STUDIES IN AEROSOL DRUG FORMULATION, ANALYSIS, AND MODELING

by

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I would like to thank my parents for their support and encouragement throughout my time away. You all have helped me grow as a person, allowing me the foundation to build everything I do from here forth. I have missed you in person, but never in thought.

To my wife Ryan, you are my best friend, my biggest motivation, and the most wonderful person I could ever have the pleasure of knowing.

To my advisor Dr. Myrdal, I learned a lot more than just the science in my extended time working with you. I don't know that I would have continued on to graduate school without your guidance early on. Next round is on me, I'll always owe you one.

DEDICATION

For all my favorite people: Ryan, Mom, Dad, Drew, Kristi, Rylee, Kyle, Tres, Will, Joe, Tony, Chris, Jodie, Bianka, and Team Zong.

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ABSTRACT

A recently mandated change in the use of pharmaceutical propellants spurred the development and reevaluation of aerosolized pharmaceuticals. Chlorofluorocarbon (CFC) propellants were commonly used in pressurized metered dose inhalers (MDIs), but were unfortunately linked to the depletion of the ozone layer. As such, a search for new propellants was initiated and ultimately resulted in the implementation of hydrofluoroalkane (HFA) propellants in MDIs. These HFA propellants however demonstrated significantly different properties than CFCs and necessitated a considerable amount of reformulation efforts. Not only did HFAs demonstrate different physiochemical properties, but in some cases these differences necessitated reengineering of the delivery device. Unfortunately HFA propellants are considered greenhouse gasses, albeit to a lesser degree than CFCs, so the development of alternate delivery methods has been ongoing. One delivery method that has received significant attention and resources is dry powder inhalers (DPIs). DPIs are a propellant-free alternative to aerosolized drug delivery, and demonstrate some advantages and disadvantages compared to the use of MDIs and nebulizers.

In addition to the modernization of pharmaceutical agents, excipients, and delivery devices, technological advances have allowed for different and/or improved characterization of pharmaceutical aerosols. Particle size characteristics of aerosols are the primary physical measure examined and are relevant to ensure proper and reproducible drug delivery to the lung. Likewise, chemical analysis of the pharmaceutical

agent is extremely important for pharmaceutical development and monitoring, including solubility determination, stability monitoring, and ultimately, dose emitted. Because many limitations exist in characterization however, and because experimental means can be costly with regard to labor and materials, prediction of aerosol performance characteristics based on formulation and device variables are valuable.

Previous work predicting the performance of solution based MDIs has opened the door for improved prediction of suspension based MDI systems. Suspension aerosol prediction has been examined in the past, but additional information is now available to more appropriately model suspension MDI systems that include polydisperse drug material and emit polydisperse droplets.

CHAPTER 1: PHARMACEUTICAL PROPELLANTS: TRANSITION TO THE FUTURE

I – A Brief History of Inhalable Aerosols

Inhalation drug therapy has existed for thousands of years [1]. The leaves of the *Atropa belladonna* plant, which contains atropine, were smoked to help symptoms of pulmonary diseases [2]. Countless examples of various smokes and vapors used to treat a myriad of mouth, throat, and chest disorders have been cited worldwide. In the early 1800's it was noted that *Datura stramonium* relieved asthma symptoms, and was added into tobacco cigarettes [3]. Subsequently, these cigarettes were studied and shown to produce bronchodilatory effects [4].

The use of vapors became more common when the first nebulizers were developed in the 1820s [5]. Several methods were employed to create vapor or steam, but all were designed with the same purpose; aerosolize liquid. Later, the first metered dose inhaler (MDI) was developed. In 1956, Rikers Laboratories, now 3M, developed the first metered dose inhaler, Medihaler-Epi, a MDI (seen in Figure 1.1) containing epinephrine (adrenaline) which acts to relax the smooth muscle of the airway by agonizing β-receptors of the lung, effectively mimicking the 'fight or flight' response.

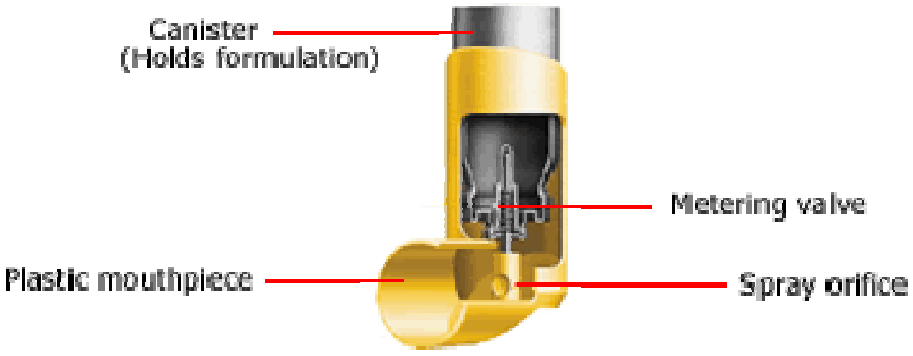


Figure 1.1. Schematic of a typical metered dose inhaler (MDI).

Inactive ingredients, which later became quite common for this application, included the chlorofluorocarbon (CFC) propellants dichlorodifluoromethane, trichlorofluoromethane, and dichlorotetrafluoroethane (Figures 1.2a-c, respectively), the preservative cetylpyridinium chloride, and sorbitan trioleate to act as a suspending agent.

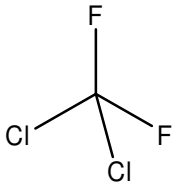


Figure 1.2a. Structure of dichlorodifluoromethane (CFC 12)

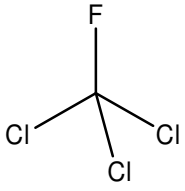


Figure 1.2b. Structure of trichlorofluoromethane (CFC 11)

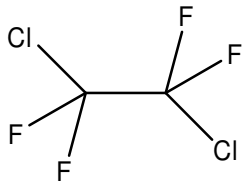


Figure 1.2c. Structure of dichlorotetrafluoroethane (CFC 114)

Unfortunately, along with the appearance of MDIs, a disturbing increase in asthma related deaths followed. Several authors documented deaths primarily cardiac in nature, associated with the overuse of MDIs [6, 7]. It had later been identified that the newly developed (at the time) CFC propellants sensitized the myocardial tissue to catecholamines. In controlled animal studies, dogs were exposed to moderate amounts of fluorocarbons and then challenged with epinephrine, which resulted in severe ventricular arrhythmias [8]. This was only the case however when significant amounts of propellants had been inhaled, and studies showed that when using a MDI in a controlled fashion, no adverse effects were noted [9].

During this time, a transition was underway when the Medihaler-Epi was marketed. Previously, CFC blends were used along with high concentrations of ethanol to dissolve the drug yielding primarily solution formulations. Medihaler-Epi was instead formulated using sorbitan trioleate, a surfactant to suspend the micronized drug in the propellants rather than solubilizing it. This was of great utility, because compounds which were either completely insoluble or insufficiently soluble were thereafter able to be

delivered via MDI [10]. This formulation technique turned out to be one that is still used very readily today.

II – Elimination of CFC Propellants

Metered dose inhalers quickly became commonly used in the medical community. In addition to their use in pharmaceuticals, CFC propellants were also utilized in a number of different manners including:

- air conditioners as refrigerants
- fire extinguishers
- household aerosol sprays
- as part of the manufacturing of foams and insulations

CFCs demonstrated many desirable properties including limited toxicity, inertness, and suitable vapor pressures to allow for storage in simple containers such as an aerosol can or MDI. Unfortunately, despite the numerous advantages offered by the CFCs including their numerous applications, they were found to be contributing to the depletion of the ozone layer and the greenhouse effect [11]. CFCs 11 and 12 (chlorofluoromethanes) were primarily indicated in these ozone depleting effects. Through photolytic dissociation caused by ultraviolet light, both CFC 11 and 12 lose a

chlorine (Figure 1.3). In the stratosphere, this chlorine acts as a catalyst and interacts with oxygen radicals, and results in the depletion of ozone (O_3), as shown in Figure 1.4, resulting in O_2, and chlorine.

$$CFCl_3 \rightarrow CFCl_2 + Cl$$

$$CF_2Cl_2 \rightarrow CF_2Cl + Cl$$

Figure 1.3. Photolytic dissociation of chlorine atoms from CFC 11 and 12, respectively.

$$Cl + O_3 \rightarrow ClO + O_2$$

$$ClO + O \rightarrow Cl + O_2$$

Figure 1.4. Reaction of chlorine catalyzed ozone depletion in the stratosphere.

Due to the environmental ramifications of CFC use, the Montreal Protocol was drafted, and then ratified in 1989, initiating the phase-out of CFC propellants, including those used in MDIs. As of 2002, the Montreal Protocol had been ratified by 183 countries [12]. However, because pharmaceutical inhalers are considered life saving for many asthmatic and COPD patients, they were exempted from the protocol as 'essential-use' pending availability of suitable alternatives [13].

As a result of the Montreal Protocol, it was apparent that significant resources needed to be invested for the development of alternative non-CFC containing products, namely dry powder inhalers (DPIs) and nebulized solutions, in addition to finding other suitable propellants to replace CFCs for use in MDIs.

Two alternate propellant candidates for CFC replacement were identified, HFA 134a (1,1,1,2-tetratfluoroethane, Figure 1.5a) and HFA 227 (1,1,1,2,3,3,3-heptafluoropropane, Figure 1.5b).

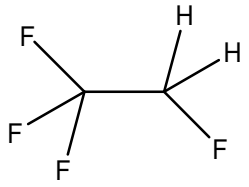


Figure 1.5a. Structure of HFA 134a

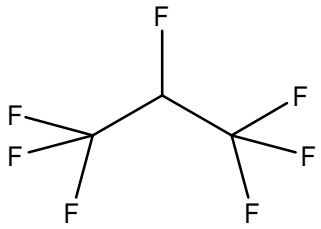


Figure 1.5b. Structure of HFA 227

These hydrofluoroalkanes lack the ozone depleting characteristics of their predecessors, as they are not chloro-substituted. However, they still contribute to the greenhouse effect, albeit to a lesser degree than their CFC counterparts, as displayed in Table 1.1 [14]. Additionally, the half-life of these HFA propellants in the atmosphere is a fraction of that of the CFCs they would ultimately replace [15].

Propellant	***Ozone Depletion Potential****	***Atmospheric Life (Years)***	***Global Warming Potential****
CFC 11	1	60	1
CFC 12	1	125	3
CFC 114	0.7	200	3.9

HFA 134a	0	16	0.3
HFA 227	0	33	0.7

Table 1.1. Characteristics of pharmaceutical propellants in the atmosphere.

Several authors have described the physiochemical properties and differences observed between CFCs and HFAs [16-19]. As Table 1.2 exhibits, the two HFA propellants that are currently utilized have boiling points and vapor pressures comparable to CFC 12 (dichlorodifluoromethane), the chief propellant used to obtain sufficient formulation vapor pressure when used in blends [15]. However, HFA 134a and 227 do not display the same solvency characteristics of the CFCs [18].

Propellant	***Liquid Density (g/ml)***	***Dipole Moment (debye)***	***Boiling Point (ºC)***	***Vapor Pressure (psig @ 20 ºC)***
CFC 11	1.49	0.46	23.7	-1.8
CFC 12	1.33	0.51	-29.8	67.6
CFC 114	1.47	0.50	3.6	11.9
HFA 134a	1.21	2.06	-26.3	82.3
HFA 227	1.41	0.93	-16.5	56.6

Table 1.2. Physical and chemical properties of pharmaceutical propellants.

This difference in solvency characteristics is presumably due to the lack of polarizability of the fluorinated hydrocarbons as compared to the highly malleable electron clouds of chlorines on the partially chloro-substituted CFCs [16]. This decrease in polarizability relative to CFC propellants could help explain some solubility

differences of solutes and excipients in HFA based systems, despite their increased polarity over CFCs. Another major difference between the propellants is the hydrogen(s) on the HFAs, resulting in an increased dipole moment relative to CFC propellants which are completely chloro- and fluoro-substituted. As a result of this dipole, the highly electropositive hydrogen(s) appear to make the environment much less amiable to nonpolar solutes, while potentially enabling a degree of hydrogen bonding. This will be discussed in further detail in later chapters with regard to excipients designed specifically for HFA propellants.

Although these characteristics may begin to explain the differences in observed propellant-excipient/drug interactions, it is arguably academic, as CFC propellants are not a propellant option for future therapeutics. Thus, when formulating MDIs, the only currently available options for propellants are HFA 134a and HFA 227.

III – Comparison of HFA Propellants; Advantages and Disadvantages

Differences in the physical properties of HFAs, although minor, may warrant using 134a versus 227, or vice versa for a given formulation. Purity profiles of both propellants show a very low degree of impurity (both >99.9% pure) [20], and would not significantly impact the choice of propellant. Compared to CFC propellants, both HFAs have relatively low boiling points (as seen in Table 1.2) which afford sufficient vapor pressure at lower temperatures without compromising efficiency [21]. Stein and Stefely displayed that upon reaching -10ºC, HFA based MDIs reached the full respirable dose

emitted at room temperature, whereas the CFC based (suspension) MDI displayed respirable dose increasing as a function of temperature up to at least room temperature [21]. This implies that in cold weather, HFA based MDIs will perform as expected, unlike CFC based systems. At the other end of the temperature scale, Hoye *et al* showed that when HFA based systems were actuated at higher temperatures, respirable mass (droplets < 4.7µm) increased by ~14% and ~46% for Proventil HFA and Ventolin HFA, respectively (shown in Figure 1.6). Conversely the shot weight emitted by the MDIs decreased by ~9% and ~24%, respectively (shown in Figure 1.7), as temperature increased and ultimately dose emitted decreased by the same factor [22]. The end result is that though less formulation is being emitted, a higher proportion results as respirable droplets, and ultimately the fine particle dose is close to unchanged. Though the temperatures at the high end of this study were rather high (~140ºF), it again showed relatively consistent doses ultimately being administered [22].

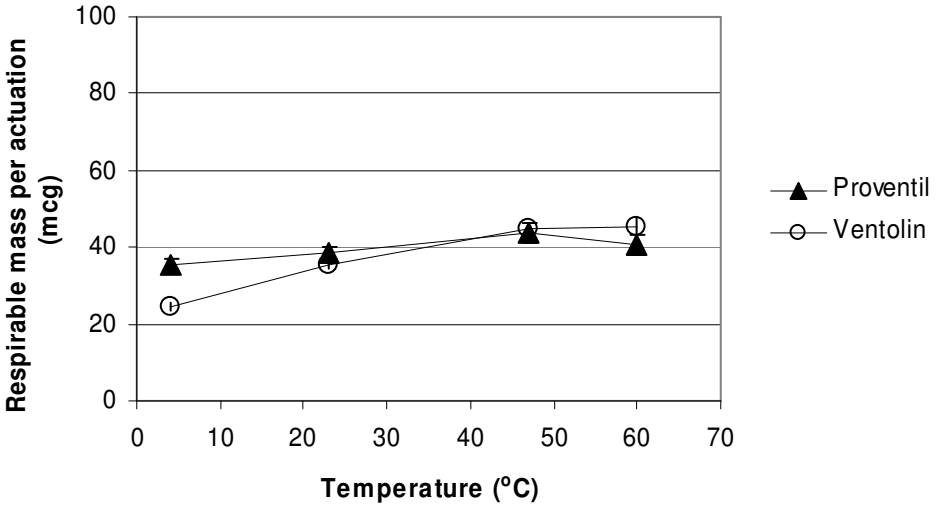


Figure 1.6. Respirable mass as a function of temperature for two HFA formulations.

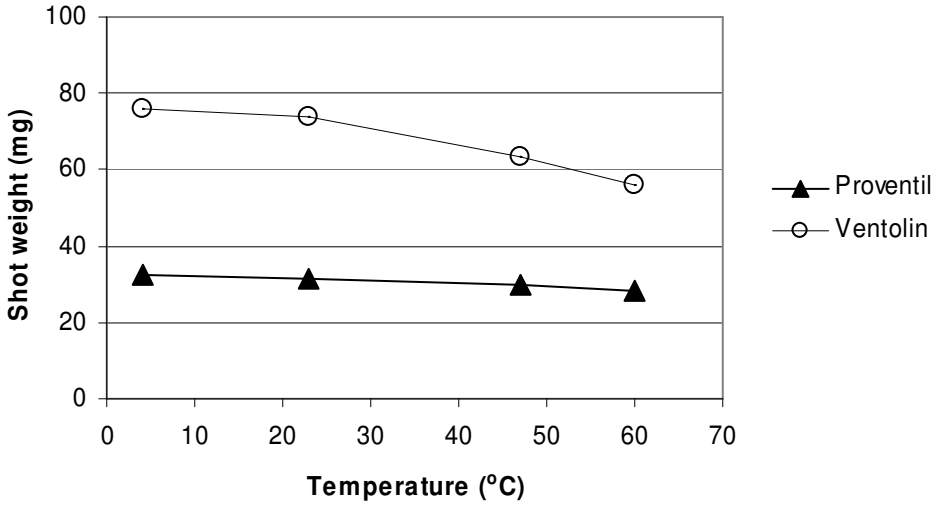


Figure 1.7. Shot weight as a function of temperature for two HFA formulations.

HFA 134a and 227 are completely miscible in one another and vapor pressure upon mixing behaves nearly ideally, thus they may be blended in different proportions to obtain a specific vapor pressure or density [23]. There is no toxicological advantage to either propellant, nor is there a degradation concern of one over the other, under relatively normal storage conditions [20]. That said, some potential differences which may persuade a formulator to choose one or the other mainly relate to chemical structure and resultant physiochemical properties. HFA 134a has a $\log K_{ow}$ of 1.06 versus 2.05 for 227, and as such, water has nearly four-fold increased solubility in HFA 134a versus 227 (2200 and 610ppm, respectively), though both HFAs are not considered to be hydrophilic. Of note, both HFA 134a and 227 uptake significantly greater amounts of

water as compared to the aforementioned CFC propellants (all ~120ppm), likely due to the relatively increased polarity [20]. Thus, when formulating a suspension of a compound, physical stability as a function of water may require addressing. Likewise, if in a solution formulation the compound of interest is water labile, HFA 227 may offer a slight advantage, though the formulation may still be susceptible to water migration. Williams and Hu confirmed these findings experimentally, though did not obtain the same magnitude of difference [24]. They also showed that depending on the drug, emitted particle size could change, likely due to Ostwald ripening and as result, fine particle fraction would change as well (fraction of aerosol less than 4.7 microns). Additionally, they indicated container lining and storage temperature for impacting increased water content.

In conclusion, inhaled medications have been used for centuries, but only recently have more controlled methods of delivery developed. The primary ailments treated remain similar to those in times past, but the advent of MDIs, DPIs, and more advanced nebulizers have allowed for simpler disease management. MDIs were very broadly used, but contributed to the depletion of the ozone layer, thus needed to be reformulated with alternative propellants. HFAs were the propellant of choice for the replacement of CFCs in MDIs, but it was apparent that alternative delivery methods needed to be sought. As such, more advanced DPIs were designed in addition to advancing nebulizer technology and further investigations into MDI reformulation.

CHAPTER 2: UPDATE IN EXCIPIENT USAGE FOR PHARMACEUTICAL AEROSOLS

I – Metered Dose Inhalers

As mentioned in the previous chapter, a significant shift in MDI formulation has taken place in the recent past. As result of the Montreal Protocol, though MDIs were considered 'essential-use', but a need for non-ozone depleting propellants was still needed. Hydrofluoroalkane propellants (134a and 227) were discovered to have many necessary properties to be successful replacements for CFC propellants. Per the descriptions in the previous chapter however, HFAs displayed significantly different solvency characteristics than CFCs, and in general, they solubilize both solutes and solvents comparatively much poorer.

Table 2.1 shows many previously approved excipients for use in MDIs (excluding surfactants). As mentioned previously, ethanol was a commonly used excipient to solubilize compounds into CFC-based systems prior to the advent of suspension formulations.

Excipient	***Product***	***Function***	***Maximum Approved Concentration***
Ethanol/Dehydrated Alcohol/Alcohol	Azmacort, Isuprel, Primatene Mist, Tornalate, Qvar, Atrovent HFA, Proventil HFA, Xopenex HFA, Aerospan HFA, Alvesco	Co-solvent	34.5%
Water	Atrovent HFA	Co-solvent	
Menthol	Aerobid, Tornalate	Flavoring agent	0.05%
Saccharin sodium		Flavoring agent	0.045%
Saccharin	Tornalate	Flavoring agent	0.112%
Citric acid (anhydrous)	Atrovent HFA	Flavoring agent	0.00022%
Hydrochloric acid		pH adjustment	1.72%
Nitric acid		pH adjustment	1.67%
Ascorbic acid	Primatene Mist, Isuprel, Tornalate	Antioxidant	1.02%

Table 2.1. Previously approved excipients used in MDIs excluding surfactants.

In addition to enhancing solute solubility, particularly ethanol as a cosolvent has been used to alter the vapor pressure of MDI systems. According to Raoult's law, the vapor pressure of the system is the sum of the vapor pressures of its parts, based on fractional concentrations. With the addition of ethanol to HFA systems (both in 134a and 227), vapor pressure has shown to deviate positively compared to ideality [23, 25]. Regardless of this effect, Gupta *et al* (2003) has shown that despite increasing ethanol concentrations enhancing solute solubility, there is also a correlating decrease in overall

performance (respirable deposition) caused by a decrease in vapor pressure of the system, resulting in limited gains in total respirable dose, as can be seen in Figure 2.1 [26]. Ultimately, when using high proportions of ethanol for solubilization enhancement, the increase in ethanol concentration must show adequate improvements in solute solubility such that the concentration is warranted.

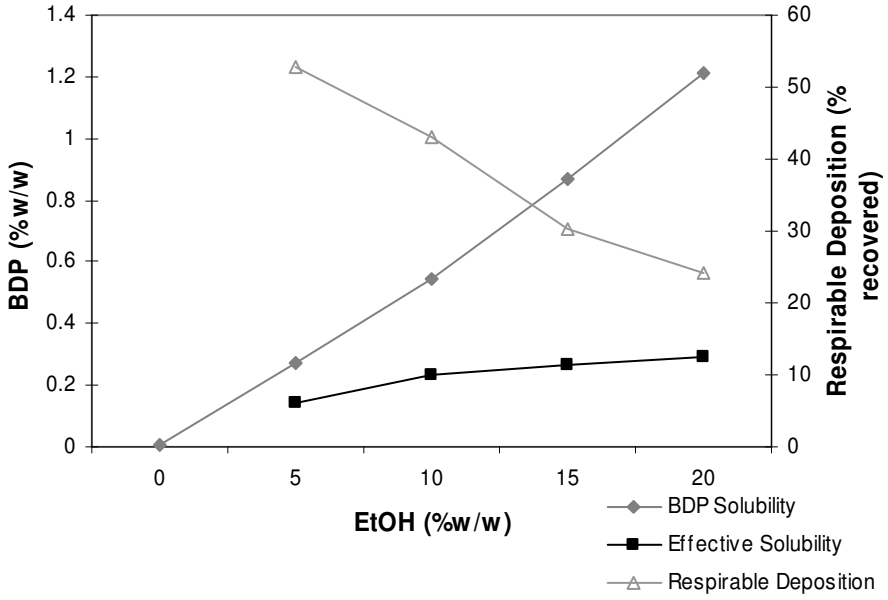


Figure 2.1. Balancing solubility and performance using ethanol as a cosolvent.

One advantage to using ethanol as a cosolvent compared to others such as water, glycerin, or propylene glycol is its volatility. Despite decreasing the vapor pressure of a MDI system, once aerosolized the ethanol evaporates relatively quickly, so no significant concentrations of ethanol should build up in the lung with chronic use of an inhaler. Water has been used in an approved MDI (Atrovent HFA), and it is obviously well

tolerated in the body, but lacks the volatility of ethanol, and may not evaporate at all seeing as though the aerosol is being administered into the highly humid mouth and respiratory tract. This can be used as an advantage however. In the case of dilute solution MDIs, water or the other slowly evaporating cosolvents can increase the particle size of the emitted aerosol to the desirable respirable range of roughly 1-5µm by both altering the vapor pressure of the MDI system, decreasing the force that drives the expansion to an aerosol, and by maintaining the size of the droplet by slowing or eliminating the rate of evaporation.

Another important application of cosolvents recently has been to improve the solubility of commonly used surfactants. Table 2.2 lists traditionally used surfactants in CFC-based systems, some of which have also been utilized in some HFA-based systems.

Excipient	***Product***	***Function***	***Maximum Approved Concentration***
Sorbitan trioleate (Span 85)	Aerobid, Alupent, Intal, Maxair, Tilade	Surfactant Dispersion Suspension Solubilization agent	0.069%
Soya lecithin	Atrovent, Combivent	Dispersion	0.28%
Lecithin	Flovent, Serevent	Dispersion Solubilization	0.00025%
Oleic acid	Beclovent, Proventil, Proventil HFA, Vanceril, Ventolin, Xopenex HFA	Dispersion Emulsification	0.267%
Cetylpyridinium chloride	Asthmahaler Mist, Bronkaid Mist	Preservative Surfactant	

Table 2.2. Traditionally used surfactants in CFC-based systems.

Surfactants have several functions in MDIs:

- seal lubrication
- valve lubrication
- solubilization
- dispersion
- emulsification
- preservation

As mentioned in previous sections, the physical and chemical properties exhibited by the HFAs are significantly different compared to CFCs. These differences became

very evident when previously miscible surfactants were discovered to exhibit relatively poor solubility in HFAs. As such, cosolvents (primarily ethanol), have been included in more recent formulations to enhance the solubility of the surfactant in the formulation. Several examples of this can also be seen in Table 2.2, where Proventil HFA and Xopenex HFA both utilize oleic acid (octadenenoic acid), but also contain ethanol to solubilize it in the HFA system. Using this technique could in some cases be risky, or at least warrant investigation, as the ethanol may also increase the solubility of the (suspended) solute, which again could lead to Ostwald ripening.

Several authors point out that surfactant polarity, indicated by their respective hydrophilic-lipophilic balance (HLB) correlates with the incompatibility of the aforementioned surfactants in the more polar HFA environment [18, 27]. Other authors point out that it is not only the HLB that matters, but because the HFAs contain (a) highly electropositive hydrogen(s), the potential for interaction with certain groups may be enhanced [28]. As such, new surfactants have been designed specifically to overcome the poor interactions with HFAs. One such example of these is oligolactic acids, the structure as seen in Figure 2.2.

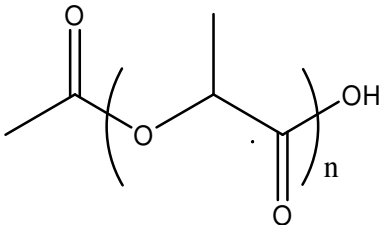


Figure 2.2. Structure of OLAs.

The oligolactic acids (OLAs) are short chain polymers of lactic acid (terminally acetylated). There were several reasons rationalizing the use of this surfactant. First, OLAs demonstrated much improved solubility in HFAs. Because the OLAs contain a relatively high concentration of electronegative oxygens (as esters) to interact with the electropositive hydrogen of the HFAs, they resulted in significantly improved solubility. They resulted with solubilities >5% w/w in both HFA 134a and 227 without the need to add ethanol to the formulation [28]. Another attractive reason rationalizing the use of OLAs is the fact that it degrades primarily to lactic acid by nonspecific esterases, which are omnipresent in the body. Lactic acid is an endogenous substance, and while the OLAs are the polymeric form, the chain length only consists of 6-15 repeating units. Toxicological studies have found no measureable toxicity for many measured parameters including in human Phase I clinical studies [28]. Likewise, polylactic acid has already been FDA (United States Food and Drug Administration) approved, so a degree is already known about it, unlike other synthetic surfactants that may have more to prove toxicologically.

Obviously to be successfully used in MDIs, OLAs must be good suspending agents, which has been shown [28]. An additional benefit is its possible solubilization of amine containing compounds through ion pairing. This has also been demonstrated by two other surfactants designed to be more soluble in HFAs, as can be seen in Figures 2.3 and 2.4, respectively.

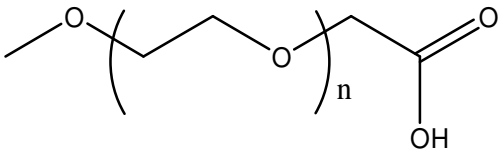


Figure 2.3. Structure of functionalized PEG

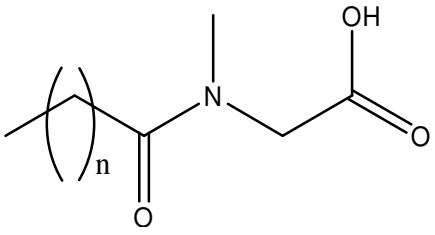


Figure 2.4. Structure of acyl amide-acid.

A significant advantage to the OLAs is the ability to easily form sustained release aerosol droplets in either solution or suspension-based systems. Because OLAs are relatively insoluble in water and break down over time, if added to a solution formulation, aerosol droplets can form drug-surfactant matrices *in situ*. Likewise, for suspension formulations, upon actuation the propellant will rapidly evaporate, leaving surfactant coated drug particles. Again, this is a simple means to develop sustained release aerosol particles [28].

Granted, if a more water soluble surfactant was needed, functionalized PEG would be more adequate. Though they may not display the same sustained-release properties as OLAs, they could offer benefit depending on the solute in question.

Other possible modifications that can be made to alter MDI output via excipient utilization are through the use of carrier particles. As will be discussed in a future chapter, solid particles in suspension based systems only occupy a fraction of the aerosolized droplets. Acknowledging this fact, when utilizing a solution based system, solid particles which are insoluble in HFA propellants, such as lactose, or other active medications like albuterol sulfate can be used as combination or carrier particles to alter the particle size distribution. Figure 2.5 demonstrates the utility of using insoluble carrier particles to modify particle size distributions.

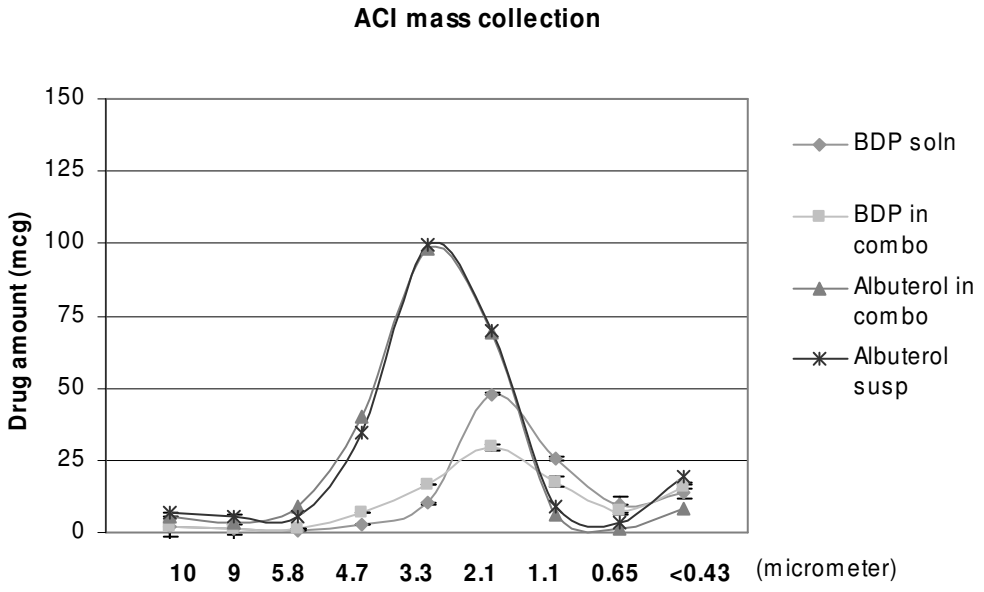


Figure 2.5. Andersen Cascade Impactor data displaying the alteration of particle size distributions in a combination MDI.

	MMAD (μm)	GSD	FPF
Albuterol (susp)	2.50	1.53	0.508
Albuterol (combo)	2.52	1.51	0.491
BDP (soln)	1.26	1.78	0.594
BDP (combo)	1.30	2.60	0.570

Table 2.3. Mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), and fine particle fraction (FPF) for solo and combination MDIs.

Albuterol sulfate and beclomethasone diproprionate (BDP) were tested individually using the Andersen Cascade Impactor (ACI, methods detailed in following chapters), the albuterol alone as a suspension, and the BDP alone as a solution. They were then combined into the same formulation. Because the albuterol already exists as a suspension, the only change to the particle size distribution was a very subtle increase in particle size (as seen in Table 2.3 above), as BDP in solution coats the outside of the particle upon evaporation of all other excipients. However, because BDP (in solution) is evenly dispersed throughout the liquid phase of the formulation, and subsequently in the aerosol droplets, the presence of albuterol particles within an aerosol droplet will significantly impact the particle deposition with regard to BDP. This is noted through the significant change in GSD for BDP. Though there is only a small upward shift in particle size, and a slight decrease in the corresponding fine particle fraction (FPF), the GSD is vastly affected. Depending on the size of the micronized solid, the GSD could be affected

differently. The smaller the material, the less change it will cause to the particle distribution of the solution component. Likewise, the smaller the micronized solid, the more its apparent distribution will change as the "coating" created by the solution component will have a smaller surface area to cover, thus will result in a relatively thicker coating of the particle. Nonetheless, solid suspended particles only occupy a fraction of the total droplets, thus most aerosol droplets (containing drug in solution) will behave and deposit as normal, but those droplets containing solid particulate albuterol (or a given carrier) will be subject to said suspended particle, altering the deposition thereof.

II – Excipients for Nebulization

Another manner in which to deliver pharmaceutical aerosols is via nebulization. Nebulizers offer some advantages compared to MDIs and DPIs in that they enable the administration of larger doses over time, and are easier to use for uncoordinated patients. Though the use of spacers has been recommended in many cases with MDIs, the dose load is generally quite small relative to nebulizers and dry powder inhalers. Like dry powder inhalers, nebulizers can offer administration of multiple milligrams of medication, though because nebulizers do not offer bolus dosing, dosing consistency over time may be relatively improved [29].

Nebulizers use mechanical means to aerosolize solutions or suspensions of micronized droplets. Unlike MDIs, the excipients included in the formulation do not

significantly impact the aerosolization process, but can play a role in final particle size via evaporation. The solutions are primarily aqueous in nature, with water being the most overwhelmingly used non-active ingredient. As with most aqueous systems, solubility and/or stability could be problematic depending on the compound. As such, cosolvents are commonly used to improve solubility and/ or stability in nebulized systems as can be seen in Table 2.4.

Excipient	***Product***	***Function***	***Maximum Approved Concentration***
Alcohol (ethanol)	Tornalate	Co-solvent	25%
Glycerin	Isuprel	Co-solvent Humectant Preservative Tonicity agent	7.3%
Propylene glycol	Tornalate	Co-solvent Preservative	25%
Methylparaben		Preservative	0.07%
Propylparaben		Preservative	0.037%
Chlorobutanol	Isuprel	Preservative	0.5%
Sodium meta bisulfite	Isuprel	Preservative	1%
Sodium bisulfite		Preservative	0.32%
Sodium sulfite		Preservative	0.1%
Sodium sulfate (anhydrous)		Tonicity agent	0.025%
Thymol		Preservative	0.01%
Benzalkonium chloride	Alupent, Proventil, Ventolin	Preservative Wetting agent Solubilizing agent	20%
Sodium chloride	Airet, Proventil, Isuprel, Xopenex, Atrovent, Duovent	Tonicity	3.16%
Sodium citrate/ Citric acid	Airet, Isuprel, Tornalate	Buffering agent Chelating agent Flavoring agent	0.6%/ 0.44%

Edetate sodium/ Edetate disodium/ EDTA	Airet, Alupent, Atrovent	Chelating agent Buffering agent Preservative	0.02%/ 0.03%
Saccharin sodium		Flavoring agent	
Hydrochloric acid	Airet, Atrovent, Duovent	pH adjustment	3.5%
Sulfuric acid	Proventil, Ventolin, Xopenex	pH adjustment	12.5%
Sodium hydroxide	Tornalate	pH adjustment	8%
Ascorbic acid		Antioxidant	1.02%
Tromethamine (TRIS)	Ventavis	Buffering agent	
Water			

Table 2.4. List of commonly used excipients in aqueous solutions for nebulization.

In addition to commonly used cosolvents such as ethanol, glycerine, and propylene glycol, other very commonly used excipients in nebulized solutions are preservatives. Many solutions for nebulization are manufactured in unit dose vials, but in some instances, preservative are still necessary. Some example preservatives can also be seen in Table 2.4 [30].

One of the most heavily utilized preservatives in nebulized solutions is EDTA, (ethylenediaminetetraacetic acid, or salts thereof), as seen in Figure 2.6. EDTA offers advantages beyond acting as a preservative however, including acting as a chelating agent, and as a buffer [29].

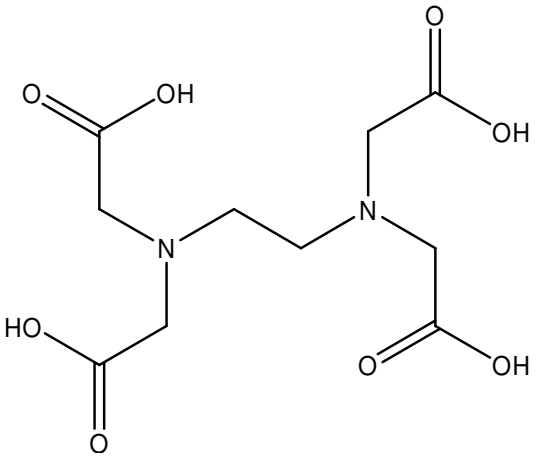


Figure 2.6. Structure of EDTA.

Buffers are another class of excipient commonly used in nebulized solutions. Again, because they are aqueous systems, buffering the system to improve solubility or stability is another technique that can be used instead of including excipients such as cosolvents. Human lungs do not have the buffering capacity of the gut or blood, but are still capable of tolerating somewhat adjusted, yet relatively neutral pH ranges [31]. Some other buffers used in more recent formulations include tromethamine (also known as TRIS, or trishydroxymethylaminomethane), citric acid, or sodium citrate. Likewise, simple acids and bases such as sodium hydroxide, hydrochloric acid, or sulfuric acid are commonly used to adjust the pH of the system [30].

Again, seeing as though these are aqueous solutions primarily containing water, upon nebulization, the evaporation rate of the aerosolized droplets approaches zero upon entering the mouth and deeper within the respiratory tract, where humidity approaches one hundred percent. Because the evaporation rate will be so poor, most formulations are designed to be isotonic, or close to, as very high doses of medication can be given, and if

hyper or hypotonic, could cause tissue irritation. As such, sodium chloride, calcium chloride, sodium sulfate, or other salts have been used to adjust tonicity [30].

III – Excipients for Dry Powder Inhalers

Dry powder inhalers (DPIs) have received much attention as an alternate to MDIs while undergoing reformulation from chlorofluorocarbon to hydrofluoroalkane propellants. While HFA propellants do not cause ozone depletion as CFCs do, as described above, they are still greenhouse gasses which are clearly still not ideal environmentally. Being able to deliver medication via dry powder is highly desirable because there is generally no need for environmentally deleterious materials. Because nebulizers are also cumbersome and are generally used in either the home or acute care setting, DPIs offer the advantage of portability, while also allowing for larger bolus doses to be administered.

Dry powder inhalers are significantly different than both MDIs and nebulized solutions in that the source or energy is again different, which gives rise to some unique characteristics. In general, DPIs are actuated by active or passive means, utilizing either the patient's inspiratory efforts, or the patient's inspiration causing some mechanism activation (depending on the device) which then aerosolizes the dry powder. In general, DPIs are designed in one of two ways. Either agglomerates of micronized drug, or drug and excipient are administered, or carrier based systems, which are more common are

used. In carrier based systems, large carrier particles roughly of 40-80 micrometers in size are coated by micronized materials bound by adhesive forces, typically including micronized drug, or drug and other micronized excipients [32].

Lactose is by far the most commonly used excipient. Lactose, is a disaccharide composed of D-glucose (upper right) and β-D-galactose (lower left), and is seen in Figure 2.7. It is the primary sugar found in milk, and is a readily available material [33].

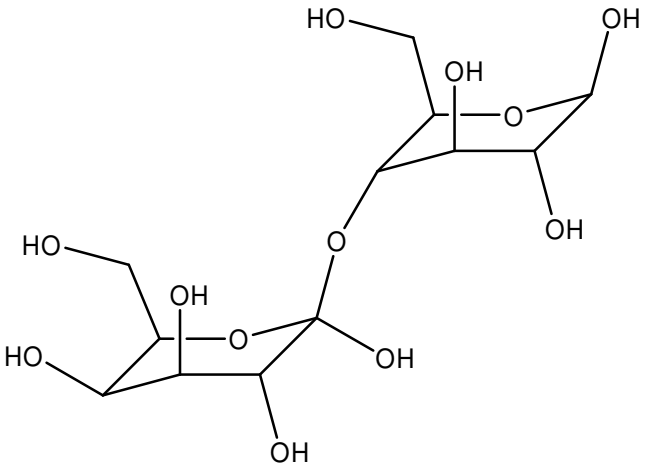


Figure 2.7. Structure of lactose.

Some other advantages of lactose include its high purity, established safety profile, its limited hygroscopisity compared to other sugar alcohols, and its workability. Examining the literature, several authors take advantage of the many ways to work with the material. Often spray drying, milling, spray freeze drying, or controlled crystallization are used to obtain micronized material of different morphologies. Surface characteristics are paramount for lactose and other carrier particles, as adhesive forces keep the

medication in contact with the carrier until purposefully agitated whereupon ideally all adhered particles deagglomerate and are available for deposition in the lungs [32].

Unfortunately, lactose is not compatible will all compounds. It has been well documented that it is incompatible with amine containing compounds, including amine containing small molecules such as amphetamines, in addition to proteins and peptides [33]. Fortunately an alternate sugar alcohol just received approval by the USFDA for pulmonary delivery. Mannitol, as seen in Figure 2.8, was recently approved as part of the Exubera® formulation, which is the first approved form of inhalable insulin.

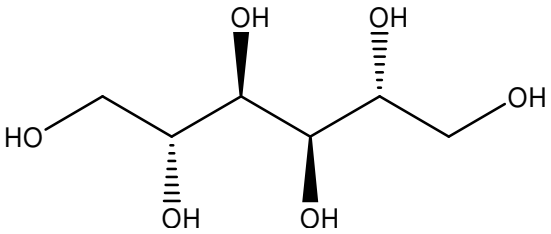


Figure 2.8. Structure of mannitol.

Because mannitol is a linear sugar alcohol, there is no potential for Maillard type browning reactions as seen with lactose and other pentose and hexoses. The reaction occurs when a reducing sugar such as lactose, in addition to glucose, fructose, maltose, and arabinose, in the presence of a basic environment has the potential to form an aldehyde or a ketone, ultimately acting as a reducing agent. Alternate sugars such as sucrose (which is interestingly a disaccharide of glucose and fructose) or sorbitol have been noted to not cause this reaction. Mannitol has also been approved as a stand alone therapeutic injectable. Mannitol is dosed greater than 100 grams per dose to be used as an

osmotic diuretic used commonly in patients with head trauma. As such, it has an established safety profile, though this was the first approval being administered pulmonarily. Other sugars that also appear in the literature include maltilol, sorbitol, and maltodextrin, some of which have the advantage of being sweeter than the more commonly used lactose [34].

In addition to mannitol being a newly approved excipient for inhalation, an amino acid, glycine was also part of the Exubera® formulation. Amino acids have also been found elsewhere in the literature including arginine, threonine, phenylalanine, aspartic acid, and trileucine [35]. In all cases the amino acids were used to improve yields from spray drying, or to improve solid-state properties such as particle flow and/or adhesion, resulting in increased emitted dose.

In conclusion, the change from CFC propellants to HFA propellants not only spurred improvements and necessary changes in MDIs, but also reinforced the need for alternate delivery methods such as DPIs. Significant reformulation was needed in MDIs due to the significantly different physiochemical properties, and resulted in the development of new types of surfactants in addition to new techniques allowing the use of previously used surfactants. Though improvements have been made in MDI systems, DPIs and nebulizers both still offer other advantages in the utilization of pharmaceutical aerosols. Neither has a need for greenhouse gasses, though require more technologically savvy delivery instruments, unlike MDIs, where propellants are the driving force behind aerosolization. Based on the very different technologies, the ultimate emitted dose and

respirable dose can be quite different between instruments, but depending on the medication, one or another may provide some significant advantage.

CHAPTER 3: ADVANCES IN AEROSOL CHARACTERIZATION

I – Advances in Chemical Analysis

Chemical analysis is a necessary part of development for any method of drug delivery. Previous studies have detailed methods to measure the solubility in a MDI [36, 37]. The traditional method utilized to evaluate the solubility of an API in a pressurized metered dose inhaler is labor intensive, requires a large number of MDI vials, and a great deal of time. This process involves one MDI vial per sample point, which must be compromised as the contents are analytically transferred to a volumetric flask, where the propellant is evaporated off. At this time, the API is reconstituted with an appropriate diluent and then assayed for content. Traini *et al.* (2006) recently described another method that does not require decrimping of the MDI vial. These methods, however, do not allow for direct injection of the MDI into the HPLC [38].

Gupta and Myrdal [39-41] have developed and tested a novel method for direct injection of a MDI into an HPLC. The new online direct inject method, schematic shown in Figure 3.1, allows for the direct injection of an MDI into the manual inject port of the HPLC. This method potentially provides numerous advantages over the traditional method in analysis of MDIs. These advantages include decreased number of sample vials, decreased sample preparation time and the ability to acquire multiple sample points from one MDI vial [41].

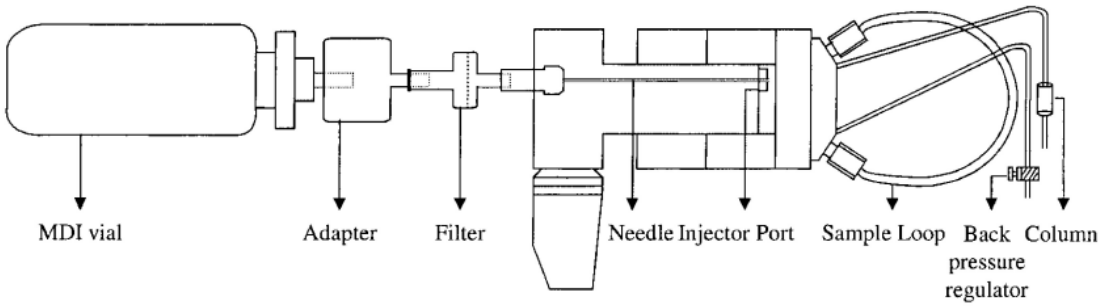


Figure 3.1. Schematic of the online direct inject method.

Characterizing the degradation of an API in different formulations is vital in early formulation. In order to determine the stability of an API in an MDI, the methods of Dalby *et al.* (1991) and Williams *et al.* (1999) are utilized to quantify the amount of API at a given time [36, 37]. These methods are repeated on separate MDI vials over time to characterize the degradation of the API.

Conducting stability studies in this manner requires that the laborious process be repeated numerous times on a large number of MDI vials. Logically, the online direct inject method would provide several advantages when conducting stability studies in an MDI; specifically, decreased number of vials, decreased amount of API and other formulation components required, and a significant decrease in the amount of time required for analysis.

As such, one of the focal points of this work was to evaluate the utility of the online direct inject method for characterizing the stability of an API in an MDI. For this evaluation, Imexon was chosen as the model drug. Imexon, Figure 3.2, was selected as

its stability has been well characterized in aqueous environments and analytical methodologies have been established [42].

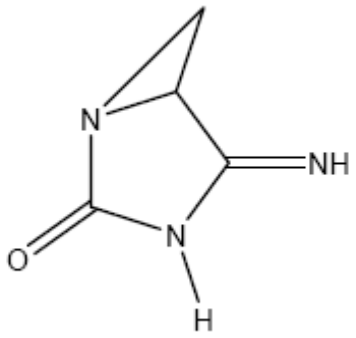


Figure 3.2. Structure of Imexon.

Traditional methods detailed above allow for quantitative analysis of MDIs crimped with either metered or continuous valves, as the MDIs are ultimately decrimped prior to analysis. Currently, the online direct inject method has only been evaluated for use with MDIs crimped with continuous valves. The second focus of this study was to extend the applicability of this online direct inject HPLC method to include analyzing drug content of inhalers crimped with metered valves. As the original validation of the online direct inject method was conducted with beclomethasone diproprionate (BDP) these studies similarly utilize BDP (Figure 3.3).

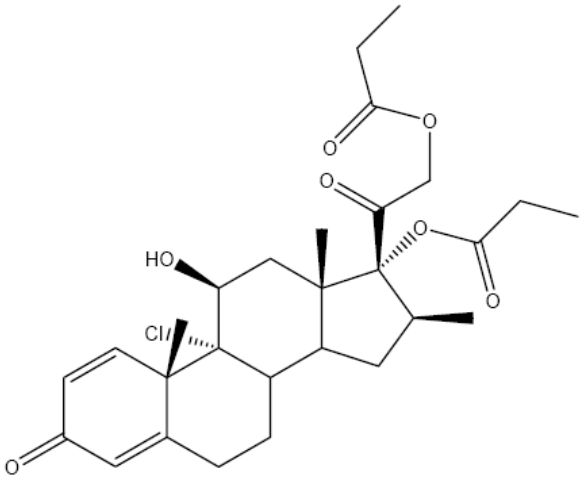


Figure 3.3. Structure of beclomethasone dipropionate (BDP).

Because MDIs are subject to dynamic changes in liquid component concentration due to the decreasing liquid volume and increasing gaseous space, a through-life trend must first be examined to ensure that this phenomenon does not affect drug concentration throughout the use of the inhaler. To examine that listed above for the 'through-life' portion of the study, the following materials and methods were used.

Materials

Beclomethasone dipropionate and continuous valves were provided by 3M Drug Delivery Systems (St. Paul, MN, USA). Pressure resistant glass aerosol vials were purchased from Research Products International Corporation (Mt. Prospect, IL, USA). 1,1,1,2-Tetrafluoroethane (HFA-134a) and ethanol (200 proof) were obtained from

DuPont Chemicals (Wilmington, DE, USA) and Aaper Alcohol and Chemical Company (Shelbyville, KY, USA), respectively. HPLC grade acetonitrile (ACN) was obtained from EMD (Gibbstown, NJ, USA). A Millipore (Billerica, MA, USA) Milli-Q Ultrapure Water purification system with a 0.22 μm filter was utilized for water.

Methods

Through-life analysis was conducted using a solution MDI containing 0.1% w/w BDP, 9% w/w ethanol, and HFA-134a, crimped with a continuous valve. Canister weight was measured before and after each injection, and HPLC measurements were taken throughout the life of the canister (21.2g formulation). The online direct inject method was employed to allow for direct injection of the MDIs into a Waters 600E multisolvent delivery module (Waters, Milford, MA, USA) coupled with a Waters 2487 diode array (Dual) detector. Analysis was performed by reverse phase HPLC, using a 150 mm x 4.6 mm Apollo C_{18} 5 μ column (Alltech Associates, Deerfield, IL). Ultraviolet detection was set at 240 nm. Mobile phase consisted of 90:10 (v/v) ACN:H_2O at a flow rate of 0.6 mL/min. Injection volume was 5 μL. BDP exhibited a retention time of 5.2 minutes.

Results

Single HPLC measurements were taken for the life of an inhaler and are shown in Figure 3.4. On average, each injection used 580 mg of formulation, which correlates to 30+ possible injections for every 20 grams of formulation. This was calculated using the

average weight emitted over the series of consecutive injections (from ~12-20 grams formulation used). Because no significant deviations were expected early in the life of the inhaler, multiple actuations were sampled to waste, accounting for the gaps noted in Figure 3.4. Percent of theoretical concentration did not appear to change as a function of formulation used and is thus of limited concern when repeatedly sampling an MDI vial. Of note is that this analysis was conducted in a single day, subsequently propellant leak over time may be a factor to consider, as in the case of stability studies. Additionally, care should be taken to avoid assaying canister contents when minimal formulation remains, as end-of-life issues are documented with MDIs [43, 44].

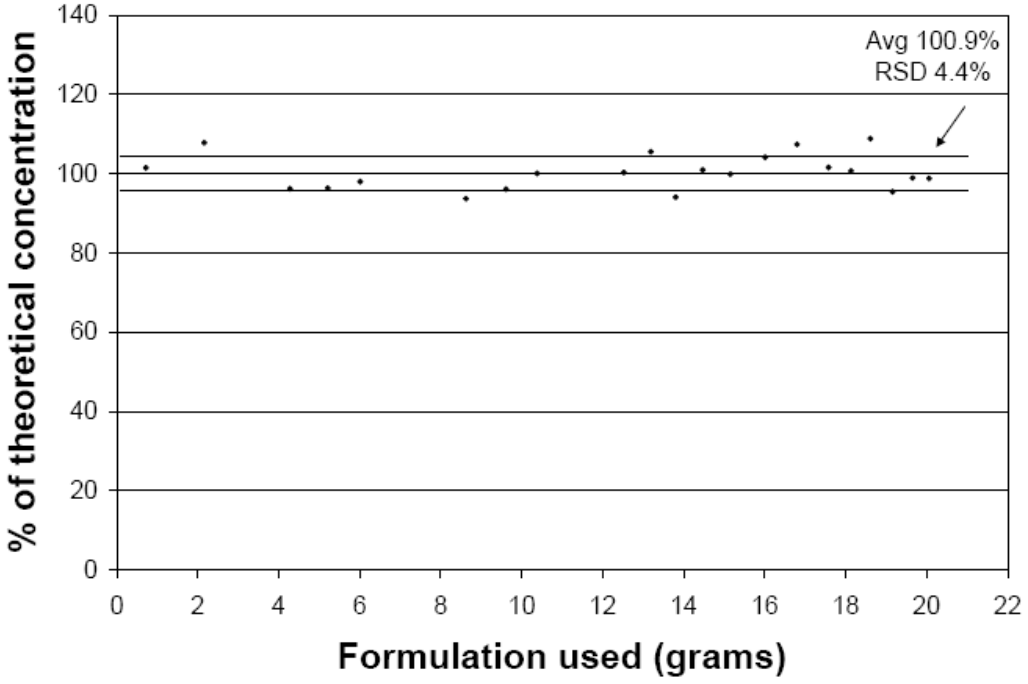


Figure 3.4. Through-life concentration analysis of a BDP solution MDI, 21.2g total formulation.

Upon successful completion of the through-life studies, stability studies could then ensue to allow for the monitoring of chemical content of an inhaler over time, with no concentration changes attributable to through-life phenomenon. The following materials and methods were used for the stability monitoring section of the study.

Materials

Imexon was provided by AmpliMed Corp., Tucson, AZ, USA. Valves were provided by 3M Drug Delivery Systems (St Paul, MN,USA) and pressure resistant glass aerosol vials were purchased from Research Products International Corporation (Mt. Prospect, IL, USA). Ammonium acetate was obtained from Sigma Aldrich (St. Louis, MO, USA). All other materials were as described in the through-life analysis materials section.

HPLC Method

The online direct inject method was employed to allow for direct injection of the MDI into a Waters 600E multisolvent delivery module (Waters, Milford, MA, USA) coupled with a Waters 2487 diode array (Dual) detector. Analysis was performed by a normal phase HPLC assay, using a 150 mm x 4.6 mm Apollo Silica 5 µ column (Alltech Associates, Deerfield, IL). Ultraviolet detection was set at 234 nm. Mobile phase

consisted of 90:10 (v/v) ACN:H_2O at a flow rate of 0.6 mL/min. Water was buffered with ammonium acetate at 0.1 M, with a pH of approximately 6.5. Injection volume was 5 µL. The parent compound had a retention time of 7.8 minutes.

Solubility of Model Drug, Imexon

The solubility of Imexon was determined in pure HFA-134a propellant at 23°C. The solubility of Imexon was also determined as a function of the cosolvent ethanol at 5, 10, 15, 20 and 25 % (w/w). Preformulation work has also shown that Imexon has favorable water solubility (~25 mg/mL, at 23°C), thus the solubility of Imexon was also explored as a function of water. Using ethanol as a cosolvent for water, solubility was determined for formulations containing 0.5 and 1% H_2O (w/w) with 10% EtOH, and 0.5, 1, 1.5, 2, and 3% H_2O (w/w) with 20% ethanol (w/w).

Stability of Model Drug, Imexon

The stability of Imexon in an MDI environment was measured as a function of temperature at 11, 23 and 37°C, with a MDI containing 80:20 HFA-134a:EtOH and 80 µg/g of Imexon. The effect of EtOH was evaluated at 80:20 and 75:25 HFA-134a:EtOH with a drug concentration of 80 µg/g. The stability as a function of H_2O was determined with compositions of 80:20:0, 79:20:1 and 78:20:2 (HFA-134a:EtOH:H_2O, % w/w) with 80 µg/g. Because initial drug concentration had an effect on stability in aqueous studies, the effect of initial drug concentration on stability was determined at concentrations of

80, 150 and 250 μg/g with an inhaler composition of 78:20:2 (HFA-134a:EtOH:H_2O, % w/w). Four injections were used for each sample condition.

Results

Based on aqueous preformulation studies of Imexon, several factors contributed to the stability of the compound, including temperature, water, ethanol, and initial drug concentration [42]. As such, those factors were examined over time in the MDI environment using the online direct inject HPLC method for analysis. Prior to evaluating the stability of the model drug in a MDI, the solubility of Imexon was determined as a function of several different variables.

The solubility of Imexon in pure HFA-134a was found to be 0.00022 % (w/w). The solubility of Imexon as a function of the cosolvent EtOH is presented in Figure 3.5. As the figure indicates, the solubility of Imexon increases linearly as a function of EtOH.

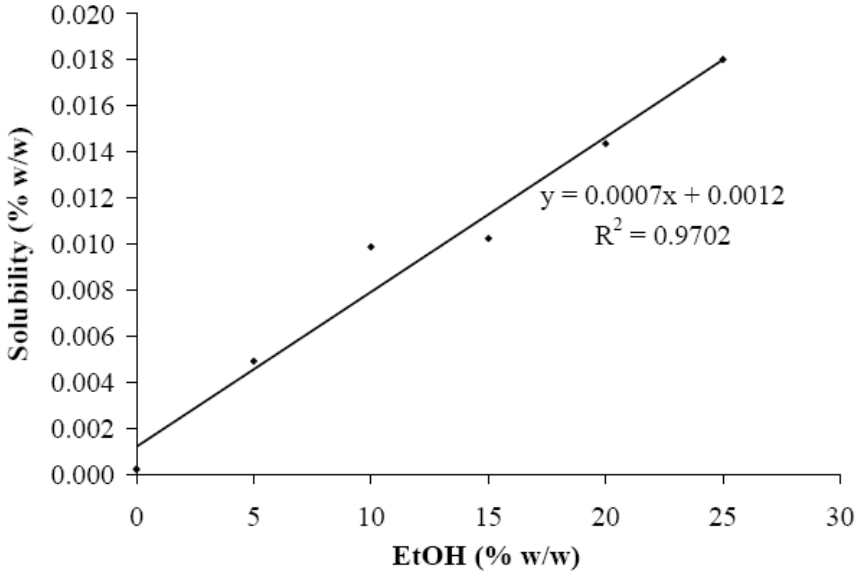


Figure 3.5. Solubility of Imexon in a MDI as a function of EtOH in HFA 134a.

Given that Imexon is relatively polar, the use of water to increase the solubility Imexon was also evaluated. However, in view of the fact that water is relatively immiscible with HFA-134a alone, the presence of either 10 or 20% (w/w) ethanol was incorporated to solubilize the water. Figure 3.6 displays the solubility increase for Imexon with 10% ethanol and water concentrations of 0, 0.5, and 1% (w/w) as well as for 20% ethanol with 0, 0.5, 1, 1.5, 2, and 3% (w/w) water concentrations. The solubility of Imexon increases linearly as a function of H_2O concentration for both ethanol concentrations.

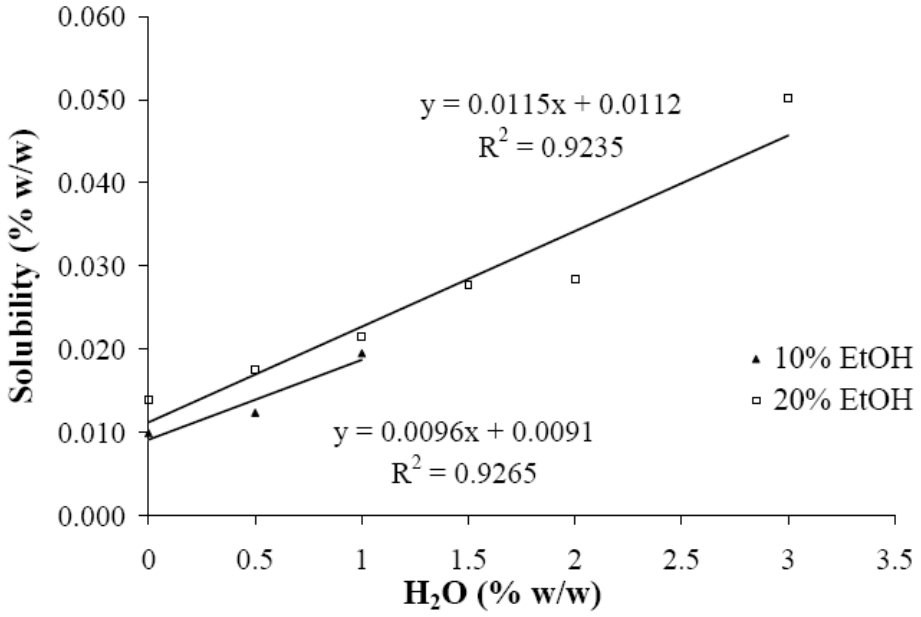


Figure 3.6. Solubility of Imexon as a function of water and EtOH in HFA 134a.

These solubility data, along with preformulation data collected under aqueous conditions, were utilized to determine the formulation combinations for evaluating the utility of the direct inject method for chemical stability. Namely, these were the effect of temperature, H_2O, EtOH and initial drug concentration on the degradation of Imexon in a pMDI.

Prior to formulation of stability vials, the effectiveness of the direct inject method to function as a stability indicating method was established. The HPLC conditions (column, mobile phase, flow rate, etc.) previously used to characterize the stability of Imexon under aqueous conditions were combined with the direct injection method [42]. Importantly, the mobile phase contains a high percentage of organic (90:10, ACN: H_2O)

which facilitates the direct inject of the non aqueous formulation [40]. For an initial screen, a representative formulation was prepared (80:20 HFA-134a:EtOH, 80 µg/g) and crimped with a continuous valve. The vial was stored at 37°C to facilitate the degradation of the Imexon parent drug. The chromatography of the Imexon and degradant from the non aqueous system was similar to that observed from analysis of Imexon under aqueous conditions. As can be seen from the four sequential injections represented in Figure 3.7, the Imexon peak is well resolved from Degradation Peak A. The degradation product is proposed to be the same degradation product observed in aqueous media [42, 45, 46].

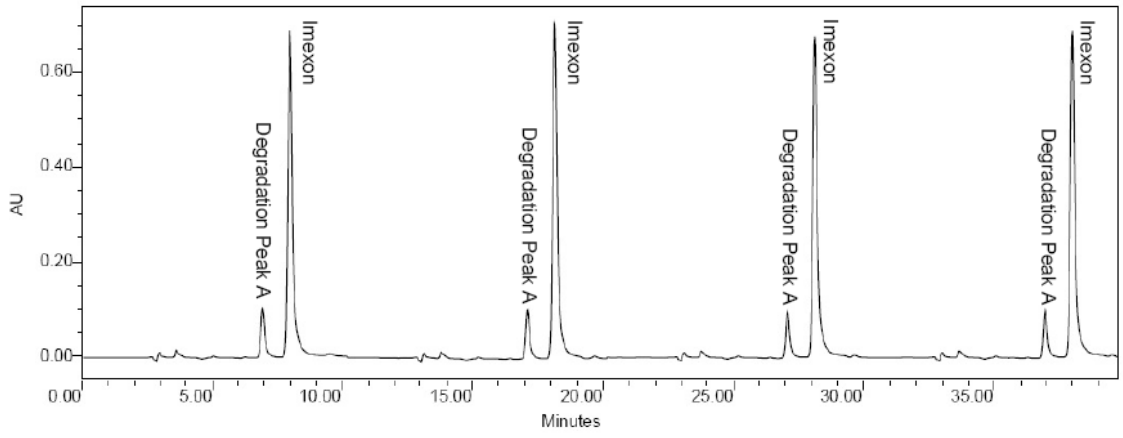


Figure 3.7. Representative chromatograms of an Imexon stability sample showing the separation of a degradant.

The overall method capabilities were in alignment with aqueous analysis, having a linear range from 5 to 500 µg/mL. Collectively, repeated analyses from individual vials

were found to afford a RSD of 4% (under all conditions tested). From these data it was concluded that the direct inject method is feasible for use in preformulation stability studies. In order to accurately determine the concentration at each time point, each vial was injected four times. The reported concentrations are averages of these four injections.

The effect of temperature on the degradation of Imexon was assessed with formulations conditions of 80:20 (HFA-134a:EtOH), 80 μg/g initial Imexon concentration stored at three different temperatures (11, 23 and 37°C). To maintain consistency, the inhalers were brought to room temperature for analysis and then immediately returned to the appropriate storage condition. Analysis of Log percent drug remaining as a function of time (Figure 3.8) indicate that Imexon undergoes apparent 1^{st} order degradation in an MDI environment, which was ultimately observed to be the same for all MDI conditions evaluated. These studies were conducted over a period of ~3 months. During that time, the most degradation Imexon went through was ~2 half-lives; however, due to the log-linear nature of the degradation, they were identified as apparent 1^{st} order processes.

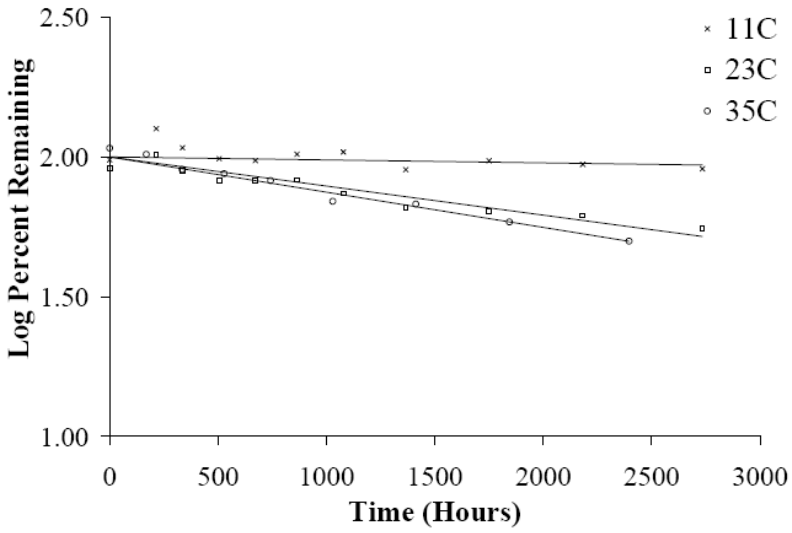


Figure 3.8. Log percent remaining of Imexon in 80:20 134a:EtOH with 80µg/g Imexon, stored at 11, 23, or 35ºC.

Analysis of the effect of temperature on the degradation of Imexon indicates that the degradation rate of Imexon increases as the temperature increases. An Arrhenius plot for the three temperatures evaluated is shown in Figure 3.9, which results in a calculated activation energy for Imexon of 110.04 kJ/mol in the propellant system. Additional relevant degradation parameters for all conditions evaluated in a MDI are shown in Table 3.1 (A-C).

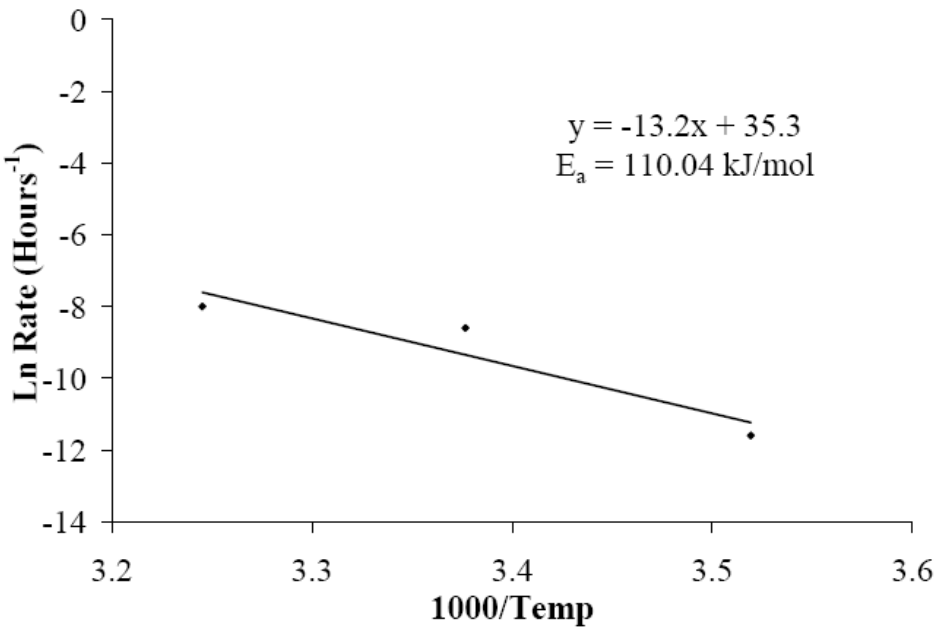


Figure 3.9. Arrhenius plot for Imexon in an MDI environment.

Row #	HFA-134a (%)	EtOH (%)	H_2O (%)	Imexon (µg/g)	Temp (°C)	k (Hours^{-1})	T_{50} (Hours)	T_{95} (Hours)
A	80	20	0	80	11	0.000009	75228	5591
B	80	20	0	80	23	0.000184	3761	279.5
C	80	20	0	80	37	0.000336	2061	153.2
D	79	20	1	80	23	0.000576	1204	89.4
E	78	20	2	80	23	0.000649	1067	79.3
F	78	20	2	150	23	0.000415	1672	124.2
G	78	20	2	250	23	0.000394	1760	130.8
H	75	25	0	80	23	0.000157	4425	328.9

Table 3.1. Calculated degradation parameters for Imexon in an MDI environment.

The effect of EtOH concentration on the degradation of Imexon in an MDI environment was evaluated at EtOH concentrations of 20 and 25% (w/w). Referring to

Table 3.1 (B, H), it can be seen that an increase in EtOH concentration decreases the degradation rate of Imexon.

In order to elucidate the effect water concentration has on the degradation of Imexon in a MDI, formulations were prepared with water concentrations of 0, 1 and 2% (w/w). As would be expected based on aqueous preformulation stability, an increase in the concentration of water resulted in an increase in the degradation rate of Imexon as can be seen in Table 3.1 (B, D, E).

Under aqueous conditions the initial concentration of Imexon was determined to have a direct correlation to the degradation rate [42]. In order to determine if this was true in an MDI, formulations were prepared at 80, 150 and 250 μg/g. Analysis of these formulations indicates that when the concentration is increased the degradation rate is decreased as can be seen in Table 3.1 (E-G).

Overall, these data not only describe the stability of the model drug, as a function of several different formulation variables, they more importantly establish the ability of direct inject method to determine the stability in a non aqueous propellant system. Specifically, the data collected for the model drug indicated that Imexon displayed first-order degradation for all variables tested, and the degradation product was chromatographically resolved from the parent compound just as it was in aqueous studies.

It is important to note that these data were conducted with 8 MDI vials, assayed four times at each time point. As each vial was sampled at approximately eight different time points, these 8 MDI vials were utilized to generate over 250 data points. Collecting

similar data with the traditional method (Dalby *et al*, 1991, 36) would have required one MDI vial for each data point, or in excess of 250 MDI vials. This reduction in the number of vials required affords a considerable decrease in material and drug supplies, as well as a significant reduction in analyst time.

A separate, but useful add-on to this information being collected above is the ability to monitor the life of a MDI taken straight off a production line. To do this, the following section of the study aims to assess the feasibility of sampling MDIs capped with metered instead of continuous-flow valves. The following methods were utilized for this section of the study, as all materials were used as described in the 'through life analysis' portion listed above.

Experimental Method

A stock solution formulation (0.078% w/w BDP, 9% w/w ethanol, HFA-134a) was prepared, chilled, and then transferred into 8 glass vials via cold transfer. These eight inhalers were crimped (2 each) with continuous-flow valves or with 25, 50, or 100µL metered valves.

Each inhaler was assayed using the online direct inject HPLC setup as mentioned in the through-life analysis methods section above, however, alterations were made to the stem-needle juncture. The adapter and filter were eliminated to decrease losses upon actuation as seen in Figure 3.10. A pre-column filter was used to capture any particulate matter from the inhalers in place of the removed filter. In order to create a seal between the valve-stem and injection needle, rubber o-rings were used. These changes were done

to decrease the internal volume of the system to allow for analysis small amounts of (metered) formulation. Four injections were measured for each inhaler.

Figure 3.10. MDI with the traditional injection apparatus (left), and modified injection coupler (right).

The traditional method utilizes an adapter and filter between the stem and injection needle, as can be seen in Figure 3.1. However, upon analyzing the canister contents using the traditional setup of the direct inject method, the MDIs crimped with a 25 µL valve did not achieve the desired content, resulting in 12.5% of the theoretical

concentration. The vials crimped with 50 and 100 µL valves were comparatively improved but still not adequate, resulting at 55.8% and 82.5% of theoretical concentration, respectively. As such, it was clear that the losses from the traditional setup were too great and thus the external filter was eliminated. With the filter removed, there was no longer a need for an adapter as the injection needle fits the stem of the MDI with the help of rubber o-rings to seal the juncture. Figure 4 shows the traditional setup (including filter and adapter) and the modified setup.

Once the injection coupler was modified, measurements from MDIs with 50 and 100 µL metered valves compared favorably to the vial with a continuous-flow valve in both concentration and variability. Figure 3.11 shows the results of using the traditional setup compared to the modified setup. The MDI with a 25 µL was relatively improved with the modified setup, however, was still only at 60% of the theoretical concentration, indicating that even with the reduced adapter volume, there was not ample formulation for loop filling.

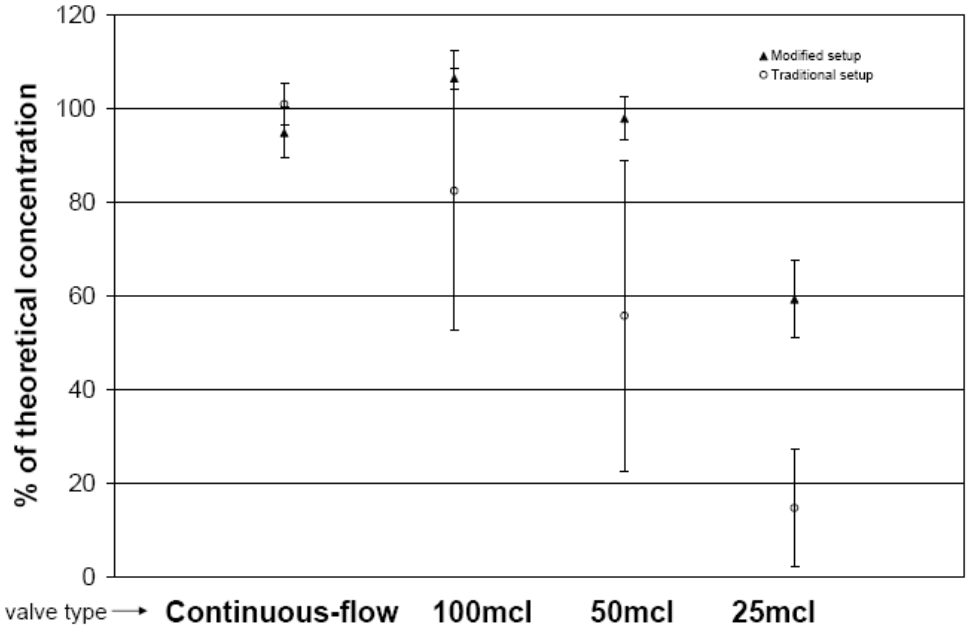


Figure 3.11. Comparison of the traditional and modified setup for different valve types.

Though the changes made to the system did allow for the analysis of contents from inhalers with larger metered volumes (50 and 100 µL), engineering an injector needle specifically for this purpose would likely improve the variability and possibly improve the efficiency such that analysis of inhalers crimped with 25 µL metered valves would be possible.

In conclusion, through-life analysis indicates that an API, in a single vial with a continuous valve, can be repeatedly analyzed via the direct inject method (30+ samples per 20 gm of formulation). Solubility analysis, as previously described using this method, was performed on the agent Imexon. Stability studies conducted on Imexon indicate that the online direct inject method is a viable and resource conserving analytical method

(using 8 versus 250+ MDI vials) for determining chemical stability as a function of several different formulation factors. Specifically, they were used to determine that Imexon undergoes apparent 1st order degradation under all propellant conditions evaluated. The degradation rate of Imexon in a MDI is increased by increasing temperature and concentration of H_2O, while increasing EtOH or initial drug concentration appears to decrease degradation rate.

Alterations to the injection coupler allowed for accurate content analysis of MDIs crimped with metered valves of larger volumes (50 and 100 µL), however was not efficient enough to accurately analyze inhalers crimped with 25 µL valves. With improvements to the engineering of the injection coupler, it should be possible to accurately analyze the range of commercially available metered valves. In summary, it has been found that the direct inject method can be a useful technique for preformulation screening in propellant based systems.

II – Advances in Physical Analysis (Particle Size Characterization)

Chemical analysis is paramount for the monitoring of any drug product. Arguably as important for aerosols however is physical characterization. Because the drug must reach part or all regions of the lung to be available therapeutically, the particle size and characteristics of the physical substance must be adequate to do so.

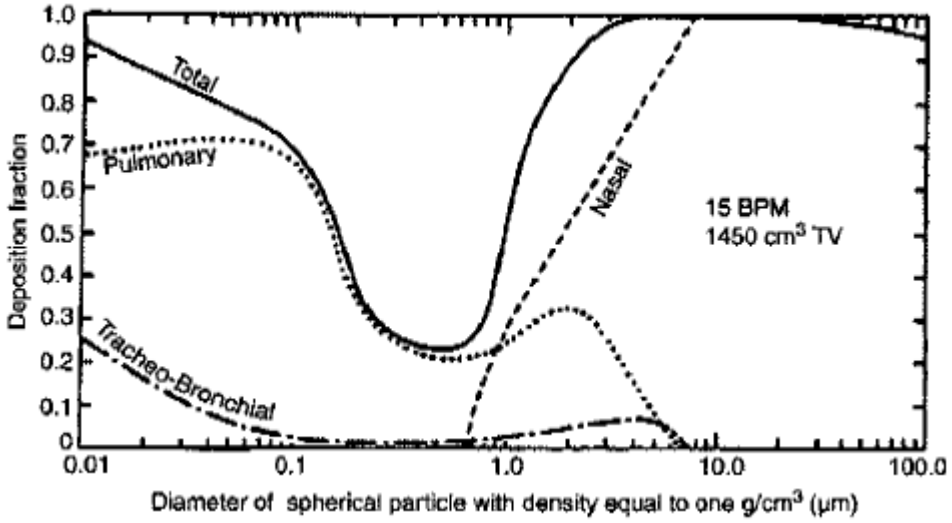


Figure 3.12. Deposition as a function of inhaled particle size in the human respiratory tract [47].

As demonstrated in Figure 3.12, the optimal particle size for aerosol delivery to the lung is in the nanometer scale. Unfortunately for particles of that size, cohesive forces are great and can cause significant agglomeration in a suspension MDI or dry powder based system. Likewise, in a solution based system, increased apparent solubility could be problematic as the relative surface area for a given amount of drug would be extremely large compared to micronized material (particle size ~1-5μm). A third problem lies in the delivery of the nanoparticles to the lung. Because of the minute size, airflow does not affect the nanoparticles as it would particles greater than roughly one micrometer (micron) in size. As such, the particles would behave more like gasses, and

Brownian motion (diffusion) would be the primary means for deposition. Other manners in which particles may deposit include impaction, sedimentation, and interception.

As with all aerosol particles less than one micron in size, diffusion is the primary means of deposition. As a given particle gets larger (mass and/or volume), airflow will contribute increasingly to inertia. This inertia based on the size of the particle will cause impaction. Particles of larger sizes are subject to great deals of inertial impaction; this is a primary principle of nasal drug delivery, where average particle sizes are routinely ~10 microns, and likewise the reason for oral deposition or aerosol droplets in the mouth from MDIs, where the size and mass of the droplets are relatively larger while the propellant and potentially cosolvents are evaporating off. Sedimentation and interception are somewhat relatively defined, and affect particles of intermediate sizes of roughly one micron, where insufficient mass exists to create a great deal of inertia, but the particles are still large enough to be affected by airflow to some degree [47].

Seeing as though all drug products are screened thoroughly by regulating agencies for dose reproducibility, acceptably delivering nanoparticles would be of great difficulty as diffusion will be the primary means of deposition. Thus, when examining Figure 3.12, a secondary local maximum can be identified for pulmonary aerosol deposition. Particles greater than 1 micron and less than 5 microns deposit with decent efficiency. As such, these are generally accepted as ideal particle sizes for pulmonary drug delivery, as they can be reproducibly administered by nebulizers, MDIs, or DPIs, and are also of adequate size for solid particle engineering (primarily micronization) by ball milling, spray-drying, freeze drying, or other techniques.

Not only is particle size important for reproducibly delivering the drug, but also characterizing it. Because pharmaceutical aerosols are designed to fit within the aforementioned secondary maximum, they are easier to characterize as well. Traditional methods for characterizing pharmaceutical aerosols started with impaction based methods. The Andersen Cascade Impactor (ACI, Figure 3.13a-c) is a very commonly used aerosol characterization instrument.

Figure 3.13a. Photograph of an Andersen Cascade Impactor (ACI).

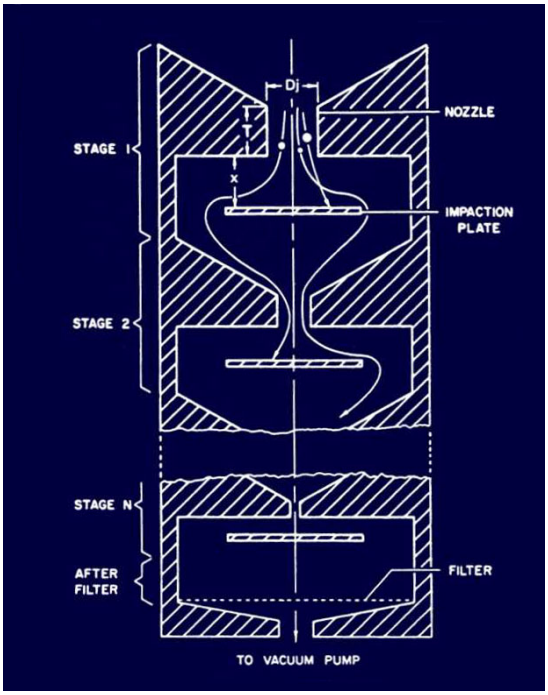


Figure 3.13b. Schematic of the inner working of an ACI.

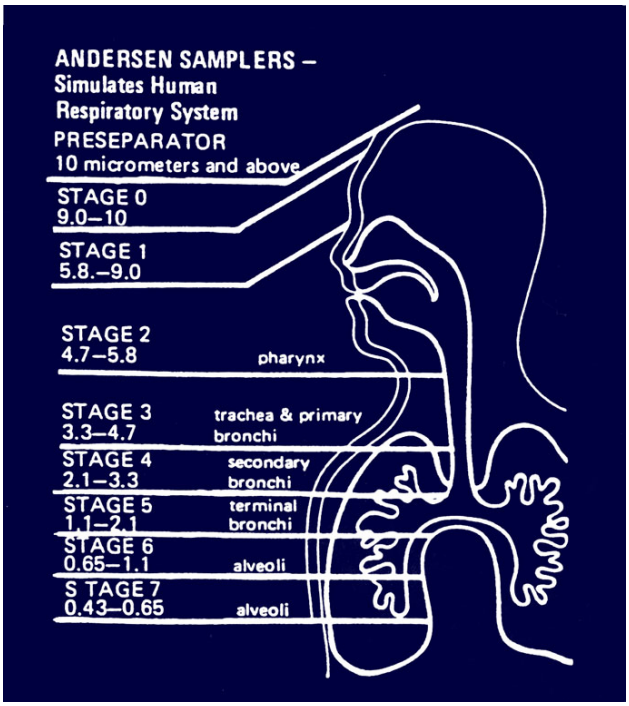


Figure 3.13c. Theoretical in vivo correlation of impactor plates of an ACI.

The ACI relies primarily on inertial impaction of aerosol particles to effectively characterize pharmaceutical aerosols, as all cascade impactors do (shown in Figure 3.13b). Upon actuation of an aerosol device into the USP inlet ("throat", as seen in Figure 3.13a), the aerosol particles are pulled by a vacuum created airflow typically of 28.3L/min at the inlet terminus through jets of specific size created to accelerate the particles to a fixed velocity, where based upon their size, may or may not impact on the given plate. This process is repeated throughout the instrument with increasingly smaller jet orifices, creating higher and higher velocity through the jet causing more and more inertia (mass x velocity) for a given particle, ultimately causing deposition on one plate or another.

The use of this instrument also requires gravitometric or more likely chemical analysis of the contents of each plate, which can then help determine a particle size distribution, based on the distribution of drug mass throughout the instrument. Unfortunately, because of this, analysis via cascade impaction is an extremely laborious and time consuming, though well accepted technique.

Though cascade impactors are generally well accepted, they are not without drawbacks. As mentioned above, cascade impactors in general are very labor intensive. A well-trained and efficient operator will only be able to complete one run every 45

minutes. The operation of the instrument only requires (specifically referring to the ACI) a few minutes per run, but then the apparatus must be disassembled, each portion rinsed in diluent for quantification, then washed to remove any residual compound, allowed to dry, then reassembled. Another issue that has been documented in the literature is particle bounce [49]. Particle bounce can be a significant issue with cascade impactors depending on a formulation under examination. Particle bounce, just like the name implies is when a given particle impacts onto an impactor's collection plate, but does not stick. Instead, it "bounces", and continues onto further stages and ultimately deposits further down in the impactor. Because aerosols are characterized by 1) mass fractions, which are used to assess the total respirable dose available for a patient (in theory) and to assess the efficiency of drug delivery, and 2) broad size characterizations such as mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). If the mass fraction is incorrect, that could falsely imply that a recommended therapeutic dose may be larger than it appears. Likewise, it could lead to false characterization of a material itself, generally labeling an aerosol product as having a smaller MMAD than is actually the case.

Less labor intensive alternates have been investigated, and one such instrument that has proven adequate for at least early phase screening is the Aerodynamic Particle Sizer (TSI, Inc. 3321) coupled with the Impactor Inlet (TSI, Inc. 3306), seen in Figure 3.14.

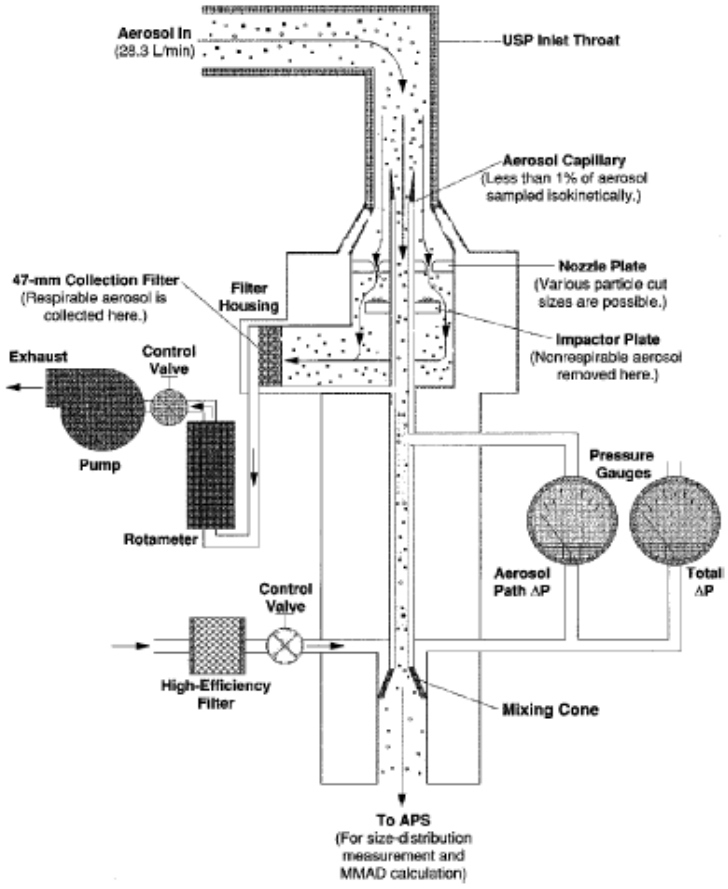


Figure 3.14. Schematic of the TSI 3306 Impactor Inlet.

The 3306 Impactor Inlet is a single stage impactor that works similarly compared to that described above by the ACI, however the sole impactor plate collects all particles greater than 4.7µm (considered 'course' or 'non-respirable'). All particles smaller than the 4.7µm cut-point (or equivalent given that each cut point is considered to have a 50% collection efficiency), continue on to the end filter. As above, this instrument was designed to operate with the same USP throat and at 28.3L/min. This instrument allows

for analysis of the mass balance of the dose administered, but does not account for particle size characterization.

Particle size characterization is completed with the Aerodynamic Particle Sizer (Model 3320, 3321, sizing mechanism seen in Figure 3.15), which uses laser time-of-flight to determine particle size [48]. Aerosol particles are accelerated to a fixed velocity and continue through two overlapping lasers which create an impulse from a particle passing through each laser. The size is then calculated based on the time between and the size of electrical impulses, assuming the particles are spherical and have a unit density of 1g/ml. The results are somewhat confirmed by an independent means with light scattering measurement, such that small particles are not expected to scatter much light, and larger particles will correspondingly scatter more.

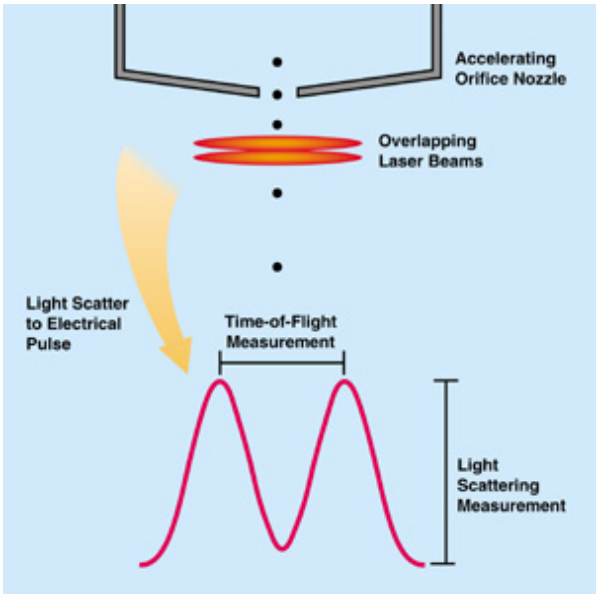


Figure 3.15. Schematic of the particle sizing mechanism for the TSI Aerodynamic Particle Sizer [48].

Because the 3306/3321 (modified from the original Model 3320, collectively referred to as the TSI system) only contains a single stage impactor, it is a tremendous time saver, as only three (or four, discussed below) parts need to be rinsed and quantified, including the USP throat, the lone impactor plate, and the collection filter. Comparing against the ACI, an operator must also rinse for chemical quantification the USP throat, eight impactor plates, and a collection filter. This alone is a tremendous time savings, not to mention the additional time and materials savings of (typically HPLC) analysis of the solute.

Another significant advantage to using the TSI system is the availability of real-time results. Because the particle sizes are measured electronically, a full size analysis is available upon completion of a run. As alluded to above when using the ACI, particle size characterization is dependent upon the relative placement of the drug mass throughout the instrument, which is only available after chemical analysis and back calculations (from various dilutions) are completed. This benefit is particularly useful if no mass fractions are needed for a given research project, and particle size analysis can be completed as quickly as your given run time.

Most importantly however is the correlation of the TSI system compared to well accepted, traditionally used instruments such as the ACI. If no accurate correlation is noted, then it could severely limit the utility of the TSI system. As such, investigations

into this correlation were initiated, though some issues arose. The following is a detailed accounting of these problems, and potential solutions which may allow for better use of the instruments.

As mentioned above, determination of drug mass distribution and particle size characterization are the two most important measures determined by pharmaceutical aerosol characterization instruments. The TSI Model 3306 is the Impactor Inlet portion that works in conjunction with the sizing apparatus that is the Model 3321. The Model 3306 is a single stage impactor, as mentioned above, which offers significant analyst time savings, but still completes the function of determining drug mass distribution by offering a cut-point comparably used on the ACI. However, due to physical differences in the two devices, experimentation was necessary to ensure proper correlation.

In the ACI, an aerosol plume travels into the USP throat, into the device, where it is immediately met by a jet and subsequent impactor plate, then on through the following jets and impactor plates as determined by the aerodynamic size of the material. In the Model 3306, an aerosol plume with travel through the very same USP throat, and as the manufacturer provides the device, through the jet, by the impactor plate (as determined by aerodynamic size) and to the collection filter, which collects all drug less than 4.7μm. This is a seemingly unnoticeable difference until further review. The impactor plate in the Model 3306 was designed to mimic the stage on the ACI where aerosol particles are determined to be respirable, or non-respirable (less than or greater than 4.7μm, respectively). This correlates to stage 3 of the ACI, the 4^{th} impactor plate, as the device starts at stage 0. Lastly, in the TSI system, the aerosol is passing through three jets,

compared to the ACI, where depending on the stage could contain tens or hundreds of jets.

After acknowledging these differences and again comparing the two instruments, a noticeable difference can be identified. The aerosol droplets entering the ACI are subject to significant mixing with surrounding air through four series of jets, impactor plates, and the associated path therein. This could yield potentially different results depending on the aerosol formulation and method of delivery into the Model 3306, where the aerosol has a straight path to the jet and impactor plate. This will not be an issue with aerosols emitted from DPIs, as they are emitted at their final particle size and will deposit (theoretically) identically in either instrument. This is not the case however when examining aerosols emitted from MDIs or nebulizers, where they are propellant or aqueous based liquid formulations subject to evaporation, and based on the fixed distance and airflow from the inlet entry, evaporation rate.

Because this instrument is used much more commonly with DPIs, and MDIs, the evaporation rate of MDIs becomes important. Likewise, this could be of concern with regard to the particle sizing apparatus (Model 3321), which will be described later. To examine this potential problem, inlet extensions were added between the USP inlet and the impactor inlet to allow more time and interaction with surrounding carrier air to more closely match drug mass distributions close to the ACI. Figure 3.16 shows photographs of the TSI system as the manufacturer supplies the instrument, with no inlet extension (left), and modified with a 20 and 40cm throat extension to improve potential problems

caused by the evaporation, or lack thereof of aerosol droplets (middle and right, respectively).

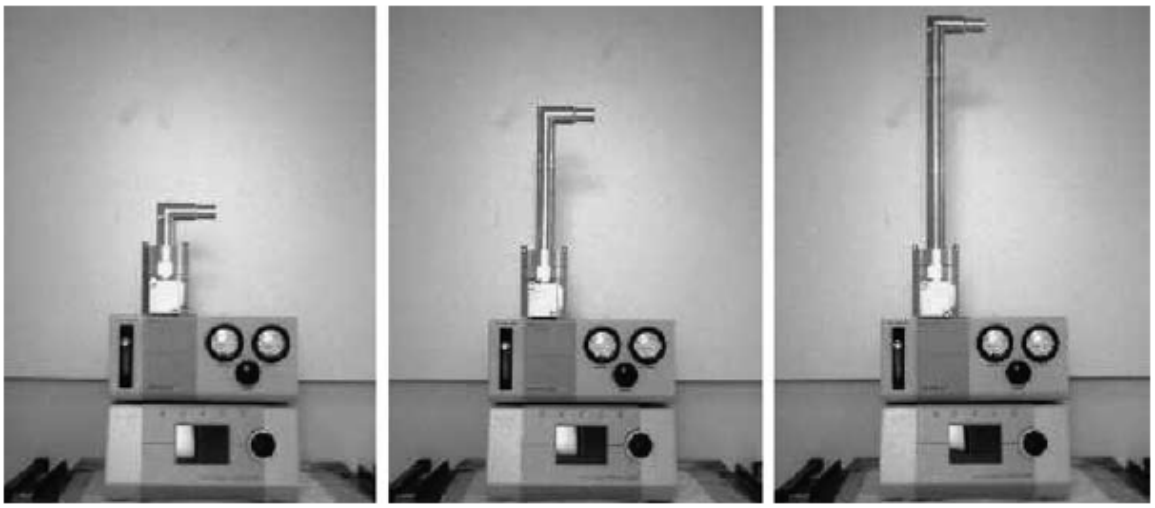


Figure 3.16. Photos of the TSI system as the manufacturer provides (left, without throat extension), and modified with throat extensions of 20 or 40cm (middle and right).

Seven formulations were examined to evaluate the need for throat extensions. Each contained varying concentrations of BDP in solution, ethanol and HFA 227, as seen in Table 3.2. Due to solubility limitations, 0.4% BDP at 5% ethanol was not examined. Likewise, a third series containing 0.8% BDP was to be examined, but only the formulation containing 20% ethanol was sufficient to solubilize the BDP, thus the series was not included.

BDP % w/w	Ethanol % w/w	HFA 227 % w/w
0.08	5	94.92
0.08	10	89.92
0.08	15	84.92
0.08	20	79.92
0.4	10	89.6
0.4	15	84.6
0.4	20	79.6

Table 3.2. Formulations examined to evaluate need for throat extensions.

The need for the throat extensions were confirmed when ethanol sensitive paper was placed on the impactor plate of the Model 3306, as displayed in Figure 3.17. This technique was used to offer a qualitative look into the potential issue at hand.

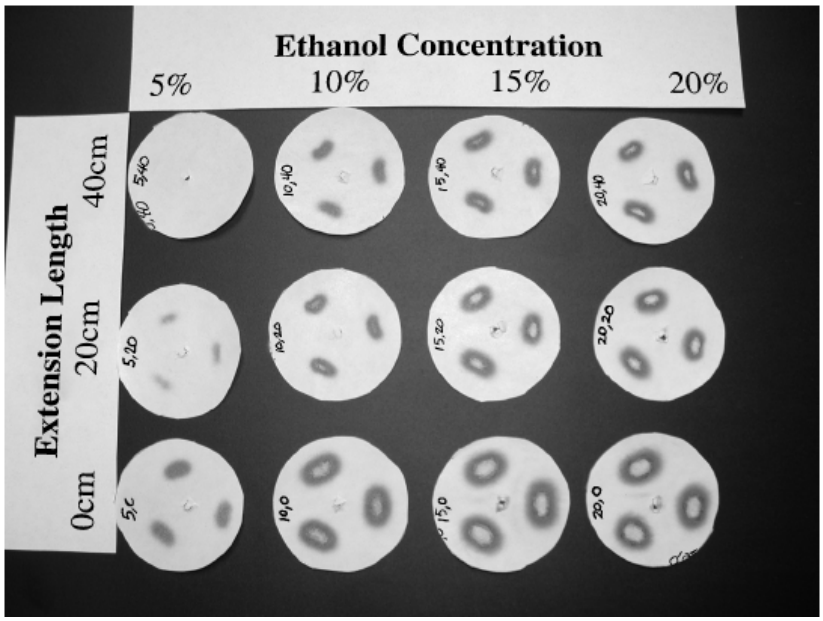


Figure 3.17. Photo of ethanol sensitive paper placed on the impactor plate of TSI Model 3306 after actuation by ethanol containing MDIs with or without throat extensions.

Ethanol sensitive paper contains a reagent that causes the paper to turn blue upon contact with ethanol. This paper was fitted over the impaction plate of the Model 3306 to identify ethanol containing droplets at the impactor plate. As shown in the above figure, when no throat extensions were added, even in the best case scenario examined of a formulation containing 5% ethanol, ethanol remains in the droplets upon deposition on the impactor plate. This trend becomes more apparent with increasing concentrations of ethanol, which makes sense as described above. The addition of non- or semi-volatile components into a MDI formulation effectively dilutes the propellant and its ability to aerosolize the formulation. This tends to result in increased aerosol droplet size upon actuation, and also extends the length of time needed to fully evaporate the ethanol.

Again, the qualitative data collected suggested just this. As the extension length increased, the apparent amount of ethanol remaining in the droplets decreased for all formulations examined.

The addition of the throat extension did not adversely affect the aerodynamics or the USP inlet as expected. Figure 3.18 displays multiple formulations examined containing varying concentrations of a model drug in solution, BDP, with varying proportions of ethanol in HFA 227. This figure displays that the addition of throat extensions results in deposition no different than the module without extensions or the ACI, which is sensical seeing as though the USP inlet (throat) is identical across the systems and configurations examined, and is upstream from any changes made to the system.

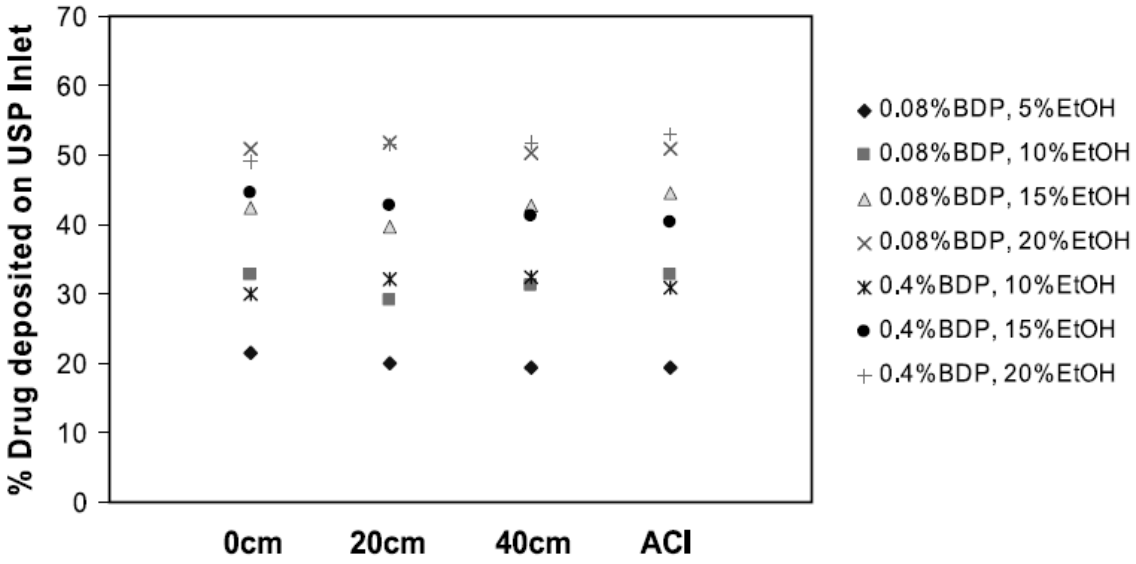


Figure 3.18. Throat deposition (mass %) as examined in the ACI or TSI Model 3306 with or without extensions.

The differences noted in this figure are due primarily to the different formulations, as the slope across instruments and configurations was maintained at roughly zero. The formulations containing the most dilute amount of ethanol and secondarily dilute amount of drug displayed the lowest deposition in the throat, whereas formulations containing larger amounts of ethanol resulted in increased throat deposition.

Fine particle fraction (FPF) was examined to assess the changes in drug mass distribution throughout the instrument. FPF more specifically refers to the fraction of total drug less than 4.7μm relative to the total drug emitted. FPF is determined by the following equations, given for each instrument, each portion listed refers to the mass of drug collected:

$$FPF_{TSI} = \frac{filter}{filter + plate + throat} \qquad (1)$$

$$FPF_{ACI} = \frac{Stages\,3 \rightarrow filter}{Stages\,0 \rightarrow filter + throat} \qquad (2)$$

As seen in Figure 3.19, there was a significant decrease in FPF noted as ethanol concentrations increased, which is notable for both drug concentration groups. Examining both drug concentrations, no apparent differences were noted for equivalent ethanol concentrations. More importantly with regard to this investigation, the formulations examined without throat extensions demonstrated decreased FPF relative to the other two

impactor inlet groups, which were more difficult to decipher, also indicating the need for further drying, as this data suggests a higher proportion of droplets were relatively larger at the impaction site.

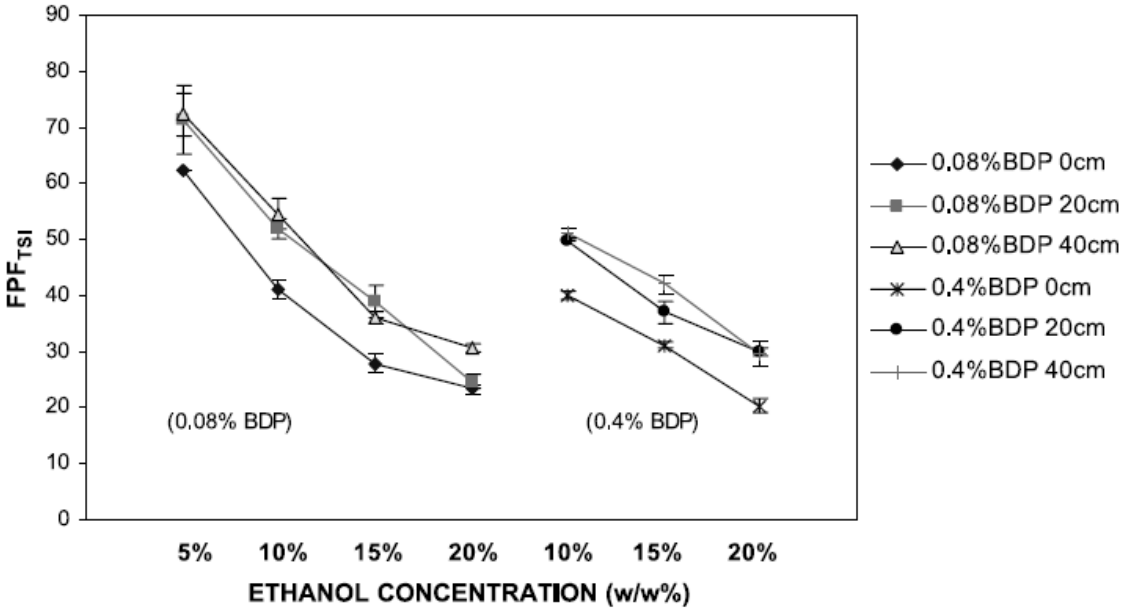


Figure 3.19. FPF as a function of ethanol measured with or without throat extensions.

To assess the improvements against the most important measurement, the ACI, the ratio of FPFs were examined, as the following equation displays, and the results can be seen in Figure 3.20.

$$ratio\,TSI/ACI = \left[\frac{FPF_{TSI}}{FPF_{ACI}}\right] \qquad (3)$$

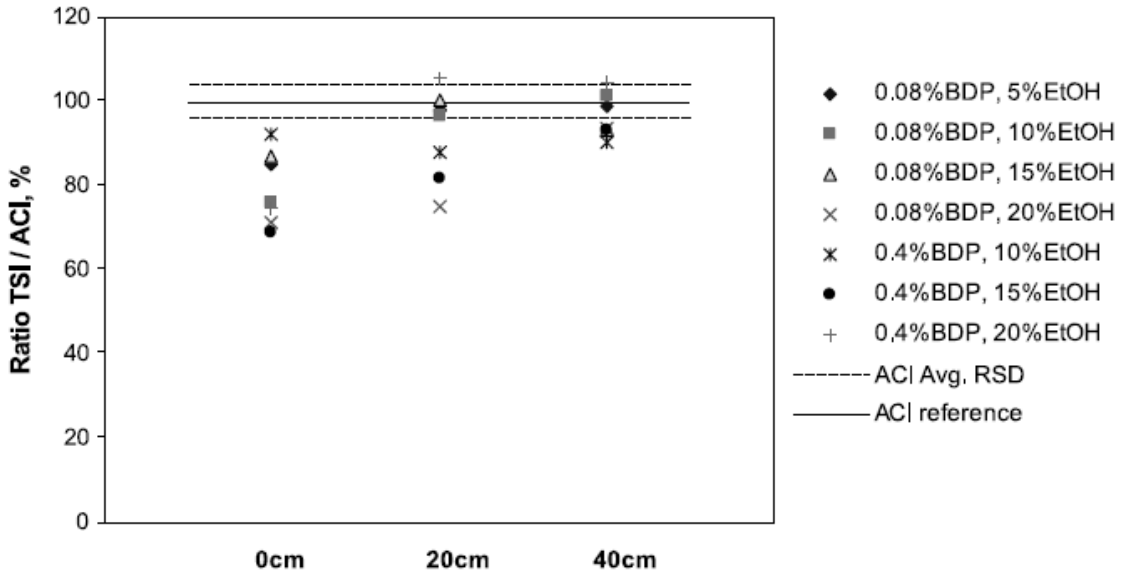


Figure 3.20. FPF (expressed as a percentage) for the TSI configurations relative to the ACI.

As shown in the above figure there is a clear improvement in the ratio, indicating that the TSI 3306 configuration more closely matches the ACI with the 20 and 40cm extensions across the formulations examined, and the 40cm extension more consistently resulted closer to the ACI.

In addition to assessing the mass balance within the given instruments, it is imperative to ensure that the changes to the system do not affect the particle sizing function of the Aerodynamic Particle Sizer (Model 3320, 3321). To assess this, MMAD was examined for each configuration and compared against MMAD calculated from the ACI. The results were graphed and displayed in Figure 3.21.

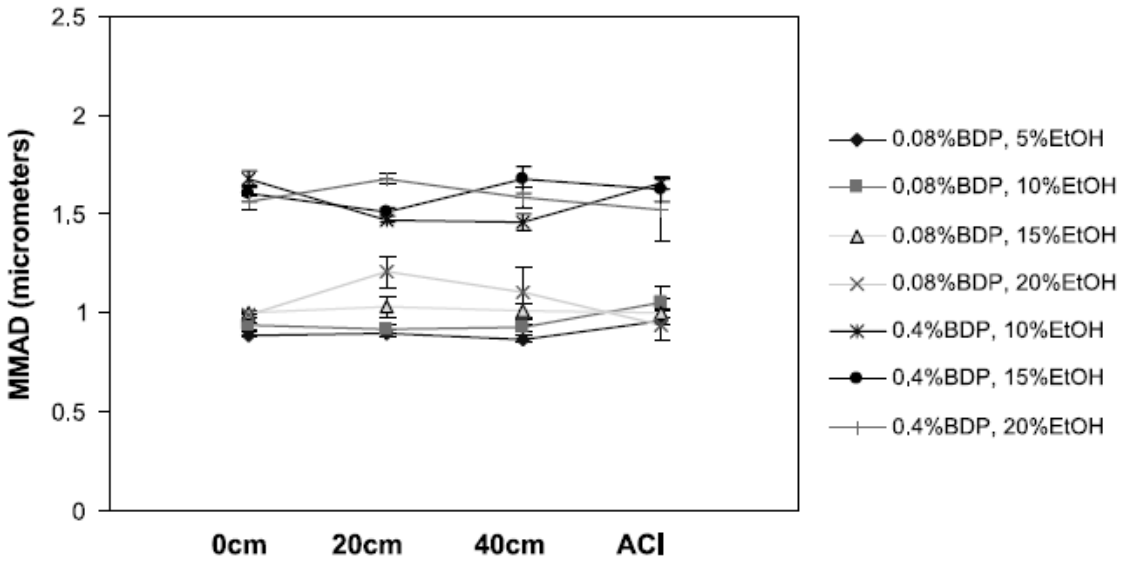


Figure 3.21. MMAD comparison between ACI and impactor inlet configurations.

No systematic or statistical differences were noted in the particle size characterization after the addition of throat extensions. This is imperative, as particle size characterization must also closely match the ACI to ensure that the TSI system is of comparable performance, allowing for its use and benefit from the time and labor savings. In the 3306 Impactor Inlet, a sampling port exists which samples a small fraction of the aerosol (less than 1%) for particle size. The aerosol particles that enter this sampling port are again diluted by sheath air and are accelerated to a fixed velocity prior to being sized by the time-of-flight mechanism as described above. Because there is additional mixing with air, it is possible, as the case of the ACI, that the particles 'dry' prior to being characterized, potentially explaining the lack of statistical differences noted in MMAD between the configurations of the TSI instrument ($p>0.05$, ANOVA).

In conclusion, the addition of throat extensions to the TSI system showed improved correlation to the ACI, the instrument widely considered a standard for aerosol measurements. There appeared to be no deleterious effects on the aerodynamics of the TSI system, as there was no change in throat deposition, no significant drug deposition on the walls of the extensions, and no significant changes in particle size. By all accounts, the addition of throat extensions improved the correlation to the ACI. Unfortunately, using a 40cm extension can be difficult for vertically challenged operators, and more recent instruments, such as the Next Generation Impactor (NGI) were recently introduced. As such, a further investigation was completed, attempting to address the same issue, but with a different approach. In this study, heated throat extensions were added, 20cm or 28cm in length, which is from an operator standpoint, much easier to work with than a 40cm extension, though in the last study, it appeared as though the 40cm extension did offer some advantage over the unheated 20cm extension. Comparisons were again made to the ACI, being a well-accepted instrument, but also comparing against the NGI, a recently introduced cascade impactor. The modifications to the TSI system can be seen in Figure 3.22, and compared against the standard configuration as shown above in Figure 3.14.

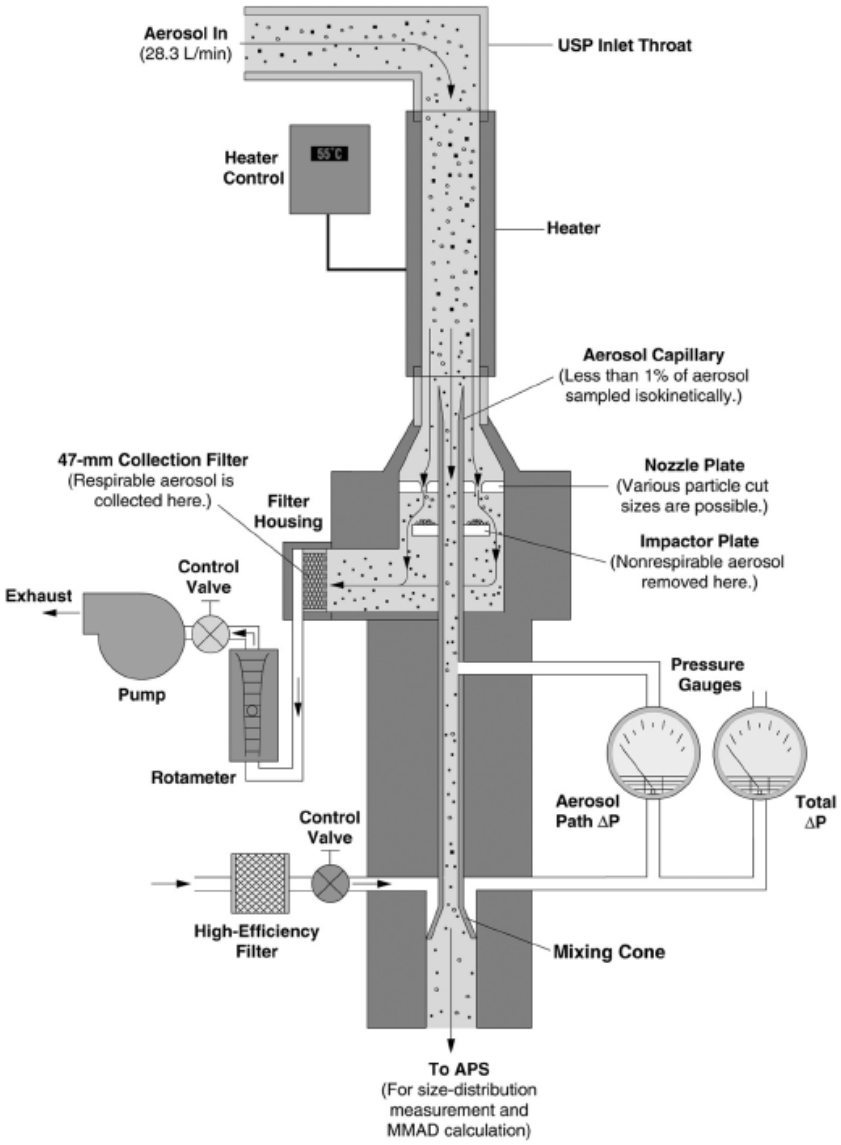


Figure 3.22. Modified schematic of TSI Model 3306 including a heated throat extension.

This study only utilized two formulations containing, 0.167% BDP w/w, 8% or 20% w/w ethanol in HFA 134a as the variables to be measured were the temperature settings of the throat extensions in comparison to the two cascade impactors. The heated throat extensions had the same internal diameter as the rest of the system and were

designed not to alter the aerodynamics of the inlet. Around the exterior, an electrically controlled heating element was wrapped around the length of the extension. A thermally insulating sleeve was fitted over the exterior of the extension so the analyst would not burn themselves, as the set temperatures of interest were at 60, 80, and 100ºC (140-212ºF), though in the experiment more modest set temperatures of 40, 55, and 70ºC were used. The heating element was electronically controlled and was allowed to stabilize at given temperatures prior to testing. Figure 3.23 displays the internal temperature within the extension.

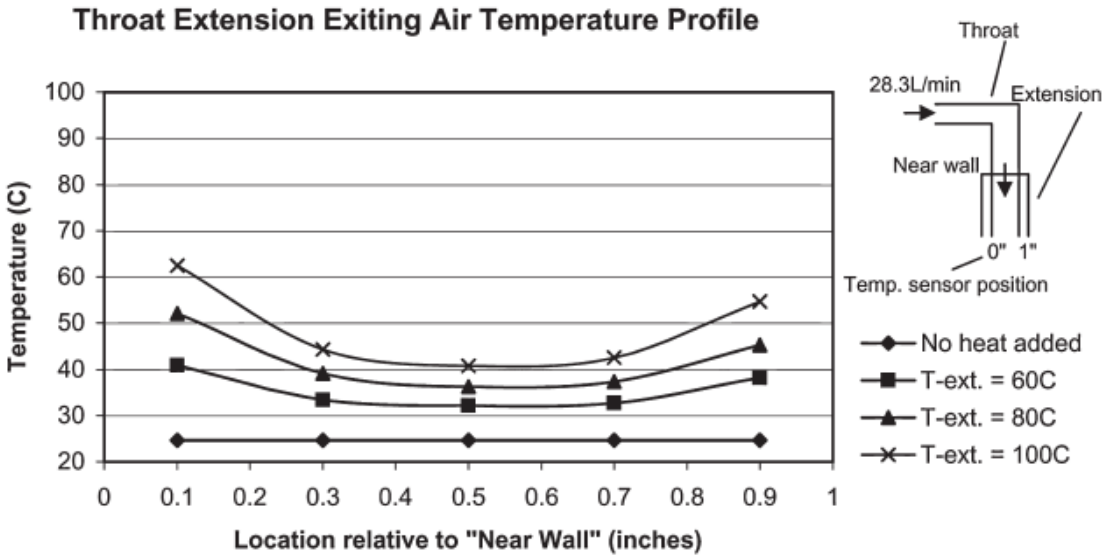


Figure 3.23. Exiting air temperature of 20cm throat extension heated to 60, 80, or 100ºC.

A temperature probe was placed at different points across the internal diameter of the throat to asses the heat at different points as the air was exiting. Because the air is flowing at 28.3L/min, the above figure suggests that heating across the extension is not

even, but rather appears to be laminar in nature. Obviously higher temperatures were achieved nearest the walls of the extension where the heat was entering the system, and were lowest in the center. Again, FPF was used to examining the mass distribution of the drug entering the system.

As was the case in the last study, the fraction of drug collected in the throat was not statistically different for the 20% ethanol containing formulation ($p > 0.05$, ANOVA). Statistical differences were noted in the 8% ethanol containing formulation ($p < 0.05$), but were small enough differences to be considered practically insignificant. Of note is the fact that the NGI resulted in slightly lower Throat Fraction (TF) in both cases. This is likely due to the fact that the NGI is calibrated to operate at 30L/min suction compared to the 28.3L/min of the TSI system and the ACI. This increased velocity decreases the difference between the aerosol rapidly expanding from the orifice and the surrounding air, causing less turbulent airflow. Figures 3.24 and 3.25 display the fraction of drug collected in the throat for the given formulations.

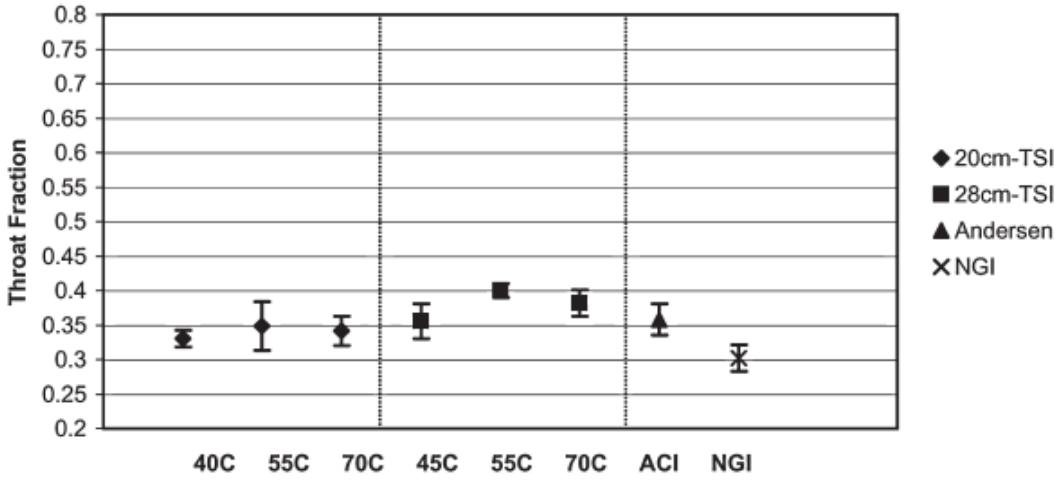


Figure 3.24. Fraction of drug collected in the throat for the 8% ethanol formulation.

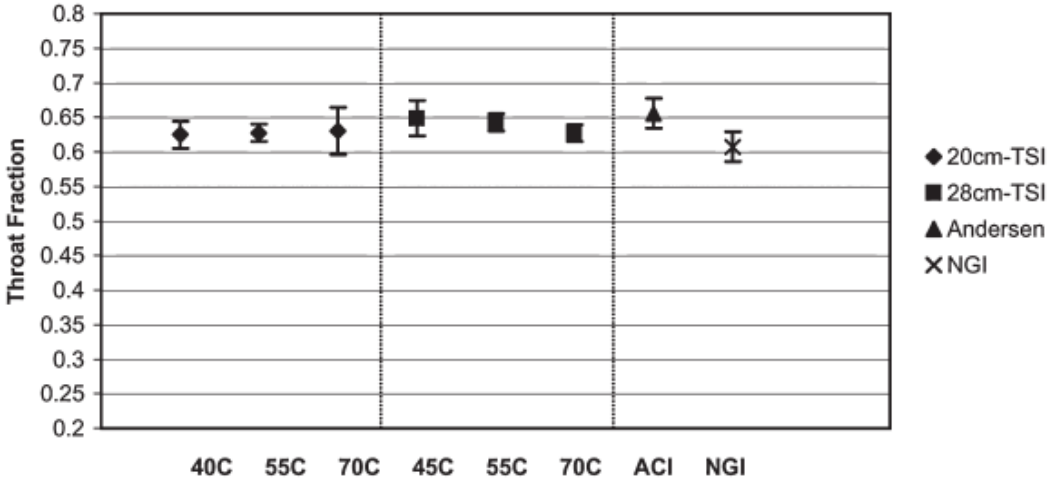


Figure 3.25. Fraction of drug collected in the throat for the 20% ethanol formulation.

As mentioned above, FPF was again used to assess the distribution of drug throughout the system and can be seen in Figures 3.26 and 3.27. These figures display the FPF and it is clear that the fraction of drug collected in the throat highly influences the results seen in these figures. Notable from Figures 3.24 and 3.25 is the fact that 30-60%

of the total drug mass collected was in the throat. As such, it is somewhat difficult to determine the effect that heating the throat has on the distribution of the aerosol within the impactors. Thus, a second, more sensitive measurement (Impactor Fine Particle Fraction, IFPF) was used. IFPF is the same calculation as listed above, only it excludes drug collected on the throat, as no changes would be expected as the changes to the system are downstream. IFPF ultimately allows for a more sensitive view of what is happening or changing within the impactors. Figures 3.28 and 3.29 display the IFPF for the 8 and 20% w/w ethanol respectively.

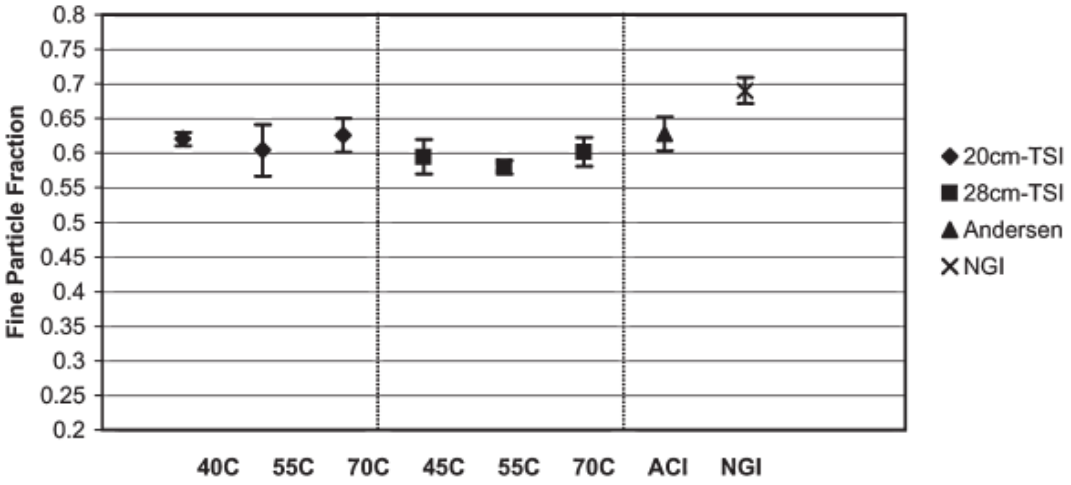


Figure 3.26. FPF for 8% ethanol containing formulation for different heated extension configurations and instruments.

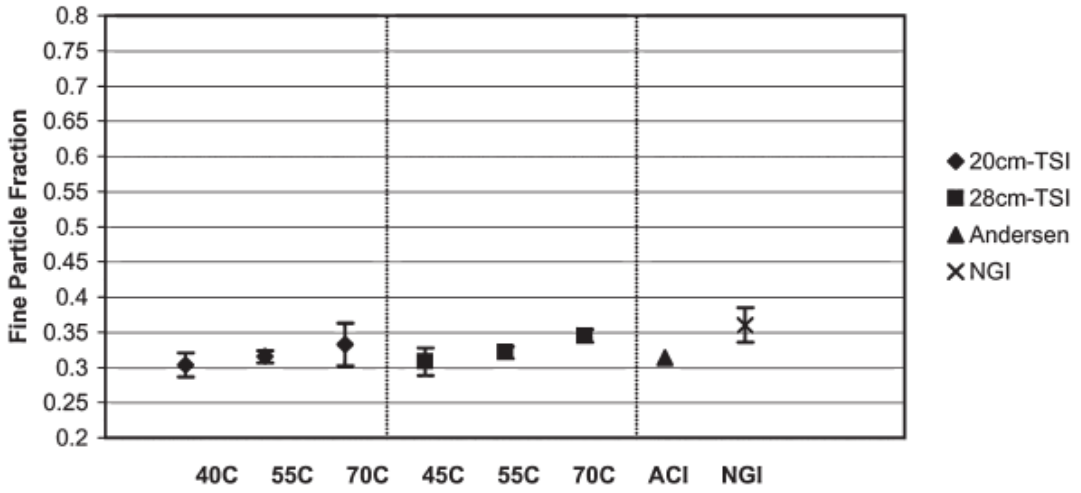


Figure 3.27. FPF for 20% ethanol containing formulation for different heated extension configurations and instruments.

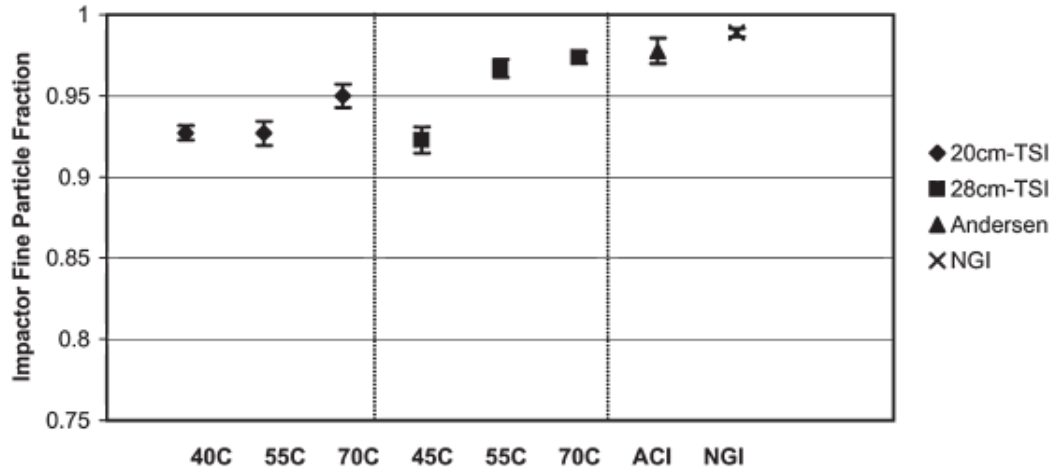


Figure 3.28. IFPF for formulation containing 8% ethanol for different instruments and configurations.

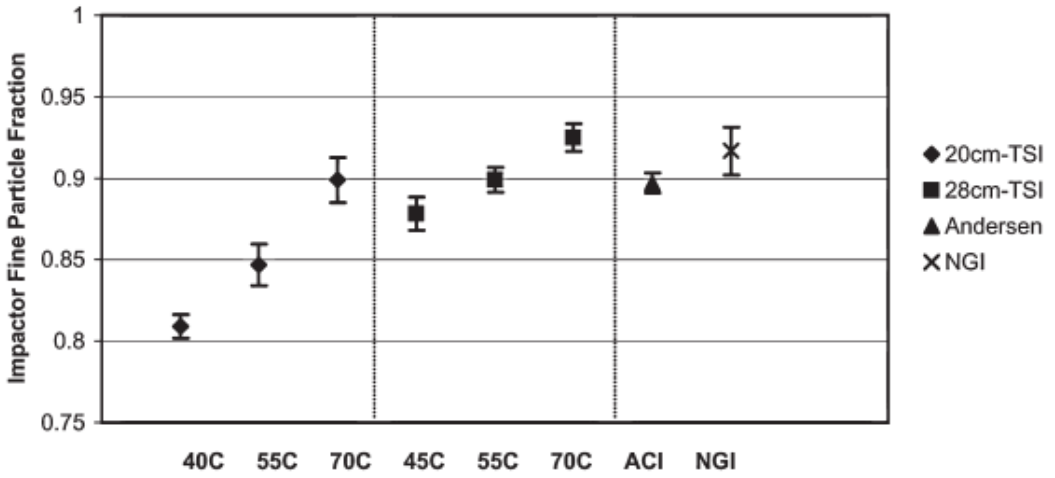


Figure 3.29. IFPF for formulation containing 20% ethanol for different instruments and configurations.

In both cases, the addition of heated throat extensions improved the FPF. It did not appear to matter if a 20 or 28cm extension was utilized, and the temperature setting of the heated extension at 40 or 55ºC showed the closest correlation with the ACI, while the highest temperature showed a better correlation to the NGI which also resulted with a higher FPF than the ACI. Regardless, care must be taken, particularly in the case of formulations containing higher proportions of semi-volatile components such as ethanol not to over-dry the droplets, making for a less than optimal correlation. However, upon examination of IFPF, it appears as though specific combinations would be more appropriate to match the ACI. The two most similar in this case were the 28cm heated to 55 or 70ºC for the lower concentration of ethanol, or 20cm heated to 70ºC and 28cm extension heated to 55ºC for the 20% ethanol formulation. Keep in mind however that

throat deposition, which was not appreciably different, contributes to FPF which may ultimately be affecting the FPF results.

As in the last study, drug mass distribution is important, but particle size characterization also must be taken into consideration. Figures 3.30 and 3.31 present cumulative mass size distributions as a function of aerodynamic particle size.

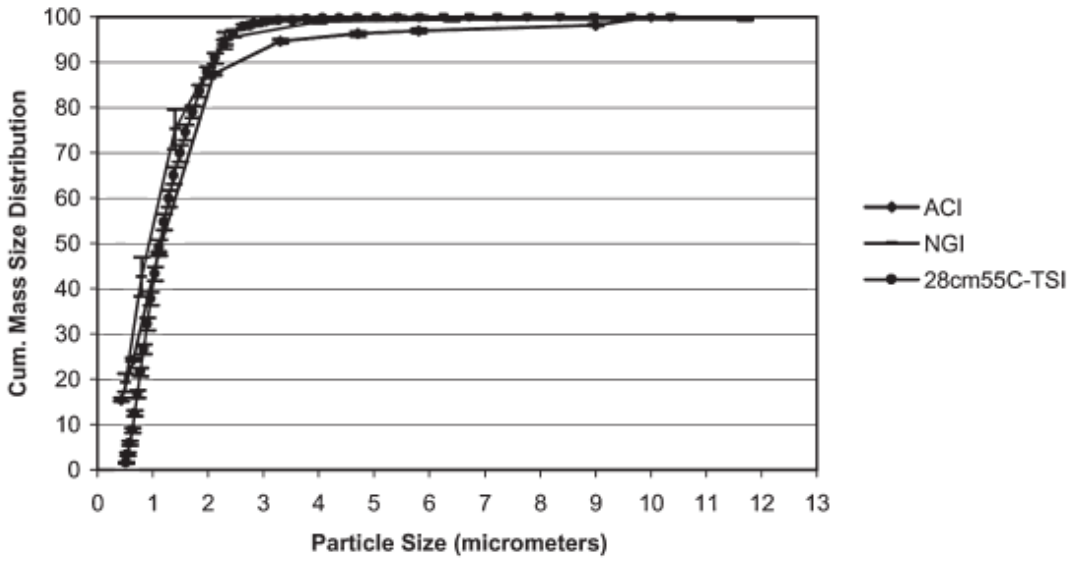


Figure 3.30. Particle size comparison between ACI, NGI, and a representative TSI sample for the 8% ethanol formulation.

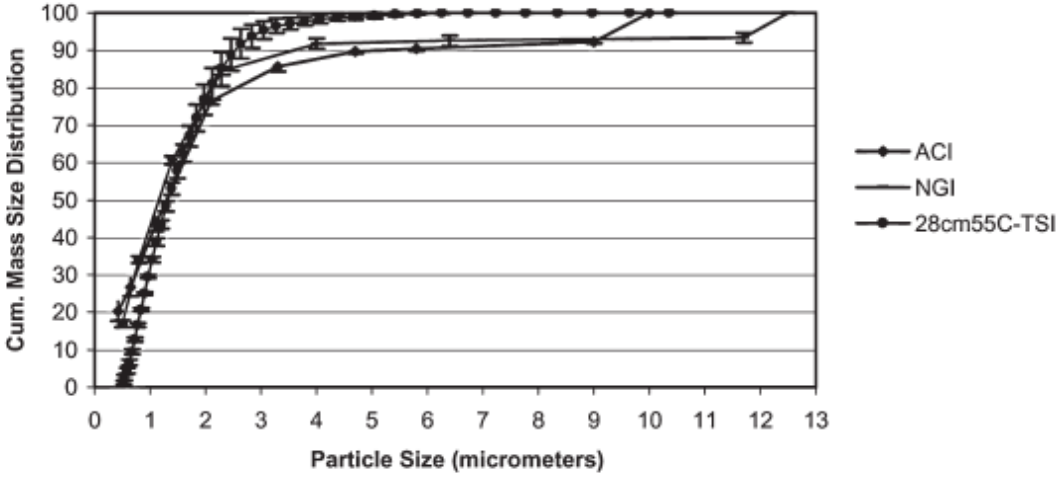


Figure 3.31. Particle size comparison between ACI, NGI, and a representative TSI sample for the 20% ethanol formulation.

Figure 3.30 notes the fact that no significant differences in particle size characterization were shown between any of the systems with regard to cumulative mass percent. This will correspondingly lead to no differences in MMAD or GSD measurements, which could also be identified by using this graphing technique.

Figure 3.31 however shows some differences in that the two cascade impactors resulted with drug impacting on early stages, likely from larger aerosol droplets which will take longer to evaporate. This is not the case for the TSI system however, because the sizing mechanism is downstream from the heated extension which will at least partially dry the droplets, in addition to the sheath air also introduced to accelerate the particles immediately prior to sizing as described above. Both of these physical differences will lead to the drying of the droplets prior to particle sizing, and will likely

lead to poor comparative correlation for formulations containing larger quantities of semi-volatile substances such as cosolvents.

Bringing this study to a conclusion, when examining formulations containing more moderate concentrations of semi-volatile components, the use of a 20cm extension appears to be sufficient, and heating this extension appears to provide a better correlation compared to commonly used cascade impactors. Because of the physical difference in the devices and different sizing mechanisms, caution must be taken when examining formulations containing higher proportions of semi-volatile substances, as droplets that would appear to impactor early after actuation instead are over-dried relative to what they should be.

III- Conclusions

The above studies have shown improvements in the chemical and physical analysis of pharmaceutical aerosols. The ability to inject samples administered from an MDI demonstrates a significant advance in previous analytical methods, though operator variability may still be less than ideal. Fortunately, the tradeoff is in the fact that there are fewer steps, thus less analyst manipulation, and potentially fewer chances for making dilution or calculation errors. Overall, the variability noted in the more recent method is less than that achieved using traditional methods, which is an advantage in addition to the significant material and time savings already noted.

Several different methods of characterizing pharmaceutical aerosols exist. The methods proposed above improve an instrument that offers the competitive advantage of significant time savings compared to traditionally used methods such as cascade impaction. As the instrument is provided from the manufacturer, only aerosols containing very low or no semi-volatile substances may have the potential to be accurately characterized. Utilizing some fairly straight forward techniques, a better correlation was achieved for MDI formulations containing higher concentrations of cosolvents without affecting the particle size characterization. This was not the case however when heated extensions were added, when some droplets that impacted early in the cascade impactors were allowed significant drying time prior to being sized in the TSI system. Likewise, due to the mechanism of sizing in the TSI system, it must also be recognized that other non-volatile excipients may also be measured as real aerosol particles, potentially skewing the results. However, being aware of the formulation under examination, its composition, and using what is known about the different instruments, an educated decision can be reached on which device is most appropriate for *in vitro* measurements. All offer different advantages, and have specific drawbacks, but depending on the formulation and experiment, an appropriate decision should be rather easily met.

CHAPTER 4: UPDATE IN SUSPENSION AEROSOL MODELING AND PREDICTION

Earlier in this manuscript, much attention was paid to the rebirth of MDIs as CFCs were phased out due to deleterious effects on the ozone layer. Reformulations ensued, and as such a review of excipient utilization was presented as the MDI systems have changed considerably since their inception in the 1950's. As previously discussed, HFA propellants are much poorer cosolvents themselves compared to their CFC counterparts, and as such, the bulk of the recently approved HFA formulations are suspensions. This sections will attempt to build upon past methods of modeling suspension aerosols given what is more recently known about formulation and device parameters and their effect on aerosol output.

Raabe (1968) attempted to calculate and experimentally verify the dilutions necessary for monodisperse polystyrene latex spheres to be nebulized and result in primarily aerosol droplets containing a single latex sphere per droplet [50]. In this study, the commercial nebulizers used emitted aerosol droplets in a log-normal manner, as has also been noted with MDIs. He used a Poisson probability distribution, given by the following equation, to estimate the number of latex spheres that would likely occupy a given droplet described by the droplet volume and concentration of spheres assuming they are perfectly dispersed throughout the suspending media.

$$P(x) = \frac{e^{-m} \bullet m^{x}}{x!} \qquad (1)$$

This Poisson function essentially determines the probability (p) of a number (x) of drug particles, or in this case polystyrene latex spheres existing in a droplet, given a certain average (m) particles per unit volume, and could yield droplets containing no drug particles, a single particle, or multiple. For more concentrated suspensions, more particles would likely inhabit a given droplet. Likewise, for larger droplets, more particles would likely inhabit the given droplet. Another interesting and useful piece of information that came from this study was the fact that no particles existed in droplets smaller than the given particles, nor did particles tend to exist in droplets up to 25% larger than the particle, because the particle to droplet volume ratio is so large, the probability of the event occurring is quite small.

Gonda (1985) furthered this work by assuming that both the raw drug substance and the droplets are monodisperse, seeing as though most droplets and micronized drug have a geometric standard deviation (GSD) of less than 2 [51]. The relationship describing GSD is as follows:

$$GSD = \sqrt{\frac{d_{84\%}}{d_{16\%}}} \qquad (2)$$

Though monodisperse distributions indicate that all particles or droplets are of the same size (GSD = 1), materials with GSD less than 2 have oft been considered close

enough to be adequately described by calling them monodisperse [50, 51]. Chan and Gonda built on this work further, developing a model that attempted to account for clusters of monodisperse particles in an aerosol of polydisperse droplets, which is actually the case for most aerosols emitted using nebulizers or MDIs [52].

The utility of these works were only available to be applied to MDIs, if the size of initial droplets emitted from MDIs was known. Unfortunately, upon actuation, propellant evaporates from the emitted droplets far too fast to be able to measure. As such, no reliable methods have been employed successfully determining the size of droplets initially emitted from MDIs, and attention has only been paid to the resulting characteristics of the final particle distribution. Noted by Chan and Gonda, depending on the concentrations of the particles in the suspension media and depending on their relative proportions, the resulting particle size characterizations may correlate very differently when dilute or concentrated suspensions exist [52]. Thus, there was no apparent way to calculate initial droplet sizes based on final size characteristics of suspension aerosols.

Stein and Myrdal helped alleviate this deficit when analyzing formulation and device parameters and their effect on solution MDI size distribution [53]. Amongst several other findings of the study, they were able to back calculate the initial droplet size of particles emitted from solution MDIs, based on their measured characteristics, and composition based on the following equation:

$$D_{residual} = D_{droplet} \sqrt[3]{\frac{\rho_{droplet} \bullet C_{NV}}{\rho_{residual}}} \qquad (3)$$

which can be rearranged to yield an equation to calculate initial droplet size, $D_{droplet}$.

$$D_{droplet} = \frac{D_{residual}}{\sqrt[3]{\frac{\rho_{droplet} \bullet C_{NV}}{\rho_{residual}}}} \qquad (4)$$

In addition to establishing the size of initial droplets emitted from MDIs, they also found that the primary factor influencing the size of the droplets is the nonvolatile concentration of the formulation. Because the drug (and possibly surfactant) only accounts for a very small fraction formulation composition, generally less than 1% combined, they exert negligible effects on the emitted droplet sizes. However, when cosolvents are added, only then are significant deviations in initial droplet size noted. This phenomenon was already described in detail in previous chapters, where the vapor pressure of the propellant is effectively diluted by the addition of significant proportions of less volatile material in the formulation, in this case cosolvents. Ultimately, it was determined that the initial droplet sizes emitted from MDIs is log-normal, demonstrates an identical GSD compared to the initial droplets, and is relatively constant at ~10μm [53]. Table 4.1 displays three example solution based formulations and their

experimentally derived particle sizes with the corresponding calculated initial droplets sizes.

BDP (%w/w)	HFA 134a (%w/w)	Formulation density (g/ml)	$D_{residual}$ (μm)	$D_{droplet}$ (μm)
0.08	84.92	1.147	1.18	13.25
0.40	84.60	1.147	1.54	10.11
0.80	84.20	1.147	1.90	9.90

Table 4.1. Example formulations using solution based formulations for the calculation of initial droplet size. All formulations contain 15% ethanol w/w.

As demonstrated, the initial droplet sizes are all close to 10μm, though note the slightly increased initial droplet size ($D_{droplet}$) calculated from $D_{residual}$ (or $MMAD_{residual}$ for a distribution of droplets) of the most dilute solution formulation. This could be an example of a limitation of the instrument used to measure the aerosols, the TSI system. The TSI system loses resolution and less accurately sizes particles significantly less than 1 micron. As such, for all particles that go through the time-of-flight mechanism and are less than 0.523 microns, they are all grouped together and are generically called particles <0.523 microns. This becomes problematic for dilute solution based aerosols or other smaller nanoparticles, as they are somewhat misrepresented. As such, particles smaller than 0.523 microns contribute to MMAD calculations as if they are 0.523 microns, which

does not seem overly significant, but when back extrapolating to initial droplets, the sizes ($D_{droplet}$) could also be falsely inflated.

Nonetheless, it appears as though most solution based MDIs result in initial droplet diameters of 10 microns, though another propellant or device configuration (different valve size, actuator geometry, etc.) could affect this number. This information is of great value, because in the studies mentioned above (Raabe, Gonda, Chan and Gonda), the principles of suspension modeling were laid out, but no information was available noting the initial droplet size in MDIs. Because they where using nebulized suspensions, and less volatile aqueous media, initial droplet size was easily acquired and calculations ensued. Given the data presented by Stein and Myrdal stating that initial droplet sizes emitted from MDIs is a property influenced by the bulk of the formulations and is relatively consistent at 10 microns, one could use this as a surrogate measurement for suspension MDIs, where the formulation composition is relatively similar with regard to drug and excipient concentrations. If anything, formulations will contain less nonvolatile mass in suspension MDI systems compared to solution based MDIs, and as such, should yield relatively similar initial droplet sizes.

Thus, using Equation 3, one could estimate the initial droplet size distribution of a suspension aerosol, by experimentally determining the particle size distribution of a comparable solution based MDI, as depicted in Figure 4.1.

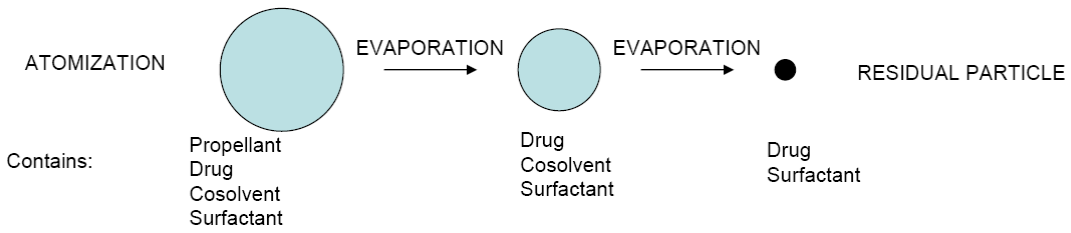


Figure 4.1. Schematic of solution based aerosol droplet.

Suspension systems are different however, as the initial droplet size does not correlate directly to the initial droplet size of the aerosol. As shown in Figure 4.2, suspension based aerosol droplets can contain no suspended particles, which would yield residual droplets containing only nonvolatile components of the formulation including impurities, dissolved drug, and more likely surfactant, as described in detail above, is used quite commonly in suspension based aerosols.

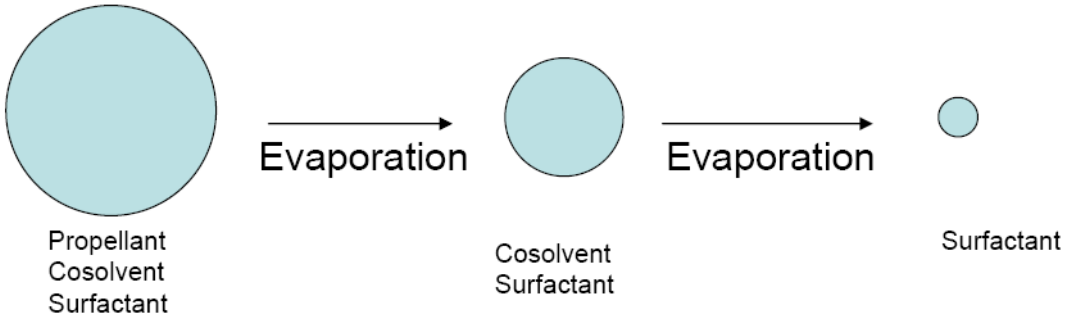
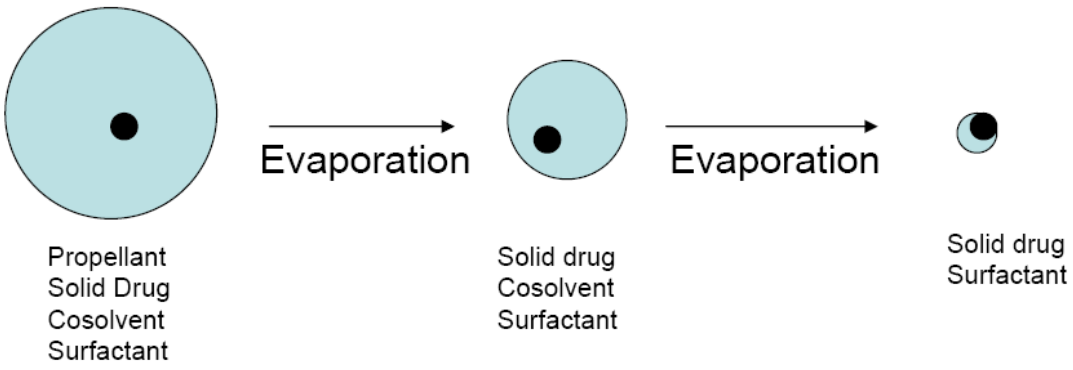


Figure 4.2. Schematic of suspension based aerosol droplet containing no solid particles.

As identified above in the workings of Raabe, depending on the concentration of the suspension, and the relative size of the droplets and the particles themselves, one or

more particles could also exist in a given droplet, as depicted in Figure 4.3. These droplets will also clearly contain the other nonvolatile material that exists in the formulation, as shown in Figure 4.2, but in this case they will coat the suspended particles and not affect their size distribution to any great degree. Data of this nature was shown in previous sections (Figure 2.5) where a combination inhaler containing BDP in solution and suspended albuterol sulfate resulted in a large shift in size related deposition for the BDP, but effectively no change at all for the suspended albuterol sulfate, as the nonvolatiles merely coat the surface of the solid particles.



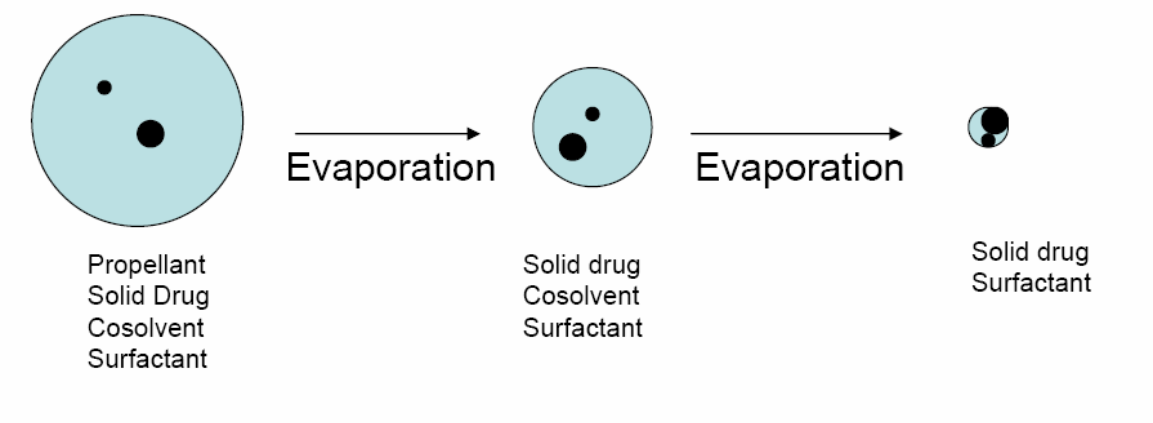


Figure 4.3. Schematic of suspension based aerosol droplet containing a single or multiple drug particles.

Before the Poisson probability function can be applied however, the composition of raw drug that will be in the system must also be characterized. In order to figure out an average number of particles per unit volume, a description of the material is necessary, because unlike the polystyrene latex spheres used in Raabe's research, most drug supplied for suspension MDI administration is polydisperse, and the primary techniques for obtaining micronized material are milling and spray-drying, both of which yield polydisperse powders (by definition, GSD >1). As both Raabe and Gonda noted, the probability of a particle existing in a given droplet (or volume) is dependent upon its size relative to that of the droplet it would occupy. Thus, for polydisperse material in polydisperse droplets, the probability is determined on a per droplet basis, and cannot be singly determined by assuming the material or the droplets to be monodisperse. Given a description of the raw drug substance however (MMAD or MMD + ρ and GSD), it would be possible to develop a series of theoretical droplets of a volume described by the

MMAD and GSD of a comparable solution MDI as described above. The initial droplet size distribution could then be calculated, and at that time, knowing the description of the raw drug substance, an average number of drug particles per unit volume could be calculated.

If this process is repeated thousands of times (entering polydisperse raw material into polydisperse droplets based on the probability as a function of their relative sizes and concentration), a distribution of aerosol droplets containing no drug particles, a single drug particle, or multiple drug particles of various sizes could be determined.

This process was completed, where using inputs described as above, and the known relationships established, a particle size distribution was able to be created using polydisperse drug being aerosolized into polydisperse droplets. A sample group can be seen in Table 4.2 which displays the random assignment of initial droplet sizes based on the description from the solution based MDI formulation. Based on the diameter (and composition) of the droplet, the volume can be calculated, by the following equation, which determines the volume of a sphere.

$$V_{droplet} = \frac{\pi \bullet D_{droplet}^{3} \bullet \rho_{droplet} \bullet C_{NV}}{6} \qquad (5)$$

Diameter of Droplet (micron)	Droplet Volume (cc)	Number of Drug Particles in Droplet	Volume of Drug Particles (cc)	Mass of Drug Particles (g)	Mass of Surfactant (g)	Mass Of Residual Particle (g)	Aerodynamic Diameter (micron)
12.49516	1.02E-09	3	4.97E-12	6.47E-12	1.18E-13	6.58E-12	2.337276
12.49406	1.02E-09	1	3.21E-13	4.17E-13	1.19E-13	5.36E-13	1.051224
5.138914	7.11E-11	1	8.14E-13	1.06E-12	8.15E-15	1.07E-12	1.323946
10.42128	5.93E-10	1	8.19E-13	1.07E-12	6.87E-14	1.13E-12	1.351049
9.028472	3.85E-10	1	2.76E-12	3.59E-12	4.44E-14	3.64E-12	1.992794
15.41741	1.92E-09	1	1.35E-12	1.75E-12	2.23E-13	1.98E-12	1.625221
3.781739	2.83E-11	1	5.63E-13	7.31E-13	3.22E-15	7.35E-13	1.169453
11.06436	7.09E-10	1	2.21E-12	2.87E-12	8.21E-14	2.95E-12	1.858723
9.143127	4E-10	4	3.38E-12	4.4E-12	4.61E-14	4.45E-12	2.013819
9.201072	4.08E-10	1	4.06E-12	5.27E-12	4.69E-14	5.32E-12	2.262592
8.582103	3.31E-10	1	5.77E-13	7.5E-13	3.84E-14	7.88E-13	1.196978
13.67964	1.34E-09	2	3.64E-12	4.73E-12	1.55E-13	4.89E-12	2.175292
3.114085	1.58E-11	1	3.28E-12	4.26E-12	1.45E-15	4.26E-12	2.10161
4.084987	3.57E-11	1	4.84E-13	6.3E-13	4.09E-15	6.34E-13	1.113257
12.01871	9.09E-10	1	2.08E-12	2.7E-12	1.05E-13	2.8E-12	1.827494
11.0723	7.11E-10	1	9.5E-13	1.24E-12	8.24E-14	1.32E-12	1.420341
4.84695	5.96E-11	1	1.73E-13	2.25E-13	6.9E-15	2.32E-13	0.795751
8.32152	3.02E-10	1	4.67E-12	6.07E-12	3.45E-14	6.1E-12	2.368336
4.873598	6.06E-11	1	2.43E-12	3.16E-12	6.75E-15	3.17E-12	1.903255
4.064648	3.52E-11	1	6.1E-14	7.93E-14	4.07E-15	8.34E-14	0.566155
12.1594	9.41E-10	1	1.95E-12	2.53E-12	1.09E-13	2.64E-12	1.790363
3.070802	1.52E-11	1	5.15E-14	6.7E-14	1.75E-15	6.87E-14	0.530789
9.630075	4.68E-10	1	3.37E-13	4.38E-13	5.42E-14	4.92E-13	1.022782
8.935156	3.74E-10	3	2.13E-12	2.77E-12	4.31E-14	2.82E-12	1.760953
9.074563	3.91E-10	1	2.89E-12	3.75E-12	4.51E-14	3.8E-12	2.022254
12.79149	1.1E-09	3	1.5E-11	1.95E-11	1.25E-13	1.97E-11	3.366031
11.86324	8.74E-10	2	1.29E-12	1.67E-12	1.01E-13	1.77E-12	1.551248
4.490816	4.74E-11	1	6.65E-13	8.65E-13	5.43E-15	8.7E-13	1.2375
21.5015	5.2E-09	5	9.26E-12	1.2E-11	6.03E-13	1.26E-11	2.802138
4.551428	4.94E-11	1	3.13E-13	4.07E-13	5.69E-15	4.13E-13	0.964789
8.258303	2.95E-10	1	1.65E-12	2.15E-12	3.4E-14	2.18E-12	1.680607
7.132786	1.9E-10	1	2.42E-13	3.14E-13	2.2E-14	3.36E-13	0.901093
4.806452	5.81E-11	1	1.27E-12	1.65E-12	6.6E-15	1.65E-12	1.532832
6.887962	1.71E-10	1	1.22E-12	1.59E-12	1.97E-14	1.61E-12	1.51817
10.02436	5.27E-10	1	6.03E-13	7.84E-13	6.12E-14	8.45E-13	1.224892
5.792133	1.02E-10	1	1.43E-12	1.86E-12	1.16E-14	1.87E-12	1.597777
9.056161	3.89E-10	1	8.46E-14	1.1E-13	4.51E-14	1.55E-13	0.695122
4.0022	3.36E-11	1	4.92E-13	6.4E-13	3.84E-15	6.44E-13	1.119314
4.673779	5.35E-11	1	6.54E-13	8.5E-13	6.13E-15	8.56E-13	1.230533
13.78656	1.37E-09	2	1.99E-12	2.58E-12	1.59E-13	2.74E-12	1.793361
5.778582	1.01E-10	1	1.28E-12	1.66E-12	1.16E-14	1.67E-12	1.538968
8.120271	2.8E-10	1	3.4E-13	4.42E-13	3.25E-14	4.74E-13	1.010272
11.82317	8.65E-10	1	8.7E-12	1.13E-11	9.94E-14	1.14E-11	2.918301
8.141237	2.83E-10	1	2.01E-12	2.62E-12	3.26E-14	2.65E-12	1.793963

3.600362	2.44E-11	1	7.9E-14	1.03E-13	2.83E-15	1.06E-13	0.612456
6.589483	1.5E-10	1	2.79E-13	3.63E-13	1.74E-14	3.81E-13	0.939017
6.174336	1.23E-10	2	2.69E-12	3.5E-12	1.4E-14	3.51E-12	1.948641
6.366984	1.35E-10	1	6.62E-13	8.61E-13	1.56E-14	8.77E-13	1.240294
4.269914	4.08E-11	2	1.44E-12	1.87E-12	4.56E-15	1.87E-12	1.58039
5.082634	6.87E-11	2	1.84E-13	2.39E-13	7.96E-15	2.47E-13	0.804212

Table 4.2. Example of theoretical droplets formed with accompanying information.

Once the volume of the droplet is determined, and knowing the number of drug particles in the suspension per unit volume, the number of particles existing in the droplet is assigned per the probability of the Poisson function. Only particle containing droplets are recorded, thus many droplets are potentially cycled to waste, as they do not contribute to the ultimate particle size distribution. The size of the drug particle is described by its distribution, and is randomly drawn upon to be "placed" in a given droplet. The mass of the residual droplet also takes into account the concentration of surfactant used.

In all cases, the same solution based formulation was used to create the theoretical initial droplet distribution. Suspension formulations do not tend to have significant amounts of nonvolatile excipients compared to solution formulations that may when cosolvents are used to solubilize the drug substance. As such, bulk properties of suspensions are not that different from one another, thus using a single solution formulation to model initial droplet size seems sufficient, especially when the $D_{droplet}$ will likely calculate to roughly 10 microns in most cases anyway. As such, a solution formulation was made and tested on the TSI system, the resulting particle size

characteristics and formulation variables were used to calculate the initial droplet size and distribution of the formulation.

This solution formulation contained a drug concentration of 0.1667% w/w (density 1.3g/ml), ethanol concentration of 8% w/w (density 0.789g/ml), surfactant concentration of 0% w/w (density 1g/ml), and HFA 134a concentration of 91.833% w/w (density 1.21g/ml). The formulation as a whole resulted with a density of 1.16g/ml. The resultant residual particle had an overall density of 1.3g/ml and experimentally resulted in a MMD of 1.01 microns, MMAD of 1.16 microns, and GSD of 1.8. This calculated to a $D_{droplet}$ of 10.16 microns with a GSD of 1.8, which again from a solution formulation remains unchanged from aerosol formation, to ultimate evaporation.

Having this information, and having characterized raw drug, theoretical modeling could ensue. Prior to correctly generating data however, the reproducibility of the calculations needed to be assessed. Because the computer is picking random droplet sizes based on the description of the solution formulation and data, and then entering drug particles based on the Poisson probability function based on the suspension formulation and raw drug substance data, one must ensure that enough theoretical droplets are created to effectively and reproducibly describe the data inputs. As such, a random formulation was created to examine the reproducibility, and is displayed in Figure 4.4.

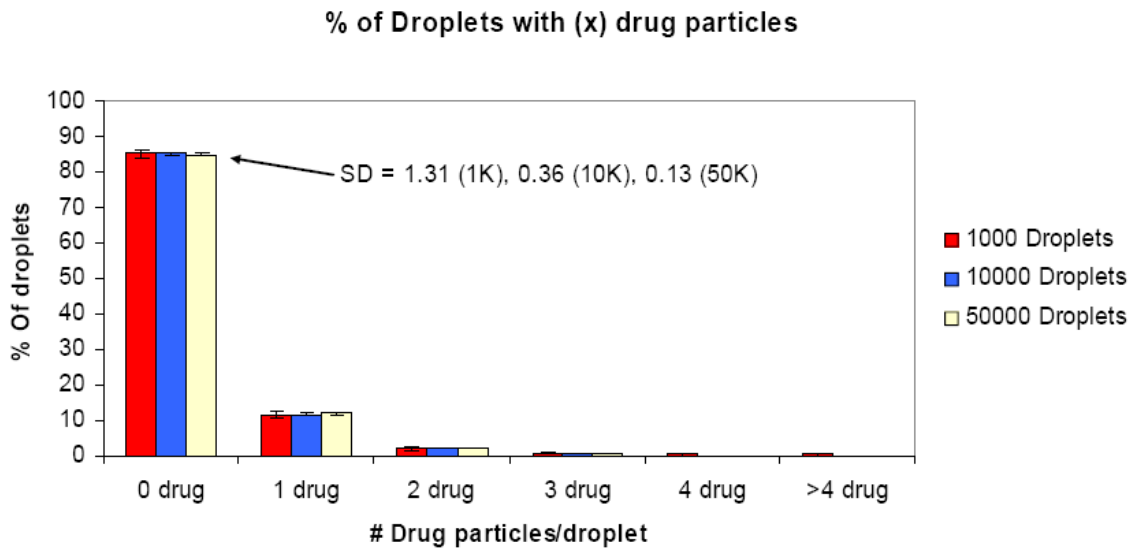


Figure 4.4. Reproducibility of theoretical calculations for random suspension formulation.

In this figure, a random formulation was input and 1,000, 10,000, and 50,000 theoretical droplets were produced, and each number was repeated five times. As expected, the more droplets that were theoretically calculated, the more tightly the standard deviations resulted, as the value of the few random small or large particles would be diminished as more and more particles of an expected size appear. Examining cumulative mass distribution graphs (not shown here), a more consistent result was seen when 50,000 droplets were theoretically created. Because these calculations are done on the computer in a looping function, it only costs extra time to complete the additional droplets, thus 50,000 particle containing droplets were created for the following experiments.

Once the reproducibility of the system was assessed, testing formulation factors and their affect on drug distribution and particle size characterization could ensue. First, a formulation was created to assess the effect of drug concentration on the distribution of drug particles throughout the droplets. The suspension formulation inputs were as follows. The drug concentrations were varied at 0.05, 0.15, and 0.45% w/w (density 1.3g/ml), 0.01% w/w surfactant (density 1.25g/ml) was used, which is a fairly standard concentration for surfactants in HFA systems. Ethanol was included in the formulation at 8% w/w (density 0.789g/ml) and was in a HFA 134a base (91.59%, density 1.21g/ml). The MMAD of the raw drug substance was 1.5 with a GSD of 1.8. This information yielded results which can be seen in Figure 4.5.

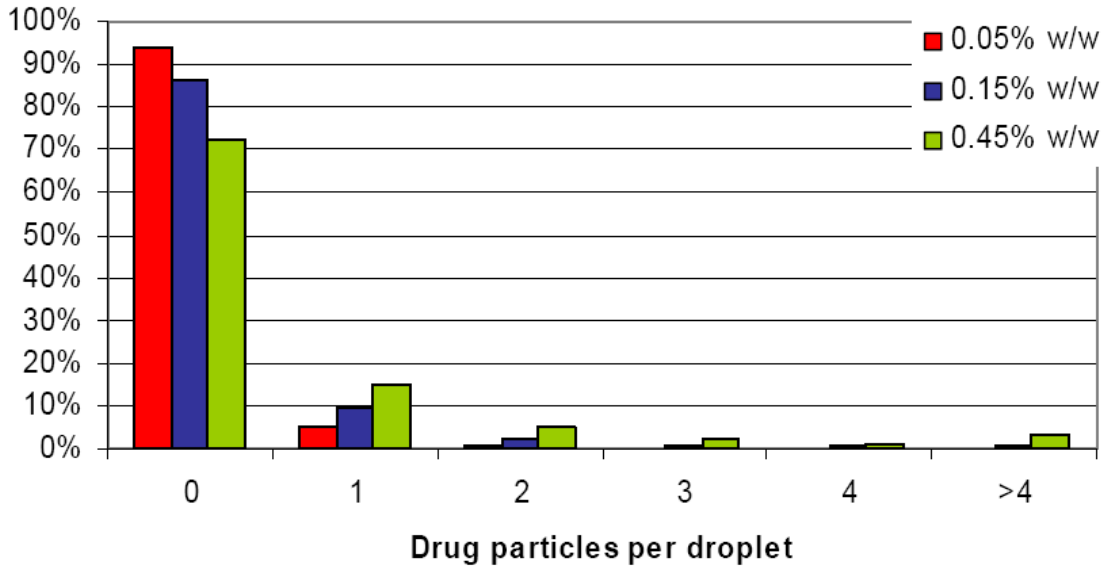


Figure 4.5. Calculation of theoretical number of drug particles per droplet.

Interestingly, at a drug concentration of 0.05%, which is a rather dilute suspension, roughly 95% of the droplets formed upon aerosolization do not have any drug particles in them. At a suspended drug concentration of 0.45% w/w, which is a fairly average concentration, and somewhat representative of many marketed products, fewer than 30% of the aerosolized droplets contain drug. In all cases, when a droplet does contain drug, more often than not it contains a single drug particle, though as expected with a more concentrated suspension or 0.15% and 0.45% w/w, respectively, the higher concentration containing formulations also have a higher incidence of multiplets as can be seen in Figure 4.6, which contains the same data as Figure 4.6, but is plotted without the drugless droplets for improved clarity.

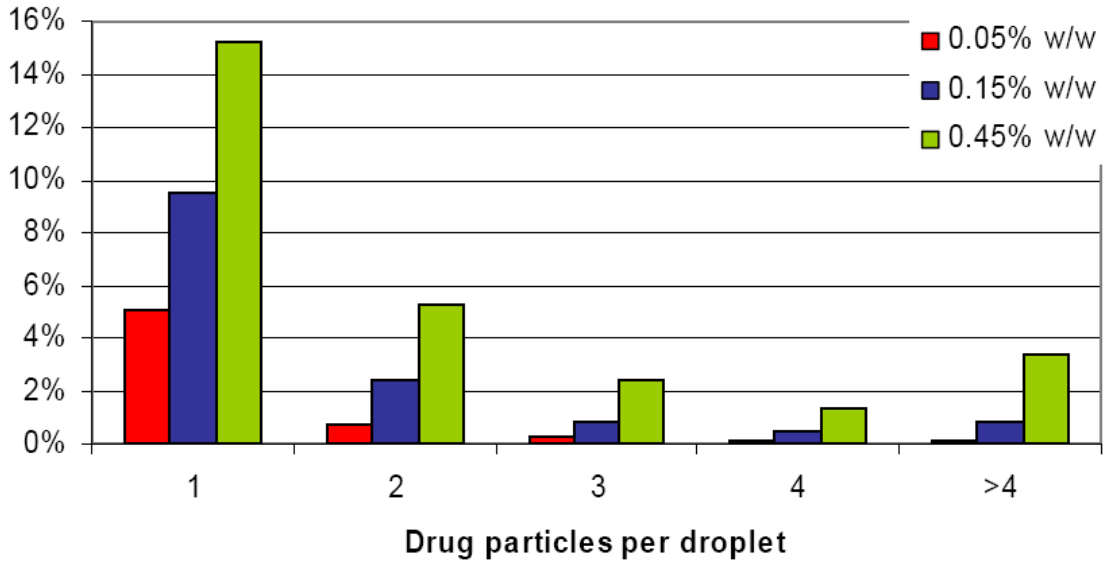


Figure 4.6. Number of drug particles per droplet for varying drug concentration formulations (excluding non-drug containing droplets).

Examining these figures, it is clearly evident that increasing drug concentration will increase the number of drug containing droplets. It also increases the number of doublets, triplets, and further multiplets. Table 4.3 displays additional data from this series.

drug conc. (% w/w)	0.05	0.15	0.45
# drug particles per 50,000 droplets	4230	12881	37771
% droplets with drug particles	6.2	13.9	27.4

Table 4.3. Number particles per droplet and percentage of drug containing droplets.

Table 4.3 displays the number of drug particles that were counted after developing 50,000 theoretical droplets. The formulation containing 0.15% w/w is three fold in drug concentration compared to that of the first column, yet yielded 3.05x the number of drug particles counted. The third formulation only contained 2.93x of the second formulation (again a 3-fold increase). Because the Poisson probability function is considered a random chance model, this explains the differences noted in the number of drug particles counted in the given 50,000 droplets, and not an exact three-fold change. Examining the total number of droplets containing any drug particles (singles or

multiplets), a linear increase does not exist. This was explained previously by Raabe (1968), and has been linked to a cube root function relating to the relative volume of the droplet compared to the number and volume of the drug particles.

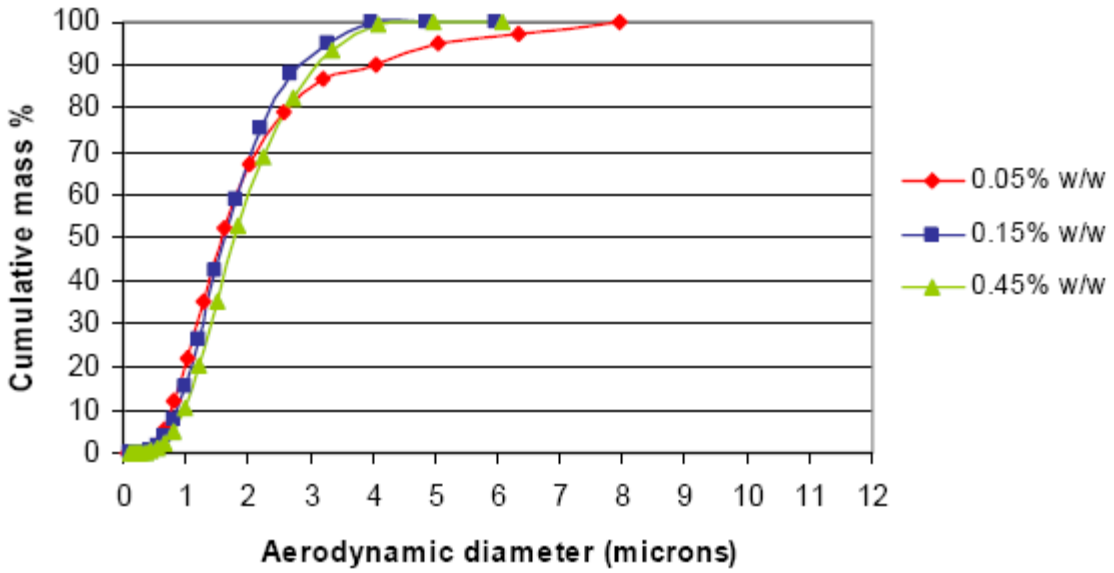


Figure 4.7. Drug concentration effect on cumulative mass size distributions.

Figure 4.7 shows the above formulations presented graphically, assessing cumulative mass as a function of aerodynamic diameter. As noted in the figure, the drug concentration itself does not account for a huge difference in the particle distribution, as was shown to be the case with solution based inhalers where a direct correlation existed between the total concentration of nonvolatile components and residual particle size. In a suspension system, as noted by Raabe, the particle size of the emitted aerosol will depend on the size of the raw material and the concentration that it is used in. At increased

concentrations, more multiple drug particle containing droplets will exist, however, even in somewhat dilute suspensions, multiplets still account for a fairly large portion of all aerosolized droplets. This again was a cube-root relationship due to the volume differences between the droplets and particles, but it is statistically difficult to achieve an unusually high concentration of single drug containing droplets without increasing the probability of multiplets.

Of note, for the formulations containing 0.05% w/w drug, because the amount of drug is more dilute in the given formulation, the cumulative mass distribution will be more susceptible to outlier particles of large size, which appears to be affecting the distribution presented above.

Examining the effect of raw drug particle size on particle size distributions emitted from a suspension formulation, particle size distributions were assessed. All suspension inputs were as above, with the raw drug material particle size (MMAD), varied. Drug concentration was held constant at 0.15% w/w, again with 0.01% w/w surfactant, and 8% w/w ethanol in a HFA 134a based system. GSD was held constant, while particle size was varied from 1-2.5 microns. Recognized that though the GSD is constant, it is a ratio of sizes, and the absolute numbers change based on the MMAD of the material. This can be seen in Figure 4.8.

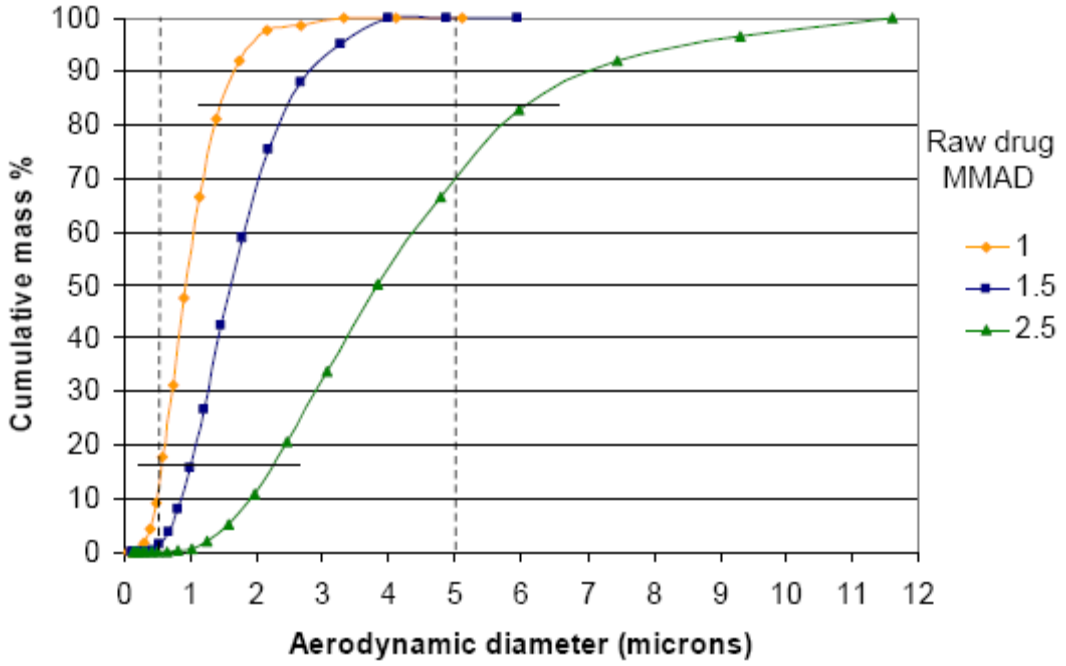


Figure 4.8. Varied raw drug particle sizes and their affect on particle size distributions emitted from a suspension MDI system.

The solid horizontal lines (not standard graduations) are at the 16th and 84th percentiles of the cumulative mass distribution. The cumulative mass distribution as a function of aerodynamic diameter (microns), shows the total amount of drug collected that is greater or less than a given size (0-100%). As mentioned above, the GSD is a relative ratio, and this can be seen graphically in the above figure. With a MMAD of 1, and a GSD of 1.8, there is a much tighter range of particle sizes compared to the material 2.5 microns in size. As expected, when graphed on a log-normal scale, the lines are parallel between these points (16th and 84th percentile), as shown in Figure 4.9.

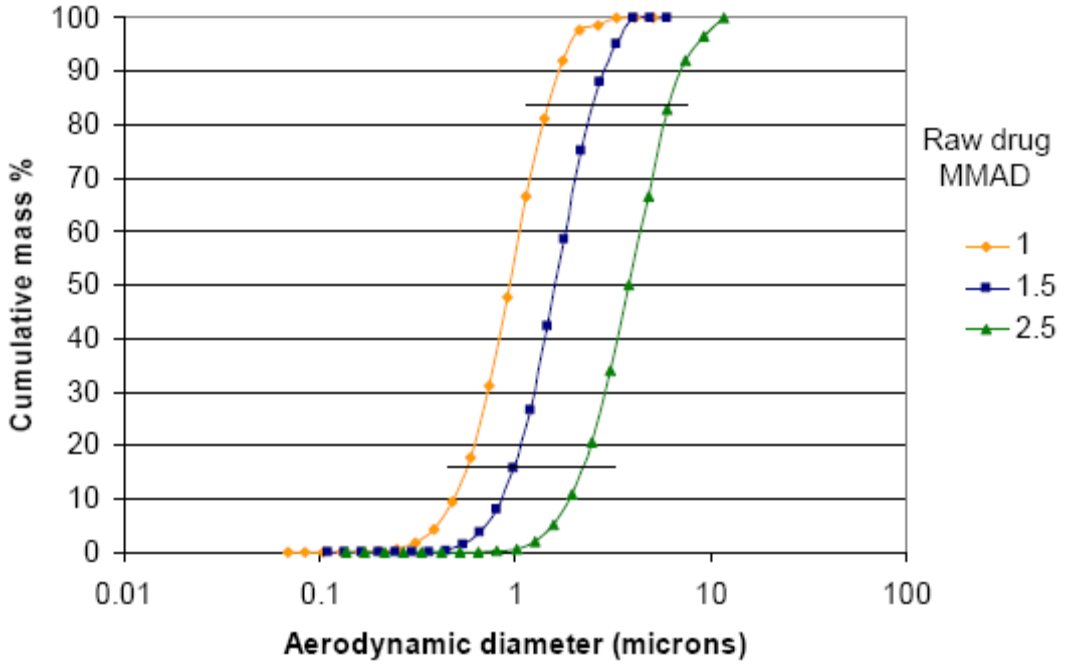


Figure 4.9. Log-normal plot displaying parallel regions of different sized material, with identical GSDs.

The two dashed vertical lines in Figure 4.8 represent the desired particle sizes that tend to result in the most efficient deposition in the lungs, 1-5 microns. Again, even though the materials have identical GSDs, the larger particle sized material results in a larger absolute range of particle sizes, which could be desirable or not depending on the purpose of the aerosol, which may result in improved whole lung coverage, including that of the upper airways, which is important in lung diseases such as asthma where smooth muscle inflammation primarily constricts the upper airways. In addition to the raw drug particle size distribution being wider for larger particle sizes, when doublets, triplets, and other multiplets exist, they can also be increasingly large, thus the graduation of slope

noted on the linear plot. When particle agglomerates form, if the particles are of a relatively small size, they will only create an agglomerate fractionally as large as a when two or more larger particles form an agglomerate. MMAD can also be determined from this plot, because it is a cumulative mass size distribution, the MMAD exists at the 50^{th} percentile. As noted, the 1 and 1.5 micron material display an MMAD close to expected, but for the 2.5 micron material, the MMAD appears to be more than 1 micron larger than expected. This indicates the presence of multiple particle containing droplets accounting for a significant portion of the cumulative mass.

Investigations examining the effect of GSD on cumulative size distributions were conducted. Again, similar theoretical suspension formulations were input and examined, as can be seen in Figure 4.10. In this scenario, the drug concentration was held constant at 0.15% w/w, the other formulation variables remained unchanged, surfactant concentration of 0.01% w/w, and ethanol concentration of 8% w/w in a HFA 134a based system. The raw drug substance was held constant at 1.5 micron, and the GSD was varied from 1 to 2.2. Again, because the GSD is a ratio between the 16^{th} and 84^{th}, with a GSD of 1, the particles will be monodisperse, though calculation errors arose, so 1.00000001 was instead used. This can be seen in Figure 4.10 with the formulation containing monodisperse material.

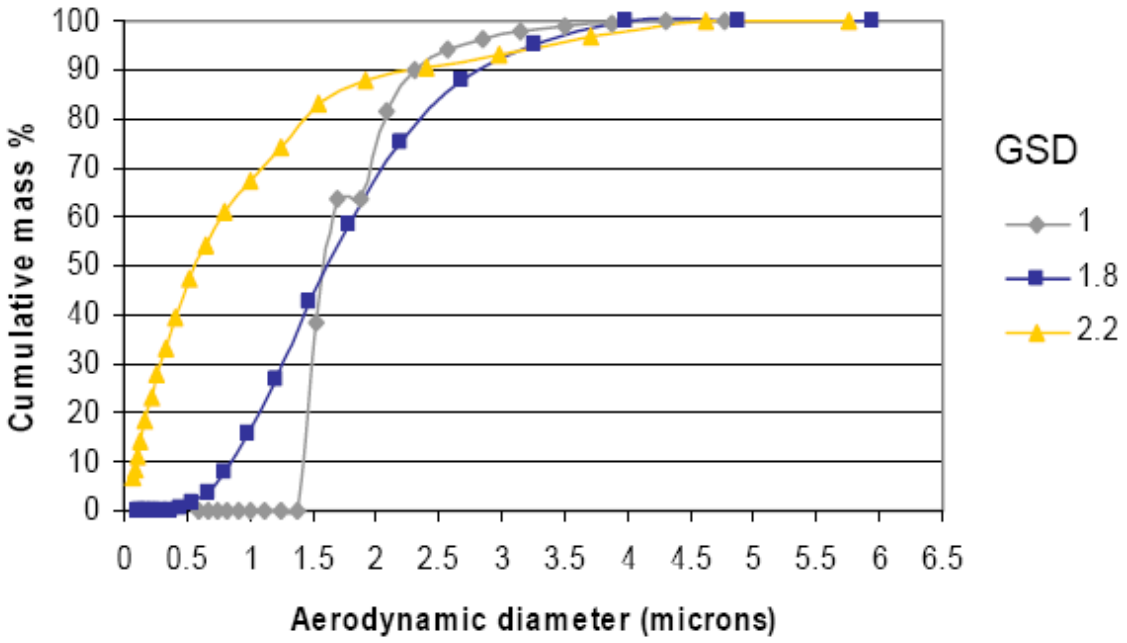


Figure 4.10. Varied GSD for suspension formulations containing 1.5 micron material at 0.15% w/w.

Again, the raw drug substance is 1.5 microns in size, and as shown by the GSD = 1 data, no droplets exist that carry drug particles less than 1.5 microns (or close due to a mathematical error). From the graph, one is able to elucidate the fact that roughly 65% of the droplets contained singlets, then a successively smaller fraction containing doublets, triplets, and so forth. Theoretically, the line describing this data should only contain completely vertical portions, where a particle(s) containing droplet contributes to the cumulative mass all of a single size, then completely horizontal portions where no particle containing droplets or a given size exist. Thus, a singlet is of one exclusive size, a double is of one exclusive aerodynamic size, and so forth. Rigorously, this line should be saw-tooth in nature, with perfectly horizontal lines between vertical lines where another

multiplet exists. This argument is somewhat academic however, because creating truly monodisperse drug material is very difficult, and is not likely to happen.

More likely to occur is the second scenario, where the drug (again 1.5 micron in size) has a GSD of 1.8, and demonstrates a smooth distribution. This is because the single drug containing droplets all carry particles of different sizes. Thus there is no clear line between the sizes of singlets and multiplets. A larger drug particle may be in a droplet with a significantly smaller drug particle, which will end up being somewhat irrelevant compared to the larger particle. However, because singlets exist small, large, and all sizes in between, and likewise doublets will be in combinations of small-small, small-medium, small-large, medium-large, large-large combinations, and larger multiplets will contain permutations of particles, the distribution is dependent upon the entire distribution of particle sizes and combinations thereof.

Examining the particle distribution of the material with a GSD of 2.2, the cumulative mass distribution is even wider, as expected. There is a larger proportion of drug mass accounted for at a smaller size (again, MMAD = 1.5, GSD = 2.2), while the cumulative mass does not reach one hundred percent until after the others, as larger singlets exist.

Suspension MDIs were formulated to experimentally back the theoretical calculations presented. Unfortunately, the availability of raw drug materials of different sizes was difficult to obtain. Upon acquisition of adequate micronized materials, sizing of the material was difficult, as the best apparent method to deliver the materials to the TSI system for sizing was through the use of an MDI. Fortunately, the through the help of a

collaborator, the materials were characterized using the TSI 3321 APS, coupled with a small scale powder disperser, an apparatus that sucks the sample powder into a chamber, and ultimately forces it out, and on to the TSI 3321 where the material is sized.

Figure 4.11 presents experimentally derived data, which presents the cumulative mass distribution, again as a function of aerodynamic particle size. Albuterol sulfate was used as the model drug, which was previously established to have a raw drug size (MMAD) of 1.53 microns and GSD of 2.11. The formulations contained 0.01%w/w the surfactant oleic acid, 8% ethanol w/w, in a base of HFA 134a. Drug concentration was varied, as was examined in the theoretical formulations above at 0.05, 0.15, and 0.45% w/w.

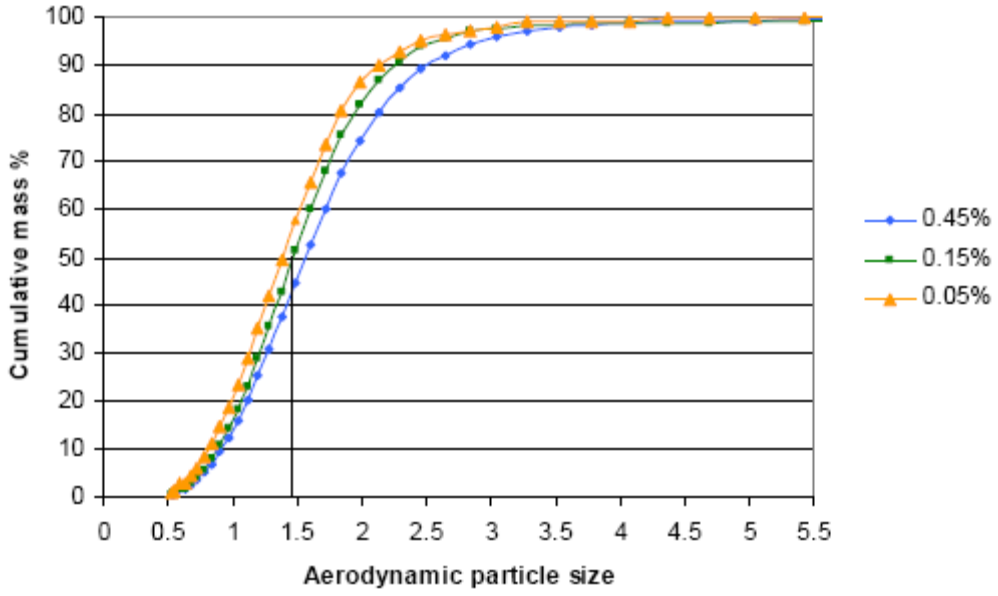


Figure 4.11. Cumulative mass distribution of albuterol sulfate formulations varying drug concentration.

As seen with the theoretical data from Figure 4.7, very little difference was noted when changing drug concentration, using the same raw drug material. Table 4.4 displays particle size data based on drug concentration. MMAD increased very modestly with drug concentration. GSD did not change significantly as expected, and per the theoretical data, a modest shift in cumulative mass distributions was noted.

Drug conc. (% w/w)	0.5	0.15	0.45
MMAD (μm)	1.37	1.44	1.55
GSD	1.49	1.45	1.56

Table 4.4. Particle size data of albuterol sulfate formulations varying drug concentration.

The average MMAD obtained from the aerosolization with the small scale powder disperser did however result larger than two of the three formulations, which indicates that full atomization was possibly not completed by the small scale powder disperser. However, the sizing was conducted on two different TSI systems, so the differences could also lie in the calibration, or operator, though the sizing mechanism in the TSI system does not generally tend to be very operator sensitive.

At this point, several potential formulation series need to be tested to more comprehensively validate the utility of the theoretical backing suggested here. Unfortunately, micronization techniques make it difficult to obtain drug with specified

particle sizes and geometric standard deviations. Nonetheless, all the data extracted from the theoretical formulations appears to follow expected outcomes, and the experimental validation to follow will likely better reveal systematic differences. Though the need exists for additional experimental validation, it appears as though this theoretical backing may sufficiently describe the principles affecting suspension MDI size distributions based on formulation variables.

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