

Capsule Based Aerosol Generation-Test Article Conservation during the Conduct of in vivo Lead Optimization Respiratory Safety Assessment Studies

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Abstract

In both clinical and non-clinical environments the inhaled delivery of drug substances to conscious subjects will typically require larger quantities of the test article than any other route of administration. This difference is greatest for dry powder formulations in which charge and proximity effects are most pronounced, but it is also due to the losses and inefficiencies in the delivery device and aerosol delivery system. Minimizing such losses, particularly when conducting in vivo lead optimization studies in rodents, provides a means of reducing the cost of drug development. Decreasing the amount of test article requirement enables the conduct of investigations utilising inhaled route earlier in a development programme, when the test article is most likely to have limited availability and greatest cost. Over the last three years GSK and Envigo have successfully collaborated to identify alternative aerosol generation methods to minimize powder usage for inhaled delivery to conscious non-clinical species. The principle alternative delivery methodology, intratracheal insufflation, achieves particulate deposition dissimilar to conscious inhaled delivery and can produce artefactual toxicological and pharmacological results. Results of work conducted by Envigo and GSK to characterize and redesign a capsule-based aerosol generator are presented. If further work is successfully concluded, the doser should facilitate the use of the inhaled route earlier in in vivo lead optimization studies, so increasing the quality of candidate drugs and reducing compound attrition precipitated by findings in later, resource intensive in vivo studies.

Keywords: Aerosol; Capsule; Envigo capsule doser; Test articles

(Envigo capsule doser) was selected for further evaluation and refinement. The primary benefit identified for this doser was the potential to utilize the entirety of any test article dispensed. This contrasts with commercially available particulate aerosol generators that incur losses to dead volume as a consequence of their relatively large test article reservoirs. These losses contribute significantly to overall test article usage, particularly for aerosol production in shorter studies of 1-7 exposures. The doser sequentially loads, single-pierces and then discharges the contents from individual hydroxypropyl methyl cellulose (HPMC) size 2 capsules through a pneumatically operated cycle. A small compressed air pulse and venturi effect are used to aerosolize the contents of each capsule.

Envigo Capsule Doser

The results presented (Tables 1 and 2) for this doser showed good potential to significantly reduce overall test article usage even though it had been originally designed to operate at a significantly higher airflow (50 L/minute) than used for the evaluation.

The doser generated atmospheres with a mass median aerodynamic diameter (MMAD) that was within the respirable range for rodents (1-3 μm)² for both blended and undiluted test article (Table 2). The achieved values were similar to those found for the same test article when used in studies using commercially available powder generators. The UPLC-UV data were consistent, however, the duration of aerosol sampling exceeded 3 minutes and was not of a suitable duration to characterize the pulstile delivery (three capsule/minute) of aerosol into the chamber.

Powder Aerosol Generator Assessment

Following a review of available powder aerosol generators, a capsule-based doser designed and manufactured by Envigo

Table 1: Analytical aerosol concentration of Envigo capsule doser.

Sample number	Analytical concentration ($\mu\text{g/L}$)	
	Undiluted micronised test article	40% test article with lactose
1	25.8	8.60
2	23.4	7.56
3	27.9	0
4	26.7	0
Overall mean	26.0	8.08
Coefficient variation	7.3	9.1

Table 2: Analytical particle size distribution data of the Envigo capsule doser.

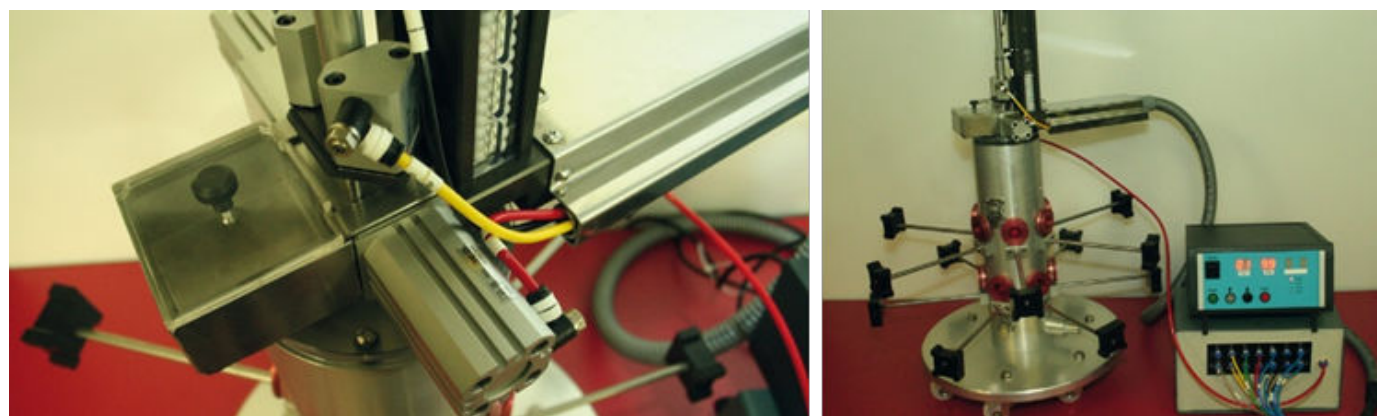
Test article	MMAD (μm)	Geometric standard deviation
1 mg undiluted	2.3	2.03
1 mg 40% blend	2.5	2.24

The other issue with this device was the aerosol generation efficiency (calculated as the ratio of analyzed to nominal test article concentrations) was lower than anticipated when compared with some standard commercial powder generators. Consequently, the doser was redesigned and a new prototype produced to improve utilization of the test article.

Redesigning the Envigo Capsule Doser

The Capsule Based Aerosol Generator (CBAG) The doser (Figures 1 and 2) was completely redesigned and rebuilt to

include the following features: +In-line double piercing of the capsules+ Capsule contents discharged downwards rather than upwards+ Actual capsule feed Greater flexibility in the control systems to permit variation of the air discharge volume and pulse timeframe to maximize capsule content ejection+ Greater actuation rate flexibility (time between capsules discharges) +And, critically, a lower overall operating airflow to reduce test article usage

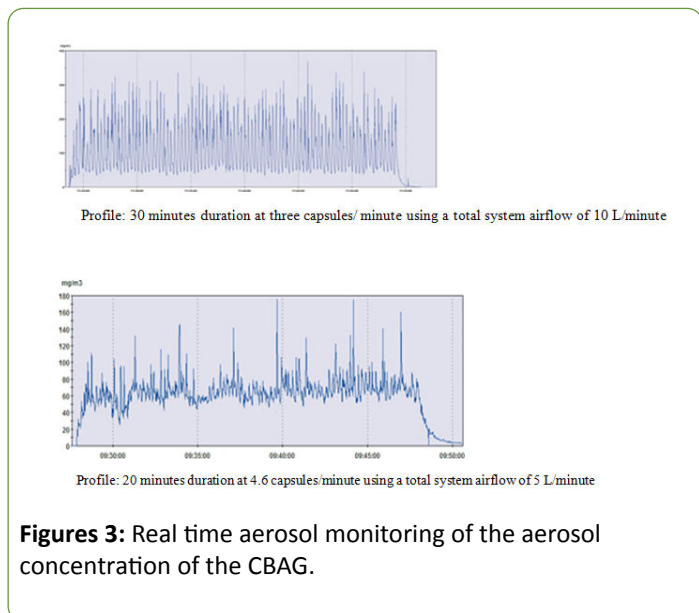


Figures 1 and 2: The CBAG mounted on top of a Flow-Through inhalation exposure system for snout-only delivery to rodents.

The prototype CBAG was evaluated³ with three drug materials that were selected based on their dissimilar physical characteristics. These were: +Fluticasone propionate (FP) +Tiotropium bromide +GSK-CMPD: 3-{8-(2,6-difluorophenyl)-2-[(1H-imidazol-2-yl) methyl] amino-7-oxo-7,8-dihydropyrido[2,3-D]pyrimidin-4-yl}-4-methyl-N-2-thiazolylbenzamide monomethanesulfonate For these trials HPMC size 2 capsules were again used and the capsule fill weight employed was varied between 0.1 and 1 mg (for FP only) to investigate the relationship between the two dosers. However, for these trials,

the capsules were filled using a semi-automated system for capsule loadings greater than 0.3 mg, as well as manually. Aerosol concentration was determined using an HPLC-UV method of analysis. Additionally, a continuous real-time dust monitor was connected to the exposure system and used to specifically monitor the consistency of aerosol generation at one second intervals by providing qualitative assessment. As expected for a capsule based system, a pulsatile powder aerosol concentration was observed, which was particularly marked when delivering three capsules/minute with an airflow of 10 L/

minute (**Figure 3**). The operating parameters were then modified by increasing the capsule delivery rate and reducing the airflow to 5 L/minute. These conditions achieved a more consistent and less pulsatile aerosol concentration.



Figures 3: Real time aerosol monitoring of the aerosol concentration of the CBAG.

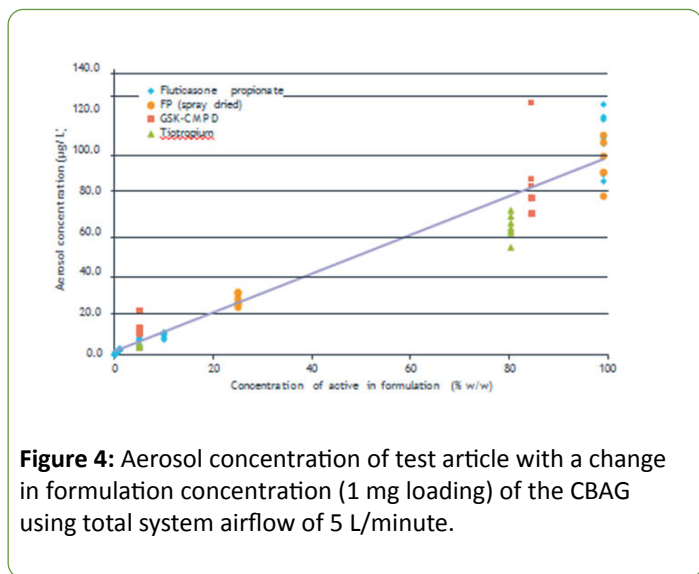


Figure 4: Aerosol concentration of test article with a change in formulation concentration (1 mg loading) of the CBAG using total system airflow of 5 L/minute.

Further trials were conducted over a broader range of target aerosol concentrations (0.07 to 119 µg/L) using an airflow of 5 L/minute and, like the Envigo capsule doser, the redesigned doser gave a linear relationship (**Figure 4**) with respect to content of drug substance. However, this observation is of greater significance due to the dissimilar physical properties of the individual test articles.

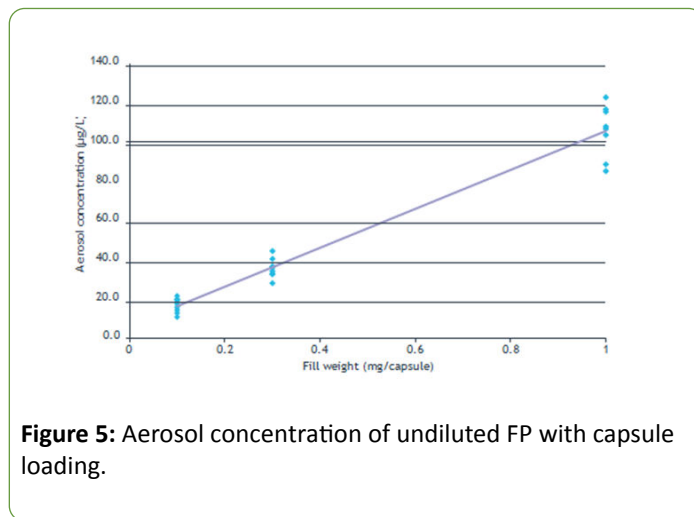


Figure 5: Aerosol concentration of undiluted FP with capsule loading.

Varying the capsule loading of undiluted FP from 0.1 to 1 mg/capsule (**Figure 5**) also gave a linear relationship with respect to aerosol concentration (20 to 119 µg/L). This was consistent with the previously presented results¹ using the original Envigo capsule doser. The mean MMAD for these three test articles varied between 2.7 and 5.0 µm under various operating conditions³, which was larger than expected and greater than was observed with the original Envigo capsule doser. These data are presented in **Table 3**. No explanation is currently available for the larger than expected particle size, however, the authors suspect it is related to the low exposure system airflow rather than the CBAG doser. Another factor that may have impacted results is the static charge associated with generation of aerosol from capsules, this may have resulted in increased particle aggregation as evidence of charge accumulation was readily observed during trials with the spray dried FP test article.

Table 3: Blend strengths, airflow, analytical particle size distribution and generation efficiency data for the three different test articles.

Test article	GSK-CMPD		Tiotropium	FP	Spray dried FP
Doser	Envigo capsule	CBAG	CBAG	CBAG	CBAG
Blend strengths Used(w/w)	36% and 91%	5% and 85%	5% and 80%	0.01%, 0.1%, 1%, 5%, 10% and 99%	25% and 99%
Airflow (L/minute)	10 and 20	5 and 13	5, 7 and 10	5	5
MMAD (µm)	2.3-2.5	2.7-4.4	3.3-5.0	3.3-4.9	5.6-5.8
Average generation efficiency	12%	24%	19%	21%	11%

¹In terms of active and correcting for purity

Comparison between EnvigoCapsule Doser and CBAG Dosers

Although over 80 trials have been conducted with these two dosers, a direct comparison between the two is currently difficult to make as modifications were made to the dosers during the course of the trials and the number and nature of operating parameter.

These include:

- Type of chamber (modified large commercially available, medium sized and narrow Flow-Through chamber)
- A wide range of airflows
- Piercing and airburst time could not be varied for the Envigo capsule doser
- Capsule actuation rate per minute (one capsule delivered every 20, 30 or 40 seconds for the Envigo capsule doser and one capsule delivered every 10, 13 or 20 seconds for the CBAG)
- Different batches of blended test material

However, when comparing the Envigo capsule doser and CBAG, it is clear that the new doser provided greater consistency between and within trials, and improved generation efficiency with the same test article (Table 3).

The reason for the low generation efficiency of the spray dried FP was considered to be due to static charge with the capsules.

Reduction of the Particle Size

Based on the particle size results, further modifications to the doser were made to reduce the particle size and additional preliminary trials were conducted. The generator outlet nozzle was modified to include the option of a baffle and to permit the introduction of a jet to aid particle deaggregation (Table 4). This reduced the particle size for most samples to an acceptable MMAD range for rodents².

Table 4: Analytical particle size distribution data with and without the modified nozzle, jet and baffle.

Formulation	% blend strength Mass	Median Aerodynamic Diameter (μm)		
		Nozzle only	Nozzle with jet (no baffle)	Nozzle with jet and baffle
-	-			
Tiopium	5%	4.7	3.7	3.5

Compound Requirement Assessment

The data in Figure 6 represents a comparison between two commonly used powder generators, Wright Dust Feed (WDF) and Rotating Brush Generator (RBG) with an Envigo-modified WDF (reduced dead space) and the CBAG. This represents the

minimum undiluted test article usage for a single exposure of 30 minutes duration with 250 g animals and the respiratory minute volume equation derived by Alexander et al⁴.

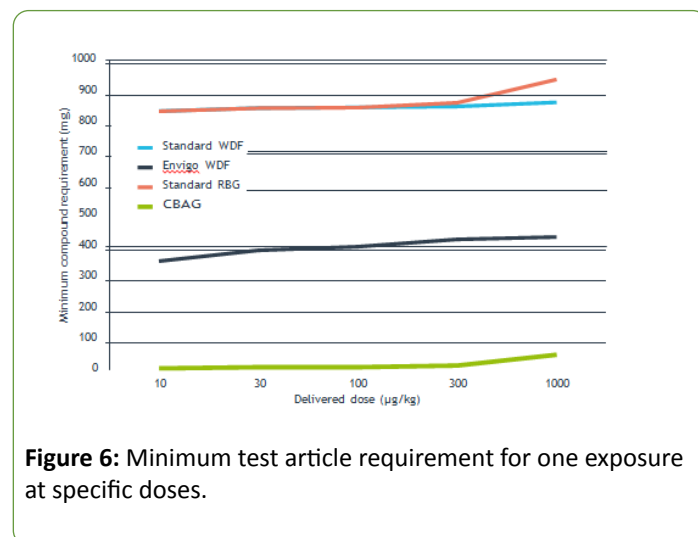


Figure 6: Minimum test article requirement for one exposure at specific doses.

The mass of test article that would be required for standard WDF and RBG generators is significantly greater than that required for the modified WDF and for the Envigo capsule doser. The lower requirement for the modified WDF generator when compared with the standard WDF is due to a decrease in the canister depth and thus a lower overall unusable test article 'dead space' (reduced from 8 mm to 2 mm in depth). The higher usage for the RBG generator is principally due to the required higher operating airflow for generation relative to the other dosers (even when minimal operating settings are used) and the relatively high test article dead volume in the powder reservoir. The significant advantage of the CBAG is its ability to utilize the entire dispensed test article, other generators require that a proportion of the test article is used to fill dead volume in a powder reservoir.

Conclusion

The capsule based aerosol generator (CBAG) successfully generated aerosols from three test articles with dissimilar physical characteristics. There was a linear and proportional relationship between aerosol concentration and formulation loading of the test article when capsules are filled with 1 mg of total formulation. There was a linear and proportional relationship between aerosol concentration and capsule loading. Following system modifications to include a jet and baffle option in the nozzle, the particle size, for most samples was within the respirable range acceptable for rodents². The test article requirement to achieve a delivered dose of 300 $\mu\text{g/kg}$ has decreased from 950 mg using standard commercial generators, to 15 mg using the CBAG for a single exposure. This means the limiting factor for supply is the amount required for chemical validation and analysis rather than technical dosing considerations. The CBAG offers a significant cost saving in test

article production over commercially available dosers as it minimizes dead volume waste

Acknowledgement

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