FORMULATION, CHARACTERIZATION AND PHARMACOKINETIC MODELLING OF EXCIPIENT ENHANCED GROWTH SPRAY-DRIED INHALATION POWDERS

Serena Bonasera
Virginia Commonwealth University

Follow this and additional works at: https://scholarscompass.vcu.edu/etd

Part of the Pharmaceutics and Drug Design Commons

© The Author

Downloaded from
https://scholarscompass.vcu.edu/etd/6809

This Dissertation is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of VCU Scholars Compass. For more information, please contact libcompass@vcu.edu.
FORMULATION, CHARACTERIZATION AND PHARMACOKINETIC MODELLING OF EXCIPIENT ENHANCED GROWTH SPRAY-DRIED INHALATION POWDERS

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

by

Serena Bonasera,
M.Pharm., University of Messina, Italy, 2016

Director: Michael Hindle, Ph.D.,
Professor, Department of Pharmaceutics

Virginia Commonwealth University
Richmond, Virginia,
October, 2021
ACKNOWLEDGMENT

I am extremely grateful for reaching this step in my personal and professional development and I want to thank everyone who has been of help and support along this journey.

I am and always will be thankful to my advisor and mentor, Professor Michael Hindle for the incredible guidance, support and patience provided during my doctorate pathway. He gave me the great opportunity to work in a very collaborative team. He allowed me to become a scientist by challenging but guiding and supporting my research ideas.

I would also like to thank my Graduate Advisory Committee members: Dr. Worth Longest, Dr. Jürgen Venitz, Dr. Qingguo Xu, Dr. Thomas Roper, Dr. Aaron May for their expert support and guidance. I would like to thank Dr. Masahiro Sakagami for the comments and challenges provided during the aerosol research meetings.

I would like to thank previous and current members of the Aerosol Research Group Dr. Amr Hassan, Dr. Mohammad Momin, Dr. Susan Boc, Dr. Xiangyin Wei, Dr. Sneha Dhapare, Dr. Abhinav Mohan, Varsha Nair, Yuan Xiao for directing and guiding me during these four years and for becoming great friends. Also, I would like to thank the members of the Mechanical and Nuclear Engineering for their tremendous practical and scientific help: Dr. Dale Farkas, Dr. Karl Bass, Ben Spence, Ghali Aladwani, Sarah Strickler, Morgan Thomas, Connor Howe.

Finally, I would like to thank my family and friends for the emotional support and the strength provided me during the past four years, despite the challenging distance and time zone difference. A special thanks goes to my mum, dad, and brother for being my rock in life, and to my grandad for being a great model of resilience. Great thanks to Claudio for always being there for me.
3.2 Materials and methods---------------------------------------------------------------27
3.2.1 Materials ...........................................................................................................27
3.2.2 Preparation of AS EEG powders .......................................................................27
3.2.3 Particle size characterization............................................................................28
3.2.4 Scanning electron microscopy and powder density ........................................29
3.2.5 Dynamic vapor sorption analysis .....................................................................29
3.2.6 X-ray powder diffraction analysis ..................................................................30
3.2.7 In vitro aerosol performance testing .................................................................30
3.2.8 Excipient enhanced growth aerosol characterization.........................................32
3.2.9 Statistical analysis ............................................................................................32
3.3 Results.....................................................................................................................33
3.3.1 Evaluation of the effects of spray drying and formulation variables ..............33
3.3.2 EEG powder characterization ...........................................................................39
3.3.2.1 SEM and powder bulk characterization .........................................................39
3.3.2.2 Dynamic vapor sorption analysis ................................................................40
3.3.2.3 X-ray powder diffraction analysis ...............................................................41
3.3.2.4 Excipient enhanced growth aerosol characterization ....................................45
3.4 Discussion and conclusions......................................................................................46

CHAPTER 4: ADVANCEMENTS FOR A TOBRAMYCIN EXCIPIENT ENHANCED GROWTH SPRAY-DRIED POWDER FORMULATION DELIVERED BY A NOVEL POSITIVE PRESSURE DRY POWDER INHALER..........................................................51
4.1 Introduction ............................................................................................................51
4.2 Materials and methods ..........................................................................................52
4.2.1 Materials ...........................................................................................................52
4.2.2 Assessment of the spray drying and storage conditions for tobramycin excipient enhanced growth (EEG) spray-dried powder formulations ..........................................................52
4.2.2.1 Preparation of tobramycin EEG spray-dried powders ..................................52
4.2.2.2 Solid-state characterization of tobramycin EEG spray-dried powders ..........53
4.2.2.3 In vitro aerosol performance of tobramycin EEG spray-dried powders ........54
4.2.3 Optimization of composition of tobramycin EEG spray-dried powder formulation ..........................................................55
4.2.3.1 Preparation of tobramycin EEG spray-dried powders ..................................55
4.2.3.2 Solid-state characterization of tobramycin EEG spray-dried powders ..........56
4.2.3.3 In vitro aerosol performance of tobramycin EEG spray-dried powders ........57
4.2.3.4 In vitro aerosol performance of tobramycin EEG spray-dried powders using realistic airway conditions ..........................................................58
5.2.4.1.2

5.2.4.1.1

5.2.4.1

5.2.4

5.2.3

5.2.2

5.2.1

5.2

5.1

DIFFRACTION TESTING

ENHANCED GROWTH SPRAY

CHAPTER 5: RAPID SCREENING OF THE AEROSOL PERFORMANCE OF EXCIPIENT

4.4.2

4.4.1

4.4

4.3.3.5

4.3.3.4

4.3.3.3

4.3.3.2

4.3.3.1

4.3.3

4.3.2.2

4.3.2.1

4.3.2

4.3.1.3

4.3.1.2

4.3.1.1

4.3.1

4.3

4.2.4

Aerosol performance testing using laser diffraction

In vitro

Solid

Preparation of the spray

Materials

Materials and methods

Introduction

Optimization of composition of tobramycin EEG spray

Assessment of the optimized spray drying and storage conditions for a model

dried powder formulations

Dynamic vapor sorption analysis

Primary particle size, powder polydispersity properties, bulk density and morphology

Dynamic vapor sorption analysis

X-ray powder diffraction analysis of tobramycin EEG powder formulations

In vitro aerosol performance using cascade impaction method

In vitro aerosol performance of tobramycin EEG powders using realistic airway conditions

Discussion and conclusions

Assessment of the optimized spray drying and storage conditions for a model tobramycin EEG

dried powder

Optimization of composition of tobramycin EEG spray-dried powder formulation

CHAPTER 5: RAPID SCREENING OF THE AEROSOL PERFORMANCE OF EXCIPIENT

ENHANCED GROWTH SPRAY-DRIED POWDER FORMULATION USING LASER

DIFFRACTION TESTING

Introduction

Materials and methods

Materials

Preparation of the spray-dried powder formulations

Solid-state characterization of EEG powders

In vitro aerosol particle sizing of EEG powder formulations

Aerosol performance testing using laser diffraction

AS and BUD EEG formulation testing

Tobramycin excipient enhanced growth spray-dried powder formulation testing

vi
CHAPTER 6: DEVELOPMENT AND VALIDATION OF PHARMACOKINETIC MODEL TO PREDICT THE SYSTEMIC EXPOSURE OF PEDIATRIC CYSTIC FIBROSIS SUBJECTS .......... 128

6.1 Introduction .................................................................................................................. 128
6.2 Methods ....................................................................................................................... 128
6.2.1 PK study selection ..................................................................................................... 128
6.2.2 Body weight imputation and Non-compartmental PK analysis .................................. 129
6.2.2.1 Non-compartmental analysis: IV administration ...................................................... 129
6.2.2.2 Non-compartmental analysis: INH administration .................................................. 130
6.2.2.3 Non-compartmental analysis acceptance criteria .................................................... 131
6.2.3 In vitro DTL determination for nebulization of tobramycin in pediatric subjects .......... 132
6.2.4 Determination of AUC∞IV and AUC∞INH following administration to pediatric subjects ..... 133
6.2.4.1 Determination of AUCα IV following IV administration of tobramycin in pediatric subjects ..... 133
6.2.5 Determination of AUC∞INH following nebulized administration of tobramycin in pediatric subjects............................................................. 134
6.2.6 Prediction of estimated local and systemic exposure for tobramycin excipient enhanced growth formulation in the novel positive air gas source dry powder inhaler in pediatric subjects .......... 135
6.3 Results .......................................................................................................................... 136
6.3.1 In vitro DTL determination for nebulization of tobramycin in pediatric subjects .......... 136
6.3.2 Summary and discussion of IV and INH PK study selection ....................................... 137
6.3.2.1 Pediatric studies ..................................................................................................... 137
6.3.2.2 Adult studies ......................................................................................................... 140
6.3.3 Non-compartmental PK analysis for pediatric IV studies............................................. 143
6.3.4 Non-compartmental PK analysis for adult IV study .......................................................... 147
6.3.5 Dose proportionality and effect of age and body weight on clearance and volume of distribution ...................................................................................................................................... 150
6.3.6 Non-compartmental PK analysis for pediatric INH nebulizer study .................................... 153
6.3.7 Non-compartmental PK analysis for adult INH nebulization studies ........................................ 158
6.3.8 Prediction of AUC∞IV for adult and pediatric subjects ......................................................... 161
6.3.9 Prediction of AUC∞INH for adult and pediatric subjects following inhaled nebulizer administration .................................................................................................................... 163
6.3.10 Estimated AUC∞INH for tobramycin excipient enhanced growth formulation in the novel positive air gas source dry powder inhaler in pediatric subjects and FPUL sensitivity analysis 165
6.4 Discussion and Conclusions ........................................................................................................ 168
6.4.1 Review of the studies employed in the pharmacokinetic analysis ........................................... 168
6.4.2 In vitro DtL determination of tobramycin in pediatric subjects .................................................. 169
6.4.3 Comparison of systemic exposures following tobramycin IV administration in pediatric and adult CF subjects ........................................................................................................... 170
6.4.4 Comparison of systemic exposures following nebulized tobramycin INH administration in pediatric and adult CF subjects ........................................................................................................... 172
6.4.5 Prediction of the systemic exposure in pediatric CF subjects following inhalation of a tobramycin excipient enhanced growth formulation in the novel positive air gas source dry powder inhaler and sensitivity analysis ........................................................................................................... 174

CHAPTER 7: SUMMARY AND CONCLUSIONS ............................................................................... 178

LIST OF REFERENCE .................................................................................................................. 182
LIST OF FIGURES

Figure 1.1 Schematic settings of laser diffraction measurements. Adapted from Mitchell et al., 2006......5

Figure 1.2 Schematic representation for semi-PBPK model for adult CF and healthy subjects............ 10

Figure 3.1 Schematic representation of the CC_{90}-3D dry powder inhaler. ................................ 31

Figure 3.2 Spray rate (mL/min) as a function of different peristaltic pump rate and spray head vibration. 36

Figure 3.3 Scanning electron micrographs of the (a) L-leucine and (b) trileucine AS EEG powder formulations. ................................................................. 39

Figure 3.4 Dynamic vapor sorption profiles for the L-leucine and the trileucine AS EEG powder formulations. ...................................................................................... 41

Figure 3.5 XRPD diffractograms for albuterol sulfate, mannitol, trileucine, and L-leucine raw materials. 44

Figure 3.6 XRPD diffractograms of L-leucine and trileucine AS EEG powder formulations.............. 44

Figure 3.7 XRPD diffractograms of L-leucine and trileucine AS EEG powder formulations after moisture exposure. ................................................................. 45

Figure 4.1 Schematic representation of the DAC v3 positive pressure DPI. ........................................ 55

Figure 4.2 Schematic representation of the modified positive pressure DAC v3 DPI with the mouthpiece adaptor containing the 3D rod array. Adapted from Farkas et al., 2020. ................................ 58

Figure 4.3 Schematic representation of the realistic in vitro aerosol performance test setup used for modified DAC v3 DPI - TOBI EEG formulation combination. ........................................ 59

Figure 4.4 Particle size frequency distribution at t=0 for the tobramycin EEG powder formulations spray-dried at 11.9 g/m^3 water vapor density and stored at 15, 35 and 60 %RH and room temperature (RT). Markers represent mean values and error bars represent standard deviation (SD) (n = 3). ..................................... 62

Figure 4.5 Particle size frequency distribution at t=0 for the tobramycin EEG powder formulations spray-dried at 4.2 g/m^3 water vapor density and stored at 15, 35 and 60 %RH and RT. Markers represent mean values and error bars represent SD (n = 3)......................................................... 62

Figure 4.6 Dynamic vapor sorption profiles for tobramycin EEG powder formulations spray-dried at 11.9 g/m^3 outlet water vapor density and initially (t=0) stored at 15, 35 and 60 %RH and room temperature, respectively. ........................................................................................................... 65

Figure 4.7 Dynamic vapor sorption profiles for tobramycin EEG powder formulations spray-dried at 4.2 g/m^3 outlet water vapor density and initially (t=0) stored at 15, 35 and 60 %RH and room temperature, respectively. ........................................................................................................... 65
Figure 4.8 Emitted dose (% nominal dose) for the tobramycin EEG powder formulations spray-dried at 4.2 and 11.9 g/m$^3$ outlet water vapor density and initially stored (t=0) at 15, 35 and 60 %RH and room temperature, respectively. Markers represent mean values and error bars represent SD (n = 3).

Figure 4.9 Pre-separator drug deposition (% nominal dose, left plot) and mass median aerodynamic diameter (right plot) for the tobramycin EEG powder formulations spray-dried at 4.2 and 11.9 g/m$^3$ outlet water vapor density and initially stored (t=0) at 15, 35 and 60 %RH and room temperature, respectively. Markers represent mean values and error bars represent SD (n = 3).

Figure 4.10 Fine particle fraction smaller than 5 µm (left plot) and 1 µm (right plot) reported as % of impactor dose for the tobramycin EEG powder formulations spray-dried at 4.2 and 11.9 g/m$^3$ outlet water vapor density and initially stored (t=0) at 15, 35 and 60 %RH and room temperature, respectively. Markers represent mean values and error bars represent SD (n = 3).

Figure 4.11 Dynamic vapor sorption profiles for tobramycin EEG powder formulation spray-dried at 11.9 g/m$^3$ outlet water vapor density following storage at 15 %RH and room temperature measured at t=0 and 6 months.

Figure 4.12 Dynamic vapor sorption profiles for tobramycin EEG powder formulation spray-dried at 4.2 g/m$^3$ outlet water vapor density following storage at 15 %RH and room temperature measured at t=0 and 6 months.

Figure 4.13 Dynamic vapor sorption profiles for tobramycin EEG powder formulation spray-dried at 11.9 g/m$^3$ outlet water vapor density following storage at 35 %RH and room temperature measured at t=0 and 6 months.

Figure 4.14 Dynamic vapor sorption profiles for tobramycin EEG powder formulation spray-dried at 4.2 g/m$^3$ outlet water vapor density following storage at 35 %RH and room temperature measured at t=0 and 6 months.

Figure 4.15 Emitted dose (% nominal dose) over time for the tobramycin EEG powder formulations spray-dried at 11.9 (circle) and 4.2 g/m$^3$ (square) outlet water vapor densities and stored at 15 %RH and room temperature. Markers represent mean values and error bars represent SD (n = 3).

Figure 4.16 Pre-separator deposition (% nominal dose, left plot) and mass median aerodynamic diameter (right plot) over time for the tobramycin EEG powder formulations spray-dried at 11.9 (circle) and 4.2 g/m$^3$ (square) outlet water vapor densities and stored at 15 %RH and room temperature. Markers represent mean values and error bars represent SD (n = 3).

Figure 4.17 Fine particle fraction (% impactor dose) smaller than 5 µm (left plot) and 1 µm (right plot) over time for the tobramycin EEG powder formulations spray-dried at 11.9 (circle) and 4.2 g/m$^3$ (square) outlet water vapor densities and stored at 15 %RH and room temperature. Markers represent mean values and error bars represent SD (n = 3).

Figure 4.18 Emitted dose (% nominal dose) over time for the tobramycin EEG powder formulations spray-dried at 11.9 (circle) and 4.2 g/m$^3$ (square) outlet water vapor densities and stored at 35 %RH and room temperature. Markers represent mean values and error bars represent SD (n = 3).
Figure 4.19 Pre-separator deposition (% nominal dose, left plot) and mass median aerodynamic diameter (right plot) over time for the tobramycin EEG powder formulations spray-dried at 11.9 (circle) and 4.2 g/m$^3$ (square) outlet water vapor densities and stored at 35 %RH and room temperature. Markers represent mean values and error bars represent SD ($n=3$). ..............................................................78

Figure 4.20 Fine particle fraction (% impactor dose) smaller than 5 µm (left plot) and 1 µm (right plot) over time for the tobramycin EEG powder formulations spray-dried at 11.9 (circle) and 4.2 g/m$^3$ (square) outlet water vapor densities and stored at 35 %RH and room temperature. Markers represent mean values and error bars represent SD ($n=3$)...............................................................79

Figure 4.21 Particle size frequency distribution of the tobramycin EEG powder formulations determined using the Sympatec at a dispersion pressure of 0.5 bar. Markers represent mean values and error bars represent SD ($n=3$).............................................................................................................82

Figure 4.22 Particle size frequency distribution of the tobramycin EEG powder formulations determined using the Sympatec at a dispersion pressure of 4 bar. Markers represent mean values and error bars represent SD ($n=3$).............................................................................................................82

Figure 4.23 Scanning electron micrographs of the tobramycin EEG formulations (a) T1, (b) T2, (c) T3, (d) T4 and (e) T5........................................................................................................................................84

Figure 4.24 Dynamic vapor sorption profiles for tobramycin EEG powder formulation containing TOBI:MAN:LL:POL188 as 60:18:20:2 %w/w (T1). .............................................................................................................87

Figure 4.25 Dynamic vapor sorption profiles for tobramycin EEG powder formulation containing TOBI:MAN:TL:POL188 as 60:18:20:2 %w/w (T2). .............................................................................................................87

Figure 4.26 Dynamic vapor sorption profiles for tobramycin EEG powder formulation containing TOBI:SO$_4$^2-:MAN:TL:POL188 as 49:18:15:16:2 %w/w (T3).............................................................................................................88

Figure 4.27 Dynamic vapor sorption profiles for tobramycin EEG powder formulation containing TOBI:LL as 80:20 %w/w (T4).............................................................................................................88

Figure 4.28 Dynamic vapor sorption profiles for tobramycin EEG powder formulation containing TOBI:SO$_4$^2-:TL as 62:23:15 %w/w (T5).............................................................................................................89

Figure 4.29 XRPD diffractograms for tobramycin base, tobramycin sulfate, mannitol, trileucine, and L-leucine raw materials. .............................................................................................................92

Figure 4.30 XRPD diffractograms of T1 and T4 tobramycin EEG powder formulations containing L-leucine.............................................................................................................92

Figure 4.31 XRPD diffractograms of T2, T3 and T5 tobramycin EEG powder formulations containing trileucine. .............................................................................................................93

Figure 4.32 XRPD diffractograms of T1 and T4 tobramycin EEG powder formulations containing L-leucine after moisture exposure. .............................................................................................................93
Figure 4.33 XRPD diffractograms of T2, T3 and T5 tobramycin EEG powder formulations containing trileucine after moisture exposure..............................................................94

Figure 5.1 Schematic representation of air inlet aperture position 1 (left) and 2 (right). Adapted from Behara et al., 2014..................................................................................................................106

Figure 5.2 Set up for the LD – realistic inhalation testing using Spraytec with inhalation cell system... 107

Figure 5.3 Schematic representation of the realistic mouth-throat model testing using the CC90-3D dry powder inhaler. ..........................................................................................................................109

Figure 5.4 Schematic setting for the CI – realistic inhalation testing using Next Generation Impactor and breath simulator. Adapted from Wei et al., 2017...........................................................................................................110

Figure 5.5 Typical time history profile of the aerosol emission for each of the two capsule air inlet positions plotted as the % transmission (top) and Dv50 (bottom) against time for the BUD-EEG (left) and AS-EEG (right) aerosols........................................................................................................................113

Figure 5.6 Capsule retention and filter deposition for BUD EEG (left) and AS EEG (right) when aerosolized using AIP 1 and AIP 2 using laser diffraction and mouth-throat model methods......................115

Figure 5.7 Isotherm profiles of AS EEG batch 1, 2, 3, and 4 obtained using dynamic vapor sorption analysis..................................................................................................................................................117

Figure 5.8 Emitted dose (% of nominal dose) for AS EEG batch 1, 2, 3, and 4 using CI and LD methods (n = 3).............................................................................................................................................119

Figure 5.9 Fine particle fraction smaller than 5 µm (% of emitted dose) for AS EEG batch 1, 2, 3, and 4 using CI and LD methods (n = 3). ..............................................................................................................119

Figure 5.10 Fine particle fraction smaller than 1 µm (% of emitted dose) for AS EEG batch 1, 2, 3, and 4 using CI and LD methods (n = 3). ..............................................................................................................120

Figure 5.11 Linear relationship between the volume concentration obtained from the laser diffraction and the emitted dose obtained from the cascade impactor. Values are reported as means (n = 3).................123

Figure 5.12 Linear relationship between the Dv50 obtained from the laser diffraction and the MMAD obtained from the cascade impactor. Values are reported as means (n = 3)..............................123

Figure 5.13 Linear relationship between the percentages of fine particle fraction smaller than 5 µm obtained from the laser diffraction and the cascade impactor. Values are reported as means (n = 3) ..... 124

Figure 5.14 Linear relationship between the percentages of fine particle fraction smaller than 1 µm obtained from the laser diffraction and the cascade impactor. Values are reported as means (n = 3) ..... 124

Figure 6.1 In vitro test setup to estimate Dtl using a realistic airway model and the PARI-LC® PLUS nebulizer..................................................................................................................................................133
Figure 6.2 Cystic Fibrosis Foundation weight percentile vs. subject age (years) over 1989-2018 years used for body weight imputation.

Figure 6.3 Reported and imputed body weight (kg) vs age (years) proportionality for the 13 pediatric subgroups (blue circles) obtained from 5 IV studies and the single adult IV study (red diamond).

Figure 6.4 NCA and reported total clearance (left) and body weight normalized clearance (right) vs. age (years) for the 13 pediatric subgroups (blue circles) obtained from 5 tobramycin pediatric studies and the single adult IV study (red diamond). Dashed line on the right plot represents the mean value obtained from all IV pediatric studies.

Figure 6.5 NCA and reported total clearance (left) and body weight normalized clearance (right) vs. body weight for the 13 pediatric subgroups (blue circles) obtained from 5 tobramycin pediatric studies and the single adult IV study (red diamond). Dashed line on the right plot represents the mean value obtained from all IV pediatric studies.

Figure 6.6 NCA and reported volume of distribution (left) and body weight normalized volume of distribution (right) vs. age (years) for the 13 pediatric subgroups (blue circles) obtained from 5 tobramycin pediatric studies and the single adult IV study (red diamond). Dashed line on the right plot represents the mean value obtained from all IV pediatric studies.

Figure 6.7 NCA and reported volume of distribution (left) and body weight normalized volume of distribution (right) vs. body weight for the 13 pediatric subgroups (blue circles) obtained from 5 tobramycin pediatric studies and the single adult IV study (red diamond). Dashed line on the right plot represents the mean value obtained from all IV pediatric studies.

Figure 6.8 IV infusion dose escalation plots of AUC vs. dose of tobramycin infused (left plot) and body weight normalized dose (right plot) for the 13 pediatric subgroups (blue circles) obtained from 5 tobramycin pediatric studies and the single adult IV study (red diamond).

Figure 6.9 Predicted AUC$_{\text{IV}}$ vs. IV nominal dose (left plot) and IV BW-normalized dose (right plot) for pediatric (blue line) and adult (red line) subjects following IV administration. Blue circles represent the observed AUC$_{\text{IV}}$ from 13 pediatric subgroups obtained from 5 IV studies; red diamond represents the observed AUC$_{\text{IV}}$ from a single adult IV study.

Figure 6.10 Predicted AUC$_{\text{INH}}$ vs nominal dose loaded in the device (left plot) and BW-normalized dose (right plot) for pediatric (blue line) and adult (red line) subjects following nebulization. Blue circle represents the observed AUC$_{\text{INH}}$ from a single pediatric inhalation study; red diamonds represent the observed AUC$_{\text{INH}}$ from 3 adult inhalation studies.

Figure 6.11 Predicted AUC$_{\text{INH}}$ vs dose to lung (left plot) and BW-normalized dose to lung (right plot) for pediatric (blue line) and adults (red line) subject following nebulization. Blue circle represents the observed AUC$_{\text{INH}}$ from a single pediatric inhalation study; red diamonds represent the observed AUC$_{\text{INH}}$ from 3 adult inhalation studies.

Figure 6.12 Predicted AUC$_{\text{INH}}$ vs nominal dose (left plot) and body weight-normalized nominal dose (right plot). Circles represent the predicted AUC$_{\text{INH}}$ for nebulized delivery. Triangles represent the predicted AUC$_{\text{INH}}$ for delivery using the EEG formulation/device combination. Mean and standard
deviation values obtained from the NCA obtained in study PINH, are reported as dark solid and dot lines, respectively.

Figure 6.13 Predicted $\text{AUC}_e^{\text{INH}}$ vs nominal dose (left plot) and body weight-normalized nominal dose (right plot). Circles represent the predicted $\text{AUC}_x^{\text{INH}}$ for nebulized delivery. Triangles, squares and asterisk represent the predicted $\text{AUC}_x^{\text{INH}}$ for the EEG formulation/device combinations $F_{\text{PUL}} = 16.8\%$, 33.7$\%$ and 67.4$, $respectively. Mean and standard deviation values obtained from the NCA obtained in study PINH, are reported as dark solid and dot lines, respectively.
LIST OF TABLES

Table 3.1 Spray drying conditions and dispersion enhancer used to produce AS EEG powder formulations ................................................................. 28

Table 3.2 Effect of nebulizer mesh hole size on particle characteristics and in vitro aerosol performance of AS EEG powder formulation (values are reported as means (SD), n ≥ 3) .................................................................................. 34

Table 3.3 Effect of % peristaltic pump and % spray head vibration on particle characteristics and in vitro aerosol performance of AS EEG powder formulations (values are reported as means (SD), n ≥ 3). ........................................... 36

Table 3.4 Effect of inlet gas drying temperature on particle characteristics and in vitro aerosol performance of AS EEG powder formulations (values are reported as means (SD), n ≥ 3) .................................................................................. 38

Table 3.5 Effect of dispersion enhancer on particle characteristics and in vitro aerosol performance of AS EEG powder formulations (values are reported as means (SD), n ≥ 3) .................................................................................. 38

Table 3.6 Dynamic vapor sorption analysis for the L-leucine and trileucine AS EEG powder formulations .................................................................................................................................. 41

Table 3.7 Aerosol characteristics of EEG powder formulations following exposure to ambient and simulated airway conditions (values are reported as means (SD), n ≥ 3).................................................................................................................................. 46

Table 4.1 Measured outlet water vapor density during spray drying and storage conditions for tobramycin EEG spray-dried powder formulations ........................................................................................................ 53

Table 4.2 Tobramycin EEG spray-dried powder formulations, components and component ratio .............................................................................. 56

Table 4.3 Particle size and Span measurements at t=0 for tobramycin EEG powder formulations spray-dried at 11.9 g/m³ and 4.2 g/m³ outlet water vapor density and stored at 15, 35 and 60 %RH and room temperature (RT). Values are reported as means (SD), n = 3. ........................................................................................................ 61

Table 4.4 Dynamic vapor sorption analysis for tobramycin EEG powder formulations spray-dried at 11.9 and 4.2 g/m³ outlet water vapor densities and initially stored (t=0) at 15 and 35 %RH and room temperature. ........................................................................................................ 66

Table 4.5 Dynamic vapor sorption analysis for tobramycin EEG powder formulations spray-dried at 11.9 and 4.2 g/m³ outlet water vapor densities and stored for 6 months at 15 and 35 %RH and room temperature. ........................................................................................................ 73

Table 4.6 Mean (SD) particle size characteristics measured at 0.5 and 4 bar dispersion pressure and bulk density measurements of the tobramycin EEG powder formulations (n= 3) ........................................................................................................ 81

Table 4.7 Dynamic vapor sorption analysis for tobramycin EEG powder formulations T1 to T5. ........................................................................ 89

Table 4.8 Mean (SD) aerosol performance characteristics of the tobramycin EEG powder formulations T1 to T5 (n = 3) .................................................................................................................................. 95
Table 4.9 Aerosol characteristic of tobramycin EEG powder formulation T5 following exposure to ambient and simulated airway conditions. Values represent mean (SD), \((n = 3)\) ..................................96

Table 5.1 Albuterol and budesonide excipient enhanced growth spray-dried powder formulations total solids content, components and component ratio. .........................................................103

Table 5.2 Mean (SD) particle size characteristics of the emitted aerosol from the two spray-dried EEG formulations measured using the laser diffraction method \((n>3)\). ......................................................112

Table 5.3 Particle size characteristics at 1 and 4 bar, true density measurements (reported as mean (SD), \(n = 3\)), and percentage weight loss \((n = 1)\) for AS EEG batch 1, 2, 3, and 4. ......................................................116

Table 5.4 Particle size characteristics of emitted aerosols measured using CI and LD methods \((n = 3)\). 121

Table 5.5 Mean (SD) particle size characteristics and concentration volume of the emitted aerosols from the tobramycin EEG formulations measured using the laser diffraction method \((n = 3)\). ......................................................122

Table 6.1 Mean (SD) tobramycin deposition in realistic in vitro testing using the PARI-LC\textsuperscript{®} PLUS Reusable nebulizer to determine the DtL .................................................................136

Table 6.2 Summary of identified pediatric IV tobramycin pharmacokinetic studies. Tabulated key study design elements: number of subjects per study \((n)\), year of study, subject disease state (CF-Cystic Fibrosis, or other), subject age (years), body weight (kg), dose strength, analytical method (LLOQ if reported), sampling schedule, availability of \(C(p(t))\) profile, and the availability of \(C(s(t))\) profile ..............................................................139

Table 6.3 Summary of identified pediatric INH nebulizer pharmacokinetic study. Tabulated key study design elements: number of subjects per study \((n)\), year of study, subject disease state (CF-Cystic Fibrosis, or other), subject age (years), body weight (kg), dose, analytical method (LLOQ if reported), inhalation device used, sampling schedule, availability of \(C(p(t))\) profile, availability of \(C(s(t))\) profile, and information about in vivo aerosol deposition ..............................................................140

Table 6.4 Summary of the identified adult IV tobramycin pharmacokinetic study. Tabulated key study design elements: number of subjects per study \((n)\), year of study, subject disease state (CF-Cystic Fibrosis, or other), subject age (years), body weight (kg), dose strength, analytical method (LLOQ if reported), sampling schedule, availability of \(C(p(t))\) profile, and the availability of \(C(s(t))\) profile ..............................................................142

Table 6.5 Summary of identified adult INH nebulized tobramycin pharmacokinetic studies. Tabulated key study design elements: number of subjects per study \((n)\), year of study, subject disease state (CF-Cystic Fibrosis, or other), subject age (years), body weight (kg), dose, analytical method (LLOQ if reported), inhalation device used, sampling schedule, availability of \(C(p(t))\) profile, availability of \(C(s(t))\) profile, and information about in vivo aerosol deposition ..............................................................143

Table 6.6 Pharmacokinetic NCA estimated and reported parameters (RP) for each of the individual data sets from the identified tobramycin pediatric IV studies. Tabulated PK metrics: number of subjects per subgroup \((n)\), total clearance (mL/min), body weight-normalized total clearance (mL/min/kg), volume of distribution at steady state (L), body weight-normalized volume of distribution at steady state (L/kg), \(AUC_{\infty}\) (mg*min/L), half-life (min), % extrapolated, obtained \(R^2\), subject age (years), body weight (kg), the PK analysis conducted, dose (mg and mg/kg) ..............................................................145
Table 6.7 Summary PK metrics statistics obtained from NCA estimates and reported parameters from the identified tobramycin pediatric IV studies (133 subjects). Tabulated mean, standard deviation (SD), coefficient of variation (COV, %), median, max and min, and fold change for $CL_{TOT}$ (mL/min), BW-normalized $CL_{TOT}$ (mL/min/kg), $V_{dss}$ (L), BW-normalized $V_{dss}$ (L/kg), AUC$_{0\rightarrow\infty}$ (mg*min/L), AUC$_{\infty}$ (mg*min/L), half-life (min), age (years), body weight (kg), and dose (in mg and mg/kg). *n=9

Table 6.8. Pharmacokinetic NCA estimated and reported parameters (RP) for each of the individual data sets from the identified tobramycin adult IV study. Tabulated PK metrics: $C_{\text{max}}$ (mg/L), $t_{\text{max}}$ (min), total clearance (mL/min), body weight-normalized total clearance (mL/min/kg), volume of distribution at steady state (L), body weight-normalized volume of distribution at steady state (L/kg), AUC$_{0\rightarrow\infty}$ (mg*min/L), % extrapolated, AUC$_{\infty}$ (mg*min/L), half-life (min), obtained $R^2$, the PK analysis conducted, dose (mg and mg/kg). 

Table 6.9. Summary PK metrics statistics obtained from NCA estimates from 6 subjects in the adult IV study. Tabulated mean, standard deviation (SD), coefficient of variation (COV, %), median, max and min, fold range, and interquartile range for $C_{\text{max}}$ (mg/L), $t_{\text{max}}$ (min), total clearance (mL/min), body weight-normalized total clearance (mL/min/kg), volume of distribution at steady state (L), body weight-normalized volume of distribution at steady state (L/kg), AUC$_{0\rightarrow\infty}$ (mg*min/L), AUC$_{\infty}$ (mg*min/L), half-life (min).

Table 6.10 Pharmacokinetic NCA estimated parameters for 14 subjects in the single pediatric tobramycin nebulizer inhalation study. Tabulated PK metrics: $C_{\text{max}}$ (mg/L), $t_{\text{max}}$ (min), AUC$_{0\rightarrow\infty}$ (mg*min/L), % extrapolated, AUC$_{\infty}$ (mg*min/L), half-life (min), $R^2$

Table 6.11 Estimated $F_{\text{INH}}$ and $F_{\text{PUL}}$ obtained from NCA for 14 subjects in the single pediatric tobramycin nebulizer inhalation study. Tabulated PK metrics: $F_{\text{INH}}$ (%) obtained using AUC$_{0\rightarrow\infty}$ and AUC$_{\infty}$, and $F_{\text{PUL}}$ (%) obtained using AUC$_{0\rightarrow\infty}$ and AUC$_{\infty}$.

Table 6.12 Summary PK metrics statistics obtained from NCA for 13 subjects in the single pediatric tobramycin nebulizer inhalation study. Tabulated mean, standard deviation (SD), coefficient of variation (COV, %), median, max and min, and fold range for $C_{\text{max}}$ (mg/L), $t_{\text{max}}$ (min), AUC$_{0\rightarrow\infty}$ (mg*min/L), AUC$_{\infty}$ (mg*min/L), half-life (min).

Table 6.13 Summary statistics for estimated $F_{\text{INH}}$ and $F_{\text{PUL}}$ obtained from NCA for 13 subjects in the single pediatric tobramycin nebulizer inhalation study. Tabulated PK metrics: mean, standard deviation (SD), coefficient of variation (COV, %), median, max and min, and fold range for $F_{\text{INH}}$ (%) and $F_{\text{PUL}}$ (%).

Table 6.14. Pharmacokinetic NCA estimated and reported parameters (RP) for the three adult tobramycin inhalation nebulizer studies. Tabulated PK metrics: $C_{\text{max}}$ (mg/L), $t_{\text{max}}$ (min), AUC$_{0\rightarrow\infty}$ (mg*min/L), % extrapolated, AUC$_{\infty}$ (mg*min/L), half-life (min), $R^2$.

Table 6.15. Estimated $F_{\text{INH}}$ and $F_{\text{PUL}}$ obtained from the NCA and reported parameters (RP) for the three adult tobramycin inhalation nebulizer studies. Tabulated PK metrics: $F_{\text{INH}}$ (%) and $F_{\text{PUL}}$ (%) obtained using AUC$_{0\rightarrow\infty}$ and AUC$_{\infty}$.

Table 6.16 Summary PK metrics statistics obtained for the three adult tobramycin inhalation nebulizer studies. Tabulated mean, standard deviation (SD), coefficient of variation (COV, %), median, max and
min, and fold range for \( C_{\text{max}} \) (mg/L), \( t_{\text{max}} \) (min), AUC\(_{0-t} \) (mg*min/L), AUC\(_{\infty} \) (mg*min/L), half-life (min).

*\( n=2 \) .................................................................................................................................................................. 161

**Table 6.17** Summary statistics for estimated \( F_{\text{INH}} \) and \( F_{\text{PUL}} \) obtained for the three adult tobramycin inhalation nebulizer studies. Tabulated PK metrics: mean, standard deviation (SD), coefficient of variation (COV,%), median, max and min, and fold range for \( F_{\text{INH}} \) (%) and \( F_{\text{PUL}} \) (%). *\( n=2 \) .............. 161

**Table 6.18** Dose metrics and estimated inhalation bioavailability for pediatric CF subjects using the novel EEG formulation/device combination. Tabulated PK metrics: nominal dose (mg), *in vitro* determined dose to lung (DtL, mg and %), body weight normalized dose to lung (mg/kg), pulmonary bioavailability and inhalation bioavailability (\( F_{\text{INH}} \) and \( F_{\text{PUL}} \), %). .......................................................................................................................... 166

**Table 6.19** Comparison of pediatric and adult PK summary statistics following intravenous administration generated from 13 pediatric studies and a single IV study with 6 subjects. Tabulated mean±standard deviation (SD) or median, range (min-max) for \( C_{\text{max}} \) (mg/L), \( t_{\text{max}} \) (min), CL\(_{\text{TOT}} \) (mL/min), BW-normalized CL\(_{\text{TOT}} \) (mL/min*kg), Vd\(_{ss} \) (L), BW-normalized Vd\(_{ss} \) (L/kg), AUC\(_{ss} \) (mg*min/L), half-life (min), age (years), body weight (kg), dose (mg), BW-normalized dose (mg/kg). ........................................... 172

**Table 6.20** Comparison of pediatric and adult PK summary statistics following inhalation administration generated from 13 subjects of a single inhalation study and 3 adult studies. Tabulated mean±standard deviation (SD) or median, range (min-max) for \( C_{\text{max}} \) (mg/L), \( t_{\text{max}} \) (min), CL\(_{\text{TOT}} \) (mL/min), BW-normalized CL\(_{\text{TOT}} \) (mL/min*kg), Vd\(_{ss} \) (L), BW-normalized Vd\(_{ss} \) (L/kg), AUC\(_{ss} \) (mg*min/L), half-life (min), age (years), body weight (kg), dose (mg), BW-normalized dose (mg/kg). ........................................... 174

**Table 6.21** Predicted systemic exposure for 2, 5, and 12-year-old pediatric CF subject when administering tobramycin a nominal dose of 300 mg using the PARI-LC\textsuperscript{®} PLUS nebulizer and the EEG formulation/device combination. .......................................................................................................................... 176

**Table 6.22** Nominal doses of the EEG formulation – device combination for 2, 5 and 12-year-old pediatric CF subjects with varying pulmonary bioavailabilities, required to achieve comparable systemic exposure to nebulized delivery. ..................................................................................................................... 177
**GLOSSARY OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B</td>
<td>apical to basolateral</td>
</tr>
<tr>
<td>AINH</td>
<td>adult inhalation study</td>
</tr>
<tr>
<td>AIV</td>
<td>adult intravenous study</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>API</td>
<td>active pharmaceutical ingredient</td>
</tr>
<tr>
<td>AS</td>
<td>albuterol sulfate</td>
</tr>
<tr>
<td>ASL</td>
<td>airway surface liquid</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t&lt;/sub&gt;</td>
<td>estimated area under the curve obtained by trapezoidal rule</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;t-∞&lt;/sub&gt;</td>
<td>extrapolated area under the curve from last point to infinity</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;∞&lt;/sub&gt;</td>
<td>total area under the curve</td>
</tr>
<tr>
<td>B-A</td>
<td>basolateral to apical</td>
</tr>
<tr>
<td>BSA</td>
<td>body surface area</td>
</tr>
<tr>
<td>BW</td>
<td>body weight</td>
</tr>
<tr>
<td>CaCC</td>
<td>calcium-activated channels</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CF</td>
<td>cystic fibrosis</td>
</tr>
<tr>
<td>CFF</td>
<td>Cystic Fibrosis Foundation</td>
</tr>
<tr>
<td>CFTR</td>
<td>cystic fibrosis transmembrane conductance regulator</td>
</tr>
<tr>
<td>CI</td>
<td>cascade impactor</td>
</tr>
<tr>
<td>CL&lt;sub&gt;TOT&lt;/sub&gt;</td>
<td>total clearance</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>maximum concentration</td>
</tr>
<tr>
<td>COV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>$C_p(t)$</td>
<td>plasma concentration-time profile</td>
</tr>
<tr>
<td>$C_s(t)$</td>
<td>sputum concentration-time profile</td>
</tr>
<tr>
<td>DPI</td>
<td>dry powder inhaler</td>
</tr>
<tr>
<td>$D_{v10}$</td>
<td>volume diameter at the 10th percentile</td>
</tr>
<tr>
<td>$D_{v50}$</td>
<td>volume diameter at the 50th percentile</td>
</tr>
<tr>
<td>$D_{v90}$</td>
<td>volume diameter at the 90th percentile</td>
</tr>
<tr>
<td>DtL</td>
<td>dose to lung</td>
</tr>
<tr>
<td>DVS</td>
<td>dynamic vapor sorption</td>
</tr>
<tr>
<td>ED</td>
<td>emitted dose</td>
</tr>
<tr>
<td>EEG</td>
<td>excipient enhanced growth</td>
</tr>
<tr>
<td>ELITE</td>
<td>Early Inhaled Tobramycin for Eradication</td>
</tr>
<tr>
<td>EMC</td>
<td>equilibrium moisture content</td>
</tr>
<tr>
<td>ENaCs</td>
<td>epithelial sodium channels</td>
</tr>
<tr>
<td>EPIC</td>
<td>Early <em>Pseudomonas</em> Infection Control</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>$F_{\text{INH}}$</td>
<td>inhaled bioavailability</td>
</tr>
<tr>
<td>FPF</td>
<td>fine particle fraction</td>
</tr>
<tr>
<td>$F_{\text{PUL}}$</td>
<td>pulmonary bioavailability</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GFR</td>
<td>glomerular filtration</td>
</tr>
<tr>
<td>GI</td>
<td>gastrointestinal</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HPMC</td>
<td>hydroxypropyl methylcellulose</td>
</tr>
<tr>
<td>INH</td>
<td>inhalation</td>
</tr>
<tr>
<td>IV</td>
<td>intravenous</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>kPa</td>
<td>kilopascal</td>
</tr>
<tr>
<td>L</td>
<td>liter</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LD</td>
<td>laser diffraction</td>
</tr>
<tr>
<td>LL</td>
<td>L-leucine</td>
</tr>
<tr>
<td>LLOQ</td>
<td>lower limit of quantification</td>
</tr>
<tr>
<td>logP</td>
<td>partition coefficient</td>
</tr>
<tr>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>minimum inhibitory concentration required to inhibit 50% of the growth</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>minimum inhibitory concentration required to inhibit 90% of the growth</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>mL</td>
<td>milliliter</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter</td>
</tr>
<tr>
<td>MMAD</td>
<td>mass median aerodynamic diameter</td>
</tr>
<tr>
<td>MN</td>
<td>mannitol</td>
</tr>
<tr>
<td>MT</td>
<td>mouth-throat</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>$n$</td>
<td>number of experimental replicates</td>
</tr>
<tr>
<td>NCA</td>
<td>non-compartmental analysis</td>
</tr>
<tr>
<td>NGI</td>
<td>Next Generation Impactor</td>
</tr>
<tr>
<td>ORCC</td>
<td>outward rectifying chloride channels</td>
</tr>
<tr>
<td>$Pa$</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>PBPK</td>
<td>physiologically-based pharmacokinetic</td>
</tr>
<tr>
<td>$Pe$</td>
<td>Peclet number</td>
</tr>
<tr>
<td>PINH</td>
<td>pediatric inhalation study</td>
</tr>
<tr>
<td>PIV</td>
<td>pediatric intravenous study</td>
</tr>
<tr>
<td>PK</td>
<td>pharmacokinetic</td>
</tr>
<tr>
<td>POL188</td>
<td>poloxamer 188</td>
</tr>
<tr>
<td>$R^2$</td>
<td>coefficient of determination</td>
</tr>
<tr>
<td>RDD</td>
<td>Respiratory Drug Delivery</td>
</tr>
<tr>
<td>RH</td>
<td>relative humidity</td>
</tr>
<tr>
<td>$RH_c$</td>
<td>crystallization relative humidity</td>
</tr>
<tr>
<td>$RH_g$</td>
<td>glass transition relative humidity</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>RTV</td>
<td>room temperature vulcanizing</td>
</tr>
<tr>
<td>$s$</td>
<td>second</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy or standard error of mean</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>half-life</td>
</tr>
<tr>
<td>TGA</td>
<td>thermogravimetric analysis</td>
</tr>
<tr>
<td>TIP</td>
<td>tobramycin inhalation powder</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>TIS</td>
<td>tobramycin inhalation solution</td>
</tr>
<tr>
<td>$t_{\text{max}}$</td>
<td>time to reach maximum concentration</td>
</tr>
<tr>
<td>TL</td>
<td>trileucine</td>
</tr>
<tr>
<td>TOBI</td>
<td>tobramycin</td>
</tr>
<tr>
<td>Tukey’s HSD</td>
<td>Tukey’s honest significant difference test</td>
</tr>
<tr>
<td>USP</td>
<td>United States Pharmacopeia</td>
</tr>
<tr>
<td>VCU</td>
<td>Virginia Commonwealth University</td>
</tr>
<tr>
<td>Vd</td>
<td>volume of distribution</td>
</tr>
<tr>
<td>Vd$_{\text{ss}}$</td>
<td>volume of distribution at steady-state</td>
</tr>
<tr>
<td>w/v</td>
<td>gram of solute per 100 mL of solution</td>
</tr>
<tr>
<td>w/w</td>
<td>gram of solute per 100 g of solid</td>
</tr>
<tr>
<td>XRPD</td>
<td>X-ray powder diffraction</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>µm</td>
<td>micrometer</td>
</tr>
<tr>
<td>µM</td>
<td>micromolar</td>
</tr>
<tr>
<td>3D</td>
<td>three-dimensional rod array</td>
</tr>
<tr>
<td>%P</td>
<td>percent peristaltic pump rate</td>
</tr>
<tr>
<td>%S</td>
<td>percent spray head vibration</td>
</tr>
</tbody>
</table>
ABSTRACT

FORMULATION, CHARACTERIZATION AND PHARMACOKINETIC MODELLING OF EXCIPIENT ENHANCED GROWTH SPRAY-DRIED INHALATION POWDERS

By Serena Bonasera, M.Pharm.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

Virginia Commonwealth University, 2021

Major Director: Michael Hindle, Ph.D., Professor, Department of Pharmaceutics

The overall aim of this research project was to evaluate effects of spray drying parameters and storage conditions on solid-state characteristics and aerosol performance of excipient enhanced growth (EEG) powders and to formulate a stable and highly dispersible tobramycin EEG powder to be delivered to pediatric cystic fibrosis subjects using a novel positive pressure dry powder inhaler.

Micrometer-sized albuterol sulfate EEG powders were produced using a medium-sized nebulizer and optimized spray drying conditions. Using trileucine as the dispersion enhancer improved the aerosol performance but showed increased moisture absorption compared to the L-leucine formulation.

The optimized spray drying conditions were then implemented together with controlling the drying gas water vapor density (11.9 g/m³) and storage conditions (15 %RH) for hygroscopic tobramycin EEG powders. A stable tobramycin EEG powder with 75.7% emitted dose and 71.1% respirable fraction was obtained using L-leucine as the dispersion enhancer. The optimized formulation containing trileucine had a respirable fraction of 96.0% and when aerosolized in a 5-year-old mouth-throat model, the mouth-throat airway losses were negligible (~1 %).

Aerosol performance of spray-dried combination formulations was evaluated by laser diffraction. Results indicated this method could accurately characterize emitted aerosols with reduced testing times compared to cascade impactor methods.
A pharmacokinetic model was developed and validated, dose reductions based on age and body weight were found to be required for the tobramycin EEG formulation in pediatric patients to ensure equivalent systemic exposure compared to the nebulized delivery due to the predicted increased dose to lung for this new formulation-device combination.
CHAPTER 1: BACKGROUND AND INTRODUCTION

1.1 Dry powder inhalers and spray drying technology

Initially developed as a propellant-free alternative to metered dose inhalers, dry powders inhalers (DPIs) represent a valuable and efficient choice in the pharmaceutical aerosol delivery device arena (Lavorini et al., 2017). Innovations in DPI platforms continue to be developed (Mehta, 2020, de Boer et al., 2006, Behara et al., 2014) from the simple unit dose devices that were initially developed such as the Spinhaler and Rotahaler. DPI devices are used in combination with a variety of carrier-based and carrier-free powder formulations for the treatment of systemic and local diseases within the lungs. However, despite the importance of device design improvements, a successful DPI is still reliant on powder formulation properties to produce respirable particles (1-5 µm) that are dispersible (Islam and Cleary, 2012).

Spray drying is an increasing important aerosol powder formulation technology that enables the production of engineered particles to efficiently target lung delivery (Geller et al., 2011). In the spray drying process, a feed formulation (solution or suspension) is atomized into fine droplets containing dissolved or suspended drug and excipients. These liquid droplets are converted to solid particles as the formulation solvent/s evaporate due to the heated airflow within the drying chamber. The particle size and surface characteristics being dependent on the spray drying conditions and the excipients included in the spray-dried engineered particles, including the use of surface modifying agents such as L-leucine. Several studies have investigated the effect of spray drying and formulation variables on the powder yield, particle size and the in vitro aerosol characteristics of spray-dried powder formulations, with the aim of improving their inhalation performance and solid-state stability (Littringer et al., 2013, Focaroli et al., 2019). Focaroli and colleagues studied the effect of airflow rate, pump, aspirator and L-leucine concentration in a combination of D-trehalose and L-leucine formulations produced using the Büchi Mini spray dryer B-290 (Focaroli et al., 2019). Similar studies using the Büchi Nano spray dryer B-90 were performed by Son et al., and Littringer et al., where particles in the range of 1-2 µm were obtained by optimizing mesh size, inlet gas drying temperature, solids
concentration, vehicle composition and added excipients (Littringer et al., 2013; Son et al., 2013). These studies were performed with an early version of the Büchi B-90 Nano spray dryer that used spray caps containing a vibrating stainless-steel membrane. Recently, the B-90 HP was introduced that uses a mesh nebulizer design and a piezoelectric actuator to produce droplets for spray drying. Investigators have also evaluated the effect of water vapor density of the drying gas during the spray drying process of a hygroscopic tobramycin EEG formulation as a tool to control the rate of solvent evaporation during the formation of dried particles (Hassan et al., 2020). They found that increasing the water vapor density of the drying gas was believed to slow the droplet drying kinetics and resulted in improved solid-state stability with the powder formulation having higher glass transition and crystallization relative humidities compared to using a lower water vapor density during spray drying to produce the spray-dried powder (Hassan et al., 2020).

1.1.1 Excipient enhanced growth spray-dried powder formulations

The promise of engineered spray-dried powder formulations is the possibility to modify particle surface properties such that highly dispersible composite small particles can be produced that can effectively overcome the high extrathoracic losses of carrier-based dry powder formulations and improve regional lung deposition (Newman and Busse, 2002). The excipient enhanced growth (EEG) technology is a novel dry powder aerosol platform that uses micrometer-sized spray-dried powder formulations and subsequent hygroscopic growth in the airway to minimize mouth-throat deposition, promote targeted lung deposition and minimize exhalation of the small particle aerosol (Hindle and Longest, 2012, Longest and Hindle, 2011). The EEG approach has been successfully employed to formulate a number of active pharmaceutical ingredients (APIs) (Boc et al., 2021; Hassan et al., 2020; Hindle and Longest, 2012; Son et al., 2013). Compendial testing of EEG powder formulations aerosolized with a novel DPI resulted in emitted doses of >75% of the nominal dose, respirable fractions >80% of the nominal dose and a significant fraction (>20%)
of submicrometer particles to target the small airways (Hassan et al., 2020; Longest and Hindle, 2017; Son et al., 2013).

1.1.1.1 Dispersion enhancers used in the EEG platform

The EEG formulations employ excipients such as mannitol and sodium chloride to enhance hygroscopic growth in the airways and L-leucine to enhance dispersion and protect formulations from moisture sorption under ambient storage conditions. L-leucine has been used in several spray-dried powder formulations to protect against moisture uptake due to its surface enrichment and shell forming properties (Chang et al., 2014, Li et al., 2016). During the drying phase of the spray drying process, excipients with low water solubility will reach saturation earlier than more soluble excipients and drug, therefore precipitating on the surface layer of the droplet (Feng et al., 2011). Several authors have outlined the role of L-leucine as a surface shell forming agent, which is related to a drying parameter defined as Peclet number \( (P_e) \) (Longest et al., 2020, Vehring, 2008, Vehring et al., 2007). The \( P_e \) is calculated by dividing the solvent evaporation rate, \( K \), by 8 times the solute diffusion coefficient, \( D_i \) (Vehring, 2008). For a spray-dried formulation containing a 10 %\(^w\)/w concentration of L-leucine in spray-dried herbal extract powder, it was shown that L-leucine reduced moisture uptake and had an anti-hygroscopic effect (Chang et al., 2014). Li et al., showed that the addition of 2 %\(^w\)/w L-leucine in a spray-dried disodium cromoglycate formulation can significantly improve the aerosol performance under ambient conditions and limit the aerosol performance decline after exposure to 60 %RH, compared to pure spray-dried disodium cromoglycate (Li et al., 2016). The moisture protectant at ambient humidities and ability to enhance the powder dispersion properties of spray-dried powders make L-leucine an important component of spray-dried EEG formulations (Eedara et al., 2018, Boraey et al., 2013). It is important to recognize that at simulated airway humidities of >90% RH, the surface protection effects of L-leucine are negated due to the overwhelming bulk absorption that takes place at this high humidity.
Several studies have investigated other amino acids employed as dispersion enhancing and moisture protecting agents in spray-dried powders (Lechuga-Ballesteros et al., 2008a, Sibum et al., 2019, Carrigy et al., 2019, Gomez et al., 2021). Trileucine is an L-leucine trimer, its aqueous solubility is pH dependent (Vehring, 2008). It is believed that trileucine can also enrich the droplet surface during spray drying, but this occurs on larger droplets and forms particles that are amorphous and collapse in a wrinkled-morphology (Ordoubadi et al., 2021a; Vehring, 2008; Wang et al., 2021). The use of trileucine as a dispersion enhancer has been shown to result in spray-dried powders with improved aerosol performance characteristics, compared to L-leucine (Gomez et al., 2021). There is inconclusive data available with respect to trileucine’s ability to act as a moisture protecting agent (Wang et al., 2021, Sibum et al., 2019).

1.2 Particle analysis techniques for the characterization of aerosols

The *in vitro* aerosol performance testing of orally inhaled drug products relies mostly on cascade impactors (CI) and multi-stage liquid impingers that operate based on the principles of inertial impaction. The methods employed are described in pharmacopeial compendia and have been used for many decades. Many types of impactors and impingers have been used to characterize the aerosol particle size distribution of inhaled formulations. However, they are labor intensive and difficult to automate. CI’s and impingers can provide aerodynamic size information in the range of 0.1-15 µm while allowing the direct quantification of the active pharmaceutical ingredient using analytical methods (e.g., HPLC, LC-MS). Several newer methods have been used to characterize particles delivered by inhalation devices however, these methods often lack acceptance by the pharmaceutical regulators. The pharmaceutical industry therefore often relies on the time-consuming CI methods accepted by the regulators rather than more innovative technologies.

Laser diffraction (LD) methods are the most common alternative to impactor-based methods for inhalation aerosols characterization. A schematic representation of the set-up of a laser diffraction-based instrument is reported in Figure 1.1. The particles going through the measuring zone will scatter the light from the laser
beam source at different angles depending on their size. Before reaching the diode detector, which is placed on the opposite side of the laser source, the beam goes through a Fourier lens that is used to focus the diffraction pattern onto the detector. Volume-weighted aerosol particle size is determined based on the diffraction pattern, for which particles with large diameter scatter light energy at low angles, while particles with small diameters scatter at higher angles. Fraunhofer and Lorenz-Mie theories are the most common principles behind the particle size determination with laser diffraction (Mitchell et al., 2011).

**Figure 1.1** Schematic settings of laser diffraction measurements. Adapted from Mitchell et al., 2006.

Laser diffraction based instruments characterize the size of an aerosol bolus, defined as the cloud present in the measurement zone (Mitchell and Nagel, 2004). This alternative method to rapidly evaluate the aerosol size distribution of orally inhaled drug products, especially spray-dried products, are of current interest to accelerate the development of new pharmaceutical inhalers (Mitchell et al., 2011).
1.2.1 Advantages and limitations of laser diffraction techniques for pharmaceutical aerosol characterization

There are several advantages in the use of laser diffraction techniques for the assessment of pharmaceutical inhalation aerosols. Laser diffraction instruments are capable of sampling and sizing thousands of particles in an aerosol cloud within a few seconds. Additionally, the dynamic aerosol cloud measurements in a real-time manner allow measurement of size during the time course of the spray generation and the characterization of transient events during the plume development from a nasal spray device or inhaler (Mitchell and Nagel, 2004). Depending on the selected instrument, the laser diffraction systems can measure particle size from 0.5 up to 200 µm, which is a wider size range compared to CIs, and they can also be modified to achieve up to 1,000 µm for nasal spray testing (Mitchell and Nagel, 2004). Finally, newer laser diffraction instruments have reduced the issue of “vignetting”, in which small particles scatter light at wider angles than the range of the lens, thus resulting in an inaccurate particle size determination (Mitchell and Nagel, 2004).

In contrast to CI methods, however, laser diffraction does not allow a direct link between particle size and mass of the specific API. This limitation is of particular importance when testing multicomponent-based formulations for which a single droplet/particle contains multiple compounds. More complications arise when evaluating drug and lactose carrier-based powder formulations using a laser diffraction system, as it is not possible to discriminate between the carrier and drug particles. Additional challenges can come from the presence of propellant-based formulations delivered by meter dose inhalers, as chlorofluorocarbon and hydrofluoroalkane propellants have different refractive indices compared to air (Mitchell and Nagel, 2004).

As with CI methods, droplet evaporation needs to be taken in consideration when testing nebulized solutions or nasal sprays, as inaccurate droplet size determination can result if evaporation takes place during measurement. However, controlled testing conditions can potentially limit this drawback (Mitchell and Nagel, 2004).
1.2.2 Laser diffraction for the characterization of dry powder formulations – device combinations

Laser diffraction has been used to evaluate the aerosol performance of several inhaler devices, including pressurized metered dose inhalers, nebulizers and dry powder inhalers. Several authors have used such methods to characterize powder dry formulation – device combinations.

Behara and colleagues used the laser diffraction-based instrument Spraytec to conduct studies of emitted aerosol concentration – time profiles to evaluate the kinetics of emission from a lactose-based formulation using Rotahaler, Monodose Inhaler and Handihaler devices (Behara et al., 2011). Similarly, Huynh and colleagues studied the emptying and the dispersion of mannitol powders emitted from high and low resistance dry powder inhalers, using laser obscuration - time profiles (Huynh et al., 2015). The Spraytec was also employed to evaluate how the dynamics of aerosol emission was affected under unsteady flow conditions. Dorosz et al. used two adult model inhalation profiles to actuate passive dry powder inhalers. From the time – history profile, the authors observed that the aerosol emission was concluded before the peak inspiratory flow rate for these specific powder- device combinations (Dorosz et al., 2016).

Laser diffraction characterization can also be employed in line with cascade impaction quantification. Krarup et al. used the Spraytec with the inhalation cell system in a vertical orientation. The USP induction port was connected to the entrance of the inhalation cell and to the inhaler. The Andersen Cascade Impactor was connected to the exit of the inhalation cell and attached to a vacuum system to pull a fixed flow rate through the inhaler and actuate the device (Krarup et al., 2004). Lactose-blended formulations obtained by micronization and supercritical fluid precipitations were compared for their time – history profiles. These studies indicated improved dispersion for the supercritical fluid formulation compared to the micronized formulation. Similarly, Pilcer et al. have shown correlations between the aerosol size distribution measured by the Spraytec and an inline Multi-stage Liquid Impinger, using breath-actuated dry powder inhalers at different flow-rates (Pilcer et al., 2008).
1.3 Pharmacokinetic modelling for orally inhaled medications

Despite the importance of pharmacokinetic (PK) modelling, only a limited number of PK models for orally inhaled medications are available (Borghardt et al., 2015). Predictions of systemic and pulmonary drug concentrations after inhalation administration has become relevant for innovator as well as for the generic drug development processes. Overall, PK modelling can be developed on physiologically based parameters (PBPK models), on clinically relevant data (empirical models) or it can be non-empirically based (Borghardt et al., 2015). Several considerations need to be taken in account when developing a PK model for orally inhaled medications, which make it more complicated than orally administered medications.

An example of a developed and validated semi-physiologically based model for a tobramycin orally inhaled product was reported by Hanna (Hanna, 2018). The PBPK model was developed to estimate tobramycin disposition in plasma and lung compartments in both healthy adults and cystic fibrosis (CF) adult patients, after intravenous (IV) and inhaled administration (Hanna, 2018). The model was built based on 4 relevant intravenous studies and 4 inhalation studies. From non-compartmental PK analysis of tobramycin IV studies, Hanna observed a small volume of distribution at steady-state (Vdss) of 16-18 L, suggesting that tobramycin mostly stays in the central compartment once administered by the IV route (Hanna, 2018). As a consequence of the small volume of distribution, the obtained half-life (t1/2) ranged from 125-167 min. The observed total clearance (CLTOT) approached the glomerular filtration of 120 mL/min, suggesting that tobramycin is primarily eliminated by the kidney and that there is some tubular reabsorption occurring. Despite observing no difference when comparing CLTOT and Vdss between healthy adult and CF subjects, once CLTOT and Vdss were normalized per body weight (BW), both parameters were higher in CF adult subjects compared to healthy adults. These findings support the observation that CF adult subjects have a lower body weight than healthy adults and that body weight is a relevant covariate when accounting for the disease state. Hanna additionally found no evidence of non-linear PK when administering tobramycin within the 1.5-10 mg/kg dosing range for both healthy and CF subjects (Hanna, 2018).

The adult oral inhalation PK model (Figure 1.2) included 4 lung compartments. Two unbound
compartments represent the peripheral and central lung surface for pulmonary absorption of deposited drug. A second layer for each absorption compartment (the sequestration compartment) accounts for intracellular sequestration and release into blood. In adults, the sequestrated peripheral lung compartment appeared to have high drug capacity with a prolonged duration compared to other compartments. Previous *in vivo* evidence supports this observation (Li and Byron, 2013). All processes are bi-directional, i.e., the lung absorption (after inhalation) and elimination (after IV administration). Therefore, the lung serves as an absorption, elimination and sequestration compartment for tobramycin. A major assumption was that sputum concentration reflects the tobramycin concentration in the central lung unbound compartment. Additional model assumptions include the oral bioavailability of tobramycin to be zero, due to the drug’s low permeability (Hombach et al., 2008). Body weight adjustment is performed by allometric scaling and is used to incorporate the effects of CF into relevant model parameters (Vd, CL_TOT, total lung volume). Sensitivity analysis was conducted on the validated model for adults both for IV and inhaled tobramycin administration. For the IV administration, analysis indicated a substantial decrease in plasma area under the curve (AUC) and maximum concentration (C_max) (13 and 15-fold, respectively) when increasing CL_TOT (by 25-fold) and some minimum reduction when pulmonary model parameters were increased (micro-rate constants). Similarly, after IV tobramycin administration, sputum AUC and C_max were again sensitive to changes in CL_TOT but also to mucociliary clearance rate and absorption rate from the central unbound to the sequestered lung compartment. These results suggest that an increase in CL_TOT and a decrease in mucociliary clearance and absorption from the central unbound to the sequestered lung compartment rates would maximize pulmonary exposure and minimize systemic exposure after IV administration. No evidence of different pulmonary disposition was found between adult CF and healthy adult subjects when administering tobramycin by inhalation using the same device.

When tobramycin was administered by inhalation route, decreased plasma AUC and C_max were found when an increased clearance was simulated. Interestingly, not much change was observed in sputum AUC and C_max when simulating changes in CL_TOT. Sputum exposure metrics were substantially decreased when increasing absorption micro-rate constants from peripheral and central unbound lung compartments toward
the sequestered lung compartment. Therefore, increased sputum exposure could be achieved by reducing such absorption rates toward the sequestered lung compartment. Sputum exposure was insensitive to changes in systemic parameters.

Overall, when administering tobramycin to adults by the inhalation route, systemic exposure is reduced as the CL_{TOT} increases while pulmonary exposure increases as the mucociliary clearance and the absorption rate to the sequestered compartment reduce.

Hanna also simulated the administration in adults of a 200 mg tobramycin dose as a 10 min IV infusion, and found that tobramycin sputum concentration was 100-fold lower compared to plasma concentration, as a consequence of the low and slow plasma to lungs distribution (Hanna, 2018). Additionally, the sputum concentration vs. time profile was observed to peak later compared to the plasma peak. This lag-time between sputum and plasma is due to the sequestration compartment. The sequestration compartment is also the reason why the drug is slowly eliminated from the lungs. When simulating a 1000 mg oral

Figure 1.2 Schematic representation for semi-PBPK model for adult CF and healthy subjects.
administration, no drug is observed in the sputum. Finally, when simulating a 500 mg tobramycin dose administered by inhalation in adults, the sputum concentration vs. time profile showed high initial drug concentration, as the drug is deposited directly in the lungs. However, absorption from the central unbound to the sequestered compartment is fast, and so the concentration declines rapidly. A secondary peak is observed as the drug is recirculated in the peripheral lung sequestered compartment, which acts as a tobramycin reservoir. Following this secondary increase in concentration in the lung sputum, tobramycin equilibrates between the sequestered and the unbound compartment, from where it gets slowly eliminated by mucociliary clearance. The drug release from the sequestration compartment is therefore the rate limiting step in the elimination of the drug from the body after inhaled administration.

1.4 Cystic fibrosis

1.4.1 Demographics, epidemiology, pathophysiology and effect on lung function

Cystic fibrosis is a genetic disease caused by mutations in a single gene that encodes for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. More than 2,000 CFTR mutations have been identified so far (Emerson et al., 2002; Robinson et al., 2009, Cystic Fibrosis Mutation Database: Statistics). These mutations could result from a premature stop codon in the protein synthesis (non-sense mutations), from a degradation of the newly formed CFTR (protein-processing mutations), from a formed but malfunctioning CFTR (gating mutations), or more rare mutations like splicing or conductance mutations (Gibson et al., 2003a). The Cystic Fibrosis Foundation (CFF) Patient Registry reports that in 2019 more than 30,000 individuals were affected by CF in the United States, with about 1,000 subjects diagnosed each year with a median age of 3 months (2019 Patient Registry Annual Data Report, 2019). While the number of pediatric subjects (<18 years old) remained consistent throughout the last years, the percentage of CF patients who are adults (>18 years old) increased from 31.1% in 1989 to 56.0% in 2019 (2019 Patient Registry Annual Data Report, 2019). Despite such improved life expectancy, subjects affected by CF are
still only expected to have a median survival age of 46.2 years (2019 Patient Registry Annual Data Report, 2019).

As an adenosine triphosphate (ATP)-binding cassette transporter, CFTR is a glycoprotein involved in ion transport regulation on the apical membrane of epithelial cells, and consequently in the homeostasis of fluids (Bergeron and Cantin, 2019). The CFTR protein is formed by two transmembrane domains, that result in a central pore for the ion transport, a regulatory domain, and two nucleotide binding domains (Bergeron and Cantin, 2019). When a protein kinase A phosphorylates the regulatory domain, the ATP binds to the nucleotide domains causing them to dimerize, and therefore activating the channel (open channel) to allow ion transport (Bergeron and Cantin, 2019). Conversely, the hydrolysis of ATP causes monomer formation and channel closure (Rey et al., 2019). CFTR protein mainly regulates the chlorine ions transport across membranes, but it is also involved in the transport of the bicarbonate anion, and in the activity of other ion channels like epithelial sodium channels (ENaCs), calcium-activated channels (CaCC), outward rectifying chloride channels (ORCC), and other intracellular proteins (Bergeron and Cantin, 2019). Abnormal ion transport is observed for absent or malfunctioning CFTR, consequently resulting in a multi-organ disease condition that involves pancreas, sweat glands, gastrointestinal tract, liver, reproductive tract, and lungs. All organs present with an accumulation of a layer of thick and sticky mucus (Bergeron and Cantin, 2019).

Dysfunctional CFTR on the lung level results in abnormal chloride and sodium ion transport across the apical membrane of epithelial cells, and consequently in airway surface liquid depletion (Ratjen, 2009). The airway surface liquid (ASL) is the fluid layer that covers the airway epithelial cells. The ASL consists of the periciliary liquid layer formed by the cilia and the mucus layer. Mucus and cilia are involved in mucociliary clearance, the main mechanism of defense from inhaled particles in the airways. However, the mucus layer has a major role in regulating the liquid volume on the airway surfaces (Boucher, 2004). In healthy airways with functioning CFTR, proper ion transport and water content results in low-viscosity mucus and correct cilia beating movements. Conversely, CF subjects present de-hydrated, thick mucus with
an unpaired cilia beating mechanism. It is hypothesized that the more viscous mucus and the altered ionic composition could explain the reduced mucociliary clearance when the CFTR protein is not present or is not properly working (Gibson et al., 2003a). Additionally, the more viscous and dehydrated mucus facilitates the presence of pathogens, that cannot be easily cleared by the mucociliary escalator (Bergeron and Cantin, 2019).

Airways disease is one of the main causes of mortality in CF patients. Despite CF subjects being born with normal lungs, they eventually progress to initial small airways obstruction and ultimately to chronic infection (Cutting et al., 2019). Chronic infections are virtually impossible to eradicate and they are frequently due to *Pseudomonas aeruginosa* (Pa) bacteria. Therefore early eradication is necessary to prevent the recurrent Pa infection becoming chronic (Ratjen, 2009).

### 1.4.1.1 Standard of care for early eradication treatment of *Pseudomonas aeruginosa* infection

*Pseudomonas aeruginosa* is a common Gram-negative bacteria found in CF subjects’ airways. Untreated Pa infection has been shown to lead to faster lung deterioration and to be associated with increased morbidity and mortality (Emerson et al., 2002; Robinson et al., 2009). Lack of early Pa eradication leads to chronic infection, ultimately resulting in more rapid progression to death if compared to CF subjects with no chronic Pa infection (Parkins et al., 2018). It was shown that early Pa treatment results in improved survival and delayed chronic infection (Frederiksen et al., 1996; Hansen et al., 2008). Multi-drug resistance is a serious concern regarding Pa. The CDC reports that in 2017 more than 32,000 infections in nosocomial environment were caused by multi-drug resistant Pa (*Pseudomonas aeruginosa Infection | HAI | CDC, 2019*).

Consequently, the effectiveness of several antibiotics was tested to treat early Pa infection. Valerius and colleagues compared the efficacy of oral ciprofloxacin together with inhaled colistin to that of no antibiotic treatment in children (2-9 years) (Valerius et al., 1991). The study revealed that only 14% of the treated
subjects vs. 58% of the untreated subjects developed chronic Pa infection. Gibson et al. have shown that inhaled tobramycin solution (300 mg, twice a day) administered to CF pediatric subjects (< 6 years) for 28 days resulted in 8 out of 8 Pa-free cultures, which was a significant improvement compared 1 out of 13 for the placebo group (Gibson et al., 2003b). In the Early Pseudomonas Infection Control (EPIC) study, Treggiari and colleagues compared interventions for CF pediatric patients (1-12 years) using tobramycin inhalation solution (300 mg, twice a day) and oral ciprofloxacin (15-20 mg/kg, twice a day) or placebo, and found Pa-free cultures for the treatment groups and no differences among the treatments (Treggiari et al., 2011). In the Early Inhaled Tobramycin for Eradication (ELITE) trial 90% of CF subjects (> 6 months) with early Pa infection were treated with tobramycin inhalation solution (300 mg, twice a day) for either 28 or 56 days, and results showed the absence of Pa infection 1 month after the treatment was completed (Ratjen et al., 2010). This study also concluded that only 28 days treatment was required to eradicate early Pa infection.

The FDA recently approved a tobramycin inhalation powder (TIP) formulation, delivered by the T-326 dry powder inhaler, for the treatment of Pa infection in CF subjects ≥ 6 years. When the TIP formulation was delivered by the passive T-326 DPI to healthy subjects, 34% of the dose was deposited in the lungs with 44% of the dose recovered as mouth-throat losses, and 22% of the dose being retained in the DPI (Geller et al., 2011). The prescribed regimen requires the delivery of 112 mg tobramycin dose delivered from 4 capsules, per each administration. However, this breath-actuated dry powder inhaler is not suitable for pediatric subjects <6 years old.

No unified early Pa treatment protocol has been approved for pediatric CF subjects yet. However, based on the results obtained from trials, the CFF guidelines recommend the use of inhaled antibiotic therapy to treat early Pa infection in pediatric patients, defining tobramycin inhalation solution (300 mg, twice a day) for 28 days as the favored antibiotic regimen (Mogayzel et al., 2014). Tobramycin inhalation solution (TIS) contains 300 mg tobramycin in a 5 mL nebulizer solution. It is approved by the FDA for the treatment of early Pa infection in CF subjects, and it is recommended by the CFF guidelines. TIS needs to be stored in
refrigerator, and it is recommended to be delivered by the PARI-LC© PLUS jet nebulizer using a compressor over 15-20 min. After completion of the administration, the equipment requires careful cleaning to prevent the risk of additional bacterial infection.

However, compared to the TIS, the use of TIP has reduced the administration time burden required for inhaled tobramycin (6 min for TIP vs. 20 min for TIS), and has also allowed the use of a more portable device with no need for additional cleaning procedures (Geller et al., 2011). A study by Konstan et al., concluded that the safety and the efficacy of TIP vs. TIS treatment over a 28-days period were comparable both in terms of lung function and sputum Pa density (Konstan et al., 2011). During the same clinical trial, patient satisfaction with the treatment was assessed, and the results indicate that TIP was more convenient compared to TIS. However, this breath-actuated dry powder inhaler is not approved for pediatric subjects <6 years old.

1.5 Aminoglycosides overview: mechanism of action, pharmacokinetics and pharmacodynamics, toxicity

Aminoglycosides are low molecular weight, weakly basic compounds that are primarily excreted by glomerular filtration, with no protein binding and no metabolism (Pechere and Dugal, 1979). Gentamicin and tobramycin have a similar structure and pharmacokinetic behavior, but tobramycin seemed to be more potent against Pa (de Groot and Smith, 1987). The aminoglycosides bind to the 30S subunit of bacterial ribosomes, resulting either in inappropriate protein formation or synthesis. They have bactericidal activity against Gram-negative pathogens, and some Gram-positive as well.

Aminoglycosides are poorly absorbed from the gastrointestinal tract and are mainly excreted unchanged by the kidneys, with only ~10% elimination by non-renal pathways. They are concentration-dependent bactericidal antibiotics, therefore as the drug concentration increases the rate and the extent of the bactericidal activity increase as well (Ambrose et al., 2007). Also, a post-antibiotic effect against the Pa
infection has been observed, resulting in prolonged ability to exert bactericidal activity despite the drug concentration falling below the minimum inhibitory concentration (MIC) (Geller, 2009). The activity of several antibiotics has been tested using *in vitro* samples obtained from CF patients affected by *Pa* infection, and tobramycin showed the highest activity with minimum inhibitory concentrations required to inhibit 50% of the growth (MIC<sub>50</sub>) and 90% of the growth (MIC<sub>90</sub>) of 1 and 8 µg/mL, respectively (Shawar et al., 1999).

Due to their narrow therapeutic window and the CF patients’ prolonged exposure, aminoglycosides can cause nephrotoxicity and ototoxicity (Prayle et al., 2010). It was reported that intravenous administration of aminoglycosides achieves sputum concentrations (representative of local lung concentrations) that only correspond to 20% of the plasma concentration (Klastersky et al., 1981). Thus, IV administration to target airway exposure would require high doses that would ultimately increase the risk for systemic toxicity. Therefore, the main advantage of delivering tobramycin through the inhalation route is to achieve high and well distributed lung doses, while limiting the systemic exposure and consequently the potential risk for toxicity (Geller, 2009).

1.6 Tobramycin Pharmacokinetics

1.6.1 Tobramycin Relevant Biopharmaceutical Properties for Systemic and Pulmonary Disposition.

Tobramycin (free base) is an aminoglycoside antibiotic with 467.5 g/mol molecular weight (MW), -7.32 experimentally determined logP, and 1 g/mL aqueous solubility (Stigliani et al., 2016). An apparent permeability of 1.78 ± 0.23x10<sup>-6</sup> cm/s was found in a Caco-2 cell model at a drug concentration of 5 mg/mL (Hombach et al., 2008). The Caco-2 cell model mimics the gastrointestinal (GI) permeability. Negligible GI absorption is expected both in cystic fibrosis patients and healthy subjects (Touw et al., 1997). Tobramycin permeability has also been tested in Calu-3 cells as a lung cell model (Stigliani et al., 2016). In this study, a bi-directional permeability experiments (apical to basolateral- A-B, and basolateral to apical-
B-A) was conducted. While the A-B permeability experiment mimics the permeation from lung to plasma, the B-A direction mimics the plasma to lung permeability. Tobramycin was administered on the basolateral side at a concentration of 100 µM (46.7 mg/L) and after 3 hours, no drug was found in the apical side. This result suggests a very low and slow rate of permeation of the drug. This study also underlines that once tobramycin is administered by IV route, elimination from plasma into lung is limited. As mentioned above, tobramycin permeability in a Calu-3 cell model has also been tested in A-B direction (lung-plasma permeability) (Stigliani et al., 2016). After 3 hours, only 10% of the dose was found intracellularly. Therefore, despite the semi-quantitative analysis conducted, it seems that the lung-plasma permeability is also characterized by a low rate and extent of permeation. Such low permeability is explained by the drug hydrophilicity and polycationic nature. The drug’s hydrophilic nature also explains its distribution into the extracellular space. Tobramycin was reported to be mainly excreted by glomerular filtration and to have negligible plasma protein binding (Gordon et al., 1972; Jaffe et al., 1974).

Based on the described biopharmaceutical properties, it is expected that when administering tobramycin by IV route, the drug will poorly penetrate the lung via the bronchial membrane or the alveoli capillary membrane. Valcake et al., reported that passive diffusion (concentration gradient based) is the most likely mechanism that allows the permeation of these antibiotics (Valcke et al., 1990). They also reported that several factors can affect this permeation, including the presence of damaged barriers due to the inflamed status. In the inflammatory state, vasodilation would occur, resulting in enhanced permeation. However, in the presence of intact barriers, hydrophilic, low MW molecules appear to have a low ability to permeate, therefore active-mediated transport mechanisms could potentially be involved. Patton et al. described that small, hydrophobic molecules are quickly absorbed by a transcellular diffusion mechanism, as they can integrate into the membrane lipids (Patton et al., 2004). Conversely, small and hydrophilic molecules, like tobramycin, usually cross pulmonary membranes by paracellular diffusion, occurring through aqueous pores in the tight junctions. However, the authors also hypothesized that additional energy-related transport or vesicle-mediated endocytosis mechanisms may contribute to tobramycin absorption from the lungs.
Furthermore, due to its polycationic nature, the compound has been shown to bind to acidic phospholipids on kidney cell membranes and initiate drug accumulation via a transporter-mediated mechanism which is nephrotoxic (Nagai and Takano, 2014). Such transporters were also found in the lungs (Assémat et al., 2005).

1.6.2 Tobramycin pharmacokinetic differences in healthy subjects and cystic fibrosis patients

1.6.2.1 Systemic disposition

A few studies have compared tobramycin in healthy subjects and CF patients from a pharmacokinetic perspective. Levy et al., evaluated tobramycin PK in healthy and CF adolescents and young adults. When 55.7±14.7 mg/m² of tobramycin (body surface area (BSA) = 1.6±0.4 m²) was administered by 5 min IV infusion to healthy subjects, the volume of distribution (Vd) corresponded to 0.23±0.07 L/kg and the total body clearance was 102.2±18.9 mL/min/1.73 m² (Levy et al., 1984). Similarly, when a dose of 75.5±10.2 mg/m² was administered to CF subjects (BSA = 1.2±0.3 m²), Vd and CL were 0.31±0.08 L/kg and 121.2±14.9 mL/min/1.73 m², respectively. For an average body weight of 75 kg, CF subjects have a higher CLTOT and Vd when normalized to body weight compared to healthy subjects. The difference is even more pronounced when considering that CF subjects usually have lower body weight compared to healthy subjects (mean±SD of 37.1±12.5 kg and 57.8±18.4 kg, respectively). The higher total body clearance is not associated with a higher glomerular filtration rate (GFR) in CF subjects as reflected by the creatinine clearance in these patients. Therefore, given that glomerular filtration is the primary renal elimination route for tobramycin and that the drug is not significantly metabolized, the authors explained that the higher CLTOT was possibly due to non-renal excretion routes, which accounted for ~10% of the total clearance. The hydrophilic nature of tobramycin results in extracellular fluid distribution which is inversely related to body weight, and therefore the authors reported that the higher Vd was a result of the higher tobramycin distribution in the extracellular fluids. No difference was observed in half-life between CF and healthy
subjects, likely because Vd and CL\textsubscript{TOT} had comparable fold increase in CF subjects compared to healthy ones. In another study, Mann et al., evaluated the PK behavior of tobramycin in CF subjects 16.0±6.3 years of age after a one hour IV infusion, and compared to age-matched healthy subjects (Mann et al., 1985). After a 1.5 mg/kg dose, a higher CL\textsubscript{TOT} (2.19 mL/min/kg) was observed in CF subjects. However, no difference between CF patients and control was found in volume of distribution (0.18 L/kg). As a consequence of the increased CL\textsubscript{TOT} and the unvaried Vd, t\textsubscript{1/2} was shorter in CF patients (0.98 hours) compared to healthy subjects. The authors found no correlation between t\textsubscript{1/2}, Vd, CL\textsubscript{TOT}, weight or age.

Distribution to the systemic compartment has also been compared following administration of tobramycin by inhalation (INH) route. Lenney et al., administered 300 mg nebulized tobramycin to both healthy and CF adults (18-65 years) using two different nebulizers (Lenney et al., 2011). Results showed that healthy subjects had comparable time to reach the maximum plasma concentration (t\textsubscript{max}, ~1 h), lower maximum plasma concentration (C\textsubscript{max} = 0.6±0.2 µg/mL healthy vs. 1.2±0.6 µg/mL CF) and area under the curve (AUC\textsubscript{0-8}, 3.3±0.7 µg*h/mL for healthy vs. 5.0±3.0 µg*h/mL for CF). However, a longer t\textsubscript{1/2} (5.8±2.0 h healthy vs. 2.7±1.1 h CF) was observed in healthy subjects compared to CF subjects following nebulized administration using the PARI-LC\textsuperscript{®} PLUS nebulizer. In contrast, when using the Pari eFlow rapid nebulizer, AUC\textsubscript{0-8} was higher in healthy subjects, while C\textsubscript{max} was comparable, and t\textsubscript{1/2} remained longer. No mechanistic explanation was provided by the authors.

1.6.2.2 Pulmonary disposition

There is limited healthy vs. CF subjects’ comparison of sputum concentrations after either IV or inhaled administration available in literature. In part, this is likely due to the inability of healthy subjects to expectorate. However, when CF adult (mean age = 30.3, age range = 18-50 years) subjects were treated with 8 mg/kg as 10 min IV infusion, the C\textsubscript{max} reported for the sputum was much lower compared to that of the plasma (mean = 3.88 µg/mL and 29.4 µg/mL, respectively) (Moriarty et al., 2007). Similarly, Mombelli
et al., treated CF subjects with 2 or 3.5 mg/kg tobramycin IV infusion over 8 h (Mombelli et al., 1981). Serum antibiotic concentrations ranged from 2.3 to 5.6 µg/mL (mean = 3.6 µg/mL), as opposed to sputum concentrations that ranged from 0.1 to 2.2 µg/mL (mean = 0.71 µg/mL). A linear relationship (R² = 0.894) was found between sputum vs. serum tobramycin concentration. Lower sputum concentrations were likely due to the limited permeability of the drug. In order to achieve relevant tobramycin concentration in the lungs, high serum concentration is necessary, with consequent increased risk for toxicity.

The pulmonary disposition of tobramycin after inhaled administration is dependent upon several factors related to the device - formulation combination used to deliver the medications, including the amount of drug reaching the lungs. Although, Lenney et al. did not report pulmonary PK exposure metrics, they observed comparable whole and regional lung deposition of tobramycin with healthy and CF subjects when tobramycin was administered by PARI-LC® PLUS nebulizer (Lenney et al., 2011). Therefore, it might be reasonable to extrapolate that no difference in sputum exposure metrics is expected between healthy and CF subjects. In a CF patient study, Geller et al. administered 300 mg/5 mL nebulized tobramycin and obtained serum and sputum exposure metrics (Geller et al., 2003). The serum Cₘₐₓ was 1.12±0.44 µg/mL occurring at a tₘₐₓ of 1.05±0.38 h and the AUC₀₋₈ was 4.96±2.24 µg/h/mL. The sputum Cₘₐₓ was 985.7±839.3 µg/g achieved at a tₘₐₓ of 0.26±0.38 h and the AUC₀₋₈ was 1471±1278 µg/h/g. Rapid sputum peak concentrations were achieved after INH administration. Additionally, the authors reported a higher median serum t₁/₂ of 3.14 h, compared to the median sputum t₁/₂ of 2 h. However, highly variable results were obtained when examining the sputum exposure metrics, as expected by the complexity of the employed nebulization method. Despite a lack of direct comparisons between healthy and CF sputum exposure following inhalation, the studies available further underline the limited tobramycin concentration present in the sputum when administered by IV compared to the inhaled route, an observation that was already made during in vitro studies (Stigliani et al., 2016).
1.6.3 Differences in tobramycin pharmacokinetics between adults and children

1.6.3.1 Systemic disposition

There is no literature available on IV and inhaled administration of tobramycin to the same cohort of pediatric healthy subjects. In addition, there is also limited literature comparisons available for tobramycin PK in pediatric and adult CF subjects. However, it is observed that, as in the adult population, the pediatric CF population showed lower body weight when compared to age-matched healthy subjects (Hennig et al., 2013).

Kelly and colleagues evaluated CF patients with ages between 3-25 years treated with 2.5 mg/kg IV tobramycin, and observed no age-related trend for volume of distribution and total clearance (Kelly et al., 1982). Similarly, Touw et al., treated CF subjects (mean age = 23.8, range = 16-32 years) with 3.3 mg/kg dose of tobramycin and no relationship was found between age and clearance (Touw et al., 1994). Hoecker et al., evaluated the PK of tobramycin in pediatric subjects using different dosing regimen and compared a toddler (2-8 years) with a young adult (11-18 years) subgroup (Hoecker et al., 1978). In contrast to previous studies, their findings show a higher BW-normalized volume of distribution for the younger subgroup compared to the young adults (0.49±0.06 L/kg vs. 0.40±0.04 L/kg). The same authors also reported a total body clearance of 123.5±10.2 mL/min/1.73 m² and 195.0±16 mL/min/1.73 m² for the toddler and the young adult group, respectively. However, when recalculating per body weight, their CL\textsubscript{TOT} is higher for the 2-8 years old group compared to the 11–18-year-olds (3.30 mL/min/kg vs. 2.97 mL/min/kg, respectively). Therefore, given larger fold difference in volume of distribution compared to clearance, the t\textsubscript{1/2} was reported to be longer for the toddlers compared to the young adults. In a separate population PK study, Touw et al retrospectively evaluated CF pediatric (5-15 years) and adult (16-50 years) subjects that were treated with either 3.3 mg/kg or 10 mg/kg tobramycin IV infusion (Touw et al., 2007). Assuming a one compartment body model, a trend was observed between Vd and age for which a lower volume of distribution was found for older subjects. The Vd reported were 0.363±0.081 L/kg for pediatrics and 0.294±0.038 L/kg for adults.
The smaller adult Vd was again explained due to the hydrophilicity of tobramycin that tends to distribute in the extracellular fluid, which decreases as the age progresses.

As with IV administration, there were no inhalation studies comparing systemic disposition between adult and pediatric subjects. However, as for adults there is also evidence of tobramycin permeation from the lungs to the systemic circulation following inhalation in pediatric CF patients (Lenney et al., 2011). Rosenfeld et al (2001) treated pediatric CF subjects (3.6±1.6 years), with 300 mg tobramycin inhalation solution and found a C<sub>max</sub> of 0.6±0.5 µg/mL occurring at a t<sub>max</sub> of 1 h (Rosenfeld et al., 2001).

### 1.6.3.2 Pulmonary disposition

There are no direct adult-pediatric comparisons of tobramycin pulmonary disposition available in literature. The presence of tobramycin within the lungs of adult subjects after inhalation administration was already discussed, in addition, study of tobramycin disposition within the lung is also available in pediatric CF subjects (Mombelli et al., 1981; Moriarty et al., 2007). Levy et al. reported both plasma and sputum concentrations over time after tobramycin administration by IV infusion to pediatric CF patients with ages between 8 to 21 years (Levy et al., 1982). Using a one-compartment body model analysis, authors reported a Vd of 19.6±5.9 L/1.73 m<sup>2</sup>, CL<sub>TOT</sub> of 195±50 mL/min/1.73 m<sup>2</sup>, and t<sub>1/2</sub> of 1.7±4 h. Additionally, the authors reported a peak serum level (mean±standard error of mean, SEM) of 3.10±0.25 mg/L and peak sputum level of 0.45±0.05 mg/L taken 70 min after the end of infusion. Similarly to observations in adults, this study revealed >6-fold lower C<sub>max</sub> for sputum compared to plasma when administering tobramycin IV. Only one study was found in which tobramycin was administered to pediatric CF subjects (mean age 3.3 years) by inhalation route (Rosenfeld et al., 2001). However, only a single time point (43 min) sputum concentration was provided. The sputum concentration observed was 90±54 µg/mL. Despite the huge variability, it was significantly higher compared to the serum C<sub>max</sub> of 0.6±0.5 µg/mL (t<sub>max</sub> of 1 h). Therefore, even for pediatric CF subjects there is evidence of improved lung exposure when administering tobramycin by inhalation.
1.7 Objective

This research focuses on the understanding of the spray drying technology and the development of rapid screening characterization methods to produce a new tobramycin excipient enhanced growth spray-dried powder administered with a novel positive pressure dry powder inhaler for the treatment of *Pseudomonas aeruginosa* infection in cystic fibrosis pediatric patients. Treatment of *Pseudomonas aeruginosa* infection in pediatric cystic fibrosis patients is currently reliant on nebulized delivery of an inhaled antibiotic such as tobramycin. A tobramycin inhalation powder has been recently developed but is not indicated for children younger than 6 years old due to concerns about breath actuation of the dry powder inhaler in these young patients. A novel combination of a tobramycin EEG powder formulation and a positive pressure inhaler could allow the effective and rapid treatment of *Pa* infection in pediatric CF patients as young as 2 years old, therefore potentially delaying the appearance of chronic infection. The EEG technology is designed to produce micrometer size particles with low extrathoracic and exhalation losses together with a uniform regional lung deposition. Delivery using a positive pressure dry powder inhaler will ensure patient inhalation independent aerosolization with high lung dose delivery, while minimizing systemic exposure and administration time. A pediatric pharmacokinetic model will be developed to assess the systemic exposure and safety of a novel tobramycin EEG formulation - device combination administered by inhalation route. The proposed model will be employed to predict tobramycin systemic exposure for the novel inhaler formulation – device combination and estimate exposure changes using body weight as a surrogate for pediatric patient age and pulmonary bioavailability.
CHAPTER 2: HYPOTHESIS AND SPECIFIC AIMS

The overall aim of this research project was to evaluate the effect of spray drying process parameters and the storage conditions on the solid-state characteristics and aerosol performance of EEG powders to formulate a stable and highly dispersible tobramycin EEG powder to be delivered to pediatric cystic fibrosis subjects using a novel positive pressure dry powder inhaler. The aerosol performance screening process was conducted using cascade impactor and rapid laser diffraction methods, together with state-of-the-art solid-state characterization. Finally, the pediatric systemic exposure of the novel tobramycin EEG formulation – device combination was evaluated using a newly developed and validated pediatric pharmacokinetic model. The research objectives were addressed by the following hypotheses and specific tasks:

**Hypothesis 1: Spray drying conditions and excipients affect the particle size, the aerosol performance, the solid-state stability, and the hygroscopic growth capacity of EEG powders.**

Task 1-1: Investigate the effect of spray-drying conditions on the particle size and the aerosol performance of spray-dried EEG powders.

Task 1-2: Investigate the effect of dispersion enhancer on the characteristics and the aerosol performance of spray-dried EEG powders.

Task 1-3: Evaluate the effect of dispersion enhancer when delivering spray-dried powders under simulated airway conditions.

**Hypothesis 2: Tobramycin can be formulated as an EEG spray-dried powder and delivered by a novel positive pressure airflow dry powder inhaler with a large respirable fraction and low mouth-throat losses.**

Task 2-1: Evaluate the effects of outlet water vapor density during the spray-drying process and the storage conditions on tobramycin EEG spray-dried powders in terms of aerosol performance and solid-state characteristics at baseline and up to 6 months storage.
Task 2-2: Formulate and optimize tobramycin EEG spray-dried powders to improve the aerosol performance when delivered by a novel positive pressure airflow dry powder inhaler.

Task 2-3: Investigate the aerosol performance of a tobramycin EEG spray-dried powder under simulated airways condition in a realistic in vitro pediatric model.

**Hypothesis 3: Rapid screening laser diffraction-based method can be used to understand the dynamic aerosol performance of EEG spray-dried powders delivered using passive and active dry powder inhalers.**

Task 3-1: Use laser diffraction laser methods to investigate the influence of capsule air inlet aperture positioning on the aerosol performance of albuterol and budesonide model spray-dried EEG powders.

Task 3-2: Use laser diffraction laser methods to investigate the batch-to-batch reproducibility and incorporation of a realistic inhalation profile on the aerosol performance of spray-dried EEG powders of multiple batches of EEG formulations.

Task 3-3: Compare the aerosol performance of tobramycin EEG spray-dried powders using laser diffraction and cascade impaction methods.

**Hypothesis 4: A validated pharmacokinetic model to predict the systemic exposure of pediatric cystic fibrosis subjects treated with a novel tobramycin EEG spray-dried powder – positive pressure dry powder inhaler combination can be developed.**

Task 4-1: Collect available literature studies reporting plasma and sputum concentration time profiles for tobramycin administered by IV and INH route to pediatric CF subjects.

Task 4-2: Develop and validate a pharmacokinetic model including relevant disease state covariate.

Task 4-3: Estimate the systemic exposure for a novel tobramycin EEG formulation- dry powder inhaler combination using the validated pediatric PK model.
CHAPTER 3: ALBUTEROL SULFATE EXCIPIENT ENHANCED GROWTH (EEG) DRY POWDERS: FORMULATION AND SPRAY DRYING PROCESS CONDITIONS OPTIMIZATION

3.1 Introduction

This chapter evaluates the effects of spray drying and formulation variables on an excipient enhanced growth spray-dried powder formulation produced using the updated B-90 HP spray dryer with the goal of optimizing the aerosol performance of the powders. Previously, Son et al., investigated the effect of drying chamber length, spray mesh size, inlet gas drying temperature, % L-leucine content, % ethanol concentration in the spray drying vehicle, and % solids concentration using a Büchi Nano spray dryer B-90 (Büchi LaboratoryTechniques, Flawil, Switzerland) (Son et al., 2013). Son et al., 2013 evaluated the effect of 4 µm and 5.5 µm mesh size, as well as compared the effect of 70 °C vs. 85 °C inlet gas drying temperature. In this study, the effect of the Büchi Nano spray dryer B-90 HP new generation nebulizers will be evaluated, in addition to the characterization of spray-dried powders obtained at a higher inlet gas drying temperature (120 °C). The effect of the adjustable settings for peristaltic pump rate and spray head vibration will also be evaluated. Finally, L-leucine and trileucine will be compared as dispersion enhancers and moisture protecting agents to evaluate the powder dispersion properties and the solid-state stability of AS EEG powder formulations. The in vitro aerosol performance of these EEG powder formulations will be assessed following aerosolization using the CC90-3D inhaler, a high-efficiency, capsule-based dry powder inhaler, and the optimized capsule aperture orientation (Behara et al., 2014; Behara et al., 2014).
3.2 Materials and methods

3.2.1 Materials

Pearlitol® PF-Mannitol was donated from Roquette Pharma (Lestrem, France). Poloxamer 188 (Leutrol F68) was donated from BASF Corporation (Florham Park, NJ). L-leucine, albuterol sulfate, and all other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Trileucine was purchased from Bachem Americas, Inc. (Torrance, CA). Quali-V size 3 hydroxypropyl methylcellulose (HPMC) capsules were donated from Qualicaps (Whitsett, NC). Molykote®316 silicone release spray was purchased from Dow Corning Corporation (Midland, MI).

3.2.2 Preparation of AS EEG powders

Multicomponent spray-dried powders formulations were prepared using the Büchi Nano spray dryer B-90 HP in the open loop configuration (Büchi Corporation, New Castle, DE). Albuterol sulfate, mannitol (MN), either L-leucine (LL) or trileucine (TL), and poloxamer 188 were selected as model drug, hygroscopic excipient, dispersibility enhancer and surfactant, respectively. A solid concentration of 0.5 % w/v in 80:20 % v/v water:ethanol was used to prepare the feed solutions, with a relative component ratio of 30:48:20:2 % w/w for AS:MAN:LL/TL:poloxamer 188, respectively. The effects of different spray drying and formulation variables on the properties of the produced AS EEG powders was investigated as shown in Table 3.1. The feed solution was recirculated with the fraction of the liquid feed that was not sprayed return to the bulk liquid. The drying airflow gas rate was held constant at 120 L/min. The total solids concentration and relative component ratios were also unvaried. The effects of nebulizer mesh hole size, inlet gas drying temperature and the dispersion enhancer together with different combinations of peristaltic pump rate (%P), defined as the amount of feed solution pumped to the spray head over time and spray head vibration (%S), defined as the amplitude of the nebulizer vibration, were evaluated. Each powder formulation was collected from the electrostatic precipitator and stored in capped vials placed in a desiccator at room temperature and
~ 0% relative humidity (RH). In a stepwise study, the AS EEG powder formulation having best aerosol performance was evaluated for further optimization. Albuterol sulfate, L-leucine, and trileucine content uniformities were determined using a validated HPLC method. During the primary screening, the EEG powders were characterized for their particle size, powder uniformity, as well as for their in vitro aerosol performance when delivered by the high-efficiency CC90-3D dry powder inhaler. The lead powders will then be further characterized using a range of solid-state physico-chemical methods to evaluate the powders as described below.

### Table 3.1 Spray drying conditions and dispersion enhancer used to produce AS EEG powder formulations.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Spray drying conditions</th>
<th>Dispersion enhancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nebulizer mesh hole size</td>
<td>% P</td>
</tr>
<tr>
<td>1</td>
<td>small</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>medium</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>large</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>medium</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>medium</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>medium</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>medium</td>
<td>100</td>
</tr>
</tbody>
</table>

% P=percent peristaltic pump rate, %S=percent spray head vibration

### 3.2.3 Particle size characterization

The primary particle size of each spray-dried AS EEG powder formulation was determined by laser diffraction using the Sympatec HELOS (submicron R1 lens with 20 mm focal length) with RODOS/M disperser and ASPIROS sample feeder (Sympatec GmbH, Clausthal-Zellerfeld, Germany). The RODOS/M used compressed air to disperse the powders at 4 bar. The ASPIROS sample feeder was set to 60 mm/s. The minimum optical concentration (C opt) was set to 2.1%, for 2 s. Volume-based size distributions were
calculated by WINDOX software, based on Fraunhofer theory, and the particle size below which 50% of the particles lie (Dv50) was reported. Span was calculated as (Dv90-Dv10)/Dv50 and reported for each formulation. Each measurement was performed in triplicate.

3.2.4 Scanning electron microscopy and powder density

AS EEG powder morphology was evaluated using Zeiss EVO 50 XVP (Carl Zeiss AG, Germany) scanning electron microscopy. Powder formulations were separately spread on double adhesive carbon tape, then mounted onto an SEM aluminum stub, followed by sputter coating with gold using Electron Microscopy Sciences (EMS) 550x sputter coater.

Powder bulk density for samples with limited powder mass was measured using a 1 mL plastic syringe (Sansone et al., 2009). Bulk density was calculated as the weight difference between the weight of the empty syringe and the weight of the syringe containing powder divided by the volume occupied by the powder. Each powder bulk density was measured in triplicate.

3.2.5 Dynamic vapor sorption analysis

Dynamic vapor sorption (DVS Adventure, Surface Measurement Systems Ltd., UK) was used to evaluate the water vapor uptake at different %RH values. At isothermal conditions of 25 °C, about 10 mg of sample was initially equilibrated at 0 %RH until no mass change was observed based on mass equilibration criterion of \( \frac{dm}{dt} = 0.002 \%/\text{min} \). Once the drying step was completed, the humidity was set to increase from 0 %RH to 90 %RH at a rate of 2 %RH/h. Powders were exposed to one cycle of moisture sorption. The rates of surface water vapor adsorption and bulk water vapor absorption were compared. Rates of mass change were obtained from the slope of the best fitting tangent of the data points before and after the RH glass transition.
inflection (Hassan et al., 2020). Weight change is expressed as a function of % of the reference weight, defined as the equilibrium weight at 0 %RH.

3.2.6 X-ray powder diffraction analysis

Powder crystallinity was evaluated by X-ray powder diffraction (XRPD) and it was conducted using the Rigaku Miniflex 6G (Rigaku, Japan). Samples were exposed to Cu-Kα radiation source at 40 kV and 15 mA and they were scanned using a continuous mode in the range of 5-50 °2θ with a 0.05 °2θ step width and a speed rate of 1.5 °2θ /min. The D/tx Ultra scintillation detector was used. The Rigaku Miniflex 6G was equipped with an automatic sample changer (ASC-8) and spinner, thus all samples were subjected to 80 rpm rotation during each experiment. Samples were placed in a 5 mm x 0.2 mm zero background sample holder. Raw materials and spray-dried powders obtained after DVS moisture exposure were used to evaluate the crystallinity of AS EEG powder formulations.

3.2.7 In vitro aerosol performance testing

Aerodynamic particle sizing of AS EEG powder formulations was evaluated by cascade impactor using a Next Generation Impactor (NGI, MSP Co., Shoreline, MN). About 2 mg of the AS EEG formulation was filled into size 3 HPMC capsules, pierced, and placed in the CC90-3D inhaler. The CC90-3D inhaler (Figure 3.1) was created using Accura ClearVue stereolithography resin (3D Systems Inc.) (Behara et al., 2014). The NGI pre-separator and stages were coated with Molycote® 316 silicone release spray to avoid particle bounce. The CC90-3D inhaler was positioned in the NGI pre-separator using a mouthpiece adapter made from a RTV (room temperature vulcanizing) silicone mold rubber (Micro-mark Ten-to-One, Berkeley Heights, NJ). The NGI was in the upright position to ensure vertical orientation of the CC90-3D inhaler. A
constant flow of 45 L/min for 5.3 s was used in order to obtain 4 kPa pressure drop across the inhaler. A three-way valve and timer was employed to deliver the air flow.

![Figure 3.1 Schematic representation of the CC90-3D dry powder inhaler.](image)

No USP induction port was included in the NGI experiments with the aim of determining the particle size for the total dose of powder formulation delivered by the inhaler. Powder remaining in the CC90-3D inhaler, capsule, and powder deposited on pre-separator, and each NGI stage was dissolved in water and then quantitatively analyzed using a validated HPLC method. Emitted dose (ED) and fine particle fractions (FPF) were determined as following:

\[
\text{Emitted dose, } \% = \frac{1 - (\text{device + capsule}, \text{mg})}{\text{nominal drug dose, mg}} \times 100 \tag{Equation 3.1}
\]

\[
\text{Fine particle fraction, } \% = \frac{\text{mass of drug < stated size, mg}}{\text{pre-separator + impactor drug dose, mg}} \times 100 \tag{Equation 3.2}
\]

The nominal dose of AS in the AS EEG formulations was determined by taking known masses of the formulation which were dissolve in known volumes of water to determine the AS powder content uniformity. The mean amount of AS per milligram of formulation was determined using the HPLC analysis.
Mass median aerodynamic diameters (MMADs) were calculated as the size associated with 50% of the cumulative mass count relative to the impactor dose, including the pre-separator. Each experiment was performed in replicate \( n \geq 3 \).

### 3.2.8 Excipient enhanced growth aerosol characterization

The *in vitro* aerosol performance of AS EEG powder formulations was additionally evaluated using simulated airways conditions using the growth tube methods previously described (Hindle and Longest, 2012). The CC\(_{90-3D}\) inhaler was attached to the inlet of the growth tube and the exit was attached to the NGI to allow for aerosol particle size determination. The inhaler was actuated and sized using a flow rate of 45 L/min which produces a 1.5 second exposure as the aerosol transits through the growth tube to simulate inhaled delivery. The growth tube and impactor were equilibrated in simulated airway conditions of 37 °C and 99 %RH in an environmental chamber. A control experiment was performed under ambient conditions (22 °C and 45 %RH). The %FPF<1 µm, MMAD, and MMAD ratio, defined as the ratio between the MMAD obtained under simulated airway conditions and MMAD obtained under ambient conditions, were evaluated.

### 3.2.9 Statistical analysis

Analysis of variance (ANOVA) or Student’s *t*-test were conducted to evaluate the effect of different variables on the particle size characteristics and the aerosol performance of AS EEG powder formulations. Significant difference was considered for *p*-value< 0.05 for all statistical analyses. JMP Pro 15 (SAS Institute Inc., Cary, NC) was used to perform the statistical analysis.
3.3 Results

3.3.1 Evaluation of the effects of spray drying and formulation variables

The AS EEG powder formulations shown in Table 3.1 were spray-dried using the Büchi Nano B-90 HP spray dryer. The feed solutions have identical solvent composition, % solids content and relative component ratio. Table 3.1 shows the different variables selected for investigation, the nebulizer mesh hole size, inlet gas drying temperature and the dispersion enhancer together with different combinations of peristaltic pump rate and spray head vibration.

Overall, it can be observed from Table 3.2, Table 3.3, Table 3.4, Table 3.5 that all of the AS EEG powder formulations produced had small primary particle sizes, reported as Dv50, ranging from a mean (SD) value of 0.85 (0.01) µm and 1.25 (0.01) µm. The mean (SD) Span ranged from 1.29 (0.01) to 1.60 (0.03). All of the powder formulations had acceptable aerosol performance when delivered by the CC90-3D dry powder inhaler. The emitted dose was higher than 60% and the MMAD was ≤2 µm for each of the powder formulations. In addition, the respirable and submicron fractions were greater than 90% and 20% of the nominal dose, respectively, for most of the powder formulations.

The nebulizer mesh hole sizes are characterized by nominal labels of “small”, “medium” and “large”. The manufacturer provides no additional information regarding the actual mesh hole size. An initial study using laser diffraction was performed to estimate the droplet size generated by each of the nebulizer meshes. An AS EEG feed formulation was sprayed by small, medium and large nebulizers directly into the laser beam and size of the droplets was measured by laser diffraction (Spraytec®, Malvern Instruments Inc., MA). The mean droplet size observed for the small, medium and large mesh hole sizes were 3 µm, 5.5 µm, and 7 µm, respectively. Analysis of variance confirmed that the difference observed in the droplet size measurements translated into differences in the primary particle size, span, and aerodynamic diameter measurements. It was observed that as the droplet size increased, the dried particles were larger and less uniform. Despite having about 70% of the powder emitted from the dry powder inhaler, spray drying with the large mesh
hole produced a relatively large MMAD (mean (SD) = 2.18 µm (0.01)) that consequently resulted in 10.6% submicrometer aerosol size fraction. Spray drying using the small mesh hole size produced an aerosol size of 1.19 µm, the %FPF<5 µm was 91.8% and the %FPF<1 µm was 37.6%, which was the highest submicrometer aerosol fraction. However, using the small mesh hole size, the aerosol powder emitted dose was significantly lower compared to medium mesh hole size nebulizer. The medium mesh hole size nebulizer provided the highest emitted dose while maintaining the MMAD below 1.5 µm.

**Table 3.2** Effect of nebulizer mesh hole size on particle characteristics and *in vitro* aerosol performance of AS EEG powder formulations (values are reported as means (SD), n ≥ 3).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dv50, µm</th>
<th>Span</th>
<th>ED, %</th>
<th>MMAD, µm</th>
<th>% FPF&lt;5 µm</th>
<th>% FPF&lt;1 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (small mesh hole size)</td>
<td>0.85 (0.01)</td>
<td>1.29 (0.01)</td>
<td>62.4 (1.9)</td>
<td>1.19 (0.05)</td>
<td>91.8 (1.3)</td>
<td>37.6 (1.9)</td>
</tr>
<tr>
<td>2 (medium mesh hole size)</td>
<td>1.07* (0.01)</td>
<td>1.37* (0.02)</td>
<td>76.0* (0.7)</td>
<td>1.49* (0.04)</td>
<td>98.4* (0.9)</td>
<td>22.9* (1.8)</td>
</tr>
<tr>
<td>3 (large mesh hole size)</td>
<td>1.25*# (0.01)</td>
<td>1.60*# (0.03)</td>
<td>70.7*# (1.8)</td>
<td>2.18*# (0.01)</td>
<td>90.9* (0.9)</td>
<td>10.6*# (0.2)</td>
</tr>
</tbody>
</table>

*Significantly different from small mesh hole size (One-way ANOVA, Tukey’s HSD, p-value< 0.05).  
*Significantly different from medium mesh hole size (One-way ANOVA, Tukey’s HSD, p-value< 0.05).  
Dv50=volume diameter at the 50th percentile, ED=emitted dose, MMAD=mass median aerodynamic diameter, FPF=fine particle fraction

Following selection of the medium mesh hole size for continued investigation, Table 3.3 shows the effect on the particle size and aerosol performance of AS EEG powder formulations after altering the peristaltic pump rate and spray head vibration from the initial conditions of 100 %P and 80 %S. It was observed that as the peristaltic pump rate decreases from 100% to 5%, the Dv50 was statistically smaller with values of 1.07 and 1.01 µm, respectively. Although this size difference appears small, it did appear to have a significant effect on the aerosol performance. Once aerosolized, a lower emitted dose of 68.5% compared to 76.0% (p = 0.0197) and a lower respirable fraction of 91.5% compared to 98.4% (p = 0.0378) was observed for the AS EEG powder formulation sprayed at 5% pump rate, while no difference was observed in span, MMAD, and submicron fraction.
When the spray head vibration was reduced from 80% to 10%, the Dv50 decreased by 0.11 µm, with a mean value of 0.96 µm compared to 1.07 µm. Again, this small but statistically significant decrease in primary particle size produced a significant effect on the aerosol performance in the CC90-3D inhaler. The powder formulation produced using the 10% spray head vibration had a lower emitted dose (p-value < 0.0001) and fine particle fraction smaller than 5 µm (p-value = 0.0002) by ~15% and ~6%, respectively, compared to the 80% spray head vibration.

Changing the spray parameters in this study resulted in changes to the measured spray rate. The spray rate was defined as the volume of feed solution sprayed through the nebulizer mesh per minute. Figure 3.2 shows the measured spray rate as the %S and %P were varied. As pump rate and spray head vibration decreased, so did the spray rate, and a linear correlation was observed (R² = 0.999).

In this study, it was observed that changing the pump and spray variables resulted in a relatively large range of spray rates (0.2 – 0.7 mL/min) for the AS EEG feed formulations. Despite the changes in spray rate, the measured droplet size emitted from the medium mesh hole size did not vary when changing the pump and spray variables with a size range of 5.5-5.6 µm. This resulted in only small differences in Dv50 that were observed ranging from 0.96 – 1.07 µm across the pump and spray conditions. Despite the small measured differences in droplet and primary particle sizes there were significant differences in the aerosol performance, high %P and %S were found to produce powders with improved aerosol characteristics compared to lower %P and %S. Primarily, these effects were believed to be associated with the larger size primary particles, despite having differences only in the range of 0.1-0.2 µm, having a positive effect on aerosol dispersion and emptying from the used inhaler.
Table 3.3 Effect of % peristaltic pump and % spray head vibration on particle characteristics and *in vitro* aerosol performance of AS EEG powder formulations (values are reported as means (SD) n ≥ 3).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dv50, µm</th>
<th>Span</th>
<th>ED, %</th>
<th>MMAD, µm</th>
<th>FPF&lt;5 µm, %</th>
<th>FPF&lt;1 µm, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effect of %P</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (100%P)</td>
<td>1.07 (0.01)</td>
<td>1.37 (0.02)</td>
<td>76.0 (0.7)</td>
<td>1.49 (0.04)</td>
<td>98.4 (0.9)</td>
<td>22.9 (1.8)</td>
</tr>
<tr>
<td>4 (5%P)</td>
<td>1.01* (0.02)</td>
<td>1.38 (0.03)</td>
<td>68.5* (4.5)</td>
<td>1.48 (0.06)</td>
<td>91.5* (5.0)</td>
<td>24.7 (2.4)</td>
</tr>
<tr>
<td><strong>Effect of %S</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (80%S)</td>
<td>1.07 (0.01)</td>
<td>1.37 (0.02)</td>
<td>76.0 (0.7)</td>
<td>1.49 (0.04)</td>
<td>98.4 (0.9)</td>
<td>22.9 (1.8)</td>
</tr>
<tr>
<td>5 (10%S)</td>
<td>0.96* (0.00)</td>
<td>1.44* (0.02)</td>
<td>60.9* (1.3)</td>
<td>1.49 (0.02)</td>
<td>92.0* (0.8)</td>
<td>25.3 (0.9)</td>
</tr>
</tbody>
</table>

*Significantly different from 100%P-80%S (Student’s *t*-test, *p*-value < 0.05).

ED=emitted dose, MMAD=mass median aerodynamic diameter, FPF= fine particle fraction

Figure 3.2 Spray rate (mL/min) as a function of different peristaltic pump rate and spray head vibration.
Following selection of the 100 %P and 80 %S spray drying conditions, Table 3.4 shows the effect of increasing the inlet gas drying temperature from 70 °C to 120 °C. It was observed that increasing the inlet gas drying temperature results in an increased outlet temperature from 40°C to 50 °C. One potential issue resulting from this increase in temperature was degradation of the drug in the powder both during spraying and collection on the electrostatic precipitator. Prior to characterization, both the AS content and the L-leucine content was assayed and confirmed to be in agreement with their nominal concentrations. The AS content for the 120 °C powder was close to the nominal value of 30% and not significantly different from the powder sprayed at 70 °C. It was found that the mean (SD) L-leucine content was 21.4% (1.9) for powder sprayed at 70 °C and 20.4% (2.0) for the powder spray-dried at 120 °C. Despite an increase in inlet gas drying temperature, no statistically significant L-leucine degradation was observed.

Table 3.4 shows that increasing the inlet gas drying temperature results in slightly smaller particle size ($p = 0.0058$), which is also reflected in a smaller MMAD ($p < 0.0001$) and small increases in the fine particle fractions when considering aerosol performance. There was no significant difference observed in the aerosol emitted dose. The powder obtained at 70 °C was selected as the lead powder given the lack of practical differences between the powders and potential issues regarding degradation at higher temperatures for both albuterol sulfate and L-leucine. These optimized conditions will be employed to compare the effects of dispersion enhancer between L-leucine and trileucine.
Table 3.4 Effect of inlet gas drying temperature on particle characteristics and in vitro aerosol performance of AS EEG powder formulations (values are reported as means (SD), n ≥ 3).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dv50, µm</th>
<th>Span</th>
<th>ED, %</th>
<th>MMAD, µm</th>
<th>FPF&lt;5 µm, %</th>
<th>FPF&lt;1 µm, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (70 °C)</td>
<td>1.07 (0.01)</td>
<td>1.37 (0.02)</td>
<td>76.0 (0.7)</td>
<td>1.49 (0.04)</td>
<td>98.4 (0.9)</td>
<td>22.9 (1.8)</td>
</tr>
<tr>
<td>6 (120 °C)</td>
<td>1.03* (0.01)</td>
<td>1.38 (0.03)</td>
<td>73.4 (3.1)</td>
<td>1.46* (0.03)</td>
<td>97.2* (0.6)</td>
<td>25.1* (1.4)</td>
</tr>
</tbody>
</table>

*Significantly different from 70 (Student’s t-test, p-value< 0.05).
ED=emitted dose, MMAD=mass median aerodynamic diameter, FPF=fine particle fraction

Table 3.5 reports the particle characteristics and the aerosol performance of AS EEG powder formulations obtained using L-leucine and trileucine as dispersion enhancers. The formulation with trileucine has a larger Dv50 and it is a more uniform powder compared to the L-leucine powder (p = 0.0007). When aerosolized, the trileucine powder formulation had a slightly lower emitted dose (p = 0.0025) and respirable fraction (p < 0.0001), compared to the L-leucine powder. However, the trileucine formulation resulted in a 1.01 µm MMAD, which was 0.5 µm smaller (p-value < 0.0001) compared to L-leucine powder. Impressively, it had a significantly higher submicron fraction by more than 20% (p-value = 0.0001), compared to L-leucine powder, with almost 50% of the powder being less than 1 µm.

Table 3.5 Effect of dispersion enhancer on particle characteristics and in vitro aerosol performance of AS EEG powder formulations (values are reported as means (SD), n ≥ 3).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dv50, µm</th>
<th>Span</th>
<th>ED, %</th>
<th>MMAD, µm</th>
<th>FPF&lt;5 µm, %</th>
<th>FPF&lt;1 µm, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (L-leucine)</td>
<td>1.07 (0.01)</td>
<td>1.37 (0.02)</td>
<td>76.0 (0.7)</td>
<td>1.49 (0.04)</td>
<td>98.4 (0.9)</td>
<td>22.9 (1.8)</td>
</tr>
<tr>
<td>7 (trileucine)</td>
<td>1.15* (0.01)</td>
<td>1.31* (0.02)</td>
<td>73.7* (1.3)</td>
<td>1.01* (0.01)</td>
<td>96.0 (1.3)</td>
<td>48.9* (0.9)</td>
</tr>
</tbody>
</table>

*Significantly different from L-leucine (Student’s t-test, p-value< 0.05).
ED=emitted dose, MMAD=mass median aerodynamic diameter, FPF=fine particle fraction
The spray drying and formulation conditions that resulted in the best performing AS EEG powder formulation were achieved using the medium nebulizer size together with 70 °C inlet gas temperature, 100%P and 80%S settings and trileucine as dispersion enhancing agent. The optimized L-leucine and trileucine formulation powders were further characterized using a range of solid-state physico-chemical methods to compare the powders as described below.

### 3.3.2 EEG powder characterization

#### 3.3.2.1 SEM and powder bulk characterization

Powder morphology showed that the L-leucine AS EEG powder formulation resulted in particles having a spherical shape and a slightly corrugated surface (Figure 3.3a). Conversely, the trileucine EEG powder appeared as collapsed particles with a highly corrugated surface, as shown in Figure 3.3b. The morphology difference between the two powders was reflected in the bulk density with the L-leucine powder having a mean (SD) bulk density of 0.136 (0.007) g/cm³, while the trileucine powder was significantly lower with a value of 0.102 (0.009) g/cm³ (p-value= 0.0068; n = 3).

![Figure 3.3](image)

**Figure 3.3** Scanning electron micrographs of the (a) L-leucine and (b) trileucine AS EEG powder formulations.
3.3.2.2 Dynamic vapor sorption analysis

Figure 3.4 shows the moisture sorption profiles for the L-leucine and trileucine AS EEG powder formulations. After drying at 0 %RH, both AS EEG powder formulations were exposed to increasing RH (%) at a rate of 2 %RH/h starting from 0 %RH to 90 %RH. Both powders absorbed less than 3% moisture on the particle surface, as shown by the inflection point obtained between the end of the surface absorption and the beginning of both surface and bulk sorption (Burnett et al., 2004). As shown in Table 3.6, the inflection point that defines the glass transition RH (RH_g) occurred at a lower RH for the L-leucine EEG powder, compared to trileucine EEG powder (47% vs. 43%, respectively). There was only a ~2% mass change difference observed in the surface moisture sorption between the powders. However, the rate at which moisture was absorbed by the trileucine powder was twice the rate of the L-leucine powder (0.1 vs. 0.05 Δm %dried mass/h). There was a clear difference between the two powders in the surface and bulk sorption region until the crystallization RH (RH_c). Surface and bulk moisture sorption occur after the inflection point at a rate that was calculated to be slower for the L-leucine EEG powder compared to the trileucine EEG powder (0.23 Δm %dried mass/h and 0.32 Δm %dried mass/h, respectively) (Burnett et al., 2004). In summary, the surface and bulk sorption rates were 4 and 3 folds faster than the initial surface absorption for the L-leucine and trileucine powders, respectively. The mass change observed at RH_c was 2.8-fold higher for the trileucine powder compared to the L-leucine powder, suggesting increased hygroscopicity for the trileucine EEG powder. Despite its tendency to absorb more moisture at a faster rate, the trileucine powder recrystallized at a higher RH (78% RH) compared to L-leucine powder, suggesting prolonged stability even with the increased moisture content. Overall, the AS EEG powder formulation spray-dried using L-leucine as dispersion enhancer absorbed less moisture but recrystallized at a lower RH compared to the AS EEG powder sprayed with trileucine.
Table 3.6 Dynamic vapor sorption analysis for the L-leucine and trileucine AS EEG powder formulations.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$\text{RH}_{g}$, % RH</th>
<th>$\text{RH}_{c}$, % RH</th>
<th>$\Delta m$ at 50 % RH, %</th>
<th>$\Delta m$ at RH$_c$, %</th>
<th>Surface ad, $\Delta m$ % dried mass/h</th>
<th>Surface+bulk, $\Delta m$ % dried mass/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (L-leucine)</td>
<td>43</td>
<td>57</td>
<td>2.4</td>
<td>2.8</td>
<td>0.05</td>
<td>0.23</td>
</tr>
<tr>
<td>7 (trileucine)</td>
<td>47</td>
<td>78</td>
<td>3.6</td>
<td>7.9</td>
<td>0.10</td>
<td>0.32</td>
</tr>
</tbody>
</table>

$\text{RH}$=relative humidity, $\text{RH}_{c}$=crystallization relative humidity, $\text{RH}_{g}$=glass transition relative humidity

3.3.2.3 X-ray powder diffraction analysis

Powder crystallinity was assessed using x-ray powder diffraction as shown in Figure 3.5, Figure 3.6 and Figure 3.7. The XRPD diffractograms for the L-leucine and trileucine AS EEG powder formulations (Figure 3.6 and Figure 3.7) as well as for albuterol sulfate, mannitol, L-leucine, and trileucine raw materials (Figure 3.5) are reported. All raw materials showed characteristic crystalline features, as reported in the literature (Haghi et al., 2012; Sou et al., 2013). The spray-dried powder formulations revealed a degree of amorphicity, represented by the characteristic halos. The amorphous nature of the spray-dried powders was
confirmed by comparing the diffractograms obtained before and after moisture exposure and recrystallization (Figure 3.7). Following exposure to moisture (90%RH), both of the AS EEG powder formulations revealed a higher degree of crystallinity compared to the raw material standards.

Despite the complexity of the diffractograms due to the multicomponent EEG platform, some characteristic peaks can be recognized. Figure 3.5 shows the albuterol sulfate raw material, the diffractogram indicates that the raw material was crystalline. In contrast, none of the characteristic albuterol sulfate crystal lattice peaks were observed in both of the EEG spray-dried powder formulations, which suggests that albuterol sulfate is in the amorphous phase after spray drying. A similar observation was made by Chawla et al. (Chawla et al., 1994). Following exposure to relative humidity and induced crystallization during the DVS experiments, characteristic albuterol sulfate crystalline peaks were identified at 15.2 °2θ, and 17.7 °2θ (Figure 3.7) for both the L-leucine and trileucine EEG powders. For the L-leucine powder, an additional characteristic peak at 12.7 °2θ was observed, however this peak co- incidental with a characteristic trileucine peak in the trileucine EEG powder formulation. From this study, it appears that following spray drying of both powder formulations, albuterol sulfate is present in an amorphous form that does crystallize following exposure to moisture during the DVS experiment. Zellnitz et al., reported a similar observation of albuterol crystallization following moisture exposure (Zellnitz et al., 2015).

Previous studies have shown that both raw material and spray-dried mannitol are crystalline in nature however mannitol exists in a number of polymorphic forms (Sou et al., 2013, Li et al., 2014). In this study, the reference mannitol raw material used was found to be the β polymorph, which is the most thermodynamically stable form of mannitol (Li et al., 2014). Following spray drying of the L-leucine EEG formulation, β-mannitol was also found in the EEG powder before and after moisture exposure, as confirmed by 14.5 °2θ and 16.7 °2θ peaks, indicating that mannitol retains a crystalline phase even after spray drying (Sou et al., 2013, Lee et al., 2011). In addition, the presence of a less stable mannitol polymorph (α) was observed in both EEG powders with a characteristic peak at 9.7 °2θ (Li et al., 2014).
For the L-leucine EEG powder, it was observed that the intensity of this peak decreased after moisture exposure, suggesting the conversion to a more stable polymorphic form.

The powder diffractogram for the L-leucine raw material revealed a crystalline profile (Figure 3.5), however following spray drying, this amino acid was reported to result in a partially crystalline system (Sou et al., 2013, Hassan et al., 2020, Leung et al., 2017). The L-leucine EEG powder formulation showed broad and less intense L-leucine peaks at 6 °2θ and at 18.6 °2θ compared to the raw material (Lamy et al., 2019). It is suggested that L-leucine does not fully recrystallize during the spray drying process, but it forms a crystalline shell on the particle surface (Sou et al., 2013). This is supported by the observed d-spacing value of 1.47 nm for the 6 °2θ peak in the L-leucine EEG powder diffractogram. Additionally, it was observed that while the L-leucine raw material had a preferred orientation toward the (001) plane at 6 °2θ, the partially crystalline L-leucine in the AS EEG powder formulation also showed a preferred orientation toward the (110) plane at 18.6 °2θ (Raula et al., 2008). Trileucine raw material is crystalline as shown in Figure 3.5. However, separate studies not reported here showed that trileucine when spray-dried alone was amorphous given the absence of characteristic crystalline peaks and it remained amorphous even after moisture exposure (data not shown). No crystalline peaks characteristic for trileucine were observed in its EEG powder before and after moisture exposure, suggesting an amorphous state in this multicomponent spray-dried powder that did not appear to change following exposure to elevated humidity.
Figure 3.5 XRPD diffractograms for albuterol sulfate, mannitol, trileucine, and L-leucine raw materials.

Figure 3.6 XRPD diffractograms of L-leucine and trileucine AS EEG powder formulations.
3.3.2.4 Excipient enhanced growth aerosol characterization

Given the observed differences for the L-leucine and trileucine formulations in terms of their dispersion enhancing and moisture protection effects, the final study examined the aerosol performance following exposure to simulated airways conditions. L-leucine or trileucine EEG powders were aerosolized by the CC\textsubscript{90}-3D DPI and exposed to ambient and simulated airway conditions (37 °C and 99 %RH) in a growth tube. Table 3.7 shows the aerosol characteristics for each EEG powder formulation. Irrespective of the dispersion enhancer used, the MMAD obtained under simulated airway conditions was larger than the MMAD obtained under ambient conditions ($p = 0.0004$ in both cases). The MMAD growth ratio, defined by the ratio of the simulated airway MMAD to the ambient MMAD was also calculated for the two powder formulations. The L-leucine powder formulation had a growth ratio of 1.54 compared to the trileucine powder which had a larger growth ratio of 1.81. The trileucine EEG powder formulation showed more than 30% decrease in percentage of submicrometer fraction compared to a 12% decrease for the L-leucine EEG.

![Figure 3.7 XRPD diffractograms of L-leucine and trileucine AS EEG powder formulations after moisture exposure.](image-url)
powder. In both cases, there were less than 10% of the particles less than 1 micrometer in size following exposure to simulated airway conditions which will improve aerosol retention following inhalation.

**Table 3.7** Aerosol characteristics of EEG powder formulations following exposure to ambient and simulated airway conditions (values are reported as means (SD), n ≥ 3).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Ambient MMAD, µm</th>
<th>Simulated Airway MMAD, µm</th>
<th>Ambient FPF&lt;1 µm, %</th>
<th>Simulated Airway FPF&lt;1 µm, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (L-leucine)</td>
<td>1.60 (0.02)</td>
<td>2.46* (0.14)</td>
<td>16.1 (1.3)</td>
<td>4.1* (1.5)</td>
</tr>
<tr>
<td>7 (trileucine)</td>
<td>1.08 (0.01)</td>
<td>1.96* (0.14)</td>
<td>43.5 (1.1)</td>
<td>10.1* (1.6)</td>
</tr>
</tbody>
</table>

* Significantly different from initial MMAD or %FPF<1 µm (Student’s t-test, p-value< 0.05).
MMAD=mass median aerodynamic diameter, FPF=fine particle fraction

**3.4 Discussion and conclusions**

This study investigated the effect of nebulizer mesh hole size, peristaltic pump rate, spray head vibration, inlet gas drying temperature as spray drying variables that could potentially have an effect on the particle size and the aerosol performance of EEG powder formulations produced by the Büchi Nano B-90 HP spray dryer. Additionally, the effect of L-leucine and trileucine as dispersion enhancing and moisture protecting agents was conducted by testing the particle size, the solidstate characteristics and the aerosol performance both under environmental and lung simulated conditions. Feed solution solvent, solids concentration, and relative component ratios were maintained unvaried for AS EEG powder formulations. Previous studies have shown that the best performing AS EEG powder formulation produced using the original Büchi Nano B-90 and tested with the Aerolizer DPI was obtained using the 4 µm mesh size (Son et al., 2013a). Recently, the Nano B-90HP has implemented new spray drying nebulizers which are available in small, medium and large mesh hole sizes. For this study, the droplet size measured for the medium nebulizer mesh hole size was 5.5 µm, as previously reported by Longest et al., 2020, and it resulted in the best performing AS EEG powder (Longest et al., 2020). The optimized L-leucine powder produced in this study had a comparable
aerosol performance to that obtained by Behara et al., 2014 using the CC<sub>90</sub>-3D DPI. Measured particle size differences for the three new nebulizer mesh hole sizes investigated were in agreement with the measured droplet sizes for the nebulizers. The powder Dv50 was found to increase as the nebulizer mesh hole size increased from small to large. The same trend was observed for the EEG powders during aerosolization in the CC<sub>90</sub>-3D DPI. A larger MMAD and decreased %FPF<1 µm were observed as the nebulizer mesh hole size increased. Due to the lower emitted dose for the small mesh hole size nebulizer and the low submicrometer size fraction (10%) for the large mesh hole size nebulizer, the medium nebulizer was selected to proceed during the process conditions optimization experiments. Using the medium nebulizer, the effects of peristaltic pump rate and spray head vibration on the particle size and the aerosol performance of AS EEG powders were studied. At higher peristaltic pump rates and spray head vibration setting there was an observed increase in the formulation spray rate. However, irrespective of changes in the spray drying settings and the resultant spray rate, the measured droplet size remained in the range of 5.5-5.6 µm. There is a literature report of an increase in spray rate as a function of increased peristaltic pump rate, in that study it results in larger droplets being formed due to an increased pressure on the nebulizer (Suryaprakash et al., 2014). It has been also reported that increasing the spray head vibration, increased the number of droplets sprayed per time causing an increased spray rate but only minor droplet size change were observed (Littringer et al., 2013). Despite changes in the spray rate observed in this study when changing the %P or %S, there were no differences found in sprayed droplet size and only small differences observed in the Dv50s. However, the aerosol performance was significantly affected with statistical differences observed in emitted dose and MMAD when changing the spray drying condition variables. When aerosolized by the CC<sub>90</sub>-3D DPI, the L-leucine EEG powders obtained with low peristaltic pump rate and spray head vibration did not perform as well as the EEG powder obtained by 100%P-80%S. Increasing the inlet gas drying temperature has been reported to have a direct effect on the solvent evaporation rate (Vehring, 2008). In this study, small differences in particle size and aerosol performance were observed when comparing inlet gas drying temperatures of 70 °C and 120 °C. However, it has been likely that the tested temperature range
was too narrow to obtain significant changes in the drying process, and consequently on the particle size and the aerosol performance of AS EEG powders (Littringer et al., 2013, Lee et al., 2011).

It has been observed that due to its lower aqueous solubility, trileucine will reach supersaturation earlier in the drying process and therefore onto the surface layer of a larger droplet compared to L-leucine (Wang et al., 2021). However, due to a lack of mechanical strength, the surface shell of these trileucine particles will ultimately collapse as wrinkled morphology particles (Wang et al., 2021). The wrinkle morphology results in decreased powder bulk density compared to the spherical L-leucine particles as observed in this study. Wrinkled particles are known to have reduced points of contact and lower interparticle interactions which may be beneficial for aerosol performance and dispersion (Lechuga-Ballesteros et al., 2008b). In addition, trileucine is known to be amorphous after spray drying (Gomez et al., 2021; Wang et al., 2021). This physicochemical property together with the wrinkled morphology were observed for the trileucine EEG formulation which had an improved aerosol performance in terms of smaller MMAD (1.46 µm) compared to the L-leucine formulation (1.49 µm). It was also found that the trileucine EEG powder produced double the submicrometer fraction compared to the L-leucine EEG powder. A similar increase in the submicrometer fraction for a trileucine formulation was reported by Gomez et al. (Gomez et al., 2021). The combination of a small MMAD and high submicrometer faction are key characteristics that will result in improved performance of an EEG powders, as it minimizes mouth-throat deposition and increases in size in the airways due to hygroscopic growth and increasing airway retention.

Sibum et al. previously studied the aerosol performance of isoniazid spray-dried powders containing L-leucine or trileucine that were exposed to different RH storage conditions (Sibum et al., 2019). They observed that trileucine offered improved moisture protection over three months, resulting in a larger respirable fraction when aerosolized. Conversely, Wang et al., 2021 showed the superiority of L-leucine as humidity protecting agent for trehalose spray-dried powders stored at 90 %RH and 25 °C, and aerosolized after 1 hour, compared to trileucine (Wang et al., 2021). In this study, the powder moisture protection effects of L-leucine and trileucine were evaluated using a dynamic moisture absorption experiment. The trileucine
powder had a faster surface moisture sorption rate compared to L-leucine (0.10 %/h and 0.05 %/h, respectively). This finding agreed with the observation that an amorphous trileucine phase will be more prone to absorb moisture, compared to a crystalline L-leucine shell. As reported by Hassan et al., the faster surface sorption rate implies that the trileucine EEG powder is more susceptible to moisture uptake than the L-leucine powder, as confirmed by the moisture uptake at 50 %RH (3.6% vs. 2.4%, respectively) (Hassan et al., 2020). Despite the 5% higher mass change observed in trileucine EEG powder at crystallization, crystallization was delayed until reaching a humidity of 78 %RH as opposed to crystallization at only 57 %RH for the L-leucine EEG powder. Therefore, despite the increased tendency to absorb moisture, trileucine delayed the recrystallization event compared to L-leucine.

The L-leucine and trileucine EEG powders were aerosolized and exposed to simulated inhalation (1.5 s) and airway conditions in an aerosol growth tube. As expected from the DVS experiment, the MMAD growth ratio was larger for the trileucine EEG powder compared to L-leucine powder formulation. Importantly, for the trileucine powder which was observed to produce around 50% of the particles less than 1 µm in size, there was a significant reduction in the submicrometer fraction. The large fraction of small particles is required to effectively penetrate the extrathoracic regions and reach the lungs and the hygroscopic growth then prevents their exhalation and allows lung retention. Overall, trileucine produced improved dispersion compared to L-leucine for the AS EEG powder formulation. An amorphous trileucine phase may potentially be problematic from a stability perspective, however despite larger moisture absorbed as a function of increasing RH, there was no evidence of recrystallization of the trileucine and any observed recrystallization of other formulation components took place at an elevated RH of 76%. Observation of effects of different spray drying variables and alternative dispersion enhancers on the aerosol performance and the solid-state stability characteristics of the albuterol sulfate EEG powder will be useful for the development of other novel EEG powders.

In conclusion, multi-component spray-dried powders containing drug and excipients were produced using the Büchi Nano B-90 HP spray dryer. These powders were obtained with different nebulizer mesh hole
sizes, spray drying settings, inlet gas drying temperature, and dispersion enhancer but they all produced micrometer size particles that were potentially suitable for the EEG application. High emitted dose and fine particle fractions were obtained from the best performing L-leucine powder and compared to a trileucine EEG powder, which resulted in improved \textit{in vitro} aerosol performance but showed increased moisture absorption. For the trileucine formulation, the combination of an increased submicron particle fraction and the ability to absorb moisture while maintaining solid-state stability could offer a potentially advantageous formulation option compared to the L-leucine formulation.
4.1 Introduction

In this chapter, the development of a tobramycin EEG spray-dried formulation will be described. The core constituents of the EEG formulation have been previously established to include about 20-30 % drug together with a hygroscopic excipient and dispersion enhancer. A challenge for antibiotic formulations is the relatively large doses required to be inhaled for therapeutic efficacy, therefore a goal of this study is to maximize the amount of drug in the powder formulation while maintaining formulation stability and aerosol performance. This chapter will continue the development and understanding of the spray drying process for EEG formulations and expand knowledge with respect to the optimal storage conditions for a tobramycin EEG formulation. Tobramycin is a hygroscopic drug molecule and this offers both opportunities and challenges for its use as dry powder aerosol formulation. Due to its hygroscopicity, exposure to moisture during storage may affect both its solid-state stability and aerosol performance. Conversely, the hygroscopic nature of the molecule may allow reduction of amount of hygroscopic excipient required for EEG growth in the simulated airways and increase the drug payload. The composition of the formulation with respect to selection of a dispersion enhancer will also be investigated and its effects both on solid-state stability and aerosol performance will be described. A key outcome of these studies will be a dispersible and stable dry powder tobramycin EEG formulation for further development.
4.2 Materials and methods

4.2.1 Materials

Pearlitol® PF-Mannitol was donated from Roquette Pharma (Lestrem, France). Poloxamer 188 (Leutrol F68) was donated from BASF Corporation (Florham Park, NJ). Tobramycin base was purchased from ACROS Organics (Carlsbad, CA). L-leucine and all other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Trileucine was purchased from Bachem Americas, Inc. (Torrance, CA). Sulfuric acid was purchased from Thermo Fisher Sci. (Waltham, MA). Molykote®316 silicone release spray was purchased from Dow Corning Corporation (Midland, MI).

4.2.2 Assessment of the spray drying and storage conditions for tobramycin excipient enhanced growth (EEG) spray-dried powder formulations

4.2.2.1 Preparation of tobramycin EEG spray-dried powders

Based on the studies for the optimized AS EEG spray-dried powders, the medium nebulizer mesh hole size, 120 L/min air gas flow, 70 °C inlet temperature and 0.5 mL/min spray rate were used as based conditions for preparation of the tobramycin EEG spray-dried powders. The multicomponent spray-dried formulation included the drug, tobramycin (TOBI) and mannitol, L-leucine and poloxamer 188 (POL188) as hygroscopic excipient, dispersion enhancer and surfactant, respectively. Two tobramycin EEG powders were prepared using the spray drying conditions reported by Hassan et al. (Hassan et al., 2020). Briefly, the two formulations were produced using the nominal inlet drying gas absolute water vapor densities of 10 g/m³ and 1 g/m³, respectively (Hassan et al., 2020). The measured outlet absolute water vapor density to which the EEG powders were exposed in the collector was measured as 11.9 g/m³ and 4.2 g/m³, respectively. Tobramycin EEG powders were obtained using the Büchi Nano spray dryer B-90 HP in the open loop configuration (Büchi Laboratory Techniques, Flawil, Switzerland). The spray drying feed
solution for both formulations had a solid concentration of 0.5 %"/\, and was prepared in 80:20 %"/\, water:ethanol. Once spray-dried and collected, each EEG powder was subdivided into three batches and stored uncapped in a desiccator at 22 °C (room temperature; RT) exposed to a saturated salt solution to produce relative humidities of 15 %, 35 %, and 60 %, respectively (Table 4.1). Prior to initial testing at time zero (t=0), each TOBI EEG powder formulations was equilibrated for 7 days. Initially, the effect of spray drying and storage conditions was evaluated based on formulation particle size characteristics, dynamic vapor sorption analysis and *in vitro* aerosol performance. Based on the initial results at t=0, selected TOBI EEG powder formulations and storage conditions were further evaluated for their moisture sorption behavior and *in vitro* aerosol performance following storage for 6 months.

**Table 4.1** Measured outlet water vapor density during spray drying and storage conditions for tobramycin EEG spray-dried powder formulations.

<table>
<thead>
<tr>
<th>Measured outlet water vapor density, g/m³</th>
<th>Storage relative humidity at 22 °C, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.9</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>60</td>
</tr>
<tr>
<td>4.2</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>60</td>
</tr>
</tbody>
</table>

**4.2.2.2 Solid-state characterization of tobramycin EEG spray-dried powders**

The two TOBI EEG spray-dried powder formulations stored at RT under different RH conditions were initially characterized at t=0 for their primary particle size using the Sympatec HELOS (submicron R1 lens with 20 mm focal length) with RODOS/M disperser and ASPIROS sample feeder (Sympatec GmbH,
Clausthal-Zellerfeld, Germany), as described in Chapter 3. Volume-based size distributions were calculated by WINDOX software and the particle size below which 50% of the particles lie (Dv50) was reported. Span was calculated as (Dv90-Dv10)/Dv50 and reported for each formulation. Each measurement was performed in triplicate.

Dynamic water vapor sorption studies were conducted on the two TOBI EEG powders stored at RT under different RH conditions using the DVS Adventure (Surface Measurement Systems, PA) as reported in Chapter 3. Moisture sorption profiles were reported at t=0 and following 6 months storage. Weight change was expressed as a function of % of the reference weight, defined as the equilibrium weight at 0 %RH.

### 4.2.2.3 In vitro aerosol performance of tobramycin EEG spray-dried powders

A novel positive pressure DPI (Figure 4.1) was used to evaluate the in vitro aerosol performance of each TOBI EEG spray-dried powder formulation following storage at RT under different RH conditions. The powders were aerosolized in a Next Generation Impactor with a steady flow of 45 L/min. The positive pressure airflow DPI was used to deliver the tobramycin EEG powders. The positive pressure DAC v3 DPI was actuated for 4.64 s at 9.7 L/min and delivered a volume of air of 750 mL (Farkas et al., 2020). No USP induction port was included in the NGI experiments. About 10 mg of each TOBI EEG powder formulation was filled into the DAC v3 DPI. The NGI pre-separator and stages were coated with Molycote® 316 silicone release spray to avoid particle bounce. The NGI was in the upright position to ensure vertical orientation of the inhaler. The powder remaining in the DAC v3 DPI and deposited on pre-separator and each NGI stage was dissolved in water and then the tobramycin content was analyzed using a validated LC-MS method. Emitted dose and fine particle fractions were determined as reported in Equations 3.1 and 3.2. Mass median aerodynamic diameters were calculated as the size associated with 50% of the cumulative mass count relative to the impactor dose, excluding the pre-separator. Replicates of each experiment was performed (n ≥ 3). The in vitro aerosol performance was assessed following storage for t=0, 2, 4 and 6 months. The
nominal dose of tobramycin in each powder formulation was determined by dissolving a known amount of powder formulation in known water volume and tobramycin was quantified using LC-MS analysis.

![Schematic representation of the DAC v3 positive pressure DPI.](image)

**Figure 4.1** Schematic representation of the DAC v3 positive pressure DPI.

### 4.2.3 Optimization of composition of tobramycin EEG spray-dried powder formulation

#### 4.2.3.1 Preparation of tobramycin EEG spray-dried powders

After the preliminary assessment of the spray drying and storage conditions described above, further studies were conducted to optimize the composition of the tobramycin EEG powder formulation. Powders were prepared with the Büchi Nano spray dryer B-90 HP in the open loop configuration using the optimized conditions reported in Section 4.2.2.1. Tobramycin base, mannitol, either L-leucine or trileucine, and poloxamer 188 were selected as drug, hygroscopic excipient, dispersion enhancer and surfactant, respectively. The solids concentration in the feed solutions was maintained within 0.50 - 0.65 % w/v, and were prepared in an 80:20 % v/v water:ethanol solution. Five tobramycin EEG powder formulations (T1-T5) were spray-dried as shown on Table 4.2. The basic pH (pH = 9.2) of the T3 and T5 feed solutions was modified using sulfuric acid to achieve a pH ~6.2. The purpose of the pH adjustment was to reduce trileucine solubility (Lechuga-Ballesteros et al., 2008b). After spray-drying, each tobramycin EEG powder formulation was equilibrated in uncapped vials during storage at 15 %RH and 22 °C before characterization. The effect of dispersion enhancer and the replacement of the hygroscopic excipient mannitol with tobramycin were evaluated. Tobramycin EEG powders were evaluated for their physico-chemical
characteristics as well as for their in vitro aerosol performance. Finally, the lead tobramycin EEG powder formulation was tested in a realistic in vitro airway model. The aerosol performance of the formulation in the DAC v3 DPI was evaluated using a 5-year-old mouth-throat model under simulated airway and flow conditions (Farkas et al., 2020).

Table 4.2 Tobramycin EEG spray-dried powder formulations, components and component ratio.

<table>
<thead>
<tr>
<th>Formulation ID</th>
<th>Components</th>
<th>Component ratio, %/w</th>
<th>Total solids content, %/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>TOBI:MAN:LL:POL188</td>
<td>60:18:20:2</td>
<td>0.50</td>
</tr>
<tr>
<td>T2</td>
<td>TOBI:MAN:TL:POL188</td>
<td>60:18:20:2</td>
<td>0.50</td>
</tr>
<tr>
<td>T3</td>
<td>TOBI:SO₄²⁻:MAN:TL:POL188</td>
<td>49:18:15:16:2</td>
<td>0.61</td>
</tr>
<tr>
<td>T4</td>
<td>TOBI:LL</td>
<td>80:20</td>
<td>0.50</td>
</tr>
<tr>
<td>T5</td>
<td>TOBI:SO₄²⁻:TL</td>
<td>62:23:15</td>
<td>0.65</td>
</tr>
</tbody>
</table>

TOBI=tobramycin, MAN=mannitol, LL=L-leucine, TL=trileucine, POL188=poloxamer 188

4.2.3.2 Solid-state characterization of tobramycin EEG spray-dried powders

Formulation particle size was evaluated using the Sympatec HELOS (submicron R1 lens with 20 mm focal length) with RODOS/M disperser and ASPIROS sample feeder (Sympatec GmbH, Clausthal-Zellerfeld, Germany) at 4 bar and 0.5 bar dispersion pressures. Volume-based size distributions were calculated by WINDOX software and the particle size below which 50% of the particles lie (Dv50) was reported. Span was calculated as (Dv90-Dv10)/Dv50 and reported for each formulation. Fine particle fractions smaller than 5 and 1 µm were reported. Each measurement was performed in triplicate. Scanning electron microscopy was conducted as reported on Chapter 3.

Powder bulk density was measured using a 0.1 cm³ sample cup insert and calculated as the weight difference between the weight of the empty insert and the weight of the insert containing powder divided by the volume occupied by the powder (0.1 cm³). Powder bulk density measurements were conducted in triplicate.
Dynamic vapor sorption analysis was conducted on each tobramycin EEG powder formulation as reported on Chapter 3. Weight change is expressed as a function of % of the reference weight, defined as the equilibrium weight at 0 %RH.

Tobramycin EEG powder formulations were evaluated for their crystallinity using the Rigaku Miniflex 6G. The radiation source was the Cu-Kα at 40 kV and 15mA. Continuous scanning mode in the range of 5-50 2θ with a 0.05 °2θ step width and a speed rate of 1.5 °2θ /min were used. Tobramycin EEG powders were loaded in a 5 mm x 0.1 mm zero background sample holder and placed in the automatic sample changer and spinner (80 rpm) prior the analysis. Tobramycin EEG powders and raw materials were analyzed. Samples were analyzed following humidity exposure in the dynamic vapor sorption analysis to assess the effects of moisture exposure on powder crystallinity.

4.2.3.3 *In vitro* aerosol performance of tobramycin EEG spray-dried powders

The novel positive pressure DAC v3 DPI used to determine the optimized spray drying and storage conditions was also used to evaluate the *in vitro* aerosol performance during the tobramycin EEG powder formulation composition optimization study. A modified version of the DAC v3 DPI was implemented with a mouth piece adaptor containing a 3D rod array that further disperses the powder, as shown in Figure 4.2 (Farkas et al., 2020). The inhaler was employed as reported in Section 4.2.2.3 and the aerosol size characteristics of the tobramycin EEG powders were measured using the NGI at a steady flow of 45 L/min. About 10 mg of each TOBI EEG powder formulation was filled into the modified DAC v3 DPI and the sizing was performed as described previously. Each experiment was performed in replicate (n = 3).
4.2.3.4 *In vitro* aerosol performance of tobramycin EEG spray-dried powders using realistic airway conditions

To evaluate the aerosol growth and the dose to lung achieved with the lead tobramycin EEG powder formulation, aerosol testing in a 5-year-old pediatric mouth-throat model and growth chamber using simulated airway and flow conditions was performed (Bass et al., 2021; Farkas et al., 2020). The measured dose to lung will be used in PK simulations described in Chapter 6.

The experimental setup is shown in Figure 4.3. A mouth-piece adaptor was placed between the modified DAC v3 DPI and the pediatric mouth-throat model. A pediatric trachea through bifurcation B3 model was attached at the end of the mouth-throat model and it was placed inside the growth chamber. The exit of the growth chamber was attached to the NGI. The growth chamber was designed to allow an aerosol residence time of ~2 s. The positive pressure modified DAC v3 DPI was actuated as previously described in the absence of a simulated inhalation, as the DPI is not breath-actuated. The mouth-throat model, trachea and growth chamber were equilibrated at 37 °C and 99 %RH to simulate airways conditions. The inner walls of the airway model and growth chamber were saturated with heated and humidified air at 37 °C and...
99%RH to ensure saturation of the airways. The impactor was also equilibrated at 37 °C to ensure isotherm conditions. A control experiment was conducted under ambient conditions (22 °C and 45 %RH). The drug retained in the device and the mouth-piece adaptor, deposited on the mouth-throat and trachea models and the growth chamber, and on each stage of the NGI were quantified using a validated LC-MS method and the recovered amount of tobramycin was reported as percentage of the nominal dose.

![Diagram](image)  
**Figure 4.3** Schematic representation of the realistic in vitro aerosol performance test setup used for modified DAC v3 DPI - TOBI EEG formulation combination.

### 4.2.4 Statistical analysis

The effects of spray drying and storage conditions at t=0 were evaluated using analysis of variance (ANOVA, Tukey’s HSD). The effects of spray drying and storage conditions over time were evaluated using analysis of variance (ANOVA, Dunnet’s post hoc analysis) or Student’s t-test. One way ANOVA (Tukey’s HSD) statistical analysis was used to determine the effect of formulation variable.
Significant difference was established for \( p \)-value< 0.05 for all statistical analyses. JMP Pro 15 (SAS Institute Inc., Cary, NC) was used to conduct the statistical analysis.

4.3 Results

4.3.1 Initial stability and performance assessment of the effects of spray drying and storage conditions on tobramycin formulations at t=0

4.3.1.1 Primary particle size and powder dispersion properties

The primary particle size for the six tobramycin EEG batches obtained from the spray drying and storage state stability study (from Table 4.2) was characterized at t=0 using 4 bar dispersion pressure. Table 4.3 shows the mean volumetric diameter (Dv50) and the Span of each tobramycin EEG powder, prepared at 4.2 and 11.9 g/m\(^3\) outlet water vapor density, and stored under the varied RH conditions. Figure 4.4 and Figure 4.5 report the frequency distribution vs. particle diameter for the powder formulations prepared at 11.9 and 4.2 g/m\(^3\) outlet water vapor density, respectively, and stored at 15, 35, and 60 %RH. Irrespective of the spray drying conditions, tobramycin EEG powders stored at 15 and 35 %RH were less polydisperse compared to those stored at 60 %RH. Overall, the powder formulations stored at 15 and 35 %RH have a primary particle size smaller than 1 \( \mu \)m and good powder uniformity with Span ranges from 1.23 (0.01) to 1.48 (0.07) (mean (SD)), irrespective of the outlet water vapor density during the spray drying process. Larger values for Dv50 and Span were observed for both powders when exposed to 60 %RH storage conditions at t=0.

Two-way ANOVA (Tukey’s HSD) analysis was conducted to evaluate the main effects (spray drying and storage conditions) as well as their interaction effect. The statistical model used showed a good fit for Dv50 and Span (\( R^2 \) of 0.999 and 0.996, respectively), and revealed that both main effects and interaction effects were significant (\( p \)-value< 0.0001). When comparing by the storage relative humidity, Dv50 and span were smaller when spray drying was performed with an outlet water vapor density of 4.2 g/m\(^3\) and when the
powder formulation was stored at either 15 or 35 %RH at t=0. However, when the powder was stored at 60 %RH, the 4.2 g/m$^3$ outlet water vapor density condition resulted in larger Dv50 and Span compared to the higher outlet water vapor density (mean Dv50 and Span of 1.79 µm and 3.38 (4.2 g/m$^3$) vs 1.35 µm and 1.83 (11.9 g/m$^3$), respectively). When evaluating the effect of the outlet water vapor density during spray drying, no difference was observed between the Dv50 and the Span at 15 and 30 %RH storage conditions, irrespective of the spray drying outlet water vapor density. Conversely, the tobramycin EEG powders produced at both 11.9 and 4.2 g/m$^3$ and stored at 60 %RH had a larger Dv50 and Span compared to those produced under the same spray drying conditions and stored at 15 and 35 %RH.

Table 4.3 Particle size and Span measurements at t=0 for tobramycin EEG powder formulations spray-dried at 11.9 g/m$^3$ and 4.2 g/m$^3$ outlet water vapor density and stored at 15, 35 and 60 %RH and room temperature (RT). Values are reported as means (SD), n = 3.

<table>
<thead>
<tr>
<th>Storage condition at RT</th>
<th>Measured outlet water vapor density, g/m$^3$</th>
<th>11.9</th>
<th>4.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dv50, µm</td>
<td>Span</td>
<td>Dv50, µm</td>
</tr>
<tr>
<td>15 %RH</td>
<td>0.95 (0.01)</td>
<td>1.48 (0.07)</td>
<td>0.90 (0.01)</td>
</tr>
<tr>
<td>35 %RH</td>
<td>0.96 (0.03)</td>
<td>1.43 (0.11)</td>
<td>0.92 (0.01)</td>
</tr>
<tr>
<td>60 %RH*</td>
<td>1.35 (0.01)</td>
<td>1.83 (0.02)</td>
<td>1.79 (0.01)</td>
</tr>
</tbody>
</table>

*Significantly different from 15 and 35 %RH, RT (Two-way ANOVA, Tukey’s HSD, p-value< 0.05)
Figure 4.4 Particle size frequency distribution at t=0 for the tobramycin EEG powder formulations spray-dried at 11.9 g/m³ water vapor density and stored at 15, 35 and 60 %RH and RT. Markers represent mean values and error bars represent SD ($n = 3$).

Figure 4.5 Particle size frequency distribution at t=0 for the tobramycin EEG powder formulations spray-dried at 4.2 g/m³ water vapor density and stored at 15, 35 and 60 %RH and RT. Markers represent mean values and error bars represent SD ($n = 3$).
4.3.1.2 Dynamic vapor sorption analysis

The moisture sorption profiles obtained at t=0 for the TOBI EEG formulations prepared at 11.9 and 4.2 g/m³ outlet water vapor density are shown in Figure 4.6 and Figure 4.7, respectively after storage at 15, 35 and 60 %RH. During the moisture sorption study, initially all powder formulations were dried at 0 %RH. Once achieving equilibrium at 0 %RH, they were exposed to increasing RH at a rate of 2 %RH/h from 0 %RH to 90 %RH.

Overall, both spray-dried powders absorbed more than 14 % of their initial dried weight as moisture uptake when the RH was increased to 90% RH, irrespective of their storage conditions. As shown in Figure 4.6 and Figure 4.7, both powders stored at 15 and 35 %RH and room temperature showed crystallization irrespective of the outlet water vapor density used during the spray drying process. Conversely, the TOBI EEG powders stored at 60 %RH did not appear to demonstrate a crystallization event, suggesting an already crystalline powder, this was further confirmed by X-ray diffraction analysis (data not shown). Additionally, these powders did not have a characteristic inflection point that differentiates the surface moisture adsorption, which happens initially, from the combined surface and bulk absorption, which takes place at higher RH’s. Therefore, due to their apparent crystalline form, the tobramycin EEG powders stored at 60% RH were not further evaluated using DVS.

Differences were observed in the percentage of moisture absorbed on the particle surface for the TOBI EEG powder formulations following initial storage at 15 and 35 %RH (Burnett et al., 2004). Table 4.4 shows that the inflection point, reported as RHₐ, occurred at 25 %RH for the powders stored at 15 %RH, irrespective of the outlet water vapor density during the spray drying process, as opposed to 31 %RH and 32 %RH for the powders stored at 35 %RH and spray-dried at 11.9 g/m³ and 4.2 g/m³, respectively. Despite this 5-6 %RH difference, there was only 1 % mass change difference, as the water uptake for both powders was between 4-5% at the RHₐ for the powders stored at 15 %RH and 35 %RH. The rate of surface moisture adsorption ranged between 0.24 %/h and 0.26 %/h, irrespective of storage and spray drying conditions. In contrast, the rate of combined surface and bulk sorption was ~0.02 %/h slower for the TOBI EEG powder
formulations stored at 35 %RH compared to the powders stored at 15 %RH, irrespective of the outlet water vapor density used during the spray drying process.

Table 4.4 also shows the crystallization RH (RHc) for the TOBI EEG powder formulations evaluated. The RHc was observed at 46 % and 47 %RH for the powders stored at 15 %RH and spray-dried at an outlet water vapor density of 4.2 g/m³ and 11.9 g/m³, respectively. A higher RHc was reported for the powders stored at 35 %RH (49 %RH for both 11.9 g/m³ and 4.2 g/m³ powders). Despite difference in RHc for these samples, the moisture content at crystallization (9%) was the same for both powders stored at 15 %RH and 35 %RH.

In summary, TOBI EEG powder formulations stored at 15 %RH resulted in a glass transition occurring at a lower RH compared to the powders stored at 35 %RH. Similarly, a lower RHc was found for the powders stored at 15 %RH. However, only small differences were observed when comparing the rates of surface and bulk moisture sorption for the TOBI EEG powders stored at 15 and 35 %RH and spray-dried at 11.9 and 4.2 g/m³. The equilibrium moisture contents at RHg and RHc were similar despite occurring at different relative humidities following initial storage at 15 and 35 %RH. Finally, the TOBI EEG powder formulations stored at 60 %RH and room temperature had a higher degree of crystallinity compared to those stored at lower RH, irrespective of the outlet water vapor density used during the spray drying process. These results indicate that the initial storage conditions, can have significant effects on the physico-chemical properties of the spray-dried powders during the initial equilibration period immediately following spray drying. Samples were equilibrated for only 1 week and changes in the moisture uptake and crystallinity were observed which could affect future aerosol performance and stability.
Figure 4.6 Dynamic vapor sorption profiles for tobramycin EEG powder formulations spray-dried at 11.9 g/m³ outlet water vapor density and initially (t=0) stored at 15, 35 and 60 %RH and at room temperature, respectively.

Figure 4.7 Dynamic vapor sorption profiles for tobramycin EEG powder formulations spray-dried at 4.2 g/m³ outlet water vapor density and initially (t=0) stored at 15, 35 and 60 %RH and at room temperature, respectively.
Table 4.4 Dynamic vapor sorption analysis for tobramycin EEG powder formulations spray-dried at 11.9 and 4.2 g/m$^3$ outlet water vapor densities and initially stored (t=0) at 15 and 35 %RH and at room temperature, respectively.

<table>
<thead>
<tr>
<th>Outlet water vapor density, g/m$^3$</th>
<th>Storage RH, %RH</th>
<th>RH$_{c}$ %RH</th>
<th>Am at RH$_{c}$, %</th>
<th>Surface ad, Am % dried mass/h</th>
<th>Surface+bulk, Am % dried mass/h</th>
<th>RH$_{c}$ %RH</th>
<th>Am at RH$_{c}$, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.9</td>
<td>15</td>
<td>25</td>
<td>4</td>
<td>0.24</td>
<td>0.46</td>
<td>47</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>31</td>
<td>5</td>
<td>0.26</td>
<td>0.44</td>
<td>49</td>
<td>9</td>
</tr>
<tr>
<td>4.2</td>
<td>15</td>
<td>25</td>
<td>4</td>
<td>0.25</td>
<td>0.47</td>
<td>46</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>32</td>
<td>5</td>
<td>0.25</td>
<td>0.44</td>
<td>49</td>
<td>9</td>
</tr>
</tbody>
</table>

RH= relative humidity, RH$_{c}$=crystallization relative humidity, RH$_{g}$=glass transition relative humidity

4.3.1.3 Initial assessment of in vitro aerosol performance of tobramycin EEG spray-dried powders at t=0

The initial aerosol performance of TOBI EEG powders was characterized at t=0 using the DAC v3 DPI, a novel positive air pressure inhaler. The emitted dose, mass median aerodynamic diameter (MMAD), pre-separator drug losses, and %FPF<5 µm and 1 µm were the dependent variables evaluated for the two powders exposed to varying RH conditions. Two-way ANOVA (Tukey’s HSD) analysis was conducted to determine the effect of the independent variables (outlet water vapor density during the spray drying process and storage conditions) as well as their interaction. Figure 4.8 shows the emitted dose as a function of drying gas water vapor density for each of the three storage conditions following the initial equilibration period (t=0). Statistics revealed that the model has a good fit (R$^2$ of 0.962), but only the storage conditions had an effect on the emitted dose ($p$-value< 0.0001). In fact, no difference was found in the emitted dose between the formulation produced with the 4.2 g/m$^3$ and the 11.9 g/m$^3$ outlet water vapor density spray drying conditions, (mean 77.4±1.0% vs. 74.3±3.2%, respectively). In contrast, one-way ANOVA (Tukey’s HDS) revealed that storage at 35 %RH produced a higher ED than storage at 15 %RH ($p$-value= 0.0031) during the initial equilibration period, and that storage at 60 %RH produced the highest ED ($p$-value<
Emitted doses following the initial storage at 35% RH were 87.3±4.4% and 79.7±1.5% using 4.2 g/m³ and 11.9 g/m³ dry gas water vapor density, respectively, at t=0.

![Figure 4.8 Emitted dose (% nominal dose) for the tobramycin EEG powder formulations spray-dried at 4.2 and 11.9 g/m³ outlet water vapor density and initially stored (t=0) at 15, 35 and 60 %RH and room temperature, respectively. Markers represent mean values and error bars represent SD (n = 3).](image)

Figure 4.8 shows the pre-separator drug loss and the MMAD for the two TOBI EEG powders stored initially (t=0) at different RH conditions. Statistical analysis models for pre-separator drug deposition and MMAD had a good fit (R² of 0.989 and 0.881, respectively) and both main effects and interaction effect had a significant impact on both the pre-separator drug deposition and the aerosol MMAD (p-value< 0.005).

When comparing by the storage RH conditions at t=0, the TOBI EEG powder formulation spray-dried at 4.2 g/m³ water vapor density has a higher pre-separator drug loss and larger aerosol MMAD (p-value = 0.0208 and 0.0012) compared to powder produced at 11.9 g/m³ when stored at 15 %RH (23.3±1.6 % and 2.14±0.18 µm vs. 9.4±2.1% and 1.68±0.01 µm, respectively). Initially storing the powder formulations at 35 %RH resulted in higher pre-separator drug loss (p-value = 0.0002) for the 4.2 g/m³ spray drying condition (44.3±6.6%), compared to 11.9 g/m³ (20.2±3.5%), while no difference was observed for the aerosol MMAD at 35% RH storage. In contrast, when both powders were stored initially at 60 %RH, no difference was found in pre-separator drug loss and aerosol MMAD. When comparing the effect of the
spray drying conditions (water vapor density during spray drying), initial storage at 35 %RH storage produced significantly higher pre-separator drug loss ($p$-value = 0.0008) compared to the 15 %RH storage conditions for the 4.2 g/m$^3$ spray drying water vapor density powder formulation. Additionally, initial storage at 60 %RH storage produced higher pre-separator drug loss and the largest MMAD compared to storage at 35 and 15 %RH for both spray drying conditions ($p$-value< 0.0001). No difference in pre-separator drug loss and MMAD were observed between initial storage at 15 and 35 %RH when spray drying using a water vapor density of 11.9 g/m$^3$.

Figure 4.9 shows the %FPF<5 µm and 1 µm for the TOBI EEG formulations initially (t=0) stored at 15, 35 and 60 %RH and room temperature, respectively. Markers represent mean values and error bars represent SD ($n = 3$).

Figure 4.10 shows the %FPF<5 µm and 1 µm for the TOBI EEG formulations initially (t=0) stored at 15, 35 and 60% RH. The statistical model has a good fit for both metrics ($R^2$ of 0.988), and both main effects and interaction effect have a significant effect on %FPF<5 µm and <1 µm ($p$-value< 0.005 and <0.0001, respectively). When comparing by the storage RH, the formulation produced at 11.9 g/m$^3$ water vapor density had a higher fraction of respirable and submicron particles following initial storage at 15 and 35
%RH, compared to formulation produced using 4.2 g/m$^3$ ($p$-value< 0.0001). No difference in %FPF<5 µm and 1 µm was observed for both powders after initial storage at 60 %RH. When comparing the formulations by the water vapor density during spray drying, initial storage at 15 %RH produced a higher respirable and submicron fractions compared to 35 and 60 %RH under both spray drying conditions. Also, storage at 35 %RH also resulted in higher respirable and submicron fractions compared to 60 %RH for both TOBI EEG formulations.

![Figure 4.10](image)

**Figure 4.10** Fine particle fraction smaller than 5 µm (left plot) and 1 µm (right plot) reported as % of impactor dose for the tobramycin EEG powder formulations spray-dried at 4.2 and 11.9 g/m$^3$ outlet water vapor density and initially stored (t=0) at 15, 35 and 60 %RH and room temperature, respectively. Markers represent mean values and error bars represent SD ($n = 3$).

### 4.3.2 Long-term stability and performance assessment of the effects of spray drying and storage conditions on tobramycin formulations over 6 months

#### 4.3.2.1 Dynamic vapor sorption analysis

The moisture sorption profiles measured at t=0 and 6 months for the TOBI EEG formulations spray-dried with 11.9 and 4.2 g/m$^3$ water vapor density and stored at 15 %RH and room temperature are shown in Figure 4.11 and Figure 4.12, respectively. Similar plots for the two TOBI EEG formulations stored at 35
%RH are shown in Figure 4.13 and Figure 4.14, respectively. All powders were equilibrated at 0 %RH before being exposed to increasing RH (%) at a rate of 2 %RH/h from 0 %RH to 90 %RH. Powders stored for 6 months showed crystallization events similar to those observed for the initial storage samples, indicating that recrystallization had not taken place during the 6-month storage period. Similar moisture sorption profiles were obtained after 6 months for the formulations stored at 15% RH and room temperature. However, changes in the moisture sorption profiles were more evident for the formulations stored at 35 %RH and room temperature.

Figure 4.11 and Table 4.5 revealed that following 6 months storage at 15 %RH, the TOBI EEG powder formulation spray-dried using 11.9 g/m³ water vapor density had only a 1% change in the RHₑ compared to t=0 (25 %RH and 26 %RH, respectively) and no change in the equilibrium water content at the RHₑ. The rate of surface moisture adsorption was 0.20 %/h after 6 months storage at 15 %RH compared to 0.24 %/h at t=0. The slower surface adsorption rate was compensated by a faster surface and bulk absorption rate of 0.63 %/h after 6 months. Finally, the RHₑ was 47 %RH even after 6 months storage and was identical to the value observed initially at t=0. Similar small changes were observed for the TOBI EEG powder formulation spray-dried at 4.2 g/m³ water vapor density and stored at 15 %RH (Figure 4.12). As reported in Table 4.5, the RHₑ increased by 2%RH to 27%RH and there was no change in the equilibrium moisture content at the RHₑ following 6 months storage at 15 %RH compared to t=0. Additionally, rates of surface adsorption and surface and bulk absorption observed after 6 months storage were lower by 0.05 %/h and higher by 0.11 %/h, respectively, compared to the t=0 values. The RHₑ increased by 1% to 47%RH with no difference in the equilibrium moisture content at the RHₑ after 6 months storage. Overall minimal changes were observed for both TOBI EEG formulations stored for 6 months at 15 %RH, indicating the excellent stability of the formulations in terms of their solid-state properties.
**Figure 4.11** Dynamic vapor sorption profiles for tobramycin EEG powder formulation spray-dried at 11.9 g/m³ outlet water vapor density following storage at 15 %RH and room temperature measured at t=0 and 6 months.

**Figure 4.12** Dynamic vapor sorption profiles for tobramycin EEG powder formulation spray-dried at 4.2 g/m³ outlet water vapor density following storage at 15 %RH and room temperature measured at t=0 and 6 months.
When stored at 35 %RH, RT, tobramycin EEG powder formulations obtained at 11.9 g/m³ and 4.2 g/m³ outlet water vapor densities did not show a similar moisture sorption behavior as previously observed for the 15 %RH, RT storage conditions. After 6 months storage, the 11.9 g/m³ spray-dried powder resulted in 37 % RH and 6.0% mass change, which corresponded to 6% RH and 1 % mass change increase compared to t=0. As well as the 15 %RH storage conditions, this tobramycin EEG powder had a slower surface adsorption and a faster bulk absorption rates after 6 months storage compared to t=0 (0.22 and 0.26 %/h and 0.45 and 0.44 %/h, respectively). However, this powder crystallized at 52 %RH with consistent mass change (9 %) after 6 months storage, compared to t=0. Tobramycin EEG powder formulation obtained at 4.2 g/m³ and stored at 35 %RH, RT resulted in 37 % RH and 5 % mass change. Both surface and bulk sorption rates for this powder were slower compared to t=0, but a 1 % higher RH was found.

Figure 4.13 Dynamic vapor sorption profiles for tobramycin EEG powder formulation spray-dried at 11.9 g/m³ outlet water vapor density following storage at 35 %RH and room temperature measured at t=0 and 6 months.
Table 4.5 Dynamic vapor sorption analysis for tobramycin EEG powder formulations spray-dried at 11.9 and 4.2 g/m³ outlet water vapor densities and stored for 6 months at 15 and 35 %RH and room temperature, respectively.

<table>
<thead>
<tr>
<th>Outlet water vapor density, g/m³</th>
<th>Storage RH, %RH</th>
<th>RHₜ, %RH</th>
<th>Δm at RHₜ, %</th>
<th>Surface ad, Δm % dried mass/h</th>
<th>Surface+bulk, Δm % dried mass/h</th>
<th>RHₜ, %RH</th>
<th>Δm at RHₜ, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.9</td>
<td>15</td>
<td>26</td>
<td>4</td>
<td>0.20</td>
<td>0.63</td>
<td>47</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>37</td>
<td>6</td>
<td>0.22</td>
<td>0.45</td>
<td>52</td>
<td>9</td>
</tr>
<tr>
<td>4.2</td>
<td>15</td>
<td>27</td>
<td>4</td>
<td>0.20</td>
<td>0.58</td>
<td>47</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>37</td>
<td>5</td>
<td>0.19</td>
<td>0.41</td>
<td>50</td>
<td>8</td>
</tr>
</tbody>
</table>

RH=relative humidity, RHₜ=crystallization relative humidity, RHₜ=glass transition relative humidity
4.3.2.2 Long-term stability assessment of *in vitro* aerosol performance of tobramycin EEG spray-dried powders over 6 months

The aerosol performance of the tobramycin EEG powders spray-dried using an outlet water vapor density of 11.9 and 4.2 g/m³ and stored at 15 and 35 %RH were evaluated after 2, 4 and 6 months and compared with the performance at t=0. One-way ANOVA and Dunnet’s post hoc statistical analysis was used and results at t=0 were set as control. Additionally, Student’s t-test was conducted after 2, 4 and 6 months storage to directly compare the aerosol performance parameters for the two tobramycin EEG powders.

The emitted dose (Figure 4.15), the MMAD (Figure 4.16) and the %FPF<1 µm (Figure 4.17) of the TOBI EEG spray-dried powder that was spray-dried using 11.9 g/m³ outlet water vapor density and stored at 15 %RH did not show any significant difference over time compared to its performance at t=0. However, there was a significantly higher pre-separator drug loss after 2 (p-value= 0.0289), 4 (p-value= 0.0006), and 6 months (p-value< 0.0001) storage compared to t=0 (Figure 4.16). Consequently, the %FPF<5 µm (Figure 4.17) was significantly lower after 2, 4 and 6 months storage compared to t=0 (p-values of 0.0039, 0.0003, and <0.0001, respectively).

In contrast, the TOBI EEG spray-dried formulations produced using 4.2 g/m³ outlet water vapor density and stored at 15 %RH showed no changes in emitted dose (Figure 4.15) after 2- and 4- months storage and a small but significant increase in ED at 6 months compared to t=0 (p-value< 0.0001). The MMAD (Figure 4.16) and %FPF<1 µm (Figure 4.17) remained unchanged over the 6 months study period compared to t=0, while the pre-separator drug loss (Figure 4.16) increased over time (p-value< 0.05).

When comparing the TOBI EEG powders obtained at 11.9 and 4.2 g/m³ outlet water vapor density and stored at 15 %RH at each storage interval, it was found that for each time interval, the formulation produced using 11.9 g/m³ water vapor density resulted in lower emitted doses with lower pre-separator drug deposition, smaller MMAD’s and larger respirable and submicron fractions compared to the spray-dried powder produced using the 4.2 g/m³ water vapor density (Figure 4.15 - Figure 4.17).
Figure 4.15 Emitted dose (% nominal dose) over time for the tobramycin EEG powder formulations spray-dried at 11.9 (circle) and 4.2 g/m³ (square) outlet water vapor densities and stored at 15 %RH and room temperature. Markers represent mean values and error bars represent SD (n = 3).
*Significant difference compared to t=0 (One-way ANOVA, Dunnet’s post-hoc).

Figure 4.16 Pre-separator deposition (% nominal dose, left plot) and mass median aerodynamic diameter (right plot) over time for the tobramycin EEG powder formulations spray-dried at 11.9 (circle) and 4.2 g/m³ (square) outlet water vapor densities and stored at 15 %RH and room temperature. Markers represent mean values and error bars represent SD (n = 3).
*Significant difference compared to t=0 (One-way ANOVA, Dunnet’s post-hoc).
The tobramycin EEG powder formulation spray-dried using 11.9 g/m³ outlet water vapor density and stored at 35 %RH showed higher variability over time compared to the formulation stored at 15 %RH and spray-dried under the same conditions. For this formulation the emitted doses (Figure 4.18) increased following storage at 35% RH for 4 and 6 months (p-values of 0.0036 and 0.0084, respectively) compared to t=0. Similarly, there was a significant increase in MMAD (Figure 4.19) over the same time period of 4 and 6 months compared to t=0 (p-values of 0.0147 and 0.0087, respectively). Additionally, pre-separator drug loss (Figure 4.19), %FPF<5 and 1 µm (Figure 4.20) were different at all-time points (p-values< 0.05) compared to t=0.

In contrast, the TOBI EEG formulation spray-dried using 4.2 g/m³ outlet water vapor density and stored at 35 %RH had no measured difference in emitted dose (Figure 4.18), pre-separator drug deposition (Figure 4.19), MMAD (Figure 4.19) and %FPF<1µm (Figure 4.20) at each storage time point compared to t=0. However, the %FPF<5 µm did show a significant difference at 4 months (p-value = 0.0279), but this was not significantly different following storage for 6 months compared to t=0.

*Significant difference compared to t=0 (One-way ANOVA, Dunnet’s post-hoc).
When comparing the TOBI EEG powders produced with 11.9 and 4.2 g/m$^3$ outlet water vapor density and stored at 35 %RH at each storage interval, it was found that at all time points, the powder formulation produced using 11.9 g/m$^3$ water vapor density had lower emitted doses and lower pre-separator drug deposition together with a larger %FPF<5 µm compared to the other formulation. The MMAD for the two formulations showed no significant difference after storage for 4 and 6 months, with a significant difference only being observed at 2 months. Finally, the %FPF<1 µm was different at 2 and 4 months, while no difference was found at 6 months storage between the two formulations.

Overall, this study showed that storing at 35% RH compared to 15%RH increased the emitted dose of the TOBI EEG formulation and that these storage conditions did not negatively affect the emitted dose of the formulations. Pre-separator drug deposition was minimized by storage at 15% RH and the formulation prepared using 11.9 g/m$^3$ water vapor density had the lowest pre-separator drug deposition. In general, storage over 6 months increased the pre-separator deposition. The aerosol MMAD of the TOBI EEG formulations was stable during the duration of the stability experiments with the formulation prepared using 11.9 g/m$^3$ water vapor density having the lower MMAD. The optimized outlet water vapor density and effect of storage conditions on the TOBI EEG formulations will be utilized in the tobramycin EEG formulation composition optimization study.
Figure 4.18 Emitted dose (% nominal dose) over time for the tobramycin EEG powder formulations spray-dried at 11.9 (circle) and 4.2 g/m$^3$ (square) outlet water vapor densities and stored at 35 %RH and room temperature. Markers represent mean values and error bars represent SD ($n = 3$).

*Significant difference compared to $t=0$ (One-way ANOVA, Dunnet’s post-hoc).

Figure 4.19 Pre-separator deposition (% nominal dose, left plot) and mass median aerodynamic diameter (right plot) over time for the tobramycin EEG powder formulations spray-dried at 11.9 (circle) and 4.2 g/m$^3$ (square) outlet water vapor densities and stored at 35 %RH and room temperature. Markers represent mean values and error bars represent SD ($n = 3$).

*Significant difference compared to $t=0$ (One-way ANOVA, Dunnet’s post-hoc).
4.3.3 Optimization of composition of tobramycin EEG spray-dried powder formulation

4.3.3.1 Primary particle size, powder polydispersity properties, bulk density and morphology

Table 4.6 shows the particle size and bulk density characteristics of the TOBI EEG powder formulations evaluated in the composition optimization study measured after a seven-day equilibration period at 15% RH. Overall, particle size ranged from a mean of 0.90 (0.03) µm to 1.34 (0.53) µm and all the tobramycin EEG powder formulations had a smaller Dv50 when dispersed at 4 bar compared to 0.5 bar (Student’s t-test, p-values< 0.05) indicating that dispersion to primary particles required the higher dispersion pressure. Reflecting the relatively small size of the formulations, the % FPF<5 µm was ~100% for all the powders and no difference in the % FPF<5 µm was found when dispersing the formulations using 0.5 and 4 bar dispersion pressure. The percentage of submicron particles ranged from 37.5% to 59.3% for the formulations when tested at 0.5 and 4 bar dispersion pressure. Formulations T1, T3 and T5 produced a significantly higher % FPF<1 µm when dispersed at 4 bar compared to 0.5 bar (Student’s t-test, p-values< 0.05). Powder formulation polydispersity is shown in the frequency distribution graphs plotted in Figure 4.20.

**Figure 4.20** Fine particle fraction (% impactor dose) smaller than 5 µm (left plot) and 1 µm (right plot) over time for the tobramycin EEG powder formulations spray-dried at 11.9 (circle) and 4.2 g/m³ (square) outlet water vapor densities and stored at 35 %RH and room temperature. Markers represent mean values and error bars represent SD (n = 3).

*Significant difference compared to t=0 (One-way ANOVA, Dunnet’s post-hoc).
4.21 and Figure 4.22 for the TOBI EEG powders dispersed at 0.5 and 4 bar, respectively. A higher degree of polydispersity was found for the T4 and T5 powders, these powders are formulated without mannitol, their Span was calculated as 1.59 (0.09) and 1.35 (0.02) at 0.5 bar and 1.51 (0.02) and 2.13 (0.10) at 4 bar. The remaining tobramycin EEG powders had Span values <1.5.

When comparing all 5 tobramycin EEG powders with varying compositions, no difference was found in the Dv50 and %FPF<5 µm when sized using a dispersion pressure of 0.5 bar. However, at the same dispersion pressure, formulations T3 and T5 were observed to have a significantly lower %FPF<1 µm compared to formulations T1, T2, and T4 powders (p-values< 0.0001). Similar observations regarding formulations T3 and T5 were made when the dispersion pressure was increased at 4 bar, these formulations had the largest primary particle size (Dv50) and smallest %FPF<1 µm compared to T1, T2, and T4 (p-values< 0.0001), respectively. Dispersion using 4 bar revealed that the formulation T1 had the smallest primary particle size (Dv50) and the highest %FPF<1 µm compared to all the other tobramycin EEG powder formulations. This formulation was the formulation previously developed in Section 4.3, containing 60% tobramycin, mannitol and L-leucine as the dispersion enhancer produced using the optimized spray drying conditions. Interestingly, the tobramycin EEG powders containing trileucine as the dispersion enhancer and sulfate for pH adjustment resulted in a largest primary particle and lowest percentage of submicron particles.

The bulk density of the formulations ranged from 0.098 (0.006) g/cm³ to 0.288 (0.009) g/cm³ with the lowest density being produced from the formulation containing TOBI:MAN:TL:POL:188 with the ratio of 60:18:20:2 %w/w (T2) and the highest density being for the sample TOBI:LL (80:20 %w/w) formulation (T4). The bulk density of the other formulations ranged from 0.168 to 0.195 g/cm³. When L-leucine was replaced with trileucine (T1 vs. T2), the bulk density reduced from 0.186 to 0.098 g/cm³. Addition of sulfate to this formulation (T3) to reduce the solubility of trileucine doubled the bulk density to 0.195 g/cm³. Finally, it was observed that replacing mannitol in the powder formulations with additional tobramycin (T1 vs. T4) increased the bulk density by ~ 0.1 g/cm³ for the L-leucine formulations, but this effect was not observed.
for trileucine powders (T3 vs. T5). Changes in bulk density reflect changes in the particle formation process due to the differing formulation compositions which will alter both the solid-state stability and aerosol performance properties of the formulations.

**Table 4.6** Mean (SD) particle size characteristics measured at 0.5 and 4 bar dispersion pressure and bulk density measurements of the tobramycin EEG powder formulations (n= 3).

<table>
<thead>
<tr>
<th>Formulation ID</th>
<th>0.5 bar</th>
<th>4 bar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dv50, µm</td>
<td>FPF&lt;5 µm, %</td>
</tr>
<tr>
<td>T1</td>
<td>1.00 (0.04)</td>
<td>100.0 (0.0)</td>
</tr>
<tr>
<td>T2</td>
<td>1.34 (0.53)</td>
<td>98.9 (1.8)</td>
</tr>
<tr>
<td>T3</td>
<td>1.18 (0.01)</td>
<td>100.0 (0.1)</td>
</tr>
<tr>
<td>T4</td>
<td>0.97 (0.00)</td>
<td>99.7 (0.2)</td>
</tr>
<tr>
<td>T5</td>
<td>1.19 (0.00)</td>
<td>100.0 (0.0)</td>
</tr>
</tbody>
</table>

*Significant difference compared to T3 and T5 (One-way ANOVA, Tukey’s HSD, p-value< 0.05).
# Significant difference compared to T1 (One-way ANOVA, Tukey’s HSD, p-value< 0.05).
¶ Significant difference compared to all powders (One-way ANOVA, Tukey’s HSD, p-value< 0.05).
¶¶ Significant difference compared to T1, T3 and T5 (One-way ANOVA, Tukey’s HSD, p-value< 0.05).
FPF=fine particle fraction
Figure 4.21 Particle size frequency distribution of the tobramycin EEG powder formulations determined using the Sympatec at a dispersion pressure of 0.5 bar. Markers represent mean values and error bars represent SD ($n = 3$).

Figure 4.22 Particle size frequency distribution of the tobramycin EEG powder formulations determined using the Sympatec at a dispersion pressure of 4 bar. Markers represent mean values and error bars represent SD ($n = 3$).
Powder morphologies were reported in Figure 4.23 for each tobramycin EEG powder formulations. The L-leucine containing powder T1 and T4 showed spherical shaped particles with slightly corrugated surface (shown in (a) and (d), respectively). Except for T2, the trileucine containing powders T3 (shown in (c)) and T5 (shown in (e)) resulted in particles with characteristic wrinkled morphology. As the pH was not adjusted to 6.2, powder T2 (shown in (a)) did not result in wrinkle morphology. The wrinkle morphology resulted in powders with low bulk density compared to the spherical shaped particles. In fact, despite T3 and T5 had a higher total solids content compared to T1, their bulk density was lower than 0.2 g/cm³.
Figure 4.23 Scanning electron micrographs of the tobramycin EEG formulations (a) T1, (b) T2, (c) T3, (d) T4 and (e) T5.
4.3.3.2 Dynamic vapor sorption analysis

The moisture sorption profiles for each of the tobramycin EEG powders following storage at 15 %RH are shown in Figure 4.24, Figure 4.25, Figure 4.26, Figure 4.27 and Figure 4.28, respectively. All powders were initially dried at 0 %RH, and then exposed to increasing RH (%) at a rate of 2 %RH/h starting from 0 %RH to 90 %RH.

Overall, powder formulations containing L-leucine had lower moisture uptake and exhibited a crystallization during moisture sorption. In comparison, the trileucine powder formulations had higher moisture uptake and showed no visible crystallization. However, the characteristic desorption following transition from an amorphous powder to its crystalline form may have been obscured by the extensive water uptake in these hygroscopic trileucine powders. Table 4.7 reveals significantly higher moisture sorption for the tobramycin EEG powders containing trileucine (T2, T3 and T5) compared to the L-leucine (T1 and T4) formulations during the moisture sorption studies. For example, the maximal moisture sorption at 90% RH for the L-leucine containing formulations, T1 and T4, were 17% and 16% of the dry weight, respectively. Similar values at 90 % RH for the trileucine formulations, T2, T3 and T5, were 35%, 44% and 45% of the dry weight, respectively. Additionally, the rates of surface and bulk moisture sorption ranged from 0.26 to 0.80 %/h for L-leucine formulations compared to values ranging from 0.26 to 1.30 %/h, for the trileucine formulations.

When comparing the L-leucine containing powders T1 and T4 (with and without mannitol), it was observed that the glass transition RH occurred at 11% higher relative humidity when mannitol was replaced with additional tobramycin, improving the moisture stability of the formulation. Although the initial surface absorption rate was similar between the two formulations (0.28 %/h), the secondary bulk adsorption rate was double for the drug/L-leucine formulation in the absence of mannitol (0.43 %/h vs. 0.80 %/h), reflecting the increased hygroscopicity of tobramycin compared to mannitol. Despite the slower rate of bulk adsorption, the crystallization for the EEG formulation containing mannitol occurred at a lower RH compared to the more hygroscopic drug/L-leucine formulation (47 %RH vs. 55 %RH). Crystallization for
the mannitol containing formulation (T1) occurred with a water content of 9% compared to the dry weight and 14% water content for the more hygroscopic drug/L-leucine formulation. Similarly, the mannitol containing trileucine powder T3 was less hygroscopic compared to T5, in which mannitol was replaced with additional tobramycin.

Replacing L-leucine with trileucine (T1 vs. T2) as the dispersion enhancer, slightly increased the RHg which was observed at 29 %RH for T2. The rate of surface moisture adsorption was comparable between the two formulations, however the trileucine formulation (T2) had a faster bulk absorption rate (0.55 %/h vs. 0.43 %/h).

For the trileucine powder formulations, it was observed that the RHg occurred at a higher humidities and the powders had higher water contents at the transitions when tobramycin sulfate was formed (T3 and T5) when compared to the EEG powders with tobramycin base (T1, T2 and T4). Also, there were higher surface and bulk moisture sorption rates with tobramycin sulfate EEG powder formulations.

Overall, the L-leucine formulations appeared less hygroscopic, however displayed instability at elevated relative humidities. In contrast, the trileucine formulations were very hygroscopic but did not show evidence of any phase changes during moisture exposure.
Figure 4.24 Dynamic vapor sorption profiles for tobramycin EEG powder formulation containing TOBI:MAN:LL:POL188 as 60:18:20:2 %w/w (T1).

Figure 4.25 Dynamic vapor sorption profiles for tobramycin EEG powder formulation containing TOBI:MAN:TL:POL188 as 60:18:20:2 %w/w (T2).
Figure 4.26 Dynamic vapor sorption profiles for tobramycin EEG powder formulation containing TOBI:SO$_4^2$-MAN:TL:POL188 as 49:18:15:16:2 %w/w (T3).

Figure 4.27 Dynamic vapor sorption profiles for tobramycin EEG powder formulation containing TOBI:LL as 80:20 %w/w (T4).
Table 4.7 Dynamic vapor sorption analysis for tobramycin EEG powder formulations T1 to T5.

<table>
<thead>
<tr>
<th>Formulation ID</th>
<th>RHg, % RH</th>
<th>Δm at RHg, %</th>
<th>Surface ad, Δm % dried mass/h</th>
<th>Surface+bulk, Δm % dried mass/h</th>
<th>RHc, % RH</th>
<th>Δm at 90 % RH, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>27</td>
<td>4</td>
<td>0.28</td>
<td>0.43</td>
<td>47</td>
<td>9</td>
</tr>
<tr>
<td>T2</td>
<td>29</td>
<td>5</td>
<td>0.26</td>
<td>0.55</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T3</td>
<td>44</td>
<td>9</td>
<td>0.32</td>
<td>1.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T4</td>
<td>38</td>
<td>8</td>
<td>0.28</td>
<td>0.80</td>
<td>55</td>
<td>14</td>
</tr>
<tr>
<td>T5</td>
<td>56</td>
<td>16</td>
<td>0.44</td>
<td>1.30</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**4.3.3.3 X-ray powder diffraction analysis of tobramycin EEG powder formulations**

The crystallinity of the TOBI EEG powder formulations after equilibration at 15% RH was evaluated using x-ray powder diffraction analysis as shown in Figure 4.30 and Figure 4.31. Raw materials diffractograms
obtained with tobramycin base, tobramycin sulfate, mannitol, L-leucine and trileucine are reported in Figure 4.29. The profiles observed for the raw materials were in agreement with literature reports (Haghi et al., 2012; Miller et al., 2015; Sou et al., 2013). The spray-dried powders containing L-leucine (T1 and T4) showed some crystalline peaks characteristic of L-leucine and together with a characteristic halo indicating amorphous properties. For the tobramycin EEG powders containing trileucine (T2, T3, and T5), there was no evidence of crystalline peaks and a halo was present. Similar x-ray diffractograms were performed after moisture exposure in the DVS experiment, where the powders were exposed to increasing humidity up to 90 %RH as shown in Figure 4.32 and Figure 4.33 for the L-leucine and trileucine formulations, respectively. All the tobramycin EEG powders showed some degree of crystallinity after moisture exposure.

The crystalline peaks observed in T1 and T4 represented partially crystalline L-leucine (Sou et al., 2013, Hassan et al., 2020, Leung et al., 2017). Both L-leucine EEG powders showed intense peaks at 6°2θ and 19°2θ, suggesting partial crystallization of L-leucine during the spray drying process. As reported in Chapter 3 and supported by the d-spacing value of 1.5 and 1.49 for T1 and T4, respectively, L-leucine forms a crystalline shell on the particle surface (Sou et al., 2013) and reduces the moisture sorption of these formulations as shown in the DVS studies. When compared to L-leucine raw material, the preferred orientation toward the (110) plane at 19°2θ as opposed to the (001) plane at 6°2θ was also found (Raula et al., 2008). As previously reported in Chapter 3, the trileucine containing powders (T2, T3 and T5) did not show any peaks corresponding to the crystalline trileucine raw material.

Following moisture exposure up to 90 %RH, both the L-leucine formulations (T1 and T4) showed an increase intensity of crystalline peaks and new peaks assigned to crystalline tobramycin base compared to the formulations measured immediately following spray drying. The T1 formulation appeared to have additional crystalline peaks due to the presence of mannitol (Figure 4.32) and higher intensity crystalline peaks. The peaks observed at 9.7, 20.3, 21.1, 22.0 and 25.2°2θ in T1 are characteristic of mannitol (Li et al., 2014).
The trileucine containing powder formulations (T2, T3 and T5) also showed characteristic crystalline peaks after exposure to 90 %RH, despite clear crystallization events not being seen in the moisture sorption profiles. The mannitol containing powders (T2 and T3) showed the presence of a characteristic peak at 9.7 °2θ corresponding to crystalline mannitol. However, additional mannitol peaks could not be discriminated from trileucine crystal peaks. These powder formulations following exposure to moisture had characteristic peaks for crystalline trileucine (7.8 °2θ and 18.6 °2θ). Additionally, T2 had characteristic tobramycin base peaks at 6.7, 17.6 and 18.3 °2θ that were also found in L-leucine formulation (T4). These tobramycin base peaks were not observed in both powder formulations in which tobramycin sulfate is believed to have been formed (T3 and T5). The lack of characteristic tobramycin base peaks and the highly amorphous nature of the sample even after exposure to moisture, suggest the presence of amorphous tobramycin sulfate (Figure 4.29) in the trileucine formulations (T3 and T5) which has sulfuric acid added to alter the trileucine solubility. The powder formulation containing TOBI:SO₄²⁻:TL at 62:23:15 %%/w (T5) appeared mostly amorphous after exposure to 90 %RH indicating the stability of this formulation compared to the other powder formulations in which crystalline peaks were evident following moisture exposure.
Figure 4.30 XRPD diffractograms of T1 and T4 tobramycin EEG powder formulations containing L-leucine.
Figure 4.31 XRPD diffractograms of T2, T3 and T5 tobramycin EEG powder formulations containing trileucine.

Figure 4.32 XRPD diffractograms of T1 and T4 tobramycin EEG powder formulations containing L-leucine after moisture exposure.
4.3.3.4 *In vitro* aerosol performance using cascade impaction method

The aerosol performance of the tobramycin EEG powders spray-dried during the composition optimization study was evaluated using the modified DAC v3 DPI attached to the mouth-piece adaptor with the 3D rod array and sized using the NGI. Results of the aerosol performance studies are shown in Table 4.8.

Unacceptably high device retention was observed for formulations T2 and T4 with values of 53.3 (2.3) % and 66.1 (2.7) %, respectively. Consequently, the emitted doses for these two tobramycin EEG powders were 32.7 % and 30.4 %, respectively. Although these formulations had high fractions of fine particles, the low emitted doses indicated poor aerosol performance. Interestingly, these formulations had the extreme (low and high) values of bulk density among the studied formulations. Replacing L-leucine (T1) with trileucine (T2) in the mannitol EEG formulation reduced the emitted dose significantly. Similarly, removing mannitol in the Tobi/L-leucine (T4) formulation also significantly reduced the emitted dose during

![Figure 4.33 XRPD diffractograms of T2, T3 and T5 tobramycin EEG powder formulations containing trileucine after moisture exposure.](image)
In contrast, the previously optimized L-leucine formulation (T1), and the two formulations with tobramycin sulfate and trileucine (T3 and T5) had emitted doses of ~70%, with respirable and submicron fractions ranging from 89.6 (0.3) % to 96.0 (0.1) % and 24.1 (2.5) % to 19.2 (1.3) %, respectively. Due to its larger %FPF<5 µm compared to T1 and T3 and due to its apparent stability following exposure to high relative humidities, T5 (TOBI:SO₄²⁻:TL at 62:23:15 \%) was selected as tobramycin EEG lead powder formulation to be tested in the in vitro aerosol performance study using realistic airway conditions. This formulation also meets the goals of this study to increase the drug payload in the spray-dried powder with 85% of tobramycin sulfate in the formulation.

**Table 4.8** Mean (SD) aerosol performance characteristics of the tobramycin EEG powder formulations T1 to T5 (n = 3).

<table>
<thead>
<tr>
<th>Formulation ID</th>
<th>Device retention, %</th>
<th>ED, %</th>
<th>MMAD, µm</th>
<th>FPF&lt;5 µm, %</th>
<th>FPF&lt;1 µm, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>21.5 (1.3)</td>
<td>69.2 (1.4)</td>
<td>1.89 (0.02)</td>
<td>88.8 (0.8)</td>
<td>12.5&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td>53.3* (2.3)</td>
<td>32.7* (3.1)</td>
<td>1.98 (0.16)</td>
<td>79.4&lt;sup&gt;‡&lt;/sup&gt; (2.4)</td>
<td>12.7&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>T3</td>
<td>17.5 (4.5)</td>
<td>72.9 (4.1)</td>
<td>1.47&lt;sup&gt;#&lt;/sup&gt; (0.06)</td>
<td>89.6 (0.3)</td>
<td>24.1 (2.5)</td>
</tr>
<tr>
<td>T4</td>
<td>66.1* (2.7)</td>
<td>30.4* (2.8)</td>
<td>1.43* (0.07)</td>
<td>94.8&lt;sup&gt;‡&lt;/sup&gt; (3.4)</td>
<td>28.7 (3.1)</td>
</tr>
<tr>
<td>T5</td>
<td>25.6 (4.0)</td>
<td>69.6 (4.2)</td>
<td>1.61&lt;sup&gt;#&lt;/sup&gt; (0.03)</td>
<td>96.0&lt;sup&gt;‡&lt;/sup&gt; (0.1)</td>
<td>19.2&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*</sup>Significant difference compared to T1, T3 and T5 (One-way ANOVA, Tukey’s HSD, p-value< 0.0001).
<sup>‡</sup>Significant difference compared to T1 and T2 (One-way ANOVA, Tukey’s HSD, p-value< 0.05).
<sup>‡</sup>Significant difference compared to all powders (One-way ANOVA, Tukey’s HSD, p-value< 0.05).
<sup>‡</sup>Significant difference compared to T1 and T3 (One-way ANOVA, Tukey’s HSD, p-value< 0.05).
<sup>x</sup>Significant difference compared to T1, T2 and T5 (One-way ANOVA, Tukey’s HSD, p-value< 0.05).

ED=emitted dose, MMAD=mass median aerodynamic diameter, FPF=fine particle fraction.
4.3.3.5 *In vitro* aerosol performance of tobramycin EEG powders using realistic airway conditions

The excipient enhanced growth aerosol characterization was evaluated in 5-year-old mouth throat chamber model under simulated airways conditions at 37 °C and 99 %RH. A control study was also conducted under ambient condition at 22 °C and 45 %RH. Results from these two conditions were reported in Table 4.9. The simulated airways experiments were validated as the device retention and the emitted dose were comparable irrespective of the conditions used. The mass median aerodynamic diameter was significantly larger under simulated airway condition with a mean of 2.44 (0.05) µm compared to the aerosol size of 1.76 (0.01) µm under ambient conditions. The MMAD growth ratio corresponded to 1.4. While no difference was found in the respirable fraction, the FPF<1 µm decreased by almost 10 % under simulated airways conditions, decreasing from 15.6 % to 6.7 %.

Table 4.9 Aerosol characteristic of tobramycin EEG powder formulation T5 following exposure to ambient and simulated airway conditions. Values represent mean (SD), (n = 3).

<table>
<thead>
<tr>
<th></th>
<th>Ambient</th>
<th>Simulated airway</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Device retention, %</strong></td>
<td>21.6 (2.3)</td>
<td>24.0 (3.1)</td>
</tr>
<tr>
<td><strong>ED, %</strong></td>
<td>71.6 (2.3)</td>
<td>70.6 (2.8)</td>
</tr>
<tr>
<td><strong>MT, %</strong></td>
<td>1.2 (0.1)</td>
<td>1.0 (0.1)</td>
</tr>
<tr>
<td><strong>Trachea, %</strong></td>
<td>0.7 (0.0)</td>
<td>0.6 (0.1)</td>
</tr>
<tr>
<td><strong>Growth chamber, %</strong></td>
<td>2.3 (1.2)</td>
<td>0.8* (0.1)</td>
</tr>
<tr>
<td><strong>MMAD, µm</strong></td>
<td>1.76 (0.01)</td>
<td>2.45* (0.04)</td>
</tr>
<tr>
<td><strong>FPF&lt;5 µm, %</strong></td>
<td>95.4 (1.7)</td>
<td>94.2 (1.0)</td>
</tr>
<tr>
<td><strong>FPF&lt;1 µm, %</strong></td>
<td>15.6 (0.3)</td>
<td>6.2* (0.6)</td>
</tr>
</tbody>
</table>

*Significant difference from ambient conditions (Student’s t-test, p-value < 0.05)

ED=emitted dose, MT=mouth-throat, MMAD=mass median aerodynamic diameter, FPF=fine particle fraction
The percentage of tobramycin that overcame the mouth-throat region was considered as the total lung dose and it corresponded to a mean value of 71.1 (1.1) % of the nominal dose. The DtL will be used for pharmacokinetic predictions in Chapter 6.

4.4 Discussion and conclusions

4.4.1 Assessment of the optimized spray drying and storage conditions for a model tobramycin EEG powder

This study investigated the effect of spray drying and storage conditions on tobramycin EEG powder formulations produced using the Büchi Nano B-90 HP spray dryer. While feed solution solvent, solids concentration and relative component ratios were unvaried in this study, the selection of medium mesh nebulizer size and 70 °C drying inlet temperature was based on previous findings obtained from the AS EEG powder formulations (Chapter 3). Particle size and powder dispersion characteristics, moisture sorption behavior and aerosol performance were evaluated immediately following spray drying following a brief equilibration period exposed to different RH conditions. A previous study has investigated the effect of the drying gas water content during the particle formation process (Hassan et al., 2020). It was hypothesized that the surface enriching agent L-leucine had a thicker shell on the particle surface when using a slow droplet drying rate, as opposed to a thinner shell in the case of fast drying rate. A thicker L-leucine shell could result in more stable powder formulation as it would retard the uptake of water due to its hydrophobicity. Hassan et al., modified the drying rate by altering the drying gas water vapor density. In this chapter, the same technique was employed to alter the surface characteristics of a series of tobramycin EEG formulations and those powders were exposed to a range of storage relative humidities to investigate the effects of moisture uptake on solid-state stability and aerosol performance with the goal of identifying optimal spray drying and storage conditions.
Following spray drying using different drying gas water vapor densities, the TOBI EEG formulations were exposed to humidities between 15-60%RH. At t=0, initial particle size analysis revealed that powders stored at 15 and 35 %RH and room temperature had a small primary particle size (Dv50) of ~0.9 µm and were relatively less polydisperse compared to the same EEG formulations stored at 60 %RH and room temperature. When evaluated from a solid-state stability perspective immediately following spray drying (t=0) using dynamic vapor sorption analysis, the formulations stored at 15 and 35 %RH exhibited a crystallization event, as opposed to the powders stored at 60 %RH which showed no crystallization. Powder x-ray diffraction confirmed the powders were amorphous when stored at 15 and 35 %RH, while the powders stored at 60 %RH was already crystalline prior to the moisture sorption study. In addition, the powders stored at 15 and 35 %RH were both in the amorphous form after 6 months storage.

Dynamic vapor sorption analysis showed differences in the tobramycin EEG powders spray-dried using drying gas water vapor densities of 11.9 g/m³ and 4.2 g/m³ which was used to alter the particle drying rate. Formulations characterized at t=0 stored at both 15 % and 35% RH showed comparable glass transition and crystallization RHs as well as moisture uptakes at RH_g and RH_c for both drying gas water vapor densities, indicating that the drying rate did not affect the initial particle characteristics, this was in contrast to the observations of Hassan et al. After 6 months storage, the tobramycin EEG powders stored at 15 %RH and room temperature again showed comparable RH_g and RH_c together with moisture uptakes, irrespective of the drying rate employed. The formulation stored at 35 %RH had the same RH_g but higher RH_c and faster absorption rates indicating improved stability for the formulation produced using conditions to generate the slower drying rate (11.9 g/m³). For these studies, the DVS analysis showed mixed results regarding the influence of drying rate on the solid-state stability of tobramycin EEG powders. It was not possible to select the optimal drying conditions and storage conditions for the tobramycin EEG powder based on dynamic vapor sorption analysis alone. From an aerosol performance perspective, the initial (t=0) aerosol performance of the tobramycin EEG powders stored at 15 and 35 %RH was good, irrespective of the spray drying conditions. Emitted doses ranged between 74% and 87%, the aerodynamic size was ~1.8 µm, and
the fine particle fractions were >40%. Following long-term storage (up to 6 months), the powders stored at 15 %RH and room temperature had a better aerosol performance compared to those stored at 35 %RH. Although emitted doses were slightly lower, enhanced dispersion was observed for the powders stored at 15 %RH having a smaller MMAD and higher FPFs compared to long-term storage at 35 %RH. The tobramycin EEG powders initially stored at 60 %RH had high emitted doses when aerosolized using the novel positive pressure DAC v3 DPI. However, the high emitted doses reflect agglomeration of particles at this storage humidity and resulted in high pre-separator drug losses and large mass median aerodynamic diameters. For these formulations, >90% of the nominal dose was found in the pre-separator, the MMADs were larger than 3 μm, and the respirable and submicron fractions were <5% when initially exposed to 60 %RH at room temperature, irrespective of the spray drying conditions. The tobramycin EEG powders stored at 60 %RH were not included in the long-term solid-state stability and aerosol performance evaluation. Despite the inconclusive results obtained from a moisture sorption perspective, a better aerosol performance was observed from the tobramycin EEG powders produced using a 11.9 g/m³ drying gas water vapor density compared to 4.2 g/m³, when compared for the same storage conditions.

Overtime tobramycin EEG powders stored at 15 %RH and room temperature resulted in better aerosol performance compared to those stored at 35 %RH, irrespective of the spray drying conditions. When comparing the two drying gas water vapor densities used during the spray drying process, it was found that the 11.9 g/m³ maintained a lower pre-separator loss, smaller MMAD and larger fine particle fractions compared to 4.2 g/m³ at almost all time points. Therefore, the optimized spray drying and storage conditions employed a 11.9 g/m³ drying gas water vapor density and storage at 15 %RH and room temperature for the tobramycin EEG powder formulation.

4.4.2 Optimization of composition of tobramycin EEG spray-dried powder formulation

Further studies were performed to consider the composition of the tobramycin EEG powder formulation by evaluating trileucine as dispersion enhancing agent as an alternative to L-leucine and to maximize the
tobramycin payload in the powder. A selection of tobramycin EEG powder formulations was spray-dried and characterized from in vitro aerosol performance perspective as well as solid-state stability.

Due to its surface enrichment in the drying droplet phase, trileucine is known to result in wrinkled shaped particles with effective aerosol performance (Lechuga-Ballesteros et al., 2008b; Ordoubadi et al., 2021b; Wang et al., 2021). Additionally, results obtained from the albuterol sulfate EEG powders (Chapter 3) also showed the enhanced aerosol performance and solid-state stability of trileucine AS EEG powders compared to L-leucine AS EEG powders. In this present study, when comparing the initial L-leucine and trileucine powders (T1 and T2), it was found that addition of trileucine as dispersion enhancer resulted in a larger particle size and worse aerosol performance compared to L-leucine. The emitted dose for the L-leucine formulation was 69.2 % and was reduced to 32.7 % when using trileucine together with a low respirable fraction. Trileucine is known to have pH-dependent aqueous solubility and with tobramycin in the formulation produced a feed formulation with a pH of ~9 and higher aqueous solubility compared to previous studies with the acidic albuterol sulfate feed formulation and lower trileucine solubility (Lechuga-Ballesteros et al., 2008). This difference in the aqueous solubility may be responsible for changes in the particle formation for the tobramycin powder and the absence of a shell. In order to reduce the feed solution pH and reduce the trileucine aqueous solubility, the pH was adjusted using sulfuric acid to 6.2 which decreased the trileucine solubility to 7 mg/mL. The tobramycin EEG powder sprayed using the modified pH feed solution (T3) had an improved aerosol performance with an emitted dose of 72.9 % and high respirable and submicron fractions (89.6 % and 24.1 %, respectively). High emitted dose and fine particles are key aerosol properties that minimize mouth-throat loss and therefore deliver high tobramycin lung doses for the EEG powder formulations. The pH modification did not only improve the aerosol performance of the tobramycin EEG powder, but also resulted in particle morphology and crystallinity differences. The spherical shaped particles observed for formulation T2 was converted in the typical trileucine wrinkle morphology (T3), as reported by others (Lechuga-Ballesteros et al., 2008b). Both trileucine powders (T2 and T3) were completely amorphous after the spray drying process. However, following exposure to 90
%RH during DVS, the T3 formulation appeared to have a lower crystalline component compared to T2, perhaps reflecting the intact trileucine shell for the powder sprayed from the solution with the lower trileucine solubility.

Addition of sulphuric acid to modify the pH of formulation T3 resulted in a decreased tobramycin payload in the powder. Further studies (T4 and T5) were performed removing mannitol and increasing tobramycin to use its hygroscopic nature to facilitate the hygroscopic growth. When aerosolized with the positive pressure DAC v3 inhaler, the L-leucine powder T4 resulted in unacceptable aerosol performance with only 30.4 % emitted dose, which is likely due to its relatively high bulk density of 0.288 g/cm$^3$ compared to the bulk density of other tobramycin EEG powders. From the XRPD analysis, T4 was more crystalline compared to the trileucine T5 powder, especially after exposure to 90 %RH. Conversely, the pH adjusted trileucine T5 powder without mannitol resulted in amorphous particles that achieved 69.6 % emitted dose and the highest respirable fraction among all the tobramycin EEG powder tested.

Overall, tobramycin EEG powders containing trileucine resulted in faster moisture sorption rate and higher hygroscopicity compared to the L-leucine containing powders. However, the glass transition and crystallization RHs occurred at much higher RH in the presence of trileucine compared to L-leucine in these tobramycin EEG powders, which was indicative of improved solid-state stability (Hassan et al., 2020). The XRPD analysis further confirmed this finding, as the trileucine containing powders maintained a higher degree of amorphicity even after exposure to 90 %RH. Finally, the pH adjusted trileucine powders showed improved aerosol performance compared to the corresponding L-leucine tobramycin EEG powders.

The tobramycin EEG powder formulation T5 containing trileucine and with modified pH was selected as the lead powder for its high tobramycin payload and enhanced aerosol performance and hygroscopicity, compared to all the other tobramycin EEG powders evaluated. T5 was therefore tested under simulated airways condition and resulted in growth aerodynamic size as well as reduced percentage of submicron particles. Therefore, the replacement of mannitol with additional tobramycin still resulted in a tobramycin EEG powder that can grow under simulated airways conditions.
CHAPTER 5: RAPID SCREENING OF THE AEROSOL PERFORMANCE OF EXCIPIENT
ENHANCED GROWTH SPRAY-DRIED POWDER FORMULATION USING LASER
DIFFRACTION TESTING

5.1 Introduction

This chapter evaluates the suitability of Malvern Spraytec as a laser diffraction-based method to characterize the aerosol performance of excipient enhanced growth spray-dried powders using active and passive dry powder inhalers at a steady flow rate as well as breathing profile. The dynamics of the albuterol sulfate and budesonide EEG powders aerosolized using different air inlet aperture positions were evaluated with respect to realistic *in vitro* mouth-throat model, using a breath-actuated DPI. The batch to batch reproducibility of albuterol sulfate EEG formulations was conducted using laser diffraction method coupled with realistic breathing profile and compared to cascade impactor methods used with steady flow and inhalation profile. Relationship between laser diffraction characterization and steady-flow cascade impaction data was conducted for a series of tobramycin EEG powders delivered using a novel positive pressure DPI.

5.2 Materials and methods

5.2.1 Materials

Albuterol sulfate (USP), budesonide (USP), and tobramycin base (ACROS Organics, CA) were used as the active pharmaceutical ingredients for three spray-dried excipient enhanced growth formulations. The AS and TOBI formulations also included Pearlitol® PF-Mannitol (Roquette Pharma, Lestrem, France), poloxamer 188 (Leutrol F68) (BASF Corporation, Florham Park, NJ), L-leucine (Sigma Chemical Co., St. Louis, MO) or trileucine (Bachem). The budesonide (BUD) EEG formulation contained sodium chloride, in addition to L-leucine. Quali-V size 3 hydroxypropyl methylcellulose (HPMC) capsules were donated from Qualicaps (Whitsett, NC). Molykote®316 silicone release spray was purchased from Dow Corning Corporation (Midland, MI).
5.2.2 Preparation of the spray-dried powder formulations

Spray-dried AS EEG and TOBI EEG powder formulations were obtained using the Büchi Nano spray dryer B-90 HP (Büchi LaboratoryTechniques, Flawil, Switzerland), as reported in Chapters 3 and 4. Mannitol was used as the hygroscopic excipient, L-leucine or trileucine as dispersion enhancing agents, and poloxamer 188 as surfactant, respectively. Table 5.1 summarizes the formulations used in this study. Four batches of the AS EEG powder were produced to evaluate the batch to batch reproducibility by comparing the particle characteristics of the four replicate batches.

BUD EEG was spray-dried at Lonza’s Bend facility using a custom lab-scale spray dryer (BLD-35), and it contained L-leucine as dispersion enhancer and sodium chloride.

**Table 5.1** Albuterol, budesonide and tobramycin excipient enhanced growth spray-dried powder formulations with total solids contents, components and component ratio.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Total solids content, %</th>
<th>Components</th>
<th>Component ratio, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS EEG</td>
<td>0.50</td>
<td>albuterol sulfate:mannitol:L-leucine:poloxamer 188</td>
<td>30:48:20:2</td>
</tr>
<tr>
<td>BUD EEG</td>
<td>1.0</td>
<td>budesonide:sodium chloride:L-leucine</td>
<td>8:52:40</td>
</tr>
<tr>
<td>TOBI EEG</td>
<td>0.50</td>
<td>tobramycin:mannitol:L-leucine:poloxamer 188</td>
<td>60:18:20:2</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>tobramycin:mannitol:trileucine:poloxamer 188</td>
<td>60:18:20:2</td>
</tr>
<tr>
<td></td>
<td>0.61</td>
<td>tobramycin:sulfate:mannitol:trileucine:poloxamer 188</td>
<td>49:18:15:16:2</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>tobramycin:L-leucine</td>
<td>80:20</td>
</tr>
<tr>
<td></td>
<td>0.65</td>
<td>tobramycin:sulfate:L-leucine</td>
<td>62:23:15</td>
</tr>
</tbody>
</table>

AS=albuterol sulfate, BUD=budesonide, TOBI=tobramycin, EEG=excipient enhanced growth

103
5.2.3 Solid-state characterization of EEG powders

Primary particle size of the bulk powder was measured at 1 and 4 bar dispersion pressures by laser diffraction using the Sympatec HELOS (submicron R1 lens with 20 mm focal length) with RODOS/M disperser and ASPIROS sample feeder (Sympatec GmbH, Clausthal-Zellerfeld, Germany). Volume-based particle size distributions were obtained by WINDOX software and the particle size below which 50% of the particles lie was considered as the Dv50.

True and bulk densities were measured for the powder formulations. The true density corresponds to the mass of a substance divided by its volume, excluding open and closed pores. The true density measurement was performed by gas pycnometer (AccuPyc II 1340, Micromeritics, Norcross, GA). Helium was the inert gas used as the displacement medium. Calibration was performed daily to ensure the accuracy of the experiments. The 0.1 cm³ sample cup insert was employed due to the small mass of sample available for measurement. Approximately 20 mg of spray-dried formulation was weighed in the previously tared sample cup insert, and then positioned in the sample chamber. Measurements were conducted in triplicate.

Thermogravimetric analysis (TGA) was performed using the Pyris 1 TGA (PerkinElmer, Covina, CA) with TAC 7/DX Thermal Analysis Controller. 6-7 mg of spray-dried powder were held at 22 °C for 2 min, then heated from 22 °C to 50 °C at a rate of 25 °C/min, and then finally held at 50 °C for 30 min. A nitrogen purge gas was employed at a flow rate of 20 mL/min. The % weight loss at 50 °C was expressed as percentage of the dried weight and was considered as loosely bound surface water.

Dynamic vapor sorption analysis was conducted under isothermal condition (25 °C), while the humidity was set to increase from 0 %RH to 90 %RH in 9 steps of 10 %RH each. The equilibrium moisture content for each step was considered to be achieved based on dm/dt ≤ 0.002%. Weight change was expressed as a percentage of the reference weight, defined as the equilibrium weight at 0 %RH and isotherm profiles were reported.
5.2.4  *In vitro* aerosol particle sizing of EEG powder formulations

5.2.4.1  Aerosol performance testing using laser diffraction

All EEG powders were characterized using the Malvern Spraytec® (Malvern Instruments, Ltd., Worcestershire, UK) laser diffraction-based instrument. Spraytec measurements were conducted using the 100 mm lens focal length, which can measure particles with a size within the 0.5 – 200 µm range. A transmission of less than 98-99% was used to trigger the initiation of Spraytec data collection. Data was collected for a maximum of 4 s and at a sampling frequency of 2500 Hz. Media refractive index and particulate refractive index were set to 1.00 + 0.00i (air) and 1.50 + 0.50i (opaque), respectively. Background measurement was performed before each data collection.

5.2.4.1.1  AS and BUD EEG formulation testing

AS and BUD EEG powders were delivered using the breath-actuated CC90-3D dry powder inhaler and loaded with 1-2 mg of powder in a size 3 HPMC capsule. The effects of two previously used air inlet positions (AIPs) to pierce the capsules were investigated for the AS and BUD EEG powder formulations (Srinivas R.B. Behara et al., 2014; Longest et al., 2014). The piercing with a 0.5 mm hole diameter in the head of the capsule was varied while the 0.5 mm hole in the base remained constant. The air inlet aperture was positioned in the side wall (AIP 1) or in the perpendicular axis (AIP 2) of the capsule head as shown in Figure 5.1.
In order to test the AS and BUD EEG powders emitted from the CC$_{90}$-3D inhaler, the Spraytec inhalation cell system was employed to enable the breath actuation of the DPI using a breath simulator (ASL 5000-XL, IngMar Medical, Pittsburgh, PA). The CC$_{90}$-3D inhaler was connected using an airtight mouthpiece adaptor to the inlet of the inhalation cell, while the breath simulator was attached to the outlet of the cell as shown in Figure 5.2. The delivered aerosol was collected on a PulmoGuard II™ filter that was positioned inline between the outlet of the inhalation cell and the breath simulator. The breath simulator was used to actuate the DPI using the realistic medium breathing profile previously reported by Delvadia et al., which was characterized with an average flow of 45 L/min over 4 s of inhalation and a peak inspiratory flow rate of 65 L/min, for a total inhaled volume of 2.8 L (Delvadia et al., 2016). The drug retained in the inhaler capsule and deposited on the PulmoGuard II™ filter was quantified using validated HPLC methods and reported as a percentage of nominal dose. The time-history profiles of the emitted dose aerosol particle size distributions were compared with respect to the % transmission of the aerosol, reflecting laser obscuration, and aerosol cloud density. The RTSizer software was used to obtain particle size information, reported as time averaged volumetric diameter (Dv) for the 10$^{th}$, 50$^{th}$, and 90$^{th}$ percentile of the volumetric diameter frequency, respectively. The percentage of particle smaller than 5 and 1 μm was obtained from the cumulative volume distribution. All measurements were conducted in triplicate.

Figure 5.1 Schematic representation of air inlet aperture position 1 (left) and 2 (right). Adapted from Behara et al., 2014.
5.2.4.1.2  Tobramycin excipient enhanced growth spray-dried powder formulation testing

For the TOBI EEG powders, 10 mg of formulation was loaded in the novel positive pressure dry powder inhaler DAC v3 attached to the mouthpiece adaptor. For the positive pressure device, the Spraytec open bench system was employed as a realistic breathing profile was not required for device actuation. The DAC v3 inhaler was actuated for 4.64 s at 9.7 L/min to deliver a volume of air of 750 mL, as mentioned in Chapter 4. The inhaler was aligned vertically with the center of the laser beam and it was positioned 5 cm horizontally from the Spraytec detector and 8 cm vertically from the Spraytec laser beam, distances similar to the inhalation cell system. The RTSizer software was used to obtain particle size information, reported
as time averaged volumetric diameter (Dv) for the 50th percentile of the volumetric diameter frequency. The percentage of particle smaller than 5 and 1 µm was obtained from the cumulative volume distribution. The volume concentration (Cv) was reported to characterize the aerosol cloud concentration emitted from the DPI. Linear relationships with impactor results obtained in Chapter 4 will be evaluated. All measurements were conducted in triplicate.

5.2.4.2 Aerosol performance testing using a realistic mouth-throat airway model

The aerosol performance of the AS and BUD EEG formulations and the capsule air inlet positions was assessed using a realistic mouth-throat airway model. As described previously, each powder formulation was loaded into a size 3 HPMC capsule and placed in the CC90-3D dry powder inhaler. The emitted aerosol was delivered through a medium sized VCU mouth-throat (MT) model using the same inhalation profile reported in Chapter 3 (Wei et al., 2018). The inhaler was connected to the inlet of the mouth-throat model while the outlet was attached to a respiratory filter (PulmoGuard II™, Quset Medica, North Easton, MA) placed inline with the breath simulator as shown in Figure 5.3. Particle bounce was prevented by coating the MT model with Molykote® 316 Silicone Release Spray. Aerosol deposited in the filter, retained in the capsule and deposited in the MT model was collected and quantified by validated HPLC method for AS and BUD, respectively. Drug collected on the respiratory filter positioned at the exit of the trachea was labeled as the estimated total lung dose. Results were expressed as percentage of the nominal dose. All measurements were conducted in triplicate.
5.2.4.3 Aerosol performance testing using cascade impaction

The four batches of AS EEG powder formulation were assessed using cascade impaction methods. For these studies, 2 mg of AS EEG was loaded into a size 3 HPMC capsule and delivered from the CC\textsubscript{90-3D} dry powder inhaler. The Next Generation Impactor with the Westech W7 attachment was employed to size the EEG powders under steady flow conditions and using a simulated realistic inhalation. The steady flow conditions were conducted using 45 L/min flow rate through the NGI and used the same methods reported in Chapter 3. This method was referred to as “CI – 45 L/min”.

The simulated inhalation, shown in Figure 5.4 and referred to as “CI – realistic inhalation” method, required modification of the impactor method to include use of the Nephele Mixing Inlet (RDD Online) to allow operation of the cascade impactor at the constant flow rate while actuating the inhaler with the realistic...
inhalation profile (Wei et al., 2017). The NGI was placed in the upright position to correctly actuate the dry powder inhaler, and the Westech W7 attachment was used to allow a flow rate through the impactor of 90 L/min. The Nephele Mizing Inlet has the function to mix non-homogeneous aerosols and was placed between the NGI and the CC$_{90}$-3D inhaler, with the side inlet attached to the dilution air at 90 L/min. The ASL breath simulator was connected between the dilution air and the Nephele Mixing Inlet. The realistic inhalation profile used to actuate the CC$_{90}$-3D inhaler was the same as previously reported in Chapter 3.

Emitted dose, mass median aerodynamic diameter and fine particle fraction smaller than 5 and 1 µm were reported as a percentage of the emitted dose. All measurements were conducted in triplicate.

![Figure 5.4 Schematic setting for the CI – realistic inhalation testing using Next Generation Impactor and breath simulator. Adapted from Wei et al., 2017.](image)

5.2.5 Statistical analysis

One-way ANOVA (Tukey’s HSD) or Student’s $t$-test were conducted to evaluate the effect of different variables on the particle size characteristics and the aerosol performance of EEG powder formulations when
using the study characterization methods. Significant difference was considered for \( p\)-value\(< 0.05 \) for all statistical analyses. JMP Pro 15 (SAS Institute Inc., Cary, NC) was used to perform the statistical analysis.

5.3 Results

5.3.1 The effect of capsule air inlet position on the aerosol performance of AS and BUD EEG spray-dried powders when delivered by a novel capsule-based dry powder inhaler

5.3.1.1 Aerosol performance testing using laser diffraction and realistic mouth-throat airway model

Table 5.2 shows the aerosol duration and the particle size characteristics obtained using Spraytec from the aerosolization of AS and BUD EEG spray-dried powders using AIP 1 and AIP 2, respectively. The Dv10 was found to be constant at 0.4±0.0 \( \mu m \) for both of the EEG powder formulations tested with AIP 1 and AIP 2. Aerosolization of the BUD EEG powder formulation resulted in a comparable mean volumetric diameter of 1.4±0.0 \( \mu m \) and 1.5±0.0 \( \mu m \) when using AIP 1 and AIP 2, respectively. Conversely, the mean volumetric diameter obtained from the aerosolization of the AS EEG powder formulation was found to be influenced by the air inlet aperture position, resulting in a smaller Dv50 of 1.2±0.0 \( \mu m \) when using AIP 1 compared to 1.5±0.0 \( \mu m \) when using AIP 2. This result suggests that the AS EEG powder was more effectively aerosolized when the air inlet aperture was positioned in the side wall of the capsule head. Additionally, a significantly larger Dv90 of 38.2±5.1 \( \mu m \) was observed for the AS EEG powder aerosolized using the AIP 2, compared to all the other Dv90 values obtained from BUD EEG and from AS EEG aerosolized using AIP 1. The poor performance of the AS EEG formulation in combination with AIP 2 was also evident from the reduced %FPF< 5 and 1 \( \mu m \) when compared to the AS EEG powder formulation aerosolized using AIP 1 (81.2±1.8 % and 31.7±1.0 % vs. 96.8±0.4 % and 43.1±0.4 %, respectively).
Table 5.2 Mean (SD) particle size characteristics of the emitted aerosol from the two spray-dried EEG formulations measured using the laser diffraction method (n>3).

<table>
<thead>
<tr>
<th>Formulation-AIP</th>
<th>Aerosol Duration, s</th>
<th>Dv10, µm</th>
<th>Dv50, µm</th>
<th>Dv90, µm</th>
<th>FPF&lt;1 µm, %</th>
<th>FPF&lt;5 µm, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUD AIP 1</td>
<td>1.9 (0.1)</td>
<td>0.4 (0.0)</td>
<td>1.4 (0.0)</td>
<td>3.6 (0.0)</td>
<td>34.1 (0.2)</td>
<td>96.0 (0.0)</td>
</tr>
<tr>
<td>BUD AIP 2</td>
<td>1.5 (0.3)</td>
<td>0.4 (0.0)</td>
<td>1.5 (0.0)</td>
<td>3.8 (0.2)</td>
<td>34.3 (1.0)</td>
<td>95.1 (1.1)</td>
</tr>
<tr>
<td>AS AIP 1</td>
<td>0.7 (0.1)</td>
<td>0.4 (0.0)</td>
<td>1.2 (0.0)</td>
<td>3.0 (0.1)</td>
<td>43.1 (0.4)</td>
<td>96.8 (0.4)</td>
</tr>
<tr>
<td>AS AIP 2</td>
<td>0.6 (0.0)</td>
<td>0.4 (0.0)</td>
<td>1.6* (0.1)</td>
<td>38.2* (5.1)</td>
<td>31.7* (1.0)</td>
<td>81.2* (1.8)</td>
</tr>
</tbody>
</table>

*Significantly different from AIP 1 (Student’s t-test, p-value< 0.05).

AIP=air inlet aperture position, Dv10=volume diameter at the 10th percentile, Dv50=volume diameter at the 50th percentile, Dv90=volume diameter at the 90th percentile, FPF=fine particle fraction.

The effects of AIP on the aerosolization of the AS and BUD EEG was also observed from the time history profiles shown in Figure 5.5 as percentage transmission (top) and Dv50 (bottom) reported as a function of time (msec). The duration of aerosol emission was shorter for the AS formulation compared to the BUD formulation. The BUD EEG powder formulation was delivered from the CC90-3D inhaler over 1.5±0.3 - 1.9±0.1 s, compared to emptying of the AS EEG powder formulation which took place over 0.6±0.0 - 0.7±0.1 s. In order to explain these differences, the powder density of the two formulations was investigated. The BUD EEG formulation bulk density was 0.252±0.007 g/cm³ while the AS EEG formulation bulk density was 0.197±0.006 g/cm³. It is hypothesized that density differences between the powder formulations was responsible for the differences in the device emptying profiles. Additionally, it was observed that the AIP 2 resulted in a higher and more variable laser obscuration, when compared to the AIP 1 % transmission profile of both AS and BUD EEG powder formulations. However, the more uniform Dv50 time profile was observed for the BUD EEG powder formulation when aerosolized using the AIP 2. Conversely, AS EEG powder formulation showed a highly variable Dv50 signal over time which further suggests worse powder dispersion. It is believed that higher obscuration is reflective of an increased emitted dose from the inhaler.
Figure 5.6 shows the capsule drug retention and the filter deposition at the exit of the inhalation cell for the BUD EEG (left) and AS EEG (right) powder formulations obtained from the Spraytec – laser diffraction experiment. The drug retention in the capsule for both formulations using the AIP 2 method was lower compared to AIP 1, indicative of a higher emitted dose, as reflected in the higher obscuration previously observed. These results indicated that the % transmission could be used as a surrogate to compare inhaler emitted doses and that for this study, for both formulations, using the AIP 2 capsule piercing position increased the emitted aerosol dose of the EEG powder formulations.

In order to further validate these observations, the laser diffraction results were compared with the similar measurements of capsule retention and filter deposition when aerosolizing BUD and AS EEG powder formulations using AIP 1 and 2 in a realistic in vitro mouth-throat model. Figure 5.6 shows that no difference was observed for BUD EEG in capsule retention and filter deposition when comparing laser
diffraction and mouth-throat model results. However, a higher filter deposition was found for the laser
diffraction method compared to mouth-throat model for the AS EEG powder formulation and irrespective
of the selected AIP. This difference was likely due to the slightly larger fraction of MT deposition for the
AS EEG formulations compared to the BUD EEG formulation. From the MT experiments, it was observed
that the BUD EEG formulation aerosolized using AIP 1 resulted in high capsule retention (23.3±4.0 %) and
low MT deposition (2.6±0.6 %) compared to 13.2±1.7 % and 6.3±1.9 %, respectively, for AIP 2. An
efficient dispersion was found when BUD EEG formulation was aerosolized using AIP 2, as the estimated
in vitro lung deposition was 73.5±1.1 %, compared to only 59.8±3.1 % for AIP 1. Similarly, the AS EEG
formulation resulted in higher capsule retention when aerosolized using AIP 1 compared to AIP 2 (27.0±5.0
% and 6.1±0.2 %, respectively). The ineffective performance of AS EEG powder formulation aerosolized
with AIP 2 was further confirmed by the higher mouth-throat model loss compared to the BUD EEG powder
formulation aerosolized with the same AIP. In fact, AS EEG aerosolized with AIP 2 had low capsule
retention (6.1±0.2 %) that resulted in high mouth-throat model deposition (16.2±2.5 %). Despite this
increased MT deposition loss for AS EEG powder formulation, the in vitro lung deposition was 63.1±1.6
% with AIP 2, compared to 45.2±4.5 % with AIP 1.

Overall, both the laser diffraction method and MT studies showed that the AIP 2 resulted in improved
capsule emptying and higher filter doses reflective of improved dispersion of the powder formulation
compared to AIP 1. However, the higher mouth-throat depositional losses observed when aerosolizing AS
EEG with AIP 2 could be of concerns, despite the higher emitted dose.
5.3.2 Assessment of AS EEG spray-dried powders batch to batch reproducibility and realistic in vitro testing conditions using laser diffraction and cascade impaction methods

5.3.2.1 Solid-state characterization

The solid-state characteristics of the four AS EEG powder formulations are shown in Table 5.3. The powder formulations had a median volumetric diameter of 1 µm, irrespective of the applied dispersion pressure. Similarly, the Dv90 ranged between 1.9 and 2.1 µm at 1 and 4 bar. Thus, no differences were observed in the Dv50 and Dv90 when comparing the four batches, indicating both reproducibility and effective dispersibility of the batches that would be assessed using the aerosol performance testing.

Table 5.3 also shows the weight loss (% of dried weight) for each of the four batches of AS EEG powders that was obtained from thermogravimetric analysis. Each of the AS EEG powders had a low amount of loosely bound water as the weight loss ranged from 1.4 to 1.8 % of the dried weight.

Triplicate measurement of the true density was conducted for each batch of AS EEG powder formulation, as shown in Table 5.3. All batches had a true density of 1.4±0.0 g/cm³ with no statistical difference observed when compared using One-way ANOVA test.
Table 5.3 Particle size characteristics at 1 and 4 bar, true density measurements (reported as mean (SD), \(n = 3\)), and percentage weight loss (\(n = 1\)) for AS EEG batch 1, 2, 3, and 4.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Particle size</th>
<th>1 bar</th>
<th>4 bar</th>
<th>Weight loss, %</th>
<th>True density, g/cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dv50, (\mu m)</td>
<td>Dv90, (\mu m)</td>
<td>Dv50, (\mu m)</td>
<td>Dv90, (\mu m)</td>
<td></td>
</tr>
<tr>
<td>Batch 1</td>
<td>1.0 (0.0)</td>
<td>2.1 (0.0)</td>
<td>1.0 (0.0)</td>
<td>2.0 (0.0)</td>
<td>1.6</td>
</tr>
<tr>
<td>Batch 2</td>
<td>1.0 (0.0)</td>
<td>2.0 (0.0)</td>
<td>1.0 (0.0)</td>
<td>1.9 (0.0)</td>
<td>1.4</td>
</tr>
<tr>
<td>Batch 3</td>
<td>1.0 (0.0)</td>
<td>2.0 (0.0)</td>
<td>1.0 (0.0)</td>
<td>2.0 (0.0)</td>
<td>1.5</td>
</tr>
<tr>
<td>Batch 4</td>
<td>1.0 (0.0)</td>
<td>2.1 (0.0)</td>
<td>1.0 (0.0)</td>
<td>2.0 (0.0)</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Dv50=volume diameter at the 50th percentile, Dv90=volume diameter at the 90th percentile

The batches were also evaluated for their moisture sorption profile. Figure 5.7 shows the isotherm plot that reports the change in mass as percentage of the dried mass, as a function of relative humidity. Increasing the humidity to 40 %RH resulted in ≤ 2% weight change for each of the batches. However, at ~50 %RH, a recrystallization process was observed for each of the batches, as shown by the weight loss occurring after 50 %RH. Moisture uptake at 90 %RH resulted in a weight change that ranged from 2.6% to 3.1% of the dried weight for the four batches of AS EEG powder formulation.
5.3.2.2 Aerosol performance testing using laser diffraction and cascade impaction

The aerosol performance for the four AS EEG batches emitted from the CC<sub>90</sub>-3D dry powder inhaler was evaluated using the laser diffraction and cascade impactor methods with a realistic breathing profile. Histograms in Figure 5.8, Figure 5.9 and Figure 5.10 show the results obtained from the aerosol performance evaluated using cascade impaction and laser diffraction methods for the four AS EEG batches. The emitted dose reported in Figure 5.8 ranged from 72 to 80% of the nominal dose across the AS EEG spray-dried powder batches. Figure 5.9 and Figure 5.10 report the respirable and submicron fractions as percentage of the emitted dose for each of the AS EEG batches. A high mean %FPF<5 and 1 µm was found for all AS EEG batches (>90% and >25%, respectively).

The batch to batch reproducibility of the AS EEG formulation was initially evaluated using the same characterization method (LD – realistic, CI – realistic, CI – 45 L/min). The mean emitted doses showed no difference across the four AS EEG batches when evaluated with the same characterization method. One-way ANOVA statistical test revealed that the %FPF<5 µm was comparable for the four batches when testing the AS EEG powders using the CI – 45 L/min and the CI – realistic inhalation methods. However,
Batch 4 resulted in lower % of respirable particles compared to Batch 2 and 3 when aerosolized using the LD – realistic method (p = 0.0132 and 0.0266, respectively). Higher variability was found for the %FPF<1 µm. Batch 2 had a higher percentage of submicron particles compared to Batch 1, 3, and 4 when aerosolized using the CI – realistic inhalation method (p = 0.0006, 0.0047, and 0.0043, respectively). A small but significant difference was also observed between Batch 3 and 4 (p = 0.0161) when tested using the LD – realistic method. No difference across batches was observed when aerosolized using the CI – 45 L/min method.

Additionally, the aerosol performance of each AS EEG batch was compared when tested using the CI and LD realistic inhalation methods. Student’s t-test revealed that the emitted dose did not show any difference between the two methods except for Batch 4 that resulted in higher ED when tested with the LD compared to the CI method (p = 0.0453). A higher percentage of respirable particles was observed for Batch 2 and 4 when comparing the LD and CI methods (p = 0.0021 and 0.0088, respectively). Conversely, Batch 1 and 3 showed no difference in the %FPF<5 µm irrespective of the realistic inhalation test used. %FPF<1 µm and particle size were certainly the two most variable parameters. A lower submicron fraction was found for the LD method for Batch 1, 2, and 4 (p = 0.0046, 0.0021, and 0.0021, respectively).
Figure 5.8 Emitted dose (% of nominal dose) for AS EEG batch 1, 2, 3, and 4 using CI and LD methods ($n=3$).

*Significantly different from CI – realistic inhalation (Student’s $t$-test, $p$-value< 0.05).

Figure 5.9 Fine particle fraction smaller than 5 µm (% of emitted dose) for AS EEG batch 1, 2, 3, and 4 using CI and LD methods ($n=3$).

*Significantly different from Batch 2 and 3 (One-way ANOVA, Tukey’s HSD, $p$-value< 0.05).

*Significantly different from CI – realistic inhalation (Student’s $t$-test, $p$-value< 0.05).
The particle size characteristics for each of the AS EEG batches evaluated with the three analytical methods are reported in Table 5.4. Mass median aerodynamic diameter was reported for the CI characterizations, while Dv50 was reported for the LD characterization.

When comparing the four AS EEG batches aerosolized using the same method, the MMAD was smaller for Batch 2 compared to Batch 1, 3, and 4 when tested using the CI – realistic method ($p = 0.0020$, $0.0069$, and $0.0205$, respectively). Also, Batch 4 showed a larger Dv50 compared to Batch 2 and 3 when aerosolized with the LD – realistic inhalation method ($p = 0.0118$ and $0.0030$, respectively). Instead, when comparing the aerosolization of each batch using the LD and CI realistic inhalation methods, the MMAD resulted to be smaller than Dv50 for all batches.

**Figure 5.10** Fine particle fraction smaller than 1 µm (% of emitted dose) for AS EEG batch 1, 2, 3, and 4 using CI and LD methods ($n = 3$).

*Significantly different from Batch 1, 3 and 4 (One-way ANOVA, Tukey’s HSD, $p$-value< 0.05).

†Significantly different from Batch 4 (One-way ANOVA, Tukey’s HSD, $p$-value< 0.05).

§Significantly different from CI – realistic inhalation (Student’s $t$-test, $p$-value< 0.05).
Table 5.4  Particle size characteristics of emitted aerosols measured using CI and LD methods \((n = 3)\).

<table>
<thead>
<tr>
<th></th>
<th>MMAD, µm CI - 45 L/min</th>
<th>MMAD, µm CI – realistic inhalation</th>
<th>Dv50, µm LD – realistic inhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1</td>
<td>1.53 (0.01)</td>
<td>1.43 (0.04)</td>
<td>1.66 (0.04)</td>
</tr>
<tr>
<td>Batch 2</td>
<td>1.41 (0.01)</td>
<td>1.25* (0.04)</td>
<td>1.58 (0.01)</td>
</tr>
<tr>
<td>Batch 3</td>
<td>1.51 (0.10)</td>
<td>1.39 (0.05)</td>
<td>1.55 (0.05)</td>
</tr>
<tr>
<td>Batch 4</td>
<td>1.53 (0.03)</td>
<td>1.37 (0.02)</td>
<td>1.74* (0.08)</td>
</tr>
</tbody>
</table>

*Significantly different from batch 1, 3, and 4 (One-way ANOVA, Tukey’s HSD, \(p\)-value< 0.05).

Significantly different from batch 2 and 3 (One-way ANOVA, Tukey’s HSD, \(p\)-value< 0.05).

MMAD=mass median aerodynamic diameter, Dv50=volume diameter at the 50th percentile

5.3.3  Rapid screening of Tobramycin EEG spray-dried powders using a novel positive pressure dry powder inhaler using laser diffraction method and comparing with cascade impaction method

Tobramycin EEG powder formulations evaluated in Chapter 4 were analyzed using the Spraytec laser diffraction technique in the open system. Powders were aerosolized using the DAC v3 dry powder inhaler, actuated as reported in Chapter 4. No quantitative measurements were conducted to determine device retention, emitted dose, aerodynamic size and fine particle fractions. All measurements reported in Table 5.5 were laser diffraction based. Powders were compared using one-way ANOVA statistical analysis.

Table 5.5 shows the Dv50, fine particle fractions smaller than 5 and 1 µm, and the concentration volume. The Dv50 ranged from a mean of 1.2 (0.1) µm to 2.9 (0.8) µm. The tobramycin EEG powder formulation T2 containing trileucine resulted in the largest Dv50, while the powders containing the largest amount of tobramycin resulted in the two smallest Dv50. No difference was observed in the concentration volume across all powders. However, it was observed that all tobramycin EEG powders had a larger percentage of respirable particles compared to T2, and T4 resulted in the highest %FPF<1 µm.
Table 5.5 Mean (SD) particle size characteristics and concentration volume of the emitted aerosols from the tobramycin EEG formulations measured using the laser diffraction method (n = 3).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Dv50, µm</th>
<th>%FPF&lt;5, µm</th>
<th>%FPF&lt;1, µm</th>
<th>Cv, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>2.0 (0.3)</td>
<td>80.5* (5.4)</td>
<td>27.8§ (4.2)</td>
<td>25.1 (8.3)</td>
</tr>
<tr>
<td>T2</td>
<td>2.9 (0.8)</td>
<td>65.2 (7.0)</td>
<td>21.4§ (4.1)</td>
<td>15.9 (3.3)</td>
</tr>
<tr>
<td>T3</td>
<td>1.9 (0.2)</td>
<td>83.0* (2.4)</td>
<td>29.1§ (3.5)</td>
<td>19.3 (2.3)</td>
</tr>
<tr>
<td>T4</td>
<td>1.2* (0.1)</td>
<td>96.1* # (2.4)</td>
<td>44.2 (4.9)</td>
<td>12.0 (2.2)</td>
</tr>
<tr>
<td>T5</td>
<td>1.7* (0.3)</td>
<td>86.1* (5.9)</td>
<td>31.2§ (5.7)</td>
<td>21.5 (5.1)</td>
</tr>
</tbody>
</table>

*Significantly different from T2 (One-way ANOVA, Tukey’s HSD, p-value< 0.05). 
*Significantly different from T1 (One-way ANOVA, Tukey’s HSD, p-value< 0.05). 
§Significantly different from T4 (One-way ANOVA, Tukey’s HSD, p-value< 0.05). 
Dv50=volume diameter at the 50th percentile, MMAD=mass median aerodynamic diameter, FPF=fine particle fraction, Cv=volume concentration

Despite the differences observed from the overall laser diffraction analysis, the purpose of this study was to evaluate the relationship between the results obtained from the cascade impactor tests (Chapter 4) to the laser diffraction data shown in Table 5.5. Therefore, scatter plots reported in Figure 5.11, Figure 5.12, Figure 5.13, Figure 5.14 show the linear relationship between the concentration volume and the emitted dose, the Dv50 and the MMAD, the %FPF<5 and 1 µm. Given the large difference between emitted doses found from the tobramycin EEG powders when aerosolized using the DAC v3 inhaler during the CI – 45 L/min experiment, it was observed that the concentration volume resulted in a comparable trend, as shown in Figure 5.11. In fact, a linear relationship with an R² of 0.7324 was found between these two parameters. Conversely, despite the MMAD resulted larger as the Dv50 increased, no linear relationship was found between these two outcomes (Figure 5.12). A strong positive relationship was instead observed between the percentage of respirable particles determined using the laser diffraction and the cascade impactor method at 45 L/min, as demonstrated by the R² of 0.8561 in Figure 5.13. Similarly, the percentage of submicron particles obtained from the LD and CI – 45 L/min methods was following the same trend (R² of 0.7096), as found in Figure 5.14.
Figure 5.11 Linear relationship between the volume concentration obtained from the laser diffraction and the emitted dose obtained from the cascade impactor. Values are reported as means \((n = 3)\).

Figure 5.12 Linear relationship between the Dv50 obtained from the laser diffraction and the MMAD obtained from the cascade impactor. Values are reported as means \((n = 3)\).
Figure 5.13 Linear relationship between the percentages of fine particle fraction smaller than 5 \( \mu \text{m} \) obtained from the laser diffraction and the cascade impactor. Values are reported as means \((n = 3)\).

Figure 5.14 Linear relationship between the percentages of fine particle fraction smaller than 1 \( \mu \text{m} \) obtained from the laser diffraction and the cascade impactor. Values are reported as means \((n = 3)\).
5.4 Discussion and conclusions

In vitro aerodynamic particle size distribution is usually evaluated using impactors and impingers instruments as established by pharmacopeial guidance. Impaction methods are also employed during the product development phase of inhalation aerosol – device combinations, despite the undoubtedly laborious and time consuming effort required. This arises the need for a rapid screening method that could support and guide the early stage development of formulation – device combinations. The suitability of Malvern Spraytec as laser diffraction method was therefore evaluated to rapidly screening and characterize the aerosol performance of EEG spray-dried powders. Active and passive dry powder inhalers as well as steady flow rate and realistic breathing profile were used to aerosolize the EEG powders. Albuterol sulfate and budesonide EEG powders, delivered by the breath actuated and capsule-based CC90-3D DPI, were evaluated for the dynamic emitted aerosol characteristics when aerosolized using different capsule air inlet aperture positions. The results obtained from the laser diffraction analysis were compared with realistic in vitro mouth-throat model characterization using the same DPI and AIPs. Albuterol sulfate EEG powders were evaluated for their batch to batch reproducibility using laser diffraction method coupled with realistic breathing profile and then compared to cascade impactor methods employed with steady flow rate as well as realistic inhalation profile. Finally, the relationship between laser diffraction characterization and steady-flow cascade impaction data was conducted for a series of tobramycin EEG powders delivered using a novel positive pressure DPI.

Laser diffraction testing showed that while the BUD EEG volumetric diameter (Dv50) was not influenced by the AIP used, a smaller Dv50 of 1.2 µm was found when aerosolizing the AS EEG powder using the AIP 1 compared to 1.5 µm when using the AIP 2, as dynamically shown in the highly variable Dv50 - time history profile for this powder – AIP. The ineffective AS EEG powder aerosolization using the AIP 2 was further confirmed from the large Dv90 of 38.2 µm and from the low percentage of particles smaller than 5 and 1 µm of 81.2% and 31.7%, compared to the corresponding values obtained for the other powders. Capsule retention and filter deposition obtained from the laser diffraction experiment were compared with
realistic *in vitro* mouth-throat model to evaluate the validity of Spraytec results. Despite unchanged Dv50 values, BUD EEG resulted in a more variable time – history profile when aerosolized with AIP 2 which was reflected by a higher capsule retention (23.3%) and a lower filter deposition (59.8%) compared to the aerosolization using AIP 1. This finding was confirmed by the MT experiment as no difference was found between the two methods. AS EEG aerosolized with AIP 2 had low capsule retention (6.1±0.2 %) and slightly reduced *in vitro* lung deposition (63.1 %) compared to AIP 1 (73.5 %). Despite differences between laser diffraction and MT results were found for AS EEG due to a larger MT deposition compared to the BUD EEG powders, the two methods agreed on determining the optimized AIP required to aerosolize this powder.

An additional set of experiments was conducted using the Spraytec inhalation cell system to evaluate the batch to batch reproducibility of four AS EEG spray-dried replicates, characterized with laser diffraction and cascade impaction methods. Each testing methods confirmed the reproducibility of the AS EEG powder in terms of %FPF<5 µm, as no differences were found when quantifying the fraction of emitted drug in the impactor and only small differences were observed when using Spraytec. Conversely, higher variability was observed for the %FPF< 1 µm in the characterization methods using a realistic breathing profile. It was also relevant to observed that despite the significant differences found when comparing the respirable and submicron fraction for the same batch using the LD and CI realistic methods, there was virtually no difference between the results obtained for these methods. As expected by the different nature of the measurement, Dv50 and MMAD were found to result in ~0.2 µm difference between the LD and CI methods.

Finally, a series of tobramycin EEG powders aerosolized by an active DPI were characterized in the Spraytec open configuration system and linear relationships were evaluated with respect to steady flow cascade impactor measurements. Interestingly, it was found that the volume concentration parameter obtained from the laser diffraction technique had a significant linear relationship with the emitted dose found from the CI experiment. Additionally, respirable and submicron fractions were also found to correlate
between the two characterization methods. Also, as a larger Dv50 corresponded to a larger aerodynamic particle size, despite lack of linear relationship between MMAD and Dv50 was observed.

In conclusion, despite the inability of laser diffraction methods to characterize the aerodynamic size distribution of inhalation powders, this study suggests the suitability of Spraytec as rapid screening method to evaluate the effect of air inlet aperture position, the batch to batch reproducibility and the aerosol performance of a selection of EEG powders. The usefulness of the laser diffraction characterization was shown to be especially relevant when characterizing EEG powders using a realistic breathing profile, as expressed by the different results obtained in the batch to batch study between the cascade impactor data obtained using steady and variable flow. Reduced testing times and therefore higher throughput are a major advantage during the development of pharmaceutical aerosols.
CHAPTER 6: DEVELOPMENT AND VALIDATION OF PHARMACOKINETIC MODEL TO PREDICT THE SYSTEMIC EXPOSURE OF PEDIATRIC CYSTIC FIBROSIS SUBJECTS

6.1 Introduction

This chapter describes the development and application of a pharmacokinetic model used to predict systemic exposure after tobramycin inhalation powder administration to pediatric cystic fibrosis subjects. The developed model will be employed to estimate systemic exposure in pediatric cystic fibrosis patients following inhalation of the novel tobramycin EEG formulation administered using the positive pressure dry powder inhaler. The model will evaluate changes in systemic exposure as a function of dose, patient age, body weight and changes in pulmonary bioavailability.

6.2 Methods

6.2.1 PK study selection

Acceptable and available in vivo PK literature of tobramycin administered to pediatric CF population by both IV and INH routes were collected. Studies were screened and identified based on the following criteria: (1) population age (pediatric when <18 years old, adult when ≥18 years old), size, disease state, body weight or body surface area; (2) dose and dosing regimen; (3) reported mean or individual patient plasma concentration time profile (C_p(t)) or (4) reported plasma PK exposure metrics; (5) reported mean or individual patient sputum concentration time profile (C_s(t)) or (6) reported sputum PK exposure metrics; (7) appropriate analytical method used to quantify tobramycin content and respective lower limit of quantification (LLOQ); (8) appropriate PK sampling schedule. Given the limited availability of pediatric CF inhalation studies, the amount of tobramycin deposited in the lung (DtL) was evaluated in vitro using realistic airway models.
Pediatric CF subjects were compared to adult CF subjects. Relevant *in vivo* PK literature of tobramycin delivered to adult CF population administered by both IV and INH routes previously used to develop and validate the semi-PBPK model for adults were selected (Hanna, 2018). Studies were screened based on the same criteria reported above. Only the studies reporting the same inhalation device used for the pediatric subject studies were included. For the adult CF inhalation studies, the *in vivo* determined DtL was measured by gamma scintigraphy and it was reported, when available. When unavailable, DtL was estimated from reference studies conducted using the same inhalation device in adult subjects of comparable age range.

**6.2.2 Body weight imputation and Non-compartmental PK analysis**

When unavailable, body weight was imputed using CFF report (2017 Patient Registry Annual Data Report, 2017) and the CDC growth charts (Growth Charts - Clinical Growth Charts, 2019). When body surface area was reported, imputation was conducted by Du Bois formula using the CFF report (2017 Patient Registry Annual Data Report, 2017) and the CDC growth charts (Growth Charts - Clinical Growth Charts, 2019) for pediatric studies. When unknown, gender distribution was assumed. If the gender ratio was provided, then body weight or body surface area were imputed based on that.

Digitization of \( C_p(t) \) and \( C_s(t) \) was performed using GetData© Graph Digitizer Version 2.26 software. The mean concentration values per time obtained from the digitization were then evaluated by non-compartmental PK analysis (NCA) on MS Excel, Analysis ToolPak add-in.

**6.2.2.1 Non-compartmental analysis: IV administration**

Non-compartmental analysis was conducted on available \( C_p(t) \) after IV administration. Log-linear regression was performed on the slope of the terminal phase (\( \lambda \)) of the plasma concentration vs. time profiles to estimate the area under the curve (\( \text{AUC}_{0-t} \)) by the trapezoidal rule. Extrapolated AUC from last point to infinity (\( \text{AUC}_{t-\infty} \)) was calculated by dividing the last measured concentration by \( \lambda \), and the total area under
the curve (AUC$_{\infty}$ or AUC$^\text{IV}$) was obtained as the sum of AUC$_{0,t}$ and AUC$_{t,\infty}$. NCA was accepted based on visual inspection of the log-linear regression performed on the terminal points of the Cp(t). CL$_{TOT}$ was obtained from the administered dose divided by AUC$^\text{IV}$, as reported by the following equation:

$$\text{CL}_{\text{TOT}} = \frac{\text{Dose}^{\text{IV}}}{\text{AUC}^{\text{IV}}}$$  \hspace{1cm} (Equation 6.1)

Vd$_{ss}$ was calculated as CL$_{TOT}$ multiplied by the systemic mean residence time. For pediatric studies, PK dose proportionality was evaluated by AUC vs. dose (mg and mg/kg) plots. Regression analysis was conducted on age and BW vs. CL$_{TOT}$ and Vd$_{ss}$.

### 6.2.2.2 Non-compartmental analysis: INH administration

The bioavailability after inhalation ($F_{\text{INH}}$) was calculated as shown in Equation 6.2, with the dose administered in the inhalation studies (Dose$^{\text{INH}}$). The AUC$_{0,t}$ and AUC$_{t,\infty}$, obtained from the NCA PK analysis from INH studies was used to calculate the AUC$_{\text{INH}}$ and the CL$_{TOT}$ calculated as shown in Equation 6.1:

$$F_{\text{INH}} = \frac{\text{AUC}_{\text{INH}} \cdot \text{CL}_{\text{TOT}}}{\text{Dose}^{\text{INH}}}$$  \hspace{1cm} (Equation 6.2)

Dose$^{\text{INH}}$ was replaced by DtL to calculate the pulmonary bioavailability ($F_{\text{PUL}}$) as following:

$$F_{\text{PUL}} = \frac{\text{AUC}_{\text{INH}} \cdot \text{CL}_{\text{TOT}}}{\text{DtL}}$$  \hspace{1cm} (Equation 6.3)

The relationship between $F_{\text{INH}}$ and $F_{\text{PUL}}$ is obtained by the following equation:

$$F_{\text{INH}} = \text{DtL} \cdot F_{\text{PUL}}$$  \hspace{1cm} (Equation 6.4)
DtL was used to calculate $F_{\text{PUL}}$ in order to account for the actual amount of tobramycin deposited in the lungs. In contrast, $\text{Dose}^{\text{INH}}$ represents the dose loaded in the inhalation device, without considering the actual amount of drug reaching the lungs. The DtL, conversely, accounts for inhalation device efficiency and the potential drug losses due to exhaled or device retained aerosol fractions. Furthermore, the systemic PK contribution for the fraction of the drug depositing in the mouth-throat region and then reaching the gastrointestinal site was considered negligible due to the low absorption of the drug, as previously described.

6.2.2.3 Non-compartmental analysis acceptance criteria

Several criteria were considered when evaluating NCA: (1) visual inspection on the log-linear regression performed on the terminal points of $C_p(t)$ and conducted to obtain the slope of the terminal phase ($\lambda$), was performed to ensure that concentrations were evenly distributed above and below the regression line; (2) a minimum of 3 data points were used to perform the log-linear regression; (3) concentrations below the LLOQ were not included in the terminal slope calculation (4) the coefficient of determination ($R^2$) obtained from the log-linear regression analysis was greater than 0.7; (5) preferably, $\text{AUC}_{\infty}$ was taken into consideration if the extrapolated AUC resulted to be <20% of the total AUC. However, both $\text{AUC}_{0-t}$ and $\text{AUC}_{\infty}$ were compared and further used to obtain respective $F_{\text{INH}}$ and $F_{\text{PUL}}$.

Confidence in the NCA was obtained when the analysis results were in agreement with reported literature PK exposure metrics. The studies and each respective subgroups were individually evaluated and, if acceptable, summary statistics was used to establish relevant point estimates for each exposure metric. Mean, standard deviation, coefficient of variation (COV), median, max, min, and fold change were calculated. Summary statistics were obtained for each exposure metric for further exposure predictions or comparisons. Mean and SD were preferred. Median and ranges were considered when COV was <50%.
In vitro DtL determination for nebulization of tobramycin in pediatric subjects

An assessment of the DtL for pediatric subjects was conducted in vitro using the current standard of care tobramycin nebulization delivery for the treatment of *Pseudomonas aeruginosa* infection in pediatric patients with cystic fibrosis. These studies were performed with the PARI-LC® PLUS Reusable Nebulizer (Pari, Richmond, VA) with a DeVilbiss Pulmo-aide compressor loaded with 5 mL solution for inhalation containing 300 mg of Tobramycin (TOBI® Cardinal Health, Inc., Greensboro, NC).

The experimental setup is shown on Figure 6.1. Compressed air was supplied to the jet nebulizer at a flow rate of 6 L/min and the aerosol was delivered to the realistic airway model from the mouthpiece. A PulmoGuard II™ filter (Queset Medical, Brockton, MA) was positioned above the mouthpiece one-way valve of the breath enhanced nebulizer to collect the exhaled aerosol fraction. The PARI-LC® PLUS mouthpiece was connected to a mouthpiece adapter made from a RTV (room temperature vulcanizing) silicone mold rubber (Micro-mark Ten-to-One, Berkeley Heights, NJ). The aerosol was aerosolized into the same pediatric realistic mouth-throat model used in Section 4.2.3.4, that was pre-wetted with humidified air to mimic the realistic humid airway environment. The nebulized aerosol penetrating the mouth-throat model was collected on an inhalation PulmoGuard II™ filter positioned at the exit of the airway model. A realistic breathing profile was generated using the Dual Adult TTL (Michigan Instruments, Grand Rapids, Michigan). The breathing cycle was characterized by a 155 mL tidal volume, 25 breath cycles/min, and an inhalation:exhalation ratio of 1:2, based on USP <1601> for pediatric breath enhanced nebulizer testing. A compliance of 10 L/cm H$_2$O was used with an airway resistance of 50 cm H$_2$O/L/s.

After 5 min aerosolization, tobramycin deposited in the inhalation filter, exhalation filter, mouth throat model, mouthpiece adaptor, and Pari mouthpiece was quantified using a validated HPLC method (Hassan et al., 2020). The solution remaining in the nebulizer was quantified gravimetrically, and this value was used to calculate the dose of tobramycin remaining in the nebulizer based on the nominal dose of 60 mg/mL. In a separate experiment, the time to nebulize the solution to sputtering was determined. The mean time
was found to be 15 minutes. The results obtained were normalized to a 15 minute delivery time for use in the PK analysis. All results are reported as a percentage of the nominal dose.

![Diagram of test setup for estimating Dtl](image)

**Figure 6.1 In vitro test setup to estimate Dtl using a realistic airway model and the PARI-LC® PLUS nebulizer.**

### 6.2.4 Determination of AUC∞<sub>IV</sub> and AUC∞<sub>INH</sub> following administration to pediatric subjects

#### 6.2.4.1 Determination of AUC<sub>∞</sub><sup>IV</sup> following IV administration of tobramycin in pediatric subjects

The pharmacokinetic exposure for a range of administered IV doses was predicted based on the data obtained from all the pediatric and adult IV studies and each exposure metric is presented in a summary statistics table. Using the assumption of normal distribution, mean values were used to make these predictions. In the case of non-normal distribution, the median values were used. The target IV dose range was selected based on literature reported doses.

Prediction of AUC<sub>∞</sub><sup>IV</sup> was calculated by rearranging Equation 6.1 as follows:
\[
\text{AUC}_{\infty}^{IV} = \frac{\text{Dose}^{IV}}{\text{CL}_{\text{TOT}}}
\]

Nominal dose (mg) and BW-normalized nominal dose (mg/kg) predictions were calculated and reported. When using the nominal dose (mg), CL_{TOT} (mL/min) was used. Conversely, when using the BW-normalized nominal dose (mg/kg), BW-normalized CL_{TOT} (mL/min/kg) was used.

6.2.5 Determination of AUC_{\infty}^{INH} following nebulized administration of tobramycin in pediatric subjects

The pharmacokinetic exposure for a range of inhaled doses was predicted based on the data obtained from all IV and INH pediatric and adult studies and each exposure metric was presented in a summary statistics table. As with the IV predictions, when using an assumption of normal distribution, mean values were used to make predictions. In the case of non-normal distribution, the median was considered. Pulmonary exposures were investigated using both Dose^{INH} and DtL. The inhalation dose was evaluated using doses up to the standard of care dose of 300 mg, while the DtL was set to 40% of the nominal dose.

Prediction of AUC_{\infty}^{INH} using the nominal dose loaded in the device and the respective F_{INH} for pediatric and adult CF subjects, was calculated by rearranging Equation 6.2 as follows:

\[
\text{AUC}_{\infty}^{INH} = \frac{F_{INH} \cdot \text{Dose}^{INH}}{\text{CL}_{TOT}}
\]

Prediction of AUC_{\infty}^{INH} using the DtL and the respective F_{PUL} for pediatric and adult CF subjects, was calculated by rearranging Equation 6.3 as follows:

\[
\text{AUC}_{\infty}^{INH} = \frac{F_{PUL} \cdot \text{DtL}}{\text{CL}_{TOT}}
\]
Dose\textsuperscript{INH} and DtL predictions were reported in mg and mg/kg. When the dose is expressed in mg, CL\textsubscript{TOT} (mL/min) was used. Conversely, for the BW-normalized dose (mg/kg), BW-normalized CL\textsubscript{TOT} (mL/min/kg) was used.

6.2.6 Prediction of estimated local and systemic exposure for tobramycin excipient enhanced growth formulation in the novel positive air gas source dry powder inhaler in pediatric subjects

Using the systemic exposures determined in the IV and INH pediatric studies, estimates of the local and systemic exposure for the novel positive air gas source dry powder inhaler, DAC v3, when delivering a tobramycin EEG spray-dried powder were made. Predicted F\textsubscript{INH} was calculated with Equation 6.4, using the \textit{in vitro} determined DtL obtained by realistic deposition testing and the F\textsubscript{PUL} obtained from the IV and INH NCA for the standard of care nebulizer device. In addition, estimated exposures were obtained using 2-fold and 4-fold higher F\textsubscript{PUL}. Estimated AUC\textsubscript{\infty}\textsuperscript{INH} was calculated rearranging Equation 6.2 as follows:

\[
\text{AUC}_{\infty}^{\text{INH}} = \frac{\text{Predicted } F_{\text{INH}} \times \text{Dose}_{\text{INH}}}{\text{CL}_{\text{TOT}}}
\]

Estimate for AUC\textsubscript{\infty}\textsuperscript{INH} were reported when varying the nominal dose (mg) and BW-normalized nominal dose (mg/kg) as a surrogate for patient age. When using the nominal dose (mg), CL\textsubscript{TOT} (mL/min) was used. Similarly, when using the BW-normalized nominal dose (mg/kg), BW-normalized CL\textsubscript{TOT} (mL/min/kg) was used. A sensitivity analysis was performed to estimate systemic exposure when pulmonary bioavailability were varied. These estimates of systemic exposure will be used to suggest nominal doses of the EEG formulation to be employed for delivery to pediatric patients in the age range of 2-12 years old.
6.3 Results

6.3.1 *In vitro* DtL determination for nebulization of tobramycin in pediatric subjects

The dose to lung was determined following nebulization of 300 mg of tobramycin in a 5ml nebulizer solution using the PARI-LC® PLUS Reusable Nebulizer with a DeVilbiss Pulmo-aide compressor. The study used a realistic *in vitro* pediatric airway model and a pediatric breathing profile to determine the dose to lung, as the drug deposited on the inhalation filter at the exit of the airway model. Table 6.1 shows the results for the *in vitro* study as percentage of the nominal dose loaded into the nebulizer. The emitted dose was determined to be 40.1 % of the nominal dose, with the remaining dose being retained in the nebulizer. Over 50% of the emitted dose was exhaled through the valve on the mouthpiece of the nebulizer, this accounted for 23.7% of the nominal dose. Reflecting the inhalation:exhalation ratio of 1:2, 14.9% of the nominal dose was delivered to the inhalation filter and was determined as the DtL for pediatric subjects and was used in the PK analysis. Mouth-throat deposition of this nebulized aerosol was surprisingly low (1.2%) in these pediatric testing.

**Table 6.1** Mean (SD) tobramycin deposition in realistic *in vitro* testing using the PARI-LC® PLUS Reusable nebulizer to determine the DtL.

<table>
<thead>
<tr>
<th>% Nominal dose</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emitted dose</td>
<td>40.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Exhaled fraction</td>
<td>23.7</td>
<td>1.9</td>
</tr>
<tr>
<td>MP loss</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>MT deposition</td>
<td>1.2</td>
<td>0.2</td>
</tr>
<tr>
<td>DtL</td>
<td>14.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

MP=mouthpiece, MT=mouth-throat, DtL=dose to lung
6.3.2 Summary and discussion of IV and INH PK study selection

6.3.2.1 Pediatric studies

An extensive literature search resulted in the identification of five pediatric IV studies (reported in Table 6.2) that were consistent with the established screening criteria. The pediatric age of interest ranged from 0.5 to <18 years in all studies. Studies PIV2, PIV3, PIV5 were considered for the NCA, as they reported Cp(t) profiles (Bragonier and Brown, 1998; Hoecker et al., 1978; Levy et al., 1982). However, the PK metrics in studies PIV1 and PIV4 were also evaluated (Arends and Pettit, 2018; Hsu et al., 1984). For all the studies, the population had a CF diagnosis or bacterial infection. For studies PIV1, PIV2 and PIV4, all subgroup ages were taken into consideration. Subgroup B in study PIV5 had a mean age of 16 years, therefore it was used despite the age range of 5-27 years. Study PIV5 was the only IV study reporting sputum information in a relatively young population age range.

The overall infused dose ranged from 0.9 mg/kg to 15 mg/kg. Of note, studies PIV1, PIV2, PIV5 had lower doses (0.9 mg/kg to 3.0 mg/kg) compared to studies PIV3 and PIV4 (8.0 mg/kg to 15 mg/kg). Studies PIV1, PIV2, and PIV 4 investigated population ages and dosing regimens. Study PIV1A ranged from 0.1-1 years old, while Study PIV1B ranged from 5-27 years. Study PIV2 was divided into 3 dosage subgroups and each of them had a younger (2-8 years) and an older (11-18) subgroup. Study PIV4 investigated 2 subgroups, ranging from less <1 years to 6 years. In most of the studies, tobramycin quantification was conducted by antibiotic activity assay, which is not considered a preferred analytical method due to its lack of sensitivity, but given the scarcity of relevant literature they were considered for use in this review. In summary, the PK exposure metrics reported in studies PIV1 and PIV4 PK were used for comparison purposes, while studies PIV2, PIV3, and PIV5 reported Cp(t) and were evaluated by NCA.

Except for study PIV4, no study reported body weight information. Therefore, body weight was imputed from CFF report (2017 Patient Registry Annual Data Report, 2017) based on the year in which each study was conducted, the subjects age, and the corresponding weight percentile (Figure 6.2). The CDC growth charts (Growth Charts - Clinical Growth Charts, 2019) for females and males were then used to obtain the
corresponding weight. Body surface area was imputed by Du Bois formula using the CFF report and the CDC growth charts.

Table 6.3 shows the single study that was identified for inhaled administration of tobramycin in pediatric CF subjects. Plasma (PINH-Plasma) and sputum (PINH-Sputum) information together with key study elements are shown in the table (Rosenfeld et al., 2001). This study was conducted in CF pediatric subjects with an average age of 3.6 years treated with a 300 mg tobramycin solution that was administered using PARI-LC® PLUS nebulizer and Pulmo-Aide Devilbiss compressor. Body weight was not reported for this study, therefore it was imputed as described in Section 6.2.2
Table 6.2 Summary of identified pediatric IV tobramycin pharmacokinetic studies. Tabulated key study design elements: number of subjects per study \((n)\), year of study, subject disease state (CF-Cystic Fibrosis, or other), subject age (years), body weight (kg), dose strength, analytical method (LLOQ if reported), sampling schedule, availability of \(C_p(t)\) profile, and the availability of \(C_s(t)\) profile.

<table>
<thead>
<tr>
<th>n</th>
<th>Year of the study</th>
<th>Subject Disease State</th>
<th>Age, years (range)</th>
<th>Body Weight, kg (mean, range)</th>
<th>Dose Strength (mean, range)</th>
<th>Assay Method (LLOQ)</th>
<th>PK Sampling, hours</th>
<th>Cp(t) Profile</th>
<th>Cs(t) Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIV1 (Hsu et al., 1984)</td>
<td>52</td>
<td>CF</td>
<td>A: 0.1-1, B: 5-27</td>
<td>×</td>
<td>A: 3 mg/kg</td>
<td>FPI</td>
<td>0.5, 2</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>PIV2 (Hoecker et al., 1978)</td>
<td>41</td>
<td>bacterial infection</td>
<td>A: 2-6, D: 12-18</td>
<td>×</td>
<td>A: 8 mg/kg q24h 30 min</td>
<td>radioenzymatic assay</td>
<td>0, 0.25, 0.5, 1, 2, 4</td>
<td>✓</td>
<td>×</td>
</tr>
<tr>
<td>PIV3 (Bragonier and Brown, 1998)</td>
<td>7</td>
<td>CF</td>
<td>A: 3, E: 11-18</td>
<td>×</td>
<td>A: 8 mg/kg q24h 60 min</td>
<td>FPI (&lt; 0.6 mg/L)</td>
<td>0.25, 0.5, 1, 3, 8, 12, 24</td>
<td>✓</td>
<td>×</td>
</tr>
<tr>
<td>PIV4 (Arends and Pettit, 2018)</td>
<td>23</td>
<td>CF</td>
<td>A: 1-3, B: 3-6</td>
<td>×</td>
<td>A: 12 (11.7-12) mg/kg</td>
<td>FPI</td>
<td>2, 6</td>
<td>×</td>
<td>✓</td>
</tr>
<tr>
<td>PIV5 (Levy et al., 1982)</td>
<td>10</td>
<td>CF</td>
<td>A: 8-21</td>
<td>×</td>
<td>2 mg/kg q8h 10 min</td>
<td>FPI</td>
<td>Serum: 1, 4, 8 Sputum: 1, 2, 4, 8</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Table 6.3 Summary of identified pediatric INH nebulizer pharmacokinetic study. Tabulated key study design elements: number of subjects per study (n), year of study, subject disease state (CF-Cystic Fibrosis, or other), subject age (years), body weight (kg), dose, analytical method (LLOQ if reported), inhalation device used, sampling schedule, availability of Cp(t) profile, availability of Cs(t) profile, and information about in vivo aerosol deposition.

<table>
<thead>
<tr>
<th></th>
<th>PINH-Plasma (Rosenfeld et al., 2001)</th>
<th>PINH-Sputum (Rosenfeld et al., 2001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Year of the study</td>
<td>1998-1999</td>
<td>1998-1999</td>
</tr>
<tr>
<td>Subject Disease State</td>
<td>CF</td>
<td>CF</td>
</tr>
<tr>
<td>Age, years</td>
<td>3.6 ± 1.6 (0.6-5.8)</td>
<td>3.3 (±1.7, 0.9-5.9)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>Dose, mg</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Assay Method (LLOQ)</td>
<td>Immunoassay (0.2 µg/mL)</td>
<td>HPLC (0.2 µg/mL)</td>
</tr>
<tr>
<td>Device</td>
<td>PARI-LC® PLUS nebulizer Pulmo-Aide Devilbiss compressor ~15 min</td>
<td>PARI-LC® PLUS nebulizer Pulmo-Aide Devilbiss compressor ~15 min</td>
</tr>
<tr>
<td>PK Sampling, hours</td>
<td>0, 0.5, 1, 2, 4</td>
<td>0.7</td>
</tr>
<tr>
<td>Cp(t) Profile</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Cs(t) Profile</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>Deposition</td>
<td>×</td>
<td>×</td>
</tr>
</tbody>
</table>

6.3.2.2 Adult studies

Table 6.4 shows the relevant IV adult study that was obtained from the literature and had been previously used for the developed and validated adult semi-PBPK model (Hanna, 2018). Study AIV (Amininamizani et al., 2002) was the IV study used to obtain relative bioavailability information (F\textsubscript{INH} and F\textsubscript{PUL}) in adult CF subjects. The mean age was 29 years old and the mean body weight was reported to be 55 kg. Study AIVA
evaluated the dose regimen of 3.3 mg/kg q8h over 30 min infusion, while AIVB 10 mg/kg q24h over 60 min infusion. Single subjects Cp(t) reported in study AIVA have been evaluated using NCA.

Table 6.5 shows the relevant adult INH studies. Studies AINH1, AINH2, and AINH3 were the three available studies conducted on CF adult subjects (≥18 years old) with the administration of tobramycin inhalation solution by the PARI-LC® PLUS nebulizer (Geller et al., 2007, 2003; Lenney et al., 2011). Studies AINH1 and AINH3 did not report any Cp(t), therefore PK exposure metrics were considered only for comparison. However, NCA was conducted on the Cp(t) mean data points available for study AINH2. Studies AINH1 and AINH3 did not provide any tobramycin lung deposition information. However, study AINH1 provided gamma scintigraphy in vivo measurement of tobramycin lung deposition. Given the similar age range and the fact that all studies included CF subjects, the same lung deposition estimate was used for studies AINH2 and AINH3.
Table 6.4 Summary of the identified adult IV tobramycin pharmacokinetic study. Tabulated key study design elements: number of subjects per study ($n$), year of study, subject disease state (CF-Cystic Fibrosis, or other), subject age (years), body weight (kg), dose strength, analytical method (LLOQ if reported), sampling schedule, availability of $C_p(t)$ profile, and the availability of $C_s(t)$ profile.

<table>
<thead>
<tr>
<th></th>
<th>AIVA (Aminimanizani et al., 2002)</th>
<th>AIVB (Aminimanizani et al., 2002)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Year of the study</td>
<td>2002</td>
<td>2002</td>
</tr>
<tr>
<td>Subject Disease State</td>
<td>CF</td>
<td>CF</td>
</tr>
<tr>
<td>Age, years</td>
<td>29.0±4.6 (23-34)</td>
<td>29.0±4.6 (23-34)</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>55.3±13.8 (45-75)</td>
<td>55.3±13.8 (45-75)</td>
</tr>
<tr>
<td>Dose Strength</td>
<td>3.3 mg/kg q8h 30 min</td>
<td>10 mg/kg q24h 60 min</td>
</tr>
<tr>
<td>Assay Method (LLOQ)</td>
<td>FPI (&lt; 0.1 mg/L)</td>
<td>FPI (&lt; 0.1 mg/L)</td>
</tr>
<tr>
<td>PK Sampling, hours</td>
<td>0, 0.2, 0.3, 0.5, 0.8, 1, 1.5, 2, 4, 7.5</td>
<td>0, 0.2, 0.3, 0.5, 0.8, 1, 1.5, 2, 4, 7.5</td>
</tr>
<tr>
<td>$C_p(t)$ Profile</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>$C_s(t)$ Profile</td>
<td>✗</td>
<td>✗</td>
</tr>
</tbody>
</table>
Table 6.5 Summary of identified adult INH nebulized tobramycin pharmacokinetic studies. Tabulated key study design elements: number of subjects per study (n), year of study, subject disease state (CF-Cystic Fibrosis, or other), subject age (years), body weight (kg), dose, analytical method (LLOQ if reported), inhalation device used, sampling schedule, availability of Cp(t) profile, availability of Cs(t) profile, and information about in vivo aerosol deposition.

<table>
<thead>
<tr>
<th></th>
<th>AINH1 (Lenney et al., 2011)</th>
<th>AINH2 (Geller et al., 2003)</th>
<th>AINH3 (Geller et al., 2007)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>49</td>
<td>20</td>
</tr>
<tr>
<td>Year of the study</td>
<td>2010</td>
<td>2003</td>
<td>2007</td>
</tr>
<tr>
<td>Subject Disease State</td>
<td>CF</td>
<td>CF</td>
<td>CF</td>
</tr>
<tr>
<td>Age, years</td>
<td>21</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>×</td>
<td>59</td>
<td>×</td>
</tr>
<tr>
<td>Dose, mg</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>PK Assay Method (LLOQ)</td>
<td>FPI</td>
<td>FPI (0.18 ug/mL)</td>
<td>FPI (0.05 ug/mL)</td>
</tr>
<tr>
<td>Device</td>
<td>PARI-LC® PLUS nebulizer</td>
<td>PARI-LC® PLUS nebulizer, Pulmo-Aide Devilbiss compressor</td>
<td>PARI-LC® PLUS nebulizer, Pulmo-Aide Devilbiss compressor</td>
</tr>
<tr>
<td>PK sampling, hours</td>
<td>0, 0.5, 1, 2, 4, 8</td>
<td>0, 0.2, 1, 2, 4, 8</td>
<td>0, 0.5, 1, 2, 4, 8, 12</td>
</tr>
<tr>
<td>Cp(t) Profile</td>
<td>×</td>
<td>✓</td>
<td>×</td>
</tr>
<tr>
<td>Cs(t) Profile</td>
<td>×</td>
<td>✓</td>
<td>×</td>
</tr>
<tr>
<td>Deposition</td>
<td>✓</td>
<td>×</td>
<td>×</td>
</tr>
</tbody>
</table>

6.3.3 Non-compartmental PK analysis for pediatric IV studies

Table 6.6 shows the PK metrics obtained from the reported parameters (RP) in studies PIV1 and PIV4 and the values estimated by NCA for studies PIV2, PIV3, and PIV5. NCA was performed for each subgroup of the three pediatric IV studies found. Visual inspection of the log-linear regression analysis conducted on the terminal concentration vs. time data points used to estimate the terminal slope, were acceptable. Additionally, R² was greater than 0.94 in each of the analyses.
There was a 6-fold and a 3-fold inter-subgroup variability observed in CL_{TOT} and V_{dss}, respectively. Values ranged from 28.4-128.5 mL/min for CL_{TOT} and 6.32 – 18.47 L for V_{dss}. However, both exposure metrics were comparable once normalized per body weight. The extrapolated area obtained when calculating AUCs from each subgroup in studies PIV3 and PIV5 did not exceed 5% of the total area. However, the extrapolated AUC area for all subgroups in study PIV2 was above 20%, except PIV2F (15%). Therefore, both AUC_{0t} and AUC_{\infty} will be used for further comparisons. In studies PIV1, PIV2, and PIV5, the reported values and NCA resulted in AUC_{\infty}’s ranging from 540-1228 mg*min/L, when the lower dose of tobramycin was administered. This was in contrast to the higher doses used in studies PIV3 and PIV4 in which the AUC_{\infty} ranged from 4387-9907 mg*min/L. There was a similar trend for t_{1/2}, which ranged from 77.9 min to 116.9 min for the lower tobramycin dose, compared to more than 300 min for the higher dose studies. C_{max} and t_{max} were within 3.0-4.4 mg/L and 30-60 min, respectively for the lower dose studies, while higher values for C_{max} and shorter t_{max} were observed for the higher dose studies. Exposure metrics obtained from NCA were in agreement with reported values from the literature (Bragonier and Brown, 1998; Hoecker et al., 1978).

Summary statistics was generated using all 13 subgroups from the 5 IV pediatric studies, as shown on Table 6.7. Mean, standard deviation (SD), coefficient of variation (COV), median, max, min, and fold change for each exposure metrics were calculated. A non-normal distribution was found for C_{max}, t_{max}, CL_{TOT} and t_{1/2}, given the COV >50%, the fold-change obtained from max and min, the >20% variability seen between mean and median and the difference observed between min and max with respect to the mean, which suggested a right-tailed distribution. Therefore, C_{max}, t_{max}, CL_{TOT}, and t_{1/2} were expressed as median and min-max for further comparisons. The large variability observed in AUCs was due to the different dosing regimens used, therefore median and min-max values were also used for further comparison. Using the same criteria, a normal distribution can be assumed in the case of BW-normalized CL_{TOT}, and V_{dss}, therefore mean±SD was used for these exposure metrics.
Table 6.6 Pharmacokinetic NCA estimated and reported parameters (RP) for each of the individual data sets from the identified tobramycin pediatric IV studies. Tabulated PK metrics: number of subjects per subgroup \((n)\), total clearance (mL/min), body weight-normalized total clearance (mL/min/kg), volume of distribution at steady state (L), body weight-normalized volume of distribution at steady state (L/kg), AUC\(\infty\) (mg*min/L), half-life (min), % extrapolated, obtained \(R^2\), subject age (years), body weight (kg), the PK analysis conducted, dose (mg and mg/kg).

<table>
<thead>
<tr>
<th></th>
<th>PIV1A</th>
<th>PIV1B</th>
<th>PIV2A</th>
<th>PIV2B</th>
<th>PIV2C</th>
<th>PIV2D</th>
<th>PIV2E</th>
<th>PIV2F</th>
<th>PIV3A</th>
<th>PIV3B</th>
<th>PIV4A</th>
<th>PIV4B</th>
<th>PIV5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>4</td>
<td>48</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>(C_{\text{max}}, \text{mg/L})</td>
<td>×</td>
<td>×</td>
<td>3.9</td>
<td>3.1</td>
<td>4.3</td>
<td>4.2</td>
<td>3.1</td>
<td>4.4</td>
<td>38.2</td>
<td>76.3</td>
<td>×</td>
<td>×</td>
<td>3.0</td>
</tr>
<tr>
<td>(t_{\text{max}}, \text{min})</td>
<td>×</td>
<td>×</td>
<td>30.0</td>
<td>60.0</td>
<td>30.0</td>
<td>30.0</td>
<td>60.0</td>
<td>60.0</td>
<td>14.7</td>
<td>15.2</td>
<td>×</td>
<td>×</td>
<td>71.7</td>
</tr>
<tr>
<td>(\text{CL}_{\text{TOT}}, \text{mL/min})</td>
<td>17.6</td>
<td>128.5</td>
<td>48.8</td>
<td>46.1</td>
<td>46.4</td>
<td>79.7</td>
<td>90.8</td>
<td>93.8</td>
<td>51.8</td>
<td>55.0</td>
<td>28.4</td>
<td>44.7</td>
<td>109.3</td>
</tr>
<tr>
<td>BW-normalized (\text{CL}_{\text{TOT}}, \text{mL/min*kg})</td>
<td>2.4</td>
<td>2.5</td>
<td>3.3</td>
<td>2.6</td>
<td>2.8</td>
<td>1.7</td>
<td>1.9</td>
<td>2.0</td>
<td>1.4</td>
<td>1.5</td>
<td>2.4</td>
<td>2.7</td>
<td>3.1</td>
</tr>
<tr>
<td>(\text{Vd}_{\text{ss}}) (L)</td>
<td>×</td>
<td>×</td>
<td>7.14</td>
<td>7.32</td>
<td>6.32</td>
<td>9.96</td>
<td>11.70</td>
<td>9.29</td>
<td>10.69</td>
<td>10.44</td>
<td>×</td>
<td>×</td>
<td>18.47</td>
</tr>
<tr>
<td>BW-normalized (\text{Vd}_{\text{ss}}, \text{L/kg})</td>
<td>×</td>
<td>×</td>
<td>0.48</td>
<td>0.41</td>
<td>0.38</td>
<td>0.21</td>
<td>0.25</td>
<td>0.20</td>
<td>0.29</td>
<td>0.29</td>
<td>×</td>
<td>×</td>
<td>0.52</td>
</tr>
<tr>
<td>(\text{AUC}_{0\rightarrow\infty}, \text{mg*min/L})</td>
<td>×</td>
<td>×</td>
<td>475</td>
<td>475</td>
<td>621</td>
<td>503</td>
<td>421</td>
<td>556</td>
<td>5492</td>
<td>9755</td>
<td>×</td>
<td>×</td>
<td>635</td>
</tr>
<tr>
<td>% Extrapolated</td>
<td>×</td>
<td>×</td>
<td>23</td>
<td>27</td>
<td>24</td>
<td>19</td>
<td>22</td>
<td>15</td>
<td>2</td>
<td>1</td>
<td>×</td>
<td>×</td>
<td>5</td>
</tr>
<tr>
<td>(\text{AUC}_{\infty}, \text{mg*min/L})</td>
<td>1228</td>
<td>808</td>
<td>619</td>
<td>655</td>
<td>813</td>
<td>615</td>
<td>540</td>
<td>653</td>
<td>5595</td>
<td>9907</td>
<td>4959</td>
<td>4387</td>
<td>667</td>
</tr>
<tr>
<td>(t_{1/2}, \text{min})</td>
<td>×</td>
<td>×</td>
<td>111.3</td>
<td>116.9</td>
<td>107.4</td>
<td>96.8</td>
<td>98.1</td>
<td>77.9</td>
<td>348.5</td>
<td>335.2</td>
<td>×</td>
<td>×</td>
<td>103.2</td>
</tr>
<tr>
<td>(R^2)</td>
<td>×</td>
<td>×</td>
<td>0.98</td>
<td>1.00</td>
<td>0.99</td>
<td>1.00</td>
<td>0.97</td>
<td>0.98</td>
<td>0.94</td>
<td>0.95</td>
<td>×</td>
<td>×</td>
<td>0.99</td>
</tr>
<tr>
<td>Age, years</td>
<td>0.5</td>
<td>16</td>
<td>4</td>
<td>5.5</td>
<td>5</td>
<td>15</td>
<td>14.5</td>
<td>14.5</td>
<td>12</td>
<td>12</td>
<td>2</td>
<td>4.5</td>
<td>12</td>
</tr>
<tr>
<td>Body Weight, kg</td>
<td>7.2</td>
<td>50.5</td>
<td>14.8</td>
<td>17.8</td>
<td>16.8</td>
<td>46.5</td>
<td>46.5</td>
<td>46.5</td>
<td>36.3</td>
<td>36.3</td>
<td>11.8</td>
<td>16.8</td>
<td>35.6</td>
</tr>
<tr>
<td>PK analysis</td>
<td>RP</td>
<td>RP</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
<td>RP</td>
<td>RP</td>
<td>NCA</td>
</tr>
<tr>
<td>Dose, mg</td>
<td>22</td>
<td>104</td>
<td>30</td>
<td>30</td>
<td>38</td>
<td>49</td>
<td>49</td>
<td>61</td>
<td>290</td>
<td>545</td>
<td>141</td>
<td>196</td>
<td>73</td>
</tr>
<tr>
<td>BW-normalized dose, mg/kg</td>
<td>3.0</td>
<td>2.1</td>
<td>2.0</td>
<td>1.7</td>
<td>2.3</td>
<td>1.1</td>
<td>0.9</td>
<td>1.1</td>
<td>8.0</td>
<td>15.0</td>
<td>12.0</td>
<td>11.7</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Table 6.7 Summary PK metrics statistics obtained from NCA estimates and reported parameters from the identified tobramycin pediatric IV studies (133 subjects). Tabulated mean, standard deviation (SD), coefficient of variation (COV, %), median, max and min, and fold change for CL\textsubscript{TOT} (mL/min), BW-normalized CL\textsubscript{TOT} (mL/min/kg), V\textsubscript{dss} (L), BW-normalized V\textsubscript{dss} (L/kg), AUC\text{0-t} (mg*min/L), AUC\text{∞} (mg*min/L), half-life (min), age (years), body weight (kg), and dose (in mg and mg/kg). \text{*n=9}

<table>
<thead>
<tr>
<th>Metric</th>
<th>Mean</th>
<th>SD</th>
<th>COV (%)</th>
<th>Median</th>
<th>Max</th>
<th>Min</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{max}, mg/L\text{*}</td>
<td>15.6</td>
<td>25.5</td>
<td>163</td>
<td>4.2</td>
<td>76.3</td>
<td>3.0</td>
<td>25.0</td>
</tr>
<tr>
<td>t\textsubscript{max}, min\text{*}</td>
<td>41.3</td>
<td>21.7</td>
<td>52</td>
<td>30.0</td>
<td>71.7</td>
<td>14.7</td>
<td>4.9</td>
</tr>
<tr>
<td>CL\textsubscript{TOT}, mL/min</td>
<td>64.7</td>
<td>32.8</td>
<td>51</td>
<td>51.8</td>
<td>128.5</td>
<td>17.6</td>
<td>7.3</td>
</tr>
<tr>
<td>BW-normalized CL\textsubscript{TOT}, mL/min/kg</td>
<td>2.3</td>
<td>0.6</td>
<td>25</td>
<td>2.4</td>
<td>3.3</td>
<td>1.4</td>
<td>2.3</td>
</tr>
<tr>
<td>V\textsubscript{dss}, L\text{*}</td>
<td>10.1</td>
<td>3.6</td>
<td>36</td>
<td>10.0</td>
<td>18.5</td>
<td>6.3</td>
<td>2.92</td>
</tr>
<tr>
<td>BW-normalized V\textsubscript{dss}, L/kg\text{*}</td>
<td>0.3</td>
<td>0.1</td>
<td>34</td>
<td>0.3</td>
<td>0.5</td>
<td>0.2</td>
<td>2.60</td>
</tr>
<tr>
<td>AUC\text{0-t}, mg<em>min/L\text{</em>}</td>
<td>2104</td>
<td>3307</td>
<td>157</td>
<td>556</td>
<td>9755</td>
<td>421</td>
<td>23</td>
</tr>
<tr>
<td>AUC\text{∞}, mg*min/L</td>
<td>2419</td>
<td>2921</td>
<td>121</td>
<td>808</td>
<td>9907</td>
<td>540</td>
<td>18</td>
</tr>
<tr>
<td>t\text{1/2}, min\text{*}</td>
<td>155.0</td>
<td>106.5</td>
<td>69</td>
<td>107.5</td>
<td>348.5</td>
<td>77.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Age, years</td>
<td>9.0</td>
<td>5.5</td>
<td>61</td>
<td>12.0</td>
<td>16.0</td>
<td>0.5</td>
<td>29.5</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>29.5</td>
<td>15.6</td>
<td>53</td>
<td>35.6</td>
<td>50.5</td>
<td>7.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Dose, mg</td>
<td>125.2</td>
<td>148.2</td>
<td>118</td>
<td>61.3</td>
<td>544.5</td>
<td>21.6</td>
<td>25</td>
</tr>
<tr>
<td>BW-normalized dose, mg/kg</td>
<td>4.8</td>
<td>5.0</td>
<td>103</td>
<td>2.1</td>
<td>15.0</td>
<td>0.9</td>
<td>17.4</td>
</tr>
</tbody>
</table>
6.3.4 Non-compartmental PK analysis for adult IV study

Table 6.8 shows the PK metrics obtained after NCA on individual subject Cp(t) from the adult IV study (AIVA) which were used to estimate bioavailability after inhalation administration. Visual inspection of the log-linear regression analysis conducted on the terminal concentration vs. time data points used to estimate the terminal slope, were acceptable. Additionally, the obtained $R^2$ values were larger than 0.87.

The $C_{\text{max}}$ and $t_{\text{max}}$ ranged from 10.4-29.7 mg/L and 29.3-40.8 min, respectively. Despite some inter-subject variability in $\text{CL}_{\text{TOT}}$ and $\text{Vd}_{\text{ss}}$, both exposure metrics were comparable between subjects once normalized per body weight. The extrapolated areas for the AUCs obtained from each subject did not exceed 11%. However, both $AUC_0$ and $AUC_{\infty}$ will be considered to obtain the calculated bioavailabilities. The observed $t_{\frac{1}{2}}$ ranged from 118.6 min to 167.2 min.
Table 6.8. Pharmacokinetic NCA estimated and reported parameters (RP) for each of the individual data sets from the identified tobramycin adult IV study. Tabulated PK metrics: $C_{\text{max}}$ (mg/L), $t_{\text{max}}$ (min), total clearance (mL/min), body weight-normalized total clearance (mL/min/kg), volume of distribution at steady state (L), body weight-normalized volume of distribution at steady state (L/kg), $\text{AUC}_{0-t}$ (mg*min/L), % extrapolated, $\text{AUC}_{\infty}$ (mg*min/L), half-life (min), obtained $R^2$, the PK analysis conducted, dose (mg and mg/kg).

<table>
<thead>
<tr>
<th></th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
<th>Subject 5</th>
<th>Subject 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$, mg/L</td>
<td>14.1</td>
<td>9.7</td>
<td>10.4</td>
<td>29.7</td>
<td>16.0</td>
<td>13.9</td>
</tr>
<tr>
<td>$t_{\text{max}}$, min</td>
<td>40.8</td>
<td>40.6</td>
<td>39.6</td>
<td>32.8</td>
<td>35.1</td>
<td>29.3</td>
</tr>
<tr>
<td>$\text{CL}_{\text{TOT}}$, mL/min</td>
<td>91.0</td>
<td>131.7</td>
<td>107.2</td>
<td>68.9</td>
<td>128.2</td>
<td>82.1</td>
</tr>
<tr>
<td>BW $\text{CL}_{\text{TOT}}$, mL/min*kg</td>
<td>1.7</td>
<td>2.4</td>
<td>1.9</td>
<td>1.3</td>
<td>2.3</td>
<td>1.5</td>
</tr>
<tr>
<td>$V_{\text{dss}}$, L</td>
<td>14.7</td>
<td>23.2</td>
<td>22.7</td>
<td>8.8</td>
<td>17.5</td>
<td>13.6</td>
</tr>
<tr>
<td>BW $V_{\text{dss}}$, L/kg</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>$\text{AUC}_{0-t}$, mg*min/L</td>
<td>1926</td>
<td>1285</td>
<td>1542</td>
<td>2599</td>
<td>1290</td>
<td>2122</td>
</tr>
<tr>
<td>% Extrapolated</td>
<td>6</td>
<td>9</td>
<td>11</td>
<td>4</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>$\text{AUC}_{\infty}$, mg*min/L</td>
<td>2052</td>
<td>1417</td>
<td>1742</td>
<td>2709</td>
<td>1456</td>
<td>2273</td>
</tr>
<tr>
<td>$t_{\frac{1}{2}}$, min</td>
<td>127.5</td>
<td>136.0</td>
<td>167.2</td>
<td>108.1</td>
<td>118.6</td>
<td>126.8</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.99</td>
<td>0.87</td>
<td>0.94</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>PK analysis</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
<td>NCA</td>
</tr>
<tr>
<td>Dose, mg</td>
<td>186.7</td>
<td>186.7</td>
<td>186.7</td>
<td>186.7</td>
<td>186.7</td>
<td>186.7</td>
</tr>
<tr>
<td>Dose, mg/kg</td>
<td>3.39</td>
<td>3.39</td>
<td>3.39</td>
<td>3.39</td>
<td>3.39</td>
<td>3.39</td>
</tr>
</tbody>
</table>

Summary statistics was generated using all 6 subjects from the single IV adult study, as shown on Table 6.9. Mean, standard deviation (SD), coefficient of variation (COV), median, max, min, and fold change for each exposure metrics have been calculated. All estimated PK metrics resulted in a COV lower than 50%,
therefore a normal distribution is assumed and mean±SD will be used for further comparison. Results from the NCA on the single IV study in adult CF subjects were all in agreement with previously reported analysis in the literature (Geller et al., 2003; Hanna, 2018).

**Table 6.9.** Summary PK metrics statistics obtained from NCA estimates from 6 subjects in the adult IV study. Tabulated mean, standard deviation (SD), coefficient of variation (COV, %), median, max and min, fold range, and interquartile range for $C_{\text{max}}$ (mg/L), $t_{\text{max}}$ (min), total clearance (mL/min), body weight-normalized total clearance (mL/min/kg), volume of distribution at steady state (L), body weight-normalized volume of distribution at steady state (L/kg), $\text{AUC}_{0-t}$ (mg*min/L), $\text{AUC}_{\infty}$ (mg*min/L), half-life (min).

<table>
<thead>
<tr>
<th>Metric</th>
<th>Mean</th>
<th>SD</th>
<th>COV (%)</th>
<th>Median</th>
<th>Max</th>
<th>Min</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$, mg/L</td>
<td>15.6</td>
<td>7.3</td>
<td>47</td>
<td>14.0</td>
<td>29.7</td>
<td>9.7</td>
<td>3.1</td>
</tr>
<tr>
<td>$t_{\text{max}}$, min</td>
<td>36.3</td>
<td>4.7</td>
<td>13</td>
<td>37.4</td>
<td>40.8</td>
<td>29.3</td>
<td>1.39</td>
</tr>
<tr>
<td>$CL_{\text{TOT}}$, mL/min</td>
<td>101.5</td>
<td>25.3</td>
<td>25</td>
<td>99.1</td>
<td>131.7</td>
<td>68.9</td>
<td>1.91</td>
</tr>
<tr>
<td>BW $CL_{\text{TOT}}$, mL/min*kg</td>
<td>1.8</td>
<td>0.5</td>
<td>25</td>
<td>1.8</td>
<td>2.4</td>
<td>1.3</td>
<td>1.91</td>
</tr>
<tr>
<td>$Vd_{\text{ss}}$, L</td>
<td>16.7</td>
<td>5.6</td>
<td>33</td>
<td>16.1</td>
<td>23.2</td>
<td>8.8</td>
<td>2.63</td>
</tr>
<tr>
<td>BW $Vd_{\text{ss}}$, L/kg</td>
<td>0.3</td>
<td>0.1</td>
<td>33</td>
<td>0.3</td>
<td>0.4</td>
<td>0.2</td>
<td>2.63</td>
</tr>
<tr>
<td>$\text{AUC}_{0-t}$, mg*min/L</td>
<td>1794</td>
<td>519</td>
<td>29</td>
<td>1734</td>
<td>2599</td>
<td>1285</td>
<td>2.02</td>
</tr>
<tr>
<td>$\text{AUC}_{\infty}$, mg*min/L</td>
<td>1942</td>
<td>502</td>
<td>26</td>
<td>1897</td>
<td>2709</td>
<td>1417</td>
<td>1.91</td>
</tr>
<tr>
<td>$t_{1/2}$, min</td>
<td>130.7</td>
<td>20.2</td>
<td>15</td>
<td>127.2</td>
<td>167.2</td>
<td>108.1</td>
<td>1.55</td>
</tr>
</tbody>
</table>
6.3.5 Dose proportionality and effect of age and body weight on clearance and volume of distribution

Body weight was plotted against age as shown in Figure 6.3, there was a linear relationship observed for the IV CF pediatric studies. As expected, body weight increased with increasing age ($R^2 = 0.9899$) as reported in the literature (Mann et al., 1985). The value obtained in the adult IV study is also plotted for reference, but not included in the regression analysis. The reported adult body weight was lower than expected, but it was characterized by a relatively large variability, as expressed by the reported standard deviation. Given that most of the body weight values were imputed but all the age values were available from the respective literature studies, the observed BW-age proportionality allows the use of BW as a surrogate for age for future comparisons.

![Body weight vs. Age](image)

**Figure 6.3** Reported and imputed body weight (kg) vs age (years) proportionality for the 13 pediatric subgroups (blue circles) obtained from 5 IV studies and the single adult IV study (red diamond).

Total body clearance and BW-normalized clearance were expressed as a function of age and body weight. As expected by the evolving maturation process, $CL_{TOT}$ increased linearly as a function of age ($R^2 = 0.7347$), and therefore body weight ($R^2 = 0.7369$), as shown in Figure 6.4 and Figure 6.5 (left plots). However, no change when body weight normalized $CL_{TOT}$ across pediatric ages and different body weights was observed, as shown in Figure 6.4 and Figure 6.5 (right plots). The mean value obtained from the summary statistics
for all 13 subgroups of the 5 pediatric IV studies was reported as the dashed line in both BW-normalized CLTOT plots. An even distribution above and below the mean value was observed.

Figure 6.4 NCA and reported total clearance (left) and body weight normalized clearance (right) vs. age (years) for the 13 pediatric subgroups (blue circles) obtained from 5 tobramycin pediatric studies and the single adult IV study (red diamond). Dashed line on the right plot represents the mean value obtained from all IV pediatric studies.

Figure 6.5 NCA and reported total clearance (left) and body weight normalized clearance (right) vs. body weight for the 13 pediatric subgroups (blue circles) obtained from 5 tobramycin pediatric studies and the single adult IV study (red diamond). Dashed line on the right plot represents the mean value obtained from all IV pediatric studies.
Volume of distribution and BW-normalized volume of distribution were expressed as a function of age and body weight. Studies PIV1 and PIV4 did not report any Vd<sub>ss</sub> metrics, therefore only the Vd<sub>ss</sub> obtained from the NCA of studies PIV2, PIV3, and PIV5 were plotted. No relationship was observed when reporting Vd<sub>ss</sub> as a function of age (R<sup>2</sup> = 0.3026) and body weight (R<sup>2</sup> = 0.2481), as shown in Figure 6.7 and Figure 6.6 (left plots). Comparable values were seen when BW-normalized Vd<sub>ss</sub> was reported (right plots). The mean...
value obtained from the summary statistics for all 9 subgroups of the 3 IV pediatric studies was reported as dashed line in both BW-normalized Vdss plots.

Linear relationship was also observed between the AUC and dose (actual and BW normalized), as shown in the escalation graphs in Figure 6.8. Only pediatric AUC values were included in the linear regression analysis. No deviation from linearity was observed within a 25-fold dose range of IV tobramycin administration to pediatric subjects. R² values were 0.913 and 0.8892, respectively for plots vs dose (left plot) and BW normalized dose (right plot). The visual inspection of these plots confirmed dose proportionality. These results were in agreement with the previously developed adult model (Hanna, 2018).

![AUC vs. Dose](image1.png)\[y = 19.045x\quad R^2 = 0.913\]

![AUC vs. BW-normalized Dose](image2.png)\[y = 551.67x - 245.65\quad R^2 = 0.8892\]

**Figure 6.8** IV infusion dose escalation plots of AUC vs. dose of tobramycin infused (left plot) and body weight normalized dose (right plot) for the 13 pediatric subgroups (blue circles) obtained from 5 tobramycin pediatric studies and the single adult IV study (red diamond).

### 6.3.6 Non-compartmental PK analysis for pediatric INH nebulizer study

The NCA was conducted on the single CF pediatric study in which tobramycin was administered by the inhalation route using nebulization. Study PINH reported single subjects Cp(t) after nebulized administration of 300 mg tobramycin, therefore NCA was conducted on 14 subjects, as shown on Table 6.10. Two subjects were not included in the NCA given that only two sampling data point were available. Visual inspection of the log-linear regression analysis conducted on the terminal concentration vs. time data
points used to estimate the terminal slope was acceptable. Additionally, all R² values were greater than 0.9, except for subject 14 (R² = 0.5), who was excluded from the summary statistics reported in Table 6.12.

The Cmax varied from 0.30 mg/L to 1.20 mg/L, tmax ranged from 30.0 min to 120.8 min, and t1/2 substantially varied from 111.8 min to 455.9 min. The extrapolated areas of the AUCs obtained for each subject were in the range of 24-58%, which was greater than the established 20% criteria, therefore both AUC₀₄ and AUCₜ was considered to calculate bioavailability.

Table 6.11 reports F_INH and F_PUL obtained for each subject using Equations 6.2 and 6.3 and using the median IV pediatric CLTOT reported in Table 6.7. The estimated F_INH, calculated using AUC₀₄, ranged from 0.6 to 2.2%, which was similar to the F_INH calculated using AUCₜ (1.5-4.8%). As well as F_INH, F_PUL ranged from 4.4 to 15.0% when calculated using AUC₀₄, and 10.1 to 32.0% when using AUCₜ. Therefore, F_INH and F_PUL calculated from AUCₜ will be used for further comparisons and predictions.
Table 6.10 Pharmacokinetic NCA estimated parameters for 14 subjects in the single pediatric tobramycin nebulizer inhalation study. Tabulated PK metrics: $C_{\text{max}}$ (mg/L), $t_{\text{max}}$ (min), $AUC_{0-t}$ (mg*min/L), % extrapolated, $AUC_{\infty}$ (mg*min/L), half-life (min), $R^2$.

<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$, mg/L</td>
<td>1.20</td>
<td>0.90</td>
<td>1.00</td>
<td>0.80</td>
<td>0.70</td>
<td>0.80</td>
<td>0.70</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
<td>0.50</td>
<td>0.60</td>
<td>0.60</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>$t_{\text{max}}$, min</td>
<td>59.8</td>
<td>29.7</td>
<td>120.1</td>
<td>63.8</td>
<td>30.0</td>
<td>59.1</td>
<td>59.2</td>
<td>60.5</td>
<td>40.6</td>
<td>61.1</td>
<td>62.5</td>
<td>120.8</td>
<td>120.4</td>
<td>59.5</td>
<td></td>
</tr>
<tr>
<td>$AUC_{0-t}$, mg*min/L</td>
<td>201</td>
<td>165</td>
<td>190</td>
<td>142</td>
<td>113</td>
<td>147</td>
<td>131</td>
<td>103</td>
<td>101</td>
<td>102</td>
<td>88</td>
<td>111</td>
<td>109</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>% Extrapolated</td>
<td>44</td>
<td>34</td>
<td>56</td>
<td>28</td>
<td>41</td>
<td>57</td>
<td>32</td>
<td>24</td>
<td>28</td>
<td>43</td>
<td>56</td>
<td>41</td>
<td>71</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>$AUC_{\infty}$, mg*min/L</td>
<td>359</td>
<td>252</td>
<td>431</td>
<td>196</td>
<td>191</td>
<td>342</td>
<td>193</td>
<td>136</td>
<td>140</td>
<td>178</td>
<td>199</td>
<td>188</td>
<td>372</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>$t_{1/2}$, min</td>
<td>183.1</td>
<td>150.1</td>
<td>267.8</td>
<td>123.8</td>
<td>178.3</td>
<td>268.8</td>
<td>141.8</td>
<td>111.8</td>
<td>136.5</td>
<td>175.8</td>
<td>254.5</td>
<td>175.9</td>
<td>455.9</td>
<td>281.9</td>
<td></td>
</tr>
<tr>
<td>$R^2$</td>
<td>1.0</td>
<td>1.0</td>
<td>0.7</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>1.0</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>0.5</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>
Table 6.11 Estimated $F_{\text{INH}}$ and $F_{\text{PUL}}$ obtained from NCA for 14 subjects in the single pediatric tobramycin nebulizer inhalation study. Tabulated PK metrics: $F_{\text{INH}}$ (%) obtained using $\text{AUC}_{0\rightarrow t}$ and $\text{AUC}_{\infty}$, and $F_{\text{PUL}}$ (%) obtained using $\text{AUC}_{0\rightarrow t}$ and $\text{AUC}_{\infty}$.

<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{\text{INH}}$ ($\text{AUC}_{0\rightarrow t}$)</td>
<td>2.2</td>
<td>1.8</td>
<td>2.1</td>
<td>1.6</td>
<td>1.2</td>
<td>1.6</td>
<td>1.4</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.0</td>
<td>1.2</td>
<td>1.2</td>
<td>0.6</td>
</tr>
<tr>
<td>$F_{\text{INH}}$ ($\text{AUC}_{\infty}$)</td>
<td>4.0</td>
<td>2.8</td>
<td>4.8</td>
<td>2.2</td>
<td>2.1</td>
<td>3.8</td>
<td>2.1</td>
<td>1.5</td>
<td>1.6</td>
<td>2.0</td>
<td>2.2</td>
<td>2.1</td>
<td>4.1</td>
<td>1.6</td>
</tr>
<tr>
<td>$F_{\text{PUL}}$ ($\text{AUC}_{0\rightarrow t}$)</td>
<td>15.0</td>
<td>12.3</td>
<td>14.2</td>
<td>10.5</td>
<td>8.4</td>
<td>10.9</td>
<td>9.7</td>
<td>7.7</td>
<td>7.5</td>
<td>7.6</td>
<td>6.5</td>
<td>8.2</td>
<td>8.1</td>
<td>4.4</td>
</tr>
<tr>
<td>$F_{\text{PUL}}$ ($\text{AUC}_{\infty}$)</td>
<td>26.7</td>
<td>18.7</td>
<td>32.0</td>
<td>14.6</td>
<td>14.2</td>
<td>25.4</td>
<td>14.4</td>
<td>10.1</td>
<td>10.4</td>
<td>13.2</td>
<td>14.8</td>
<td>14.0</td>
<td>27.7</td>
<td>10.4</td>
</tr>
</tbody>
</table>
Table 6.12 reports the summary statistics obtained from individual subjects PK metrics shown in Table 6.10, excluding subject 14. For all exposure metrics, the coefficient of variation is <50%, and <10% variability was found between mean and median. Additionally, max and min results were equally spaced from the median and ~4 fold-change was obtained from max and min. Assumption of normal distribution can be done for these PK metrics, therefore mean and standard deviation was used as measure of central tendency and variability for future comparisons.

Table 6.13 shows the summary statistics for \(F_{\text{INH}}\) and \(F_{\text{PUL}}\) obtained both from AUC\(_{0-t}\) and AUC\(_{\infty}\) following nebulized administration in pediatric CF subjects. Assumption of normal distribution was concluded in all cases, as the COV was <50%, the mean and the median differed by less than 20%, and max and min were equally distanced from the median (low fold-range), suggesting that no outlier was observed. Mean and SD will be reported for future comparisons.

**Table 6.12** Summary PK metrics statistics obtained from NCA for 13 subjects in the single pediatric tobramycin nebulizer inhalation study. Tabulated mean, standard deviation (SD), coefficient of variation (COV, %), median, max and min, and fold range for \(C_{\text{max}}\) (mg/L), \(t_{\text{max}}\) (min), AUC\(_{0-t}\) (mg*min/L), AUC\(_{\infty}\) (mg*min/L), half-life (min).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>COV (%)</th>
<th>Median</th>
<th>Max</th>
<th>Min</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_{\text{max}}), mg/L</td>
<td>0.72</td>
<td>0.23</td>
<td>32</td>
<td>0.70</td>
<td>1.20</td>
<td>0.30</td>
<td>4.0</td>
</tr>
<tr>
<td>(t_{\text{max}}), min</td>
<td>63.6</td>
<td>27.9</td>
<td>44</td>
<td>59.8</td>
<td>120.8</td>
<td>29.7</td>
<td>4.1</td>
</tr>
<tr>
<td>AUC(_{0-t}), mg*min/L</td>
<td>127</td>
<td>41</td>
<td>32</td>
<td>113</td>
<td>201</td>
<td>59</td>
<td>3</td>
</tr>
<tr>
<td>AUC(_{\infty}), mg*min/L</td>
<td>226</td>
<td>93</td>
<td>41</td>
<td>193</td>
<td>431</td>
<td>136</td>
<td>3</td>
</tr>
<tr>
<td>(t_{1/2}), min</td>
<td>188.5</td>
<td>59.6</td>
<td>32</td>
<td>175.9</td>
<td>455.9</td>
<td>111.8</td>
<td>4.1</td>
</tr>
</tbody>
</table>
Table 6.13 Summary statistics for estimated \( F_{\text{INH}} \) and \( F_{\text{PUL}} \) obtained from NCA for 13 subjects in the single pediatric tobramycin nebulizer inhalation study. Tabulated PK metrics: mean, standard deviation (SD), coefficient of variation (COV, %), median, max and min, and fold range for \( F_{\text{INH}} \) (%) and \( F_{\text{PUL}} \) (%).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>COV (%)</th>
<th>Median</th>
<th>Max</th>
<th>Min</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F_{\text{INH}} (\text{AUC}_0-t) )</td>
<td>1.4</td>
<td>0.5</td>
<td>32</td>
<td>1.2</td>
<td>2.2</td>
<td>0.6</td>
<td>3.4</td>
</tr>
<tr>
<td>( F_{\text{INH}} (\text{AUC}_\infty) )</td>
<td>2.5</td>
<td>1.0</td>
<td>41</td>
<td>2.1</td>
<td>4.8</td>
<td>1.5</td>
<td>3.2</td>
</tr>
<tr>
<td>( F_{\text{PUL}} (\text{AUC}_0-t) )</td>
<td>9.5</td>
<td>3.0</td>
<td>32</td>
<td>8.4</td>
<td>15.0</td>
<td>4.4</td>
<td>3.4</td>
</tr>
<tr>
<td>( F_{\text{PUL}} (\text{AUC}_\infty) )</td>
<td>16.8</td>
<td>6.9</td>
<td>41</td>
<td>14.4</td>
<td>32.0</td>
<td>10.1</td>
<td>3.2</td>
</tr>
</tbody>
</table>

6.3.7 Non-compartmental PK analysis for adult INH nebulization studies

The NCA was conducted on one of the CF adult studies that reported \( C_p(t) \) after tobramycin nebulized inhalation administration (Table 6.14). Study AINH2 reported mean subjects \( C_p(t) \) after administration of 300 mg tobramycin, therefore the NCA was conducted on the mean profile. There was agreement between estimated NCA data performed in this analysis and the reported literature data. The \( \text{AUC}_0-t \) was determined as 291 mg*min/L from the NCA and 298 mg*min/L study by Geller et al (Geller et al., 2003). Similarly for \( C_{\text{max}} \) and \( t_{\text{max}} \), values of 1.10 mg/L and 63.2 min, respectively, from the NCA compared to literature values of 1.12 mg/L and 63.0 min, respectively (Geller et al., 2003). In addition, visual inspection of the log-linear regression analysis conducted on the terminal concentration vs. time data points used to estimate the terminal slope, was acceptable. The calculated \( R^2 \) was 1.00 and the extrapolated area AUC was 13%.

The NCA was not conducted on the other two adult inhalation nebulizer studies due to the absence of available \( C_p(t) \) data. The reported PK metrics for these two studies (AINH1 and AINH3) are also reported in Table 6.14. These three studies revealed comparable PK metrics. This resulted in comparable estimates for \( F_{\text{INH}} \) and \( F_{\text{PUL}} \), as shown in Table 6.15 for each study. No \( F_{\text{INH}} \) and \( F_{\text{PUL}} \) were calculated for AINH1 using \( \text{AUC}_\infty \), as no information was provided for this exposure metric. \( F_{\text{INH}} \) ranged from 10.5% to 12.2%,
irrespective of the AUC used. Similarly, \(F_{PUL}\) ranged from 69.2% to 80.7%. These results are comparable to those obtained by Hanna in their PK analysis (Hanna, 2018).

**Table 6.14.** Pharmacokinetic NCA estimated and reported parameters (RP) for the 3 adult tobramycin inhalation nebulizer studies. Tabulated PK metrics: \(C_{\text{max}}\) (mg/L), \(t_{\text{max}}\) (min), \(\text{AUC}_{0-t}\) (mg*min/L), % extrapolated, \(\text{AUC}_{\infty}\) (mg*min/L), half-life (min), \(R^2\).

<table>
<thead>
<tr>
<th></th>
<th>AINH1</th>
<th>AINH2</th>
<th>AINH3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_{\text{max}}), mg/L</td>
<td>1.20</td>
<td>1.10</td>
<td>1.04</td>
</tr>
<tr>
<td>(t_{\text{max}}), min</td>
<td>60.0</td>
<td>63.2</td>
<td>60.0</td>
</tr>
<tr>
<td>(\text{AUC}_{0-t}), mg*min/L</td>
<td>300</td>
<td>291</td>
<td>288</td>
</tr>
<tr>
<td>% Extrapolated</td>
<td>✗</td>
<td>13</td>
<td>✗</td>
</tr>
<tr>
<td>(\text{AUC}_{\infty}), mg*min/L</td>
<td>✗</td>
<td>336</td>
<td>318</td>
</tr>
<tr>
<td>(t_{1/2}), min</td>
<td>162.0</td>
<td>159.8</td>
<td>180.0</td>
</tr>
<tr>
<td>(R^2)</td>
<td>✗</td>
<td>1.00</td>
<td>✗</td>
</tr>
<tr>
<td>PK analysis</td>
<td>RP</td>
<td>NCA</td>
<td>RP</td>
</tr>
</tbody>
</table>
Table 6.15. Estimated $F_{\text{INH}}$ and $F_{\text{PUL}}$ obtained from the NCA and reported parameters (RP) for the 3 adult tobramycin inhalation nebulizer studies. Tabulated PK metrics: $F_{\text{INH}}$ (%) and $F_{\text{PUL}}$ (%) obtained using $\text{AUC}_{0-t}$ and $\text{AUC}_{\infty}$.

<table>
<thead>
<tr>
<th></th>
<th>AINH1</th>
<th>AINH2</th>
<th>AINH3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{\text{INH}} (\text{AUC}_{0-t})$</td>
<td>10.9</td>
<td>10.6</td>
<td>10.5</td>
</tr>
<tr>
<td>$F_{\text{INH}} (\text{AUC}_{\infty})$</td>
<td>-</td>
<td>12.2</td>
<td>11.5</td>
</tr>
<tr>
<td>$F_{\text{PUL}} (\text{AUC}_{0-t})$</td>
<td>72.3</td>
<td>69.9</td>
<td>69.2</td>
</tr>
<tr>
<td>$F_{\text{PUL}} (\text{AUC}_{\infty})$</td>
<td>-</td>
<td>80.7</td>
<td>76.5</td>
</tr>
</tbody>
</table>

Table 6.16 and Table 6.17 report the summary statistics obtained from adult INH nebulizer studies. All the estimated exposure metrics resulted in a COV <10%, comparable values of mean and median, and max and min equally distanced from the median. In addition, the calculated $F_{\text{INH}}$ and $F_{\text{PUL}}$ also resulted in <5% coefficient of variation and comparable mean and median. Therefore, a normal distribution was assumed both for PK metric and calculated bioavailabilities and mean±SD was used for further comparisons. This observation was expected as the values calculated are means of means, in contrast to the pediatric INH nebulizer study, in which single subject $C_p(t)$’s were evaluated.
Table 6.16 Summary PK metrics statistics obtained for the 3 adult tobramycin inhalation nebulizer studies. Tabulated mean, standard deviation (SD), coefficient of variation (COV, %), median, max and min, and fold range for C<sub>max</sub> (mg/L), t<sub>max</sub> (min), AUC<sub>0-t</sub> (mg*min/L), AUC<sub>∞</sub> (mg*min/L), half-life (min). *n=2

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>COV (%)</th>
<th>Median</th>
<th>Max</th>
<th>Min</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, mg/L</td>
<td>1.11</td>
<td>0.08</td>
<td>7.26</td>
<td>1.10</td>
<td>1.20</td>
<td>1.04</td>
<td>1.15</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt;, min</td>
<td>61.1</td>
<td>1.9</td>
<td>3.0</td>
<td>60.0</td>
<td>63.2</td>
<td>60.0</td>
<td>1.1</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t&lt;/sub&gt;, mg*min/L</td>
<td>293</td>
<td>6</td>
<td>2</td>
<td>291</td>
<td>300</td>
<td>288</td>
<td>1</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;∞&lt;/sub&gt; (mg<em>min/L)</em></td>
<td>327</td>
<td>12.5</td>
<td>4</td>
<td>327</td>
<td>336</td>
<td>318</td>
<td>1</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (min)</td>
<td>167.3</td>
<td>11.1</td>
<td>6.6</td>
<td>162.0</td>
<td>180.0</td>
<td>159.8</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Table 6.17 Summary statistics for estimated F<sub>INH</sub> and F<sub>PUL</sub> obtained for the 3 adult tobramycin inhalation nebulizer studies. Tabulated PK metrics: mean, standard deviation (SD), coefficient of variation (COV, %), median, max and min, and fold range for F<sub>INH</sub> (%) and F<sub>PUL</sub> (%). *n=2

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>COV (%)</th>
<th>Median</th>
<th>Max</th>
<th>Min</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>F&lt;sub&gt;INH&lt;/sub&gt; (AUC&lt;sub&gt;0-t&lt;/sub&gt;)</td>
<td>10.6</td>
<td>0.2</td>
<td>2</td>
<td>10.6</td>
<td>10.9</td>
<td>10.5</td>
<td>1.0</td>
</tr>
<tr>
<td>F&lt;sub&gt;INH&lt;/sub&gt; (AUC&lt;sub&gt;∞&lt;/sub&gt;)*</td>
<td>11.9</td>
<td>0.5</td>
<td>4</td>
<td>11.9</td>
<td>12.2</td>
<td>11.5</td>
<td>1.1</td>
</tr>
<tr>
<td>F&lt;sub&gt;PUL&lt;/sub&gt; (AUC&lt;sub&gt;0-t&lt;/sub&gt;)</td>
<td>70.5</td>
<td>1.6</td>
<td>2</td>
<td>69.9</td>
<td>72.3</td>
<td>69.2</td>
<td>1.0</td>
</tr>
<tr>
<td>F&lt;sub&gt;PUL&lt;/sub&gt; (AUC&lt;sub&gt;∞&lt;/sub&gt;)*</td>
<td>78.6</td>
<td>3.0</td>
<td>4</td>
<td>78.6</td>
<td>80.7</td>
<td>76.5</td>
<td>1.1</td>
</tr>
</tbody>
</table>

6.3.8 Prediction of AUC<sub>∞-IV</sub> for adult and pediatric subjects

The AUC<sub>∞-IV</sub> was predicted over an IV dose range selected based on the literature (Aminimanizani et al., 2002; Arends and Pettit, 2018; Bragonier and Brown, 1998; Hoecker et al., 1978; Hsu et al., 1984; Levy et al., 1982). The median CL<sub>TOT</sub> and mean body weight normalized CL<sub>TOT</sub> were used to predict AUC<sub>∞-IV</sub> for
pediatric CF subjects, while the mean $\text{CL}_{\text{TOT}}$ and body weight normalized values were used for adult predictions.

Figure 6.9 (left panel) shows the predicted and observed $AUC_{\infty}^{\text{IV}}$ for pediatric and adult CF subjects. The $AUC_{\infty}^{\text{IV}}$ vs dose plots revealed that pediatric CF subjects have a $\sim$2-fold higher exposure compared to adults for the same dose. The steeper slope obtained from the pediatric prediction was expected, as pediatric CF subjects resulted to have a lower $\text{CL}_{\text{TOT}}$ (51.8, 17.6-128.5 mL/min) compared to adult CF subjects (101.5±25.3 mL/min). Most of the observed values for the pediatric subjects matched with the predictions, and the adult predictions were found to match the observed data for the single study investigated.

When normalizing the IV dose by the body weight (Figure 6.9, right plot), the predicted $AUC_{\infty}^{\text{IV}}$ shows that pediatric CF subjects are expected to have lower exposure (by $\sim$1.2-fold) compared to adults for the same dose. The shallower slope obtained from the pediatric prediction was again expected, as pediatric CF subjects have a higher BW-normalized $\text{CL}_{\text{TOT}}$ (2.3±0.6 mL/min/kg) compared to adult CF subjects (1.8±0.5 mL/min/kg). However, the pediatric-adult difference in BW-normalized $\text{CL}_{\text{TOT}}$ was reduced compared to unnormalized $\text{CL}_{\text{TOT}}$, and this explains the lower predicted exposure difference when pediatric and adult CF subjects are administered the same IV dose. Again, most of the pediatric observed values matched the predicted values, and the adult observation perfectly matched the prediction.

![Figure 6.9 Predicted $AUC_{\infty}^{\text{IV}}$ vs. IV nominal dose (left plot) and IV BW-normalized dose (right plot) for pediatric (blue line) and adult (red line) subjects following IV administration. Blue circles represent the observed $AUC_{\infty}^{\text{IV}}$ from 13 pediatric subgroups obtained from 5 IV studies; red diamond represents the observed $AUC_{\infty}^{\text{IV}}$ from a single adult IV study.](image-url)
6.3.9 Prediction of AUC∞INH for adult and pediatric subjects following inhaled nebulizer administration

The AUC∞INH was predicted for a nominal nebulized inhaled dose range up to 300 mg. The dose used in these predictions is the nominal label claim dose loaded into the nebulizer. The median CLTOT and mean body weight normalized CLTOT were used to predict AUC∞INH for pediatric CF subjects, while mean CLTOT and body weight normalized values were used for the adult predictions.

Figure 6.10 shows predicted and observed AUC∞INH for pediatric and adult CF subjects following nebulization of tobramycin. The predicted tobramycin AUC∞INH vs dose, with and without body weight normalization show that pediatric CF subjects are expected to have lower exposure compared to adults for the same nominal nebulized dose. A shallower slope obtained with the non-normalized dose (left plot) from the pediatric prediction was expected, as pediatric CF subjects have a 4.8-fold and 2-fold lower FINH and CLTOT (2.5±1.0%, 51.8, 17.6-128.5 mL/min, respectively) respectively, compared to adult CF subjects with FINH of 11.9±0.5% and CLTOT of 101.5±25.3 mL/min. The observed pediatric AUC∞INH had a mean value that was 1.5-fold higher than the predicted value for the 300 mg nebulized dose. However, the predictions was still within the observed variability, as expressed by the error bars. Less than a fold difference was instead found between observed and predicted adult exposures for a 300 mg dose. Overall, when administering the same IV dose to adult and pediatric CF subjects, a 2.3-fold lower exposure in pediatrics was predicted.

For AUC∞INH predictions using the BW-normalized dose (Figure 6.10, right plot), although FINH between pediatric and adult CF subjects was unchanged in this analysis, the reduced fold difference (1.3) between pediatric and adult BW-normalized CLTOT (2.3±0.6 and 1.8±0.5 mL/min/kg, respectively) produced a much larger difference in the observed exposure for the same dose. The observed pediatric AUC∞INH was in good agreement with the predicted values. A 6-fold exposure difference was predicted based on this analysis when administering the same BW-normalized dose to adult and pediatric CF subjects.
Figure 6.10 Predicted $\text{AUC}_{\infty}^{\text{INH}}$ vs nominal dose loaded in the device (left plot) and BW-normalized dose (right plot) for pediatric (blue line) and adult (red line) subjects following nebulization. Blue circle represents the observed $\text{AUC}_{\infty}^{\text{INH}}$ from a single pediatric inhalation study; red diamonds represent the observed $\text{AUC}_{\infty}^{\text{INH}}$ from 3 adult inhalation studies.

Figure 6.11 shows predicted and observed $\text{AUC}_{\infty}^{\text{INH}}$ for pediatric and adult CF subjects obtained using estimated dose to lung (DtL) following nebulization with (right plot) and without (left plot) body weight normalization. The DtL is hypothesized to reflect the dose actually delivered to the subject rather than the dose nominally available from the nebulizer. Pediatric CF subjects were expected to have lower exposure compared to adults for the same dose. When using the non-normalized DtL, the shallower slope obtained from the pediatric prediction was expected, as described previously, due to lower values for $F_{\text{PUL}}$ and $CL_{\text{TOT}}$ in pediatric subjects compared to adult CF subjects. As with the predicted $\text{AUC}_{\infty}^{\text{INH}}$ using the nominal nebulizer dose, the predictions using the non-normalized DtL showed some deviations from the observed exposures for pediatric and adults CF subjects, but these were again within the accepted variability range. The observed pediatric $\text{AUC}_{\infty}^{\text{INH}}$ had a mean value that was 1.5-fold higher than the predicted value for a nominal nebulized dose of 300 mg. There was closer agreement between the observed and predicted value for adult exposure following a nominal 300 mg inhaled dose.

The BW-normalized DtL $\text{AUC}_{\infty}^{\text{INH}}$ predictions show a much larger difference between pediatric and adult CF subjects for the same dose, with predicted pediatric exposure being lower at a comparable body weight normalized dose to lung. This difference was again explained by the reduced fold difference between pediatric and adult BW-normalized $CL_{\text{TOT}}$ as compared to the non-normalized $CL_{\text{TOT}}$ (mL/min).
6.3.10 Estimated AUC$r_{\infty}^{\text{INH}}$ for tobramycin excipient enhanced growth formulation in the novel positive air gas source dry powder inhaler in pediatric subjects and $F_{\text{PUL}}$ sensitivity analysis

Following the development and validation of the PK model, systemic exposure metrics estimates in pediatric subjects were produced for the novel tobramycin EEG formulation – device combination. It is important to recognize that high systemic exposures have the potential to result in toxic effects (ototoxicity and nephrotoxicity). This analysis was conducted using the data obtained from the PK analysis described above for the delivery of inhaled tobramycin to pediatric subjects by nebulization using the PARI-LC® PLUS/TIS combination. It should be noted that the predictions and observations regarding nebulized delivery are specific for the PARI-LC® PLUS nebulizer used in combination with the tobramycin inhalation solution. The novel EEG formulation/device combination has an in vitro determined DtL of 71.1 % of the nominal dose (Section 4.3.3.5), this compares to 14.9 % for the nebulizer (PARI-LC® PLUS/TIS). From this in vitro study, the novel EEG formulation/device combination achieved ~5 times higher DtL compared to the nebulized delivery method. $F_{\text{INH}}$ was calculated using Equation 6.2 and initially hypothesizing that the EEG formulation/device combination has the same pulmonary bioavailability ($F_{\text{PUL}} = 16.8\%$) as the
nebulized delivery. From this analysis, the estimated inhalation bioavailability was 12.0% for EEG formulation/device combination compared to only 2.5% for the nebulizer as shown in Table 6.19.

Table 6.18 Dose metrics and estimated inhalation bioavailability for pediatric CF subjects using the novel EEG formulation/device combination. Tabulated PK metrics: nominal dose (mg), *in vitro* determined dose to lung (DtL, mg and %), body weight normalized dose to lung (mg/kg), pulmonary bioavailability and inhalation bioavailability (\( F_{\text{INH}} \) and \( F_{\text{PUL}} \), %).

<table>
<thead>
<tr>
<th>Device – formulation</th>
<th>Nominal dose (mg)</th>
<th>DtL (mg)</th>
<th>DtL (%)</th>
<th>BW-normalized DtL (mg/kg)</th>
<th>( F_{\text{PUL}} ) (%)</th>
<th>( F_{\text{INH}} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nebulizer</td>
<td>300</td>
<td>45</td>
<td>14.9</td>
<td>3.1</td>
<td>16.8</td>
<td>2.5</td>
</tr>
<tr>
<td>EEG formulation/device combination</td>
<td>300</td>
<td>213</td>
<td>71.1</td>
<td>15.0</td>
<td>16.8</td>
<td>12.0</td>
</tr>
</tbody>
</table>

The estimated systemic exposure for the tobramycin EEG formulation – positive pressure device combination was predicted based on nominal doses ranging from 5 to 300 mg. Figure 6.12 shows the estimated \( \text{AUC}_{\infty}^{\text{INH}} \) for nominal doses of 5 to 300 mg (left plot) and the corresponding BW-normalized dose (right plot). The blue dash line represents the predicted \( \text{AUC}_{\infty}^{\text{INH}} \) when administering nebulized tobramycin using PARI-LC\textsuperscript{®} PLUS/ TIS combination, while the green dash line represents the predicted \( \text{AUC}_{\infty}^{\text{INH}} \) when administering different tobramycin doses using EEG formulation/device combination. Additionally, the mean and standard deviation values obtained from the NCA performed using the data from the pediatric nebulizer study (PINH) are reported for reference as dark solid and dot lines, respectively. The higher \( F_{\text{INH}} \) for the EEG formulation/device combination was expected to result in a 5-fold increase in estimated systemic exposure compared to the nebulizer. From this analysis, it is clear that a decrease in the
nominal dose for the EEG formulation/device combination would be required to limit the systemic exposure and possible toxic effects.

For this analysis, the EEG formulation/device combination DtL was determined by realistic in vitro experiment, however it was not possible to experimentally determine $F_{\text{PUL}}$. The PK model can be used to evaluate the effects of changes in $F_{\text{PUL}}$ that could potentially occur for the novel EEG formulation - device combination. Initial estimates were obtained based on the $F_{\text{PUL}}$ of 16.8% determined for nebulized delivery. A sensitivity analysis was performed using 2-fold and 4-fold increases in $F_{\text{PUL}}$ to investigate the effect of this parameter on the estimates of $AUC_{\infty}^{\text{INH}}$ for the tobramycin EEG formulation/device combination. Figure 6.13 shows the predicted $AUC_{\infty}^{\text{INH}}$ as a function of nominal dose (left plot) and of the BW-normalized nominal dose (right plot) using $F_{\text{PUL}}$ values of 16.8, 33.7 and 67.4%. The predicted $AUC_{\infty}^{\text{INH}}$ increased by more than 10-fold and 20-fold, respectively with the 2-fold and 4-fold increases in $F_{\text{PUL}}$. 

---

**Figure 6.12** Predicted $AUC_{\infty}^{\text{INH}}$ vs nominal dose (left plot) and body weight-normalized nominal dose (right plot). Circles represent the predicted $AUC_{\infty}^{\text{INH}}$ for nebulized delivery. Triangles represent the predicted $AUC_{\infty}^{\text{INH}}$ for delivery using the EEG formulation/device combination. Mean and standard deviation values obtained from the NCA obtained in study PINH, are reported as dark solid and dot lines, respectively.
6.4 Discussion and Conclusion

6.4.1 Review of the studies employed in the pharmacokinetic analysis

For pharmacokinetic analysis it is important to recognize that confidence in predictions and conclusions for the exposure PK metrics determined in both the pediatric and adult CF subjects is highly dependent on literature studies used in the analysis. Therefore, a critical evaluation of the studies employed in this analysis is described below.

Studies PIV1 and PIV4 obtained PK metrics based on only 2 sample plasma concentrations, which may offer challenges when determining PK metrics. All pediatric IV studies used non-selective methods (radioenzymatic assay, polarization fluoro-immunoassay, and TDA immunofluorescent assay) to quantify tobramycin concentrations, thus potentially leading to variable results. Study PIV4 was a retrospective study, and therefore not designed to study PK metrics. In addition, study PIV5 reports some tobramycin concentrations below the LLOQ, however this was a key study as it was the only study reporting tobramycin sputum concentration after IV administration in pediatric CF subjects. Due to the scarcity of pediatric studies of tobramycin administered by IV, all these studies were employed in the analysis despite their potential shortcomings. Study PINH was the only pediatric INH study found in literature that reported
individual subjects Cp(t) and a single time point tobramycin sputum concentration after administration of tobramycin by inhalation route. Additionally, body weight information was lacking in almost all pediatric studies. Despite the limited sampling schedule used, and the overlapping and not well defined single Cp(t) profiles, this study was also evaluated in the analysis. Repeated IV or INH repeated dose studies would also have been useful to evaluate any drug accumulations effect, however no such studies were available.

Study AIV was the only study for intravenous administration of tobramycin in adult CF subjects and was employed in the previously developed PK model (Hanna, 2018). Studies AINH1 and AINH3 had no information about body weight and, most importantly, they had no measurement of tobramycin lung deposition. Therefore, it was assumed that body weight and lung dose were comparable to those reported in study AINH2, as the population age and disease state were comparable.

6.4.2  *In vitro* DTL determination of tobramycin in pediatric subjects

Pediatric lung dose assessment for nebulized tobramycin using PARI-LC® PLUS Nebulizer and DeVilbiss PulmoAide air compressor was determined using realistic *in vitro* experiments due to lack of reliable *in vitro* and *in vivo* literature data. Wee et al. evaluated the aerosol performance of PARI-LC® PLUS with DeVilbiss PulmoAide air compressor and compared it to the TOBI Podhaler in a tracheostomized *in vitro* system (Wee et al., 2016). Limited information was provided for the experimental conditions, however the results indicated that a 6 and 12 years old tracheostomy model had virtually the same lung dose deposition (~23% of the nominal dose), despite different model dimensions. In contrast to the limited pediatric studies, both *in vitro* and *in vivo* studies in adults are available. Schneiders (2008) and Poli (2007) evaluated the *in vitro* performance using impactor experiments (Poli et al., 2007; Schneiders et al., 2008). Schneiders and colleagues observed that Bramitob® (75 mg/mL tobramycin for 4 mL solution) delivered by the PARI-LC® PLUS device with TurboBOY compressor resulted in fine particle dose of 50.4 mg (Schneiders et al., 2008). This was equivalent to a fine particle fraction of 16.8% of the nominal dose. Poli et al. compared the *in
vitro aerosol performance of Bramitob® and Tobi® delivered by PARI-LC® PLUS/TurboBOY and Systam 290 LC (ultrasonic nebulizer) using an adult realistic breathing profile and sizing the aerosol in a multi-stage liquid impinger (Poli et al., 2007). They reported a respirable dose ranging from 98.25-127.45 mg for both devices, which is equivalent to a fine particle fraction of 33-40%. It should be noted that the use of a different compressor and/or formulation could result in differences in aerosol performance that would affect the estimated DtL. Lenney et al. and Denk et al. reported 15.1% and 15.4% lung deposition, respectively, when radiolabeled tobramycin inhalation solution was administered using the PARI-LC® PLUS nebulizer to adult CF subjects (Denk et al., 2009; Lenney et al., 2011).

In this current study, a realistic pediatric mouth-throat model of a 5 year old was used to determine the in vitro DtL following nebulization using the PARI-LC® PLUS nebulizer. The DtL was measured as 14.9% of the nominal dose and was found to be comparable with the in vivo adult lung dose observed elsewhere (15.1%) (Lenney et al., 2011).

6.4.3 Comparison of systemic exposures following tobramycin IV administration in pediatric and adult CF subjects

Five pediatric IV studies, divided in 13 subgroups, were evaluated to predict pediatric exposure after IV administration. The NCA was conducted on 9 subgroups, while reported exposure metrics were used for remaining 3 studies. Based on the findings of Hanna, 2018, a single adult IV study was evaluated using individual subject NCA and compared with the pediatric CF subjects (Hanna, 2018). All NCA resulted in comparable data with reported literature values (Aminimanizani et al., 2002; Bragonier and Brown, 1998; Hoecker et al., 1978; Levy et al., 1982).

Table 6.19 summarizes the main PK exposure metrics obtained from the pediatric and adult IV studies. The median pediatric Cmax is lower compared to the mean adult value, however the Cmax pediatric range overlaps with the mean adult value due to the wider dosing regimen used for the pediatric patients. Similarly, the
observed $t_{\text{max}}$ was highly variable for pediatric subjects although median and mean values for pediatric and adult subjects, respectively, were similar. The mean pediatric CL$_{\text{TOT}}$ was found to be higher in adults compared to the median in pediatric CF subjects, however when normalizing per body weight, adults showed a relatively lower mean CL$_{\text{TOT}}$ compared to pediatrics. Given that the wide pediatric age range (0.5-16.0 years) included in the analysis, the non-normalized CL$_{\text{TOT}}$ range observed for the pediatric subjects overlapped with mean adult clearance. This result was in agreement with the findings of Hoecker et al. who compared toddlers with young adults and saw no difference in clearance (Hoecker et al., 1978). Increased CL$_{\text{TOT}}$ is only a function of body weight difference, however there was no obvious explanation for the decreased BW-normalized CL$_{\text{TOT}}$ clearance in adults compared to pediatric subjects. Based on literature and the validated semi-PBPK model, tobramycin is mainly excreted unchanged by glomerular filtration (~90%) and the remaining portion is excreted by non-renal pathways (Hanna, 2018). An energy-mediated transport mechanism has been advocated to be responsible for the nephrotoxicity of tobramycin (Nagai and Takano, 2014). Thus, a potential age-related, incomplete transporter maturation or increased rate in non-renal pathways could potentially explain the differences in pediatric and adult subjects.

Similar to CL$_{\text{TOT}}$, the V$_{\text{ss}}$ was observed to be higher in adults compared to pediatric subjects. However, the BW-normalized V$_{\text{ss}}$ (L/kg) showed a slightly higher value (0.34 L/kg) in pediatrics compared to adult subjects (0.3 L/kg). No correlation for V$_{\text{ss}}$ was found with respect to age (Figure 6.6), and this was in agreement with the limited literature in this area (Kelly et al., 1982). Touw et al., (2007) observed that pediatric subjects had higher BW-normalized V$_{\text{ss}}$ compared to adults (Touw et al., 2007) as seen in the present study. The most reliable explanation for the slightly larger V$_{\text{ss}}$ of pediatric patients relates to the hydrophilic nature of tobramycin that distributes in the extracellular fluid which is lower in adults compared to pediatric subjects.

As a consequence of the higher CL$_{\text{TOT}}$, the pediatric subjects have a shorter median $t_{1/2}$ compared to the mean of the adult subjects. However, the pediatric range overlaps with the mean adult half-life. The high
variability within the exposure of pediatric CF subjects could be due to the wide dosing regimen used in study literature.

**Table 6.19** Comparison of pediatric and adult PK summary statistics following intravenous administration generated from 13 pediatric studies and a single IV study with 6 subjects. Tabulated mean±standard deviation (SD) or median, range (min-max) for $C_{\text{max}}$ (mg/L), $t_{\text{max}}$ (min), $CL_{\text{TOT}}$ (mL/min), BW-normalized $CL_{\text{TOT}}$ (mL/min*kg), $V_{\text{dss}}$ (L), BW-normalized $V_{\text{dss}}$ (L/kg), $AUC_{\infty}$ (mg*min/L), half-life (min), age (years), body weight (kg), dose (mg), BW-normalized dose (mg/kg).

<table>
<thead>
<tr>
<th>Metric</th>
<th>Pediatric</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$, mg/L</td>
<td>4.2 (3.0-76.3)</td>
<td>15.6±7.3</td>
</tr>
<tr>
<td>(median, range/ mean±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{\text{max}}$, min (median, range/ mean±SD)</td>
<td>30.0 (14.7-71.7)</td>
<td>36.3±4.7</td>
</tr>
<tr>
<td>$CL_{\text{TOT}}$, mL/min</td>
<td>51.8 (17.6-128.5)</td>
<td>101.5±25.3</td>
</tr>
<tr>
<td>(median, range/ mean±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW-normalized $CL_{\text{TOT}}$, mL/min*kg</td>
<td>2.3±0.6</td>
<td>1.8±0.5</td>
</tr>
<tr>
<td>(mean±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{\text{dss}}$, L</td>
<td>10.15±3.62</td>
<td>16.75±5.56</td>
</tr>
<tr>
<td>BW-normalized $V_{\text{dss}}$, L/kg</td>
<td>0.34±0.12</td>
<td>0.30±0.10</td>
</tr>
<tr>
<td>$AUC_{\infty}$, mg*min/L</td>
<td>808 (540-9907)</td>
<td>1942±502</td>
</tr>
<tr>
<td>(median, range/ mean±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{1/2}$, min (median, range/ mean±SD)</td>
<td>107.5 (77.9-348.5)</td>
<td>130.7±20.2</td>
</tr>
<tr>
<td>Age, years (mean±SD/median, range)</td>
<td>9.0 (0.5-16.0)</td>
<td>29.0±4.6 (23-34)</td>
</tr>
<tr>
<td>Body weight, kg (mean±SD, range)</td>
<td>29.5±15.6 (7.2-50.5)</td>
<td>55.3±13.8 (45-75)</td>
</tr>
<tr>
<td>Dose, mg (mean/median, range)</td>
<td>61.3 (21.6-544.5)</td>
<td>186.7 (140-240)</td>
</tr>
<tr>
<td>BW-normalized dose, mg/kg</td>
<td>2.1 (0.9-15.0)</td>
<td>3.3</td>
</tr>
<tr>
<td>(mean/median, range)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.4.4 **Comparison of systemic exposures following nebulized tobramycin INH administration in pediatric and adult CF subjects**

Table 6.20 reports the systemic exposure metrics of pediatric and adults CF subjects when administered tobramycin using the same nebulizer inhalation device-formulation combination, and for a comparable dose to lung (~15% of the nominal dose). Pediatric subjects had a lower systemic $C_{\text{max}}$ compared to adults, but
comparable time to reach the peak concentration of around 1 hour post inhalation. The large inter-subject variability within the pediatric INH study resulted in comparable systemic exposure, based on the range of AUC, between pediatric and adult subjects are nebulization. However, there was a difference in bioavailability observed between the two age populations. Both $F_{\text{INH}}$ and $F_{\text{PUL}}$ are lower in pediatric CF subjects compared to adults by ~5 fold. Based on the IV studies, it is known that pediatric subjects have a higher BW-normalized clearance, which could explain this difference. However, other explanations including multiple mechanisms for crossing the airways epithelium have been suggested in literature for tobramycin. Valcke et al. and Pennington reported that inflamed airways contribute to vasodilation and therefore to enhanced permeation (Pennington, 1981; Valcke et al., 1990). Clearly, as the CF disease progresses into adulthood, a worsening of the inflammation status within the airways is expected which may be responsible for the increased systemic exposure. In addition, Patton et al. and Nagai and Tanano described the involvement of an active transport-related mechanism for tobramycin crossing (Nagai and Takano, 2014; Patton et al., 2004). Incomplete transporter maturation in pediatric subjects could potentially reduce the uptake of tobramycin from the airways. Finally, Laube et al. observed a 30% decrease in the mucociliary clearance of CF subjects as their age progressed from $9.8 \pm 2.1$ years to $15.8 \pm 3.2$ years (Laube et al., 2020). The reduced mucociliary clearance is again a consequence of worsening of the CF disease and may affect drug clearance in adults compared to pediatric subjects. Hanna reported using a sensitivity analysis that following inhalation administration in adult subjects, decreasing the mucociliary clearance rate result in an increase in the serum AUC (Hanna, 2018). It is reasonable to hypothesize that higher adult bioavailability compared to pediatric subjects following inhalation could also be explained by this mechanism.
Table 6.20 Comparison of pediatric and adult PK summary statistics following inhalation administration generated from 13 subjects of a single inhalation study and 3 adult studies. Tabulated mean±standard deviation (SD) or median, range (min-max) for C\text{\textsubscript{max}} (mg/L), t\text{\textsubscript{max}} (min), CL\text{\textsubscript{TOT}} (mL/min), BW-normalized CL\text{\textsubscript{TOT}} (mL/min*kg), Vd\text{\textsubscript{ss}} (L), BW-normalized Vd\text{\textsubscript{ss}} (L/kg), AUC\text{\textsubscript{\infty}} (mg*min/L), half-life (min), age (years), body weight (kg), dose (mg), BW-normalized dose (mg/kg).

<table>
<thead>
<tr>
<th></th>
<th>Pediatric</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\text{\textsubscript{max}}, mg/L (mean±SD)</td>
<td>0.72 (0.23)</td>
<td>1.11 (0.08)</td>
</tr>
<tr>
<td>t\text{\textsubscript{max}}, min (mean±SD)</td>
<td>63.6 (27.9)</td>
<td>61.1 (1.9)</td>
</tr>
<tr>
<td>AUC\text{\textsubscript{\infty}}, mg*min/L (mean±SD)</td>
<td>226±93 (136-431)</td>
<td>327±12</td>
</tr>
<tr>
<td>t\text{\textsubscript{1/2}}, min (median, range/mean±SD)</td>
<td>188.5±59.6</td>
<td>167.3±11.1</td>
</tr>
<tr>
<td>Age, years (mean±SD/median, range)</td>
<td>3.6 ± 1.6 (0.6-5.8)</td>
<td>21.5±2.3 (19.5-24)</td>
</tr>
<tr>
<td>Body weight, kg (mean±SD, range)</td>
<td>14.2</td>
<td>59</td>
</tr>
<tr>
<td>Dose, mg</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>BW-normalized dose, mg/kg (mean)</td>
<td>21.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Dose to lung (DtL), %</td>
<td>14.9</td>
<td>15.1</td>
</tr>
<tr>
<td>F\text{\textsubscript{INH}}, % (mean±SD)</td>
<td>2.5 (1.0)</td>
<td>11.9 (0.5)</td>
</tr>
<tr>
<td>F\text{\textsubscript{PUL}}, % (mean±SD)</td>
<td>16.8 (6.9)</td>
<td>78.6 (3.0)</td>
</tr>
</tbody>
</table>

6.4.5 Prediction of the systemic exposure in pediatric CF subjects following inhalation of a tobramycin excipient enhanced growth formulation in the novel positive air gas source dry powder inhaler and sensitivity analysis

An experimentally determined in vitro DtL for the tobramycin excipient enhanced growth formulation delivered using the novel positive air gas source dry powder inhaler was measured as 71.1 % of the nominal dose. Because of the increased tobramycin lung dose deposition using the novel drug formulation – inhaler combination, the predicted system exposure observed in pediatric CF subjects will be ~5-fold higher when using DAC v3/TOBI EEG compared to PARI-LC\textsuperscript{®} PLUS/ TIS. In comparison, when 300 mg of tobramycin inhalation solution was administered using the PARI-LC\textsuperscript{®} PLUS nebulizer, the systemic exposure corresponded to 226±93 mg*min/L for 3.6±1.6 years old pediatric CF subjects with a body weight of 14.2
kg. Assuming the same pulmonary bioavailability, when a nominal dose of 300 mg tobramycin is loaded in the DAC v3 device, the predicted systemic exposure for the same age and body weight CF subject corresponds to 1100 mg•min/L. In order to obtain the same systemic exposure as the nebulizer in this subject, a nominal dose to 63 mg of tobramycin in the novel dry powder inhaler would be required, as shown in Figure 6.12.

The utility of the PK model allows estimates of systemic exposure when changes to the pulmonary bioavailability occur. It is important to recognize that high systemic exposures have the potential to result in toxic effects (ototoxicity and nephrotoxicity). It is reasonable to hypothesize that the EEG powder formulation that is predicted by computational fluid dynamic models to deposit more uniformly in the lungs compared to a nebulized aerosol may have a different pulmonary bioavailability. The TIS formulation is characterized by a 2-fold larger MMAD (~4 vs. 1.6 µm) compared to the tobramycin EEG spray-dried powder (Schneiders et al., 2008, Poli et al., 2007, Awad and Berlinski, 2018). The smaller diameter aerosol and the EEG formulation technology is expected to increase both the total lung exposure and to produce a more uniform airway distribution. The micrometer-sized aerosol obtained for the DAC v3/ TOBI EEG combination is predicted to increase in size within the humid airway environment producing a low exhaled fraction, and it will potentially deposit in the central as well the peripheral lung regions. It was shown that a 20/30-fold increase in alveolar deposition can be achieved with novel DPI and EEG powders, as opposed to conventional inhalers, which may affect pulmonary bioavailability (Tian et al., 2013). Increases in the total lung deposition for the EEG formulation would be expected to produce higher systemic exposure. Pulmonary bioavailability may also be affected by the effects of tobramycin sequestration which is released over time results in prolonged exposure at the site of infection compared to IV administration (Gibson et al., 2003b; Newhouse et al., 2003; Valcke and Pauwels, 1991). In addition, it is noted that the estimated values of F_{PUL} were significantly different between adult CF patients (78.6%) and pediatric CF patients (16.5%). This estimate for pediatric patients represents a study population with a mean age of 3.6 years, it may be reasonable to expect that pulmonary bioavailability will increase with the age of the child.
In this study, the effects of 2-fold and 4-fold increase in $F_{\text{PUL}}$ on systemic exposure of pediatric CF subjects was predicted. For subjects with a mean age of 3.6 years, increasing the $F_{\text{PUL}}$ to 33.7%, resulted in a reduction of the nominal dose to 31 mg of tobramycin in the novel DAC v3 DPI to achieve the same systemic exposure as a 300 mg nebulized dose. When further increasing the pulmonary bioavailability to 67.4 %, the tobramycin dose for the EEG formulation and device combination was only 15 mg. Clearly, if the EEG formulation – device combination is able to improve both the total delivered dose to the lung and increase the pulmonary bioavailability of tobramycin in the pediatric population, the nominal doses of tobramycin required to achieve equivalent systemic exposure to the nebulized delivery will be significantly lower.

In addition, the PK model can assess the effect of the pediatric subject age, using a body weight normalized dose, on the predicted systemic exposure. Table 6.21 shows that when administering a nominal dose of 300 mg of tobramycin via the nebulizer and EEG device, there is a lower exposure for the nebulizer compared to the EEG device across the age range (2-12 years). These predictions support the need for reducing the dose of tobramycin for the EEG formulation – device combination for all pediatric ages.

**Table 6.21** Predicted systemic exposure for 2, 5, and 12-year-old pediatric CF subject when administering tobramycin a nominal dose of 300 mg using the PARI-LC® PLUS nebulizer and the EEG formulation/device combination.

<table>
<thead>
<tr>
<th>Age, years</th>
<th>Body weight, kg</th>
<th>Body weight normalized dose, mg/kg</th>
<th>Predicted $AUC_\infty$, mg*min/L – Nebulizer</th>
<th>Predicted $AUC_\infty$, mg*min/L – EEG formulation/device combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11.8</td>
<td>25.4</td>
<td>271</td>
<td>1301</td>
</tr>
<tr>
<td>5</td>
<td>17.3</td>
<td>17.3</td>
<td>185</td>
<td>888</td>
</tr>
<tr>
<td>12</td>
<td>36.3</td>
<td>8.3</td>
<td>88</td>
<td>423</td>
</tr>
</tbody>
</table>

Table 6.22 reports the estimated doses required for a 2, 5, and 12 year old subject assuming pulmonary bioavailabilities of 16.8, 33.7 and 67.4 %. These results suggest that lower nominal doses can be used to
deliver TOBI EEG with the DAC v3 dry powder inhaler maintaining safe systemic levels while potentially achieving a more uniform airway disposition in pediatric CF subjects compared to nebulized delivery of 300 mg.

Table 6.22 Nominal doses of the EEG formulation – device combination for 2, 5 and 12-year-old pediatric CF subjects with varying pulmonary bioavailabilities, required to achieve comparable systemic exposure to nebulized delivery.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>TOBI EEG Nominal dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F_{PUL}= 16.8%</td>
</tr>
<tr>
<td>2</td>
<td>11.8</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>17.3</td>
<td>76</td>
</tr>
<tr>
<td>12</td>
<td>36.3</td>
<td>160</td>
</tr>
</tbody>
</table>

In conclusion, a pediatric PK model used to predict systemic exposure after IV and INH tobramycin administration was developed and validated. The model has allowed the prediction of systemic exposure for an EEG device-formulation combination adjusted based on age and body weight and is a practical tool that can be used to assess the effects of pulmonary bioavailability changes on systemic exposure.
CHAPTER 7: SUMMARY AND CONCLUSIONS

Spray drying process parameters and storage conditions have major effects on the solid-state characteristics and the aerosol performance of spray-dried powders. Thus, it was hypothesized that they need to be considered when formulating stable and highly dispersible EEG powders. The evaluation of the aerosol performance characterization of EEG powders using rapid screening laser diffraction methods facilitated the development of a novel tobramycin EEG formulation to be delivered to pediatric cystic fibrosis subjects using a novel positive pressure dry powder inhaler. The need for efficient tobramycin powder formulation arises from the inefficient standard of care nebulized formulation and the unsuitable commercially available dry powder formulation for children aged less than 6 years old. The novel tobramycin EEG powder formulation – positive pressure dry powder inhaler combination was ultimately evaluated from a pharmacokinetic perspective. A pediatric pharmacokinetic model was developed to estimate the pediatric systemic exposure of a novel tobramycin EEG formulation – device combination.

Initially, this research project investigated the effect of spray drying critical process parameters and formulation characteristics on the primary particle size, aerosol performance and solid-state characteristics of model albuterol sulfate EEG powder formulations. Small, medium and large mesh nebulizer sizes were evaluated and when the EEG powders were aerosolized by the CC90-3D DPI, a larger MMAD and decreased %FPF<1 µm were found as the nebulizer mesh hole size increased. The medium mesh hole size nebulizer had an emitted dose of 76.0% and a submicrometer fraction of 22.9% and it was selected due to the unacceptably low emitted dose for the formulation produced by the small mesh nebulizer (62.4%) and the low submicrometer size fraction (10.6%) for the formulation produced by the large mesh nebulizer. Using the medium mesh sized nebulizer, the effect of peristaltic pump rate, spray head vibration and inlet gas drying temperature showed only small differences in the particle size and aerosol performance of AS EEG powders. However, when trileucine was used as dispersion enhancer, the AS EEG formulation had smaller MMAD (1.01 µm) and double submicrometer fraction (48.9%) compared to the L-leucine formulation (1.49
μm and 22.9%, respectively). Despite having faster and higher moisture sorption, trileucine powder crystallized at 78% RH compared to 57% RH for L-leucine formulation, thus suggesting improved stability. The hygroscopicity of trileucine EEG formulation also translated in a ~40% decrease of submicron particles under simulated airway conditions. Small particles are required to effectively penetrate the extrathoracic regions and reach the lungs. Overall, trileucine showed improved dispersion compared to L-leucine for the AS EEG powder formulation but there were concerns about its long-term stability due to the presence of an amorphous trileucine phase.

The optimized spray drying process parameters were applied for the development of a novel tobramycin EEG formulation but unlike albuterol sulfate, tobramycin is a hygroscopic molecule and controlling its exposure to moisture during spray drying and storage was important to maintain the stability and aerosol performance of the formulation. High moisture content during the spray drying process (11.9 g/m³ water vapor density) and 15% RH and room temperature storage conditions (up to 6 months) resulted in the most stable and best performing tobramycin EEG powder with 75.5% emitted dose and 1.73 μm aerodynamic diameter. The hygroscopicity of tobramycin was exploited to allow replacement of mannitol with additional drug to increase the formulation drug payload while maintaining the EEG properties of the formulation. At the same time, given the improved performance with trileucine in the AS EEG powders, trileucine was evaluated as an alternate dispersion enhancer in the tobramycin EEG formulations. Tobramycin EEG powders containing trileucine were fully amorphous and were characterized by faster moisture sorption rates and higher hygroscopicity compared to the L-leucine containing powders, despite delayed crystallization RHs, which was indicative of improved solid-state stability (Hassan et al., 2020). Feed formulations containing trileucine required pH modification (~6.2) to result in EEG powders with efficient aerosol performance. However, trileucine powders with acidified pH showed improved aerosol performance but lower tobramycin payload compared to the corresponding L-leucine powders. Thus, it was necessary to replace mannitol with additional tobramycin. Despite this modification, when the trileucine containing powder was aerosolized by the novel positive pressure DPI (DAC v3), it maintained 69.9%
emitted dose compared to only 30.4% for the L-leucine powder. The lead EEG formulation containing tobramycin and trileucine was evaluated under simulated airway conditions in a 5-year-old airway model to characterize the hygroscopic growth and estimate the total lung dose. A 1.4 growth ratio in the aerodynamic diameter and a 10% decrease in submicron fraction confirmed the hygroscopic growth of this EEG powder. Additionally, a total lung dose of 71.1% was found.

The estimated systemic exposure of the novel tobramycin EEG formulation – positive pressure DPI combination was evaluated using a pediatric pharmacokinetic model developed based on the commercially available nebulized tobramycin inhalation solution. Due to the higher lung dose found for the novel TOBI EEG combination, the PK analysis revealed that a range of 423 – 1301 mg*min/L systemic exposure resulted in pediatric subjects between 2-12 years old, compared to 88 – 271 mg*min/L for the nebulized formulation. Thus, ~5-fold decrease in administered dose is required to maintain safety exposure. Furthermore, the EEG combinations is expected to achieve a more uniform airway distribution that will require further tobramycin dose adjustments. It was estimated that for a 2-fold and 4-fold higher pulmonary bioavailability, a tobramycin loading dose in the range of 13 - 80 mg is required for pediatric subjects between 2 to 12 years old.

The characterization of EEG powders was evaluated using cascade impactor as well as laser diffraction methods. Impaction methods are laborious and time consuming, therefore there was the need for a rapid screening method. Malvern Spraytec resulted in valuable laser diffraction method to characterize the aerosol performance of EEG spray-dried powders using active and passive dry powder inhalers as well as steady flow rate and realistic breathing profile. The results obtained from the laser diffraction analysis were overall in accordance with realistic in vitro mouth-throat model characterization and cascade impactor data.

Several limitations characterized this research project, including that the stability studies were conducted on bulk EEG powders as opposed to appropriately packaged formulations. Long-term studies of spray-dried EEG powders when packaged in capsules and with primary blister packaging could provide additional and more realistic information about their stability when comparing the effect of dispersion enhancing agents
or when evaluating the optimized spray drying and storage conditions. Formulation is maintained at elevated temperature for 4-6 hours during collection on the electrostatic precipitator. Further insights into the stability of the formulation during the spray drying process could have been gained by performing moisture sorption studies at exit drying temperature during exposure to relative humidity. Lastly, the pharmacokinetic model was employed to estimate the systemic exposure of tobramycin after intravenous and inhalation administration but gave no information about the pulmonary exposure of the drug, which would be of particular interest from an efficacy and safety perspective. Such analysis was not possible due to the lack of lung fluid or mucus drug concentrations in the pediatric study population.

In conclusion, these studies have shown the effects of spray drying and storage conditions on solid-state stability and aerosol performance of EEG formulations. These formulations have been screened using both conventional impactor methods and by a rapid screening method. A tobramycin EEG powder formulation that can be delivered with high efficiency to pediatric CF subjects using a novel positive pressure dry powder inhaler was developed and characterized. A pediatric pharmacokinetic model was developed and employed to predict the nominal doses of formulation required to produce systemic drug levels equivalent to the current standard of care delivered by nebulizer.
LIST OF REFERENCE


Denk, O., Coates, A., Keller, M., Leung, K., Green, M., Chan, J., Ribeiro, N., Charron, M., 2009. Lung delivery of a new tobramycin nebuliser solution (150 mg/1.5 ml) by an investigational eFlow® nebuliser is equivalent to TOBI® but in a fraction of time.


Li, M., Byron, P.R., 2013. Tobramycin Disposition in the Rat Lung Following Airway Administration. *Journal of Pharmacology and Experimental Therapeutics* 347, 318–324.


VITA

EDUCATION

Doctor of Philosophy, Pharmaceutical Sciences 2017 - current
School of Pharmacy, Virginia Commonwealth University
Richmond, VA

Combined Bachelor of Science and Master of Science in Pharmaceutical Chemistry and Technology 2009 - 2016
School of Pharmacy, University of Messina, Messina
Italy

PUBLICATIONS


CONFERENCE ABSTRACTS


**ORAL PRESENTATIONS**


AWARDS AND RECOGNITION

- Selected as Moderator for Poster on the Podium, RDD 2021
- 2021 PCEU Leadership Award, for distinction in leadership, research and service, School of Pharmacy, VCU
- Selected as Poster on the Podium Presenter, RDD 2020
- 2018 Joseph P. Schwartz Award for distinction in scholarship, research and service, School of Pharmacy, VCU
- 2018 Rector & Rorrer travel award, School of Pharmacy, VCU
- Erasmus+, EU mobility studentship, Italy, 2015