Drug Development and Industrial Pharmacy, 33:155–162, 2007

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040600814650

informa
healthcare

Particle Size Coarsening Induced by Valve Silicone in a Metered Dose Inhaler

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ABSTRACT The objective of this study was to evaluate the effect of valve silicone on the delivered particle size distribution of a suspension metered dose inhaler (MDI). Valves were manufactured with distinct levels of silicone, which could be differentiated with Fourier transform infrared spectroscopy (FT-IR). The amount of silicone in the valve was proportional to the amount of silicone that entered the formulation and the subsequent decrease in fine particle fraction (FPF) of the active pharmaceutical ingredient (API) measured by Andersen cascade impaction. The effect of silicone content was not linear as even small amounts of silicone made a significant contribution to particle size coarsening. This coarsening was also a function of storage time and temperature. Accelerated stability conditions greatly increased coarsening kinetics as 1 month at 40°C and 75% RH induced significantly more coarsening than 12 months at room temperature. Field emission scanning electron micrograph images suggest that the primary mechanism of particle size change may be aggregation as particle clusters were seen. This study indicates that silicone can be a critical process parameter for particle size distribution of a suspension MDI product. Thus, the amount of silicone in the valves needs to be minimized and controlled.

KEYWORDS Aerosol, Metered dose inhalers, Particle size coarsening, Silicone, Pulmonary drug delivery, Stability, Formulation, Leachables, Aerodynamic particle size distribution, Andersen cascade impaction, Valve lubricant

INTRODUCTION

Suspension aerosols for the treatment of pulmonary diseases, such as asthma, contain a distribution of particle sizes of suspended active pharmaceutical ingredient (API). Generally, only particles less than approximately 5 μm reach the lungs and are effective; these are referred to as fine particles (Courrier et al., 2002). Thus, product efficacy is a function of the fine particle fraction (FPF) in a metered dose inhaler (MDI) and the lung deposition patterns of these fine particles.

Suspension formulations are subject to aggregation and crystal growth, each of which increases the median API particle size (i.e., decrease in FPF). Crystal

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growth can be caused by temperature fluctuations, which can form supersaturated solutions and cause crystallization in rod-like structures (Martin, 1993). Aggregation is thermodynamically favored as the suspension tries to approach a stable state by reducing interfacial surface area (Adamson, 1982). HFA-based (1,1,1,2,3,3,3-heptafluoropropane) systems are particularly prone to aggregation since HFA is highly self-associating thereby reducing interparticle distances which increases van der Waals forces (Tzou et al., 1997; Smyth, 2003).

Preliminary studies suggest that leachables from the can or valve could have a detrimental effect on particle size stability owing to agglomeration induced by charges on the API particle surface (Wyatt & Vincent, 1992; Bower et al., 1995), or crystal growth, both of which lead to changes in the aerodynamic particle size distribution (APSD) (Berry et al., 2003, 2004a). Silicone is a nonvolatile residue that is often used as a valve lubricant on specific valve components or the valve as a whole (Fig. 1). Different valve manufacturers not only use different types of silicone oil but siliconize different parts of the valve and use different siliconization techniques. Depending on the silicone application technique, silicone can enter the valve stem. In addition, silicone may be used on the rubber valve gaskets to aid in their movement through assembly lines. When the can is stored valve down (inverted), the liquid formulation is in constant contact with valve components and the applied silicone can leach out. Therefore, small quantities of silicone can get into the formulation and effect the APSD of suspension MDI products.

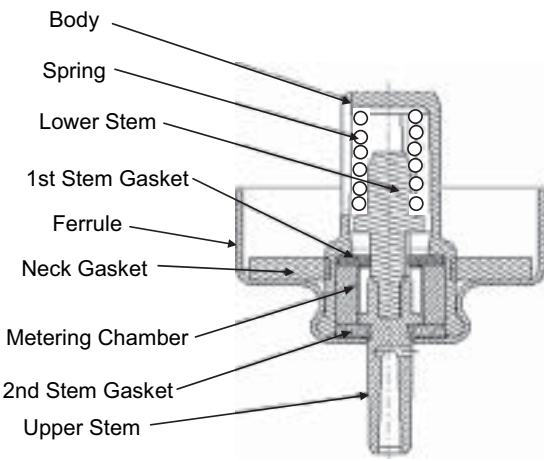

FIGURE 1 **Drawing of Valve.**

Previous work is extremely limited and has only investigated the effect of silicone on the particle size distribution of a suspension at a single time point and at a single storage condition. In one study, 2 weeks after manufacture a 13–28% difference in FPF was seen between MDIs with valves containing low (~50 μg/valve) and high (~350 μg/valve) levels of silicone (Berry et al., 2004a). This study did not investigate the APSD stability at later time points or at different storage conditions. In another study, samples were made with valves containing a single level of silicone. After 6 months at 40°C and 75% RH, cans stored inverted had ~35% more nonvolatile leachables and a ~20% reduction in fine particle dose than cans stored upright (initial samples not analyzed) (Berry et al., 2003). However, this study did not distinguish silicone from other leachables or a silicone-free control. In addition, inverted samples showed wall adhesion of API that affected the API recovery. These studies showed that increasing the amount of nonvolatile residue in the formulation decreases the fine particle dose at a single point in time but changes over time were not investigated, as there was no initial data. In addition, these preliminary studies did not explore the effect of silicone on APSD as a function of storage temperature. Furthermore, these studies did not investigate the relationship between the amount of silicone in the valve and the amount of silicone that enters the formulation.

The study presented in this paper investigated the effect of valve silicone on particle size coarsening as a function of amount of silicone in the valve, time and storage temperature. We hypothesized that increasing amounts of silicone in the valve would cause increasing particle size coarsening; and that temperature would accelerate the kinetics of coarsening. The work presented here was a controlled experiment with a single variable, the amount of silicone in the valve. Valves were manufactured identically except with three levels of silicone oil, ~50 μg, ~100 μg and ~300 μg per valve. Silicone-free valves were used as a control for other leachables from the valve elastomers. The data presented show that valves can be manufactured with controlled low levels of silicone and that Fourier transform infrared spectroscopy (FT-IR) can differentiate these levels of silicone on the valve. Two methods were evaluated for determining the amount of silicone that enters the formulation. This work also goes beyond previous studies by investigating the

effects of different levels of silicone oil in valves on particle size coarsening of MDIs as a function of time and storage temperature. Samples stored at 40°C and 75% RH were evaluated after 1, 3, and 6 months, and samples stored at room temperature (RT) were assessed after 12 months. Upright and inverted samples were evaluated after 3 months at 40°C and 75% RH. In addition, a primary mechanism is proposed for the particle size coarsening as seen by SEM.

MATERIALS AND METHODS

Determination of Silicone on the Valve Components

Silicone oil levels were measured on individual components (e.g., valve stems) and on the whole valve assembly. Silicone oil was extracted from the surface of the components with 1,1,1-trichloro-1-fluoromethane (immersion and rinsing) and the resulting solution was analyzed for silicone oil content by FT-IR using a Spectrum 1000 from Perkin Elmer.

Determination of Silicone in the MDI Formulation

The amount of silicone in the formulation of 16-month old samples was determined. A Thermo Nicolet Avatar 370 FT-IR spectrophotometer (Thermo Electron Corp., Madison, WI) was equipped with a KBr beamsplitter, HP-DTGS-KBr detector, an Ever-Glo source, a spectral range of 7800 – 375 cm^{-1}, and OMNIC software. Also used was Sigma-Plot 4.0 software program capable of performing linear regression analysis. Samples consisted of the contents from three cans to ensure silicone levels were above the lower limit of detection (LOD ~ 1 μg/g formulation or 10 μg/can) and limit of quantification (LOQ ~ 17 μg/can). Samples were tested in duplicate ($n = 2$). The samples were collected in 20-mL scintillation vials (Wheaton, VWR, Batavia, IL) and then passed through pre-packed RP-18 solid phase extraction (SPE) columns (Merck, Gibbstown, NJ). All extracts were analyzed on the FT-IR using a 0.5-mm sodium chloride (NaCl) sealed cell (Perkin Elmer Instruments, Boston, MA). The solvent used was HPLC grade (EMD, Gibbstown, NJ). Other chemicals and reagents used were USP grade (AAPER Alcohol, Shelbyville, KY), ACS-reagent grade (Aldrich, St. Louis, MO).

Effect of Silicone Level on FPF Study

For this study, Valois DF32 RCU valves containing nonextracted polychloroprene elastomers and acetal resin plastic parts were manufactured by Valois using three levels of silicone oil (polydimethylsiloxane; 300 cps; Silbione V300 from Rhodia), see Table 1. This silicone oil was selected primarily on its viscosity (300 cps) as it needs to remain fluid enough so that it can be deposited on the valves during assembly yet viscous enough to be retained on the valve mechanism during filling and use. The silicone oil was applied to the valve stem and some silicone may have entered the inner diameter of the valve stem. The three valve prototypes used in this study were: no silicone/valve, ~50 μg silicone/valve, and ~300 μg silicone/valve.

The MDI batch used in this study contained 100 μg/actuation of a micronized corticosteroid API suspended in a HFA-227 propellant (Solvay Fluor, Hannover, Germany) along with a small amount of ethanol (<5% w/w) and oleic acid (<0.2% w/w). A 10-mL fluorinated ethylene propylene copolymer (FEP)-coated can was used with a 10-g fill. This commercial-scale MDI batch was made by a process in which the valve was crimped onto the can and then the formulation was pressure-filled through the valve stem.

TABLE 1 Valve Silicone Levels

Applied Silicone (μg)	Measured Silicone Per Valve Before Valve Assembly[1]				Measured Silicone Per Valve After Valve Assembly[3]			
	Mean (μg)	Max (μg)	Min (μg)	SD (μg)	Mean (μg)	Max (μg)	Min (μg)	SD (μg)
0	<LOD[2]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
50	55[2]	107	31	15	<LOQ	<LOQ	<LOQ	<LOQ
100	89	100	80	5	72	87	51	14
300	286	347	237	20	219	260	157	35

[1]$n = 60$, [2]$n = 30$, [3]$n = 20$, LOQ = 40μg, LOD = 11μG.

Andersen Cascade Impaction

Inverted and upright samples were stressed under accelerated ICH stability conditions (40°C and 75% RH) for up to 6 months. Samples were also stored inverted at room temperature for 12 months. The resulting APSD was then evaluated using a Mark II Andersen cascade impaction (1 AFCM nonviable ambient particle sizing sampler, Graseby-Andersen, Smyrna, GA) using a 1-L glass entry port and high-performance liquid chromatography (HPLC) (Milosovich, 1992; Stein, 1999; US Pharmacopeia, 2003).

The MDIs were equilibrated in a 25°C water bath and then shaken and primed four times, with one minute between each prime to reduce cooling effects. The room was controlled at 21 ± 1°C and 30 ± 5% RH (generally 30 ± 1% RH). After priming, an initial weight was taken and then two shots were sprayed into the impactor and the final can weight was then taken. The drug was then allowed to follow the 28.3 L/min air stream through the impactor. The entry port, casings and plates were rinsed with HPLC-grade sample solvent and analyzed using HPLC (Waters, Model 2690, Milford, MA) with an ultraviolet detector. Samples were tested in triplicate ($n = 3$). The FPF (<4.7 μm) was calculated by dividing the sum of plates 3, 4, 5, 6, 7, and filter by the sum of the entry port, casings, and plates 0, 1, 2, 3, 4, 5, 6, 7, and filter.

Scanning Electron Microscopy

All samples were consistently prepared for field emission scanning electron microscopy (FESEM) observation by spraying two actuations of MDI formulation onto a vitreous carbon planchet (Ted Pella, Inc., Redding, CA) in a chemical fume hood. The samples were blinded so as to not bias the SEM analyst. Secondary electron images of the uncoated sample were obtained empirically at various magnifications with a JEOL model 6330F FESEM (JEOL, Peabody, MA). Over 25 fields of view per sample were randomly selected and qualitatively assessed in real time for aggregation and representative fields were captured. A slow capture scan was used with a 1–2 kV accelerating voltage, 12 μA emission current, a 7.0 spot size, a 5–8 mm working distance, and a slow capture scan.

X-ray microanalysis spectra were obtained using the EDAX X-ray analyzer (EDAX Inc., Mahwah, NJ). The detector for this system was a super-ultra thin window. The optimized take-off angle, set by the vendor, was 41.42. Elemental spectra were collected with the following parameters: 500–2500 counts per second, dead time of 20–40%, and 100 μs amp time. Emitted X-ray peaks were identified from the acquired spectrum using the EDAX peak identification chart and identified elements were verified using halographic peak deconvolution. Samples were evaluated in 10–20 areas/sample for silicone.

RESULTS AND DISCUSSION

Determination of Silicone on the Valve Components

The amount of silicone per valve was determined in two ways; the individual stems were tested, and assembled valves were tested (Table 1). Valves targeted for 50, 100, and 300 μg/valve had a mean of 55, 89, and 286 μg of silicone per valve before assembly, respectively. These results show that the valve manufacturer was successful in producing valves with controlled levels of silicone. This ability to select the level of valve silicone gives the formulator more flexibility. The FT-IR method was able to differentiate the low levels of silicone extracted from the valves. Higher levels of silicone were detected (with lower standard deviations) by testing the components before assembly rather than after assembly. However, testing the valves after assembly may be more representative of the amount of silicone that can leach out when in contact with the formulation. Furthermore, this FT-IR method allows for quality control of the valves.

Amount of Silicone in the MDI Formulation

The amount of silicone that gets into the formulation is ultimately what is important for product stability. Thus, aged samples (16 months) that were inverted at standard accelerated ICH conditions (40°C and 75% RH) and then at RT were analyzed by FT-IR for silicone content in duplicate ($n = 2$). Three cans were tested per sample to ensure that amounts of silicone would be above the limit of quantification. The amount of silicone that was in the formulation was proportional to the amount that was originally in the valve (Fig. 2). Valves that contained 55, 89, and 286 μg

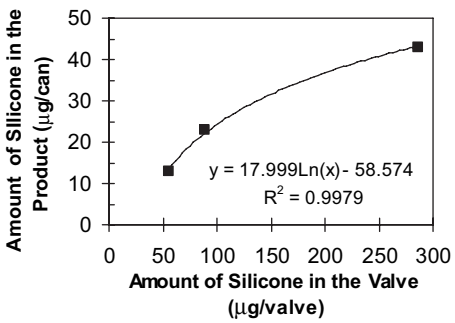

FIGURE 2 **Relationship of Valve Silicone Levels to Silicone in the Formulation.**

of silicone per valve stem before assembly resulted in 13, 23, and 43 µg of silicone in the formulation per can. Thus, only a fraction of the silicone in the valve gets into the formulation. Silicone-free valves were also tested as a control and did not have any detectable levels of silicone in the formulation. The relationship between silicone in the valve and the amount of silicone in the formulation was logarithmic with a 99.8% correlation for this system. Having established this relationship, we can now meaningfully investigate the correlation between valve silicone levels and changes in FPF.

Samples Stored at 40°C and 75% RH for up to 6 Months

Samples made with 50 µg of silicone/valve, 300 µg of silicone/valve or no silicone controls were placed on accelerated ICH stability for up to 6 months inverted and up to 3 months upright. Figure 3 shows the cascade results for samples that were inverted at 40°C and 75% RH for 1, 3, and 6 months (error bars represent the standard deviation of $n = 3$). The average

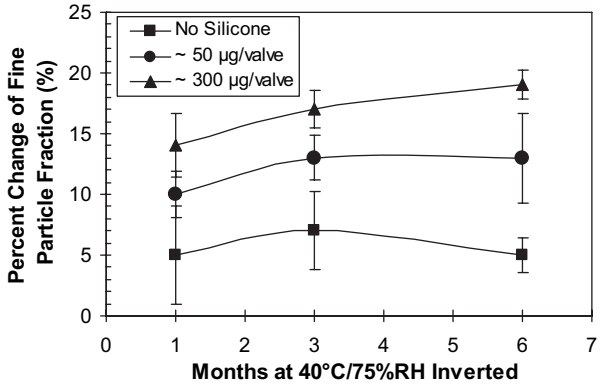

FIGURE 3 **Effect of Silicone on the Percent Change of Fine Particles ($n = 3$).**

recovery was 97 ± 5% of label claim, and the average percentages recovered on the throat and casings were 9 ± 2% and 4.5 ± 0.5%, respectively. The results indicate that there was a relationship between the amount of silicone on the valve and the FPF. Increasing amounts of silicone in the valve resulted in decreasing FPF. The silicone-free configuration changed approximately 5–7% over 6 months. This 2% difference in FPF is within the variability of the cascade method. Since this sample was silicone-free, this change in FPF was due to other factors, such as other leachables or physical properties of the suspended API (Berry et al., 2004b), with the most likely factor being Ostwald ripening. This silicone-free configuration serves as a control and baseline for the siliconized valves. Therefore, changes in FPF above approximately 7% are above baseline and can be attributed to the amount of silicone in the valve.

After 6 months at 40°C and 75% RH, samples that contained 50 µg of silicone/valve decreased as much as 13% in FPF (MMAD changed from 2.54 to 2.77 µm), and those that contained ~300 µg/valve had a 19% decrease in FPF (MMAD changed from 2.53 to 3.01 µm). Thus, the degree of change in the particle size distribution as seen in the decrease of FPF is a function of the amount of silicone used in the valves.

The data also showed that this decrease in FPF was exacerbated with time. Samples with 50 and 300 µg of silicone per valve had a 10% and 14% decrease in FPF after 1 month at 40°C and 75% RH, respectively. After 3 months, a 13% and 17% decrease was seen for these samples, respectively. Thus, most of the change occurred in the first month with a small change occurring between 1 and 3 months, after which the coarsening effect seems to have reached a plateau. This plateau may be useful to the formulator in quickly determining maximum coarsening effects using accelerated ICH conditions.

Statistical analysis was performed on the FPF for samples with 0, 50, and 300 µg of silicone per valve at the initial time point. A statistical difference was seen between the valve configurations (i.e., silicone content) initially with the *p*-value being 0.0045. Thus, silicone can affect the particle size distribution from the start. Since unique cans were tested at each time point, only the averages of three cans could be compared. This average data did not have enough statistical power to perform a statistical analysis between silicone levels within a time point, although the decrease in

FPF with increasing silicone level is clear. Looking at the data as whole, an ANOVA was run to examine the effects of silicone, time, and silicone-by-time interaction on the FPF change from baseline. Silicone and time were statistically significant ($p < 0.05$), while the interaction of silicone level with time was marginally significant ($p = 0.08$). The changes from baseline in FPF were statistically different from each other at all levels of silicone ($p < 0.01$). In addition, there was a rank order correlation between silicone and decreases in FPF.

Samples that were stored upright (valve up), showed a smaller change in the FPF than inverted samples (Table 2, $n = 3$). The average recovery was 97 ± 3% of label claim, and the average percentages recovered on the throat and casings were 10 ± 2% and 4.4 ± 0.6%, respectively. Even the small change in FPF was proportional to the amount of silicone in the valve. Since these upright samples did not have the formulation contacting the valves, only minimal leachables from the valve (including silicone) are likely to have migrated into the formulation over time. However, samples with 50 and 300 μg/valve did show a 5% and 9% change in FPF, which was more of a change in FPF than the 3% change seen with the silicone-free control. Since the 300 μg silicone/valve samples did show a noticeable change from 50 μg silicone/valve samples, the amount of silicone in the valve seems to be a coarsening factor in upright samples as well. This is likely due to the fact that silicone enters the valve stem during the silicone application process used in this study and is then flushed into the formulation during filling through the valve. Thus, when silicone is present inside the valve stem, the amount of silicone on the valve affects the particle size distribution of the formulation regardless of the can orientation if the formulation is filled through the valve. If the stem is not siliconized (or siliconized in a way that silicone is only on the outside of the stem) and/or the formulation is not filled through the valve, cans stored upright would not be expected to show this clear dependence of particle size coarsening on valve silicone level.

Inverted Samples Stored at Room Temperature for 12 Months

Table 3 shows the cascade results after 12 months inverted at RT ($n = 3$). The average recovery was 97 ± 7% of label claim, and the average percentages recovered on the throat and casings were 10 ± 0.8% and 4.6 ± 0.2%, respectively. The silicone-free control had a 7% decrease in FPF. Samples with 50 μg of silicone per valve had an 8% decrease in FPF, which is not significantly different than the silicone-free control. Samples which had 300 μg of silicone per valve, had an 11% decrease in FPF after 12 months at RT. This is markedly less change than seen after 6 months at 40°C and 75% RH (19% decrease). Samples that were at RT for twice as long as those at 40°C and 75% RH showed only about half the amount of coarsening. Therefore, increased temperature and humidity changed the reaction kinetics of coarsening and therefore 6 months at 40°C and 75% RH is a particularly harsh condition for this system and does not mimic extended times at RT. Thus, stability of this type of product may have to be performed at 30°C and 60% RH or room temperature rather than at 40°C and 75% RH, which does not seem to mimic long-term RT conditions. Even at room temperature, the coarsening was a function of silicone level. However, at room temperature, higher levels of silicone (~300 μg/valve) were needed to induce a change in the particle size distribution more than that seen in the control sample.

Scanning Electron Microscopy and Energy Dispersive Spectroscopy

The SEM analyst assessed blinded samples for aggregation by viewing more than 25 fields for each

TABLE 2 **Change in FPF of Upright Samples Over 3 Months at 40°C and 75% RH ($n = 3$)**

Silicone (μg)	Initial %FP	3 Months	
		%FP	%Δ
0	75.2 ± 0.3	73 ± 6*	–3%
50	76.8 ± 0.7	73 ± 2	–5%
300	78.2 ± 0.8	71 ± 1*	–9%

*$n = 2$.

TABLE 3 **Cascade Results After 12 Months Inverted at Room Temperature ($n = 3$)**

Silicone (μg)	Initial %FP	12 Months at RT	
		%FP	%Δ
0	75.2 ± 0.3	70 ± 4	–7%
50	76.8 ± 0.7	71 ± 1*	–8%
300	78.2 ± 0.8	69 ± 2	–11%

*$n = 2$.

sample, including control samples. A clear difference in aggregation could be seen between the control samples and those containing silicone; thus, the results were not due to inherent cluster formation owing to propellant vaporization. In fact, a direct correlation was seen between the amount of silicone in the sample and the amount of aggregation. SEMs of emitted API particles from silicone-free and 300 μg of silicone/valve samples after 3 months at 40°C and 75% RH +7 months at RT are shown in Fig. 4. No needle or rod-like crystal structures were seen that would indicate crystal growth. The high silicone sample appears to have more particle clusters than the silicone-free control sample, which suggests aggregation or growth on existing particles. Excipients and leachables can interact with the suspended API particles and change their surface properties (Dalby & Byron, 1988). In particular, silicone is insoluble in HFA-227 and may coat the API particles causing them to become more adhesive, thereby increasing particle-particle interactions that lead to aggregation. When silicone is spiked into samples an increase in the settling rate is seen (Smyth, 2003; unpublished data), which is characteristic of flocculation or aggregation. It appears that the primary mechanism for particle size coarsening is aggregation but a secondary mechanism of growth of existing particles (e.g., Ostwald ripening) could also be occurring to a lesser extent. Further studies with more sensitive methods are needed to understand the coarsening process.

No silicone associated with the API was detected by EDAX for any of the samples. Thus, silicone was in the formulation in very low levels and only in discrete areas (as EDAX does a spot check). Thus, the FT-IR method was more sensitive than EDAX.

CONCLUSIONS

This study showed that valves can be manufactured with controlled levels of silicone, from 50 – 300 μg/valve, and these different levels of silicone were distinguishable with FT-IR, which can be used for quality control. In addition, a logarithmic correlation was seen between the amount of silicone in the valve and the amount that enters the formulation. Cascade impaction data showed that the coarsening effect was

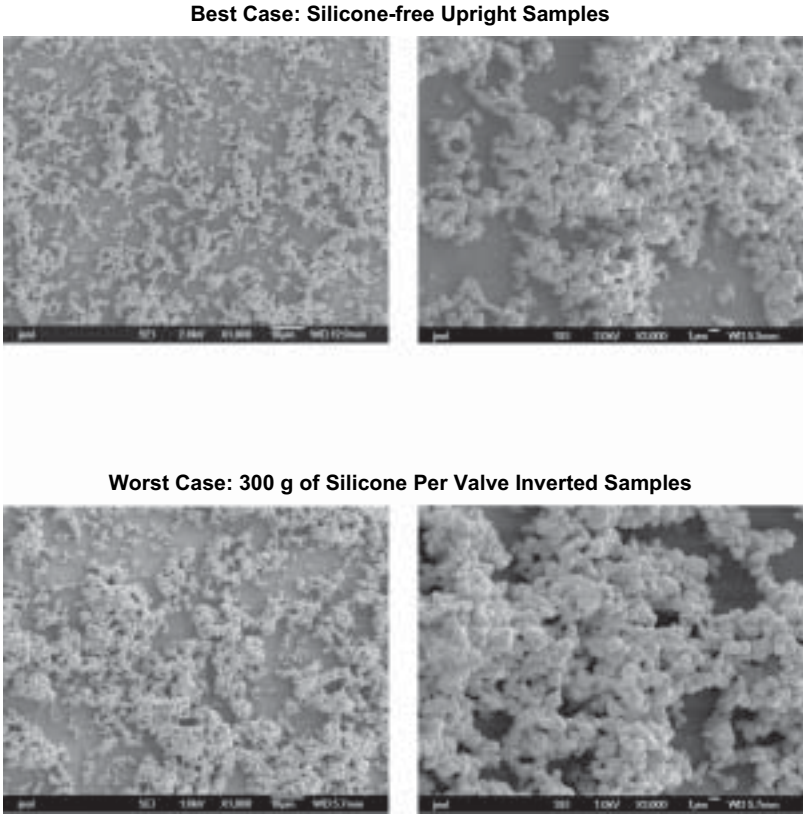

FIGURE 4 SEMs Showing Various Samples.

dependant on the amount of silicone in the valve, storage time, temperature and orientation. This study indicated that the delivery characteristics of a suspension MDI product could be affected by even small amounts of silicone in the valves. Thus, the amount of silicone in the valves needs to be tightly controlled. Accelerated stability conditions greatly increased the coarsening kinetics; and 40°C and 75% RH is an exceptionally harsh condition for this MDI system and does not mimic long-term stability at room temperature, which showed significantly less coarsening. At room temperature, larger amounts of silicone were needed to stimulate coarsening beyond the control sample. The largest influence of time was seen during the first month. Cans that were stored inverted, and therefore with the formulation in constant contact with the valve, had a more significant change in particle size distribution than upright cans. The primary coarsening mechanism appears to be aggregation as particle clusters were seen by SEM compared to control samples.

ACKNOWLEDGMENTS

The authors express their thanks to the Schering-Plough colleagues in Kenilworth and Heist for the manufacture of the MDI batches, Monarch Analytical Laboratories for determination of the amount of silicone in the formulation and to Michael Lusty and SI Associates including Tom Jones, Colin Andrews, and Dale Roberts.

REFERENCES

Adamson, A. W. (1982). *Physical Chemistry of Surfaces*, Fourth Edition. John Wiley & Sons, New York.

Berry, J., Kline, L. C., Hart, J. L., & Sequeira, J. (2003). Influence of the storage orientation on the aerodynamic particle size of a suspension metered dose inhaler containing propellant HFA-227. *Drug Dev. Ind. Pharm.*, *29*, 631–639.

Berry, J., Kline, L. C., Naini, V., Chaudhry, S., Hart, J. L., & Sequeira, J. (2004a). Influence of the valve lubricant on the aerodynamic particle size of a suspension metered dose inhaler. *Drug Dev. Ind. Pharm.*, *30*, 267–275.

Berry, J., Kline, L. C., Sherwood, J. K., Chaudhry, S., Obenauer-Kutner, L., Hart, J. L.. & Sequeira, J. (2004b). Influence of the size of micronized active pharmaceutical ingredient on the aerodynamic particle size and stability of a suspension metered dose inhaler. *Drug Dev. Ind. Pharm.*, *30*, 705–714.

Bower, C., Washington, C., & Purewal, T. S. (1995). Fractal morphology of drug aggregates in aerosol propellant suspensions. *Int. J. Pharm.*, *118*, 229–235.

Courrier, H. M., Butz, N., & Vandamme, T.F. (2002). Pulmonary drug delivery systems: recent developments and prospects. *Crit. Rev. Ther. Drug Carrier Syst.*,*19*, 425–498.

Dalby, R. N., & Byron, P. R. (1988). Comparison of output particle size distributions from pressurized aerosols formulated as solutions or suspensions. *Pharm. Res.*, *5*, 36–39.

Martin, A. (1993). *Physical Pharmacy*, Fourth Edition. Lippincott Williams & Wilkins, Philadelphia.

Milosovich, S. M. (1992). Particle-size determination via cascade impaction. *Pharm. Technol., 16*, 82–86.

Smyth, H. D. C. (2003). The influence of formulation variables on the performance of the alternative propellant-driven metered dose inhalers. *Adv. Drug Deliv. Rev.*, *55*, 807–828.

Stein, S. W. (1999). Size distribution measurements of metered dose inhalers using andersen mark II cascade impactors. *Int. J. Pharm.*,*186*, 43–52.

Tzou, T. Z., Pachuta, R. R., Coy, R. B., & Schultz, R. K. (1997). Drug form selection in albuterol-containing metered-dose inhaler formulations and its impact on chemical and physical stability. *J. Pharm. Sci*, *86*, 1352–1357.

United States Pharmacopeia (2003). *General Chapter* 601, 2105–2123.

Wyatt, D. A., & Vincent, B. (1992). Electrical effects in non-aqueous systems. *J. Biopharm Sci.*, *3*, 27–31.