Curriculum Vitae

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Kelkar MS, Gokhale AS, Dalby RN, Cascade Impaction Testing of Solution and Suspension-type Metered Dose Inhalers Prepared Using Effervescent Aerosol Technology, Drug Development and Industrial Pharmacy (*in preparation*)

Kelkar MS and Dalby RN, Plume Geometry Evaluation of Solution-type Metered Dose Inhalers Formulated Using Effervescent Aerosol Technology, Respiratory Drug Delivery 2016 (*in preparation*)

Kelkar, MS, Dalby, RN: Formulation of suspension-type pressurized metered dose inhalers using effervescent aerosol technology. In *Respiratory Drug Delivery Europe 2015.* DHI Publishing; River Grove, IL: 2015: 493-96

Kelkar, MS, Dalby, RN: Formulation of Solution-type Pressurized Metered Dose Inhalers Using Effervescent Aerosol Technology. In *Respiratory Drug Delivery Asia 2014.* Edited by Dalby, RN, Byron, PR, Peart, J., Suman, JD, Farr, SJ, Young, PM and Traini, D. DHI Publishing; River Grove, IL: 2014: 229-232

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Abstract

Title of Dissertation: Effervescent Aerosols: A Novel Formulation Technology for Solution and Suspension-Type Metered Dose Inhalers

Mukul Kelkar Doctor of Philosophy, 2016

Dissertation Directed by: Richard N. Dalby, Ph.D.
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Pressurized metered dose inhalers are complex drug delivery systems which have revolutionized the treatment of asthma and chronic obstructive pulmonary disease since the 1950s. Since the early 1990s, solution and suspension metered dose inhalers have been formulated using HFA-134a and HFA-227 as the propellants of choice, along with other excipients such as ethanol and oleic acid. Performance of an inhaler is partially dependent upon the size of particles it generates, which is partially dictated by intra-canister pressure.

In this dissertation, a novel method of formulation of inhalers- the 'effervescent aerosol technology' has been introduced. A propellant consisting of HFA-134a containing dissolved carbon dioxide has been used to formulate solution and suspension inhalers. We hypothesized that dissolution of carbon dioxide in HFA-134a will lead to higher degree of aerosol particle size reduction upon actuation, caused due to the process of effervescence i.e. escape of dissolved carbon dioxide from HFA -134a droplet.

Since this is a novel technology, the initial part of the dissertation tested the hypothesis in a custom-designed pressure vessel capable of holding and spraying solution

inhaler formulations. Particle size analysis was done using Malvern Spraytec®, a laser diffractometry instrument. Formulations prepared using a mixture of HFA-134a and carbon dioxide showed significantly lower particle sizes compared to analogous, pressure-controlled formulations.

In the later part of the dissertation, a method to transfer the formulation from the pressure vessel to commercially viable canisters has been described. Further laser diffractometry experiments showed that solution and suspension inhalers prepared using effervescent aerosol technology consistently produce particles with significantly lower particle sizes than HFA-134a-only inhalers.

Cascade impaction testing showed that inhalers prepared using a mixture of HFA-134a and carbon dioxide cause significantly lower throat deposition and show improvements in other crucial performance parameters, when compared to HFA-134a-only formulations. Additionally, spray characterization studies such as spray force and plume geometry have also been conducted.

This thesis demonstrates that using a combination propellant of liquefied HFA-134a and dissolved CO_2 to prepare solution and suspension-type metered dose inhalers is a viable formulation strategy to achieve improved inhaled drug delivery.

Effervescent Aerosols: A Novel Formulation Technology for Solution and Suspension-Type Metered Dose Inhalers

By

Mukul Sunil Kelkar

Dissertation submitted to the Faculty of the Graduate School of the
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Chapter 1: Background and Significance

1.0 Pulmonary drug delivery

Pulmonary drug delivery (drug delivery through the lung) has become the route of administration of choice for treating conditions, such as asthma and chronic obstructive pulmonary disease (COPD. This is due to the advantage of rapid relief from symptoms of lung conditions. Inhalation drug delivery, popularly considered a modern method of drug delivery, actually traces its origins to many ancient cultures, such as the Indians, Chinese, and Greeks[1]. These ancient cultures relied on combustion of herbal and organic materials to produce symptom-relieving fumes. In the 19th and 20th century, advances in inhalation technology led to the development of atomizers and asthma cigarettes, though these advancements, which despite being vast improvements, still failed on the fronts of efficacy, patient safety, and ease of use[2].

The modern age of inhalation medicine was ushered in with the development of the first pressurized metered dose inhaler (pMDI) in 1956, which improved efficacy and predictability of treatment, and also increased patient safety, robustness of device, and ease of carry and use compared to earlier technologies[3]. Since the 1950s, the inhalation platform portfolio has expanded to include dry powder inhalers, portable nebulizers, breath-actuated inhalers and a host of other formats. These novel drug delivery systems have not only allowed delivery of established inhaled medicines, such as bronchodilators (e.g. albuterol sulfate) and corticosteroids (e.g. beclomethasone dipropionate), but also opened up possibilities for delivery of systemic agents using the lung as a portal of entry. A good example would be the recently approved inhaled insulin, Afrezza® [4]. Despite these

advances, there remains a lot of scope to improve performance of inhalers to enhance patient adherence, increase efficacy and reduce drug wastage during administration[5].

Studies described in this dissertation are aimed at improving performance of pMDIs, still considered the 'gold standard' in the field of inhalation[6]. In the following sections, different mechanisms of aerosolized particle deposition in lungs and various pulmonary drug delivery systems are discussed. A detailed summary of specific aims for this dissertation is also provided.

1.1 Deposition of aerosolized particles in the lung- An overview

Aerosolized drugs which gain entry into the respiratory system, primarily deposit in one of the following ways- Inertial impaction, gravitational sedimentation and Brownian diffusion[7]. Inertial impaction of particles occurs primarily in the upper regions of the respiratory tract, especially at the oropharynx, which features an almost 90º bend into the larynx and trachea[8]. Upon administration from a pMDI or dry powder inhaler (DPI), the momentum of heavier particles is simply too high for them to successfully negotiate the turn within the oropharynx, and they deposit on the throat[9]. A significant amount of drug per dose is often lost in this method, representing a limitation, but also an opportunity for formulation scientists[10]. Inertial impaction is also a cause of drug deposition in central airways of the lung, where bifurcations of major airways can be observed[8]. Gravitational sedimentation, as the name suggests, is the deposition of particles under the influence of gravity. This type of deposition predominates during slow inhalation of dose, or when patients hold their breath following inhalation. Brownian diffusion, which causes deposition of particles greater than 1µm due to molecular interactions in the ambient air, is a minor mechanism of deposition in most pharmaceutical aerosols[11].

1.2 Particle size distribution of aerosols- key concepts

Many naturally occurring, and most pharmaceutical aerosols such as those produced from inhalers are heterodisperse in nature i.e. they are composed of droplets or particles of various sizes, and dynamic i.e. they are moving and in many cases changing in size, which represents a challenge to quantification of aerosols[12]. Thus, in order to gauge the pharmaceutical viability of aerosols, it is essential to consider particle size distribution to estimate the amount of 'respirable' particles. In pulmonary drug delivery, a particle is considered 'respirable' if its diameter is less than 5μm[13,14]. In pharmaceutical aerosols the 'aerodynamic diameter' is most important- it takes into account the geometric diameter of the particle and its specific gravity[15].

1.2.1 Aerodynamic diameter

In its simplest form, aerodynamic diameter is defined as the product of physical diameter of the particle and square root of its density. Thus, if D is the diameter of the particle, and ρ is its density, D_a, the aerodynamic diameter can be calculated as[16]

$$D_a = D * \sqrt{\rho}$$

From the equation, it can be easily deduced that a physically large particle with a density lesser than 1g/cm^3, will exhibit aerodynamic properties of a smaller particle, and vice versa. This is a very important concept as it has a direct bearing on settling properties of drug particles in the lung. It is, therefore, a standard industry practice to consider the particle size distribution based upon aerodynamic diameter, rather than just geometric diameter, while assessing performance of pharmaceutical aerosols[17]. Since direct measurement of aerodynamic diameters is difficult and unreliable, its value is predicted based on the

behaviors of particles in moving airstreams. This method to gauge aerodynamic diameter using settling behavior will be discussed later in this dissertation.

1.2.2 Mass median aerodynamic diameter and geometric standard deviation

Aerodynamic particle size distribution (APSD) is the parameter used to gauge performance of pharmaceutical aerosols. This distribution can often be approximated to a log-normal distribution. APSD is usually plotted on a log scale in terms of mass i.e. it determines the mass frequency of particles corresponding to a particular aerodynamic diameter value. Mass median aerodynamic diameter (MMAD), a commonly used metric, is defined as a "value of aerodynamic diameter above and below which half the aerosol mass resides"[16]. Geometric standard deviation (GSD) describes the 'spread' of the distribution, and thus, a higher GSD value indicates a high degree of heterodispersity[16,18]. Although, in theory, a monodisperse aerosol should have a GSD value of unity, for practical purposes, an aerosol is considered monodisperse if it has a GSD value less than 1.22[16]. An approximate log-normal distribution from an aerosol is depicted in Figure 1.1.

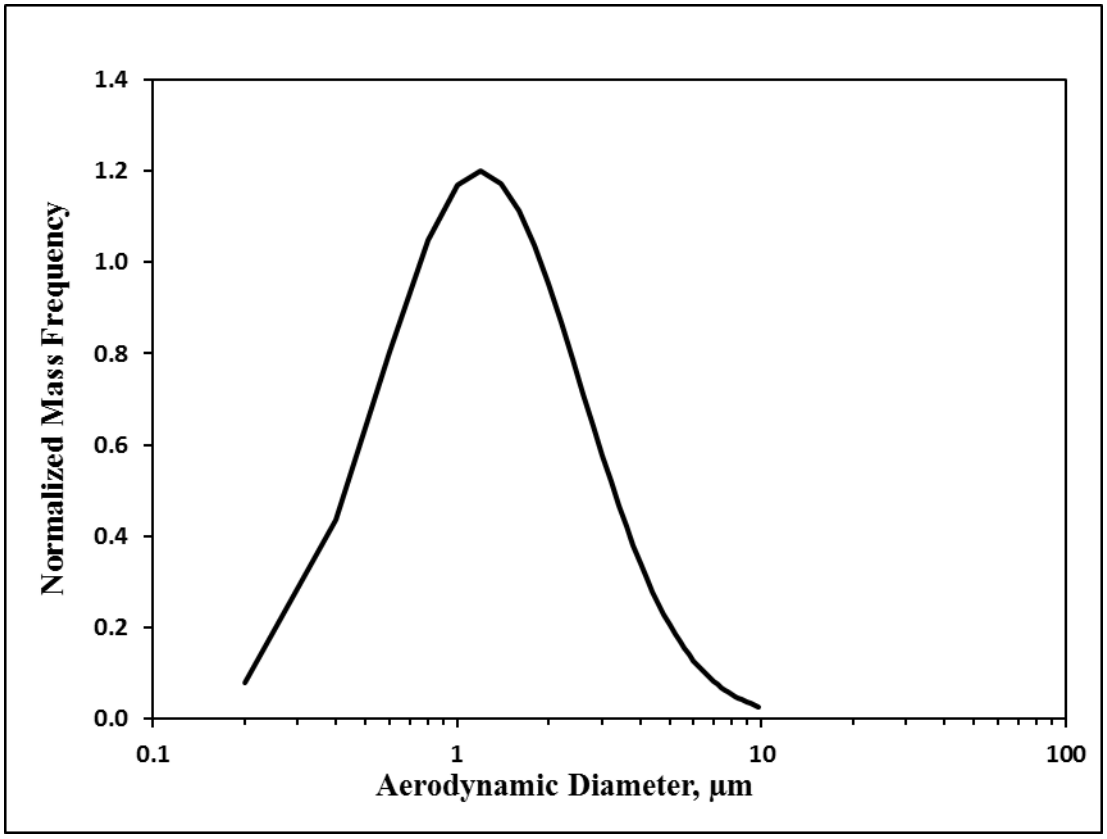


Figure 1.1: Log-normal aerodynamic particle size distribution- Figure 1.1 shows a graph representing an approximate log-normal aerodynamic particle size distribution of aerosol produced by a pMDI, in terms of mass frequency. X axis is a log scale representing aerodynamic diameter values; Y axis represents mass frequency. (Source: In-house data)

1.3 Pulmonary drug delivery platforms

Most of the currently marketed pulmonary drug delivery systems can be classified into 3 types: nebulizers, dry powder inhalers, and pressurized metered dose inhalers. This section explores all 3 in detail.

1.3.1 Nebulizers

Nebulizers, an earlier drug delivery platform, are used to deliver mostly aqueous solutions of drugs. They are an excellent method to deliver drugs such as albuterol (Ventolin®) and tobramycin (TOBI®) in a pediatric, geriatric or emergency room setting[19,20], where a patient in either unconscious or unable to use other devices such as pMDIs and DPIs. Nebulizers also have distinct advantages of delivering a much higher dose of the drug compared to other devices and that the dose is delivered during tidal breathing[20,21]. This might reduce dosing variability caused by incorrect patient technique.

Nebulizers can be broadly classified into jet nebulizers, ultrasonic nebulizers and vibrating mesh nebulizers. Jet nebulizers, which operate based on Bernoulli's principle, use the energy of compressed gases to create an aerosol from the bulk solution in the drug reservoir[22]. In a hospital setting, a jet nebulizer may be attached to a constant pressurized air supply. As air is forced through a nozzle, it gains kinetic energy, and the resulting pressure drop causes drug from the reservoir to be drawn into the airstream and be sheared into droplets. However, the resulting aerosol has a wide particle size distribution range. A baffle system is used to separate out larger droplets, which are then recycled for nebulization[23,24]. Advancements in jet nebulizers include breath enhanced (e.g. Ventstream® by Philips Respironics) and breath actuated jet nebulizers (e.g. AeroEclipse® by Trudell), which reduce drug wastage by coordinating patient's breath to aerosol generation and dispensation[25,26].

Ultrasonic nebulizers use a rapidly vibrating piezoelectric element that is connected to drug reservoir by a coupling system. The vibrations produce droplets over a wide size range, which requires ultrasonic nebulizers to have a baffle system similar to jet

nebulizers, in order to separate out larger droplets[27,28]. Although being a quieter and quicker method than jet nebulizers, ultrasonic nebulizers have disadvantages of producing droplets with larger MMAD and limited functionality with viscous and proteinaceous formulations[29].

Development of vibrating mesh nebulizers represented a significant advancement in nebulizer technology. They were developed to overcome problems that arose during ultrasonic nebulizer operation. As the name suggests, vibrating mesh nebulizers consist of a mesh with multiple orifices, which vibrates at a high frequency over the drug reservoir causing droplet formation. Diameter of orifices on the mesh might be varied in order to suit the formulation being nebulized. eFlow® by PARI is a good example of a nebulizer using this mode of vibration[30,31].

1.3.2 Dry powder inhalers

Dry powder inhalers usually consist of a powdered mixture of the drug with a carrier, packaged into devices designed for rapid inhalation by the patient. In recent times, and especially after the success of Advair®[32], dry powder inhalers have emerged as an excellent alternative to pMDIs and nebulizers. Being breath actuated, DPIs do not need coordination between patient's breathing cycle and actuation to function properly. Due to lack of any liquid propellants, DPIs offer an advantage of increased drug formulation stability, and hence are more suitable for delivery of delicate molecules such as proteins[33]. The recent launch of inhaled insulin (Afrezza®) in a DPI format is an excellent example[4]. A typical dry powder inhaler formulation consists of a jet milled active pharmaceutical ingredient mixed with a carrier excipient, mostly commonly, lactose[33,34]. Carrier excipients are used to avoid aggregation of micronized drug particles.

Dry powder inhalers can be classified based upon the type of drug reservoir or their actuation mechanism. Drug reservoir type can either be single dose or multiple dose, while actuation mechanism can be either passive or active[33]. Spinhaler® for cromolyn sodium and Rotahaler® for albuterol, both passive single dose inhalers with a similar working principle, were one of the first products to be introduced. Both consisted of a mechanism to pierce or open a capsule containing powder formulation. Upon inhalation by a patient, turbulence in the generated airflow caused formulation to exit the capsule and enter patient's airways[35].

Passive multiple dose DPIs consist of drug reservoir designed to hold up to 100 blistered doses and are meant to increase patient convenience[35]. Advair Diskus®, which contains 60 blistered doses of bronchodilator and corticosteroid, is the best example of a multidose DPI, and has attained 'blockbuster' status many times over[33]. Active DPIs were developed to overcome variability introduced by patient's capacity to inspire. A good example is Pfizer's Exubera®, which was the first commercial inhalable insulin product. Its mechanism consisted of a compressed air pump, which dispersed formulation from a blister into a chamber attached to a mouthpiece, allowing patient to inhale insulin by tidal breathing[35,36]. Similar to ultrasonic nebulizers, some DPIs use vibrational energy produced by a piezoelectric element to disaggregate the drug prior to inhalation by the patient (e.g. MicroDose® inhaler)[32,37].

1.3.3 Pressurized metered dose inhalers

Pressurized metered dose inhalers were first introduced in 1956, and quickly became the 'gold standard' in asthma and COPD treatment due to their size, apparent ease of use, and robustness[38]. From the 1950s to late 1990s, chlorofluorocarbons (CFCs) were the propellants of choice in formulation of solution and suspension-type pMDIs[39]. However, after evidence suggested that the ozone layer was being harmed by medical and commercial use of CFCs, the Montreal Protocol was adopted in the 1990s, setting up a timetable for their phase-out[40]. In order to comply with the protocol, the pharmaceutical industry had to come up with not only new propellants to replace CFCs, but also new hardware amenable to changes in formulation technology. This was a challenge to formulation scientists, but also an opportunity to introduce improvements in pMDI structure and function[41].

Since the 1990s, hydrofluoroalkanes 134a and 227 (HFA-134a and HFA-227, respectively) were introduced as replacements for CFCs for formulation of solution and suspension pMDIs[42]. In inhaler hardware, improvements in valve and canister design were realized. CFC formulations were notorious for producing poor dosing consistency at the end of a canister's life. This was addressed in HFA formulations by introducing changes in valve design and material. This led to development of products showing greatly improved dosing uniformity regardless of storage orientation of the canister[41].

Currently, a large number of solution and suspension pMDI formulations are on the market. QVAR®, a solution formulation of beclomethasone dipropionate is an example of HFA technology improving pMDI performance because QVAR is capable of producing

aerosols with a MMAD as low as 1.0 µm, leading to better lung deposition and reduced wastage at the oropharynx[43]. Main components of a pMDI are depicted in Figure 1.2.

1.3.3.1 Structure of a pMDI

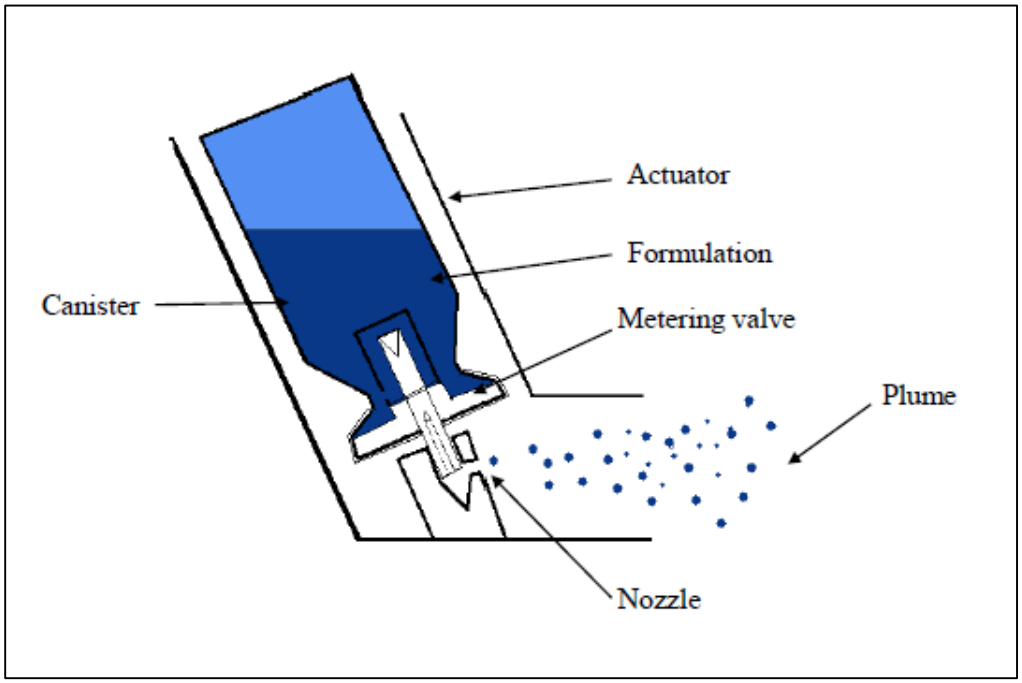


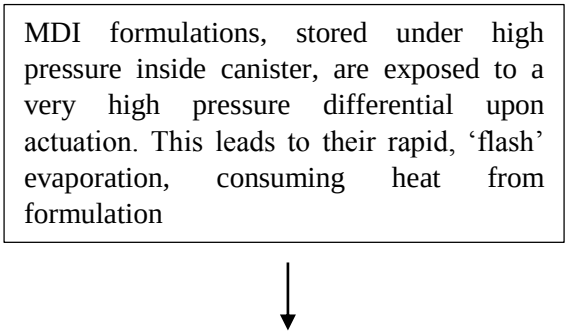
Figure 1.2: Main components of a pMDI- Canister, metering valve and actuator with nozzle (Adapted from Respiratory Drug Delivery: Essential Theory and Practice; Chapter 6)

Pressurized metered dose inhalers are a highly complex drug delivery system, as their function is dependent upon interplay of formulation, device and patient variables. In its simplest form, a pMDI consists of a canister containing a formulation, fitted with a metering valve and an actuator with a nozzle. The formulation may be a solution or suspension depending upon the API intended for delivery. Excipients such as ethanol and other surfactants can be used to enhance solubility or suspendability of the drug. Formulation and device variables affect the particle size of the aerosol being produced[44].

A higher percentage of HFA-134a means a higher vapor pressure in the canister, which translates to lower particle size[45,46]. Additives, such as ethanol, tend to decrease vapor pressure of the formulation and alter droplet evaporation behavior. Choice of nozzle diameter, which is often dependent on the type of formulation, also has a direct impact on particle size distribution of aerosols. QVAR®, a solution formulation containing ethanol, is able to produce 'softer', extra-fine aerosols due to a 0.30 mm nozzle[43,47] producing high shear in combination with a lower vapor pressure formulation. On the other hand, Ventolin®, which is a suspension of albuterol sulfate in HFA-134a, uses a 0.5 mm diameter nozzle[48] in order to avoid clogging by suspended particles, yet still produces an acceptable aerosol due to its relatively higher vapor pressure.

1.3.3.2 Spray formation from a pMDI- Primary and secondary atomization

Evolution of a spray from a metered dose inhaler is a highly complex process, which is notoriously hard to study due to its transient nature. Spray formation from a metered dose inhaler can be divided into primary and secondary atomization. The following flow chart depicts important stages in development of a plume from a formulations stored under pressure in a canister. Depiction shown in Figure 1.3 summarizes findings from separate studies on pMDI spray formation by Clark and Dunbar[49-51].



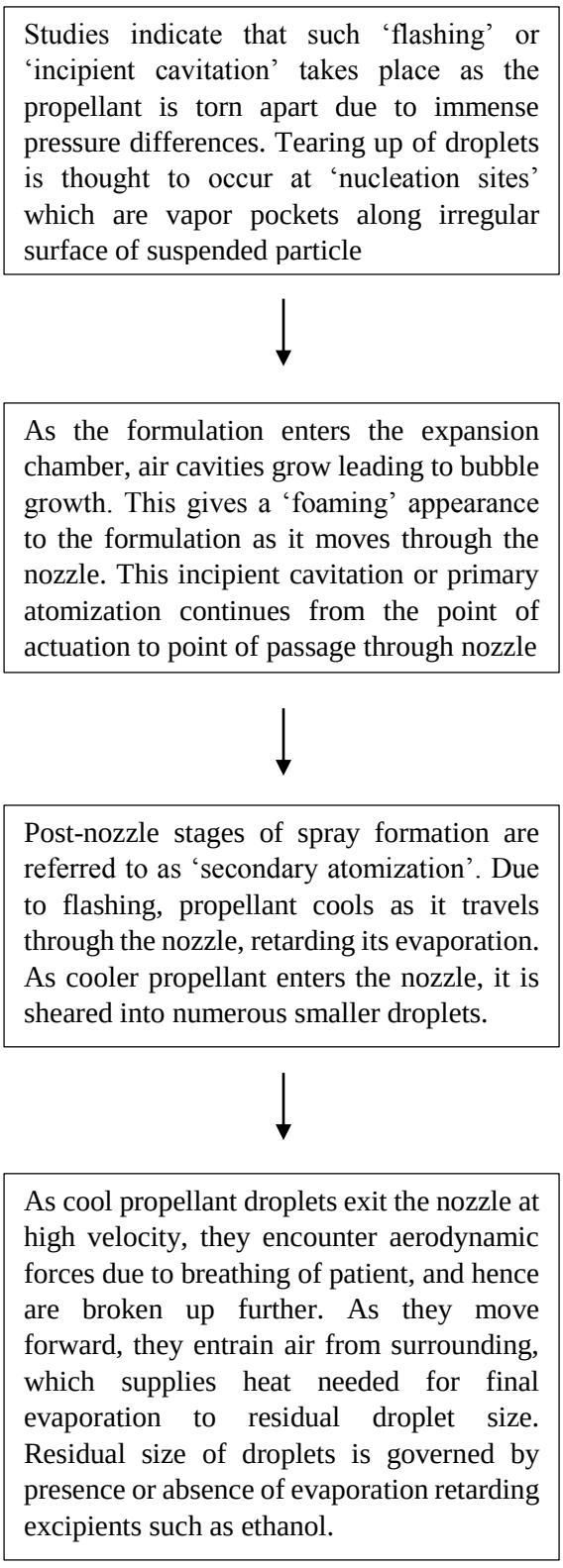


Figure 1.3: Primary and secondary atomization from a metered dose inhaler

Imaging studies on spray formation by pMDIs conducted by Versteeg et al. clearly revealed the process of flash evaporation and 'foaming' by bubble formation in the expansion chamber of an MDI actuator[52]. Original research described in this dissertation investigates the effect of dissolved carbon dioxide (CO_2) in HFA-134a based solution and suspension formulations on droplet formation and evaporation behavior and the resulting aerosol particle size. It might be hypothesized that the process of cavitation by nucleation may be enhanced due to presence of dissolved CO_2 in propellant, thus affecting residual particle size.

1.3.4 Previous research on use of HFA-134a/dissolved gas as propellant for pMDI formulation

Since their invention, a formulator working on pMDI formulations has had a limited number of tools to influence the final particle size of the aerosol produced. The most widely used approach is varying the percent of propellant (typically HFA-134a or HFA-227) by dilution with ethanol. Any variation in composition of propellant system leads to a change in vapor pressure, which ultimately affects the particle size distribution of the aerosol droplets[46]. The use of compressed gases such as CO_2 and a solvent system in place of liquefied HFAs (with or without ethanol) is an attractive option to pressurize formulations. However, this option suffers a gradual loss of canister pressure during emptying[44], and the inability to benefit from fast droplet size reductions due to flash evaporation of solvents (which have a lower vapor pressure than liquefied propellants).

Keller and Herzog, in US Patent # 6461951 B1, have described solution and suspension pMDI formulations prepared using a combination of liquefied HFA-134a, HFA-227 and dissolved CO_2. The resulting formulations exhibited smaller particle size

distribution, wettability and stability. The patent proposes that increased vapor pressure in the container headspace due to addition of CO_2 is responsible for these improved properties. The patentees do not address the possible influence of CO_2 dissolved in liquefied HFA-134a on sprayed particle size[53].

This dissertation proposes a novel method of producing a HFA/CO_2 combination propellant system suitable for use in pressurized metered dose inhalers and investigates the mechanism of droplet size reduction that results from dissolving CO_2 in HFAs. Because a gas is dissolved in a liquefied propellant under pressure, much as carbon dioxide is dissolved in a soda, we have come to refer to the aerosol formulations we investigated as "effervescent aerosol". Various investigative experimental methods such as laser diffractometry, cascade impaction studies, and spray characterization methods have been conducted. Research described in this dissertation served the following specific aims.

1.4 Specific aims

1.4.1 Specific aim 1- To develop novel, 'effervescent' HFA-134a/CO_2 combination propellant based solution inhaler formulations and to perform laser diffractometry experiments to determine volume diameter of the resulting spray.

Using a custom designed pressure vessel, a novel method of producing 'effervescent' HFA-134a/CO_2 combination propellant based solution inhaler formulations has been described. Beclomethasone dipropionate (BDP) has been used as a model drug, with ethanol being the only other excipient. The pressure vessel used for producing these formulations has been designed so that a continuous plume of aerosol can be produced using a nozzle of desired diameter. The setup is amenable to laser diffractometry by

Malvern Spraytec® (Malvern Instruments, Malvern, UK) in order to measure volume diameter of the droplets. Appropriate pressure-controlled, analogous formulations containing HFA-134a and insoluble helium have been used to compare differences in volume diameters of droplets produced by the two types of formulations. An evidence based model for HFA-134a droplet size reduction due to dissolved CO_2 has been proposed.

1.4.2 Specific aim 2- To translate the novel, 'effervescent' propellant system concept to solution and suspension aerosol formulations filled intro canisters appropriate for patient use and to conduct laser diffractometry experiments following spraying from metering valves

In order to make effervescent aerosols commercially viable, it is necessary to translate novel HFA-134a/CO_2 combination propellant based formulations from an expensive, heavy and cumbersome pressure vessel to 19 ml aluminum canisters, widely used in commercialized pMDI products. A filling line using Pamasol 2016 (Pamasol Willi Mäder AG, Switzerland) propellant filler and an intermediate stage to mix HFA-134a and CO_2 has been designed and successfully used. Along with BDP solution formulations, suspension formulations containing jet-milled albuterol sulfate (AS) have been prepared. Further, laser diffractometry experiments with Spraytec using an inhalation cell configuration have been conducted to investigate the effects of formulation and device variables on *in vitro* inhaler performance.

1.4.3 Specific aim 3- To conduct Andersen Cascade Impactor tests on various solution and suspension formulations in order to determine critical quality attributes

Cascade impaction studies using Andersen Cascade Impactor (ACI), as prescribed in the United States Pharmacopeia (USP), have been performed on solution and suspension-type pMDI formulations. APSD, MMAD, fine particle fraction (FPF) and fine particle dose (FPD) have been calculated. Other compendial studies such as inhaler performance at beginning (B stage), middle (M stage), and end (E stage) have also been conducted.

1.4.4 Specific aim 4- To conduct spray characterization studies on 'effervescent' solution and suspension-type inhalers.

Spray characterization studies utilizing impaction force testing and plume geometry techniques have been conducted on novel 'effervescent' solution and suspension-type metered dose inhalers.

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Chapter 2: Formulation of novel, 'effervescent' solution aerosol formulations using a custom-designed pressure vessel, subsequent laser diffractometry experiments, and elucidation of mechanism of droplet size reduction[A]

2.0 Introduction

Effervescence is the escape of gas from a liquid accompanied by foaming and hissing. A soda can, which contains CO_2 mixed with flavored water, is a good daily life example. Upon opening of a soda can, bubbles of CO_2 can be seen rising and bursting at the surface, giving rise to a fine mist of water droplets. Bursting CO_2 bubbles are clearly able to produce droplets from the surface of flavored water bulk.

In this part of the dissertation, we have explored the application of the observation above to droplet size reduction due to CO_2 induced effervescence in HFA-134a based solution pMDI formulations[1]. A custom-designed pressure vessel, capable of holding HFA-134/CO_2 based formulations under constant pressure, and spraying it continuously from a nozzle of desired diameter, has been used for these proof-of-concept experiments.

Malvern Spraytec, a state-of-the-art laser diffractometry instrument, has been used to measure volume diameter of the aerosol produced by the 'effervescent' formulations[2]. Pressure-controlled formulations, containing HFA-134a and insoluble helium, but otherwise similar to test formulations, have also been prepared and tested for volume diameter of aerosol produced. A hypothetical mechanism for observed droplet size reduction due to dissolved CO_2 has also been proposed and discussed.

[A]Kelkar, MS, Dalby, RN: Effervescent aerosols: A novel formulation technology for pressurized metered dose inhalers. In *Respiratory Drug Delivery 2014. Volume 3.* DHI Publishing; River Grove, IL: 2014: 669-672.

2.1 Hypothesis

Particles produced by solution aerosol formulations containing HFA-134a/CO_2 combination propellant have lower volume diameter values than those produced by an analogous, pressure-controlled negative control formulation due to the effervescence caused by CO_2.

2.2 Materials

A custom-designed pressure vessel was purchased from Parr Instrument Co. (Moline, IL). The pressure vessel (depicted in Figure 2.1) consists of a chamber to hold a formulation, a pressure gauge to monitor the pressure and an inlet to supply CO_2 during formulation. The formulation can be sprayed through a dip tube fed outlet to which a nozzle can be attached. The formulation grade HFA-134a and CO_2 was purchased from Airgas Co. (Radnor, PA). 200 proof ethanol and beclomethasone dipropionate (BDP) were sourced from Sigma-Aldrich (St. Louis, MO). Spraying nozzles were acquired from AeroMist (Phoenix, AZ). Table 2.1 lists all types of BDP solution formulations prepared.

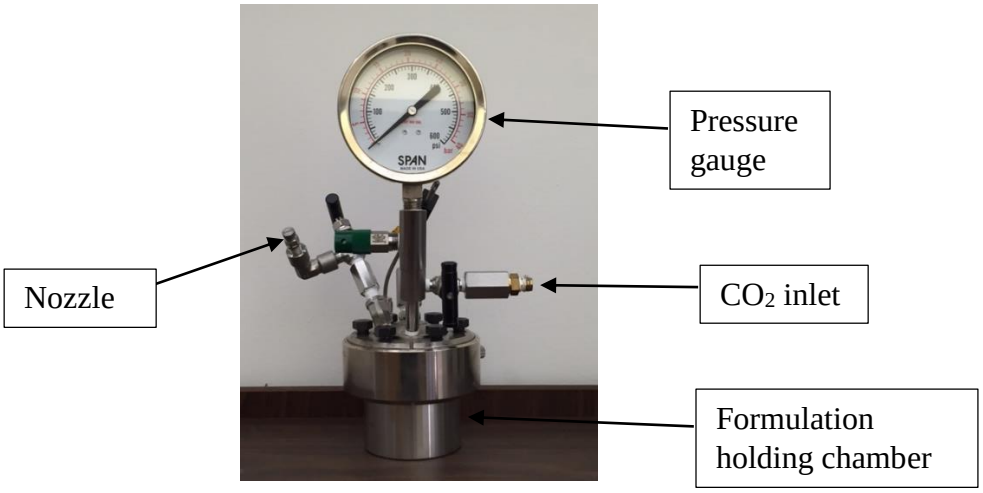


Figure 2.1: Pressure vessel used to prepare HFA-134a/CO_2 combination formulations

2.3 Methods

2.3.1 Preparation of formulations

ID	HFA-134a (% w/w)	Ethanol (% w/w)	Beclomethasone dipropionate (% w/w)	Final formulation pressure with CO_2 or Helium
A	**89.975%**	**10%**	**0.025%**	**140 psi**
B	**89.950%**	**10%**	**0.050%**	**140 psi**
C	**89.920%**	**10%**	**0.080%**	**140 psi**

Table 2.1: Composition of 3 HFA-134a/CO2 based solution aerosol formulations- Table 2.1 shows the composition of 3 HFA-134a/CO_2 based solution aerosol formulations prepared and sprayed using the custom-designed pressure vessel

For formulation A, initially, BDP equivalent to 0.025% w/w and ethanol equivalent to 10% w/w of the final formulation were added to the pressure vessel. This was followed by the addition of HFA-134a by the cold-filling method[3]. The cold-filling method involved the use of a mixture of dry ice and acetone to cool the pressure vessel's holding chamber below the boiling point of HFA-134a then pouring in a pre-weighed quantity of HFA-134a. The pressure vessel was then sealed off immediately to prevent loss of propellant by evaporation. The vessel was then allowed to equilibrate to room temperature (RT). As the temperature rose, the liquid HFA-134a partially turned to vapor and started exerting its vapor pressure, which was recorded by the pressure gauge. Equilibration was judged to

have happened when the pressure gauge showed a pressure of approximately 80 psi, the native vapor pressure of HFA-134a at room temperature.

After equilibration was attained, the vessel was connected to a continuous supply CO_2 cylinder. The CO_2 was supplied at a constant pressure of 140 psi and the gas was in direct contact with the liquefied HFA-134a. Prior to this experiment, a pressure of 140 psi was judged to be the minimum pressure necessary to achieve a continuous aerosol plume using this setup. CO_2, being highly soluble in HFA-134a[4], was allowed to dissolve and saturate the liquid formulation, aided by manual mechanical shaking. Saturation was confirmed to have happened when the internal pressure of the vessel was stable even after cutting off the CO_2 supply. A 0.6 mm spray nozzle was then screwed on to the dip tube fed exit valve. Such a formulation was deemed to be ready for spraying, and analysis using Spraytec. Formulations B and C were also prepared in a similar manner. Identical, pressure-controlled formulations using insoluble helium (He) instead of CO_2 were prepared in a similar fashion. Figure 2.2 depicts the process of saturation of HFA-134a by CO_2. By the virtue of being insoluble in HFA-134a, He almost immediately attains equilibrium. The next section, with help of Figure 2.3 explains functionality of Malvern Spraytec, used in all experiments described in this chapter.

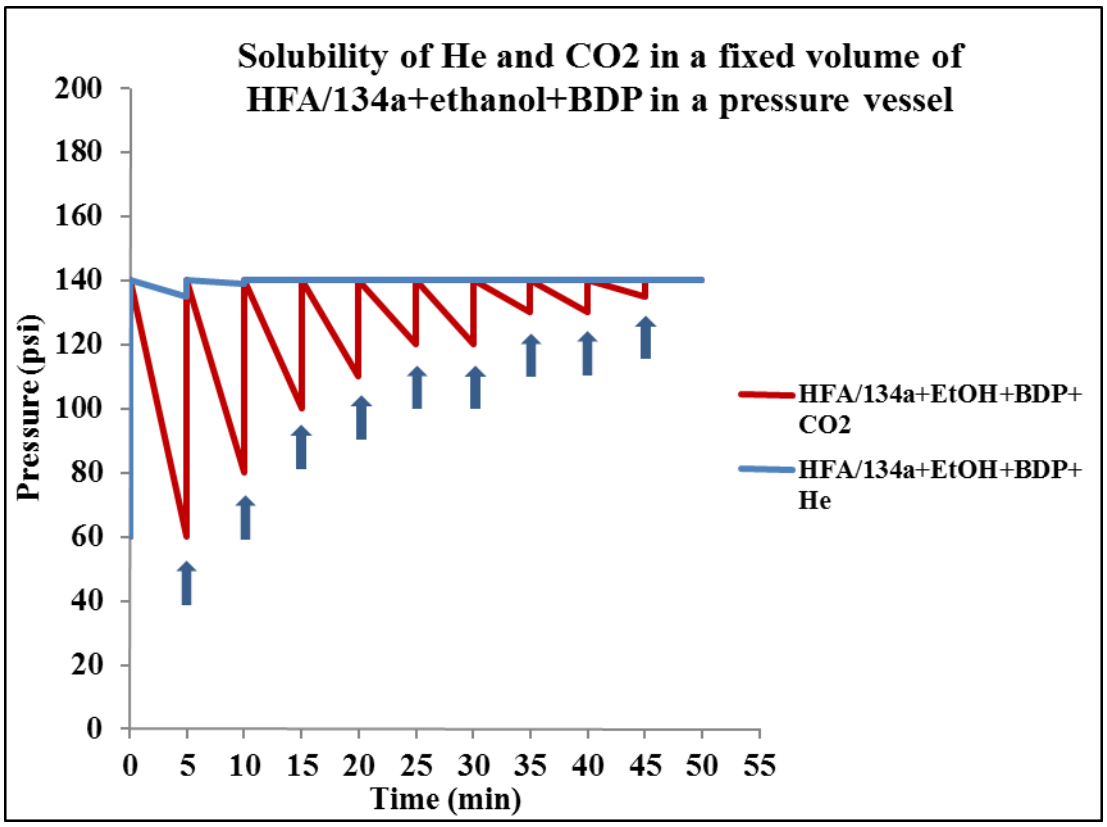


Figure 2.2: Process of saturation of HFA-134a by CO_2- Figure 2.2 depicts the process of saturation of HFA-134a by CO_2 using mechanical shaking. Blue arrows show the point of addition of CO_2. When CO_2 supply is turned on, a pressure of 140 psi is attained almost immediately. Upon closing of supply and mechanical shaking, the CO_2 gradually dissolves in liquid HFA-134a and pressure falls. Upon 9-10 addition and shaking cycles, the HFA-134a begins to saturate, and a point is reached when cutting off CO_2 does not alter internal vessel pressure. In case of He, saturation occurs almost immediately

2.3.2 Malvern Spraytec®

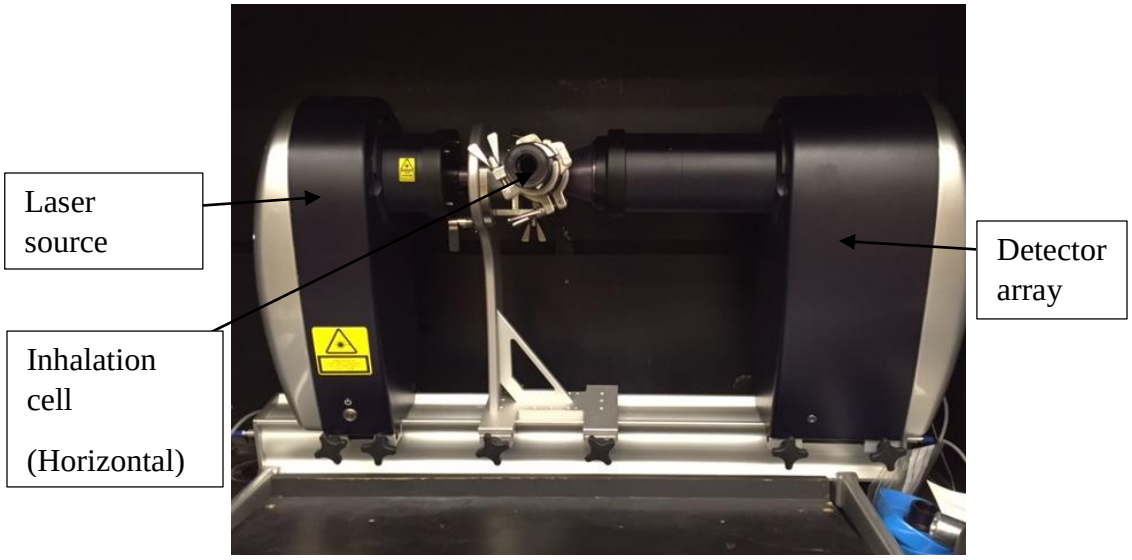


Figure 2.3: Malvern Spraytec with inhalation cell attachment in horizontal configuration

The Spraytec is a state-of-the-art laser diffractometry system meant for volume diameter measurements of sprayed particles. Its structure consists of a laser source, an inhalation cell attachment (for metered dose inhaler measurements) and a detector array (containing 36 concentric detectors), all aligned on an optical bench (Spraytec manual).

When a sample is sprayed in the measurement zone, the laser hits the particles and diffracts at various angles. The complex diffraction pattern generated by thousands of particles is detected by the detector array. Using an algorithm, the Spraytec system is able to create a particle size distribution chart for the sprayed material. The Spraytec system is operated in either a continuous mode, which is ideal for continuous sprays or in a rapid mode, which is ideal for pMDI measurements. For the purpose of this study, the Spraytec was operated in continuous mode. Figure 2.4 describes a Spraytec method setup.

2.3.3 Spraytec method setup

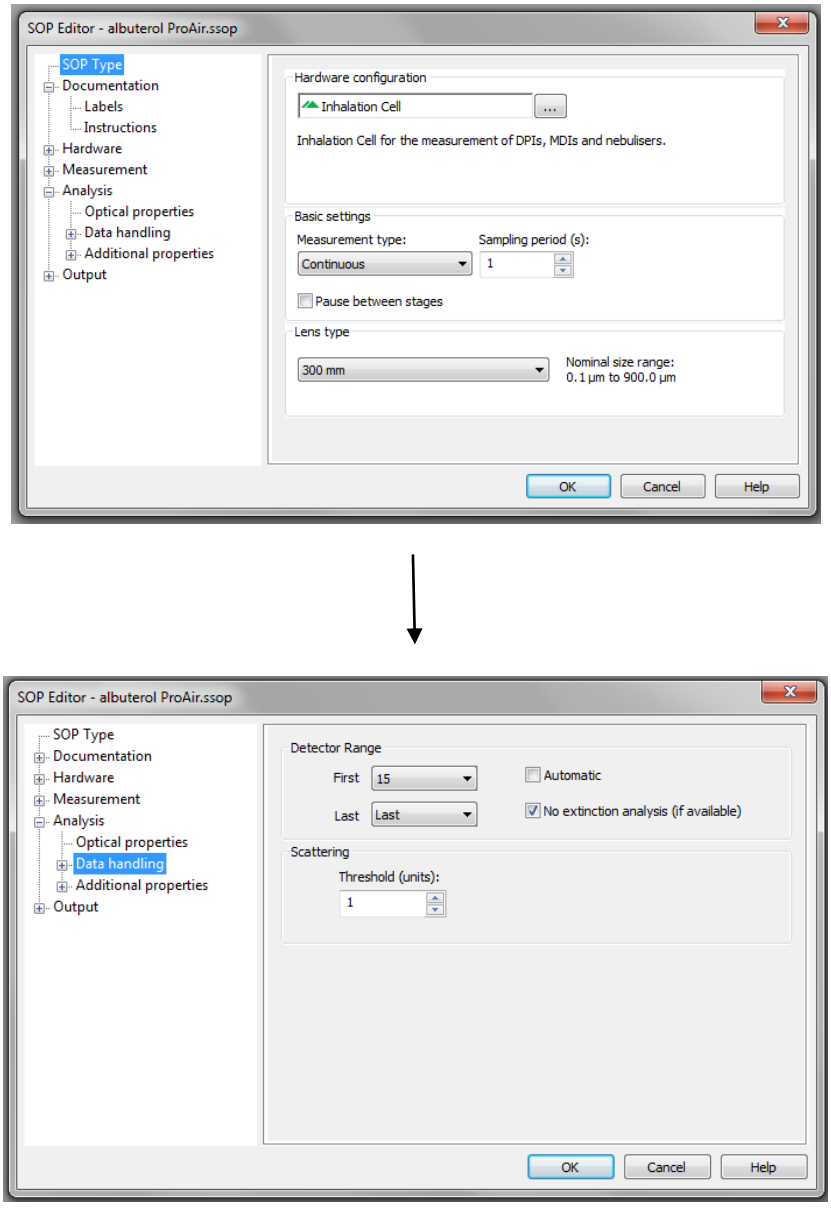


Figure 2.4: Spraytec method setup for a horizontal inhalation cell configuration used for the proof-of-concept experiments. Beam steering adjustment has been done

For experiments conducted with the custom-designed pressure vessel, the continuous mode of Spraytec was used, which allows data collection throughout the

duration of spray. A horizontal configuration of inhalation cell was used to allow direct entry of sprayed particles into the laser measurement zone.

A problem which arises when sizing aerosols dispersed in evaporating propellant vapor is beam steering. Beam steering occurs when swirling currents of propellant vapor in air lead to a constantly changing and inhomogeneous background, which complicates laser diffractometry from sprays generated from some liquefied gas based formulations; including those based on HFA-134a. When the laser enters a measurement zone filled with vaporized HFA-134a, it encounters an environment with an unstable refractive index locally different than air. This difference in refractive indices leads to the beam being 'steered'. The steered beam hits detectors corresponding to a larger particle volume diameter, which leads to phantom readings for large size particles (Beam steering explanation from Spraytec application note). In order to adjust for the error that may be caused due to beam steering, Spraytec User's Manual has suggested taking the first 12-15 detectors offline. In this experimental setup, first 14 detectors were taken offline to prevent the occurrence of phantom peaks, which might skew particle size distribution (Figure 2.4). The next section (Figure 4.5) describes an experimental setup for laser diffractometry on aerosols produced by the pressure vessel.

2.3.4 Setup for actuation of experimental HFA-134/CO_2 based solution formulations

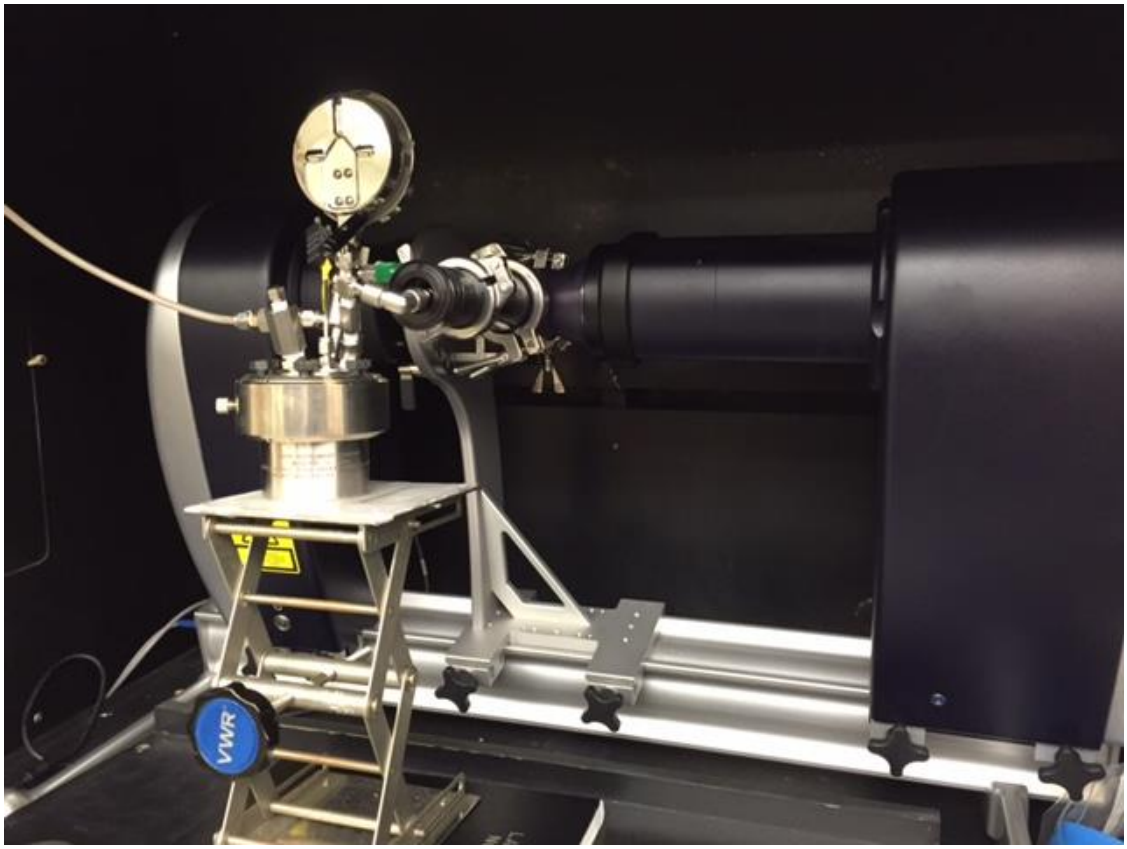


Figure 2.5: Pressure vessel setup for laser diffractometry experiments using Spraytec

The pressure vessel containing HFA-134/CO_2 based solution aerosol formulations was placed on an elevated platform such that its nozzle was aligned with the entrance of the horizontal inhalation cell. A pressure vessel was connected to a CO_2 or He cylinder source in order to maintain constant pressure during the process of actuation. A continuous plume was obtained by turning the tap connected to the dip-tube. All runs were performed in triplicate in order to gather enough data for statistical analysis.

2.3.5 Key metrics obtained from this study

In a laser diffractometry experiment using Spraytec, the objective is to determine particle size distribution based upon volume diameter of the particles. Volume diameter might be defined as the diameter of a sphere that has the same volume as a given particle[5]. Spraytec also calculates derived parameters such as Dv (50), Dv (10), and Dv (90). Dv (50) is the median volume diameter value i.e. 50% of all measured particles have a volume diameter value lower than Dv (50). Similarly, 10% and 90% of total measured particles have a volume diameter value lower than Dv (10) and Dv (90) respectively.

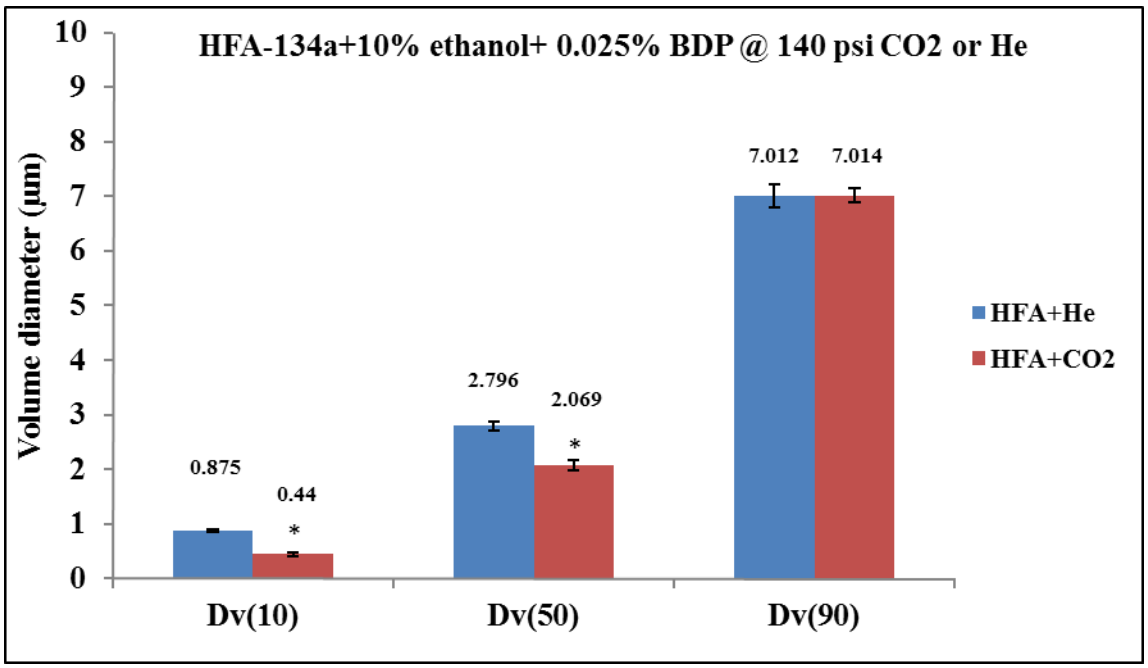
2.3.6 Statistical analysis

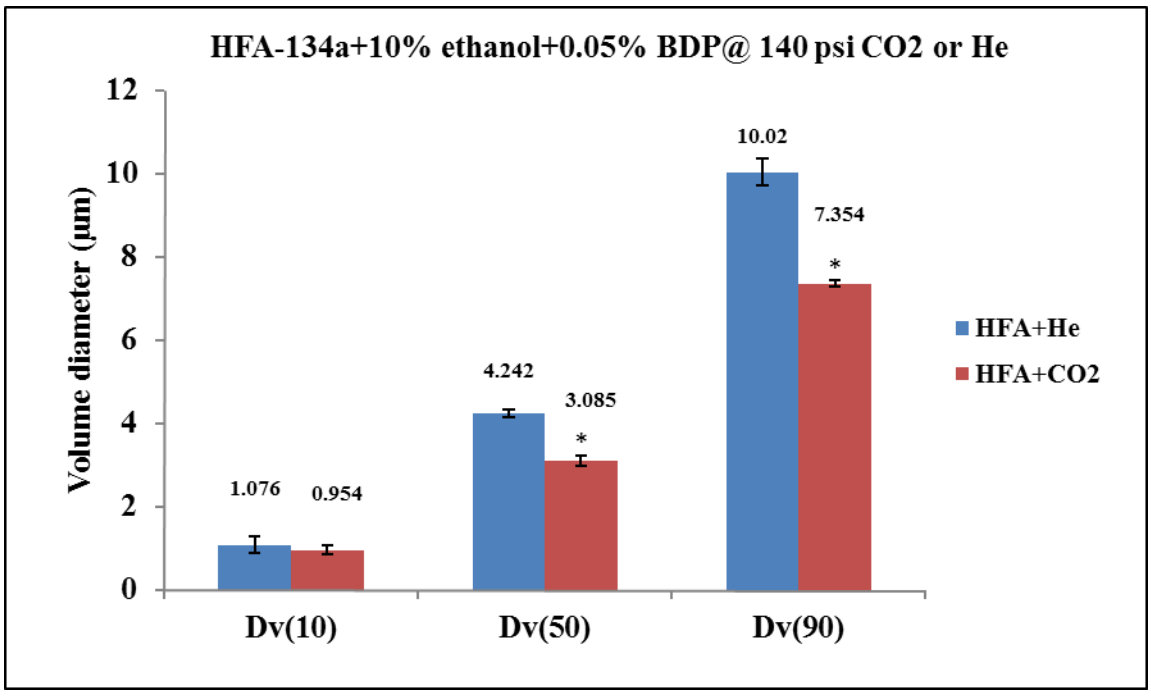
Values of Dv (50) ± SD, Dv (10) ± SD and Dv (90) ± SD data obtained from test and control runs were compared using Student's t-test. A difference was deemed significant if $p < 0.05$. Statistical significance was denoted by asterisk (*).

2.4 Results and discussion

2.4.1 Dv (10), Dv (50), and Dv (90) values obtained from test and control experiments

Figure 4.6 and Table 2.2 shows data obtained from proof of concept experiments.





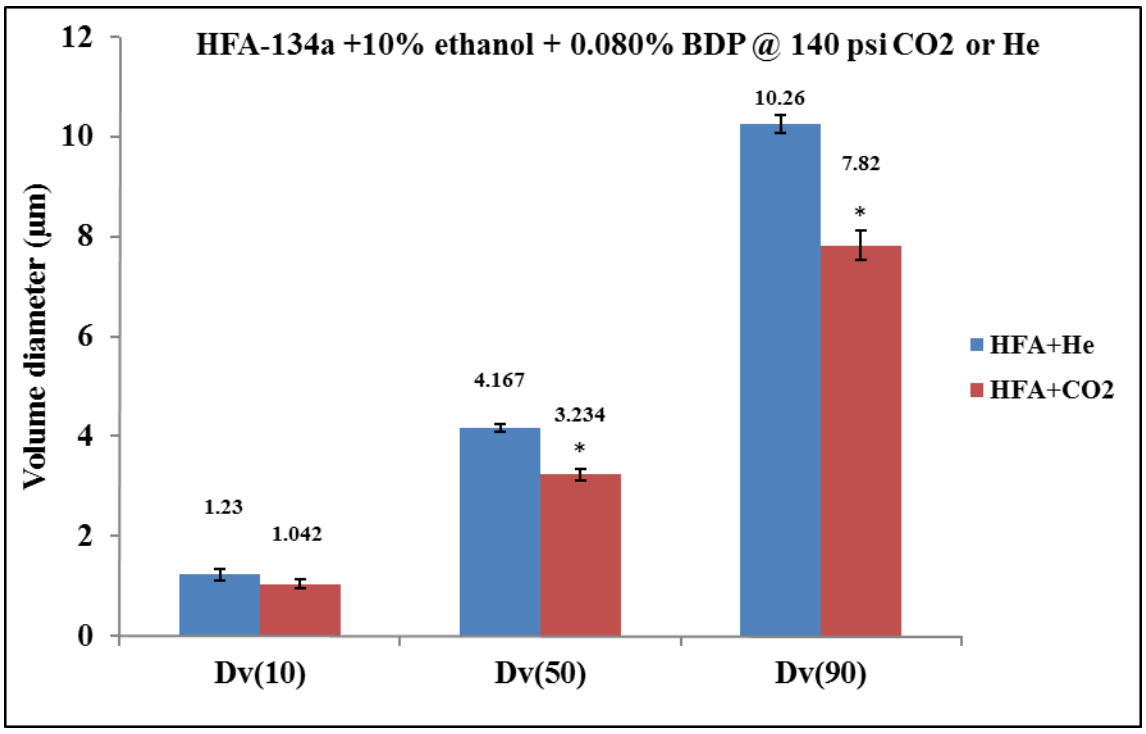


Figure 2.6: Dv (10), Dv (50) and Dv (90) values obtained from proof of concept experiments- Above graphs represent Dv (10), Dv (50) and Dv (90) values obtained by spraying HFA-134a/CO_2 and HFA-134a/He based solutions containing three different concentrations of BDP. All runs were performed in triplicate and statistical analysis was done using t-test. Error bars denote SD. * denotes $p < 0.05$

BDP concentration	Formulation	Particle Size (µm)		
		Dv(10)± SD	Dv(50)± SD	Dv(90)± SD
0.025% w/w	HFA-134a-He	0.88 ± 0.03	2.80 ± 0.08	7.01 ± 0.20
	HFA-134a-CO_2	0.44 ± 0.04	2.07 ± 0.10	7.01 ± 0.12
0.05% w/w	HFA-134a-He	1.08 ± 0.19	4.24 ± 0.09	10.02 ± 0.32
	HFA-134a-CO_2	0.95 ± 0.11	3.09 ± 0.12	7.35 ± 0.06
0.08% w/w	HFA-134a-He	1.23 ± 0.12	4.17 ± 0.08	10.30 ± 0.18
	HFA-134a-CO_2	1.04 ± 0.10	3.23 ± 0.11	7.82 ± 0.29

Table 2.2: Summary of Dv (10), Dv (50) and Dv (90) values- Table 2.2 lists all volume diameter values from the proof-of-concept experiments

It is apparent that BDP solution formulations prepared using HFA-134a/CO_2 combination propellant, and sprayed using the custom-designed pressure vessel, produce finer aerosols than control formulations prepared using HFA-134a/He (Figure 2.6).

In the case of formulation containing 0.025% w/w BDP, significantly lower Dv (10) and Dv (50) values are observed in the CO_2 batches. The overall particle size range for this formulation is understandably lower due to a lower drug concentration. For formulations containing 0.05% and 0.08% w/w BDP, median volume diameter and Dv (90) for CO_2 batches is significantly lower than corresponding He batches. A higher overall particle size is also observed compared to 0.025% w/w formulation, presumably due to increased drug concentration.

Despite being formulated at a same pressure of 140 psi, CO_2 based batches produce finer aerosols than He based batches. This strongly suggests that not only increased vapor pressure, but the dissolved CO_2 component in HFA-134a might have an effect on droplet evaporation behavior.

In this study, a 0.6 mm nozzle, which is not a typical nozzle diameter for marketed preparations, has been used. It is notable that despite using a larger diameter nozzle for spraying, particles with diameters considered suitable for inhalation have been produced. A low diameter nozzle (0.3 mm)[6], which is used in marketed solution preparations such as QVAR®, could not be used in this setup because it greatly increases the minimum pressure required to operate the pressure vessel. However, it may be surmised that if such a HFA-134a/CO_2 based BDP solution formulation is translated to a 19ml aluminum canister, and actuated using a 0.3 mm actuator, a great improvement in particle size might be seen.

Median particle size data obtained from test and control formulations across all three BDP concentration levels (Figure 2.7). Figure 2.8 depicts a representative particle size distribution, along with transmission data and derived parameters obtained from a Spraytec run.

In the next section, we propose a mechanism by which 'effervescence' in HFA-134a droplet due to dissolved CO_2 leads to its size reduction.

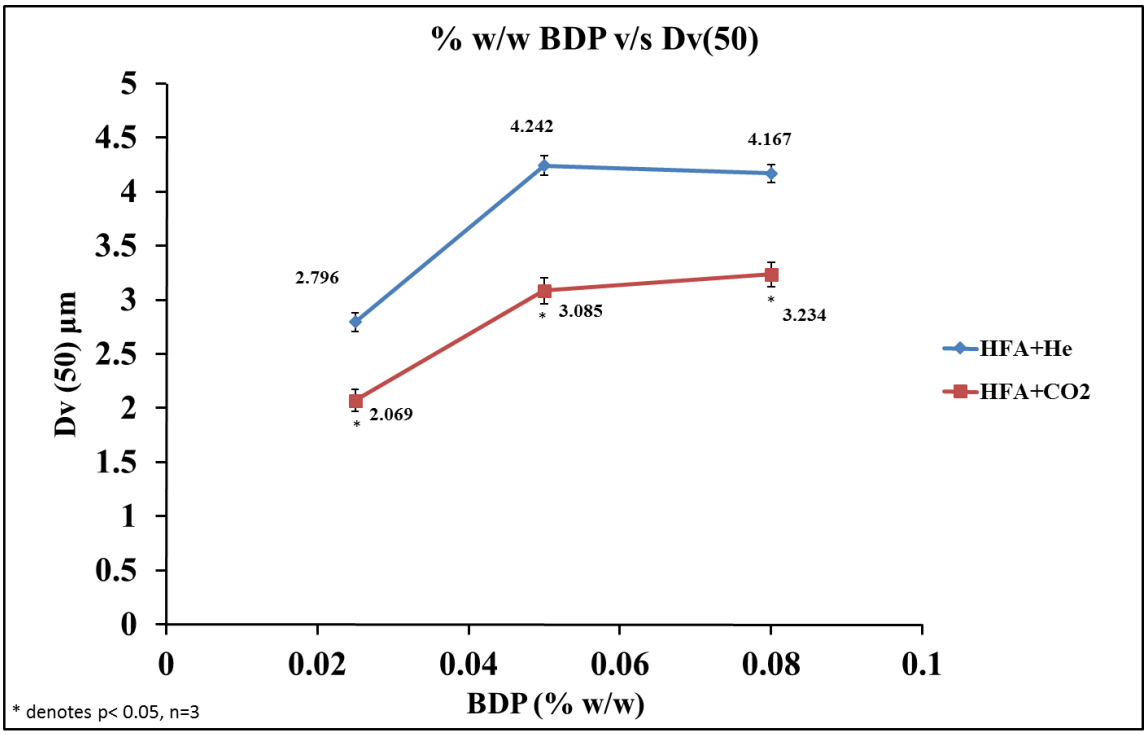


Figure 2.7: Dv (50) data of HFA-134a/CO_2 and HFA-134a/He batches obtained across 3 BDP concentration levels

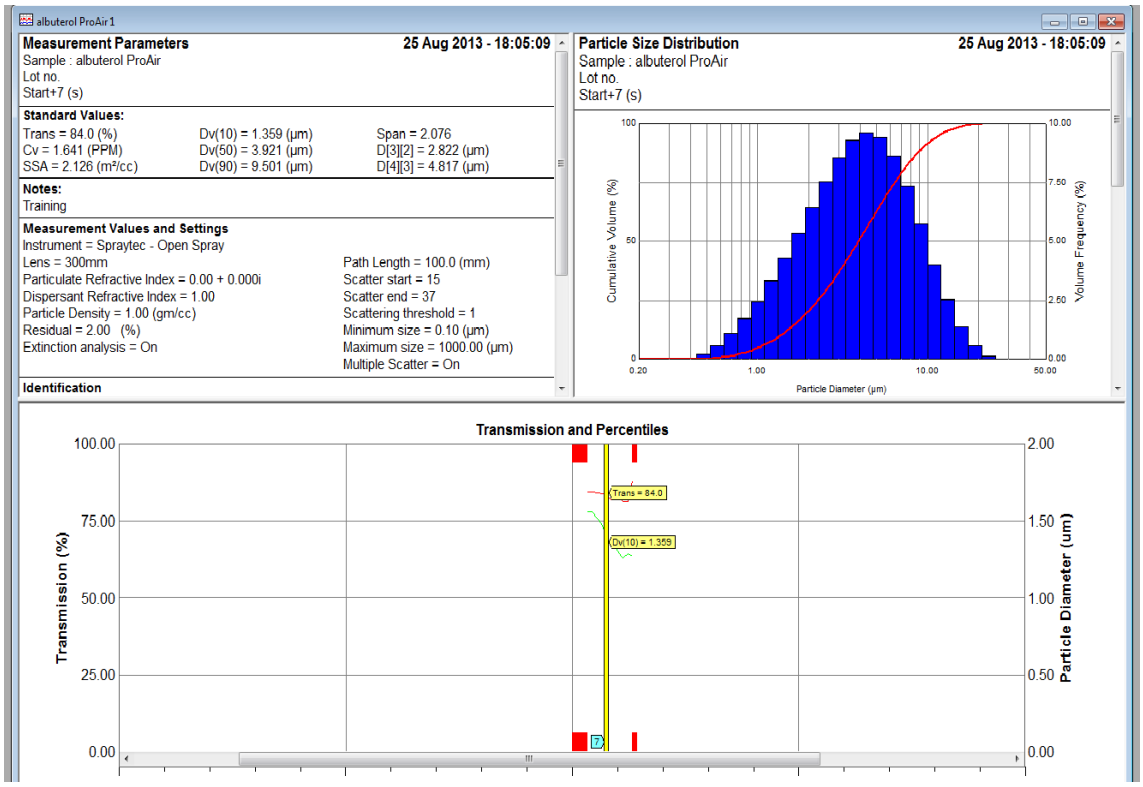


Figure 2.8: Bell-shaped particle size distribution curve obtained from Spraytec run- Figure 2.8 shows a typical log scale particle size distribution curve obtained from a Spraytec run. Output also includes laser transmission data. According to Spraytec user manual, for a run to be deemed successful, transmission value should be less than 95% i.e. obscuration should be more than 5%. Also obtained are derived parameters such as Dv values and span

2.4.2 Proposed mechanism of HFA-134a/ethanol droplet size reduction caused by dissolved CO_2

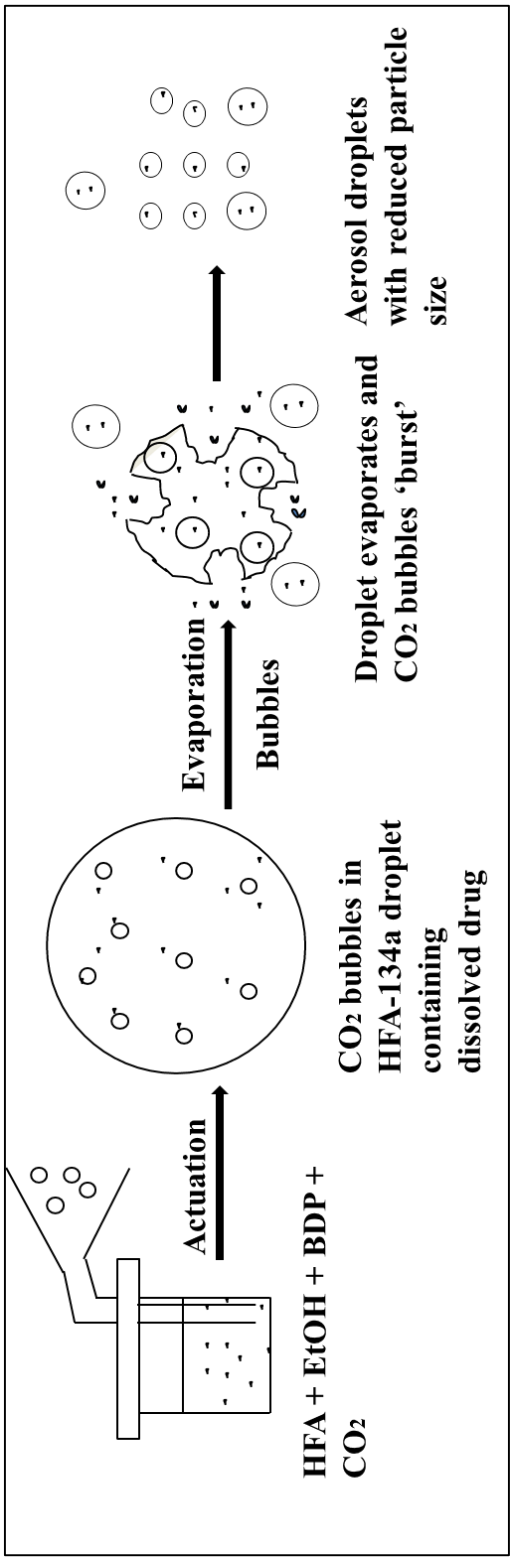


Figure 2.9: Proposed mechanism for size reduction of HFA-134a/ethanol droplet caused due to dissolved CO_2 component. We hypothesize that upon actuation, dissolved CO_2 forms bubbles inside the HFA droplet, which 'burst' to cause droplet size reduction. In the diagram, white circles represent CO_2 bubbles, while black dots represent drug particles

Figure 2.9 illustrates a hypothetical mechanism by which 'effervescence' caused due to CO_2 might bring about size reduction in a HFA-134a/ethanol droplet. We speculate that upon actuation of the solution formulation, dissolved CO_2 forms bubbles inside HFA-134a droplets as it equilibrates with a drastically lower atmospheric pressure. As the droplet moves further, its size is reduced by two occurrences- evaporation of HFA-134a and some ethanol, and rapid expansion and bursting of CO_2 bubbles. Expansion of CO_2 bubbles also causes mechanical fracturing of the droplet, giving rise to satellite droplets. As this is a BDP solution formulation, all satellite droplets can be expected to contain the drug. In the case of HFA-134a/He formulations, even though bubble formation is possible, it will be much lesser than HFA-134a/CO_2 formulations, due to difference in solubility of the two gases in HFA-134a.

We further speculate, that this formulation approach can be successful in suspension formulations, where 'effervescence' might play a role in keeping individual suspended particles discrete and prevent agglomerations upon evaporation of HFA-134a/ethanol droplet. However, a custom-designed pressure vessel experimental setup is not amenable to a proof-of-concept experiment for suspensions, as keeping the particles suspended by agitation is difficult due to structure of the pressure vessel.

2.5 Conclusion

In this chapter, a custom-designed pressure vessel has been used to prepare novel BDP solution formulations containing dissolved CO_2 in liquefied HFA-134a. Aerosols generated from such a formulation have been shown to be significantly finer than those generated from a HFA-134a/He based system.

In order to be commercially viable, it is crucial that 'effervescent' aerosol technology is translated to pMDI aluminum canisters fitted with metering valves. It is fortuitous that formulations prepared in the proof-of-concept experiments are pressurized at 140 psi, a pressure which can be easily contained in an aluminum canister at room temperature.

Studies described in the next chapter of this dissertation focus on translation of effervescent solution and suspension formulations to pMDI aluminum canisters. Translation to a canister-actuator format also opens up possibilities of further laser diffractometry, cascade impaction and spray characterization testing. It also makes it possible to introduce device variables by using actuators of various nozzle diameters.

2.6 References

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Chapter 3: Translation of 'effervescent' aerosol technology to metered dose inhaler format; formulation of solution and suspension-type metered dose inhalers, and laser diffractometry measurements using Spraytec [A, B]

3.0 Introduction

As discussed in Chapter 1, metered dose inhalers (pMDIs) have become the gold standard in asthma treatment due to their ease of use, robustness, and portability. Thus, it is crucial that any pMDI formulation technology in the proof-of-concept stage be successfully translated to the recognizable canister-actuator format.

In the previous chapter, a novel, 'effervescent' formulation technology for solution and suspension aerosols was introduced, in which a pressure vessel was used to prepare formulations based on a HFA-134a/CO_2 combination propellant system. However, in order to be commercially viable, it is essential that this technology be translated to standard aluminum pMDI canisters using a propellant filling machine. As this technology uses an extra ingredient, CO_2, it becomes necessary to modify the filling process to incorporate a stage for mixing of HFA-134a and CO_2.

In this chapter, a novel method to manufacture solution and suspension-type inhalers based on effervescent aerosol technology has been described. For the purpose of mixing of HFA-134a and CO_2, the use of an intermediate mixing stage between the

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[A]Kelkar, MS, Dalby, RN: Formulation of solution-type pressurized metered dose inhalers using effervescent aerosol technology. In *Respiratory Drug Delivery Asia 2014.* DHI Publishing; River Grove, IL: 2014: 229-32.

[B]Kelkar, MS, Dalby, RN: Formulation of suspension-type pressurized metered dose inhalers using effervescent aerosol technology. In *Respiratory Drug Delivery Europe 2015*. DHI Publishing; River Grove, IL: 2015: 493-96

HFA-134a source and the propellant filler has been described. Solution formulations containing BDP and suspension formulations containing AS have been prepared; containing varying concentrations of ethanol. Formulation variables include the concentration of API and ethanol, and CO_2 supply pressure to the mixing vessel in the filling line. The rationale behind varying CO_2 supply pressure is the hypothesis that increase in pressure of gas will affect its solubility in a liquid, which in turn might influence effervescent behavior.

Finally, laser diffractometry experiments, to determine impact of formulation variables on volume diameter of aerosol particles, have been performed. Volume diameter data of 'effervescent' aerosol samples has been compared against control samples containing HFA-134a only[1,2].

3.1 Materials

BDP and 200 proof ethanol were purchased from Sigma-Aldrich (St. Louis, MO) and AS and from Spectrum Chemical Mfg. Corp., USA. CO_2 was supplied by Airgas Co. (Radnor, PA). The propellant filling machine used was Pamasol 2016 (Pamasol Willi Mäder AG, Switzerland), and the pressurized mixing vessel used to mix CO_2 with HFA-134a was acquired from Alloy Products Corp. (Waukesha, WI). 19 ml pMDI canisters were sourced from Presspart (Lancashire, UK) and 50µl valves from Bespak (Norfolk, UK). A manual crimper (Type 555G; Pamasol Willi Mäder AG, Switzerland) was used to crimp valves onto canisters. A Jet-o-mizer 00 jet milling apparatus of Fluid Energy (Telford, PA) was used mill to AS to a desired particle size range. Particle size analysis of jet milled AS was performed using Horiba laser scattering instrument LA-910 (Horiba; Kyoto, Japan).

QVAR® (IVAX Laboratories, Miami, FL) and Ventolin® (GSK, UK) actuators were used to actuate solution and suspension-type formulations respectively.

3.2 Methods

3.2.1 Setup for mixing HFA-134a and CO_2 and filling of metered dose inhaler canisters

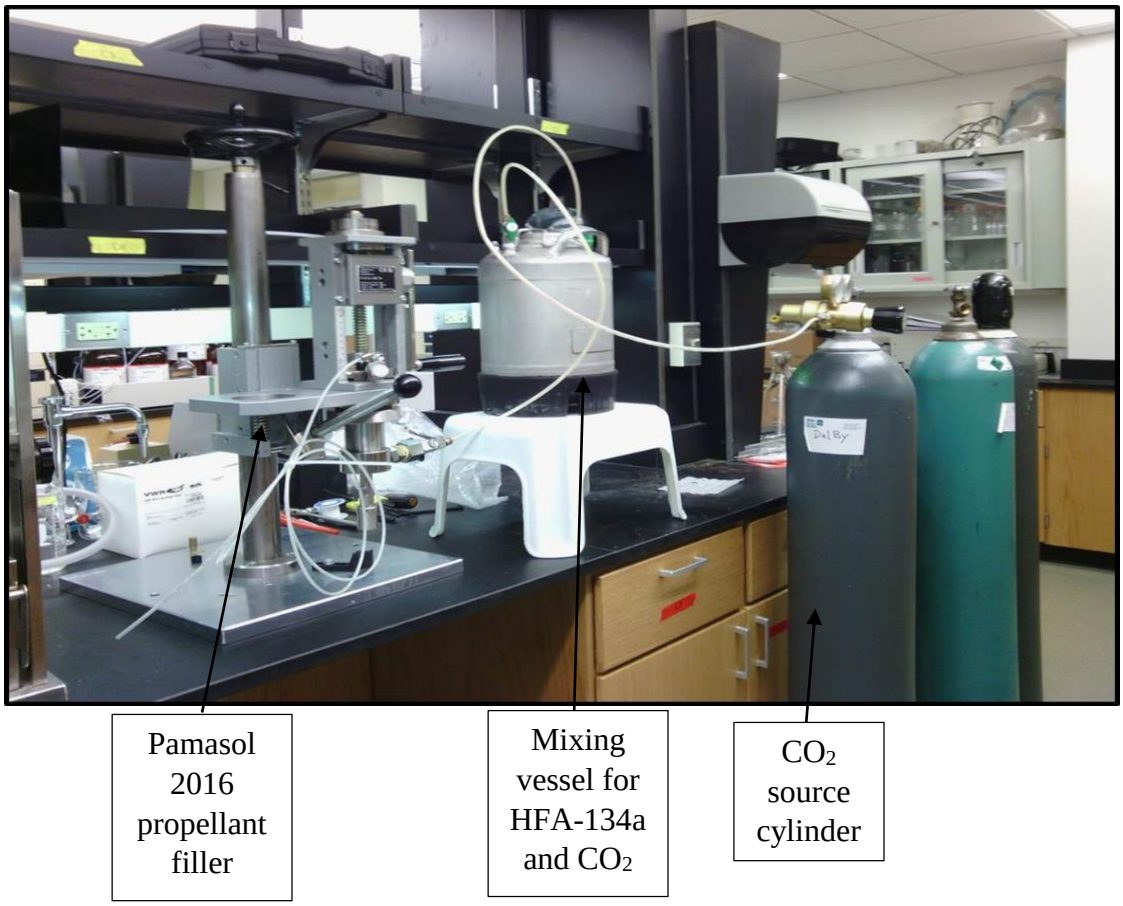


Figure 3.1: Filling line setup which incorporates a 'mixing' stage for HFA-134a and CO_2 using a pressure vessel. The dip-tube fed outlet of the vessel is connected to the propellant filler. Another inlet serves as entry point for CO_2

Figure 3.1 depicts a Pamasol 2016 propellant filler used to fill pMDI canisters. In a normal industrial operation, the propellant filler is directly connected to a HFA-134a source, but in this case, due to addition of an extra ingredient CO_2, a modification was

made in the filling assembly; a mixing stage in the form of a pressure vessel was added. The pressure vessel used consists of an entry port for CO_2, and a dip-tube fed exit which connects to the filling machine. The addition of HFA-134a to the pressure vessel was done using a combination of cold filling and pressure filling[3,4]. Initially, the pressure vessel was placed in a cold trap consisting of a dry ice and acetone and was allowed to cool below the boiling point of HFA-134a, -26.3º C. Subsequently, the vessel was connected to a HFA-134a source cylinder and the supply was turned on. The low temperature of the vessel facilitated instant conversion of HFA-134a vapors to liquid. After filling up, the supply was turned off and the vessel was instantly transferred to its place between the CO_2 cylinder and filler and sealed. Overnight equilibration was allowed to happen, which brought HFA-134a to its native, partially vaporized state.

Using this setup, CO_2 could be supplied to the pressure vessel at a desired pressure level. For the purpose of formulation of solution and suspension pMDIs, three pressure levels- 100, 130 and 160 psi were used. The rationale behind using three pressure levels being Henry's law, which states that solubility of a gas in a liquid is directly proportional to partial pressure of the gas over that liquid. CO_2 was allowed to mix with HFA-134a assisted with mechanical shaking, and saturation was judged to have occurred when no loss in pressure was observed even after closing CO_2 supply. The pressure vessel was then deemed ready for filling operations, the pressure of CO_2 cylinder being used as a driver of liquid HFA-134a to the filler. For manufacture of control pMDIs containing only HFA-134a, insoluble He was used to drive HFA-134a in to the filler.

Figure 3.2 illustrates the actual process of filling of canisters using a Pamasol 2016 filler. A pMDI canister, fitted with a metering valve, containing all ingredient except HFA-

134a, is placed below the filler head. Using the handle, propellant is drawn in to a desired volume, and the filler head is lowered on to the canister using a lever. The crank handle is then rotated in a clockwise direction to transfer propellant to canister. Figure 3.3 in the next section describes various BDP solution pMDIs with or without CO_2 addition.

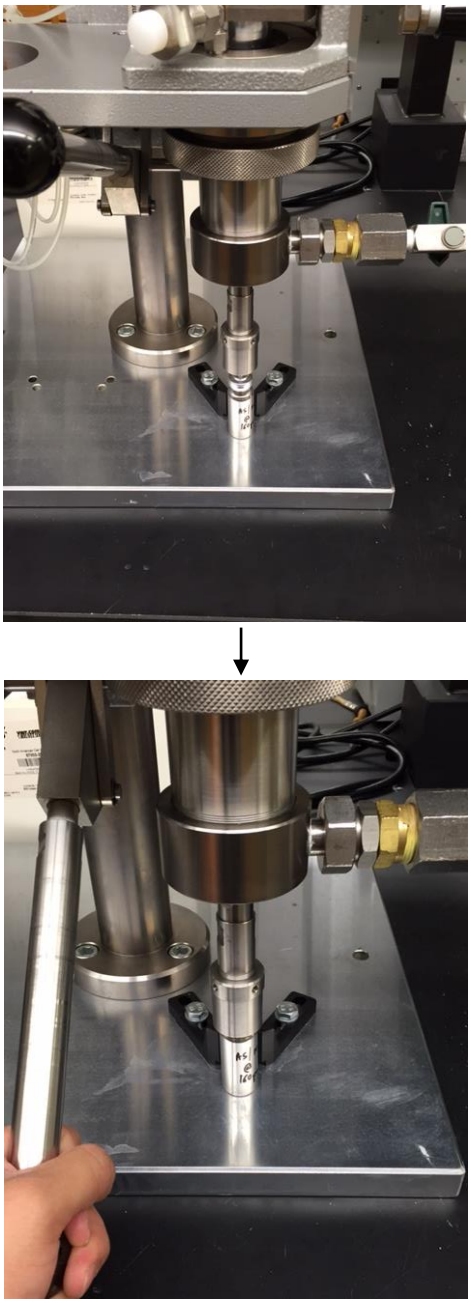


Figure 3.2: pMDI filling using a Pamasol 2016 propellant filler

3.2.2 Formulation of solution-type pMDIs containing BDP and ethanol

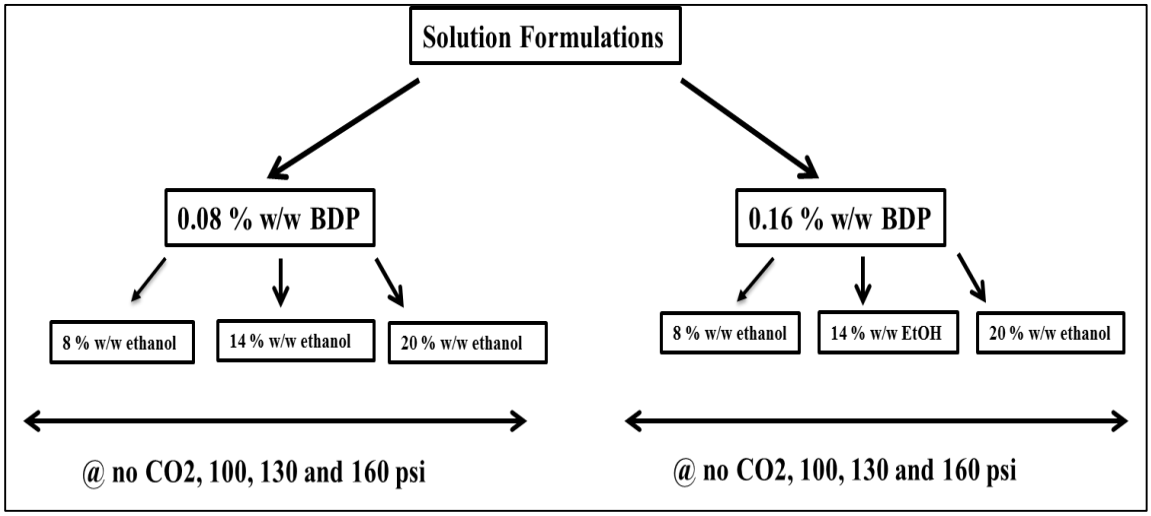


Figure 3.3: Experimental plan to formulate solution inhalers containing 2 concentration levels of BDP, and 3 different concentration levels of ethanol, all pressurized at either 100, 130 or 160 psi with CO_2. HFA-134a only inhalers also prepared using same drug and ethanol concentration levels

The target fill weight for all solution-type pMDIs was 13 g. Initially, BDP equal to 0.08% or 0.16% w/w of the final formulation weight was added to the canister. Ethanol equivalent to 8%, 14% or 20% w/w of the final weight was added, and the canister was then crimped using a manual crimper. After that, mixture of HFA-134a and CO_2 equilibrated at 100, 130 or 160 psi were transferred to the canisters to make up to the desired weight. Control formulations containing HFA-134a were filled with the propellant using pressurized He as a driver. All test and control inhalers were prepared in triplicate. QVAR actuator was used to discharge all formulations during laser diffractometry testing.

3.2.3 Jet milling and particle size measurement of albuterol sulfate

Albuterol sulfate purchased from a vendor is not micronized, and it is not uncommon for its median particle size to be 50-60 µm. In order to formulate inhalers, it is necessary that the median particle size of API be in the 'respirable' range i.e. <5 µm. The most common method used to generate such fine particles is jet milling, which uses energy of compressed air to cause collision of particles which causes their communition. In this study, a lab scale jet mill, Jet-o-mizer 00 has been used (Figure 3.4). Table 3.1 summarizes conditions used in the operation of the mill.

Feed Rate	60-80 grams/hour
Feed Pressure (Pusher nozzle)	80 psi
Milling Pressure	80 psi
Milling Gas	Compressed Air
Number of milling passes	2

Table 3.1: Jet milling conditions for albuterol sulfate

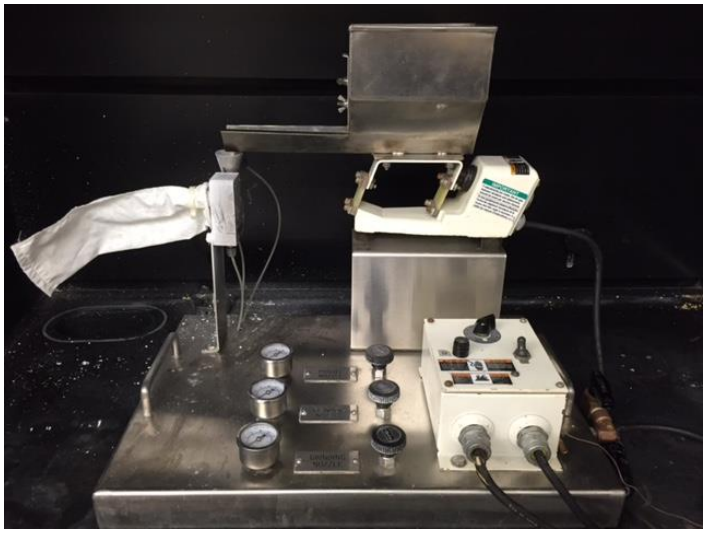


Figure 3.4: Jet-o-mizer 00 mill

In this study, the starting material purchased from the vendor had a median particle size of approximately 50-60 µm. After one pass through the mill, the particle size was reduced to about 7 µm, which was still too large for respiratory delivery. The powder was then again milled to achieve a median particle size of 3.9 µm, which was deemed acceptable for experimental purposes.

Particle sizing was done by liquid dispersion particle sizer by Horiba Laser Scattering Instrument. Milled AS was suspended in isopropyl alcohol in order to conduct laser scattering experiments. A detailed plan to formulate suspension-type inhalers with or without CO_2 is depicted in Figure 3.5 in the following section.

3.2.4 Formulation of suspension-type pMDIs containing AS with or without ethanol

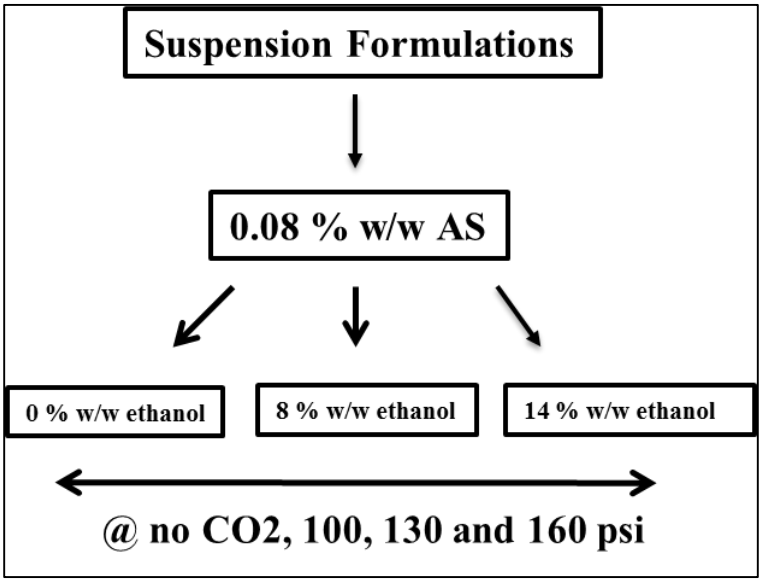


Figure 3.5: Experimental plan to formulate suspension inhalers containing 0.08% w/w AS, and 3 different concentration levels of ethanol, all pressurized at either 100, 130 or 160 psi with CO_2. HFA-134a only inhalers also prepared using same drug and ethanol concentration levels

The procedure for mixing and transfer of mixture of HFA-134a and CO_2 was similar to that described in section 3.2.1. For suspension formulations, the target fill weight was 13 g. All formulations contained 0.08% w/w AS of the final formulation weight. Ethanol concentration was varied at 0%, 8% and 14% w/w of the final formulation weight. After adding drug and ethanol, canisters were crimped with 50µl Bespak® valves, and mixture of HFA-134a and CO_2 equilibrated at 100, 130 or 160 psi was added to make up the weight. The pressure of CO_2 was used to drive the contents from pressure vessel to filler. In case of control formulations containing HFA-134a only, He was used to drive propellant into filler. All canisters, including control, were made in triplicate. A Ventolin actuator, with orifice diameter (OD) 0.5 mm[5] was used to actuate all formulations. At this

point, solution and suspension-type inhalers were deemed ready for laser diffraction testing. Figure 3.6 shows the setup for pMDI laser diffractometry studies using Spraytec.

3.2.5 Spraytec configuration, method and metrics

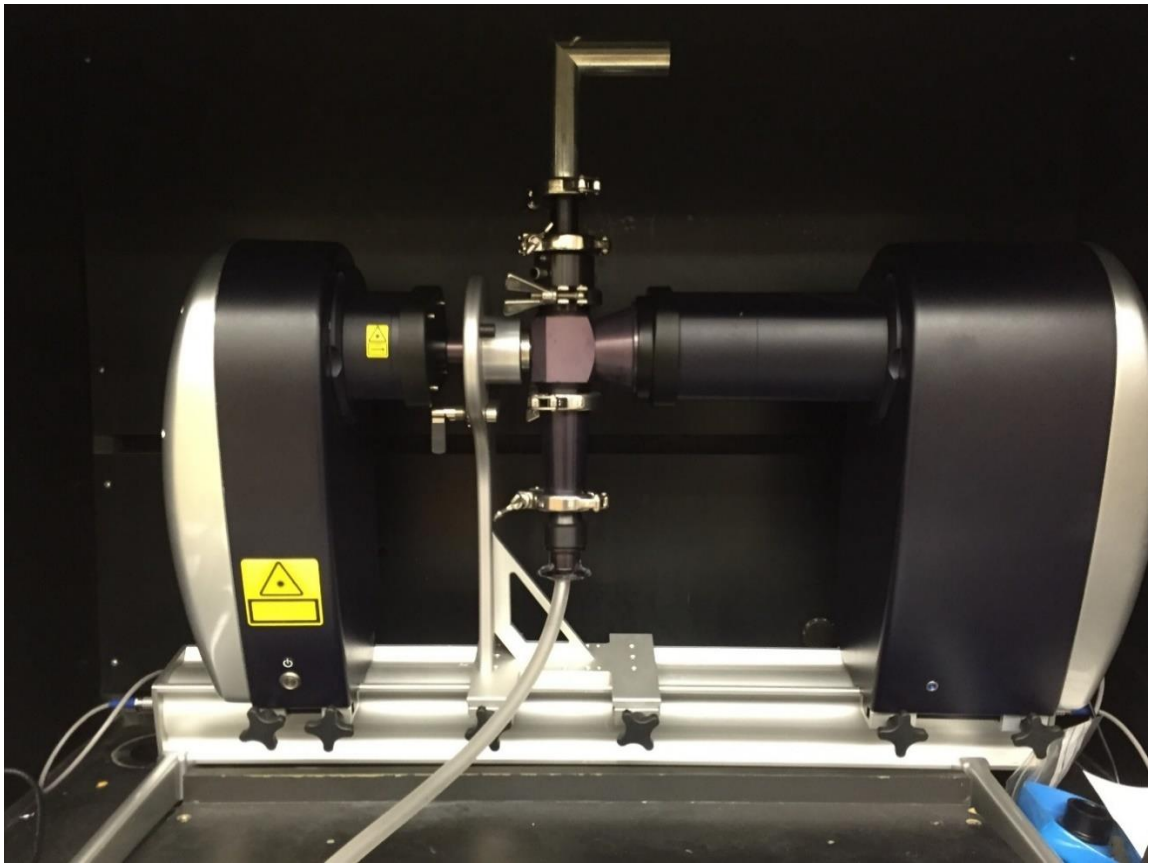


Figure 3.6: Spraytec used in vertical configuration, with USP throat and suction for solution and suspension pMDI testing

For pMDI testing purposes, Spraytec was used in vertical configuration and in rapid mode, in which initiation of measurement is dependent upon actuation stimulus[6]. Beam steering adjustment was done. A USP throat was attached to the vertical inhalation cell as per published literature. A continuous suction was maintained at 28.3 $Lmin^{-1}$ using a vacuum pump. All canisters were primed before testing and a single actuation was used per reading.

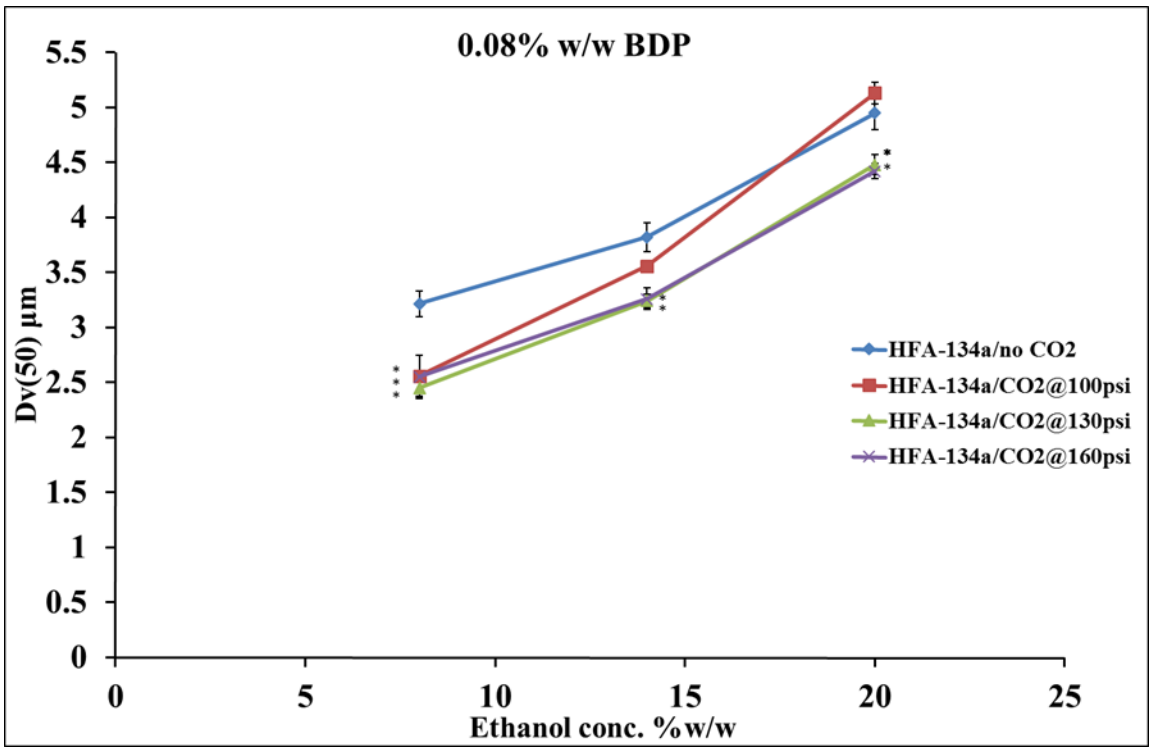
Median volume diameter i.e. Dv (50) was the chief deliverable metric from these experiments.

3.2.6 Statistical analysis

Values of Dv (50) ± SD data obtained from test and control runs were compared using Student's t-test. A difference was deemed significant if $p < 0.05$. Statistical significance was denoted by asterisk (*). Dv (50) data from solution-type inhalers is shown in graphs of Figure 3.7.

3.3 Results and discussion

3.3.1 Dv (50) data from test and control solution-type formulation inhalers



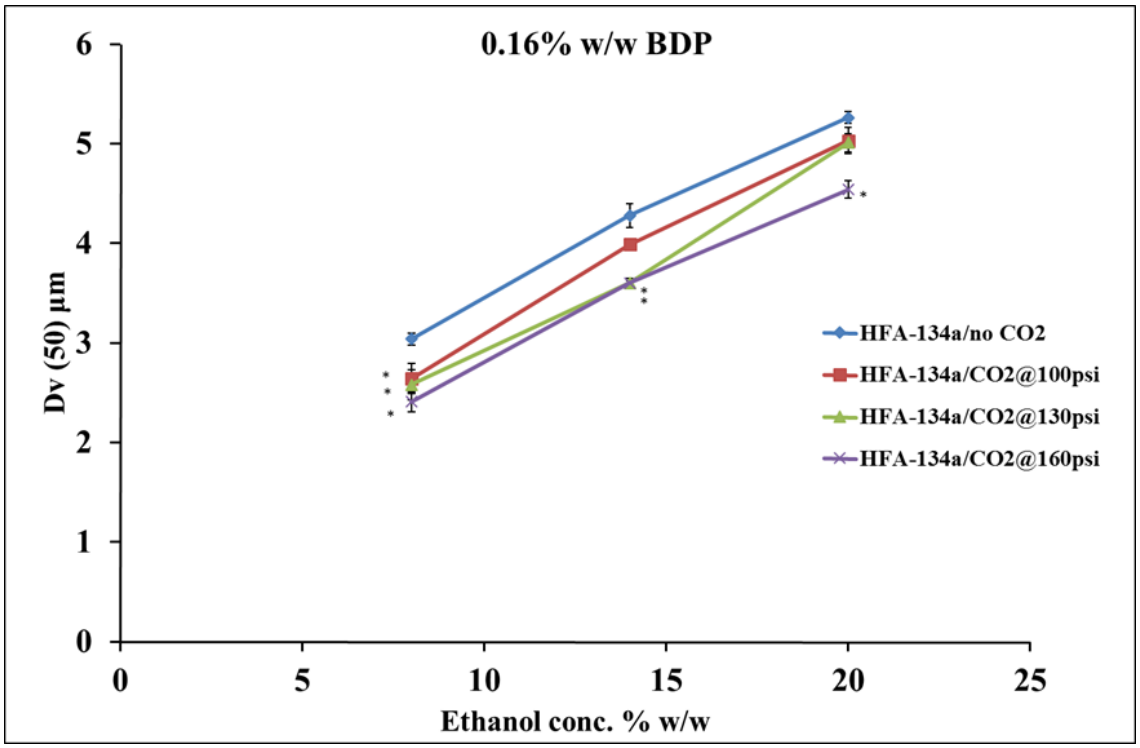


Figure 3.7: Dv (50) data from test and control solution formulation inhalers across various BDP and ethanol concentrations, and various CO_2 supply pressures * denotes $p < 0.05$

Formulations prepared using a mixture of HFA-134a and CO_2, produce finer aerosols than analogous HFA-134 only aerosols. Significant droplet size reduction in formulations containing 0.08% w/w BDP and 8% w/w ethanol at all three pressure levels (100, 130 and 160 psi) is understandably similar due to lower non-volatile content (NVC). However, as the ethanol concentration is increased to 14 % and 20% w/w, a CO_2 pressure of 100 psi is no longer sufficient to cause significant size reduction; significant reduction is only seen at 130 and 160 psi CO_2 pressure. This observation lends credibility to our initial theory that increased partial pressure of the gas over a liquid causes more gas to go into solution, thus increasing effervescent behavior and overcoming resistance due to NVC.

At 0.16% w/w BDP concentration, similar set of results is observed except at 20 % w/w ethanol content, where even a CO_2 pressure of 130 psi is insufficient to cause significant droplet size reduction due to very high NVC levels.

The following section describes a hypothetical explanation of observed droplet size reduction in HFA-134a-ethanol droplets due to effervescence, similarly described in Chapter 2.

3.3.2 Proposed mechanism of droplet size reduction in HFA-134a-ethanol droplets due to effervescence

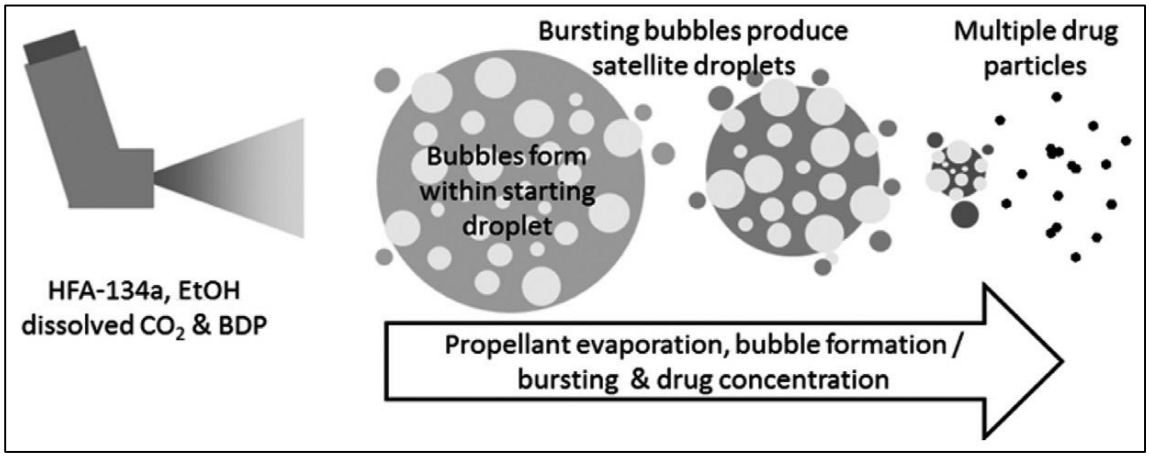


Figure 3.8: Mechanism of droplet size reduction due to effervescence[1]. Light circles represent CO_2 bubbles.

Figure 3.8[1] depicts a hypothetical explanation for observed droplet size reduction due to effervescence caused by dissolved CO_2. We speculate that upon actuation, CO_2 bubbles form inside HFA-134a-ethanol droplets. As the droplet moves forward, evaporation occurs, which causes most of propellant to vaporize. However, mechanical fracture of the droplet caused by CO_2 bubble also occurs, which causes further reduction in droplet size. In formulations containing HFA-134a only, mechanical fracture of droplets would not occur, which explains their significantly higher size than those produced by 'effervescent' formulations.

Since this a solution formulation, each satellite droplet will contain dissolved drug; unlike suspensions where droplets may be too small to hold micronized drug. Figure 3.9 in next section shows Dv (50) data from test and control suspension inhalers.

3.3.3 Dv (50) data from test and control suspension-type formulation inhalers

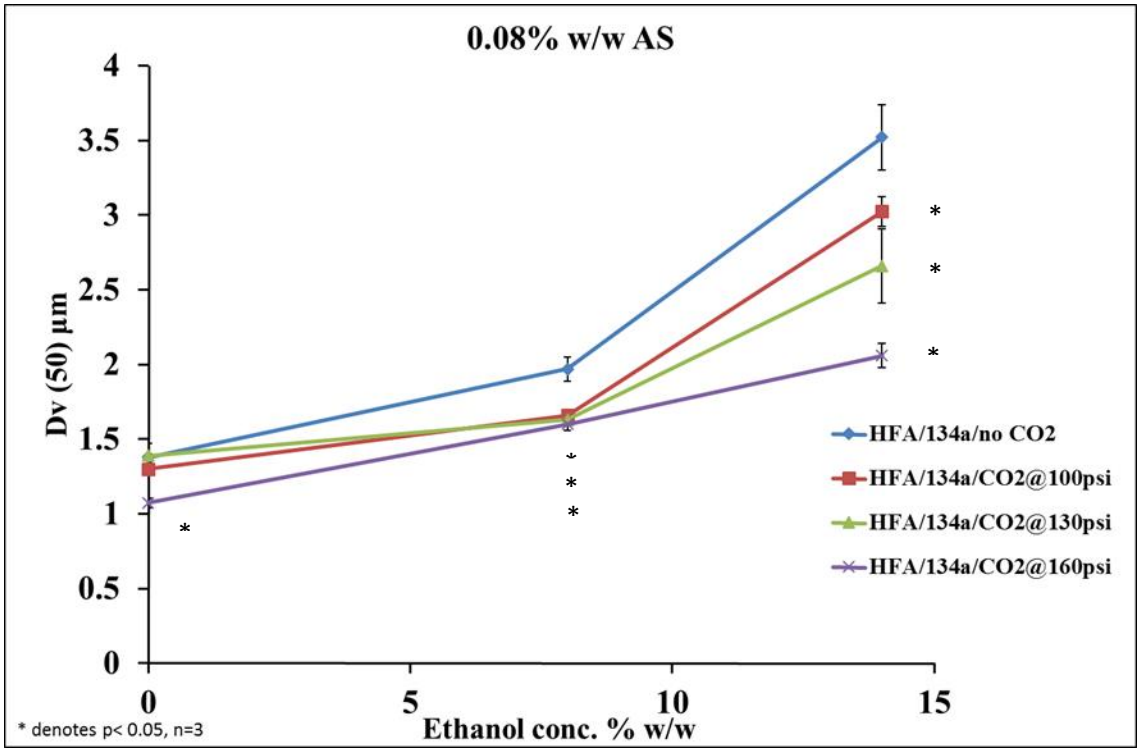


Figure 3.9: Dv (50) data from test and control AS suspension formulation inhalers across ethanol concentrations, and various CO_2 supply pressures

In formulations containing no ethanol, a significant but small size reduction is seen at 160 psi CO_2 pressure. This is interesting since we expect a larger decrease in particle size in presence of very little non-volatile content. In this case, particle sizes across all pressures are understandably lower due to absence of ethanol. In formulations containing 8% and 14% w/w ethanol, significant reduction is observed across all pressures. As ethanol concentration increases from 8% to 14%, the overall droplet size increases too.

It is interesting to note that final droplet size observed in some cases above is lesser than the original micronization grade of AS, 3.9 µm. This can be explained as following- Formulations containing ethanol and suspended AS, actuated using a Ventolin actuator,

gather considerable momentum due to their mass and velocity. As a result, larger droplets are unable to negotiate the turn of USP throat and strike the bend. However, small particles, with lesser momentum, are able to turn, enter the measurement zone, and obscure the laser beam. This can be considered as a measurement error, which unfortunately, cannot be addressed in a Spraytec setting; it can only be addressed in cascade impaction studies which will be described in the subsequent chapter. Figure 3.10 shows a proposed mechanism of action for effervescence in suspension-type inhalers

3.3.4 Proposed mechanism of size reduction in suspension- type formulation droplets due to effervescence

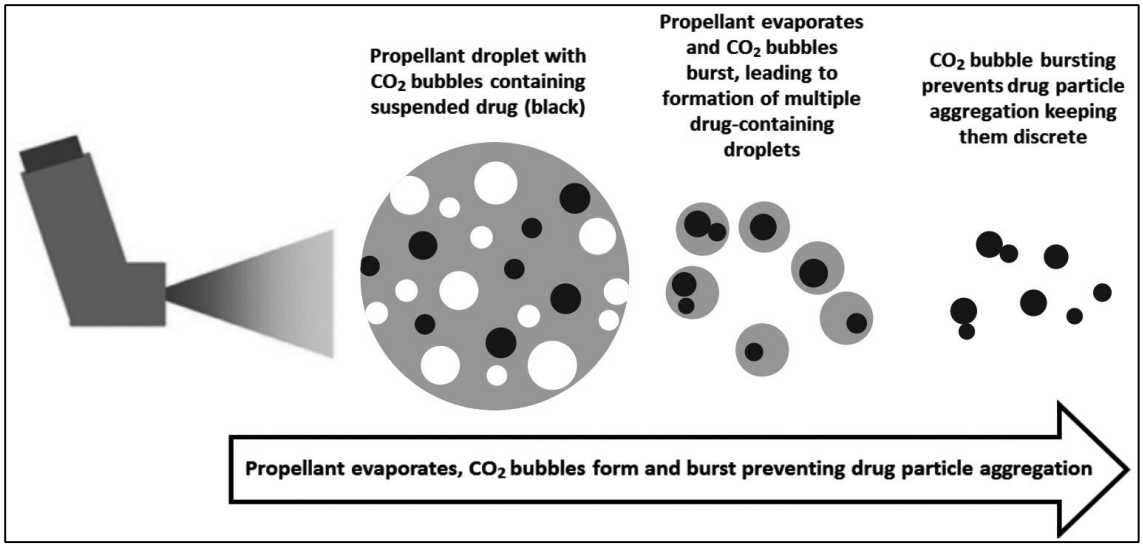


Figure 3.10: Schematic diagram of proposed mechanism of size reduction in suspension-type droplets due to effervescence[2]. White circles are CO2 bubbles. Black circles are suspended drug particles.

We hypothesize that during actuation of suspension-type inhalers, CO_2 bubbles will form inside HFA-134a-ethanol droplets that contain suspended drug particles. As the droplet travels away from the nozzle, HFA and some ethanol evaporates, bringing drug

particles closer to each other. However, the expansion and eventual bursting of CO_2 bubbles result in production of smaller droplets containing drug particles causing a reduction in the formation of particle aggregates and increase number of discrete particles. In contrast, in HFA-134a only droplets, mechanical fracture and bursting due to CO_2 bubbles will not happen, eventually resulting in particles coming together to form aggregates.

3.4 Conclusion

In the previous chapter, the concept of effervescent aerosols was introduced using a specially fabricated pressure vessel capable of holding and actuating BDP solution formulations. In order to be commercially viable and to facilitate further compendial testing, it was essential that such a novel technology be translated to the recognizable pMDI canister format. However, translating 'effervescent' aerosols posed a unique challenge of figuring out a way to bring about effective mixing of HFA-134a and CO_2, which was successfully done in studies described in this chapter by using an intermediate stage of mixing.

These studies also proved that such a formulation, whether solution or suspension, can be safely stored in a standard, commercially available pMDI canister without any safety issues. Although studies at elevated temperatures were not conducted, it might be speculated that increase in temperature might increase solubility of CO_2 in HFA-134a, thereby reducing its presence in headspace. Perhaps the greatest advantage of a translation to pMDI canisters is that it makes compendial tests such as cascade impaction and plume geometry measurements feasible.

Laser diffractometry, although a quick method to estimate volume diameters of aerosolized particles, does not provide insights into the contents of the droplets. The USP prescribes cascade impaction tests which yield valuable information regarding particle size distribution based upon aerodynamic diameter[7]. Cascade impaction testing also yields information about mass of drug contained in a particular aerodynamic size range[8], which is essential to judge whether enough drug is reaching its intended target in lungs. The following chapter describes extensive cascade impaction studies conducted on 'effervescent' solution and suspension pMDI formulation, in order to evaluate the effect of formulation and device variables on product performance and critical quality attributes.

3.5 References

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Chapter 4: Cascade impaction studies on 'effervescent' solution and suspension-type pMDI formulations using Andersen Cascade Impactor to evaluate effect of formulation and device variables on product performance

4.0 Introduction

In the previous chapter, a novel way of formulating 'effervescent' solution and suspension inhalers has been described. Laser diffractometry testing to determine effect of formulation variables on volume diameter of emitted droplets has been done. Laser diffractometry, which is a quick method to analyze aerosol particle size, is not designed to provide information on actual drug concentration in the droplet. This is a serious limitation for formulators as well as regulators interested in knowing the amount of drug contained in various aerodynamic particle size ranges. Since aerodynamic behavior of aerosolized particles dictates their eventual deposition location in the respiratory tract, an insight on the amount of drug in each size range is very useful to formulators looking to target a particular part of respiratory tract. The USFDA does not accept particle size distribution data based solely on volume diameter, but requires that NDA/ANDA submissions contain mass based particle size distribution along with other performance tests and derived parameters.

The USP Apparatus 1 attached with a 'USP throat' also called the Andersen Cascade Impactor (ACI), or the Next Generation Impactor (NGI) are standard equipment used to carry out mass-based particle size distribution analysis[1]. In this study, the ACI, (described in detail in 'methods' section) has been used to analyze various 'effervescent' solution and suspension formulations containing various levels of ethanol, and sprayed using actuators of varying orifice diameters. In addition to yielding information about

amount of drug contained in various particle size ranges, various derived parameters such as FPF, FPD and MMAD have also been calculated based on the distribution of drug particles. HPLC methods used to analyze and quantify drug on impactor stages have also been described. Other compendial tests described include performance analysis of inhalers at various stages of life in order to investigate whether canister emptying adversely affects performance of HFA-134a-CO_2 based formulations.

4.1 Materials

Two ACIs equipped with a USP throat, previously purchased from MSP Corp. (Shoreview, MN) were used in all experiments. A TSI (TSI Inc., Shoreview, MN) digital flowmeter was used to adjust air flow rate in ACI experiments. A Copley HCP 5 (MSP Corp., Shoreview, MN) vacuum pump was used to create air flow. Solution formulations contained BDP purchased from Sigma-Aldrich (St. Louis, MO), and suspension formulations contained AS purchased from Spectrum Chemical Mfg. Co. (New Brunswick, NJ). HFA-134a and CO_2 were procured from AirGas Co. (Radnor, PA). 19 ml aluminum canisters were purchased from Presspart (Lancashire, UK) and 50 µl and 63 µl metered valves were purchased from Bespak (Norfolk, UK). Waters HPLC system (Milford, MA) consisting of isocratic pump 1525, autosampler 717 and photodiode UV array detector 2996 was used in all HPLC experiments to analyze samples from ACI runs. An Agilent (Santa Clara, CA) Zorbax C18 reverse phase HPLC column was used in all experiments. Chemicals used in HPLC were as follows- HPLC grade acetonitrile and water, and acetic acid were sourced from Sigma-Aldrich (St. Louis, MO). Heptane sulfonic acid was purchased from Fischer Scientific (Fairlawn, NJ). Following section (Figure 4.1) describes structure of an ACI.

4.2 Methods

4.2.1 Structure of the Andersen Cascade Impactor

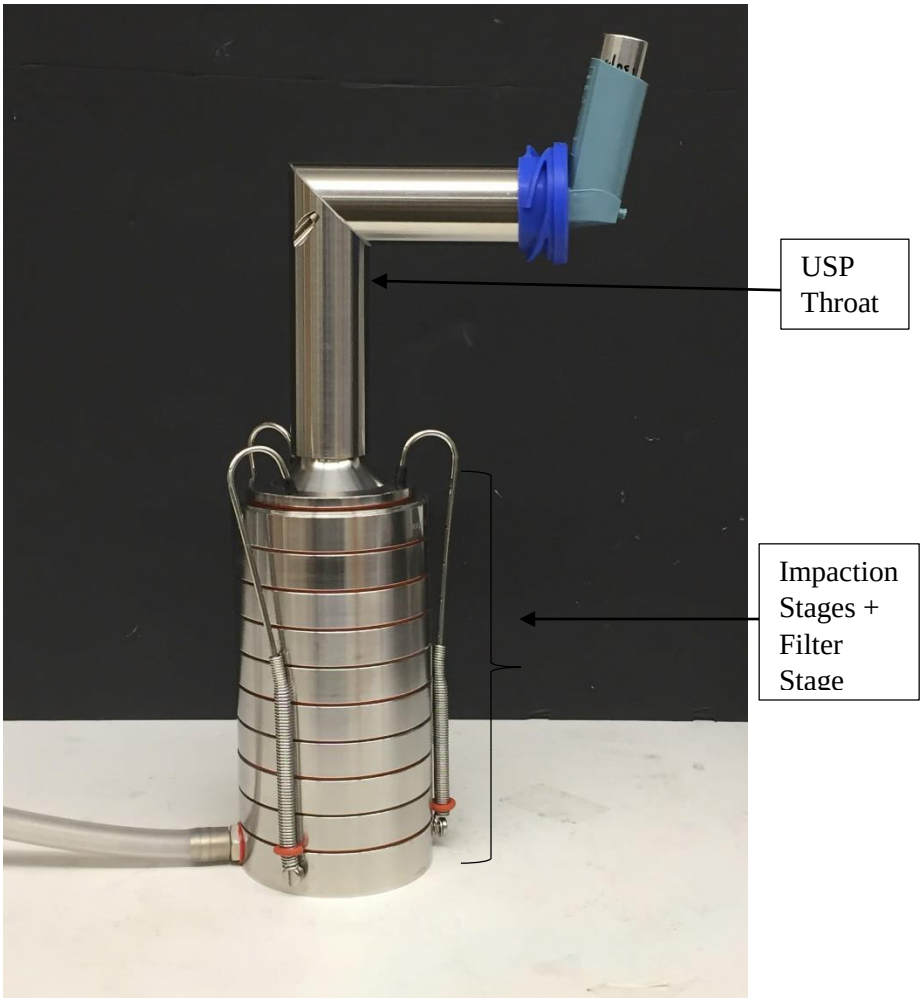


Figure 4.1: Andersen Cascade Impactor (ACI) with USP throat, attached to vacuum pump for constant air flow

The ACI is a standard instrument used in inhalation labs across the world to determine aerodynamic particle size distribution of aerosols. The ACI consists of 8 stages; each stage contains an impaction plate which is followed by numerous jets that lead to the next plate. All stages are calibrated so that only particles of a particular aerodynamic diameter pass through the jets, and those with high inertia impact the plate and settle. All

cut-offs for 8 stages have been listed in Table 4.1[2]. The USP throat is a thick steel tube with a 90º turn. An actuator can be attached to the entrance of the throat with help of a specially designed port. Upon actuation, larger and heavier droplets of sprayed formulations are unable to negotiate the turn of the throat and hit the turn. This is intended to mimic the real life problem of drug wastage due to impaction in oropharyngeal region of throat. At the filter stage, the ACI has a port, which can be connected to a vacuum pump in order to produce a constant air flow. In these set of experiments, an air flow of 28.3 $Lmin^{-1}$, representing slow tidal breathing, has been used[3].

It is important to note that the ACI is not intended to be an *in-vitro* lung, although its design resembles one, it is designed to classify and sort aerosol spray particles based upon their aerodynamic diameter. There are too many differences for it to be an in-vitro lung. Firstly, it is a rigid instrument, unlike a lung, which is soft and spongy. Also, the ACI operates at a constant air flow of 28.3 $Lmin^{-1}$, whereas a normal breathing cycle consists of inhaling and exhaling[5]. ACI cutoffs for all stages are listed in Table 4.1.

ACI Impactor Location	Cut-off aerodynamic diameter (µm)
Actuator and valve stem	-
Throat	-
Stage 0	9.0
Stage 1	5.8
Stage 2	4.7
Stage 3	3.3
Stage 4	2.1

Stage 5	1.1
Stage 6	0.7
Stage 7	0.4
Filter	0.0

Table 4.1: Cut-off diameters for various stages of ACI

4.2.2 Operation of ACI

In this set of experiments, we have performed cascade impaction experiments on solution and suspension-type inhalers. Before a run, the ACI was washed thoroughly with deionized water to remove any residual drug or contaminants from previous experiments. After drying, a filter paper was placed in the last stage of ACI, and all stages, including the USP throat, were assembled together and sealed. The vacuum pump was attached to the port on filter stage and air flow rate was set at 28.3 $Lmin^{-1}$ using the digital air flow meter. The actuator was attached to USP throat such that it was exactly parallel to it.

Before spraying into ACI, the inhalers are primed by firing 4-5 shots to waste. In the case of BDP solution formulation canisters, which are fitted with a 50 µl valve, 15 puffs were used per ACI run. For AS suspension formulation canisters, which were fitted with a 63 µl valve, 12 puffs were used. For all runs, an interval of 30 secs was maintained between each puff, and in case of suspensions, the canister was shaken between each actuation[4].

After all actuations were complete, the ACI was disassembled and the throat, actuator and stage plates were washed with a pre-determined volume of mobile phase. For all experiments, throat, actuator and filter were rinsed using 10 mL of mobile phase, whereas impactor plates were rinsed with 5 mL. mobile phase. Different mobile phases

were used for solution and suspension experiments as discussed in following sections. Clean petri dishes were used to wash impactor plates, and Ziploc® bags were used to wash actuators. In case of throat, after adding mobile phase, both openings were sealed off using Parafilm wrap and inner portion of throat was rinsed. Aliquots of mobile phase were taken from all ACI parts after washing and put in HPLC vials (VWR; Radnor, PA) for analysis.

4.2.3 Deliverables from ACI experiments

Aerodynamic Particle Size Distribution (APSD) - APSD is the chief output from an ACI experiment. APSD gives information on the amount of drug deposited on each stage on impactor stage as well as throat and actuator. A typical APSD chart, as shown in 'results' section, has ACI part name on X-axis and percent of total recovered mass of drug on Y-axis. It is important to note that percent of drug is based on recovered dose and not on total drug sprayed. Thus, attaining a sufficient mass balance in HPLC experiments is critical. For these experiments, a mass balance between 80% - 120% of total drug was considered appropriate for the data to be considered valid[1,2].

Fine Particle Fraction (FPF) - FPF can be defined as the percentage of total amount of drug sprayed contained in particles with an aerodynamic diameter less than 4.7 µm. It is percentage of total mass of drug deposited from Stage 3 to the filter.

Fine Particle Dose (FPD) – FPD can be defined as total mass of drug contained in particles with aerodynamic diameter less than 4.7 µm. As a general rule, particles smaller than 4.7 µm have the best chance of penetrating lungs. Thus, they are considered 'inhalable' particles[2].

Mass Median Aerodynamic Diameter (MMAD) – MMAD is defined as the aerodynamic diameter below which half of the aerosol mass resides. It is a metric used to judge aerosol performance; but MMAD by itself is not acceptable in NDA/ANDA submissions to the USFDA. To make it acceptable, a complete APSD profile is needed. In this study, MMAD of test and control samples has been calculated using an in-house MS-EXCEL template[2,5].

Following sections describe various types of solution and suspension formulations tested using ACI. Solution formulations made are depicted using Figure 4.2.

4.2.4 BDP solution formulations tested using ACI

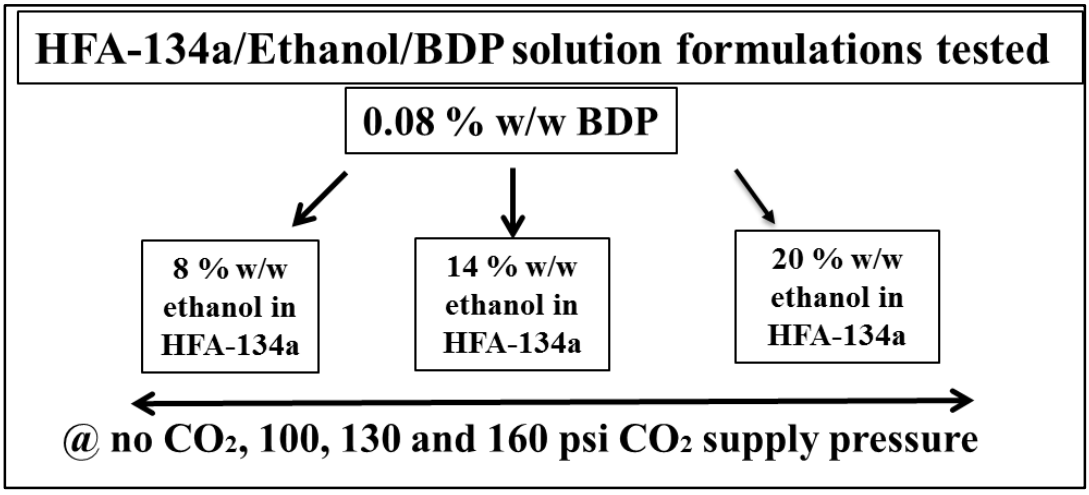


Figure 4.2: Various BDP solution formulations tested using ACI

All solution inhalers contained 0.08% w/w BDP. As a formulation variable, 3 different concentrations of ethanol, 8%, 14% and 20% w/w of the final formulation weight (13 g) were used. After adding BDP and ethanol to the canisters, a 50 µl Bespak valve was crimped onto it. The weight was made up by a mixture of HFA-134a and CO_2 manufactured in a pressure vessel as previously described in Chapter 2. As in the case of previous samples prepared for laser diffractometry experiments, CO_2 supply pressure to HFA-134a was

varied at 100 psi, 130 psi and 160 psi. Analogous, HFA-134a only canisters were also prepared for control experiments. All canisters were prepared in triplicate. During each ACI experiment, 15 actuations were made with 30 sec intervals between actuations. QVAR actuators were used in all experiments.

Apart from formulations described above following formulation was also prepared-

Solution formulations containing HFA-134a mixed with CO_2 at 85 psi supply pressure- The native pressure of HFA-134a at room temperature is approximately 80 psi. In this case, CO_2 was supplied to HFA-134a at 85 psi, an increment of only 5 psi over HFA-134a pressure. BDP concentration was 0.08% w/w and ethanol concentration was 8%. The rationale behind preparation of these samples was to investigate whether droplet size reduction due to effervescence occurs in samples prepared using an HFA-134a-CO_2 mixture, in which CO_2 supply pressure to HFA-134a represents only a minute increment over HFA-134a native pressure. Samples were prepared in triplicate and all other conditions of ACI testing and HPLC analysis were kept similar to previous experiments. Table 4.2 in following section describes HPLC conditions for testing BDP formulations, while Figure 4.3 depicts the various test and control suspension formulations tested using ACI.

4.2.5 HPLC and ACI conditions for testing solution formulations

Table 4.2: HPLC and ACI conditions for testing BDP solution formulations[6,7]

HPLC Column	Agilent Zorbax C18 RP-HPLC column
Mobile phase composition	65: 35 Acetonitrile: water
Detector	UV
UV wavelength	239 nm
HPLC pump flow rate	1.5 ml/min
Auto-injector volume	50 µl
Retention time	7.1 min
Standard BDP conc. Range	0.5-62.5 µg/ml
Calibration curve correlation R^2	1; QC samples used in all runs
Number of actuations in ACI	15
Wash volumes	Actuator, inlet port + Stage 0, filter- 10 ml All stages- 5 ml; 70:30 acetonitrile: water used as solvent

4.2.6 AS suspension formulations tested using ACI

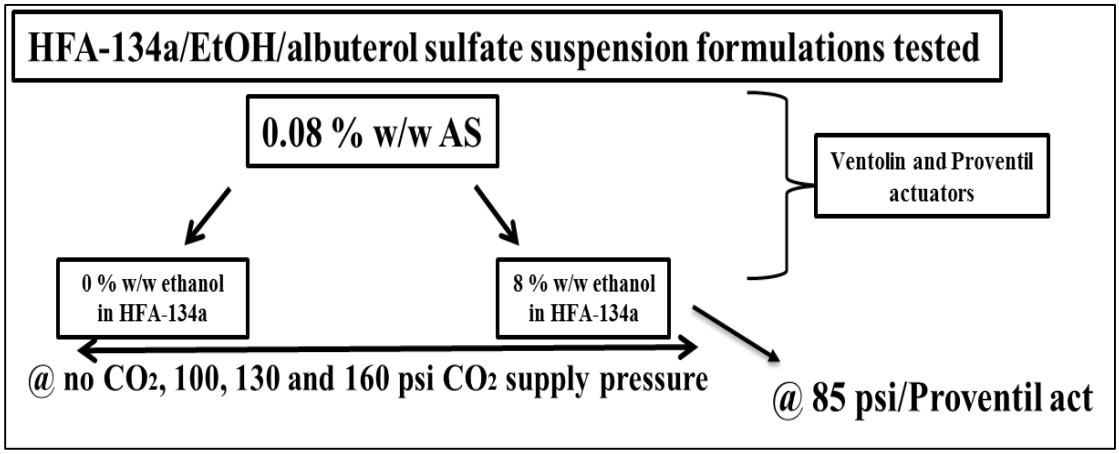


Figure 4.3: Various AS suspension formulations tested using ACI

All suspension formulations contained 0.08% w/w micronized AS. As a formulation variable, 2 different concentrations of ethanol, 0% and 8% w/w of the final formulation weight (13 g) were used. After adding AS and ethanol to the canisters, a 63 µl Bespak valve was crimped onto it. The weight was made up by a mixture of HFA-134a and CO_2 manufactured in a pressure vessel; CO_2 supply pressure to HFA-134a was varied at 100 psi, 130 psi and 160 psi. Analogous, HFA-134a only canisters were also prepared for control experiments. All canisters were prepared in triplicate. 12 actuations were used for each ACI experiment and an interval of 30 secs was maintained between each actuation.

In addition to formulation variable of 2 ethanol concentration levels, device variables in form of 2 actuators with different orifice diameters have been used. Proventil® actuators with OD of 0.25 mm[8] and Ventolin actuators with OD of 0.5 mm have been used to spray formulations with both concentrations of ethanol. The next section (Figure 4.4) describes 'B', 'M' and 'E' stage experiments performed on suspension type inhalers.

4.2.7 ‘B’, ‘M’ and ‘E’ stage performance study of formulations prepared using effervescent aerosol technology

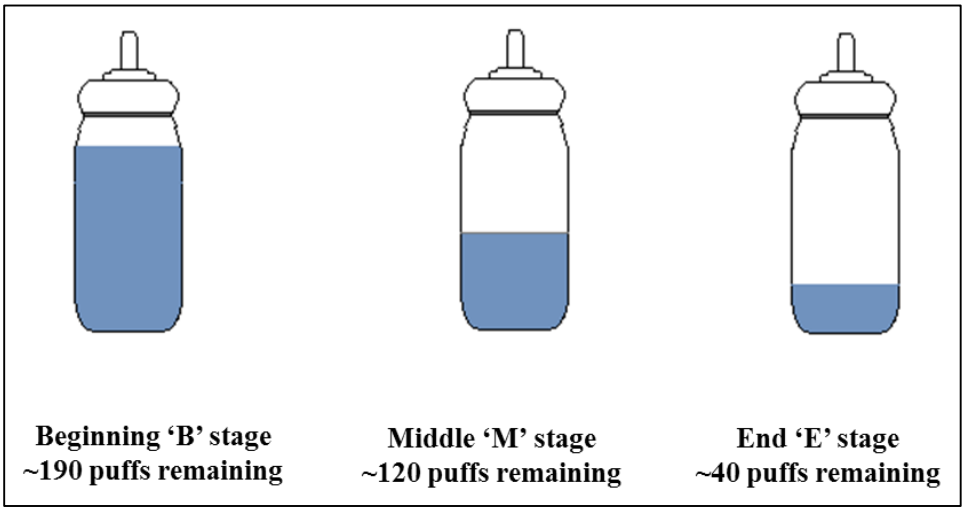


Figure 4.4: Stages of canister life- Beginning ‘B’, middle ‘M’ and end ‘E’

This study was intended to investigate the effects of gradual emptying on performance of inhalers formulated using effervescent aerosol technology. At the beginning of the life of canister, a high percentage of space is occupied by the formulation leaving only a little headspace. In this situation, in formulations containing HFA-134a and dissolved CO_2, most of CO_2 can be expected to remain dissolved; effecting effervescence upon actuation. However, as the headspace increases upon gradual emptying, there is a possibility that dissolved CO_2 moves into the headspace, thus diminishing effervescent effect. In order to investigate if such a diminishing of effervescent effect occurs upon emptying, we designed an experiment, in which canisters containing 0.08% w/w AS and 8% ethanol, pressurized at 100 psi HFA-134a-CO_2, were subjected to ACI testing at 3 stages of their life.

3 canisters containing 13 g of previously mentioned formulation were prepared. At full capacity, they contain approximately 200 puffs of formulation. For 'B' stage experiments, canisters were primed and puffs 5-17 were used for ACI testing. After that, several shots, accompanied with shaking, were actuated to waste until approximately 130 puffs remained. At 130 puffs, 'M' stage ACI experiment was performed. Similarly, 'E' stage experiments were performed when about 40 puff remained in canisters. ACI data from 'B', 'M' and 'E' stages was compared for any changes in APSD, FPF or FPD. Proventil actuators were used for these experiments. Table 4.3 lists HPLC conditions for testing suspension-type pMDIs using ACI.

4.2.8 HPLC and ACI conditions for testing suspension formulations

Table 4.3: HPLC and ACI conditions for testing AS suspension formulations (in-house method)

HPLC Column	Agilent Zorbax C18 RP-HPLC column
Mobile phase composition	Deionized water (58% v/v) , Acetonitrile (40% v/v), Glacial Acetic Acid (2 %v/v), Heptane sulfonic acid sodium (0.065% w/v)
Detector	UV
UV wavelength	276 nm
HPLC pump flow rate	0.7 ml/min
Auto-injector volume	10 µl
Retention time	3.6 min
Standard BDP conc. range	3.125-100 µg/ml

Calibration curve correlation R^2	0.999; QC samples used in all runs
Number of actuations in ACI	12
Wash volumes	Actuator, inlet port, filter- 10 ml All stages- 5 ml; Mobile phase without heptane sulfonic acid and acetic acid was used as solvent

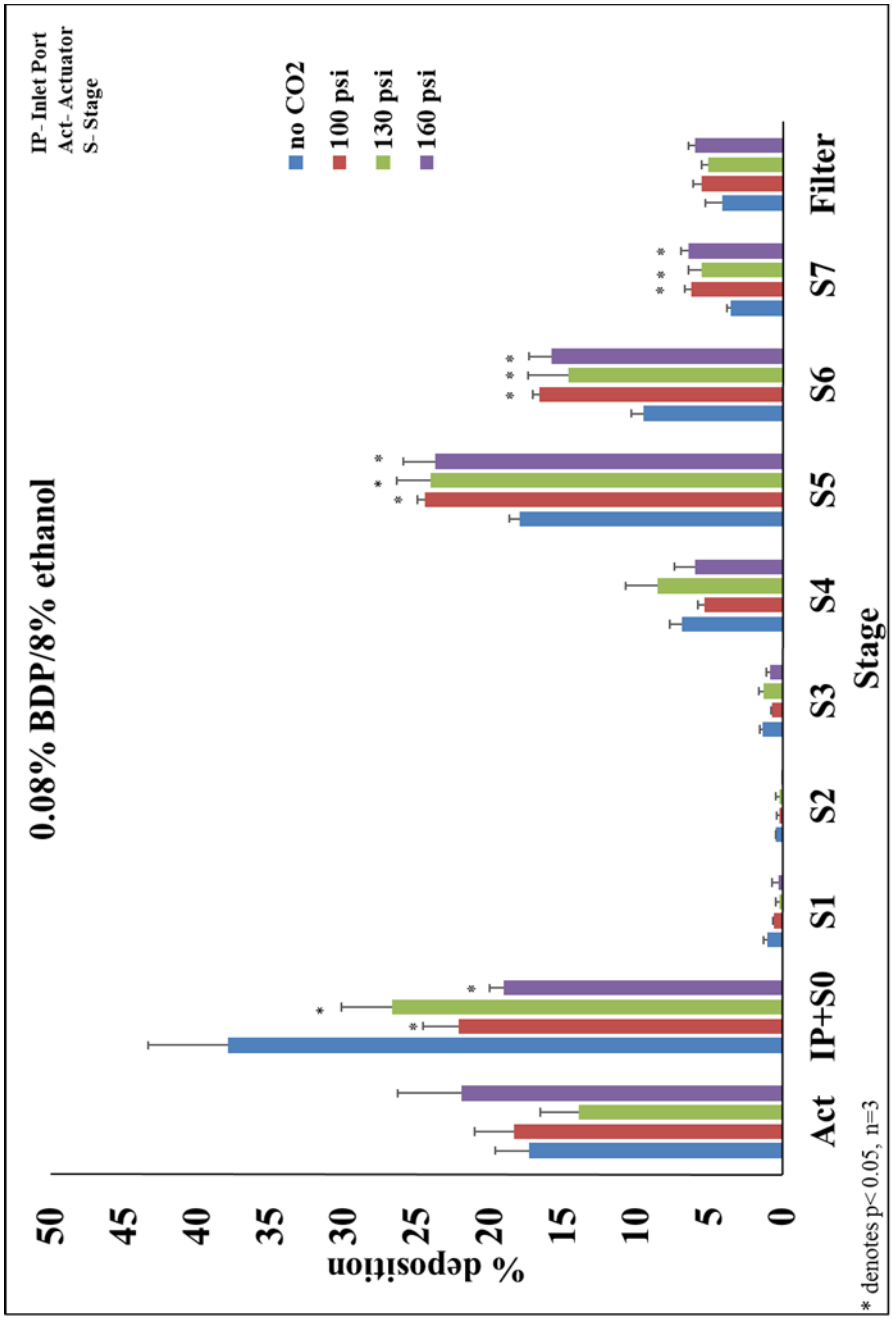
4.2.9 Statistical analysis

Values of percent mass fraction in APSD data obtained from test and control runs were compared using Student's t-test. A difference was deemed significant if $p < 0.05$. Statistical significance was denoted by asterisk (*).

4.3 Results and Discussion

4.3.1 APSD charts and derived parameters of BDP solution formulations

APSD chart and derived parameters for 0.08% w/w BDP/8% w/w ethanol formulations



0.08% w/w BDP/ 8% w/w ethanol in HFA-134a	No CO_2	100 psi CO^2 supply pressure	130 psi CO^2 supply pressure	160 psi CO^2 supply pressure
MMAD ± SD (µm)	1.23 ± 0.05	1.07 ± 0.02	1.12 ± 0.07	1.05 ± 0.05
GSD ± SD	2.06 ± 0.07	1.93 ± 0.01	1.87 ± 0.06	1.91 ± 0.04
Mass Balance ± SD (%)	115.21 ± 3.76	97.82 ± 2.17	98.97 ± 1.99	85.50 ± 3.32
FPD ± SD (µg)	23.15 ± 1.82	26.64 ± 0.74	26.86 ± 2.16	22.87 ± 0.61
FPF ± SD (%)	43.66 ± 3.35	58.91 ± 0.32	59.14 ± 5.23	58.68 ± 3.35

Figure 4.5 and Table 4.4: APSD chart and important derived parameters of 0.08% w/w BDP/8% ethanol w/w formulations

Figure 4.5 shows APSD chart and derived parameters for test and control formulations containing 0.08% w/w BDP/8% ethanol w/w. As compared to control HFA-134a only formulations, a significant decrease in throat deposition is seen in all effervescent aerosols. For example, as compared to 38% deposition in control samples, 100 psi samples show only about 23% throat deposition. However, no significant difference is seen in throat deposition within various effervescent samples i.e. 100 psi, 130 psi and 160 psi samples have approximately similar throat deposition.

It is interesting to note that significantly higher deposition on S5, S6 and S7 is seen in effervescent samples compared to control. This is therapeutically significant as these

impactor stages correspond to deeper lung levels. This data suggests that HFA-134a-CO_2 formulations are capable of producing finer aerosols than HFA-134a only formulations. Due to finer aerodynamic diameter of droplets, drug particles that may have been deposited on throat are instead shifted to lower impactor levels, increasing their 'inhalability'

In terms of derived parameters (Table 4.4), effervescent samples produce particles with smaller MMADs compared to control formulations. MMAD is reduced from about 1.23 µm for control formulations to approximately 1.10 µm for effervescent formulations. A sizeable increase in FPF is seen in effervescent aerosols compared to control formulations. FPF is increased from 44% for control formulations to up to 59% for effervescent formulations. Increases in FPF is also associated with an increase in FPD, which is a direct indicator of the amount of drug that has a high chance of making it into the lungs[5].

The next section describes APSD data from formulations containing higher (14% and 20%) ethanol content, actuated using QVAR actuators. Table 4.5 lists all the derived parameters obtained from APSD charts.

APSD charts and derived parameters for 0.08% w/w BDP/14% and 20% w/w ethanol formulations

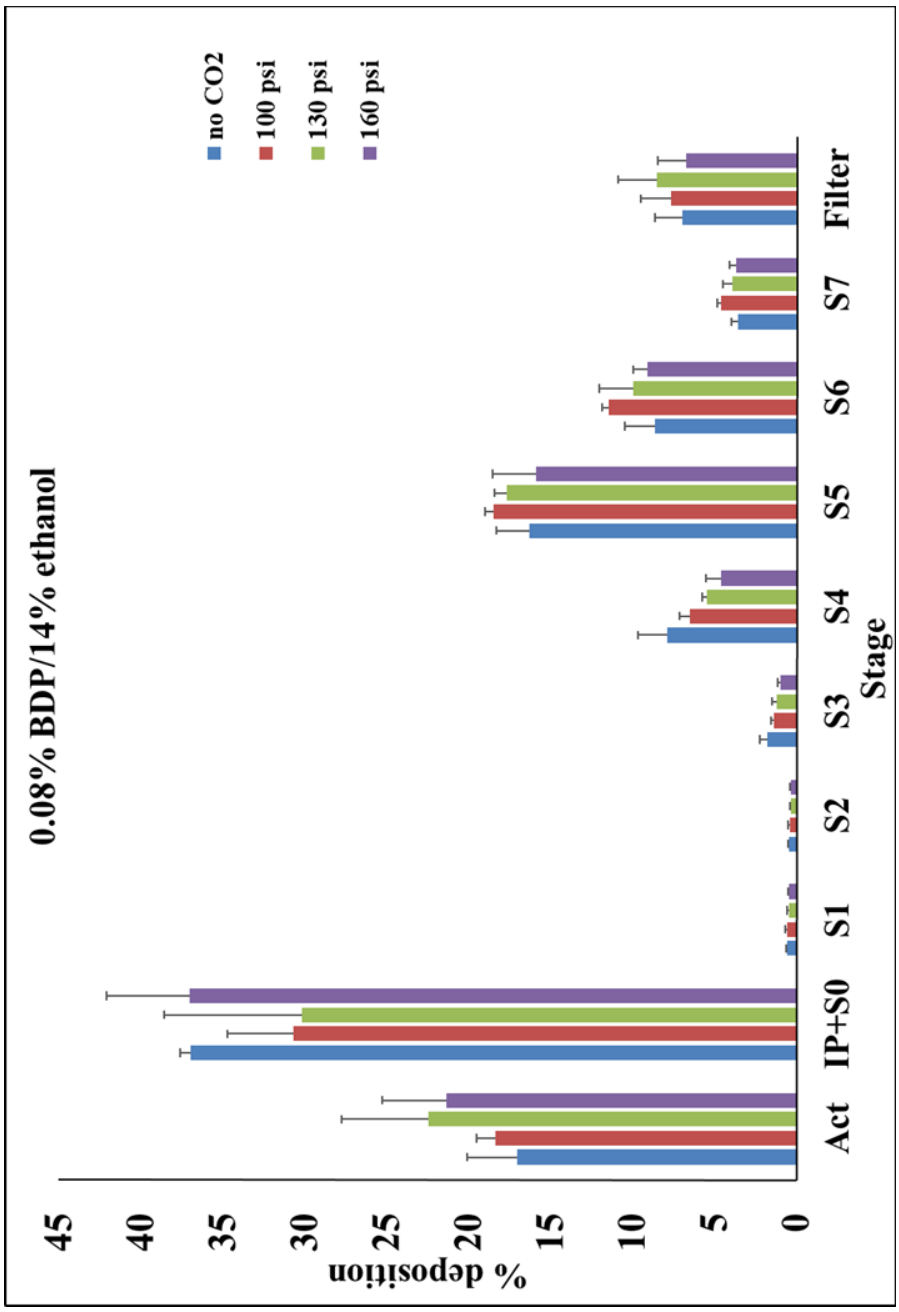


Figure 4.6a: APSD chart for pMDI formulations containing 0.08% BDP and 14% ethanol

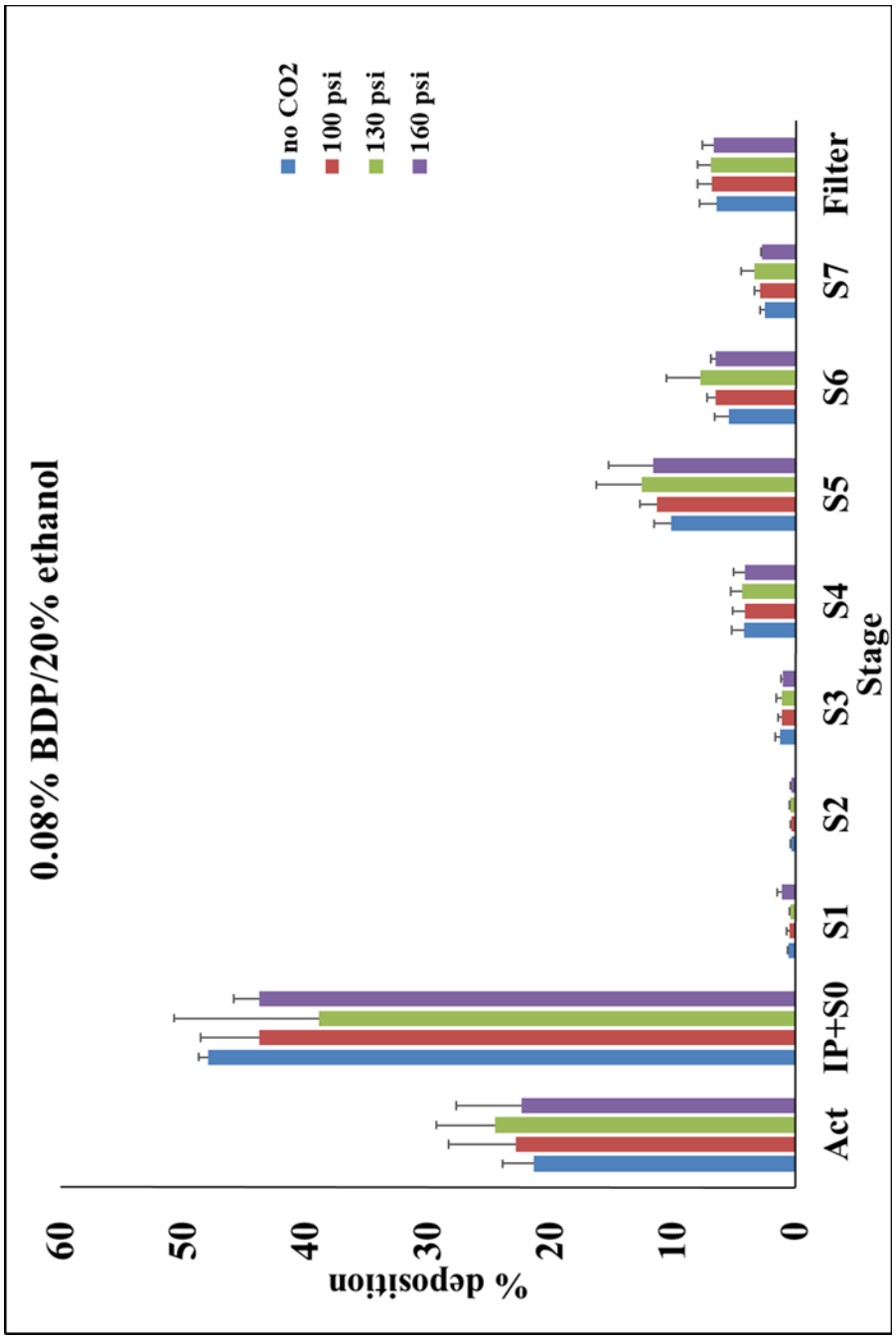


Figure 4.6b: APSD chart for pMDI formulations containing 0.08% w/w BDP and 20% w/w ethanol

0.08 % w/w BDP/14% w/w ethanol in HFA-134a	No CO_2	100 psi CO_2 supply pressure	130 psi CO_2 supply pressure	160 psi CO_2 supply pressure
MMAD ± SD (µm)	1.1 ± 0.04	1.03 ± 0.02	0.97 ± 0.05	1.00 ± 0.06
GSD ± SD	2.09 ± 0.07	2.06 ± 0.09	2.02 ± 0.04	2.04 ± 0.04
Mass Balance ± SD (%)	94.66 ± 3.75	81.11 ± 4.71	82.96 ± 4.62	87.40 ± 1.28
FPD ± SD (µg)	19.57 ± 0.70	17.5 ± 0.70	17.33 ± 1.52	16.33 ± 2.51
FPF ± SD (%)	45.32 ± 2.28	50.33 ± 2.95	46.90 ± 3.71	41.12 ± 5.31

Table 4.5: Derived parameters from APSD data for pMDI formulations containing 0.08% BDP and 14% ethanol

Data depicted in Figure 4.6 is APSD chart for 0.08% BDP formulations containing 14% and 20% w/w ethanol. In the case of control BDP formulations containing 14% w/w ethanol, inlet port deposition is roughly similar to formulations containing 8% w/w ethanol (~30-35%). However, inlet port deposition for HFA-134a-CO_2 formulations fails to show significant reduction compared to control, even though a decreasing trend is apparent. In case of 100 psi formulation, throat deposition narrowly misses attaining significant reduction. For the same kind of formulation, deposition on S5, S6 and S7 clearly trends upwards, but fails to achieve significance. 130 and 160 psi formulations show roughly the same deposition levels as the control on throat and lower levels of the impactor. In terms

of derived parameters, no appreciable reduction is seen in MMAD values in test formulations. FPF and FPD also do not show any significant difference compared to control. This data clearly shows the effect of increase in NVC in formulations on effervescent behavior. In 8% ethanol formulations, effervescent effect is very apparent due to lower NVC levels. However, as ethanol level in HFA-134a droplets increases to 14%, its evaporation is retarded, leading to larger residual droplets. It can also be deduced that presence of a higher amount of ethanol makes it harder for CO_2 bubbles to form, burst and cause size reduction of HFA droplets.

Formulations containing 0.08% w/w BDP/20% w/w ethanol have the highest amount of NVC of the three types of formulations tested. As seen in APSD panel in Fig. 4.6, ACI throat deposition for such formulations is very high (~50%). Although trending downwards in 100 and 130 psi formulations, significant reduction in throat deposition is not achieved compared to HFA-134a only formulations. Impactor stage depositions for control and test samples are roughly the same across all stages. Droplets formed upon actuation of formulations containing 20% ethanol probably have a very high momentum due to their mass, and hence are unable to negotiate the turn of USP throat, causing them to strike it. As seen in 14% ethanol formulations, high levels of NVC also make droplet size reduction due to effervescent effect difficult.

APSD chart for 0.08% w/w BDP/8% ethanol formulations containing HFA-134a mixed with CO_2 at 85 psi supply pressure

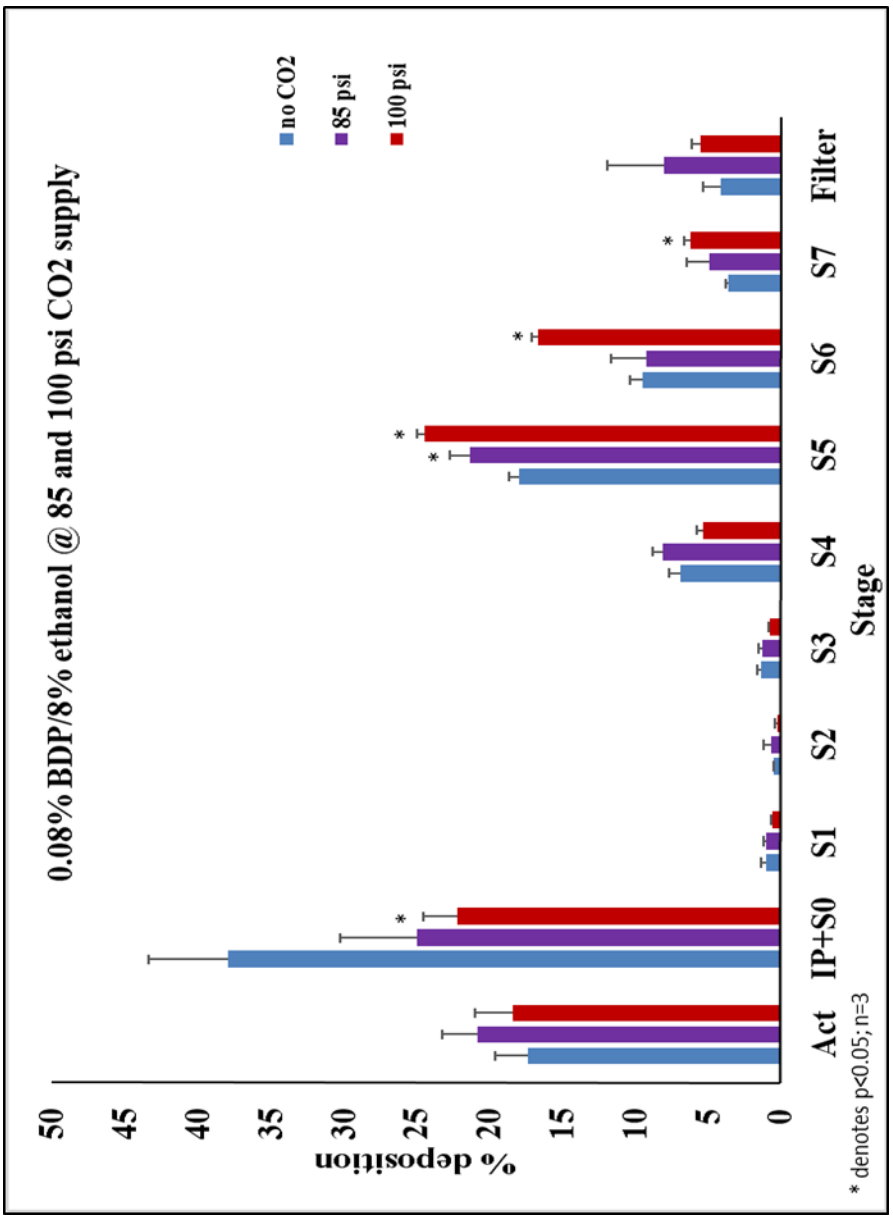


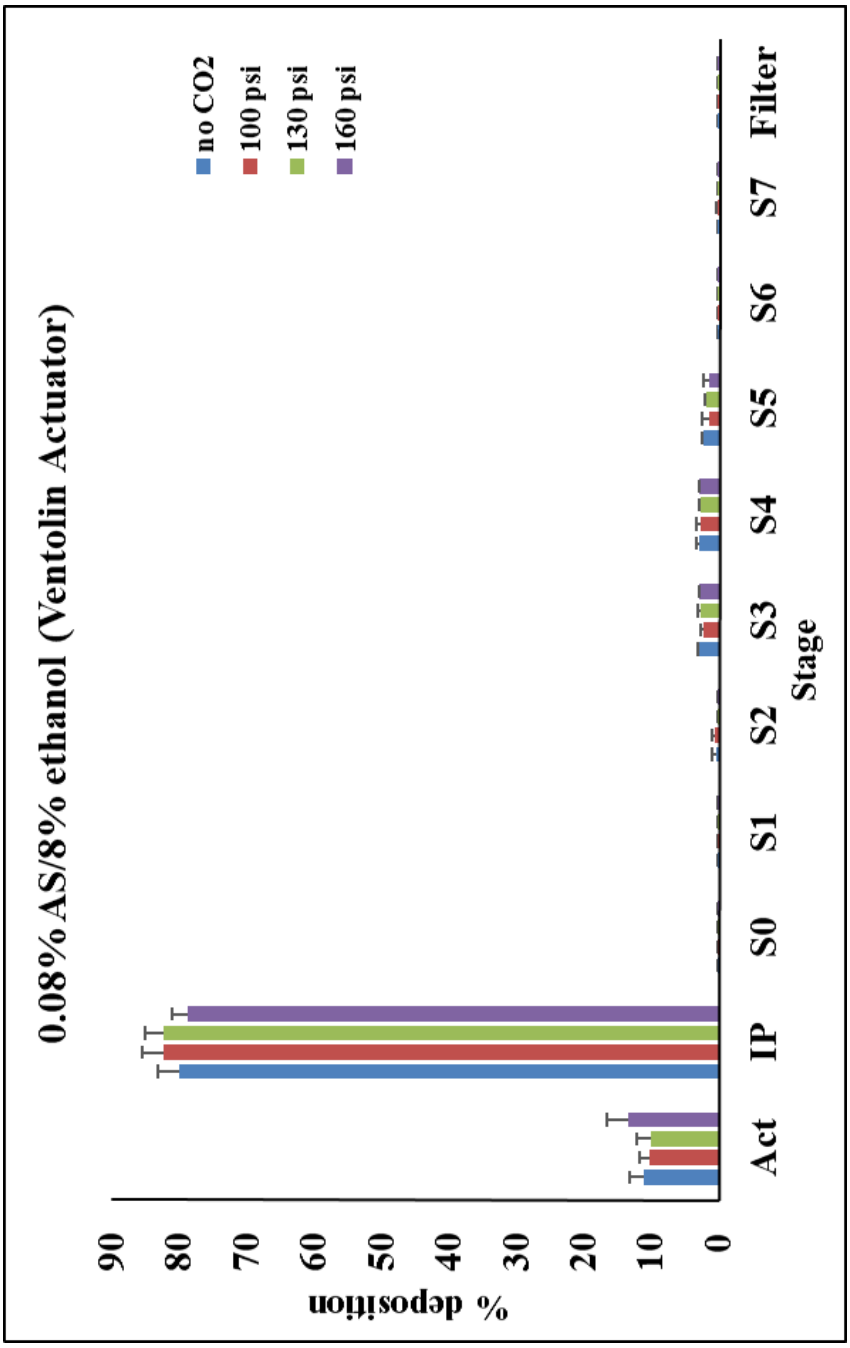
Figure 4.7: APSD chart for 0.08% BDP/8% ethanol formulations prepared using HFA-134a mixed with CO_2 at 85 and 100 psi supply pressure

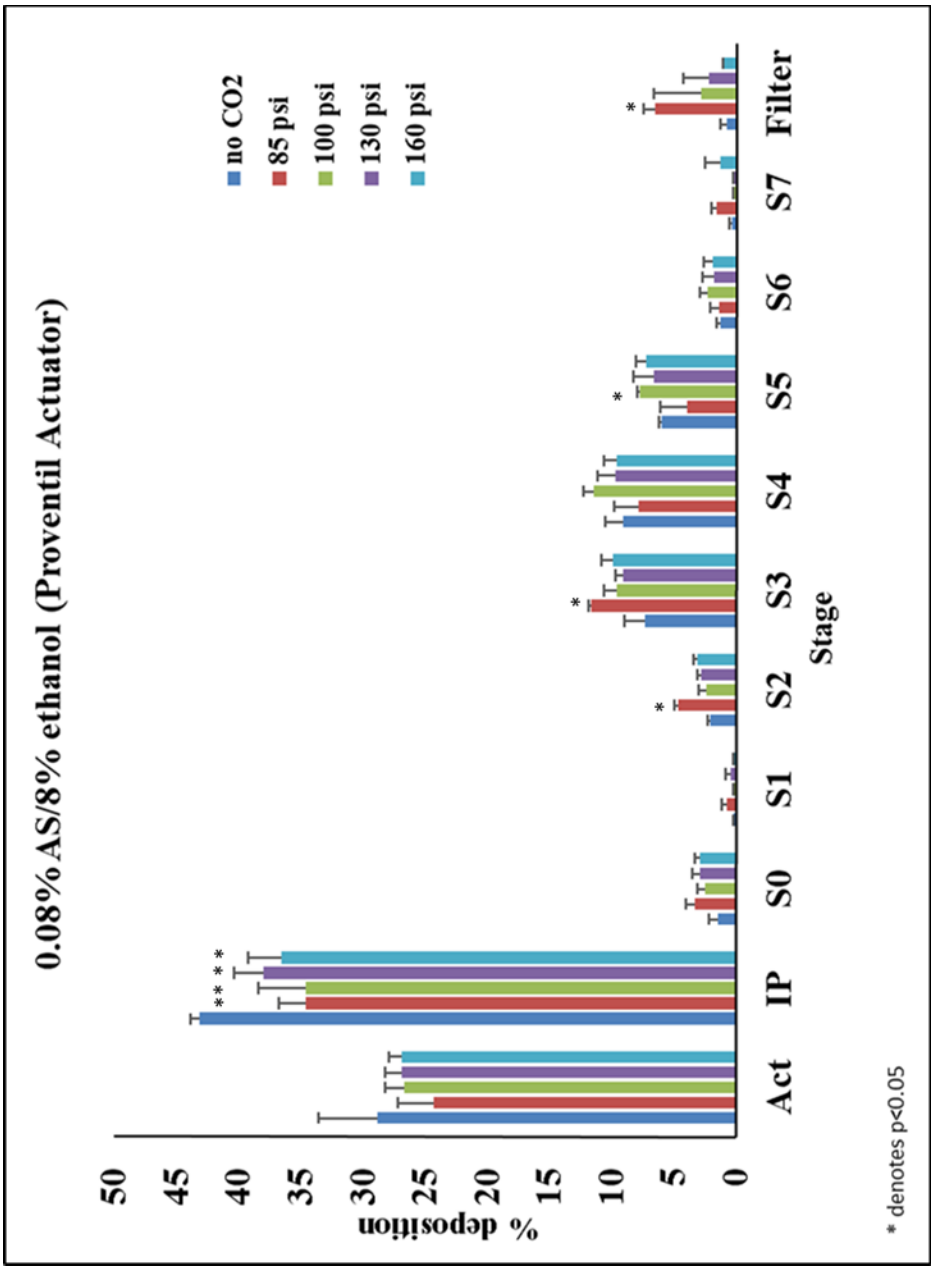
Figure 4.7 shows APSD chart for 0.08% BDP/8% ethanol formulations prepared with HFA-134a mixed with CO_2 supplied at 85 and 100 psi. The native pressure of HFA-

134a is approximately 80 psi, so 85 psi CO_2 supply pressure represents only a 5 psi increment over it. Compared to control formulation, BDP throat deposition for 85 psi formulation shows a decrease, but does not achieve significance due to high variation. On the other hand, 100 psi formulations show a significantly reduced BDP throat deposition. The 85 psi formulation fails to show a remarkable increase in drug deposition on S6 and S7 compared to control, but shows higher deposition on S4, S5, and achieving significance on the latter. In case of 100 psi, significantly high drug deposition compared to control is seen on S5, S6, and S7, which correspond to deeper lung levels. Based upon these observations, it may be concluded that BDP solution formulation canisters prepared using HFA-134a mixed with CO_2 at a supply pressure slightly above HFA's native pressure lack sufficient amount of dissolved CO_2 needed to bring about significant droplet size reduction compared to control. At a 100 psi supply pressure, enough CO_2 is driven into solution in HFA-134a, in order to cause significant droplet size reduction due to effervescence.

In the next section, ACI experimental results obtained from effervescent and control suspension aerosol formulations have been elaborated. Table 4.6 lists all derived parameters obtained from 0.08% AS/8% ethanol pMDIs sprayed using Proventil actuators.

4.3.2 APSD charts and derived parameters of 0.08% albuterol sulfate suspension pMDI formulations containing 8% ethanol actuated using Ventolin and Proventil actuators




0.08% AS/8% ethanol (Proventil Actuator)
no CO2
85 psi
100 psi
130 psi
160 psi
% deposition
50
45
40
35
30
25
20
15
10
5
0
Act
IP
S0
S1
S2
S3
S4
S5
S6
S7
Filter
Stage
* denotes p<0.05

0.08% w/w AS/ 8% w/w ethanol in HFA-134a using Proventil actuator	No CO_2	85 psi CO_2 supply pressure	100 psi CO_2 supply pressure	130 psi CO_2 supply pressure	160 psi CO_2 supply pressure
MMAD ± SD (µm)	2.41 ± 0.16	2.06 ± 0.06	2.65 ± 0.06	2.43 ± 0.11	2.49 ± 0.03
GSD ± SD	2.01 ± 0.12	2.80 ± 0.10	2.01 ± 0.13	2.33 ± 0.39	2.22 ± 0.12
Mass Balance ± SD (%)	90.81 ± 5.25	94.75 ± 7.01	91.03 ± 2.66	93.55 ± 6.01	89.27 ± 0.68
FPD ± SD (µg)	13.12 ± 0.96	17.99 ± 3.85	17.84 ± 2.80	15.84 ± 1.27	15.80 ± 1.08
FPF ± SD (%)	24.44 ± 4.11	32.53 ± 4.47	33.78 ± 5.20	29.26 ± 2.81	30.52 ± 1.87

Figure 4.8 and Table 4.6: APSD charts and derived parameters for 0.08% w/w AS/8% w/w ethanol formulations sprayed using Proventil actuator

Figure 4.8 shows APSD charts and derived parameters obtained from ACI experiments conducted on suspension pMDI formulation containing 0.08% AS w/w/ 8% ethanol, prepared using a HFA-134a/CO_2 combination, and sprayed using Ventolin and Proventil actuators. The first APSD panel represents data obtained using Ventolin actuator, which has an OD of 0.5 mm. A very high deposition is seen in throat region of the ACI, with very little amount of drug reaching impactor plates after making the turn of USP throat. No detectable deposition is seen on any plates except S3, S4 and S5. This occurrence may be explained as follows. Suspension formulations containing AS and 8% w/w ethanol,

when sprayed using a Ventolin actuator, produce droplets which attain significant momentum due to their mass and high velocity due to a bigger OD. Hence, most droplets, due to high inertia, strike the throat region of ACI and do not make it to impaction plates. One can conclude from this explanation that even though droplet size reduction due to effervescence of CO_2 is possible in this case, it does not get enough time to develop due to very high velocity of droplets produced from a Ventolin actuator. The next APSD panel shown in Figure 4.8 depicts data obtained when the same formulation is sprayed using a Proventil actuator, with an OD of 0.25mm, thus producing slower and 'softer' plumes.

Upon spraying with a Proventil actuator, approximately 45% throat deposition is seen with control HFA-134a only formulations. Throat deposition across all effervescent formulations, including 85 psi CO_2 supply pressure, is significantly reduced. Although statistically significant, this decrease is not substantial in absolute terms, unlike similar solution formulations. Moreover, no significant difference is seen within various effervescent formulations. As decrease in throat deposition is low in the first place, not enough drug is 'saved' in order to be distributed on impactor plates downstream. A few significant plate deposition increases such as that on S3 and S5 are seen, but the overall impactor plate deposition is similar for control and test formulations. It may be reasoned that since Proventil actuator produces a slower plume, there is enough time for effervescence to develop and cause droplet size reduction, unlike the Ventolin actuator.

This data highlights a fundamental difference in effervescent mechanisms in solution and suspension aerosol formulations. In the case of solutions, as the drug is completely dissolved in the propellant/ethanol, effervescence is able to produce very fine droplets. However, in case of suspensions, even though dissolved CO_2 demonstrates

effervescence in HFA-134a droplets containing suspended micronized drug, it is unable to cause diminution of drug particles below their micronization grade. This translates into effervescent solution formulations showing a greater decrease in throat deposition, compared to control, than suspension formulations.

4.3.3 APSD chart of 0.08% albuterol sulfate suspension pMDI formulations containing no ethanol actuated using Ventolin actuator

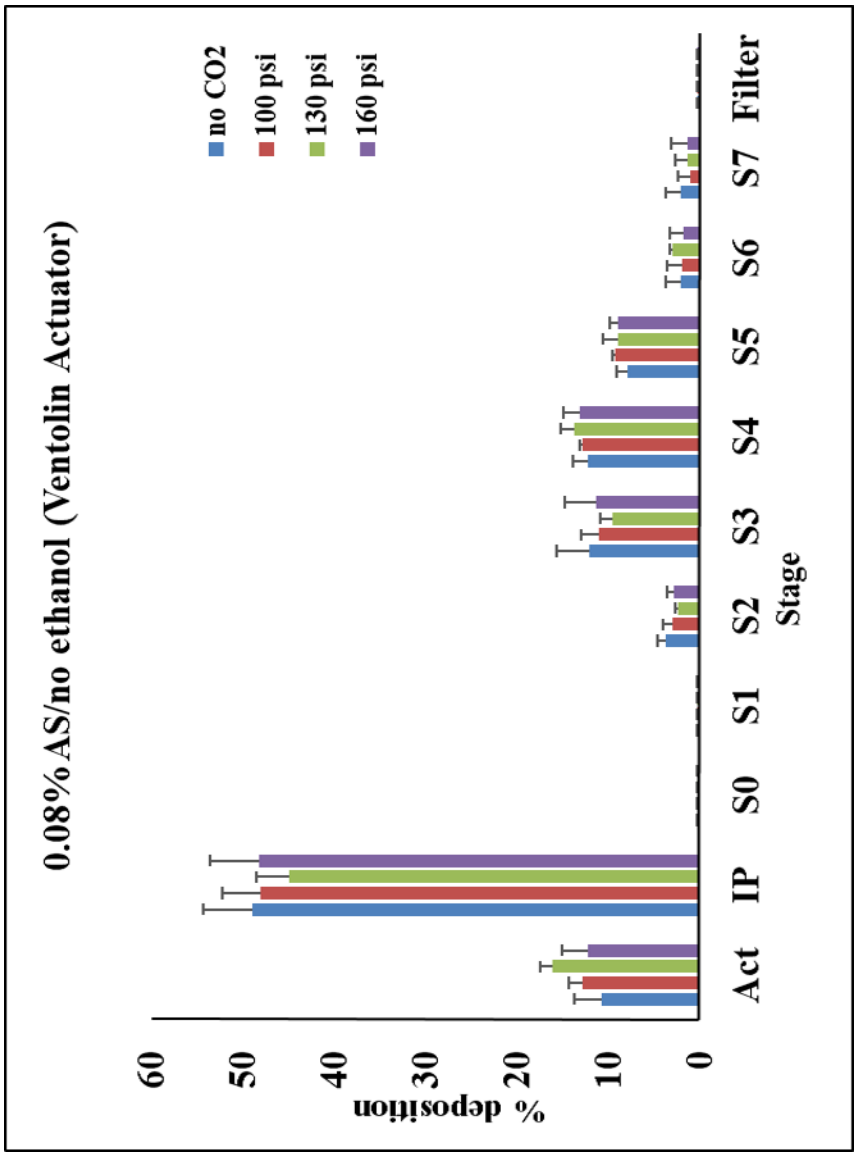


Figure 4.9: APSD chart of 0.08% albuterol sulfate suspension formulation containing no ethanol sprayed using Ventolin actuator

The APSD chart in Figure 4.9 is obtained from ACI testing of control and effervescent formulations containing 0.08% AS and no ethanol, actuated using Ventolin actuator. Unlike formulations containing 8% w/w ethanol, formulations with no ethanol show a lower throat deposition even after being sprayed with a Ventolin actuator. Suspension formulations without ethanol have lower mass value than those containing ethanol. Thus, even after being sprayed at a high velocity from a Ventolin actuator, they do not gain very high momentum and inertia, and consequently are able to successfully negotiate the turn of USP throat and reach impactor plates. However, no significant difference is seen between throat or impactor plate depositions of control and test formulations. As previously explained, the lack of time for effervescence to develop due to high plume velocity might be responsible for this outcome.

Attempts to test 0.08% w/w AS/ no ethanol formulations using a Proventil actuator were made to test the hypothesis that effervescent effect takes time to develop, and therefore manifests clearly in a slower plume. However, these experiments failed as suspension formulations without ethanol clogged Proventil actuator nozzle upon repeated spraying.

4.3.4 Comparative APSD data of 0.08% AS/8% ethanol @ 100 psi CO_2 supply pressure formulations tested at ‘B’, ‘M, and ‘E’ stage of canister life

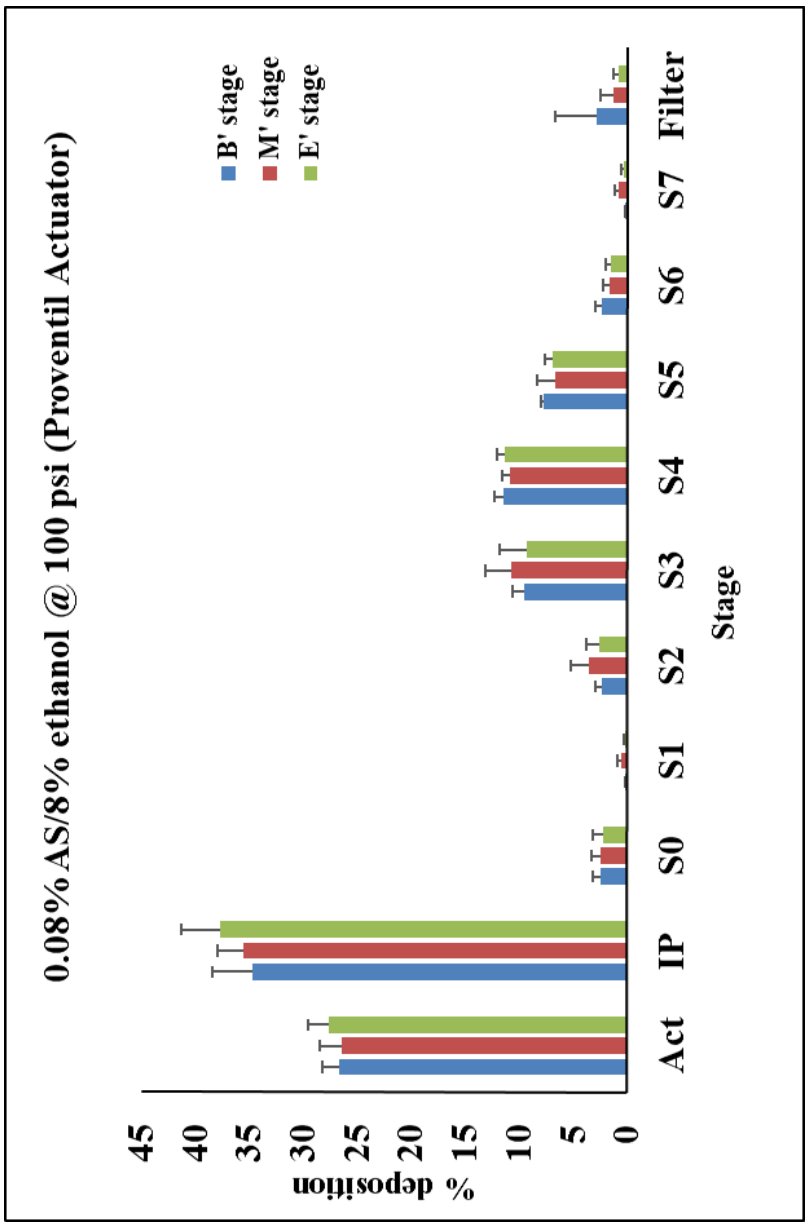


Figure 4.10: APSD data from B, M, E study of 0.08% w/w AS/8% w/w @ 100 psi ethanol formulations

Figure 4.10 shows APSD data obtained from B, M, E study of 0.08% w/w AS/8% w/w ethanol @ 100 psi sprayed using Proventil actuator. The rationale of this study, besides being an FDA required test, was to investigate whether canister emptying has an effect on effervescent behavior of pMDI formulations. A fair critique of effervescent aerosol technology is that upon gradual canister emptying, CO_2 dissolved in HFA-134a will get displaced and move into the increasing headspace. This might affect inhaler performance at end stage of canister life. In order to test this theory, 0.08% w/w AS/8% w/w ethanol @ 100 psi formulation was selected for testing. Beginning i.e. 'B' stage ACI testing was done when approximately 190 puffs remained in canister. Middle i.e. 'M' stage testing was done at 120 puffs remaining, and end i.e. 'E' stage testing was done when 40 puffs remained. All tests were performed in triplicate. APSD data from all three canister life stages was then compared. No significant difference was observed in throat deposition within three stages. Impactor plate deposition was similar for all three stages of canister life.

This data suggests that even upon gradual canister emptying, effervescent effect due to dissolved CO_2 is not diminished and pMDI performance in terms of APSD is not affected. CO_2, being highly soluble in HFA-134a, probably tends to stay dissolved in liquid HFA-134a instead of getting displaced into headspace. This data is crucial as it addresses a common critique of effervescent aerosol technology, and also proves that pMDI formulations prepared using effervescent aerosol technology perform consistently throughout the life of canister.

4.4 Conclusion

In Chapter 4, a setup to translate effervescent aerosol technology from pressure vessel to pMDI canisters was described and laser diffractometry experiments were described. As laser diffractometry experiments alone are not sufficient to characterize pharmaceutical aerosol plumes, it becomes necessary to perform cascade impaction experiments using ACI, which were described in the current chapter.

Upon analyzing ACI data obtained from solution and suspension control and effervescent formulations, the mechanism by which effervescence works in both becomes apparent. Solution formulations, which have a uniform nature, show a high degree of droplet size reduction in effervescent formulations compared to control and consequently lower throat deposition and higher impactor plate deposition. Effervescent suspension formulations containing micronized drug particles show limited droplet size reduction compared to control. This indicates that CO_2 bubbles inside HFA-134a droplets are unable to cause diminution of suspended particles beyond their micronization grade, and at best, are able to keep particles discrete due to their bursting action.

With the help of B, M, E studies, we were able to demonstrate that pMDI canisters formulated using an HFA-134a/CO_2 combination are able to perform consistently throughout the life of the canister. Overall, ACI studies described in this chapter indicate that using a combination of HFA-134a and CO_2 as propellant to formulate pMDI canisters offers distinct advantages over traditional HFA-134a only formulations in terms of improved APSD and other derived indicators.

In the next chapter, spray characterization studies such as spray impaction force and plume geometry performed on effervescent solution and suspension formulations have been described in detail.

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Chapter 5: Spray impaction force and plume geometry testing on 'effervescent' solution and suspension pMDI formulations

5.0 Introduction

Metered dose inhalers are unique drug delivery platforms as they need good patient coordination between actuation and breathing. If the MDI plume is too quick and forceful, it can lead to difficulties in such coordination. Marketed products such as Respimat® Soft Mist® inhalers, which produce a slower and 'softer' plumes have been become popular due to fact that they allow greater time for patient coordination between actuation and breathing[1]. Plume impaction force is an attribute that is most noticeable by the patients, and hence merits measurement along with other previously described tests. In this chapter, control and effervescent solution and suspension formulations have been tested for plume impaction force[2]. In addition, plume geometry experiments to investigate effect of possible increase in intra-canister pressure due to CO_2 addition on plume angle and width have also been described[3]. This data provides addition insights into MDI plume behavior upon addition of a dissolved gas to a liquid propellant.

5.1 Materials

A Copley force tester (MSP Corp., Shoreview, MN) coupled with a Mecmesin® (West Sussex, UK) force gauge was used for plume impaction force experiments. Plume geometry experiments were done on Proveris SprayView® (Marlborough, MA) run by its proprietary Viota® software. Formulations tested include- 0.08% w/w BDP/8% w/w ethanol control and effervescent formulations sprayed using QVAR actuator, and 0.08%

w/w AS/8% w/w ethanol control and effervescent formulations sprayed using Ventolin actuator. A Copley spray force tested is shown in Figure 5.1 along with impaction plate.

5.2 Methods

5.2.1 Spray impaction force testing

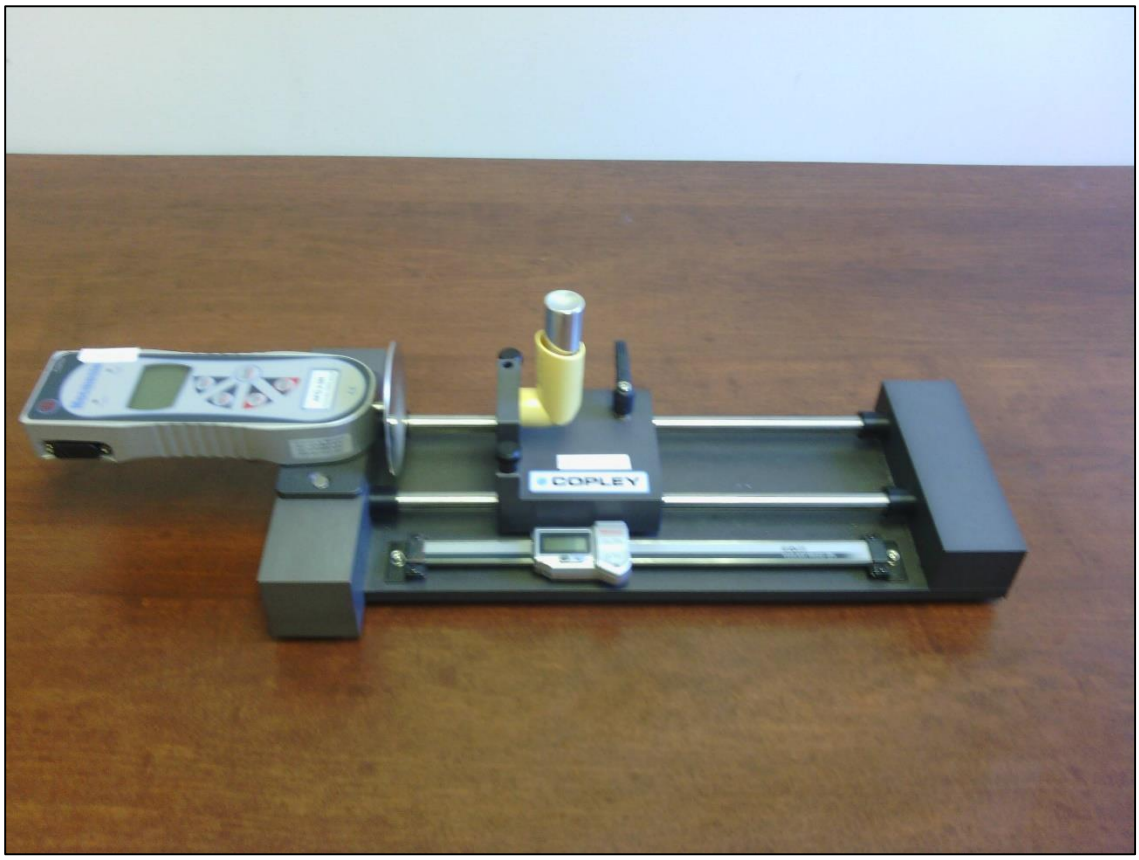


Figure 5.1: Copley spray force tester with Mecmesin digital force gauge

Spray impaction force testing was done using a Mecmesin digital force gauge, fitted with an ACI plate. The gauge was attached to a Copley spray force tester, which consists of a movable fixture which holds MDI actuators. For purpose of these experiments, a distance of 6 cm was maintained between the actuator mouthpiece and impaction plate[2]. Force was measured in gram-force (gf). All measurements were performed in triplicate.

5.2.2 Plume geometry experiments

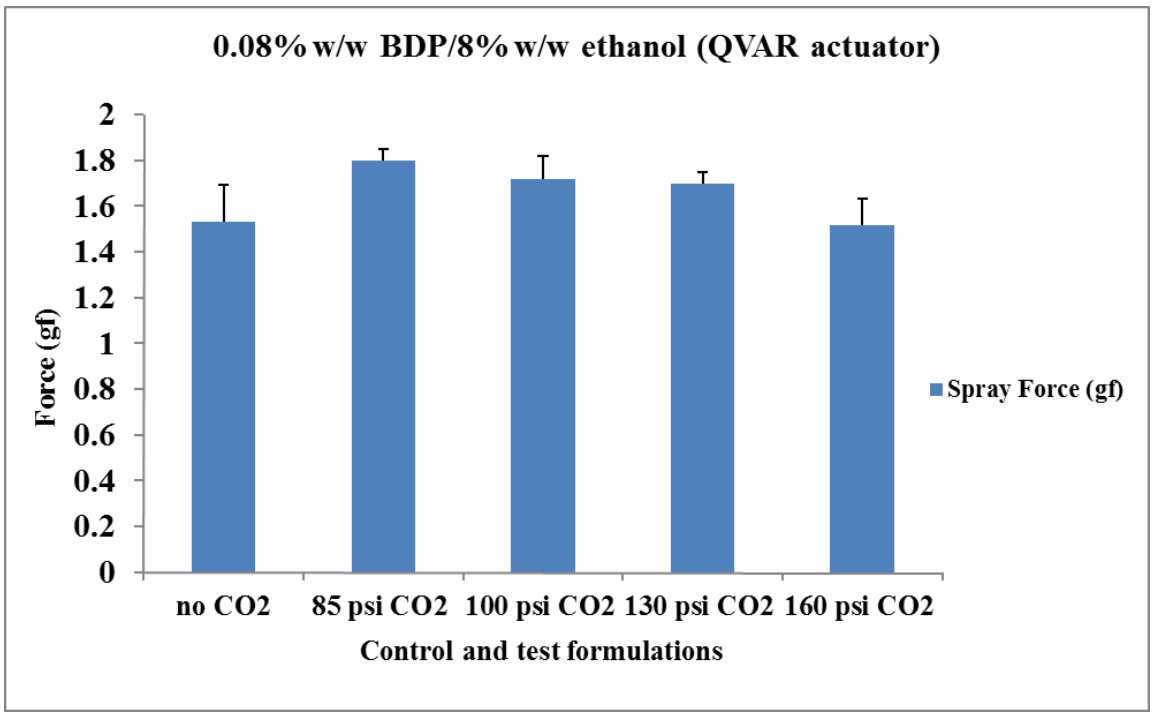
Proveris SprayView was used to conduct plume geometry experiments. It is equipped with SprayView MDx system for automatic actuation of pMDIs. A laser source is placed horizontal to the actuator and the camera is placed perpendicular. The plume distance was maintained at 70 mm. Camera distance from the actuator and camera height were 32 cm and 7 cm respectively. Actuation velocity used was 70 mm/s. A frame rate of 250 Hz was used for the camera measurements. Following formulations were tested- 0.08% w/w BDP/8% w/w ethanol solution formulation without CO_2, and with CO_2 supplied at 100 psi and 160 psi. Deliverables were plume angle (degrees) and plume width (mm). All measurements were performed in triplicate. All operational parameters are standards used in plume geometry experiments involving pMDIs at Next Breath LLC, the venue for testing.

5.2.3 Statistical analysis

Student's t-test was used to compare data obtained from plume impaction force and plume geometry experiments. A difference was deemed significant if $p < 0.05$. Statistical significance was denoted by asterisk (*).

5.3 Results and Discussion

5.3.1 Spray impaction force data obtained from solution and suspension formulations



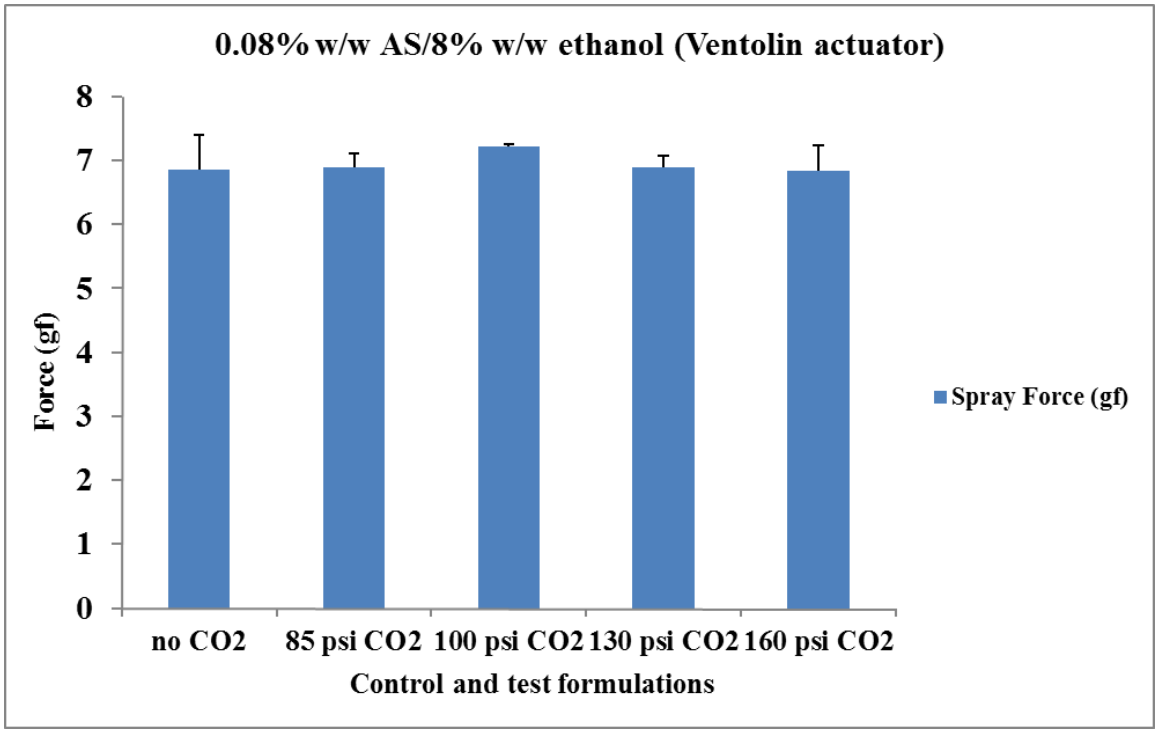


Figure 5.2: Spray impaction force data obtained from solution and suspension formulations

Figure 5.2 shows spray impaction force data for solution and suspension formulations sprayed with QVAR and Ventolin actuators respectively. QVAR actuators, which has an OD of 0.25 mm and produces slower plumes from control formulations that register an impaction force of approximately 1.5 gf on the gauge. Upon testing effervescent formulation, 85 psi, impaction force value inches upwards slightly, but not enough to achieve significance. Force values for all other effervescent samples are roughly the same as the 85 psi sample, suggesting that increase in CO_2 supply pressure does not affect impaction force of solution formulation plumes.

In case of suspension formulations, which are sprayed using a 0.5 mm OD Ventolin actuator, the plume is significantly quicker than QVAR actuator generated plumes, and hence registers a force value of approximately 7 gf, which is significantly higher than solution formulation values. However, as in case of solution formulations, no difference is seen in force values generated by control and effervescent suspension formulations.

In case of effervescent formulations, satellite droplets are probably generated due to bursting action of CO_2 bubbles inside HFA-134a droplets. Even though it is logical to reason that addition of CO_2 to inhalers increases canister pressure and hence plume velocity and impaction force, the production of much smaller satellite droplets might reduce the overall momentum of the spray. This reduced momentum might explain the lack of increase in observed force values in effervescent sprays compared to HFA-134a only sprays.

5.3.2 Plume geometry data obtained from control and effervescent solution formulations

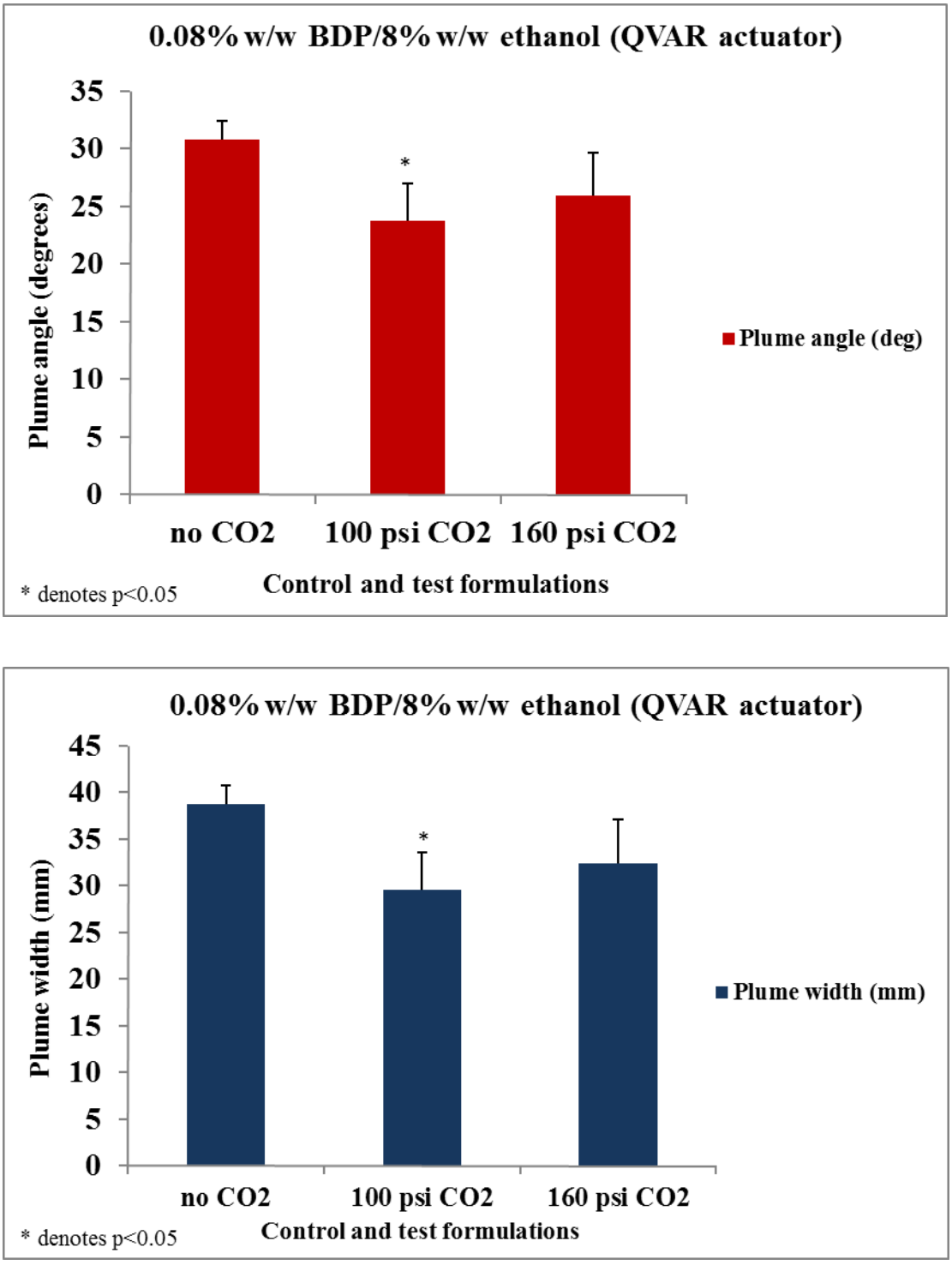
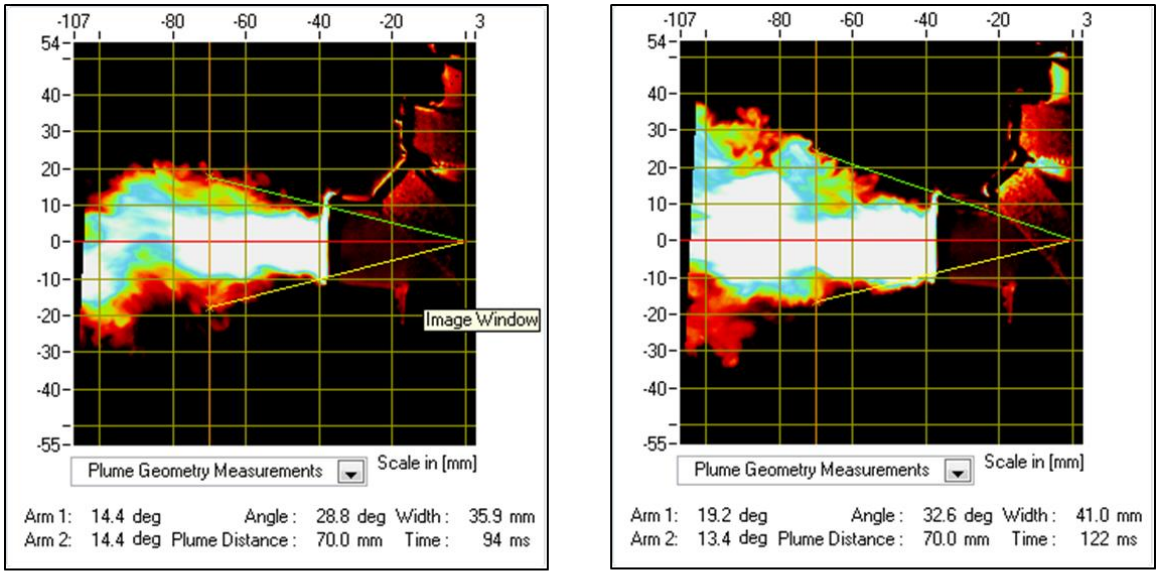


Figure 5.3: Plume geometry data from solution formulations

Figure 5.3 shows plume angle and plume width data obtained from control and 100 psi and 160 psi BDP solution formulations. In case of plume angle control formulations yield an angle of approximately 30º. Plume angle decreases significantly for 100 psi samples and increases again for 160 psi samples. Similar trend is observed in plume width-100 psi samples produce plumes with significantly lesser width than control. This decrease in plume angle and width might account for decreased throat deposition seen in effervescent samples in ACI experiments described in Chapter 4. This data is interesting as it seems to contradict the argument that adding CO_2 to HFA-134a will increase canister pressure, which will in turn increase plume angle. Further investigations are needed to understand the cause of such an observation. Figure 5.4 depicts plume photographs from SprayView experiments for control and effervescent samples. It is clearly evident that effervescent formulations produce much leaner, streamlined plumes compared to HFA-134a only samples.



Effervescent formulation HFA-134a only formulation

Figure 5.4: Plume photographs for effervescent and HFA-134a only formulations

5.4 Conclusion

Plume impaction force is an aspect of inhalation drug delivery that is most conspicuously felt by the patient. Hence, in addition to laser diffractometry and cascade impaction experiments, plume impaction force and plume geometry experiments can yield valuable information about inhaler performance.

In case of effervescent aerosol technology, a common critique is that dissolving CO_2 in HFA-134a inside the canister would increase intra-canister pressure that would lead to an increased plume impaction force or a wider plume angle. This would in turn lead to an increase in throat deposition, and hence loss in amount of drug reaching the lungs. However, based upon data our experiments generated, no appreciable increase is seen in plume impaction force generated by effervescent aerosols compared to HFA-134a only. On the other hand, plume angle and width actually decrease for effervescent samples, which is beneficial in terms of reducing throat deposition.

Overall, plume impaction force and geometry experiments yield valuable information about aerosol plume behavior and might also have clinical value in terms of predicting patient comfort and drug deposition.

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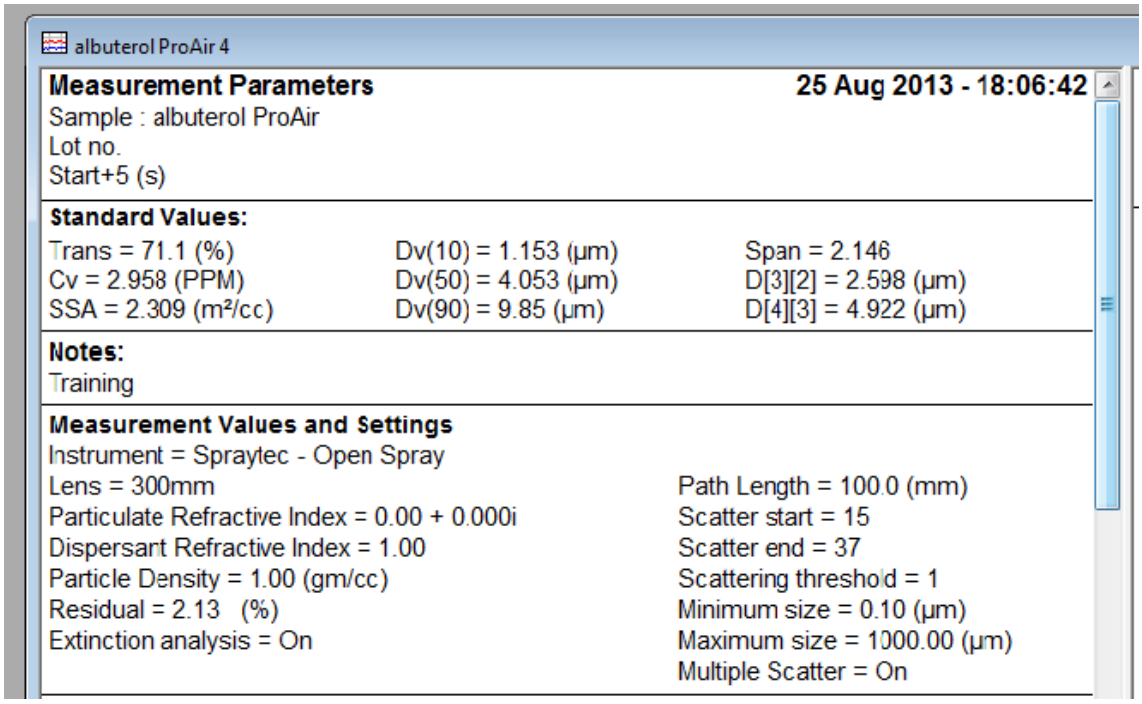
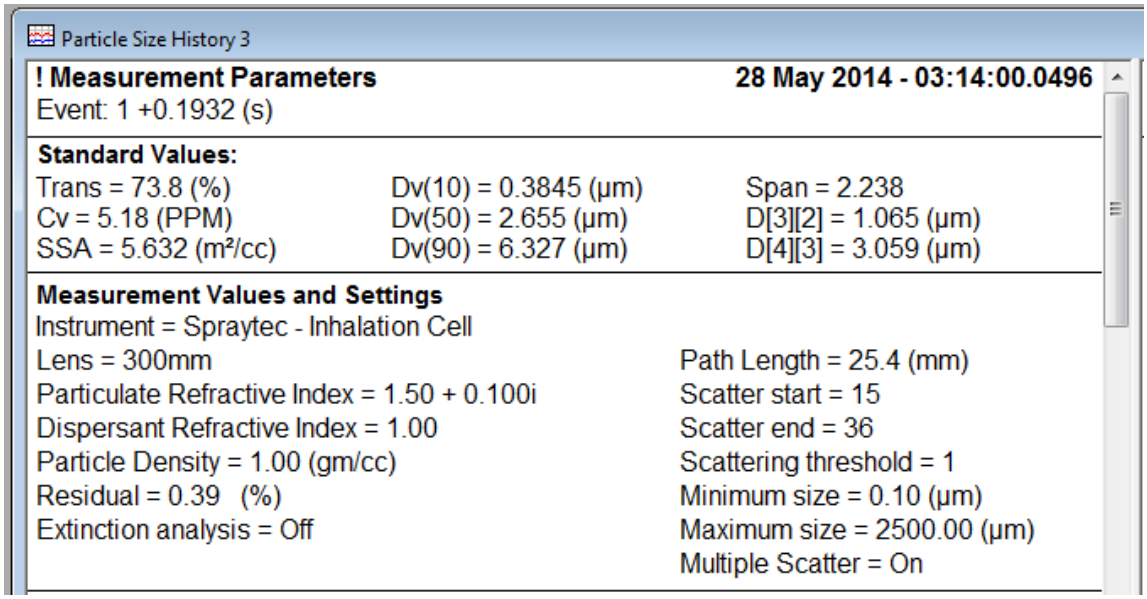
APPENDIX I: Malvern Spraytec® operational parameters

Parameters for Spraytec operation in continuous mode (Used in experiments using custom designed pressure vessel)



Measurement mode	Continuous
Sampling period	8 secs
Lens type	300 mm
Inhalation cell	Yes; horizontal configuration
Beam steering	Adjusted
Detector Range	15 to last
Multiple scattering analysis	On

Parameters for Spraytec operation in rapid mode (Used for formulations in pMDI format)



Measurement mode	Rapid
Data acquisition rate	2.5 kHz
Lens type	300 mm
Inhalation cell	Yes; vertical configuration with USP throat
Trigger type	Transmission drops to 98%
Multiple scattering analysis	On
Beam steering	Adjusted
Detector range	15 to last

APPENDIX II: Explanation for some common critique points regarding 'Effervescent Aerosol Technology'

Critique Point 1: Chemical stability of formulations prepared using effervescent aerosol technology

Questions regarding chemical stability of formulations prepared using effervescent aerosol technology are frequently posed to us by scientists who are skilled in the science of pMDI formulation. One of the main issue raised is the effect of ingress of moisture upon such canisters, which will lead to production of carbonic acid due to the presence of CO_2. Presence of carbonic acid might affect stability of APIs, leading to unstable formulations. In our opinion, this is a very valid concern. Carbonic acid generation may be reduced by controlling ingress of moisture into the canister. In order to minimize ingress of moisture, it is important to closely monitor canister filling process at every stage. Use of pressure filling, which is an industry standard, would be an effective tool in controlling moisture ingress. However, despite best efforts and precautions, some moisture will invariably enter the canister and generate carbonic acid. A detailed study would be needed to assess the impact of carbonic acid generation on formulation stability. Such a study was not feasible in this dissertation due to complexity of analytical methods involved, but can be easily commissioned in an industrial product development setting.

Critique Point 2: Effect of canister emptying on performance of effervescent formulations- As the formulation is used up, headspace in canister will increase, CO_2 dissolved in liquid formulation will get displaced into headspace, which will lead to lesser effervescence in final stages of canister

This is a common concern raised by pMDI formulation scientists regarding effervescent aerosol technology. However, in our opinion, CO_2 being highly soluble in HFA-134a, will stay dissolved in liquid formulation even at end stage of canister life. This will lead to consistent effervescent effect throughout canister life. Although it is difficult to get direct evidence on this, B, M and E stage experiments described in Chapter 4 show that effervescent inhaler performance at different stages of its life is consistent, which indicates that CO_2 stays dissolved in HFA-134a.

Critique Point 3: Effect of temperature on stability and safety of canisters prepared using effervescent aerosol technology- Increase in temperature might lead to an increase in internal canister pressure, which may cause canister to lose structural integrity

In our opinion, at elevated temperatures, capacity of HFA-134a to dissolve CO_2 will increase, leading to CO_2 in headspace getting dissolved in liquid formulation. Thus, there may not be any appreciable increase in internal canister pressure at elevated temperatures.

Critique Point 4: Regulatory aspects concerning effervescent aerosol technology

In our opinion, regulatory aspects regarding formulations prepared using effervescent aerosol technology can be categorized into formulation and device issues. All ingredients in effervescent formulations, except CO_2, are similar to other marketed pMDI products. CO_2 is included in the list of ingredients 'generally recognized as safe' by the USFDA, thereby posing minimal regulatory challenges in effervescent pMDI development.

As our experiments demonstrate, effervescent formulations can be safely packaged into existing, state-of-the-art canisters fitted with metering valves. Hence, development of new hardware suited for effervescent formulations is not necessary. Thus, we expect device related regulatory issues related to be minimal. Overall, if effervescent formulations demonstrate consistent performance and stability, we expect few regulatory hurdles in their development.

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