13,000 rpm for 5 min. The bottom layer of debris was removed from the liposome sample prior to characterization for the sonicated samples.

2.5.3. **Disintegration time for both BDP and XH drugs**

The time taken for the samples to disperse was analyzed by using the same amount of distilled water (5 ml), and the concentration of proliposome granules used in the aqueous phase was 30 mg/ml for all samples. Granules were dispersed in the water and the time taken for dispersed (disintegration) was measured for BDP. The XH drug was weighed and dissolved in distilled water of 5 ml for the 30 mg/ml proliposome sample. Each sample was measured three times by timing it with a stop watch. Samples were taken as dispersed when no solid particles were seen in the bottom. A three second shake was given as soon as the solute was added to the solvent. A mannitol non-effervescent proliposome formulation was used as a control to demonstrate the disintegration of non-effervescent proliposomes and compared time taken to the effervescent sample.
Dispersion time is somehow subjective but it was necessary to check in the initial stages of making an effervescent formulation.

2.6. **Nebulization of Liposomal Formulations**

2.6.1. **Nebulization of the effervescent formulation of drugs and liposomes for drug BDP**

The effervescent granules (30 mg/ml) were allowed to disintegrate in a beaker by the addition of 5 ml of deionized water. Following the completed disintegration of the samples and generation of liposomes from the proliposomes, the resultant dispersion was placed into a Pari Sinus nebulizer reservoir. The following studies have been carried out for both SPC- and DPPC-based effervescent formulations made as previously described in section 2.4.1.

The nebulization took place in front of a vacuum line and the time taken for achievement of ‘dryness’ (i.e. when aerosol generation completely ceased) was determined. The amount of the formulation that was not to be aerosolized was then measured by washing all the parts of the nebulizer with deionized water. The drug fraction delivered from the nebulizer was quantified using HPLC.

2.6.2. **Determination of nebulization time and generation of deliverable liposome from proliposomes for drug BDP**

A volume of 6 ml of deionized water was used to hydrate 180 mg of effervescent powder. Sugar-based and salt-based formulations were tested separately. A volume of 5 ml of water was placed in a Pari Sinus nebulizer with its mouthpiece being directed towards the round bottom flask. The nebulizer was not shaken prior to nebulization. When nebulized to ‘dryness’, sputtering time is recorded when a few minutes of erratic and intermittent nebulization is noted. The sputtering nebulizer was gently hand-tapped to maximize the aerosol output. When aerosol generation was ceased for at least 30 s the time taken for dryness was calculated.
2.6.3. Determination of aerosol size: volume median diameter (VMD), span, and fine particle fraction (% < 5.4 µm)

Aerosol size, span, and fine particle fractions were all analyzed using the Spraytec instrument (i.e. by utilizing laser diffraction to analyze the size distribution of aerosol particles). Each sample was nebulized for 2 min, which was the time at which the nebulizer was directed towards the laser beam of the Spraytec.

2.6.4. Mass output of nebulized aerosols

To calculate the mass output (%), the weight of the nebulizer was weighed before and after nebulization to dryness for the collection of the aerosol. The aerosol mass output is calculated by (mass nebulized – residual volume)/mass nebulized *100.

2.6.5. Determination of drug entrapment before and after nebulization

The entrapment efficiency of the model drugs was determined by placing the liposomes in centrifuge tubes and measuring the unentrapped amount of the drug (i.e. the drug amount that was left in the supernatant) using HPLC. The entrapment efficiency of the drug was calculated as the percentage proportion of the entrapped drug (within the liposome pellet) over the total amount of the drug originally included in the proliposome sample.

To investigate the entrapment of drug in liposomes, separation of liposomes was achieved by centrifugation. Liposomes and BDP crystals have different densities; this was helpful for effective separation via centrifugation, resulting in sedimentation of the suspended drug crystals (i.e. unentrapped drug fraction), while liposomes (with the entrapped drug fraction) was floating on the surface. Batavia et al. (2001) have investigated if deuterium oxide may help the separation of liposomes from unincorporated BDP crystals. Therefore, in this study we investigated if effervescent proliposomes, both mannitol-based and salt-based, would make a difference when suspended in H₂O and D₂O. A proliposome concentration of 30 mg/ml was used for separation. Microscopic study and HPLC analysis were conducted on the liposome formulations. After preparation of the samples,
each sample was centrifuged for 90 min and liposomes (in D$_2$O the floating layer) were removed using a Pasteur pipette. This procedure was adapted from the preliminary work conducted within Dr Elhissi’s research group (Khan et al., 2014) to effectively separate the entrapped drug from the unentrapped fraction (i.e. the free BDP crystals). A light microscope was used to observe the BDP crystals sedimented as a white spot upon centrifugation. HPLC analysis of the Beclometasone dipropionate was conducted at room temperature using the procedure explained in sections 2.5, 2.51, and 2.52.
2.7. Nasal Cast and Impinger Studies

2.7.1. Developing the novel system for aerosol deposition analysis to sinus

A unique system was developed to investigate the effectiveness of deposition in the nasal cavity and sinuses. Existing methods have several limitations due to the location of hidden pockets of sinuses and the fact that parasinuses are non-ventilated nasal pockets. A twin impinger and a transparent nasal cast model coated with Sar-Gel® (water indicating paste) (Figure 2-8 and Figure 2.9) were fixed on to the vacuum of 60 ml/l to mimic active breathing (Figure 2-10). A balloon was used to fix the nasal cast to the impinger and wrapped with cling film. Air was passed through the nasal cast and impinger while liposomes formulations were nebulized.

Figure 2-5: Pari Sinus mouth pieces that were changed in Pari Sprint (only the white diverted part that will be fixed to nostril).
(Source: http://nebology.com/pari-sinus-pulsating-aerosol-compressor-system.html)
The Pari Sinus nebulizer (Pari LC ® Sprint Sinus™ nebulizer attached to a Pari Sinus® compressor) (Figure 2-7) and Pari Sprint nebulizers (Pari LC ® Sprint Nebulizer attached to a PARI Turboboy®SX compressor) (Figure 2-6) (adapted with the parasinus mouthpiece to target the sinuses, Figure 2-4) were used and 20 ml of each sample was nebulized. A nose plug was used to close up one of the nose openings in the nasal cast and nebulized to mimic actual nebulization.

Figure 2-6: Pari Sprint nebulizer.
(Source: http://nebology.com/media/catalog/product/cache/1/image/500x500/9df78eb33525d08d6e5fb8d27136e95/vi/vios-pro-system.png)
Figure 2-7: Pari Sinus nebulizer.
2.7.2. Deposition pattern analysis for nasal cavity and sinuses of the nasal cast

Twenty ml of effervescent proliposome formulations (30 mg/ml) and a control non-effervescent liposomal formulation (based on mannitol with cholesterol and water) were hydrated and nebulized by the Pari Sinus and Pari Sprint nebulizers. A novel system was developed to study the deposition patterns in the nasal cavity and sinuses cast model using a paste Sar-Gel® that turns purple upon contact with water (Figure 5-3). This system contains the two-stage impinger that is connected to the nasal cast, which will allow analysis of both upper and lower respiratory drug deposition in one system. The Pari Sprint nebulizer was modified by replacing the mouth piece of the nebulizer with the nosepiece of the Pari Sinus; thus, aerosols can be directed into the ‘nostril’ of the nasal cast. Effervescent BDP proliposomes (DPPC: Chol coated onto mannitol or salt carrier particles) were hydrated with 20 ml of water (how much lipid concentration) and then nebulized towards the ‘nostril’ of the cast. A nose plug was used to close up one of the ‘nostrils’; hence, permitting efficient deposition via a single nostril to mimic the actual nebulization to the patient. All parameters were kept the same in terms of distance between the nosepiece and nostril, and height of the nasal cast. Nebulization was performed to ‘dryness’ and nebulization deposition patterns were then photographed and images quantified using Adobe® Photoshop (n=3). The pixels were counted and then converted into cubic centimetres for both the full nasal cavity including the sinuses and the sinuses alone. Deposition patterns for both the nasal cavity and sinus for each formulation using both nebulizers were studied (Figure 5-4). Deposition of aerosols generated by the Pari Sinus nebulizer and Pari Sprint devices were compared on the basis of their ability to deliver aerosols to the nasal cavity and specifically the sinuses.
Figure 2-8: Change in nasal cast colour is attributed to the coating with Sar Gel. Non-effervescent DPPC:Chol liposomes using mannitol as carrier following nebulization via the Pari Sinus nebulizer.

Figure 2-9: The deposition pattern is demonstrated in pixels. DPPC:Chol, non-effervescent liposomes made with mannitol carrier and nebulized via the Pari Sinus nebulizer.
Determination of BDP deposition profile in the nasal cast twin impinge model using HPLC

Effervescent mannitol-based DPPC:Chol proliposomes were hydrated to generate liposomes (30 mg/ml), which were compared with the corresponding non-effervescent liposomes. Twenty 5 ml of each for four times, a total of 20 ml of formulation was nebulized via the Pari Sinus or Pari Sprint nebulizers until ‘dryness’ status was reached. The nasal cast was not coated with Sar-Gel® this time, and the deposited contents were collected and analyzed for regional drug deposition within each compartment of the system (nebulizer reservoir, nasal cast, upper stage, and lower stage of the impinger). Collection of the contents was accomplished by washing the nasal cast with 5 ml of HPLC grade water using a pipette, making sure all areas of the cast were washed including the

Figure 2-10: Twin stage impinge and nasal cast system to analyze aerosol deposition in nasal and sinus regions.

2.7.3. Determination of BDP deposition profile in the nasal cast twin impinge model using HPLC

Effervescent mannitol-based DPPC:Chol proliposomes were hydrated to generate liposomes (30 mg/ml), which were compared with the corresponding non-effervescent liposomes. Twenty 5 ml of each for four times, a total of 20 ml of formulation was nebulized via the Pari Sinus or Pari Sprint nebulizers until ‘dryness’ status was reached. The nasal cast was not coated with Sar-Gel® this time, and the deposited contents were collected and analyzed for regional drug deposition within each compartment of the system (nebulizer reservoir, nasal cast, upper stage, and lower stage of the impinger). Collection of the contents was accomplished by washing the nasal cast with 5 ml of HPLC grade water using a pipette, making sure all areas of the cast were washed including the
sinus cavities. The same water was used over again until all parts of the nasal cast were washed thoroughly, then HPLC analysis was conducted as described in section 2.4. Aerosolized formulations to the upper and lower stage of the impinger were separately collected after washing, also for performing HPLC analysis. HPLC analysis was also carried out for residual volume of the nebulizer reservoir. Regional drug deposition was then calculated by quantifying the drug within each compartment divided by the total quantity of drug in all compartments to understand the percentage of regional drug deposition. Regional drug deposition was calculated for the nebulizer reservoir, nasal cast, upper stage, and lower stage of the impinger.

2.7.4. Data analysis

All values were expressed as the mean of three readings from three different experiments and the standard deviations (SD) were calculated. The statistical significance was assessed using the students’ t-tests for comparing two groups, and analysis of variance (ANOVA) for comparing three groups or more. When P values were lower than 0.05 (i.e. <0.05) the difference between the groups was considered statistically significant.
CHAPTER 3
CHARACTERIZATION AND ENTRAPMENT STUDIES OF EFFERVESCENT LIPOSOMES
3.1. Introduction

Liposomes are vesicles used for the delivery of drugs and nutrients. They are made artificially and consist of lipid bilayers. Liposomes were first discovered in 1961 by Dr A. Bangham (Bangham and Horne, 1964). There are many advantages in using liposomes. They provide a controlled and sustained drug delivery, are site-specific and targeted, biodegradable, increase the therapeutic effect of drugs, are able to carry both water and lipid-soluble drugs, and protect against oxidation (Goyal et al., 2005).

The drawbacks of liposomes include the tendency of phospholipids to oxidize and hydrolyze, and the susceptibility of liposomes to microbial contamination during storage (Sangare et al., 1998). Hence, stable powdered phospholipid formulations were introduced; these were referred to as proliposomes (Payne et al., 1986). Since proliposomes are solid formulations, the rate of phospholipid hydrolysis and oxidation is expected to be much lower, offering unique advantages in liposome technology.

Effervescence is an acid-based reaction occurring in water, resulting in the production of carbon dioxide. Effervescent formulations have been used in oral delivery for nearly 200 years (Eichman and Robinson, 1998). Effervescent granules are typically uncoated multiparticulate entities containing carbonates, acid substance, or hydrogen carbonate, which disperse at a rapid rate when they contact water, resulting in carbon dioxide liberation. Effervescent granules disintegrate or dissolve their ingredients and drugs in water, usually within five minutes. Modified release effervescent granules are designed to control the rate of drug release, target the desired areas, and delay the active substance from being released too quickly (Aulton and Taylor, 2013).

Effervescent Ibuprofen proliposomes produced with Soya bean lecithin, Stearylamine, and cholesterol were investigated by Katare et al. (1990). They discovered that liposomes produced from effervescent granules were regular and uniform in size (1–4 µm) with high drug encapsulation efficiency (Katare et al., 1990). High shear force produced by
effervescence gave uniform liposomes, by facilitating the hydration of lipids. Furthermore, Indomethacin effervescent proliposomes were investigated in vivo using rats, which confirmed the superior anti-inflammatory activity of liposomal indomethacin compared to plain drug administration (Katare et al., 1995).

Effervescent proliposomes containing docetaxel for delivery to the lung were produced by solid dispersion technique with docetaxel/Tween-80/Phospholipon 90H/cholesterol/citric, hydrated in NaHCO₃ solution (Zhao et al., 2010). Liposomes loaded with docetaxel were approximately 1 µm, negatively charged and had favourable lung-targeting effect. A similar study by Wei et al. (2013) using paclitaxel, also involved formulations targeted the lung. Liposomes composed of 90H and Tween-80 had a particle size of 8.166±0.459 µm and high drug entrapment efficiency and negative surface charge. These studies demonstrated that effervescent proliposomes are promising drug carriers (Wei et al., 2013).

Conventional proliposomes require vortex or vigorous shaking to disperse in water in order to form liposomes. The aim of this study was to design and compare effervescent proliposome formulations that would disintegrate in water and liberate liposomes in a matter of minutes. This would improve dosing and produce liposomes available for immediate administration. Beclometasone dipropionate (BDP) is a glucocorticoid steroid that is insoluble in water. BDP has anti-inflammatory properties that are effective in hindering inflammatory cells such as mast cells, eosinophils, basophils, lymphocytes, macrophages, and neutrophils production in the human body to produce an immune reaction. BDP also inhibits the release of inflammatory mediators such as histamines, leukotrienes, and cytokines. Monoester, 17 monopropionate (17-BMP) is activated by hydrolysis in vivo by BDP and is demonstrated by its binding affinity for humans’ glucocorticoid receptor (GR), resulting in anti-inflammatory effects (Wang et al., 2011). BDP has serious side effects during long-term oral use that could lead to headaches and
visual changes. Thus, formulation of this drug as effervescent proliposomes will not only enhance its solubility but may also shorten the therapy course by enhancing the drug’s mucoadhesiveness and absorption (Mygind, 1973; Messerli et al., 1975). The hydrophilic drug Xylometazoline hydrochloride is used for the treatment of sinusitis. XH is a topical nasal decongestant that constricts blood vessels of the nose, resulting in reduced inflammation within the nose. However, long-term use of the drug causes side effects such as rebound effect; therefore, preparation of XH in liposomes may reduce the therapeutic dose of the drug and sustain its release, thus reducing the dosing frequency (Castellano and Mautone, 2002). It could also be useful to investigate if effervescent liposomes are capable of entrapping high proportions of XH.

Dipalmitoyl phosphatidylcholine (DPPC) and Soya phosphatidylcholine (SPC) lipids were chosen to prepare separate formulations. Moreover, proliposomes based on effervescent salt carriers have not previously been investigated. In this project, effervescent proliposome formulations with sugar carrier or salt carrier were developed and compared.
3.2. Aims of the Chapter

The aim of this chapter is to investigate the suitability of carbohydrate carriers (e.g. sucrose and mannitol) by characterizing empty and BDP-loaded liposomes in terms of vesicle size, span, zeta potential, and morphology.

This chapter also explores the suitability of ethanol and chloroform as solvents during proliposome preparation by characterizing the resultant liposomes in terms of size, span, and zeta potential.

The chosen carbohydrate carrier (mannitol) and solvent (ethanol) were then used for preparation of effervescent proliposomes. Effervescent proliposomes were compared to conventional liposomes by investigating the disintegration time, and characterizing liposomes in terms of size, span, zeta potential, and vesicle morphology using drug-free and BDP-loaded formulations. SPC or DPPC were used for producing effervescent liposomes.

Effervescent liposomes were made with mannitol and effervescent salts alone (with SPC or DPPC). Liposomes produced were investigated for disintegration time and then characterized using BDP-loaded or drug-free liposomes.

All effervescent formulations made were tested with and without cholesterol to understand the influence of cholesterol on effervescent liposomes. All effervescent liposomes made with either a sugar-based carrier or salt-based carrier (with or without cholesterol) were made with SPC or DPPC and then studied for ability to entrap BDP using HPLC.

Alginic acid and chitosan were used for coating the effervescent BDP-loaded liposomes made with SPC, with or without cholesterol, followed by characterization and drug entrapment studies to understand whether the incorporation of mucoadhesive would be beneficial for improving the drug entrapment.
The hydrophilic drug XH was incorporated into the effervescent liposomes made with the sugar-based carrier (mannitol) or effervescent salt-based (effervescent salts alone) and using SPC with or without cholesterol. All formulations were studied in terms of disintegration time and liposome properties (size, span, and zeta potential).

A novel HPLC method was developed to investigate the entrapment of XH within effervescent liposomes. At the end of this chapter the most suitable carrier is decided, and the potential of effervescent salts was explored for the production of stable liposomes.
3.3. Results and Discussion

3.3.1. Characterization of sugar- and mannitol-based liposomes (conventional) manufactured with SPC lipid

3.3.1.1. Characterization of particulate-based empty liposomes (1:5 w/w lipid to carrier)

Particulate-based proliposomes were made using the slurry method as explained in method section 2.2. Sugar or mannitol particles were coated with SPC (1:5 w/w lipid to carrier), and following hydration, the resultant liposomes were characterized in terms of size, span, and zeta potential.

Drug-free and drug-loaded proliposomes were compared (Figure 3-1) and results indicated that liposomes generated from sucrose-based proliposomes had a VMD measurement of 6.21±0.81 µm. These results correlate with previous findings by Elhissi and Taylor (2005) and Elhissi et al. (2006). Empty mannitol-based liposomes had a size of 6.82±0.44 µm. No statistically significant difference was detected for VMD between sucrose- and mannitol-based proliposomes.

The span was at 2.54±0.360 for sucrose-based proliposomes and 2.21±0.79 for mannitol-based proliposomes (Figure 3-1), with a (P<0.05) significant difference between the two formulations.
Figure 3-1: Sucrose- and mannitol-based liposomes 1:5 w/w lipid to carrier ratio, size (µm), and span analysis of empty liposomes.

Elhissi (2005) stated that SPC:Chol (1:1) span, when using sucrose carrier particles, was 1.68±0.21, correlating with the results obtained with sugar-based proliposomes in the present report. Elhissi et al. (2006) employing particulate-based proliposomes with dimyristoyl phosphatidylcholine (DMPC) reported the span was 0.99±0.06; hence, different phospholipids and different methods of producing proliposomes may cause a difference in the span of the resultant liposomes.

The zeta potential for both formulations are presented in Figure 3-2. Liposomes generated from sucrose-based proliposomes showed a slightly more negative charge of -2.24±0.08 mV, while mannitol-based proliposomes have generated liposomes having a zeta potential measurement of -1.40±0.19 mV. A statistical significance (P≤0.05) was observed between the two formulations. Phospholipids used in the preparation of liposomes are neutral, but liposomes observed in both formulations were slightly negative, which may be due to impurities in SPC or sugar carriers. Literature by Yandrapati (2012) demonstrated phosphatidylcholine lipid with different concentrations
produced highly negative liposomes that were stable. Sharma and Sharma (1997) have stated that the negative surface charge of liposomes may cause them to exhibit greater uptake by the cells via endocytosis. The negatively charged liposomes were also cleared rapidly after systemic administration, helping with the release of the drug. Sharma (2009a) and Sharma et al. (2010) have also reported that positively or negatively charged liposomes tend to be taken up by the reticulo-endothelial system to greater extents, compared to neutral liposomes. Moreover, neutral liposomes tend to aggregate more than charged liposomes; therefore, having negative liposomes may help reduce liposome aggregation, and improve physical stability of the formulation.

![Sucrose and mannitol based liposomes 1:5 w/w lipid to carrier ratio](image)

**Figure 3-2:** Sucrose- and mannitol-based liposomes 1:5 w/w lipid to carrier ratio, zeta potential (mV) analysis of empty liposomes.
3.3.1.2. Characterization of particulate drug-loaded 2.5 mol% liposomes 1:5 ratio (lipid to carrier)

Both carriers were investigated with inclusion of BDP (2.5 mol %). Drug-loaded sucrose liposomes showed a VMD of 5.94±0.94, whereas mannitol-based liposomes demonstrated a VMD of 6.92±1.05, with no statistically significant difference between the two formulations. However, when compared with empty liposomes, it was observed that the size of the sucrose liposomes has slightly decreased, while mannitol-based liposomes showed a slight size increase.

No statistically significant difference was seen between empty and drug-loaded liposomes for both sucrose- and mannitol-based formulations. Elhissi et al. (2006) have stated that liposomes generated from sucrose-based proliposomes had a VMD of 5.23±0.10 and a span of 1.09±0.01 when incorporated with 2.5 mol% BDP, correlating with the results obtained in this study.

The span for sucrose-based liposomes (2.5 mol% BDP) was 2.13±0.22 whereas mannitol-based proliposomes had a span of 2.69±0.40 and the differences were insignificant (Figure 3-3).

![Figure 3-3: Sucrose and mannitol based liposomes (1:5 w/w lipid to carrier ratio) loaded with 2.5 mol% BDP, liposome size distribution (µm) analysis and span of BDP loaded liposomes.](image-url)
The zeta potential of liposomes was also investigated. The surface charge for sucrose-based drug-loaded liposomes was -2.57± 0.98 mV while mannitol-based liposomes had a charge of -1.29± 0.11 mV (Figure 3-4). Sucrose-based liposomes were slightly more negative than mannitol-based liposomes for drug-containing formulations; this was also observed in non-effervescent proliposome formulations.

Considering all of the above results between the two carriers, drug-loaded and empty, mannitol was more appropriate as a carrier, since after loading the drug, liposome size was increased only slightly. It is also widely known that mannitol gives a cooling effect; patients with ‘hot’ inflamed sinuses would probably feel more comfortable when the cooling effect of mannitol occurs in their nasal cavity. Therefore, mannitol was the carrier of choice for the subsequent studies in this report.

Since better treatment for sinuses and reducing nasal irritation is an objective, the use of chloroform might not be appropriate, since any unsuccessfully removed solvent residues may cause epithelial irritation. Ethanol may comprise an alternative to disperse mannitol and dissolve lipids in the preparation of the particulate-based proliposomes. Therefore, 1:5 lipid to carrier ratio using mannitol as carrier and ethanol as solvent was employed to manufacture liposomes, and the resultant vesicles were studied.

Subsequent changes were made to the ratio of lipid and carrier (1:10 ratio) utilizing mannitol with 2.5 mol% BDP. Further investigations were conducted to ascertain whether the 1:10 ratio with 5 mol% BDP has exhibited any change.
Figure 3-4: Sucrose- and mannitol-based liposomes 1:5 w/w lipid to carrier ratio loaded with 2.5 mol% BDP, liposome zeta potential (mV) analysis of BDP-loaded liposomes.

3.3.1.3. Characterization of mannitol-based empty liposomes 1:5 ratio with ethanol as a solvent

Ethanol was compared as a solvent to chloroform. Ethanol is known to be less toxic and cheaper than chloroform. Liposomes using a mannitol carrier and BDP were characterized. The size analysis (Figure 3-5) of empty liposomes was measured at 5.52±2.89 µm, compared to liposomes made with chloroform, which gave a mean value of 6.82 µm. Liposomes with BDP included showed a size of 6.27±1.51 µm.

The span was 2.89±0.44 for empty liposomes, which is very similar to the results achieved when chloroform was used as a solvent, and size distribution was not affected by the change of solvent. Drug-loaded liposomes were observed to have a span of 3.11±0.67.

No significant difference in the span measurements was seen between empty and drug-loaded liposomes for ethanol-based formulation.

The zeta potential of empty liposomes was -1.81±0.101 mV, while drug-loaded liposomes had a zeta potential of -2.07±0.59 mV (Figure 3-6). Similar to results achieved previously, no significant difference was seen between the results. Since size, span, and zeta potential
all correlated with previous results and especially since the size was slightly smaller when ethanol was used, it was decided to continue further research with mannitol as carrier and ethanol as solvent to cast the lipid film on the carrier particles.

**Figure 3-6:** Mannitol liposomes 1:5 w/w lipid to carrier ratio made with ethanol as a solvent, loaded with 2.5 mol% BDP. Liposome sizes (µm) and span analysis of empty and BDP-loaded liposomes.

**Figure 3-5:** Mannitol liposomes 1:5 w/w lipid to carrier ratio made with ethanol as a solvent, 2.5 mol% BDP, liposome zeta potential (mV) of empty and BDP-loaded liposomes.
3.3.1.4. Characterization of mannitol-based empty liposomes (1:10 ratio) using ethanol as a solvent

Since the research now achieved has served to finalize the thorough results that mannitol works best as a carrier and ethanol would be a better solvent, it was decided to change the lipid to carrier ratio to explain the comparisons. The size of empty liposomes was 5.71±0.519 µm correlating with the results obtained by the 1:10 ratio liposomes made with chloroform as a solvent; this indicates that ethanol is a suitable solvent to make liposomes. Size appeared to increase following entrapment of BDP, being 7.71±0.68 µm (Figure 3-7).

The span was observed to be 4.26±0.89 for empty liposomes and 4.01±0.44 for liposomes loaded with the drug for mannitol-based liposomes made using liposomes. The span has increased minimally when using chloroform. In addition to its higher safety and lower cost, ethanol produced liposomes with a smaller size than chloroform. The zeta potential of empty liposomes was seen at -1.72±0.69 mV while liposomes with drug were -3.02±0.70 mV (Figure 3-8). Liposomes with the drug elicited a trend for a more negative charge than empty liposomes (P>0.05).
Figure 3-7: Mannitol-based liposomes 1:10 w/w lipid to carrier ratio with 2.5 mol% BDP, liposome size (µm) and span analysis of empty and BDP loaded liposomes.

Figure 3-8: Mannitol-based liposomes 1:10 w/w lipid to carrier ratio with 2.5 mol% BDP, liposomes zeta potential (mV) of empty and BDP-loaded liposomes.
3.3.1.5. Characterization of mannitol-based empty and drug-loaded liposomes
1:10 ratio with ethanol as a solvent with 5% BDP

Liposome size shown with 2.5 mol% in 1:10 ratio and consequently 1:10 ratio was
chosen. Liposomes were characterized with 5 mol% BDP in 1:10 lipid to carrier ratio. A
range of studies have focused on using BDP in 5 mol% as the maximum concentration
for the drug to be entrapped in liposomes. Earlier experiments were also conducted with
2.5 mol% in order to reduce drug wastage, if no greater entrapment would be achieved.
On formulation of effervescent liposomes, it was believed that 1:10 lipid to mannitol
carrier ratio using ethanol as the solvent for casting the thin film and the drug in 5 mol%
would possibly be most appropriate for subsequent characterization.
The size of empty liposomes was 5.27±0.16 µm, whereas after loading the drug it was
observed that the size was 8.25±0.50 µm (Figure 3-9). A significant difference (P≤0.05)
was seen when the size of empty and drug-loaded liposomes was compared. There was
no significant difference observed for the size and span between liposomes
accommodating 2.5 and 5 mol% BDP.
The zeta potential for empty liposomes was -1.62±0.42 mV while after loading the drug
it became -3.72±0.25 mV (Figure 3-10) with a significant difference between empty and
drug-loaded vesicles.
Figure 3-9: Mannitol-based liposomes 1:10 w/w lipid to carrier ratio with 5 mol% BDP, liposome size (µm) and span analysis of empty and BDP-loaded liposomes.

Figure 3-10: Mannitol-based liposomes 1:10 w/w lipid to carrier ratio, with 5 mol% BDP, liposome zeta charge (mV) of empty and BDP-loaded liposomes.
3.3.2. Scanning electron microscopy (SEM) for conventional liposome

3.3.2.1. Surface morphology of sucrose-based proliposomes

Scanning electron microscopy pictures looked at the surface morphology of sucrose-based proliposomes. This was observed at x 500 magnification for both sucrose particles (Figure 3-11) and drug-loaded proliposomes (Figure 3-12). Both samples showed uniform phospholipid coating. The surface of sucrose particles appeared glossy; this may be explained by the sticky nature of phospholipid. Particles were irregular but more cubed in shape and some of the particles had aggregated. Similar SEM pictures were observed for sucrose in a study done by (Wulkersdorfer et al. 2010). There was no difference observed between drug-free proliposome particles and BDP-loaded proliposomes.

Figure 3-12: Sucrose-based proliposomes. A typical observation of three different experiments.

Figure 3-11: Sucrose-based proliposomes particles with 2.5 mol % BDP drug and sucrose-based proliposomes. A typical observation of three different experiments.
3.3.2.2. Surface morphology of mannitol-based proliposomes

In comparison to the morphology of sucrose particles (Figure 3-13) that are irregular and have cube shapes, mannitol-based proliposomes were cylindrical crystals. Yan-yu et al. (2006) compared the difference of mannitol crystal to mannitol-based proliposomes. The results shown in Figure 3-14 correlate with their finding, showing a glossy appearance when compared to typical mannitol crystals, indicating uniform coating with phospholipid was achieved.

3.3.3. Effervescent BDP proliposome using SPC phospholipid

3.3.3.1. Investigation of effervescence of formulations

To the knowledge of the author of this report, no work has been done with BDP effervescent proliposomes formulation for targeting the sinuses via aerosolization.

As mentioned in chapter 2, using 1:10 lipid to carrier ratio (section 2.2.3), and mannitol as carrier particles, proliposomes were manufactured. This was achieved without drug or with inclusion of BDP (5 mol%) followed by characterization studies (size, span, and zeta potential).
3.3.3.2. Drug-free effervescent formulations

Effervescent formulations were prepared without drug to compare conventional liposomes to effervescent liposomes loaded with drug. Mannitol-based effervescent formulation and liposomes without a carbohydrate-based (effervescent salts alone used as carrier) carriers were investigated. Both formulations generated liposomes upon liberation of carbon dioxide that was induced by the acid-base reaction in the formulation. Mannitol-based liposomes had a size measurement of 5.06+0.12 µm (Figure 3-15); results correlated with the previous results showing a similar size to liposomes without effervescent. In fact, span was smaller (span = 3.07) compared to normal liposomes, indicating slightly less aggregated liposomes (Figure 3-16); hence, effervescence may have contributed to disaggregating the liposomes, resulting in narrower size distribution. Sodium chloride salt was used as a possible carrier previously by other investigators (Payne et al., 1986; Yan-yu et al., 2006). Since salts might be used in formulating proliposomes, the possibility of formulating effervescent liposomes using salts as a carrier was investigated in this report. Salt-based liposomes generated liposomes having a very large size (20.60+3.74 µm) with a relatively high span value of 3.74 (Figure 3-16), indicating aggregation of the liposomes.

The zeta potential values were also different compared to mannitol-based liposomes (Figure 3-16). While liposomes generated from mannitol-based proliposomes were negatively charged (-1.86±0.60 mV), correlating with non-effervescent liposomes, salt-based proliposomes generated vesicles with positive zeta potential measurements (+1.19±0.16 mV).
**Liposome size of SPC lipid based effervescent formulations**

![Graph showing liposome size of SPC lipid based effervescent formulations](image)

**Figure 3-15:** Liposome size of SPC lipid-based effervescent formulations, 5 mol% BDP-loaded.
Figure 3-16: Liposome span analysis of SPC lipid-based effervescent formulations, 5 mol% BDP-loaded.

Figure 3-17: Zeta potential (mV) analysis of effervescent liposome formulations of SPC lipid-based formulations, 5 mol% BDP-loaded liposomes.
3.3.3.3. Drug-loaded effervescent formulations

BDP-loaded effervescent formulations were studied for size, span, and zeta potential. Drug-loaded effervescent mannitol-based liposomes had a size of $5.14 \pm 0.49 \, \mu m$ while effervescent salt-based liposomes had a size of $20.27 \pm 6.55 \, \mu m$. When comparing mannitol-based to salt-based formulations a significant difference was observed in terms of measured size. Moreover, salt-based liposomes had a span of $2.96 \pm 0.77$ while mannitol-based samples had a span of $4.41 \pm 2.72 \, mV$. Zeta potential for mannitol-based formulations correlated with all previous mannitol-based formulations, having a measurement of $-1.86 \pm 0.67 \, mV$, while salt liposomes were positively charged ($+0.89 \pm 0.85 \, mV$). Since the size of salt-based liposomes was relatively large, it was decided to omit cholesterol from the formulation to investigate if this would affect the results. Mannitol-based liposomes showed that the size of $7.33 \pm 0.45 \, \mu m$ slightly increased with drug when cholesterol was omitted. The size of vesicles was relatively large compared to liposomes with cholesterol ($P < 0.05$).

Salt-based proliposomes also showed a drastic difference in size when investigated without cholesterol. Size was observed to be $6.04 \pm 0.19 \, \mu m$ when cholesterol was omitted. Moreover, when cholesterol was excluded from the formulations, there was no significant difference between mannitol samples and salt samples. Therefore, it is justified to state that the presence of cholesterol as an effervescent ingredient can result in a huge increase in the size of liposomes when salt was used as a carrier in preparing proliposomes.

The span of liposomes using mannitol as a carrier without using cholesterol was $2.59 \pm 0.41$, and salt-based liposomes without cholesterol had a span of $2.74 \pm 0.16$. There was no significant difference between the samples with cholesterol and without cholesterol when mannitol was used as carrier.

The zeta potential for mannitol-based proliposomes was $-0.68 \pm 0.24$ while salt-based proliposomes generated liposomes having positive surface charge ($1.19 \pm 0.50 \, mV$).
(Figure 17). Omitting cholesterol has not changed the charge of liposomes generated from salt-based proliposomes (Kokkona et al., 2000).

Tseng et al. (2007) found that by increasing cholesterol concentration, liposomal stability increased. Liposomes with higher cholesterol concentrations were able to withstand more shear stress compared to liposomes with lower cholesterol concentrations. Cholesterol has an impact on the integrity of liposomes (Tseng et al., 2007). Cholesterol molecules are positioned between the free spaces of the unsaturated lipids of the liposome bilayers, leading to decreased flexibility of the surrounding lipid chains (Figure 3-18). Thus, the difference of size as a result of cholesterol inclusion might be attributed to the different packing profile of the liposome bilayers.

![Figure 3-18: Location of cholesterol molecules within phospholipids bilayer.
(Source: http://www.uic.edu/classes/bios/bios100/lectf03am/cholesterol.jpg)](http://www.uic.edu/classes/bios/bios100/lectf03am/cholesterol.jpg)

Liposomes are usually stable at different pH values, but in pancreatic lipase they rapidly lose their entrapped material. Liposomes made with phosphatidylcholine and cholesterol immersed in cholate salts were investigated at different concentrations of cholate salts to see if any changes would be observed in the size of liposomes. A decrease of 20% in size after immediate addition of the salts was observed for liposomes that are free from
cholesterol (Kokkona et al., 2000). The protective characteristics of cholesterol may help in the presence of sodium cholate salt, which is attributed to the rigidity of the bilayers upon incorporation of cholesterol, preventing reduction in liposome size (Kokkona et al., 2000).

Liposomes exhibit properties similar to that of the biological membrane. One such property is the ability of water to pass in and out through the liposome’s semi-permeable membranes. Bangham and co-workers (1967) illustrated that liposomes are a good model to study the osmotic properties and water permeability of phospholipid liquid crystals. When liposomes were immersed in a hypertonic surrounding (e.g. because of the effervescent salts) and the inside of the liposomes have more water (hypotonic) than outside, this may result in water movement from inside the vesicles to outside, possibly causing liposomes to shrink (Hupfeld et al., 2010). Therefore, without the protective function of cholesterol in effervescent samples, liposomes may not be able to withstand the external osmotic pressure, resulting in a size decrease of liposomes because of shrinkage. This was further reported in 2006, when the stability of liposomes was studied by introducing a hydrated radius of the adsorbed ions on to the liposome’s surface by extending Derjaguin-Landau-Verwey-Overbeek (DLVO) theory. The theory explains force changes when interacting between a liquid medium and a charged surface. The double layer electrostatic repulsion and Van der Waals attraction combined and resulted in the DLVO effect. The presence of ions resulted in a decrease in liposome size when certain ions did not pass through the membrane, resulting in an osmotic force leading water to pass from inside to outside the liposome vesicles (Sabin et al., 2006). Ions present in effervescent content may also result in a decreased size when the protective effect of cholesterol is not present.

In summary, when only taking into account the characteristics of liposomes in the presence of effervesce, it is reasonable to say mannitol-based liposomes with cholesterol
would be the best choice of effervescent samples for sugar-based formulations, while salt-based samples without cholesterol would be more suitable for further investigations in terms of size, span, and zeta potential. Physical characterization alone cannot decide if a formulation would be suitable for therapeutic use; therefore, further studies are needed in the future.

3.3.3.4. Appearance of effervescent formulations

A study by (Katare et al., 1990; Katare et al., 1995a) have hypothesized that Ibuprofen effervescent proliposomes, upon addition of water, have generated liposome dispersions as a result of shaking provided via the liberation of carbon dioxide. These results correlated with the study in this report using BDP, resulting in the formation of liposomes having narrow size distribution.

Disintegration of proliposome granules was carried out as described earlier (chapter 2; section 2.5.3) via the determination of time taken for the sample to disperse in distilled water (5 ml; 30 mg/ml). The end point for full disintegration is achieved when no solid particles are seen in the bottom of the vial. Three seconds of shaking was provided as soon as the proliposomes were added to water. Mannitol non-effervescent proliposome formulation was used as a control for comparison with effervescent proliposome disintegration.

Table 3-1: Dispersion time analysis of SPC-based effervescent proliposomes

<table>
<thead>
<tr>
<th></th>
<th>Mannitol with cholesterol</th>
<th>Salt with cholesterol</th>
<th>Mannitol without cholesterol</th>
<th>Salt without cholesterol</th>
<th>Mannitol non-effervescent proliposomes (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>1.21±0.22</td>
<td>0.53±0.04</td>
<td>1.23±0.15</td>
<td>0.4±0.03</td>
<td>56.51±1.87</td>
</tr>
</tbody>
</table>

The non-effervescent formulation took about one hour to fully disperse. Without any shaking, solids were observed at the bottom and shaking was necessary for all solids to dissolve completely in the control sample. By contrast, the effervescent sample took less
than 1.5 min to completely disintegrate, without observing any solids at the bottom of the vial.

Mannitol-based proliposomes with cholesterol was observed to be dispersed after 1.21 min of water addition. Mannitol-based proliposomes without cholesterol also had a similar dispersion time at 1.23 min (Table 3-1). Both mannitol-based samples (Figure 3-20, Figure 2-22) showed comparatively less form compared to salt-based samples and was shown to have less agitation compared to salt-based samples. Both samples showed a milky colour after dispersion observed, with very little foam.

Salt-based samples (Figure 3-20), on the other hand, showed a significant difference in disintegration time compared to mannitol-based samples. Less than half the time was taken to disperse effervescent salt-based samples. A very large amount of foam was observed upon hydration, which immediately diminished (Figure 3-21).
Figure 3-19: Appearance of mannitol-based effervescent liposome sample (more foam), milky appearance.

Figure 3-20: Appearance of salt-based effervescent liposome sample (more foam) milky appearance.

Figure 3-21: Appearance of mannitol and salt–based effervescent liposome samples together, foam goes down after a while giving both the samples similar appearance.
3.3.4. **SEM picture of effervescent proliposomes (mannitol- and salt-based)**

As seen in Figure 3-22 and Figure 3-23, effervescent mannitol-based proliposomes had needle-like glossy crystals. Effervescent salt-based samples shown in Figure 3-24 and Figure 3-25 had less spiky structures compared to mannitol-based formulations, and uniformly distributed phospholipid coating was observed.

![Figure 3-22: SEM picture of mannitol-based sample with cholesterol.](image)

![Figure 3-23: SEM picture of mannitol-based sample with cholesterol.](image)

![Figure 3-24: SEM picture of mannitol-based sample without cholesterol.](image)

![Figure 3-25: SEM picture of salt-based sample without cholesterol](image)
3.3.5. HPLC results of BDP-loaded effervescent samples

3.3.5.1. Entrapment studies of BDP for SPC-based formulations

BDP entrapment of effervescent formulations was tested with water and D$_2$O as described in chapter 2, section 2.4. In Figure 3-26, BDP entrapments are determined as a percentage in both water and D$_2$O.

Table 3-2: Drug entrapment efficiency for BDP-loaded liposomes (%) in water and D$_2$O

<table>
<thead>
<tr>
<th></th>
<th>Mannitol with cholesterol</th>
<th>Salt with cholesterol</th>
<th>Mannitol without cholesterol</th>
<th>Salt without cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>98±0.69</td>
<td>97±1.46</td>
<td>86±13.19</td>
<td>81±15.81</td>
</tr>
<tr>
<td>D$_2$O</td>
<td>20.54±12.02</td>
<td>29±13.49</td>
<td>8±1.79</td>
<td>11±3.40</td>
</tr>
</tbody>
</table>

The HPLC study demonstrated that all peaks of BDP were symmetrical and had a retention time of 4.5 min. A calibration curve was constructed for BDP using drug concentrations of 5-40 µg/ml. The highest level of entrapment was observed for mannitol-based proliposomes with cholesterol effervescent at 98±0.69%. Salt-based proliposomes with cholesterol caused the entrapment to become 97±1.46%. No significant difference was observed between these two formulations. However, mannitol-based proliposomes without cholesterol demonstrated a lower entrapment of 86±13.19%. Moreover, salt-based proliposomes without cholesterol also displayed an entrapment of 81±15.81%.

Entrapment studies using D$_2$O as a separation medium displayed completely different results, but cholesterol was still advantageous at enhancing BDP entrapment. In contrast to water, samples dispersed using D$_2$O exhibited that salt with cholesterol formulations was able to entrap more BDP with an entrapment of 29±13.49%. By contrast, mannitol-based samples with cholesterol exhibited an entrapment of 20.54±12.02%
Liposomes without cholesterol based on mannitol as a carrier in effervescent formulations resulted in an entrapment of 8.39±1.79%, while salt-based proliposomes presented a slightly higher trend for entrapment of 11.28±3.40%, with no significant difference between the two samples. Moreover, lower entrapment in the salt-based formulations in the absence of cholesterol was observed. No significant difference was observed when comparing mannitol-based samples with and without cholesterol for samples dispersed in D₂O. Results demonstrated that the presence of cholesterol did not affect BDP entrapment. When comparing salt-based samples with and without cholesterol, no significant difference in entrapment was observed.

Radhakrishnan (1991) stated that achieving 100% encapsulation efficiency is challenging due to the encapsulation percentage having dependence on the lipid properties and concentration, and on the encapsulation method used. It was further discussed that liposomes made without cholesterol have less flexibility to encapsulate the drug; only 1–3 mol% of steroidal drugs was seen entrapped in formulations without cholesterol (Radhakrishnan, 1991). This correlates with the results obtained in the present study. Formulations without cholesterol demonstrated lower encapsulation efficiency of BDP. It was also elaborated that fluffy hydrogenated soy phosphatidylcholine may not be the most suitable candidate in the lipid encapsulation of steroidal drugs. Liposomes made with egg phosphatidylcholine (EPC) and BDP have shown an entrapment of 75% using water as a dispersion medium. However, the experiments undertaken obtained a higher entrapment efficiency of BDP with SPC phospholipid.

BDP entrapment efficiency of 100% was noted when water was the dispersion medium for liposomes prepared using the ethanol injection method (Jaafar-Maalej et al., 2010). Moreover, the amounts of lipid and cholesterol have affected the size and encapsulation efficiency of liposomes. Studies done by Jaafar-Maalej et al. (2011) also reported an entrapment efficiency of 98% for BDP.
Darwis and Kellaway (2001) have reported that liposomes have a low encapsulation efficiency of steroidal drugs, due to the geometric structure of steroidal drug molecules which offer a limited interaction with the liposome bilayers. Liposome ability to accommodate BDP was reported to be inversely proportional to the transition temperature \((T_m)\) of the phospholipid employed. The higher the \(T_m\) of the phospholipid the lower the entrapment efficiency. The size of liposomes may also affect drug encapsulation, since small multilamellar vesicles offered lower drug entrapment compared to larger multilamellar vesicles.

When using D\(_2\)O to investigate entrapment there is a need to separate the liposomes from the suspension. This process may lead to disruption of liposomal bilayers, with concomitant leakage of BDP. Batavia et al. (2001) have reported that using several stages of separation may be disadvantageous and the use of D\(_2\)O can affect the partitioning behaviour of the drug between the liposome bilayers and the surrounding aqueous environment.

### 3.3.5.2. Microscopy investigation of BDP crystal sediment

Steroids and steroid esters have low solubility in phospholipid formulations (Fildes and Oliver, 1978). Batavia et al. (2001) found an alternative method of separating entrapped liposomes from BDP crystals. Excess BDP tends to crystallize due to their incompatible steric fit with the liposome bilayers. Batavia et al. (2001) investigated the separation of liposomes and BDP crystals, stating that BDP crystals have a size and density that are comparable to those of liposomes, hence separation is difficult; therefore, density gradient centrifugation is necessary for the reliable separation of liposomes (with entrapped drug) and free drug that tends to sediment as crystals. The density difference between D\(_2\)O and water facilitates the separation of BDP crystals and liposomes. BDP crystals were investigated using light microscopy. Crystalline spots of BDP appeared at the bottom of the centrifuge tubes (Figures 3-26 and 3-27, 3:28). This indicates that by using D\(_2\)O,
effective separation of BDP-entrapped liposomes (floating at the surface) from BDP crystals (sedimenting at the bottom) was achieved.

Figures 3-26 and 3-28 represent light microscopy pictures of BDP spot showing the presence of BDP crystals alone, with no significant observation of liposomes. Salt-based liposome samples and mannitol-based liposomes appeared to have similar types of BDP crystals.

**Figure 3-26:** Microscopy investigation of BDP crystal sedimentation of effervescent mannitol based with cholesterol formulation 40X.

**Figure 3-27:** Microscopy investigation of BDP crystal sedimentation of effervescent mannitol based without cholesterol formulation 40X.

**Figure 3-28:** Microscopy investigation of BDP crystal sedimentation of effervescent salt based with cholesterol 40X.
3.3.6. Mucoadhesives in SPC formulations

Formulations with 1:10 w/w lipid to carbohydrate carrier (mannitol) with cholesterol formulations and 1:10 w/w lipid to salt without cholesterol formulations were chosen to be tested with mucoadhesive. Samples were made according to chapter 2, section 3.3.3 using the slurry method and were hydrated in alginic acid and chitosan solutions (0.2% w/v or 1% w/v). In this part, the effervescent formulations that demonstrated overall suitability (size, span, zeta potential, and entrapment) of previous studies for targeting the paranasinuses were chosen. Mucoadhesive polymers such as alginic acid or chitosan were incorporated to improve the bioadhesive properties of the formulations.

3.3.6.1. Mannitol-based formulation containing alginic acid

Non-effervescent liposomes with the 1:10 w/w lipid to carrier ratio generated liposomes with alginic acid (0.2% w/v), with size (17.99±0.56 μm), while both drug-free effervescent liposomes (9.47±0.12 μm) and drug containing effervescent liposomes (6.15±0.04 μm) had smaller size. There was no significant difference between drug-loaded non-effervescent liposomes and effervescent liposomes. Non-effervescent liposomes with 1% w/v alginic acid produced smaller liposomes (7.26±0.60 μm) compared to non-effervescent liposomes with 0.2% w/v alginic acid. Effervescent liposome size generated by alginic acid (0.2% w/v) is smaller compared to 1% w/v alginic acid, which produced liposomes with a much larger size (12.25±0.07 μm) in the presence of BDP (Table 3-3).

The span of mannitol-based formulations for alginic acid (0.2% w/v) seem to be more suitable compared to non-effervescent liposomes in terms of size distribution (span = 3.763±0.1), indicating less aggregation. Mannitol-based effervescent formulations with alginic acid (1% w/v) have significant span values in comparison to formulations hydrated in 0.2% w/v alginic acid (P≤0.05) (Table 3-3). Non-effervescent and effervescent formulations with the drug also demonstrated significant differences in span.
for 0.1% w/v formulation (P≤0.05). Incorporation of alginic acid appears to increase the negative charge of the liposomes when compared to samples that did not contain alginic acid. Alginic acid with a concentration of 0.2% w/v was deemed to increase the negative charge of the effervescent liposomes in general when compared to 1% w/v concentration (Table 3-3). The zeta potential in liposome charge was observed for non-effervescent liposomes with 0.2% w/v (-17.76±1.05 mV) while 1% w/v formulations had a zeta potential of (-42.03±4.0). Formulations of 0.2 w/v seem to be the most suitable for mannitol-based formulations in terms of size, span, and zeta potential.

**Table 3-3: Characterization of non-effervescent and effervescent mannitol-based liposomes made with SPC, coated with mucoadhesive alginic acid**

<table>
<thead>
<tr>
<th>Alginic acid concentration</th>
<th>Size (μm)</th>
<th>Span</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2% W/V (With drug, non-effervescent liposomes)</td>
<td>17.99±0.56</td>
<td>3.87±0.11</td>
<td>-17.76±1.05</td>
</tr>
<tr>
<td>0.2% W/V (Empty, effervescent liposomes)</td>
<td>9.47±0.12</td>
<td>2.47±0.04</td>
<td>-16.1±0.5</td>
</tr>
<tr>
<td>0.2% W/V (with drug, effervescent liposomes)</td>
<td>6.15±0.04</td>
<td>3.76±0.14</td>
<td>-18.63±0.9</td>
</tr>
<tr>
<td>1% W/V non-effervescent liposomes (with drug)</td>
<td>7.26±0.60</td>
<td>5.35±0.6</td>
<td>-42.03±4.0</td>
</tr>
<tr>
<td>1% W/V (empty, effervescent liposomes)</td>
<td>11.0±0.07</td>
<td>2.5±0.15</td>
<td>-12.4±0.65</td>
</tr>
<tr>
<td>1% W/V (with drug, effervescent liposomes)</td>
<td>12.25±0.07</td>
<td>2.9±0.15</td>
<td>-12.5±0.5</td>
</tr>
</tbody>
</table>
3.3.6.2. **Salt-based formulation containing alginic acid**

Liposomes produced with effervescent salts alone, without mannitol and without cholesterol, were analyzed after incorporation of mucoadhesive alginic acid. Unlike mannitol-based formulations there was no significant size difference between non-effervescent liposomes loaded with drug and effervescent liposomes loaded with drug BDP with 0.2% w/v alginic acid. No size difference was observed between effervescent empty and drug-loaded liposomes for 0.2 %w/v alginic acid concentration. A significant size difference was observed for effervescent salt-based liposomes without the drug (P≤0.05) between 0.2% and 1% concentration of alginic acid. Salt-based formulations with 1% w/v formulation demonstrated a significant (P≤0.05) difference between drugs loaded and empty (Table 3-4). Size distribution differences between 0.2% w/v empty and drug-loaded liposomes were observed to be significant (P≤0.05) (Table 3-4). Liposomes hydrated in 0.2% w/v concentration without drug seem to be less aggregated compared to 1% w/v formulation (P≤0.05). Both concentrations seem to be giving negative values with similar size and span distribution, demonstrating that in salt-based formulations both concentrations may be suitable in terms of size, span, and zeta. However, 1% w/v would be the most suitable overall due to its smaller size compared to 0.2% w/v formulation.
3.3.6.3. **Entrapment study using HPLC for alginic mucoadhesive**

Entrapment studies were conducted according to chapter 2, section 2.4. Coating the liposomes with bioadhesive agents highly affected the entrapment of BDP. Non-effervescent liposomes were used as a control and coated with alginic acid for comparison. Entrapment was very high for conventional liposomes upon coating with alginic acid. Alginic acid with 2% w/v concentration for mannitol-based formulations demonstrated an entrapment efficiency of 63.53±7.10% while 1% w/v bioadhesive demonstrated an entrapment of 66.34±5.43%. However, this was not observed to be the same for improved effervescent formulations.
Entrapment results for alginic acid (0.2% w/v) in the mannitol formulation showed a very low entrapment efficiency (1.04±1.05%) while 1% showed no drug entrapment at all. However, alginic acid did demonstrate an increase in entrapment with salt-based formulations, 0.2 w/v alginic acid demonstrated 30.07±31.11% entrapment while salt-based 1 w/v% concentration demonstrated an entrapment of 46.41±34.33, but both concentrations demonstrated high stranded deviations with variable results and the results were not reproducible. As a whole this indicates that alginic acid does not improve the entrapment of drugs in liposomes. In fact, it reduces the ability to entrap drugs in the presence of effervescent salts.

Alginic acids are naturally occurring polysaccharide polymers (Tønnesen and Karlsen, 2002). Tønnesen and Karlsen reported that alginates can go through proton-catalyzed hydrolysis depending on time, PH, and temperature. Alginates also tend to increase solution viscosity due to intermolecular binding upon hydration. Data indicate liposome-improving entrapment upon coating with alginic acid in conventional non-effervescent liposomes; however, this was not observed in effervescent formulations. Effervescent mannitol and effervescent salt formulations caused alginic acid to behave differently compared to its behaviour in conventional liposome formulations (Tønnesen and Karlsen, 2002). Another possible reason for reduced entrapment of the drug in the presence of alginic acid could be due to alginic acid alone being entrapped in the core of the liposomes, causing the liposomes to burst, resulting in leakage of BDP from the bilayers (Hong et al., 2008). Alginic acid also forms soluble salts in the presence of monovalent metal ions while divalent and multivalent cations (except Mg$^{2+}$) result in the formation of gels. Alginic acid results in swelling, transmittancy, and viscoelasticity depending on its M residue and G residue ratio (Tønnesen and Karlsen, 2002). The swelling property is usually used in activating the release of drugs trapped in alginic acid gel. However, in this case amplified swelling in the presence of salts may possibly have disrupted the
liposomes, causing drug leakage. Alginic acid itself could be in competition with the drug for entrapment in the liposomes, resulting in reduced drug association with the lipid bilayers.

3.3.6.4. Chitosan as a mucoadhesive used in effervescent mannitol-based liposomes and salt-based liposomes

As drug entrapment in effervescent liposomes was not improved with the addition of alginic acid, chitosan was employed as an alternative mucoadhesive polymer. The liposome size was different when chitosan was included, with high aggregation being observed in comparison to alginic acid. In general, chitosan (0.2%) caused increases in liposome size; however, mannitol formulation exhibited smaller size compared to the rest of the formulations (21.63±5.57 μm) (Table 3.5). Liposome size was found to be significantly larger (P<0.05) (130.47±15.05 μm) for liposomes coated with 1% chitosan. Highly negative zeta potential measurements were observed for all formulations with chitosan in comparison to those incorporating alginic acid. Significant differences were observed for the zeta potential when the two concentrations of chitosan were compared in mannitol- and salt-based formulations (Table 3-5). Literature indicate uncoated conventional DPPC liposomes are generally negatively charged (Klein et al., 1987; Law et al., 1988; Mady and Darwish, 2010). Studies indicate chitosan carries a highly positive charge; hence, the coating of liposomes with chitosan shift slightly negatively charged liposomes to positively charged liposomes (Guo et al., 2003; Mady and Darwish, 2010). Liposomes become increasingly positive as chitosan concentration increases; hydrogen bonding between polysaccharide and phospholipid head groups results in this positive zeta potential (Guo et al., 2003; Perugini et al., 2000). However, in contrast, effervescent liposomes coated with chitosan demonstrates the zeta potential of liposomes to be highly negative. Studies suggest zeta potential of chitosan was close to zero at alkaline pH of the amino group. Researchers further suggests the surface charge of chitosan is related to the
chemical groups present in the formulation (Kim, 2013). This could explain the unexpected negative charge observed on effervescent proliposomes. Doubling and crystalline of chitosan in the presence of effervescent salts may also affect surface charge of the liposomes, resulting in negatively charged liposomes.

Table 3-5: Characterization of BDP-loaded effervescent liposomes coated with mucoadhesive chitosan

<table>
<thead>
<tr>
<th>Chitosan concentration (w/v)</th>
<th>Carrier</th>
<th>Size (μm)</th>
<th>Span</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2% Mannitol</td>
<td>21.63±5.57</td>
<td>10.78±0.57</td>
<td>-20.9±1.53</td>
<td></td>
</tr>
<tr>
<td>1% Mannitol</td>
<td>130.47±15.05</td>
<td>2.81±0.11</td>
<td>-28.33±2.63</td>
<td></td>
</tr>
<tr>
<td>0.2% Salt</td>
<td>42.22±9.51</td>
<td>5.39±0.29</td>
<td>-10.06±7.78</td>
<td></td>
</tr>
<tr>
<td>1% Salt</td>
<td>114.50±11.68</td>
<td>2.24±0.14</td>
<td>-32.03±1.96</td>
<td></td>
</tr>
<tr>
<td>0% Mannitol</td>
<td>5.14±0.49</td>
<td>4.4±2.72</td>
<td>-1.86±0.67</td>
<td></td>
</tr>
<tr>
<td>0% Salt</td>
<td>6.04±0.19</td>
<td>2.74±0.16</td>
<td>1.19±0.50</td>
<td></td>
</tr>
</tbody>
</table>

3.3.6.5. Entrapment studies in liposomes generated from proliposomes using chitosan polymer

For mannitol-based formulations, the entrapment of BDP using chitosan (0.2%) was as low as 3.91±6.5.2% while with 1% chitosan it was as high as 37.10±34.38. Chitosan was observed to be swelling in the presence of effervescent salts. Salt-based formulations demonstrated no entrapment with both concentrations of chitosan. These results indicate that the addition of a mucoadhesive results in aggregated liposomes with intense negative charge and lower drug entrapment for salt-based effervescent formulation. It can be concluded that effervescent formulations, both mannitol- and salt-based formulations, are best kept without the addition of a mucoadhesive. Therefore, it was decided to exclude mucoadhesives in subsequent investigations.
3.3.7. Characterization of DPPC-based empty liposomes

Dipalmitoyl phosphatidylcholine (DPPC) was investigated as a replacement for SPC, and the resultant liposomes were characterized. Forty per cent of lung surfactants are made from DPPC, which is also an essential component of mammalian membranes (Chono et al., 2009; Schmitz and Müller, 1991; Veldhuizen et al., 1998). Schmitz and Müller (1991) have reported DPPC to be an essential constituent of the human respiratory system, including the nose. DPPC liposomes produced have been previously demonstrated to be advantageous in pulmonary drug delivery due to the similarity of this phospholipid to the lung surfactants; hence, it was shown to be highly biodegradable and biocompatible (Kellaway and Farr, 1990). Therefore, the potential of effervescent formulation for nasal delivery was explored in this report using DPPC.

3.3.7.1. Size of liposomes generated from DPPC-based proliposomes

These experiments were conducted using DPPC liposomes generated from mannitol-based proliposomes with 1:10 lipid to carrier ratio. The proliposomes were manufactured using ethanol as a lipid solvent, and BDP was included in 5 mol% concentration. Non-effervescent liposomes were first made with DPPC with or without cholesterol, followed by size analysis. Cholesterol containing DPPC liposomes generated from non-effervescent mannitol-based proliposomes had a size of 11.87±0.4 µm when no drug was included and 8.32±0.1 µm when the drug was incorporated. Formulation significantly affected the resultant size of liposomes (P≤0.5) (Table 3-6). Mannitol-based effervescent liposomes were slightly smaller than non-effervescent liposomes (Figure 3-29). Additions of cholesterol in effervescent formulations seem significant when comparing mannitol formulations (P≤0.5). Compared to SPC formulations, DPPC liposomes demonstrated a significant difference in size. SPC-based mannitol formulations with cholesterol demonstrated smaller sized liposomes (5.143±0.50 µm) compared to DPPC-based (7.540±0.15 µm). Salt-based formulations demonstrated completely opposite results to
SPC-based formulations. Salt formulations, DPPC-based and loaded with drug in the presence of cholesterol had smaller size (7.047±0.45 µm) compared to formulations without cholesterol (17.81±0.04 µm). SPC-based formulations had large liposome sizes when cholesterol was included, 20.27±6.55 µm, while when cholesterol was omitted liposome size was reduced.
Figure 3-29: Size (µm), analysis of 5 mol% BDP-loaded effervescent liposomes and non-effervescent liposomes made with DPPC lipid.
3.3.7.2. Size distribution (span) of liposomes generated from DPPC-based formulations

These experiments were conducted using DPPC liposomes generated from mannitol-based proliposomes with 1:10 lipid to carrier ratio. The proliposomes were manufactured using ethanol as the lipid solvent, and BDP was included in 5 mol% concentration. DPPC-based effervescent liposomes tended to form less aggregates, and size distribution was more uniform compared to non-effervescent liposomes; hence, the span of effervescent vesicles was significantly smaller (P≤0.05). Non-effervescent formulations using mannitol as the carrier without the drug had a similar span value to formulations containing the drug. However, a significant difference (P≤0.05) was observed between the drug-loaded non-effervescent mannitol formulation (span = 1.93±0.04) and effervescent mannitol formulations (span = 1.51±0.07) (Figure 3-30). Inclusion of cholesterol in the drug-loaded effervescent mannitol-based formulations with the drug did not affect the span value.

Salt-based formulations with cholesterol and drug had a span measurement of 1.56±0.12 in comparison to those without cholesterol and drug, which demonstrated a significantly higher span of 2.65±0.04, possibly indicating vesicle aggregation. However, for drug-containing mannitol-based formulations, cholesterol did not affect the span value (Table 3-6).
Figure 3-30: Span value, analysis of 5 mol% BDP-loaded effervescent liposomes and non-effervescent liposomes made with DPPC.
**Table 3-6: Size, span, and zeta charge of liposomes made with SPC and DPPC as phospholipids prepared with cholesterol and cholesterol-free form by slurry method**

<table>
<thead>
<tr>
<th>Formulation (10 mol%)</th>
<th>Soya phosphatidylcholine-based (SPC)</th>
<th>Dimyristoyl phosphatidylcholine (DPPC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VMD (µm)</td>
<td>Span</td>
</tr>
<tr>
<td>Effervescent liposomes prepared with mannitol, and cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Empty</td>
<td>5.065 ± 0.12</td>
<td>3.076 ± 0.71</td>
</tr>
<tr>
<td>Drug-loaded</td>
<td>5.143 ± 0.50</td>
<td>4.411 ± 2.72</td>
</tr>
<tr>
<td>Effervescent liposomes prepared with mannitol, without cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug-loaded</td>
<td>7.32 ± 0.45</td>
<td>2.590 ± 0.41</td>
</tr>
<tr>
<td>Effervescent liposomes prepared with effervescent salts alone as carrier, and cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Empty</td>
<td>20.60 ± 1.61</td>
<td>2.320 ± 0.38</td>
</tr>
<tr>
<td>Drug-loaded</td>
<td>20.27 ± 6.55</td>
<td>2.969 ± 0.77</td>
</tr>
<tr>
<td>Effervescent liposomes prepared with effervescent salts alone as carrier, without cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug-loaded</td>
<td>6.045 ± 0.19</td>
<td>2.745 ± 0.16</td>
</tr>
<tr>
<td>non-effervescent (Control) prepared with mannitol and cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Empty</td>
<td>5.275 ± 0.16</td>
<td>4.472 ± 0.73</td>
</tr>
<tr>
<td>Drug-loaded</td>
<td>8.254 ± 0.50</td>
<td>4.138 ± 0.19</td>
</tr>
</tbody>
</table>
3.3.7.3. Zeta potential of liposomes generated from DPPC-based formulations

These experiments were performed using DPPC liposomes generated from mannitol-based or salt-based proliposomes with 1:10 lipid to carrier ratio with 5 mol% BDP. The zeta potential of DPPC-based liposomes was different to the zeta potential of SPC-based liposomes. DPPC liposomes had positive zeta potential measurements, while SPC liposomes were negatively charged except for salt-based formulations, while in DPPC only mannitol-based non-effervescent and mannitol-based effervescent with cholesterol had negative zeta potential measurements (Table 3-6). These findings indicate that different lipid composition can lead to different zeta potential values of liposomes. Non-effervescent mannitol-based formulations with cholesterol demonstrated a significant difference in charge between empty and drug-loaded (P≤0.05) (Table 3-6). However, no significance was seen between empty and drug-loaded effervescent mannitol-based formulations with cholesterol. In general, the drug seems to have an effect on the surface charge of the liposome. A study by Mady and Darwish (2010) demonstrates empty DPPC-based liposomes were negatively charged; a similar result was observed with DPPC-based liposomes from research done by Imura et al. (2003). DPPC liposomes prepared by the high pressure homogenizer method was 0.51±0.79 mV slightly positive (Hasanovic et al., 2010). DPPC/cholesterol liposomes were observed to be around 5 mV positively charged (Szczęs, 2013). Research on ethanol passed proliposomes formulation (drug-free) prepared with DPPC and SPC demonstrated DPPC-based liposomes were observed to have a zeta potential of -2.8 mV while SPC-based formulations were observed at -1.9 mV. Both lipids produced negatively charged liposomes (Jain, 2012). It can be observed that the preparation method also has an effect on DPPC-based liposomes’ zeta potential. SPC-based liposomes made via the thin film hydration method was compared to ethanol-based proliposomes. Research indicated that regardless of the preparation method, liposomes were negatively charged with values between -3.5 to -6.5 mV; however, a
significant difference in liposome charge was observed between the two preparation methods (P≤0.05). Researchers further state negativity maybe attributed to the presence of negatively charged lipids in lipoid S-100 (Jaiswal, 2013). Liposomes made with SPC used in a study to deliver curcumin demonstrated a zeta potential of −12.88±1.38 mV (Chen et al., 2012). A study comparing tamoxifen citrate demonstrated empty liposomes to be highly negative -56.09±4.3 mV compared to liposomes loaded with tamoxifen with a zeta potential of −36.88±3.8 mV. Researchers state the reduced negativity of the drug-loaded formulation is due to the cationic charge present on the drug having neutralized the surface charges that existed on the formulation surface (Layek and Mukherjee, 2010).

Literature indicates SPC is lipid and is mostly negativity charged.

The zeta potential of cholesterol containing effervescent formulations is affected by carrier type (P≤0.05). However, for salt-based DPPC:Chol liposomes, no significant differences in the zeta potential were observed as a result of drug inclusion. Furthermore, cholesterol did affect the charge of liposomes by making them slightly less positive for salt-based formulations (P<0.05) (Figure 3-31). Inclusion or exclusion of cholesterol affected the zeta potential of SPC formulations as well.

Positively charged liposomes were found to have a high entrapment efficiency compared to neutral liposomes. Positively charged liposomes were also reported to have prolonged circulation and reduced toxicities in the body (Brigham et al., 1989; Bailey and Sullivan, 2000). Positively charged liposomes tend to behave more like neutral liposomes and maintain higher entrapment efficiencies compared to neutral liposomes (Brigham et al., 1989; Bailey and Sullivan, 2000; Yadav et al., 2011)

Lipid composition and lipid phase transition temperature can affect the surface charge of liposomes (Liu, 2011). Neutral liposomes are mostly based on DPPC, DMPC, Distearoyl phosphatidylcholine (DSPC). However, charged liposomes are observed in an electric field when they are dispersed in a solution of PH 7.4 (Liu, 2011). The zeta potential in
low ionic strength is negative and reduces in magnitude. Change of zeta potential caused by an increase in ionic strength due to a structural change in the head group of liposomes, reversal in this charge is observed due to change in direction of the dipole connecting the negative charge of liposomes phosphatidyl group and the positive charge of the choline group in the head group of the molecule (Liu, 2011).
Figure 3-31: Zeta potential, analysis of 5 mol% BDP-loaded effervescent liposomes and non-effervescent liposomes made with DPPC.
3.3.8. **SEM images of DPPC proliposomes**

Non-effervescent DPPC proliposome formulations had similar SEM morphology to corresponding SPC formulations (Figure 3-36 and 3.37). Both lipids resulted in particles that are irregular in shape, with crystalline structures. SEM images demonstrated a glossy appearance, indicating that mannitol was equally coated with phospholipid. SEM pictures were similar to those published by Yan-yu et al. (2006). DPPC effervescent formulations also demonstrated a similar morphology to corresponding SPC formulations. However, non-effervescent and effervescent liposomes for both SPC and DPPC formulations were observed to be different due to the needle-like structure protruding outwards from the mannitol surface once effervescent salts were incorporated (Figure 3-32 and 3-34). Effervescent samples for DPPC were also observed to be glossy and irregular in shape, correlating with SPC-based formulation morphology. Salt-based formulations without mannitol and without cholesterol with DPPC incorporation had needle-like structures (Figure 3-37) while SPC formulations looked smoother (Figure 3-33).
Figure 3-33: SEM picture of mannitol-based effervescent proliposomes with SPC.

Figure 3-32: SEM picture of mannitol-based effervescent proliposomes with DPPC.

Figure 3-35: SEM picture of salt-based effervescent proliposomes with SPC.

Figure 3-34: SEM picture of salt-based effervescent proliposomes with DPPC.

Figure 3-36: SEM picture of mannitol-based non-effervescent proliposomes with SPC.

Figure 3-37: SEM picture of mannitol-based non-effervescent proliposomes with DPPC.
3.3.9. Disintegration time of DPPC and SPC effervescent proliposome formulations

DPPC formulations took slightly longer time to disintegrate compared to SPC proliposomes. Disintegration of proliposomes without effervescent property took over 50 min. Non-effervescent DPPC or SPC formulations also failed to fully disintegrate without vigorous hand shaking. Effervescent formulations took significantly less time to disintegrate (P≤0.05) compared to non-effervescent formulations (Table 3-7). Salt-based DPPC in general took slightly more time than mannitol-based formulations, while for SPC formulations made with salt carrier only half the time for disintegration to happen was needed compared to the corresponding mannitol-based formulations. Formulations without cholesterol using mannitol or salt carrier took less time to disintegrate compared to the corresponding formulations with cholesterol, indicating that cholesterol may mask effervescent ingredients from being in efficient contact with water. However, considering the characterization findings, formulations with cholesterol might be generally better using DPPC effervescent liposomes. The most appropriate formulation for further studies should be decided by considering the properties of liposomes, drug entrapment, nebulization performance, etc.

Table 3-7: Disintegration time for DPPC-based effervescent proliposome formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Carrier</th>
<th>Cholesterol</th>
<th>Drug (BDP)</th>
<th>Disintegration Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-effervescent</td>
<td>Mannitol</td>
<td>Yes</td>
<td>-</td>
<td>53.66 ±4.28</td>
</tr>
<tr>
<td>Non-effervescent</td>
<td>Mannitol</td>
<td>Yes</td>
<td>Yes</td>
<td>61.04 ±5.75</td>
</tr>
<tr>
<td>Effervescent</td>
<td>Mannitol</td>
<td>Yes</td>
<td>-</td>
<td>2.63 ±0.48</td>
</tr>
<tr>
<td>Effervescent</td>
<td>Mannitol</td>
<td>Yes</td>
<td>Yes</td>
<td>2.65 ±0.41</td>
</tr>
<tr>
<td>Effervescent</td>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
<td>3.69±0.21</td>
</tr>
<tr>
<td>Effervescent</td>
<td>Mannitol</td>
<td>-</td>
<td>Yes</td>
<td>4.10 ±0.58</td>
</tr>
<tr>
<td>Effervescent</td>
<td>Salt</td>
<td>Yes</td>
<td>-</td>
<td>3.70 ±0.36</td>
</tr>
<tr>
<td>Effervescent</td>
<td>Salt</td>
<td>Yes</td>
<td>Yes</td>
<td>4.09 ±0.45</td>
</tr>
<tr>
<td>Effervescent</td>
<td>Salt</td>
<td>-</td>
<td>-</td>
<td>3.5 ±0.53</td>
</tr>
<tr>
<td>Effervescent</td>
<td>Salt</td>
<td>-</td>
<td>Yes</td>
<td>3.74±0.35</td>
</tr>
</tbody>
</table>
3.3.10. HPLC analysis of DPPC formulations

BDP entrapment in DPPC liposomes tended to be higher than that in SPC liposomes. DPPC:Chol non-effervescent formulation using mannitol carrier had a much less entrapment efficiency of 51.07±23.5% compared to SPC liposomes, which offered an entrapment efficiency of 75.35±1.4%. However, DPPC:Chol effervescent mannitol-based formulations offered an entrapment of 82.15±8.29 (Table 3-8). DPPC effervescent formulations with mannitol carrier have improved the entrapment compared to SPC, which offered BDP entrapment of 18.12±12.02 (P≤0.05) (Table 3-8). Cholesterol-free DPPC mannitol-based formulations offered a slightly lower trend for drug entrapment but that was not statistically significant (Table 3-8). Cholesterol-free DPPC mannitol formulation offered higher drug entrapment compared to corresponding SPC liposomes (Table 3-8). The use of mannitol as a proliposome carrier had a very mild effect on BDP entrapment efficacy, while changing lipid choice from DPPC to SPC greatly improved the entrapment.

These observations were not consistent with salt-based formulations. DPPC:Chol salt-based formulations offered a high drug entrapment of 90.60±13.51% while entrapment using the corresponding cholesterol-free formulations was as low as 36.3±7.0% (P≤0.05). SPC:Chol salt-based formulations entrapment was 29.64±12.35% compared to 11.36±3.41% for the corresponding cholesterol-free preparations (Table 3-8). DPPC formulations using salt as carrier and cholesterol as bilayer constituent offered the highest drug entrapment, while mannitol-based formulations with cholesterol proved to be second best.

Incorporation of cholesterol may reduce bilayer fluidity (by positioning between the phospholipid molecules), and reduce permeability (Alberts et al., 2009; Xiang et al., 2000). The addition of cholesterol gives liposomes the elasticity to accommodate 1 mol%
of steroidal drugs; this is observed for both SPC and DPPC liposomes (Jaafar-Maalej et al., 2011).

It has been reported that entrapment of the drug is dependent on the properties and concentration of the lipid used in the liposome formulation, with achievement of 100% entrapment efficiency being extremely difficult (Jaafar-Maalej et al., 2011). The entrapment efficiency of BDP in DPPC and SPC liposomes as found in the present study contradicts with the results demonstrated previously by Darwis and Kellaway (2001) who found that BDP entrapment is inversely propositional to the $T_m$ of phospholipid used in formulation. However, in effervescent formulations this was not observed to be the case, possibly due to the difference in liposome size or zeta potential. DPPC liposomes in general had a slightly larger size, which may have enhanced the drug entrapment compared to SPC liposomes. Darwis and Kellaway (2001) have also shown larger liposomes to entrap greater proportions of BDP.

DPPC and SPC drug entrapment without a coating of mucoadhesives when compared to results of SPC-based formulation with the mucoadhesive polymers alginic acid and chitosan, demonstrated very low entrapment. Data agree with our previous conclusion that effervescent formulations are better at drug entrapment without mucoadhesives. Looking at the DPPC results, it can be observed that DPPC is the better lipid, which improves drug entrapment when compared to both SPC alone and SPC alone with mucoadhesive formulations.
### Table 3-8: Comparison of entrapment efficacies by HPLC for effervescent proliposomes with BDP formulations, with lipids SPC and DPPC

<table>
<thead>
<tr>
<th>Formulation (10 mol %)</th>
<th>Entrapment efficacies of formulations %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPC</td>
</tr>
<tr>
<td>Effervescent liposomes prepared with mannitol, and cholesterol</td>
<td>18.12±12.02</td>
</tr>
<tr>
<td>Effervescent liposomes prepared with mannitol, without cholesterol</td>
<td>8.25±1.75</td>
</tr>
<tr>
<td>Effervescent liposomes prepared with effervescent salts alone as carrier, and cholesterol</td>
<td>29.64±12.35</td>
</tr>
<tr>
<td>Effervescent liposomes prepared with effervescent salts alone without addition of cholesterol</td>
<td>11.36±3.41</td>
</tr>
<tr>
<td>Non-effervescent (control) prepared with mannitol and cholesterol</td>
<td>75.35±1.4</td>
</tr>
</tbody>
</table>
3.4. Conclusion for Characterization of BDP-Loaded Liposomes With Both SPC and DPPC Liposomes

The addition of effervescent property to liposomes and improving disintegration time did not have a negative impact on the liposomes generated by both SPC-based and DPPC-based formulations. A model drug (Beclometasone dipropionate: BDP) was used to understand the capability of effervescent formulations to carry hydrophobic drugs within effervescent liposomes. The comparison of results from formulations based on both lipids demonstrated that factors such as carrier choice (mannitol or salt), choice of lipid (DPPC or SPC), the addition of cholesterol, and the presence of effervescent ingredients can all effect liposome size, span, zeta potential, and drug entrapment efficiency. Mucoadhesives such as alginic acid and chitosan in effervescent liposome formulations did not prove to be beneficial due to the fact that drug entrapment has decreased.

SPC and DPPC formulations produced liposomes with similar size and span. However, SPC liposomes in general were smaller in size. However, as these novel formulations are targeted to be nebulized to the sinus, liposomes from both formulations are proved beneficial. Research suggested that nasal drug delivery is done through liposomes that are typically large in size (>10 µm), while smaller liposomes particles that are 2 to 10 µm can be reached through the trachea and the lungs. Particles less than 1 µm are believed to be exhaled (Saari, 2003; Debjit Bhowmik et al., 2010). Therefore, DPPC-based liposomes with slightly larger liposome size, smaller span, and better drug entrapment have proved to be the better formulation. DPPC would be a better lipid of choice in terms of drug entrapment in liposomes.

Mannitol-based formulations with cholesterol were proven to be the best formulations for further research in SPC-based formulations, while salt-based formulations without cholesterol proved to be the second best. DPPC-based formulations, on the other hand, demonstrated salt-based formulations with cholesterol to have the highest entrapment.
Considering the size, span, and zeta charge, mannitol with cholesterol liposomes is also proven to be a good candidate formulation for further studies. SPC based with cholesterol was excluded from further studies due to its large liposome size; this issue of liposome size was by excluding cholesterol, which brought down the liposome size drastically. However, this also resulted in reduced drug entrapment.

Interactions of cholesterol with different lipids (DPPC and SPC) were observed by characterization data of liposomes for both SPC- and DPPC-based lipids. Size, span, and zeta charge were observed to be different with DPPC when compared to SPC with cholesterol due to packaging structure and transition temperature. Mannitol as a sugar-based carrier demonstrated similar results when used with both SPC and DPPC. Salt-based formulations (sugar-free) demonstrated promising results with the DPPC lipid. Entrapment of BDP was therefore dependent on choice of lipid and cholesterol. Further work is necessary to understand the stability of liposomes in nasal delivery and the entrapment of hydrophilic drugs. Effervescent formulations made with SPC lipid based on mannitol and cholesterol, SPC-based salt-based formulations without cholesterol, DPPC-based mannitol-based formulations with cholesterol, and DPPC-based salt-based formulations with cholesterol were chosen for nebulization studies. All formulations of SPC were tested with the hydrophilic drug Xylometazoline hydrochloride to understand the capacity of effervescent liposomes to entrap hydrophilic drugs. In conclusion of the characterization data for BDP drugs with different lipids, effervescent liposomes reduced the time taken for disintegration and improves dosing while not having a negative impact on size, span, zeta potential, and entrapment of BDP. This suggests that with further improvement is required for the effervescent formulations.
3.5. **Effervescent Proliposomes Formulations for Xylometazoline hydrochloride (XH) With SPC**

Xylometazoline hydrochloride is a hydrophilic drug that is commonly used as a nasal decongestant. In general, hydrophilic drugs have a lower entrapment efficiency in liposomes compared to hydrophobic drugs. Hydrophilic molecules are entrapped in the aqueous core and spaces of the liposomes, unlike hydrophobic drugs which are entrapped within the liposome bilayers. Entrapment of hydrophilic drugs is dependent on the preparation method, liposome size, lipid concentration, and liposome lamellarity (Bangham et al., 1965; Bangham and Papahadjopoulos, 1966). Various preparation methods have been used to maximize the entrapment of hydrophilic molecules in liposomes such as reverse phase evaporation, dehydration-rehydration of preformed empty liposomes, and freeze-thaw cycles (Xu et al., 2012).

The difficulty of entrapment of hydrophilic drugs such as Tenofovir and superoxide dismutase in liposomes has been previously discussed, with entrapment efficiencies being in the range of 20–50% (Xu, 2012). Both of the above researchers indicate the need for the improvement of the capacity of liposomes to entrap the hydrophilic drug. To the best knowledge of the author of this thesis, no liposome entrapment studies for the drug XH have yet been reported. XH was chosen due to its nasal decongestant property and its use in the treatment of sinusitis. In the present investigation, effervescent liposomes were prepared using the ethanolic slurry method (1:10 w/w lipid to carrier) using mannitol or salt carrier particles, SPC phospholipid, and 5 mol% XH (chapter 2, section 2.2).

Formulations were tested with mannitol or salt as carriers, with or without cholesterol. Characterization of liposomes by size, span, and zeta potential was conducted without probe-sonication and following probe-sonication for 20 sec to reduce liposome size (Figure 3-38). The drug entrapment (EE) of the resulting liposomes was determined using HPLC.
Figure 3-36: Preparation of effervescent liposomes with hydrophilic drug.
3.5.1. Characterization of mannitol- and salt-based liposomes loaded with XH

Effervescent liposomes were characterized for size before and after sonication. Formulations tested were 1:10 w/w lipid to carrier ratio with 5 mol% XH loaded on liposomes manufactured with both carriers, mannitol and salt, with and without addition of cholesterol. Formulations tested listed in chapter 2, section 2.3. Non-effervescent conventional liposomes were used as control.

3.5.1.1. Liposomes size before sonication

Drug-free SPC:Chol liposomes made with mannitol carrier had a measured size of 7.24±0.15 µm, while the corresponding cholesterol-free formulation had a smaller size (5.11±0.09 µm). However, upon inclusion of XH, large size was noted for both non-effervescent formulations. Mannitol with the cholesterol drug demonstrated a vast difference in liposome size upon addition of the drug (18.63±0.17 µm) (Figure 3-39). Drug-containing effervescent mannitol formulations with cholesterol had slightly smaller size measurements compared to conventional liposomes with drug. Mannitol-based formulations with or without cholesterol both had a similar size of around 7.6 and 7.9 µm respectively. Salt-based effervescent formulations with cholesterol had a slightly larger size (9.53±0.18 µm); however, it was not significantly different in comparison to liposomes with mannitol and cholesterol. Furthermore, upon investigation of liposomes based on salt without cholesterol, a difference in reduction of size was observed, which was also observed to be similar in the case of hydrophobic drug BDP. The Mass Median Dimeter (MMD) of Pari Sinus aerosols is noted to be around 3.2 µm. Even though upon nebulization liposome size can be reduced, we sonicated the formulations for 20 sec to reduce its liposome size in order to improve its size fitness into the nebulizer droplets.
Figure 3-37: Size (µm), analysis of XH-loaded effervescent liposomes SPC-based formulations before sonication.

![Graph showing size analysis of liposomes before sonication.](image)

Figure 3-38: Size (µm), analysis of XH-loaded effervescent liposomes SPC-based formulations after sonication.

![Graph showing size analysis of liposomes after sonication.](image)
3.5.1.2. Liposome size after sonication

Non-effervescent liposomes after sonication, mannitol-based formulations with cholesterol, conventional liposomes reduced its liposome size drastically before and after sonication to (4.10±0.16 µm). Non-effervescent mannitol-based formulations with cholesterol liposomes did not seem to have changed liposome size significantly, indicating that the addition of cholesterol did not have an impact on liposome size for conventional liposomes. However, the addition of a drug into mannitol based with and without cholesterol for conventional liposome has significantly increased liposome size for both formulations (P≤0.05). Addition of a drug resulted in increases in liposome size compared to its empty counterpart. Mannitol effervescent liposomes reduced in size drastically before and after sonication (Figure 3-39) (Figure 3-40). Effervescent liposomes mannitol with cholesterol, before and after sonication, went from large size liposomes (18.63±0.17 µm) to (2.347±1.03 µm) (P≤0.05). Non-effervescent liposomes with drug by contrast to effervescent mannitol-based liposomes appeared to have large liposome sizes (4.48±0.25 µm) (P<0.05). Mannitol-based formulations without cholesterol effervescent formulations were significantly different when compared to conventional liposomes (P≤0.05). Salt-based formulations with cholesterol effervescent liposomes and drug-loaded were reduced to half the size compared to effervescent mannitol-based formulations with cholesterol with drug (P≤0.05). Effervescent salt-based formulations, with and without cholesterol, did not seem to have any significant difference in liposome size (Figure 3-40). Looking at the results before and after sonication it can be concluded that the liposome size with sonication would be more appropriate in terms of size for effervescent mannitol- and salt-based with cholesterol formulations, which seem to be liposomes with a smaller liposome size. However, entrapment studies would be a better indication of the liposome’s capability to entrap
drugs for effervescent formulations to understand overall which formulations would best suit for the transport of XH.

3.5.2. **Size distribution of liposomes generated from mannitol- and salt-based liposomes**

3.5.2.1. **Liposomes span before sonication**

Non-effervescent liposomes before sonication, mannitol-based formulations with and without cholesterol both had a similar significance. However, they had a significant difference in span comparison for empty and drug-loaded formulations (P≤0.05). Mannitol-based effervescent formulations with cholesterol, both empty and drug-loaded, were not deemed significant. Mannitol-based formulations without cholesterol, on the other hand, did have a significantly different liposome span when drug-free and drug-loaded formulations were compared (P≤0.05) (Figure 3-41). Drug-free salt-based formulations with cholesterol, however, had a larger span compared to the corresponding mannitol-based formulations (P≤0.05). No difference in span was observed for salt-based formulations with cholesterol, both for empty and drug-loaded liposomes. Both empty (4.96±0.10) and drug-loaded (5.63±0.05) and salt-based effervescent formulations without cholesterol seemed to have a slightly larger span, indicating aggregation of liposomes or a difference in the polydispersity of liposomes (P≤0.05).

3.5.2.2. **Liposome span after sonication**

The span measurements of non-effervescent liposomes with cholesterol were independent of drug inclusion. Furthermore, drug incorporation has affected the span of cholesterol containing effervescent mannitol formulations (P≤0.05). Non-effervescent drug-loaded liposomes with cholesterol and effervescent mannitol-based formulations with cholesterol did not have a difference in span values (Figure 3-42). The addition of effervescent characteristics to the liposomes has not had an impact on size distribution. Mannitol-based effervescent formulations without cholesterol, both empty (10.22±1.05)
and drug-loaded (1.72±0.33) demonstrated a significant (P≤0.05) difference in span values. Salt-based liposomes with cholesterol, empty and drug-loaded, have similar span values. However, results were significantly different (P≤0.05). The same trend of significant differences was observed with cholesterol-free, salt-based formulations. Comparison of mannitol-based formulations to salt-based formulations with cholesterol loaded with drug did not show a significant difference. A general comparison of sonicated vs unsonicated formulations indicates that sonicated liposomes tend to have more stable span values due to the breakdown of liposome layers making all liposomes similar in size. Mannitol-based formulations without cholesterol may not be suitable due to its high span value and that without the rigidity provided by cholesterol liposomes they seem to be breaking or fusing under the pressure of sonication and may not be suitable for nebulization. Considering size and span values, further studies on sonicated liposomes would be more appropriate for drug XH since liposomes around 5 µm are expected to fit into droplets produced by the Pari Sinus nebulizer.
Figure 3-39: Span, analysis of XH-loaded effervescent liposomes SPC-based formulations before sonication.

Figure 3-40: Span, analysis of XH-loaded effervescent liposomes SPC-based formulations after sonication.
3.5.3. Characterization of zeta potential mannitol- and salt-based liposomes

3.5.3.1. Liposome zeta potential before sonication

The zeta potential of empty and drug-loaded formulations seems to be very different from each other. The addition of the drug for all formulations has given a highly positive charge to the liposomes. Non-effervescent liposomes with cholesterol seem to be the only formulations that gave liposomes a negative charge. The addition of the drug to the same formulations made the liposomes positively charged. Non-effervescent liposomes without cholesterol and empty liposomes were around 2.07±0.05 mV. The addition of the drug resulted in highly positively charged liposomes of 36.4±0.87 mV. Mannitol-based formulations, with or without cholesterol, empty or drug-loaded seem to have similar trends, with no significant differences between the formulations (Figure 3-43). Salt-based formulations with cholesterol and without cholesterol resulted in positive liposomes. Empty and drug-loaded liposomes were not significant for both formulations. All formulations with the drug and without cholesterol seem to have a higher positive charge when compared to formulations with cholesterol.

3.5.3.2. Zeta potential analysis after sonication

Non-effervescent liposome formulations, with cholesterol, without drug and after sonication were negatively charged. However, additions of the drug gave it highly positively zeta potential (Figure 3-44). The addition of the drug for all formulations resulted in highly positive liposomes. The same trend was seen in effervescent mannitol formulations regardless of cholesterol incorporation in the liposome formulation. No significant difference was observed as a result of drug inclusion in the formulations. However, drug-loaded liposomes tended to be more positively charged compared to empty liposomes. Drug-free salt-based liposomes with cholesterol had a zeta potential measurement of 2.25±0.16 mV and drug-loaded had a value of 7.03±0.9 mV, and the difference was significant (P≤0.05).
Mannitol and salt-based formulations with cholesterol and loaded with the drug were not significantly different (Figure 3-44). Salt-based formulations without cholesterol, empty and drug-loaded, were also similarly positively charged and not significantly different. Liposomes before sonication and after sonication for effervescent mannitol-based formulations with cholesterol and loaded with the drug were found to be significantly different. Cationic liposomes are positivity charged liposomes made with positively charged lipids.

Cationic liposomes have been shown to be promising candidates in targeting negatively charged DNA and cell membranes (Krasnici et al., 2003; Simões et al., 2005; Villasmil-Sánchez et al., 2010). Cationic liposomes have been used to target the angiogenic vesicle in tumour sites and sites of chronic inflammation (Thurston et al., 1998). This suggests that maybe positively charged liposomes loaded with XH drug can be suitable for inflammation of the sinuses. The study found that when comparing (1,2-dioleoyl-3-trimethyl ammonium propane [DOTAP]/cholesterol or dimethyl dioctadecyl ammonium bromide [DDAB]/cholesterol) or liposome-DNA complexes, liposomes-DNA complexes were taken up to a greater extent compared to anionic liposomes (Thurston et al., 1998). Anionic and cationic liposomes were observed to have similar span values, similar encapsulation efficiencies, and reduced cytotoxicity when compared to neutral liposomes. Therefore, it can be concluded that charged liposomes (positive or negative), would be potentially more appropriate for drug delivery (Nie et al., 2012).
Figure 3-41: Zeta potential, analysis of XH-loaded effervescent liposomes SPC-based formulations before sonication.

Figure 3-42: Zeta potential, analysis of XH-loaded effervescent liposomes SPC-based formulations after sonication.
3.5.4. HPLC results of effervescent samples for XH drug (sonicated)

Entrapment efficiency for XH was very high compared to the hydrophobic drug BDP. There was only one available method for HPLC analysis of XH, which was time consuming (Milojevic et al., 2002). Therefore, a novel HPLC method was developed that was demonstrated to be faster, easier, and more specific. Considering size, span, and zeta potential data, it was decided to investigate the HPLC results of sonicated liposomes for drug entrapment. Non-effervescent liposomes without cholesterol (89.43±6.13%) or with cholesterol (69.64±7.88%) demonstrated their entrapment efficiency (P≤0.05). Effervescent mannitol with cholesterol formulations seemed to have slightly less drug entrapment when compared to conventional non-effervescent liposomes (P≤0.05). However, they demonstrated high entrapment when compared to the BDP hydrophobic drug. The use of mannitol as carrier did not affect the entrapment of XH. Salt-based liposomes with cholesterol demonstrated less ability to entrap this hydrophilic drug. On the other hand, salt with cholesterol in DPPC-based formulations offered very high entrapment (93%) for the hydrophobic drug BDP. Salt-based formulations, with or without cholesterol in comparison did not show any significant difference, further proving in effervescent formulations that the addition of cholesterol did not have any effect on drug entrapment (Figure 3-45). Even though non-effervescent liposome formulations proved to have high entrapment compared to effervescent formulations, the non-effervescent formulation has high span value (Figure 3-45) and is not suitable in terms of liposome size for drug delivery to the sinus. The size of the liposomes of non-effervescent is too large for fitting into the aerosol droplets generated by the Pari Sinus nebulizer, indicating that effervescent mannitol-based formulations and salt-based formulations are potentially suitable for aerosolization to the nasal cavity. Considering the rigidity and reduced permeability
given by cholesterols to liposomes bilayers, the formulations with cholesterol may be more suitable to withstand the high shear pressure of nebulization.

Hydrophilic drugs have slightly lower entrapment efficiency in liposomes compared to hydrophobic drugs (Uster, 1989). Salbutamol sulphate, a hydrophilic bronchodilator, had an entrapment efficiency of 50% and that was further increased by using high cholesterol concentrations (Bendas and Tadros, 2007).
Figure 3-43: Drug entrapment analysis of XH-loaded effervescent liposomes SPC-based formulations.
3.5.5. **Conclusion for Xylometazoline hydrochloride (hydrophilic drug)**

Effervescent formulations appeared to be better candidates for the delivery of hydrophilic drugs compared to our previous findings on the hydrophobic drug BDP. Mannitol-based and salt-based formulations both were promising; however, mannitol with cholesterol formulation may be more suitable in terms of liposome size and span values after sonication. Overall sonicated liposomes tended to be better candidates for delivery via the Pari Sinus nebulizer. The addition of cholesterol did not affect the entrapment efficiency for effervescent formulations. However, rigidity provided to liposomes may help in protecting the drug from leakage during nebulization. The addition of the drug has made all formulations highly positively charged. It can be concluded that effervescent formulations could enhance the entrapment of hydrophilic drugs such as XH.
3.6. Conclusion for Characterization of Effervescent Liposomes

Mannitol was more suitable as a proliposome carrier than sucrose in terms of liposome size, span, and zeta potential. Ethanol was a suitable solvent in the preparation of proliposomes using the slurry method. The addition of effervescent ingredients to conventional proliposomes proved successful with effervescent proliposomes, producing stable liposomes similar to conventional liposomes in terms of size, span, and zeta potential. The time taken for disintegration was drastically reduced from over 50 min to less than 5 min for all formulations prepared with effervescence. SEM images demonstrated difference in morphology of conventional liposomes and effervescent liposomes; however, effervescent proliposomes had an evenly coated phospholipid layer and glossy appearance.

Effervescent formulations were made with either SPC or DPPC. Mannitol-based formulations with cholesterol liposomes made with SPC were found to be more appropriate at generating liposomes for sinus delivery in terms of liposome size, span, zeta potential, and high BDP entrapment efficiency, while salt with cholesterol proliposomes generated liposomes that were relatively large. Mucoadhesive alginic acid and chitosan in effervescent formulations did not prove to be beneficial due to reduced drug entrapment and interaction and swelling of the mucoadhesive agents in the presence of effervescent salts.

SPC- and DPPC-based formulations both produced liposomes with a similar size and span. However, SPC-based formulations in general were comparatively smaller in size. However, DPPC liposomes have shown to be beneficial by providing high entrapment efficiency of BDP. DPPC proved to be better choice of lipid, while mannitol and salt formulations with cholesterol proved to be appropriate in terms of size, span, and zeta potential with high BDP entrapment.
Cholesterol was found to be interacting differently in the presence of the different lipids due to packing structure and difference in the transition temperatures of the lipids. It can be concluded that mannitol with cholesterol and salt with cholesterol DPPC formulations would be used for further testing. Choice of lipid was the deciding factor for entrapment efficiency of BDP.

Hydrophilic drug Xylometazoline hydrochloride was loaded into SPC-based effervescent formulations and demonstrated high entrapment in liposomes. Sonicated XH-loaded liposomes were potentially more promising for delivery via the Pari Sinus nebulizer. Unlike BDP-loaded SPC liposomes, XH-loaded liposomes did not have an effect on entrapment efficiency upon addition of cholesterol. A novel HPLC method that was developed to investigate entrapment efficiency of XH within liposomes proved to be successful, efficient, highly sensitive, and less time consuming.

It can be concluded that effervescent liposomes based on SPC or DPPC can produce stable liposomes similar to conventional liposomes with improved disintegration times. DPPC is a better choice of lipid. Effervescent salts alone without the presence of a sugar-based carrier were able to produce stable liposomes. The addition of a mucoadhesive hampered the entrapment efficiencies in effervescent liposomes. Cholesterol affected the entrapment efficiency and stability of liposomes depending on the choice of lipid.
CHAPTER 4
DELIVERY OF EFFERVESCENT PROLIPOSOMES VIA PARI SINUS NEBULIZER
4.1. Introduction

Nebulization is a method of transporting medical aerosols for the treatment of respiratory diseases. One major drawback of nebulization is drugs being stored in the nebulizer ampoules during storage. Ampoules need to be shaken to re-disperse the drug prior to performing nebulization. Issues are often observed due to the lack of space for the drug to disperse (Iyer and Uster, 2013). Effervescent granules with the drug loaded in the liposomes would potentially overcome this drawback and improve drug dosage. Conversional liposomes need vortexing and manual shaking to produce liposomes; if not, the proliposome particles would not all dissolve, leaving solid in the bottom of the ampoules. Effervescent proliposomes, on the other hand, do not need manual shaking or vortexing and can disintegrate proliposomes without any solid particles within a matter of minutes, generating liposome formulation ready for nebulization.

Liposomes for aerosol delivery have proven to be advantageous. Liposomes are compatible aqueous preparations that provide sustained drug delivery coupled with aerosol technology, reduced local irritation, decreased toxicity, increased therapeutic benefit, reduced drug clearance, and increased retention with the effective delivery of lipophilic or hydrophilic compounds to the site of action (Cullis et al., 1989; Schreier et al., 1993; Smola et al., 2008; Taylor and Fan, 1993).

Little research has been done on the nebulization of sustained release formulations of corticosteroid drugs such as Beclometasone dipropionate (BDP). Older patients who used nebulization as a method of delivering corticosteroids have been observed to have less visits to emergency services when compared to patients who were systemically administrating corticosteroids (Marcuos et al, 2006). It is hypothesized that liposomes loaded with the drug BDP can be efficiently transported to the paranasal sinuses for the treatment of sinusitis using a Pari Sinus nebulizer, improving the treatment of conditions such as sinusitis.
To understand the suitability of liposomes and their ability to withstand the shearing stress during nebulization, the formulations were nebulized using the Pari Sinus nebulizer. Nebulization of liposomal formulations will help to recognize change in the behaviour of liposomes upon nebulization. Limited research has been done using the Pari Sinus technology, and research on the use of this nebulizer with liposomal formulations has not yet been conducted. There are no records of studies being conducted on effervescent liposome formulations for paranasal sinus targeting.

Traditional nebulizers, such as jet nebulizers, were initially used with liposomal drug formulations (Taylor et al., 1990). However, research indicates that high shear stress from the nebulizer causes liposomes to leak their contents. Additionally, in some instances the baffles inside the nebulizers damaged the vesicles, resulting in leakage of the liposome contents. In this particular part of the study, the ability of effervescent proliposome formulation to deliver BDP-loaded liposomes to the nasal cavity through the Pari Sinus nebulizer was investigated. The following data are on the performance (nebulization time, sputtering time, output, aerosol size) of the Pari Sinus nebulizer with effervescent liposomal formulations.
4.2. Aims of the Chapter

The aim of this chapter is to investigate the suitability of effervescent liposome formulations made with SPC and DPPC lipids to produce liposomal aerosols suitable for drug delivery to the sinuses.

In this chapter, the Pari Sinus nebulizer with its pulsation technology was employed to deliver effervescent nanotechnology-based formulations.

SPC- and DPPC-based effervescent formulations were nebulized and compared in terms of their nebulization time, sputtering time, aerosol size distribution, and aerosol volume median diameter (VMD), span, fine particle fraction (FPF), and mass output (%).

Liposome size, span, and zeta potential of the effervescent formulations upon nebulization, and the influence of cholesterol and its impact on the stability of the liposomes upon nebulization have also been investigated.

At the end of this chapter, the most suitable effervescent liposome formulations and most suitable lipid for effervescent liposome production were decided and chosen for further studies with a nasal cast.
4.3. Results and Discussion

4.3.1. Nebulization of BDP-loaded SPC liposomes using Pari Sinus nebulizer

4.3.1.1. Nebulization and sputtering time

Nebulization times may differ when different nebulizers are used. A study compared the corticosteroids BDP, Flunisolide, Fluticasone Propionate and Budesonide. The drugs were nebulized via different nebulizers to compare nebulization time, nebulizer output, compressor pressure needed, and aerosol characteristics. Research indicated a significant difference in drug output and nebulization time between the nebulizers. It was concluded that obtaining the best possible characteristics with nebulizers, such as nebulization time, adds positively to the resultant clinical benefit (Terzano et al., 2007). There are a number of factors affecting efficiency of a nebulizer, such as the design of the device, characteristics of the drug solution, cleaning and maintenance procedures of the nebulizer, which may all affect the nebulizer performance including the time needed to deliver the nebulizer solution (Brun et al., 2000).

One of the drawbacks of nebulization is the low deposition efficiency of the drug, with only 10% of the drug nebulized reaching the target area of the respiratory tract. This is considerably low compared to drug deposition using dry powder inhalers (20–30%). However, for corticosteroids and β-2 agonists, since the therapeutic dose is less than 1 mg, the desired drug amount would still reach the targeted site via nebulizers (Selroos et al., 1996). Another disadvantage of nebulizers would be the longer time taken for nebulization. In clinical practice, 15–30 min of nebulization is considered acceptable (Brun et al., 2000). However, the nebulization time could be affected by the solution characteristics and nebulizer design. Therefore, different effervescent formulations that have been characterized were also nebulized to investigate whether they are appropriate for targeting the paranasal airways. The nebulization time was investigated by using the Pari Sinus nebulizer as previously described (section 4.1).
Nebulization times between the samples (mannitol based with or without cholesterol and salt-based with or without cholesterol) were insignificant ($P \geq 0.05$) with respect to the control sample of deionized water. Nebulization of different samples consumed time ranging from 21–23 mins to reach ‘dryness’. No significant difference in nebulization time was observed between the mannitol- and salt- based formulations, both with and without cholesterol. Thus, the inclusion of cholesterol did not affect nebulization time when mannitol was used as a proliposome carrier. A similar trend was observed for the effervescent salt-based formulations.

The sputtering duration was significantly different between the control and salt-based solutions without cholesterol ($P \leq 0.05$). However, other formulations (effervescent mannitol with cholesterol, effervescent salt with cholesterol, and effervescent mannitol without cholesterol) were not significantly different. The sputtering times between the mannitol and salt formulations with cholesterol did not indicate a significant difference. However, differences in sputtering times for salt formulations with and without cholesterol proved to be significant ($P \leq 0.05$). Furthermore, unpublished data by Papanou (2011) stated that the Pari Sinus nebulizer demonstrated a sputtering time of 30 sec. In contrast, the data collected in the current study indicated a longer sputtering time of 1–2 min.

Low patient compliance in nebulization is usually attributed to nebulization time. Most children and adults become impatient if the time taken to administer drugs is long, resulting in ceasing the administration before the whole dose is delivered. Factors such as drug nebulized, concentration, amount of solution to be nebulized, and surface tension and viscosity of the solution may affect time the required for nebulization to be completed (Iyer and Uster, 2013). Differences in solution physicochemical properties can affect nebulization time (Brun et al., 2000). However, preparations used in this study demonstrated similarity in nebulization time indicating that effervescence and
incorporation of lipids did not hugely affect the nebulization performance when the Pari
Sprint nebulizer was used. Air-jet nebulizers are less affected by formulation properties
compared to other types of nebulizers like vibrating-mesh devices (Ghazanfari et al.,
2007).

Pari (Jet), Liberty (ultrasonic), and Omron (vibrating-mesh) nebulizers were used to
deliver liposomes in order to compare efficacy and the impact on the delivery of drugs
loaded in liposomes via nebulizers (Elhissi and Taylor, 2005). The research demonstrated
that all nebulizers took approximately 12–30 min to nebulize to ‘dryness’, depending on
the nebulizer type. Sputtering duration ranged between 2–3 min, showing no significant
difference between the samples when compared to sputtering time using water, for each
nebulizer. Elhissi and Taylor (2005) concluded that the nebulization time had some
influence on the size of liposomes delivered. Moreover, when shearing was applied for a
longer duration, liposomes were reduced in size within the nebulizer. The findings here
agree with the results of Elhissi and Taylor (2005).

Two studies done on improving nebulization time with the AeroEclipse® II breath-
actuated nebulizer by modifying it has improved the time needed for nebulization, leading
to a 31% reduction in therapy time for administration and a 20% reduction in total costs
for the year 2008 at Forsyth Medical Centre. Studies done in 2007 further stated that
breath-actuated nebulizers have reduced nebulization time by 19% and decreased the total
costs of therapy by 18% (Wilson, 2011). Studies done in 2007 also demonstrated a
reduction in total cost with shortened nebulization time (St. Dominic Hospital et al., 2007)
Table 4-1: Nebulization time and aerosol characteristics for effervescent formulations prepared using SPC as a lipid for nebulization via the Pari Sinus nebulizer

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nebulization time (min)</th>
<th>Sputtering duration (min)</th>
<th>Mass median diameter (VMD) of droplets (µm)</th>
<th>Fine particle fraction (% &lt; 5 µm)</th>
<th>Span</th>
<th>Aerosol mass output (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol with cholesterol</td>
<td>22.93±1.68</td>
<td>2.61±0.38</td>
<td>4.65±0.16</td>
<td>59.75±3.58</td>
<td>1.81±0.03</td>
<td>74.28±4.90</td>
</tr>
<tr>
<td>Salt with cholesterol</td>
<td>22.3±0.20</td>
<td>2.71±0.67</td>
<td>3.40±0.12</td>
<td>77.44±3.24</td>
<td>1.64±0.10</td>
<td>85.22±12</td>
</tr>
<tr>
<td>Mannitol without cholesterol</td>
<td>22.67±2.68</td>
<td>2.0±0.73</td>
<td>4.05±0.42</td>
<td>64.75±6.13</td>
<td>2.28±0.25</td>
<td>75.49±3.20</td>
</tr>
<tr>
<td>Salt without cholesterol</td>
<td>21.51±1.80</td>
<td>1.81±0.10</td>
<td>3.485±0.53</td>
<td>80.77±12.15</td>
<td>1.378±0.23</td>
<td>78.24±1.83</td>
</tr>
<tr>
<td>Water</td>
<td>21.85±0.53</td>
<td>2.61±0.38</td>
<td>4.09±0.78</td>
<td>70.78±14.18</td>
<td>1.40±0.15</td>
<td>66.68±1.58</td>
</tr>
</tbody>
</table>
4.3.1.2. Determination of size and size distribution (span) of the generated aerosols

Aerosol particle size is a fundamental factor in the determination of aerosol suitability for nasal delivery. Predominantly, aerosol size influences the deposition on the targeted site of the sinus. To ensure aerosols get deposited to the sinus, the aerosols should reach the posterior nasal cavity. Aerosols below 5 µm (aerodynamic diameter) have been found to be the most suitable for the deposition in sinuses (ICRP Publication 66, 1994). Current nasal sprays have been found to be able to deposit 100% of the administrated drug into the nasal cavity; however, only minimal deposition has been observed in the sinuses due to the large size emitted from nasal sprays (50–200 µm) (Hwang et al., 2006; Möller et al., 2010).

Möller et al. (2009) studied ventilation efficiency of the sinuses of three volunteers using dynamic 81mKr-gas imaging along with pulsating air flow. Radiolabel DTPA (Diethylenetriaminepentacetic acid) and retention of 99tc-DTPA aerosol particles were monitored over a period of four hours (Moeller et al., 2009). No significant difference was observed in the central nasal cavity due to pulsating air flow. However, a fivefold increase in the proportion deposited was observed in the four sinuses with pulsating air flow. A total deposition of 25±16% without pulsation was observed, while a significant difference of 58±17% in the nasal cavity was observed with the Pari Sinus nebulizer. A value of 4.2±0.3% penetration of the aerosol to the sinuses region was observed with the pulsating aerosol technology, while only 1% deposition was observed without pulsation (Moeller et al., 2009).

The supplier manual of the Pari Sinus nebulizer indicates that the VMD of aerosol droplets for 0.9% sodium chloride solutions was 3.6 µm in VMD when analysis was conducted via laser diffraction (Schuschnig et al., 2008). Therefore, the Pari Sinus nebulizer was used for the following part of the study to explore the deposition profile of aerosols of containing effervescent liposomes loaded with BDP.
The nebulization of liposomes using this nebulizer was performed in vitro using laser diffraction. Droplets were generated as a cloud passing through the laser beam, which allowed the determination of the VMD.

The VMD analysis of aerosols showed the droplet size to range from 3–4 µm. Aerosol VMD measurements were taken after 2 min of commencing nebulization for each formulation and compared with the control sample of deionized water. The VMD was observed to be smaller in the salt-based formulation in comparison to the mannitol-based formulations. On comparing these formulations to the control, no significant difference was observed. However, the mannitol sample in comparison to the salt sample with cholesterol demonstrated a large droplet size with a significant difference (p≤0.05). A significant size difference was only observed when comparing the two carrier types in the presence of cholesterol.

Span values were significantly larger (p≤0.05) in comparison to the control when using a mannitol without cholesterol formulation. These findings may be explained by the ability of cholesterol to increase the rigidity of vesicles. The span was higher for aerosols generated from cholesterol containing mannitol-based samples compared to the control.

The size of aerosol droplets generated from all formulations was less than 5 µm, demonstrating that liposome formulations could deposit in the sinus. The results are in agreement with Möller et al., (2008, 2010) and Schuschnig et al., (2008). SPC-based liposome formulations loaded with BDP were found to have a liposome size of 5.14±0.49 µm. Mannitol-based effervescent formulations with cholesterol were found to be the most appropriate formulations in terms of size, span, zeta potential entrapment of liposomes, and also aerosol size. Liposomes would be able to fit in the aerosols of the Pari Sinus nebulizer. Salt formulations without cholesterol have a vesicle size of 6.04± 0.19 µm, also appropriate in terms of liposome size. However, the salt-based formulation with
cholesterol was observed to have a very low entrapment compared to the mannitol with cholesterol formulations.

Investigations conducted using a cadaver nasal cast to collect aerosols generated from the pulsating nebulizer revealed that the appropriate aerosol size for deposition in the maxillary sinus was 3–10 µm (Saijo et al., 2004). However, only 3% of particles in this size range were found to deposit in the maxillary sinus. Effervescent liposomes and aerosol VMD of the Pari Sinus nebulizer fall within this size range. A post–ESS nasal cast model was also used along with the Pari Sinus nebulizer to investigate the aerosol deposition profile. Findings indicated that aerosol droplets with a particle size of 5.63 µm were deposited in the maxillary sinus, while larger aerosol droplets (e.g. 16.37 µm) failed to deposit in the maxillary sinus. Moreover, the insertion angle of the nose adaptor (45°) has been found to influence the deposition profile (Saijo et al., 2004).

Many investigations (Djupesland et al., 2006b; Frank et al., 2012; Möller et al., 2011, 2008; Negley et al., 1999; Sato et al., 1981) have indicated a large particle size (>10 µm) produced by nasal sprays and plume for deposition in the nose. However, the deposition of aerosols via the Pari Sinus have been shown to be more advantages due to the smaller particle size of the droplets generated by this device (e.g. < 5 µm) (Moeller et al., 2009). Frank et al. (2012) have reported that when nebulizers were compared to nasal sprays (generated droplets size >10 µm), findings showed that nasal sprays deposited 100% of the sprayed material in the nasal passage while nebulizers had an aerosol size of <6.42 µm causing more than 50% of the aerosolized material to bypass deposition in the nasal passages. Thus, nebulizer particles <10 µm were more likely to be respirable.

Significant (p≥0.05) differences in aerosol span values were not observed between mannitol and salt formulations. No considerable difference in size between formulations and control was found, indicating that the effervescent property did not affect the size distribution of aerosols, which might be an indication of a stable liposomal formulation.
and consistent nebulizer performance when different formulations are used. Unpublished data by Papanou (2011) observed a span value of 1.563 using this nebulizer, which is in agreement with the present findings in this report.

Non-effervescent proliposomes were hydrated in situ within the nebulizer and nebulized by Elhissi and Taylor (2005), who compared nebulizers operating at different mechanisms such as the Pari Plus (air jet), Liberty (ultrasonic) and Omron (vibrating-mesh) nebulizers. They found that the Pari Plus nebulizer (similar but not identical to the Pari Sinus) produced smaller aerosol droplets (2.50 µm) compared to the other two nebulizers. Elhissi and Taylor (2005) stated that rigid liposomes made with cholesterol had no effect on aerosol particle size. Furthermore, Elhissi and Taylor (2005) also stated that large aerosol particles delivery to the deep lung would be unlikely, whereas smaller aerosol particles were delivered to the lower impinger. Even though the focus of this research is not on pulmonary delivery, the proposed theory could be extended, i.e. that small aerosols may be easier to transport to the sinus, while larger liposomes may deposit in the nasal passages. Research by Elhissi et al. (2012) indicate smaller aerosols contain smaller liposomes while larger liposomes contain larger aerosols. This suggests that smaller aerosol containing smaller liposomes would be delivered to the sinuses and the larger aerosol would deposit in the nasal cavity. Larger liposomes could also penetrate to nasal epithelium and help with decongestion.

Darwis and Kellaway, (2001) have investigated BDP liposomes prepared from various lipids, dilauryl phosphatidylcholine (DLPC), dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), and hydrogenated soy bean phosphatidylcholine (HSPC). DLPC liposomes had the smallest VMD aerosol measurements (3.31 µm), indicating that formulation may affect the performance of jet nebulizers. However, using the Pari Sinus nebulizer, the effervescent property of liposomes had no significant effect on the aerosol VMD, suggesting droplet size can be
affected by nebulizer type and formulation characteristics. Both mannitol- and salt-based liposomes had comparative aerosol sizes when non-effervescent liposomes were nebulized. Results indicated that salt-based liposomes with cholesterol were able to produce the smallest aerosol droplets with a VMD of 3.40 µm, indicating the effect of salt content on aerosol droplet size.

The Pari Sinus nebulizer (according to the supplier’s manual) when the temperature is 23 °C, the relative humidity is 50% and the fill volume is 5 ml using 0.9% sodium chloride solution, the laser diffraction measured VMD should be around 3.6 µm. This correlates with the results obtained in this report. Salt-based effervescent aerosols were only slightly smaller in size than sodium chloride aerosols, possibly because of the larger salt concentration used in the liposome formulations. Moreover, mannitol-based aerosols were slightly larger compared to the result achieved by some other investigators (Schuschnig et al., 2008), suggesting that a slight difference in the environmental conditions may slightly affect the droplet size of the nebulizer.

Hyo et al. (1989) have simulated the deposition of aerosolized particles to paranasal sinuses using a nasal cast model. Their research has stated three factors may affect particle deposition, with the size of ostia being the most significant factor that had an impact on the size of the particles deposited on the maxillary sinus (observed in healthy individuals).

In summary, mannitol with cholesterol effervescent formulations may be the most suitable in terms of particle size and particle distribution (span). Liposomes in this formulation also had a smaller size and offered higher drug entrapment, and established suitability for delivery by the Pari Sinus nebulizer.
4.3.1.3. Aerosol mass output (%)

Although droplet size is an important factor in nebulization, the amount of drug that is put into the nebulizer and the amount that is delivered to the patient, as well as the amount of drug that can be delivered at a given time, are all important factors that help evaluate nebulization efficiency (Finlay, 2001). The amount of drug released from the nebulizer depends on nebulizer output, particle size, and amount of liquid within the nebulizer (Wanger, 2011). The simplest way to measure nebulizer output is to study aerosol mass output. Given that the Pari Sinus nebulizer has a fixed output rate of 6 l/min with a pulsation frequency of 44.5 Hz, the aerosol mass output and output rate can be calculated. Higher drug output rates help reduce long treatment times, which may have an effect on patient compliance. Mass and drug output rates of a nebulizer can be affected by the viscosity increase or decrease in accordance with the nebulizer solution temperature.

The Pari Sinus brochure indicates that the mass output percentage below 5 µm is 71% using 0.9% NaCl (5 ml) solution. Samples (5 ml) of each effervescent formulation were nebulized to ‘dryness’; however, complete atomization of the liposome formulation was not observed in all formulations and in the control. This indicates that the aerosol mass output was less than 100% due to the residual volume remaining in the nebulizer after the completed nebulization.

The aerosol mass output between the control (66.68±1.58%) and mannitol effervescent formulations with cholesterol loaded with the drug (74.28± 4.9%) were significantly different to each other. Salt with cholesterol demonstrated a significantly higher aerosol mass output rate in comparison. Carriers, mannitol, and salt loaded with the drug, without cholesterol, had a significantly higher aerosol mass output rate compared to formulations with cholesterol. This indicates that the effervescent ingredients have helped increase the aerosol mass output rate. No significant difference of aerosol mass output (%) between mannitol and salt formulations with cholesterol was observed. Salt and cholesterol as
formulation ingredients gave the highest aerosol mass output (85.22±12.0). Both mannitol formulations exhibited similar results for aerosol mass output. For each carrier, the incorporation of cholesterol had no effect on the aerosol output.

Moeller et al. (2009) investigated the aerosol mass output within the sinuses using three healthy subjects. Results demonstrated a total aerosol deposition of 25±16% of the nebulized dose being deposited within the nasal cavity when no pulsation technology was used. However, when pulsation technology was employed the nebulized dose increased significantly (p<0.01) to 58±17%. Furthermore, without pulsation only 1% of the nebulized dose was found to reach the sinuses, while with pulsation 4.2±0.3% reached the sinuses (Table 4-1). It can be hypothesized that a higher percentage of the drug may be reached with a large amount of the drug being nebulized to the sinus when the pulsation technology is employed. Therefore, with high aerosol mass output (%) and with effervescent liposomal formulations, a higher content of drug reaching the sinuses can be expected.

Literature indicates that higher phospholipid concentration in liposome formulations may lead to enhanced packing of drug molecules within the liposome bilayers (Manca et al., 2012). Hence, increased phospholipid and cholesterol content may improve the stability of liposomes during nebulization by reducing drug leakage from the liposomes. Liposomes with cholesterol tend to have higher nebulization efficiency compared to formulations without cholesterol. By contrast, formulations with phosphatidylcholine had a nebulization efficiency of 35±2.3%, the same formulations with cholesterol had a nebulization efficiency of 48.39%. A similar increase was observed for other the formulations tested (Manca et al., 2012). A similar observation was observed for effervescent formulations prepared in this study with and without cholesterol.

Elhissi et al. (2006) investigated the aerosol mass output rate of non-effervescent liposomes using the Omron (mesh), Pari (Jet), and Liberty (ultrasonic) nebulizers.
Aerosol output was the highest, with the Omron nebulizer exhibiting an output value of 91%. In comparison to these nebulizers, the Pari Sinus gave an average amount of aerosol output, being in the range of 74–85% depending on the formulation. Both salt formulations elicited a lower aerosol mass output (Elhissi et al., 2006).

4.3.1.4. Fine particle fraction (FPF)

The deposition profile of the drug in the nasal cavity and lung differ drastically depending on the nebulizer model used (Hickey et al., 1996; Newman, 1993). In this study, the FPF was determined as the aerosol fraction with an aerodynamic diameter less than % <5 µm using the Pari Sinus nebulizer. The Pari Sinus nebulizer has been identified as having an FPF of 71%, according to the brochure supplied by the manufacturer (PARI Respiratory Equipment, Inc., 2012; Schuschnig et al., 2008).

Sodium chloride trace solution was nebulized with the Pari Sinus to study the in vitro effect of the deposition using a silicon nasal cast. The nasal cast consisted of four cylindrical chambers representing the sinuses with equal volumes. The ostium diameter varied from 0.5 mm to 3 mm in the nasal cast (Boehm et al. 2004, Karn et al., 2011).

Particles below 5 µm were deposited in the sinus chambers and particles with VMD of 5-10 µm deposited in the nasal region of the cast. By contrast, particles with a VMD greater than 10 µm were deposited in the frontal region of the nasal cast, indicating the influence of the aerosol particle size on the deposition profile. Thus, based on the aerosol droplet size, it is expected that the majority of the aerosol with effervescent formulation can deposit in the sinus region, resulting in the expected therapeutic effect when the drugs are administrated. Further investigations are needed to study the validity of this assumption.

In the present study, nebulization of the effervescent formulations via the Pari Sinus nebulizer would generate aerosols with FPF dependent on the carrier type and lipid composition of the effervescent formulation. All effervescent formulations in comparison to the water control sample did not demonstrate a difference in FPF, except for the
cholesterol containing the mannitol-based formulation, which had significantly lower FPF by 11.03% when compared to the control water.

The effervescent formulation made from mannitol and using cholesterol had an FPF of 59.75% ±3.59, whereas the formulation made with salt and cholesterol had a significantly higher FPF (77.44% ±3.25). The difference in FPF might be attributed to different physicochemical properties such as viscosity and surface tension (Ghazanfari et al., 2007).

No significant differences in FPF were observed between samples of mannitol and salt formulations when cholesterol was omitted from the formulations (Table 4-1). It is possible that the rigidity given to the liposome structures owing to the inclusion of cholesterol has affected the FPF of the aerosol. Both mannitol formulations with and without cholesterol had lower FPF when compared to the relevant counterpart in salt formulations (Table 4-1).

According to a study conducted by Ghazanfari et al. (2007), using the Omron MicroAir NE-U22 nebulizer and Aeroneb Pro nebulizer with deionized water, glycerol and NaCl solutions, the Omron MicroAir NE-U22 nebulizer had a FPF of 41.00±1.68 for deionized water, 30.23±6.36 FPF for the glycerol solution, while sodium chloride solution had a FPF of 39.40±0.21. Thus, the formulation may markedly influence the FPF of aerosols. Salt-based formulations showed an increase in the FPF compared to the control as in the case of using deionized water. Overall, the effervescent formulations using mannitol or salt as proliposome carriers nebulized with the Pari Sinus demonstrated higher FPF values than those in the literature using the Aeroneb Pro mesh nebulizer. All formulations showed that aerosols could potentially be deliverable to the sinuses using the Pari Sinus nebulizer. Darwis and Kellaway (2001) worked on liposomal formulations for pulmonary delivery. This research states that aerosols produced by the Pari LC Plus nebulizer with
sizes >10 μm were deposited in the ‘throat’ of the impinger, and particles having a size
cut between 10 and 5.8 μm have deposited in stages 0 and 1 (Darwis and Kellaway, 2001).

4.3.1.5. Determination of drug output from the nebulizer

The drug output (%) was determined based on the reservoir volume and HPLC analysis
of the drug in the residual volume left in the reservoir of the nebulizer after nebulization.
High drug output was observed for all formulations after nebulization. Drug output (%) was shown to be 82% for mannitol with cholesterol and 79% for salt with cholesterol formulation. Drug output of 76% was found for mannitol without cholesterol and 66% in the salt sample without cholesterol. Samples without cholesterol demonstrated lower nebulization efficiency (Figure 4-1). There was a significant difference observed (P≤0.05) between mannitol with cholesterol samples and salt without cholesterol samples. Mannitol without cholesterol gave a high standard deviation, showing inconsistencies in the results. From this data it can be concluded that mannitol with cholesterol seems to be the most suitable formulation for nebulization.

![Figure 4-1: Determination of drug output (%) of SPC-based liposomes from Pari Sinus nebulizer.](image_url)
Slightly higher drug output was noted when compared to aerosol mass output. In contrast, Papanou (2011) observed that non-effervescent liposomes have excess aerosol mass output over drug output (P≤0.05), which is consistent with other findings (Elhissi and Taylor, 2005). Thus, it is possible that the influence of effervescent ingredients on liposomes have enhanced the drug output, causing it to exceed the aerosol mass output.

4.3.1.6. Liposome size after nebulization

Results for VMD, span, and zeta potential, before and after nebulization, were studied (Table 4-2). The nebulized aerosols were collected in a flask fitted in front of the nebulizer during aerosolization. Compared to the size prior to nebulization, the measurements differed upon the release of the aerosols to the flask. When mannitol with cholesterol formulation was used, no difference in VMD of liposomes was observed upon nebulization (P≥0.05). Similarly, salt with cholesterol samples exhibited no change in VMD, span, and zeta potential upon nebulization. Comparison of mannitol and salt with cholesterol formulations after nebulization showed no significant difference between the two formulations (P≥0.05), indicating that atomization within the nebulizer may not affect the physical integrity of the liposomes.

In the absence of cholesterol, liposomes generated from mannitol proliposomes had inconsistent VMD measurements, which might be attributed to inconsistent aggregation. The zeta potential was positive for liposomes before nebulization and slightly negative after nebulization, without significant difference (P≥0.05). Salt without cholesterol also showed aggregation in samples after nebulization with a high VMD measurement, but no significant difference (P≥0.05) was observed for both size and span when formulation before and after aerosolization were compared. However, the zeta potential showed a significant difference (P≤0.05). The zeta potential was positive before nebulization and negative after nebulization. A study carried out using the Aeroneb Pro and Omron MicroAir vibrating-mesh nebulizers and the Pari LC Sprint air-jet nebulizer demonstrated
that nebulization causes fragmentation of the vesicles. This fragmentation as a result of nebulization may cause the vesicles to aggregate and may change the charge distribution on the vesicle. The side reduction of vesicles change the surface charge of vesicles (Elhissi, et al., 2013).
<table>
<thead>
<tr>
<th></th>
<th>Before nebulization</th>
<th></th>
<th></th>
<th>After nebulization</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size (µm)</td>
<td>Span</td>
<td>Zeta potential (mV)</td>
<td>Size (µm)</td>
<td>Span</td>
<td>Zeta potential (mV)</td>
</tr>
<tr>
<td>Effervescent mannitol with cholesterol</td>
<td>6.29±0.45</td>
<td>3.03±0.26</td>
<td>-1.15±1.07</td>
<td>26.4±18.43</td>
<td>2.72±0.85</td>
<td>-1.34±1.48</td>
</tr>
<tr>
<td>Effervescent salt with cholesterol</td>
<td>12.07±6.08</td>
<td>3.88±0.90</td>
<td>-0.54±0.51</td>
<td>12.77±11.58</td>
<td>8.25±7.63</td>
<td>-8.89±5.34</td>
</tr>
<tr>
<td>Effervescent mannitol without cholesterol</td>
<td>6.75±0.71</td>
<td>3.16±1.20</td>
<td>0.18±1.12</td>
<td>49.48±27.68</td>
<td>1.51±0.95</td>
<td>-2.54±1.11</td>
</tr>
<tr>
<td>Effervescent salt without cholesterol</td>
<td>4.87±1.09</td>
<td>3.96±2.56</td>
<td>0.53±0.58</td>
<td>39.62±32.92</td>
<td>1.56±0.16</td>
<td>-5.23±1.86</td>
</tr>
</tbody>
</table>

**Table 4-2: Characterization of SPC-based liposomes before and after nebulization**
4.3.1.7. Conclusions for SPC-based liposome nebulization

Novel BDP effervescent liposome formulations were able to produce stable liposomes for nebulization using the Pari Sinus nebulizer. Effervescent liposome formulations had a higher nebulization efficiency compared to water samples, suggesting that the influence of liposomes on the nebulizer fluid characteristics was advantageous. Nebulization time and sputtering of all the formulations was similar. SPC-based formulations loaded with BDP had a size of 5.14±0.49 µm (mean ± SD), which was also the most appropriate formulation in terms of span, zeta potential, and drug entrapment. This formulation also performed very well in terms of aerosol droplet size, and aerosol mass output. However, the formulation had less of a FPF rate at 59%.
4.3.2. Nebulization of BDP drug loaded with DPPC-based formulations using Pari Sinus nebulizer

The lipid composition used for the preparation of liposomes has been found to influence nebulization efficiency (Niven and Schreier, 1990; Niven et al., 1991;). Furthermore, the lipid concentration and liposome preparation method have also been found to influence the nebulization efficacy. Liposomes are known to fragment upon application of shear forces or as a result of impact on the baffles during nebulization, resulting in drug leakage and loss of the controlled release characteristics (Elhissi et al., 2006; Niven et al., 1991; Taylor et al., 1990). Therefore, optimization of the effervescent formulations with the most suitable lipid composition that would result in more stable liposomes during nebulization is needed.

SPC, Hydrogenated SPC, or Dipalmitoyl Phosphatidylglycerol were made in order to understand the effect of lipids on liposome stability during nebulization. 5(6)-Carboxyfluorescein was used as a model hydrophilic marker which had an entrapment dependent on the lipid composition of the liposomes (Niven and Schreier, 1990).

In a previous study, nine combinations of lipid and cholesterol ratios were used for preparing liposomes, in order to understand the effects of lipid composition on drug entrapment and nebulization efficiency. The research indicated that retention of Rifampicin after nebulization and the entrapment efficiency of the drug was affected by lipid composition. Liposomes made from DPPC or DSPC were found to offer a higher entrapment efficiency for Rifampicin when compared to liposomes made with a natural PC (Zaru et al., 2007). Cholesterol was found to have an effect on the entrapment. DSPC with cholesterol (2:1) proved to be the most appropriate for maximizing drug entrapment and reducing leakage during nebulization (Zaru et al., 2007). This study correlated with the findings using SPC and DPPC effervescent liposomes (chapter 3). Both lipids we used acted differently with cholesterol and both lipids were compared in a 2:1 lipid to cholesterol ratio. As demonstrated earlier in chapter 3, it was found that effervescent
DPPC formulations have offered a higher BDP entrapment compared to effervescent SPC formulations. When considering size, span, zeta, and entrapment, mannitol with cholesterol and salt with cholesterol effervescent formulations proved to be the most appropriate for subsequent studies. This chapter has identified how DPPC-based effervescent liposome formulations using mannitol or salt and via incorporation of cholesterol may affect nebulization efficiency using the Pari Sinus nebulizer.

4.3.2.1. Nebulization and Sputtering time for DPPC-based formulations

Proliposomes were made using DPPC as a lipid and mannitol or salt as carriers and using BDP as the model drug. Proliposomes were then disintegrated in deionized water and the drug entrapment efficiency was investigated (section 2.3). This was followed by performing the nebulization studies as described earlier in section 2.5. DPPC formulations using mannitol as the carrier with cholesterol as a lipid ingredient had prolonged the time for the completed nebulization (26.72±1.57 min). DPPC-based salt-based formulations with cholesterol and a non-effervescent mannitol formulation demonstrated a similar nebulization time to SPC-based formulations (22–23 min). The mannitol-based formulation also demonstrated a significantly different (p≤0.05) nebulization time compared to the non-effervescent mannitol-based formulation with cholesterol and water. The salt-based effervescent formulation demonstrated no significant difference when compared to non-effervescent formulations and water (Table 4-2). It is possible that mannitol had an interaction with DPPC and cholesterol in a different manner when compared to other formulations, resulting in different viscosities and physiochemical properties. This might be the reason behind the difference in time taken for nebulization (McCallion et al., 1995; Steckel and Eskandar, 2003).

Mannitol and salt formulation with cholesterol had differences in nebulization time. Different carriers have affected nebulization in the presence of effervescent ingredients. Effervescent mannitol with cholesterol made with DPPC demonstrated a significant
difference (p≥0.05). Compared to the DPPC:Chol formulation, the mannitol-based SPC:Chol formulation had a shorter nebulization time.

The nebulization time of 5 ml of liposome formulation to dryness for all formulations using the Pari Sinus for both DPPC and SPC was less than 30 min. This was in agreement with the time taken for nebulization using the Pari Plus (air jet) nebulizer (Elhissi et al., 2007). This indicates that the effervescent properties and the use of DPPC did not considerably affect nebulization time.

Sputtering time differed between the non-effervescent mannitol with cholesterol formulation and the corresponding effervescent preparation (p≤0.05). The effervescent salt-based formulation, however, did not show any significant difference compared to the control non-effervescent mannitol with cholesterol. The difference in carrier was found to affect sputtering time when mannitol- and salt-based effervescent formulations were compared. The sputtering time of the DPPC-based formulations were slightly different compared to the SPC-based formulations. The sputtering time for effervescent mannitol-based formulations and effervescent salt-based formulations with cholesterol between SPC-based and DPPC-based formulations were similar in time and were not found to be significantly different.
Table 4-3: Nebulization data for effervescent formulations prepared using DPPC as a lipid using the Pari Sinus nebulizer

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nebulization time (min)</th>
<th>Sputtering time (min)</th>
<th>Mass median diameter (VMD) of Droplet(µm)</th>
<th>Fine particle fraction (% &lt; 5 µm)</th>
<th>Span</th>
<th>Aerosol mass output rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol with cholesterol</td>
<td>26.72±1.57</td>
<td>2.98±0.34</td>
<td>6.37±0.40</td>
<td>50.07±5.60</td>
<td>20.22±5.97</td>
<td>84.47±9.01</td>
</tr>
<tr>
<td>Salt with cholesterol</td>
<td>22.26±1.15</td>
<td>2.22±0.12</td>
<td>6.11±0.68</td>
<td>50.67±6.1</td>
<td>21.60±2.63</td>
<td>82.29±6.34</td>
</tr>
<tr>
<td>Non-effervescent liposomes (mannitol with cholesterol)</td>
<td>22.53±1.57</td>
<td>1.96±0.35</td>
<td>5.79±0.41</td>
<td>54.57±0.41</td>
<td>22.43±1.80</td>
<td>76.96±4.53</td>
</tr>
<tr>
<td>Water</td>
<td>21.85±0.53</td>
<td>2.61±0.38</td>
<td>4.09±0.78</td>
<td>70.78±14.18</td>
<td>1.40±0.15</td>
<td>66.68±1.582</td>
</tr>
</tbody>
</table>
### 4.3.2.2. Determination of size distribution of the generated aerosols

The DPPC-based formulations demonstrated an aerodynamic diameter that was slightly larger compared to the SPC formulations. However, they were still in the range of 5–6 µm (Table 4-1 and Table 4-3). The mannitol- and salt-based effervescent formulations with cholesterol did not have a significant difference in aerosol size compared to the control, or to each other. The effervescent property and carrier type did not affect the aerosol size (Table 4-3).

The data on effervescent formulations with the SPC lipid suggest that the change in lipid had a significant effect on aerosol size \((p \leq 0.05)\) in effervescent mannitol-based formulations with cholesterol. The same observation about aerosol size was found to be the case for salt with cholesterol formulations using SPC or DPPC formulations \((p \leq 0.05)\). Data also suggest that aerosol size was smaller for the SPC formulations for both mannitol and salt effervescent formulations, compared to DPPC.

A study on an air-jet nebulizer suggested that the size of aerosol droplets was more dependent on the type of nebulizer than on the composition of liposomes (Bridges and Taylor, 1998). Liposomes were reduced in size during nebulization, resulting in leakage of the originally entrapped drug, and greater instability was observed for larger liposomes (Elhissi et al., 2007; Niven et al., 1991; Taylor et al., 1990). Even though DPPC formulations produced slightly larger liposomes compared to SPC formulations, these liposomes might be fragmented to smaller sizes, so that they may fit into the aerosol droplets released by the nebulizer. However, this fragmentation is usually accompanied by loss of the drug from the liposomes during nebulization.

The span of the DPPC formulations was notably high compared to the SPC formulations (Table 4-3). The DPPC formulations had a span in the range of 5–7, while the SPC formulations had span values in the range of 1–3. The aerosol generated from all
effervescent and non-effervescent DPPC formulations demonstrated significantly different span values in comparison to water. However, when effervescent formulations were compared to non-effervescent formulations no significant difference was observed. The difference in aerosol characteristics using liposomes with a range of compositions might be attributed to the different fluid physicochemical properties. Fluid properties have been previously shown to influence the nebulized aerosol performance (Ghazanfari et al., 2007). However, the carrier type (mannitol or salt) had no effect on aerosol size distribution.

4.3.2.3. Fine particle fraction (FPF)

The effervescent formulation made with DPPC demonstrated FPF values in the range of 50–55%; this was slightly higher than FPF using the earlier used SPC formulations. The FPF of aerosols generated from non-effervescent DPPC:Chol formulations using mannitol or salt carriers were similar to FPF generated from water. Non-effervescent formulations and effervescent DPPC formulations both had similar FPF values. A change of carrier between mannitol and salt did not seem to have any effect on FPF of the generated aerosol (Table 4-3).

Mannitol formulations with SPC:Chol or DPPC:Chol demonstrated similar FPF values. The FPF of salt formulations with cholesterol when compared to formulations made with two different lipids demonstrated nearly 20% compared to SPC (p≤0.05). Therefore, lipid composition may affect FPF only when salt was used as carrier.

Previous studies have shown that lipid composition may greatly affect the FPF of aerosols generated from liposome formulations (Darwis and Kellaway, 2001b). Effervescent liposomes made with DPPC had lower FPF than conventional non-effervescent liposomes made in this study. However, the FPF of aerosols generated from salt-based effervescent formulations with SPC was higher (Table 4-3).
4.3.2.4. Mass output for DPPC effervescent formulations

Effervescent formulations with DPPC when nebulized through the Pari Sinus nebulizer to ‘dryness’ did not result in atomization of all the formulation; therefore, some residual fluid was left behind within the reservoir of the nebulizer. Hence, aerosol mass output and drug output were less than 100% (Table 4-3). The study done by Elhissi et al. (2006) indicated formulation differences in liposomes affecting the mass output of several liposomes. Therefore, understanding the effect of effervescent formulations on the Pari Sinus nebulizer and how changes of effervescent formulations, such as type of carrier and lipid used affect the mass output rate, was an interesting part of the investigation.

Improved aerosol mass output percentages for effervescent formulations were found compared to formulations without effervescence (conventional liposomes) and water samples (Table 4-3). Effervescent mannitol (84.47±9.01%) and salt formulations (82.29±6.34%) with cholesterol were significantly different in aerosol mass output compared to deionized water (66.68±1.582%) (p≤0.05). However, no significant difference was observed when effervescent formulations of mannitol and salt with cholesterol were compared to non-effervescent formulation (mannitol with cholesterol). This indicates that the effervescent property of liposomes has not hampered aerosol mass output. Formulations made with either carrier, mannitol or salt, when compared to each other did not demonstrate a significant difference in terms of aerosol output.

Both mannitol formulation and salt formulation with cholesterol made with DPPC as a lipid were compared to mannitol and salt formulation with cholesterol made with SPC. Both formulations demonstrated no significant differences in results (Table 4-1 and Table 4-3). This indicates that the type of lipid did not affect aerosol mass output.

4.3.2.5. Determination of drug entrapment after nebulization

After nebulization the drug outputs for all formulations were relatively low, both for non-effervescent and effervescent formulations (Figure 4-2). This can be explained by the
stress liposomes undergo during nebulization. Non-effervescent liposomes demonstrated low entrapment before nebulization with high SD, and low entrapment 35.04 ±5.39% after nebulization (Figure 4-2). This indicates that non-effervescent liposomes made with DPPC:Chol and mannitol were less stable during nebulization compared to effervescent liposomes. Effervescent liposomes had greater drug entrapment after nebulization, and when salt was used as a carrier and cholesterol as one of the lipid constituents, the entrapment was further enhanced.

When comparing drug entrapment after nebulization between non-effervescent (35.04±5.39%) and effervescent (61.27±3.90%) they demonstrated a significant difference in drug entrapment (p≥0.05) for mannitol-based formulations with cholesterol. The effervescent property has increased the stability of the liposomes towards shear stress. Salt-based effervescent formulations were also found to offer significantly different entrapment (p≤0.05) compared to non-effervescent liposomes. However, they were not found to be significantly different with regard to drug entrapment when compared to mannitol-based effervescent formulations. This indicates that the differences in carrier did not have any impact on the physical stability of liposomes during nebulization.

The incorporation of cholesterol in liposomes has been found to increase rigidity of the liposome membrane, which can reduce drug losses during jet nebulization (Moribe et al., 1999; Subczynski et al., 1994).
It is suggested that including cholesterol and other rigidifying agents increases the resistance of conventional liposomes and also reduces leakage of the drug during nebulization (Elhissi et al., 2012). Low entrapment of drugs within liposomes of steroid drugs is due to the geometric structure of steroid molecules, which reduce interaction of the drug with lipid bilayers. Research also indicates that BDP encapsulation is inversely proportional to the transition temperature of the phospholipid (Darwis, 2000; Darwis and Kellaway, 2001b; Szoka and Papahadjopoulos, 1980). Liposomes made with lipids with an increased length of acryl chains were found to have lower entrapment of BDP (Darwis and Kellaway, 2001b). The rigidity of liposomes has also been found to increase with higher transition temperature lipids. However, this was in contrast to this study, which concluded that DPPC was a better choice of lipid for enhancing the entrapment of BDP before and after nebulization, compared to SPC.

BDP forms complexes with the lipid head groups of the liposomes. However, these complexes have been observed to break during extrusion and sonication of liposomes.
through the polycarbonate membranes. Similarly, BDP complexes that could have been formed with the lipid head group could be broken during nebulization and that may result in the reduction of drug entrapment observed post-nebulization (Darwis and Kellaway, 2001b; Stamp and Juliano, 1979; Taniguchi et al., 1987). High entrapment observed before nebulization could be the result of these complexes between the drug and the lipid head group.

Overall, DPPC:Chol effervescent formulations, made both with mannitol or salt carriers were suitable for nebulization. While salt with cholesterol effervescent formulation demonstrates high entrapment, mannitol with cholesterol demonstrates itself to be more suitable overall for nebulization in terms of entrapment and nebulization performance.

4.3.2.6. Size of DPPC liposomes after nebulization

Non-effervescent mannitol-based formulations with cholesterol were observed to aggregated upon nebulization since the particle size measured was very high (37.48±0.33 µm), while effervescent formulations had a smaller size, possibly indicating less liposome aggregation (Table 4-4). When mannitol or salt were used as carriers and cholesterol as a lipid constituent, effervescent and non-effervescent liposomes did not massively aggregate during nebulization (Table 4-4).

Non-effervescent liposomes before nebulization (8.32±01 µm) drastically increased in size and demonstrated high aggregation after nebulization (37.48±0.33 µm). Similar increased liposome sizes were observed for both effervescent formulations upon nebulization. The effervescent property was not found to have a negative effect on liposomes upon nebulization. However, both effervescent formulations, mannitol and salt with cholesterol, were observed to be not significantly different in size before and after nebulization, even though drastic size differences are observed. A change of carrier in effervescent formulation did not seem to affect liposome size after nebulization. SPC-based formulations were also observed to increase in liposome size and an aggregation of
liposomes was found. This indicates that a change of lipid does not have any effect on reducing aggregation and the increase in liposome size post-nebulization.

Research done on the Pari air-jet nebulizer with conventional non-effervescent liposomes demonstrates similar results with smaller liposomes before nebulization (Elhissi et al., 2012). Moreover, smaller liposomes were incorporated into smaller aerosols while larger liposomes were incorporated into larger aerosol droplets during nebulization. The liposome size of the residual volume was not found to be changed in the Pari Air-jet nebulizer (Elhissi et al., 2012). This indicates liposome accumulation and aggregation is observed during nebulization (Bridges and Taylor, 2000). Solvent evaporation by compressed gas employed during jet nebulization has been found to cause aggregation of liposomes. This could also be the case with the Pari Sinus nebulizer, which demonstrates that large liposomes aggregate post-nebulization (Clay et al., 1983).
Table 4-4: Characterization of DPPC-based liposomes before and after nebulization with the Pari Sinus nebulizer

<table>
<thead>
<tr>
<th></th>
<th>Before nebulization</th>
<th></th>
<th>After nebulization</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size (µm)</td>
<td>Span</td>
<td>Zeta potential (mV)</td>
<td>Size (µm)</td>
</tr>
<tr>
<td>Non-effervescent mannitol with cholesterol</td>
<td>8.32±0.1</td>
<td>1.93±0.42</td>
<td>2.47±0.36</td>
<td>37.48±0.33</td>
</tr>
<tr>
<td>Effervescent mannitol with cholesterol</td>
<td>7.54±0.155</td>
<td>1.51±0.071</td>
<td>2.53±0.18</td>
<td>17.79±0.03</td>
</tr>
<tr>
<td>Effervescent salt with cholesterol</td>
<td>7.19±0.11</td>
<td>1.56±0.12</td>
<td>3.83±0.62</td>
<td>22.12±0.05</td>
</tr>
</tbody>
</table>
However, the result of the effervescent liposomes were in contrast with the results of Taylor et al., (1990) in that the increased reduction of liposome size after nebulization was a result of the breakage of liposome aggregates during nebulization within the reservoir. Darwis and Kellaway (2001b) indicate that it is assumed that liposome size within the reservoir is an indication of the liposome size of the nebulized liposomes. Literature also indicates that liposomes could aggregate within the nebulizer reservoir due to the transition temperature of the lipid being higher than the temperature in the nebulizer reservoir (Waldrep et al., 1993). The research indicates BDP-DPPC liposomes demonstrated efficient nebulization of liposomal aerosols, with the liposome size before nebulization $15.78\pm1.62 \mu m$ increasing in size post-nebulization to $47.51\pm8.32 \mu m$. Effervescent DPPC-BDP liposomes demonstrated much smaller liposomes post-nebulization, indicating potentially better aerosols and drug output compared to liposomes of research by Waldrep et al. (1993).

The span of liposomes is similar and not significant for non-effervescent liposomes, both before and after nebulization. Effervescent mannitol and salt with cholesterol formulations demonstrate similar span values. Mannitol with cholesterol effervescent formulation demonstrated significant differences ($P<0.05$), while salt with cholesterol does not seem to have significant differences in span value when compared to non-effervescent liposomes (Table 4-4). No significant differences in span were observed between effervescent formulations. Similarly, Waldrep et al.’s (1993) results demonstrate DPPC-BDP liposomes ($1.76\pm0.25$) and other lipids such as DMPC ($1.40\pm0.26$) and DLPC ($0.85\pm0.19$) demonstrate similar values post-nebulization. Research also indicated that span values post-nebulization are slightly smaller compared to pre-nebulization. This result correlates with effervescent DPPC-BDP liposomes.
Non-effervescent liposomes demonstrated negative zeta potential values post-nebulization, while effervescent formulations were both charged positively (Table 4-4). Positive charges were slightly increased for effervescent formulations after nebulization but were not deemed significant. However, both formulations when compared to non-effervescent formulations demonstrated significantly different changes in charge (P<0.05).

In general, both DPPC-based effervescent and non-effervescent liposomes were stable and produced deliverable liposomes. However, the effervescent property seems to have a positive effect on the stability of liposomes and their ability to withstand shear stress during nebulization. Overall, mannitol with cholesterol seemed to have better characteristics more suitable for further research in terms of characterization, entrapment, and nebulization, while salt with effervescent seems to be the best formulation when only considering the nebulization aspect of the research and entrapment post-nebulization.

4.4. Conclusion for Nebulization

Novel BDP effervescent proliposomes for both SPC and DPPC lipids generated deliverable and stable liposomes via the Pari Sinus nebulizer. Liposomes produced with effervescent properties did not seem to hamper the formulation’s ability to efficiently nebulize the drug when compared to BDP loaded into conventional liposomes for both SPC and DPPC.

The nebulization time and sputtering times for all SPC-based formations were similar. The VMD of aerosol droplets for SPC-based formulations was around 3–4 µm. The droplet size of the aerosol was significantly (P≤0.05) different when mannitol and salt effervescent formulations were compared for SPC-based formulations. Furthermore, the aerosol mass output did not seem to be affected by the addition of cholesterol; however, the omission of cholesterol did seem to affect the span values in mannitol-based effervescent formulations compared to mannitol-based formulations with cholesterol.
(P≤0.05). Formulations without cholesterol demonstrated lower nebulization efficiency. The findings of the SPC-based formulations demonstrated that mannitol as a carrier increased aerosol droplet size, whereas salt formulations made with SPC reduced aerosol droplet size. Furthermore, mannitol with cholesterol was found to produce liposomes with a size of 5.14±0.49 µm (mean + SD), with the appropriate liposomal characteristics in terms of size, span, and zeta charge. The mannitol with cholesterol formulation also performed well in terms of the aerodynamic diameter and aerosol mass output (%), but demonstrated a lower FPF rate of 59%.

DPPC-based formulations of mannitol with cholesterol and salt with cholesterol were chosen for nebulization due to their suitability in terms of liposomes size, span, zeta potential, and entrapment of BDP. Nebulization of both samples demonstrated a similar nebulization time and sputtering time to SPC-based formulations and conventional formulations without effervescent property, indicating no negative impact of nebulization and sputtering time performance by the effervescent ingredients. However, mannitol and salt effervescent formulations demonstrated significant differences in nebulization time (P≥0.05). The difference in carriers seems to have an effect on nebulization time. A change of lipid also had an impact on nebulization time when SPC took less time and DPPC-based mannitol with cholesterol formulations were investigated. However, due to the high entrapment of the drug by the DPPC formulations compared to SPC it provided an answer for the differences in time and increased patient compliance.

Drug entrapment post-nebulization of non-effervescent formulations and effervescent mannitol with cholesterol, demonstrated significant increases in drug entrapment for DPPC-based effervescent formulations (61.27±3.90%). The effervescent property was found to improve the liposomes’ ability to withstand shear stress. However, carrier choice of mannitol or salt did not seem to have any impact on the mechanical strength of liposomes.
When looking at both lipids, mannitol with cholesterol effervescent formulations seemed to have the greatest potential for drug delivery to the sinus via the Pari Sinus nebulizer. It can be concluded that the nebulizer mechanism was the deciding factor affecting aerosol mass output compared to other factors in effervescent liposomal formulations. In general, the Pari Sinus nebulizer has performed well compared to research done using different nebulizers. Mannitol and salt with cholesterol and DPPC would be most appropriate for further research. DPPC helped to increase the drug entrapment and both effervescent formulations performed very well in all aspects of nebulization. Therefore, it is recommended that further work be done on effervescent formulations made with mannitol with cholesterol and salt with cholesterol for the drug BDP for delivery to the sinuses via the Pari Sinus nebulizer.
CHAPTER 5
DRUG DEPOSITION
STUDY IN NASAL CAST
AND IMPINGER
SYSTEM
5.1. Introduction

Intranasal delivery is a common approach for the administration of peptides, proteins, and polar drugs, which have a low oral bioavailability, poor stability, poor intestinal absorption, or extensive hepatic first pass degradation (Pires et al., 2009). Non-invasiveness, large surface area, permeable/vascularized mucosa, rapid systemic drug absorption, quick onset of action, painless administration, and favourable tolerability are among the advantages offered by the nasal route of drug administration (Rapoport and Winner, 2006).

Even though nasal drug administration has been used for decades, only a limited amount of research has been conducted on factors influencing drug deposition patterns within the nasal cavity (Kundoor and Dalby, 2011) let alone sinuses, due to the inaccessible location and difficulty in delivery of aerosols to the targeted site. Literature indicates that the device used for drug administration, device handling by the patient, inhalation of spray, delivered dose, and formulation effects on spray plumb and droplet size are key factors affecting drug delivery to the nasal cavity (Newman et al., 1994; Kublik and Vidgren, 1998; Kundoor and Dalby, 2011).

Aerosol particles larger than 10 µm are likely to deposit in the nasal cavity while particles smaller than 5 µm may reach the lung (Kundoor and Dalby, 2011). However, due to limited ventilation and the hidden location of the sinuses, particles larger than 10 µm are unlikely to deposit into the sinuses (Sato et al., 1981; Möller et al., 2008; Keller et al., 2010). The Pari Sinus nebulizer generates a pulsating aerosol that is especially designed to deliver aerosols to the sinus through its special ‘snake-like’ aerosols manoeuvre (Keller et al., 2010). The Pari Sinus nebulizer brochure describes the ability of this device to deliver aerosols directly to the sinus via generating aerosols with a mass median diameter of 3.2 µm, hence a lower medication dose is needed and less side effects are elicited (Schuschnig et al., 2006). The Pari Sinus nebulizer was also found to deposit more
medication in the sinus cavity compared to nasal sprays (Schuschnig et al., 2006). The viscosity of formulation may affect aerosol droplet size, plume angles, and angle of the device during administration, and all may affect the deposition profile of the spray in the nose and generally in the respiratory system (Harris et al., 1988; Cheng et al., 2001; Foo et al., 2007).

Very limited research on aerosol deposition patterns in the sinuses has been conducted, due to the anatomical position of the sinuses. Many devices have been used for aerosol drug delivery to the nasal cavity. Deposition patterns within the sinus and nasal cavity through nebulization has not been thoroughly investigated.

In this report, a fast, less expensive colour-based method was designed by assembling a unique system (Figure 5-1: by using a transparent nasal cast connected to a two-stage impinger via a balloon). The nasal cast was coated with water indicating paste Sar-Gel®, and the nasal cast impinger system was fixed onto a vacuum system of 60 l/min to comply with the inspiration flow rate used in pulmonary delivery studies (Figure 5-1 and Figure 5-2). This study has investigated the effectiveness of incorporating the effervescent ingredients in liposomes along with the drug, to increase drug deposition in parasinuses. The Pari Sinus nebulizer (designed for drug delivery to the sinuses) and Pari Sprint nebulizer (designed for pulmonary delivery) were compared on the basis of their ability to deliver aerosols to the in vitro model of nasal cavity, sinuses, and lower respiratory airways using the nasal cast two-stage impinge model.
Figure 5.1: Nasal cast coated with water indicating paste Sar-Gel® to an impinger system (vacuum of 60 l/min to mimic normal breathing).

Figure 5.2 Nasal cast was coated with water indicating paste Sar-Gel®.
5.2. Specific Aims

This study was designed to analyze the delivery of BDP-loaded effervescent liposomes and the non-effervescent liposome deposition profile of aerosols in the aforementioned nasal cast model using the Pari Sinus (pulsating aerosol system) and Pari Sprint (non-Pulsating aerosol system) air-jet nebulizers.

The deposition was also compared between the different effervescent formulations. A high-performance liquid chromatography (HPLC) analysis was conducted to quantify the deposition profile of the drug BDP within the nasal cast and the impinger (upper stage and lower stage) using a range of liposome formulations and by employing the two aforementioned jet nebulizers.
5.3. Results and Discussion

The deposition profile of nebulized particles in the nasal cavity is largely dependent on airflow through the nose (Giroux et al., 2005). The flow of air in the upper posterior region of the nasal cavity is very difficult; this part allows access to the paranasal sinuses (Giroux et al., 2005). Nebulizers were originally designed for pulmonary delivery; however, targeting the nasal cavity using nebulizers is now an established approach in nasal delivery. Targeting the paranasinuses via nebulization has been improved by generating smaller aerosol particles and particularly by using the pulsating aerosolization technology, which generates aerosol clouds having ‘snake-like’ manoeuvres, helping access to the hidden sinus pockets (PARI Respiratory Equipment, Inc., 2012).

Mannitol-based and salt-based DPPC:Chol effervescent formulations have generated liposomes potentially suitable for drug delivery to the sinuses, as concluded from HPLC analysis. These results are further supported by the previous positive findings of size analysis (VMD and span), zeta potential measurements, and BDP entrapment efficiency. Therefore, these two formulations were chosen for the last part of the research.

5.3.1. Nebulization deposition patterns of BDP-loaded liposomes with DPPC-based formulations

5.3.1.1. Deposition analysis of full nasal cavity for Pari Sinus Nebulizer

In this study, the deposition profiles of the two effervescent formulations, mannitol-based and salt-based DPPC:Chol liposomes, were studied and compared with those of deionized water and the corresponding non-effervescent liposomes using the two aforementioned nebulizers. Investigating deposition areas with different formulations will help to study if different viscosities, ingredients, addition of liposomes, and incorporation of the effervescent property to liposomes impact deposition area and pattern. Distilled water was nebulized through the Pari Sinus nebulizer and the distribution area was compared to liposomal formulations nebulized with the Pari Sprint nebulizer. It was observed that
control samples (water) had the most deposition area in the nasal cavity when nebulized via the Pari Sinus 132.71±47.42 cm² (Table 5-1). This was the largest deposition area observed with both nebulizers and formulations. The mannitol-based effervescent formulation had a total deposition area of 107.16±5.50 cm² and the salt-based effervescent preparation had a deposition area of 107.94±12.05 cm²; thus, the carrier type did not affect the deposition profile of the effervescent formulations. Furthermore, since there is no difference in deposition between the two effervescent formulations it is safe to hypothesize that for patients (e.g. diabetics) who need sugar-free medication, DPPC-based salt with cholesterol formulations may be used.

Incorporation of liposomes showed a trend of lower deposition (P≥0.5) nasal cast deposition compared to the control water (Table 5-1). The total area of the nasal cast used in this study is 158.99 cm², and the total area of the respiratory zone of a human nasal cavity has been found to vary in the range of 120–150 cm² (Grassin-Delyle et al., 2012). When analyzing the deposition area as a percentage of the total nasal cavity as a whole, it can be observed that water has a deposition of 83.47% while non-effervescent formulations demonstrated a deposition of 65.57%. By contrast, both effervescent formulations demonstrated a deposition of 67.40% (mannitol with cholesterol) and salt demonstrating 67.89% (salt with cholesterol) (Figure 5-4). Previous investigations with nasal sprays having different designs have shown lower deposition (Kundoor and Dalby, 2011) compared to the values obtained in the present study. This highlights the advantage of using the Pari Sinus nebulizer for nasal delivery.

The lower deposition of liposomes compared to water might be explained by the different physicochemical properties of liposomes. Results from the Pari Sinus Nebulizer were in agreement with the nasal cast findings previously published by Kundoor and Dalby (2011). Researchers found that Zicam nasal spray with increased viscosity demonstrated significantly lower nasal deposition compared to the nasal sprays with lower viscosity.
values. Furthermore, they have reported that high viscosity of formulations may produce larger droplets, with greater deposition in the anterior part of the nasal cavity. Recently, two nebulizers (Atomisor Sonique® and Easynose®) with a droplet size of 5.6 \( \mu m \) were compared for nasal drug deposition by nebulizing \(^{99m}\)Tc-DTPA tagged aerosols in healthy volunteers. A human plastinated head model and its replica constructed from CT scans was used for this study (Guellec et al., 2014). Deposition was determined in the upper nasal cavity and maxillary sinus (MS) regions. Results indicated no significant difference between volunteers and human plastinated head model (NC1). However, a significant difference in low aerosol deposition was observed in the nasal model made from the CT scan compared to volunteers. They concluded that nasal cast models are suitable for the prediction of aerosol deposition but the reliability of the model is actually dependent on its design. Therefore, further testing of the transparent nasal cast used in the present research as compared to different nasal casts used in literature may constitute an essential part of the future research of nasal delivery.

One drawback observed in using a nasal cast in this experiment is the overload on the location of the deposition due to the quantity needed for testing (20 ml was nebulized by nebulizing 5 ml each, four times). The same drawback was observed with nasal casts in the study of Guellec and co-workers (Guellec et al., 2014). Furthermore, findings suggested that the radioactive count gamma images (in vitro) used for deposition investigation may have been affected by the materials used for building the two nasal casts. Considering this aspect, the colour-based method used with the transparent nasal cast could provide a clear advantage, adding to the fact that it is fast, cheap and highly convenient to use for analyzing nasal drug deposition in vitro.
Table 5-1: Deposition area analysis of effervescent liposomal formulations in the nasal cast (overall) and the sinuses region when nebulized via the Pari Sinus and Pari Sprint nebulizers, n=3

<table>
<thead>
<tr>
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<th>Pari Sinus nebulizer</th>
<th>Pari Sprint nebulizer</th>
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<tbody>
<tr>
<td></td>
<td>Full nasal cavity (cm²)</td>
<td>Sinus alone (cm²)</td>
</tr>
<tr>
<td>Water</td>
<td>132.71±47.42</td>
<td>43.57±6.39</td>
</tr>
<tr>
<td>Non-effervescent liposomes (mannitol with cholesterol)</td>
<td>104.26±7.07</td>
<td>46.43±4.28</td>
</tr>
<tr>
<td>Effervescent mannitol with cholesterol</td>
<td>107.16±5.50</td>
<td>48.45±2.75</td>
</tr>
<tr>
<td>Effervescent salt with cholesterol</td>
<td>107.94±12.05</td>
<td>47.09±1.92</td>
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5.3.1.2. Deposition analysis of full nasal cavity for Pari Sprint nebulizer

The air-jet Pari Sprint nebulizer is typically used to generate aerosols for the treatment of lower lung diseases. However, in this study it was used for the nebulization of effervescent liposomes to the sinuses and compared with the Pari Sinus nebulizer in order to evaluate the influence of pulsating aerosol technology on drug deposition in the nasal cavity in general, and access to the parasinuses in particular. A nasal cast deposition study using the Pari Sprint nebulizer demonstrated a lower deposition area for all formulations compared to the Pari Sinus nebulizer (Table 5-1), indicating that the ‘snake-like’ manoeuvre of aerosols generated by the Pari Sinus was advantageous at maximizing deposition in the nasal cast. Distilled water was used as the control fluid, which when nebulized with the Pari Sprint had a deposition area of 73.05±4.68 cm², which was significantly less that that using the Pari Sinus nebulizer (132.71±47.42 cm²).
Non-effervescent liposomes demonstrated higher deposition than distilled water (P≤0.05), while effervescent mannitol-based liposome formulations demonstrated a similar deposition to water. The salt-based DPPC:Chol liposomes have performed really well, with similar deposition (P>0.05) compared to the non-effervescent liposomes formulation (Table 5-1). The difference in carrier did not significantly affect the total deposition area in the nasal cast for the Pari Sprint nebulizer. Inclusion of the effervescent ingredients has reduced the deposition area for the mannitol-based formulation; however, the deposition of the salt-based formulation was unaffected.

The mannitol-based effervescent formulation was observed to have a significantly lower deposition area by the Pari Sprint nebulizer (82.66±10.29 cm²) compared to the Pari Sinus nebulizer (107.16±5.50 cm²) (P≤0.05). However, for salt-based proliposomes no significant difference between the two nebulizers was observed when deposition in the whole nasal cast was considered. Results indicate, in general, that the Pari Sprint nebulizer was less suitable for liposome delivery to the nasal cast when compared to the Pari Sinus nebulizer, which is possibly attributed to the mechanism of aerosol delivery rather than the aerosol size produced by the two nebulizers. The Pari Sprint nebulizers generate droplets with a size around 3.5 µm, while the Pari Sinus nebulizer generates droplets having a median diameter around 3.2 µm (Pari Gmbh brochures).

5.3.1.3. Deposition analysis of aerosols in the sinuses using Pari Sinus nebulizer

The difficulty of drug delivery to the paranasal sinuses is attributed to their anatomical position, resulting in difficulty to access them. Active ventilation is not reported in the ostiomeatal complex and sinuses. The ostiomeatal complexes connect nasal passages through small orifices called ostia (typically 0.5–2 mm in diameter). Only very limited air flows into the ostia during inhalation through the nose and, in fact, most air passes to the trachea rather than the sinuses (Keller et al., 2014). However, aerosol deposition in the sinuses is possible, even though difficult, due to low ventilated cavities and right of
entry to the nose only via narrow ducts. Creation of a pressure gradient between nasal and paranasal cavities is the general principle that allows aerosols to access the inactively ventilated areas of the paranasal sinuses (Mainz et al., 2011). Mathematical modelling of drug deposition into the sinuses is ascribed to three factors: particle size, pressure gradient, and size of the ostium (Martin et al., 2007).

Using the Pari Sinus device, deionized water was found to have the lower area of deposition (43.57±6.39 cm²), compared to the liposome formulations (P≤0.05). Liposomal formulations had a similar deposition area, while effervescent mannitol-based formulations demonstrated the largest deposition area in the paranasal sinuses (48.45±2.75 cm²) (Table 5-1). No significant difference in results was observed between the non-effervescent liposomal formulation when compared to both mannitol- and salt-based effervescent formulations. This indicates that the effervescent property did not have a negative effect on the drug deposition area. The carrier type (mannitol or salt) also did not affect the drug deposition area for the Pari Sinus nebulizer (Table 5-1).

Comparatively, a fairly large amount of liposome formulation (48.45±2.75 cm²) (Table 5-1) has been deposited in the paranasal sinuses, especially the effervescent mannitol formulations, suggesting that DPPC liposomes generated from effervescent mannitol-based proliposomes was highly appropriate for targeting the parasinuses of the nasal cast, and hence, future in vivo investigations should consider this particular formulation. The effervescent mannitol-based formulation was on the lower side of deposition within the nasal cavity; however, results of sinus deposition indicate that this liposome formulation has maximized the sinus targeting.

The pulsating aerosol technology has been studied by Möller et al. (2008), investigating sinus deposition by pulsating airflow Kr-gas ventilation. The study indicated that pulsating aerosols increase the volume of sinus, which lead to an increase in sinus
deposition. The pulsating air flow increased deposition by 8% compared to non-pulsating aerosols, which had deposition as low as 0.2%.

According to the findings of this report, loading the drug into effervescent liposomes followed by nebulization using the Pari Sinus nebulizer can be successful for aerosol deposition into the parasinuses, and it certainly merits future in vivo investigations especially by conducting studies on patients having sinusitis.

Sinusitis is associated with inflamed nasal linings and infected mucous, reduced mucosal drainage, and infections. Disease conditions may make reaching the paranasal hidden pockets more difficult compared to patients with healthy sinuses. The hypothesis of enhanced drug penetration into the sinuses following drug-loaded liposomes into the nasal cavity via nebulization merits investigation.

A nasal cast model designed by Pari GmbH with four sinus cavities and four front and maxillary positions was used for the nebulization of aerosols with the VibrENT™ nebulizer. The VibrENT™ system has demonstrated the ability to deposit 10% of the aerosol in the sinuses, while 10–15% was expected to travel to the lung (Joseph, 2002). Even though this nasal cast model was possibly better designed to suit the investigation of deposition in the sinuses, it only represented the upper respiratory airways; thus, deposition in the lower airways was not possible to investigate. By contrast, the in vitro nasal deposition model used in the present report represents a nasal cast that was attached to a widely established in vitro model for quantification of ‘deep lung’ deposition, namely the two-stage impinge.

To the best knowledge of the author of this report, non-effervescent or effervescent liposomes have not yet been used as a delivery system for treating sinusitis. Salt-based DPPC:Chol proliposomes have actually generated liposomes with highly desirable properties for nebulization, since BDP entrapment efficiency was as high as 90.60±13.51% within the vesicles. Moreover, mannitol-based DPPC:Chol liposomes
were also suitable since BDP entrapment was as high as 82.15±8.29% and liposome size, span, and zeta potential were in the acceptable ranges and nebulization performance was superior. The effervescent properties of DPPC were also found to improve the liposomes’ ability to withstand shear stress during nebulization as explained in chapter 4.

5.3.1.4. Drug deposition analysis in the sinus of the nasal cast using Pari Sprint nebulizer

The Pari Sprint nebulizer was used for delivery of liposomal formulations to the sinuses of the nasal cast. Pari LC Sprint nebulizer is a nebulizer designed originally for delivering the drug to the ‘deep lung’. In this study, the potential of this nebulizer for targeting the paranasal sinuses was explored using the nasal cast model, and the findings were compared with those of the Pari Sinus device. The Pari Sprint nebulizer demonstrated different results from those observed with the Pari Sinus. With the Pari Sprint, aerosolized water covered a surface of 29.71±3.61 cm², which is half of that shown by the Pari Sinus nebulizer (43.57±6.39 cm²). However, non-effervescent liposomes have improved the deposition area (P≤0.05) when compared to water (Table 5-1). The deposition area for non-effervescent liposomes was similar for both nebulizers (P>0.05). This possibly suggests that the influence of the phospholipid on the nebulizer fluid’s physicochemical properties (viscosity, surface tension, etc.) has made the conventional Pari Sprint as capable as the Pari Sinus for targeting aerosols to the sinuses of the nasal cast. Mannitol-based effervescent liposomes demonstrated a trend for a lower deposition area but this was not significant; thus, no difference was observed in the deposition area as a result of including effervescent ingredients in the liposome formulation. Furthermore, when mannitol-based effervescent liposomes were used, a lower sinus deposition was observed with the Pari Sprint (35.52±11.11 cm²) compared to the Pari Sinus (48.45±2.75 cm²). The change of carrier has affected the deposition area only when the Pari Sprint
nebulizer was employed; thus, the area for the salt-based effervescent formulations was 42.17±8.63 cm² (Table 5-1).

Due to the poor air flow into the paranasal sinuses, very little or no aerosols can be deposited to the sinuses. By contrast, aerosols generated via vibrations of the pulsating technology nebulizers may cause periodic transient pressure gradients from ventilated nasal cavities through ostia, resulting in deposition into the sinuses by allowing a convective flow of air into the sinuses; this achieves equal air pressure in the nasal cavity and sinuses (Keller et al., 2014).

Schuschnig et al. (2008) have compared drug delivery using two human nasal cast models. Non-pulsating aerosols were found to have a chance of 85% to be expelled from the nostril of the nasal cast with minimal aerosol deposition in the sinus. In the present report, the Pari Sinus nebulizer performed better than the Pari Sprint nebulizer in terms of drug deposition in the nasal cast as well as the sinuses. Moreover, Schuschnig et al. (2008) have reported that pulsating nebulizer technology can localize the deposited aerosols in the sinuses for prolonged periods of time compared to non-pulsating aerosols.

An in vivo study was conducted using the Pari Sinus pulsating system using healthy subjects. The nasal cavities of the subjects were ventilated for 10 sec of breath-holding through 81mKr-gas in front of a planar gamma camera head. The study has reported that without pulsation only the nasal passage was ventilated, while with pulsation the sinuses were also ventilated. Moreover, the gamma camera images identified maxillary sinuses being ventilated using the pulsating aerosols (Möller et al., 2010).

5.3.2. Regional drug deposition analysis of Pari Sinus and Pari Sprint nebulizers

The efficiency of the Pari Sinus and Pari Sprint nebulizers to deliver mannitol-based DPPC:Chol effervescent liposomes or non-effervescent vesicles to the nasal cast and twin impinger was investigated. Understanding regional drug deposition within the three stages of the cast (nasal cast, upper stage, and lower stage) may help identify which
nebulizer and formulation are most suitable in terms of the potential deposition in the nasal and sinus region and upper respiratory airways.

5.3.2.1. Drug remaining within the residual volume of the Nebulizer reservoir

Figures 5-5 and 5-6 have shown that both nebulizers had a large proportion of drug remaining within the nebulizer reservoir for non-effervescent liposomes. Non-effervescent formulation demonstrated a drug distribution of 63.57±10.5% within the Pari Sinus nebulizer, while the Pari Sprint nebulizer had a drug proportion of 53.58±1.5% remaining undelivered. This demonstrated that the Pari Sprint nebulizer delivered a higher proportion of drug compared to the Pari Sinus nebulizer (Figure 5-5 and Figure 5-6). In contrast, the effervescent mannitol-based formulation was found to deliver a higher percentage of the drug compared to the non-effervescent formulation for both nebulizers. The Pari Sinus nebulizer demonstrated a drug distribution of 46.47±7.3%, while the Pari Sprint demonstrated 51.15±2.8% remaining undelivered within the nebulizers. Data indicate that effervescent formulations are able to entrap a higher amount of drugs within vesicles compared to non-effervescent formulations, which also correlates with the HPLC entrapment studies done in chapter 3. The addition of effervescent ingredients proved to be beneficial in terms of improving the vesicles’ capacity to deliver more drug from the nebulizer, to reduce leakage and in its capacity to withstand the high shear pressure of nebulization. Comparison of the mannitol-based effervescent formulations to the salt-based effervescent formulations demonstrated that the salt-based formulations are more prone to drug leakage with both nebulizers (Figure 5-2 and 5-3). The result indicates that vesicles produced by effervescent formulations delivered higher proportions of drug compared to non-effervescent formulations, regardless of the nebulizer.
5.3.2.2. Drug deposition in the nasal cast

The Pari Sinus is a specially designed nebulizer with a pulsating aerosol system that is designed to allow aerosols to reach the non-ventilated hollow cavities of the paranasal sinuses. Unlike other nebulizers that target the nasal cavity, aerosols nebulized via the Pari Sinus nebulizer are smaller, and are similar to the size of liposomes generated from the Pari Sprint nebulizer. Nasal cast drug deposition indicated that the Pari Sinus nebulizer deposited 7.3±2.9% of the original drug amount with non-effervescent liposomes while the Pari Sprint nebulizer deposited as low as 3.62±1.5% BDP to the nasal cast. Effervescent liposomes demonstrated promising results of 10.47±2.9%, while the Pari Sprint delivered as low as 4.6±1.4% to the nasal cast. Thus, effervescent liposomes delivered significantly (P≤0.05) higher drug amounts compared to the non-effervescent formulation with the Pari Sinus nebulizer. The degree of drug loss was higher with conventional liposomes in the Pari Sinus nebulizer indicating that the degree of bilayers disruption was dependent on the formulation (Figure 5-5). The addition of effervescent ingredients has increased the ability of liposomes to withstand pressure and increase drug entrapment and the liposomes’ ability to retain the drug during nebulization, regardless of the nebulizer.

Effervescent salt formulations had better drug delivery to the nasal region with the Pari Sinus (9.43±2.3%) while the Pari Sprint had a nebulization efficiency of 1.7±1.4% (P≤0.05). Effervescent mannitol formulation had a better trend of performance with both nebulizers compared to effervescent salt formulations (Figure 5-5 and Figure 5-6), but with no significant difference.

A change of carrier from mannitol to salt has hampered its ability to keep drugs entrapped without drug leakage during nebulization. Mannitol-based liposomes demonstrated less entrapment efficiency prior to nebulization compared to salt-based preparations. However, it seems to be able to withstand pressure, making it a better performing
formulation in terms of entrapment efficiency post-nebulization within the nasal cavity. The addition of effervescent ingredients has increased its ability to withstand pressure and increases both drug entrapment and the liposomes’ ability to retain the drug during nebulization, regardless of nebulizer type.

The Pari Sinus nebulizer delivered a higher proportion of drug to the nasal cast with all three formulations compared to the Pari Sprint nebulizer. The Pari Sinus nebulizer mechanism of aerosol movement (pulsating aerosol technology) proved beneficial compared to the Pari Sprint. Mannitol-based effervescent formulations were demonstrated to have the highest potential for delivering the drug. Vesicles made with mannitol-based effervescent liposomes demonstrated to be more flexible compared to liposomes made from salt-based formulations, which suffered from fragmentation during nebulization.

Studies done with nasal casts and vibrating air flow technology conclude that vibration technology, apart from helping the aerosol reach paranasal sinuses, also enhances the retention of material deposited within the nasal cavity up to a threefold longer time compared to aerosols delivered via nasal sprays (Möller et al., 2010).

A drawback of using a nasal cast to understated deposition and drug entrapment efficiency is that it could be hampered by the horizontal position of the nasal cast when the drug was nebulized (Möller et al., 2010); thus, patients are informed to observe an appropriate inhalation technique. In real life, the patient should administer drugs via the Pari Sinus through one nostril, while the other nostril should be closed. This was done with the nasal cast impinger system introduced in this study.

Literature indicates that pulsation increases nasal drug deposition compared to a non-pulsating drug delivery system by a factor of three (Möller et al., 2008). Möller et al., (2008) have stated that Kr-gas ventilation and aerosol deposition has improved drug deposition within sinus cavities by 8% while only 0.2% drugs were deposited with non-
pulsation. The study concluded that topical drug delivery to paranasal sinuses with relevant quantities is possible. The results of nasal cavity drug deposition in this study are in correlation with the results of Möller et al. (2008). Drug deposition by the Pari Sinus has improved, indicating that effervescent liposome technology, coupled with pulsation technology, is a very good candidate for further studies to improve drug deposition to the sinuses and nasal cavity.

5.3.2.3. Drug deposition in the upper stage of the twin impinger

The upper stage of the impinger was demonstrated to have a lower drug distribution compared to the nasal region of the system with the Pari Sinus nebulizer. This was expected, as larger liposomes and aerosols would be settling within the upper region, while smaller liposomes in smaller aerosol droplets would be delivered to the upper stage and lower stage of the system. The Pari Sprint nebulizer had a higher drug content delivered to the upper stage compared to the Pari Sinus. The Pari Sprint nebulizer targeted drug delivery to the lungs while the Pari Sinus nebulizer targeted drug delivery to the nasal region, and sinuses to be specific. Non-effervescent liposomes demonstrated a drug delivery of 0.86±1.2% while the Pari Sprint was observed to have a drug delivery of 8.05±2.1% to the impinger’s upper stage. Effervescent mannitol-based formulations and salt-based formulations were demonstrated to have a much less drug deposition into the upper stage with the Pari Sinus nebulizer compared to the Pari Sprint nebulizer, which demonstrated a higher drug deposition in the upper stage. However, results were not deemed significant. Adverse effects of BDP deposition in the upper respiratory tract has been observed such as hoarseness of voice, oral candiditis, cough, and Dysphonia (Barnes, 2007).

One of the techniques to improve drug delivery is to introduce drug delivery via one nostril while the other nostril is tightly closed, via a nose piece sealing it from extra air entering. This helps to maintain a high pressure amplitude of pulsating aerosols in the
nasal cavity (Keller et al., 2014; patent WO 2004/020029). This was done accordingly in our study by closing one nostril of the nasal cast. However, it is advised to keep the soft palate of the patient closed to improve drug deposition with the mouth closed, but the nasal cast does not have an oral cavity opening. In a situation of drug delivery to a patient, if the soft palate is not closed, aerosols may enter the oral cavity, reducing the amount of drug that can be deposited within the nasal cavity (Keller et al., 2014). The patient is also advised to hold their breath. Therefore, the nasal cast drug delivery system may be overlooking this potential loss of drug that may be seen if the patient does not keep the soft palate closed. Drug deposition by non-vibrating devices may also improve drug delivery by directing the drug via one nostril while the other is closed (Keller et al., 2014).

A study done on comparisons of BDP-loaded conventional and ultradeformable vesicles with drug entrapment of 50.3% and 39.5% respectively (P≤0.05), demonstrated that upon nebulization via an Aeroneb Pro nebulizer to a two-stage twin impinger, drug entrapment deceased drastically to 10.8% and 15.1% in the upper stage of the impinger (Subramanian et al., 2014). A similar study conducted compared liposome drug delivery in an impinger via delivery through an air-jet nebulizer (7.57%) and Aeroneb Pro (10.87%) in the upper stage of the impinger (Subramanian et al., 2014). This indicates that nebulizer drug delivery may also affect the liposomes’ capability to keep the drug entrapped during nebulization.
Figure 5-2: Regional drug deposition study via Pari Sinus nebulizer.

Figure 5-3: Regional drug deposition study via Pari Sprint nebulizer.
5.3.2.4. Drug deposition in the lower stage of the twin impinger

Higher drug deposition in the lower impinger’s stage was observed, regardless of formulation composition and nebulizer type. However, the Pari Sprint nebulizer has a higher drug deposition in the lower stage. Non-effervescent formulations offered lower drug deposition in the lower impinger using the Pari Sinus nebulizer compared to effervescent formulations. Mannitol and salt effervescent formulations demonstrated a similar drug delivery to the lower stage with the Pari Sinus nebulizer (Figure 5-5). The Pari Sprint nebulizer has a significantly higher proportion of drug delivered to the lower stage of the impinger than to the upper stage (P≤0.05) when compared with the Pari Sinus. Results correlate with the ‘size fraction’ indication, with smaller liposomes deposited within the lower stage while larger liposomes are deposited in the nasal cast and upper stage of the impinger (Figure 5-5 and Figure 5-6).

A study done with liposomes to understand pulmonary drug delivery using a two-stage twin impinger (Elhissi et al., 2012) demonstrated larger particles being deposited within the upper stage of the impinger, while smaller liposomes deposited in the lower stage of the impinger.

Considering the desired region for drug delivery it can be concluded that the Pari Sinus nebulizer is potentially more appropriate for drug delivery using the effervescent mannitol formulations. Even though drug delivery was less in the nasal cast compared to the lower stage using the Pari Sinus nebulizer, the desired dose for nasal deposition was achieved. Furthermore, the continuous air flow using the cast impinger model may have overestimated the drug deposition in the lower impinger.
5.4. Conclusion

In conclusion, the unique system of a nasal cast coated with water indicating paste, Sar-Gel® fixed onto a two-stage impinger to analyze drug deposition within the nasal cavity proved to be efficient, simple, and greatly convenient, to predict drug deposition in the respiratory tract. Overall, the Pari Sinus Nebulizer performed better with its pulsating aerosol technology compared to the non-pulsating Pari Sprint nebulizer.

The largest deposition area for the nasal cavity was observed when water was nebulized via the Pari Sinus Nebulizer at 132.71±47.42 cm². Liposome formulations, effervescent and non-effervescent, did not show a significant difference in the deposition area demonstrated for effervescent property. Also, differences in carrier, mannitol and salts alone, were not observed to affect the nebulization deposition area when nebulization took place via the Pari Sinus nebulizer. Overall, due to the high standard deviation (standard error) observed with salt-based effervescent formulations, even though the deposition area is similar to mannitol-based formulation, the latter proved to be a better drug carrier in terms of liposome stability. Notably, even though no difference in deposition was observed for non-effervescent liposomes and effervescent liposomes within the nasal cavity, it was observed that the addition of effervescent liposomes and mannitol combination (mannitol-based effervescent formulation) improved targeting of the sinuses, bypassing nasal cavity deposition, and resulting in increased deposition within the sinuses 48.45±2.75 cm² via the Pari Sinus nebulizer.

Deposition within the sinuses proved to be better with pulsation 48.45±2.75 cm² compared to non-pulsation 35.52±11.11 cm² for an effervescent mannitol formulation. Overall, the nasal cast data demonstrated that the Pari Sinus nebulizer performed better overall with all formulations when compared to the Pari Sprint nebulizer.

Mannitol-based effervescent liposomes were observed to have the highest drug distribution in the nasal cast, indicating the maximum drug will be deposited through this
formulation when nebulized via the Pari Sinus. It can be concluded that even though drug deposition to the sinuses through BDP-loaded liposomes nebulized via a non-pulsating Pari Sprint nebulizer was possible, the pulsating technology of the ‘zig zag’ aerosol generated by the Pari Sinus improved the drug deposition within the nasal cavity and the sinuses.

A change in viscosity and the addition of liposomes changed the deposition area within the nasal cavity of the cast employed in this study. The addition of liposomes did not prove to improve deposition within the nasal cavity; however, it did improve depositing within the sinuses. The nasal cast Sar-Gel® method, coupled with the impinger, a unique and novel system introduced in this project, can be used for further studies as an efficient, simple, colour-based method of studying nasal cavity deposition. An improved version of the nasal cast to include all sinuses would one day help analyze sinus delivery accurately via this simple colour-based method.
CHAPTER 6
GENERAL CONCLUSION
6.1. General Conclusions

Effervescent proliposomes formulations using BDP as a model hydrophobic drug have demonstrated the capability to disintegrate in water in less than 5 min, compared to conventional proliposomes, which took longer than 50 min without manual shaking or vortex mixing. Effervescent proliposomes have disintegrated with no solid particles being visible in the bottom of the flask. By contrast, conventional (non-effervescent) proliposomes had visible solid particles at the bottom of the flask even after 50 min of ‘stagnant’ hydration.

6.1.1. Carrier choice for effervescent proliposomes

Two different carbohydrate carriers, sucrose and mannitol, were investigated in 1:5 w/w and 1:10 w/w lipid to carrier ratio with combinations of chloroform and ethanol. This was done in order to find the most suitable type of carrier that produces stable liposomes with desirable characteristics such as size, span, zeta potential of liposomes, and morphology of the carrier’s particles. Drugs with different percentages from 2.5 mol% to 5 mol% were tested with the best formulations using SPC and DPPC as the choice of lipid. Data indicated that mannitol is a better carbohydrate carrier than sucrose. Liposomes loaded with 2.5 mol% SPC:Chol with mannitol as a carrier was observed to have a VMD of 6.92±1.05 µm, span (1.09±0.01), and zeta potential (-1.293± 0.11 mV). Comparatively, mannitol seems to be producing better liposomes that can entrap more drug, and also due to the cooling effect that is produced through mannitol, which would be beneficial for the treatment of hot, inflamed sinuses because of the cooling effect within the nasal cavity.

6.1.2. SPC-based effervescent proliposomes (BDP- and XH-loaded)

6.1.2.1. BDP-loaded effervescent proliposomes

The liposome formulations are 1:10 lipid to carrier (mannitol) ratio with SPC lipid loaded with the BDP formulation, which were further improved with the addition of the effervescent property to improve the liposomes disintegration time. The effervescent
property was improved with the addition of sodium bicarbonate, citric acid, and sodium benzoate. Samples were tested with and without cholesterol to understand how disintegration time, size, span, zeta potential, morphology, and drug entrapment were investigated. The disintegration time of conventional mannitol-based liposomes with cholesterol was improved (56.51±1.87 min), while effervescent mannitol with cholesterol formulation disintegrated in (1.21±0.22 min). Cholesterol did not seem to affect disintegration time. Salt-based formulations with or without cholesterol also demonstrated a superior disintegration property compared to mannitol (P≤0.05).

Mannitol with cholesterol formulation demonstrated the most ideal liposomes with high drug entrapment, size, span, and zeta potential suitable for drug delivery to the sinuses. Effervescent mannitol with SPC:Chol liposomes had a size of 5.14±0.49 µm. The addition of cholesterol did not seem to have a major impact on mannitol formulations. Effervescent salt-based liposomes with cholesterol appeared to have large size liposomes that were unsuitable for drug delivery to the sinuses. However, upon excluding cholesterol, liposome size decreased to 6.04±0.19, and a significant difference was seen between the size of liposomes with cholesterol and without cholesterol. The zeta potential of mannitol-based proliposomes were mostly negative, while salt-based proliposome formulations were positively charged. Cholesterol did not affect the charge of liposomes in salt-based formulations. Therefore, considering size, span, and zeta potential, effervescent mannitol formulations with cholesterol were more suitable for further studies.

Jaafar-Maalej et al. (2010) stated that archiving 100% BDP drug entrapment efficiency was difficult, and lipid composition may have an effect on size and encapsulation efficiency of BDP in liposomes, which was further proved to be true with novel effervescent liposomes in the present report. Drug entrapment in mannitol-based liposomes with SPC:Chol was found to be 20.54±12.02%. However, cholesterol-free
formulations offered lower BDP entrapment efficiency compared to samples containing cholesterol. Cholesterol may increase stability, improve rigidity of liposomes, reduce drug leakage and affect osmosis (Sabin et al., 2006; Tseng, 2007).

It can be concluded that mannitol may help stabilize the liposomes and reduce drug leakage. The salt-based formulation without cholesterol had a lower drug entrapment of 11.28±3.40%, but was still suitable in terms of liposome size; therefore, it was decided to use mannitol with cholesterol and salt without cholesterol formulations for further studies. Thus, novel effervescent formulations able to entrap BDP with improved disintegration property were successfully produced. In the second stage of the study, the addition of mucoadhesives and changing the lipid from SPC to DPPC was done to improve drug entrapment efficiency.

6.1.2.2. Mucoadhesive-coated effervescent proliposomes

Alginic acid and chitosan mucoadhesives were used for the coating of effervescent liposomes. The best formulations, mannitol with cholesterol and salt without cholesterol, were coated by hydrating proliposomes with alginic acid and chitosan solutions (0.2% w/v or 1% w/v). The liposome size of non-effervescent liposomes was increased to 17.99±0.56 μm, while effervescent mannitol-based formulations with cholesterol were noted at 6.15±0.04 μm (P≥0.05). Alginic acid 1% w/v formulations have produced much larger effervescent mannitol-based liposomes. Liposomes were seen to be less aggregated with 0.2/v% formulations. Chitosan-based formulations in the presence of cholesterol increased the liposome size, with the smallest liposome size noted at 21.63±5.57 μm. Similar liposome size increases were observed for both mannitol- and salt-based formulations and 1% w/v concentration had high polydispersity compared to 0.2% w/v for chitosan-coated formulations.

Drug entrapment with alginic acid or chitosan proved to be unsuccessful, with very low drug entrapment when compared to mucoadhesive-free formulations. Effervescent
mannitol coated with chitosan (0.2% w/v) was observed to have an entrapment efficiency of 1.04±1.05%, while 1% showed no entrapment of the drug at all. Results indicated that alginic acid did improve drug entrapment in non-effervescent liposomes; therefore, it was concluded in the presence of effervescent salts the alginic acid did not improve drug entrapment. Possible reasons for this could be proton-catalyzed hydrolysis, and the alginic acid itself being deposited within the liposomes core, while BDP is being deposited within bilayers resulting in bursting of liposomes (Tønnesen and Karlsen, 2002). Alginic acid was also seen to be swelling in the presence of effervescent salts, leading to drug leakage. It is also possible that carbon dioxide liberation during effervescence may promote the swelling of alginic acid. Chitosan also acted similarly to the alginic acid by swelling in the presence of effervescent salts. Therefore, the addition of a mucoadhesive was not deemed successful to improve drug entrapment. Effervescent liposomes, regardless of carrier type, had higher drug entrapment in the absence of the mucoadhesive agent.

6.1.3. DPPC lipid-based effervescent proliposomes (BDP-loaded)

Effervescent formulations made with a DPPC lipid were able to generate stable and similar liposomes to conversional liposomes (non-effervescent) and SPC-based effervescent liposomes. The presence of effervescence improved the DPPC liposomes’ disintegration time when compared to conversional (i.e. non-effervescent) liposomes. Mannitol-based formulations with cholesterol had a size of 8.32±0.1, which was significantly different (P≤0.5) compared to formulations without cholesterol. Unlike SPC-based formulations, salt formulations with cholesterol had smaller liposomes. Salt formulations made using DPPC loaded with drug in the presence of cholesterol had smaller size liposomes 7.047±0.45 µm in comparison to formulations without cholesterol 17.81±0.04 µm. Cholesterol affected packing density by reducing the area per phospholipid in DPPC, reduced surface tension, and increased mechanical strength (New,
Liposome size was also seen to be dependent on the transition temperature of the lipid. The zeta potential of DPPC-based liposomes had positive surface charge, while SPC formulations mostly had negative surface charge. The size and zeta potential of effervescent formulations were affected by lipid type and drug inclusion. Salt-based effervescent formulations with DPPC lipid demonstrated the possibility of having stable liposomes with high drug entrapment without the presence of a carbohydrate carrier.

Drug entrapment in DPPC formulations was quite high compared to SPC formulations. Effervescent mannitol with cholesterol formulations using a DPPC lipid offered a drug entrapment of $82.15\pm8.29\%$ while SPC-based formulations had a much lower entrapment of $20.54\pm12.02\ (P<0.05)$. Addition of cholesterol did not create a significant difference for mannitol. However, salt-based formulations had a high entrapment of $90.60\pm13.51$ with cholesterol, while salt-based formulations without cholesterol had entrapment of $36.3\pm7.0\ (P<0.05)$. A change of lipid did have an impact on entrapment for both mannitol and salt formulations, while the addition of cholesterol made a difference in the effervescent salt formulations only.

Using mannitol as a carrier was important with SPC-based liposomes. DPPC-based formulations produce better liposomes with higher entrapment and stability. Cholesterol acts differently towards the packing structure of the two different lipids, producing liposomes with different characteristics when lipids where changed. The inclusion of cholesterol was beneficial for enhancing the stability and entrapment of effervescent formulations made with DPPC. Effervescent liposomes with both DPPC and SPC seemed to improve disintegration time when compared to conversional liposomes.

### 6.1.4. Xylometazoline hydrochloride-loaded effervescent proliposomes

Xylometazoline hydrochloride is a hydrophilic drug used as a nasal decongestant. Hydrophilic drugs generally have low entrapment in liposomes. Liposomes made with an
SPC lipid were used for investigation. Formulations were made with mannitol and salt with and without cholesterol to understand liposome characteristics and capacity to entrap 5 mol% XH. Liposomes were tested with or without sonication prior to characterization. Before sonication, the addition of XH to mannitol-based, non-effervescent liposomes without cholesterol, produced large liposomes (18.63±0.17 µm) compared to conventional liposomes with cholesterol (7.24±0.15 µm). However, effervescent mannitol-based liposomes, with cholesterol, had smaller liposome size measurements with cholesterol compared to the corresponding formulations containing no cholesterol. Salt-based effervescent liposomes with cholesterol had slightly larger liposomes (9.53±0.18 µm) compared to those free from cholesterol. Effervescent liposomes were capable of producing stable liposomes loaded with XH for both mannitol and salt formulations. Sonication was done to the same formulations to reduce liposome size. Upon sonication, mannitol with cholesterol effervescent liposomes were drastically reduced in size (2.347±1.03 µm) (P≤0.05) compared to the non-sonicated liposomes. Mannitol formulations without cholesterol were also found to differ significantly (P≤0.05) in size compared to mannitol with cholesterol upon sonication. Salt-based formulations produced liposomes that were reduced by half in size after sonication compared to mannitol-based samples. The zeta potential of liposomes upon addition of the drug was highly positive. The addition of cholesterol has slightly reduced the positive zeta potential. Drug entrapment in sonicated liposomes was studied. The entrapment efficiency of the hydrophilic drug was surprisingly very high. SPC-based liposomes had a low entrapment of BDP compared to DPPC-based liposomes. However, in the case of XH, this hydrophilic drug had very high drug entrapment. Non-effervescent liposomes with cholesterol and mannitol-based had an entrapment of 89.43±6.13%, while without cholesterol the entrapment efficiency was 69.64±7.88%.
Effervescent formulations had slightly lower entrapment of XH but the values were actually higher than BDP entrapment. Cholesterol was beneficial for enhancing the entrapment of the hydrophilic drug in mannitol formulations but did not show an effect with the salt formulations. Unlike BDP, for XH, mannitol or cholesterol did not make a significant difference in entrapment. The effervescent formulation proved to be beneficial for enhancing XH entrapment and lowering the span values compared to conventional liposomes. The size of non-effervescent liposomes was not suitable for drug delivery via the Pari Sinus nebulizer due to the large size of liposomes. Thus, formulations using mannitol or salt as carriers with cholesterol would be more suitable due to the rigidity provided by the liposomes when high shear pressure of nebulization is applied. Effervescent liposomes produced stable liposomes with a high entrapment of XH that could be potentially suitable for nasal drug delivery.

6.1.5. Nebulization of BDP-loaded effervescent liposomes with Pari Sinus nebulizer

The nebulization of BDP-loaded effervescent liposomes made with two different lipids based liposomes (SPC or DPPC) and nebulized via the Pari Sinus or the Pari Sprint nebulizers to target sinuses, was evaluated in vitro using a nasal cast attached to a twin impinger.

6.1.5.1. Nebulization of SPC-based formulations

Effervescent liposomes made with SPC and loaded with BDP produced stable liposomes, with an improved disintegration time compared to conventional non-effervescent proliposomes. Effervescent formulations using mannitol or salt carriers with or without cholesterol were investigated for their suitability for nebulization. The nebulization time to dryness ranged from 21–23 min. The nebulization time was not affected by cholesterol regardless of carrier type. Sputtering time ranged from 1–2 min for all formulations.
The VMD of aerosols ranged from 3–4 µm. The VMD of salt formulations were smaller when compared to mannitol formulations. Mannitol-based formulations with cholesterol compared to salt formulations with cholesterol were significantly different (P≤0.05). The aerosol mass output for control water was (66.68±1.582) while for effervescent mannitol with cholesterol formulations it was (74.28± 4.90) (P≤0.05). Salt with cholesterol gave the highest aerosol mass output. The effervescent property was observed to improve aerosol mass output rate. Cholesterol did not seem to have any impact on aerosol mass output. Effervescent mannitol with cholesterol liposomes loaded with BDP with liposome size of 5.14± 0.49 µm, was the most appropriate in terms of the liposome’s zeta potential, span, aerosol size, and liposome entrapment. However, it had less FPF of 59%, while salt with cholesterol showed an FPF of 77.44±3.25 (P≤0.05). Therefore, it can be concluded that SPC-based effervescent proliposomes produce liposomes and aerosols suitable for drug delivery to the sinuses via a Pari Sinus nebulizer and does not show any negative impact compared to conversional liposomes.

6.1.5.2. Nebulization of DPPC-based formulations

BDP effervescent liposomes made with a DPPC lipid also produced stable liposomes suitable for delivery via a Pari Sinus nebulizer. The effervescent property and improved disintegration property did not hamper liposomal formulation to produce aerosols with characteristics to deliver drug to the sinuses of the cast. DPPC-based liposomal formulations made with mannitol and salt incorporating cholesterol were used due to high drug entrapment and a suitable liposome size to compare with liposomes and aerosols produced by SPC-based formulations.

The nebulization time of DPPC-based mannitol with cholesterol effervescent liposomes formulation took slightly longer to nebulize compared to a salt-based formulation and control water. Effervescent mannitol with cholesterol formulation compared to mannitol with cholesterol conversional formulation demonstrated a significant difference in
nebulization time \( (p \leq 0.05) \). A change in lipid from SPC to DPPC affected nebulization time, with the DPPC sample taking a longer time to dry.

The aerosol size of liposomes was between 5–6 µm and not hampered by effervescent property or choice of carrier. However, the aerosol size was slightly higher than SPC-based formulations. The span of DPPC-based formulations was notably high compared to SPC-based formulations.

Choice of carrier, mannitol or salt, did not affect the fine particle fractionation of DPPC-based liposomal formulations aerosols. However, a change of lipid from SPC to DPPC caused a lowering in FPF of DPPC to 50–55%. A difference in carrier did not affect the aerosol mass output (%). SPC-based formulations performed better in terms of aerosol mass output (%) compared to SPC formulations. However, SPC liposomes had less drug entrapment compared to DPPC liposomes; thus, DPPC formulations, owing to the high drug entrapment, would be more appropriate for drug delivery to the sinuses.

Effervescent proliposomes of mannitol with cholesterol retained a higher entrapment of 61.27±3.90%, when compared to non-effervescent liposomal formulations. This indicates that the effervescent property can improve the ability of liposomes to withstand shear stress. The choice of carrier did not affect the liposome’s ability to withstand shearing. The mass output rate was mainly affected by nebulizer type. Both mannitol with cholesterol and salt with cholesterol made with a DPPC lipid were chosen for further studies with the nasal cast, due to high performance in drug entrapment and aerosol characteristics. DPPC was overall a better choice of lipid for novel effervescent BDP-loaded formulations, while the SPC lipid performed well with the hydrophilic drug XH.

Data from salt-based effervescent liposomes made with DPPC indicate the possibility of a sugar-free, stable liposome formulation with high drug entrapment for further work. The Pari Sinus nebulizer produced aerosols for drug delivery with effervescent liposomal
formulations; therefore, nasal cast work would help identify if the deposition area was affected by carrier type, cholesterol inclusion and nebulizer type.

6.1.6. Nasal cast and impinger studies using Pari Sinus and Pari Sprint nebulizer

Chapter 5 of this thesis focused on factors affecting nasal drug deposition patterns within the nasal cavity and sinuses. The Pari Sinus pulsating aerosol system’s snake-like movement was compared to a nebulizer with the non-pulsating aerosol system of the Pari Sprint nebulizer. A novel system of a Sar-Gel® (water indicating paste) coated clear nasal cast fixed to a two-stage impinger system was set up to analyze drug deposition within the nasal cavity. This system proved to be simple, effective, and a cheap colour-based method to help identify deposition patterns in a matter of minutes. DPPC lipid-based mannitol with cholesterol and salt with cholesterol effervescent liposomes were nebulized via both nebulizers to compare drug deposition within the nasal cavity as a whole and the sinuses.

Data indicate that drug deposition with the Pari Sinus nebulizer indicates a large nasal cavity deposition area of 132.71±47.42 cm² while both non-effervescent and effervescent formulations demonstrated less drug deposition area. Effervescent liposomal formulations based on mannitol (107.16±5.50 cm²) and salt (107.94±12.05 cm²) demonstrated similar drug deposition areas within the nasal cavity, indicating that the choice of carrier did not affect the deposition patterns. The effervescent property tended to improve nasal drug deposition when compared to non-effervescent formulations using the nasal cast model. However, mannitol with cholesterol effervescent formulation was observed to increase drug deposition within sinuses to 48.45±2.75 cm² via the Pari Sinus nebulizer.

Similarly, the difference in carrier, mannitol or salt, did not affect drug deposition within the nasal cavity via the non-pulsating aerosol system Pari Sprint nebulizer. By contrast, the Pari Sprint nebulizer had a lower drug deposition than the control water and had a
significant increase in drug deposition when a non-effervescent mannitol with cholesterol formulation was nebulized (P≤0.05). Effervescent formulations based on salt and cholesterol were noted to have a higher drug deposition with the Pari Sprint nebulizer. Sinus drug deposition was observed to be highest at 48.45±2.75 cm² with pulsation in the Pari Sinus nebulizer, compared to a drug deposition of 35.52±11.11 cm² for effervescent mannitol with cholesterol formulation for the Pari Sprint nebulizer. This indicates that even though the drug deposition within the nasal cavity was possible with the non-pulsating system of the Pari Sprint nebulizer, the “zigzag” movement of the Pari Sinus nebulizer improves drug deposition within the sinuses compared to the Pari Sprint nebulizer. It can be concluded that the Pari Sinus nebulizer with pulsating aerosol system had a higher drug deposition when compared to the non-pulsating nebulizer Pari Sprint. Drug entrapment studies within the nasal cast and two-stage impinger study demonstrated that the effervescent mannitol with cholesterol liposome formulation had a high entrapment efficiency of 47.6±6.60% compared to the non-effervescent liposome formulations with entrapment efficiency of 33.06±2.06 % (P≤0.05). The addition of effervescent liposomes and mannitol as a carrier improved drug deposition in the sinuses when nebulization took place via the Pari Sinus nebulizer; however, drug deposition in the nasal cavity as a whole was not improved. Liposomes may improve drug deposition overall by penetration through the nasal cavity; this cannot be shown using the in vitro cast model. A nasal cast with the addition of sinus cavities in all areas will improve demonstrations of sinus drug deposition as a fast, efficient, and cheap alternative to existing technologies; this should constitute part of the future studies. Overall, the whole study demonstrated that mannitol was a better choice as a proliposome carrier compared to salt. Novel effervescent liposome formulations were made with mannitol or salt effervescent to generate stable deliverable liposomes. Effervescence did not have a negative effect on liposome size, drug entrapment, or aerosol characteristics.
compared to non-effervescent liposomes. In fact, effervescence greatly shortened the disintegration time of the formulation with no need for shaking or vortex mixing to generate liposomes. DPPC demonstrated to be a better phospholipid for effervescent proliposomes loaded with BDP. Cholesterol improved liposomes’ stability, physical strength of liposome bilayers, and drug deposition profile in vitro. Inclusion of the mucoadhesive agent’s alginic acid or chitosan hampered drug entrapment of effervescent liposomes; thus, effervescent liposome formulations performed better when no mucoadhesive was included. Effervescent liposomes made with SPC proved able to entrap hydrophilic drugs such as Xylometazoline hydrochloride. A colour-based, simple, cheap, efficient, and unique system was developed for investigations of drug deposition within the nasal cavity and sinuses by incorporating a transparent nasal cast coated with Sar-Gel® (water indicating paste) attached to a two-stage impinger. Proliposome technology was established for drug delivery to the nasal cavity and sinuses of the nasal cast used. The pulsation aerosol system of the Pari Sinus nebulizer proved to be more appropriate for drug deposition within the sinuses compared to the non-pulsating Pari Sprint nebulizer. Effervescent liposomes made with mannitol and cholesterol with DPPC performed best overall, out of all formulations tested in this study.
6.2. Future Studies

Effervescent liposomes made with SPC or DPPC lipids were analyzed upon disintegration for liposomes size, span, zeta potential, and drug entrapment of BDP. The physical and chemical stability of liposomes should be evaluated in three different temperatures over a duration of three months (Panwar et al., 2010).

Isotonicity of a solution is an important indicator to understand its suitability within the body for drug delivery. Osmole concentration of a solution should ideally be the same as the solute concentration of a cell; if not, the cells could either swell or shrink. Formulations, therefore, should be tested with a haemolysis test as described in Das (1980).

Investigation of the effervescent liposome behaviour in vivo is necessary to understand its capacity to deliver drugs to the sinuses. Fluorescent tagging or 81mKr-gas inhalation imaging could be used to understand the deposition within the nasal and sinuses cavity with effervescent liposomes similar to the study done by Moeller et al. (2009).

A drug release study of effervescent liposomes in vitro with dialysis method or any other method should be conducted (Hua, 2014). A drug release study could help identify if rate of drug release is affected by the presence of salts, and if addition of cholesterol is favourable for sustaining the drug release from liposomes in the presence of salt or mannitol.

Finally, the nasal cast impinger system could be further improved by the addition of all sinuses to the nasal cast and coating of sinuses alone, to understand drug deposition within the sinus more accurately. Drug deposition could further be tested in different head tilting positions to understand if nebulization and drug delivery to the sinuses might be affected by position of the head when nebulization is performed.
CHAPTER 7
REFERENCES
7.1. Reference


n of rehydrated freeze-dried beclomethasone
dipropionate liposomes. Int. J. Pharm. 215, 113–121. doi:10.1016/S0378-5173(00)00670-0

Pharmacokinet. 42, 1107–1128. doi:10.2165/00003088-200342130-00003

Debjit Bhownik, Rakesh Kharel, Jyoti Jaiswal, Chiranjib, Biswajit, K.P. Sampath Kumar,
2010. Innovative approaches for nasal drug delivery system and its challenges and

Djupesland, P.G., 2013. Nasal drug delivery devices: characteristics and performance in
012-0108-9

improves delivery to target sites beyond the nasal valve. The Laryngoscope 116, 466–
472. doi:10.1097/01.MLG.0000199741.08517.99

Eccles, R., Eriksson, M., Garreffa, S., Chen, S.C., 2008. The nasal decongestant effect of

Eggesbø, H.B., 2006. Radiological imaging of inflammatory lesions in the nasal cavity

Eggesbø, H.B., Ringertz, S., Haanaes, O.C., Dølvik, S., Erichsen, A., Stiris, M.,
Kolmannskog, F., 1999. CT and MR imaging of the paranasal sinuses in cystic fibrosis.
Correlation with microbiological and histopathological results. Acta Radiol. Stockh.

permeability enhancement. Pharm. Res. 15, 925–930.

and aerosol properties of liposomes delivered using an air-jet nebulizer and a novel
micropump device with large mesh apertures. Int. J. Pharm. 334, 62–70.
doi:10.1016/j.ijpharm.2006.10.022


Hasanovic, A., Hollick, C., Fischinger, K., Valenta, C., 2010. Improvement in physicochemical parameters of DPPC liposomes and increase in skin permeation of


Schuschnig et al., 2006. Comparison of delivery efficiency in a nasal cast model of fluticasone (Flutide®) nasal spray versus a novel solution aerosolized via the PARI SINUS™. Presented at the OTO Conference Poster.


St. Dominic Hospital, Jackson, MS, L. Simmons, K. Thigpen, 2007. Reduction of nebulization time, number of treatments and length of stay can be achieved with breath-actuated nebulizer. Resp Care 52, 1519.


Torchilin, V.P., 2005. Recent advances with liposomes as pharmaceutical carriers. Nat. Rev. Drug Discov. 4, 145–160. doi:10.1038/nrd1632


