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The Deposition of Spray-Dried β-Galactosidase from Dry Powder Inhaler Devices

J. Broadhead,[1,*] S. K. Edmond Rouan,[2] and C. T. Rhodes[1]

[1]*Department of Applied Pharmaceutical Sciences, University of Rhode Island, Kingston, Rhode Island 02881*
[2]*SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, Pennsylvania 19406*

ABSTRACT

The object of this study was to evaluate the in vitro deposition properties of a model spray-dried protein (β-galactosidase) from a dry powder inhaler device (ISF inhaler). The stabilized spray-dried protein was evaluated alone and when blended with equal weights of (a) Avicel and (b) mannitol. The deposition properties were studied after exposure to environments of varying humidity using a twin impinger and a cascade impactor. The spray-dried material was extremely sensitive to humidity, with large reductions in respirable fraction occurring after storage at 43% relative humidity. The presence of a nonhygroscopic carrier (mannitol) did not prevent this reduction. There was no significant difference between the estimates of respirable fraction obtained using the cascade impactor and twin impinger for the material which had been stored in a dessicator. However, for the powders which had been exposed to environments of 43% relative humidity, the twin impinger was more reliable, as the cascade impactor results were adversely affected by entrainment of aggregates in the air stream.

*Present address: Creative BioMolecules, 35 South Street, Hopkinton, MA 01748.

INTRODUCTION

The pulmonary route has traditionally been used for the delivery of locally acting drugs such as bronchodilators. These can be administered using metered dose inhalers (MDIs), nebulizers, or dry powder inhalers (DPIs). Currently considerable attention is being focused on the possible exploitation of the pulmonary route for the delivery of peptides and proteins (Byron, 1990). Inhalation may provide a means by which this type of drug can be administered systematically without the need for injection. Insulin (51 amino acids) and growth hormone (192 amino acids) are known to be absorbed from the alveolar region of the lung (Patton and Platz, 1992), and bioavailabilities of 35–55% were obtained when leuprolide acetate (nine amino acids) was administered to healthy volunteers via metered dose inhalers (Adjei and Garren, 1990). Thus, there is a clear potential for absorption of some macromolecular drugs from the lungs, provided that the protein can be successfully delivered to the alveoli.

Although metered dose inhalers have been the most popular pulmonary delivery system in the past, these are now losing favor. Presently available MDIs contain chlorofluorocarbon (CFC) propellants. These are currently being phased out worldwide because of environmental concerns, which means that alternative propellants must be found (Newman, 1990). Hydrofluorocarbons are being considered although their properties make MDIs containing them more difficult to formulate than those containing CFCs. Additionally, many patients, especially children, are unable to use MDIs correctly since the discharge of the device must be synchronized with inspiration (Ganderton and Kassem, 1991). These two factors have led to increased interest in dry powder devices since they do not require propellants and are "actuated" by the patients themselves. Also, they can deliver much higher doses than can MDIs, which was the initial reason for their invention.

If the pulmonary route is to be used as a means of delivering macromolecular drugs systemically, it is important that the particles are small enough to reach the alveoli, where absorption can occur (Patton and Platz, 1992). Ideally, particles should be in the range 1–3 μm to reach the lower airways, with particles of around 5 μm probably depositing in the upper regions of the lung (Gupta and Hickey, 1991). The micronized drug material in a dry powder device is usually mixed with a carrier of a much larger size, typically lactose. The function of the carrier is threefold. It improves the flow of the powder blend during the filling process and facilitates the dispersion of the cohesive drug particles on inhalation. In addition, it acts as a diluent when low dose drugs are used (Ganderton and Kassem, 1991).

Spray drying can be used to produce spherical particles of controlled size in a one-step process (Broadhead et al., 1992). These features render it suitable for producing dry powders for inhalation. Vidgrén et al. (1987, 1988, 1989) have carried out extensive investigations into the deposition properties of spray disodium cromoglycate. They observed that during in vitro testing, a greater portion of spray-dried material was in the therapeutically important size range compared with mechanically micronized material (Vidgrén et al., 1987). Spray drying is particularly suited to the processing of protein drugs, since it is possible to produce small spherical particles from a solution of the drug without any activity losses occurring (Broadhead et al., 1994; Masters, 1985).

One problem associated with dry powders is that they are often hygroscopic, which can result in apparent particle size changes if they are exposed to humid environments (Byron, 1990). This phenomenon was encountered by Vidgrén et al. (1989), who also found that the in vitro deposition of spray-dried disodium cromoglycate was adversely affected by moisture uptake whereas that of the micromized material was relatively unaffected. We have previously developed an optimized spray-dried formulation of β-galactosidase of a size suitable for evaluation in dry powder inhaler devices (Broadhead et al., 1994). The objective of the present study was to evaluate the effect of the formulation of the powder blend on the deposition properties of spray-dried β-galactosidase after exposure to environments of varying humidities. Materials of very low (mannitol) and higher (microcrystalline cellulose) hygroscopicity were evaluated as model carriers for the micromized protein, so that the effect of the inherent hygroscopicity of the carrier on the behavior of the powder blend could be examined.

MATERIALS AND METHODS

Materials

Lyophilized β-galactosidase derived from *Aspergillus oryzae* was obtained from Enzyme Development Corporation (New York). Reagents and standard for the BCA and micro-BCA protein assays were obtained from Pierce Chemical Company. Mannitol was obtained from

ICI Chemicals. Avicel PH 101 was obtained from FMC. Trehalose dihydrate and all other chemicals were obtained from Sigma Chemical Company.

Spray Drying

A 60-mg/ml solution of β-galactosidase was prepared by dispersing the manufacturer's material in deionized water, centrifuging, and dialyzing the supernatant against deionized water. Trehalose, at a concentration of 50 mg/ml, was added to this solution prior to spray drying to prevent activity loss during drying and subsequent storage. Spray drying was carried out using a Büchi 190 mini-spray dryer at an inlet temperature of 140°C and an outlet temperature of approximately 95°C. The solution was fed to the dryer at a rate of 2 ml/min. An air flow rate of 600 liters/hr and aspirator vacuum of 35 mbar were used. The geometric mean size of the material was determined by image analysis to be 2.89 μm (Magiscan, Joyce Loebl); the size distribution is shown in Fig. 1.

In Vitro Inhalation Behavior

The in vitro inhalation behavior of formulations with and without a carrier was evaluated after 2 weeks exposure to environments of varying humidities. The humidities were maintained using saturated salt solutions as shown in Table 1.

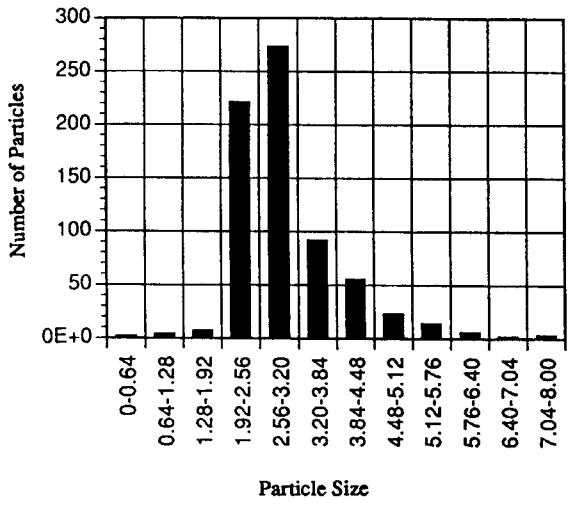


Figure 1. Particle size distribution of the spray-dried β-galactosidase formulation determined using image analysis.

Table 1

Saturated Salt Solutions Used to Maintain Controlled Humidities

Salt Solution	Humidity (%)
Silica gel	0
Potassium acetate	22.5
Potassium carbonate	43.2
Sodium chloride	75.3

Source. Greenspan, 1977.

Carrier formulations were prepared by mixing equal weights of the spray-dried material with (a) microcrystalline cellulose (Avicel PH 101) and (b) mannitol. Lactose was not used as a carrier because it is a substrate for β-galactosidase. The mean size of the Avicel was 24.3 μm and that of the mannitol was 18.4 μm as determined by image analysis. Number 3 hard gelatin capsules were filled with either 20 mg of the spray-dried powder or 40 mg of the carrier blends after 2 weeks storage at the appropriate humidity. In both cases each capsule contained approximately 11–12 mg of protein. The in vitro deposition properties were evaluated using a twin impinger (Copley Instruments Ltd.) and a cascade impactor (Andersen 8 stage 1 ACFM Non Viable Particle Size Sampler Mark II). An ISF inhaler (Cocozza, 1976) was used throughout the study. In both cases five capsules were discharged into the apparatus per determination, and each determination was carried out five times. An air flow rate of 60 liters/min and an inhalation time of 10 sec were used. Deionized water was used as the impingement fluid for the twin impinger. The volumes in stage 1 and 2 were 5 ml and 20 ml, respectively. After each determination the throat and two stages of the twin impinger were rinsed with deionized water, and the washings analyzed for protein content. For the cascade impactor determinations, the eight glass plates of the cascade impactor were coated with a thin layer of silicone grease. This prevents particles bouncing off the plates and becoming reentrained in the air stream (Allen, 1990). A preseparator was attached to the top of the cascade impactor to prevent large particles or aggregates reaching the stages. The twin impinger throat piece was attached to the top of the preseparator. After each determination the material on each plate of the impactor was collected by rinsing with deionized water. The material which deposited in the throat piece, the preseparator, and on the metal stages was also determined. The aerodynamic diameters col-

lected with 50% efficiency at each stage of the impactor are 7.39, 4.71, 3.21, 2.21, 1.43, 0.73, 0.46, and 0.32 at an air flow rate of 60 liters/min (calibration data from Andersen). The cutoff aerodynamic diameter between stage 1 and 2 of the twin impinger is approximately 6.4 μm.

The protein content of the various samples was determined using the BCA protein assay (concentrations less than 20–300 μg/ml) or micro-BCA protein assay (concentrations less than 20 μg/ml) (Smith et al., 1985). The percentage of the protein filled into the capsules which was recovered in each region of the impinger or impactor was thus calculated. The mass median aerodynamic diameter of the samples was determined from the cascade impactor data. Student's *t* test (two sided) was used to evaluate the significance of differences in deposition results at the 95% confidence level.

Moisture Content

The moisture content of the samples was determined using a Mitsubishi Moisture Meter (model CA-06, with vaporizer VA-06) in which samples were heated to 120°C for 10 min prior to Karl Fischer titration (Johnson, 1967).

Scanning Electron Micrographs

Samples of the powders were sprinkled on SEM stubs and gold coated. Scanning electron micrographs were obtained using an Amray 1200C instrument.

RESULTS AND DISCUSSION

Moisture Content

The moisture content of the formulations following 2 weeks storage at different humidities is shown in Table 2. In addition, the moisture content of the carriers alone after exposure to the different humidities was determined. It is evident that the spray-dried material is extremely hygroscopic. Avicel is also a moderately hygroscopic material, in contrast to mannitol, which absorbed virtually no moisture even after storage in an environment of 75% relative humidity (RH). The moisture content of the blends was roughly equivalent to the mean of the moisture contents of the two components of the blend. This indicates that the presence of a nonhygroscopic excipient, such as mannitol, in an intimate mix with the spray-dried material has no effect on the moisture uptake of the latter. All the formulations became noticeably caked after storage at the higher humidity levels (43% and 75% RH). Interestingly, the Avicel formulation exhibited less caking than the mannitol formulation, despite its considerably higher moisture content.

Particle Morphology

Figures 2–4 are scanning electron micrographs of the powders after exposure to 0%, 43%, and 75% RH. The aggregate formation which occurs after exposure to elevated humidities is particularly evident for the formulation without carrier, where the transition from discrete particles to aggregates composed of large numbers of formerly distinct particles is clearly visible. After storage at 43% humidity the individual spray-dried particles are still evident for all three formulations, but after exposure to 75% humidity they can no longer be detected. The moisture uptake has presumably caused the particles in the aggregates to partially dissolve and form into new, very large agglomerates.

In-Vitro Inhalation Behavior

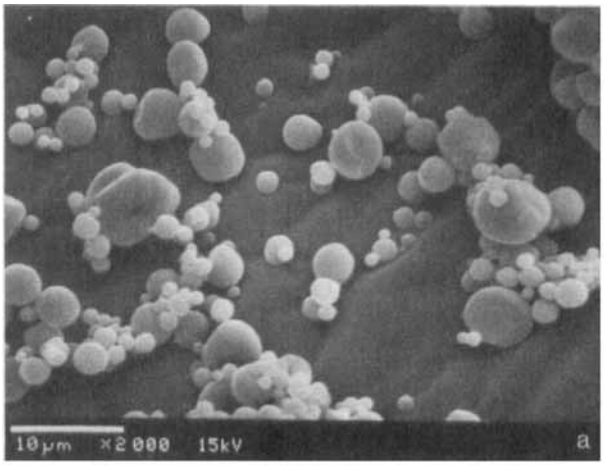
Twin Impinger

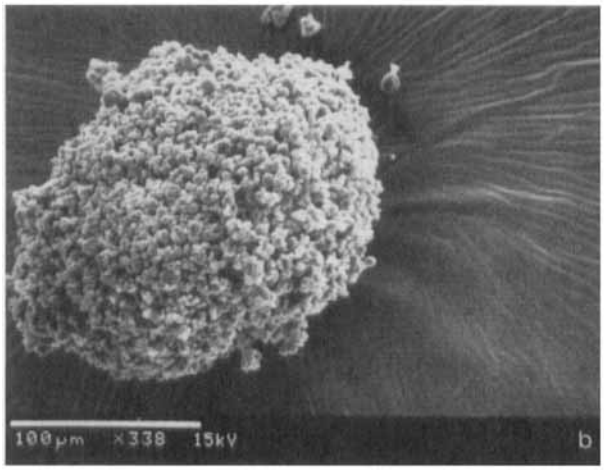
The percentage of the protein filled into the capsules which reached the second stage of the twin impinger is

Table 2

Moisture Content of Powders and Excipients

	Moisture Content %			
Humidity (%)	No Carrier	Avicel Alone	Avicel Blend	Mannitol Alone
0	4.20	4.01	4.33	0.056
22.5	5.83	4.35	5.47	0.098
43.2	8.20	5.11	6.65	0.074
75.3	13.32	8.05	9.03	0.139





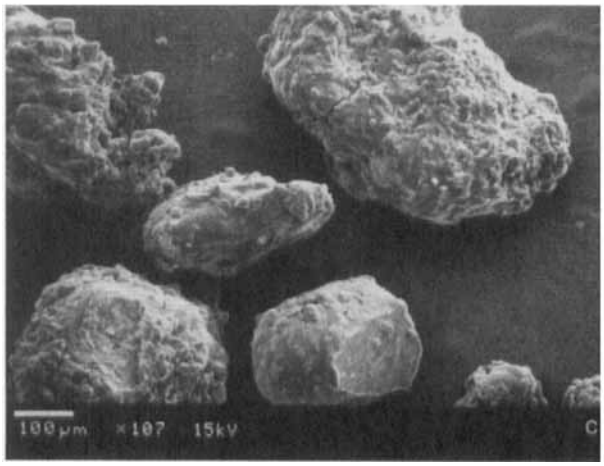
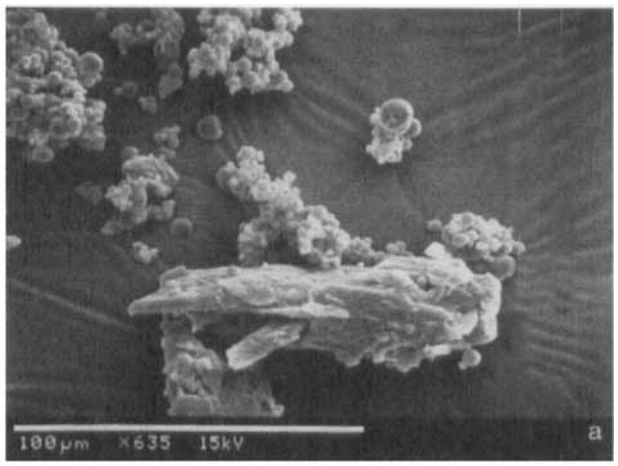
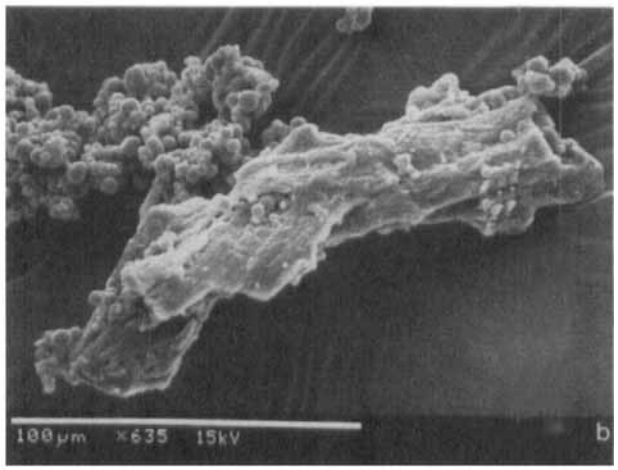


Figure 2. Scanning electron micrographs of the spray-dried material after storage at (a) 0% humidity, (b) 43.2% humidity, and (c) 75.3% humidity.





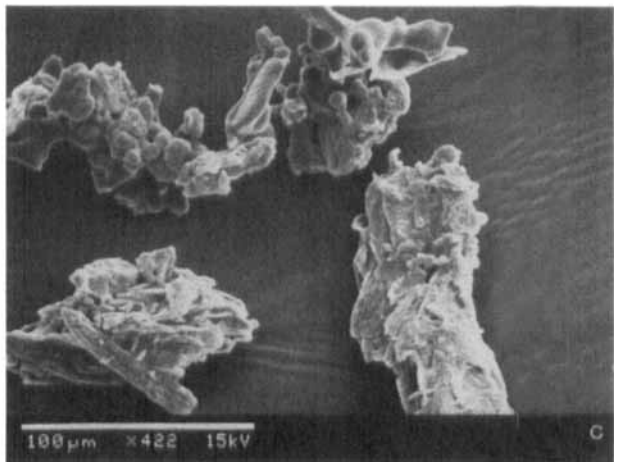


Figure 3. Scanning electron micrographs of the Avicel powder blend after storage at (a) 0% humidity, (b) 43.2% humidity, and (c) 75.3% humidity.

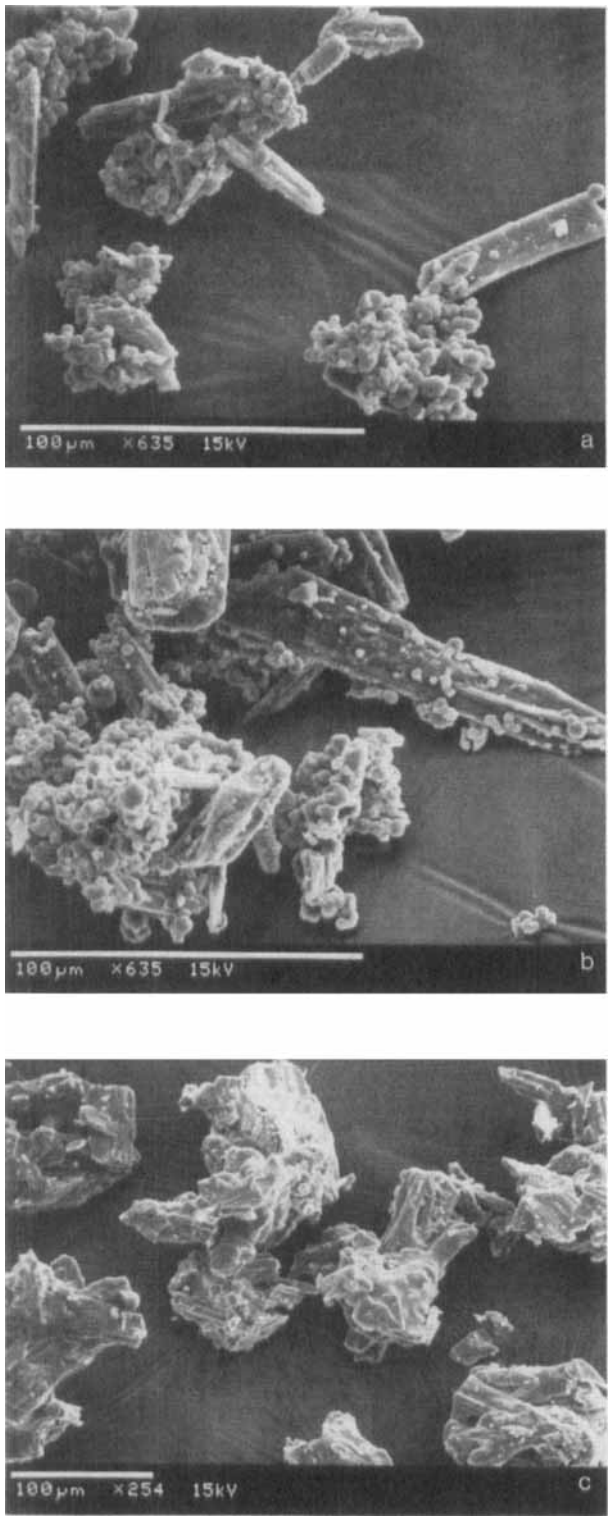


Figure 4. Scanning electron micrographs of the mannitol powder blend after storage at (a) 0% humidity, (b) 43.2% humidity, and (c) 75.3% humidity.

shown in Table 3. This represents the fraction of spray-dried material in the respirable size range and is usually termed the "respirable fraction." It is evident that the respirable portion is dramatically reduced by exposure to humid environments. Respirable fractions of the material stored at 75% humidity were not determined since the powders had formed into hard, solid cakes. It is evident from the electron micrographs that no respirable particles remained after exposure to these humidity levels. Significant caking had also occurred with the powders stored at 43% relative humidity. Evidently the powders had formed into strong aggregates which could not be dispersed in the in vitro evaluations. After storage at 0% RH, no significant differences in stage 2 deposition were observed between the three formulations. After storage at 43% humidity, a significant difference was only observed between the mannitol blend and the formulation without a carrier, but the magnitude of this difference was small and therefore of little practical significance. It is interesting to note that the moisture content of the mannitol blend after exposure to 43% RH is the same as that of the spray-dried material after storage in a dessicator. Thus the moisture content of the blend gives no indication of performance. The critical factor is the moisture content of the micromized component of the blend.

Figure 5 shows the distribution of the blends between the regions of the impinger. It can be seen that the stage 1 deposition increases after exposure to high humidities, and that the stage 2 deposition decreases. This is because the aggregates which escaped the inhaler tended not to impact on the glass surface of the throat but slipped downward into the first stage of the impinger. In an in vivo situation, however, it is likely that these aggregates wuold be retained in the mouth and throat. The relatively high level of unrecovered material can be accounted for, at least partially, by loss of material from the sides of the device during "inhalation." This is unavoidable due to the design of the ISF inhaler, which

Table 3

Percentage Deposition in Stage 2 of Twin Impinger

	Humidity[a]	
Formulation	0%	22.5%
No carrier	19.66 (1.921)	16.04 (2.745)
Avicel blend	18.97 (4.87)	17.88 (1.912)
Mannitol blend	22.44 (2.577)	24.27 (3.369)

[a]Standard deviations in parentheses $n = 5$.

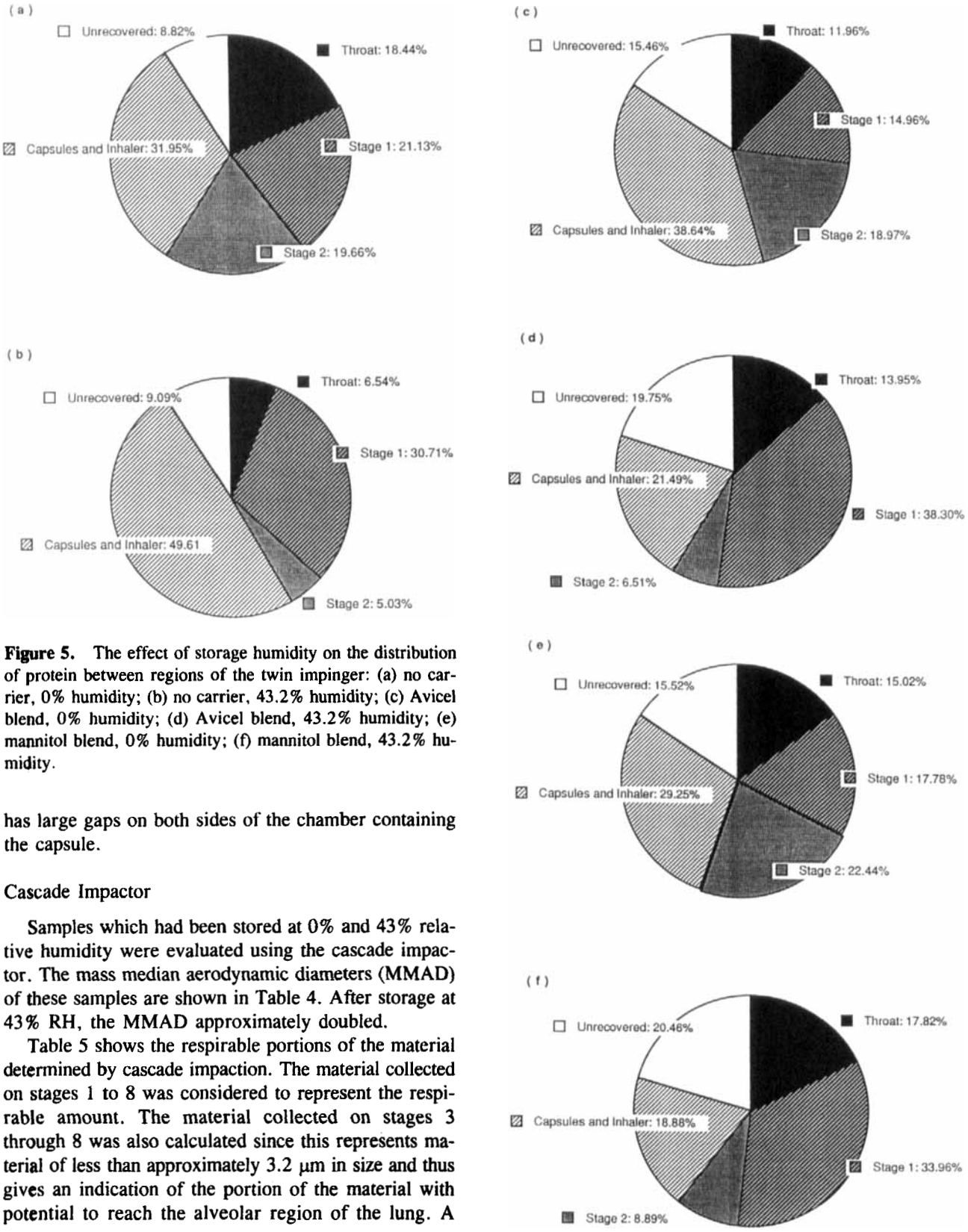


Figure 5. The effect of storage humidity on the distribution of protein between regions of the twin impinger: (a) no carrier, 0% humidity; (b) no carrier, 43.2% humidity; (c) Avicel blend, 0% humidity; (d) Avicel blend, 43.2% humidity; (e) mannitol blend, 0% humidity; (f) mannitol blend, 43.2% humidity.

has large gaps on both sides of the chamber containing the capsule.

Cascade Impactor

Samples which had been stored at 0% and 43% relative humidity were evaluated using the cascade impactor. The mass median aerodynamic diameters (MMAD) of these samples are shown in Table 4. After storage at 43% RH, the MMAD approximately doubled.

Table 5 shows the respirable portions of the material determined by cascade impaction. The material collected on stages 1 to 8 was considered to represent the respirable amount. The material collected on stages 3 through 8 was also calculated since this represents material of less than approximately 3.2 μm in size and thus gives an indication of the portion of the material with potential to reach the alveolar region of the lung. A

Table 4

Mass Median Aerodynamic Diameters of DPI Formulations

Formulation	MMAD[a] (μm) 0% Humidity	MMAD[a] (μm) 43.2% Humidity	Growth Ratio
No carrier	2.94 (0.055)	5.8 (3.54)	1.97
Avicel blend	3.32 (0.217)	6.16 (0.619)	1.86
Mannitol blend	3.32 (0.110)	6.16 (1.613)	1.86

[a]Standard deviations in parentheses (n = 5).

Table 5

Percentage Deposition in Regions of Cascade Impactor

Formulation	Stages 1–8[a] 0% Humidity	Stages 1–8[a] 43.2% Humidity	Stages[a] 0% Humidity
No carrier	18.54 (2.74)	12.75 (1.249)	9.48 (1.46)
Avicel blend	24.69 (5.15)	17.88 (2.35)	11.45 (1.89)
Mannitol blend	22.09 (1.51)	16.78 (2.167)	10.15 (0.975)

[a]Standard deviations in parentheses (n = 5).

small, but significant, improvement was observed in respirable percentage when either carrier was used at both humidity levels. However, no significant differences between the formulations were observed in terms of deposition in the lower stages of the impactor at either humidity level.

It can be seen from Tables 3 and 5 that the estimates of respirable percentage for the dessicated material obtained from the two different analytical methods are very similar. This is despite the theoretical difference in the cutoff diameter for the estimates (6.4 μm for the twin impinger; 7.39 μm for the cascade impactor). No significant differences were observed between the estimates for any of the formulations. However, the cascade impactor gave a much higher estimate of respirable percentage for the samples which had been stored at 43% RH. This seemed to be due to entrainment of aggregates in the air stream of the cascade impactor. Visual inspection of the plates following discharge of the capsules showed the presence of large powder aggregates on plates 1 to 3 and to a slight extent on plate 4. This is undoubtedly responsible for the larger than expected respirable estimates. The MMADs obtained at 43% RH were also skewed by this phenomenon. For all formulations, an approximately 60% reduction in deposition in the lower regions of the impactor was observed after exposure to high humidities. These lower stages seemed to be less affected by aggregate entrainment, and so the estimates should be more realistic. Figure 6 shows the distribution of the protein between the various stages of the cascade impactor after storage at 0% and 43% RH. After storage at 0% RH, the majority of the material is deposited in the first four stages, with only small amounts of material reaching the lower stages. This indicates that there is only a very small amount of material in the submicro size range. All three formulations showed an increase in stage 1 deposition in subsequent stages. As described above, however, the increase in stage 1 deposition can probably be accounted for by the presence of aggregates. Thus the twin impinger may be more suitable than the cascade impactor for discriminating between dry powders for inhalation, particularly when the effect of "stressing" the formulation is to be examined.

CONCLUSIONS

These results show that spray drying is a suitable method for producing respirable particles of proteins. In our in vitro experiments, approximately 20% of the protein filled into the capsules was respirable. Furthermore,

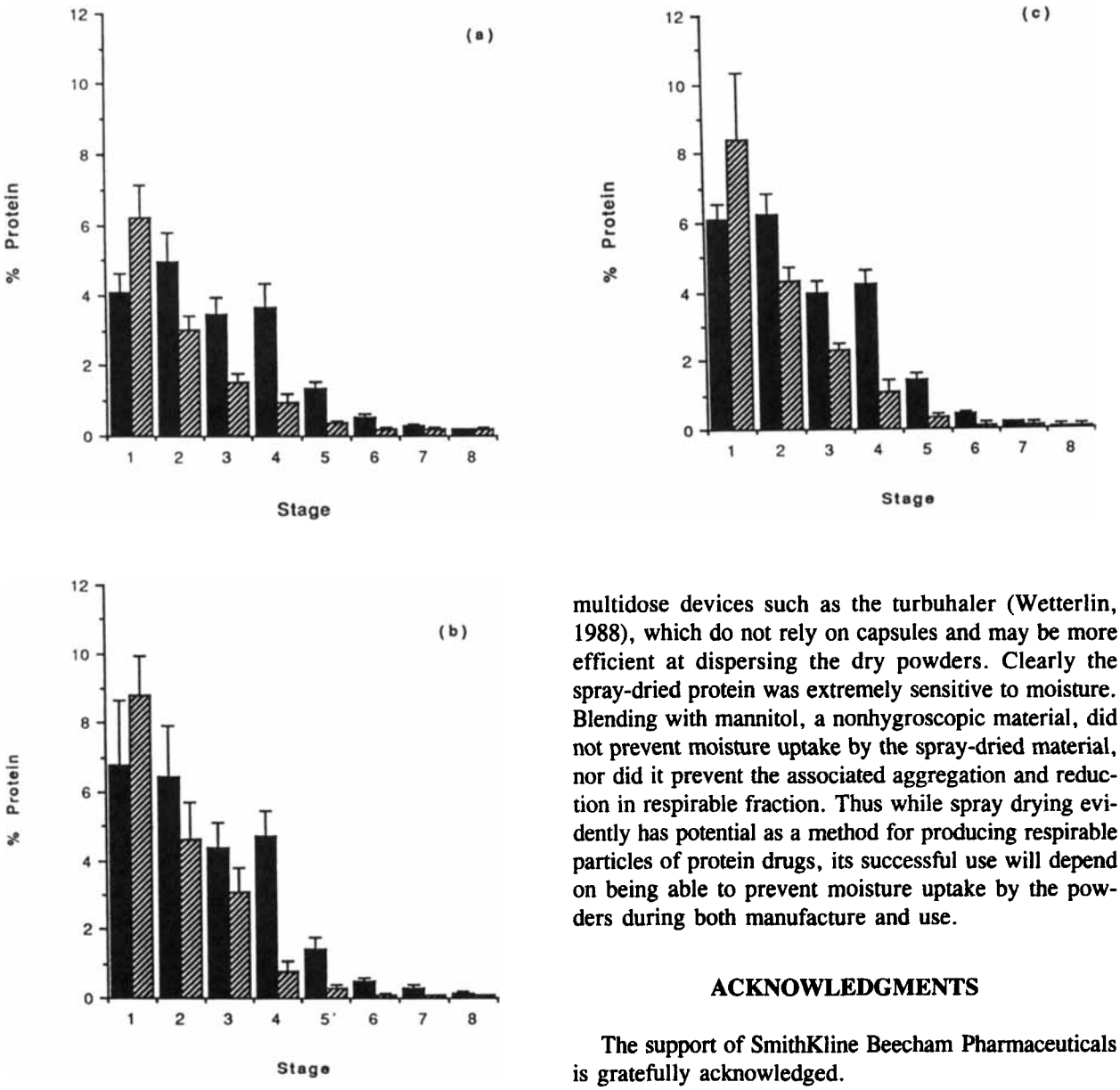


Figure 6. The effect of storage humidity on the deposition of protein in the cascade impactor: solid bars, 0% humidity; hatched bars, 43.2% humidity. (a) No carrier. (b) Avicel blend. (c) Mannitol blend.

approxinately half of this material reached the lower portion of the cascade impactor and was thus in the size range with potential for alveolar absorption. A large portion of the remaining material was retained in the inhaler and capsules. There is therefore considerable potential for improving these results by using newer multidose devices such as the turbuhaler (Wetterlin, 1988), which do not rely on capsules and may be more efficient at dispersing the dry powders. Clearly the spray-dried protein was extremely sensitive to moisture. Blending with mannitol, a nonhygroscopic material, did not prevent moisture uptake by the spray-dried material, nor did it prevent the associated aggregation and reduction in respirable fraction. Thus while spray drying evidently has potential as a method for producing respirable particles of protein drugs, its successful use will depend on being able to prevent moisture uptake by the powders during both manufacture and use.

ACKNOWLEDGMENTS

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