# The Formulation Development of Suspended Budesonide Pressurized Metered Dose Inhaler

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| **ชื่อวิทยานิพนธ์** | การพัฒนาสูตรตำรับยาสูดอัดไอกำหนดขนาดบูดีโซไนด์ชนิดแขวนลอย |
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## บทคัดย่อ


บูดีโซไนด์จัดอยู่ในกลุ่มยากลูโคคอร์ติโคสเตียร์รอยด์สังเคราะห์ ซึ่งมีฤทธิ์ต้านการอักเสบ ในรูปแบบยาพ่นสูด จัดเป็นยาที่มีประสิทธิภาพในกา รรักษาโรค หืด โดยส่วนใหญ่นิยมใช้ในรูปแบบยาสูดอัดไอกำหนดขนาดเพื่อนำส่งยาไปยังทางเดินหายใจ การวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาสูตรตำรับยาสูดอัดไอกำหนดขนาดของบูดีโซไนด์ซึ่งเป็นระบบแขวนลอยในสารขับดั นชนิดใหม่ คือ ไฮโดรฟลูออโรแอลเคน (HFAs) เนื่องจากบูดีโซไน ด์ละลาย ได้น้อยในสารขับดัน ชนิดนี้ การทดลองแบบ แฟคทอเรียลดีไซน์ ถูกประยุกต์ใช้ ในการออกแบบสูตรตำรับเพื่อประเมินผลของปัจจัย 2 ปัจจัย คือ ความดันไอของระบบสารขับดัน และ ความเข้มข้นของสารเพิ่มความคงตัว ต่อลักษณะของสูตรตำรับ ซึ่งเตรียมสูตรตำรับทั้งหมด 24 สูตร จากการวิเคราะห์โดยวิธี analysis of variance (ANOVA) บ่งชี้ว่าความดันไอของระบบสารขับดันเป็นปัจจัยสำคัญที่มีผลต่อค่าปริมาณตัวยาสำคัญที่ถูกนำส่งผ่านวาล์ววัดขนาด ($p$<0.05) โดยสูตรตำรับที่ใช้ สารขับดันชนิด HFA 134a บริสุทธิ์และ HFA 227 บริสุทธิ์ มีค่าปริมาณตัวยาสำคัญ ไม่อยู่ใน ช่วงเป้าหมาย (80-120%) ในขณะที่ปริมาณตัวยาสำคัญมีค่าใ กล้เคียงช่วงที่ยอมรับได้เมื่อใช้สารขับดันผสมระหว่าง HFA 134a และ HFA 227 ในอัตราส่วน 70 ต่อ 30 โดยน้ำหนัก ทั้งนี้ เนื่องจากสารขับดัน

ผสมของ HFA ให้ความดันไอที่เหมาะสมและความหนาแน่นของระบบสารขับดัน ใกล้เคียงกับความหนาแน่นของอนุภาคยาบูดีโซไนด์ ดังนั้น ในการศึกษาคุณสมบัติทางแอโรโซลจึงเลือกทดสอบเพียง 8 สูตรตำรับที่ใช้สารขับดันผสม จากผลการทดสอบโดย twin-stage liquid impinger (TSI) ได้ค่า fine particle fraction (FPF) อยู่ในช่วง 32-38% และค่า mass median aerodynamic diameter (MMAD) ของทุกสูตรตำรับมีค่าประมาณ 3 ไมโครเมตร เมื่อทดสอบด้วย Andersen cascade impactor (ACI) นอกจากนี้ พบว่า สูตรตำรับที่ใช้สารเพิ่มความคงตัวในความเข้มข้นที่แตกต่างกัน มีค่า FPF และ MMAD ไม่แตกต่างกันทางสถิติ ($p$>0.05) สำหรับการประเมินความคงตัวทางกายภาพ พบว่า สูตรตำรับที่ใช้สารขับดันผสม มีความคงตัวทางกายภาพที่ดี โดย ทำนายได้จากความสามารถในการกระจายตัวได้ใหม่ของอนุภาคยาบูดีโซไนด์ในสารขับดันเหลวหลังจากเก็บผลิตภัณฑ์ไว้เป็นระยะเวลา 3 เดือน และระบบแขวนลอยสามารถคงความเป็นเนื้อเดียวกันได้นานมากกว่า 20 นาที

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# Abstract

Budesonide is classified as a synthetic glucocorticosteroid with mainly anti-inflammatory activity. Inhaled budesonide is an effective medication used in persistent asthma, and the most widely used device for delivering budesonide to the respiratory tract is pressurized metered dose inhaler (pMDI). The aim of this study was to develop a pMDI of budesonide as suspension-based formulation with the new propellants, hydrofluoroalkanes (HFAs), since they were found to be poor solvent for budesonide. The factorial design was applied to investigate the effects of two factors (vapor pressure of propellant system and concentration of stabilizing agents) on formulation performances. The 24 designed formulations of budesonide suspension-based pMDI were prepared. The analysis of variance (ANOVA) results indicate that the vapor pressure of propellant system is important factor affecting the content of active ingredient ($p<0.05$). The formulations containing both pure HFA 134a and pure HFA 227 gave budesonide contents that far from the target doses (80-120%), while the contents of active ingredient were close to the acceptable range when those two propellants were used as a mixture (HFA 134a and HFA 227 in the weight ratio of 70:30). This may due to the fact that the HFA mixture provides suitable vapor pressure, and the density of propellant blend match with the density of micronized

budesonide. Consequently, the 8 formulations containing HFA mixture were chosen for aerosol property assessments. The results reveal that the fine particle fraction (FPF) measured by twin-stage liquid impinger (TSI) ranged between 32-38%, and the mass median aerodynamic diameter (MMAD) obtained from Andersen cascade impactor (ACI) were around 3 μm for all formulations. It was observed that 8 formulations containing different concentration of stabilizing agents were not significantly different in FPF and MMAD ($p$>0.05). Further studies of physical stability testing indicate that the formulations containing HFA mixture had good physical stability which was predicted by the ability to redisperse of micronized budesonide in liquefied propellant after 3 month storage, and the homogeneous suspension which was maintained over 20 min.

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# ABBRIEVIATIONS AND SYSMBOLS

| | | |
|---|---|---|
| ∇ | = | Alpha |
| η | = | Beta |
| ° C | = | Degree Celsius |
| ° K | = | Degree Kelvin |
| µg | = | Microgram (s) |
| µl | = | Microliter (s) |
| µm | = | Micrometer (s) |
| % | = | Percentage |
| %RSD | = | Percentage relative standard deviation |
| 3-D | = | Three dimensions |
| ACI | = | Andersen cascade impactor |
| ANOVA | = | Analysis of variance |
| A.R. | = | Analytical grade |
| BP | = | British Pharmacopoeia |
| CFC | = | Chlorofluorocarbon |
| DPI | = | Dry powder inhaler |
| DSPC | = | Diestearoylphosphatidyl choline |
| EP | = | European Pharmacopoeia |
| $FEV_1$ | = | Force expiratory volume in one second |
| FPF | = | Fine particle fraction |
| GC | = | Cytoplasmic glucocorticoid receptor |

# **ABBRIEVIATIONS AND SYSMBOLS** (continued)

| | | |
|---|---|---|
| $g/cm^3$ | = | Gram/cubic centimeter |
| g/L | = | Gram/liter |
| GSD | = | Geometric standard deviation |
| HFA | = | Hydrofluoroalkane |
| HFA 134a | = | Tetrafluoroethane |
| HFA 227 | = | Heptafluoropropane |
| HP-η-CD | = | Hydroxy propyl-beta-cyclodextrin |
| HPLC | = | High performance liquid chromatography |
| ICH | = | International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use |
| kPa | = | Kilopascal |
| L/min | = | Liter/minute |
| LOD | = | Limit of detection |
| LOQ | = | Limit of quantitation |
| MI | = | Metal inlet |
| min | = | Minute (s) |
| ml | = | Milliliter (s) |
| ml/min | = | Milliliter/minute |
| mM | = | Millimolar |
| mm | = | Millimeter (s) |
| MMAD | = | Mass median aerodynamic diameter |

# **ABBRIEVIATIONS AND SYSMBOLS** (continued)

| | | |
|---|---|---|
| nm | = | Nanometer (s) |
| o.d. | = | Orifice diameter |
| PEG | = | Polyethylene glycol |
| PFOB | = | Perfluorooctyl bromide |
| pMDI | = | Pressurized metered dose inhaler |
| *R* | = | R configuration |
| $r^2$ | = | Correlation coefficient |
| *S* | = | S configuration |
| s | = | Second (s) |
| SCFM | = | Standard cubic feet per minute |
| SD | = | Standard deviation |
| SEM | = | Scanning electron microscopy |
| TEM | = | Transmission electron microscopy |
| TSI | = | Twin-stage liquid impinger |
| US | = | United State |
| USP-NF | = | United State Pharmacopoeia and National Formulary |
| UV | = | Ultraviolet |
| v/v | = | Volume/volume |
| w/w | = | Weight/weight |

# CHAPTER 1

# INTRODUCTION

## 1.1 Rationale of this study

Budesonide is a synthetic glucocorticosteroid with mainly anti-inflammatory activity (Meloche *et al.*, 2002; Sweetman, 2002). Although it is rapidly and almost completely absorbed from gastrointestinal tract, the bioavailability is low (about 10%) due to extensive first-pass metabolism in the liver. Furthermore, the metabolites have less than 1% of glucocorticoid activity compared to unchanged budesonide (Sweetman, 2002). Similar to other glucocorticosteroids, a long-term administration of systemic budesonide may cause many adverse effects, such as hypothalamic-pituitary-adrenal suppression, growth retardation and osteoporosis (Kelly and Kamada, 1999)

Inhaled glucocorticosteroids provide more advantageous than oral administration for the treatment of respiratory diseases. They are the most effective controller medications currently available as first-line therapy for persistent asthma (Global Initiative for Asthma, 2008; National Heart Lung and Blood Institute, 2007; O'Connell, 2003). By inhalation, drugs are directly delivered to the target site including the airway and lung. Thus, the high topical anti-inflammatory effect is achieved, while the systemic side effect is low (Thorsson and Geller, 2005; Global Initiative for Asthma, 2008).

Budesonide has been well documented in safety and efficacy for clinical use in both children and adult (O'Connell, 2002). It acts as an anti-inflammatory agent by binding to cytoplasmic glucocorticoid (GC) receptors that present on most cells throughout the body, and then the glucocorticosteroid-GC receptor complex alters the transcription of inflammatory mediators (O'Connell, 2003).

It has previously reported that lipophilic substitution at 16ζ and 17ζ positions on corticosteroid nucleus increases airway selectivity. The lipophilic acetal groups at the 16ζ and 17ζ positions of budesonide enhance the affinity of drug molecules to GC receptors, prolong airway retention, and increase systemic metabolism (O'Connell, 2003). Thus, budesonide exhibits higher topical potency than other glucocorticosteroids. Its topical skin blanching potency (relative to dexamethasone equal to 1) is 980, while beclomethasone dipropionate, flunisolide and triamcinolone acetonile have topical skin blanching potency of 600, 330 and 330, respectively (Kelly, 1998).

The pressurized metered dose inhaler (pMDI) is the most widely used device for delivering drugs to the respiratory tract. It consists of five key components including container, propellant, concentrated drug formulation, metering valve, and actuator. The concentrated drug formulation and propellant are filled into the container under the pressure. The metering valve is crimped onto the container. The patient uses the pMDI by fitting the container into the actuator where its nozzle connects to the metering valve. By actuation, the aerosol cloud is generated and emitted through the actuator nozzle with high velocity (Newman, 2005; Smyth, 2003).

Chlorofluorocarbons (CFC) has been previously used as propellant in pMDI because it has necessary properties, such as non-toxic, non-flammable, and sufficient vapor pressure (Newman, 2005; Smyth, 2003). However, its production was phased out under the Montreal protocol in 1987 due to it causes the depletion of atmospheric ozone layer in the stratosphere (Vastagh *et al.*, 2003; Grzelewska-Rzymowska *et al.*, 2003). Consequently, new propellants have been investigated for replacing of CFCs used in pMDI.

Two new hydrofluoroalkanes (HFAs), tetrafluoroethane (HFA 134a) and heptafluoropropane (HFA 227), are suitable alternative propellants for pMDI. They are also reached the requirement for using in pMDI as CFC, but they do not damage the ozone layer (Grzelewska-Rzymowska *et al.*, 2003; McDonald and Martin, 2000; Smyth, 2003). The physicochemical properties, such as vapor pressure, polarity, and density of HFA are significantly different from CFC (Smyth, 2003). The re-formulated pMDI with these new propellants is required to be equivalent in efficacy and safety profiles to marketed CFC products.

The re-formulations of pMDI with new propellant can be developed either in solution or suspension system. But, it was found that the HFAs are poor solvent for many anti-asthmatic drugs which are currently available (Smyth, 2003). The development of inhaled anti-asthmatic drugs as suspension formulation is an alternative way. Suspension system requires very low solubility of the drug in the formulation resulting in good chemical stability of the drug. Moreover, it is expected that the disagreeable tastes of the drug are reduced when formulate as the suspension compared to solution (Hickey, 2002; Smyth, 2003). However, the difficulty is to stabilize the dispersion system throughout the product life. The unstable suspension

results in unpredictable particle size and emitted dose (Smyth, 2003). Hence, other excipients such as stabilizing agent are required to solve this problem (Hickey, 2002).

CFC-free products of budesonide pMDI are available now. In Thailand, most of marketed products are solution-based formulation (MIMS, 2009). This may be from the reason that solution system is easy to obtain the uniformity requirement. However, the large amount of co-solvent usually ethanol is required to dissolve budesonide, leading to chemical instability of the drug substance in the formulation and disagreeable taste and odor of the formulation. Therefore, the development of suspension-based formulation is a promising way since budesonide has poor solubility in HFA propellant. In this study, the budesonide suspension-based pMDI formulations were prepared using factorial design to investigate the influences of propellant system and stabilizing agent on the formulation performances.

## 1.2 Objectives of the thesis

The aims of this study were as follows:

1. To formulate and prepare of pMDI containing suspended budesonide in HFA propellant
2. To assess the influences of propellant systems and stabilizing agents on formulation performances
3. To evaluate the aerosol properties of stable formulations
4. To evaluate the physical stability of prepared formulations

# CHAPTER 2

# LITERATURE REVIEWS

## 2.1 Characteristics of budesonide

Budesonide is categorized as glucocorticosteroid with mainly anti-inflammatory effect. It is widely used in the treatment of asthma by inhalation administration (Lacy *et al.*, 2002).

Figure 2.1 shows the chemical structure of budesonide with chemical formula of $C_{25}H_{34}O_6$ and molecular weight of 430.54. It is a white or almost white crystalline powder (BP, 2009; Krzek *et al.*, 2004). Budesonide is practically insoluble in water with solubility of 19.04 x $10^{-3}$ g/L, but it is more soluble in ethanol and ethyl acetate. The solubility of budesonide in ethanol and ethyl acetate are 14.94 and 2.49 g/L (at temperature of 298° K), respectively (Mota *et al.*, 2009).

Budesonide is an epimeric mixture which is categorized into epimer A (22*S*) and epimer B (22*R*) in the ratio of 1:1 (Meloche *et al.*, 2002). Structurally, it is the 16∇, 17∇-acetal of 16∇-hydroxyprednisolone with n-butyraldehyde. In the course of the acetal ring formation a new stereocenter is introduced leading to a mixture of 22*S* and 22*R* epimers (Ferraboschi *et al*., 2008).

Both epimer A (22*S*) and epimer B (22*R*) exhibit the similar pharmacological effects, but epimer B (22*R*) has been reported to have 2- to 3-fold of potency greater than epimer A (22*S*) based on anti-inflammatory activity. This is

possibly due to the configuration of epimer B (22*R*) has greater affinity for glucocorticoid receptor (Meloche *et al*., 2002).

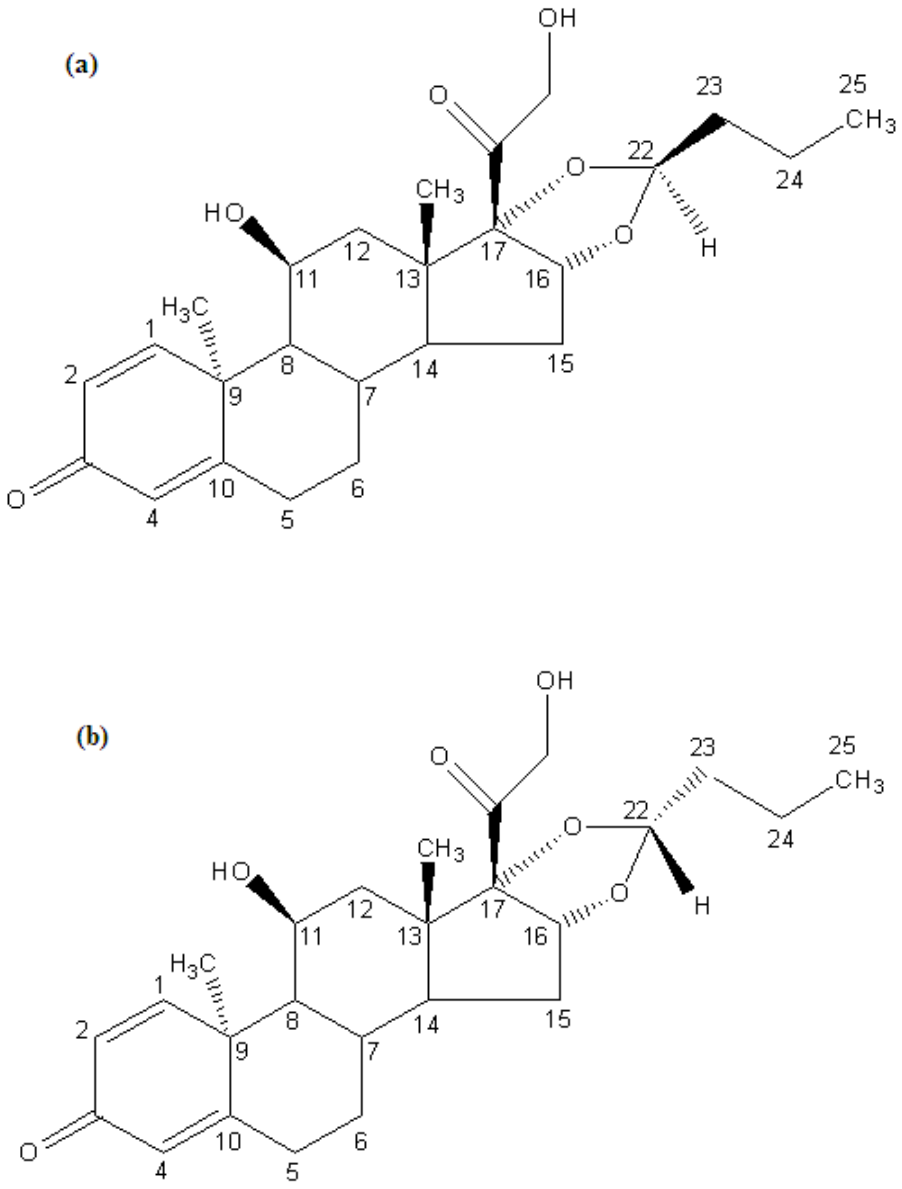


Figure 2.1 Structural formula of budesonide epimers: (a) epimer A (22*S*) and (b) epimer B (22*R*)

The stability study of budesonide has been employed under various stress conditions. The results showed that there was very little degradation of budesonide either by heat, oxidation, or acid hydrolysis. However, budesonide was degraded completely by alkali hydrolysis with heating temperature of 85° C (Naikwade and Bajaj, 2008).

## 2.2 Targeting of drugs to the respiratory tract

The respiratory system is known as the gas exchange system. The primary function of this system is to supply the body with oxygen and to get rid of carbon dioxide. Molecules of oxygen and carbon dioxide are passively exchanged between the air-filled spaces in the lung and blood by diffusion. This process occurs during breathing (Porth, 2007).

By functionally, the respiratory system can be divided into two parts. First is the conducting zone which consists of the nasal passages, mouth and pharynx, larynx, trachea, bronchi and bronchioles. This zone serves as the ways which air passes between the atmosphere and lung. Second, the respiratory zone, where the gas exchange takes place, consists of terminal respiratory bronchioles, alveolar ducts and alveolar sacs (Hickey and Thompson, 1992; Kleinstreuer *et al.*, 2008; Porth, 2007).

The model of the human airway according to Weibel (Figure 2.2) represents that each airway divides to form two smaller airways. As a result, the number of airways at each generation is double that of the previous generation. The model proposes that the number of airway generation is 24 in total, beginning with the trachea (generation 0) and terminating with alveolar sacs (generation 23). These

exhibit the important physical characteristics of the airway. Indeed, the branches become smaller with more numerous of the generation, and the surface area of the airway increases at each generation (Hickey and Thompson, 1992; Porth, 2007).

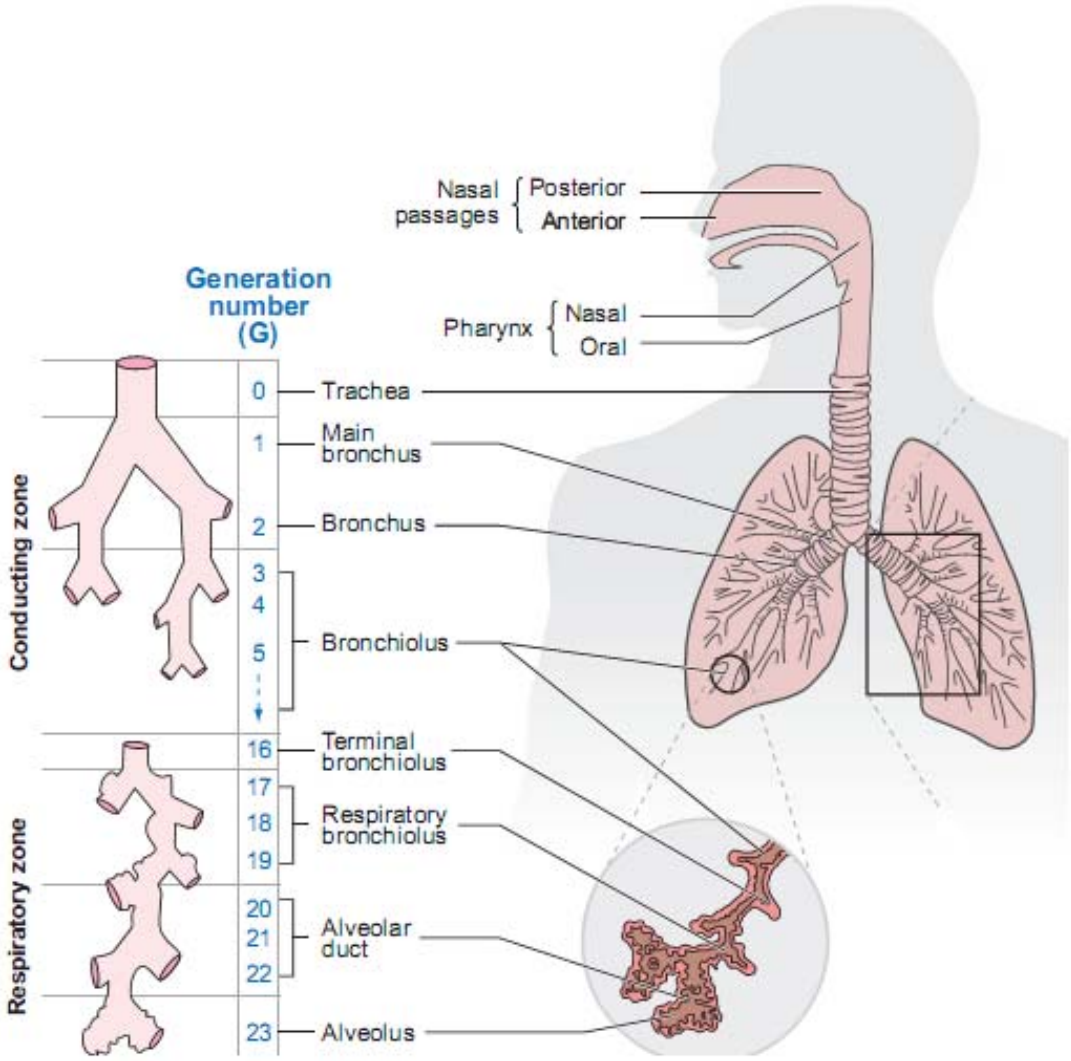


Figure 2.2 Schematics of human respiratory system (Kleinstreuer *et al.*, 2008)

Targeting of drugs to the respiratory tract is the concept of delivering the aerosolized drugs into the airway by inhalation. The drugs can be administered by either nasal inhalation or oral inhalation. The site of action can be proposed by the deposition of drugs into the airway. If the local action is expected, the drugs should be designed to deposit on the bronchial tree. But, the drugs must be penetrated to the deep lung (alveolus) if systemic effects are intended (Rau, 2002).

The aerosol delivery is a non-invasive method of drug administration which exhibits many advantages over other administration routes. First, the dose delivered via inhalation is allowed to lower than those for systemic treatment due to the drugs are localized to the target site. Consequently, the systemic side effects are reduced and less severe. Second, the potential benefit of inhalation route is rapid onset of drug action comparing to oral routes. The inhalation of short acting $\eta_2$-agonists provides the most useful in management of acute asthma. The bronchodilatory effect is achieved within 5-15 minutes after inhalation. Furthermore, the respiratory tract presents the appropriated characteristics for drug absorption. These are large lung surface area with excellent blood perfusion, large capacity for solute exchange, and a thin epithelium of alveolar region (Kelly and Kamada, 1999; Kleinstreuer *et al.*, 2008; National Asthma Council Australia, 2006; Rau, 2002)

The efficiency of asthma drug delivered to the lung via current inhaler device is varied in the range of 10-30% for adult users and 3-5% for children (Kleinstreuer *et al.*, 2008). These are depended on physicochemical properties of drug particles, the delivery devices, and the physiology and anatomy of the respiratory tract (Hickey, 2002).

## 2.3 Components of pressurized metered dose inhaler (pMDI)

The delivery of medications to the respiratory tracts is necessary to use the special devices which are able to generate the aerosols. The appropriate aerodynamic diameters of therapeutic aerosols are ranged between 1 to 5 µm because they can reach to the lower airways. The larger particles, 5 to 10 µm in size, are deposited in the trachea and large bronchi. Whilst the particles which are smaller than 0.5 µm do not deposit in the airway, they act as gas and are exhaled (Hickey, 2002; Kelly and Kamada, 1999).

The pMDI is the most widely used device for delivering anti-asthmatic drugs. This is due to its favorable feature, such as easy handling, high reliability, accurate metering dose, and less expensive (Ganderton *et al.*, 2003; Grzelewska-Rzymowska *et al.*, 2003; Malcolmson and Embleton, 1998).

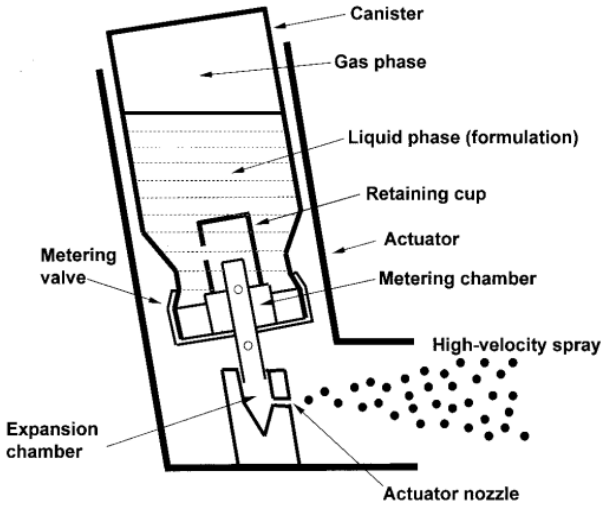


Figure 2.3 The key component parts of the pressurized metered dose inhaler (Newman, 2005)

The key components of pMDI are concentrated drug formulation, propellant, metering valve, actuator, and container (Figure 2.3). All play role in the formation of aerosol plume and in determining amount of drug to the lung (Grzelewska-Rzymowska *et al.*, 2003; Smyth, 2003).

### 2.3.1 Concentrated drug formulations

The pMDI formulation can be developed into two types either as suspension or as solution systems. To select of which the physicochemical properties of the active ingredient, such as solubility, physical/chemical stability, and disagreeable taste must be considered. At present, various anti-asthmatic drugs are available as CFC-free pMDIs, and some commercial products in Thailand are shown in Table 2.1.

Table 2.1 Commercial products of CFC-free pressurized metered dose inhalers and their composition (MIMS, 2009)

| **Therapeutic group/ Brand name** | **Active ingredient** | **Dosage** | **Formulation type** |
|---|---|---|---|
| *Bronchodilators* | | | |
| Aerodual | Ipratropium Br | 20 µg/dose | Solution |
| | Fenoterol HBr | 50 µg/dose | |
| Aeromol | Salbutamol sulfate | 100 µg/dose | Solution |
| Asthalin | Salbutamol sulfate | 100 µg/dose | Suspension |
| Salbutamol Inhalation-CFC-free | Salbutamol | 100 µg/dose | Solution |

Table 2.1 Commercial products of CFC-free pressurized metered dose inhalers and their composition (continued)

| **Therapeutic group/ Brand name** | **Active ingredient** | **Dosage** | **Formulation type** |
|---|---|---|---|
| *Corticosteroids* | | | |
| Aeronide | Budesonide | 200 µg/dose | Solution |
| Budecort-100 HFA inhaler | Budesonide | 100 µg/dose | Suspension |
| Budecort-200 HFA inhaler | Budesonide | 200 µg/dose | Suspension |
| Budesonide inhalation | Budesonide | 200 µg/dose | Solution |
| CFC-free | | | |
| Flixotide | Fluticasone propionate | 125 µg/dose | Solution |
| | | 250 µg/dose | |
| Qvar | Beclomethasone dipropionate | 50 µg/dose | Solution |
| | | 100 µg/dose | |

#### 2.3.1.1 Solution-based formulations

In solution formulations, the drug substance and other excipients are dissolved in propellant system. They provide advantages of improved dose uniformity when compared to suspensions. However, the major concerns with solution systems are the possible reduction in chemical stability and the sufficient solubility of drug that must be allowed the therapeutic doses to be delivered with a few actuations. In addition, the physical stability may also be a problem, in case of moisture ingress, the precipitation of drug may occur. This is due to the fact that the available propellant, hydrofluoroalkane (HFA), exhibits a high affinity to water.

The solubility of drug substance in formulation can be improved by modifying the solvency property of propellant system. This can be done by the use

of co-solvent (usually ethanol) or propellant mixture. Furthermore, the complexing excipients have been reported to increase drug solubility in propellants with minimizing the quantity of ethanol in the formulation (Atkins *et al.*, 1992; Newman, 2005).

#### 2.3.1.2 Suspension-based formulations

Suspension system requires very low solubility of drug in the formulation resulting in good chemical stability of drug substance. It is widely used for anti-asthmatic drugs due to those drugs have poor solubility in available HFA propellants. Thus, the suspension is formed by dispersing of micronized drug with the particle size of 2-5 μm in propellant. In addition, the delivery of greater amount of drug per actuation can be obtained from suspension rather than solution system. However, the physical stability is a critical problem of suspension formulations. The difficulty is to stabilize the dispersion throughout the products' life. If suspensions are unstable, the emitted dose and particle size characteristics may be unpredictable leading to poor therapy (Atkins *et al.*, 1992; Smyth, 2003). The factors affecting the stability of suspension-based pMDI are described as follow:

*1) Concentration and properties of active ingredient*

Drug concentration is also affected the stability of suspension system and the uniformity of emitted dose. The recommended concentrations are ranged between 0.1-10 %w/w. The higher drug amount may lead to valve clogging. Furthermore, it has been reported that the increase in particle size was observed at

high drug concentration. This is due to the decreasing of propellant fraction and efficiencies of atomization at the nozzle.

Drug properties such as particle size, lipophilicity, and crystal form also influence the stability of suspensions. Crystal form of drug, amorphous content, and stability of drug polymorphs are important parameters in minimizing drug solubility and crystal growth in suspension systems (Mistry and Gibson, 2003; Smyth, 2003).

The conventional size-reduction techniques, such as milling and micronization have been shown the effect on crystallinity of the material. The energy input during processing can induce disorder, defects or amorphous regions in the drug particles resulting in physicochemical instability of the formulations (Telko and Hickey, 2005; Zhao *et al.*, 2009).

*2) Density property of dispersed phase and continuous phase*

In suspension-based pMDI the density and viscosity of the propellant system can influence the physical stability of formulation according to Stokes' law. A difference in density between the drug particles and the propellants causes the drug particles either to rise to the liquid surface or to sink under the force of gravity. As a consequent, one approach to create the stable suspension is minimizing the difference in density between propellant system and suspended drug by using the propellant blend (Newman, 2005; Steckel and Wehle, 2004). The study of triamcinolone acetonide suspension pMDI was previously reported. According to this study, the physical stability and aerodynamic performance were optimized by varying the relative composition of the mixture of HFA 134a and HFA 227. The

results revealed that blending the propellant so that the density of the mixture approached that of the suspended drug improved dose uniformity. Moreover, the lower aerodynamic diameter and higher respirable fraction were achieved (McDonald and Martin, 2000).

The use of large porous or hollow particles is an alternative principle to minimize the difference in density between the particles and the medium. In the study of Dellamary *et al*. (2000), the microsphere of cromolyn sodium, albuterol sulfate, and formoterol fumarate were prepared by a spray-drying method. The scanning electron microscopy (SEM) studies and the transmission electron microscopy (TEM) studies exhibited the hollow porous particle morphology (Figure 2.4). They proposed that this characteristic allowed the propellant to permeate freely within the particles creating a form of suspension termed a homodispersion. This technology improved the physical stability of drugs suspended in HFA 134a propellant, content uniformity, and aerosolization efficiency with fine particle fraction of approximately 70%.

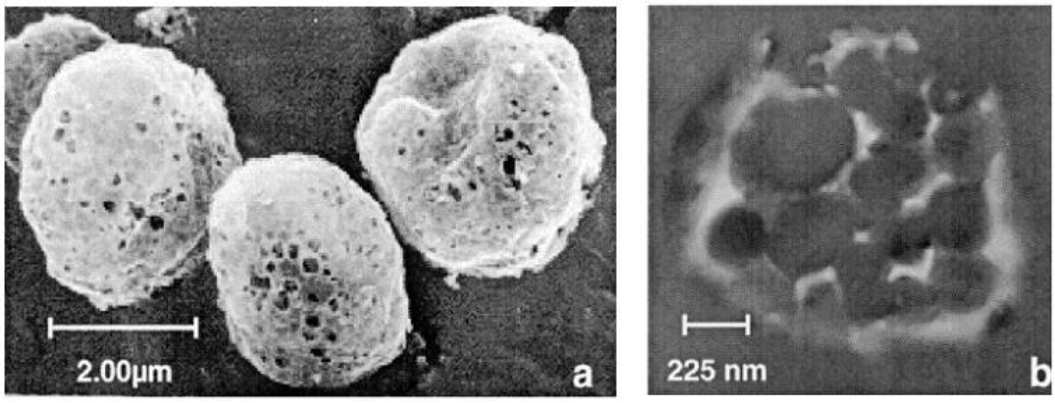


Figure 2.4 Electron microscopy images of (a) hollow porous cromolyn sodium by SEM, and (b) by TEM (Dellamary *et al*., 2000)

*3) Stabilizing agent*

The use of nonionic surfactants as stabilizing agent is one approach to prevent aggregation and rapid flocculation of suspension system. The dominant mechanism is steric effect interaction of hydrophobic surfactant chains. Indeed, the surfactant molecules adsorb on particle surfaces, and their chains are projected from the particles leading the repulsion between particles. In addition, the surfactants in formulations act as a valve lubricant for preventing valve clogging (Atkins *et al.*, 1992; Smyth, 2003).

The surfactants approved for using in pMDIs are soya lecithin, sorbitan trioleate, and oleic acid. These traditional surfactants have been used in CFC pMDIs in the concentrations ranging from 0.1% to 2%, but the concentrations are much reduced in HFA pMDIs because of their poor solubility in those new propellants. The solubility of these in HFA 134a and HFA 227 compared to CFC-11 are shown in Table 2.2. The new surfactants including polyethylene glycol (PEG), propoxylated PEG and perfluoroalkonic acids are under investigation. They exhibit more soluble in HFAs, but they are not available due to there is no sufficient data concerning safety and toxicity profiles in chronic respiratory administration (McDonald and Martin, 2000).

The use of co-solvent such as ethanol to increase the solubility of traditional surfactants in HFA formulations can overcome this problem. In the study of Tzou *et al*. (1997), suspension of salbutamol sulphate containing oleic acid without co-solvent exhibited rapid flocculation and settling, but the inclusion of ethanol improved the physical stability of the formulation. The stable suspension of salbutamol sulphate formed three-dimension flocculated network, and the particle size

measured by laser diffraction technique was maintained in the desired range of 2-3 μm over 12 months in both real time and accelerated testing. However, the level of co-solvent must be limited. Higher amount of co-solvent causes higher solubilization of drug substance, leading to chemical degradation and particle size increase upon storage (Govind *et al*., 2000).

Table 2.2 The apparent solubilities of surfactants in propellants (Adapted from Vervaet and Byron, 1999)

| **Surfactant** | **Apparent solubility (%w/w)** | | |
|---|---|---|---|
| | **CFC 11** | **HFA 134a** | **HFA 227** |
| Oleic acid | ∞ | <0.02 | <0.02 |
| Sorbitan trioleate | ∞ | <0.02 | <0.01 |
| Lecithin | ≈ 22.7 | <0.01 | <0.01 |
| Proproxylated PEG | ∞ | 3.6 | 1.5-15.3[a] |
| | | | 32.0-60.3[a] |
| Sorbitan monooleate | ∞ | <0.01 | <0.01 |
| Tween 80 | ≈0.1 | <0.03 | 0-10.0[a] |
| | | | 25.0-89.8[a] |
| PEG 300 | <0.01 | ≈4.0 | 1.5-4.3[a] |
| | | | 16.1-100[a] |

[a] Existed as one clear phase only in this concentration range
∞ means appeared soluble in all proportion
≈ means approximately equal to

### 2.3.2 Propellant

The propellant system is the main ingredient in pMDI formulations. It serves as a solvent or dispersion medium for drug substance and other excipients in formulations. Moreover, it is the energy source for generating the aerosol cloud during actuation (Steckel and Wehle, 2004). The propellants used in pMDIs are liquefied compressed gases that are in the gaseous phase at atmospheric pressure, but form liquids when compressed. They are required to be nontoxic, non-flammable, compatible with drug formulation, and to have appropriate boiling point and density. In addition, the vapor pressure must be constant throughout the product's life to ensure consistent dosing (Newman, 2005).

CFCs meet all requirements. The dichlorodifluoromethane (CFC-12), the highly volatile propellant, is used as a major component, and either trichlorofluoromethane (CFC-11) or dichlorotetrafluoroethane (CFC-114) which has higher boiling points is used to modify the vapor pressure (Newman, 2005). However, the production of CFCs was eliminated under the Montreal protocol (1987) due to the depletion of atmospheric ozone layer in the stratosphere. Consequently, pMDI was developed and reformulated as CFC-free formulations (Vastagh *et al*., 2003).

Two new hydrofluoroalkanes (HFAs), tetrafluoroethane (HFA 134a) and heptafluoropropane (HFA 227), are suitable alternative propellants for pMDI. They are also reached the requirement for use in pMDI as CFCs, but they do not damage the ozone layer (Grzelewska-Rzymowska *et al.*, 2003; McDonald and Martin, 2000; Smyth, 2003). However, the physicochemical properties of HFAs are

significantly different from CFCs as shown in Table 2.3. This may impact the pMDI designs and formulations.

Table 2.3 Physicochemical properties of pMDI propellants (Adapted from Smyth, 2003)

| Property | CFC-11 | CFC-12 | CFC-114 | HFA 134a | HFA 227 |
|---|---|---|---|---|---|
| Boiling point ($^{o}$C) | 24 | -30 | 4 | -26 | -16 |
| Vapor pressure (kPa) | 89 | 566 | 182 | 572 | 390 |
| Dielectric constant | 2.3 | 2.1 | 2.2 | 9.5 | 4.1 |
| Dipole moment | 0.45 | 0.51 | 0.58 | 2.1 | 1.2 |
| Density (g/cm$^3$) | 1.49 | 1.33 | 1.47 | 1.23 | 1.42 |

Figure 2.5 shows the structures of common CFCs and HFAs used in pMDIs. The physicochemical properties of each propellant relate to its chemical structure. Both HFA 134a and HFA 227 exhibit high vapor pressures and low boiling points. These properties cause by the strong electronegative repulsive interactions between HFA molecules. The dielectric constants and dipole moments reflect the polar natures of each propellant. According to these values, HFAs are relatively polar than CFCs (Smyth, 2003).

Many pMDI formulations use propellant blends or low volatility excipients to modify the characteristics of propellant systems. The mixtures of HFA 134a/ethanol, HFA 227/ethanol, and HFA 134a/HFA227 have been studied, and reported that these three miscible components may provide appropriate densities and vapor pressures (Newman, 2005; Smyth, 2003).

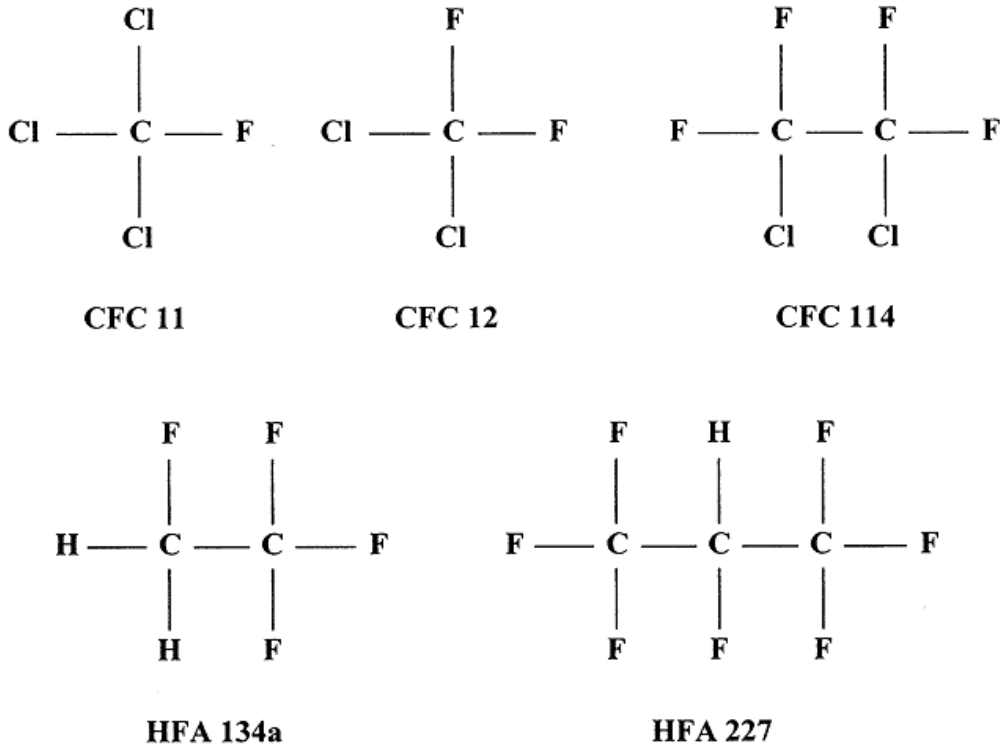


Figure 2.5 Chemical structures of CFC and HFA propellants
(Adapted from Vervaet and Byron, 1999)

### 2.3.3 Container

The pMDI container must be able to withstand the high pressure generated by the propellant, and must be made of inert materials. Unlike glass, aluminum container is now preferred because it is lighter, more compact, less fragile, and light proof. Coatings on the internal container surfaces may be useful to prevent drug adsorption, corrosion of the aluminium, and drug degradation. Common coatings include epoxy resins, anodized aluminium, epoxy-phenol, or perfluoroalkoxyalkane (Newman, 2005; Smyth, 2003).

### 2.3.4 Metering valve

The metering valve is a potential component for a good reproducible emitted dose. The function of metering valve is to deliver the metered volume of liquid formulation which is typically ranged between 25 and 100 σl. The metering valves are designed for use in the inverted position. They also form the seal upon the containers to prevent the loss of the pressurized contents. By inverted position, the valve is filled under gravity. Then only the metered content is discharged under vapor pressure of propellant during actuation (Atkins *et al.*, 1992; Newman, 2005; Smyth, 2003).

The valves must be constructed from a variety of inert materials to ensure the compatibility of the formulation with the valve components. The elastomer seals and the gaskets are important components of the metering valve. They form the barrier to the external environment, and they prevent the leakage of the product. The solvency properties of the propellant can affect the degree of swelling of valve elastomer. If elastomeric seals swell, it will loss the barrier function (Atkins *et al.*, 1992; McDonald and Martin, 2000).

### 2.3.5 Actuator

The actuator of a pMDI is generally made from polyethylene or polypropylene materials. The design of actuator is important for the production of appropriate aerosols including the particle size of the droplets and the characteristics of the aerosol plume emitted from a pMDI (Atkins *et al.*, 1992; Newman, 2005;

Smyth, 2003). The reduction of actuator orifice diameter has been shown to reduce the particle size and alter the fine particle fraction (FPF) of pMDIs. In the study of Brambilla *et al*. (1999), the effect of actuator orifice diameter on aerosol performance was investigated with the formulation of solution-based budesonide pMDI in HFA 134a. The orifice diameter of actuator was decreased from 0.42 to 0.25 mm. The results showed that the fine particle dose was significantly increased, whereas mass median aerodynamic diameter (MMAD) was slightly decreased with a reduction in the orifice diameter.

## 2.4 Performance testing of pMDIs

The transition of pMDIs from CFCs to HFAs system must be considered to re-evaluate inhaler performances. The re-formulated pMDIs are purposed to be therapeutic equivalence or improve in product performances compared with the original CFCs containing products. However, the measurement and assessment of inhaled drug delivery is very complicate due to the variation in drug delivery, such as drug formulations, delivery devices, administration skill, breathing pattern, and lung pathology.

The *in vitro* methods have been developed by understanding the mechanism of drug deposition within the airway, such as inertial impaction, sedimentation, and diffusion. As already mentioned, the particle or droplet size of aerosol is the most important parameter that reflects the lung deposition efficiency. The particle size data is used in the formulation development processes as the comparison parameter among developed formulations (Hickey, 1992; McDonald and

Martin, 2000). However, the drug deposition study in volunteers that provides the data of clinical responses, efficacy, and safety should be employed in parallel with *in vitro* size characterization. There are some evidences to demonstrate that the *in vitro* results were significantly deviated from *in vivo* experiments. For example, Weda *et al*. (2002) studied on salbutamol dry powder formulations. The three formulations were prepared and measured of the aerodynamic particle size distribution and fine particle doses (FPDs) using four impactors (Glass impinger, Metal impinger, Multistage-liquid impinger and Andersen cascade impactor). The same formulations were administered to 12 asthmatics. The force expiratory volume in one second ($FEV_1$) was measured at baseline and 15 min after each dose (50 μg). The *in vitro* results of all impactors showed large differences between the FPDs of the three preparations, but the *in vivo* measurements did not show significant differences in efficacy of those preparations. The results were in agreement with other studies (Fishwick *et al.*, 2001; Melchor *et al.*, 1993). In addition, it has been reported that the less correlation between the *in vitro* and *in vivo* experiments was a result from the unrealistic inlet tubes or throats that are usually used in most cascade impaction and liquid impinger systems according to the Pharmacopoeia (BP, 2009; USP30-NF25, 2007). Therefore, the use of anatomic throats can improve *in vitro* and *in vivo* correlations. The human oral-throat cast has been investigated about the applicability of replacing the glass throat from a twin-stage liquid impinger (TSI). The results indicated that the replacement of the glass throat in the TSI by the human oral-throat cast lead to a change in deposition efficiency. The cast exhibited higher filter efficiency. It trapped more drug than the glass throat model with a dry powder inhaler (DPI) formulation that employed the large carrier (63-90 μm) (Srichana *et al.*, 2000).

## 2.5 *In vitro* measurement of aerosol properties

*In vitro* methods have been developed to measure the particle size and emitted dose characteristics of aerosol formulations. A wide variety of methods have been used in the size measurements of pharmaceutical aerosols. Thus, the particular diameter may be defined according to principle underlying each measurement. However, the particle or droplet size of aerosol is usually represents in the term of aerodynamic diameter that is defined as the diameter of a unit density sphere having the same settling velocity, generally in air, as the particle. Therefore, the aerodynamic behavior of the particle can be influenced by particle shape, density, and physical size (Hickey, 1992).

The most common aerodynamic parameter is the mass median aerodynamic diameter (MMAD), which is statistical measure of the aerodynamic size of aerosol and represents the diameter that divides the particle size distribution into two halves with respect to mass. MMAD is often measured by using inertial impaction techniques via the impactor devices, such as cascade impactor and multistage-liquid impinger. These methods have been adopted as pharmacopoeial standard for aerodynamic assessment of fine particle of inhalation dosage form (BP, 2009).

The distribution of aerosol particle size can be expressed in other terms described as follow:

1) Geometric standard deviation (GSD) is the quotient of the ratio (84% undersize or 16% oversize)/(50%size) or (50%size)/(16% undersize or 84% oversize) when the particle size in logarithmic

scale is plotted against the cumulative percent frequency on a probability scale. In practice, the GSD of 1 indicates a monodispersion of aerosol, however, and accepted limit is 1.2 (Martin, 1993).

2) Fine particle fraction (FPF) is the percentage of particles that are smaller than 5 μm in diameter. This value also terms respirable fraction which is predicted amount of drug reached the lower airway (Thorsson and Geller, 2005).

### 2.5.1 Andersen cascade impactor (ACI)

The Andersen cascade impactor or apparatus D according to British Pharmacopoeia (BP, 2009) is a commonly used impactor for pharmaceutical aerosol characterization. It consists of eight stages (stage 0-7) together with a final filter. These stages are preceded by a preseparator that removes large particles (>9.9 μm). The plates contain multi-hole which are different in number and size of each stage. The plates are clamped together and sealed with the O-ring. Beneath the plate is situated a stainless steel collection plate (80 mm in diameter). The nominal cut off diameters at the operated air flow of 28.3 L/min for stage 0-7 are 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7, and 0.4 μm, respectively (BP, 2009).

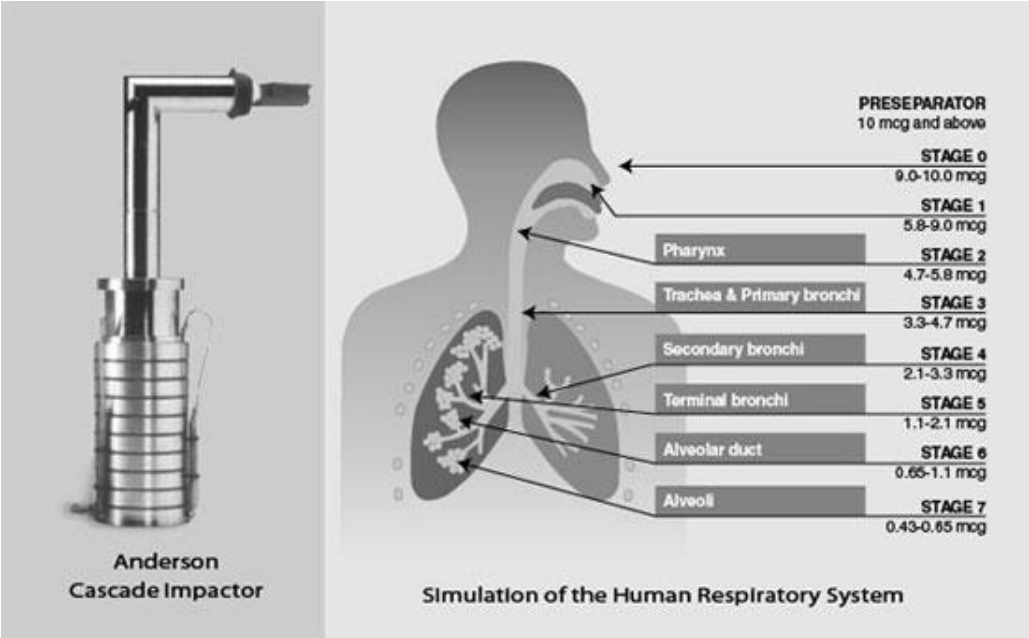


Figure 2.6 Apparatus D: Andersen cascade impactor

(http://www.asthmadevices.com/revolizer/pharmaceutics.html)

This device is designed to mimic the human respiratory tract and the method of collection the different size fractions of aerosol within the device bears similarities to the respiratory tract. In principle, when the air stream changes the direction of moving, the suspended particles continue to move in the original direction until they lose inertia. Then they will relax into the new direction of gas flow. On the other hand if their inertia overcome the drag forces, suspended particles tend to retain in the original direction and impact the surface of collection plate which is placed closer to the jets. The ACI discriminates aerosol particles according to their size. Large particles will impact on the collection surface at early stage, while small particles relax more quickly into a new direction of gas flow that let them pass around the collection plate and deposit on the lower stages.

### 2.5.2 Twin-stage liquid impinger (TSI)

Twin-stage liquid impinger has been adopted in BP as apparatus A (Figure 2.7 and Table 2.4). It consists two collection chambers, upper impingement chamber and lower impingement chamber, which are filled with a suitable solvent to collect the aerosols. The particles passing to the lower chamber are considered to be a respirable fraction.

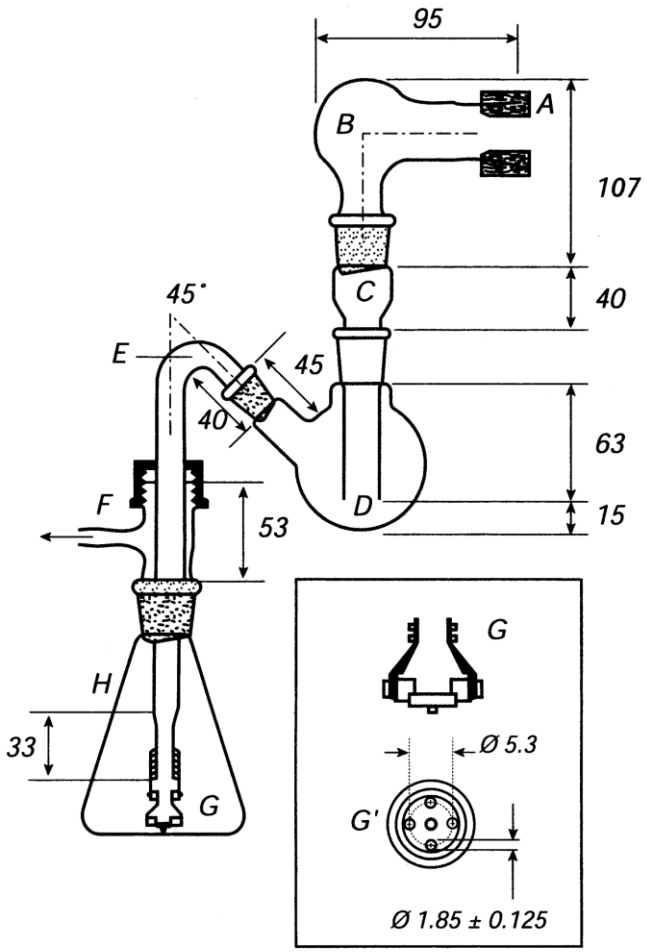


Figure 2.7 Apparatus A: Twin-stage liquid impinger (BP, 2009)

Table 2.4 Component specification for apparatus A (BP, 2009)

| Code | Item | Description | Dimensions* |
|---|---|---|---|
| A | Mouthpiece adaptor | Moulded rubber adapter for actuator mouthpiece. | |
| B | Throat | Modified round-bottomed flask: | 50 ml |
| | | – *ground-glass inlet socket* | 29/32 |
| | | – *ground-glass outlet cone* | 24/29 |
| C | Neck | Modified glass adapter: | |
| | | – *ground-glass inlet socket* | 24/29 |
| | | – *ground-glass outlet cone* | 24/29 |
| | | Lower outlet section of precision-bore glass tubing: | |
| | | – *bore diameter* | 14 |
| | | Selected bore light-wall glass tubing: | |
| | | – *external diameter* | 17 |
| D | Upper | Modified round-bottomed flask | 100 ml |
| | impingement | – *ground-glass inlet socket* | 24/29 |
| | chamber | – *ground-glass outlet cone* | 24/29 |
| E | Coupling tube | Medium-wall glass tubing: | |
| | | – *ground-glass cone* | 14/23 |
| | | Bent section and upper vertical section: | |
| | | – *external diameter* | 13 |
| | | Lower vertical section: | |
| | | – *external diameter* | 8 |
| F | Screwthread, | Plastic screw cap | 28/13 |
| | side-arm | Silicone rubber ring | 28/11 |
| | adaptor | PTFE washer | 28/11 |
| | | Glass screwthread: | |
| | | – *thread size* | 28 |
| | | Side-arm outlet to vacuum pump: | |
| | | – *minimum bore diameter* | 5 |
| G | Lower jet assembly | Modified polypropylene filter holder connected to lower vertical section of coupling tube by PTFE tubing. | see Figure 2.9.18.-1 |
| | | Acetal circular disc with the centres of four jets arranged on a projected circle of diameter 5.3 mm with an integral jet spacer peg: | 10 |
| | | – *peg diameter* | 2 |
| | | – *peg protrusion* | 2 |
| H | Lower | Conical flask | 250 ml |
| | impingement chamber | – *ground-glass inlet socket* | 24/29 |

* Dimensions in millimetres, unless otherwise stated.

## 2.6 Development of budesonide suspension-based pMDI

### 2.6.1 Preparation of medicinal aerosol formulations containing budesonide suspended in propellant mixtures

The preparation of medicinal aerosol formulations containing budesonide which were suitable for administration to the respiratory system has been described in US patent 6,039,932 (Govind *et al*., 2000). According to this invention, a pharmaceutical aerosol formulation consists of a suspension of particulate budesonide, a HFA propellant and optionally one or more of 1) one or more additional HFA propellants, 2) surfactant selected from oleic acid, sorbitan oleate, and 3) adjuvant having a Kauri-butanol value[1] of at least 10.

It was found that it is possible to achieve stable suspension of particulate budesonide by employing up to 3% of an adjuvant having a Kauri-butanol value greater than 10. The preferred adjuvant is ethanol, but other adjuvants such as isopropyl alcohol and polyethylene glycol (PEG) may be used. The level of adjuvant is selected to decrease the propensity for rapid formation of coarse flocs and for deposition of drug on manufacturing equipment and on the internal surfaces of the container closure system of the inhaler. However, the level is not so high as to cause significant solubilization of drug, leading to problem of chemical degradation and particle size increase on storage.

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[1] Kauri-butanol value is used as a measure of solvent power of hydrocarbon solvents. High Kauri-butanol values indicate relatively strong solvency (Drews, 1998)

In addition, it has been found that it is possible to match the density of budesonide using propellant mixture of HFAs. Suitable propellant mixtures comprise from 15-35% of HFA 227 and correspondingly 65-85% by weight of HFA 134a. The density matched mixtures of HFA propellants provide improved formulations of suspended budesonide compared with the use of single propellant.

Small amount of surfactant was recommended in this invention. The use of surfactant at the concentration ranging 0.0005-0.01% may provide improved properties including the prevention of drug particle adhering to surfaces and providing lubrication for valve components in contact with the formulations.

### 2.6.2 In situ-precipitation technique for preparation of budesonide pMDI suspension

The preparation of a physically stable micro-suspension of budesonide by an in situ-precipitation process has been described in the study of Steckel and Wehle (2004). The invention is based on the use of hydrophilic stabilizer. The preparation started with a real solution of drug substance and excipients which were prepared by mixing budesonide, PEG 300, hydroxypropyl-η-cyclodextrin (HP-η-CD), and dried ethanol. An aliquot was dispensed in an aerosol container. The 50 µl metering valve was immediately crimped onto the container and the HFA 227 propellant was then filled through the valve.

The addition of the non-solvent HFA 227 propellant led to the rapid precipitation of both drug substance and cyclodextrin resulting in the formation of a milky and homogeneous suspension. A stable flocculated dispersion with sediment

volume equal to the total volume of the liquid propellant was obtained. It was assumed that the hydrophilic PEG rapidly covered the surface of both particulate ingredients and thereby reduced the interfacial energy between the propellant and particles. The high sediment volume and the good physical stability of the dispersion was explained by the cyclodextrin acting as a kind of bulking agent where the drug and the cyclodextrin formed a network-like structure by non-covalent bonding, probably hydrogen bonds.

Table 2.5 The compositions of 200 μg/shot budesonide formulations (Steckel and Wehle, 2004)

| **Excipient** | **Product code** | | | | | |
|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI* |
| HP-η-CD (mg) | 83 | 83 | 26 | 68 | 26 | 68 |
| Ethanol (mg) | 300 | 880 | 880 | 1300 | 1000 | 1300 |
| PEG 300 (mg) | 1000 | 760 | 760 | 300 | 300 | 300 |
| Budesonide (mg) | 50 | 50 | 50 | 50 | 50 | 50 |
| HFA 227 (g) | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |

* Actuator type IN 5 with 0.5 mm o.d. was used.

The 6 formulations which were different in the concentrations of each ingredient (Table 2.5) were characterized according to dosing properties and aerosolization behavior. All formulations exhibited the excellent content uniformity through the container life of 200 doses. The influences of formulation composition on

FPF and MMAD are shown in Figure 2.8. The highest FPF resulted from compositions IV-VI with a value of approximately 16% for formulation IV and V and approximately 30% for formulation VI. An increase in the PEG 300 content and decreasing the ethanol content reduced the FPF to 6.4% as would be expected due to the increased level of the non-volatile compound. Furthermore, the influence of device on FPF was illustrated by formulation VI. When an actuator with a smaller orifice diameter was used, the higher FPF was received.

The MMAD values behaved inversely to FPF, formulation I exhibited MMAD of 7.8 µm that decreased to 4.0 µm for formulation V. Comparing formulation II and III indicated that the concentration of HP-η-CD slightly affected the FPF. However, it was observed that the composition with lower solid content gave higher FPF.

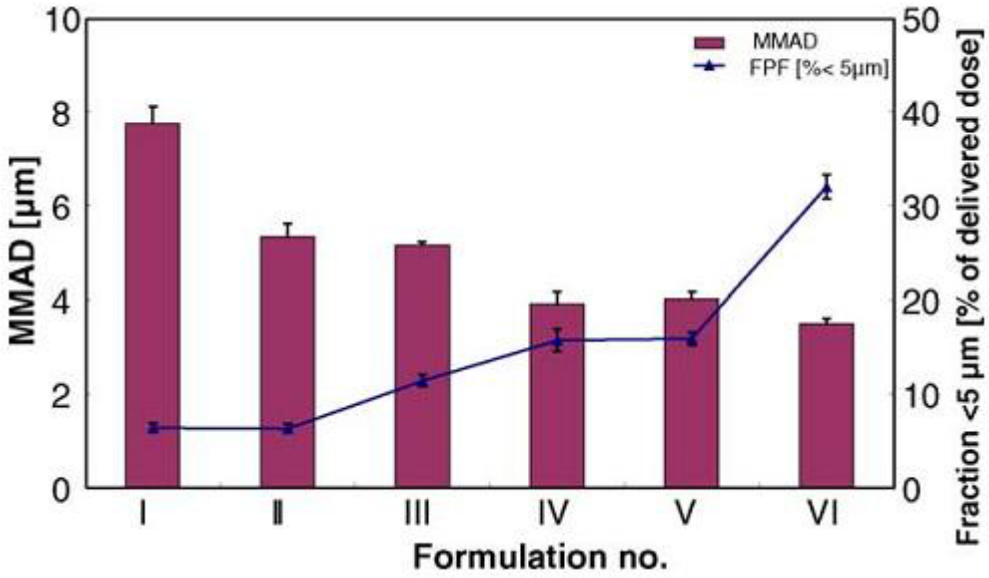



Figure 2.8 MMAD and FPF of the 6 investigated budesonide pMDIs (Steckel and Wehle, 2004)

### 2.6.3 Spray-dried budesonide microcrystals dispersed in HFA 134a

Tarara *et al*. (2004) studied on the particle engineering of budesonide for using in suspension-based pMDI. They prepared the lipid-coated budesonide microcrystals by spray-drying an emulsion-based feedstock. In brief, the emulsion-based feed stock was prepared by dispersing distearoylphosphatidylcholine (DSPC) and calcium chloride dihydrate in hot water with the mole ratio of 2:1, respectively. During mixing the perfluorooctyl bromide (PFOB) was added dropwise at a rate of 20-50 ml/min to create the PFOB-in-water emulsion. The weight ratio of PFOB/DSPC was 24.3. After that, the coarse emulsion was mixed and homogenized to create submicron emulsion droplets with median diameter of 0.19 μm measured by photosedimentation. The micronized budesonide was then added to the fine emulsion, and the complex dispersion was mixed and homogenized. Finally, the concentrated dispersion was diluted by roughly 4-fold with water creating the final feedstock concentration of 1.1 %w/w.

For spray-drying process, the multi-particulate dispersion was spray-dried using the conditions as follow: inlet temperature = 110° C, outlet temperature = 64° C, atomization pressure = 120 psi, pump flow rate = 31 ml/min, total gas flow = 110 standard cubic feet per minute (SCFM). The final compositions of the spray-dried powder contained 58.2 %w/w budesonide, 38.5 %w/w DSPC, and 3.3 %w/w $CaCl_2$.

The surface morphology of lipid-coated budesonide compared to neat micronized budesonide is shown in Figure 2.9. The spray-dried particles appeared to be spheroidal in shape with a rough surface. The geometric sizes were ranged between

1 and 3 σm. In contrast, neat micronized budesonide microcrystals were characterized by smooth plate-like crystals with a broad particle size distribution from tens of nanometers to a few micrometers. High agglomeration was observed. Indeed, the smaller microcrystals adhered to the surface of larger microcystals owing to their planar shape. However, the lipid-coated budesonide exhibited a little tendency to agglomerate because high degree of surface roughness could prevent close contact of particles within Van der Waals force. Moreover, the low surface energy of the hydrophobic phosphatidylcholine also contributed to decreasing interparticle attractive forces.

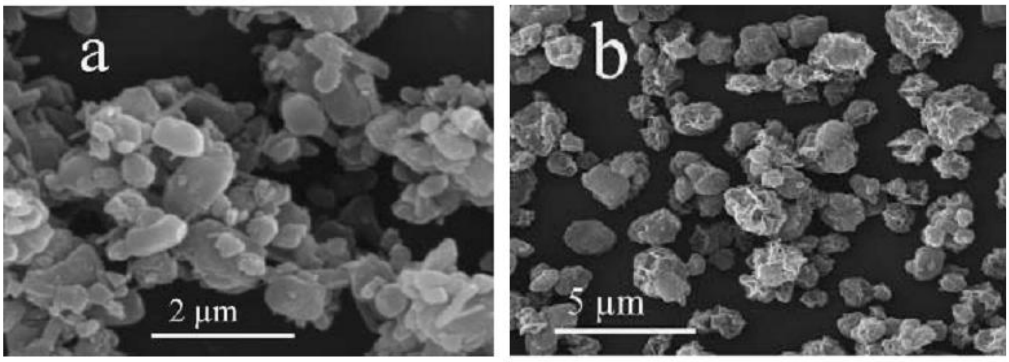



Figure 2.9 SEM photomicrographs of neat micronized budesonide microcrystals (a) and lipid-coated budesonide microcrystals (b) (Tarara *et al.*, 2004)

The particle size distributions measured by laser diffraction are shown in Figure 2.10. The lipid-coated budesonide exhibited the narrow particle size distribution with median geometric size of 1.7 µm and GSD of 1.6, while the neat budesonide microcrystals exhibited the median geometric size and GSD of 2.5 µm and 2.3, respectively.

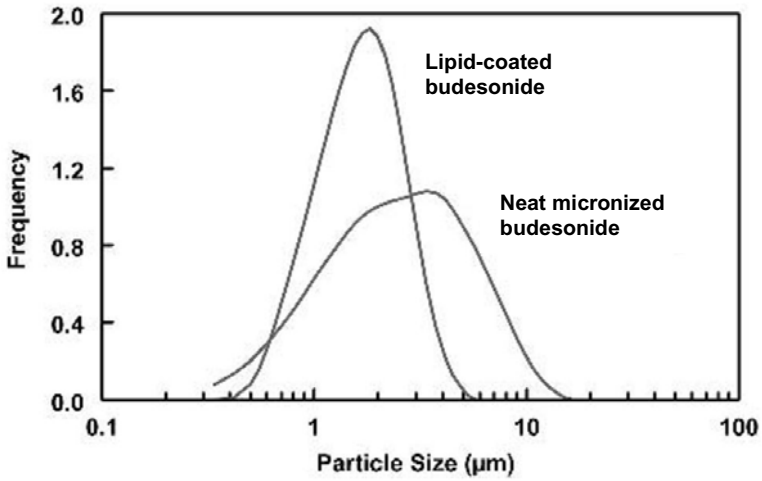



Figure 2.10 Particle size distribution determined by laser diffraction (Tarara *et al.*, 2004)

The suspension of lipid-coated budesonide microcrystals in HFA 134a propellant were prepared at the concentration of 0.82 %w/v which was corresponded to metered dose of 300 µg budesonide per shot. The stable suspension was formed with slow creaming time (>20 min). This translated into good uniformity in metered dose across the contents of inhaler (mean value of metered dose was 313.7 µg, RSD = 3.6%, n=4) and acceptable aerodynamic particle size distributions (MMAD = 3.2-3.4 σm).

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Materials

Acetonitrile A.R. (Lab-Scan Analytical Sciences, Bangkok, Thailand)

Methanol A.R. (RCI Lab-Scan limited, Bangkok, Thailand)

Ortho-phosphoric acid 85% (Merck, Danstadt, Germany)

Potassium dihydrogen orthophosphate (Ajax Finechem Pty Ltd, NSW, Australia)

Micronized budesonide (New Chem, Milano, Italy)

HFA 134a (ZEPHEX$^{TM}$ 227ea, INEOS Fluor Limited, Cheshire, United Kingdom)

HFA 227 (ZEPHEX$^{TM}$ 227ea, INEOS Fluor Limited, Cheshire, United Kingdom)

HFA 227/HFA 134a blend (ZEPHEX$^{TM}$ 227ea/134a blend, INEOS Fluor Limited, Cheshire, United Kingdom)

Oleic acid (Panreac, Barcelona, Spain)

Sorbitan trioleate (Span$^{®}$85, Fluka, Buchs, Switzerland)

Absolute ethanol (Lab-Scan Analytical Sciences, Bangkok, Thailand)

Polyamide membrane 0.45 µm (Sartorius stedim, Goettingen, Geramany)

## 3.2 Equipments

High performance liquid chromatography (HPLC) system consists of components as follow;

- Spectra System SCM 1000
- Spectra System Pump P2000
- Spectra System AS 3000 auto sampler
- Spectra System SN4000
- Spectra System UV 1000 detector
- Finigan Spectra System UV 6000 LP diode-array detector
- BDS Hypersil C18 column (150 x 4.6 mm id, 5 µm) (Thermo Scientific, United Kingdom)

Ultrasonic bath (Bandelin Sonorex, Berlin, Germany)

Vortex-2 Genie$^{TM}$ (Scientific Industries, New York, USA)

Twin-stage liquid impinger (Copley instrument, Nottingham, United Kingdom)

Andersen cascade impactor (Atlanta, Georgia, USA)

## 3.3 Experimental design

The three levels factorial design was applied to investigate the effects of two factors (vapor pressure of propellant system and concentration of stabilizing agent) on formulation performances. The three levels of vapor pressure were obtained from the different propellant systems which were HFA 134a, HFA 227, and HFA mixture (HFA 134a : HFA227 in the weight ratio of 70:30). Oleic acid or sorbitan

trioleate was selected and used as stabilizing agent. Three levels of concentration for oleic acid and sorbitan trioleate were studied in each formulation (0.01, 0.03, 0.05 % w/w and 0.003, 0.005, and 0.010 %w/w, respectively). The combination use of the two stabilizing agents was also investigated. The high level of oleic acid combined with low level of sorbitan trioleate, and the low level of oleic acid combined with high level of sorbitan trioleate. The code and composition of designed formulations are shown in Table 3.1. The content of active ingredient and aerosol properties of the pressurized metered dose inhaler formulations at 1 and 3 months storage were measured as a response.

## 3.4 Preparation of budesonide suspension-based pMDI

The micronized budesonide was first dispersed in dried ethanol (99.9%). The stabilizing agents were then added and mixed by Vortex-2 Genie$^{TM}$ to obtain the concentrated formulation. Each aliquot was pipetted into an aluminum canister. Then 50 µl metering valves were crimp-sealed onto the canisters and the canisters were filled with desired amount of liquefied propellant under the pressure through a crimped valve. The fill volume for each canister was 10 ml. Completed dispersion of micronized budesonide in propellant system was warranted by sonicating the canisters for 60 s in an ultrasonic bath. The concentration of budesonide was adjusted to 100 µg per actuation.

Table 3.1 Code and composition of 24 formulations from factorial design

| Code | Budesonide (%w/w) | Stabilizing agent (%w/w) | | Dried ethanol (%w/w) |
|---|---|---|---|---|
| | | Oleic acid | Sorbitan trioleate | |
| **HFA 134a (Vapor pressure 572 kPa)** | | | | |
| 134a-F1 | 0.15 | 0.01 | - | 1.0 |
| 134a-F2 | 0.15 | 0.03 | - | 1.0 |
| 134a-F3 | 0.15 | 0.05 | - | 1.0 |
| 134a-F4 | 0.15 | - | 0.003 | 1.0 |
| 134a-F5 | 0.15 | - | 0.005 | 1.0 |
| 134a-F6 | 0.15 | - | 0.010 | 1.0 |
| 134a-F7 | 0.15 | 0.01 | 0.010 | 1.0 |
| 134a-F8 | 0.15 | 0.05 | 0.003 | 1.0 |
| **HFA mixture (Vapor pressure 535 kPa)** | | | | |
| Mix-F1 | 0.15 | 0.01 | - | 1.0 |
| Mix-F2 | 0.15 | 0.03 | - | 1.0 |
| Mix-F3 | 0.15 | 0.05 | - | 1.0 |
| Mix-F4 | 0.15 | - | 0.003 | 1.0 |
| Mix-F5 | 0.15 | - | 0.005 | 1.0 |
| Mix-F6 | 0.15 | - | 0.010 | 1.0 |
| Mix-F7 | 0.15 | 0.01 | 0.010 | 1.0 |
| Mix-F8 | 0.15 | 0.05 | 0.003 | 1.0 |
| **HFA 227 (Vapor pressure 390 kPa)** | | | | |
| 227-F1 | 0.15 | 0.01 | - | 1.0 |
| 227-F2 | 0.15 | 0.03 | - | 1.0 |
| 227-F3 | 0.15 | 0.05 | - | 1.0 |
| 227-F4 | 0.15 | - | 0.003 | 1.0 |
| 227-F5 | 0.15 | - | 0.005 | 1.0 |
| 227-F6 | 0.15 | - | 0.010 | 1.0 |
| 227-F7 | 0.15 | 0.01 | 0.010 | 1.0 |
| 227-F8 | 0.15 | 0.05 | 0.003 | 1.0 |

*The concentration of budesonide is equivalent to 100 µg/ actuation

## 3.5 Validation of HPLC method

### 3.5.1 Chromatographic conditions

The mobile phase consists of 25 mM phosphate buffer, pH 3.2 and acetonitrile in the ratio of 65:35 (v/v). The mobile phase was set at a flow rate of 1.5 ml/min at ambient temperature. The UV detector was operated at 240 nm. The injection volume was 50 µl.

### 3.5.2 Preparation of stock solution and standard solution

The stock solution of budesonide was prepared by dissolving 10 mg of budesonide standard in 50 ml methanol. This solution was diluted with mobile phase as needed to prepare various concentrations of standard solution.

### 3.5.3 Specificity

Specificity is the ability to assess unequivocally of the analyte in the presence of other components. Typically these might include impurities, degradation products, and matrix components (ICH, 2005).

For HPLC system, the specificity was conducted by using diode array detector. The standard solution with the concentration of 1 µg/ml was analyzed. The detector was employed at the scanned wavelength ranging 200-380 nm. The contour

plot and 3-D chromatogram was performed and determined the degree of selectivity and peak purity of each epimer peak.

### 3.5.4 Linearity

The series of standard solution of budesonide were prepared by diluting the stock solution into 5 concentrations including 0.1, 0.5, 1, 3, and 5 µg/ml. The standard solutions were analyzed for the concentration of budesonide by injecting each standard solution into HPLC system in triplicates. Peak areas were plotted against theoretical concentrations of standard. The method of linear regression was used for data evaluation, and the linearity was expressed as a correlation coefficient ($r^2$).

### 3.5.5 Accuracy

The intra-day accuracy was performed by injecting three standard solutions of budesonide (1, 3, and 5 µg/ml) for five times on the same day, and inter-day accuracy was obtained from the injection of standard solutions once of each on four consecutive days. The accuracy of an analysis was determined by comparing the amount of drug measured by this analytical method and the theoretical amount. The data were presented as a percentage recovery.

### 3.5.6 Precision

The intra-day precision was determined by performing five repeated analyzes of three standard solutions (1, 3, and 5 µg/ml) on the same day. The inter-day precision was received from an analysis of the same set of standard solution for four consecutive days. The system precision was expressed as percentage relative standard deviation (%RSD). The results must be less than 2% RSD.

### 3.5.7 Determination of limit of detection and quantitation

The limit of detection (LOD) is the lowest amount of analyte in a sample which can detect but not necessarily quantitated as an exact value. The limit of quantitation (LOQ) is the lowest amount of analyte which can be quantitatively determined with suitable precision.

In this study the LOD and LOQ were determined by using an approach based on the standard deviation of the response and the slope. The LOD and LOQ may be expressed as equation 3.1 and equation 3.2, respectively.

$$LOD = 3.3\sigma / S \quad \text{-----} \quad (3.1)$$

$$LOQ = 10\sigma / S \quad \text{-----} \quad (3.2)$$

Where $\sigma$ = the standard deviation of the response

S = the slope of the calibration curve

To obtain the LOD and LOQ the series of standard solution of budesonide ranged 0.1-5 µg/ml were injected into HPLC system, in triplicates of each concentration. The calibration curves were performed. The S was obtained from the slope of regression line, and the σ was the standard deviation of y-intercepts of regression lines (ICH, 2005).

## 3.6 Content of active ingredient delivered by actuation of the valve

The 24 designed formulations were determined for the content of active ingredient delivered by actuation of the valve after 1 month and 3 months storage (25° C at ambient condition). The test was employed by using the stainless steel base plate specified in BP (2009) as a circular disc which has three legs and 1.5 mm in diameter of a central circular hole.

Before testing, the pressurized inhaler was shaken for 30 s, and the first two doses were discharged to waste. Then, the stainless steal base plate was placed in a suitable vessel and filled with the specified volume of methanol. The inhaler was discharged in inverted position under the surface of solvent. Ten delivered doses at the beginning (3-12), the middle (76-85), and the end (141-150) of the calculated number of dose were collected and analyzed the amount of active ingredient using HPLC. The result was calculated as the amount of active ingredient delivered from each actuation of the valve.

## 3.7 Assessment of fine particle fraction

After 1month and 3 months storage, the developed formulations were assessed for the fine particle fraction (FPF) by using the twin-stage liquid impinger (TSI), apparatus A, according to BP (2009). The experimental set up is shown in Figure 3.1.

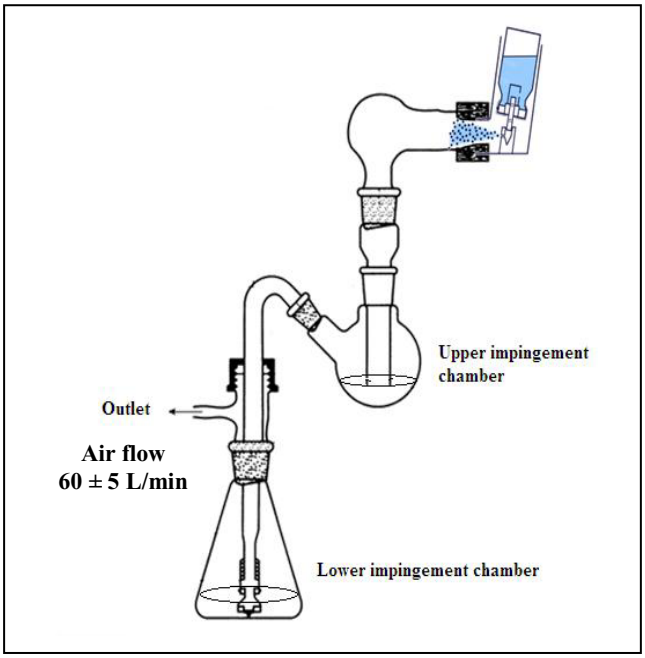



Figure 3.1 The experimental set up for fine particle assessment using twin-stage liquid impinger

The volume of 7 ml and 30 ml of the mobile phase were introduced into upper and lower impingement chambers of the apparatus, respectively. All component parts of the apparatus were assembled, and then connected the outlet to a suitable pump. The pressurized container was shaken for 5 s, and 5 doses were

discharged to waste. The pMDI was connected to the TSI using actuator adapter. Five consecutive doses were discharged into the impinger at the operation flow rate of 60±5 L/min. The drug deposited on the inner surface of the throat and neck was rinsed with the mobile phase into the upper impingement chamber. Whereas, the drug deposited in the inlet tube was rinsed into the lower impingement chamber. The amount of active drug collected in the upper and lower impingement chambers were analyzed by HPLC. The percentage of drug that reached the lower impingement chamber with the cut-off diameter of 6.4 μm was defined as %FPF of emitted dose. The measurements were performed three times for all formulations.

## 3.8 Assessment of aerodynamic particle size distribution

The measurement of aerodynamic particle size distribution was performed on an eight-stage Andersen cascade impactor (ACI) stated in BP (2009) as apparatus D. The set up of the apparatus for pMDI assessment is shown in Figure 3.2.

The formulations were assessed after 1 month and 3 months storage. The pressurized inhaler was shaken for 5 s and discharged one delivery to waste. The pMDI was connected to the induction port of ACI using an adaptor. Air was drawn through the apparatus by connecting with a vacuum pump and adjusted the flow to 28.3 L/min (±5 percent). Then, the inverted inhaler was discharged into the apparatus for 2 consecutive doses. The shaking of 5 s was required between each delivery. The induction port and stages were washed with the mobile phase. Each fraction was adjusted to specified volume and analyzed of drug amount by HPLC with UV detector.

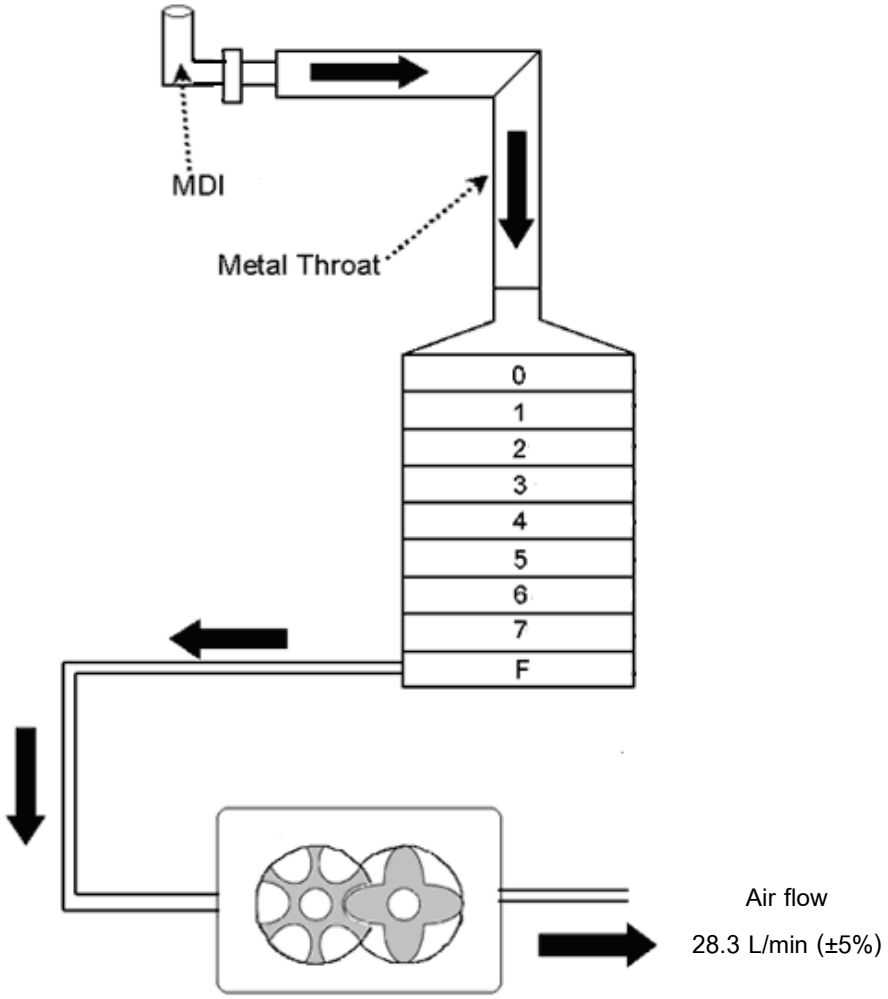



Figure 3.2 The experimental set up for aerodynamic particle size distribution mearurement using Andersen cascade impactor

The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated according to the method described elsewhere (Srichana *et al*., 2005). Briefly, the cumulative percentage of drug deposited on each stage was transformed to the Z-value and was plotted against cut-off diameter of each stage in logarithmic scale. The MMAD was obtained from the

particle diameter at Z-value of zero. The GSD was calculated from the equation 3.3. The measurements were performed three times for all formulations.

$$GSD = \sqrt{(\text{size at z of -1}) / (\text{size at z of 1})} \quad \text{----- (3.3)}$$

The FPF was calculated stated on emitted dose. It was defined as the percentage of drug that was deposited on stage 2-7. Thus, the FPF obtained from ACI reflected the amount of drug with aerodynamic diameter ranging 0.43-5.8 μm.

## 3.9 Physical stability assessment of budesonide suspension-based pMDI

The physical stability of suspension formulation is generally determined by visual examination for the appearance of sedimentation, creaming, and caking. But, the aluminum canister used in this study did not allow to visualize the containing formulation. Thus, the assessment of dose uniformity described as follow was used to predict the physical stability of the developed formulations at short time storage (3 months, 25° C at ambient condition).

### 3.9.1 Redispersibility of budesonide suspension-based pMDI

The assessment was carried out by allowing the pMDI formulations to be stored for a period of 3 months in order that complete sedimentation could take place. In process, the stainless steel base plate according to BP (2009) was placed in a

small vessel, and a volume of 25 ml of methanol was then added. The test container was handled in upright position and gentle twisted the container to the inverted position. The container was then put on the stainless steel base plate and pressed the valve through the circular hole of that plate to deliver a single dose. Each actuation sample was transferred to a volumetric flask and adjusted the volume to 50 ml. The process was repeated until nine inversions were obtained. The amount of budesonide was analyzed by injecting 50 µl of collected solution to HPLC system.

### 3.9.2 Sedimentation testing of budesonide suspension-based pMDI

This test was performed to assess the sedimentation of micronized budesonide in the continuous phase. The container was shaken about 30 s prior the test to complete the dispersion. The container was inverted and placed in a small vessel which was added 25 ml of methanol. The formulation was discharged through the circular hole of stainless steel base plate at 0, 5, 10, 15, and 20 min by maintaining the container in the vertical plane. The fraction collected at each time interval was transferred into a volumetric flask and adjusted the volume to 50 ml. Finally, each fraction was determined the amount of budesonide by injecting 50 µl of the sample solution to HPLC system. The measurements were performed three times for all formulations.

## 3.10 Statistical analysis

Data, when applicable, are presented as mean ± standard deviation (SD) from at least three samples. The linear regression analysis was performed by using Microsoft Excel Version-2007. All statistical comparisons were calculated using the SPSS software version 16.0. The analysis of variance (ANOVA) and paired-sample t-test were used in the comparison of data among formulations. A significance level of $p$-value < 0.05 was considered statistically significant.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Formulation design and preparation of budesonide suspension-based pMDI

The 24 formulations of budesonide suspension-based pMDI were prepared according to factorial design. The samples of finished products are shown in Figure 4.1.

Figure 4.1 Finished products of budesonide suspension-based pMDI formulation

The key components of pMDI including formulation, propellant, container, metering valve, and actuator play important roles in the formation of aerosol cloud (Newman, 2005). Although, the effects of container, metering valve, and actuator did not evaluate in this study, the compatibility of these component parts with the formulations was observed. The leakage of volatile components and loss of prime can occur when the valve components are incompatible with the formulation (McDonald and Martin, 2000; Smyth, 2003). In this study, it was observed that 240 containers of 24 designed formulations exhibited constant weight up to 3 months on storage, and the sticking valve did not occur. This indicates the compatibility between designed formulations and valve components.

## 4.2 Validation of HPLC method

Although budesonide has been widely used in the treatment of asthma, the monograph of budesonide only appears in the European Pharmacopoeia (EP). The official assay for this drug according to the $6^{th}$ edition of the EP (2007), which is incorporated in the BP 2009, is reversed phase HPLC method. The chromatographic procedure is carried out using a C18 column and mobile phase of acetonitrile : phosphate buffer solution, pH 3.2.

The ratio of acetonitrile and 25 mM phosphate buffer, pH 3.2 was adjusted to achieve a rapid assay with suitable retention time and acceptable resolution. The ratio of 35: 65 v/v of acetonitrile and 25 mM phosphate buffer, pH 3.2 provided the separated sharp peaks of epimer A and epimer B with the retention time of 10.20 and 9.36 min, respectively (Figure 4.2).

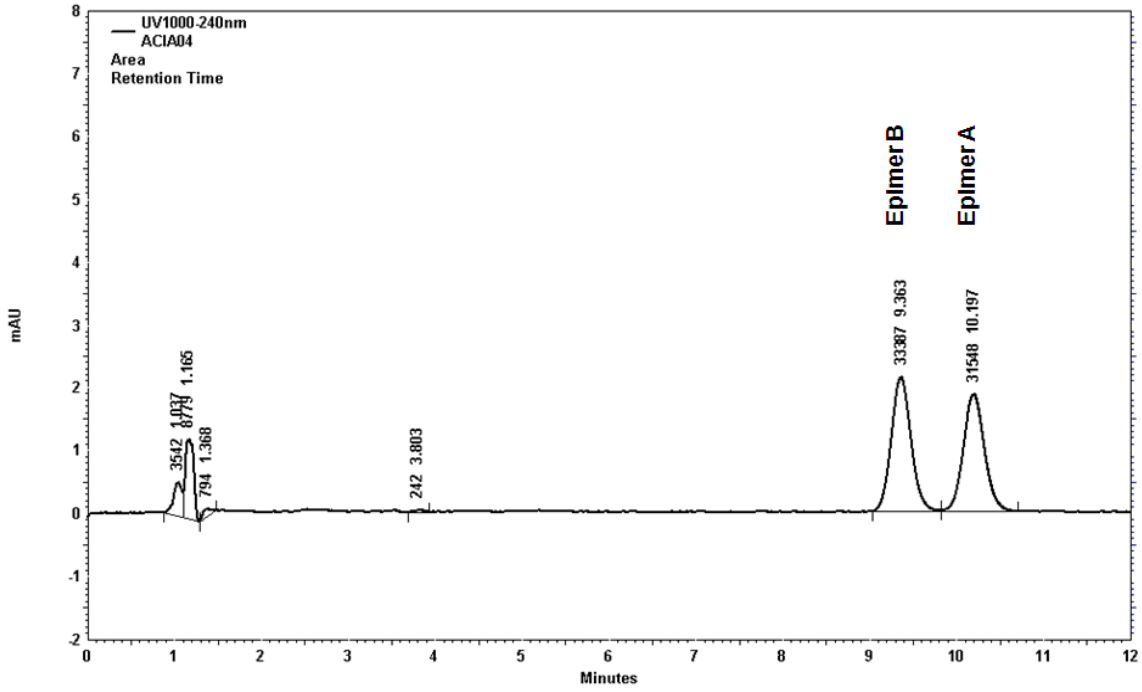



Figure 4.2 HPLC chromatogram of budesonide: first peak is epimer B and the second peak is epimer A

### 4.2.1 Specificity

The chromatographic condition was validated for its suitability to quantify budesonide. A diode-array detector was used to determine the specificity of the analytical method. The detector was employed to scan the wavelength ranging 200-380 nm during analyzing the drug substance. The contour plot and 3-D chromatogram were constructed and shown in Figures 4.3 and 4.4, respectively. At the wavelength of 240 nm the highest absorbance was obtained, and there are no any interference peaks appearing in the range of scanned wavelength. The results indicate that this analytical method exhibits the ability to identify the budesonide by its specificity.

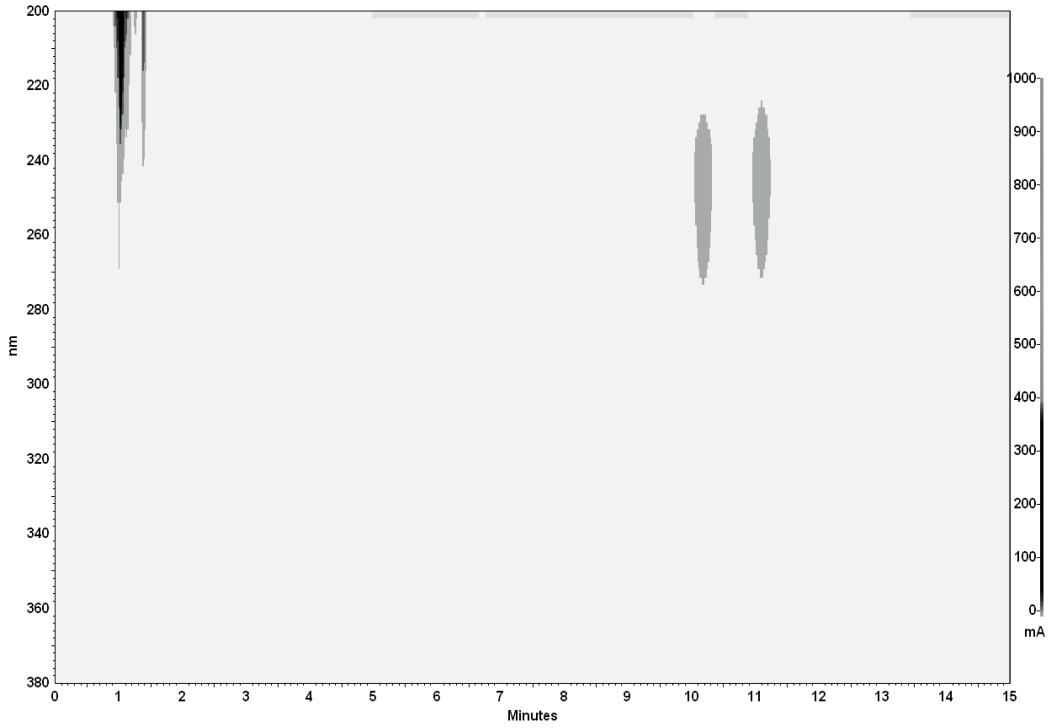



Figure 4.3 Contour plot of budesonide (scanned wavelength 200-380 nm)

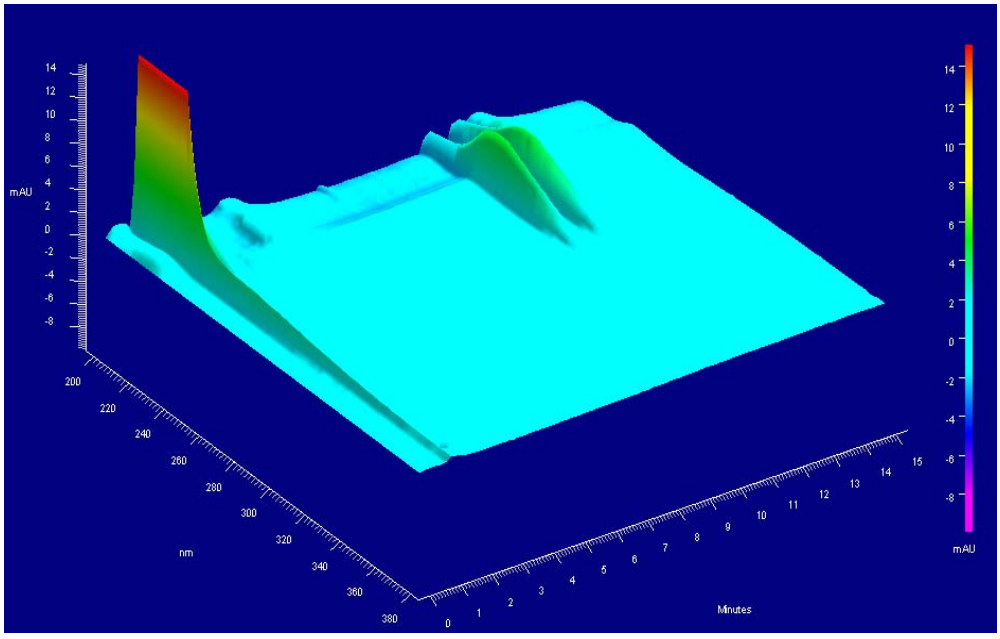



Figure 4.4 3-D chromatogram of budesonide (scanned wavelength 200-380 nm)

### 4.2.2 Linearity

Figure 4.5 and 4.6 show the linear regression analysis of standard curve. It was observed that the standard curves were linear over the investigated range (0.1-5 µg/ml) with the correlation coefficient ($r^2$) over 0.9999 for both epimers. Therefore, these ranges of concentration were suitable for preparing the standard solution and sample for analysis.

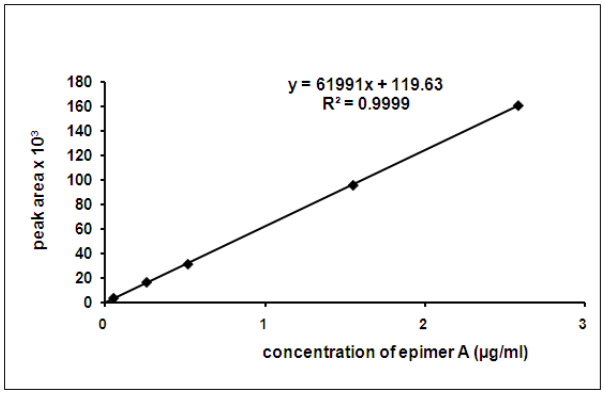



Figure 4.5 Standard curve of budesonide epimer A (22*S*)

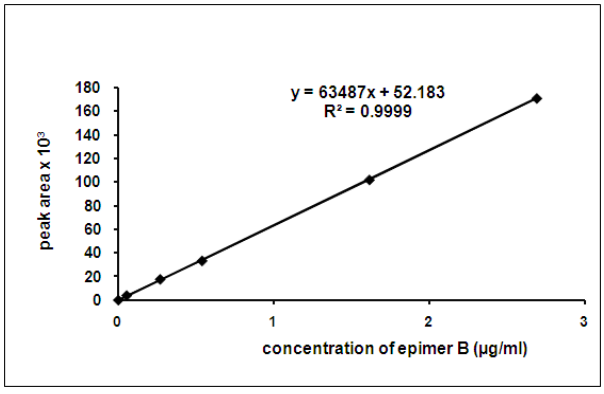



Figure 4.6 Standard curve of budesonide epimer B (22*R*)

### 4.2.3 Accuracy and precision

The results of accuracy and system precision tests performed on each concentration of budesonide are shown in Table 4.1. The accuracy and precision were presented as % recovery and %RSD, respectively.

Table 4.1 Percent recovery and RSD for accuracy and precision

| Concentration | Accuracy (% Recovery ± SD) | | Precision (% RSD) | |
|---|---|---|---|---|
| (μg/ml) | Intra-day (n=5) | Inter-day (n=4) | Intra-day (n=5) | Inter-day (n=4) |
| 1.06 | 98.4 ± 1.1 | 99.6 ± 1.7 | 1.16 | 1.63 |
| 3.19 | 98.9 ± 0.5 | 99.9 ± 0.7 | 0.55 | 0.66 |
| 5.32 | 99.9 ± 0.4 | 100.4 ± 0.4 | 0.44 | 0.44 |

For accuracy, both intra-day run and inter-day run gave the values of %recovery varied between 98-100% which were lined in the acceptable range. In the same way, the system precision was considered to be satisfactory since the %RSD values were less than 2% for both intra- and inter-day run.

### 4.2.4 Limit of detection and quantitation

Under the HPLC conditions, the limit of detection were found to be 3 ng/ml and 6 ng/ml for epimer A and epimer B, respectively. In addition, the limit of quantitation were determined to be 9 ng/ml and 17 ng/ml for epimer A and epimer B, respectively.

## 4.3 Content of active ingredient delivered by actuation of the valve

The 24 designed formulations were tested for content of active ingredient delivered by actuation of the valve after one month storage. The results are shown in Figure 4.7. The formulations containing HFA 134a propellant gave drug content over the maximum limit (>120%). However, the content of active ingredient tended to be decreased as the dosing numbers increased. The drug content of the end delivered doses (141-150) were in the acceptable range (80-120%) (BP, 2009) for all 134a containing formulations. The good uniformity of delivered doses was obtained from the HFA 227 formulations, but those values were lower than that of the lower limit. The content of active ingredient values in every interval of dosing were approximately 35-50%. Whereas, the formulations formulated with the propellant mixture gave drug content that were close to the acceptable range.

The analysis of variance (ANOVA) was employed to investigate the effect of vapor pressure of propellant system and concentration of stabilizing agent on content of active ingredient delivered by actuation of the valve. The results reveal that the vapor pressure of propellant system is important factor affecting the content of active ingredient. These values were significantly different ($p$<0.05) when the formulations exhibited the different level of vapor pressure. However, the formulations containing different types and concentrations of stabilizing agent gave no significant difference on the content of budesonide ($p$>0.05).

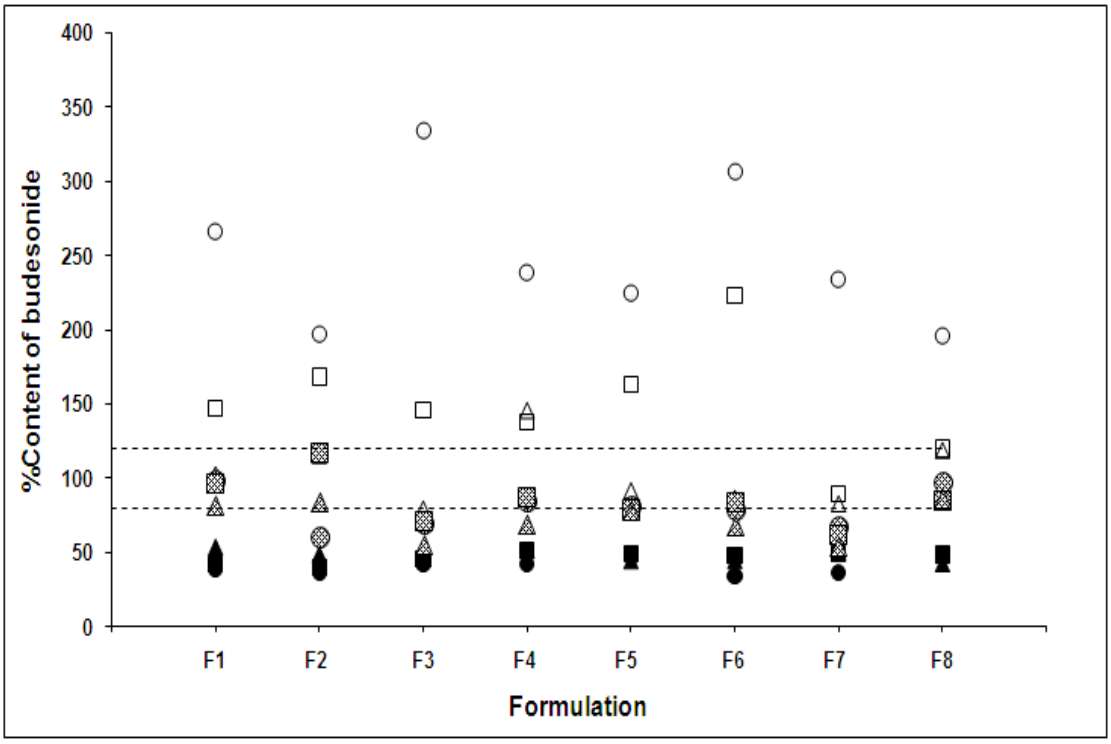



Figure 4.7 Content of active ingredient delivered by actuation of the valve of 24 designed formulations. Dose collection at beginning, middle, and end of the total doses are represented as round, square, and triangle symbols, respectively (○, □, △ for HFA 134a, ●, ■, ▲ for HFA 227, and ⊛, ⊠, ◬ for HFA mixture). The dash line is upper limit (120%) and lower limit (80%) of drug content stated in BP, 2009.

The propellant mixture of HFA 134a and HFA 227 in the weight ratio of 70:30 is more preferable than either pure HFA 134a or pure HFA 227. The HFA mixture exhibits potentially suitable properties for using as pMDI propellant. First is appropriate vapor pressure. According to Dalton's law and Raoult's law, the vapor pressure of propellant blend can be calculated from partial pressure of the individual propellant. By the weight ratio of 70:30, the HFA 134a : HFA 227 blend has the predicted vapor pressure of 535 kPa which is close to the recommended range of vapor pressure used in pMDI (Newman, 2005). The suitable vapor pressure is

expected to provide satisfied MMAD and FPF. Among three propellant systems, the highest vapor pressure (572 kPa) was obtained from HFA 134a. Although, the high vapor pressure provided fine aerosol due to rapid propellant evaporation, the velocity of plume discharge resulted in large amount of drug impacting in the oropharynx region (McDonald and Martin, 2000). In contrast, the HFA 227 exhibited the lowest vapor pressure (390 kPa) compared to those two propellant systems. This may result in insufficient vapor pressure for generating respirable fraction.

In addition, the propellant blend exhibited the change in density property. The density of propellant mixture can be predicted from equation 4.1 (Vervaet and Byron, 1999).

$$1/d_{mixture} = ((g_a/g_{mixture})/d_a) + ((g_b/g_{mixture})/d_b) \quad \text{----- (4.1)}$$

Where, $d$ and $g$ represent density and mass, respectively, and subscripts a and b represent the components of the propellant mixture. According to equation 4.1, the predicted density of HFA 134a : HFA 227 (70:30) was 1.254 g/ml when the density of HFA 134a and HFA 227 were 1.205 and 1.385 g/ml, respectively (Vervaet and Byron, 1999). Since the micronized budesonide had true density of 1.27 g/ml, the density match was obtained with the system of HFA mixture. When the density of drug powder closes to the density of propellant system the sedimentation of dispersed phase was reduced (Steckel and Wehle, 2004), and the stable suspension was achieved.

The difference in density between liquefied propellant and suspended drug exhibited much effect on the content and uniformity of delivered doses. As

clearly seen in the formulations containing HFA 134a as single propellant, poor uniformity was observed (80-330%). This may be resulted from the sedimentation of micronized drug in HFA 134a system since the density of micronized budesonide (1.27 g/ml) was higher than liquefied propellant (1.205 g/ml). When drug particles sedimented under gravitational acceleration, high drug amount was loaded into metering system. As a consequence, drug content was higher than 80-120% at the beginning of dosing, but the delivered doses were significantly decreased at the last 10 doses.

For the propellant system of HFA 227 although the delivered doses were uniformed, the content of active ingredient delivered through the metering system (35-50%) was lower than the target doses (80-120%). This was due to the formulation of HFA 227 exhibited the phase separation. The budesonide powder floated when suspended in HFA 227 propellant because the density of micronized budesonide (1.27 g/ml) was lower than that of propellant (1.385 g/ml) leading to small amount of drug could fill into the metering valve.

Due to the propellant blend exhibited the density matching to the micronized drug, the stable suspension was obtained resulting in reproducible of dosing, and the content of active ingredient was close to the acceptable range according to specification of BP (2009). Thus, the eight formulations of HFA mixture were chosen for full aerosol properties testing.

## 4.4 Assessment of fine particle fraction of budesonide suspension-based pMDI containing HFA mixture

The pMDI formulations of budesonide suspended in HFA mixture were determined for fine particle fraction (FPF). This value could reflect the lung deposition of developed formulations. The TSI was used for this purpose and the results are shown in Figure 4.8.

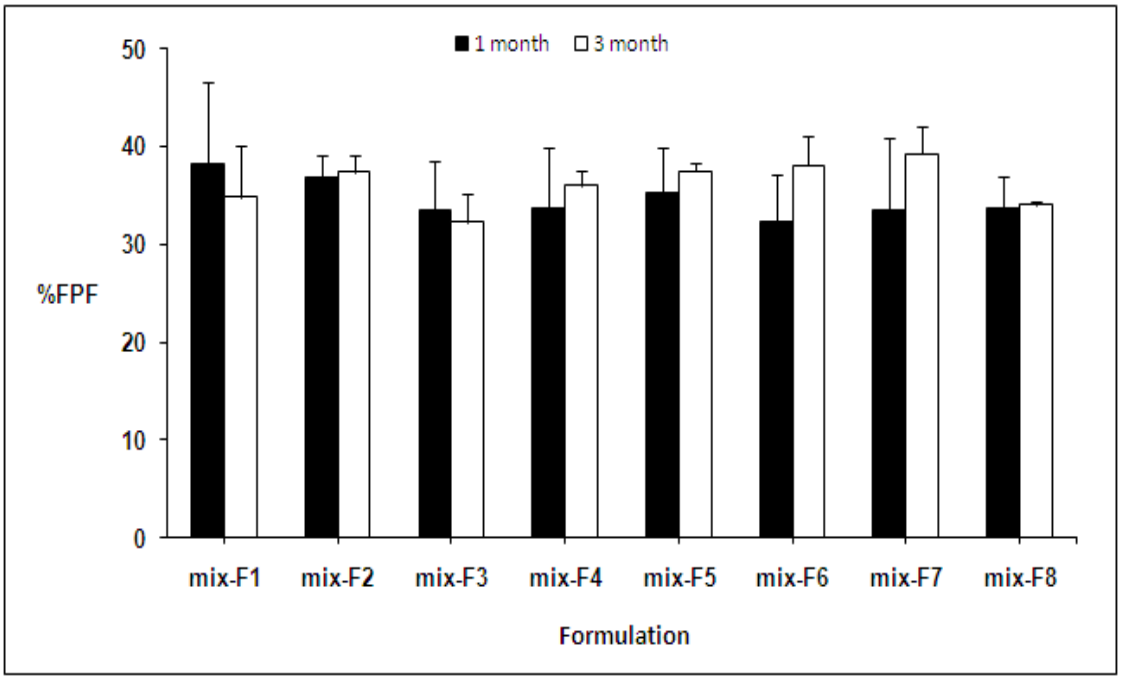



Figure 4.8 Fine particle fraction (FPF) of formulations containing HFA 134a : HFA 227 mixture (70:30) as propellant after 1 month and 3 months storage (mean ± SD, n=3)

The tests were performed at 1 month storage (25° C at ambient conditions). It was observed that all formulations exhibit %FPF ranging between

32-38%, and the ANOVA results indicate that the formulations with different types and concentrations of stabilizing agent gave no significant difference on % FPF ($p$>0.05). After 3 month storage (25° C at ambient conditions), the %FPF of all formulations were not significantly different from the %FPF of 1 month testing ($p$>0.05). It can predict that the deposition behavior of developed formulations was maintained over 3 months.

The FPF of formulations developed as budesonide suspension-based pMDIs reported in the literature are controversial (14-40%) (Robin *et al.*, 2007; Steckel and Wehle, 2004; Tarara *et al.*, 2004; Thorsson and Geller, 2005), and there is no criteria of this value specified in the Pharmacopoeia. This cause some difficulty and complexity to decide the optimal value. The highest amount of drug deposited in the lung is not the goal in the development of inhaled glucocorticosteroid because the local effect is required for the treatment of asthma. The higher amount of drug reached the lung resulting in higher systemic drug concentration that leads to occurrence of systemic side effects (Acerbi *et al.*, 2007). However, the beclometasone dipropionate which is classified in the same category as budesonide has individual monograph for pressurized inhalation dosage form (BP, 2009). According to the monograph, the FPF of pressurized inhalation containing 50 µg and 100 µg of anhydrous beclometasone dipropionate is not less than 35%. Based on the same guidance, some developed formulations in this study were good candidates.

## 4.5 Assessment of aerodynamic particle size distribution

The formulations containing HFA mixture were assessed for particle size distribution after the short time storage. The tests were employed by using the eight-stage ACI. Figure 4.9 shows the deposition pattern of each formulation after 1 month storage. It was observed that similar pattern was achieved. Large amount of drug emitted from pMDI was deposited on metal inlet which reflected the amount of drug would retain in the oropharynx region. In addition, the drug that passed through the metal inlet could reach the lower stage of ACI, and approximately 50%w/w of this fraction was deposited on stage 3 and 4.

The MMAD and GSD results are shown in Table 4.2. The 8 formulations were not significantly different in their MMAD ($p$>0.05). All formulations exhibited the MMAD around 3 μm that was suitable for pulmonary delivery. Moreover, the aerodynamic particle size did not change after 3 month storage. It can postulate that the stable suspension was obtained over 3 months. For the GSD results, it indicated that the polydispersion was obtained since the GSD values were higher than that of 1.2 (Hickey, 1992).

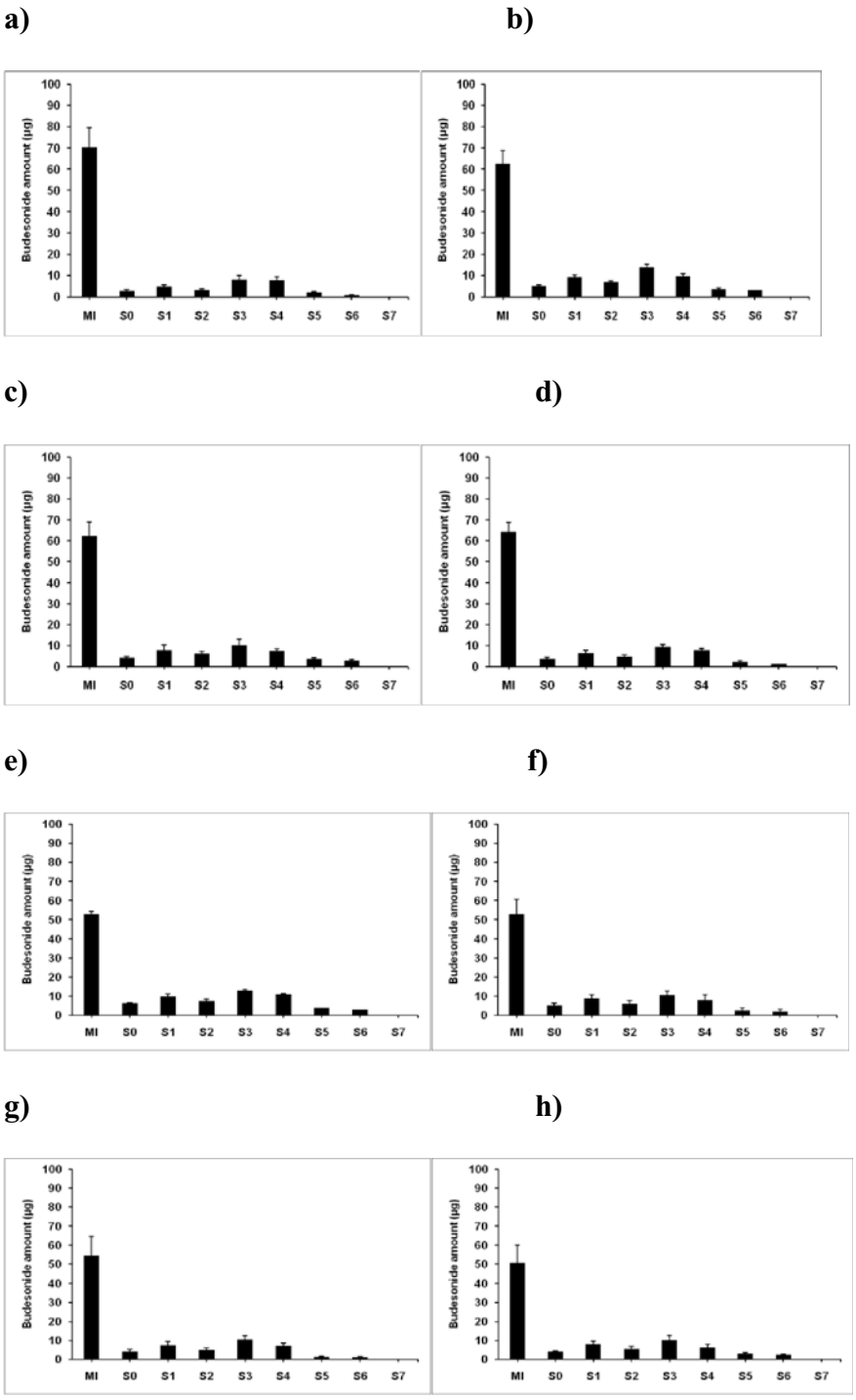
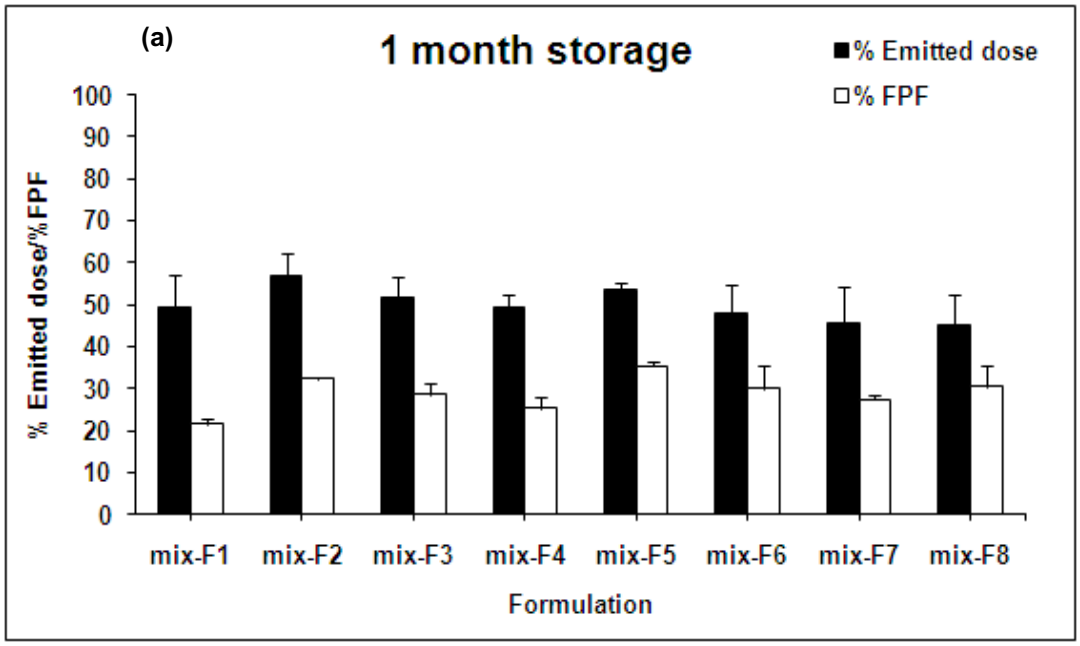



Figure 4.9 Drug deposition on each stage of Andersen cascade impactor a) Mix-F1, b) Mix-F2, c) Mix-F3, d) Mix-F4, e) Mix-F5, f) Mix-F6, g) Mix-F7, and h) Mix-F8 (Data performed in triplicate and represent as mean±SD, MI refers to metal inlet, and S0-S7 refer to stage 0-stage 7 of ACI)

Table 4.2 The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the formulations containing propellant mixture after 1 month and 3 months storage (mean ± SD, n=3)

| Formulation | 1 month storage | | 3 months storage | |
|---|---|---|---|---|
| | MMAD (μm) | GSD | MMAD (μm) | GSD |
| Mix-F1 | 3.04 ± 0.01 | 2.23 | 3.04 ± 0.01 | 2.24 |
| Mix-F2 | 3.02 ± 0.01 | 2.26 | 3.04 ± 0.01 | 2.25 |
| Mix-F3 | 3.01 ± 0.02 | 2.27 | 3.05 ± 0.01 | 2.25 |
| Mix-F4 | 3.04 ± 0.01 | 2.24 | 3.06 ± 0.01 | 2.23 |
| Mix-F5 | 3.03 ± 0.01 | 2.25 | 3.06 ± 0.01 | 2.23 |
| Mix-F6 | 3.05 ± 0.02 | 2.24 | 3.07 ± 0.01 | 2.23 |
| Mix-F7 | 3.07 ± 0.01 | 2.23 | 3.06 ± 0.01 | 2.24 |
| Mix-F8 | 3.02 ± 0.01 | 2.27 | 3.04 ± 0.01 | 2.26 |

The emitted dose and FPF of developed formulations at 1 month and 3 months storage are shown in Figure 4.10. The percentage emitted dose was varied between 45-70%. This may be due to loss of aerosols on the device. The FPF from ACI were approximately 21-35%. It was observed that the FPF values obtained from ACI were slightly lower than the FPF obtained from TSI (32-38%). The difference is from the cut-off diameter of the two apparatuses (cut-off diameter of TSI = 6.4 μm, cut-off diameter of ACI = 5.8 μm). However, the FPF obtained from TSI can be used as a parameter in a selection of candidate formulation since it is simple and less time consuming, whereas the ACI is more complicated for using in research work.



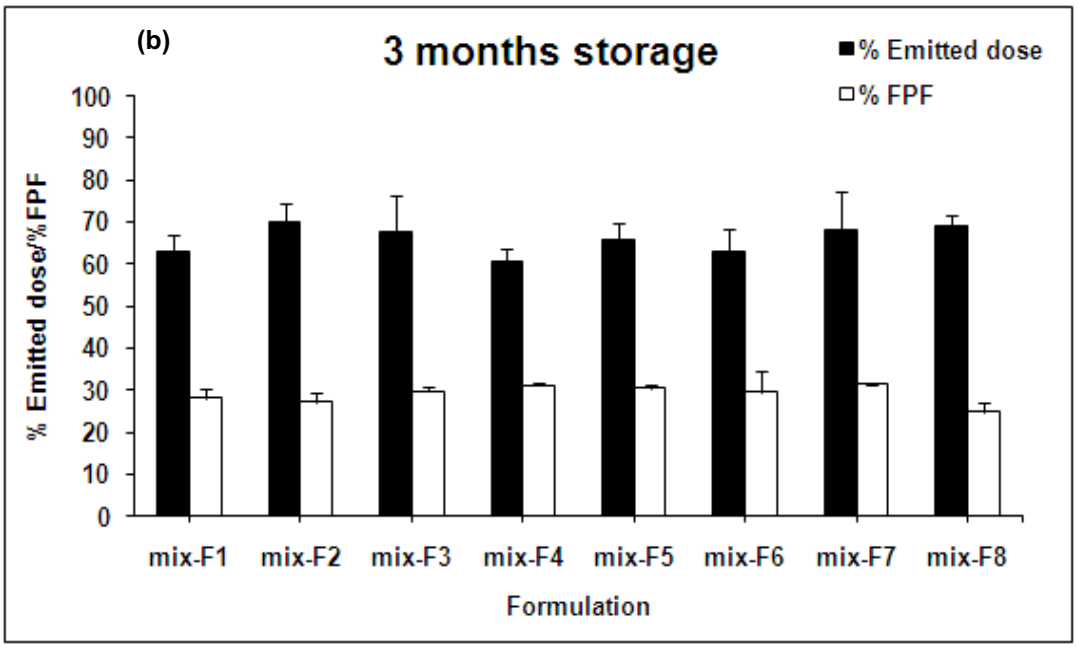




Figure 4.10 Emitted dose and fine particle fraction (FPF) obtained from Andersen cascade impactor of formulations containing HFA mixture (a) after 1 month storage and (b) after 3 months storage

When the results of aerosol properties including MMAD and FPF were combined to the data of budesonide delivered by actuation of the valve, the answer about the effect of stabilizing agent on formulation performances was clearly seen. The results exhibited that 8 formulations which were different in type and concentration of stabilizing agent showed no significant difference in aerosol properties ($p$>0.05). As a consequent, both oleic acid and sorbitan trioleate are preferable for using as stabilizing agent in the suspension-base pMDI of budesonide, and only small amount of stabilizing agent is required to stabilize the particles of micronized drug in liquefied propellant. Moreover, there is no synergetic effect between oleic acid and sorbitan trioleate. The combination use of these two stabilizing agents did not significantly improve the formulation performances.

## 4.6 Physical stability assessment

The physical stability assessments were employed on some formulations (134a-F1, 134a-F4, 227-F1, 227-F4, Mix-F1, and Mix-F4) which were chosen as a representative of the three propellant systems. The samples were determined the redispersibility and sedimentation behavior of dispersed phase in liquefied propellant after 3 months storage. The effect of density characteristic of different propellant system on physical stability was also observed.

### 4.6.1 Redispersibility of budesonide suspension-based pMDI

The formulations were assessed the ability of redispersion because this characteristic is essential for dose uniformity. Figure 4.11 shows redispersibility of tested formulations. The plots represent the amount of budesonide emitted from pMDI formulations according to the number of inversion. It was observed that after the first inversion both 134a-F1 and 134a-F4 gave high emitted dose over the designed range (80-120%), but the lower trend was received as the number of inversion increased. For formulations containing HFA 227, the different trend was observed. Both 227-F1 and 227-F4 gave low emitted dose at the first inversion, and the values tended to be increased when the number of inversion increased. However, the emitted doses were rather constant after inverting more than 5 times for both propellant systems. This could expect that the homogeneous suspension was obtained after 5 times redispersion.

The formulations containing HFA mixture gave the most favorable results. There was no trend in emitted dose data, and the values were close to the acceptable range at the first inversion. It was postulated that the micronized budesonide suspended in HFA mixture was easy to redispersed and the dose uniformity was obtained. In addition, it was observed that formulation containing oleic acid (F1) and formulation containing sorbitan trioleate (F4) showed no difference in ability of redispersion. Thus, both stabilizing agents are preferred for use in suspension-based pMDI.

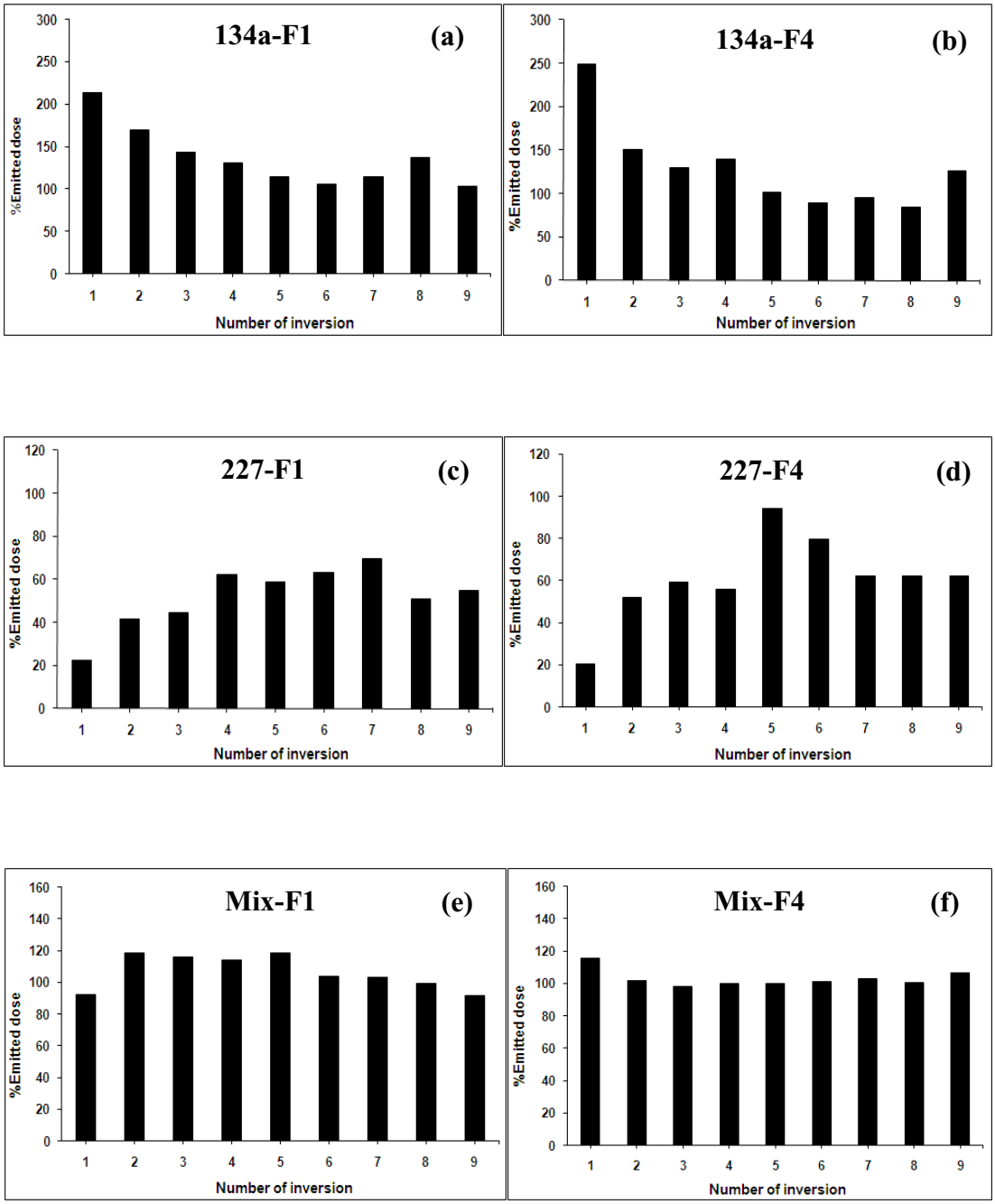



Figure 4.11 Redispersibility of formulations: (a) 134a-F1, (b) 134a-F4, (c) 227-F1, (d) 227-F4, (e) Mix-F1, and (f) Mix-F4 represented as % emitted dose according to the number of inversion

### 4.6.2 Sedimentation testing of budesonide suspension-based pMDI

The sedimentation of suspended budesonide in each formulation was observed for 20 min. The formulations with complete dispersion were left to sediment under the force of gravity, and the doses were sampling at designed interval. The results are shown in Figure 4.12. In case of formulation containing HFA134a, the sampling dose at initial exhibited the emitted dose around 120% for both 134a-F1 and 134a-F4, but the emitted doses found to be increased 2 fold higher than the target dose (>250%) after the formulations were left for sediment for 5 min. However, the other formulations provided the different observation. The formulations 227-F1 and 227-F4 gave the emitted dose approximately 80% at initial, and the values tended to be decreased when the sampling time increase. Whereas, the consistent emitted doses were obtained from formulations using the HFA mixture as propellant. These may be from the influence of density that can describe by Stokes' law. The factors affecting the sedimentation velocity according to Stokes' law are shown in equation 4.2 (Smyth, 2003).

$$\tau = 2gr^2(d_2-d_1) / 9\xi \quad \text{----- (4.2)}$$

where $\tau$ is the sedimentation rate of a spherical particle, $g$ is acceleration due to gravity, $r$ is particle radius, $d_1$ and $d_2$ are the densities of the continuous phase and dispersed phase, respectively, and the $\xi$ is the viscosity of continuous phase. Thus, the particle size reduction and density matching between

drug particle and liquefied propellant are crucial approaches to reduce sedimentation rates.

In this case the difference in densities between dispersed phase and continuous phase showed much effect on emitted doses. The formulations of HFA 134 a system formed homogenous suspension after shaking, but the micronized drug started to sediment in a few minutes because the drug particles exhibited higher density (1.27 g/ml) than that of HFA 134a propellant (1.205 g/ml). As a result, high amount of drug was loaded into metering system by gravitational force, leading to the increasing of emitted doses at extended sampling time.

The effect of density was also found in formulations containing HFA 227. Since the micronized budesonide had lower density than the HFA 227 propellant (1.385 g/ml), the drug particles tended to be floated and the phase separation of suspension would occur. As a consequence, the emitted doses of both 227-F1 and 227-F4 found to be decreased when the sampling time increased.

The propellant blend of HFA 134a and HFA 227 in the weight ratio of 70:30 provided the propellant density (1.254 g/ml) which match to the density of micronized budesonide. Consequently, the drug particles could suspend in this propellant system and the suspension exhibited the homogeneity over the observed time of 20 min.

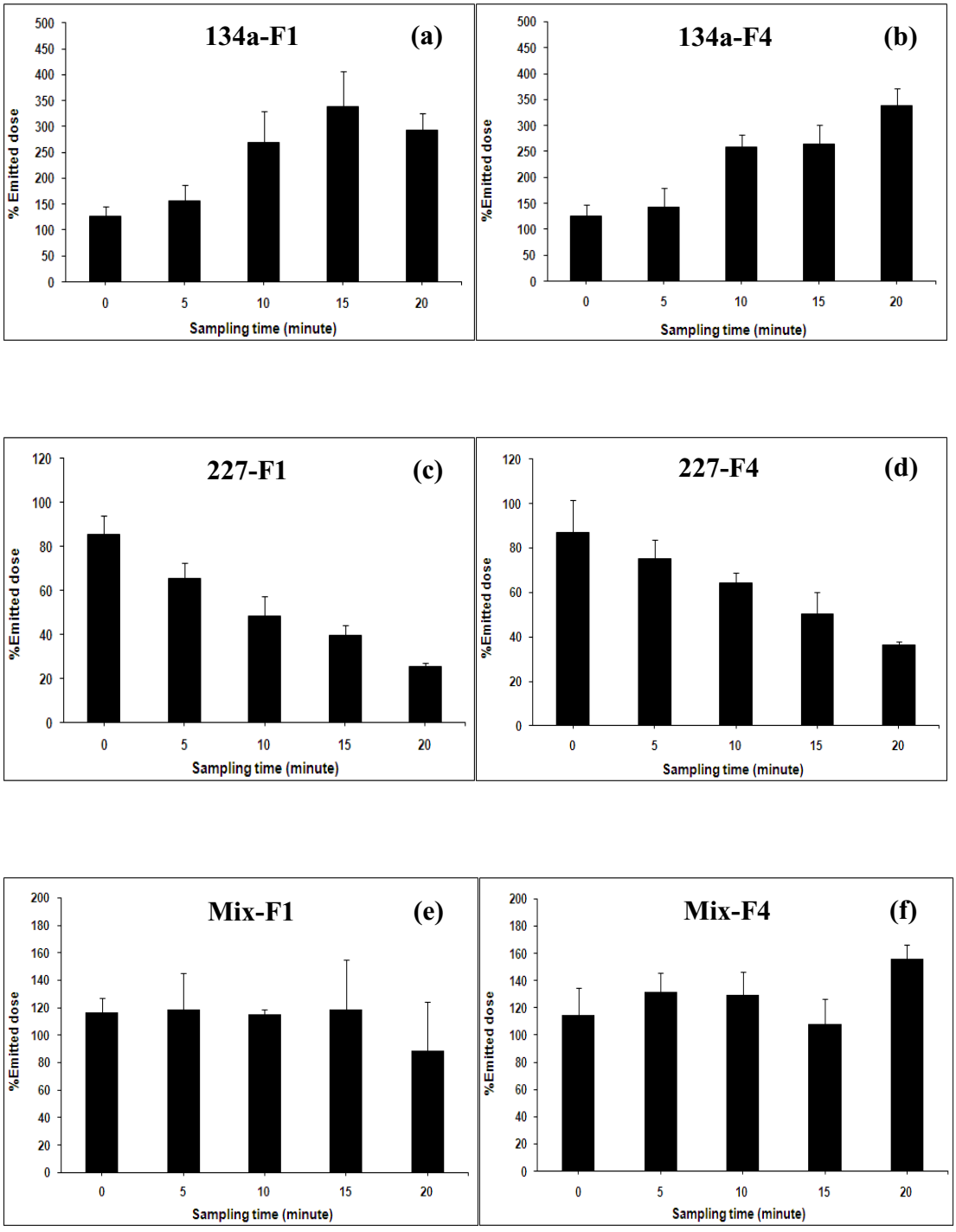



Figure 4.12 Sedimentation of formulations: (a) 134a-F1, (b) 134a-F4, (c) 227-F1, (d) 227-F4, (e) Mix-F1, and (f) Mix-F4 represented as % emitted dose versus sampling time (n = 3)

# CHAPTER 5

# CONCLUSIONS

In this study, the budesonide was developed as suspension-based pMDI. The 24 designed formulations were performed on the factorial design experiment. Analysis of variance results indicate that the vapor pressure of propellant system highly affected the content of active ingredient delivered by actuation of the valve ($p<0.05$). The formulations containing pure HFA 134a gave high drug content over maximum limit (>120%), while the formulation using pure HFA 227 gave lower values, approximately 50%. The preferred propellant system was a mixture of HFA 134a and HFA 227 in the weight ratio of 70:30. The budesonide contents of formulations containing HFA mixture closed to the acceptable range (80-120%). It can be explained that the HFA mixture exhibits potentially suitable properties for using in suspension-based pMDI including the sufficient vapor pressure for generating aerosol cloud and the density matching of liquefied propellant to suspended micronized budesonide.

The most favorable formulations containing HFA mixture were chosen for full *in vitro* aerosol properties testing. All formulations provided satisfied FPF and MMAD. After 1 month storage, the FPFs were ranged between 32-38%, and MMADs were around 3 μm. These properties were maintained over 3 months, indicating the stable suspensions were received. However, it was observed that the FPF and MMAD were not significantly improved when the stabilizing agents were used at higher

concentrations. We propose that small amount of either oleic acid or sorbitan trioleate is sufficient to stabilize the dispersion of budesonide suspension-based pMDI.

The pMDI formulations of budesonide dispersing in the three propellant systems were sampled for redispersibility and sedimentation testing after 3 months storage. The selected formulations containing pure HFA 134a, pure HFA 227, and HFA mixture exhibited good redispersibility. The particles of micronized budesonide were completely resuspended in both pure HFA 134a and HFA 227 after 5 times of inversion, while the formulations containing HFA mixture gave homogeneous suspensions at the first redispersion. However, when the formulations with complete dispersion were allowed to stand for 20 min, the phase separation and sedimentation were observed in the system of HFA 227 and HFA 134a, respectively. This phenomenon was not found in formulations containing HFA mixture. The homogeneous suspensions were maintained over 20 min in this propellant system.

The 8 developed formulations of budesonide suspension-based pMDI which were formulated by dispersing micronized budesonide in HFA mixture provided satisfied aerosol properties and good physical stability. Although the results of statistical analysis indicated that there was no significant difference in formulation performances among those formulations, the formulation containing oleic acid of 0.01%w/w with the presence of co-solvent (1%w/w of dried ethanol) was more preferable because it reached all requirements of pressurized inhaler including accurate and reproducible dosing, suitable aerodynamic diameter, and acceptable FPF. However, large amount of drug deposited in metal inlet must be concerned. This problem can be solved by minimizing the particle size distribution of micronized budesonide. Thus, further study of long-termed stability and drug release of the

candidate formulation should be carried out to obtain more information before entering the clinical trial.

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# APPENDIX

# APPENDIX

# CHEMICAL COMPOUNDS

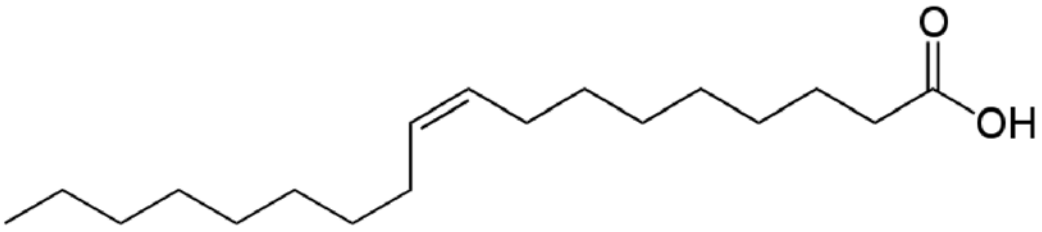
**OLEIC ACID**

**Chemical name:** (Z)-9-Octadecenoic acid

**Molecular formula:** $C_{18}H_{34}O_2$

**Molecular weight:** 282.47 g/mol

**Chemical structure:**




**Description:** A yellow to pale brown, oily liquid with a characteristic lard-like odor and taste. Oleic acid consists chiefly of (Z)-9-octadecenoic acid together with varying amounts of saturated and other unsaturated acids.

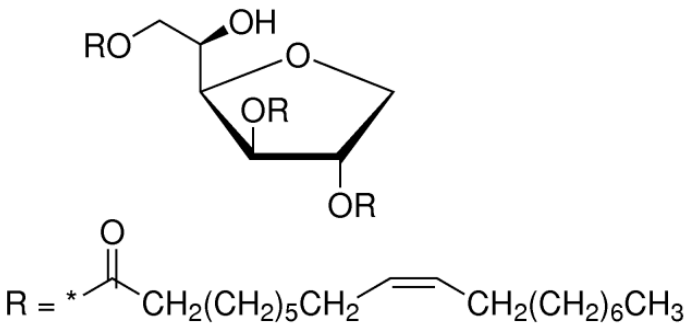
**Acidity/alkalinity:** pH 4.4 (saturated aqueous solution)

| | |
|---|---|
| **Boiling point:** | 286° C at 13.3 kPa (100 mmHg) (decomposition at 80-100° C) |
| **Melting point:** | 4° C |
| **Density:** | 0.895 g/ml |
| **Flash point:** | 189° C |
| **Viscosity (dynamic):** | 26 mPa s (26 cP) at 25° C |
| **Vapor pressure:** | 133 Pa (1 mmHg) at 176.5° C |
| **Solubility:** | Miscible with benzene, chloroform, ethanol (95%), ether, hexane, and fixed and volatile oils; practically insoluble in water. |
| **Stability:** | On exposure to air, oleic acid gradually absorbs oxygen, darkens in color, and develops a more pronounced odor. At atmospheric pressure, it decomposes when heated 80-100° C. |
| **Storage conditions:** | Oleic acid should be stored in a well-filled, well-closed container, protected from light, in a cool, dry place. |

**SORBITAN TRIOLEATE**

| | |
|---|---|
| **Chemical name:** | (Z,Z,Z)-Sorbitan tri-9-octadecenoate |
| **Molecular formula:** | $C_{60}H_{108}O_8$ |
| **Molecular weight:** | 958 g/mol |

**Chemical structure:**




| | |
|---|---|
| **Description:** | An amber viscous liquid with a distinctive odor and taste. |
| **Acid value:** | 10-14 |
| **Density:** | 0.95 g/ml |
| **HLB value:** | 1.8 |
| **Hydroxy value:** | 55-60 |
| **Viscosity:** | 200-250 mPa s at 25° C |
| **Solubility:** | Sorbitan trioleate is generally soluble or dispersible in oils, also soluble in most organic solvents, but insoluble in water. |
| **Stability:** | Gradually soap formation occurs with strong acids and bases. |
| **Storage conditions:** | Sorbitan trioleate should be stored in a well-closed container in a cool, dry place. |

# ANALYSIS OF VARIANCE RESULTS

Two-way ANOVA was performed to investigate the effect of two factors on the response described as follow:

**Factor 1: Vapor pressure of propellant system**

HFA 227 = Low level of vapor pressure

HFA mixture = Intermediate level of vapor pressure

HFA 134a = High level of vapor pressure

**Factor 2: Concentration of propellant system**

F1 = Formulation containing 0.01%w/w oleic acid

F2 = Formulation containing 0.03%w/w oleic acid

F3 = Formulation containing 0.05%w/w oleic acid

F4 = Formulation containing 0.003%w/w sorbitan trioleate

F5 = Formulation containing 0.005%w/w sorbitan trioleate

F6 = Formulation containing 0.010%w/w sorbitan trioleate

F7 = Formulation containing 0.01%w/w oleic acid and 0.01%w/w Sorbitan trioleate

F8 = Formulation containing 0.05%w/w oleic acid and 0.003%w/w Sorbitan trioleate

**Response: Content of active ingredient delivered by actuation of the valve**

## Univariate Analysis of Variance (SPSS version 16.0)

**Between-Subjects Factors**

| | | Value Label | N |
|---|---|---|---|
| Vapor pressure | 1 | low level | 8 |
| | 2 | intermediate level | 8 |
| | 3 | high level | 8 |
| Stabilizing agent | 1 | F1 | 3 |
| | 2 | F2 | 3 |
| | 3 | F3 | 3 |
| | 4 | F4 | 3 |
| | 5 | F5 | 3 |
| | 6 | F6 | 3 |
| | 7 | F7 | 3 |
| | 8 | F8 | 3 |

**Tests of Between-Subjects Effects**

Dependent Variable:Drug content

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 367042.667 | 1 | 367042.667 | 456.089 | .000 |
| | Error | 5633.333 | 7 | 804.762[a] | | |
| Vapor pressure | Hypothesis | 197868.083 | 2 | 98934.042 | 106.660 | .000 |
| | Error | 12985.917 | 14 | 927.565[b] | | |
| Stabilizing agent | Hypothesis | 5633.333 | 7 | 804.762 | .868 | .554 |
| | Error | 12985.917 | 14 | 927.565[b] | | |

a. MS(formulation)

b. MS(Error)

**Expected Mean Squares[a,b]**

| Source | Variance Component | | |
|---|---|---|---|
| | Var(formulation) | Var(Error) | Quadratic Term |
| Intercept | 3.000 | 1.000 | Intercept, vp |
| Vapor pressure | .000 | 1.000 | vp |
| Stabilizing agent | 3.000 | 1.000 | |
| Error | .000 | 1.000 | |

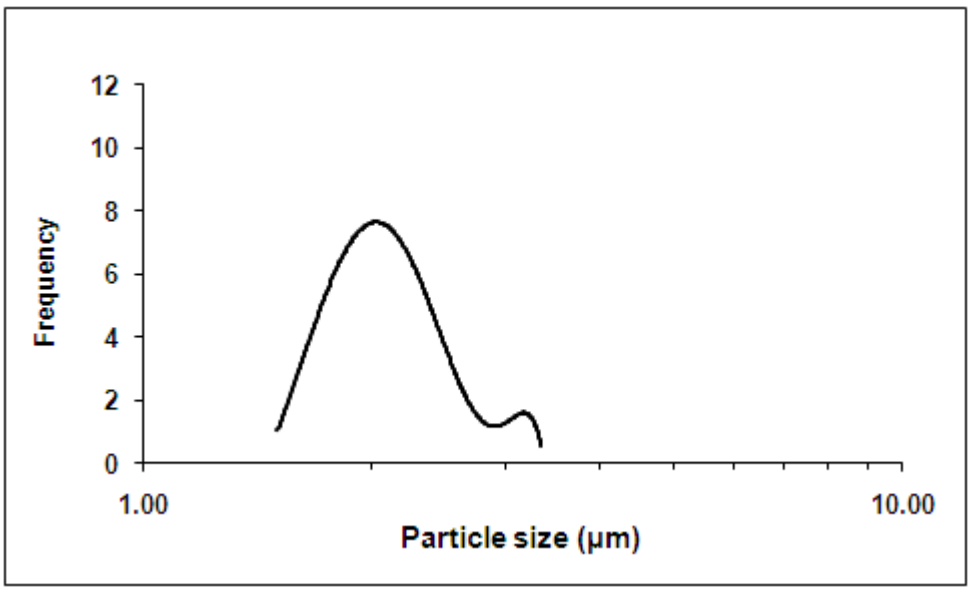
a. For each source, the expected mean square equals the sum of the coefficients in the cells times the variance components, plus a quadratic term involving effects in the Quadratic Term cell.

b. Expected Mean Squares are based on the Type III Sums of Squares.

## Particle size distribution of micronized budesonide determined by microscopic method

Material: Micronized budesonide dispersed in water

Instrument: Olympus BX61 Microscope with Cell$^{P}$ Imaging Software



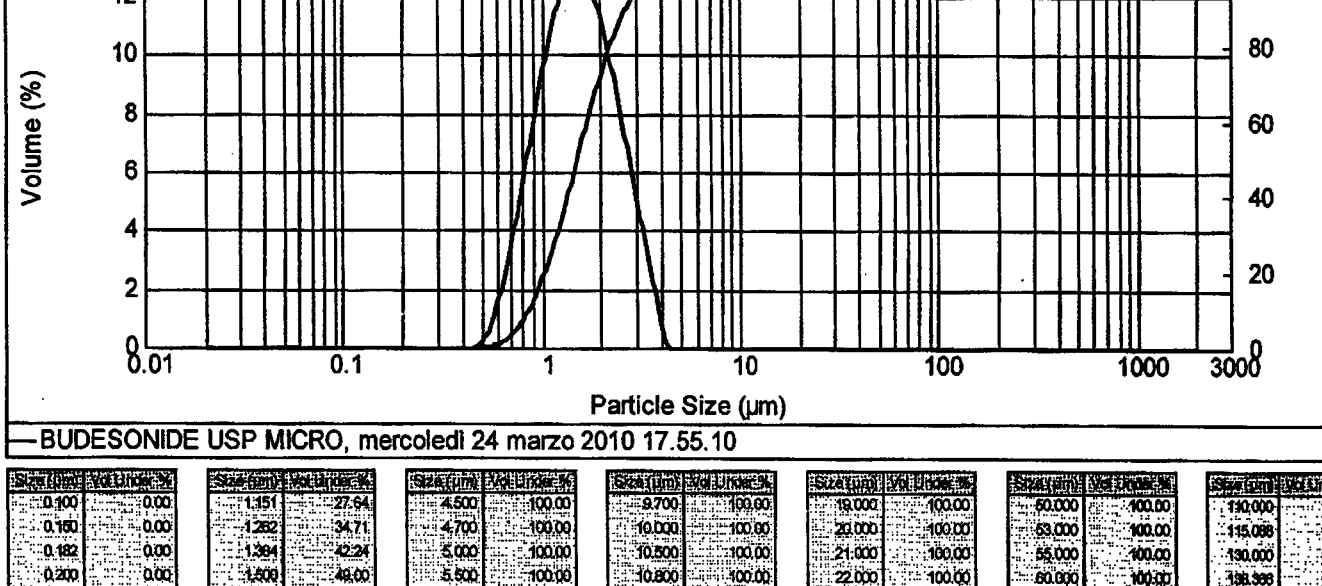
# NEWCHEM



## Particle Size Distribution Report

MASTERSIZER 2000

| | | | |
|---|---|---|---|
| **Sample Name:** BUDESONIDE USP MICRO | **SOP Name:** | **Measured:** mercoledì 24 marzo 2010 17.55.10 | |
| **Sample Source & type:** PF | **Measured by:** operatore | **Analysed:** mercoledì 24 marzo 2010 17.55.12 | |
| **Sample bulk lot ref:** NP0328M | **Result Source:** Averaged | | |
| **Particle Name:** Fraunhofer | **Accessory Name:** Hydro 2000μP (A) | **Analysis model:** General purpose (spherical) | **Sensitivity:** Normal |
| **Particle RI:** 0.000 | **Absorption:** 0 | **Size range:** 0.020 to 2000.000 μm | **Obscuration:** 8.12 % |
| **Dispersant Name:** Water | **Dispersant RI:** 1.330 | **Weighted Residual:** 1.134 % | **Result Emulation:** Off |
| **Concentration:** 0.0029 %Vol | **Span :** 1.205 | **Uniformity:** 0.369 | **Result units:** Volume |
| **Specific Surface Area:** 4.33 m²/g | **Surface Weighted Mean D[3,2]:** 1.384 μm | **Vol. Weighted Mean D[4,3]:** 1.660 μm | **RPM Pump Speed:** 2500 RPM |
| **D(0.10) : 0.86 μm** | **D(0.50) : 1.52 μm** | **D(0.90) : 2.69 μm** | **D(1.00) : 4.12 μm** PASS |
| **D(0.995) : 3.78 μm** PASS | | | |




| Size (μm) | Vol Under % | Size (μm) | Vol Under % | Size (μm) | Vol Under % | Size (μm) | Vol Under % | Size (μm) | Vol Under % | Size (μm) | Vol Under % | Size (μm) | Vol Under % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.100 | 0.00 | 1.151 | 27.64 | 4.500 | 100.00 | 9.700 | 100.00 | 19.000 | 100.00 | 50.000 | 100.00 | 130.000 | 100 |
| 0.150 | 0.00 | 1.282 | 34.71 | 4.700 | 100.00 | 10.000 | 100.00 | 20.000 | 100.00 | 53.000 | 100.00 | 135.088 | 100 |
| 0.182 | 0.00 | 1.384 | 42.24 | 5.000 | 100.00 | 10.500 | 100.00 | 21.000 | 100.00 | 55.000 | 100.00 | 140.000 | 100 |
| 0.200 | 0.00 | 1.500 | 49.00 | 5.500 | 100.00 | 10.800 | 100.00 | 22.000 | 100.00 | 60.000 | 100.00 | 145.388 | 100 |
| 0.240 | 0.00 | 1.664 | 57.71 | 6.000 | 100.00 | 11.000 | 100.00 | 25.000 | 100.00 | 65.000 | 100.00 | 148.000 | 100 |
| 0.348 | 0.00 | 1.824 | 65.19 | 6.500 | 100.00 | 11.500 | 100.00 | 28.000 | 100.00 | 70.000 | 100.00 | 148.000 | 100 |
| 0.381 | 0.00 | 2.000 | 72.25 | 7.000 | 100.00 | 12.000 | 100.00 | 30.000 | 100.00 | 72.500 | 100.00 | 150.000 | 100 |
| 0.500 | 0.02 | 2.183 | 78.89 | 7.500 | 100.00 | 13.000 | 100.00 | 31.000 | 100.00 | 75.000 | 100.00 | 165.000 | 100 |
| 0.551 | 0.26 | 2.500 | 86.48 | 8.000 | 100.00 | 14.000 | 100.00 | 32.000 | 100.00 | 80.000 | 100.00 | 180.000 | 100 |
| 0.604 | 0.92 | 2.600 | 88.46 | 8.200 | 100.00 | 15.000 | 100.00 | 35.000 | 100.00 | 85.000 | 100.00 | 200.000 | 100 |
| 0.726 | 4.15 | 2.800 | 91.76 | 8.500 | 100.00 | 16.000 | 100.00 | 37.000 | 100.00 | 90.000 | 100.00 | 250.000 | 100 |
| 0.796 | 7.00 | 3.000 | 94.31 | 8.700 | 100.00 | 16.500 | 100.00 | 40.000 | 100.00 | 95.000 | 100.00 | | |
| 0.873 | 10.80 | 3.509 | 98.32 | 9.000 | 100.00 | 17.000 | 100.00 | 42.000 | 100.00 | 97.000 | 100.00 | | |
| 0.957 | 15.52 | 4.000 | 99.94 | 9.300 | 100.00 | 18.000 | 100.00 | 45.000 | 100.00 | 100.000 | 100.00 | | |
| 1.000 | 18.12 | 4.200 | 100.00 | 9.500 | 100.00 | 18.500 | 100.00 | 48.000 | 100.00 | 103.000 | 100.00 | | |

**Operator notes:** *Average of 5 measurements from 46 to 50*

# VITAE

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**Student ID:** 5110720006

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| Bachelor of Pharmacy | Prince of Songkla University | 2003 |

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2. Academic Excellence Program in Pharmaceutical Sciences, Prince of Songkla University, 2008-2009

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1. Pharmacist in Department of Technical Pharmacy, Sirindhorn College of Public Health, Yala, 2004-2005.
2. Pharmacist in Songkhla Provincial Public Health Office, 2006-2007.

**List of Publication and Proceedings**

Sukasame, N. and Srichana, T. 2010. The formulation development of suspended budesonide pressurized metered dose inhaler. Proc. of the 1st Current Drug

Development International Conference, Phuket, Thailand, 72-75. (Full text proceeding).

Sukasame, N., Nimnoo, N., Suwandecha, T. and Srichana, T. 2009. *In vitro* size characterization, urinary pharmacokinetics and pharmacodynamics of salbutamol dry powder inhaler. Proc. of 6th Asian Aerosol Conference, Bangkok, Thailand, E-proceeding. (Full text proceeding).