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Metered dose inhalers III: metaproterenol sulphate; particle size distribution and dose uniformity

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Abstract

Three American products and one Canadian product were examined for content uniformity and particle size distribution. The results showed that not all products performed equally well. Some of the products exhibited high sprays early in the canister lifetime and all products demonstrated loss of prime. The particle size distributions were determined using the Andersen cascade impactor (USP Induction Port) and the fine particle fraction was determined using the twin impinger. The results showed that three of the four products had similar particle size distribution profiles. Both the Andersen cascade impactor and the twin impinger yielded the same trends in the amount of drug substance delivered to the fine particle fraction. © 1997 Published by Elsevier Science B.V.

Keywords: Metaproterenol sulphate; Metered-dose inhaler; Content uniformity; Particle size distribution

1. Introduction

The usual method of assessing the drug particle size distribution of metered dose inhalers (MDIs) has been to determine the mass median aerodynamic diameter (MMAD), geometric standard deviation (G.S.D.) and fine particle dose or fine particle fraction [1,2]. Of the instruments commonly used, only multi-stage impactors such as the Andersen cascade impactor (ACI) and Marple-Miller are capable of yielding data on the first two parameters. Other devices such as the twin impinger (TI) are limited to information about the fine particle fraction or dose only.

The USP XXIII [1] currently describes particle size test methods for aerosols, General Chapter ⟨601⟩ and content uniformity requirements, General Chapter ⟨905⟩. Three devices are recognized for particle size testing. However, the Advisory Panel on Aerosols [3] recently recommended that only the ACI be accepted for official use in the USP due to the contention that simpler devices may not be appropriate for conclusive product testing. The removal of the TI as an official test device may lead to the perception that it is unsuit-

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able for finished product testing. This would be unfortunate as its operation is less resource intensive than the ACI. The proposed adoption by the USP of the ACI has not as yet led to the development of specifications, based on testing with this device, for particle size distribution requirements for MDIs. Conversely, the BP [4], in its initial monograph applicable to pressurized inhalers, has developed minimum delivery requirements for the deposition of the emitted dose of MDIs using the TI.

The content uniformity of the delivered dose [5–8] of MDIs has also been shown to be a potential problem. In particular, abnormally high drug content of initial primed sprays and a generally higher drug content of primed sprays in comparison to unprimed sprays have often been observed. The current proposed requirement from the USP Advisory Panel on Aerosols [3] states that the drug content for at least 9 of 10 initial dosage units collected must be between 75.0 and 125.0% of the label claim, and the drug content of no more than one dosage unit must be outside 65.0 and 135.0% of label claim. (The proposed amendment to ⟨601⟩ refers to 'dose'. The proposed amendment to ⟨905⟩ refers to 'dosage unit'. From the description of the experimental method in ⟨601⟩ the 'dosage unit' variation limits specified in ⟨905⟩ refer to the variation of the sum of the number of recommended 'puffs'.) The EP [9] has developed a similar set of requirements but the limits of variation are based not on the label claim but the mean of the determined delivered dose.

We report here the results of comparative testing of four metaproterenol MDI products for both content uniformity and particle size. The products used in this study were generously provided by manufacturers in both Canada and the USA. Three of the four products were commercially available. The other is anomalous and should not be construed as being representative of commercially available products. Nor should it be considered to reflect the characteristics of products deemed acceptable from either a regulatory or clinical perspective. This product was included to widen the range of variability of the test samples. The particle size analysis was performed using both the ACI and the TI. Electron microscopy was used to determine whether the various products were significantly different in particle composition.

2. Materials and methods

2.1. Test samples

Two lots of one Canadian product were obtained locally (product F). The american products were procured by a third party (products G[1], H and J). Products were coded by a capital letter to represent the manufacturer, the first arabic number represents the lot and the second represents the canister number. The American products were labeled to deliver 650 μg/puff, the Canadian product 750 μg/puff. The American patient insert recommended 2–3 inhalations per dose, every 3 to 4 h, not to exceed 12 inhalations in 24 h. The insert in the Canadian package stated 1–2 inhalations over the same dosing interval, not to exceed 12 inhalations in 24 h. The same canisters were used for both particle size determination and uniformity of dose. All products were within their expiry periods when analyzed. Product F was chosen as a standard product for parameter calculations and discussion purposes.

2.2. Sample collection—spray content uniformity/particle size

A modified version of the single spray content uniformity experimental design [5] was used to evaluate the following parameters: drug content at the beginning of the canister lifetime; drug content of primed sprays, (those collected immediately after discharging a spray to waste); and drug content of unprimed sprays, (those collected after an appropriate period of rest). The sample handling procedure has been described elsewhere [10].

Four sets of two primed sprays into the ACI were collected daily for seven days in a randomized cross-over design. The same cross-over design

[1] anomalous product

was used for sampling on the TI except the number of collection days was decreased to two days. The procedure for collecting two primed sprays on the ACI is described in a previous publication [10]. Collection of sprays in the TI was as follows: the upper and lower impingement chambers were filled with 7 and 30 ml of methanol respectively and the flow rate adjusted to 60 l/min. The procedure for sample handling was the same as for the ACI. Stage 1 (upper) was washed into a 25 ml volumetric flask and stage 2 (lower, representing the particles < 6.4 μm) was washed into a 50 ml volumetric flask. Values in the tables have been normalized to single sprays.

2.3. HPLC conditions

The method used for the metaproterenol work was the salbutamol method developed in our laboratory [11]. Preliminary work showed that metaproterenol would chromatograph well on the salbutamol system since the two drugs are similar in structure. The system suitability criteria were those in the salbutamol method. No metaproterenol related compounds were available to demonstrate resolution from the drug peak.

2.4. Equipment

The HPLC system was equipped with an auto sampler (Varian 9095) with a 20 μl loop (Valco Instruments), a variable wavelength detector set at 214 nm (Varian 9050 UV-VIS detector) and a 3 μm hexyl bonded phase column (CSC-Spherisorb, 150×4.6 mm). The system was operated at ambient temperature with a mobile phase flow rate of 1 ml/min (Varian 9010). The mobile phase consisted of acetonitrile–water–*o*-phosphoric acid (60:40:0.1, v/v/v) passed through a 0.45 μm filter.

2.5. Solutions

Methanol was used to prepare all solutions. Resolution solution: 1 μg/ml each of salbutamol (Cipla, India (L)D-71049) and [1-(4-hydroxy-3-methylphenyl)-2-(*t*-butylamino)ethanol] (Huhtamaki Oy, Finland). Standard solutions: a 5 point calibration curve consisting of solutions with concentrations ranging from 1 to 20 μg/ml of metaproterenol sulphate for content uniformity; a 7 point calibration curve ranging from 0.05 to 5 μg/ml for the ACI work; and a five point calibration curve from 10 to 60 μg/ml for the TI work. The monitoring standard for content uniformity was 5 μg/ml and for particle size measurement, 1 μg/ml. Test solution: collect MDI spray as outlined in Section 2.2.

2.6. System suitability

The resolution solution (20 μl) was injected. Column efficiency, calculated using the salbutamol peak, was greater than 30 000 plates/m; the resolution was greater than 6; and the tailing factor less than 1.5. The retention times of the two peaks were about 6 (salbutamol) and 10 min. Six replicate injections of the monitoring standard, 1 or 5 μg/ml solution, yielded a coefficient of variation of less than 3%. The retention time of metaproterenol was about 6 min.

2.7. Procedure

Each of the solutions in the calibration curve and the test solution, 20 μl (content uniformity)/ 100 μl (particle size), was injected and the chromatogram recorded for 8 min. The amount, in μg, of metaproterenol sulphate in the test solution was calculated using the equation of the line derived from a weighted linear regression analysis of the calibration curve.

2.8. Calculations

The LIFEREG™ procedure from SAS® [12] was used to estimate the MMAD and GSD as discussed in [13]. Other parameters are estimated and are defined as follows:

MMAD $\pm$ 2 stages: the sum of the amount of drug deposited on the stages where the MMAD of the reference product (F) lay and the two stages on either side, a total of five stages.

$\text{Stage}_{\text{MAX}}$: the stage where the maximum amount of drug was deposited for the reference product (F).

MAX1 and MAX2: the sum of the drug on $Stage_{MAX}$ and the appropriate number of stages on either side. The $Stage_{MAX}$ used in the parameters MAX1 and MAX2 was the most frequently observed (mode) for the reference product.

Fine particle dose (FPD): the total mass of particles found on the stages ranging in particle size between 1 and 5 μm. For the ACI, the FPD comprises stages 3 through 5, (1.1 to 4.7 μm), for the TI, the FPD included particles less than 6.4 μm.

2.9. Electron microscopy

Samples were obtained from all of the stages of the ACI. The samples were collected onto 2×2 cm sheets of aluminum foil that had been placed directly on the ACI plates and were then cut into 1/2 cm squares before mounting on stubs and coating. Specimens were stored in a non vacuum desiccator.

The samples were platinum coated (21–45 nm) using an Edwards Sputtercoater. The micrographs were produced on a NanoLab LE2100 scanning electron microscope with a LaB_6 emitter (Lanthanum Hexaboride crystal). Samples were photographed between $200\times$ and $5000\times$ or $200\times$ and $10\,000\times$.

3. Results and discussion

3.1. Spray content uniformity

Fig. 1 shows the drug content in all the sprays collected. Three types of sprays were collected: primed sprays (P); unprimed sprays after the canister stood overnight (UPO); and unprimed sprays after the dosing interval (UPT). The data for all cans tested are presented in Tables 1–3, as percent of label claim. Values outside the 75–125% limits are in bold type.

Some initial spray values for products G and H were significantly higher than subsequent sprays. This phenomenon is common with MDIs and was not unexpected [5]. The data also are consistent with the previously observed trend that the drug content for primed sprays is higher than unprimed sprays [5–8].

Product F had the most reproducible values with only one primed spray of forty-eight collected sprays above 125% of the label claim but below 135%, none were less than 75%. In the case of Product G, seventy-one sprays were collected and analyzed; five lay above 125% of the label claim and these same five were greater than 135%. In addition there were six unprimed sprays and four primed sprays which delivered less than 75%; two of these were less than 65% of the label claim. Product H yielded the greatest variation. Twenty-four sprays were collected and nine (4 unprimed overnight, 3 unprimed dosing interval and 2 primed) were outside the range 75–125% and eight ($6 < 65\%$, $2 > 135\%$) of these fell outside of 65–135% of the label claim. Most of this variation could be attributed to one canister coded 12 (six outside the 75–125% limit). Product J had six sprays (all types, all less than 75%) outside the 75–125% range of the twenty-four collected; four were within 65–75% of the label claim and two were below 65%.

Applying the proposed USP criteria for content uniformity to the sampling scheme used only product F would pass. On the basis of a single actuation, products G, H and J would not meet the USP requirements. However, if the content uniformity is based on two puffs (unprimed and primed) which is the recommended lowest dosage for the american products, product G would pass. It has been suggested [14,15] that basing content uniformity requirements on more than one actuation for products with a minimum dosage of greater than one puff may be justified. It more accurately reflects patient use and actual delivered dosage.

3.2. Particle size: ACI

The particle size distribution profiles (Fig. 2) show that the four products tested were not equivalent in vitro. All of these profiles differed significantly from those of beclomethasone dipropionate products [10] in that a considerable mass of delivered particles was captured on stages with ECDs greater than 5.8 μm. Products H and J

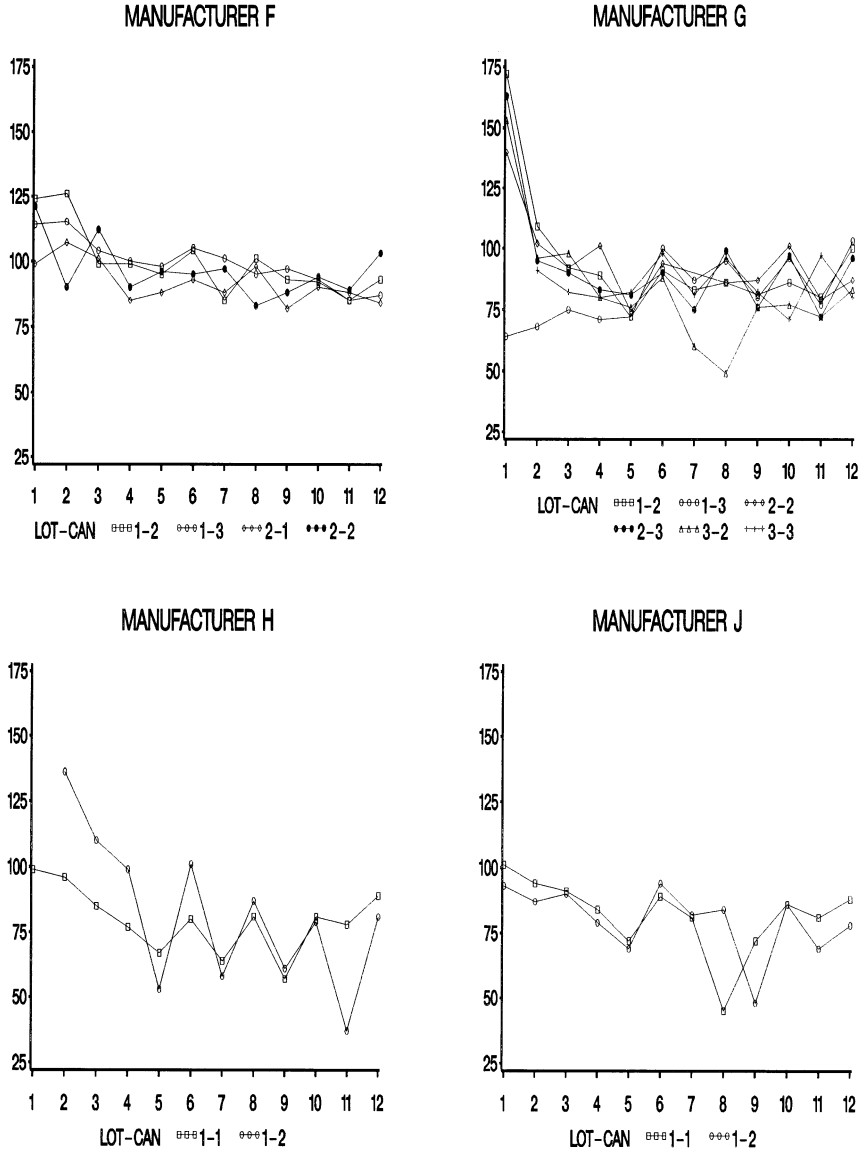

Fig. 1. Single spray content uniformity. Each point represents the drug content in % label claim (y-axis) of the nth spray (x-axis).

were the most similar. The profile of product F had the same shape as these two but demonstrated less deposition on the stage with an ECD of 3.3 μm. The profile for product G clearly demonstrated a lack of delivery of drug particles in the presumably necessary particle size range [2].

Particle size distribution parameters for all four products are shown in Table 4. In addition to the traditional parameters, MMAD and GSD, several additional parameters such as MMAD $\pm$ 2 stages, MAX1 and MAX2 are shown. We developed and introduced these parameters as potentially useful in our previous report on beclomethasone [10] products. We also recognized that further evaluation of these parameters were required to support their use in characterizing MDIs.

Table 1
Content uniformity results: manufacturer F (% label claim)

Spray	Type	F12	F13	F21	F22	Mean	R.S.D.
1	P	124	114	99	121	114	10
2	P	**126**	115	107	90	110	14
3	P	99	104	101	112	104	5
4	P	99	100	85	90	94	8
6	P	104	105	93	95	99	6
8	P	101	95	98	83	94	8
10	P	92	93	90	94	92	2
12	P	93	87	84	103	92	9
7	UPT	85	101	88	97	93	8
11	UPT	85	85	88	89	87	2
5	UPO	95	98	88	96	94	5
9	UPO	93	97	82	88	90	7
Mean	P	105	102	95	99	100	4
	UPT	85	93	88	93	90	4
	UPO	94	98	85	92	92	6

A review of the MMAD values in the table shows an upward shift when products H and J are compared to products F and especially G. This shift reflects the differences in the particle size distributions shown in Fig. 2. The MMAD for products F, H and J ranged from 4.74 to 5.18 μm whereas the MMAD for G was 6.91 μm, a 40% increase. This shift in MMAD indicates a greater proportion of large particles in the plume. For all values, the coefficient of variation about the mean MMAD values was less than 10%. The GSD values ranged from 1.4 to 1.7 and did not provide any discriminatory information about the distribution profile. This is consistent with our previous findings [10].

The significance of the MMAD for comparison of metaproterenol products is questionable particularly in the light of the resources required to calculate it. In its calculation, large particles with aerodynamic diameters greater than 5.8 μm are included which can not be expected to be delivered to the lower respiratory tract. The MMADs of all of the products may therefore be largely irrelevant. In addition, it also is unable to reflect the absolute amount of drug substance delivered.

When we introduced the use of MMAD ± 2 stages and MAX1, MAX2, we recognized the need to define these parameters according to the in vitro performance characteristics of a standard product, that is, a product demonstrated to be effective in extensive clinical studies. This ensures that identical particle size fractions are used in comparisons. We invoke this principle again in defining these ad hoc parameters. In this instance product F was arbitrarily chosen as the standard. All mass based parameter calculations were made using the experimentally determined values of MMAD and $\text{Stage}_{\text{MAX}}$ for this product.

Table 2
Content uniformity results: manufacturer G (% label claim)

Spray	Type	G12	G13	G22	G23	G32	G33	Mean	R.S.D.
1	P	**172**	**64**	**140**	**163**	**153**	**196**	148	30
2	P	109	**68**	102	95	96	91	94	15
3	P	92	75	92	90	98	82	88	9
4	P	89	**71**	101	83	80	80	84	12
6	P	91	100	94	90	88	98	94	5
8	P	86	95	86	99	**49**	96	85	22
10	P	86	96	101	97	77	**71**	88	14
12	P	100	103	87	96	83	80	92	10
7	UPT	83	87	—[a]	75	**60**	81	66	43
11	UPT	80	77	79	**72**	**72**	97	80	12
5	UPO	**72**	**72**	**74**	81	76	82	76	6
9	UPO	81	80	87	76	76	82	80	5
Mean	P	103	84	100	102	91	99	96	8
	UPT	82	82	79	74	66	89	73	22
	UPO	77	76	81	78	76	82	78	3

[a] Volumetric flask spilt.

Table 3
Content uniformity results: manufacturers H and J (% label claim)

Spray	Type	H11	H12	Mean	RSD	J11	J12	Mean	R.S.D.
1	P	99	**258**	178	63	101	93	97	6
2	P	96	**136**	116	25	94	87	90	5
3	P	85	110	98	18	91	90	90	1
4	P	77	99	88	18	84	79	82	4
6	P	80	101	91	16	89	94	92	4
8	P	81	87	84	5	**45**	84	65	43
10	P	81	79	80	2	86	86	86	0
12	P	89	81	85	7	88	78	83	9
7	UPT	**64**	**58**	61	7	81	82	82	1
11	UPT	78	**37**	58	50	81	**69**	75	11
5	UPO	**67**	**53**	60	16	**72**	**69**	71	3
9	UPO	57	61	59	5	**72**	**48**	60	28
Mean	P	86	119	102	23	85	86	86	1
	UPT	71	48	60	27	81	76	78	4
	UPO	62	57	60	6	72	58	65	15

In this instance the MMAD fell on stage 2 (effective cutoff diameter (ECD) 4.7 μm). MMAD $\pm$ 2 stages therefore would include particles collected on stage 0 (ECD 9 μm) to stage 4 (ECD 2.1 μm). The inclusion of the large particles on stage 0 for the meaningful characterization of MDIs is not consistent with current aerosol science. In the case of metaproterenol, which exhibits such a large MMAD, the use of MMAD $\pm$ 2 stages should be avoided. Restricting this parameter to one stage on either side of the MMAD would exclude from consideration the smaller range of particles, e.g. 2.1 μm, which are considered relevant to product efficacy.

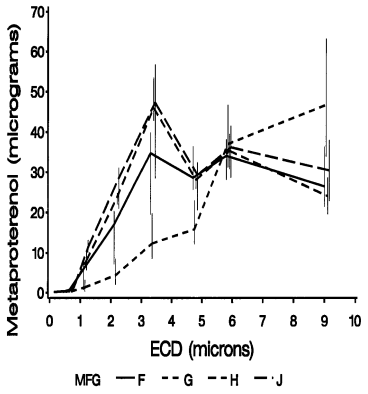

Fig. 2. Metaproterenol MDI particle distribution profiles. The plot contains the micrograms deposited on the stages on the *y*-axis and the ECD of each stage on the *x*-axis. The vertical lines on the curves indicate the range of observed values at each of the stages and have been shifted slightly to permit the visual identification of the range of values.

A similar obstacle may arise in the calculation of MAX1 and MAX2. The data presented in Fig. 2 indicate that standard product F had the greatest mean mass of deposition on stage 3 of the ACI. We had fortuitously defined $\text{Stage}_{\text{MAX}}$ as the mode (most frequently observed maximum) of the stage number with the maximum deposition of drug particles. Clearly a definition based on an experimentally determined $\text{Stage}_{\text{MAX}}$ is dangerous in the case of bimodal particle size distributions. It may be inappropriate in all situations, as the experimentally determined maximum may be unrelated to product performance.

The FPD is defined as the amount of drug on stages 3 through 5 (1.1–4.7 μm) and is independent of both the MMAD and the experimental approach to particle size range determination used to generate $\text{Stage}_{\text{MAX}}$. The FPD values shown in Table 4 and graphically presented in Fig. 3 clearly demonstrate the differences among products. Product G has, on average, one third to one quarter the amount of drug delivered to the FPD as the other products. The coefficient of variation for these totals ranges from 4 to 39%.

Table 4
Manufacturer comparison

Paramter	F	G	H	J
FPD (μg)	58 (22)	18 (39)	78 (4)	87 (21)
MMAD (μm)	5.18 (8)	6.91 (6)	4.80 (4)	4.74 (4)
GSD	1.56 (4)	1.44 (5)	1.62 (2)	1.69 (5)
MMAD $\pm$ 2 stages (μg)	141 (6)	116 (14)	157 (7)	171(14)
MAX1(μg)	80 (10)	32 (31)	98 (5)	104 (22)
MAX2 (μg)	120 (11)	71 (21)	141 (5)	152 (19)
Total in impactor (μg)	147 (8)	118 (15)	166 (6)	185 (13)
TI, stage two ($<$6.4 μm, μg)	136 (16)	68 (24)	164 (12)	206 (10)
Total in impinger (μg)	550 (8)	617 (9)	576 (5)	604 (3)

CV, F $n=8$, G $n=11$, H $n=5$, J $n=4$.

It is interesting to note that of the more than 600 μg of drug expelled from the device, only a small fraction, $<30\%$, actually passes the inlet system of the ACI (see total in impactor in Table 4). This is similar to other results we have obtained [10] and highlights the amount of drug substance impacted in the inlet throat of the ACI.

3.3. Particle size: TI

Results of the work on this device are also shown in Table 4. The amounts delivered to the second stage of this device (<6.4 μm) for product G are quite clearly indicative of inferior drug delivery. The differences in the results are not as striking as those for FPD of the ACI but clearly indicate the ability of the TI to identify unusual in vitro product performance.

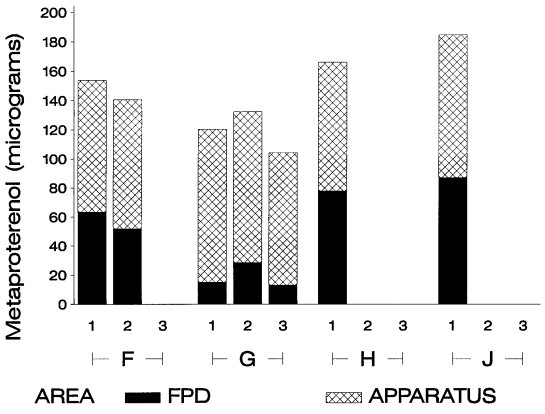

Fig. 3. Drug particle distributions of primed sprays of metaproterenol. Area definitions: FPD (1.1–4.7 μm); Apparatus, the amount deposited in the ACI that was not in the FPD. The sum of these two amounts gives the total deposited in the ACI.

3.4. Particle size: electron microscopy

The most striking feature of virtually all of the micrographs (Fig. 4), at magnifications of greater than $1000\times$, is that the particles of drug are agglomerates of finer particles. These fine particles were irregular in shape and were composed of smaller particles having lengths, along their longest axis dimension, of less than 1 micron. The agglomerates' longest dimension ranged from 3 to 10 microns. This may imply that the size of the primary drug particles contained in the canisters do not differ among the products.

The other feature of all the micrographs is that the edges of the particles become increasingly less defined at a higher plate number, that is, at a decreased particle size. This may be seen from the examples included. This may be related to the exposure of the smaller deposited particles to the higher linear flow velocities characteristic of the higher stages of the ACI, resulting in the hydration of the crystals with a consequent loss of edge definition. This hypothesis is supported by the fact that the difference in the qualitative degree of roughness of the particles between products at a given ACI stage was much less than the difference between plates for the same product.

Fig. 4. Electron microscope photographs of product G. Top left, stage 1 (5.8–9.0 μm); top right, stage 3 (3.3–4.7 μm); and bottom, stage 5 (1.1–2.1 μm). Bar = 5 μm.

4. Conclusions

The single spray content uniformity testing indicated that products from both manufacturer G and manufacturer H exhibited high sprays at the beginning of the lifetime of the canister. Product H also gave results characteristic of the loss of prime phenomenon; seven of the eight unprimed sprays delivered amounts of drug outside accepted limits. Canister 2 in particular exhibited poor performance. These results indicate that consideration should be given to incorporating unprimed sprays into dose uniformity testing of MDIs and to evaluating products on the basis of the dosage actually received by the patient.

The FPD as determined using the ACI showed greater differences among the four products tested than did the fraction determined using the TI. Nevertheless, the same trend was seen with both devices. The results presented demonstrate the ability of the TI to discriminate among MDI products of variable quality and support its continued use in quality control of MDI products. However, validation must be carried out in the product development stage against the ACI to ensure the TI's adequate performance [15].

The results further show that the ad hoc parameters we had developed during previous studies have serious limitations. Whereas MMAD $\pm$ 2 stages and MAX1 are readily able to

discriminate among products, the general application of predetermined parameters such as these may lead to the definition of particle distributions for comparison purposes that are not consistent with current aerosol theory. The results presented suggest that the performance characteristics of each particular drug product should be reviewed and used to develop specific definitions of particle size ranges.

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