2012

INFLUENCE OF ELECTROSTATIC CHARGE UPON THE DEPOSITION BEHAVIOR OF PHARMACEUTICAL AEROSOLS WITHIN CASCADE IMPACTORS

Megha Mohan
Virginia Commonwealth University

Follow this and additional works at: https://scholarscompass.vcu.edu/etd
Part of the Pharmacy and Pharmaceutical Sciences Commons

© The Author

Downloaded from https://scholarscompass.vcu.edu/etd/423

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.
INFLUENCE OF ELECTROSTATIC CHARGE UPON THE DEPOSITION BEHAVIOR OF PHARMACEUTICAL AEROSOLS WITHIN CASCADE IMPACTORS

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

by

MEGHA MOHAN
B.Pharm., Delhi Institute of Pharmaceutical Sciences and Research, University of Delhi, India, 2007

Director: JOANNE PEART, Ph.D.
ASSOCIATE PROFESSOR, DEPARTMENT OF PHARMACEUTICS, SCHOOL OF PHARMACY

Co-Director: PETER R. BYRON, PhD.
PROFESSOR, DEPARTMENT OF PHARMACEUTICS, SCHOOL OF PHARMACY

Virginia Commonwealth University
Richmond, Virginia
August, 2012
DEDICATIONS

To My Precious Parents
and Sai
ACKNOWLEDGEMENT

There are a lot of people to whom I owe a debt of thanks for helping and encouraging me along the path of my Ph.D. endeavor.

First and foremost, I am deeply grateful to my advisors, Dr. Joanne Peart and Dr. Peter Byron, for their guidance and support throughout this dissertation project. Dr. Peart has been my mentor, in every sense, from the day I arrived in Richmond. I would like to thank her for her never-ending patience, motivation and continual belief in my abilities, which has helped me grow as a scientist. I have learnt a lot from her dedication to work and the attitude to take every challenge in stride. Dr. Byron has been a wonderful source of knowledge and inspiration throughout my Ph.D. He has been instrumental in teaching me a lot more than good science. I am thankful to him for instilling in me the ability to reason, visualize future path to traverse and confidence, which have helped me mature and evolve as a better person today. I take with me fond memories of ‘Plumbing 101’ learnt from him. Words might be feeble to express their kindness and my sense of gratitude towards both my advisors.

I would also like to thank the members of my Graduate Advisory Committee: Dr. Philip.W. Longest, Dr. Masahiro Sakagami and Dr. Sarah Rutan, for their helpful insights during my dissertation project. I specifically want to thank Dr. Longest for helping me understand the computational fluid dynamics part of my project and patiently answering all my queries. I am also grateful to Dr. Sakagami for his practical approach and ideas, which helped me stay focused at various times during my Ph.D.
I thank VCU School of Pharmacy for the financial support during my Ph.D and the Pharmaceutics faculty for their help in the course of my graduate studies. Thanks are also due to Laura, Keyetta, Scott, Chris and Romano for being so efficient and wonderful at all times.

I sincerely thank all the current and past ARG members, specially Dr. Michael Hindle, for their help and useful criticism at numerous occasions, which helped me learn a lot of things. A special thank you to all my friends in the department, for being there through the good and the bad times. I will truly cherish the memories of VCU forever.

I have been very fortunate to have wonderful roommates like Pragya and Anila, who were my family away from home. Thank you for your love and for making life in Richmond a time I will never forget. A special thank you to Min, for enduring my insane routine during the dissertation writing phase.

Finally, I want to thank my family in India: Mom, Dad, my brother, Mukul, my grandmother, Chaiji, my grandfather, papaji, and my best friend, Kamalpreet, for their unconditional love, encouragement and support. Words cannot express everything that they have done for me. I am forever indebted to my mom for always lending an ear to my frustrations, being my support system and her frequent visits. This dream has become a reality because of her prayers and untiring efforts to keep me motivated at all times. I am thankful to Kamal, for his love, understanding and support all these years. Thank you for bringing the smile back on my face everytime I seemed to have lost it.

Above all, I express my gratitude to the Almighty for providing me this opportunity and the determination to keep moving forward, all along this journey.
# Table of Contents

Acknowledgement ........................................................................................................ iii

List of Tables .................................................................................................................. x

List of Figures .................................................................................................................. xiii

List of Abbreviations ....................................................................................................... xvii

Abstract ........................................................................................................................... xx

Chapters

I. Introduction ..................................................................................................................... 1

   I.A Significance and Background ................................................................................ 1
   I.B Cascade Impactor Calibration and Role of Electrostatic Charge in Sizing .......... 2
   I.C Mechanisms of Aerosol Charging ......................................................................... 7
   I.D Influence of Electrostatic Charge upon Aerosol Deposition in the Respiratory Tract .................................................................................................................. 9
   I.E Computational Fluid Dynamics (CFD) Model of the Andersen Cascade Impactor (ACI) ................................................................................................................. 12
   I.F Characterization of Inherent Electrostatic Charge on Pharmaceutical Aerosols .... 15
      I.F.1 Electrical Low Pressure Impactor (ELPI) ........................................................ 18
      I.F.2 Modified Twin Stage Impinger ....................................................................... 22
      I.F.3 Electrical Next Generation Impactor (eNGI) .................................................. 23
   I.G Overview .................................................................................................................. 25

II. Hypotheses and Research Plan .................................................................................... 26

   II.A Hypothesis I ........................................................................................................... 26
   II.B Hypothesis II ......................................................................................................... 27
   II.C Hypothesis III ....................................................................................................... 27
III. Deposition Behavior of Commercial Pressurized Metered Dose Inhalers Within Cascade Impactors following the Application of Electrostatic Charge

III.A  Introduction

III.B  Materials and Methods

III.B.1  Commercially Available Pressurized Metered Dose Inhalers

III.B.2  Determination of Electrostatic Charge and Apparent Aerodynamic Particle Size Distribution (APSD) of Commercial pMDIs using the Electrical Low Pressure Impactor (ELPI)

III.B.2.1  ELPI Configuration for the Application of Different Charge Levels on the Aerosol produced by pMDIs

III.B.2.2  Validation of the External Voltage Source against the Internal Corona Discharge of the ELPI

III.B.2.3  Influence of Applied Voltage on Electrostatic Charge and Mass Distribution of QVAR pMDI

III.B.2.4  Comparison of the Electrostatic Charge Distribution and Mass Distribution of Commercially Available pMDIs Following the Application of Charge

III.B.3  High-Pressure Liquid Chromatography Analyses

III.B.3.1  Analysis of Beclomethasone Dipropionate (BDP) using HPLC coupled with UV Detection

III.B.3.2  Analysis of Albuterol using HPLC coupled with Fluorescence Detection

III.C  Results and Discussion

III.C.1  Validation of the External Voltage Source against the Internal ELPI Corona Discharge

III.C.2  Influence of Applied Voltage on Electrostatic Charge and Mass Distribution of QVAR pMDI

III.C.3  Electrostatic Charge and Apparent Aerodynamic Particle Size Distribution (APSD) of Commercial pMDIs as a Function of Charge

III.C.3.1  Electrostatic Charge Distribution of Commercial pMDIs as a Function of Charge

III.C.3.2  Apparent Aerodynamic Particle Size Distribution (APSD) of Commercial pMDIs as a Function of Charge

III.D  Summary

IV. Deposition Behavior of Charge Neutralized and Charged Aerosols within the Andersen Cascade Impactor

IV.A  Introduction

IV.B  Materials and Methods

IV.B.1  Production of Charge Neutralized and Charged Fluorescein Disodium Aerosol

IV.B.2  Determination of Electrostatic Charge Distribution and Mass Distribution of Fluorescein Disodium Aerosol using the Electrical Low Pressure Impactor (ELPI)
IV.B.3 Determination of Mass Distribution and Apparent Aerodynamic Particle Size Distribution (APSD) of Fluorescein Disodium Aerosol as a Function of Charge within the Andersen Cascade Impactor (ACI) ........................................72
IV.B.4 Comparison of Fluorescein Disodium Aerosol used for Measurement of Electrostatic Charge and Apparent APSD in the ELPI and the ACI ....................74
IV.B.5 High Pressure Liquid Chromatography Analysis of Fluorescein using HPLC coupled with Fluorescence Detection ......................................................77
IV.B.6 Comparison of the In Vitro Study with the CFD Model Predictions for the ACI - Estimation of Number of Elementary Charges Per Particle ...............77
IV.C Results and Discussion ........................................................................78
   IV.C.1 Production of Fluorescein Disodium Aerosol ..................................78
   IV.C.2 Electrostatic Charge Distribution and Mass Distribution of Fluorescein Disodium Aerosol Obtained using the Electrical Low Pressure Impactor (ELPI) ..........................................................79
   IV.C.3 Comparison of Fluorescein Disodium Aerosol used for Measurement of Electrostatic Charge and Apparent APSD in the ELPI and the ACI ..........84
   IV.C.4 Mass Distribution and Apparent APSD of Fluorescein Disodium Aerosol within the Andersen Cascade Impactor (ACI) .................................88
   IV.C.5 Comparison of the CFD Model of the ACI with the In Vitro Study - Estimation of Number of Elementary Charges Per Particle .........................95
IV.D. Summary ............................................................................................99

V. Electrostatic Effects and Aerosol Deposition within the Andersen Cascade Impactor: Computational Versus In Vitro Effects .........................................................101
   V.A Introduction .........................................................................................101
   V.B Materials and Methods .......................................................................105
      V.B.1 Production of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol and Investigation of the Influence of Different Variables upon Deposition within the ACI .........................................................105
         V.B.1.1 Influence of Grounding upon the Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within the ACI ..........................................................107
         V.B.1.2 Influence of Aerosol Size upon the Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within the ACI ..........................................................107
         V.B.1.3 Influence of Stage Coating and Stage Loading upon the Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within the ACI ..........................108
         V.B.1.4 Characterization of the Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within an Abbreviated ACI ..........................................................109
   V.C Results and Discussion .......................................................................111
V.C.1 Influence of Grounding upon the Deposition Behavior of Model Charge Neutralized and Artificially Charged Fluorescein Disodium Aerosol within the ACI ................................................................. 111
V.C.2 Deposition Behavior of < 1 µm Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within the ACI ................................................................. 115
V.C.3 Influence of Stage Coating and Stage Loading upon the Deposition Behavior of Model Charge Neutralized and Artificially Charged Fluorescein Disodium Aerosol within the ACI ................................................................. 121
V.C.4 Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Aerosol within an Abbreviated ACI ................................................................. 132
V.D Summary ........................................................................................................................................... 135

VI. Overall Discussion and Conclusions ........................................................................................................... 137

References ......................................................................................................................................................... 149

Appendix ............................................................................................................................................................... 159

A.I Intra- and Inter-Run Accuracy and Precision Data for HPLC Analyses ......................................................... 160
A.I.1 Intra- and Inter- Run Accuracy and Precision Data for Beclomethasone dipropionate (BDP) HPLC Assay ........................................................................................................... 160
A.I.2 Intra- and Inter- Run Accuracy and Precision Data for Albuterol HPLC Assay................................................................. 161
A.I.3 Intra- and Inter- Run Accuracy and Precision Data for Fluorescein HPLC Assay......................................................... 161

A.II Use of ELPI Recalibration for Determining the Apparent Aerodynamic Particle Size Distribution (APSD) of Inherently and Artificially Charged Aerosols ........................................................................................................... 162
A.II.1 Introduction ............................................................................................................................................. 162
A.II.2 Materials and Methods ............................................................................................................................................. 162
A.II.3 Results and Discussion ............................................................................................................................................. 165

A.III Example of Scientist Model and Output Files ........................................................................................................... 170
A.III.1 Scientist Model File and an Example of the Parameter File ........................................................................................................... 170
A.III.2 Example of a SCIENTIST Plot of the Overlay of Experimental and Calculated Data ........................................................................................................... 171
A.III.3 Example of the SCIENTIST Statistical Report for the Non-linear Regression Analysis ........................................................................................................... 172

A.IV Normalized Impactor Doses for Charge Neutralized and Charged Fluorescein Disodium Dry Powder Aerosols ........................................................................................................... 174
A.IV.1 Percent Impactor Deposition for Fluorescein Aerosol (> 1 µm) as a Function of Charge Level Obtained using the ACI ........................................................................................................... 174
A.IV.2 Percent Impactor Deposition for > 1 µm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using a Grounded ACI ........................................................................................................... 174
A.IV.3 Percent Impactor Deposition for < 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using a Grounded ACI

A.IV.4 Percent Impactor Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using a Grounded ACI with Uncoated Impaction Plates

A.IV.5 Percent Impactor Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using a Grounded ACI Following a Sampling Time of 5 Seconds

A.V Influence of Percent Water Content on the Particle Size of Fluorescein Dry Powder Aerosol

A.V.1 Assumptions

A.V.2 Theoretical Calculation and Results

A.V.3 Summary
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.1</td>
<td>CFD predicted Stage Cut-off Diameters for Neutral and Saturation Charged Powder Aerosol Particles compared to the Manufacturer’s Data</td>
<td>15</td>
</tr>
<tr>
<td>III.1</td>
<td>Commercially Available Pressurized Metered Dose Inhaler Products (pMDIs)</td>
<td>32</td>
</tr>
<tr>
<td>III.2</td>
<td>Mass deposition of BDP discharged from QVAR 80 pMDIs at +5 kV applied via the Internal ELPI Corona Discharge and the External Voltage Source</td>
<td>41</td>
</tr>
<tr>
<td>III.3</td>
<td>Manufacturer (Dekati Ltd.) Reported and Recalibrated Aerodynamic Cut-off Diameters of the ELPI Stages 1-13 at a Flow Rate of 29 Lmin⁻¹ (corrected to two decimal places)</td>
<td>43</td>
</tr>
<tr>
<td>III.4</td>
<td>Mass Deposition of BDP discharged from QVAR 80 pMDI as a Function of Applied Voltage Determined using the ELPI with an External Voltage Source</td>
<td>47</td>
</tr>
<tr>
<td>III.5</td>
<td>Mass Deposition for Inherently Charged (0 kV) and Artificially Charged (+5 kV) QVAR 80, Proventil HFA and Ventolin HFA pMDIs aerosol clouds obtained using the External Voltage Source</td>
<td>56</td>
</tr>
<tr>
<td>IV.1</td>
<td>Mass Deposition for Fluorescein Aerosol obtained using the ELPI as a Function of Charge</td>
<td>81</td>
</tr>
<tr>
<td>IV.2</td>
<td>Mass Deposition for Fluorescein Aerosol obtained using the ACI and the ELPI as a Function of Charge</td>
<td>84</td>
</tr>
<tr>
<td>IV.3</td>
<td>Numerical Results of Curve Fitting the Cumulative Percent Undersize Data from the ACI and the ELPI for the Four Charge Levels. Best Estimates for MMAD and GSD are shown with 95% Confidence Intervals</td>
<td>87</td>
</tr>
<tr>
<td>IV.4</td>
<td>Mass Deposition for Fluorescein Aerosol obtained using the ACI as a Function of Charge</td>
<td>89</td>
</tr>
<tr>
<td>IV.5</td>
<td>Impactor Deposition for Fluorescein Aerosol as a Function of Charge Level Obtained using the ACI</td>
<td>92</td>
</tr>
</tbody>
</table>
Table IV.6  Comparison of the Number of Electrons per Particle for the Fluorescein Aerosol as a Function of Charge obtained using the ELPI .................................97

Table V.1   Summary of CFD Model Assumptions/Prediction and In Vitro Study  .........................102

Table V.2  Fluorescein Mass Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the Non-grounded and Grounded ACI .................................................................111

Table V.3  Mass Deposition for < 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the ACI and the ELPI as a Function of Charge.................................................................116

Table V.4  Comparison of the Number of Electrons per Particle for the Charge Neutralized and Positively Charged Fluorescein Aerosol as a Function of Aerosol Size obtained using the ELPI .................................................................120

Table V.5  Mass Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using a Grounded ACI as a Function of Stage Coating ...........................................................................121

Table V.6  Mass Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the ACI and the ELPI as a Function of Stage Loading ...........................................................................128

Table V.7  Mass Deposition of > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the Full Resolution and QC Abbreviated ACI ..................133

Table AI.1 Intra- and Inter- Run Accuracy and Precision Data for Beclomethasone dipropionate (BDP) HPLC Assay ...........................................................................160

Table AI.2 Intra- and Inter- Run Accuracy and Precision Data for Albuterol HPLC Assay .........................................................................................161

Table AI.3 Intra- and Inter- Run Accuracy and Precision Data for Fluorescein HPLC Assay .........................................................................................161

Table AII.1 Manufacturer (Dekati Ltd.) Reported and Recalibrated Aerodynamic Cut-off Diameters of the ELPI stages 1-13 at a Flow Rate of 29 L min⁻¹ ................................................................164

Table AII.2 Mass Deposition for BDP discharged from QVAR 80 pMDIs using the ELPI Without Corona Charger and With Corona Charger (at 0 kV) ................................................................165

Table AII.3 Cumulative Percent Undersize for Artificially Charged QVAR 80 Aerosol Cloud obtained using the ELPI .........................................................................................168
Table AIV.1 Percent Impactor Deposition for Fluorescein Aerosol (> 1 μm) as a Function of Charge Level Obtained using the ACI.................................................................174

Table AIV.2 Impactor Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using a Grounded ACI........................................174

Table AIV.3 Impactor Deposition for < 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using a Grounded ACI........................................175

Table AIV.4 Impactor Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using a Grounded ACI with Uncoated Impaction Plates..................................................................................................................175

Table AIV.5 Impactor Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using a Grounded ACI Following a Sampling Time of 5 Seconds.................................................................176
# List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure I.1</td>
<td>Abbreviated Impactor Measurement (AIM) Devices (a) Fast Screening Impactor (MSP Corporation) and (b) Short-Stack Fast Screening Andersen Impactor (Copley Scientific).</td>
</tr>
<tr>
<td>Figure III.1</td>
<td>Schematic Diagram of the Experimental Set Up of the ELPI to Characterize the Influence of Applied Electrostatic Charge upon the Deposition of Commercial pMDIs.</td>
</tr>
<tr>
<td>Figure III.2</td>
<td>Charge Distribution for Aerosols Generated from QVAR 80 pMDI using the ELPI Corona Discharge or an External Voltage Source operated at +5kV.</td>
</tr>
<tr>
<td>Figure III.3</td>
<td>Mass Distribution and Apparent APSD Profiles of QVAR 80 pMDI determined at +5 kV using the Internal ELPI Corona Discharge and the External Voltage Source.</td>
</tr>
<tr>
<td>Figure III.4</td>
<td>Electrostatic Charge Distribution for Aerosols Generated from QVAR 80 pMDI determined using the ELPI with the External Voltage Source at 0 kV, +1 kV, +3kV and +5 kV.</td>
</tr>
<tr>
<td>Figure III.5</td>
<td>Mass Deposition and % Impactor Deposition for QVAR 80 pMDIs determined using the ELPI as a Function of Applied Voltage.</td>
</tr>
<tr>
<td>Figure III.6</td>
<td>Apparent APSD for QVAR 80 pMDI determined using the ELPI as a Function of Applied Voltage.</td>
</tr>
<tr>
<td>Figure III.7</td>
<td>Electrostatic Charge Distribution for Inherently Charged and Artificially Charged (+5kV) QVAR 80, Proventil HFA and Ventolin HFA pMDIs determined using the ELPI with the External Voltage Source.</td>
</tr>
<tr>
<td>Figure III.8</td>
<td>Electrostatic Charge Distribution for Inherently Charged Proventil HFA determined from the Same Can using (a) ELPI with the Corona Charger and (b) Modified ELPI i.e., Without Corona Charger.</td>
</tr>
</tbody>
</table>
Figure III.9 Electrostatic Charge Distribution for Inherently Charged Ventolin HFA determined from the Same Can using (a) ELPI with the Corona Charger and (b) Modified ELPI i.e., Without Corona Charger..............................................................55

Figure III.10 Mass Deposition and % Impactor Deposition for Inherently Charged and Artificially Charged QVAR 80, Proventil HFA and Ventolin HFA pMDIs determined using the ELPI as a Function of Charge.................................................................58

Figure III.11 Apparent APSD for Inherently Charged and Artificially Charged QVAR 80, Proventil HFA and Ventolin HFA pMDI determined using the ELPI as a Function of Charge.................................................................60

Figure IV.1 Schematic Diagram of the Constant Output Atomizer Experimental Set up to Generate Charge Neutralized and Charged Fluorescein Disodium Aerosol together with the Schematic Representation of the Operating Principle of Corona Charger (Adapted from Dekati Ltd., Tampere, Finland)......................................................69

Figure IV.2 (a) Custom-built Stainless Steel Adaptor for the ACI, (b) Cross-sectional View of the Adaptor, and (c) Cross-sectional View of the Adaptor with Dimensions. All Dimensions are in Millimeters. Diagram not Drawn to Scale...............................73

Figure IV.3 Photograph of (a) ELPI with Charger Configuration and (b) ACI with the Corona Charger and the USP Induction Port Attached.................................................................74

Figure IV.4 Mean Fluorescein Aerosol Output Rate from the Constant Output Atomizer System at 1, 3, and 5 minutes.................................................................................................79

Figure IV.5 Electrostatic Charge for Fluorescein Aerosol as a Function of Charge Level Obtained using the ELPI.................................................................................................80

Figure IV.6 Mass Distribution for Fluorescein Aerosol as a Function of Charge Level obtained using the ELPI........................................................................................................82

Figure IV.7 Mean Cumulative Percent Undersize versus Aerodynamic Diameter (n=5) for the Fluorescein Aerosol at the Four Charge Levels Investigated obtained using (a) ACI and (b) ELPI. Solid Profiles are the Result of Curve Fitting to the Log Normal Distribution Function. Error Bars denote Standard Deviations...........86

Figure IV.8 Mass Distribution and Percent Impactor Dose for Fluorescein Aerosol as a Function of Charge Level obtained using the ACI.................................................................90

Figure IV.9 Apparent APSD for Fluorescein Aerosol as a Function of Charge Level determined using the ACI.................................................................................................94
Figure V.1. (a) Schematic of the Abbreviated Quality Control Impactor Configuration (Adapted from Mitchell et al. 2010a). (b) Photograph of the Abbreviated Impactor used in the Study..........................109

Figure V.2 Mass Distribution for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol as a Function of Grounding the ACI..............................112

Figure V.3 Mass Distribution and Percent Impactor Dose for > 1 μm Fluorescein Aerosol as a Function of Charge Level obtained using a Grounded ACI.................................113

Figure V.4 Apparent APSD for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using a Grounded ACI..............................114

Figure V.5 Electrostatic Charge and Mass Distribution for < 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the ELPI........................115

Figure V.6 Mass Distribution and Percent Impactor Dose for < 1 μm Fluorescein Aerosol as a Function of Charge Level determined using the ACI.........................................117

Figure V.7 Apparent APSD for < 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using the ACI..................................................119

Figure V.8 Mass Distribution and Percent Impactor Dose for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the Grounded ACI as a Function of Stage Coating.............................................122

Figure V.9 Apparent APSD for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using a Grounded ACI as a Function of Stage Coating..................................................123

Figure V.10 Mass Distribution and Percent Impactor Dose for > 1 μm Fluorescein Aerosol as a Function of Charge Level obtained using Uncoated Impaction Plates in a Grounded ACI.................................................125

Figure V.11 Apparent APSD for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using a Grounded ACI with Uncoated Impaction Plates.................................................................126

Figure V.12 Electrostatic Charge and Mass Distribution for Fluorescein Aerosol as a Function of Charge Level obtained using the ELPI Following a Sampling Time of 5 Seconds.................................................................127

Figure V.13 Percent Impactor Dose for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the Grounded ACI as a Function of Stage Loading.................................................................129
Figure V.14  Apparent APSD for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using a Grounded ACI as a Function of Stage Loading…………………………………………………………………………………..130

Figure V.15  Mass Distribution and Percent Impactor Dose for > 1 μm Fluorescein Aerosol as a Function of Charge Level obtained Following 5 Second Sampling Time using a Grounded ACI…………………………………………………………………………………………………………………………………………………..131

Figure V.16  Apparent APSD for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using a Grounded ACI Following a Sampling Time of 5 Seconds………………………………………………………………………………………………………………………………………………………………………..132

Figure V.17  Apparent APSD for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using a Full Resolution and QC Abbreviated ACI…………………………………………………………………………………………………………………………………………………………………………………..135

Figure AII.1  Schematic Diagram of the ELPI representing (a) Modified ELPI Configuration i.e., Without Corona Charger and (b) With Corona Charger, used for the Determination of Apparent APSD of QVAR 80 pMDI…………………………………………………………………………………………………………………………………………………………………………………………………………………..163

Figure AII.2  Mass Distribution and % Impactor Deposition for QVAR 80 pMDI determined using the Modified ELPI (Without Corona Charger) and ELPI With Corona Charger (at 0 kV)………………………………………………………………………………………………………………………………………………………………………………………………………………………………………..166

Figure AII.3  Apparent APSD for Inherently Charged QVAR 80 pMDI determined using the Dekati Cut-off Diameters with Modified ELPI i.e., Without Corona Charger, With Corona Charger, and using Recalibrated Cut-off Diameters with the Modified ELPI and ELPI With Corona Charger…………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………..167

Figure AII.4  Apparent APSD for Artificially Charged QVAR 80 pMDI determined using the Dekati Cut-off Diameters and Recalibrated Cut-off Diameters using the ELPI with the Corona Charger………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………………..168
## List of Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>®</td>
<td>Registered trademark</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>ρ</td>
<td>True density of the particles</td>
</tr>
<tr>
<td>ρₚ</td>
<td>Density of particle</td>
</tr>
<tr>
<td>ρₚₛ</td>
<td>Density of solute</td>
</tr>
<tr>
<td>µA</td>
<td>microampere</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>µL</td>
<td>microliter</td>
</tr>
<tr>
<td>µm</td>
<td>micrometer</td>
</tr>
<tr>
<td>% DFN</td>
<td>% Difference from nominal</td>
</tr>
<tr>
<td>% RSD</td>
<td>% Relative standard deviation</td>
</tr>
<tr>
<td>ACI</td>
<td>Andersen Cascade Impactor</td>
</tr>
<tr>
<td>AIM</td>
<td>Abbreviated Impactor Measurement</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APSD</td>
<td>Aerodynamic particle size distribution</td>
</tr>
<tr>
<td>BDP</td>
<td>Beclomethasone dipropionate</td>
</tr>
<tr>
<td>e⁻</td>
<td>Charge on an electron</td>
</tr>
<tr>
<td>E</td>
<td>Electric field</td>
</tr>
<tr>
<td>e.g.</td>
<td>For example</td>
</tr>
<tr>
<td>e/particle</td>
<td>Charge per particle</td>
</tr>
<tr>
<td><em>et al.</em></td>
<td>and others</td>
</tr>
<tr>
<td>E-SPART</td>
<td>Electrical single particle aerodynamic relaxation time</td>
</tr>
<tr>
<td>C</td>
<td>Coulomb</td>
</tr>
<tr>
<td>C/g</td>
<td>Coulomb per gram</td>
</tr>
<tr>
<td>Cₛ</td>
<td>Concentration of the solute</td>
</tr>
<tr>
<td>CDF</td>
<td>Cumulative distribution function</td>
</tr>
<tr>
<td>CFC</td>
<td>Chlorofluorocarbons</td>
</tr>
<tr>
<td>CFD</td>
<td>Computational fluid dynamics</td>
</tr>
<tr>
<td>COD</td>
<td>Coefficient of determination</td>
</tr>
<tr>
<td>D₅₀</td>
<td>Cut-off diameter</td>
</tr>
<tr>
<td>Dₐ</td>
<td>Aerodynamic diameter</td>
</tr>
<tr>
<td>Dₚ</td>
<td>Droplet diameter</td>
</tr>
<tr>
<td>Dₛ</td>
<td>Physical diameter</td>
</tr>
<tr>
<td>DPI</td>
<td>Dry powder inhalers</td>
</tr>
</tbody>
</table>

### Abbreviations
- **ACI**: Andersen Cascade Impactor
- **AIM**: Abbreviated Impactor Measurement
- **ANOVA**: Analysis of variance
- **APSD**: Aerodynamic particle size distribution
- **BDP**: Beclomethasone dipropionate
- **C**: Coulomb
- **C/g**: Coulomb per gram
- **Cₛ**: Concentration of the solute
- **CDF**: Cumulative distribution function
- **CFC**: Chlorofluorocarbons
- **CFD**: Computational fluid dynamics
- **COD**: Coefficient of determination
- **D₅₀**: Cut-off diameter
- **Dₐ**: Aerodynamic diameter
- **Dₚ**: Droplet diameter
- **Dₛ**: Physical diameter
- **DPI**: Dry powder inhalers
- **e⁻**: Charge on an electron
- **E**: Electric field
- **e.g.**: For example
- **e/particle**: Charge per particle
- ***et al.***: and others
- **E-SPART**: Electrical single particle aerodynamic relaxation time
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDA</td>
<td>Efficient data analysis</td>
</tr>
<tr>
<td>ELPI</td>
<td>Electrical Low Pressure Impactor</td>
</tr>
<tr>
<td>eNGI</td>
<td>electrical Next Generation Impactor</td>
</tr>
<tr>
<td>FPD</td>
<td>Fine particle dose</td>
</tr>
<tr>
<td>g/cm³</td>
<td>gram per cubic centimeter</td>
</tr>
<tr>
<td>GSD</td>
<td>Geometric standard deviation</td>
</tr>
<tr>
<td>HFA</td>
<td>Hydrofluoroalkanes</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-pressure liquid chromatography</td>
</tr>
<tr>
<td>IPAC-RS</td>
<td>International Pharmaceutical Aerosol Consortium on Regulation and Science</td>
</tr>
<tr>
<td>ISM</td>
<td>Impactor sized mass</td>
</tr>
<tr>
<td>$K_E$</td>
<td>Constant of proportionality</td>
</tr>
<tr>
<td>kPa</td>
<td>kiloPascal</td>
</tr>
<tr>
<td>kV</td>
<td>kiloVolt</td>
</tr>
<tr>
<td>L/min⁻¹</td>
<td>Liter per minute</td>
</tr>
<tr>
<td>LPM</td>
<td>Large particle mass</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>mbar</td>
<td>millibar</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>mg/min</td>
<td>milligram per minute</td>
</tr>
<tr>
<td>mL</td>
<td>milliliter</td>
</tr>
<tr>
<td>mL/min⁻¹</td>
<td>milliliter per minute</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter</td>
</tr>
<tr>
<td>MMAD</td>
<td>Mass median aerodynamic diameter</td>
</tr>
<tr>
<td>MMD</td>
<td>Mass median diameter</td>
</tr>
<tr>
<td>MSC</td>
<td>Model selection criteria</td>
</tr>
<tr>
<td>MSLI</td>
<td>Multistage Liquid Impinger</td>
</tr>
<tr>
<td>N</td>
<td>Normal</td>
</tr>
<tr>
<td>$N_s$</td>
<td>Saturation charge</td>
</tr>
<tr>
<td>nC</td>
<td>nanocoulomb</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>ng/ml</td>
<td>nanogram per milliliter</td>
</tr>
<tr>
<td>NGI</td>
<td>Next Generation Impactor</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer</td>
</tr>
<tr>
<td>pMDI</td>
<td>Pressurized metered dose inhalers</td>
</tr>
<tr>
<td>pA</td>
<td>picoampere</td>
</tr>
<tr>
<td>pC</td>
<td>picocoulomb</td>
</tr>
<tr>
<td>particles/cm³</td>
<td>particles per cubic centimeter</td>
</tr>
<tr>
<td>PQRI</td>
<td>Product Quality Research Institute</td>
</tr>
<tr>
<td>psig</td>
<td>pound-force per square inch gauge</td>
</tr>
<tr>
<td>q/m</td>
<td>charge per mass</td>
</tr>
<tr>
<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>RH</td>
<td>Relative Humidity</td>
</tr>
<tr>
<td>sec</td>
<td>second</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SPM</td>
<td>Small Particle Mass</td>
</tr>
<tr>
<td>St</td>
<td>Stokes number</td>
</tr>
</tbody>
</table>
TSI  Twin Stage Impinger
USP  United States Pharmacopoeia
UV   ultraviolet
V    Volume
VHC  Valved Holding Chambers
VOAG Vibrating Orifice Aerosol Generator
vs   versus
w/v  weight per volume
w/w  weight per weight
INFLUENCE OF ELECTROSTATIC CHARGE UPON THE DEPOSITION BEHAVIOR
OF PHARMACEUTICAL AEROSOLS WITHIN CASCADE IMPACTORS

By Megha Mohan, B.Pharm.

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

Virginia Commonwealth University, 2012

Major Director: Joanne Peart, Ph.D.
Associate Professor, Department of Pharmaceutics

Co-Director: Peter R. Byron, Ph.D.
Professor, Department of Pharmaceutics

Cascade impactors, routinely used for in vitro particle size characterization of pharmaceutical aerosols, are calibrated using dilute, charge-neutralized, monodisperse aerosols. But pharmaceutical aerosols are known to generate concentrated, inherently charged, polydisperse aerosol clouds. A computational model of the Andersen Cascade Impactor (ACI) suggested that the presence of charge on aerosol particles may influence their deposition within the ACI, but experimental validation of the model is warranted. This dissertation investigates the
influence of electrostatic charge upon the deposition behavior of aerosols within cascade impactors, to address the impact of charge on particle size characterization.

The influence of applied charge upon the deposition pattern and aerodynamic particle size distribution (APSD) of commercially available pressurized metered dose inhalers (pMDIs) within the Electrical Low Pressure Impactor (ELPI) was examined. Electrostatic properties were modified using an external voltage source in conjunction with the ELPI corona charger and observed to be dependent on the formulation and device packaging. Induced artificial charge on the aerosol particles influenced the deposition pattern within the impactor, but did not result in a significant change in the apparent APSD.

An experimental apparatus capable of producing charge neutralized and charged aerosol, with targeted deposition on the CFD predicted ‘charge sensitive’ ACI stages, was developed. In vitro results were observed to be in partial agreement with the CFD predictions. While charge influenced the deposition pattern in the ACI with increased deposition observed in the charger and on the upper stages of the ACI, it did not influence the apparent APSD of the aerosol. Electrostatic charge effects on deposition behavior within cascade impactors were delineated with respect to space charge and image charge effects by investigating the influence of impactor grounding, particle size, stage coating and loading. While the deposition pattern within the ACI was influenced by charge, only stage coating and stage loading resulted in a small, significant difference in the apparent APSD, which may not be practically relevant due to the variability associated with in vitro aerosol testing. Similar trends were observed in the deposition behavior of charge neutralized and charged aerosol within an abbreviated ACI system compared to the full resolution ACI.
I. INTRODUCTION

I.A Significance and Background

Aerosol electrostatics is an important property of pharmaceutical aerosols (Mitchell et al. 2007). Aerosols produced from pressurized metered dose inhalers (pMDIs), dry powder inhalers (DPIs) and nebulizers carry electrostatic charge, which is known to be a function of both the formulation and the packaging components (Byron et al. 1997, Peart et al. 1998, Glover and Chan 2004, Orban and Peart 2004, Kwok et al. 2005, Telko et al. 2007, Young et al. 2007, Kwok and Chan 2008, Kwok et al. 2010). Electrostatic charge has been reported to influence the dosing reproducibility of pMDIs and influence their deposition within valved holding chambers (VHCs), either due to the charge generated on the aerosol particles or the charge present on the insulating surface of VHCs (Dolovich et al. 2000, Bisgaard et al. 2002). Electrostatic charge has been shown to influence deposition of aerosols in animals, humans, hollow-cast lung models and numerical models (Vincent et al. 1981, Melandri et al. 1983, Balachandran et al. 1991, Hashish et al. 1994a, Balachandran et al. 1997, Bailey et al. 1998, Cohen et al. 1998). However, the influence of charge upon particle deposition within cascade impactors, which are routinely used to characterize the aerodynamic particle size distribution (APSD) of pharmaceutical aerosols, is unknown.

Cascade impactors are used to characterize the particle size distribution of pharmaceutical aerosols by fractionating them based on their aerodynamic size (USP 29 / NF 24 First Supplement 2006) and are conventionally calibrated using dilute, charge neutralized,
monodisperse aerosols of known size (Mitchell and Nagel. 2003, Marple and Olson 2009). Electrostatic charge has been suggested as one of the many factors which may account for variability in mass balance and APSD measurements obtained using conventional cascade impactors (Christopher et al. 2003, Bonam et al. 2008), but the evidence is largely anecdotal and there are no in vitro studies mapping aerosol deposition within compendial cascade impactors as a function of charge. A computational fluid dynamics (CFD) model was developed to investigate the airflow pattern and particle deposition within the Andersen Cascade Impactor (ACI), and extended to predict the influence of charge upon deposition within the ACI (Vinchurkar et al. 2009). The CFD model predicted increased deposition across the stages of the ACI as a result of particle charging (Vinchurkar et al. 2009). While computational models offer the potential to predict the deposition behavior of aerosols based upon their aerodynamic size and electrostatic charge, they are limited by the lack of comprehensive in vitro experimental validation.

This dissertation will focus on the investigation of aerosol deposition behavior and the resultant apparent APSD, of charged aerosols compared with charge-neutralized aerosol, within cascade impactors, while also evaluating the accuracy of the CFD model of the ACI.

I.B Cascade Impactor Calibration and Role of Electrostatic Charge in Sizing

Cascade impactors are the pharmacopoeial instruments of choice for in vitro particle size analysis of pharmaceutical aerosols produced by medical inhalers (USP 29 / NF 24 First Supplement 2006). Briefly, a cascade impactor consists of a series of size classification stages consisting of a jet plate and an impaction plate. As the aerosol stream passes through a stage, particles with sufficient inertia impact and deposit on the plate, while finer particles follow the airstream to the next stage (Hinds 1982). The jet sizes decrease so that the jet velocities increase
for stages successively, thereby causing progressively smaller particles to deposit on each stage and, finally on a filter beyond the last stage (Hinds 1982). Each stage is characterized by a cut-off diameter which is defined as the diameter at which the collection efficiency is observed to be 50% (Marple et al. 1998). Collection efficiency curves, typically obtained by plotting the square root of Stokes number (St) [Stokes number (St), is a dimensionless entity described as the ratio of the stopping distance of a particle to the jet diameter] against the efficiency (%) of particles collected, are not perfectly sharp step functions (Marple et al. 1998). Consequently, the sample mass collected on a particular stage of the cascade impactor may not directly correspond to the sample mass in the size fraction denoted by the cut-off diameter for the impactor stage, since each stage of the impactor collects a fraction of those particles that should have ideally been collected on the stage above, and passes a fraction of those particles, which should have ideally been collected (Marple et al. 1998). Calibration of cascade impactors is necessary to evaluate their performance in accord with impaction theory and to accurately determine their particle collection characteristics.

During cascade impactor calibration, the collection efficiency characteristics of the impactor are determined by sampling stable, fluorescent-labeled, dilute, charge neutralized monodisperse aerosols of known size at a nominal flow rate, followed by determination of the ratio of mass of particles deposited on the stage being calibrated to those that approached the stage, by chemical analysis (Marple et al. 1998). An advantage of using fluorescent-labeled monodisperse aerosols is that interstage losses, within the impactor can also be determined. Alternative methods, such as that adopted in the calibration of the multistage liquid impinger (MSLI), involve counting the number of particles upstream and downstream of the stage.
However this method does not characterize interstage losses and relies on the accuracy of the optical instrument used for counting (Asking and Olsson 1997, Marple et al. 1998).

The Andersen Cascade Impactor (ACI, sometimes called the Andersen Eight Stage Non-Viable Sampler or Mark II ACI), though not originally designed for pharmaceutical aerosols, is widely used for the characterization of inhaled products and consists of eight stages, a filter, and can be used with a preseparator (Andersen 1958). The Mark II ACI was originally calibrated by counting and sizing wax spheres using optical microscopy together with the numerical methods described by Ranz and Wong (Ranz and Wong 1952). The Mark II ACI has also been calibrated using monodisperse fluorescent-tagged particles, monodisperse polystyrene latex particles and polydisperse aerosols (Mitchell et al. 1988, Vaughan 1989). The Next Generation Pharmaceutical Impactor (NGI) was specifically designed for pharmaceutical inhaler testing and can operate at any flow rates between $15-100 \text{ Lmin}^{-1}$ because the aerodynamic properties of the impactor followed established scientific principles, (Marple et al. 1998, Marple et al. 2003a, Marple et al. 2003b, Marple et al., 2004). In the archival NGI calibration, collection efficiency was experimentally determined for particles in the size range of $0.05-20 \mu m$, for each stage using monodisperse, charge neutralized oleic acid particles tagged with uranine dye.

Abbreviated impactor measurement (AIM) has recently been proposed as an alternative strategy to full resolution cascade impactors for routine quality control testing (Tougas et al. 2009, Tougas et al. 2011). AIM-based systems (Figure I.1) divide the aerosol cloud directly into two fractions: the small (fine) and large (coarse) particle mass and, therefore, eliminate stages where little or no drug deposits. A number of options exist for performing abbreviated impactor measurements (AIM) including the modification of an existing ACI or NGI by reducing the number of stages to commercially available AIM systems including the Fast Screening Impactor.
(MSP Corporation, Shoreview, MN) and the Short Stack Fast Screening Andersen Impactor (Copley Scientific, Nottingham, United Kingdom). Calibration data is not available for all the abbreviated systems.

![Image](a) Fast Screening Impactor (MSP Corporation) and (b) Short-Stack Fast Screening Andersen Impactor (Copley Scientific)

Figure I.1 Abbreviated Impactor Measurement (AIM) Devices (a) Fast Screening Impactor (MSP Corporation) and (b) Short-Stack Fast Screening Andersen Impactor (Copley Scientific)

Impactor calibration is undertaken using monodisperse droplets generated using instruments such as a vibrating orifice aerosol generator (VOAG) (Berglund and Liu 1973). Because such droplets have been shown to possess electrostatic charge (Berglund and Liu 1973, Liu and Pui 1974), the droplets are neutralized prior to use with a Kr-85 charge neutralizer to avoid unwanted electrostatic effects on aerosol deposition. The importance of charge neutralization of the generated aerosol during impactor calibration was observed during the calibration of PC-2 Quartz Crystal cascade impactor (Horton and Mitchell 1992). Preliminary experiments undertaken during the calibration in the absence of a neutralizer showed that the presence of electrostatic charge on non-neutralized monodisperse 5 \( \mu \text{m} \) oleic acid droplets...
caused them to deposit on stages 1 and 2 of the impactor and not the expected stages 3 and 4 based on the manufacturer’s collection efficiency data (California Measurements, 1982). Since the droplets were confirmed to be monodisperse, within 5% of the target size, and deposited on the periphery of stages 1 and 2, electrostatic charge was suspected as the cause for the deposition of droplets on higher stages. Droplets were sampled into a Faraday-cup electrometer (Model 3068, TSI Inc.), and an electrostatic charge of about 3500 to 4000 electrons per particle was recorded. Following charge neutralization, the average charge per droplet decreased to approximately 100 electrons per particle and droplets deposited on stages 3 and 4 i.e., the expected location. While the results of this study suggested that electrostatic charge may influence deposition of aerosols within cascade impactors, a systematic study exploring the effect of electrostatic charge in inertial impactors is warranted because pharmaceutical aerosols are known to carry inherent charge in contrast to the charge neutralized calibration aerosols (Byron et al. 1997, Peart et al. 1998).

Electrostatic charge has also been suggested to cause variability in mass balance and aerodynamic particle size distribution (APSD) measurements obtained using cascade impactors. The Product Quality Research Institute (PQRI) Particle Size Distribution Mass Balance Working Group suggested that charge accumulation may influence mass balance and APSD, due to wall losses, resulting in a possible mass balance failure (Christopher et al. 2003). A working group of the International Pharmaceutical Aerosol Consortium on Regulation and Science (IPAC-RS) reported that electrostatic charge acquired by non-conducting or electrically insulated cascade impactor components may influence deposition of aerosols within the impactor, resulting in variability in the measured APSD of aerosols and recommended exploratory studies (Bonam et al. 2008).
Further evidence of the influence of charge upon deposition of aerosols within cascade impactors is provided by the recalibration of the Electrical Low Pressure Impactor (ELPI, Dekati Ltd, Finland) (Kotian et al. 2009). The ELPI was originally designed to be used with a corona charger to enable electrical detection, and was calibrated with corona charged, monodisperse aerosols (not charge neutralized aerosols) (Keskinen et al. 1992, Keskinen et al. 1999). However, the ELPI is frequently used to simultaneously characterize the size and inherent charge of pharmaceutical aerosols, with the charger removed. Kotian et al. reported that the ELPI undersized commercially available pharmaceutical aerosols when used in this modified configuration i.e., without the corona charger, compared to the ACI, with chemical detection used to determine the size distribution (Kotian et al. 2009). The modified ELPI was recalibrated with four polydisperse commercially available pMDIs, using the ACI as a reference impactor. The recalibrated mean cut-off diameters for ELPI appeared to deviate increasingly from those supplied by the manufacturer as the aerodynamic diameter decreased, suggesting that the deposition of charged particles may differ from charge neutral particles of the same size. It is possible therefore, that the deposition and apparent APSD of inherently charged aerosols may be a function of electrostatic charge, in addition to aerodynamic size. A well-controlled in vitro study quantifying the effects of electrostatic charge upon deposition within cascade impactors is warranted to gain a better understanding of the regulatory implications of aerosol electrostatics.

I.C  Mechanisms of Aerosol Charging

Aerosols acquire charge by three primary mechanisms: triboelectrification, diffusion charging and field charging, the latter two being dependent on particle size (Cross 1987). The development of electrostatic charge on aerosol particles occurs primarily through
triboelectricity, wherein charge transfer takes place as a result of contact and frictional separation of dissimilar materials resulting in the transfer of charges between surfaces to equalize their Fermi levels (Cross 1987). During triboelectrification, the polarity, as well as the magnitude of charge developed, depends upon the physical and chemical properties of the materials involved. However, triboelectrification is uncontrolled and not well understood (Cross 1987).

Diffusion charging of aerosol particles occurs in the presence of unipolar or bipolar ions as a result of collisions between ions and particles due to the random thermal motion of the ions in the gas (Cross 1987). It does not depend on the nature of the material and does not require an external electric field. The acquired electrostatic charge is proportional to the diameter of particles and is an important charging mechanism for particles smaller than 1 µm. With increasing diffusion charging, accumulation of charge produces a field around the particle which slowly reduces the charging rate (Cross 1987).

In contrast to diffusion charging, field charging occurs in the presence of a strong electric field, which causes rapid movement of ions and hence frequent collisions between ions and particles (Cross 1987). The acquired charge developed by field charging, in the presence of unipolar ions, is proportional to the square of the particle diameter and is considered to be an important charging mechanism for particles larger than 1 µm. As particles become charged, they repel incoming like-charged ions thus reducing the rate of particle charging (Cross 1987).

Once developed, the presence of charge on a particle may influence its interaction either with other particles or, with a nearby surface resulting in space charge and image charge effects, respectively (Bailey 1997, Bailey et al. 1998). Space charge effects involve repulsion between like charged particles in a dense aerosol cloud (typical particle concentration $> 10^5$ particles/cm$^3$) resulting in deposition, whereas image charge effects involve attraction between a charged
particle and an equal and opposite induced image charge on a neutral surface causing deposition of the particle on the surface (Bailey et al. 1998). Image charge effects are considered to be more relevant for deposition within the smaller airways where charged particles are close to the airway surface (Bailey et al. 1998), while space charge effects may be more pronounced for pharmaceutical aerosols, which are highly concentrated, bolus aerosol clouds.

I.D Influence of Electrostatic Charge upon Aerosol Deposition in the Respiratory Tract

Electrostatic attraction is one of five mechanisms considered to influence particle deposition in the lung airways (Hinds 1982). Several studies have been undertaken in order to address the influence of charge on the site or extent of deposition of aerosols within the respiratory tract using animals, humans, hollow-cast lung models as well as theoretical models (Fraser et al. 1966, Yu and Chandra 1977, Yu 1978, Vincent et al. 1981, Melandri et al. 1983, Balachandran et al. 1991, Hashish et al. 1994a, Hashish et al. 1994b, Balachandran et al. 1997, Bailey 1997, Bailey et al. 1998, Cohen et al. 1998)

Fraser et al. reported that the total deposition in the respiratory tract doubled for charged dust particles (carrying approximately 1000 elementary charges/particle) compared to uncharged particles in rabbits (Fraser et al. 1966). Vincent et al. reported an increase of 40% in the deposition for asbestos particles carrying a net charge of approximately 60 electrons per particle compared to neutral particles in rats, which was attributed to image charge effects (Vincent et al. 1981).

Melandri et al. showed that the deposition of monodisperse 0.3-1.1 μm carnauba wax particles increased by approximately 15 to 30%, when the charge level was increased from 30 to approximately 100 electrons per particle in human subjects (Melandri et al. 1983). The authors
ascribed the enhanced deposition to image charge effects rather than space charge effects since the deposition increased with increasing particle charge, while particle concentration had no effect on deposition. However, only net deposition was measured in the study and the specific location of deposition within the respiratory tract was not established.

Cohen et al. used a hollow ‘plastic’ airway cast lined with a conducting layer, based on dimensions obtained from a human cadaver, extending from the trachea to the sixth generation of the lung to investigate the influence of electrostatic charge upon deposition within the lungs (Cohen et al. 1998). In vitro deposition of monodisperse ultrafine fluorescein aerosol particles, 20 and 125 nm in size, carrying single positive electronic charges was significantly higher when compared to zero-charged as well as charge neutralized particles. (Cohen et al. 1998).

In addition to the experimental studies, a number of theoretical models have been developed to investigate the role of electrostatic attraction in the deposition of aerosol particles in the airways. Yu and Chandra developed a theoretical model of the airways to predict the deposition of charged particles compared to neutral particles during a complete inspiration period. It was predicted that at a low particle concentration, only image charge effects caused any increase in deposition and space charge effects were negligible. The authors also reported that image charge effects were negligible for aerosols carrying less than 10 elementary charges per particle (Yu and Chandra 1977).

Computational models to predict the influence of electrostatic charge upon the deposition of aerosols have also been described. Balachandran et al. developed a computational model to predict the deposition of aerosols in the respiratory tract based upon impaction, sedimentation, diffusion, space charge and image charge effects (Balachandran et al. 1991, Balachandran et al. 1997). The model predicted that the deposition of 2.2 μm particles carrying 200 electrons per
particle increased in the upper airways due to space charge effects and in the lower airways due to image charge effects (Balachandran et al. 1997). The authors also predicted a shift in the deposition pattern for highly charged particles holding charge equal to half of Gauss’ limit, which is the maximum charge a particle can hold. It was speculated that under these conditions, maximum deposition would occur in the upper respiratory tract, perhaps due to space charge effects dominating over image charge effects. The computational model developed by Balachandran et al. included only the inhalation phase. Computational models have also been developed to account for the complete respiratory cycle i.e., inhalation as well as exhalation (Hashish et al. 1994a, Hashish et al. 1994b, Bailey 1997, Bailey et al. 1998). Image charge effects were considered to become more pronounced in the peripheral regions of the respiratory tract, where image charges of equal magnitude and opposite polarity to approaching charged particles may be induced on the surface of smaller airways, due to the proximity of the charged particle to the surface (Bailey 1997, Bailey et al. 1998). A similar increase in airway deposition was predicted by Bailey et al. for 0.5 μm and 5 μm particles carrying 200 and 3000 electrons per particle respectively, compared to neutral particles (Bailey et al. 1998). The authors speculated that increasing the charge levels on aerosol particles may be useful in providing improved control over the clinical administration of aerosols (Bailey et al. 1998). In another study, it was reported that the human scintigraphic results of inhaled radiolabelled droplets deposition was in agreement with computational predictions of total deposition and deposition in the ‘head’ i.e., the extrathoracic region, but the in vivo alveolar deposition was observed to be higher than that predicted by the computational model (Bailey 1997). However, the charges on the radiolabelled droplets used in the study were not reported.
An important limitation of the computational studies discussed is the lack of experimental validation. These studies assume stable, monodisperse particles to describe the deposition behavior of charged aerosols, whereas pharmaceutical aerosols are known to be polydisperse and may undergo size changes due to evaporation or moisture absorption. Another limitation with the theoretical and computational models is that the predicted results were primarily empirical and specific deposition location was not discussed. This could be attributed to the lack of knowledge of the charge distribution among different particle size fractions when these numerical models were developed. More recently, the computational approach has been employed to understand the influence of electrostatic charge upon the deposition behavior of aerosols within a cascade impactor.

I.E Computational Fluid Dynamics (CFD) Model of the Andersen Cascade Impactor (ACI)

The influence of charge upon the deposition of pharmaceutical aerosols within the ACI has been quantified using computational fluid dynamics (CFD) (Vinchurkar et al. 2009). The CFD model of the ACI simulated incompressible and laminar transitional flow within the USP induction port and eight stages of the ACI at 28.3 Lmin\(^{-1}\) (Vinchurkar et al. 2009). Particle trajectories and deposition were evaluated using a well-tested Lagrangian tracking approach that accounted for impaction, sedimentation, diffusion and electrostatic attraction, and corrected for near-wall interpolation effects. The effect of electrostatic charge was assumed to influence deposition through the interaction of the particle and a neutral conducting wall i.e., the image charge was considered, but space charge effects were ignored based upon the assumption of dilute aerosol conditions and the fact that the aerosol particles were considered to be in close
proximity to the impaction stages of the ACI, and were more likely to deposit by image charge, rather than space charge effects. Space charge effects within an impactor, may be described as particle-particle interactions, which may lead to repulsion during the deposition of like charged particles as ‘heaps’ below the jets on an impactor stage. Monodisperse, neutral, spherical particles in the size range 0.3 to 12 μm were assumed for the prediction of the deposition fraction and hence, the cut-off diameter for each stage of the ACI. The CFD model of the ACI was validated by comparing the CFD predicted cut-off diameters for the ACI stages with the existing experimental calibration (Vaughan 1989) as well as the USP specifications based on the manufacturer supplied cut-off diameters (Andersen Inc. 1985). While the CFD predicted stage cut-off diameters were found to be within 11% difference of the experimentally determined stage cut-off diameters, deviations of up to approximately 20% were observed for the CFD predicted cut-off diameters compared to the manufacturer data.

The CFD model was extended to quantify the influence of the saturation charge level, which was assumed to be a reasonable approximation of realistic charge levels taken from the literature, on deposition within the ACI. Since triboelectrification is not well understood and cannot be modeled, field charging of the particles was assumed in the CFD study. During field charging, particles accept ions from the gases in the surrounding electric field. The charge on the particle increases to a point when it can no longer accept incoming ions, and is said to be at saturation charge level. The saturation charge level ($N_s$) is defined by Equation I.1.

$$N_s = \left( \frac{\varepsilon}{\varepsilon+2} \right) \left( \frac{Ed^2}{4K_Ee} \right)$$

Equation I.1

where, $\varepsilon$ is the relative permittivity of the material comprising the particle, $E$ is the electric field strength, $K_E$ is a constant of proportionality, $e$ is the charge on an electron, and $d$ is the diameter of the particle. Different charge levels were used to approximate the size dependent charges for
the modeled aerosol particles. Predicted deposition in a simplified tubular geometry was compared with the analytical expression of Chen and Yu to ensure that the electrostatic image force was correctly modeled. Particles in the size range 0.05 - 10 μm, carrying 500 elementary charges were assumed in this validation study and good agreement was found between the theoretical predictions and the CFD model for charged aerosol deposition (Chen and Yu 1972). It was concluded that the CFD model was able to predict aerosol deposition as a function of image charge effects, in a way that was consistent with other theoretical treatments of electrostatic deposition accuracy. Monodisperse, spherical, charged particles in the size range 0.3 - 12 μm were then assumed to predict the influence of charge on deposition within the ACI. The saturation charge level was assumed to be representative of DPI aerosols. For stages 0 and 1 of the ACI, the CFD model predicted an approximately 5% increase in the deposition fraction of charged, compared to neutral, aerosol particles. An approximately 30% increase in the deposition fraction of charged particles compared to neutral particles was predicted for Stages 2 and 3. For Stages 4 and 5, the model predicted a 90% increase in deposition fraction, while on Stages 6 and 7, 100% increase in deposition of charged aerosol particles in the submicron range was predicted. Numerical predictions of cut-off diameters (d_{50}) for neutral and charged aerosols were determined from the linear section of S-shaped deposition curves for each impactor stage, and are shown in Table I.1.
Table I.1  CFD predicted Stage Cut-off Diameters for Neutral and Saturation Charged Powder Aerosol Particles compared to the USP Specifications.

<table>
<thead>
<tr>
<th>ACI Stage</th>
<th>USP $d_{50}$ (µm)</th>
<th>CFD Predicted Neutral $d_{50}$ (µm)</th>
<th>% Difference$^b$</th>
<th>CFD Predicted Charged$^a$ $d_{50}$ (µm)</th>
<th>% Difference$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>9.0</td>
<td>8.79</td>
<td>2.3</td>
<td>8.29</td>
<td>7.9</td>
</tr>
<tr>
<td>Stage 1</td>
<td>5.8</td>
<td>5.43</td>
<td>6.3</td>
<td>5.30</td>
<td>8.6</td>
</tr>
<tr>
<td>Stage 2</td>
<td>4.7</td>
<td>5.67</td>
<td>20.6</td>
<td>5.38</td>
<td>14.5</td>
</tr>
<tr>
<td>Stage 3</td>
<td>3.3</td>
<td>3.3</td>
<td>0</td>
<td>3.05</td>
<td>7.6</td>
</tr>
<tr>
<td>Stage 4</td>
<td>2.1</td>
<td>1.86</td>
<td>10.9</td>
<td>0.14</td>
<td>93.3</td>
</tr>
<tr>
<td>Stage 5</td>
<td>1.1</td>
<td>1.00</td>
<td>9.0</td>
<td>0.56</td>
<td>49.1</td>
</tr>
<tr>
<td>Stage 6</td>
<td>0.7</td>
<td>0.59</td>
<td>15.7</td>
<td>NA$^d$</td>
<td>NA$^d$</td>
</tr>
<tr>
<td>Stage 7</td>
<td>0.4</td>
<td>0.44</td>
<td>10.0</td>
<td>NA$^d$</td>
<td>NA$^d$</td>
</tr>
</tbody>
</table>

$^a$Charge believed representative of DPI particles,

$^b$Percent Difference compared to USP $d_{50}$,

$^c$Percent Difference compared to Neutral $d_{50}$,

$^d$Cut-off diameter could not be calculated because all particles deposited

The resulting CFD predicted stage cut-off diameters obtained for charged aerosol particles were used to approximate the deviation in the ACI measured aerodynamic size compared to charge neutralized aerosols. Correction curves to be used along with the particle size distribution data obtained using the ACI were provided, to account for the effect of particle charge on deposition and sizing. CFD model predictions suggested that the presence of inherent charge on pharmaceutical aerosols may be a confounding factor during in vitro testing using cascade impactors, which are calibrated with charge neutralized aerosols.

I. F  Characterization of Inherent Electrostatic Charge on Pharmaceutical Aerosols

Over the last decade, the literature concerning the inherent electrostatic properties of pharmaceutical aerosols and their characterization methods has greatly expanded. Charge generation in pharmaceutical aerosols has been reported to be a function of a number of factors including the drug properties (particle size, shape, surface roughness, area of contact),

A number of instruments have been used to characterize electrostatic properties of pharmaceutical aerosols, which can be divided into static and dynamic techniques depending upon the principle of operation used.

Dynamic methods e.g., bipolar charge measurement system and the Electrical Single Particle Aerodynamic Relaxation Time (E-SPART) analyzer, rely on the measurement of the electrical mobility of the individual charged particles (Balachandran et al. 2003, Ali et al. 2007)). In general, the electrical mobility of a particle depends on the charge that it carries, particle diameter and its migration velocity in an applied external electric field. Therefore, each charged particle possesses a unique electrical mobility, which can be calculated by measuring its migration velocity in an electric field. Furthermore, since this method characterizes the electrostatic properties of individual particles, bipolar charge on an aerosol cloud can also be assessed.

In the bipolar charge measurement system, aerosol is drawn through a sample inlet vertically at a flow rate of 60 Lmin$^{-1}$ into two electrostatic precipitators, with electrodes of opposite polarity in each of the precipitators (Balachandran et al. 2003). When a charged particle enters the precipitator, which is divided into five sections, it is subjected to an electric field generated between the electrode and the grounded cylindrical wall of the precipitator. The charge on the particle is measured at the site of deposition depending upon its electrical mobility. While the bipolar charge measurement system is capable of measuring the bipolar charge within
an aerosol cloud, its drawback lies in the fact that it requires size determination to be obtained by some other technique for the same aerosol (Balachandran et al. 2003).

The E-SPART enables simultaneous measurement of aerosol size and charge. Aerosol particles move vertically downwards in the air stream, and cross the path of two converging laser beams, while being simultaneously exposed to an alternating electric field, which causes charged particles to oscillate horizontally (Ali et al. 2007). The phase lag of the particle with respect to the field is used to determine the particle size, because the phase lag is proportional to the aerodynamic diameter of the particle (Ali et al. 2007). The field causes migration of charged particles in a certain direction depending on the polarity of the particle. Therefore, polarity and magnitude of charge on the particle can be derived from the migration velocity of the particle in the electric field, as well as its aerodynamic diameter. The E-SPART has been used to characterize electrostatic properties of commercial inhaler products (Ali et al. 2009). It was reported that for Atrovent® (pMDI) and Spiriva® (DPI), 6.4 and 42.2% of the total number of particles emitted were charged respectively, within which, 46% of the particles emitted from Atrovent® were estimated to be positively charged while 54% were negatively charged. In the case of Spiriva®, 60% of the particles were positively charged while 40% were observed to be negatively charged (Ali et al. 2009). One of the limitations with E-SPART is that the size distribution obtained is number based, whereas mass based distribution are used for pharmaceutical aerosols. Furthermore, the widespread use of E-SPART may be limited due to the requirement of a continuous aerosol for size and charge measurement whereas typical commercial inhaler products (pMDIs or DPIs) produce a short lived bolus of particles (Mitchell et al. 2007). It should also be noted that the size as well as charge distribution from both the E-
SPART and bipolar charge measurement system is not obtained from a drug specific mass based assay.

Static methods involve the transfer of charge from, or induction of charge, by the aerosol particles to the measurement device. Static measurement instruments include the Faraday pail, the aerosol electrometer sampling apparatus, the electrical low pressure impactor (ELPI), the modified twin stage impinger and the electrical next generation impactor (eNGI) (Keskinen et al. 1992, Peart et al. 1998, Orban and Peart 2004, Zhu et al. 2008, Hoe et al. 2009a, Hoe et al. 2009b, Hoe et al. 2010, Hoe et al. 2011).

Electrostatic properties of pressurized metered dose inhalers (pMDIs) and dry powder inhalers (DPIs) were first characterized using the aerosol electrometer sampling apparatus (Byron et al. 1997, Peart et al. 1998). The aerosol electrometer only characterizes the electrostatic properties of the < 5 μm size fraction, does not differentiate bipolar aerosol clouds and chemical analysis of the fine particle dose is not possible due to the method of dose capture (Peart et al. 1998). Hence, the relationship between aerosol charge and the drug mass cannot be elucidated using this technique.

I.F.1 Electrical Low Pressure Impactor (ELPI)

The Electrical Low Pressure Impactor (ELPI) overcomes some of the above-mentioned limitations as it enables the measurement of the net inherent charge of aerosols as a function of particle size. The ELPI is a 13 stage low pressure impactor, which fractionates aerosol particles, based on their aerodynamic diameter, in the range of 0.03 - 10 μm, with the lower 12 stages connected to a multichannel electrometer (Keskinen et al. 1992, Keskinen et al. 1999). The ELPI was originally designed with a corona charger atop the impactor stack, in order to charge
the sample aerosol by subjecting it to a high voltage of approximately +5 kV, following which real time size measurement was achieved using electrical detection (Keskinen et al. 1992). The induced current measured from each stage is converted into particle concentration as well as the particle size distribution. The ELPI may be considered as a vertical arrangement of Faraday wells, with each of its 13 electrically isolated stages, acting as a Faraday well.

The majority of studies using the ELPI for pharmaceutical aerosols, have utilized the ELPI in a modified configuration, wherein the corona charger is removed such that the inherent electrostatic charge on the pMDIs, DPIs and nebulizers can be characterized, while simultaneously determining the particle size distribution by chemical analysis of the drug deposited on each impactor stage, typical of cascade impaction experiments (Glover and Chan 2004, Orban and Peart 2004, Kwok et al. 2005, Telko et al. 2007, Kwok and Chan 2008, Kwok et al. 2010).

Electrostatic properties of pMDIs have been characterized using the modified ELPI. Ventolin CFC pMDI was observed to carry a net fine particle dose (FPD) charge of -0.18 ± 0.04 nC (defined as charge on particles < 4.04 μm) that was in agreement with data reported using the aerosol electrometer (-0.17 ± 0.01 nC) (Orban and Peart 2004). In addition, the aerosol cloud was determined to be bipolar. Kwok et al. characterized the charge and size distribution of commercially available HFA pMDIs including Intal®, Tilade®, Flixotide® and Ventolin® HFA suspension pMDIs as well as QVAR®, a solution pMDI, Qvar (Kwok et al. 2005). The authors reported that Flixotide (mean net charge=0.45±0.03 nC), Intal (mean net charge=1.15±0.08 nC) and Tilade (mean net charge=1.12±0.12 nC) produced bipolar aerosol clouds irrespective of the testing conditions i.e., single or continuous shots. Ventolin HFA (mean net charge= -1.10±0.22 nC), was shown to be more sensitive to the testing conditions and produced bipolar aerosol
clouds when single actuations were used while it produced net electronegative aerosol when continuous actuations were used. The authors attributed this to relaxation of counter charges generated within the formulation and the device components, by the actuation of albuterol sulfate formulation of Ventolin HFA, suggesting that their presence or absence in case of continuous and single actuations respectively, may influence the electrostatic charge measured with Ventolin HFA. The mean net charge values for Ventolin HFA were lower than those reported by Orban and Peart (Orban and Peart 2004); no explanation was provided. QVAR was observed to produce unipolar positive aerosol clouds (0.29 ± 0.23 nC) and showed agreement between charge and mass determined using the ELPI. It was suggested that the nature of formulation, i.e., solution or suspension, is likely to influence the charge to mass correlation.

The modified ELPI has also been used to characterize the electrostatic charge on DPIs (Young et al. 2007, Telko et al. 2007, Kwok and Chan 2008). One of the challenges associated with the use of the ELPI for testing DPIs remains its operational flow rate. The ELPI has been designed to operate at 10 Lmin⁻¹ or 30 Lmin⁻¹ whereas most of the DPIs are required to be tested at higher flow rates. Young et al. investigated the effect of storage relative humidity (ranging from 0-84% RH) on the electrostatic properties, as well as the aerosol performance, of experimental dry powder formulations delivered using a Cyclohaler® device using the ELPI at 30 Lmin⁻¹ and the NGI at a flow rate of 60 Lmin⁻¹. However, the pharmacopoeial specification for in vitro testing of DPIs requires that flow rate equivalent to a pressure drop of 4 kPa across the device should be used for aerosolization and testing protocol. In the case of the Cyclohaler, this flow rate is expected to be above 100 Lmin⁻¹ based on the relationship between device resistance and pressure drop (Clark and Hollingworth 1993). Not surprising then, less efficient device emptying using the ELPI at 30 Lmin⁻¹ to test the carrier based formulation delivered by
Cyclohaler compared to that attained using the NGI at 60 Lmin\(^{-1}\) was reported by Young et al. (Young et al. 2007).

Further studies have attempted to overcome the flow rate limitation of the ELPI such that a DPI formulation could be aerosolized at 60 Lmin\(^{-1}\) but sampled into the ELPI at 30 Lmin\(^{-1}\). Telko et al. investigated the influence of lactose type (milled vs. sieved), capsule fill weight (15 vs. 30 mg), capsule material (gelatin vs. carrageenan), drug load (0.5 vs. 1% w/w), and inhaler type (Inhalator vs. Rotahaler) on the electrostatic properties of albuterol sulfate - lactose blends by aerosolizing the DPI at 60 Lmin\(^{-1}\), followed by dividing the flow and sampling simultaneously into the ELPI and the ACI, each operated at 30 Lmin\(^{-1}\). The authors reported that lactose type, capsule material, drug load and inhaler type affected the magnitude of net charge while the polarity of charge was influenced by lactose type, capsule material and inhaler type.

Kwok and Chan investigated the influence of relative humidity (15-90\%) during aerosolization of Pulmicort\(^{®}\) and Bricanyl\(^{®}\) Turbohalers, using the ELPI. The DPIs were aerosolized at 60 Lmin\(^{-1}\) following which, the flow was divided using a custom-made Y-piece, thereby enabling sampling into the ELPI and a unit dose sampler, both connected to vacuum pumps operated at 30 Lmin\(^{-1}\) each. Charge profiles obtained for both Bricanyl and Pulmicort turbohalers were observed to be bipolar with the Bricanyl FPD charge found to decrease as the relative humidity increased. In contrast, a high specific charge was observed for Pulmicort at 15 and 90\% RH, with a minimum value recorded at 40\% RH.

The modified ELPI, operated at 30 Lmin\(^{-1}\), has also been used to characterize the electrostatic properties of droplets generated from commercial Ventolin, Bricanyl and Atrovent nebules, using a PARI LC Plus jet nebulizer at 50\% RH for a run time of 30 seconds (Kwok et al. 2010). The electrostatic charge of the three products investigated was dependent on the
concentration and physicochemical properties of the active drug and all three products produced net negatively charged aerosols. Additionally, it was observed that the charge magnitude decreased with increasing duration of nebulization. The numbers of elementary charges per droplet estimated in the study were deemed to be low to potentially affect lung deposition, based on previous numerical studies. It is important to note that the size distribution determined in the study may not be accurate due to the higher flow rate used and droplet evaporation effects within the impactor, since the testing protocol used is not in conformance with the current compendial specification as well as literature reported methods for in vitro testing of nebulizers (CEN 2001).

The use of the ELPI for simultaneous determination of charge and size is limited due to the fact that it is not a pharmacopoeial impactor. Moreover, since the ELPI was originally designed to sample industrial aerosols, it can only be operated at a low flow rate of 30 Lmin⁻¹ unlike the NGI, which has been designed to operate at flow rates between 15-100 Lmin⁻¹. Recently, attempts have been made to modify the existing compendial impactors such that simultaneous measurement of charge and size is feasible.

### I.F.2 Modified Twin Stage Impinger

The Twin Stage Impinger (TSI) was modified by Zhu et al (Zhu et al. 2008). The glass twin stage impinger has two stages with 6.4 μm as the aerodynamic cutoff diameter of the lower stage at an operational flow rate of 60 Lmin⁻¹. The two stages of the TSI were connected to an electrometer to enable measurement of induced charge by the particles deposited on these stages. While the charge measurements by the modified Twin Stage Impinger were shown to be comparable with those of the Faraday pail, it still suffered from the drawback of providing charge as a function of only two size fractions.
I.F.3 Electrical Next Generation Impactor (eNGI)

The concept of modifying an existing pharmacopoeial impactor to enable simultaneous measurement of electrostatic charge and size has been extended to the Next Generation Impactor (NGI) (Hoe et al. 2009a). The NGI includes a pre-separator for removing larger carrier particles, seven stages followed by a micro-orifice collector. The NGI was modified by enclosing it in an earthed steel cage and electrically isolating the USP throat from the body of the NGI using polypropylene adaptors (Hoe et al. 2009a). The outer surface of the collection cups was coated with acrylic latex to provide electrical isolation, except for a small region at the center of the cup base, where a connection with an electrometer probe was established (Hoe et al. 2009a). The electrometer was connected to a computer for processing the data in real time.

The accuracy of the eNGI was assessed by comparing the charge and size distributions of three commercial pMDIs including Flixotide, Ventolin HFA and QVAR obtained using the eNGI with that obtained using the ELPI and the NGI, respectively (Hoe et al. 2009a). The size distribution profiles were similar between the eNGI and the NGI for each of the three products with the MMAD statistically comparable between the two impactors, thus verifying that the modification of the collection cups in the eNGI did not affect the sizing capabilities of the NGI. Barring the discrepancy observed in the charge distribution obtained using the eNGI and ELPI for the top stages, there was reasonable similarity between the charge data obtained using the eNGI and the ELPI for the pMDI products investigated. However, no attempt was made to compare the data with the existing literature for these products. Nevertheless, the study demonstrated the advantages that the eNGI offers over the ELPI in terms of flow rate flexibility and simpler design.
Electrostatic properties of Pulmicort and Bricanyl Turbohalers were characterized using the eNGI while comparing the charge and size distribution against the ELPI and the NGI respectively at 30 Lmin\(^{-1}\) (Hoe et al. 2009b). The size distribution profiles for both Pulmicort and Bricanyl turbohalers were similar between the eNGI and the NGI at 30 Lmin\(^{-1}\). However, there were discrepancies between the size distribution obtained using the ELPI compared with both the eNGI and the NGI. The mass of drug deposited on stage 13 (10.08 µm) of the ELPI was observed to be lower than stage 1 (cut-off diameter = 11.7 µm) in both the NGI and eNGI. Interestingly, in accordance with the compendial specifications for testing of DPIs and the specific resistance of turbohalers, the recommended flow rate for \textit{in vitro} testing is approximately 45 Lmin\(^{-1}\). Therefore, the size distribution obtained at 30 Lmin\(^{-1}\) may not be completely accurate. In terms of electrostatic charge, similar results were obtained using the the eNGI and the ELPI. The charge profile for Pulmicort Turbohaler obtained using both the ELPI and eNGI was net positive, which did not agree with the previous results reported by Kwok and Chan using the modified ELPI (Kwok and Chan 2008); Kwok and Chan had reported a bipolar charge profile for Pulmicort. However, the authors did not address this discrepancy in the study. For Bricanyl, a bipolar charge profile was obtained using the ELPI with stages 1 to 6 being net electropositive and stages 6 to 12 were negatively charged, and was comparable between the eNGI and the ELPI. Hoe \textit{et al.} have further improvised the eNGI by modifying the original design to include electrometer connections for the USP throat and the preseparator as well to allow charge measurement for the coarse particles in addition to the fine particles (Hoe \textit{et al.} 2010, Hoe \textit{et al.} 2011).
I.G Overview

This dissertation is organized to investigate the influence of different levels of charge on the aerosol deposition behavior within cascade impactors and apparent APSD, in comparison with the charge neutralized aerosol, while also evaluating the accuracy of the CFD model of the ACI. The ELPI will be used to measure the electrostatic charge on the aerosols. Chapter II states the individual hypotheses and the specific aims that will be addressed in the successive chapters. Each of the chapters consists of a brief introduction, a materials and methods section, followed by a results and discussion section. Chapter III investigates the influence of different levels of applied electrostatic charge on the deposition behavior and apparent APSD of aerosol clouds generated from commercial pMDI products. Chapter IV describes the development of an in vitro experimental set up to generate model charge neutralized and charged aerosols, < 2 µm in size, and the measurement of their apparent APSD within the ACI. The influence of charge polarity upon the deposition behavior of aerosol will also be investigated in Chapter IV. Chapter V investigates the differences between the CFD model assumptions and the in vitro study with respect to aerosol deposition within the ACI as a function of charge. Finally, Chapter VI summarizes the results from each chapter as it relates to the original hypotheses.
II. HYPOTHESES AND RESEARCH PLAN

Cascade impactors are widely used to characterize the aerodynamic particle size distribution (APSD) of pharmaceutical aerosols. However, the role of electrostatic charge on aerosol deposition within cascade impactors is poorly understood. The underlying hypothesis of this dissertation is that the presence of electrostatic charge on aerosol particles or droplets within an aerosol may influence its deposition within a cascade impactor. This, in turn, may influence the apparent aerodynamic size measurements attributed to the aerosol in vitro. Accordingly, this project is designed to test the following hypotheses:

II.A Hypothesis I

The deposition pattern of aerosol particles or droplets from commercially available pressurized metered dose inhalers within a cascade impactor is a function of the electrostatic properties of the aerosol, in addition to the aerodynamic particle size distribution.

To test this hypothesis, the deposition behavior and apparent APSDs of pharmaceutical aerosols from commercial inhalers will be determined following the application of additional electrostatic charges, beyond those created during aerosol cloud creation by triboelectrification, using the Electrical Low Pressure Impactor (ELPI). The aerosol clouds generated by commercial solution and suspension-based pressurized metered dose inhalers (pMDIs) will be modified using several methods to apply electrostatic charges to the clouds emitted from the inhalers. Following
this, the electrostatic charge profiles and drug deposition profiles at different charge levels will be assessed and the apparent APSD determined, and compared, within and across products.

II.B  Hypothesis II

The presence of significant electrostatic charge on aerosols with aerodynamic sizes < 2 μm can influence the deposition behavior of aerosol particles within a cascade impactor, when this behavior is compared to similarly sized charge-neutralized aerosols. The foundation for this hypothesis is a published Computational Fluid Dynamics (CFD) model of the Andersen Cascade Impactor (ACI) that predicted significantly increased deposition across the stages of the ACI for charged aerosols so that aerosol charge appeared to influence the apparent APSD.

The deposition behavior of well-characterized, charge neutralized and charged aerosols will be studied in an ACI. An experimental apparatus capable of producing a comparable charge neutral and charged aerosol with the required aerodynamic size distribution (MMAD < 2 μm) will be developed with the purpose of targeting analyte deposition preferentially to the ‘charge sensitive’ stages of the ACI, as these were defined by the CFD model. Stage deposition and apparent APSD will be evaluated and compared for the different aerosol charge states and, in an attempt to assess the importance of charge polarity, the behavior of net electropositive, electronegative and charge-neutralized aerosols will be evaluated and compared to the results from the CFD model of the ACI.

II.C  Hypothesis III

Method variations commonly employed during the experimental use of the ACI may also influence the deposition behavior of charged aerosols during APSD assessments in the impactor.
Charge neutralized and charged aerosol deposition behavior will be assessed and compared for several method variations under constant airflow conditions:

**II.C.1.** The effects of grounding the stages of the ACI will be evaluated since the CFD model, on which hypothesis II is founded, assumed that electrical sink conditions prevailed so that electrostatic stage charges due to deposited charged aerosol particles dissipated rapidly, a feature that may be influenced by grounding the impactor. In order to assess this, the deposition behavior of charge neutralized and charged aerosols will be investigated within a grounded ACI.

**II.C.2.** The effects of aerosol size will also be evaluated. Since the CFD model predicts greater effects for smaller aerosols, the deposition behavior of charge neutralized and charged aerosols with MMADs > 1 µm and < 1 µm will be evaluated and compared.

**II.C.3.** The effects of coating the impaction plates of the ACI and stage loading will be evaluated. Coating to prevent particle bounce is conventional practice, but the CFD model of the ACI neglected this factor, as well as the possible effects of particle mass per stage while simply assuming direct contact between charged aerosol particles and the metal stages of the separator. In order to determine the influence of stage coating upon image charge formation, and deposition behavior of aerosols, the study will be performed with uncoated impaction plates of the ACI. To investigate the stage loading effect, the sampling time in the impactor will be reduced and the deposition behavior of charge neutralized and charged model aerosol within the ACI compared.
II.C.4. The effects of charge in an abbreviated Anderson Cascade Impactor will be evaluated, in part because the use of a shorter stack of stages is becoming commonplace in the industry but also because the CFD model predictions were based largely on deposition predictions in isolated stages of the ACI. An abbreviated ACI will be used to compare deposition of charge neutralized and charged aerosol using efficient data analysis.
III. DEPOSITION BEHAVIOR OF COMMERCIAL PRESSURIZED METERED DOSE INHALERS WITHIN CASCADE IMPACTORS FOLLOWING THE APPLICATION OF ELECTROSTATIC CHARGE

III.A Introduction

Aerosol clouds produced by pressurized metered dose inhalers (pMDIs), dry powder inhalers (DPIs) and nebulizers are known to carry electrostatic charge of up to 200 electrons per particle, which is a function of both the formulation and the device (Byron et al. 1997, Peart et al. 1998, Glover and Chan 2004, Orban and Peart 2004, Kwok et al. 2005, Telko et al. 2007, Young et al. 2007, Kwok and Chan 2008, Kwok et al. 2010). Electrostatic charge has been shown to influence deposition behavior in the respiratory tract and valved holding chambers (Vincent et al. 1981, Melandri et al. 1983, Cohen et al. 1998, Bisgaard et al. 2002). Despite the fact that cascade impactors are used routinely for the in vitro characterization of aerosols and are calibrated using dilute, charge-neutralized droplets, the impact of aerosol electrostatics upon deposition within a cascade impactor, and the measured aerodynamic particle size distribution (APSD) is unknown. Electrostatic charge has been suggested as one of many factors which may account for variability in mass balance and APSD measurements obtained using conventional cascade impactors (Christopher et al. 2003, Bonam et al. 2008), but the evidence is largely anecdotal and there are no in vitro studies mapping aerosol deposition within compendial cascade impactors as a function of charge. The recalibration of the Electrical Low Pressure
Impactor (ELPI) by Kotian et al. reported that the mean cut-off diameters for the ELPI appeared to deviate increasingly from those supplied by the manufacturer as the aerodynamic diameter decreased, suggesting that the deposition of charged particles may differ from uncharged particles of the same size (Kotian et al. 2009).

An in vitro study was designed to investigate the influence of different levels of electrostatic charge on the deposition of commercially available pMDIs within a cascade impactor. The ELPI was used to assess the deposition behavior of pMDIs following the application of artificial charge using an external voltage source, which was validated against the internal ELPI corona discharge prior to its use. Three marketed pMDIs including HFA-based solution and suspension pMDIs were characterized with respect to the apparent APSD in relation to the electrostatic properties.

III.B Materials and Methods

III.B.1 Commercially Available Pressurized Metered Dose Inhalers

Three commercially available HFA propelled pMDIs were chosen to represent solution and suspension based marketed formulations. QVAR 80®, Proventil HFA® and Ventolin HFA®, were obtained commercially and tested prior to their expiry dates. Table III.1 summarizes these three pMDIs including their formulation and packaging components.
Table III.1  Commercially Available Pressurized Metered Dose Inhaler Products (pMDIs).

<table>
<thead>
<tr>
<th>Product</th>
<th>Formulation</th>
<th>Active Drug (Labeled Dose)(^a)</th>
<th>Supplier</th>
<th>Propellant System</th>
<th>Inactive Ingredient(s)</th>
<th>Valve Material(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QVAR 80</td>
<td>Solution</td>
<td>Beclomethasone Dipropionate (BDP), 80 µg</td>
<td>Teva Specialty Pharmaceuticals, PA, USA</td>
<td>HFA 134a</td>
<td>Ethanol</td>
<td>Stainless Steel</td>
</tr>
<tr>
<td>Proventil HFA</td>
<td>Suspension</td>
<td>Albuterol Sulfate, 108 µg equivalent to 90 µg albuterol base</td>
<td>Schering-Plough Corporation (Subsidiary of Merck), NJ, USA</td>
<td>HFA 134a</td>
<td>Ethanol and oleic acid</td>
<td>Stainless Steel</td>
</tr>
<tr>
<td>Ventolin HFA</td>
<td>Suspension</td>
<td>Albuterol Sulfate, 108 µg equivalent to 90 µg albuterol base</td>
<td>GlaxoSmithKline, NC, USA</td>
<td>HFA 134a</td>
<td>-</td>
<td>Polymer</td>
</tr>
</tbody>
</table>

\(^a\) Labeled dose represents the dose delivered from the mouthpiece actuator

\(^b\) QVAR 80 and Proventil HFA utilizes a 3M stainless steel metering valve; Ventolin HFA utilizes a metering valve supplied by Aptar Pharma. Specific details are proprietary.
III.B.2 Determination of Electrostatic Charge and Apparent Aerodynamic Particle Size Distribution (APSD) of Commercial pMDIs using the Electrical Low Pressure Impactor (ELPI)

The apparent APSD of the pMDIs was determined following size classification using the Electrical Low Pressure Impactor (ELPI).

III.B.2.1 ELPI Configuration for the Application of Different Charge Levels on the Aerosol produced by pMDIs

The ELPI (Dekati Ltd., Tampere, Finland) is a 13-stage low pressure impactor with the lower 12 stages connected to a multichannel electrometer with a corona charger atop the impactor (Keskinen et al. 1992). The ELPI corona charger consists of concentric tungsten, corona needle housed in a cylindrical metal geometry; a Teflon block separates the corona needle from the outer geometry. In the original ELPI configuration, the corona charger applies a fixed current of 1 μA, which corresponds to a voltage of approximately +5 kV (varying between +4 and +6 kV) to the aerosol cloud. The ELPI fractionates aerosol particles, based upon their aerodynamic diameter, in the range of 0.03 to 10 μm.

In order to investigate the deposition behavior and the apparent APSD of pMDI aerosol clouds within the ELPI as a function of charge, it was necessary to generate aerosol clouds, which exhibited different levels of electrostatic charge. This was achieved by applying variable voltages to the aerosol cloud by connecting the corona charger placed atop the ELPI impactor stack, via a BNC coaxial cable, to an external voltage source (Brandenburg Voltage Source, Alpha II series, Zi-Tech Corporation, CA). Figure III.1 illustrates the experimental set up used in the study.
Figure III.1 Schematic Diagram of the Experimental Set Up of the ELPI to Characterize the Influence of Applied Electrostatic Charge upon the Deposition of Commercial pMDIs.
The ELPI was allowed to stabilize for an hour before operation and set-up requirements including air leakage testing were performed according to the manufacturer’s instructions before each experiment. Silicone-coated aluminum substrates (DS-515 grease spray, Dekati Ltd., Tampere, Finland) were used on the impaction plates to minimize bounce and to collect drug particles for chemical analysis. The aerosol clouds were introduced via the USP induction port into the corona charger before sampling into the ELPI. The ELPI was operated at 100 mbar (below the lowest stage i.e., Stage 1) to produce a flow rate of approximately 29 Lmin⁻¹, verified using an external flow meter (Sierra Instruments Inc., Monterey, CA).

The electrical current induced by the charged aerosol particles, on each of the ELPI stages 1-12, was measured as a function of time for 20 seconds. Baseline current measurements were performed to ensure zeroing of the electrometer current readings before and after each experiment. An aerosol current (pA) versus time (sec) profile was generated for each stage of the impactor. The area under each aerosol current vs. time profile represented the net inherent aerosol charge in picocoulombs (pC) for that stage. The total net charge was calculated as the sum of the net charge on each ELPI stage.

III.B.2.2 Validation of the External Voltage Source against the Internal Corona Discharge of the ELPI

Prior to use, the external voltage source was verified against the internal corona discharge of the ELPI at +5 kV voltage using QVAR 80 pMDI as a model inhaler. Validation involved the comparison of the charge and mass distribution of QVAR 80 pMDIs determined using the external voltage source and the internal corona discharge of the ELPI at +5 kV voltage. Following the stabilization of the ELPI, the electrostatic properties and apparent APSD of
aerosols produced by QVAR 80 pMDIs were determined using the ELPI. Corona charging was achieved using either the current controlled internal corona discharge of the ELPI, which resulted in a voltage of approximately +5 kV (varying between +4 and +6 kV) being applied to the aerosol cloud or the external voltage source (Brandenburg, Alpha II series, Zi-Tech Corporation, CA) which applied a fixed voltage of +5kV. Single shots of QVAR 80 pMDI were actuated (n=5) using a randomized block design through a USP induction port into the ELPI operated at 29 Lmin$^{-1}$ (Figure III.1). The canister was shaken for 5 seconds and primed by firing 2 shots to waste in accordance with the manufacturer’s recommendations. The shot weight was recorded prior to, and following each actuation into the ELPI to ensure that it was within 5% of the target shot weight for that product.

The electrical current induced by the charged aerosol particles, on each of the ELPI stages, was measured as a function of time for 20 seconds. The total net charge was calculated as the sum of the net charge on each ELPI stage. Following each experiment, the ELPI was disassembled and the actuator, the USP induction port, corona charger and the 13 impactor stages were washed with 60% acetonitrile / 40% water solution. Drug deposition in the impactor was quantified using a validated HPLC assay described in Section III.B.3.1., following which the Mass Median Aerodynamic Diameter (MMAD) was calculated. The MMAD of the pMDIs were calculated based on the recent recalibration of the ELPI for use with pMDIs (Kotian et al. 2009). All experiments were performed with laboratory temperatures ranging from 22.9 - 24.6°C and relative humidity between 33 - 45%.
III.B.2.3 Influence of Applied Voltage on Electrostatic Charge and Mass Distribution of QVAR pMDI

The electrostatic properties and apparent APSD of aerosols produced by QVAR 80 pMDIs were characterized at 0, +1, +3 and +5 kV using the ELPI in conjunction with an external voltage source (Brandenburg, Alpha II series, Zi-Tech Corporation, CA). The experimental procedure described in Section III.B.2.2 was followed to actuate single shots of QVAR 80 pMDI (n=5) using a randomized block design into the ELPI via the USP induction port (Figure III.1). The shot weight was recorded prior to, and following each actuation into the ELPI to ensure that it was within 5% of the target shot weight for that product. The net charge and the mass deposition of the drug on each ELPI stage were determined at each applied voltage using the methods described in Section III.B.2 and the MMAD were calculated based on the recent recalibration of the ELPI for use with pMDIs (Kotian et al. 2009). All experiments were performed with laboratory temperatures ranging from 22.9 - 24.6°C and relative humidity between 33 - 45%.

III.B.2.4 Comparison of the Electrostatic Charge Distribution and Mass Distribution of Commercially Available pMDIs Following the Application of Charge

The electrostatic properties and apparent APSD of aerosols produced by commercially available solution (QVAR 80) and suspension (Proventil HFA and Ventolin HFA) based pMDIs were characterized at 0 kV (inherently charged) and +5 kV (artificially charged) using the external voltage source (Brandenburg, Alpha II series, Zi-Tech Corporation, CA). The experimental procedure described in Section III.B.2.2 was followed to actuate single shots of commercial pMDIs (n=5) using a randomized block design into the ELPI via the USP induction
port (Figure III.1). The shot weight was recorded prior to, and following each actuation into the ELPI to ensure that it was within 5% of the target shot weight for that product. The net charge and the mass deposition of the drug on each ELPI stage were determined at each applied voltage using the methods described in Section III.B.2 and the MMAD were calculated based on the recent recalibration of the ELPI for use with pMDIs (Kotian et al. 2009). All experiments were performed with laboratory temperatures ranging from 22.9 - 24.6°C and relative humidity between 33 - 45%.

III.B.3 High-Pressure Liquid Chromatography Analyses

Validated high-pressure liquid chromatography (HPLC) assays were used for the detection of BDP and albuterol in the samples from the ELPI experiments. Beclomethasone dipropionate and albuterol base were purchased from Sigma Aldrich (St. Louis, MO). HPLC grade methanol, acetonitrile and ammonium acetate were obtained from Fisher Scientific (Swannee, GA). Water was obtained in-house using a Nanopure diamond water system (Barnstead International, IA, USA).

III.B.3.1 Analysis of Beclomethasone Dipropionate (BDP) using HPLC coupled with UV Detection

A validated HPLC assay was used for the detection of BDP. The mobile phase, comprised of 60% acetonitrile / 40% water, with a flow rate of 1 mLmin⁻¹ and UV detection at 238 nm for BDP (2996 Photodiode Array Detector, 1515 Isocratic HPLC Pump, 717 Autosampler, Waters, Milford, MA). The samples (100 µL) were injected onto a C-18 Symmetry Column (3.5 µm, 4.6 x 75 mm, Waters Corporation, MA). Calibration curves of peak
area vs. concentration of BDP were linear \( (r^2 > 0.999) \) over the range of concentrations used i.e., 0.075 - 2 µg/mL. Typical precision (%RSD) and accuracy (%DFN) values were less than 1.2% and 3.7%, respectively (Appendix I, Table AI.1).

III.B.3.2 Analysis of Albuterol using HPLC coupled with Fluorescence Detection

A validated HPLC assay was used for the detection of albuterol. The mobile phase comprised of 225 mL of 0.1% ammonium acetate solution and 800 mL methanol, with a flow rate of 0.8 mL/min. Fluorescence detection was used with an excitation and emission wavelength of 276 and 609 nm, respectively for albuterol (Fluorescence Detector, 1515 Isocratic HPLC Pump, 717 Autosampler, Waters, Milford, MA). The samples (100 µL) were injected onto a C-18 Spherisorb ODS-2 Column (5 µm, 4.6 x 150 mm, Waters Corporation, MA). Calibration curves of peak area vs. concentration of albuterol were linear \( (r^2 > 0.999) \) over the range of concentrations used i.e., 50 - 800 ng/mL. Typical precision (%RSD) and accuracy (%DFN) values were less than 0.9% and 2.2%, respectively (Appendix I, Table AI.2).

III.C Results and Discussion

III.C.1 Validation of the External Voltage Source against the Internal ELPI Corona Discharge

Figure III.2 compares the mean net charge of QVAR 80 pMDI on stages 1-12 of the ELPI at +5 kV applied voltage, applied using either the internal corona discharge of the ELPI or the external voltage source.
The total net charge for the ELPI corona discharge was observed to be 1.69±0.54 nC, which was statistically comparable to 1.67±0.43 nC observed with the external voltage source (p-value = 0.9260, Student’s t-test) at +5 kV. These results confirmed the ability of the external voltage source to apply electrostatic charge in an equivalent manner to the internal ELPI corona discharge.

Table III.2 summarizes the mass deposition of BDP from the QVAR pMDI obtained using the internal ELPI corona discharge and the external voltage source at +5 kV, including the mass of BDP deposited from the QVAR 80 pMDI aerosol cloud in the actuator (Actuator Dose), USP throat (Throat Dose), corona charger (Charger Dose) and the impactor (Impactor Dose). The total delivered doses, actuator doses, throat doses, charger doses as well as the impactor doses were statistically comparable for both configurations (p-value > 0.05, Student’s t-test).
Table III.2  Mass deposition of BDP discharged from QVAR 80 pMDIs at +5 kV applied via the Internal ELPI Corona Discharge and the External Voltage Source. Data represents Mean±SD, n = 5.

<table>
<thead>
<tr>
<th>Voltage Source</th>
<th>Delivered Dose (µg)</th>
<th>Actuator Dose (µg)</th>
<th>Throat Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>Impactor Dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELPI Corona Discharge</td>
<td>91.0±1.9</td>
<td>31.0±3.1</td>
<td>23.4±2.3</td>
<td>12.8±2.1</td>
<td>22.7±1.9</td>
</tr>
<tr>
<td>External Voltage Source</td>
<td>91.8±3.2</td>
<td>32.9±2.8</td>
<td>24.8±2.2</td>
<td>11.3±2.1</td>
<td>22.0±3.2</td>
</tr>
</tbody>
</table>

Figure III.3 summarizes the mass distribution and the apparent APSD for QVAR 80 aerosol cloud obtained using the recalibrated cut-off diameters of the ELPI following the application of +5 kV via the internal ELPI corona discharge and the external voltage source.

BDP predominantly deposited on stages 2 - 10 of the ELPI. No significant difference was observed in the deposition of BDP discharged from the QVAR 80 aerosol cloud across the ELPI stages irrespective of the voltage source i.e., the external voltage source and the ELPI corona discharge (p-value > 0.05, Student’s t-test). Consequently, no significant difference was observed between the MMAD for the two configurations (Brandenburg, MMAD = 0.99±0.02).
μm, ELPI corona discharge, MMAD = 0.98±0.04 μm; p-value = 0.5370, Student’s t-test). The MMAD of QVAR 80 pMDI determined using the recalibrated ELPI cut-off diameters were in good agreement with the literature reported value for QVAR (Mitchell et al. 2003). These results confirmed the use of the external voltage source to apply a voltage to the aerosol clouds during in vitro assessment, in an equivalent manner to the internal ELPI corona discharge.

The use of recalibrated ELPI cut-off diameters was deemed appropriate for the determination of the apparent APSD (Kotian et al. 2009). Kotian et al. reported that the modified ELPI undersized commercial pMDIs, thus making recalibration necessary in order to use the modified ELPI to measure size of pharmaceutical aerosols (Kotian et al. 2009). This discrepancy in size measurement was ascribed to the calibration method adopted for the ELPI. The ELPI was originally calibrated based upon electrical detection of corona charged, monodisperse aerosols; the induced current was reported to be proportional to the number and mass concentrations (Marjamaki et al. 2000). Table III.3 shows the manufacturer calibrated as well as the recalibrated stage cut-off diameters for the ELPI and illustrates that ELPI stages with smaller cut-off diameters deviated more markedly than the upper stages, from the manufacturer reported cut-offs for corona-charged aerosols. Kotian et al. demonstrated the size equivalence for Ventolin HFA, after recalibration was performed, between the recalibrated ELPI and the reference calibration instrument - the ACI (Kotian et al. 2009). It should be noted that the recalibration did not involve the use of corona charger (i.e., the corona charger was removed) and the recalibrated stage cut-off diameters were recommended for sizing of inherently charged (or tribocharged) pharmaceutical aerosols (Kotian et al. 2009). In contrast, the present study involved using the corona charger to apply +5 kV to pMDI aerosols to investigate the influence of charge upon deposition within the ELPI. Satellite experiments were undertaken to compare
the apparent APSD of inherently charged QVAR 80 aerosol at 0 kV determined with the charger in place with that obtained using the modified ELPI (i.e., without a corona charger). It was confirmed that the recalibrated ELPI cut-off diameters were also valid in the present study when the ELPI has been used with the corona charger (Appendix II). Furthermore, the APSD for artificially or corona charged QVAR 80 aerosol (at +5 kV) was compared using the manufacturer reported stage cut-offs and the recalibrated stage cut-offs of the ELPI (Appendix II). In agreement with the previous findings by Kotian et al., the use of manufacturer reported stage cut-off diameters caused under sizing of the inherently charged QVAR 80 aerosol (Kotian et al. 2009). It was considered appropriate, therefore, in the present study to use the recalibrated stage cut-off diameters for the ELPI, for all the applied charge levels investigated, in order to avoid bias in particle sizing due to the use of charged calibration aerosol and the subsequent cut-off diameters.

Table III.3 Manufacturer (Dekati Ltd.) Reported and Recalibrated Aerodynamic Cut-off Diameters of the ELPI stages 1-13 at a Flow Rate of 29 Lmin^{-1} (Corrected to Two Decimal Places).

<table>
<thead>
<tr>
<th>ELPI Stage Number</th>
<th>Dekati Cut-off Diameter (µm)</th>
<th>Cut-off Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>10.08</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>6.67</td>
<td>6.37</td>
</tr>
<tr>
<td>11</td>
<td>4.05</td>
<td>4.75</td>
</tr>
<tr>
<td>10</td>
<td>2.42</td>
<td>3.03</td>
</tr>
<tr>
<td>9</td>
<td>1.62</td>
<td>2.12</td>
</tr>
<tr>
<td>8</td>
<td>0.96</td>
<td>1.40</td>
</tr>
<tr>
<td>7</td>
<td>0.62</td>
<td>1.01</td>
</tr>
<tr>
<td>6</td>
<td>0.39</td>
<td>0.70</td>
</tr>
<tr>
<td>5</td>
<td>0.27</td>
<td>0.56</td>
</tr>
<tr>
<td>4</td>
<td>0.16</td>
<td>0.44</td>
</tr>
<tr>
<td>3</td>
<td>0.10</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>0.03</td>
<td>-</td>
</tr>
</tbody>
</table>
III.C.2 Influence of Applied Voltage on Electrostatic Charge and Mass Distribution of QVAR pMDI

Figure III.4 summarizes the mean net charge of QVAR 80 aerosols following the application of 0, +1, +3 and +5 kV, via the external voltage source, on stages 1-12 of the ELPI.

The inherent charge profile (0 kV) for QVAR 80 i.e., was net electropositive, which was in agreement with the literature reported electrostatic charge profile for QVAR 80 pMDIs (Kwok et al. 2005, Keil 2005). The total inherent net charge of 1.19±0.35 nC on the QVAR 80 aerosol was statistically comparable to the net charge, measured at both +1 kV (1.00±0.18 nC) and +3 kV (1.19±0.26 nC, p-value > 0.05, ANOVA). The net charge on the QVAR 80 aerosol cloud was 1.67±0.43 nC following the application of +5 kV applied voltage and was not significantly higher than the inherent charge.
Figure III.4  Electrostatic Charge Distribution for Aerosols Generated from QVAR 80 pMDI determined using the ELPI with the External Voltage Source at 0 kV, +1 kV, +3kV and +5 kV. Each bar represents Mean ± SD, n=5.

The inherent net electrostatic charge produced by QVAR 80 aerosol cloud (0 kV) was in good agreement with the results previously obtained in our laboratory (mean total net charge for QVAR 80 determined using a modified ELPI: +1.31±0.35 nC, Keil 2005). However, it should be noted that in the present study, the corona charger was included in the experimental set up while in Keil’s study the corona charger was removed similar to the commonly used modified ELPI configuration for the characterization of pharmaceutical aerosols i.e., Kwok et al. have also reported the electrostatic charge characteristics of QVAR 80 pMDIs obtained using the modified
ELPI (Kwok et al. 2005). The polarity of QVAR 80 aerosol reported by Kwok et al. was also net electropositive, however, they reported a much lower mean total net charge (0.30±0.24 nC) than that observed in the present study (Kwok et al. 2005). Kwok et al. reported both inter- and intra-inhaler variability in charge magnitude for QVAR. The authors speculated the presence of ethanol in the QVAR formulation and water as an impurity, as well as the lack of time for the relaxation of counter charges generated on inhaler components between actuations for the ‘continuous shot’, may have caused the variability in charging (Kwok et al. 2005). However, no follow up study was performed to investigate this phenomenon.

As the applied voltage was increased from 0 to +5 kV, the net charge per stage increased significantly for the QVAR 80 aerosol cloud on ELPI stages 1-5 at +5 kV (p-value < 0.05, ANOVA, Dunnet’s test with 0 kV as the control). In contrast, for stages 8 and above, the net charge per stage decreased significantly at +5 kV compared to the control voltage i.e., 0 kV for the QVAR 80 aerosol cloud (p-value < 0.05, ANOVA, Dunnet’s test with 0 kV as the control). Stages 10-12 of the ELPI were observed to be net negative at +5 kV for the QVAR 80 aerosol cloud due to induction effects (Dekati Ltd. Technical Note 2002). In the ELPI, the recorded induced current is a result of the difference between incoming and outgoing flux of charged particles on a particular stage. If the concentration changes rapidly, as observed in the present study for commercial pMDIs, the difference between the incoming and outgoing amount of charge is not equal during this time period, and thus a negative current signal may be recorded.

The present study was undertaken with 0 kV as the control voltage, corresponding to the inherent charge measurement of the aerosol clouds and a high positive voltage of +5 kV, which is the maximum voltage that can be applied with the ELPI corona charger. In addition, two intermediate voltages, +1 kV and +3 kV, were also investigated in order to assess the influence
of different charge levels upon the deposition behavior of QVAR 80 aerosol cloud. Based upon these results, it appears that field charging of the aerosol cloud owing to the corona discharge does not occur below +5 kV applied voltage within the corona charger of the ELPI. Corona discharge, or the production and flow of ions, occurs in the charger at this high applied voltage, producing unipolar positive ions. This unipolar positive ion wind moves from the needle tip towards the charger walls and is responsible for field charging of the aerosol particles introduced in the corona charger in a direction perpendicular to the applied field. Field charging occurs for aerosol particles larger than 1 µm, while diffusion charging is considered to be the primary charging mechanism of charging for particles smaller than 1 µm (Cross 1987). Diffusion charging occurs as a result of collisions due to Brownian motion of ions, even in the presence of an electric field (Cross 1987). However, both require the presence of unipolar ions (Cross 1987).

Table III.4 summarizes the mass deposition of BDP discharged from QVAR 80 pMDI as a function of the applied voltage (0, +1, +3 and +5 kV).

Table III.4  Mass Deposition of BDP discharged from QVAR 80 pMDI as a Function of Applied Voltage Determined using the ELPI with an External Voltage Source. Data represents Mean±SD, n = 5.

<table>
<thead>
<tr>
<th>Applied Voltage</th>
<th>Delivered Dose (µg)</th>
<th>Actuator Dose (µg)</th>
<th>Throat Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>Impactor Dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 kV</td>
<td>89.4±2.4</td>
<td>29.1±5.0</td>
<td>28.0±3.1</td>
<td>3.2±0.8</td>
<td>28.6±3.5</td>
</tr>
<tr>
<td>+1 kV</td>
<td>91.9±3.7</td>
<td>27.5±3.0</td>
<td>24.4±4.2</td>
<td>2.9±0.7</td>
<td>36.9±4.9</td>
</tr>
<tr>
<td>+3 kV</td>
<td>93.7±4.9</td>
<td>32.0±2.7</td>
<td>21.5±3.6</td>
<td>3.2±1.1</td>
<td>36.7±6.3</td>
</tr>
<tr>
<td>+5 kV</td>
<td>91.8±3.2</td>
<td>32.9±2.8</td>
<td>24.8±2.2</td>
<td>11.3±2.1*</td>
<td>22.0±3.2*</td>
</tr>
</tbody>
</table>

*Significant difference within each pMDI, ANOVA p-value < 0.05

Delivered doses were statistically comparable for all experiments (ANOVA p-value > 0.05). ANOVA showed that as the applied voltage was increased from 0 to +5 kV, the charger
dose significantly increased from 3.2±0.8 μg at 0 kV to 11.3±2.1 μg at +5 kV (p-value < 0.0001, ANOVA, Dunnet’s test with 0 kV as the control). Consequently, the impactor dose significantly decreased from 28.6±3.5 μg at 0 kV to 22.0±3.2 μg at +5 kV (p-value = 0.0002, ANOVA, Dunnet’s test with 0 kV as the control). Figure III.5 shows the mass distribution for BDP discharged from QVAR 80 pMDI as a function of applied voltage. Within the ELPI, BDP deposition on the inlet and stages 4 (p-value = 0.0023), 5 (p-value = 0.0172), 6 (p-value = 0.0004), 7 (p-value = 0.0005), 8 (p-value = 0.0058), and 9 (p-value = 0.0144), was observed to be significantly decreased when +5 kV was applied compared to the 0 kV experiments (ANOVA, Dunnet’s test with 0 kV as the control, Figure III.5). Since the impactor doses were observed to be significantly different for the two charge levels investigated, the normalized impactor deposition for the QVAR 80 aerosol collected in the ELPI was calculated for the four applied voltages as shown in Figure III.5.

Figure III.5  Mass Deposition and % Impactor Deposition for QVAR 80 pMDIs determined using the ELPI as a Function of Applied Voltage. Each bar represents Mean ± SD, n=5.

No significant difference was observed between the impactor deposition across the ELPI stages for the QVAR 80 aerosol cloud as a function of applied voltage (p-value > 0.05, ANOVA,
Dunnet’s test with 0 kV as the control). This may be attributed to the fact that the QVAR 80 aerosol cloud already carried a high inherent net charge at 0 kV, which was not significantly different from that of the QVAR 80 aerosol at +5 kV applied voltage. Figure III.6 shows the apparent APSD profile of QVAR 80 pMDI obtained by plotting the mean cumulative percent undersize against the recalibrated ELPI cut-off diameters for each applied voltage.

![Figure III.6](image)

**Figure III.6** Apparent APSD for QVAR 80 pMDI determined using the ELPI as a Function of Applied Voltage. Data represents Mean ± SD, n=5.

Although the impactor dose was significantly lower at +5 kV, compared to 0 kV, no significant difference was observed between the MMAD for QVAR 80 pMDI for the four voltage levels investigated (MMAD 0 kV = 1.02±0.03 μm, MMAD +1 kV = 1.02±0.03, MMAD +3 kV = 1.01±0.07, MMAD +5 kV = 0.99±0.02 μm, ANOVA p-value > 0.05). The MMAD of QVAR 80 aerosol determined in the present study was in good agreement with the literature reported value for QVAR (Mitchell et al. 2003).

Based upon these results, the deposition behavior as well as the apparent APSD of the commercial pMDIs was then compared following the application of charge at two charge levels -
inherently charged level corresponding to the 0 kV applied voltage and the artificially charged level corresponding to +5 kV applied voltage.

III.C.3 Electrostatic Charge and Apparent Aerodynamic Particle Size Distribution (APSD) of Commercial pMDIs as a Function of Charge

III.C.3.1 Electrostatic Charge Distribution of Commercial pMDIs as a Function of Charge

Figure III.7 illustrates the electrostatic charge distribution for inherently charged (0 kV) and artificially charged (+5 kV) QVAR 80, Proventil HFA and Ventolin HFA aerosols. The electrostatic charge profile for inherently charged QVAR 80 was unipolar net electropositive compared to the negligibly charged Proventil HFA and Ventolin HFA clouds.
Figure III.7  Electrostatic Charge Distribution for Inherently Charged and Artificially Charged (+5kV) QVAR 80, Proventil HFA and Ventolin HFA pMDIs determined using the ELPI with the External Voltage Source. Inset shows the Charge Distribution on an Expanded Scale. Each bar represents Mean ± SD, n=5.
The electrostatic charge profile for inherently charged and artificially charged QVAR 80 aerosol have been discussed in Section III.C.2. The inherent electrostatic charge on QVAR 80 aerosol (1.19±0.35 nC) was in good agreement with the results previously obtained in our laboratory (mean total net charge for QVAR 80 determined using a modified ELPI: +1.31±0.35 nC, Keil 2005). QVAR 80 contains beclomethasone dipropionate, ethanol as a cosolvent and HFA 134a as the propellant. It is packaged with a stainless steel metering valve manufactured by 3M Drug Delivery Systems (specific valve construction details are proprietary). The total net charge on QVAR 80 aerosol increased from 1.19±0.35 nC to 1.67±0.43 nC, following the application of +5kV (discussed in Section III.C.2). However, this increase in charge level was not statistically significant (p-value = 0.0919, Student’s t-test).

In contrast to QVAR 80 aerosol cloud, the total net charge observed for the inherently charged Proventil HFA aerosol cloud was negligible and variable (0.09±0.18 nC). While Proventil HFA is packaged with a similar metering valve to QVAR 80, the active drug and formulation are different. Proventil HFA, a suspension based product, contains micronized albuterol sulfate, oleic acid, ethanol and HFA 134a. The total net inherent charge for Proventil HFA aerosol cloud was observed to be negligible but net electropositive (0.09±0.18 nC), shown inset in Figure III.7. Application of 5 kV resulted in a significantly increased net charge (0.69±0.26 nC) (p-value = 0.003, Student’s t-test) with particles depositing on stages 2 - 9 demonstrating net electropositive charge and particles depositing on stages 11 - 12 exhibiting net electronegative charge. The net charge per stage was observed to be significantly higher on the stages 1 through 6, 11 and 12 of the ELPI, for the artificially charged aerosol compared to the inherently charged Proventil HFA aerosol (p-value < 0.05, Student’s t-test).
The inherent charge on Proventil HFA was observed to be net electropositive, which was not in agreement with previous studies where net electronegative and highly variable electrostatic charge properties for Proventil HFA were reported (Canister 1: -0.19±0.06 nC, Canister 2: -0.07±0.03 nC, Keil, 2005).

Electrostatic properties of Ventolin HFA, an alternative albuterol sulfate suspension product which contains micronized albuterol sulfate in HFA 134a alone and packaged with a metering valve supplied by Aptar Pharma with a polymeric metering chamber and valve stem, were observed to be different compared to Proventil HFA aerosol. The electrostatic charge profile for inherently charged Ventolin HFA aerosol showed shot to shot variability and was bipolar (-0.12±0.06 nC) compared to the significantly higher artificially charged aerosol (0.85±0.26 nC) (shown inset Figure III.7, p-value = 0.0002, Student’s t-test). Within the ELPI, the net charge per stage was significantly higher on stages 2 through 10 of the ELPI, for the artificially charged aerosol compared to the inherently charged Ventolin HFA aerosol (p-value < 0.05, Student’s t-test). However, the charge profile of inherently charged Ventolin HFA was not clearly bipolar as reported in the previous studies using the modified ELPI (Keil 2005, Kwok et al. 2005). Previously, it has been reported that the aerosol cloud produced by Ventolin HFA is bipolar, with particles larger than 0.96 µm (i.e., stages 8 - 12 of the ELPI) net electropositive, while particles smaller than 0.96 µm (i.e., stages 1 - 7 of the ELPI) were net electronegatively charged (Keil 2005, Kwok et al. 2005).

One of the possible reasons for the differences in the electrostatic charge profiles compared to previous studies for Proventil HFA and Ventolin HFA, may be the presence of corona charger in the experimental set up. Previous studies by Keil and Kwok et al. used the modified ELPI i.e., the corona charger was removed from top of the impactor, whereas in the
present study, the corona charger was present atop the impactor in the ELPI. The presence of corona charger in the present study may have influenced the polarity of the electrostatic charge profile of Proventil and Ventolin HFA. In order to verify this hypothesis, electrostatic charge measurements (n=10) were performed using the same canister of Proventil and Ventolin HFA in a satellite experiment using the modified ELPI i.e., with the corona charger removed. Mass deposition analysis was not performed for these experiments. A net electronegative charge profile (Mean net charge = -0.17±0.10 nC, Figure III.8.b) was observed for Proventil HFA, which was in agreement with that reported previously (Keil 2005).

![Electrostatic Charge Distribution](image)

**Figure III.8** Electrostatic Charge Distribution for Inherently Charged Proventil HFA determined from the Same Can using (a) ELPI with the Corona Charger and (b) Modified ELPI i.e., Without Corona Charger. Inset shows the Charge Distribution on an Expanded Scale. Each bar represents Mean ± SD, n=10.

However, the electrostatic charge profile for Ventolin HFA was still observed to vary between Figure III.9 (a), which was similar to the bipolar charge profile observed in the present study discussed previously and Figure III.9 (b), which was similar to that reported by Keil (Keil 2005) (Figure III.9 (a), n = 6, Mean net charge = -0.01±0.05 nC; Figure III.9 (b), n = 4, Mean net charge = -0.29±0.08 nC). Therefore, Ventolin HFA was observed to demonstrate more
variability compared to the other pMDIs investigated, which has been previously reported (Keil 2005, Kwok et al. 2005).

Figure III.9  Electrostatic Charge Distribution for Inherently Charged Ventolin HFA determined from the Same Can using (a) ELPI with the Corona Charger (Mean ± SD, n=6) and (b) Modified ELPI i.e., Without Corona Charger (Mean ± SD, n=4).

III.C.3.2  Apparent Aerodynamic Particle Size Distribution (APSD) of Commercial pMDIs as a Function of Charge

Table III.5 summarizes the mass deposition data for inherently charged and artificially charged QVAR 80, Proventil HFA and Ventolin HFA aerosols obtained using the ELPI. The delivered doses i.e., the metered doses and the throat doses were not significantly different between the two charge levels investigated (p-value > 0.05, Student’s t-test) for the three commercial pMDIs investigated.
Table III.5  Mass Deposition for Inherently Charged (0 kV) and Artificially Charged (+5 kV) QVAR 80, Proventil HFA and Ventolin HFA pMDIs aerosol clouds obtained using the External Voltage Source. Data represents Mean±SD, n = 5.

<table>
<thead>
<tr>
<th>Product</th>
<th>Charge Level</th>
<th>Delivered Dose (µg)</th>
<th>Actuator Dose (µg)</th>
<th>Throat Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>Impactor Dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QVAR 80</td>
<td>Inherently Charged</td>
<td>89.4±2.4</td>
<td>29.1±5.0</td>
<td>28.0±3.1</td>
<td>3.2±0.8</td>
<td>28.6±3.5</td>
</tr>
<tr>
<td></td>
<td>Artificially Charged</td>
<td>91.8±3.2</td>
<td>32.9±2.8</td>
<td>24.8±2.2</td>
<td>11.3±2.1*</td>
<td>22.0±3.2*</td>
</tr>
<tr>
<td>Proventil HFA</td>
<td>Inherently Charged</td>
<td>96.7±8.9</td>
<td>20.4±1.2</td>
<td>37.0±3.1</td>
<td>0.5±0.8</td>
<td>38.6±4.8</td>
</tr>
<tr>
<td></td>
<td>Artificially Charged</td>
<td>103.4±3.2</td>
<td>23.1±6.1</td>
<td>38.9±6.6</td>
<td>13.1±1.4*</td>
<td>28.0±2.2*</td>
</tr>
<tr>
<td>Ventolin HFA</td>
<td>Inherently Charged</td>
<td>89.4±4.6</td>
<td>19.6±3.3</td>
<td>37.6±2.4</td>
<td>1.8±0.2</td>
<td>30.2±2.3</td>
</tr>
<tr>
<td></td>
<td>Artificially Charged</td>
<td>95.3±3.4</td>
<td>17.5±3.3</td>
<td>36.9±4.4</td>
<td>14.0±1.7*</td>
<td>26.5±3.0</td>
</tr>
</tbody>
</table>

*Significant difference within each pMDI, p-value < 0.05, Student’s t-test

The mass deposition data for QVAR 80 has been discussed in Section III.C.2. Similar to QVAR 80, the charger dose significantly increased from 0.5±0.8 µg to 13.1±1.4 µg, for the inherently charged and artificially charged for Proventil HFA, respectively (p-value = 0.0005, Student’s t-test). In the case of Ventolin HFA aerosol, the charger dose increased from 1.8±0.2 µg for the inherently charged aerosol to 14.0±1.7 µg for the artificially charged Ventolin HFA aerosol respectively (p-value = 0.0004, Student’s t-test). Thus, an approximately 15% aerosol deposition was observed to occur in the corona charger for the artificially charged aerosols. Such increased charger deposition has been reported with the use of corona charger. Visual observation confirmed that drug deposited in the charger during transit into the impactor downstream, owing to the premature deposition of the artificially charged aerosol.

Impactor doses were observed to decrease significantly as a result of the increased charger deposition as discussed previously for QVAR 80 pMDI in Section III.C.2. For Proventil HFA aerosol cloud, the impactor dose significantly decreased from 38.6±4.8 µg for the inherently charged aerosol to 28.0±2.2 µg for the artificially charged aerosol (p-value = 0.0040,
Student’s t-test) while in the case of Ventolin HFA aerosol cloud, the impactor dose was also observed to decrease from 30.2±2.3 μg for the inherently charged aerosol to 26.5±3.0 μg for the artificially charged aerosol, though this decrease was not statistically significant (p-value = 0.0616, Student’s t-test).

The mass distribution of the three commercial pMDIs aerosol clouds as a function of charge within the ELPI is shown in Figure III.10.

Since the impactor doses were observed to be significantly different for the two charge levels investigated, the normalized impactor deposition for the three pMDI aerosols collected in the ELPI was calculated and is shown in Figure III.10. The mass deposition data for QVAR 80 has been discussed in Section III.C.2.
Figure III.10  Mass Deposition and % Impactor Deposition for Inherently Charged and Artificially Charged QVAR 80, Proventil HFA and Ventolin HFA pMDIs determined using the ELPI as a Function of Charge. Each bar represents Mean ± SD, n=5.
In the case of Proventil HFA, albuterol deposition on the ELPI inlet (p-value = 0.0053), stages 8 (p-value = 0.0022), 9 (p-value = 0.0015) and 10 (p-value = 0.0355) of the ELPI was observed to be significantly decreased for the artificially charged aerosol compared to the inherently charged aerosol (Student’s t-test). Similar to QVAR 80 (discussed in Section III.C.2), no significant difference between the impactor deposition across the ELPI stages for the Proventil HFA aerosol cloud as a function of charge level was observed (Figure III.10, p-value > 0.05, Student’s t-test).

For Ventolin HFA pMDIs, albuterol deposition on the inlet (p-value = 0.0014), stages 9 (p-value = 0.0372) and 10 (p-value = 0.0172) of the ELPI was observed to be significantly decreased for the artificially charged aerosol compared to the inherently charged aerosol (Student’s t-test). However, once again, no significant difference was observed between the impactor deposition across the ELPI stages for the Ventolin HFA aerosol cloud as a function of charge level (p-value > 0.05, Student’s t-test).

Figure III.11 shows the particle size distribution profiles obtained by plotting the mean cumulative percent undersize against the recalibrated ELPI cut-off diameters, as a function of charge level for the three commercial pMDIs investigated.
Figure III.11  Apparent APSD for Inherently Charged and Artificially Charged QVAR 80, Proventil HFA and Ventolin HFA pMDI determined using the ELPI as a Function of Charge. Data represents Mean ± SD, n=5.
As discussed previously in Section III.C.2, no significant difference was observed between the MMAD for the +5 kV configuration (0.99±0.02 μm) and 0 kV configuration (1.02±0.03 μm) as shown in the overlapping apparent APSD profiles in Figure III.11, even though the impactor dose was significantly lower for the artificially charged QVAR 80 aerosol compared to the inherently charged aerosol (p-value = 0.0739, Student’s t-test).

Similarly for Proventil HFA, no significant difference (p-value = 0.8404, Student’s t-test) was observed between the MMAD for the inherently charged aerosol (2.53±0.10 μm) and the artificially charged aerosol (2.54 ±0.03μm) as shown in the overlapping apparent APSD profiles in Figure III.11. The MMAD of Proventil HFA aerosol determined in the present study, for both the inherently charged aerosol as well as the artificially charged aerosol, was in good agreement with the literature reported value for Proventil HFA (Dellamary et al. 2003).

No significant difference (p-value = 0.0788, Student’s t-test) was observed between the MMAD for the inherently charged aerosol (2.71±0.03μm) and the artificially charged aerosol (2.67±0.02μm) Ventolin HFA aerosol. The MMAD of Ventolin HFA aerosol determined in the present study, for both the inherently charged aerosol as well as the artificially charged aerosol, was in good agreement with the literature reported value for Ventolin HFA (Kotian 2008).

**III.D Summary**

In summary, the potential of using an external voltage source was established for use with the ELPI corona charger in order to apply variable voltages to aerosol clouds during in vitro characterization using the ELPI. The use of an external voltage source provided a unique opportunity to understand corona discharge in the ELPI corona charger. Based upon the electrostatic charge distribution obtained for QVAR 80 aerosol cloud at different applied
voltages, it is speculated that an applied voltage less than 5 kV did not result in the electrical breakdown of air, and hence corona discharge in the ELPI corona charger. At +5 kV applied voltage, corona discharge was observed to occur, followed by unipolar positive ion formation and field charging of the pMDI aerosol tested. The systematic experimental design demonstrated that the electrostatic properties of commercial pMDIs could be modified by the artificial modification of charge. Different electrostatic charging characteristics were observed for the three commercial pMDIs investigated, based on their formulation and the device packaging components.

The present study confirmed that the induced electrostatic charge on the aerosol particles influenced the deposition pattern of aerosol produced by the pMDIs within the impactor, although it did not result in a significant change in the apparent APSD. It should be emphasized that the present study compared the deposition of inherently charged aerosols to that of artificially corona charged aerosols within an impactor. The inherent charge on the commercial pMDIs tested, especially QVAR, was observed to be high, in fact statistically comparable to the charge on artificially charged aerosol. However, calibration aerosols used for cascade impactors, and other particle sizing instruments, are generally charge neutralized aerosols. Therefore, it seems appropriate to investigate the influence of charge upon deposition and apparent APSD within an impactor, by comparing a charged aerosol with a charge neutralized aerosol. However, the use of a neutralizer in order to remove the inherent charge of the commercial inhalers investigated in the study is likely to result in low impactor dose due to deposition losses within the neutralizer itself. Another limitation observed with the experimental set up used in this study was the high aerosol deposition in the USP throat. Further, an important contribution to the inherent charge in pMDIs is due to the formulation components. It has been reported previously.
that charges generated by pMDIs are not only due to the drug, but also various formulation components, which are not quantified using the chemical assay. In order to assess the role of charge upon deposition, more control over the aerosol formulation is warranted. Future studies will address these issues.
IV. DEPOSITION BEHAVIOR OF CHARGE NEUTRALIZED AND CHARGED AEROSOLS WITHIN THE ANDERSEN CASCADE IMPACTOR

IV.A Introduction

Cascade impactors are widely used for in vitro particle size analysis of pharmaceutical aerosols during product development, as well as routine quality control testing (Mitchell and Nagel 2003). Cascade impactors are calibrated using dilute, charge neutralized, monodisperse aerosols of known size (Marple and Olson 2009) whereas pharmaceutical aerosols are known to be concentrated, electrostatically charged and polydisperse.

Electrostatic charge has been suggested as one of several factors which may account for variability in mass balance and aerodynamic particle size distribution (APSD) measurements obtained using cascade impactors, but the evidence is largely anecdotal and there are no in vitro studies mapping aerosol deposition within compendial cascade impactors as a function of charge. Liu and Pui recommended that electrostatic charge present on particles produced by laboratory aerosol generators should be neutralized to avoid unwanted electrostatic effects upon aerosol deposition (Liu and Pui 1974). Horton and Mitchell observed that the presence of electrostatic charge influenced the deposition of monodisperse oleic acid droplets within the PC-2 Quartz Crystal cascade impactor during its calibration (Horton and Mitchell 1992). Non-neutralized monodisperse oleic acid droplets deposited on higher stages of the impactor than expected, based on size and calibration data (Horton and Mitchell 1992).
The Product Quality Research Institute (PQRI) Particle Size Distribution Mass Balance Working Group suggested that accumulation of electrostatic charge may influence APSD and mass balance, due to wall losses, resulting in a possible mass balance failure for pharmaceutical aerosols (Christopher et al. 2003). While Christopher et al. acknowledged that surface charge effects in cascade impactors were related to several factors including humidity, the formulation and the impactor itself, they suggested that the presence of electrically conductive surfaces within the impactor may help avoid variability in cascade impactor measurements due to electrostatic charge (Christopher et al. 2003). A working group of the International Pharmaceutical Aerosol Consortium on Regulation and Science (IPAC-RS) also reported that electrostatic charge acquired by non-conducting or electrically insulated cascade impactor components may influence deposition of aerosols within the impactor, resulting in variability in the measured APSD of aerosols (Bonam et al. 2008).

More recently, evidence of the influence of charge upon deposition of aerosols within a cascade impactor has been provided by the recalibration of the Electrical Low Pressure Impactor (ELPI, Dekati Ltd., Tampere, Finland) using the Andersen Cascade Impactor (ACI) as a reference impactor, where it was noted that the mean cut-off diameters for the ELPI appeared to deviate increasingly from those supplied by the manufacturer as the aerodynamic diameter decreased (Kotian et al. 2009). The authors speculated that this was related to the calibration of the ELPI with corona charged monodisperse aerosols and electrical detection, as opposed to the calibration of compendial cascade impactors (e.g., ACI and the Next Generation Impactor (NGI)) with charge neutralized aerosols and chemical detection (Kotian et al. 2009) and suggested that the deposition of charged particles may differ from uncharged particles of the
same size. It is possible therefore, that the deposition and the apparent APSD of inherently charged aerosols may be a function of electrostatic charge, in addition to aerodynamic size.

A CFD model has been developed to investigate the airflow pattern and particle deposition within the ACI (Vinchurkar et al. 2009). The CFD model predicted stage cut-off diameters were in good agreement with the experimental calibration (Vaughan 1989). Subsequently, the CFD model was extended to predict the influence of the saturation charge level, which was shown to be a reasonable approximation of realistic charge levels reported in the literature, on deposition within the ACI. For stages 0 and 1 of the ACI, the CFD model predicted an approximately 5% increase in the deposition fraction of charged, compared to neutral particles. An approximately 30% increase in the deposition fraction of charged particles, compared to neutral particles, was predicted for stages 2 and 3. For stages 4 and 5, the model predicted a 90% increase in deposition fraction for suitably sized particles at saturation charge while on stages 6 and 7, 100% deposition for charged aerosol particles in the submicron range was predicted (Vinchurkar et al. 2009). However, the CFD model has not been validated with in vitro experiments.

The present study was performed to investigate the effect of electrostatic charge on the aerosol deposition behavior and the resultant apparent APSD of otherwise comparable aerosols within the ACI. An experimental apparatus was designed to produce charge neutralized as well as charged model aerosols with an aerodynamic size of $<2 \ \mu m$ targeted to deposit on ‘charge sensitive’ stages of a physical ACI, where the CFD approach predicted significant deposition changes as a result of particle charging. The CFD model described the input charge levels in terms of elementary charges per particle i.e., number of electrons per particle. However, it is recognized that aerosol clouds produced from pharmaceutical inhalers carry net electropositive
charge, net electronegative, or bipolar charges (i.e., both electropositive and electronegative) charges depending upon the formulation and the device (Kwok et al. 2005, Kwok and Chan 2008, Kwok et al. 2010). In order to gain insight into the role of charge on the deposition of pharmaceutical aerosols, both positive and negative polarities of charge were investigated. The deposition behavior and the particle size distribution of the model aerosol were characterized within the ACI, following the application of different levels of charge. A parallel series of experiments were performed using the ELPI to verify the electrostatic charge levels on the model aerosol. The aerosol deposition predictions from the CFD model of the ACI were compared with the in vitro results obtained as a function of charge.

IV.B Materials and Methods

IV.B.1 Production of Charge Neutralized and Charged Fluorescein Disodium Aerosol

An in vitro study was designed to probe the deposition behavior of aerosols in an aluminum ACI operated at 28.3 Lmin$^{-1}$ (Copley Scientific, Nottingham, United Kingdom). Based on the CFD model prediction that electrostatic attraction dominates inertial impaction below stage 4 of the ACI (2.1 µm), a target size of < 2 µm for the test aerosol was chosen. The aerosol was generated using a Constant Output Atomizer (TSI, Model 3076, Cardigan, MN, USA) using an apparatus adapted from Clark and Byron and illustrated in Figure IV.1 (Clark and Byron 1985). One of the primary advantages of the constant output atomizer is the flexibility to produce aerosols of different sizes by modifying the concentration of the feed solution. A model fluorescein disodium aerosol < 2 µm (Acros Organics, NJ, USA) was generated using the constant output atomizer from an 18.2 % aqueous solution of fluorescein disodium based on the
method by Clark and Byron (Clark and Byron 1985). The atomizer was supplied with compressed air at 35 psig for aerosol generation. Heated dilution air was provided through a copper pipe clad in a heating tape (Brisk Heat, B.H. Thermal Corporation, Ohio, USA), which converged with the atomizer output in order to dilute and partially dry the fluorescein aerosol. The temperature of the heating tape was controlled by a voltage-supplied rheostat (Fisher Scientific Co., PA). The total flow through the system was approximately 9 Lmin\(^{-1}\). The atomizer output, mixed with warm dilution air, entered a drying chamber followed by a diffusion dryer (glass chamber half-filled with self-indicating Drierite) for maximum water removal. The percent water content in the dry powder fluorescein aerosol generated was not measured.
Figure IV.1 Schematic Diagram of the Constant Output Atomizer Experimental Set up to Generate Charge Neutralized and Charged Fluorescein Disodium Aerosol together with the Schematic Representation of the Operating Principle of Corona Charger (Adapted from Dekati Ltd., Tampere, Finland).
The experimental set up was developed in order to produce fluorescein dry powder aerosol with four different charge levels: charge neutralized, inherently charged, positively charged and negatively charged. The dry fluorescein aerosol from the diffusion dryer was introduced via either a $^{85}\text{Kr}$ neutralizer (Model 3470, TSI, Cardigan, MN, USA) or a hollow metal spacer having comparable internal dimensions to the neutralizer, to obtain the charge neutralized and the inherently charged fluorescein aerosol, respectively (Figure IV.1). The previously validated external voltage source (Brandenburg, Alpha II series, Zi-Tech Corporation, CA) was used in conjunction with the corona charger atop the impactor stack to apply charge, either +5 kV or -5 kV (voltage controlled corona discharge), to the otherwise charge neutralized aerosol to produce positively charged and negatively charged fluorescein aerosol, which was then sampled into the cascade impactor via the USP inlet port. The ELPI corona charger consists of a concentric tungsten corona needle housed in a metallic cylindrical geometry with a Teflon block separating the needle and the cylinder (Figure IV.1). Corona discharge in the charger occurs at high applied voltage (approximately 5 kV, positive or negative), owing to a high, non-uniform electrostatic field generated between the corona needle and the metal cylinder, producing charged ions and electrons responsible for field charging of aerosol particles.

Initially, the reproducibility of the charge neutralized fluorescein aerosol output was assessed at 1, 3, and 5 minutes by comparing the amount of fluorescein recovered from a filter connected to a vacuum pump, at the end of these time intervals (n=5). Based on the results of these experiments, the system was operated to a period of equilibration before sampling the aerosol into the impactor. Following the equilibration period, the fluorescein aerosol output was also confirmed using a filter connected to a vacuum pump for the 20 seconds sampling time chosen for the impactor (n=5).
IV.B.2 Determination of Electrostatic Charge Distribution and Mass Distribution of Fluorescein Disodium Aerosol using the Electrical Low Pressure Impactor (ELPI)

Due to the inability of the ACI to measure charge on the aerosols, a parallel arm of the study involved the use of the ELPI to verify the charge on the charge neutralized, inherently charged, positively charged and negatively charged fluorescein aerosols investigated in the study. An additional vacuum pump operated at ~15 Lmin\(^{-1}\) diverted some of the test aerosol to waste, to prevent saturation of the ELPI electrometers; isokinetic sampling was not verified. The ELPI was operated at 100 mbar (below the lowest stage i.e., Stage 1) to produce a flow rate of approximately 29 Lmin\(^{-1}\), verified using an external flow meter (Sierra Instruments Inc., Monterey, CA). Since, the total mass inflow i.e., output of the atomizer system (~9 Lmin\(^{-1}\)) was less than the total mass outflow i.e., operational flow of impactor (~29 Lmin\(^{-1}\)) and the additional vacuum pump (~15 Lmin\(^{-1}\)), filtered room air was used as supplemental air.

The ELPI was allowed to stabilize for an hour before operation and set-up requirements such as air leakage testing were performed according to the manufacturer’s instructions and specifications before each experiment. Silicone-coated aluminum substrates (DS-515 grease spray, Dekati Ltd., Tampere, Finland) were used on the impaction plates to collect drug particles for chemical analysis and to minimize bounce. Electrostatic profiles for each charge level of fluorescein aerosol were recorded by sampling the aerosol for 20 seconds into the ELPI. Baseline current measurements were performed to ensure zeroing of the electrometer current readings before and after each experiment. The total net charge was calculated as the sum of the net charge on each ELPI stage. All experiments (n=5) were performed with laboratory temperatures ranging from 21.9 - 25.9°C and relative humidity between 30 - 44%.
Following each experiment, the ELPI was disassembled and the actuator, the USP induction port, corona charger and the 13 impactor stages were washed with 0.001 N sodium hydroxide solution. Mass distribution for fluorescein aerosol as a function of charge in the ELPI was determined using a validated HPLC assay for fluorescein (Section IV.B.5).

IV.B.3 Determination of Mass Distribution and Apparent Aerodynamic Particle Size Distribution (APSD) of Fluorescein Disodium Aerosol as a Function of Charge within the Andersen Cascade Impactor (ACI)

The influence of four charge levels namely, charge neutralized, inherently charged, positively charged and negatively charged, upon the deposition behavior of fluorescein aerosol within the ACI (Copley Scientific, Nottingham, United Kingdom), a pharmacopeial impactor, was investigated. The inlet of the ACI was fitted with a custom-built stainless steel adaptor (shown in Figure IV.2) such that the corona charger of the ELPI could be placed directly on top of the ACI impactor to enable the application of charge to the aerosol cloud. The fluorescein aerosol was sampled for 20 seconds in the ACI, operated at a flow rate of 28.3 L\text{min}^{-1} when the aerodynamic cut-off diameters of the ACI stages are 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7 and 0.4 µm. Filtered room air was used as supplemental air since the total mass inflow i.e., output of the atomizer system (~9 L\text{min}^{-1}) was less than the total mass outflow i.e., operational flow of impactor (~28 L\text{min}^{-1}) and the additional vacuum pump (~15 L\text{min}^{-1}).
Figure IV.2 (a) Custom-built Stainless Steel Adaptor for the ACI, (b) Cross-sectional View of the Adaptor, and (c) Cross-sectional View of the Adaptor with Dimensions. All Dimensions are in Millimeters. Diagram not Drawn to Scale.

The ACI stages were coated with 316 Silicone Release Spray (Dow Corning, Midland, MI) to minimize particle bounce and re-entrainment conforming to good impactor practice (Byron 1994). Following each experiment, the ACI was disassembled and the USP induction port, corona charger and the 8 impactor stages were washed with 0.001 N sodium hydroxide solution, followed by the assessment of the mass of fluorescein in the wash solution using a validated HPLC assay (Section IV.B.5). All experiments (n=5) were performed with laboratory
temperatures ranging from 21.9 - 25.9°C and relative humidity between 30 - 44%. The *in vitro* results obtained for the charge neutralized and charged aerosols were compared with the CFD model predictions for the same to assess the validity of CFD model predictions.

**IV.B.4 Comparison of Fluorescein Disodium Aerosol used for Measurement of Electrostatic Charge and Apparent APSD in the ELPI and the ACI**

The study involved the use of two impactors - the ELPI to characterize the charge distribution and the ACI to characterize the deposition behavior and the apparent APSD of the fluorescein dry powder aerosol as a function of charge, as shown in Figure IV.3. Therefore, it was important to establish that the fluorescein aerosol cloud sampled within the ELPI and the ACI was comparable.

![Photograph of (a) ELPI with Charger Configuration and (b) ACI with the Corona Charger and the USP Induction Port Attached.](image)

**Figure IV.3** Photograph of (a) ELPI with Charger Configuration and (b) ACI with the Corona Charger and the USP Induction Port Attached.
The total delivered mass of fluorescein from the aerosol for the ELPI and the ACI was determined for each charge level and compared statistically using ANOVA, to ensure the comparability of the aerosol cloud delivered to the ELPI and the ACI. Statistical significance was assessed at $p < 0.05$ level throughout.

In addition, the ELPI data was curve fitted numerically to the size distribution of the fluorescein aerosol obtained from the ACI, for each of the four charge levels investigated. In accordance with the pharmacopeial method, the mass of fluorescein, which penetrated both the ACI and the ELPI and deposited on the individual stages was tabulated to generate cumulative percent undersize profiles for the aerosol cloud at each charge level (USP 29 / NF 24 First Supplement 2006). Cumulative percent undersize data ($n=5$) obtained for the fluorescein aerosol for the four charge levels from the ACI, were fitted using least mean square nonlinear regression analysis to determine best estimates for the MMAD and the geometric standard deviation (GSD) of each cloud by fitting the data to the cumulative distribution function (CDF) (Dunbar and Hickey 2000) shown in Equation IV.1.

$$y = [100 \times (0.5 + 0.5 \ \text{ERF}[1/(2^{0.5} \times \text{SIGMA})] \times [\log_e(x) - \text{MEAN})])]$$  \hspace{1cm} \text{Equation IV.1}

ERF is the error function \(\frac{2}{\sqrt{\pi}} \int \exp\left[1/(2^{0.5} \times \text{SIGMA})\right] \times [\log_e(x) - \text{MEAN}]^2 \ \text{dx}\)

where, $e^{\text{MEAN}}$ and $e^{\text{SIGMA}}$ are the MMAD and GSD of the log-normally distributed data (Tobias 1993). No weighting factors were employed and nonlinear regression was performed using SCIENTIST (MicroMath, Inc. St. Louis, MO). The program required three input files for curve fitting: a Model File (*.EQN), a Parameter File (*.PAR) and a Data File (*.MMD). The model and parameter files are shown in Appendix III. The model file defined aerodynamic cut-off
diameter (x) of the impactor stage as the independent variable and cumulative percent undersize (y) as the dependent variable. The parameters MEAN and SIGMA were assigned initial values from linear interpolation of the cumulative percent undersize versus aerodynamic cut-off diameter curves. Nonlinear least square regression analysis was performed allowing ‘MEAN’ and ‘SIGMA’ to float in order to minimize the sum of squared deviations of the data from the line of best fit for the fluorescein aerosol at each charge level, which resulted in more precise estimates of MEAN and SIGMA. The analysis produced a SCIENTIST plot for the cumulative percent undersize vs. aerodynamic diameter along with the statistical parameters, as shown in Appendix III.

Coefficient of Determination (COD) and Model Selection Criteria (MSC) were used to assess the goodness of fit of the log normal distribution model for the curve fitting the ACI cumulative percent undersize data. COD is a measure of the fraction of the total variance accounted for by the model. The closer the value of COD to 1, the better the model fits the data. In the case of MSC, the best fit model has the highest MSC value. In general, an MSC value greater than 4 was considered to be an indication of a good model. Best estimates of MMAD and GSD were tabulated for each charge level, alongside their 95% confidence intervals.

Following this, the APSD data for the corresponding charge level in the ELPI was used to obtain the cumulative percent undersize of fluorescein aerosol penetrating below a given ELPI stage. The curves of best fit from the ACI and thus the best estimates for MMAD and GSD were assumed to describe the clouds entering the ELPI for each charge level. Hence, the MMAD and GSD obtained from the ACI, were fixed at their specific best estimates and the fit of the model for the ELPI data was then assessed depending upon the COD and the MSC value.
IV.B.5 High Pressure Liquid Chromatography Analysis of Fluorescein using HPLC coupled with Fluorescence Detection

A validated high pressure liquid chromatography (HPLC) assay was used for the detection of fluorescein in the samples from the ELPI and the ACI experiments. The mobile phase, comprised of 0.01 M phosphate buffer (pH 10)/acetonitrile/0.05 M tetrabutylammonium hydroxide (800:200:10), was used at a flow rate of 0.8 mL/min. Fluorescence detection was used with an excitation and emission wavelength of 340 and 510 nm, respectively for fluorescein (Fluorescence Detector, 1515 Isocratic HPLC Pump, 717 Autosampler, Waters, Milford, MA). The samples (10 µL) were injected onto X-bridge polymer C-18 column (4.6 x 75 mm, Waters Corporation, MA). Calibration curves of peak area vs. concentration of fluorescein were linear ($r^2 > 0.999$) over the range of concentrations used i.e., 10-100 ng/mL (Hering et al. 1979, Vanderpool et al. 1987, Sakagami et al. 2001). Typical precision (%RSD) and accuracy (%DFN) values were less than 0.4% and 2.9%, respectively (Appendix I, Table AI.3). The limit of detection was determined to be 1.10 ng/mL.

IV.B.6 Comparison of the In Vitro Study with the CFD Model Predictions for the ACI - Estimation of Number of Elementary Charges Per Particle

The in vitro deposition of the fluorescein aerosol at the four charge levels was compared with the CFD model predictions for the different stages of the ACI. Based on the electrostatic charge and mass distribution obtained using the ELPI, for each of the four charge levels of fluorescein aerosol, the specific charge, or charge-to-mass ratio (q/m), for a given size fraction was estimated. The particles were assumed to be spherical with an average true density of 1.5
g/cm³. The number of elementary charges (n) per drug particle in a particular size fraction was estimated using Equation IV.2.

\[ n = \frac{C \rho V}{e} \]  

Equation IV.2

where, \( C \) is the specific charge (C/g), \( \rho \) is the true density of the particles, \( V \) the volume of a particle, and \( e \) the elementary charge (1.602×10^{-19} C). For a given ELPI stage, the volume \( V \) was calculated from the midpoint of the recalibrated stage cut-off diameter (Kotian et al. 2009). The number of elementary charges per particle obtained experimentally was compared with input charge levels employed for the CFD model of the ACI in order to verify if the charge levels achieved in the \textit{in vitro} study were sufficient to influence the deposition behavior of fluorescein aerosols within the ACI, as predicted by the CFD model.

**IV.C  Results and Discussion**

**IV.C.1 Production of Fluorescein Disodium Aerosol**

Figure IV.4 compares the mean aerosol output rate from the constant output atomizer system after 1, 3, and 5 minutes. The constant output atomizer system produced fluorescein aerosol at a stable rate (2.4±0.2 mg/min) after 3 minutes. While the fluorescein aerosol output was observed to be statistically comparable after 1, 3 and 5 minutes (ANOVA p-value > 0.05), the output was observed to be less variable at 3 and 5 minutes compared to 1 minute. Therefore, the system was operated to equilibrium for 3 minutes before sampling the aerosol into the impactor for 20 seconds. The mean aerosol output of the fluorescein aerosol recovered from the filter after 20 seconds was 783±36 µg.
Figure IV.4  Mean Fluorescein Aerosol Output Rate from the Constant Output Atomizer System at 1, 3, and 5 minutes. Each bar represents Mean ± SD, n=5.

IV.C.2 Electrostatic Charge Distribution and Mass Distribution of Fluorescein Disodium Aerosol Obtained using the Electrical Low Pressure Impactor (ELPI)

The electrostatic charge distribution for the fluorescein aerosol for each of the four charge levels characterized using the ELPI is shown in Figure IV.5.
Figure IV.5  Electrostatic Charge for Fluorescein Aerosol as a Function of Charge Level Obtained using the ELPI.  Data represents Mean ± SD, n=5.

The total net charge on the charge neutralized fluorescein aerosol was observed to be negligible (-0.06±0.08 nC).  This mean charge was significantly lower in magnitude than the inherently charged (-5.34±0.68 nC) as well as the applied charge levels investigated, i.e., artificially positively charged (+30.64±2.72 nC) and artificially negatively charged fluorescein aerosols (-47.32±4.74 nC) (ANOVA p-value < 0.0001, Dunnet’s test with charge neutralized as the control).  Based on these results, it can be concluded that the experimental set up produced four significantly different charge levels on the fluorescein aerosol for investigation of the deposition behavior within the ACI.
The charge magnitude of the negatively charged fluorescein aerosol, produced by the application of -5 kV voltage, was significantly higher than that of the positively charged fluorescein aerosol produced by the application of +5 kV voltage. This is believed to be based on the difference between the negative and the positive corona discharge processes. Negative corona is reported to be approximately 10 times more efficient than a positive corona in terms of particle charging. In a negative corona, the corona current carriers are electrons with mobility about 1000 times higher than that of gas ions, which makes it much easier for the electrons to collide with neutral particles to produce more ions and thus makes the negative corona a more efficient ion generator compared to positive corona, where the corona current carriers are positive gas ions with relatively low mobility (Cross 1987). Since voltage-controlled corona discharge process was used in the study; it was observed that the negative corona discharge process was more efficient when compared to the positive corona discharge process. This difference in the positive and negative corona discharge processes appears to also have influenced the deposition behavior of the fluorescein aerosol within the ELPI.

Table IV.1 summarizes the mass deposition for fluorescein aerosol obtained using the ELPI and Figure IV.6 shows the mass distribution for the fluorescein aerosol, as a function of charge.

Table IV.1  Mass Deposition for Fluorescein Aerosol obtained using the ELPI as a Function of Charge. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>Charge Level</th>
<th>Delivered Dose (µg)</th>
<th>Throat Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>Impactor Dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge Neutralized</td>
<td>778.9±77.5</td>
<td>1.8±0.8</td>
<td>4.5±2.5</td>
<td>773.0±76.7</td>
</tr>
<tr>
<td>Inherently Charged</td>
<td>783.1±55.2</td>
<td>3.1±1.4</td>
<td>2.9±0.7</td>
<td>777.1±55.6</td>
</tr>
<tr>
<td>Positively Charged (+5kV)</td>
<td>827.1±70.5</td>
<td>2.3±1.4</td>
<td>112.5±13.1*</td>
<td>708.7±65.6*</td>
</tr>
<tr>
<td>Negatively Charged (-5kV)</td>
<td>771.0±51.7</td>
<td>2.4±0.7</td>
<td>225.4±25.2*</td>
<td>538.3±68.2*</td>
</tr>
</tbody>
</table>

*Significant difference, ANOVA p-value < 0.05
Figure IV.6  Mass Distribution for Fluorescein Aerosol as a Function of Charge Level obtained using the ELPI. Data represents Mean ± SD, n=5.

The total delivered doses i.e., sum of the amount of fluorescein deposited in the throat, charger and the impactor; as well as the throat doses were statistically comparable for the four charge levels investigated (ANOVA p-value > 0.05). The charger dose for the artificially charged fluorescein aerosol, both positive and negative, was significantly higher when compared to the charge neutralized and inherently charged fluorescein aerosol (ANOVA p-value < 0.0001, Dunnet’s test with charge neutralized as the control), which, consequently, decreased the fluorescein mass collected by the impactor for artificially charged aerosols (ANOVA p-value = 0.0002, Dunnet’s test with charge neutralized as the control). This deposition behavior is
believed to be a result of premature deposition of highly charged particles as they pass through the corona charger i.e., the first contact surface following charging. During realistic *in vitro* testing of pharmaceutical aerosols, aerosolization generates electrostatically charged particles, which are immediately introduced into the USP induction port before transit into the cascade impactor. In contrast, in the present study the corona charger was located downstream of the USP throat and as a result this study may neglect charge induced deposition within the USP throat and focuses on deposition within the impactor itself. Nevertheless, an important advantage of using the present experimental set up was the reduced throat deposition (< 1% of the total delivered dose) in comparison to the approximately 30% high throat deposition observed in Chapter III for the commercial pMDIs.

Furthermore, it was observed that the charger dose for the negatively charged aerosol was significantly higher, and consequently, the impactor dose was significantly lower, compared to the positively charged aerosol (p-value < 0.05). This may be attributed to the increased efficiency of the negative corona discharge compared to the positive corona discharge. Visual observation confirmed that the deposition pattern within the charger was different based on the charge polarity. Fluorescein deposition was concentrated around the base of the corona needle, with minimal deposition observed on the charger wall for the artificially positively charged aerosol, whereas deposition predominantly occurred on the charger wall with minimal deposition around the needle for the artificially negatively charged aerosol. The altered deposition pattern for negatively charged aerosol is believed to occur due to possible repulsion between the negatively charged aerosol and the Teflon block that results in deposition on the oppositely charged electrode, i.e., the charger wall. Maximum fluorescein mass deposition was observed on stages 6 through 9 of the ELPI for the four charge levels studied.
IV.C.3 Comparison of Fluorescein Disodium Aerosol used for Measurement of Electrostatic Charge and Apparent APSD in the ELPI and the ACI

Table IV.2 summarizes the mass deposition of fluorescein aerosol obtained using the ELPI (discussed in Section IV.C.2) and the ACI, across the four charge levels, in order to ensure the comparability of the fluorescein aerosol cloud sampled into the ELPI and the ACI. Upon statistical comparison, it was observed that the total mass of fluorescein deposited in the throat, charger, and within each impactor, across the four charge levels, were statistically comparable between the ELPI and the ACI (ANOVA p-value > 0.05). These results justified the use of the ELPI for the measurement of charge on each fluorescein aerosol.

Table IV.2 Mass Deposition for Fluorescein Aerosol obtained using the ACI and the ELPI as a Function of Charge. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>Charge Level</th>
<th>Impactor</th>
<th>Delivered Dose (µg)</th>
<th>Throat Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>Impactor Dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge Neutralized</td>
<td>ACI</td>
<td>790.4±68.6</td>
<td>1.7±0.6</td>
<td>4.3±1.5</td>
<td>784.4±68.9</td>
</tr>
<tr>
<td></td>
<td>ELPI</td>
<td>778.9±77.5</td>
<td>1.8±0.8</td>
<td>4.5±2.5</td>
<td>773.0±76.7</td>
</tr>
<tr>
<td>Inherently Charged</td>
<td>ACI</td>
<td>810.2±59.4</td>
<td>1.8±1.2</td>
<td>3.6±1.3</td>
<td>804.8±60.3</td>
</tr>
<tr>
<td></td>
<td>ELPI</td>
<td>783.1±55.2</td>
<td>3.1±1.4</td>
<td>2.9±0.7</td>
<td>777.1±55.6</td>
</tr>
<tr>
<td>Positively Charged</td>
<td>ACI</td>
<td>798.6±52.8</td>
<td>1.6±0.9</td>
<td>106.4±7.7</td>
<td>690.6±46.8</td>
</tr>
<tr>
<td></td>
<td>ELPI</td>
<td>827.1±70.5</td>
<td>2.3±1.4</td>
<td>112.5±13.1</td>
<td>708.7±65.6</td>
</tr>
<tr>
<td>Negatively Charged</td>
<td>ACI</td>
<td>784.2±55.0</td>
<td>2.0±0.5</td>
<td>217.1±34.6</td>
<td>565.0±43.8</td>
</tr>
<tr>
<td></td>
<td>ELPI</td>
<td>771.0±51.7</td>
<td>2.4±0.7</td>
<td>225.4±25.2</td>
<td>538.3±68.2</td>
</tr>
</tbody>
</table>

A curve fitting procedure was also undertaken to assess the comparability of the fluorescein aerosol cloud sampled into the ACI and the ELPI. The percentage of the total impactor dose was calculated for stage 0 through 7 plus filter of the ACI, based on the mass distribution data obtained from the ACI experiments, for each of the four charge levels (n=5) in accord with the pharmacopeial method (USP 29 / NF 24 First Supplement 2006). The cumulative percent undersize versus the aerodynamic diameter was obtained for each of the
charge levels. The APSD for fluorescein aerosol at each of the four charge levels investigated was log-normal, which can be described in terms of two variables, mass median aerodynamic diameter (MMAD, µm) and a dimensionless geometric standard deviation (GSD). The cumulative percent undersize data of fluorescein aerosol for each of the charge levels was fitted to the log-normal distribution function using non-linear regression analysis.

Figure IV.7(a) shows the mean results for the cumulative percent undersize versus the aerodynamic cut-off diameter of the ACI, obtained for the four charge levels. The continuous curves shown are the results of curve fitting the ACI APSD data for each charge level. The curves of best fit from the ACI, and the best estimates of MMAD and GSD were assumed to describe the fluorescein aerosol cloud sampled into the ELPI. The cumulative percent undersize data of fluorescein aerosol obtained using the ELPI was then fitted to the log-normal distribution function by fixing the MMAD and the GSD to the specific best estimates obtained for each charge level from the ACI. Figure IV.7(b) shows the mean results for the cumulative percent undersize versus the aerodynamic cut-off diameter for each of the four charge levels, obtained using the ELPI, where the continuous curves shown are the results of curve fitting for the ELPI data using ACI derived best estimates for MMAD and GSD.
Figure IV.7  Mean Cumulative Percent Undersize versus Aerodynamic Diameter (n=5) for the Fluorescein Aerosol at the Four Charge Levels Investigated obtained using (a) ACI and (b) ELPI. Solid Profiles are the Result of Curve Fitting to the Log Normal Distribution Function. Error Bars denote Standard Deviations.
Table IV.3  Numerical Results of Curve Fitting the Cumulative Percent Undersize Data from the ACI and the ELPI for the Four Charge Levels. Best Estimates for MMAD and GSD are shown with 95% Confidence Intervals.

<table>
<thead>
<tr>
<th>Charge Level</th>
<th>MMAD (µm)</th>
<th>Confidence Interval (µm)</th>
<th>GSD</th>
<th>Confidence Interval</th>
<th>Model Selection Criteria (MSC)</th>
<th>Coefficient of Determination (COD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge Neutralized</td>
<td>1.34</td>
<td>1.31-1.36</td>
<td>1.60</td>
<td>1.56-1.64</td>
<td>5.68</td>
<td>0.997</td>
</tr>
<tr>
<td>Inherently Charged</td>
<td>1.32</td>
<td>1.26-1.37</td>
<td>1.89</td>
<td>1.80-1.99</td>
<td>4.51</td>
<td>0.990</td>
</tr>
<tr>
<td>Positively Charged</td>
<td>1.32</td>
<td>1.29-1.36</td>
<td>1.74</td>
<td>1.68-1.79</td>
<td>5.38</td>
<td>0.996</td>
</tr>
<tr>
<td>Negatively Charged</td>
<td>1.36</td>
<td>1.34-1.39</td>
<td>1.86</td>
<td>1.82-1.92</td>
<td>5.69</td>
<td>0.997</td>
</tr>
</tbody>
</table>

\( ^a \),\(^b\) MSC and COD values obtained by fitting the ACI obtained data to Cumulative Distribution Function

\( ^c \),\(^d\) MSC and COD values obtained for the ELPI data using fixed best estimates obtained for the ACI
The numerical results of curve fitting the ACI data and its 95% confidence limits are shown in Table IV.3 along with the best estimates obtained for MMAD and GSD. The MSC and the COD obtained after curve fitting are reported for both the ACI and the ELPI. The curve fitting procedure produced excellent fits of the ACI data with MSC > 4 and COD > 0.99 for all charge levels. When the best estimates of MMAD and GSD obtained from the curve fitting of ACI data were used to describe the aerosol clouds sampled into the ELPI followed by curve fitting the ELPI cumulative data, excellent fits were also obtained with the MSC > 4 and COD > 0.99.

Based on the curve fitting results, it is reasonable to assume that the fluorescein aerosol sampled into the ACI and the ELPI at the four charge levels investigated are comparable. These results further corroborated the use of the ELPI to characterize the charge on the fluorescein dry powder aerosol while the deposition behavior of fluorescein aerosol was characterized in the ACI as a function of charge.

**IV.C.4 Mass Distribution and Apparent APSD of Fluorescein Disodium Aerosol within the Andersen Cascade Impactor (ACI)**

Table IV.4 summarizes the mass deposition for fluorescein aerosol obtained using the ACI as a function of the four charge levels investigated.
Table IV.4  Mass Deposition for Fluorescein Aerosol obtained using the ACI as a Function of Charge. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>Charge Level</th>
<th>Delivered Dose (µg)</th>
<th>Throat Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>Impactor Dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge Neutralized</td>
<td>790.4±68.6</td>
<td>1.7±0.6</td>
<td>4.3±1.5</td>
<td>784.4±68.9</td>
</tr>
<tr>
<td>Inherently Charged</td>
<td>810.2±59.4</td>
<td>1.8±1.2</td>
<td>3.6±1.3</td>
<td>804.8±60.3</td>
</tr>
<tr>
<td>Positively Charged</td>
<td>798.6±52.8</td>
<td>1.6±0.9</td>
<td>106.4±7.7*</td>
<td>690.6±46.8*</td>
</tr>
<tr>
<td>Negatively Charged</td>
<td>784.2±55.0</td>
<td>2.0±0.5</td>
<td>217.1±34.6*</td>
<td>565.0±43.8*</td>
</tr>
</tbody>
</table>

*Significant difference, ANOVA p-value < 0.05

Similar to the ELPI experiments, the charger dose for the artificially charged fluorescein aerosol, both positive and negative, was significantly higher when compared to the charge neutralized and inherently charged fluorescein aerosol (ANOVA p-value < 0.0001, Dunnet’s test with charge neutralized as the control), and therefore decreased the fluorescein mass collected by the impactor for artificially charged aerosols (ANOVA p-value < 0.0001, Dunnet’s test with charge neutralized as the control). Since the size of aerosol particles deposited in the corona charger is not known, it remains unclear whether the corona charger preferentially traps smaller or larger particles. It was also observed that the charger dose for negatively charged aerosol was significantly higher, and consequently, the impactor dose was significantly lower, compared to positively charged aerosol. This is considered to be related to the increased charge magnitude of the negatively charged aerosol compared to the positively charged aerosol, due to the increased efficiency of negative corona.

Deposition behavior of the fluorescein aerosols was assessed within the ACI to enable comparison with the CFD model predictions. Since the impactor doses were observed to be significantly different for the four charge levels investigated, the normalized impactor deposition for the fluorescein aerosol collected in the ACI at the four charge types was calculated. Figure IV.8 shows the mass distribution as well as the normalized impactor dose for the fluorescein
aerosol collected in the ACI at the four charge levels. It is recognized that the deposition on the ACI stages, and impactor stages in general, is interdependent such that what happens on the stage above governs deposition on the stage below it. Therefore, the data analysis to compare stage deposition trends as a function of charge as well as the multiple stages of the impactor is challenging. Furthermore, there is a lack of consensus on the metric to be used for comparison of deposition and the apparent APSD profile for inhaled products (Lee 2004, Pan et al. 2004, Christopher et al. 2007, Adams et al. 2007, Shi and Hickey 2009). One-way ANOVA has been used in the present study with posthoc analysis performed using the charge neutralized experiments as the control. Such data treatment was warranted to enable the comparison of stage deposition trends observed in the present in vitro study as a function of the presence and/or magnitude of charge, with those predicted by the CFD model of the ACI.

Figure IV.8 Mass Distribution and Percent Impactor Dose for Fluorescein Aerosol as a Function of Charge Level obtained using the ACI. Inset shows the Mass Deposition on Stages 0 through 3 on an Expanded Scale. Data represents Mean ± SD, n=5.

The presence of charge on fluorescein aerosol appeared to influence the deposition behavior within the ACI. A statistically significant difference was observed in the mass of
fluorescein deposited on stages 0 through 3, 5, 6, and 7 of the ACI (ANOVA p-value < 0.05). The artificially charged fluorescein aerosol, both positively as well as negatively charged were observed to have significantly increased fluorescein deposition on stages 0 (p-value < 0.0001), 1 (p-value < 0.0001), 2 (p-value = 0.0005) and 3 (p-value = 0.0017) of the ACI compared to the charge neutralized fluorescein aerosol (ANOVA, Dunnet’s test with charge neutralized as the control). ANOVA suggested that significantly decreased deposition was observed on stage 5 (p-value < 0.0001) and 6 (p-value = 0.0043) of the ACI. While both the positively and negatively charged fluorescein aerosol showed significantly lower deposition on stage 5, only negatively charged fluorescein aerosol showed significantly decreased deposition on stage 6 compared to the charge neutralized aerosol (ANOVA, Dunnet’s test with charge neutralized as the control). Further statistical analysis showed that the deposition on stage 5 for negatively charged fluorescein aerosol was in fact significantly lower than that of the positively charged fluorescein aerosol. The inherently charged fluorescein aerosol was observed to have significantly higher fluorescein deposition on stage 7 (p-value = 0.0018) compared to the charge neutralized aerosol (ANOVA p-value < 0.05, Dunnet’s test with charge neutralized as the control).

For the normalized percent impactor dose, significantly higher deposition (approximately 200%) was observed on stages 0 (p-value < 0.0001) and 1 (p-value < 0.0001) whereas a two-fold increase was observed on stages 2 (p-value < 0.0001) and 3 (p-value < 0.0001) of the ACI for artificially charged (both positive and negative) fluorescein aerosol, compared to the charge neutralized aerosol (Appendix IV, Table AIV.1, ANOVA p-value < 0.05, Dunnet’s test using charge neutralized as control). Such deposition behavior may be expected as the presence of charge may lead to premature deposition of particles (Hinds 1982). Since the present study was designed to produce aerosols < 2 μm in size with targeted deposition on stage 4 and below of the
ACI, the data was reanalyzed by grouping the stages 0-3 of the ACI together for the four charge levels investigated. Grouping stages 0-3 together is considered unlikely to influence the data analysis for the stages 4 and below, where maximum deposition of fluorescein aerosol was observed to occur. Furthermore, the stage to stage inter-relationships, which impose a significant challenge, were considered to be reduced. Table IV.5 summarizes the normalized impactor deposition for the fluorescein aerosol at the four charge levels investigated. Significantly increased deposition was still observed on stages 0-3 of the ACI grouped together, for the artificially charged (both positive and negative) fluorescein aerosol, compared to the charge neutralized aerosol (ANOVA p-value < 0.0001, Dunnet’s test using charge neutralized as control). Despite the increase, the deposition on these stages was less than 8% of the total impactor dose as very little fluorescein mass deposition was observed to occur on these stages of the ACI.

Table IV.5 Impactor Deposition for Fluorescein Aerosol as a Function of Charge Level Obtained using the ACI. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>ACI Stage</th>
<th>Charge Neutralized</th>
<th>Inherently Charged</th>
<th>Positively Charged</th>
<th>Negatively Charged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0-3</td>
<td>3.40±1.13</td>
<td>3.62±1.17</td>
<td>7.22±0.65</td>
<td>8.08±0.69</td>
</tr>
<tr>
<td>Stage 4</td>
<td>11.53±1.75</td>
<td>12.77±2.21</td>
<td>9.53±2.17</td>
<td>14.47±3.84</td>
</tr>
<tr>
<td>Stage 5</td>
<td>53.58±1.45</td>
<td>50.41±3.96</td>
<td>49.35±2.33</td>
<td>42.84±2.37</td>
</tr>
<tr>
<td>Stage 6</td>
<td>20.10±1.09</td>
<td>18.73±1.85</td>
<td>20.50±1.42</td>
<td>20.44±0.97</td>
</tr>
<tr>
<td>Stage 7</td>
<td>7.11±1.28</td>
<td>9.58±1.21</td>
<td>8.24±0.55</td>
<td>8.38±0.62</td>
</tr>
<tr>
<td>Filter</td>
<td>4.28±0.51</td>
<td>4.89±0.61</td>
<td>5.16±0.34</td>
<td>5.79±0.93</td>
</tr>
</tbody>
</table>

In contradiction with the CFD model predictions, significantly decreased deposition was observed on stage 5 for the artificially charged (both positive and negative) aerosols compared to charge neutralized aerosol (ANOVA p-value < 0.05, Dunnet’s test using charge neutralized as control).
control). Moreover, stage 5 was also observed to be the stage with maximum fluorescein deposition, suggesting that repulsion of incoming charge particles by the charged particles collected by stage 5 may have occurred. This hypothesis was reasonably supported by increased deposition of charged fluorescein aerosol downstream of stage 5 in the ACI. The deposition of charged fluorescein aerosol i.e., inherently charged, positively charged as well as negatively charged appeared to increase on stages 6, 7 and filter compared to the charge neutralized fluorescein aerosol, although this increase was not observed to be statistically significant on each of these stages. ANOVA indicated that the inherently charged fluorescein aerosol showed significantly higher deposition on stage 7 (p-value = 0.0095) compared to charge neutralized fluorescein aerosol, whereas negatively charged fluorescein aerosol showed significantly higher deposition on the filter stage (p-value = 0.0136) of the ACI compared to charge neutralized aerosol (ANOVA p-value < 0.05, Dunnet’s test using charge neutralized as control). It is speculated that repulsion may have resulted in increased deposition on the lower stages (6 through filter) of the ACI. However, this change in the deposition pattern within the ACI did not appear to influence the apparent APSDs obtained as a function of charge level for fluorescein aerosol, which were almost overlapping as shown in Figure IV.9.
A small, but significant difference in the apparent MMAD obtained using the ACI for the artificially negatively charged aerosol compared to the charge neutralized fluorescein aerosol was observed (ANOVA, p-value < 0.05, Dunnet’s test using charge neutralized as control, MMAD charge neutralized = 1.32±0.04 μm, MMAD inherently charged = 1.32±0.05 μm MMAD positively charged = 1.34±0.05 μm, MMAD negatively charged = 1.39±0.04 μm; Figure IV.9). However, this may not be a practically significant difference in terms of realistic *in vitro* testing. Nevertheless, it appears that the presence of charge appeared to have increased the polydispersity of the fluorescein aerosol as a result of the two opposing processes influencing the deposition of charged aerosol in the ACI - image charge effects and repulsion, or space charge effects.

One of the concerns with the use of a fluorescein aerosol in the present *in vitro* study was the reported hygroscopicity of fluorescein disodium (Material Safety Data Sheet, Acros Organics), as the water content in fluorescein disodium may influence the final size of the particles. The commercially obtained fluorescein disodium was well protected from moisture.

---

**Figure IV.9** Apparent APSD for Fluorescein Aerosol as a Function of Charge Level determined using the ACI. Data represents Mean ± SD, n=5.
during storage in the laboratory and the percent water content in the stored fluorescein disodium determined using Karl Fischer titration was observed to be 9.7±0.6%. However, since the final percent water content of the fluorescein dry powder aerosol was not determined, empirical calculations were performed to compare the MMAD of the fluorescein disodium aerosol with different water content in order to ensure that the presence of water in fluorescein aerosol did not influence the final particle size of the fluorescein aerosol (Appendix A.V). Based on these calculations, it was assumed that the final MMAD of the fluorescein disodium dry powder aerosol should not be influenced by the percent water content of fluorescein disodium.

IV.C.5 Comparison of the CFD Model of the ACI with the In Vitro Study - Estimation of Number of Elementary Charges Per Particle

The deposition behavior of the fluorescein dry powder aerosol as a function of charge in the present in vitro study was compared with the CFD model predictions. The CFD model predicted increased deposition of up to 30% for the upper stages of the ACI i.e., stage 0 to 3. While the experimental results in the in vitro study conformed with the CFD model predictions in terms of increased deposition on stages 0 to 3 of the ACI, the magnitude of increase observed in the in vitro study was higher, approximately 200% on stages 0 and 1 and approximately 110% observed on stages 2 and 3 of the ACI for artificially charged (both positive and negative) fluorescein aerosol, compared to CFD model predictions. The increased deposition of charged fluorescein aerosol on the higher stages of the ACI is expected since charged particles may deposit prematurely on contacting surfaces. This hypothesis is also supported by the observation of deposition of a significant amount of charged fluorescein aerosols depositing in the corona charger itself during their transit into the impactor. Image charge effects may be attributed for...
the increased deposition of charged aerosol on the higher stages of the ACI. Space charge effects, or the particle-particle repulsion within the charged fluorescein aerosol cloud may also play a role in the increased deposition on the upper stages of the ACI since the aerosol concentration for the fluorescein aerosol (~780 μg in 20 seconds) was estimated to be approximately 480,000 particles/cm³ in comparison to the dilute aerosol conditions simulated in the CFD study; space charge effects were neglected in the CFD model.

The CFD model predicted an approximately 90% increase in deposition for stage 5, however in contrast to the CFD predictions a significant decrease in the deposition of charged aerosol was observed on stage 5 compared to neutral aerosol. Stage 5 was observed to be the stage with the maximum mass of fluorescein and repulsion of the incoming charged aerosol particles by already deposited particles may be responsible for these observations. This phenomenon was more predominant for the negatively charged aerosol, in comparison to the positively charged aerosol, supporting this hypothesis. Moreover, since the aerosol concentration estimated in the present study was high compared to the dilute aerosol assumption used for the CFD study, repulsion of the unipolar, highly concentrated and charged fluorescein aerosol particles is also likely to occur on stage 5, within the incoming charged particles and/or by the already deposited charged particles. It is important to note that the CFD predicted cut-off diameters were observed to be in good agreement with the manufacturer and previous experimental reported cut-off diameters but even in the manufacturer and experimental calibrations of the ACI dilute calibration aerosol was used (10¹-10² particles/cm³). Nevertheless, the present in vitro study was aligned with realistic in vitro testing of pharmaceutical aerosols, which are believed to have a concentration of 10⁵-10⁶ particles/cm³. The estimated deposition efficiency for the charge neutralized aerosol on stage 5 of the ACI in the in vitro study was
observed to be approximately 65%, which was in good agreement with the CFD model prediction of approximately 70% for a charge neutral, approximately 1.3 μm sized particle. Therefore, it appears that the CFD model predictions were in agreement with realistic in vitro deposition of charge neutralized aerosols within the ACI. The CFD model simulated the ACI stages individually, while the experimental study used the entire ACI stack to reflect realistic use, thus making deposition across the stages interdependent, i.e., increased deposition on the upper stages may result in decreased deposition on the lower stages.

The number of elementary charges per particle was estimated for the four charge levels of fluorescein aerosol in an attempt to further evaluate the CFD model predictions in relation to the in vitro experimental results for stage 5. Since the maximum deposition of polydisperse (GSD~1.6) fluorescein aerosol, with an MMAD of approximately 1.3 μm, was observed on stages 5 of the ACI (corresponding to the size range of 1.1-2.1 μm), ELPI stage 8, which approximates the size range of 1.4-2.1 μm (based on recalibrated ELPI cut-off diameters), was used for the estimation of electrons per particle based on the charge and mass of the fluorescein deposited, which are shown in Table IV.6.

Table IV.6  Comparison of the Number of Electrons per Particle for the Fluorescein Aerosol as a Function of Charge obtained using the ELPI. Data represents Mean, n=5.

<table>
<thead>
<tr>
<th>Charge Level</th>
<th>Experimental&lt;sup&gt;a&lt;/sup&gt; e/particle</th>
<th>CFD&lt;sup&gt;b&lt;/sup&gt; e/particle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge Neutralized</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>Inherently Charged</td>
<td>-1</td>
<td></td>
</tr>
<tr>
<td>Positively Charged</td>
<td>216</td>
<td>~ 50</td>
</tr>
<tr>
<td>Negatively Charged</td>
<td>-502</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>e/particle calculated from Stage 8 of the ELPI following field charging of fluorescein aerosol using the stacked ELPI with the corona charger

<sup>b</sup>e/particle assumed based upon the literature reported triboelectric charges for dry powder inhalers, individual ACI stages simulated in the CFD model
The charge neutralized and the inherently charged fluorescein aerosols were observed to carry negligible number of electrons per particle, which were lower compared to those used as input for the CFD model. The positively and negatively charged fluorescein aerosol was estimated to carry a charge equivalent to approximately 200 and 500 electrons per particle, which was greater than that on the equivalent sized charged aerosol particles (approximately 50 electrons per particle) reported in the CFD study. These results verified that artificially induced charge achieved on the fluorescein aerosol in the present study was higher than that modeled in the CFD study on dry powder aerosol particles.

Increased deposition on ACI stages downstream from stage 5 for charged aerosol compared to charge neutralized aerosol was observed, though it was not observed to be statistically significant in all cases. The increase on these lower stages of the ACI i.e., stage 6 to filter was not in agreement with the CFD model predictions since the CFD model predicted almost 100% increase for these submicron sized charged aerosol particles. Increased deposition on stages 6 and below of the ACI in the in vitro study may arise due to the transit of charged aerosol particles, which were repelled on stage 5 and were forced to travel downstream in the impactor. The presence of charge appeared to increase the polydispersity of the fluorescein aerosol, while it did not influence the size of the aerosol measured in vitro using the ACI. Therefore, despite the significantly higher electrostatic charge on the artificially charged aerosol, this applied charge failed to produce a significant difference in the ACI-measured apparent APSD of the aerosol. The results observed in this study were not in agreement with the previously reported experimental study concerning the deposition of charged aerosols within the QCM PC-2 impactor (Horton and Mitchell, 1992). This may be attributed to the fact that
polydisperse and highly concentrated fluorescein aerosol was used in the present study, similar to pharmaceutical aerosols, whereas Horton and Mitchell used monodisperse and dilute aerosol in their study.

IV.D Summary

In conclusion, an in vitro experimental set up was developed to assess the deposition behavior of charged aerosol particles within the ACI, which is routinely used in in vitro testing of pharmaceutical aerosols. This study investigated the effects of electrostatic charge on the particle size distribution for pharmaceutical aerosols in the ACI, which sizes the aerosol based on inertial impaction, in an attempt to experimentally validate the CFD model predictions. The in vitro experimental set up developed generated fluorescein dry powder aerosol having aerodynamic sizes in the size range predicted to be influenced by electrostatic effects in the CFD model and enabled the modification of charge level.

CFD predicted increased deposition for charged aerosols across all the stages of the ACI induced by image charging. Higher electrostatic charge i.e., number of elementary charges per particle was achieved in the in vitro experiments for charged polydisperse fluorescein aerosols, both positively and negatively charged, than those assumed in the CFD study. The present study illustrated that upon the application of high levels of charge, increased deposition was observed in the charger and on the upper stages of the ACI, thus altering the deposition pattern of the aerosols within the ACI. The in vitro results were observed to be in partial agreement with the CFD predictions and it was observed that the presence of charge appeared to increase the polydispersity of the fluorescein aerosol due to a combined image charge and space charge
effects i.e., particle-particle interaction, while it did not influence the size of the aerosol measured in vitro using the ACI.

Differences in the CFD model predictions and the in vitro study results do not necessarily imply that the CFD model of charged particle deposition is inaccurate. Instead, the results indicate that the assumptions included in the CFD model may not have been realistic and may have excluded some of the physical phenomena that are present in the in vitro system. The present study helps gain insight into the uses and limitations of both CFD and charged aerosol impaction studies. The experiments described here cast doubt on the theoretical predictions of the effects of charge on aerosol deposition in the ACI. However, it is possible that both experimental and theoretical modifications are needed to bring predictions into agreement with practice. Future experimental studies will investigate the CFD model assumptions and the in vitro study will be modified in order to align it with the CFD model.
V. ELECTROSTATIC EFFECTS AND AEROSOL DEPOSITION WITHIN THE ANDERSEN CASCADE IMPACTOR: COMPUTATIONAL VERSUS REALISTIC IN VITRO EFFECTS

V.A Introduction

A Computational Fluid Dynamics (CFD) model of the Andersen Cascade Impactor (ACI) suggested that electrostatic charge effects in cascade impactors may be a confounding factor during in vitro testing of pharmaceutical aerosols (Vinchurkar et al. 2009). In vitro deposition behavior of charge neutralized and artificially charged fluorescein dry powder aerosols within the ACI were not in agreement with the CFD model predictions (Chapter IV). For stages 0 through 3 of the ACI, the CFD model predicted approximately 30% increased deposition for charged particles compared to neutral particles. While increased deposition was observed in the in vitro study, the experimental effects were more pronounced than the model predictions: deposition increased by approximately 200% on stages 0 and 1 and by approximately 110% on stages 2 and 3, for charged particles. For stage 5 and below of the ACI, discrepancies were also observed between the in vitro results and the CFD predictions. Deposition of charged aerosol on stage 5 decreased in the in vitro study compared to neutral aerosol, whereas the CFD model predicted 90% increased deposition for stage 5. In addition to this, little or no increase was observed in the deposition of charged particles on stage 6 and below of the ACI, compared to the
neutral aerosol. It is possible that the CFD model may have excluded some physical phenomena that are present in the *in vitro* system.

Table V.1 compares the CFD model assumptions and the *in vitro* study, which was designed to represent realistic *in vitro* testing of pharmaceutical aerosols.

Table V.1 Summary of CFD Model Assumptions and *In Vitro* Study

<table>
<thead>
<tr>
<th>CFD Model Assumption</th>
<th><em>In Vitro</em> Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particles</td>
<td>Monodisperse</td>
</tr>
<tr>
<td>Concentration</td>
<td>Low ~ $10^1$</td>
</tr>
<tr>
<td>Coincidence</td>
<td>Assumed to not occur</td>
</tr>
<tr>
<td>Impactor</td>
<td>Perfect conductor</td>
</tr>
<tr>
<td>Charge Sensitive Size</td>
<td>0.4-2.1 µm</td>
</tr>
<tr>
<td>Image Charge</td>
<td>Ideal</td>
</tr>
<tr>
<td>Space Charge</td>
<td>Neglected</td>
</tr>
<tr>
<td>Particle Deposition</td>
<td>Individual Particles</td>
</tr>
<tr>
<td>Impactor Stages</td>
<td>Simulated Individually</td>
</tr>
</tbody>
</table>

Table V.1 Summary of CFD Model Assumptions and *In Vitro* Study

While the CFD study was designed to model the deposition of monodisperse, charged, dilute aerosol with a concentration of approximately 10 particles/cm³ in accord with the aerosols used for the calibration of cascade impactors, the *in vitro* study was designed to represent realistic *in vitro* testing conditions and investigated the deposition of a polydisperse, charged aerosol with a concentration of approximately $10^5$ particles/cm³, comparable to pharmaceutical aerosols. Therefore, coincidence or particle-particle interactions, which were ignored in the CFD model, are likely to be important during *in vitro* experiments with such a dense fluorescein aerosol cloud. Moreover, it has been reported previously that upon impaction, particles deposit as ‘heaps’ or aggregates on an impactor stage due to new particles impacting on and around those that are already deposited below the jets of each impactor stage (Clarke *et al.* 2002). As a result, particle-particle repulsion may also occur either within such ‘heaps’ of unipolarly charged...
particles or between the incoming charged particles and the particles already deposited in such ‘heaps’.

The CFD model assumed that the cascade impactor was a perfect conductor and electrostatic charge due to deposited particles was not retained on the ACI impaction stage. Thus, incoming aerosol particles were assumed to interact with a neutral, metal impaction surface, leading to image charge formation, resulting in particle deposition. However, during in vitro testing, charge accumulation on the impactor stages may occur due to deposition of charged particles on the ACI impaction stage, and charge repulsion may result. Indeed, electrostatic effects have been suggested to result in erratic deposition of particles and influence mass balance as well as APSD measurements (Copley 2010). Pharmacopeial methods do not currently address whether the cascade impactor should be grounded to dissipate charge accumulation, and the influence of grounding the impactor on deposition behavior and apparent APSD measurements has not been reported. Because the in vitro study (described in Chapter IV) was performed using a non-grounded ACI, in accordance with the pharmacopeial methods, the use of a grounded impactor may align the experimental protocol with the CFD assumptions.

The CFD model predicted 90% increased deposition for the size range 1.1-2.1 µm (stage 5) and approximately 100% increased deposition for 0.4-1.1 µm (stage 6 and 7) respectively. While the in vitro study was designed to test the pharmaceutically relevant size of > 1 µm, the deposition behavior of < 1 µm aerosol is warranted to completely evaluate the experimental validity of the CFD model predictions.

A perfect image charge was assumed to form between the aerosol and a metal conducting plate in the CFD model, while in reality pharmaceutical aerosols, which are known to be insulators, do not form perfect image charges. The effects of coating the impaction surfaces on
this attractive image force were also neglected. ‘Stage coating’ is a pre-requisite during in vitro testing of aerosols to avoid bias caused by particle bounce, where a particle comes into contact with the impaction plate but is not captured (Dunbar et al. 2005). This results in the underestimation of the size of the emitted aerosol because the particles are captured on lower stages of the impactor with smaller cut-off diameters (Nasr et al. 1997, Kamiya et al. 2004, Kamiya et al. 2009). Stage coating materials, including silicone-based and glycerol-based coatings, typically provide a tacky surface ideal for particle collection and are insulating in nature (Mitchell 2003). The presence of such an insulative coating on the impaction plate eliminates direct contact between the charged aerosol particle and the metal impaction plate and may impede image charge formation. The CFD model also assumed individual particle deposition on a stage but during in vitro testing, multi-layer deposition of particles occurs, which may also modify the impaction surface i.e., as more particles deposit on a stage, incoming particles are likely to deposit on other particles rather than the impaction surface. While ‘stage loading’ has been reported to cause the deposition of incoming smaller aerosol particles on higher stages along with the larger particles (Nasr et al. 1997), the presence of electrostatic charge on the particles, may also hinder image charge formation and support space charge effects. Space charge effects were ignored in the CFD model, which was appropriate for the CFD study since the aerosol was assumed to be dilute but space charge effects are likely to play a role in the deposition of charged, bolus pharmaceutical aerosols. Moreover, within a cascade impactor, space charge effects may also result in repulsion during the deposition of like charged particles as heaps below the jets on an impactor stage. The relationship between image charge formation and stage coating and stage loading has not been investigated and the influence upon apparent APSD measurements is unknown.
Individual impactor stages were simulated in the CFD model, while in practice pharmaceutical aerosols are characterized using multi-stage impactors, where deposition on a stage is influenced by deposition on the previous stage. Recently, there has been increased interest in abbreviated impactor measurement (AIM), which is expected to provide a potential improvement to the labor-intensive full-resolution cascade impactor measurements for APSD measurement of pharmaceutical aerosols systems (Tougas et al. 2009, Mitchell et al. 2009a, Mitchell et al. 2009b, Mitchell et al. 2010a, Mitchell et al. 2010b, Mitchell et al. 2010c, Sheng et al. 2010, Chambers and Smurthwaite 2012). AIM based systems divide the aerosol cloud directly into two fractions: the small (fine) and large (coarse) particle mass and eliminate stages where little or no drug deposits. Hence, it is important to investigate the influence of electrostatic charge upon the deposition behavior within abbreviated impactors.

The *in vitro* study was extended to investigate the influence of grounding, particle size, stage coating and loading on the deposition behavior of charge neutralized and charged fluorescein aerosol within a full resolution ACI. Charge effects were also investigated in an abbreviated ACI in order to gain insight into the role of charge on the deposition of pharmaceutical aerosols.

V.B Materials and Methods

V.B.1 Production of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol and Investigation of the Influence of Different Variables upon Deposition within the ACI

The *in vitro* study designed to probe the deposition behavior of aerosols in the ACI (Copley Scientific, Nottingham, United Kingdom) described in Chapter IV.B was employed to
generate either a < 1 μm or > 1 μm fluorescein disodium aerosol using the constant output atomizer, by virtue of its flexibility to generate aerosols of different sizes. The size of the aerosol particle, $D_p$, depends on the weight fraction of the solute, $F_s$, and the droplet diameter, $D_d$, according to Equation V.1

$$D_p = D_d \times \left( \frac{F_s \times \rho_l}{\rho_s} \right)^{1/3}$$

Equation V.1

where, $D_p$ is the particle diameter ($\mu$m), $D_d$ is the droplet diameter ($\mu$m), $F_s$ is the weight fraction of the solute, $\rho_l$ is the density of the liquid (g/cm$^3$) and $\rho_s$ is the density of the solute (g/cm$^3$). A dilute 1% aqueous solution of fluorescein disodium was used to generate < 1 μm fluorescein disodium aerosol based on the above equation, whereas 18.2 % aqueous solution of fluorescein disodium was used to generate > 1 μm fluorescein disodium aerosol. The experimental set up (described in Chapter IV.B.1) was used to produce fluorescein dry powder aerosol with two extreme charge levels: charge neutralized and positively charged.

The Electrical Low Pressure Impactor (ELPI, Dekati Ltd., Tampere, Finland) was used to characterize the charge distribution of the fluorescein dry powder aerosol, when specified, using methods described in Chapter IV.B.2. The ACI was operated at 28.3 Lmin$^{-1}$ to assess the deposition behavior and apparent APSD of the fluorescein aerosol as a function of charge following a sampling time of 20 seconds as described in Chapter IV.B.3, unless specified otherwise. Whenever stage coating was used, it was performed using 316 Silicone Release Spray (Dow Corning, Midland, MI) for the ACI stages and DS-515 grease spray (Dekati Ltd., Tampere, Finland) for the ELPI aluminum substrates. Fluorescein deposition on each stage of the ACI was determined using a validated HPLC analysis as described in Chapter IV.B.5. All experiments (n=5) were performed with laboratory temperatures ranging from 20.3 to 25.7°C,
while the relative humidity varied from 27 to 43%. The testing protocol used was similar to previously described methods in Chapter IV.B, with modifications described below to investigate the influence of the following variables.

V.B.1.1 Influence of Grounding upon the Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within the ACI

To prevent the accumulation of static charge on the ACI, a grounding wire was used to connect the base of the ACI to the ELPI outer box, which is grounded when connected to an electrical socket (Dekati Ltd. 2011). The stages of a standard ACI are electrically connected, which was verified during preliminary experiments. The deposition behavior of fluorescein aerosol > 1 µm was assessed for charge neutralized and positively charged fluorescein aerosol within a grounded ACI and non-grounded ACI using coated impaction plates as described in Section V.B.1.

V.B.1.2 Influence of Aerosol Size upon the Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within the ACI

Charge neutralized and positively charged fluorescein aerosol, < 1 µm in size was generated using the methods described in Section V.B.1, following which their deposition behavior was assessed within a grounded ACI with coated impaction plates. The electrostatic charge distribution for the two charge levels of fluorescein aerosol was characterized using the ELPI with coated substrates. The total net charge was calculated as the sum of the net charge on each ELPI stage. The specific charge, or charge-to-mass ratio (q/m), for a given size fraction was calculated using the charge and mass measured using the ELPI in each size fraction as
described in Chapter IV.B.6. The total delivered dose of fluorescein aerosol for the ELPI and the ACI was determined for each charge level and compared statistically to ensure the comparability of the aerosol cloud used in the ELPI and the ACI.

V.B.1.3 Influence of Stage Coating and Stage Loading upon the Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within the ACI

The deposition behavior of charge neutralized and positively charged fluorescein aerosol, > 1 µm in size, was assessed within a grounded ACI using coated and uncoated impaction plates. The collection surface of the ACI impaction plates were coated with 316 Silicone Release Spray by holding the plates vertical, and spray coating the collection surface from a distance of approximately 6 inches for about 4-5 seconds, in a fume hood. The propellant/solvent was allowed to evaporate at room temperature for ≥ 5 minutes, following which the spraying procedure was repeated for a further 4-5 seconds. Impaction plates were removed from the fume hood after ≥ 5 minutes to continue the experimental procedure described in Section V.B.1. The uncoated ACI impaction plates were used following rinsing with acetone and deionized water and subsequent air-drying.

In order to investigate the influence of stage loading upon the deposition behavior of fluorescein aerosol within the ACI, the deposition behavior of charge neutralized and positively charged fluorescein aerosol was compared using a grounded ACI with coated impaction plates, following sampling times of either 5 seconds or 20 seconds. The electrostatic charge distribution for the charge neutralized and positively charged fluorescein aerosol was determined using the ELPI as described in Chapter IV.B.2. The total delivered dose of fluorescein sodium aerosol for
the ELPI and the ACI was determined for each charge level and compared statistically to ensure the comparability of the aerosol cloud used in the ELPI and the ACI.

V.B.1.4 Characterization of the Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within an Abbreviated ACI

Figure V.1 shows the QC metric abbreviated ACI system used, which was a reduced stack ACI system including only the inlet cone, stage 0, stage 5 and the filter stage of the full resolution ACI, based on the configuration defined by Mitchell et al. (Mitchell et al. 2010a).

![Abbreviated ACI System](image)

**Figure V.1** (a) Schematic of the Abbreviated Quality Control Impactor Configuration (Adapted from Mitchell et al. 2010a). (b) Photograph of the Abbreviated Impactor used in the Study.

The deposition behavior of > 1 µm in size, charge neutralized and positively charged fluorescein aerosol was determined using a grounded QC abbreviated ACI system, operated at 28.3 Lmin⁻¹, with coated impaction plates following a sampling time of 20 seconds (n=5).
Abbreviated QC impactor was validated by comparing the apparent APSD obtained using the abbreviated system with the full resolution ACI, in accordance with the recommendations by Mitchell et al. (Mitchell et al. 2009a, Mitchell et al. 2010a).

The single stage QC abbreviated ACI configuration enabled data analysis using the Efficient Data Analysis (EDA) metrics of Impactor Sized Mass (ISM), Large Particle Mass (LPM) and Small Particle Mass (SPM) (Tougas et al. 2009, Tougas et al. 2011, Mitchell et al. 2011, Mitchell et al. 2012). The boundary size for the QC impactor was chosen to be 1.1 μm (Stage 5) aerodynamic diameter, set at the stage cut-off diameter closest to the MMAD of the fluorescein aerosol investigated (Mitchell et al. 2010a). Both SPM and LPM were determined in a single process, where SPM was defined as the mass of fluorescein deposited below the aerodynamic particle size boundary chosen i.e., below 1.1 μm or on the filter stage while the LPM was defined as the mass of fluorescein deposited above the chosen aerodynamic particle size boundary i.e., on stage 5 of the abbreviated ACI. The large particle mass (LPM) / small particle mass (SPM) ratio was calculated together with the Impactor Sized Mass (ISM), which was defined as the sum of the fluorescein mass deposited on the filter and all impactor stages except the uppermost stage i.e., stage 0 for both the full resolution and abbreviated impactor systems.
V.C Results and Discussion

V.C.1 Influence of Grounding upon the Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within the ACI

Table V.2 summarizes the deposition summary of > 1 µm charge neutralized and positively charged fluorescein aerosol obtained using the ungrounded and grounded ACI, as a function of the two charge levels investigated.

Table V.2 Fluorescein Mass Deposition for > 1 µm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the Non-grounded and Grounded ACI. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>Charge Level</th>
<th>Impactor ACI</th>
<th>Delivered Dose (µg)</th>
<th>Throat Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>Impactor Dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge Neutralized</td>
<td>Non-grounded</td>
<td>790.4±68.6</td>
<td>1.7±0.6</td>
<td>4.3±1.5</td>
<td>784.4±68.9</td>
</tr>
<tr>
<td></td>
<td>Grounded</td>
<td>800.2±59.8</td>
<td>1.3±0.3</td>
<td>3.7±1.0</td>
<td>795.2±60.0</td>
</tr>
<tr>
<td>Positively Charged</td>
<td>Non-grounded</td>
<td>798.6±52.8</td>
<td>1.6±0.9</td>
<td>106.4±7.7*</td>
<td>690.6±46.8*</td>
</tr>
<tr>
<td></td>
<td>Grounded</td>
<td>818.7±62.4</td>
<td>1.7±0.4</td>
<td>121.1±6.7*</td>
<td>696.5±58.3*</td>
</tr>
</tbody>
</table>

*Significant difference, p-value < 0.05

It was observed that the total delivered doses, mass of fluorescein deposited in the throat, charger, and within the ACI, were statistically comparable between the non-grounded and grounded ACI measurements for both charge neutralized and positively charged fluorescein aerosol (ANOVA p-value > 0.05). As observed previously in Chapter IV.C.3, when the deposition of charge neutralized and positively charged fluorescein aerosol was compared within a non-grounded ACI, the total delivered doses and the throat doses were statistically comparable (p-value > 0.05, Student’s t-test, Table V.2), while the charger dose was observed to be significantly higher and the impactor dose was significantly decreased, for the positively charged fluorescein aerosol compared to the charge neutralized aerosol (p-value < 0.05, Student’s t-test, Table V.2).
Figure V.2 shows the mass distribution for the > 1 μm charge neutralized and positively charged fluorescein aerosol collected in the ACI as a function of grounding. No significant difference was observed between the non-grounded and grounded ACI measurements for both charge neutralized and positively charged fluorescein aerosol (p-value > 0.05, Student’s t-test).

Deposition behavior of fluorescein aerosol was compared within the grounded ACI, as a function of charge, in line with the CFD model assumption. Figure V.3 compares the mass distribution, as well as the percent impactor dose, for the charge neutralized and positively charged fluorescein aerosol collected in the ACI.

Figure V.2 Mass Distribution for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol as a Function of Grounding the ACI. Inset shows the Mass Deposition on Stages 0 through 3 on an Expanded Scale. Data represents Mean ± SD, n=5.
In agreement with the results obtained with the non-grounded ACI (discussed in Chapter IV.C.3), a statistically significant increase was observed on stages 0 through 3 of the ACI for the positively charged fluorescein aerosol compared to the charge neutralized fluorescein aerosol, for both fluorescein mass deposition (p-value = 0.0001) as well as the normalized percent impactor deposition (p-value = 0.0008) (Student’s t-test). A significant decrease was observed on stage 5 of the ACI, for the positively charged fluorescein aerosol compared to the charge neutralized fluorescein aerosol, for both fluorescein mass deposition (p-value = 0.0011) and the normalized percent impactor dose (p-value = 0.0040) (Student’s t-test). Despite this increase, the total deposition on stages 0 to 3 was < 8% of the total impactor dose (Appendix IV, Table AIV.2). As a result, no significant difference was observed in the apparent MMAD obtained using the grounded ACI for the charge neutralized and positively charged fluorescein aerosol as illustrated in Figure V.4 (p-value = 0.1959, MMAD charge neutralized = 1.32±0.05 μm, MMAD positively charged = 1.39±0.04 μm).
Figure V.4  Apparent APSD for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using a Grounded ACI. Data represents Mean ± SD, n=5.

Grounding the cascade impactor is not described in the pharmacopeial *in vitro* cascade impaction testing method (USP 29 / NF 24 First Supplement 2006). Based upon the results of the present study, it appears that grounding of the ACI did not influence the APSDs obtained as a function of charge level for fluorescein aerosol, which overlapped as shown in Figure V.4. It should be noted that fluorescein, similar to pharmaceutical powders is an insulator (Grosvenor and Staniforth 1996, Hughes 1997) and thus, the flow of charge as a result of grounding may not occur effectively throughout the ‘heaps’ of fluorescein particles. Despite the fact that grounding did not influence the deposition behavior of fluorescein aerosol as a function of charge within the ACI, it seemed prudent to use a grounded ACI for the investigation of the remaining variables in order to mirror the CFD model assumption.
V.C.2 Deposition Behavior of < 1 µm Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within the ACI

The electrostatic charge distribution and mass distribution for the < 1 µm charge neutralized and positively charged fluorescein aerosol, obtained using the ELPI is shown in Figure V.5.

![Figure V.5](image)

The total net charge on the < 1 µm charge neutralized fluorescein aerosol was observed to be negligible (-0.28±0.12 nC) and significantly lower than the positively charged fluorescein...
aerosol (17.44±2.39 nC) (p-value = 0.0040, Student’s t-test). The total net charge on the < 1 µm positively charged fluorescein aerosol was also lower compared to the > 1 µm positively charged (+30.6±2.7 nC) discussed in Chapter IV.C.2, which may be attributed to the decreased aerosol mass generated in 20 seconds for the dilute < 1 µm fluorescein aerosol compared to the more concentrated > 1 µm fluorescein aerosol produced. Maximum fluorescein mass deposition was observed on stages 5 through 7 of the ELPI for the two charge levels studied.

Table V.3 summarizes the mass deposition for < 1 µm charge neutralized and positively charged fluorescein aerosol obtained using the ACI and the ELPI as a function of charge.

<table>
<thead>
<tr>
<th>Charge Level</th>
<th>Impactor Type</th>
<th>Delivered Dose (µg)</th>
<th>Throat Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>Impactor Dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge Neutralized</td>
<td>ACI</td>
<td>124.4±13.9</td>
<td>0.9±0.4</td>
<td>2.2±0.5</td>
<td>121.3±14.3</td>
</tr>
<tr>
<td></td>
<td>ELPI</td>
<td>123.1±11.1</td>
<td>0.7±0.2</td>
<td>2.3±0.9</td>
<td>120.2±10.7</td>
</tr>
<tr>
<td>Positively Charged</td>
<td>ACI</td>
<td>111.6±14.6</td>
<td>0.9±0.1</td>
<td>14.5±1.1*</td>
<td>96.5±7.8*</td>
</tr>
<tr>
<td></td>
<td>ELPI</td>
<td>116.9±14.6</td>
<td>0.9±0.4</td>
<td>13.2±1.9*</td>
<td>102.9±15.4*</td>
</tr>
</tbody>
</table>

*Significant difference within each impactor, p-value < 0.05

It was observed that the total mass of fluorescein deposited in the throat, charger, and within the impactor, were statistically comparable for both the ACI and the ELPI (p-value > 0.05, Student’s t-test) across the two charge levels investigated. These results justified the use of the ELPI for the measurement of charge on each submicron fluorescein aerosol.

Similar to results reported for the > 1 µm positively charged fluorescein aerosol (Chapter IV.C.2 and IV.C.4), the deposition behavior of the < 1 µm fluorescein aerosol within the cascade impactor was influenced by the presence of electrostatic charge on the aerosol. The charger dose
for the positively charged fluorescein aerosol was significantly higher when compared to the charge neutralized fluorescein aerosol, which consequently, decreased the impactor dose for positively charged aerosols, for both the ACI and the ELPI (p-value < 0.05, Student’s t-test). This deposition behavior is speculated to be a result of premature deposition of highly charged particles during their transit through the corona charger.

Figure V.6 shows the mass distribution together with the normalized impactor dose for the fluorescein aerosol < 1 µm in size, collected in the ACI as a function of charge.

Figure V.6 Mass Distribution and Percent Impactor Dose for < 1 µm Fluorescein Aerosol as a Function of Charge Level determined using the ACI. Inset shows the Mass Deposition on Stages 0 through 3 on an Expanded Scale. Data represents Mean ± SD, n=5.

Positively charged fluorescein aerosol was observed to show significantly increased fluorescein deposition on stages 1 through 3 of the ACI compared to the charge neutralized fluorescein aerosol of < 1 µm in size (p-value = 0.0219, Student’s t-test). However, significantly decreased deposition was observed on stage 6 (p-value = 0.0385, Student’s t-test) and filter (p-value = 0.0110, Student’s t-test) of the ACI for the positively charged submicron fluorescein aerosol compared to the charge neutralized submicron fluorescein aerosol. For the normalized
percent impactor dose, deposition on stages 1 through 3 of the ACI increased significantly up to 200% for the positively charged aerosol (p-value = 0.0035, Student’s t-test) compared to the charge neutralized < 1 µm fluorescein aerosol, in agreement with the previous findings with > 1 µm fluorescein aerosol discussed in Chapter IV.C.4 due to image charge effects (Appendix IV, Table AIV.3). These results were in agreement with the CFD model predictions for the upper stages of the ACI. Despite this increased deposition, the deposition on stages 1 through 3 of the ACI was less than 3% of the total impactor deposition of fluorescein.

However, in contradiction with the CFD model predictions, no significant difference was observed on the deposition of < 1 µm charge neutralized and positively charged fluorescein aerosol, on the submicron stages of the ACI, i.e., stage 6 through filter (p-value > 0.05, Student’s t-test). The CFD model predicted an increase of almost 100% for charged particles compared to neutral particles on stages 6 and 7 of the ACI. These results were also not in agreement with the previous *in vitro* experiments using > 1 µm fluorescein aerosol, where deposition significantly decreased on stage 5 for the charged fluorescein aerosol compared to the neutral fluorescein aerosol. This may be attributed to the fact that the positively charged < 1 µm fluorescein aerosol carried less charge and undergoes charge dissipation as it travels further down the impactor stack, compared to the > 1 µm positively charged fluorescein aerosol. The total mass of fluorescein deposited on stages 5 and 6 for the < 1 µm fluorescein aerosol (~ 60 µg) was observed to be much less in comparison to that observed on stage 5 of the > 1 µm fluorescein aerosol, thus the repulsion of incoming charged aerosol particles may be reduced, on stages 6 and 5 respectively, where maximum fluorescein deposition was observed. The aerosol concentration for the < 1 µm fluorescein aerosol (~120 µg in 20 seconds) was estimated to be
approximately 160,000 particles/cm³, which was less concentrated than > 1 μm positively charged aerosol (~480,000 particles/cm³).

The minor shift in the deposition pattern within the ACI on the upper stages (i.e., 1 to 3) did not influence the apparent APSDs obtained for the charge neutralized and positively charged < 1 μm fluorescein aerosol, which were almost overlapping as shown in Figure V.7.

![Figure V.7](image)

**Figure V.7** Apparent APSD for < 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using the ACI. Data represents Mean ± SD, n=5.

No significant difference was observed in the MMAD obtained using the ACI for the positively charged aerosol compared to the charge neutralized fluorescein aerosol (p-value = 0.5618, Student’s t-test, MMAD charge neutralized = 0.68±0.03 μm, MMAD positively charged = 0.70±0.05 μm; Figure V.7). Since the investigated aerosol size < 1 μm, was observed to be at the tail-end of the ACI size spectrum, the size distribution was not observed to be log-normal, and numerical curve fitting of the ELPI data to the size distribution obtained using the ACI, was not performed.
The number of elementary charges per particle was estimated for the charge neutralized and positively charged fluorescein aerosol as a function of aerosol size, in order to further evaluate the CFD model predictions in relation to the in vitro experimental results. For the < 1 μm sized fluorescein aerosol, since stage 6 (size range 0.7-1.1 μm) was observed to be closest to the size of the aerosol, ELPI stage 6, which approximates the size range of 0.7-1.0 μm, was used for the estimation of electrons per particle based on the charge and mass of the fluorescein deposited, which are shown in Table V.4. The number of electrons per particle for the > 1 μm fluorescein aerosol were also estimated and have been discussed in Chapter IV.C.6.

Table V.4  Comparison of the Number of Electrons per Particle for the Charge Neutralized and Positively Charged Fluorescein Aerosol as a Function of Aerosol Size obtained using the ELPI. Data represents Mean, n=5.

<table>
<thead>
<tr>
<th>Aerosol Size</th>
<th>MMAD (µm)</th>
<th>ELPI Stage (Cut-off, µm)</th>
<th>CFD(^a) Charge Level</th>
<th>Charge(^b) Neutralized</th>
<th>Positively(^b) Charged</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 µm</td>
<td>~0.7</td>
<td>Stage 6 (0.7-1.0)</td>
<td>~ 20</td>
<td>-1.4</td>
<td>160</td>
</tr>
<tr>
<td>&gt;1 µm</td>
<td>~1.3</td>
<td>Stage 8 (1.4-2.1)</td>
<td>~50</td>
<td>0.4</td>
<td>216</td>
</tr>
</tbody>
</table>

\(^{a}\)e/particle assumed based upon the literature reported triboelectric charges for dry powder inhalers, individual ACI stages simulated in the CFD model

\(^{b}\)e/particle calculated from a single ELPI stage following field charging of fluorescein aerosol using the stacked ELPI with the corona charger

Charge neutralized fluorescein aerosol was observed to carry negligible number of electrons charges per particle irrespective of the aerosol size, whereas positively charged < 1 μm fluorescein aerosol was estimated to carry a charge equivalent to approximately 160 electrons per particle, which was less than that estimated for > 1 μm positively charged fluorescein aerosol (~200 electrons per particle). Nevertheless, the < 1 μm positively charged fluorescein aerosol was estimated to still carry more electrons per particle than that on the equivalent sized charged aerosol particles (~20 electrons per particle) used in the CFD study. Despite the higher
electrostatic charge per particle achieved in these experiments for positively charged fluorescein aerosol than those assumed in the CFD model, charge failed to bring about a significant difference in the apparent APSD of the aerosol measured by the ACI.

V.C.3 Influence of Stage Coating and Stage Loading upon the Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Disodium Aerosol within the ACI

Table V.5 summarizes the mass deposition and Figure V.8 shows the mass distribution for the > 1 µm charge neutralized and positively charged fluorescein aerosol, obtained in the grounded ACI as a function of stage coating.

<table>
<thead>
<tr>
<th>Charge Level</th>
<th>Impaction Stage</th>
<th>Delivered Dose (µg)</th>
<th>Throat Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>Impactor Dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge Neutralized</td>
<td>Coated</td>
<td>790.4±68.6</td>
<td>1.7±0.6</td>
<td>4.3±1.5</td>
<td>784.4±68.9</td>
</tr>
<tr>
<td></td>
<td>Uncoated</td>
<td>815.9±17.0</td>
<td>1.5±0.3</td>
<td>3.4±0.5</td>
<td>811.5±17.2</td>
</tr>
<tr>
<td>Positively Charged</td>
<td>Coated</td>
<td>798.6±52.8</td>
<td>1.6±0.9</td>
<td>106.4±7.7*</td>
<td>690.6±46.8*</td>
</tr>
<tr>
<td></td>
<td>Uncoated</td>
<td>813.3±63.6</td>
<td>3.0±1.5</td>
<td>123.5±14.9*</td>
<td>688.0±62.1*</td>
</tr>
</tbody>
</table>

*Significant difference within each impactor, p-value < 0.05

It was observed that the total delivered doses, mass of fluorescein deposited in the throat, charger, and within the ACI, were statistically comparable between the measurements performed using coated and uncoated impaction plates, for both charge neutralized and positively charged fluorescein aerosol (p-value > 0.05). In agreement with the previous results obtained using coated impaction plates (Chapter IV.C.4), the charger dose was significantly higher (p-value = 0.0001) and the impactor dose significantly reduced (p-value = 0.0053), for the positively
charged fluorescein aerosol compared to the charge neutralized aerosol upon using uncoated impaction plates in the ACI (Student’s t-test).

![Graphs and tables]

Figure V.8  Mass Distribution and Percent Impactor Dose for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the Grounded ACI as a Function of Stage Coating. Inset shows Mass Deposition on Stages 0 through 3 on an Expanded Scale. Data represents Mean ± SD, n=5.

No significant difference was observed in fluorescein deposition and percent impactor dose, with the exception of stage 3 (p-value = 0.0029), for charge neutralized fluorescein aerosol, between the experiments performed with uncoated and coated impaction plates (Student’s t-test).

In contrast, significantly increased fluorescein deposition was observed for positively charged fluorescein aerosol on stages 0 to 3 (p-value = 0.0032) and stage 7 (p-value = 0.0098) of the
ACI, when uncoated plates were used (Student’s t-test). In terms of normalized impactor dose, increased deposition was observed on stages 0 to 4 of the ACI (p-value = 0.0008), and significantly decreased normalized fluorescein deposition was observed on stage 7 (p-value = 0.0006), for the positively charged fluorescein aerosol between the experiments performed with uncoated plates compared to those with coated impaction plates (Student’s t-test). The increased deposition on stages 0 to 4 of the ACI accounted for approximately 23% of the total impactor dose for the experiments using uncoated impaction plates. Figure V.9 shows the apparent APSD profiles for > 1 μm charge neutralized and positively charged fluorescein aerosol obtained using the ACI, as a function of stage coating.

![Figure V.9](image)

Figure V.9  Apparent APSD for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using a Grounded ACI as a Function of Stage Coating. Data represents Mean ± SD, n=5.

No significant difference was observed in the MMAD of charge neutralized fluorescein aerosol obtained using the grounded ACI with coated and uncoated impaction plates as shown by the overlapping profiles in Figure V.9 (p-value = 0.1280, Student’s t-test, MMAD charge neutralized, coated = 1.32±0.05 μm, MMAD charge neutralized, uncoated = 1.38±0.06 μm). However, a small but significant difference was observed in the MMAD of positively charged
fluorescein aerosol obtained using the grounded ACI with coated and uncoated impaction plates (p-value = 0.0126, Student’s t-test, MMAD positively charged, coated = 1.34±0.05 μm, MMAD positively charged, uncoated = 1.43±0.04 μm, Figure V.9), although it may not be practically significant during realistic in vitro testing of aerosols.

It is speculated that the increased deposition for the positively charged aerosol, within the ACI using uncoated impaction plates, may be due to the interplay of two opposing factors, ‘bounce effects’ that cause particles to deposit on the lower stages of the impactor and ‘image charge effects’ that may increase deposition on the higher stages of the impactor. Bounce typically results in smaller MMADs but in the present study, the MMAD for positively charged fluorescein aerosol was observed to be significantly higher when uncoated impaction plates were used suggesting additional effects due to the presence of charge. It is possible that bounce may not occur in the present study since the occurrence of particle bounce is reduced when the total deposited drug mass is high, similar to the mass of fluorescein deposited in this study (~800 μg) (Kamiya et al. 2009). This observation is supported by the statistically comparable MMADs of charged neutralized fluorescein aerosol obtained using the ACI with uncoated and coated impaction plates. Therefore it is believed that the larger MMAD of positively charged fluorescein aerosol obtained using the ACI with uncoated impaction plates, compared to that obtained using coated impaction plates, may be due to image charge effects on the higher stages (0 to 4) of the ACI.

Deposition behavior of the fluorescein aerosol was then compared within the grounded ACI using uncoated impaction plates as a function of charge, in line with the CFD model assumption. Figure V.10 compares the mass distribution as well as percent impactor dose for the
> 1 μm charge neutralized and positively charged fluorescein aerosol obtained using the ACI with uncoated impaction plates.

Figure V.10 Mass Distribution and Percent Impactor Dose for > 1 μm Fluorescein Aerosol as a Function of Charge Level obtained using Uncoated Impaction Plates in a Grounded ACI. Inset shows Mass Deposition on Stages 0 through 3 on an Expanded Scale. Data represents Mean ± SD, n=5.

A statistically significant increase in fluorescein mass deposition was observed on stages 0 through 2 (p-value = 0.0001), while a significant decrease was observed on stage 5 (p-value = 0.0042) of the ACI, for the positively charged fluorescein aerosol compared to the charge neutralized fluorescein aerosol (Student’s t-test) when uncoated impaction plates were used. For the normalized data, significantly higher deposition was observed on stages 0 through 3 of the ACI (p-value < 0.0001) for positively charged fluorescein aerosol, compared to the charge neutralized aerosol, while significantly decreased deposition was observed on stage 5 (p-value = 0.0402) for the positively charged fluorescein aerosol compared to charge neutralized aerosol (Appendix IV, Table AIV.4, Student’s t-test). These results were in agreement with the previous
measurements for charge neutralized and positively charged fluorescein aerosol obtained using coated impaction plates in the ACI (Chapter IV.C.4). Despite this increase, the deposition on stages 0 through 3 was less than < 10% of the total impactor dose. Consequently, despite the absence of stage coating, the apparent APSDs obtained as a function of charge level for fluorescein aerosol, were still observed to overlap (Figure V.11). No significant difference was observed in the MMAD derived using the grounded ACI with uncoated impaction plates for the charge neutralized and positively charged fluorescein aerosol (p-value = 0.1545, Student’s t-test, MMAD charge neutralized, uncoated = 1.38±0.06 μm, MMAD positively charged, uncoated = 1.43±0.04 μm).

Stage loading has also been reported to influence the deposition behavior of aerosols within cascade impactors and result in artificially higher MMADs (Nasr et al. 1997). Therefore, this study was extended to investigate the influence of stage loading upon the deposition
behavior of charge neutralized and positively charged fluorescein aerosol by comparing 5 and 20 second sampling times. Figure V.12 shows the electrostatic charge and mass distribution of fluorescein aerosol, determined using the ELPI, at the two charge levels investigated, following 5 seconds.

The total net charge on the charge neutralized fluorescein aerosol was observed to be negligible (-0.17±0.06 nC) and significantly lower than the positively charged fluorescein...
aerosol (8.45±0.95 nC) following a 5 second sampling time, similar to the results observed for the 20 second sampling time (Chapter IV.C.2) (p-value = 0.0040, Student’s t-test). Maximum fluorescein mass deposition was again observed on stages 6 through 9 of the ELPI for the two charge levels studied. Table V.6 summarizes the mass deposition for > 1 μm charge neutralized and positively charged fluorescein aerosol obtained using the ACI and the ELPI as a function of sampling time.

Table V.6  Mass Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the ACI and the ELPI as a Function of Stage Loading. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>Charge Level</th>
<th>Cascade Impactor</th>
<th>Sampling Time</th>
<th>Delivered Dose (µg)</th>
<th>Throat Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>Impactor Dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge Neutralized</td>
<td>ACI 5 sec</td>
<td>195.8±14.7</td>
<td>1.0±0.4</td>
<td>1.1±0.2</td>
<td>194.0±14.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ELPI 5 sec</td>
<td>217.3±17.2</td>
<td>1.3±0.4</td>
<td>1.2±0.2</td>
<td>214.6±16.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACI 20 sec</td>
<td>790.4±68.6</td>
<td>1.7±0.6</td>
<td>4.3±1.5</td>
<td>784.4±68.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ELPI 20 sec</td>
<td>778.9±77.5</td>
<td>1.8±0.8</td>
<td>4.5±2.5</td>
<td>773.0±76.7</td>
<td></td>
</tr>
<tr>
<td>Positively Charged</td>
<td>ACI 5 sec</td>
<td>209.7±16.4</td>
<td>0.6±0.1</td>
<td>40.8±9.3*</td>
<td>168.5±9.7*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ELPI 5 sec</td>
<td>229.0±18.2</td>
<td>2.0±0.7</td>
<td>41.4±11.1*</td>
<td>185.3±8.9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACI 20 sec</td>
<td>798.6±52.8</td>
<td>1.6±0.9</td>
<td>106.4±7.7*</td>
<td>690.6±46.8*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ELPI 20 sec</td>
<td>827.1±70.5</td>
<td>2.3±1.4</td>
<td>112.5±13.1*</td>
<td>708.7±65.6*</td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference within each impactor and sampling time, p-value < 0.05

Similar to the results obtained for a 20 second sampling time (Chapter IV.C.3), the total delivered doses, throat doses, charger doses and impactor doses were statistically comparable for ACI and ELPI, following a sampling time of 5 seconds for both the charge levels investigated (ANOVA p-value > 0.05). These results justified the use of the ELPI for the measurement of charge on the fluorescein aerosol. The charger dose was again significantly higher and the impactor dose was significantly lower for the positively charged fluorescein aerosol when compared to the charge neutralized fluorescein aerosol (p-value < 0.05). Due to the difference in
total delivered doses for the two sampling times investigated, normalized impactor doses were compared for the two charge levels as a function of stage loading (Figure V.13)

![Graph of Percent Impactor Dose for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the Grounded ACI as a Function of Stage Loading. Inset shows the Mass Deposition on Stages 0 through 3 on an Expanded Scale. Data represents Mean ± SD, n=5.](image)

Figure V.13  Percent Impactor Dose for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the Grounded ACI as a Function of Stage Loading. Inset shows the Mass Deposition on Stages 0 through 3 on an Expanded Scale. Data represents Mean ± SD, n=5.

For charge neutralized fluorescein aerosol, significantly increased normalized fluorescein deposition was observed on stage 0 through 3 of the ACI (p-value = 0.0029), following a sampling time of 5 seconds compared to 20 seconds (Student’s t-test). For the positively charged fluorescein aerosol increased deposition was observed on stages 0 to 2 of the ACI (p-value = 0.0056) and significantly decreased normalized fluorescein deposition was observed on stage 7 (p-value = 0.0200), following a sampling time of 5 seconds compared to 20 seconds (Student’s t-test). Nevertheless, in both these cases, the total increased deposition contributed only approximately 6% to the total impactor deposition. No significant difference was observed in the MMAD of the charge neutralized fluorescein aerosol obtained using the ACI as a function of stage loading shown in Figure V.14 (p-value = 0.4764, Student’s t-test, MMAD charge neutralized, 5 seconds = 1.32±0.06 μm, MMAD charge neutralized, 20 seconds = 1.32±0.05
μm). However, a small but significant difference was observed in the MMAD of the positively charged fluorescein aerosol obtained using the ACI as a function of stage loading shown in Figure V.14 (p-value = 0.0371, Student’s t-test, MMAD positively charged, 5 seconds = 1.41±0.01 μm, MMAD positively charged, 20 seconds = 1.34±0.05 μm), although this may not be a practically significant difference during in vitro testing.

![Figure V.14](image-url)

Figure V.14  Apparent APSD for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using a Grounded ACI as a Function of Stage Loading. Data represents Mean ± SD, n=5.

The deposition behavior of fluorescein aerosol was then compared within a grounded ACI following a sampling time of 5 seconds, in line with the CFD model assumption. Figure V.15 shows the mass distribution and the percent impactor dose for the > 1 μm charge neutralized and positively charged fluorescein aerosol collected in the ACI after a sampling time of 5 seconds.
Figure V.15  Mass Distribution and Percent Impactor Dose for > 1 μm Fluorescein Aerosol as a Function of Charge Level obtained Following 5 Second Sampling Time using a Grounded ACI.  Inset shows the Mass Deposition on Stages 0 through 3 on an Expanded Scale.  Data represents Mean ± SD, n=5.

Significantly increased deposition was observed on stages 0 through 2, while a significant decrease was observed on stage 3, 5, 7 and filter of the ACI, for the positively charged fluorescein aerosol compared to the charge neutralized fluorescein aerosol (p-value < 0.05, Student’s t-test) when the sampling time was reduced to 5 seconds.  For the normalized percent impactor dose, significantly increased deposition was observed on stages 0 through 2 of the ACI (p-value = 0.0123, Student’s t-test) for positively charged fluorescein aerosol, compared to the charge neutralized aerosol, which accounted for about 9% of the total impactor dose (Appendix IV, Table AIV.5).  In contrast to the previous results obtained after a sampling time of 20 seconds, no significant decrease was observed on stage 5 for the positively charged fluorescein aerosol compared to the charge neutralized fluorescein aerosol, following a sampling time of 5 seconds.  This may be attributed to the decreased space charge effects i.e., the particle-particle repulsion, due to the decreased deposition of fluorescein mass on stage 5 following a reduced sampling time of 5 seconds.  These results strengthened the hypothesis of space charge effects or
repulsion playing an important role in the deposition of charged aerosols on the stages with high drug load. A small significant difference in the MMADs of charge neutralized and positively charged fluorescein aerosol obtained using a grounded ACI following a reduced sampling time of 5 seconds was apparent (p-value = 0.0183, Student’s t-test, MMAD charge neutralized, 5 seconds = 1.32±0.06 μm, MMAD positively charged, 5 seconds = 1.41±0.01 μm, Figure V.16), though it may not be practically significant.

Figure V.16  Apparent APSD for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using a Grounded ACI Following a Sampling Time of 5 Seconds. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>Aerodynamic diameter (μm)</th>
<th>Cumulative % Undersize</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Charge Neutralized, 5 sec</td>
<td></td>
</tr>
<tr>
<td>Positively Charged, 5 sec</td>
<td></td>
</tr>
</tbody>
</table>

V.C.4  Deposition Behavior of Charge Neutralized and Positively Charged Fluorescein Aerosol within an Abbreviated ACI

Table V.7 compares the mass deposition of > 1 μm fluorescein aerosol as a function of charge within the full resolution and the QC abbreviated ACI.
Table V.7  Mass Deposition of > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol obtained using the Full Resolution and QC Abbreviated ACI. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>Charge Level</th>
<th>Cascade Impactor</th>
<th>Delivered Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>ISM (µg)</th>
<th>LPM/SPM ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge Neutralized</td>
<td>Full Resolution ACI</td>
<td>790.4±68.6</td>
<td>4.3±1.5</td>
<td>782.2±68.2</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td></td>
<td>Abbreviated ACI</td>
<td>800.7±45.0</td>
<td>3.3±0.4</td>
<td>794.6±45.0</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>Positively Charged</td>
<td>Full Resolution ACI</td>
<td>798.6±52.8</td>
<td>106.4±7.7*</td>
<td>685.4±46.8*</td>
<td>1.9±0.1*</td>
</tr>
<tr>
<td></td>
<td>Abbreviated ACI</td>
<td>824.0±45.6</td>
<td>104.3±13.8*</td>
<td>714.1±49.3*</td>
<td>1.8±0.3</td>
</tr>
</tbody>
</table>

*Significant difference within each impactor, p-value < 0.05

Data from the full resolution ACI for both charge levels investigated, was compared with that obtained using the abbreviated QC ACI, in accordance with the recommendations by Mitchell et al. (Mitchell et al. 2009a, Mitchell et al. 2010a). The total delivered dose i.e., sum of the amount of fluorescein deposited in the throat, charger and the impactor was statistically comparable for the two charge levels investigated (p-value > 0.05, Student’s t-test), for both the full resolution and abbreviated ACI. Similar to previous experiments, the charger dose was significantly higher and hence the impactor dose was significantly lower for the positively charged fluorescein aerosol compared to charge neutralized fluorescein aerosol, for both impactors (p-value < 0.05, Student’s t-test). As a result, the fluorescein mass collected by the impactor i.e., ISM for charged aerosols was significantly lower compared to the charge neutralized fluorescein aerosol (p-value < 0.05, Student’s t-test).

The LPM/SPM ratio was observed to be smaller for the abbreviated ACI compared to the full resolution ACI, for the charge neutralized fluorescein aerosol. It was observed that the SPM of charge neutralized fluorescein aerosol obtained using the abbreviated QC configuration (323±19 µg) was significantly higher compared to that obtained using the full resolution ACI derived SPM (247±26 µg) (p-value = 0.0011, Student’s t-test). This is believed to occur because
of bounce effects, which have been reported with the abbreviated system despite the use of surface coating (Mitchell et al. 2010b). However, the investigation of these results was beyond the scope of the present study. No significant difference was observed in the LPM/SPM ratio of the positively charged fluorescein aerosol obtained using the abbreviated and the full resolution ACI. It is speculated that the presence of charge on the fluorescein aerosol particles may result in more effective ‘sticking’ on the impactor stages, thus reducing bounce effects. While a small significant difference was observed in the LPM/SPM ratio as a function of charge, for the full resolution ACI data (p-value = 0.0122, Student’s t-test), no significant difference was observed between the LPM/SPM ratio obtained for charge neutralized aerosol and positively charged fluorescein aerosol using the abbreviated QC ACI (p-value > 0.05, Student’s t-test). The LPM/SPM ratio was higher for the positively charged aerosol than the charge neutralized aerosol in the abbreviated QC impactor, which is expected due to increased deposition of charged aerosol on the higher stage of the impactor due to image charge effects.

Figure V.17 shows the cumulative percent undersize plot for the > 1 μm charge neutralized and positively charged fluorescein aerosol collected in the full resolution and QC Abbreviated ACI. Good agreement was observed between the abbreviated and full resolution data irrespective of the charge level as shown in Figure V.17.
Figure V.17  Apparent APSD for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol determined using a Full Resolution and QC Abbreviated ACI. Data represents Mean ± SD, n=5.

In summary, the results of the statistical comparisons indicate that the EDA specified metrics obtained with either the full resolution or the QC abbreviated configuration were reasonably equivalent. Therefore, in contradiction to the CFD study, which predicted increased deposition upon the individually simulated (except 2 and 3) stages of the ACI in the presence of charge, the deposition behavior of charged particles was observed to be statistically comparable to charge neutralized fluorescein aerosol particles, even in a reduced stack abbreviated ACI.

V.D  Summary

Differences in the CFD model assumptions and the in vitro study parameters were evaluated in order to assess the validity of CFD model predictions. Grounding the ACI as well as the aerosol size (< 1 μm) did not appear to influence the apparent APSD of charged aerosol compared to the charge neutralized fluorescein aerosol, even though the deposition pattern
within the ACI was observed to change. With respect to stage coating and stage loading, a small significant difference was observed in the MMAD of charged fluorescein aerosol compared to the charge neutralized fluorescein aerosol. However, the small difference observed may not be practically relevant due to the variability associated with the *in vitro* testing of aerosols.

The measurements performed with an abbreviated ACI system extended the assessment of the AIM concept to include any possible effects of electrostatic charge. The comparison of abbreviated QC ACI configuration with the full resolution ACI measurements corroborated the similarity of the abbreviated system with respect to the influence of charge upon deposition behavior of aerosols, to the full resolution ACI.
VI. OVERALL DISCUSSION AND CONCLUSIONS

Cascade impactors, which are the preferred method of particle sizing for pharmaceutical aerosols and provide a direct measurement of absolute drug mass in different aerodynamic particle size fractions (Mitchell and Nagel 2003), are conventionally calibrated using dilute, charge neutralized, monodisperse aerosols (Marple et al. 1998). However, it is now known that particles emitted from dry powder inhalers, metered dose inhalers, and nebulizers, are inherently charged as a function of formulation and packaging components (Byron et al. 1997, Peart et al. 1998, Kwok et al. 2005, Telko et al. 2007, Kwok and Chan 2008, Kwok et al. 2010). Although the electrostatic properties of some commercial inhaled products have been characterized, there is a knowledge gap concerning the influence of electrostatic charge upon the deposition of aerosols within cascade impactors which may affect the apparent APSD of aerosols sized with these instruments. Thus, the key question that needs to be addressed is whether the presence of electrostatic charge on pharmaceutical aerosols can affect their site and/or extent of deposition within an impactor, and potentially impact the reported/measured APSD. Answering this question may also add to our existing knowledge concerning the practical and regulatory implications of electrostatic charge. In this dissertation, a systematic study was pursued to determine the influence of electrostatic charge upon the deposition behavior of aerosols within cascade impactors, in light of the recent theoretical, CFD model predictions, by Vinchurkar et al. (Vinchurkar et al. 2009).
Electrical properties of pharmaceutical aerosols have been previously characterized (Kwok et al. 2005, Telko et al. 2007, Kwok and Chan 2008) using the Electrical Low Pressure Impactor (ELPI). The deposition pattern of commercially available pMDIs including QVAR, Proventil HFA and Ventolin HFA, was characterized in the present study within the ELPI, as a function of the electrostatic properties of the aerosol. It was hypothesized that the applied charge would result in (a) modified charge distribution on the aerosol and (b) altered deposition behavior within the ELPI. While the aerosol charging of pharmaceutical aerosols in practice occurs primarily through triboelectrification, in the absence of electric fields, the electrostatic properties of the aerosol clouds from commercial pMDIs were further modified using a voltage controlled charging process achieved using an external voltage source, connected to the corona charger of the ELPI.

The influence of applied voltage upon the electrostatic charge distribution and deposition behavior of the aerosol cloud emitted from QVAR 80 was assessed at applied potential differences of 0, +1, +3 and +5 kV voltage. In agreement with the literature, the QVAR cloud was observed to be unipolar positive with a total net charge of 1.19±0.35 nC at 0 kV applied voltage. This charge increased to 1.67±0.43 nC, when the aerosol was exposed to a corona discharge associated with a +5 kV applied voltage. The electrostatic charge distributions for QVAR aerosol at the intermediate voltages of +1 (1.00±0.18 nC) and +3 kV (1.19±0.26 nC) were statistically comparable to those at 0 kV. Based upon these results, it appeared that these applied voltages failed to induce a significant electrical breakdown of air; in short, the corona discharge in the ELPI corona charger was insufficient to modify the inherent charge on the QVAR cloud. At +5 kV, however, corona discharge was observed to occur, followed by unipolar positive ion formation and field charging of the aerosol cloud from the QVAR pMDI.
For QVAR, the charger dose increased significantly from 3.2±0.8 μg at 0 kV to 11.3±2.1 μg at +5 kV, resulting in a significantly decreased impactor dose from 28.6±3.5 μg at 0 kV to 22.0±3.2 μg, respectively (ANOVA p-value < 0.05). This observation related well to the conventional wisdom that charged particles undergo premature deposition upon contacting a surface. Within the ELPI, mass deposition of beclomethasone dipropionate (BDP) discharged from the QVAR pMDI was shown to be significantly decreased on the inlet and stages 4 through 9 when +5 kV was applied, compared to the 0 kV experiments. In spite of this observation however, no significant difference was observed between the impactor deposition across the ELPI stages i.e., fractional drug recovery per stage, for the QVAR aerosol cloud as a function of the degree of charge modification. The apparent APSD profile of QVAR pMDI was obtained using the recalibrated ELPI cut-off diameters (Kotian et al. 2009) for each applied voltage. Despite the impactor dose being significantly lower at +5 kV compared to 0 kV, no significant difference was observed between the MMAD for QVAR pMDI for the four voltage levels investigated (MMAD 0 kV = 1.02±0.03 μm, MMAD +1 kV = 1.02±0.03, MMAD +3 kV = 1.01±0.07, MMAD +5 kV = 0.99±0.02 μm). The MMAD of the QVAR 80 aerosol cloud determined in the present study was also in good agreement with the literature reported value for QVAR (Mitchell et al. 2003).

While the results with differently charged aerosol clouds from QVAR failed to produce dramatic differences in deposition patterns within the ELPI, this work showed that an external voltage source connected to the ELPI corona charger, enabled the comparison of apparent APSD of artificially charged QVAR, Proventil HFA and Ventolin HFA aerosols, with inherently charged aerosol clouds of the products, provided a potential difference of +5 kV was used. As expected, the charge distribution observed for all three pMDIs was shown to be significantly
different between the inherently charged and artificially charged aerosols as +5 kV. However, different inherent electrostatic charging occurred on the drug clouds emitted by the three commercial pMDIs that were investigated, based probably on their formulation and device packaging components. The total electrostatic net charge on inherently charged Proventil HFA (0.09±0.18 nC) and Ventolin HFA (-0.12±0.06 nC) aerosol clouds were significantly lower than the artificially charged Proventil HFA (0.69±0.26 nC) and Ventolin HFA (0.85±0.26 nC) aerosol clouds (p-value < 0.05). This difference in the electrostatic charge properties resulted in different mass deposition of albuterol clouds formed by the Proventil and Ventolin HFA pMDIs, within the ELPI as a function of charge. The deposition of albuterol was significantly decreased on the ELPI inlet and stages 8 through 10 for Proventil HFA as a result of +5 kV corona discharge, while significantly decreased deposition was observed on the inlet and stages 9 and 10 for Ventolin HFA. However, no statistically significant differences were observed in the impactor deposition for the three pMDI products as a function of the applied charge, making it possible to compare the apparent APSDs for both the inherently and artificially charged aerosols. Despite the differences noted above, the MMADs of inherently charged Proventil HFA (2.53±0.10 µm) and Ventolin HFA (2.71±0.03µm) were statistically comparable with their artificially charged counterparts, (Proventil HFA 2.54 ±0.03µm; Ventolin HFA 2.67±0.02µm, respectively. Both the inherently and artificially charged MMADs were in good agreement with the reported values for these pMDIs in the literature (Dellamary et al. 2003, Kotian 2008).

While the study helped to further our understanding of the influence of different levels of electrostatic charge, specifically, the realistic tribocharged or the ‘inherently charged’ condition during in vitro testing of inhalers and the artificially charged condition achieved using corona discharge, the findings from this study cast doubt over literature claims that suggested
electrostatic charge could be a cause for variability in mass balance and APSD measurements obtained using cascade impactors (Christopher et al. 2003, Bonam et al. 2008). Notably also, the electrostatically charged polydisperse, multicomponent pharmaceutical aerosols characterized as boluses in the present study should be contrasted with the dilute aerosols that are normally used to calibrate cascade impactors; these are generally charge neutralized aerosols. Therefore, in order to thoroughly evaluate the role of electrostatics upon deposition behavior of aerosols within cascade impactors, further studies were warranted.

A recently published CFD model of the ACI investigated and proposed the stage-by-stage airflow pattern and related this to the deposition of neutral particles within the ACI (Vinchurkar et al. 2009). This CFD model was extended to theoretically predict the influence of electrostatic charge on stage deposition, based on what the authors called 'representative charge levels for pharmaceutical aerosols’. The CFD model used an image-charging algorithm to predict significantly increased deposition across the stages of the ACI for charged aerosol clouds. Predicted deposition changes were most noticeable on stage 4 (Stage cut-off diameter = 2.1 µm) and stages designed to capture even smaller particles in the ACI. Such charge related effects theoretically influenced the apparent APSD of charged aerosols (Vinchurkar et al. 2009) and the authors hypothesized that the presence of significant electrostatic charge on aerosols with aerodynamic sizes < 2 µm would influence aerosol deposition behavior as compared to similarly sized charge- neutralized aerosols.

An in vitro study was performed to evaluate the CFD model predictions more closely i.e., to investigate the effect of electrostatic charge on the aerosol deposition behavior and the resultant apparent APSD of otherwise comparable aerosols within the ACI. An experimental apparatus was developed to produce charge neutralized as well as charged fluorescein aerosols
with aerodynamic sizes of < 2 μm, targeted to deposit on ‘charge sensitive’ stages of the ACI, according to the CFD model. Both positively and negatively charged aerosols were investigated, because aerosol clouds from pharmaceutical inhalers have been reported to carry net electropositive, net electronegative, or bipolar charge distributions depending upon the formulation and the device (Kwok et al. 2005, Kwok and Chan 2008, Kwok et al. 2010). The deposition behavior and the particle size distribution of the model fluorescein aerosol particles were characterized within the ACI, following the application of corona discharge using an external voltage source. The use of the external voltage source enabled the charge on the fluorescein powder aerosol to be modified such that four different charge levels - charge neutralized, inherently charged, positively charged and negatively charged (following the application of either +5 kV or -5 kV), could be studied. The electrostatic charge on these aerosols was verified using the ELPI in a parallel arm of the study. The aerosol deposition predictions from the CFD model of the ACI were compared with the in vitro results of the present study.

The electrostatic charge observed for the charge neutralized (-0.06±0.08 nC), inherently charged (-5.34±0.68 nC), positively charged (+30.6±2.7 nC), and negatively charged (-47.3±4.7 nC) fluorescein aerosol confirmed that four significantly different charge levels were obtained. It should be noted that the electrostatic charge observed for the positively and negatively charged fluorescein aerosol was much higher than that commonly seen for pharmaceutical aerosol clouds from pMDIs, restating the fact that corona discharge imparted charges of higher magnitude than those caused by triboelectrification. The negatively charged fluorescein aerosol was observed to be more charged than the positively charged fluorescein
aerosol, likely because of the higher efficiency of negative corona discharge to ionize air (Cross 1987).

Within the ACI, increased deposition of fluorescein was observed in the charger, and on stages 0 to 3, for charged aerosols compared to neutral aerosols, in agreement with the CFD model predictions. However, the increase in magnitude observed in the in vitro study on these stages (~100-200%) was much higher than that predicted by the CFD study (~30%) and the bulk of the aerosol delivered in these experiments deposited on stage 4 and below of the ACI. However, significant discrepancies between the in vitro results and the CFD model predictions occurred for stage 5 and below in the ACI. In contradiction with the CFD model predictions, a significant decrease was observed in the deposition of charged fluorescein aerosol on stage 5, compared to neutral aerosol, whereas little or no increase was observed on stages 6, 7 and filter of the ACI; the CFD model predicted maximum increase in deposition of charged aerosol particles in these regions. Image charge effects are thought to be the cause of increased deposition on the upper stages of ACI, while space charge effects, arising from the particle-particle interactions in the deposited charged aerosols, which were not incorporated in the CFD model, may have dominated and resulted in decreased deposition on stage 5, the stage with maximum experimental deposition of fluorescein. The MMAD of the fluorescein aerosol obtained using the ACI was also compared as a function of charge; there was a small but statistically significant difference observed in the MMAD of negatively charged fluorescein aerosol compared to the other charge levels investigated (MMAD charge neutralized = 1.32±0.04 μm, MMAD inherently charged = 1.32±0.05 μm MMAD positively charged = 1.34±0.05 μm, MMAD negatively charged = 1.39±0.04 μm). This small difference is unlikely to be practically
significant during *in vitro* quality control testing of aerosols, due to the inherent variability associated with cascade impaction measurements in the pharmaceutical industry.

When the charge levels attained in the *in vitro* study were compared with those assumed in the CFD study, the charge neutralized and the inherently charged fluorescein aerosol carried a very small number of electrons per ‘average’ particle, while both the positively and negatively charged fluorescein aerosol carried charges equivalent to approximately 200 through 500 electrons per particle; these latter charge states were both greater than those assumed for the equivalent sized charged aerosol particles (approximately 50 electrons per particle) in the CFD study. Thus, despite the significantly higher electrostatic charge on the artificially charged aerosol, the applied charge failed to produce a significant difference in the ACI-measured apparent APSD of these comparable fluorescein aerosols. Of note was the fact that the estimated deposition efficiency for the charge neutralized aerosol on stage 5 of the ACI in the *in vitro* study was observed to be approximately 65%, which was in good agreement with the CFD model prediction of approximately 70% for a charge neutral, approximately 1.3 µm sized particle. Therefore, it appears that the CFD model predictions may have been valid for neutral particles, while for their charged counterparts, the literature predictions were inaccurate (Vinchurkar *et al.* 2009).

Differences in the CFD model predictions and the *in vitro* study results may have arisen in part due to variations in the methods commonly employed during the experimental use of the ACI. The *in vitro* study described in this thesis was designed to mimic realistic *in vitro* testing conditions by investigating the deposition of a polydisperse, charged aerosol with a concentration comparable to pharmaceutical aerosols and sizes that were predisposed to show the effects of charging. The study was also extended to assess the effects of several common
method variations between the CFD study and the *in vitro* study. These included the effect of grounding the ACI, varying the aerosol size (<1 µm), stage coating and stage loading in a full resolution ACI. The effects of charge in an abbreviated Anderson Cascade Impactor were also evaluated, due to the present trend in the pharmaceutical industry towards the use of ‘reduced stack’ impactors (Tougas *et al.* 2009, Tougas *et al.* 2011); also because the CFD model predictions were based largely on predictions of deposition in isolated ACI stages (Vinchurkar *et al.* 2009).

The deposition of charge neutralized and positively charged fluorescein aerosol within the ACI, was statistically comparable as a function of grounding the ACI. Similar stage deposition trends were observed and, as a result, the MMADs were still statistically comparable for fluorescein aerosols with different charges (MMAD charge neutralized, grounded ACI = 1.32±0.05 µm, MMAD positively charged, grounded ACI = 1.39±0.04 µm).

Based on the CFD model predictions of maximum influence of charge upon deposition for stage 6 and 7 of the ACI, a <1 µm fluorescein powder aerosol was generated using the previously developed experimental set up. Significantly increased normalized impactor deposition of up to 200% was observed on stages 1 through 3 of the ACI for this aerosol when positively charged, compared to its charge neutralized control, in agreement with the previous findings for >1 µm aerosols. These results were in agreement with the CFD model predictions for the higher stages of the ACI. However, once again in contradiction with the CFD model predictions, no significant difference was observed on the deposition of charge neutralized and positively charged fluorescein aerosol of <1 µm size, on the submicron stages of the ACI i.e., stage 6 through filter. Furthermore, no significant difference was seen in the MMAD obtained using the ACI for the positively charged aerosol compared to the charge neutralized fluorescein.
aerosol (MMAD charge neutralized = 0.68±0.03 μm, MMAD positively charged = 0.70±0.05 μm).

The deposition behavior of charge neutralized fluorescein aerosols was statistically comparable, with or without coating. Furthermore, no significant difference was observed in the MMAD of charge neutralized fluorescein aerosol obtained using the grounded ACI with coated or uncoated impaction plates (MMAD charge neutralized, coated = 1.32±0.05 μm, MMAD charge neutralized, uncoated = 1.38±0.06 μm). However, for the positively charged fluorescein aerosol, significantly higher normalized fluorescein deposition was observed on stages 0 to 4 of the ACI and significantly decreased fluorescein deposition was observed on stage 7, between the experiments performed with uncoated plates compared to those with coated impaction plates, an increase that accounted for approximately 20% of the total impactor dose for the experiments using uncoated impaction plates. Consequently, a small but significant difference was observed in the MMAD of positively charged fluorescein aerosol obtained using the grounded ACI with coated and uncoated impaction plates, (MMAD positively charged, coated = 1.34±0.05 μm, MMAD positively charged, uncoated = 1.43±0.04 μm), perhaps due to ‘image charge effects’ in the case of coated plates and/or particle bounce, in cases without coating. However, upon comparing the MMAD obtained using the grounded ACI with uncoated impaction plates for the charge neutralized and positively charged fluorescein aerosol, no significant difference was observed (MMAD charge neutralized, uncoated = 1.38±0.06 μm, MMAD positively charged, uncoated = 1.43±0.04 μm), implying that the reported differences may not be unreliable.

Similar to the stage coating experiments, significant increases were observed in stage deposition on the upper stages of the ACI as a function of stage loading, i.e., following a sampling time of 5 and 20 seconds in the ACI, but the total increased deposition contributed only
approximately 6% to the total impactor deposition. As a result, no significant difference were seen in the MMADs of the > 1 µm charge neutralized fluorescein aerosols obtained using the ACI as a function of stage loading (MMAD charge neutralized, 5 seconds = 1.32±0.06 µm, MMAD charge neutralized, 20 seconds = 1.32±0.05 µm), while a small statistically significant difference was observed in the MMAD of the > 1 µm positively charged fluorescein aerosol obtained using the ACI as a function of stage loading (MMAD positively charged, 5 seconds = 1.41±0.01 µm, MMAD positively charged, 20 seconds = 1.34±0.05 µm). Upon comparison of fluorescein deposition within the ACI as a function of charge, following a sampling time of 5 seconds, significantly increased normalized impactor deposition was observed on stages 0 through 3 of the ACI for positively charged > 1 µm fluorescein aerosols, compared to the charge neutralized aerosols, much in agreement with the previous in vitro results. Despite the increase, the deposition on these stages accounted for about 8% of the total impactor dose; this resulted in a small but significant difference in MMADs for the charge neutralized and positively charged fluorescein aerosols collected in a grounded ACI following a reduced sampling time of 5 seconds (MMAD charge neutralized, 5 seconds = 1.32±0.06 µm, MMAD positively charged, 5 seconds = 1.41±0.01 µm); practically, such a difference is unlikely to be significant.

When the deposition behavior of charge neutralized and positively charged fluorescein aerosols were compared within an abbreviated QC impactor (Mitchell et al. 2010a), good agreement was observed between the abbreviated and full resolution data irrespective of the charge level and the EDA specified metrics (Mitchell et al. 2010c) i.e., the LPM/SPM ratio were statistically comparable for the charge neutralized and the positively charged fluorescein aerosol. In summary, the present study helped to gain insight into the uses and limitations of both CFD and charged aerosol impaction studies. It appeared that electrostatic charges, on aerosol clouds
created for pharmaceutical usage and characterized by cascade impaction, had a limited or minimal influence on deposition within the instrument. In short, impaction appeared to still be the dominant process governing deposition, irrespective of the presence and magnitude of charge on the aerosol particles, and/or whether typical method variations were employed during testing.
REFERENCES
List of References


Dekati Ltd. Tampere Finland. (2011). *Personal Communication*


volatile component - evaluation with a “droplet producing” pressurized metered dose inhaler (pMDI)-based formulation containing ethanol as co-solvent. *AAPS PharmSciTechnol.*, 10(1), 252-257.


APPENDIX I. INTRA- AND INTER-RUN ACCURACY AND PRECISION DATA FOR HPLC ANALYSES

AI.1 Intra- and Inter- Run Accuracy and Precision Data for Beclomethasone dipropionate (BDP) HPLC Assay.

<table>
<thead>
<tr>
<th></th>
<th>LQC (0.075 µg/mL)</th>
<th>MQC (0.3 µg/mL)</th>
<th>HQC (2 µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc (ng/mL)</td>
<td>% DFN</td>
<td>% RSD</td>
</tr>
<tr>
<td>Run 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.076</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>0.077</td>
<td>2.6</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>0.076</td>
<td>0.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Run 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.077</td>
<td>2.6</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>0.076</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>0.077</td>
<td>2.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Run 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.078</td>
<td>3.7</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>0.076</td>
<td>1.7</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>0.076</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>MEAN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.077</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### AI.2 Intra- and Inter-Run Accuracy and Precision Data for Albuterol HPLC Assay.

<table>
<thead>
<tr>
<th></th>
<th>LQC (50 ng/mL)</th>
<th>MQC (300 ng/mL)</th>
<th>HQC (800 ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc (ng/mL)</td>
<td>% DFN</td>
<td>% RSD</td>
</tr>
<tr>
<td>Run 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50.20</td>
<td>0.40</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>50.15</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50.96</td>
<td>1.92</td>
<td></td>
</tr>
<tr>
<td>Run 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>49.78</td>
<td>-0.44</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>49.54</td>
<td>-0.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49.67</td>
<td>-0.67</td>
<td></td>
</tr>
<tr>
<td>Run 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>51.10</td>
<td>2.21</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>50.78</td>
<td>1.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50.47</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>MEAN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50.29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SD</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### AI.3 Intra- and Inter-Run Accuracy and Precision Data for Fluorescein HPLC Assay.

<table>
<thead>
<tr>
<th></th>
<th>LQC (10 ng/mL)</th>
<th>MQC (50 ng/mL)</th>
<th>HQC (100 ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc (ng/mL)</td>
<td>% DFN</td>
<td>% RSD</td>
</tr>
<tr>
<td>Run 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.18</td>
<td>1.84</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>10.22</td>
<td>2.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.21</td>
<td>2.13</td>
<td></td>
</tr>
<tr>
<td>Run 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.99</td>
<td>-0.86</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>9.95</td>
<td>-1.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.93</td>
<td>-1.45</td>
<td></td>
</tr>
<tr>
<td>Run 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.00</td>
<td>0.03</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>10.08</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.06</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>MEAN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.07</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SD</td>
<td>0.11</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
APPENDIX II. USE OF ELPI RECALIBRATION FOR DETERMINING THE APPARENT AERODYNAMIC PARTICLE SIZE DISTRIBUTION (APSD) OF INHERENTLY AND ARTIFICIALLY CHARGED AEROSOLS

AII.1 Introduction

The recalibration of the Electrical Low Pressure Impactor (ELPI) by Kotian et al. was performed using a modified ELPI i.e., with the corona charger removed, when it was observed that the ELPI undersized the apparent aerodynamic particle size distribution (APSD) of commercial pMDIs (Kotian et al. 2009). The authors recommended the use of recalibrated stage cut-off diameters of the ELPI for the size determination of inherently charged pMDI aerosols (Kotian et al. 2009). In contrast to the study by Kotian et al., the present study involved the use of the corona charger atop the ELPI impactor stack, either at 0 or +5 kV to compare the deposition behavior of inherently and artificially charged pMDI aerosols, respectively.

The presence of the corona charger in the experimental set-up on the deposition behavior of aerosols generated by pMDIs and the use of the recalibrated ELPI stage cut-off diameters was re-evaluated.

AII.2 Materials and Methods

QVAR 80 was chosen as the model commercial pMDI for this study. A schematic diagram of the two ELPI configurations (with and without the corona charger) used in the present study is shown in Figure AII.1.
Figure AII.1 Schematic Diagram of the ELPI representing (a) Modified ELPI Configuration i.e., Without Corona Charger and (b) With Corona Charger, used for the Determination of Apparent APSD of QVAR 80 pMDI.
The deposition behavior of the inherently charged QVAR 80 aerosol cloud obtained using the modified ELPI (Figure AII.1.a) was compared with that obtained using the ELPI with the corona charger (at 0 kV) atop the impactor stack (Figure AII.1.b). The mass distribution was obtained using the methods and validated HPLC assay described in Chapter III.B.3.2, and the cumulative % undersize plotted against either the manufacturer reported ELPI cut-off diameters or the recalibrated cut-off diameters described by Kotian et al. (Kotian et al. 2009), to obtain the apparent APSD profiles. Table AII.1 shows the manufacturer as well as the recalibrated cut-off diameters for the ELPI. The study was then repeated for the artificially charged QVAR 80 aerosol (+5kV).

### Table AII.1
Manufacturer (Dekati Ltd.) Reported and Recalibrated Aerodynamic Cut-off Diameters of the ELPI stages 1-13 at a Flow Rate of 29 Lmin⁻¹

<table>
<thead>
<tr>
<th>ELPI Stage</th>
<th>Dekati Cut-off Diameter (µm)</th>
<th>Recalibrated Cut-off Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>10.08</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>6.67</td>
<td>6.37</td>
</tr>
<tr>
<td>11</td>
<td>4.05</td>
<td>4.75</td>
</tr>
<tr>
<td>10</td>
<td>2.42</td>
<td>3.03</td>
</tr>
<tr>
<td>9</td>
<td>1.62</td>
<td>2.12</td>
</tr>
<tr>
<td>8</td>
<td>0.96</td>
<td>1.40</td>
</tr>
<tr>
<td>7</td>
<td>0.62</td>
<td>1.01</td>
</tr>
<tr>
<td>6</td>
<td>0.39</td>
<td>0.70</td>
</tr>
<tr>
<td>5</td>
<td>0.27</td>
<td>0.56</td>
</tr>
<tr>
<td>4</td>
<td>0.16</td>
<td>0.44</td>
</tr>
<tr>
<td>3</td>
<td>0.10</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>0.03</td>
<td>-</td>
</tr>
</tbody>
</table>
AII.3 Results and Discussion

Table AII.2 summarizes the mass deposition for QVAR pMDI obtained using the modified ELPI i.e., without corona charger and with the corona charger in place atop the ELPI impactor stack, including the mass of BDP deposited from the QVAR 80 pMDI aerosol cloud in the USP throat (Throat Dose), corona charger (Charger Dose) and the impactor (Impactor Dose).

Table AII.2  Mass Deposition for BDP discharged from QVAR 80 pMDIs using the ELPI Without Corona Charger and With Corona Charger (at 0 kV). Data represents Mean±SD, n = 5.

<table>
<thead>
<tr>
<th>ELPI Configuration</th>
<th>Delivered Dose (µg)</th>
<th>Actuator Dose (µg)</th>
<th>Throat Dose (µg)</th>
<th>Charger Dose (µg)</th>
<th>Impactor Dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified ELPI (Without Corona Charger)</td>
<td>92.6±2.7</td>
<td>28.2±2.3</td>
<td>22.6±3.2</td>
<td>0</td>
<td>40.8±4.7*</td>
</tr>
<tr>
<td>ELPI (With Corona Charger)</td>
<td>89.4±2.4</td>
<td>29.1±5.0</td>
<td>28.0±3.1</td>
<td>3.2±0.8*</td>
<td>28.6±3.5</td>
</tr>
</tbody>
</table>

*Significant difference, p-value < 0.05, Student’s t-test

The total delivered doses and throat doses were statistically comparable for both configurations (p-value > 0.05, Student’s t-test). However, the impactor dose was observed to be significantly higher for the modified ELPI (without charger) compared to the ELPI with charger configuration (p-value < 0.05, Student’s t-test). Approximately 3 µg drug deposited in the charger when the charger was in place but switched off and coupled with an increased throat dose (albeit not statistically significant because of the variability) resulted in a reduced impactor dose compared to the without charger ELPI configuration (p-value < 0.05, Student’s t-test). The mass distribution of QVAR pMDI aerosol clouds for the two ELPI configurations investigated is shown in Figure AII.2.
Within the ELPI, deposition of BDP discharged from QVAR 80 pMDIs was observed to be significantly higher on stages 2 through 8 for the modified ELPI i.e., without corona charger configuration compared to that obtained by using the ELPI with the corona charger in place (p-value < 0.05, Student’s t-test). Since the impactor doses were observed to be significantly different for the two configurations investigated, the normalized impactor deposition for the QVAR 80 aerosol collected in the ELPI was calculated for the both configurations as illustrated in Figure AII.2. Even though the impactor deposition was observed to be significantly different on stage 2 for the two configurations investigated (Figure AII.2, p-value < 0.05, Student’s t-test), almost overlapping apparent APSD profiles of QVAR aerosol cloud were obtained using the modified ELPI and the ELPI with charger configuration, irrespective of the cut-off diameters used (Figure AII.3).
Undersizing of the QVAR aerosol was observed when the manufacturer cut-off diameters were used, which was in agreement with the findings of Kotian et al. (MMAD Without Charger Configuration = 0.64±0.02 μm, MMAD With Charger Configuration = 0.62±0.02 μm). The MMAD for inherently charged QVAR aerosol cloud obtained using the modified ELPI (without charger) and with charger (at 0 kV) configuration using the recalibrated cut-off diameters were statistically comparable (MMAD Without Charger Configuration = 1.04±0.03 μm, MMAD With Charger Configuration = 1.02±0.03 μm, p-value > 0.05, Student’s t-test) and were observed to be in good agreement with the literature reported value for QVAR (Mitchell et al. 2003).

Table AII.3 summarizes the cumulative percent undersize data obtained for artificially charged QVAR 80 aerosol, which was plotted using the Dekati calibrated and recalibrated ELPI cut-off diameters. The apparent APSD profiles for artificially charged QVAR aerosol cloud are shown in Figure AII.4, as a function of the ELPI calibration used.
Table AI.3  Cumulative Percent Undersize for Artificially Charged QVAR 80 Aerosol Cloud obtained using the ELPI. Data represents Mean±SD, n=5.

<table>
<thead>
<tr>
<th>ELPI Stage</th>
<th>Cumulative % Undersize</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 13</td>
<td>100.0±0.0</td>
</tr>
<tr>
<td>Stage 12</td>
<td>100.0±0.0</td>
</tr>
<tr>
<td>Stage 11</td>
<td>100.0±0.0</td>
</tr>
<tr>
<td>Stage 10</td>
<td>98.3±1.2</td>
</tr>
<tr>
<td>Stage 9</td>
<td>92.2±2.1</td>
</tr>
<tr>
<td>Stage 8</td>
<td>75.6±3.0</td>
</tr>
<tr>
<td>Stage 7</td>
<td>52.5±2.3</td>
</tr>
<tr>
<td>Stage 6</td>
<td>28.0±2.0</td>
</tr>
<tr>
<td>Stage 5</td>
<td>14.3±0.9</td>
</tr>
<tr>
<td>Stage 4</td>
<td>6.2±0.6</td>
</tr>
<tr>
<td>Stage 3</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>Stage 2</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Stage 1</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure AI.4  Apparent APSD for Artificially Charged QVAR 80 pMDI determined using the Dekati Cut-off Diameters and Recalibrated Cut-off Diameters using the ELPI with the Corona Charger. Data represents Mean ± SD, n=5.

Undersizing of artificially charged QVAR 80 aerosol was observed when Dekati reported stage cut-off diameters were used to determine the MMAD (0.60±0.02 μm), compared to the MMAD obtained using recalibrated ELPI cut-off diameters (0.99±0.02 μm). The original ELPI
calibration by Dekati was performed using corona charged aerosols and electrical detection since the induced electric current measured was reported to be proportional to the number and mass concentrations.

In summary, there was no statistically significant difference between the deposition behavior of QVAR 80 pMDI obtained using the ELPI with the corona charger removed and with the corona charger in place, but switched off (0 kV). Even though drug deposition was observed in the corona charger, this did not appear to cause a distortion in the apparent APSD. Furthermore, the use of Dekati reported cut-off diameters was observed to undersize the artificially charged QVAR aerosol. Therefore in order to determine the influence of charge level upon deposition of aerosols within the ELPI in the present study, it is appropriate to compare the apparent APSD of commercial pMDIs using the recalibrated cut-off diameters, which were found to be in good agreement with the literature reported size of QVAR.
A.III. EXAMPLE OF SCIENTIST MODEL and OUTPUT FILES

AIII.1 SCIENTIST Model File and an Example of the Parameter File

Model File Name: c:\scientis\model_lognmcdf.eqn

// Lognormal Cumulative distribution function - fitting Model (Mean and Sigma not conventional)
//MEAN is the mean of the (ln x) values; SIGMA is the std dev of the (ln x) values
//Take EXP (MEAN,SIGMA) for best fits for (MEAN,SIGMA G) respectively
//The ERF function below performs the integration of the usual pdf expression but the function removes
//2/SQRT(PI) from the PDF equation by using it as a coefficient in front of the integration symbol
IndVars: X
DepVars: Y
Params: MEAN, SIGMA
Y=100*(0.5+0.5*ERF((1/(SIGMA*SQRT(2)))*(LN(X)-MEAN)))
***

Parameters File Name: c:\scientis\lognmdif.par

<table>
<thead>
<tr>
<th>Parameter Name</th>
<th>Value</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN</td>
<td>0.2782</td>
<td>0.0000</td>
<td>5.0000</td>
</tr>
<tr>
<td>SIGMA</td>
<td>0.4831</td>
<td>0.0000</td>
<td>10.0000</td>
</tr>
</tbody>
</table>
AIII.2 Example of a SCIENTIST Plot of the Overlay of Experimental and Calculated Data
AIII.3 Example of the SCIENTIST Statistical Report for the Non-linear Regression Analysis

***Micromath Scientist Statistics Report***
Model: \texttt{c:\scientis\model\lognmcdf.eqn}
Data Set: \texttt{c:\scientis\acineutral.mmd}
Parameter Set: \texttt{c:\scientis\lognmidf.par}

Goodness-of-Fit Statistics

<table>
<thead>
<tr>
<th>Data Column Name: Y</th>
<th>Weighted</th>
<th>Unweighted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of squared observations:</td>
<td>2.3626E005</td>
<td>2.3626E005</td>
</tr>
<tr>
<td>Sum of squared deviations:</td>
<td>195.17</td>
<td>195.17</td>
</tr>
<tr>
<td>Standard deviation of data:</td>
<td>2.2663</td>
<td>2.2663</td>
</tr>
<tr>
<td>R-squared:</td>
<td>0.99917</td>
<td>0.99917</td>
</tr>
<tr>
<td>Coefficient of determination:</td>
<td>0.9969</td>
<td>0.9969</td>
</tr>
<tr>
<td>Correlation:</td>
<td>0.99892</td>
<td>0.99892</td>
</tr>
</tbody>
</table>

Data Set Name: \texttt{c:\scientis\acineutral.mmd}

| Weighted | Unweighted |
|---------------------|----------|------------|
| Sum of squared observations: | 2.3626E005 | 2.3626E005 |
| Sum of squared deviations: | 195.17 | 195.17 |
| Standard deviation of data: | 2.2663 | 2.2663 |
| R-squared: | 0.99917 | 0.99917 |
| Coefficient of determination: | 0.9969 | 0.9969 |
| Correlation: | 0.99892 | 0.99892 |

Model Selection Criterion: 5.6769

Confidence Intervals

Parameter Name: MEAN
Estimated Value: 0.28933
Standard Deviation: 0.010113
95% Range (Univariate): 0.26885 0.3098

Parameter Name: SIGMA
Estimated Value: 0.47234
Standard Deviation: 0.012683
95% Range (Univariate): 0.44666 0.49801

Variance-Covariance Matrix:
\[
\begin{bmatrix}
0.00010227 & 1.4343E-005 \\
1.4343E-005 & 0.00016085
\end{bmatrix}
\]

Correlation Matrix:
\[
\begin{bmatrix}
1 & 0.11183 \\
0.11183 & 1
\end{bmatrix}
\]

Residual Analysis:
The following are normalized parameters with an expected value of 0.0. Values are in units of standard deviations from the expected value.
Serial Correlation: 4.0878 indicates a systematic, non-random trend in the residuals
Skewness: -6.0978 indicates the likelihood of a few large negative residuals having an unduly large effect on the fit.
Kurtosis: -0.67098 is probably not significant.
Weighting Factor: 0
Heteroscedasticity: -1.6119
APPENDIX IV. NORMALIZED IMPACTOR DOSE FOR CHARGE NEUTRALIZED AND CHARGED FLUORESCIN DISODIUM DRY POWDER AEROSOL

Table AIV.1  Impactor Deposition for Fluorescein Aerosol (> 1 μm) as a Function of Charge Level Obtained using the ACI. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>ACI Stage</th>
<th>% Fluorescein Deposition (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Charge Neutralized</td>
</tr>
<tr>
<td>Stage 0</td>
<td>0.27±0.09</td>
</tr>
<tr>
<td>Stage 1</td>
<td>0.37±0.08</td>
</tr>
<tr>
<td>Stage 2</td>
<td>0.77±0.23</td>
</tr>
<tr>
<td>Stage 3</td>
<td>1.99±0.89</td>
</tr>
<tr>
<td>Stage 4</td>
<td>11.53±1.75</td>
</tr>
<tr>
<td>Stage 5</td>
<td>53.58±1.45</td>
</tr>
<tr>
<td>Stage 6</td>
<td>20.10±1.09</td>
</tr>
<tr>
<td>Stage 7</td>
<td>7.11±1.28</td>
</tr>
<tr>
<td>Filter</td>
<td>4.28±0.51</td>
</tr>
</tbody>
</table>

Table AIV.2  Impactor Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using Grounded ACI. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>ACI Stage</th>
<th>% Fluorescein Deposition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Charge Neutralized</td>
</tr>
<tr>
<td>Stage 0</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>Stage 1</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>Stage 2</td>
<td>0.76±0.06</td>
</tr>
<tr>
<td>Stage 3</td>
<td>2.60±0.41</td>
</tr>
<tr>
<td>Stage 4</td>
<td>11.09±1.45</td>
</tr>
<tr>
<td>Stage 5</td>
<td>54.60±1.56</td>
</tr>
<tr>
<td>Stage 6</td>
<td>18.23±2.05</td>
</tr>
<tr>
<td>Stage 7</td>
<td>7.79±0.79</td>
</tr>
<tr>
<td>Filter</td>
<td>4.39±0.87</td>
</tr>
</tbody>
</table>
Table AIV.3  Impactor Deposition for < 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using a Grounded ACI. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>ACI Stage</th>
<th>% Fluorescein Deposition</th>
<th>Charge Neutralized</th>
<th>Positively Charged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>0.42±0.29</td>
<td>0.63±0.22</td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>0.20±0.08</td>
<td>0.46±0.08</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>0.18±0.06</td>
<td>0.51±0.06</td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>0.38±0.13</td>
<td>0.71±0.06</td>
<td></td>
</tr>
<tr>
<td>Stage 4</td>
<td>2.03±1.46</td>
<td>1.70±0.38</td>
<td></td>
</tr>
<tr>
<td>Stage 5</td>
<td>20.46±2.13</td>
<td>19.39±3.93</td>
<td></td>
</tr>
<tr>
<td>Stage 6</td>
<td>26.75±1.62</td>
<td>28.08±1.96</td>
<td></td>
</tr>
<tr>
<td>Stage 7</td>
<td>24.40±2.48</td>
<td>25.05±5.38</td>
<td></td>
</tr>
<tr>
<td>Filter</td>
<td>25.18±1.62</td>
<td>23.45±2.72</td>
<td></td>
</tr>
</tbody>
</table>

Table AIV.4  Impactor Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using a Grounded ACI with Uncoated Impaction Plates. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>ACI Stage</th>
<th>% Fluorescein Deposition</th>
<th>Charge Neutralized</th>
<th>Positively Charged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>0.22±0.01</td>
<td>0.92±0.12</td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>0.43±0.10</td>
<td>1.28±0.10</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>0.94±0.04</td>
<td>1.85±0.08</td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>3.83±0.51</td>
<td>4.93±0.28</td>
<td></td>
</tr>
<tr>
<td>Stage 4</td>
<td>11.34±0.96</td>
<td>12.52±1.27</td>
<td></td>
</tr>
<tr>
<td>Stage 5</td>
<td>53.66±1.65</td>
<td>47.15±5.02</td>
<td></td>
</tr>
<tr>
<td>Stage 6</td>
<td>18.44±2.54</td>
<td>19.03±3.81</td>
<td></td>
</tr>
<tr>
<td>Stage 7</td>
<td>6.41±0.52</td>
<td>6.38±0.53</td>
<td></td>
</tr>
<tr>
<td>Filter</td>
<td>4.74±0.33</td>
<td>5.95±1.52</td>
<td></td>
</tr>
</tbody>
</table>
Table AIV.5  Impactor Deposition for > 1 μm Charge Neutralized and Positively Charged Fluorescein Aerosol Obtained using a Grounded ACI Following a Sampling Time of 5 Seconds. Data represents Mean ± SD, n=5.

<table>
<thead>
<tr>
<th>ACI Stage</th>
<th>% Fluorescein Deposition</th>
<th>Charge Neutralized</th>
<th>Positively Charged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>0.50±0.16</td>
<td>1.04±0.10</td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>0.61±0.11</td>
<td>1.29±0.17</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>1.10±0.15</td>
<td>2.40±0.69</td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>4.21±0.77</td>
<td>3.79±0.43</td>
<td></td>
</tr>
<tr>
<td>Stage 4</td>
<td>9.21±2.94</td>
<td>10.23±1.13</td>
<td></td>
</tr>
<tr>
<td>Stage 5</td>
<td>51.57±5.24</td>
<td>48.24±3.46</td>
<td></td>
</tr>
<tr>
<td>Stage 6</td>
<td>19.40±0.59</td>
<td>21.76±3.11</td>
<td></td>
</tr>
<tr>
<td>Stage 7</td>
<td>8.13±1.21</td>
<td>7.38±0.27</td>
<td></td>
</tr>
<tr>
<td>Filter</td>
<td>5.27±0.47</td>
<td>3.87±1.18</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX V. INFLUENCE OF PERCENT WATER CONTENT ON THE PARTICLE SIZE OF FLUORESCEIN DISODIUM DRY POWDER AEROSOL

The following empirical calculations were performed in order to determine if a difference in percent water content in the final ‘dry’ aerosol particles of fluorescein disodium, obtained after drying the aerosol generated using the constant output atomizer set up developed (as described previously in Chapter IV.B.1), influences the particle size of the aerosol i.e., changes the mass median aerodynamic diameter (MMAD) of the fluorescein aerosol obtained.

AV.1 Assumptions

The following assumptions were made in order to perform the above mentioned calculations:

- Two scenarios with different % water content in the final dry fluorescein aerosol particles produced.
  - Scenario I - 40% water content in the dry fluorescein aerosol particles obtained
  - Scenario II - 20 % water content in the dry fluorescein aerosol particles obtained

- No difference in collection efficiency of the fluorescein aerosol for both scenarios.

- Monodisperse aerosol was assumed for simplified calculations.

- Density calculations:
  - Density of anhydrous Disodium Fluorescein = 2.5 g/cm$^3$
  - Density of water = 1 g/cm$^3$
  - Density of the different ‘dry particles’ calculated from the proportion of water and disodium fluorescein, for example, 50% water and 50% disodium fluorescein results in a partial density = 1.75 g/cm$^3$
Equation AV.1 below provides the relationship between particle diameter and droplet diameter for fluorescein aerosol generated using the constant output atomizer.

\[ D_p = D_d \times \left( \frac{F_s \times \rho_l}{\rho_s} \right)^{1/3} \]  

Equation AV.1

where,

- \( D_p \) = Particle diameter, in µm
- \( D_d \) = Droplet diameter, in µm
- \( F_s \) = Weight fraction of the solute, in % w/w
- \( \rho_l \) = Density of the liquid, in g/cm\(^3\)
- \( \rho_s \) = Density of the solute, in g/cm\(^3\)

The droplet diameter remains constant for the two cases, as the same constant output atomizer is used, and the concentration of solute is also the same. However, the particle diameter will change depending upon the % content of water present following the drying conditions.

**AV.2 Calculation and Results**

Assuming that for a pure fluorescein sodium aerosol, with ‘\( \rho_f \)’ (density of fluorescein) = 2.5 g/cm\(^3\), Mass Median Diameter (MMD) is 1 µm.

So, Mass Median Aerodynamic Diameter (MMAD) is:

\[ \text{MMAD} = \text{MMD} \left[ \sqrt[3]{\frac{\rho_f}{\rho}} \right] \]  

Equation AV.2

Therefore,

\[ \text{MMAD} = 1 \times \left[ \sqrt[3]{2.5/1} \right] = 1.58 \mu m \]

Since, monodisperse aerosol was assumed, the calculated MMAD can be used to determine ‘\( D_p \)’:

\[ D_a = D_p \left( \sqrt[3]{\frac{\rho_f}{\rho}} \right) \]  

Equation AV.3

where,

\( D_a \) = Aerodynamic diameter, in µm
\[ D_p = \text{Physical diameter, in } \mu\text{m} \]
\[ \rho_p = \text{Density of particle, in } \text{g/cm}^3 \]
\[ \rho = \text{Unit Density} = 1 \text{ g/cm}^3 \]

Equating AV.2 and AV.3

\[ \text{MMD} \left(\sqrt{\rho_f}\right) = D_p \left(\sqrt{\rho_p}\right) \]

Hence, MMD = \(D_p = 1 \mu\text{m}\) (Since \(\rho_f\) is the same as \(\rho_p\) in this case)

Also, the mass of 1 aerosol particle = Volume \(\times\) \(\rho_f\)

\[
= \left(\frac{\pi D_p^3}{6}\right) \times \rho_f
= 1.31 \times 10^{-12} \text{ g}
\]

Assuming, there are ‘y’ number of particles, the total aerosol mass collected is -

\[
\text{Total aerosol mass} = \left[y \times (1.31 \times 10^{-12})\right] \text{ g} \quad \text{Equation AV.4}
\]

**Scenario I:**

40 % water content in the final ‘dry’ fluorescein aerosol particle

Assuming that the collection efficiency remains the same i.e., the total aerosol mass collected remains the same,

\[
\text{Total Mass} = y \left(\text{Volume} \times \rho_{p1}\right) \quad \text{Equation AV.5}
\]

where, \(\rho_{p1}\) = Density of particles in case I containing 40% water and 60% fluorescein = 1.9 g/cm\(^3\)

Substituting in Equation AV.5 and solving for \(D_p\),

\[
(y \times 1.31 \times 10^{-12}) = y \times \left(\frac{\pi D_p^3}{6}\right) \times 1.9
\]

Therefore,

\[D_p = 1.10 \mu\text{m}\]

Also,

\[\text{MMD} = 1.10 \mu\text{m}\]
So, using equation AV.3 \[ \text{MMAD} = 1.1 \left( \sqrt{1.9} \right) = 1.52 \mu m \]

**Scenario II:**

20 % water content in the final ‘dry’ fluorescein aerosol particle

Assuming that the collection efficiency remains the same i.e., the total aerosol mass collected remains the same,

\[ \text{Total Mass} = y \times (\text{Volume} \times p_{p2}) \quad \text{Equation AV.6} \]

where, \( p_{p2} \) = Density of particles in case II containing 20% water and 80% fluorescein = 2.2 g/cm\(^3\)

Substituting Equation AV.6 and solving for \( D_p \),

\[ (y \times 1.31 \times 10^{-12}) \text{ g} = y \times \left( \frac{\pi D_p^3}{6} \right) \times 2.2 \]

Therefore, \( D_p = 1.04 \mu m \)

Also, \( \text{MMD} = 1.04 \mu m \)

So, from Equation AV.2 \[ \text{MMAD} = 1.04 \left( \sqrt{2.2} \right) = 1.54 \mu m \]

As a result, the ratio of MMADs for fluorescein aerosol containing 40% water content in the final dry particles to that containing 20% water content can be obtained as follows-

\[ \text{Ratio} = \frac{\text{MMAD Scenario II (20\% water content)}}{\text{MMAD Scenario I (40\% water content)}} = \frac{1.54}{1.52} = 1.01 \]

**AV.3 Summary**

Therefore, based on the above calculations it was assumed that the difference in percent water content in the final dry fluorescein aerosol particles obtained by the constant output atomizer set up may not influence the aerodynamic diameter or the MMAD. Hence, the
variability in percent water content from between experiments was unlikely to introduce any error/bias in the size determination of the fluorescein aerosol and the subsequent data analysis.
VITA

Megha Mohan was born on May 11, 1985 in New Delhi, India and is an Indian citizen. She graduated from Delhi Institute of Pharmaceutical Sciences and Research, University of Delhi, India with Bachelors in Pharmacy in 2007. She, then, joined the Aerosol Research Group in the Department of Pharmaceutics, Virginia Commonwealth University (VCU) in 2007.

During her PhD, Megha has published 5 abstracts and given 2 podium presentations. She has presented her research at Annual Meetings of the Respiratory Drug Delivery Conference (RDD 2010, 2011, 2012), American Association of Pharmaceutical Scientists (AAPS 2010, 2011), and Graduate Research Association of Students in Pharmacy (GRASP, 2009), in addition to the poster presentations within the School of Pharmacy and VCU. She received the VCU School of Pharmacy Jyotsna and Mavji Thacker Award for academic excellence and research in Department of Pharmaceutics in 2008. Megha served as the GSA President in Department of Pharmaceutics from 2009-2010. She also served as AAPS Student Chapter at VCU Chair-elect (2009-2010) and AAPS Student Chapter at VCU Chair (2010-2011) and has successfully organized invited speaker seminars.

PUBLICATIONS:

Abstracts

Podium Presentations
2. Mohan M, Peart J, and Byron PR, Influence of Electrostatic Charge on the Particle Size Distribution of Beclomethasone Dipropionate from QVAR pMDIs using the Electrical Low Pressure Impactor, GRASP, Mercer University, Atlanta, GA, June 2009.