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**SYNTHESIS OF ROUGH-SURFACE CARRIERS FOR DRY
POWDER INHALATION**

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Doctor of Philosophy

Abstract

For dry powder inhalation (DPI), aerosolization performance depends on particle properties such as material, surface roughness, size, shape, et al. In this work, the relation of the carrier material and drug delivery performance, surface roughness and drug delivery performance, as well as carrier stability were investigated.

Mannitol and mannitol-lactose mixtures were studied initially as carrier materials. Mannitol/NaCl and mannitol/lactose microparticles that have similar size, shape, crystallinity and surface roughness were prepared successfully. The fine particle fractions (FPFs, a main parameter for the characterization of the particle aerosolization and deposition properties) of these two kinds of microparticles were studied. Mannitol was found to have lower interparticle force and better drug detachment, which resulted in a higher aerosolization performance than that of mannitol-lactose mixture.

Based on the carrier material study, mannitol was selected and the relation between carrier surface roughness and aerosolization performance was studied. Four formulations of Mannitol/LB Agar (commercial) microparticles and a formulation of mannitol/NaCl microparticles that have similar size, shape, crystallinity but different surface roughness were prepared successfully. The relation between fine particle fraction (FPF) and drug adhesion mechanism was revealed. The results indicate high surface roughness provides a balance between low drug-carrier contact area and interlocking force. It offers a good drug adhesion and detachment yielding a high FPF. An FPF of $28.6 \pm 2.4\%$ was achieved by microparticles transformed from spray-dried microparticles

using 2% mannitol(w/v)/LB Agar as feed solution. It is comparable to the highest FPF reported in the literature using lactose and spray-dried mannitol as carriers.

The results show that mannitol/LB Agar (commercial) microparticles have a short shelf-life of 3 months. Thus, the use of stabilizer for mannitol/LB Agar microparticles was investigated. The results indicate the composition of LB agar has a large influence on the stability of mannitol/LB Agar microparticles. An increase in the percentage of agar in LB Agar improved the stability of mannitol/LB Agar microparticles. Mannitol/LB Agar microparticles with an agar percentage composition of 7.26% were able to maintain the rough-surface and spherical shape for 21 months.

With the introduction of a stabilizer, it is important to be able to control the surface morphology of the microparticles. As a result, the effect of LB Agar composition (percentage of agar in LB Agar) on particle morphology was studied. The results show that different percentage of agar in LB Agar offers pollen shape as well as rough spherical shapes with surfaces covered by crevices and rods. Pollen-shape 2%mannitol/LB Agar (lab-made) microparticles with needle-like rods prepared using LB agar with an agar percentage of 77-89% have a FPF of $32.7\% \pm 2.0\%$. Which is the highest drug delivery performance reported in literatures using mannitol and lactose as carrier particles.

Abbreviations

MDI	metered dose inhalers
DPI	dry powder inhaler
CI	Carr's compressibility index
HA	hydroxyapatite
M	mannitol
Lac	lactose
LB	LB Agar
SP	spray dried
F	final
ACI	Andersen Cascade Impactor
DPI	dry powder inhalation
DSC	differential scanning calorimetry
ED	emitted dose
FDA	Food and Drug Administration
FPF	fine particle fraction
FTIR	Fourier transform infrared spectra
pMDI	pressurized metered dose inhalation
SEM	scanning electron microscopy
TGA	thermal gravimetric analysis
XRD	X-ray diffraction spectra

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Table of Contents

Abstract	i
Abbreviations	iii
Acknowledgement.....	ii
List of figures	vi
List of Tables.....	viii
Publications	ix
Chapter 1 Introduction.....	1
1.1 Introduction	1
1.2 Review of the Literature	3
1.2.1 Pulmonary Drug Delivery	4
1.2.2 Different inhalation delivery techniques.....	6
1.2.3 Factors for Deep Lung Delivery	7
1.2.4 Choice of Carrier Particles.....	10
1.2.5 Method for Preparation of carrier particles.....	17
1.2.6 Stability Study of Carrier.....	21
1.3 Objective and Scope	22
1.4 Thesis outline.....	23
Chapter 2 Experimental Design and Methods	26
2.1 Materials.....	26
2.2 Methods	26
2.2.1 Preparation of mannitol/NaCl and mannitol/lactose microparticles	26
2.2.2 Preparation of roughness-controlled mannitol/LB Agar(c) microparticles.....	27
2.2.3 Preparation of rough-surface mannitol/LB Agar(l) microparticles.....	30
2.2.4 Preparation of pollen-shape mannitol/LB Agar(l) microparticles	32
2.3 Characterization of microparticles.....	34
Chapter 3 Study of Carrier Material for Pulmonary Drug Delivery	39
3.1 Overview	39
3.2 Results and discussion	41
3.2.1 Surface properties	41
3.2.1 Morphological properties.....	43
3.2.3 Thermal Gravimetric Analysis (TGA).....	44
3.2.4 Drug Content homogeneity	45
3.2.5 In Vitro Deposition Properties.....	46
3.3 Discussion	46
3.4 Conclusion.....	48
Chapter 4 Synthesis and Roughness Study of Mannitol/LB Agar Microparticles.....	49

4.1 Overview	49
4.2 Optimization of feed solution during spray-drying process.....	50
4.2.1 Optimization of feed composition	50
4.2.2 Optimization of feed temperature	54
4.2.3 Conclusion.....	56
4.3 Characterizations	56
4.3.1 Scanning Electron Microscope (SEM)	56
4.3.2 Morphological properties.....	57
4.3.3 Differential Scanning Calorimetry (DSC)	59
4.3.4 Fourier Transform Infrared (FTIR) Spectroscopy	60
4.3.5 Thermal Gravimetric Analysis (TGA).....	61
4.3.6 Drug Content homogeneity	62
4.3.7 In Vitro Deposition Properties.....	63
4.3.8 Particle Size Distribution of BD drug	64
4.3.9 Roughness and Drug Adhesion Analysis.....	65
4.4 Discussion	66
4.5 Conclusion.....	70
Chapter 5 Synthesis and Stability Study of Mannitol/ LB Agar(lab-made) microparticles.....	72
5.1 Overview	72
5.2 Results and discussion	73
5.2.1 Morphological properties.....	74
5.2.2 Surface properties	75
5.2.3 Differential Scanning Calorimetry (DSC)	76
5.2.4 Thermal Gravimetric Analysis (TGA).....	77
5.2.5 Drug Content homogeneity	77
5.2.6 In Vitro Deposition Properties.....	78
5.2.7Stability	78
5.2.8 Stability and Agar Composition	85
5.3. Conclusion.....	86
Chapter 6 Synthesis of Pollen-shape Mannitol/LB Agar Particles with High Surface Roughness	88
6.1 Overview	88
6.2 Optimization of LB Agar Composition	88
6.3 Proposed Formation Mechanism of M2LB(77,83,86,89)-F	90
6.3.1 Morphological properties.....	90
6.3.2 Surface Morphology	92
6.3.3 Stability	94
6.3.4 Thermal Gravimetric Analysis (TGA).....	94
6.3.5 Drug Content homogeneity	95
6.3.6 In Vitro Deposition Properties.....	95

6.4 Conclusion	98
Chapter 7 Conclusion and Future Work	99
7.1 Conclusion	99
7.2 Future Work.....	102
7.2.1 Study of Relation between Carrier Size and Drug Delivery Performance	102
7.2.2 Study of Relation between Carrier Shape and Drug Delivery Performance	102
7.2.3 Animal test for mannitol/LB Agar microcarriers.....	103
Reference.....	104

List of figures

Figure 1. Schematic diagram of a human lung.	4
Figure 2 Schematic diagram demonstrating delivery process of (a) “drug-only formulation”, (b) “drug-carrier formulation”(11)	6
Figure 3 SEM of spray-dried (a) lab-scale (21) and (b)pilot-scale (17) rough particles.....	14
Figure 4 Type of drug attachment on spray-dried (a) lab-scale (21) and (b) pilot-scale (17) rough particles.	14
Figure 5 XRD of three polymorphic forms of mannitol.	17
Figure 6 Lab-scale spray dryer. (A) feed (B) atomization gas; (1) drying gas; (2) heater; (3) nozzle; (4) drying chamber; (5) connection between drying chamber and cyclone; (6) cyclone; (7) drying gas; (8) collection vessel.	19
Figure 7 Rough surface mannitol particles generated by (a) Maas et al. [19], (b) Littringeret al. [18] and (c) Littringer et al. at high magnification	20
Figure 8 SEM of (a) spray-dried M1.95Lac0.05-SP; (b) M1.95Lac0.05-F; (c) a broken M1.95Lac0.05-F ; (d) M1.875Lac0.125-F ; (e) spray-dried M2NaCl-SP.	42
Figure 9 XRD of (a) spray-dried M1.95Lac0.05-SP; (b) M1.95Lac0.05-F; (c) spray-dried M2NaCl-SP.(α : α -mannitol, β : β -mannitol, δ : δ -mannitol; α' : α -lactose, $5\alpha'/3\beta'$: $5\alpha'/3\beta$ -lactose, $4\alpha'/1\beta'$: $4\alpha'/1\beta$ -lactose).44	44
Figure 10 TGA of M1.95Lac0.05-F and oven-dried M2NaCl-SP.....	45
Figure 11 SEM of spray dried (a) M5LB-SP(c_1); (b) M5LB-SP(c_2); (c) M5LB-SP(c_3); (d) M5LB-SP(c_4); (e) M5LB-SP(c_5).	51
Figure 12 SEM of spray dried (a) M1LB-SP(c_1); (b) M1LB-SP(c_2); (c) M1LB-SP(c_3); (d) M1LB-SP(c_4).	53
Figure 13 SEM of spray dried dried (a) M5LB-SP(c_{31}); (b) M5LB-SP(c_{32}); (c) M5LB-SP(c_3); (d) M5LB-SP(c_{33});	55
Figure 14 SEM images of (a) M2LB-SP; (b)high-magnification M2LB-SP; (c) M2LB-INT; (d) high-magnification M2LB-INT; (e) M2LB-F; (f)high-magnification M2LB-F.	57
Figure 15 XRD patterns of (a) M2LB-SP; (b) M2LB-INT; and (c) M2LB-F.	58
Figure 16 DSC thermal profile of (a) M2LB-SP; (b) M2LB-INT; (c) M2LB-F microparticles.....	60
Figure 17 FTIR spectra of (a) mannitol, (b) mannitol/hexane, (c) agar, (d) agar/hexane, (e) peptone, (f) peptone/hexane, (g) yeast extract, (h) yeast extract/hexane, (i) M2LB-SP, (j) M2LB-F.	61
Figure 18 TGA curves of (a) spray-dried M2LB-SP; (b) oven-dried M2LB-F.	62
Figure 19 Size distribution of theBudesonide in terms of (a) particle length; (b) particle width.....	65
Figure 20 (i) Single particle SEM images; (ii) 2D surface plot; (iii) 3D surface plot of (a) M2LB-SP; (b) M2NaCl-SP; (c) M5LB-F; (d) M2LB-F; (e) M1LB-F.....	66

Figure 21 Five types of drug adhesion on the carrier surfaces.	66
Figure 22 XRD of (a) spray-dried M4LB-SP(86); (b) transformed M4LB-F(86).	74
Figure 23 SEM of (a) spray-dried M4LB-SP(86); (b) high-magnification image of M4LB-SP(86); (c) transformed M4LB-F(86); (d) high-magnification image of M4LB-F(86).	75
Figure 24 DSC thermal profile of (a) spray-dried M4LB-SP(86); (b) transformed M4LB-F(86).	76
Figure 25 TGA of (a) spray-dried M4LB-SP(86); (b) transformed M4LB-F(86).	77
Figure 26 SEM of M4LB(94)-F stored under room temperature and RH below 10 for (a) 0 month; (b) 3 months; (c) 9 months; (d) 15 months; (e) 21 months; (f) 24 months; (g) 27 months; (h) 30 months; (i) 33 months.	80
Figure 27 (i) Single particle SEM images; (ii) 2D surface plot; (iii) 3D surface plot of M4LB(94)-F kept at room temperature and RH below 10 for (a1) 0 month; (d1) 15 months; (e1) 21 months.	81
Figure 28 Deposition of Budesonide from Different Blends at Inhalation Rate of 60L/min.	85
Figure 29 Typical SEM of (a) spray-dried M2LB(50)-SP microparticles, (b) M2LB(50,55,66)-F, (c) M2LB(77,83,86,89)-F, (d) M2LB(92,93,94)-F, (d1) high-magnification of the rectangle area in (d).	90
Figure 30 XRD of M2LB(86)-SP after stored at 25°C for (a) 1 week, (b) 4 weeks, (c) 10 weeks, (c) 18 weeks.	90
Figure 31 SEM of M2LB(86)-SP after stored at 25°C for (a) 1 week, (b) 4 weeks (resulting particle namely M2LB(86)-INT4), (c) 7 weeks (resulting particle namely M2LB(86)-INT7), (d) 10 weeks (resulting particle namely M2LB(86)-F), (e) 13 weeks, (f) 17 weeks; (e1) high-magnification of the rectangle area in (e).	94
Figure 32 Typical TGA curves of pollen-shape M2LB(77,83,86,89)-F Microparticles.	95

List of Tables

Table 1 Composition of feed solutions and spray-drying conditions	27
Table 2 Composition of feed solutions and spray-drying conditions	29
Table 3 Components of different types of lab-made LB Agar	30
Table 4 Composition of feed solutions.....	32
Table 5 Composition of feed solutions.....	33
Table 6 BD Content and Homogeneity of the Blends	46
Table 7 Deposition of Budesonide from Different Blends at Inhalation Rate of 60L/min.	46
Table 8 Compositions of spray drying feed solutions	52
Table 9 Compositions of spray drying feed solutions	53
Table 10 Compositions of spray drying feed solutions	56
Table 11 BD Content and Homogeneity of the Blends	63
Table 12 Deposition of budesonide from different blends at inhalation rate of 60L/min.	64
Table 13 BD Content and Homogeneity of the Blends.	78
Table 14 Deposition of Budesonide from Different Blends at Inhalation Rate of 60L/min.	78
Table 15 BD Content and Homogeneity of BD-M4LB(94)-F blends	82
Table 16 Stability and agar percentage composition	86
Table 17 Area ratio of peak-I at 9.7° of 2θ to peak-II at 13.8° of 2θ.....	921
Table 18 BD Content and Homogeneity of Different Blends.....	95
Table 19 Deposition of BD from Different Blends at Inhalation Rate of 60L/min	97

Publications

Journals

1. Roughness-controlled Self-assembly of Mannitol/LB Agar Microparticles by Polymorphic Transformation for Pulmonary Drug Delivery, Zhang, Fengying; Nguyen T. Q. N.; Bao H. T.; et al. *Molecular Pharm.*, 2015(12),1: 223-231
2. Synthesis and property Study of Mannitol Based Pollen-shape Microparticles for Pulmonary Drug Delivery, Zhang, Fengying; Nguyen T. Q. N.; Raymond Lau; et al. *Molecular Pharm.*, (submitted)
3. Synthesis and Stability Study of Mannitol/LB Agar(lab-made) Microparticles for Pulmonary Drug Delivery, Zhang, Fengying; Nguyen T. Q. N.; Raymond Lau; et al *Int. J. Pharm.*, (submitted)

Conference

1. Zhang, Fengying; Nguyen T. Q. N.; Bao H. T.; et al. Roughness-controlled Self-assembly of nanorod-covered Mannitol/LB Agar Microparticles by Polymorphic Transformation for Pulmonary Drug Delivery. *IEEE NANO5*, Jul. 2015, Rome, Italy.

Chapter 1 Introduction

1.1 Introduction

Pulmonary drug delivery has gathered increasing attention for the treatment of lung diseases (1, 2), such as asthma (3), cystic fibrosis (4), and lung cancers (5) in the past decade. It targets both systemic (1, 2) and local delivery. Pulmonary drug delivery offers several benefits compared to other methods. For example, a large number of drugs can be better absorbed from pulmonary drug delivery than enteral route because lung is highly permeable and easily accessible (6). Besides, it provides direct treatment local diseases. What is more, inhaled drugs can avoid dietary complications and hepatic first-pass metabolism that reduces systemic toxicity (7). In addition, the relatively low enzymatic activity of the lung can help to maintain a higher bioavailability (8) compared to the enteral route and the non-invasive nature increases patient compliance (9).

Three aerosol techniques have been developed over the years, namely pressurized metered dose inhalation (pMDI), nebulized inhalation and dry powder inhalation (DPI). DPI formulations are more preferred due to its superior storage stability, portability, ease of operation and cost-effectiveness (10). Dry powder deposition is governed by four mechanisms e.g. inertial impaction, sedimentation, interception and diffusion (11). Among them inertial impaction and sedimentation have the major contribution. All of

these mechanisms are affected by drug and carrier properties, for example particle size, shape, density, surface energy, and surface morphology.

Extensive research has been conducted to investigate the effects of carrier size (12-14), shape (15-17), fine carrier fraction (16, 18), surface energy (19), crystallinity (20), and surface roughness (17, 18, 21) on DPI performances. Conflicting results were reported on the relation between aerosolisation performance and carrier size (2, 12, 13, 20). Both large carriers (12) and small carriers (14) were reported to improve drug deposition in the deep lung regions. For carrier particle shape, generally no consistent relation was observed between fine particle fraction (FPF, a measure of drug deposition in the lung) and elongation ratio (16). Such contradictions are large because FPF is affected by different properties of carriers, while neither the size nor shape of the carriers used in the literature were independent of other properties such as surface roughness, surface energy and fine carrier fraction (12, 14, 16). For example, a study investigating the effect of lactose carrier size, the large lactose carriers have a higher fraction of fine lactose particles and a lower surface roughness compared to the small lactose carriers used in the comparison (12). The carrier elongation ratio was demonstrated to affect the surface energy negatively (22). In the literature, parameters such as the fine carrier fraction (23), surface energy (23) and crystallinity (20) of carriers were found to have major impact on the DPI performance for certain carriers. It was reported that an increase in the fine carrier fraction (fraction of carrier that is present as fine particles) can increase the FPF due to “active site theory” and “agglomeration theory” (23, 24). The surface energy was also reported to dominate the aerosolisation performance over the carrier size distribution (19). It was shown that crystallized lactose can generate higher FPF than

commercial lactose (20). The carrier surface roughness was also found to affect the contact geometry between the drug and carrier particles, and consequently impact the drug-carrier adhesion significantly (25).

The conflicting results can be due to the inability to adjust a single carrier property independently. It is important to develop a synthesis method capable of controlling the carrier properties independently for the study of the true relation between the carrier property and aerosolisation performance. The information can provide an insight into the design criteria of drug carriers to achieve high delivery efficiencies.

Stability is an important property of carrier particles as every pharmaceutically acceptable dosage need to be efficacious and safe. It was reported during storage, spray-dried carrier particles tend to convert to a stable state by crystallization, polymorphic transformation, crystal growth et al (26). The flow behavior and aerosolization performances would change consequently. In literatures, excipients such as sugars including mannitol and sucrose etc, and polymers including polyethylene glycol (PEG) 4000, hydroxypropylcellulose (HPC), polyvinylpyrrolidone (PVP) etc can act as stabilizers (27, 28). Thus it is important to find a good excipient to stabilize carrier properties.

1.2 Review of the Literature

This literature review includes the summary of pulmonary drug delivery, different inhalation delivery techniques, choices of carriers, properties study on carriers, and methods of preparation of carriers. All these reviews provided the motivation for the design of proper carriers.

1.2.1 Pulmonary Drug Delivery

Lung is an essential respiration organ in human body, where the gas exchange of oxygen and carbon dioxide takes place. To understand the opportunities for pulmonary drug delivery, the passage of air from nose to deep lung region is necessary to be studied. Air traveling through pharynx, larynx and trachea, reaches left and right lungs, where trachea branches into two main bronchi as shown in Figure 1. Within lung, bronchi continue to branch resulting in bronchioles, alveolar sacs and alveoli arise progressively. Alveoli are wrapped in blood vessels, where oxygen is transport from the atmosphere into the blood stream, at the same time carbon dioxide is released from the blood stream into the atmosphere. This region of the lungs has the largest surface area and is able to provide the thinnest diffusion pathway for the drugs (29).

Figure 1. Schematic diagram of a human lung.

For most lung diseases, for example chronic persistent asthma, extrinsic allergic alveolitis, emphysema, sarcoidosis et al. Drugs need to reach alveolar region. Therefore

the main issue in pulmonary drug delivery is to deliver medication to the deep lung region. In order to deliver drug powder to the alveolar region, drug particles are usually micronized to be small enough to avoid being deposited too early. The typical size of drug particles used for DPI is between an aerodynamic diameter range of 1-5 μm (30). When particle aerodynamic diameter is above 5 μm , it is rare for particles reaching the deep lung region. Particles are easily exhaled out when their aerodynamic diameter is below 1 μm . Unfortunately, particles of such sizes range exhibit strong cohesive forces. That causes drug particles to aggregate and behave similar to particles of larger aerodynamic diameter. Thus the drug particles would exhibit poor dispersion behavior as illustrated in Figure 2a (11).

To improve the aerosolisation performance, drug particles are generally attached to relatively large carrier particles as Figure 2b indicates. This drug and carrier mixture exhibits better drug flow and dispersion behavior than a drug-only formulation (31). Carrier particle can help to prevent drug particles from aggregation (11). Drug would be detached from the surface of carrier particle when the mixture is inhaled. An easy detachment is resulted from a low adhesive force between drug and carrier. Carrier properties such as the material used, size, shape, porosity and surface morphology would affect the drug-carrier adhesive force (30). When drug particles are detached from carrier particles, they can continuously flow into the deep lung region while most of the carrier particles would be deposited in the initial airway. Thus it is important to study the relation between carrier property and drug delivery performance.

Moreover, mixture of drug and carrier is easier to handle than drug-only formulation during manufacturing process. Usually the amount of drug needs to be delivered one time is no more than a few milligrammes, for instance, only 20 μg to 500 μg of corticosteroids is delivered one time for asthma therapy. As the typical ratio of drug-to-carrier is 1:45 or 1:67.5, a mixture provides bulk, which is easy to handle, dispense, and meter (21, 30, 32).

Figure 2 Schematic diagram demonstrating delivery process of (a) “drug-only formulation”, (b) “drug-carrier formulation”(11).

1.2.2 Different inhalation delivery techniques

Long before the treatment of asthma, the lung had been used for drug administration, although it took a long time to get proper attention as a potentially effective way of drug administration. So far, three kinds of inhalers are commonly used. They are nebulizer, metered dose inhalers (MDI) and dry powder inhaler (DPI).

Nebulizer was first used in 1925. Asthma disease was treated with nebulizer before the invention of the MDI. Drug solution is generally broken into inhalable droplets by compressed air or ultrasound. Nebulizer is bulkier and has lower delivery efficiency than MDI and DPI. Its drug administration time is also longer than MDI and DPI.

The MDI was invented in the 1950s. Drug is usually suspended or dissolved in a solution. As such a solution or mixture passing a metering valve, a rapid volume expansion generates aerosol droplets. MDI is smaller and more portable compared to nebulizer. It also can provide a multi-dose and a relative uniform dosage. However, the use of MDI requires breath coordination and causes high oral deposition. The amount of delivered drug per actuation is limited.

DPI was invented as a good alternative of MDI in 1960. In DPI, pharmaceutical active ingredients are delivered as dry powder. The drug formulation is kept in a capsule which is manual loaded or directly stored inside the inhaler. After actuation, the patients will take the dose by deep inhalation and hold their breath for 5-10 seconds. Three different categories of DPI devices have been developed. They are single, multiple unit and multi dose devices. Single dose device is loaded with capsule or blister. In actuation, dose is released from the capsule or blister and taken inside the lung through inhalation. Multiple unit dose devices carry several capsules or blisters, e.g. Diskhaler[®] and Diskus[®] (GlaxoSmithKline). In multi dose inhalers the medications are kept in a reservoir in the inhaler and metered from there as free flowing powder, e.g. Turbuhaler[®] (Astra Zeneca).

1.2.3 Factors for Deep Lung Delivery

1.2.3.1 Aerodynamic Diameter

Aerodynamic diameter is an important factor for DPI. It is reported that particles which can be delivered into deep lung region have an aerodynamic diameter range of 1-5 μ m (30). Aerodynamic diameter is a widely used characterization method to predict drug deposition. It is defined as the diameter of a spherical particle with a density of 1 g/cm³ which has the same terminal settling velocity as tested particle:

$$d_a = d_g \sqrt{\frac{\rho_e}{\lambda \rho_s}} \quad \text{----- (1)}$$

ρ_s = unit density(1 g/cm³),

d_g = particle geometric diameter,

ρ_e = effective particle density in the same unit as ρ_s ,

λ = dynamic shape factor of the particle.

This shape factor is defined as the ratio of the drag force of tested particle to that of a spherical particle which has the same volume as tested particle. An increase in particle surface roughness can provide a high dynamic shape factor which results in a low aerodynamic diameter.

1.2.3.2 Particle Aerosolization

Particle aerosolization is also important for DPI. Aerosolization is a process of converting solid particles into aerosol which can be carried by air. Particle aerosolization in the initial airway is related to the particle–particle and particle–wall interactions. Such interactions depend on the cohesion of particles which is closely related to van der Waals force. Particle surface morphology, size, shape, electrostatic properties, and hygroscopicity are the main factors affecting the van der Waals force. Particle with low

contact area and van der Waals force exhibits low aggregation tendency which results in a good particle dispersion in the air (15). Thus a good particle aerosolization is obtained.

1.2.3.3 Powder Flowability

Powder flowability is another important factor for DPI. Flowability is not an inherent material property. It cannot be expressed as a single value or index. Flowability is an attempt to describe a combination of physical properties that affect material flow, and the equipment used for handling, storing, or processing the material.

Particle flow is similar to particle packing. During powder flow and packing, particle tends to rearrange its relative position to each other. Such particle rearrangement can be inhibited by interparticle force. Interparticle force can be characterized by the tap density (ρ_{tap}) and bulk density (ρ_{bulk}). Powder tends to aggregate and form bridges between particles. As the interparticle force in powder increases, the gap between particles increases. Tapping causes the bridges to break and the powder will collapse to a packed volume. Therefore, an increase in the difference between ρ_{tap} and ρ_{bulk} indicates an increase in interparticle force.

Carr's compressibility index (CI) is a common characterizing parameter for the particle flow behavior.

$$CI = \frac{\rho_{\text{tap}} - \rho_{\text{bulk}}}{\rho_{\text{tap}}} \times 100\% \quad \text{----- (2) (15)}$$

Low CI value which is resulted from a small difference between ρ_{tap} and ρ_{bulk} would then indicates low interparticle force and good flowability (15).

1.2.4 Choice of Carrier Particles

Various carriers have been used to improve the dispersion and delivery efficiencies of drug particles. A number of carriers such as smooth microparticles (18, 32), porous microparticles (1, 33) and rough-surface microparticles (17, 34, 35) have been explored to improve the drug delivery efficiency in the deep lung region. There are two methods for microparticles to carry drugs e.g. encapsulating drugs into microparticles and loading drugs on the surface of microparticles. By encapsulating drugs into microparticles, microspheres and porous microcarriers were utilized. Porous polymeric microparticles have gained more attention than microspheres as sustained effects can be provided (36, 37). While by loading drugs on the surface of smooth or rough microparticles, a rapid release was achieved. Loading drugs on the surface of microparticles also exhibited higher drug loading efficiency (37) than encapsulating drugs into microparticles. Furthermore, the separation of microcarriers with drugs also can facilitate the flexibility of drug encapsulation with different targeting materials and slow release reagent for treatments of different diseases.

A good pharmaceutically acceptable dosage should have good drug content uniformity and provide an easy drug detachment during inhalation. To achieve it, particle needs to provide a balance between adhesive and cohesive forces which offers sufficient drug attachment and detachment. It has been recognized that the adhesive and cohesive forces is highly dependent on carrier particle properties. Thus numerous studies have been done on carrier quality, carrier source, shape, particle size, fine carrier fraction, surface roughness, size distribution (PSD) et al. (3, 4, 7-9, 17, 18).

1.2.4.1 Size

It was reported in literatures that the particle size for DPI studies covers a diameter range of 10-220 μm . (38-62) Both large carriers (12) and small carriers (14) were reported to improve drug deposition in the deep lung regions. French et al. (58) found that large particles whose average diameter was between 90 μm to 125 μm exhibit better emission and deposition behaviour than relatively small particles in a diameter range of 38-75 μm . On the other hand, it was reported that FPF increases with a decrease in the carrier particle size (53, 61-63). Steckel and Muller (55) showed that small particles with size below 32 μm have higher fine particle fraction (FPF) than large particles in diameter range of 63-90 μm and 125-180 μm . Bell et al. (56) suggested the most suitable size range for carrier is 30-90 μm . The authors also indicated that particles with small size (less than 30 μm) aggregated easily due to the high interparticle interaction. Thus it would be difficult to disperse them. However, for granulated lactose carriers, both small particles (16 μm) and large particles (more than 196 μm) had higher aerosolisation performance than particles in a diameter range of 38.5-165 μm . On the other hand, it was suggested that small lactose particles (16 μm) can provide higher FPF than large lactose particles (38.5-275 μm). Katherine and Kunn reported small commercial and pollen-shape carriers about 14-19 μm have higher FPF than large commercial and pollen-shape carriers (50-73 μm), while large needle-shape carriers exhibit better deposition behavior than small needle-shape carriers (64).

1.2.4.2 Shape

A number of particle shapes such as rough-surface sphere, smooth sphere, elongation, plate, cube and needle shape were studied in literatures (4, 16-18, 31, 34, 42). For

elongated and other rod-like carrier particle shape, generally no consistent relation was observed between fine particle fraction (FPF) and elongation ratio (16). Kaialy et al. showed particles with large elongation ratio have high fine particle fraction (16, 65, 66). On the other hand, another study by Kaialy et al. and Nokhodchi et al. showed particles with large elongation ratio deposited large amount of drug in the deep lung region (20, 67). Katherine and Kunn suggested rough-surface spherical microparticles such as pollen-shape lactose particles had better aerosolization than rock-like commercial and needle-shape lactose particles. However large pollen-shape lactose particles exhibited poor flowability than rock-like commercial and needle-shape lactose particles while small pollen-shape lactose particles had similar flowability as rock-like commercial and needle-shape lactose particles (64). In other studies, pollen-shape microparticles, a type of rough-surface spherical microparticles, were found to have improved flowability, aerosolization and deposition performance compared to sphere, plate, cube and needle shape particles (15, 31, 34). It was anticipated that high surface roughness of pollen-shape microparticles could reduce the inter-particle interactions and adhesion properties which attribute to low aerodynamic diameter (15). Therefore it is highly possible for such particles to reach the deep lung region. Beside, pollen-shape microparticles were found to have better flowability compared to the traditional lactose (LA) particles in terms of CI index. Due to the low surface density, pollen-shape particles exhibited low inertia and could follow the geometry of the inhalation path model. Pollen-shape particles also showed high potential to travel further way in the lung airways. In addition, pollen-shape microparticles provided higher drug loading efficiency than porous, sphere, plate, cube and needle shape particles (37) due to the high surface roughness. It was reported that the

drug loading efficiency of pollen-shape Hydroxyapatite(HA) was more than 20%, while others were less than 10%.

1.2.4.3 Surface Roughness

Numerous studies have been focusing on particle surface morphology in order to enhance efficiency of drug delivery (13, 17, 18, 21, 30, 35). It was showed that smooth carrier particles were able to delivery larger amount drug than rough particles due to the small contact area (12, 18, 21). In other literatures, however, carriers with high surface roughness were reported to have high aerosolisation performance due to the accessibility of large amount of binding sites (15, 17). Among these rough surface particles, pollen-shape HA particles were reported to have excellent particle deposition properties due to high drug loading efficiency and reduced interparticular interactions (15). Moreover, rough surface carrier particles showed better drug content uniformity and stability than smooth carriers (34, 35, 68). Larhrib reported hairy lactose particles showed good drug deposition properties as they had reduced interparticular interactions and allow high drug loading (39). While Kunn suggested pollen-shape lactose particles performed low FPF since they had high adhesive force between drug and lactose particle (64).

Conflicting results were reported on rough mannitol particles (17, 21). Maas et al. showed that for lab-scale (small size) mannitol particles (1-5 μm), smooth surface generated higher FPF than rough surface (21). On the other hand, Littringer et al. reported pilot-scale (large size) rough mannitol particles (46-123 μm) deposited larger amount of drug in deep lung region than smooth mannitol carriers especially for spherical microparticles (17).

Figure 3 SEM of spray-dried (a) lab-scale (21) and (b) pilot-scale (17) rough particles.

Figure 4 Type of drug attachment on spray-dried (a) lab-scale (21) and (b) pilot-scale (17) rough particles.

It is reported that for lab-scale mannitol particles an increase in surface roughness as shown in Figure 3a resulted in an increase in contact area as illustrated in Figure 4a (21). Thus the interparticle interaction became larger. Consequently, it was difficult for drug to be detached from the particles. So there is a decrease of FPF (21). Littringer et al. (17) demonstrated that for pilot-scale mannitol particles, with the increase of surface roughness, lower contact area between the mannitol particles and the drug was obtained as illustrated in Figure 4b. Thus the interparticle forces decreased, which resulted in easier drug detachment from mannitol particles. Consequently, there is an increase in FPF (17).

It is noted that the type of drug attachment on the surface of mannitol in Figure 4b is completely different from that in Figure 4a. Therefore, Littringer et al. obtained an opposite result in FPF with the same trend of surface roughness compared to Maas et al.

Thus, the study of the relation between the type of drug attachment and the FPF has significant value.

1.2.4.4 Material

There are a variety of choices available as materials for dry powder inhalation carriers (1, 20, 30, 33, 34). Polymer based carrier particles such as synthetic poly(lactic-coglycolic) acid (PLGA), polylactides poly(lactic acid) (PLA), and poly(glycolic acid) (PGA) have been widely researched due to their excellent biodegradability and biocompatibility (1, 37). PLGA and PLA enable a relatively slow release of drug due to its hydrophobic nature, compared to other hydrophilic compounds which provide a relatively fast drug release (37, 69). However it was suggested a number of polymeric nanoparticles were able to result in toxicity via phenomena like phagocytosis by alveolar macrophages (1, 33). Furthermore, PLA/PGA/PLGA degrades quickly within weeks to months, thus they are unsuitable for administration on mucosal surfaces (37, 69). Hydroxyapatite (HA) had been employed as carrier particles (34). Unfortunately, it was reported HA had the possibility of inhibiting the biophysical function of a natural pulmonary surfactant (70). Some small molecular organic compounds, such as lactose and mannitol, have gathered growing attention as candidates for carrier material of dry powder inhalation (12, 17, 24, 30).

1.2.4.4.1 Lactose

Lactose, a reducing sugar, is the most commonly selected carrier material for DPI formulation as lactose offers several benefits. Lactose is compatible with most of drugs. It has a safe toxicological profile for human body. Lactose also possesses a physical and

chemical stability (24, 71, 72). Moreover it is inexpensive. In fact, lactose has been widely used in oral dosage forms as excipient for a long time. Nowadays various types of commercial lactose particles are available. They offer a variety of physical and chemical properties (24). However lactose particles also have some adverse effects. It can react with drugs containing primary amine moieties thus lactose is not a suitable excipient for proteins and peptides (18, 73). Besides, lactose tends to aggregate easily with the drug resulting in poor flowability and aerosolization. Consequently, a decrease of drug delivery efficiency is obtained (64, 74). Moreover, lactose has a limited range of recipients due to lactose intolerance.

1.2.4.4.2 Mannitol

Mannitol is approved by Food and Drug Administration (FDA) as a widely used pharmaceutical excipient in drug delivery. It provides cryoprotective effects to stabilize pulmonary macromolecular drugs. Mannitol powder has been produced for DPI to treat cystic fibrosis and bronchiectasis in Australia. Compared to lactose, mannitol has non-Maillard solid-state reaction property, and wider range of recipients including patients with lactose intolerance (18).

Mannitol is a crystalline sugar alcohol. It has three types of polymorphs: prismatic rod α form, prismatic rod β mannitol and needle shape δ mannitol. According to the reference X-ray diffractograms as shown in Figure 5, α -Mannitol has characteristic reflections at 2θ positions of 9.6° , 13.8° and 17.3° , while reflections at 10.6° , and 14.7° can be attributed to β -mannitol, and an intensive reflection at 9.7° ascribed to δ form.

Figure 5 XRD of three polymorphic forms of mannitol.

1.2.5 Method for Preparation of carrier particles

Several methods have been used in preparation of carrier particles. Using freeze-drying method porous carrier particles and rough surface mannitol particles were prepared (18). It was proved microemulsion method was suitable to prepare porous carrier particles and polymeric pollen-shape microcarriers (1). Double-emulsion method has been widely utilized for porous microcarriers preparation (1). Using hydrothermal method, lactose and pollen-shape inorganic microcarriers such as hydroxyapatite(HA) were obtained (34). Solvent displacement method and organic-inorganic hybridization method were reported to be able to prepare polymeric pollen-shape microcarriers for instance poly (lactic-co-glycolic acid) (PLGA) (75) and protein (76). Anti-solvent crystallization method was proved to be a potential method for the preparation of mannitol and lactose particles (64).

Although these methods provide particles with promising physical properties for inhalation, a few limitations still exist. Microemulsion method and anti-solvent crystallization method usually involve organic solvents which are not friendly to the

environment (1, 64). In addition the raw materials of polymeric microcarriers are also expensive. Using freeze-drying, large amount of liquid nitrogen is used which increases production cost (18). Some of lactose particles prepared by hydrothermal method exhibited irregular shape.

1.2.5.1 Spray-drying method

Spray-drying method is a simple technique for the preparation of dry particles from a liquid feed as Figure 6 shows. It has been widely used in food and pharmaceutical industry, for example milk powder, coffee, flavorings, vitamins, enzymes, etc (26, 77). The feed could be a solution, emulsion, suspension or slurry. During spray-drying process, the feed is sprayed into droplets through a nozzle by compressed air. When droplets pass through chamber, the solvent in droplets is vaporized by a hot gas. As the solute or suspension is separated from solvent in droplets, solid particles form. Spray drying method is able to generate a more consistent particle size distribution than other industrial techniques (26). It also can achieve a certain particle diameters with a narrow particle size distribution (PSD) which is resulted from the homogeneous droplet size and the separation by cyclone before particle collection (26). Particle size is related to temperature, feed concentration and droplet size. Increasing droplet size, feed concentration or decreasing temperature can provide an increase of particle size. Droplet size is related to nozzle orifice diameter and airflow. Moreover feed properties, such as surface tension, viscosity and density, are able to affect the break-up of the liquid feed which changes the droplet size.

Figure 6 Lab-scale spray dryer.

(A) feed (B) atomization gas; (1) drying gas; (2) heater; (3) nozzle; (4) drying chamber; (5) connection between drying chamber and cyclone; (6) cyclone; (7) drying gas; (8) collection vessel.

Spray drying method is a popular method for preparation of carrier particles. Commercial lactose particles produced by spray-drying technique exhibited excellent flowability and compactability (24, 26). These lactose particles provide a more spherical shape than that produced using other techniques. Such lactose particles also showed a narrow size distribution. Using spray-drying method, both amorphous and crystallized particles can be achieved (24, 26). For example, it was reported lactose-ethanol feed solution resulted in crystalline lactose while amorphous lactose was obtained using lactose-water feed solution (13). Another literature suggested when the solvent was a mixture of water and ethanol, the spray-dried lactose contained both amorphous and crystalline form (20). Using mannitol as solute, high crystallized particles were usually obtained as shown in literatures.

Spray drying method was also suggested to be able to produce complex structures, for instance nano-aggregates, coated particles and encapsulated materials due to the easily controllable parameters (26). Besides, it is capable of drying a product more quickly than other drying techniques.

Maas et al. (21) generated rough surface lab-scale mannitol particles as illustrated in Figure 7a using spray drying method with an outlet air temperature of 120°C. Littringer et al. (17) prepared similar rough surface pilot-scale mannitol particles as Figures 7b-c show utilizing a spray dryer at an outlet air temperature of 102°C.

However it is difficult to prepare carriers having uniform surface morphology using spray-drying method.

Figure 7 Rough surface mannitol particles generated by (a) Maas et al. (21), (b) Littringer et al. and (c) Littringer et al. at high magnification(17).

1.2.5.2 Self- assembly

In recent years, self-assembly has drawn numerous attentions for preparation of materials with organized structures (30, 78). It is a spontaneous process of organizing molecular units into ordered structures by slack interactions for example Van der Waals force, capillary, hydrogen bonds et al.

The building blocks in self-assembly process include atoms, molecules, nanostructures, micelles, and flowers for different functionalities (78, 79). Using micelles

and flowers as building blocks, it is easy to control particle distribution by modulating interparticle interactions (79). It was reported self-assembled superstructures had a number of advantageous functionalities to that of the primary, micelles, flowers, et al. Janus micelles prepared in Müller's group were able to be modified into amphiphilic Janus particles (78).

Using self-assembly or a combination of self-assembly and spray-drying method, carrier particles having uniform surface morphology and/or organized structures could be prepared.

1.2.6 Stability Study of Carrier

Stability is an important property of carrier particles as every pharmaceutically acceptable dosage need to be safe during its shelf life. The stability includes physical and chemical stability. A number of studies have been done on stability carrier particles.

Although spray drying is a simple method for the preparation of carriers, most of the spray-dried microparticles have not reached an equilibrium state due to the short spray-drying processing time (26).

A number of researches have been focused on the stability of spray-dried particles. It was reported that amorphous sucrose can start to transform to crystalline form in spray-dried sucrose/hydroxypropylmethylcellulose(HPMC) microparticles after a five-month storage at 75% relative humidity (RH) and 40 °C (80). Similarly, amorphous lactose in spray-dried lactose/rhDNase powder were found to transform to a partially crystalline material at high relative humidity (81). For polymer excipients, it was found that amorphous bendroflumethiazide (BFMT) recrystallized more quickly with an increased amount of polyethylene glycol (PEG) 4000 in spray-dried BFMT/PEG 4000

microparticles (77). Another study reported that both hydroxypropylcellulose (HPC) and polyvinylpyrrolidone (PVP) can increase the stability of amorphous valdecoxib (82). PVP was found to be a better stabilizer than HPC. It was also reported that PVP allowed the formation of more stable amorphous lactose than HPC by slightly elevating the glass transition temperature (T_g) and forming interactions such as hydrogen bonding between PVP and lactose (83). Similarly, excipients such as mannitol, sucrose, trehalose, etc. can act as stabilizers by increasing T_g . It is commonly accepted that high glass transition temperature can lead to good stability. However a study showed that humidity-induced crystallization of amorphous solids can also occur when stored at temperature below T_g (27, 28). It was suggested that the crystallization of excipients would not affect the physical stability of drugs (27). There is another study reported that the crystallization of lactose and mannitol can lead to a decrease in the activity of trypsin (84).

1.3 Objective and Scope

To improve the inhalation performance of aerosol therapeutics, the study of properties of carrier particles is essential. The objective of this study is to create a method capable of controlling carrier property independently and determine the true relation of inhalation performance and particle properties such as material and surface morphology. Based on this investigation, a method is created for preparation carrier particles with good surface roughness using proper material. A high FPF and a good stability are achieved using such carrier particles.

The scope of this research includes a number of aspects in the following,

i) Mannitol and mannitol/lactose microparticles with similar size, shape and surface morphology are prepared. The aerosolization performances of mannitol and

mannitol/lactose microparticles are investigated. Base on the results, a good choice of material is achieved.

ii) A synthesis method capable of controlling carrier surface roughness independently is created. Four kinds of mannitol/LB Agar (commercial) and a kind of mannitol/NaCl microparticles with same shape and material but different surface roughness were prepared. The true relation between the carrier surface roughness and the carrier-drug contact as well as the interlocking behavior is studied.

iii) As mannitol/LB Agar (commercial) microparticles having good surface roughness exhibit poor stability, a proper stabilizer is created for mannitol/ LB Agar microparticles. By increasing the percentage of agar in LB Agar, a 21-month stable period is obtained for mannitol/ LB Agar microparticles.

iV) Based on the investigation above, a method is created to prepare needle-like pollen-shape mannitol /LB Agar microparticles with good surface roughness and stability. The effect of LB Agar composition to the surface roughness of mannitol /LB Agar microparticles is studied.

1.4 Thesis outline

The investigations carried out in this study are demonstrated in eight chapters.

Chapter 1 is an introduction of this study and a literature review which includes the background and aim of the study. History of pulmonary drug delivery, different influencing factors of deposition and methods of preparing carrier particles are reviewed.

Chapter 2 includes material, preparation and characterization methods. Several detailed description of different experimentation methods and characterization methods are discussed.

Chapter 3 presents the study of material for spray-dried carrier particles for DPI. Mannitol, lactose and mixture of mannitol and lactose are chosen as the material candidates for this study. Spray-dried mannitol, lactose and mannitol/lactose particles with similar size range, shape and surface roughness are prepared. Aerosolization performances of these carrier particles are studied. The optimum particle material with favorable flowability and aerosolization performances would be identified.

Chapter 4 is a study of true relation between carrier surface roughness and aerosolization performances. In chapter 4, rod-covered particles with different surface roughness and similar size range, shape and CI are prepared. Their aerosolization performances are measured to determine the surface roughness effect on them. The true relation between the carrier surface roughness and the carrier-drug contact as well as the interlocking behavior is studied. The relation between carrier surface roughness and aerosolization performances would be revealed.

Chapter 5 focuses on the stability of particle properties and aerosolization performances. There is a risk for the stability of particle properties as it is possible for polymorphic transformation, crystallization, crystal growth et al. happening during storage. Particles with similar shape, size range and surface roughness are synthesized. Particle shape, surface morphology and aerosolization performances of these carrier

particles are studied during storage. A method to obtain a more stable carrier particle would be achieved.

Chapter 6 describes a method to prepare pollen-shape mannitol particles. Their surface morphology and aerosolization performance are compared with rod-covered mannitol particles respectively. The optimum particle surface roughness with favourable aerosolization performances would be identified.

Chapter 7 includes over all concluding remarks of the study and recommendations for future works.

Chapter 2 Experimental Design and Methods

2.1 Materials

D-Mannitol (ACS reagent), peptone and hexane and budesonide (BD) were purchased from Sigma-Aldrich Company, Singapore. Agar and yeast extract were obtained from Becton, Dickinson and Company, Singapore. LB Agar (peptone from casein 27.03%, yeast extract 13.51%, NaCl 27.03% and agar 32.43%) and ethanol (analytical grade) were purchased from Merck company, Singapore. HNO₃ was purchased from Fluka company, Singapore.

2.2 Methods

2.2.1 Preparation of mannitol/NaCl and mannitol/lactose microparticles

It has been reported in the literature that spray-dried rough mannitol particles can generate one of the highest FPF (17). Spray-dried rough mannitol/NaCl particles and mannitol/lactose particles that have similar surface as the literature reported rough mannitol particles were prepared in order to allow equivalent particle characterizations.

Preparation of spray-driedmannitol/NaCl and mannitol/lactose microparticles

Büchi B-290 mini spray dryer (Büchi, Switzerland) was employed to transform the feed solution into microparticles. A two-fluid flow atomizer with a nozzle diameter of 1.5

mm is used. In the present work, the gas atomizing flow rate, feed rate, inlet temperature and outlet temperature were fixed at 439 L/h, 0.246L/h, 100°C and 65-68°C respectively. The aspirator rate was set at 100%. A summary of the feed solution compositions are listed in Table 1. The resulting microparticles obtained directly from the spray dryer are denoted “SP” in the suffix and named according to the mannitol concentration in the feed solution.

Table 1 Composition of feed solutions and spray-drying conditions

Feed solution composition				Particle name		
Mannitol (w/v)	Lactose (w/v)	Water (v/v)	NaCl (w/v)	After spray- drying without other treatment	Time of treatment with ethanol gas(day)	Fully transformed particles after 14 days of polymorphic transformation
2%	–	100%	0.090%	M2NaCl-SP	–	–
1.95%	0.05%	100%	–	M1.95Lac0.05-SP	7	M1.95Lac0.05-F
1.875%	0.125%	100%	–	M1.875Lac0.125-SP	7	M1.875Lac0.125-F

Preparation of rough mannitol/lactose microparticles

The spray-dried M1.95Lac-F and M1.875Lac-F microparticles were immersed into ethanol gas to initiate self-assembly. A period of 7 days was allowed for complete self-assembly. Particle products were collected through filtering and subsequently dried in an oven at 100°C for overnight. A summary of the nomenclatures of all the respective particles are listed in Table 1.

2.2.2 Preparation of roughness-controlled mannitol/LB Agar(c) microparticles

Preparation of spray-dried mannitol/LB Agar(c) microparticles

Using the same spray dryer, method and parameters, spray-dried mannitol/LB Agar(c) microparticles were obtained. In the present work, the gas atomizing flow rate, inlet temperature and outlet temperature were fixed at 439 L/h, 100°C and 65-68°C respectively. The aspirator rate was set at 100%. A list of feed compositions, feed rate and nomenclature of the particle product is shown in Table 2. The resulting microparticles obtained directly from the spray dryer are denoted “SP” in the suffix and named according to the mannitol concentration in the feed solution.

Polymorphic transformation/self-assembly of mannitol/LB Agar(c) microparticles

The spray-dried M5LB-SP(c), M2LB-SP and M5LB-SP(b) microparticles were immersed into hexane solution to initiate polymorphic transformation and self-assembly. A period of 14 days was allowed for complete polymorphic transformation while an intermediate sample was obtained after 1 h of polymorphic transformation to study the mechanism of the transformation. Particle products were collected through filtering and subsequently dried in an oven at 100°C for 2 h. A suffix of “INT” is used to denote the intermediate particle products obtained after 1 h of polymorphic transformation and a suffix of “F” is used to denote the final microparticles obtained after 14 days of polymorphic transformation. A summary of the feed solution compositions and the nomenclatures of all the respective particles formed at different stages are listed in Table 2.

Table 2 Composition of feed solutions and spray-drying conditions

Feed solution composition				Temperature of feed (°C)	Feed rate (L/h)	After spray-drying without other treatment	Particle name	
Mannitol (w/v)	LB Agar (w/v)	Ethanol (v/v)	Water (v/v)				Intermediate particles after 1 hr of polymorphic transformation	Fully transformed particles after 14 days of polymorphic transformation
5%	0.33%	10%	90%	55	0.205	M5LB-SP(c ₁)	—	—
5%	0.33%	20%	80%	55	0.205	M5LB-SP(c ₂)	—	—
5%	0.33%	25%	75%	55	0.205	M5LB-SP(c ₃)	M5LB-INT	M5LB-F
5%	0.33%	25%	75%	25	0.205	M5LB-SP(c ₃₁)	—	—
5%	0.33%	25%	75%	40	0.205	M5LB-SP(c ₃₂)	—	—
5%	0.33%	25%	75%	70	0.205	M5LB-SP(c ₃₃)	—	—
5%	0.33%	30%	70%	55	0.205	M5LB-SP(c ₄)	—	—
5%	0.33%	40%	60%	55	0.205	M5LB-SP(c ₅)	—	—
2%	0.13%	10%	90%	55	0.246	M2LB-SP	M2LB-INT	M2LB-F
1%	0.082%	5%	95%	30	0.307	M1LB-SP(c ₁)	—	—
1%	0.066%	5%	95%	30	0.307	M1LB-SP(c ₂)	M1LB-INT	M1LB-F
1%	0.044%	5%	95%	30	0.307	M1LB-SP(c ₃)	—	—
1%	0.033%	5%	95%	30	0.307	M1LB-SP(c ₄)	—	—

Preparation of commercial lactose microparticles

Commercial lactose is a standard carrier for DPI. Comparison of the drug delivery efficiency between polymorphic transformed particles and commercial lactose was also performed. The commercial lactose particles were sieved using a 20 µm sieve on a

mechanical shaker (Retsch GmbH, Haan, Germany). The sieved lactose particles were then dried at 70 °C over night.

2.2.3 Preparation of rough-surface mannitol/LB Agar(l) microparticles

Preparation of lab-made LB Agar

Ten types of lab-made LB Agar were obtained by mixing Agar with LB Agar (commercial) at different ratio listed in table 3.

Table 3 Components of different types of lab-made LB Agar

Name	Ratio of Agar to LB Agar	Percentage Of Agar	Name	Ratio of Agar to LB Agar	Percentage Of Agar
LB Agar(50)	1:3	0.50	LB Agar(86)	4:1	0.86
LB Agar(55)	1:2	0.55	LB Agar(89)	5:1	0.89
LB Agar(66)	1:1	0.66	LB Agar(92)	7:1	0.92
LB Agar(77)	2:1	0.77	LB Agar(93)	9:1	0.93
LB Agar(83)	3:1	0.83	LB Agar(94)	11:1	0.94

Preparation of spray-dried mannitol/LB Agar(lab-made)microparticles

Using the same spray dryer, method and parameters shown in the section 2.2.1, spray-dried spray-dried mannitol/LB Agar(l) microparticles were obtained. The feed rate and temperature were fixed at 0.205L/h and 60°C respectively. The concentration of LB

Agar(w/v), mannitol (w/v), ethanol (v/v) and water (v/v) in feed solution are 0.33%, 4%, 20% and 80% respectively. A list of feed compositions and nomenclature of the particle product is shown in Table 4. The resulting microparticles obtained directly from the spray dryer are denoted “SP” in the suffix and named according to the mannitol concentration in the feed solution.

Polymorphic transformation/self-assembly of mannitol/LB Agar(lab-made) microparticles

The spray-dried M4LB-SP(86-94) microparticles were immersed into hexane solution to initiate polymorphic transformation and self-assembly. A period of 2-15 days was allowed for complete polymorphic transformation respectively. Particle products were dried in an oven at 120°C for 8h. A of “F” is used to denote the final microparticles obtained after polymorphic transformation. A summary of the feed solution compositions and the nomenclatures of all the respective particles are listed in Table 4.

Table 4 Composition of feed solutions

Type of LB Agar	Name of particles after spray-drying	Time of polymorphic transformation (days)	Name of fully transformed Particles
LB Agar(50)	M4LB(50)-SP	7	M4LB(50)-F
LB Agar(55)	M4LB(55)-SP	7	M4LB(55)-F
LB Agar(66)	M4LB(66)-SP	7	M4LB(66)-F
LB Agar(77)	M4LB(77)-SP	2	M4LB(77)-F
LB Agar(83)	M4LB(83)-SP	4	M4LB(83)-F
LB Agar(86)	M4LB(86)-SP	5	M4LB(86)-F
LB Agar(89)	M4LB(89)-SP	7	M4LB(89)-F
LB Agar(92)	M4LB(92)-SP	10	M4LB(92)-F
LB Agar(93)	M4LB(93)-SP	13	M4LB(93)-F
LB Agar(94)	M4LB(94)-SP	15	M4LB(94)-F

2.2.4 Preparation of pollen-shape mannitol/LB Agar(I) microparticles

Preparation of spray-dried mannitol/LB Agar(lab-made)microparticles

Using the same spray dryer, method and parameters shown in the section 2.2.1, spray-dried spray-dried mannitol/LB Agar(I) microparticles were obtained. The feed rate and temperature were fixed at 0.246L/h and 40°C respectively. The concentration of LB Agar(w/v), mannitol (w/v), ethanol (v/v) and water (v/v) in feed solution are 0.33%, 2% , 20% and 80% respectively. A list of feed compositions and nomenclature of the particle product is shown in Table 5. The resulting microparticles obtained directly from

the spray dryer are denoted “SP” in the suffix and named according to the mannitol concentration in the feed solution.

Polymorphic transformation/self-assembly of mannitol/LB Agar(l) microparticles

The spray-dried M2LB-SP(86-94) microparticles were put in the air at room temperature to allow polymorphic transformation and self-assembly happen. A period of 35-56 days was allowed for complete polymorphic transformation respectively. Particle products were dried in an oven at 100°C for 2h. A of “F” is used to denote the final microparticles obtained after polymorphic transformation. A summary of the feed solution compositions and the nomenclatures of all the respective particles are listed in Table 5.

Table 5 Composition of feed solutions

Type of LB Agar	Name of particles after spray-drying	Time of polymorphic transformation(week)	Name of fully transformed Particles
LB Agar(50)	M2LB(50)-SP	7	M2LB(50)-F
LB Agar(55)	M2LB(55)-SP	7	M2LB(55)-F
LB Agar(66)	M2LB(66)-SP	7	M2LB(66)-F
LB Agar(77)	M2LB(77)-SP	10	M2LB(77)-F
LB Agar(83)	M2LB(83)-SP	10	M2LB(83)-F
LB Agar(86)	M2LB(86)-SP	10	M2LB(86)-F
LB Agar(89)	M2LB(89)-SP	10	M2LB(89)-F
LB Agar(92)	M2LB(92)-SP	8	M2LB(92)-F
LB Agar(93)	M2LB(93)-SP	8	M2LB(93)-F
LB Agar(94)	M2LB(94)-SP	8	M2LB(94)-F

2.3 Characterization of microparticles

Scanning Electron Microscope (SEM)

The surface morphology of the particles is characterized using a Scanning Electron Microscope (SEM) (JEOL, JSM-6390LA). Particle samples were transferred onto carbon sticky tapes, and sputter coated subsequently using an Auto Fine Coater (JEOL, JFC-1600) with platinum under an argon atmosphere for 70 s at a current of 20 mA. The size of microparticles was determined from the SEM images taken randomly from different areas of the samples. An image analysis software, ImageJ (NIH, USA), was used to obtain the particle size distribution based on a sample of over 300 particles (15).

Crystallographic Assay

All the particle samples were analyzed using a diffractometer (D2 Phase, Bruker) with Cu radiation ($k = 1.5406$, 30 kV and 10 mA) and automatic divergence slit. Samples were scanned from 5° to 40° 2θ with a step size of 0.020242° and a scanning speed of 0.020° per second. The obtained XRD patterns were compared to references (85) for analysis of polymorph.

Thermal Gravimetric Analysis (TGA)

TGA was carried out with a TGA/SDQ600 (TA Instrument, Research Instrument). Nitrogen (99.999%) was used as purge gas at a rate of 20 mL/min. Eight to twelve mg of the samples were heated from room temperature to 400°C at $10^\circ\text{C}/\text{min}$. The reduction in the weight of the samples was measured.

Differential Scanning Calorimetry (DSC)

The DSC measurements of all the particle samples were carried out using a calorimetry (DSC822^e, Mettler Toledo). A specific weight of microparticle sample were measured on an analytical balance, and then transferred into perforated aluminum sample pans. Each sample was heated from room temperature to 200°C at 10°C/min. Nitrogen (99.999%) was used for purging at a rate of 50 mL/min.

Fourier Transform Infrared (FTIR) Spectroscopy

The Fourier transform infrared (FTIR) spectra was detected using an FTIR (Perkin-Elmer, Spectrum One) in the range of 4000-400 cm⁻¹ at room temperature. Mannitol and three components of LB Agar, namely agar, peptone and yeast extract were dried at 100°C and then casted on KBr pellet individually. Each sample was mixed with 1 mL of hexane and the transmission spectra were measured. A specific weight of spray-dried mannitol microparticles were dried at 100°C and then casted on a predetermined weight of KBr pellet. The same procedure was repeated for the measurement of fully transformed microparticles using the same weight for microparticles and KBr. The transmission spectra of these two samples were measured and compared.

Bulk and Tap Densities

Bulk and tap densities of the samples were measured according to Hassan and Lau (34, 68). Samples of 100 milligrams were placed in a 1 mL micro-syringe tube with graduated markings. The micro-syringe tube containing the sample was then tapped against a table top by hand for 2,000-2,500 times until no reduction of the particle

volume was observed. The initial and tapped particle volumes were used to determine ρ_{bulk} and ρ_{tap} , respectively.

Carr's Compressibility Index (CI)

CI is a common parameter to characterize the flow behavior of particles. Lower CI value indicates better flowability (34, 68). CI can be determined based on ρ_{tap} and ρ_{bulk} :

$$\text{CI} = \frac{\rho_{\text{tap}} - \rho_{\text{bulk}}}{\rho_{\text{tap}}} \times 100\%$$

Drug content homogeneity

The drug content homogeneity of each blend was determined by analyzing the quantity of BD in the blend. Three samples were collected randomly from different spots of each blend. The weight of each sample was about 5 mg. A mixture of 2% nitric acid (Fluka, Singapore) solution with ethanol (Merck, Singapore) in a ratio of 3:1 (v/v) was prepared as the solvent of samples. After dissolving each sample in a fixed amount of solvent, each solution was assayed using a UV spectrophotometer (Shimadzu Corporation, Kyoto, Japan) with a wavelength of 250 nm. For each blend, the degree of BD content homogeneity was expressed as coefficient of variation (CV).

Particle Size Distribution of BD drug

The BD drug sizes are characterized using a Field Emission Scanning Electron Microscope (FESEM) (JEOL, JSM-6700F) and image analysis. Particle samples were transferred onto carbon sticky tapes, and sputter coated subsequently using an Auto Fine Coater (JEOL, JFC-1600) with platinum for 80 s at a current of 20 mA under an argon atmosphere. The length and width of microparticles were determined from the SEM

images taken randomly from different areas of the samples. An image analysis software, ImageJ (NIH, USA), was used to obtain the particle length and width distribution based on a sample of over 300 particles (15).

Surface Roughness and Drug Adhesion Analysis

Surface characterization of samples was performed with Interactive 3D Surface Plot plugin (v2.33) for ImageJ. The average surface roughness values (Ra) of samples were calculated using using SurfCharJ 1q Image J. (86). A representative SEM image was selected for each sample. A rectangular area was selected at the center portion of each particle for surface characterization.

In Vitro Aerosolization and Deposition Properties

The *in vitro* aerosolization and deposition properties using Budesnoide as the model drug was determined using an eight-stage Andersen cascade impactor (ACI, COPLEY Scientific). In each experiment, the air flow rate was fixed at 60 L/min. Eight mL of a solvent mixture of 2% nitric acid solution with 70% ethanol in a volume ratio of 3:1 was poured inside the pre-separator to prevent particle de-aggregation. A solution of 1% (w/v) silicon oil in hexane was used on the impaction plates to prevent particle bounce and re-entrainment. Budesonide was mixed with different carriers at a carrier to drug weight ratio of 45 to 1 using a REAX top mixer (Heidolph, Kelheim, Germany) for 15 min at 1000 rpm. A capsule filled with carriers and drugs was loaded into a Rotahaler® (Glaxo) which was used as the inhaler to aerosolize the particles. An actuation time of 4s was allowed for each capsule to completely disperse all the particles. Particles remaining in the capsule and inhaler device together with those deposited in the throat, pre-separator, individual impaction plates and stages were extracted using the same solvent poured into

the pre-separator. The extracted solutions were examined using a UV spectrophotometer (Shimadzu, Japan, Nottingham, UK) with a wavelength of 250 nm. Emitted dose (ED) and fine particle fraction (FPF) are two main parameters to characterize the ACI experimental results. ED is defined as the mass percentage of particles leaving the inhaler. ED represents both aerosolization property and flowability of the particles. FPF is defined as the percentage of the particles deposited in stage 1 or lower in the cascade impactor. An improvement in the aerosolization properties of aerosol particles can increase the amount of particles reaching the lower stages of the impactor and improve the FPF.

Chapter 3 Study of Carrier Material for Pulmonary Drug Delivery

3.1 Overview

Numerous studies have been done on dry powder inhalation (DPI) for the treatment of lung diseases, such as asthma, in the past several decades (1, 2, 24). It was reported that the mixture of drug and carrier/excipients exhibits higher aerosolisation performance than drug-only formulation (11). Therefore a number of research work focuses on carriers synthesis and property study.

There are two methods for microparticles to carry drugs: encapsulating drugs into microcarriers (33, 87) and loading drugs on the surface of microcarriers (30, 34) . By loading drugs on the surface of microcarriers, a rapid release can be achieved. Furthermore, the separation of microcarriers with drugs also facilitates the flexibility of drug encapsulation with different targeting materials and slow release regent for different treatments of diseases. Thus in this study to convey a sufficient dose of drug to the lungs by DPI, drug particles were attached onto the surface of carrier particles.

There are two popular carrier materials for DPI with drug attached on carrier particles: lactose and mannitol. Lactose is the most commonly selected carrier material since lactose is cheap, stable, safe for human body and compatible with most of drugs (24). Lactose has been widely used in oral dosage forms as excipient for a long time. However lactose is not a suitable excipient for proteins and peptides since it can react

with drugs containing primary amine moieties (73). Moreover, lactose has a limited range of recipients due to lactose intolerance.

Mannitol is a U.S. Food and Drug Administration (FDA) approved pharmaceutical excipient. It has been well-utilized in pharmaceutical dosage form development and drug delivery for its cryoprotective effects on stabilizing pulmonary macromolecular drugs. Compared to lactose, D-mannitol has non-Maillard solid-state reaction property, and is incompatible with drugs that have primary amine moieties, such as proteins and peptides (18).

It is reported in a literature that elongated mannitol/lactose carrier particles were prepared using anti-solvent method (25). The results show that all of the crystallized mannitol, lactose and mannitol/lactose carrier particles provided similar FPFs. However the elongated carrier particles prepared by 3 types of materials exhibited different size, shape, density and surface roughness (25). No clear relation between material and aerosolization performance was shown in this paper. It was known that different carrier material would result in different adhesive force between drug and carrier particles. Adhesive force should be in a certain range which provides an enough drug loading efficiency and good drug detachment, which results in a high drug delivery performance (30). Thus it is necessary to create a method to prepare carrier particles with similar properties except material and study the adhesive force between drug and carrier materials.

In this study, mannitol/NaCl microparticles with a mannitol to NaCl ratio of 22.2 to 1 were prepared using spray-drying method. Mannitol/lactose microparticles with various ratios of mannito to lactose were prepared with ethanol gas treatment using spray-dried

mannitol/lactose microparticles as starting material. The relation between drug and carrier materials was studied.

3.2 Results and discussion

The SEM and XRD results show that ratios of mannitol to lactose have negligible influence on the preparation mechanism of the mannitol/lactose particles. Therefore spray-dried M1.95Lac0.05-SP and M1.95Lac0.05-F microparticles were chosen as the representative particles for property characterizations to study the synthesis mechanism of mannitol/lactose microparticles.

3.2.1 Surface properties

The SEM images in Figure 8 show the surface morphology of resulting microparticles. As shown in Figure 8a, the spray-dried M1.95Lac0.05-SP microparticles, at a mannitol to lactose ratio of 39:1, have a relatively smooth surface with a few defects on it. It can be seen in Figure 8b, there are numerous crevices on the surface of M1.95Lac0.05-F microparticles after ethanol gas treatment. It indicates the M1.95Lac0.05-F microparticles have higher surface roughness than M1.95Lac0.05-SP microparticles. Figure 8c illustrates a broken M1.95Lac0.05-F microparticle. It can be seen in Figure 8c, M1.95Lac0.05-F microparticles exhibit a hollow pollen-shape morphology. The shell of this broken M1.95Lac0.05-F microparticle consists of a number of petals about 1 μm long, 0.8 μm wide and 0.1 μm thick. As the mannitol to lactose ratio increasing to 15:1, an aggregation appeared in M1.875Lac0.125-F microparticles after ethanol gas treatment as Figure 8d illustrates. It is possible that as the percentage of lactose increased to 0.125%, there is a certain amount of amorphous lactose. Such

amorphous lactose absorbed ethanol moisture during preparation that resulted in aggregation of M1.875Lac0.125-F microparticles (88). M1.875Lac0.125-F microparticles, at a mannitol to lactose ratio of 15:1, are not suitable carrier particles for DPI. Same aggregation phenomenon was observed when the ratio of mannitol to lactose was below 15:1.

Figure 8 SEM of (a) spray-dried M1.95Lac0.05-SP; (b) M1.95Lac0.05-F; (c) a broken M1.95Lac0.05-F ;
 (d) M1.875Lac0.125-F ; (e) spray-dried M2NaCl-SP.

Figure 8e shows spray-dried M2NaCl-SP microparticles have similar surface morphology as M1.95Lac0.05-F microparticles. Similar crevices appeared on the surface of M2NaCl-SP microparticles. The broken particle marked with black rectangle in Figure 8e reveals M2NaCl-SP microparticles are also hollow.

In addition there are some fragments attaching on the surface of all these microparticles as shown in Figure 8, which are possibly pieces of broken microparticles.

3.2.1 Morphological properties

Peaks at 10.8° and 14.8° of 2θ shown in Figures. 9a-b indicate the presence of β -mannitol (85). Diffraction angles at 17.1° of 2θ as Figures 9a-b illustrate correspond to α form of mannitol (85). Peaks at 12.6° of 2θ as Figures 9a-b show correspond to α -lactose (25). Figures 9a-b show the presence of 5α -/ 3β -lactose with peak at 19.1° of 2θ and 4α -/ 1β -lactose with peak at 20.1° of 2θ (25). It can be seen in Figures 9a-b there are α -mannitol, β -mannitol, α -lactose, 5α -/ 3β -lactose and 4α -/ 1β -lactose coexist in spray-dried M1.95Lac0.05-SP and M1.95Lac0.05-F microparticles. It indicates that there was no polymorphic transformation happen in the mannitol/lactose microparticles with ethanol gas treatment.

As shown in Figure 9c, peak at 14.3° of 2θ indicate the presence β -mannitol (85). Peaks at 9.3° , 13.4° and 17.0° of 2θ are indications α -mannitol (85). The presence of peak at 9.7° 2θ correspond to δ form of mannitol (85). That suggests all the three polymorphic form of mannitol coexist in spray-dried M2NaCl-SP.

Figure 9 XRD of (a) spray-dried M1.95Lac0.05-SP; (b) M1.95Lac0.05-F; (c) spray-dried M2NaCl-SP. (α : α -mannitol, β : β -mannitol, δ : δ -mannitol; α' : α -lactose, $5\alpha'/3\beta'$: 5α -/ 3β -lactose, $4\alpha'/1\beta'$: 4α -/ 1β -lactose)

3.2.3 Thermal Gravimetric Analysis (TGA)

M1.95Lac0.05-F and oven-dried M2NaCl-SP microparticles exhibit similar TGA results. A typical TGA thermal degradation profile in Figure 10 shows that both M1.95Lac0.05-F and oven-dried M2NaCl-SP microparticles have the weight loss decomposition at approximately 250°C. There is no residual moisture or ethanol in the resulting microparticles.

Figure 10 TGA of M1.95Lac0.05-F and oven-dried M2NaCl-SP.

3.2.4 Drug Content homogeneity

It was reported the blending process had a significant influence on aerosolization performance. Thus in this study no optimization of blending process was conducted. As listed in Table 6, the CV values of the two blends are less than 8%. It indicates each blend has adequate homogeneity for DPI systems (35, 39, 89).

Table 6 BD Content and Homogeneity of the Blends

Blend	Run1 (%)	Run 2(%)	Run 3(%)	Average (%)	CV (%)
M1.95Lac0.05-F	2.19	2.05	2.08	2.11	3.50
M2NaCl-SP	2.73	2.51	2.45	2.55	6.25

3.2.5 In Vitro Deposition Properties

The aerosolization and deposition properties of the two blends were investigated. The particle size, bulk density, tap density and CI of the carrier particles, together with FPF and ED of the two blends are shown in Table 7. M2NaCl-SP microparticles exhibit a ED about $92.7\pm3.9\%$ which is comparable with the ED of M1.95Lac0.05-F microparticles about $88.6\pm3.8\%$. Compared to M1.95Lac0.05-F microparticles, M2NaCl-SP microparticles have a higher FPF about $15.5\pm1.1\%$.

Table 7 Deposition of Budesonide from Different Blends at Inhalation Rate of 60L/min.

Blend	average diameter (μm)	ρ_{bulk} (g/cm^3)	ρ_{tap} (g/cm^3)	CI	ED (%)	FPF (%)
M2NaCl-SP	9.71	0.247	0.556	0.556	92.7 ± 3.9	15.5 ± 1.1
M1.95Lac0.05-F	9.06	0.226	0.554	0.592	88.6 ± 3.8	13.4 ± 1.1

3.3 Discussion

XRD results indicate that there was no polymorphic transformation happen in the mannitol/lactose microparticles with ethanol gas treatment as shown in Figs 11a-b. SEM illustrates there is a morphology change from a smooth surface to a pollen shape on mannitol/lactose microparticles with ethanol gas treatment as Figs 10a-c show. It is possible the small crystals in mannitol/lactose microparticles join into each other and self-assemble into petal-like crystals with ethanol gas treatment.

Table 7 shows the bulk density of M1.95Lac0.05-F microparticles is lower than that of M2NaCl-SP microparticles. M1.95Lac0.05-F and M2NaCl-SP microparticles have similar the tap densities. The CI of M1.95Lac0.05-F microparticles is higher than that of M2NaCl-SP microparticles as table 8 indicates. It is possible that the interparticle force of M1.95Lac0.05-F microparticles is higher than that of M2NaCl-SP microparticles. More bridges formed in M1.95Lac0.05-F microparticles than in M2NaCl-SP microparticles before tapping. Thus the bulk volume of M1.95Lac0.05-F microparticles is higher than that of M2NaCl-SP microparticles. Which results in the bulk density of M1.95Lac0.05-F microparticles is lower than that of M2NaCl-SP microparticles. During tapping, the bridges were broken which resulted in the tap volume of M1.95Lac0.05-F microparticles is similar to M2NaCl-SP microparticles. Therefore M1.95Lac0.05-F microparticles have comparable tap density with M2NaCl-SP microparticles as shown in table 8. Thus the CI of M1.95Lac0.05-F microparticles is higher than that of M2NaCl-SP microparticles. Since high CI indicates poor flowability (31), the flowability of M1.95Lac0.05-F microparticles is worse than M2NaCl-SP microparticles. The FFP of M1.95Lac0.05-F blend is lower than that of M2NaCl-SP blend as listed in table 7. It is possible the adhesive force between BD drug and M1.95Lac0.05-F microparticles is larger than that

between BD drug and M2NaCl-SP microparticles. Thus it is more difficult for BD drug to be detached from than M2NaCl-SP microparticles. As the interparticle force of M1.95Lac0.05-F microparticles is higher than that of M2NaCl-SP microparticles, M1.95Lac0.05-F microparticles would exhibit high aggregation tendency. Therefore M1.95Lac0.05-F microparticles have a poor particle dispersion in the air. Thus a poor particle aerosolization of M1.95Lac0.05-F microparticles is obtained. The more difficult drug detachment, poor flowability and aerosolization result in lower FPF of M1.95Lac0.05-F than M2NaCl-SP microparticles (18).

3.4 Conclusion

Novel pollen-shape mannitol/lactose and mannitol/NaCl microparticles were successfully synthesized by spray-dried and crystal-growth methods. Ethanol used as a crystal-growth agent for preparation of pollen-shape mannitol/lactose microparticles. The two types of microparticles have similar surface morphology. However mannitol/NaCl microparticles exhibit low interparticle force, better flowability and better drug delivery performance than mannitol/lactose microparticles. In addition, mannitol/lactose microparticles have a trend to aggregation with the ratio of lactose to mannitol increasing to 1:15. Therefore for mannitol is a better material than the mixture of mannitol and lactose to prepare spherical rough carrier particles for DPI.

Chapter 4 Synthesis and Roughness Study of Mannitol/LB Agar Microparticles

4.1 Overview

The carrier surface roughness was also found to affect the contact geometry between the drug and carrier particles, and consequently impact the drug-carrier adhesion significantly (25). Some literature found smooth carriers to be able to achieve higher FPF than coarse carriers (18, 21). While in another literature, in which large size mannitol particles were used, rough surface was found to provide small contact area between drugs and carriers and improve the FPF (17). The conflicting results can be because the contact area and interlocking between drugs and carriers are not depend solely on the roughness of carriers, but also the positions of the drugs attached on the carriers (17). For instance, when drugs were caught in valley of the crevices on a carrier surface, the contact area and interlocking between drugs and carriers can be large. Similarly, if drugs were located on the peaks of carrier surface crevices, the contact area and interlocking between drugs and carriers can be small. Thus, it is possible for both rough and smooth carriers to provide small contact area, low particle interlocking, and reduce the adhesive force (17, 18, 21). However, there were few studies that can demonstrate an independent change of the carrier surface roughness without affecting other carrier properties such as size, shape, crystallinity, etc. It is important to develop a synthesis method capable of controlling the carrier surface roughness independently for the study of the true relation between the

carrier surface roughness and the carrier-drug contact as well as the interlocking behavior. The information can provide an insight into the design criteria of drug carriers to achieve high delivery efficiencies.

In this study, a novel synthesis technique using polymorphic transformation to prepare self-assembled rod-covered mannitol/LB Agar microparticles having controlled roughness was proposed. Spherical spray-dried mannitol/LB Agar particles with similar size and crystallinity were used as the starting material. Hexane is used as the polymorphic transformation reagent. Few studies have applied self-assembly method on the preparation of small molecular weight organic particles (79, 90, 91). However, it is highly promising for the synthesis of mannitol/LB Agar carriers having uniform surface morphology.

4.2 Optimization of feed solution during spray-drying process

Since the feed physical properties, such as viscosity, surface tension and density, affect the break-up of the liquid feed and thus particle property, the composition and temperature of feed solution were studied.

4.2.1 Optimization of feed composition

Mannitol/LB Agar(c) microparticles were prepared by spray drying method with feeds of 5% (w/v) mannitol, 0.33% (w/v) LB Agar(c) and different amount of ethanol. The ratio of mannitol :LB Agar : ethanol in feed solution which were used to prepare M5LB-SP(c) microparticles are shown in Table 8. SEM images of these M5LB-SP(c) microparticles are shown in Figure 11. Figure 11a and Figure 11d indicates there are a

few broken microparticles in M5LB-SP(c₁) and M5LB-SP(c₄) powder. Figure 11e illustrates that there are few spherical particles whose diameter is above 10 μm and a number of particle fragments appear in M5LB-SP(c₅) powder. That indicates most of M5LB-SP(c₅) particles (more than 10 μm) are broken. While in M5LB-SP(c₂) and M5LB-SP(c₃) powder, there are few broken microparticles as Figs . 13b-c illustrate. As shown in Table 8, the ratios of mannitol : LB agar : ethanol in feed for the preparation of M5LB-SP(c₂) and M5LB-SP(c₃) are 15:1:60 and 15:1:75 respectively. Which are the best ratios to prepare M5LB-SP(c).

Figure 11 SEM of spray dried (a) M5LB-SP(c₁); (b) M5LB-SP(c₂); (c) M5LB-SP(c₃); (d) M5LB-SP(c₄); (e) M5LB-SP(c₅).

Table 8 Compositions of spray drying feed solutions

Particle name	Ratio of mannitol : LB Agar : ethanol in feed	Temperature of feed (°C)
M5LB-SP(c ₁)	15 : 1 : 30	55
M5LB-SP(c ₂)	15 : 1 : 60	55
M5LB-SP(c ₃)	15 : 1 : 75	55
M5LB-SP(c ₄)	15 : 1 : 90	55
M5LB-SP(c ₅)	15 : 1 : 120	55

Another type of mannitol/LB Agar(c) microparticles were prepared by spray drying method to achieve an optimization of feed composition. There were 1% (w/v) mannitol, 5% (v/v) ethanol and different amount of LB Agar(c) in feed. The ratio of mannitol :LB Agar : ethanol in feed solution which were used to prepare M1LB-SP(c) microparticles are shown in Table 9. SEM images of M1LB-SP(c) microparticles are shown in Figure 12. Figure 12a and Figure 12c indicate there are a few broken microparticles in M1LB-SP(c₁) and M1LB-SP(c₃) powder. Figure 12d illustrates that there are few spherical particles and a number of particle fragments appear in M1LB-SP(c₄) powder. While in M1LB-SP(c₂) powder, there are few broken microparticles as Figures 12b illustrate. As shown in Table 9, the ratio of mannitol : LB-agar : ethanol in feed for the preparation of M1LB-SP(c₂) is 15:1:75, which is the best ratio to prepare M1LB-SP(c) microparticles.

Figure 12 SEM of spray dried (a) M1LB-SP(c₁); (b) M1LB-SP(c₂); (c) M1LB-SP(c₃); (d) M1LB-SP(c₄).

Table 3 Compositions of spray drying feed solutions

Particle name	Ratio of mannitol : LB Agar : ethanol in feed	Temperature of feed (°C)
M1LB-SP(c ₁)	12 : 1 : 60	30
M1LB-SP(c ₂)	15 : 1 : 75	30
M1LB-SP(c ₃)	22.5 : 1 : 112.5	30
M1LB-SP(c ₄)	30 : 1 : 150	30

When the feed contained 2% mannitol, different amount of LB Agar and ethanol, same phenomenon were observed. It can be deduced that to prepare mannitol/LB Agar(c) microparticles the best ratio of mannitol: LB Agar : ethanol is 15:1:75. It is possible that when the concentration of ethanol in feed solution was low, solvent would evaporate

slowly on the surface of droplets during spray drying process. Therefore there was small number of solid mannitol formed on the surface of droplets, which resulted in fragile shells formed. Such fragile shells were easily broken when they pass through chamber. When the concentration of ethanol in feed solution was high, the evaporation rate of solvent on the surface of droplets would be high (30). Thus droplets shrank fast, which result in the solid particles formed on the surface of droplets moved toward the core of droplets fast. Therefore it was different for these solid particles to form shells. A number of microparticles were broken consequently.

It is noted that compared to M5LB-SP(c₃) , there is an increase number of broken particles observed in M1LB-SP(c₂) as Figure 11c and Figure 12b indicate. It is possible that when the concentrate of mannitol in dropelts decreased, a smaller amount of solid particles formed on the surface of droplets. Which result in a number of solid particles failing to form shells. Thus the number of broken particles increased in M1LB-SP(c₂).

4.2.2 Optimization of feed temperature

M5LB-SP(c) microparticles with the feed of same composition but different temperature were prepared using spray dryer. The temperature of feed are 25°C, 40°C, 55°C and 70°C respectively as shown in Table 10.

Figures 13a-b illustrate there are smaller amount of broken particles in M5LB-SP(c₃₂) than in M5LB-SP(c₃₁). There are few broken particles in M5LB-SP(c₃) and M5LB-SP(c₃₄) as Figures 13c-d show. It is possible as the feed temperature increasing, the evaporation rate of solvent on the surface of droplets became faster. Thus there was a number of solid mannitol formed on the surface of droplets. Therefore it was easy for these solid particles to form shells.

Another possible reason is the solubility of agar at different temperature. Agar would be insoluble in water when the temperature is below 40°C. As temperature of feed decreasing, a part of agar would form solid particles together with solid mannitol on the surface of droplets during spray drying. The solid agar would stop solid mannitol form shells or make shells become fragile. Thus these shells were easily broken when they pass through chamber.

Figure 13 SEM of spray dried dried (a) M5LB-SP(c₃₁); (b) M5LB-SP(c₃₂); (c) M5LB-SP(c₃); (d) M5LB-SP(c₃₃);

Table 10 Compositions of spray drying feed solutions

Particle name	Ratio of mannitol : LB Agar : ethanol in feed	Temperature of feed (°C)
M5LB-SP(c ₃₁)	15 : 1 : 75	25
M5LB-SP(c ₃₂)	15 : 1 : 75	40
M5LB-SP(c ₃)	15 : 1 : 75	55
M5LB-SP(c ₃₄)	15 : 1 : 75	70

4.2.3 Conclusion

The ratio of mannitol :LB Agar : ethanol in feed solution which were used to prepare mannitol/LB Agar(commercial) microparticles is 15 : 1 : 75. The temperature of feed solution in spray-drying step is 55°C.

4.3 Characterizations

A change in the mannitol concentration in the feed solution was found to exhibit negligible difference in SEM, XRD, DSC FTIR and TGA measurements. Thus, the microparticles obtained from spray-drying of a feed solution composition of 2% mannitol/LB Agar (M2LB-SP), the microparticles obtained after 1 h of polymorphic transformation (M2LB-INT), and the microparticles obtained after 14 days of polymorphic transformation (M2LB-F)were chosen as the representative particles for property characterizations to study the mechanism of the transformation.

4.3.1 Scanning Electron Microscope (SEM)

The SEM images illustrate the self-assembling process of mannitol molecular into

rods on the surfaces of spray-dried mannitol/LB Agar microparticles. Figures 14a-b show that the M2LB-SP microparticles obtained straight from the spray-drying process have relatively smooth surfaces while some minor defects can also be found. After 1 h hexane treatment, it can be seen in Figures 14c-d that the intermediate microparticles, M2LB-INT have higher surface roughness compared the M2LB-SP microparticles without hexane treatment. Some nanobelts can also be observed in the magnified M2LB-INT image shown in Figure 14d. After 14 days of polymorphic transformation, the fully transformed M2LB-F microparticles presented in Figs 14e-f show that the particle surface is entirely covered with nanorods about 1 μ m long and 0.5 μ m wide. It is noted that Figures 14a and 14e also showed some broken microparticles (marked with white rectangles) that revealed the hollow nature of the M2LB-SP and M2LB-F microspheres.

Figure 14 SEM images of (a) M2LB-SP; (b) high-magnification M2LB-SP; (c) M2LB-INT; (d) high-magnification M2LB-INT; (e) M2LB-F; (f) high-magnification M2LB-F.

4.3.2 Morphological properties

The XRD patterns of particles synthesized using 2% mannitol/LB Agar feed solution are presented as the typical results in Figure 15. Peaks at 10.6° and 14.7° of 2 θ correspond to β -mannitol, and peaks at 9.4°, 13.8° and 17.3° 2 θ indicate the presence of α -mannitol (85). The presence of peak at 9.7° 2 θ is an indication of the δ form of

mannitol (85). It can be seen in Figure 15a that all the three polymorphic form of mannitol coexist in M2LB-SP. However, the XRD pattern of M2LB-INT in Figure 15b indicates the absence of the β -mannitol peaks at 10.6° and 14.7° 2θ , while peaks at 9.4° , 13.8° , 17.1° and 9.7° 2θ corresponding to the α and δ form of mannitol, respectively are still present. It shows that the polymorphic transformation of β -mannitol can happen within 1 h with the introduction of hexane. After 14 days of polymorphic transformation, the δ form peak at 9.7° 2θ also disappears in the XRD pattern of M2LB-F in Figure 15c and only peaks corresponding to α -mannitol are present. It suggests that δ -mannitol requires longer transformation time than β -mannitol and the fully transformed microparticles consist of only α -mannitol.

Figure 15 XRD patterns of (a) M2LB-SP; (b) M2LB-INT; and (c) M2LB-F.

4.3.3 Differential Scanning Calorimetry (DSC)

Amorphous mannitol is generally characterized by glass transition at 13°C together with two crystallization exothermic peaks at 25°C and 65°C. As shown in Figures 16a-c, no amorphous mannitol was detected in M2LB-SP, M2LB-INT and M2LB-F. The reference melting points of α -, β - and δ - mannitol are 166°C, 166.5°C and 155°C, respectively (85). As seen in Figures 16a-b, both M2LB-SP and M2LB-INT exhibit two overlapped endothermic peaks, while M2LB-F in Figure 18c only exhibits one endothermic peak. It was demonstrated in the XRD result that all α -, β - and δ -mannitol co-exist in M2LB-SP. It is reasonable to interpret the peak at 146.7°C shown in Figure 16a to be corresponding to mostly δ -mannitol and the peak at 162.5°C is a combination of α - and β -mannitol. On the other hand, while the peak at 147.1°C in Figure 16b is still corresponding to δ -mannitol, the peak at 162.5°C is ascribed to α -mannitol only since the XRD result in Figure 15b does not indicate the presence of β -mannitol. The single peak at 158°C in Figure 18c suggests that there is only α -mannitol present. The result of DSC is in agreement with XRD measurements that the hexane treatment causes both β -mannitol and δ -mannitol to disappear.

Figure 16 DSC thermal profile of (a) M2LB-SP; (b) M2LB-INT; (c) M2LB-F microparticles.

4.3.4 Fourier Transform Infrared (FTIR) Spectroscopy

In order to study the mechanism of polymorphic transformation, mannitol and the individual components of LB Agar, namely agar, peptone, and yeast extract were studied pair-wise with hexane using FTIR. As shown in Figure 17, mannitol/hexane, agar/hexane, peptone/hexane, and yeast extract/hexane systems exhibits light different absorption characteristics compared to pure mannitol, peptone, agar and yeast extract, respectively. With the introduction of hexane, the absorption bands of hydrogen bonds are found to be weakened in mannitol (broad peaks at $3600\text{--}3100\text{ cm}^{-1}$ in Figures 17a-b), agar (at $3700\text{--}3100\text{ cm}^{-1}$ in Figures 17c-d), peptone (at $3700\text{--}3000\text{ cm}^{-1}$ in Figure 17e-f), as well as yeast extract (at $3700\text{--}3100\text{ cm}^{-1}$ in Figures 17g-h). The absorption at $3800\text{--}3000\text{ cm}^{-1}$ and 1382 cm^{-1} corresponding to δ -mannitol (85) shown in Figure 19i disappears with the

introduction of hexane, while α -mannitol absorption (85) appears at $3800\text{--}3000\text{ cm}^{-1}$ and 1388 cm^{-1} , 1370 cm^{-1} as seen in Figure 17j. It can be inferred that the ratio of α -mannitol to δ -mannitol increases with the introduction of hexane.

Figure 17 FTIR spectra of (a) mannitol, (b) mannitol/hexane, (c) agar, (d) agar/hexane, (e) peptone, (f) peptone/hexane, (g) yeast extract, (h) yeast extract/hexane, (i) M2LB-SP, (j) M2LB-F.

4.3.5 Thermal Gravimetric Analysis (TGA)

Typical TGA thermal degradation profiles of M2LB-SP and M2LB-F microparticles are shown in Figures 18a-b, respectively. The profiles show weight loss decompositions at approximately 230°C. Figure 18a indicates the presence of about 0.7% residual moisture in M2LB-SP. After oven-drying at 100°C for 2 h, Figure 18b illustrates that there is no residual moisture or hexane in final M2LB-F microparticles.

Figure 18 TGA curves of (a) spray-dried M2LB-SP; (b) oven-dried M2LB-F.

4.3.6 Drug Content homogeneity

The drug content and CV for each blend is listed in Table 11. As the CV of all the three blends are less than 8%, the blends can be considered as sufficiently uniform (35, 39, 89).

Table 11 BD Content and Homogeneity of the Blends

Blend	Run1 (%)	Run 2(%)	Run 3(%)	Average(%)	CV(%)
M5LB-F	2.38	2.43	2.39	2.40	1.10
M2LB-F	1.99	2.3	2.26	2.18	7.72
M1LB-F	2.27	2.41	2.38	2.35	3.13
M2NaCl-SP	2.73	2.51	2.42	2.55	6.25
M2LB-SP	2.1	2.02	2.02	2.05	2.26
Commercial lactose	2.11	2.1	1.88	2.03	6.40

4.3.7 In Vitro Deposition Properties

The aerosolization and deposition properties of five microparticles namely, M5LB-F, M2LB-F, M1LB-F, M2LB-SP and M2NaCl-SP as drug carriers were investigated using BD as the model drug. The particle size, bulk and tap densities, CI, and the FPF and ED are shown in Table 12. It can be seen that the three microparticles after polymorphic transformation e.g. M5LB-F, M2LB-F and M1LB-F have comparable ED with commercial lactose carriers and M2LB-F carriers has the highest ED of about 94.1%. Nonetheless, the FPFs of all the three microcarriers are observed to be higher than commercial lactose carriers. M2LB-F particles are able to achieve a FPF of $28.6 \pm 2.4\%$.

Table 12 Deposition of budesonide from different blends at inhalation rate of 60L/min.

Blend	average diameter (μm)	ρ_{bulk} (g/cm^3)	ρ_{tap} (g/cm^3)	CI	ED (%)	FPF (%)
M5LB-F	10.76	0.222	0.541	0.589	92.5 $\pm$ 0.6	19.2 $\pm$ 1.6
M2LB-F	9.58	0.188	0.452	0.584	94.1 $\pm$ 0.1	28.6 $\pm$ 2.4
M1LB-F	8.93	0.183	0.407	0.550	90.8 $\pm$ 0.3	23.2 $\pm$ 2.8
M2NaCl-SP	9.71	0.247	0.556	0.556	92.7 $\pm$ 3.9	15.5 $\pm$ 1.1
M2LB-SP	9.47	0.175	0.408	0.565	87.7 $\pm$ 1.3	15.4 $\pm$ 2.9
Commercial lactose	<20	0.324	0.594	0.455	92.9 $\pm$ 1.5	17.8 $\pm$ 2.5

4.3.8 Particle Size Distribution of BD drug

As BD particles are not spherical, both particle length and width were determined for size distribution. As seen in Figure 19, 80 wt% of BD has a particle length between 1.4 μm to 4.8 μm and a particle width between 0.6 μm and 2.0 μm .

Figure 19 Size distribution of the Budesonide in terms of (a) particle length; (b) particle width.

4.3.9 Roughness and Drug Adhesion Analysis

ImageJ with Interactive 3D Surface Plot plugin (v2.33) was used to analyze the surface morphology of M2LB-SP, M2NaCl-SP, M5LB-F, M2LB-F and M1LB-F as shown in Figures 20(i). The rectangular area at the center of each particle was being converted into 2D and subsequently 3D surface plots individually as illustrated in Figures 21(ii) and Figures 20(iii), respectively. Figures 20a indicate the surface of M2LB-SP to be relatively smooth. It is to note that while the D90 of BD particle length and particle width are 4.8 μm and 2.0 μm , respectively, 90% by number of the BD particles have a length smaller than 2.4 μm and a width smaller than 1.2 μm . The adhesion of BD on M2LB-SP is likely to follow Type I shown in Figure 21. Similarly, Figures 20b show the highest coverage of rods on the surface of M2NaCl-SP among all the microparticles. It is reasonable to consider the adhesion of BD on M2NaCl-SP is following Type II. The rods on the surface of M5LB-F, as shown in Figures 20c, have larger width compared to those on M2NaCl-SP. On the other hand, Figures 20d show that the rods on the surface of M2LB-F become shorter than those on M2LB-F and M2NaCl-SP. When the mannitol in the feed solution was further reduced to 1%, a reduced number of rods were found on the surface of M1LB-F as can be seen in Figures 20e. Therefore, the adhesions of BD on the surface of M5LB-F, M2LB-F, and M1LB-F are anticipated to follow Types III, IV, and V as illustrated in Figure 21 (17).

Figure 20 (i) Single particle SEM images; (ii) 2D surface plot; (iii) 3D surface plot of (a) M2LB-SP; (b) M2NaCl-SP; (c) M5LB-F; (d) M2LB-F; (e) M1LB-F.

Figure 21 Five types of drug adhesion on the carrier surfaces.

4.4 Discussion

During the spray-drying process, the ethanol concentration in feed solution was found to affect the quality of the resulting particles significantly. When the ratio of ethanol to mannitol is above 5, negligible amount of broken particles were observed in SEM images. It is possible that the increase in ethanol concentration increases the droplet evaporation rate. Thus mannitol can be formed on the droplet surface rapidly and stabilizing the shell particle formation.

The spray-dried microparticles have a relatively smooth surface as shown in SEM and XRD measurements revealed that the microparticles have a mixture of α -, β - and δ -

mannitol. After the particles were immersed in hexane solution for 14 days, β - and δ -mannitol were transformed into α form as FTIR, XRD and DSC illustrated. SEM also indicates the self-assembling of mannitol molecules into rods on the surface of the microparticles. It is anticipated that the crystallization behavior of the polymorphs is affected by the presence of LB Agar. The molecules in LB Agar may have stronger affinity and can be adsorbed selectively onto certain faces of the polymorphs (92). It thereby inhibits the nucleation of β -mannitol and promotes the growth of α - and δ -mannitols (92). FTIR results show that after immersing spray-dried microparticles in hexane, -OH/hexane and -NH₂/hexane interactions are present in mannitol/LB Agar system. It is possible that new hydrogen bonds are formed between the -OH groups, -NH₂ groups and the H of hexane. XRD and DSC results illustrate that after immersing the sample into hexane solution for 1 h, β -mannitol disappeared and δ -mannitol disappeared completely only after 14 days. It is anticipated that β -mannitol and δ -mannitol were transformed into its α form while the transformation of δ -mannitol was slower than that of β -mannitol. It is likely that the new hydrogen bonds formation improved the cross-interactions among mannitol, LB Agar, residual H₂O and hexane. The improvement of cross-interactions stabilized α -mannitol and became more stable than the amorphous, β - and δ -form. The slow polymorphic transformation of δ -mannitol allowed α -mannitol crystal growth to grow in a slow and continuous manner. Moreover, the presence of hexane provided a homogeneous environment for α -mannitol crystal growth. The slow and continuous crystal growth process coupled with homogeneous environment can generate uniformly grown α -mannitol crystal pattern and lattice defects were largely avoided or covered by newly formed α -mannitol. Thus, ordered α -mannitol crystals were

obtained. With the presence of LB Agar, α -mannitol crystals self-assembled into rods with uniformly size and shape.

The proposed polymorphic transformation and self-assembly method allows the control of particle surface roughness and drug adhesion independently. As Figure 7 and Table 3 indicate, factors such as size, shape and crystallinity of all the microparticles synthesized in this study (XRD patterns of M1LB-F, M5LB-F and M2NaCl-SP not shown in this paper) are all comparable except the particle surface roughness.

The relation between particle surface roughness, drug adhesion and FPF can be obtained based on the analysis of FPF results using the microparticles synthesized in the study. Type I of drug adhesion shown in Figure 8 illustrates a small contact between drug and carrier. It resembles the low contact area between BD and the relatively smooth surface of M2LB-SP. Similar to Type I of drug adhesion, Type II also has small contact between drug and carrier owing to the high degree of surface asperity as demonstrated by the small void space between the rods present on M2NaCl-SP. Therefore, the FPFs of M2NaCl-SP and M2LB-SP indicates that both Type I and Type II drug adhesion generate similar FPF. As the space between the surface crevices on carrier surface increases, Type II drug adhesion turns into Type III drug adhesion. M5LB-F has higher rod diameter than M2NaCl-SP and provides a good representation of Type III drug adhesion. Type III drug adhesion allows most of the drugs to locate on the peaks of the crevices and certain amount can be caught in-between. As the drug-carrier contact on the peaks of the crevices in Type III drug adhesion is smaller than that in Type II drug adhesion and the space in-between crevices is relatively small, FPFs of M2NaCl-SP and M5LB-F show that Type III drug adhesion can generate a higher FPF than Type II drug adhesion. An

increase in the space in-between crevices transforms Type III drug adhesion into Type IV drug adhesion. Understandably, the drug-carrier contact area at the peaks of the crevices counteracts the space in-between crevices between Type III and Type IV drug adhesions. M2LB-F signifies a carrier surface covered by short rods. The higher FPF of M2LB-F compared to M5LB-F indicates that when the low drug-carrier contact area dominates over interlocking force, Type IV drug adhesion can have higher FPF than Type III drug adhesion. However, when interlocking force becomes dominating, Type IV drug adhesion becomes Type V drug adhesion illustrated by the low rod density on the surface of M1LB-F. The increase in the interlocking between drug and carrier on M1LB-F compared to M2LB-F thus reduces the FPF (17).

The observed relation between FPF and drug adhesion mechanism can be considered solely depends on the particle surface roughness. As the surface roughness increase and the space between the surface crevices on carrier surface increases, the drug adhesion mechanism can change from Type I to Type V. The proposed synthesis technique using polymorphic transformation allows the control of particle surface roughness simply by changing the mannitol concentration in the feed solution during the spray-drying step. A reduction of mannitol concentration from 5% (w/v) to 2% (w/v) increases the surface roughness of the fully transformed microparticles and allows the change of drug adhesion mechanism from Type III to Type IV.

It is to note that the drug adhesion mechanism involves the relation between the geometric diameter of the drug particles and the surface roughness of the carrier particles. A careful design of carriers is necessary based on the drug particles size used to achieve Type IV drug adhesion. Nonetheless, the M2LB-F microparticles used in this study is

able to provide a high FPF of 28.6 ± 2.4 using BD as drug at a drug mixing ratio of 45:1. This result is comparable with the highest reported FPFs of $29.2 \pm 4.7\%$ using mannitol as carriers with salbutamol sulfate as drug (17) and FPF of $31.8 \pm 1.3\%$ using lactose carriers with BD as drug (12).

4.5 Conclusion

Novel controlled-roughness mannitol/LB Agar microparticles were successfully prepared by polymorphic transformation and self-assembly method using spray-dried mannitol microparticles as start material. With the introduction of hexane, β -mannitol and δ -mannitol in spray-dried mannitol/LB Agar microparticles were transformed to α -mannitol. It causes the nanobelts on the surface of the spray-dried microparticles to self-assemble to nanorods and forms the uniform rod-covered shape. The particle surface roughness can be controlled independently by changing the concentration of mannitol in the feed solution during spray-drying process.

The relation between FPF and drug adhesion mechanism was revealed using 5 different microparticles as carriers, among which differ only in terms of surface roughness. Type IV drug adhesion that offers a balance between low drug-carrier contact area and interlocking force can yield the highest FPF. Among the microcarriers synthesized in the study, M2LB-F provides the FPF of $28.6 \pm 2.4\%$ using BD as drug at a drug mixing ratio of 45:1.

Chapter 5 Synthesis and Stability Study of Mannitol/ LB Agar(lab-made) microparticles

5.1 Overview

Spray drying is a widely used technique for the preparation of drug carriers as it can provide easily control of particle size, powder density and morphology (17, 21). However spray-dried microparticles are often in an unstable state. Most of the spray-dried microparticles have not reached an equilibrium state due to the short spray-drying processing time (26). A number of researches have been focused on the stability of spray-dried particles. It was reported that amorphous sucrose can start to transform to crystalline form in spray-dried sucrose/hydroxypropylmethylcellulose(HPMC) microparticles after a five-month storage at 75% relative humidity (RH) and 40 °C (80). Similarly, amorphous lactose in spray-dried lactose/rhDNase powder were found to transform to a partially crystalline material at high relative humidity (81). For polymer excipients, it was found that amorphous bendroflumethiazide (BFMT) recrystallized more quickly with an increased amount of polyethylene glycol (PEG) 4000 in spray-dried BFMT/PEG 4000 microparticles (77). Another study reported that both hydroxypropylcellulose (HPC) and polyvinylpyrrolidone (PVP) can increase the stability of amorphous valdecoxib (82). PVP was found to be a better stabilizer than HPC. It was also reported that PVP allowed the formation of more stable amorphous lactose than HPC by slightly elevating the glass transition temperature (T_g) and forming interactions such

as hydrogen bonding between PVP and lactose (83). Similarly, excipients such as mannitol, sucrose, trileucine, etc can act as stabilizers by increasing T_g . It is commonly accepted that high glass transition temperature can lead to good stability. However a study showed that humidity-induced crystallization of amorphous solids can also occur when stored at temperature below T_g (27, 28). It was suggested that the crystallization of excipients would not affect the physical stability of drugs (27). There is another study reported that the crystallization of lactose and mannitol can lead to a decrease in the activity of trypsin (84). As every pharmaceutically acceptable dosage form must remain stable for the safety of inhalation, it is important to create a method that provides a stable dosage with high aerosolization performance.

It was suggested that combining spray drying with additional processes, for example crystallization and polymorphic transformation, to convert spray-dried particles to a stable state (26). In our previous work, fully-transformed and fully-crystallized rough mannitol carriers were successfully prepared which showed a good drug delivery performance (30). However there is still a stability risk of the tendency of small crystals growth (26) since spray drying method usually produces very small mannitol crystals due to the short precipitation time. In this paper the stability of the fully-transformed and fully-crystallized mannitol carrier particles using agar as a stabilizer was studied.

5.2 Results and discussion

The spray-dried mannitol/LB Agar (lab-made) and the transformed mannitol/LB Agar (lab-made) microparticles were characterized using XRD, SEM, DSC and TGA to study the mechanism of the transformation. All the characterization results above show that different composition of LB Agar have negligible influence on the morphological,

surface, thermal and thermal gravimetric properties of the resulting particles. Hence, spray-dried M4LB-SP(86) and transformed M4LB-F(86) particles prepared using LB Agar(86) were chosen as the representative particles for detailed property characterizations.

5.2.1 Morphological properties

Figure 22 XRD of (a) spray-dried M4LB-SP(86); (b) transformed M4LB-F(86).

The XRD patterns of M4LB-SP(86) microparticles and M4LB-F(86) microparticles shown in Figure 22 illustrate the process of mannitol polymorphic transformation. Figure 22 indicates there is no β -mannitol exist in M4LB-SP(86) and M4LB-F(86) microparticles. Figure 22a shows peak at 9.7° of 2θ correspond to δ -mannitol, and peaks at 9.4° , 13.7° and 17.3° 2θ indicate the presence of α form (85). It can be seen in Figure 22a that α and δ forms of mannitol coexist in spray-dried M4LB-SP(86) microparticles. Figure 22b illustrates peaks at 9.3° , 13.7° , 17.3° of 2θ corresponding to the α -mannitol(85). It indicates there is only α form of mannitol exist in transformed M4LB-

F(86) microparticles. It suggests that after hexane treatment, the δ -mannitol transformed into α form in mannitol/LB Agar (lab-made) microparticles.

5.2.2 Surface properties

Figure 23 SEM of (a) spray-dried M4LB-SP(86); (b) high-magnification image of M4LB-SP(86); (c) transformed M4LB-F(86); (d) high-magnification image of M4LB-F(86).

The SEM images in Figure 23 show the process of surface modification of mannitol/LB Agar (lab-made) microparticles. Figures 23a-b illustrate that the spray-dried M4LB-SP(86) microparticles have a relatively smooth surface. As shown in Figure 23a, the microparticle marked with white rectangle reveals that M4LB-SP(86) microparticles are hollow and there are some crevices on the inside surface of M4LB-SP(86) microparticles. Figure 23b indicates there are some fragments attaching on the surface of M4LB-SP(86) microparticles, which are possibly pieces of broken M4LB-SP(86) microparticles. After hexane treatment, it can be seen in Figures 23c-d that the transformed M4LB-F(86) microparticles have high surface roughness. A number of crevices appear on the surface of M4LB-F(86) microparticles. Due to the short precipitation time, spray drying method usually produces very small mannitol crystals

(26). It is possible that with the hexane treatment small α -mannitol crystals on the surface of M4LB-F(86) microparticles coagulated with each other. Eventually large-size α -mannitol crystals were formed and crevices appear simultaneously.

5.2.3 Differential Scanning Calorimetry (DSC)

It was reported that the melting points of α -, β - and δ - mannitol are 166°C, 166.5°C and 155°C, respectively (85). Peaks at 168°C shown in Figure 24a and at 163°C shown in Figure 24b are possibly ascribed to α - or β -mannitol. As the XRD result illustrates that only α - and δ -mannitol coexist in spray-dried M4LB-SP(86) microparticles, peak at 168°C shown in Figure 24a is corresponding to α -mannitol and peak at 150°C is ascribed to δ -mannitol. The single peak at 163°C in Figure 24b indicates that there is only α form present in transformed M4LB-F(86) microparticles. The result of DSC suggests that with hexane treatment δ -mannitol transformed into α -mannitol which is the same as XRD measurements.

Figure 24 DSC thermal profile of (a) spray-dried M4LB-SP(86); (b) transformed M4LB-F(86).

5.2.4 Thermal Gravimetric Analysis (TGA)

Figure 25 TGA of (a) spray-dried M4LB-SP(86); (b) transformed M4LB-F(86).

TGA thermal degradation profiles in Figure 25 show that both spray-dried M4LB-SP(86) and transformed M4LB-F(86) microparticles have a weight loss decomposition at approximately 230°C. Figure 25a indicates the presence of about 1.1% residual moisture in M4LB-SP(86) microparticles. After M4LB-F(86) microparticles were oven-dried at 120°C for 8 h, there is minimal weight loss as shown in Figure 25b. That indicates there is no residual moisture or hexane in transformed M4LB-F(86) microparticles.

5.2.5 Drug Content homogeneity

As listed in Table 13, the CV values of all the three blends are less than 4%, which suggest all the blends have adequate homogeneity for DPI systems (35, 39, 89).

Table 4 BD Content and Homogeneity of the Blends.

Blend	Run1 (%)	Run 2(%)	Run 3(%)	Average(%)	CV(%)
M4LB-F(86)	2.11	2.16	2.27	2.18	3.75
M4LB-F(92)	2.07	2.15	2.22	2.15	3.50
M4LB-F(94)	2.18	2.22	2.31	2.24	2.98

5.2.6 In Vitro Deposition Properties

The aerosolization and deposition properties of three blends were investigated. The particle size, bulk density, tap density and CI of three carrier particles namely M4LB-F(86), M4LB-F(92) and M4LB-F(94), together with FPF and ED of three blends are shown in Table 14. All the three blends have comparable ED and FPF with each other as listed in Table 14. M4LB-F(86) carrier particles have the highest ED of $89.1 \pm 1.1\%$ and highest FPF of $31.1 \pm 3.4\%$, which is comparable with the best mannitol/LB Agar(commercial) microparticles reported in other literatures (12, 30).

Table 14 Deposition of Budesonide from Different Blends at Inhalation Rate of 60L/min.

Blend	average diameter (μm)	ρ_{bulk} (g/cm^3)	ρ_{tap} (g/cm^3)	CI	ED (%)	FPF (%)
M4LB-F(86)	8.76	0.224	0.481	0.533	89.1 ± 1.1	31.1 ± 3.4
M4LB-F(92)	8.82	0.229	0.480	0.523	86.1 ± 4.6	27.8 ± 2.2
M4LB-F(94)	8.94	0.234	0.481	0.514	87.8 ± 2.9	27.7 ± 1.1

5.2.7 Stability

5.2.7.1 Surface Morphology

Fig 26 shows show the SEM images of M4LB(94)-F microparticles stored under room temperature and RH below 10 for various periods. It can be seen in Figs. 26a-e and Fig. 26a1-e1 that similar spherical shape and surface morphology can be observed for all the microparticles stored for 21 months. After 24 months of storage, particles with spherical shape (marked with white ring) and rock-like shape (marked with white rectangles, triangle and trapezoid) coexisted in M4LB(94)-F microparticles as shown in Fig. 26f. That indicates that the transformed M4LB(94)-F microparticles could keep their spherical shape for about 21 months. A few large-size microrods about 5 μm long and 2 μm wide appeared on the surface of M4LB(94)-F microparticles (marked with white trapezoid). Fig. 26g indicates microrods about 5 μm long appeared on most of M4LB(94)-F microparticles and a few spherical microparticles (marked with white ring) could still be observed after particles were stored for 27 months. There were not any spherical M4LB(94)-F microparticle after a 30 months of storage as shown in Fig. 26h. The surface of M4LB(94)-F microparticles were relatively rough as Fig. 26h1 illustrates. Thirty three months later, the surface of some M4LB(94)-F microparticles became relatively smooth as Fig. 26i1 shows. It is possible the microrods about 5 μm long and 2 μm wide joined into each other and formed a relatively smooth surface.

Figure 26 SEM of M4LB(94)-F stored under room temperature and RH below 10 for (a) 0 month; (b) 3 months; (c) 9 months; (d) 15 months; (e) 21 months; (f) 24 months; (g) 27 months; (h) 30 months; (i) 33 months.

5.2.7.2 Roughness Analysis

ImageJ with Interactive 3D Surface Plot plugin (v2.33) (86) was used to analyze the surface morphology of M4LB(94)-F microparticles stored for various periods of (a1) 0 month; (d1) 15 months; (e1) 21 months as shown in Figures 27(i). The rectangular area at the center of each particle was being converted into 2D and subsequently 3D surface

plots individually as illustrated in Figures 27(ii) and Figures 27(iii), respectively. Although Figures 27(i) show that as time going on, the SEM images of M4LB(94)-F microparticles changed, Figures 27(iii) indicated the surface roughness of three microparticles are similar. The average surface roughness Ra values of three rectangular areas at the center of each particle were calculated using SurfCharJ 1q Image J. The three rectangular areas have similar Ra results as Figure 27 shows. Thus the drug detachment on M4LB(94)-F microparticles would be comparable.

Figure 27 (i) Single particle SEM images; (ii) 2D surface plot; (iii) 3D surface plot of M4LB(94)-F kept at room temperature and RH below 10 for (a1) 0 month; (d1) 15 months; (e1) 21 months.

5.2.7.3 Drug Content homogeneity

The blend homogeneity of BD drug was determined with M4LB(94)-F microparticles stored for 0-33 months. As listed in Table 15, the CV of all the BD-M4LB(94)-F blends are less than 8%. Thus all the blends can be considered as sufficiently uniform (35, 39, 89).

Table 15 BD Content and Homogeneity of BD-M4LB(94)-F blends

Time	Run1 (%)	Run 2(%)	Run 3(%)	Average(%)	CV(%)
0 month	2.18	2.22	2.31	2.24	2.98
3 months	2.08	2.25	2.13	2.15	4.06
9 months	2.13	2.23	2.25	2.20	2.92
15 months	2.11	2.26	2.24	2.20	3.70
21 months	2.21	2.22	2.1	2.18	3.06
24 months	1.98	2.13	2.26	2.12	6.60
27 months	2.07	2.29	2.31	2.22	5.99
30 months	1.99	2.17	2.26	2.14	6.42
33 months	2.01	2.27	2.22	2.17	6.37

5.2.7.4 In Vitro Deposition Properties

Figure 28a shows the bulk density, tap density and CI of M4LB(94)-F microparticles during a storage period of 33 months. The particle bulk density, tap density and CI had a minimal change when M4LB(94)-F microparticles were stored for 21 months. It is possible that during a 21-month storage period, the shape, size and

surface roughness of M4LB(94)-F microparticles were similar. Thus the interparticle forces of M4LB(94)-F microparticles had a minimal change which result in bulk density, tap density and CI were similar. That suggests the particle flowabilities were kept at the similar value during a storage period of 21 months. Twenty one months later, both of the particle bulk density and tap density decreased slightly while CI has a tendency of slightly increase. It is possible that during a storage period of 24 to 30 months, with the number of irregular particles increasing, the contact area between particles increased. There is another possibility that the interlocking force increased when small carrier particles whose diameter is below about 5 μm located between crevices of rods about 5 μm long as shown in Figures 26f–h. Both of the increased contact area and interlocking force resulted in an increase of interparticle force. After M4LB(94)-F microparticles were stored for 33 months, the relatively smooth particles shown in Figure 26i provided an increase in contact area between particles. Thus the interparticle force of M4LB(94)-F microparticles increased when the resulting particles were stored for 33 months. That indicates the particle flowability decreased slightly during a storage period of 24 to 33 months (34, 68).

The aerosolization and deposition properties of the BD-M4LB(94)-F blends were investigated. Figure 28b shows that ED values of the BD-M4LB(94)-F blends are comparable with each other when M4LB(94)-F microparticles were stored for 21 months. Similarly, all the blends have comparable FPFs about 27% with each other when M4LB(94)-F microparticles during a storage of 21 months as shown in Figure 28c. It is possible that the similar surface roughness, shape, size and density of M4LB(94)-F microparticles offered comparable aerosolization during a 21-month storage. Since the

flowability of M4LB(94)-F microparticles also had a minimal change, the EDs of the BD-M4LB(94)-F blends are similar when the particles were stored for 21 months. The similar surface roughness provided comparable drug detachment on the surface of M4LB(94)-F microparticles. Together with the similar flowability and aerosolization, comparable FPFs were obtained during a 21-month storage period. Twenty four months later, ED of BD-M4LB(94)-F blends decreased slightly but still more than 85% and the FPF of blends kept on decreasing from about 22% to 13%. It is possible that after M4LB(94)-F microparticles were stored for 24 to 30 months, as microrods about 5 μm long as shown in Figures 26f–h appeared on the surface of M4LB(94)-F microparticles, deep crevices formed simultaneously. When BD drug particles located in these deep crevices, the interlocking force between drug and carrier particles would increase (30). That result in these BD drug particles are difficult to be detached from M4LB(94)-F microparticles. When the particles were stored for 33 months, some BD drug particles would locate on smooth M4LB(94)-F microparticles as Figure 26i indicates. Which resulted in large contact area between BD and carrier particles (18, 30). The decreased flowability, increased interlocking force and contact area are all possibly resulted in FPFs keeping on decreasing during the storage of 24 to 33 months.

Figure 28 Deposition of Budesonide from Different Blends at Inhalation Rate of 60L/min.

5.2.8 Stability and Agar Composition

Percentage composition of agar in the resulting particles and carrier particle stability of shape, surface roughness and FPF are listed in Table 16. All of the three types of

mannitol/LB Agar (lab-made) microparticles have more stable FPF than mannitol/LB Agar (commercial) microparticles. Table 16 also indicated that an increase of agar percentage composition offer a better FPF Stability of blends. It is possible that agar absorbed water in the air and reduced the amount of water contacting with mannitol crystals in transformed microparticles consequently since it was reported water contained in the microparticles can accelerate crystallization (26, 93). As the percentage composition of agar increasing, its capability of water absorbability increased. Thus the rate of small crystals growth decreased which resulted in the stability of transformed microparticles increasing.

Table 16 Stability and agar percentage composition

Blend	M4LB(94)-F	M4LB(92)-F	M4LB(86)-F	M4LB(commercial)
FPF Stability of blends (month)	21	16	10	3
Agar percentage composition (%)	7.26	7.05	6.65	2.49

5.3. Conclusion

Novel rough-surface mannitol/LB Agar (lab-made) microparticles were successfully synthesized by polymorphic transformation method. Agar used as a stabilizer to prepare mannitol/LB Agar (lab-made) microparticles.

The resulting carrier particles generated drug FPF of $31.1 \pm 3.4\%$, which is as high as the best the carrier aerosolization performance reported in other literatures. Although δ -mannitol in transformed microparticles was stable, the surface morphology and shape still

changed due to crystal growth. Mannitol/LB Agar (lab-made) microparticles exhibited a better stability on surface roughness, shape and FPF than mannitol/LB Agar (commercial) microparticles. With the percentage of agar increasing in the resulting particles, the particle stability increased. A stable period of 21 months was achieved when there is 7.26% agar in mannitol/LB Agar (lab-made) microparticles.

Chapter 6 Synthesis of Pollen-shape Mannitol/LB Agar Particles with High Surface Roughness

6.1 Overview

Chapter 3 shows mannitol carrier particles provided a higher FPF than lactose particles when they have similar size, shape and surface roughness. It has been proved in chapter 4 that carrier particles with high surface roughness can provide improved aerosolization and deposition performance. In our team pollen-shape Hydroxyapatite (HA) microparticles were successfully prepared using hydrothermal method (34). It was reported that the high surface roughness of HA microparticles resulted in low inter-particle interactions, good flowability, improved aerosolization performance and high drug loading efficiency (15, 31, 34). It is reported in chapter 5 that the composition of LB Agar can affect the particle. In this study, synthesis of pollen-shape carrier particles using mannitol were carried out and the effect of LB Agar composition to carrier surface morphology were examined.

6.2 Optimization of LB Agar Composition

Figure 29a illustrates a typical SEM of spray-dried 2%mannitol/LB Agar (lab-made) microparticles prepared using LB agar with an agar percentage of 50%. As shown in Figure 29a, the spray-dried microparticles have a relatively smooth surface with a few defects. Similar morphology was observed on spray-dried 2% mannitol/LB Agar (lab-

made) obtained with an agar percentage in LB Agar up to 94%. After a storage of 7 weeks, M2LB(50,55,66)-F microparticles with a 50-66% percentage of agar in LB Agar exhibited a rough spherical shape with a few crevices on the particle surface as Figure 29b indicates. While Figure 29c shows pollen-shape microparticles with a number of rods about 4 μm long and 1 μm wide on the surface of M2LB(77,83,86,89)-F microparticles with a 77-89% percentage of agar in LB Agar when the resulting particles were stored for 10 weeks. As shown in Figure 29d, M2LB(92,93,94)-F microparticles with a 92-94% percentage of agar in LB Agar have a rod-covered rough surface. The density of rods on the surface of M2LB(92,93,94)-F microparticles is smaller than that on the surface of M2LB(77,83,86,89)-F microparticles. Figure 29d1 illustrates the size of rods on the surface of M2LB(92,93,94)-F microparticles is below 2 μm long which is also smaller than the length of rods on the surface of M2LB(77,83,86,89)-F microparticles. It can be seen in Figure 29 that transformed mannitol/LB Agar microparticles with a 77-89% percentage of agar in LB Agar have much higher surface roughness than other microparticles. Thus M2LB(77,83,86,89)-F microparticles were selected as the carrier particles for DPI.

Figure 29 Typical SEM of (a) spray-dried M2LB(50)-SP microparticles, (b) M2LB(50,55,66)-F, (c) M2LB(77,83,86,89)-F, (d) M2LB(92,93,94)-F, (d1) high-magnification of the rectangle area in (d).

6.3 Proposed Formation Mechanism of M2LB(77,83,86,89)-F

M2LB(77,83,86,89)-F microparticles with a 77-89% percentage of agar in LB Agar was found to exhibit similar results in SEM, XRD and TGA measurements. Thus M2LB(86)-SP and M2LB(86)-F microparticles were chosen as the representative particles for study the mechanism of the preparation.

6.3.1 Morphological properties

Figure 30 shows the XRD patterns of M2LB(86)-SP microparticles stored at 25°C

for 1 week, 4 weeks 10 weeks and 18 weeks. In the XRD patterns shown in Figures 30a-d, peaks at 13.8° and 17.1° of 2θ indicate α form of mannitol and the peak observed at 9.7° of is a overlapped peak interpreting as indicative of the α - and δ -mannitol (85). It can be seen, during storage there are α and δ forms of mannitol coexist in the resulting microparticles. The areas of peak-I at 9.7° of 2θ corresponding to δ -mannitol and peak-II at 13.8° of 2θ corresponding to α -mannitol as Figures 30a-d illustrate , are shown in Table 17. The area ratios of peak-I to peak-II decreased during storage. That indicates the ratio of δ to α form decreased in the resulting microparticles. It can be inferred that a part of δ -mannitol transformed to α form during storage.

Figure 30 XRD of M2LB(86)-SP after stored at 25°C for (a) 1 week, (b) 4 weeks, (c) 10 weeks, (c) 17 weeks.

Table 17 Area ratio of peak-I at 9.7° of 2 θ to peak-II at 13.8° of 2 θ .

time	area of peak-I	area of peak-II	area ratio of peak-I to peak II
1 week	30.54	10.84	2.82
4 weeks	34.50	12.46	2.77
10 weeks	30.28	12.61	2.40
17 weeks	17.60	12.80	1.38

6.3.2 Surface Morphology

A typical microparticle was selected as a representation to study the preparation of pollen-shape M2LB(86)-F microparticles. Figure 31 shows the process of rods growing on the surface of M2LB(86)-SP microparticles during storage. Figure 31a illustrates a number of nanorods about 10-100 nm long and 20 nm wide grown on the surface of the resulting microparticles after stored for 1 week. Four weeks later the rods became 1 μ m long and 200 nm wide as shown in Figure 31b. As rods keeping on growing, the surface roughness of the resulting microparticles increased. After stored for 7 weeks, the surface of the resulting microparticles was covered by rods whose length range from 0.2 to 2 μ m as Figure 31c indicates. Ten weeks later, the length of rods can grow to a range of 1-3 μ m on the resulting microparticles, namely M2LB(86)-F, as shown in Figure 31d. It is possible that as δ -mannitol transformed to α form, and α -mannitol self-assembled into rods. With the amount of α -mannitol transformed from δ -mannitol increasing, the length of self-assembled rods increased. With a storage period of 13 weeks, as the density of rods increased up to a certain degree, rods started to join in each other and surface roughness decreased, as Figure 31e illustrates. Figure 31e1 is a high-magnification of the rectangle area marked with white rectangle in Figure 31e. Figure 31e1 indicates there are

a few rods and mountain-like slabs about $0.8\ \mu\text{m}$ high. It is possible the rod density is low in this area. Some separated rods failed to join into each other. A part of rods have high density in one dimension. Which results in they joining into each other in one dimension and forming a mountain-like slab. Figure 31f indicates a relatively spherical microparticle formed after the resulting microparticles stored for 17 weeks. It is possible that as the rod density increasing on the surface of resulting particles, all of the rod joined into each other and formed a new shell.

Figure 31 SEM of M2LB(86)-SP after stored at 25°C for (a) 1 week, (b) 4 weeks (resulting particle namely M2LB(86)-INT4), (c) 7 weeks (resulting particle namely M2LB(86)-INT7), (d) 10 weeks (resulting particle namely M2LB(86)-F), (e) 13 weeks, (f) 17 weeks; (e1) high-magnification of the rectangle area in (e).

6.3.3 Stability

It can be seen in Figures 31d-e that the pollen-shape of M2LB(86)-F microparticles were stable only for 3 weeks when stored at room temperature. That suggests M2LB(86)-F microparticles are not stable at room temperature. When the pollen-shape of M2LB(86)-F microparticles shown in Figure 31d were store at 4°C, their shape were shown unchanged for 6 months. It is possible that low temperature can reduce the transformation rate and rods on the surface of M2LB(86)-F microparticles can be more stable. As a result, the stability of M2LB(86)-F microparticles can be maintained when stored at 4°C.

6.3.4 Thermal Gravimetric Analysis (TGA)

A typical TGA thermal degradation profile of M2LB(77,83,86,89)-F microparticles is shown in Figure 32. The profile shows a weight loss decomposition at approximately 250°C and there is about 1% residual moisture in pollen-shape M2LB(77,83,86,89)-F microparticles.

Figure 32 Typical TGA curves of pollen-shape M2LB(77,83,86,89)-F Microparticles.

6.3.5 Drug Content homogeneity

BD drug was blended with three pollen-shape microparticles namely M2LB(86)-INT4, M2LB(86)-INT7 and M2LB(86)-F microparticles. As is listed in Table 18, the CV of all three blends are less than 6%. Thus all the blends can be considered as sufficiently uniform (35, 39, 89).

Table 18 BD Content and Homogeneity of Different Blends

Blends	Run1 (%)	Run 2(%)	Run 3(%)	Average(%)	CV(%)
M2LB(86)-INT4	1.97	2.03	2.06	2.02	2.16
M2LB(86)-INT7	2.11	1.98	2.17	2.09	4.65
M2LB(86)-F	2.11	2.16	1.94	2.07	5.57

6.3.6 In Vitro Deposition Properties

The aerosolization and deposition properties of three blends of BD with M2LB(86)-INT4, M2LB(86)-INT7 and M2LB(86)-F microparticles were investigated. The particle size, bulk and tap densities, CI, and the FPF and ED are shown in Table 19. M2LB(86)-INT4 microparticles have a bulk density of 0.228 g/cm^3 which is lower than that of M2LB(86)-INT7 microparticles as Table 19 indicates. M2LB(86)-INT4 microparticles were covered with rods about 0.1 to $1 \mu\text{m}$ long as shown in Figure 31b. While the rods on the surface of M2LB(86)-INT7 microparticles are in a range from 0.2 to $2 \mu\text{m}$. An increase in rod size results in the surface roughness of the resulting particles increasing. It is possible that as the surface roughness of M2LB(86)-INT7 microparticles is higher than that of M2LB(86)-INT4 microparticles, the contact area between M2LB(86)-INT7 microparticles is lower than that of M2LB(86)-INT4 microparticles. Thus the interparticle force of M2LB(86)-INT7 microparticles is lower than M2LB(86)-INT4 microparticles. Smaller amount of bridges formed in M2LB(86)-INT7 microparticles than in M2LB(86)-INT4 microparticles before tapping. Thus the bulk volume of M2LB(86)-INT7 microparticles is lower than that of M2LB(86)-INT4 microparticles. Which results in the bulk density of M2LB(86)-INT7 microparticles is higher than that of M2LB(86)-INT4 microparticles. During tapping, the bridges were broken which resulted in the tap density of M2LB(86)-INT7 microparticles is similar to M2NaCl-SP microparticles as shown in Table 19. Therefore CI of M2LB(86)-INT7 microparticles is higher than that of M2LB(86)-INT4 microparticles. Similarly, as rods on the resulting microparticles growing, the bulk density of M2LB(86)-F microparticles is higher than that of M2LB(86)-INT7 microparticles as shown in Table 19. The tap densities of M2LB(86)-F and M2LB(86)-INT7 are similar. Therefore CI of M2LB(86)-F

microparticles is higher than that of M2LB(86)-INT7 microparticles. Since CI value exhibits a tendency of slightly decrease from M2LB(86)-INT4, M2LB(86)-INT7 to M2LB(86)-F microparticles, the particle flowability would increase during the storage period of 4 to 10 weeks. As the surface roughness increasing from M2LB(86)-INT4, M2LB(86)-INT7 to M2LB(86)-F microparticles, a decrease of contact area between carrier particles and drug-carrier particles is obtained. Lower drug-carrier contact results in an easier drug detachment from M2LB(86)-INT4, M2LB(86)-INT7 to M2LB(86)-F microparticles. Lower carrier-carrier contact provides a lower interparticle force from M2LB(86)-INT4, M2LB(86)-INT7 to M2LB(86)-F microparticles which results in carrier particles easily carried by air. Thus an improved aerosolization were obtained when the resulting particles stored from 4 to 7 weeks. The easier drug detachment, improved flowability and aerosolization result in increases of ED and FPF from blend of BD with M2LB(86)-INT4, M2LB(86)-INT7 to M2LB(86)-F microparticles as Table 19 indicates (18). M2LB(86)-F particles are able to achieve a FPF of $32.7 \pm 2.0\%$. This result is higher than the highest FPF reported in literatures that is $29.2 \pm 4.7\%$ using mannitol as carriers with salbutamol sulfate as drug (17). This result is also comparable with the highest reported FPF of $31.8 \pm 1.3\%$ using lactose carriers with BD as drug (12).

Table 5 Deposition of BD from Different Blends at Inhalation Rate of 60L/min

Blend	average diameter (μm)	ρ_{bulk} (g/cm^3)	ρ_{tap} (g/cm^3)	CI	ED (%)	FPF (%)
M2LB(86)-INT4	9.98	0.228	0.480	0.525	89.8 ± 2.6	23.6 ± 2.3
M2LB(86)-INT7	10.72	0.231	0.484	0.523	90.6 ± 1.6	27.5 ± 1.4
M2LB(86)-F	12.95	0.238	0.486	0.510	92.0 ± 1.6	32.7 ± 2.0

6.4 Conclusion

Novel pollen-shape M2LB(77,83,86,89)-F microparticles with a 77-89% percentage of agar in LB Agar were successfully prepared by polymorphic transformation and self-assembly method. During the storage of M2LB(77,83,86,89)-SP microparticles, δ -mannitol kept on transforming to α -mannitol and α -mannitol self-assembled into rods continuously. As the length of rods kept on increasing, the surface roughness of resulting microparticles increased during storage. After a 10-week storage period, the length of rods reached its maximum value and pollen- shape M2LB(77,83,86,89)-F microparticles were obtained. The M2LB(77,83,86,89)-F microparticles have stability period of 6 months at 4°C which is more stable than at 25°C. Pollen-shape M2LB(77,83,86,89)-F microparticles with high surface roughness can provide a FPF of $32.7 \pm 2.0\%$.

Chapter 7 Conclusion and Future Work

7.1 Conclusion

In this thesis, the relation between carrier material and drug delivery performance, relation between carrier surface roughness and drug attachment, as well as the effect of LB Agar composition to carrier stability and surface morphology are studied.

The relation between carrier material and drug delivery performance was studied using pollen-shape mannitol/NaCl and mannitol/lactose microparticles with pedal-like rods, which were synthesized by self-assembly method. SEM shows spray-dried mannitol/NaCl microparticles have a rough surface. After ethanol gas treatment, XRD indicates there is no polymorphic transformation happen in mannitol/lactose microparticles, while mannitol and lactose molecular self-assembled into petals in spray-dried mannitol/lactose microparticles as SEM images illustrate. SEM indicates mannitol/NaCl and mannitol/lactose microparticles have similar surface roughness, size and shape with each other. Both mannitol/NaCl and mannitol/lactose microparticles are highly crystallized as XRD illustrate. Mannitol/NaCl microparticles have lower interparticle force, lower CI and higher FPF than mannitol/lactose microparticles. Mannitol is proved to be a better material choice than the mixture of mannitol and lactose to prepare spherical rough carrier particles for dry powder inhalation.

The relation between carrier surface roughness and drug attachment was studied using 4 kinds of mannitol/LB Agar (commercial) microparticles and mannitol/NaCl

microparticles. Such mannitol/LB Agar (commercial) microparticles were synthesized by polymorphic transformation and self-assembly method using hexane as the polymorphic transformation reagent and spray-dried mannitol/LB Agar microparticles as the starting material. The XRD and DSC results indicate that after immersing spray-dried mannitol/LB Agar (commercial) microparticles in hexane, β -mannitol and δ -mannitol were transformed to α form. SEM shows that during the transformation, the nanobelts on the spray-dried mannitol/LB Agar microparticles become more dispersed and the contour of the individual nanobelts becomes more noticeable. Afterwards, the nanobelts self-assemble to nanorods and result in rod-covered mannitol/LB Agar microparticles. FTIR indicates new hydrogen bonds were formed among mannitol, LB Agar and hexane. SEM images coupled with image analysis software reveal that different surface morphology of the microparticles has different drug adhesion mechanisms. Comparison of ACI results and image analysis of SEM images shows that an increase in the particle surface roughness can increase the fine particle fractions (FPFs) using the rod-covered mannitol microparticles as drug carriers. Transformed microparticles show higher FPFs than commercially available lactose carriers. An FPF of $28.6 \pm 2.4\%$ was achieved by microparticles transformed from spray-dried microparticles using 2% mannitol(w/v)/LB Agar as feed solution. It is comparable to the highest FPF reported in the literature using lactose and spray-dried mannitol as carriers.

However mannitol/LB Agar (commercial) microparticles exhibit a poor stability of 3 months. A new LB Agar (lab-made) with increased percentage of agar was prepared as a stabilizer of mannitol/LB Agar (commercial) microparticles by mixing agar with commercial LB Agar. XRD indicates that the α -mannitol present in the transformed

mannitol/LB Agar (lab-made) microparticles was stable during storage. SEM indicates that the small mannitol crystals in the transformed microparticles join into each other and self-assembled into nanorods. The transformed microparticles were able to maintain the rough-surface and spherical shape for 21 months. It is substantially longer than that of the mannitol/LB Agar (commercial) microparticles. The resulting microparticles generated a FPF of $31.1 \pm 3.4\%$, which is comparable to the highest aerosolization performance reported in other literatures. FPF values are stable during a storage period of 21 months.

Based on the above mentioned investigation, novel pollen-shape carrier particles with needle-like rods using mannitol as material, LB Agar (lab-made) as a stabilizer were synthesized by polymorphic transformation and self-assembly method. The effect of LB Agar composition to surface morphology of the resulting particles was studied. The results show that a 50 - 66% percentage of agar in LB Agar offers a rough spherical shape with a few crevices on the particle surface. A 77 - 89% percentage of agar in LB Agar provides a pollen shape with needle-like rods about 0.2 - 3 μm long. A 92 - 94% percentage of agar in LB Agar offers a rod-covered surface with a rod size below 2 μm . SEM images illustrate that during storage, mannitol molecular self-assembled into needle rods on the surface of mannitol/LB Agar (lab-made) microparticles with a 77 - 89% percentage of agar in LB Agar. As the needle rods growing the particle surface roughness increased. After the resulting particles stored for 10 weeks, pollen-shape particles with the highest surface roughness were obtained. XRD indicates δ -mannitol was transformed continuously to α -mannitol during storage. As the surface roughness increasing, FPF of resulting particles increased. After a 10-week storage, a FPF of $32.7 \pm 2.0\%$ is obtained

which is the highest drug delivery performance reported in literatures using mannitol and lactose as carrier particles.

7.2 Future Work

7.2.1 Study of Relation between Carrier Size and Drug Delivery Performance

Both large and small size carrier particles were reported have high drug delivery performance. The conflicting result is possibly because the carrier size is not adjusted independently. Thus it is important to create a method to adjusted particle size independently and study the true relation between carrier size and FPF. Spherical mannitol microparticles with different size ranges can be prepared using spray-dryer having different size nozzles. Pollen-shape mannitol/LB Agar microparticles having similar surface roughness, crystallinity and different size ranges can be obtained by polymorphic transformation and self-assembly methods. The relation between particle size and drug delivery performance can be revealed.

7.2.2 Study of Relation between Carrier Shape and Drug Delivery Performance

Similar to carrier size, there is conflicting result on relation between elongation ratio and FPF. Thus it is important to create a method to adjust the particle shape independently and study the true relation between carrier shape and drug delivery performance. Spherical and elongated mannitol/LB Agar microparticles with similar size ranges can be prepared using spray-drying and freeze-drying methods. The roughness-controlled mannitol microparticles with different shapes could be obtained by polymorphic transformation and self-assembly methods. Thus carrier particles having similar surface roughness, crystallinity, size ranges and different shapes could be

obtained. The relation between particle shape and drug delivery performance would be revealed.

7.2.3 Animal test for mannitol/LB Agar microcarriers

It was reported that inhaled nanoparticles may have side effect such as acute lung injury (ALI) in vivo or worsen the progression of ALI in animal models. In the future, mannitol/LB Agar microcarriers would be prepared with proper size range, shape, surface roughness and good stability. Animal test will be carried out on such mannitol/LB Agar microcarriers.

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