# Characterization of Spray Dried Azithromycin Dry Powders for Inhalation

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## SUMMARY

Background: An inhaled formulation of azithromycin (AZ) may be advantageous for the local treatment of pulmonary infections and associated inflammation without causing the systemic side effects observed with oral formulations. Indications for this therapy include chronic obstructive pulmonary disease and cystic fibrosis. Spray dried combination particle formulations of azithromycin are described for use with the excipient enhanced growth (EEG) concept for high efficiency dry powder aerosol delivery. Methods: Solutions for spray drying using a Büchi Nano spray-dryer were prepared containing azithromycin, a hygroscopic excipient (sodium chloride or mannitol), leucine and poloxamer 188 in water. Two formulations were evaluated for their aerosol performance characteristics in a novel dry powder inhaler developed for high efficiency aerosol delivery. Results: EEG DPI formulations prepared using AZ and mannitol as the hygroscopic excipient had a higher emitted dose (89.3±9.2%) than the AZ and sodium chloride formulations (51.0±4.1%). The AZ and sodium chloride formulation had a lower MMAD (1.2±0.1 µm) than the AZ and mannitol formulation (1.6±0.1 µm), although the fine particle fraction less than 5µm was over 85% of the emitted dose for both formulations. Increasing the loaded dose of the AZ and mannitol formulation from 2 mg to 5 mg had no effect on the aerosol MMAD and fine particle fractions although the emitted dose was reduced from 89.9% to 76.9%. Conclusions: Spray dried azithromycin powders were produced with good aerosolization characteristics suitable for the delivery using the EEG DPI.

## INTRODUCTION

An inhaled formulation of azithromycin (AZ) was identified by Hickey et al.,[(1)] for administration as an antibiotic to treat pulmonary infections. It was recognized that delivery to the airways would enhance local concentrations of the drug in the lungs while minimizing the potential for systemic side effects. Over recent years, further evidence has demonstrated that AZ also has potent anti-inflammatory activity[(2)] which further enhances the drug's potential as an inhalation therapy for indications such as chronic obstructive pulmonary disease and cystic fibrosis. However for inhaled AZ therapy to be effective, it must be combined with high efficiency aerosol delivery.

A number of previous studies have considered the development of spray dried AZ formulations for inhalation[(3, 4)]. Zhang et al., reported a combination of AZ / leucine / mannitol spray dried particle with a mass median aerodynamic diameter (MMAD) of 3.82 µm[(4)]. Mansour et al. reported drug only AZ particles that had a primary particle size less than 1 µm, with MMADs between 2.16 - 5.04 µm. The MMADs of these formulations are typical of those observed with conventional dry powder inhaler (DPI) formulations, which are characterized by high mouth-throat deposition losses and low delivery efficiencies to the lungs.

One potential pathway for an inhaled AZ formulation is the use of the excipient enhanced growth (EEG) dry powder inhaler technology[(5)] in combination with spray dried submicrometer combination particles[(6)]. With this approach, the inhaler generates an aerosol from a submicrometer combination particle formulation composed of a drug and a hygroscopic excipient. The small size of the aerosol particles minimizes deposition in the device and extrathoracic airways. The particle size increases in the warm and humid lung environment due to the inclusion of a hygroscopic excipient and associated water uptake, resulting in lung deposition of the aerosol. Previous studies with spray generated aerosols and EEG delivery have demonstrated low MT deposition[(7)] and the potential for significant size increase of the aerosol in the lungs[(8)]. Son et al.[(6)] previously developed an optimized EEG formulation for use with DPIs that contained albuterol sulfate (AS; model drug), mannitol (MN; model hygroscopic excipient), and L-leucine (dispersion enhancer).

The objective of this study is to characterize the aerosol performance of spray dried combination particle formulations of AZ using a novel DPI developed for the excipient enhanced growth (EEG) concept. Two hygroscopic growth excipients will be used and the AZ EEG formulations will be investigated, together with the effect of loaded dose on performance of the DPI.

## METHODS

### Materials

Azithromycin dihydrate (AZ) was purchased from Tokyo Chemical Industries Co. (Tokyo, Japan). Pearlitol® PF-Mannitol was donated by Roquette Pharma (Lestrem, France). Poloxamer 188 (Leutrol F68) was donated by BASF Corporation (Florham Park, NJ). L-leucine, sodium chloride and all other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Hydroxypropyl methylcellulose (HPMC) capsules (size 3) were donated by Qualicaps (Whitsett, NC).

### Preparation and Characterization of Spray Dried AZ Formulations

Excipient enhanced growth formulation combination particles were engineered using methods modified from those described by Son et al. [6]. Modifications included spray drying in water rather than water/ethanol solution and the use of 80°C drying temperature compared to 70°C employed by Son et al.[6]. For the AZ formulations, the method reported by Zhang et al. was employed to dissolve the drug in water[4]. The solution used for Formulation 1 (F1) implemented sodium chloride as the hygroscopic excipient and had a total solute concentration of 0.5 %w/v. The ratio of azithromycin dihydrate : sodium chloride : leucine : poloxamer 188 was 40:28:30:2. Similarly for a mannitol formulation (Formulation 2; F2), the ratio of azithromycin dihydrate : mannitol : leucine : poloxamer 188 was 30:40:28:2, with the total solute concentration of 0.5% w/v. For F2, the amount of mannitol was increased due to its lower hygroscopic capacity compared to sodium chloride[9]. 500 mg batches of combination particles were spray dried using the Büchi Nano spray dryer B-90 (Büchi Laboratory-Techniques, Flawil, Switzerland).

Powders were collected from the electrostatic precipitator of the spray dryer and stored in a desiccator at room temperature. The physico-chemical properties of the powders were characterized using thermogravimetric analysis, differential scanning calorimeter and scanning electron microscopy, together with determination of the AZ content uniformity using an LC-MS assay for AZ.

### Aerosol Particle Size Characterization

To determine aerodynamic particle size of the emitted aerosol, 2 mg or 5 mg of EEG formulation was filled in a hydroxypropyl methylcellulose (HPMC) size 3 capsule and aerosolized into a next generation impactor (NGI; MSP Corp., Shoreview, MN) using the $CC_{90}$-3D DPI at an airflow rate of 45 L/min corresponding to a 4 kPa pressure drop across the device [5]. Two inhalations were used to ensure good emptying from the device. The DPI employed a 3D rod array for powder dispersion and uses a capsule with its long axis aligned with the incoming airflow to provide efficient emptying in the capsule chamber[5]. The inhaler was created using Autodesk Inventor and exported as .STL files to be prototyped. The files were then prepared for prototyping using 3D Lightyear Software. The parts were built using a 3D Systems Viper SLA System (3D Systems Inc., Rock Hill, SC) using Accura 60 stereolithography resin (3D Systems Inc.). The powders were aerosolized until a total air volume of 4 L was drawn through the inhalers at ambient conditions. All measurements were made with at least three replicates. The stages of the impactor were coated with silicone spray to minimize particle bounce and re-entrainment.

## RESULTS AND DISCUSSION

### Characterization of the Physico-Chemical Properties of the Spray Dried Azithromycin EEG Formulations

The majority of the combination particles produced using the spray drying technique were submicrometer in size; although there were some particles that were about 1-1.5 µm indicating the polydispersity of the powder. Figure 1 shows a scanning electron micrograph of the F2 formulation. Thermogravimetric analysis revealed that AZ was present in the anhydrous form in the combination particles and the powders had water contents of 3 and 5.7 % for F1 and F2, respectively. The mean (SD) measured content of AZ in F1 and F2 were 28.7 (3.8) % and 19.4 (4.2) %, respectively. Initial stability studies revealed no statistically significant change in the AZ content following storage for 1 month at room temperature for F1 and F2.

### Aerosolization Characteristics of Spray Dried Azithromycin EEG Formulations

Aerosolization of the spray dried powders using the EEG DPI revealed that the formulation containing sodium chloride as the hygroscopic excipient had relatively high retention within the capsule and deposition in the DPI. In contrast, replacing the excipient with mannitol significantly reduced the retention of the powder formulation both in the capsule and on the DPI device. Table 1 shows that the mean emitted doses for F1 and F2 were 51.0% and 89.9%, respectively. Increasing the loaded dose of F2, from 2mg to 5mg, had a significant effect on the amount of powder delivered from the device. The fraction of a 5mg loaded dose emitted from the device was 76.9%.

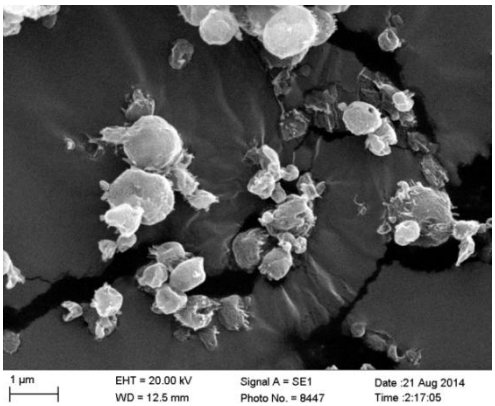


**Figure 1:** Scanning electron micrograph of the spray dried azithromycin / mannitol formulation (F2).

The aerosolization characteristics of the two formulations are also shown in Table 1. The sodium chloride / AZ formulation (F1) had a lower MMAD (1.2 µm) than the AZ / mannitol formulation (1.6 µm). This difference in MMAD is small but statistically significant, however both powders are expected to be suitable for EEG delivery. Increasing the loaded dose of F2 in the DPI did not affect the ability of the device to efficiently aerosolize the powder and there was no difference in MMAD compared to a 2 mg loaded dose of F2.

**Table 1:** Aerosolization characteristics of spray dried combination particle AZ formulations. Standard deviation is shown in parenthesis [n=3-5].

| | F1 (2mg) | F2 (2mg) | F2 (5mg) |
|---|---|---|---|
| Emitted dose (%)* | 51.0 (4.1) | 89.9 (9.2)** | 76.9 (5.6)**^ |
| $FPF_{<5\mu m/ED}$ (%) | 87.9 (6.5) | 86.4 (4.3) | 85.3 (6.4) |
| $FPF_{<1\mu m/ED}$ (%)* | 38.3 (2.0) | 23.4 (3.3)** | 21.4 (1.1)** |
| MMAD (µm)* | 1.2 (0.1) | 1.6 (0.1)** | 1.6 (0.0)** |

*P<0.05 significant effect of formulation on emitted dose, $FPF_{<1\mu m/ED}$ and MMAD (one-way ANOVA).
**P<0.05 significant difference compared F1 (post-hoc t-test).
^ P< 0.05 significant difference between F2 (2mg) and F2 (5mg)

Both powders, F1 and F2, had high fine particle fractions less than 5 µm ($FPF_{<5\mu m/ED}$), with over 85% of the emitted dose in this size range. The fraction of particles less than 1 µm ($FPF_{<1\mu m/ED}$) was higher than produced by conventional DPIs, and hygroscopic growth of these particles would prevent their exhalation and ensure deposition. Increasing the loaded dose of F2 from 2 mg to 5 mg had no significant effect on the aerosolization properties using the EEG DPI.

## CONCLUSIONS

Spray dried azithromycin powders were produced with good aerosolization characteristics suitable for delivery using the EEG DPI. Formulations containing sodium chloride as the hygroscopic excipient appeared smaller in size compared to the mannitol formulation. High efficiency aerosolization of the powders was possible using the EEG DPI for 2 mg and 5 mg loaded doses.

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**REFERENCES**


1. Hickey AJ, Lu D, Ashley ED, and Stout J: Inhaled azithromycin therapy. J Aerosol Med. 2006;19:54-60.
2. Kanoh S and Rubin BK: Mechanisms of action and clinical application of macrolides as immunomodulatory medications. Clin Microbiol Rev. 2010;23:590-615.
3. Li X, Vogt FG, Hayes D, Jr., and Mansour HM: Physicochemical characterization and aerosol dispersion performance of organic solution advanced spray-dried microparticulate/nanoparticulate antibiotic dry powders of tobramycin and azithromycin for pulmonary inhalation aerosol delivery. Eur J Pharm Sci. 2014;52:191-205.
4. Zhang Y, Wang X, Lin X, Liu X, Tian B, and Tang X: High azithromycin loading powders for inhalation and their in vivo evaluation in rats. Int J Pharm. 2010;395:205-214.
5. Behara SR, Longest PW, Farkas DR, and Hindle M: Development and comparison of new high-efficiency dry powder inhalers for carrier-free formulations. J Pharm Sci. 2014;103:465-477.
6. Son Y-J, Longest PW, and Hindle M: Aerosolization characteristics of dry powder inhaler formulations for the excipient enhanced growth (EEG) application: Effect of spray drying process conditions on aerosol performance. Int J Pharm. 2013;443:137-145.
7. Hindle M and Longest PW: Condensational growth of combination drug-excipient submicrometer particles for targeted high efficiency pulmonary delivery: Evaluation of formulation and delivery device. Journal of Pharmacy and Pharmacology. 2012;64:1254-1263.
8. Tian G, Longest PW, Li X, and Hindle M: Targeting aerosol deposition to and within the lung airways using excipient enhanced growth. J Aerosol Med Pulm Drug Deliv. 2013;26:248-265.
9. Longest PW and Hindle M: Numerical Model to Characterize the Size Increase of Combination Drug and Hygroscopic Excipient Nanoparticle Aerosols. Aerosol Sci Technol. 2011;45:884-899.