Aerosolization Studies of Zn-DTPA Using
Nebulizers for Decorporation of Internal Radioactive
Contamination

by

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Abstract

After individuals inhale radioactive particulate matter in nuclear disasters, pharmaceuticals will be essential to treat the contamination. Zn-DTPA can chelate to radionuclides, helping to accelerate the elimination of the radioactive components from the body. Although Zn-DTPA can be administered intravenously, medication in aerosol form could also be delivered directly to the lungs using commercial inhalers. This thesis will study the feasibility of aerosolizing Zn-DTPA solution by two inhalers (jet nebulizer and ultrasonic nebulizer), normally used in the treatment of asthma or bronchitis. Two evaluation parameters, the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD), were used to assess whether aerosols can reach the lower respiratory tract (particle size should be within 1 to 5 microns for ideal delivery). Inhalable Zn-DTPA aerosols were successfully generated by both of the tested nebulizers. The particle size distribution was measured by a commercial particle size analyzer using time-of-flight methods as well as a cascade impactor. Additionally, it was found that increasing the filling volume of Zn-DTPA solution in the jet nebulizer would decrease the MMAD and GSD, but this volume would not affect the MMAD of aerosols from the ultrasonic nebulizer. Zn-DTPA concentration also affected the MMAD in both nebulizers. MMAD significantly increased in the jet nebulizer along with the rising concentration. Small MMAD fluctuations were found in the ultrasonic nebulizer when the Zn-DTPA concentration was varied. Though more comprehensive work is necessary, this work could be used by health agencies around the world regarding nuclear disaster preparedness.

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Nomenclature

Symbols	Definition	Units
Abs	Absorbance	-
b	Path length, spectrophotometry	cm
B	Bias	-
Conc. or c	Concentration	mg/mL, $\mu\text{mol/L}$
d	Diameter	μm
f	Breathing frequency	-
I	Light intensity	
m	Mass	mg
M	Molecular weight	g/mol
P	Precision	-
p	Two-tailed probability	
Q	Flow rate	L/min
T	Transmittance	-
U	Uncertainty	-
Vol	Volume	mL
V_t	Tidal volume	L
x_g	Count median diameter or geometric mean diameter	μm

Greek symbols	Definition	Units
λ	Wavelength	nm
ϵ	Molar absorptivity coefficient	L/(mol·cm)
ρ	Density	g/cm ³
σ_g	Geometric standard deviation	-
ν	Stretching vibration	-

Acronyms	Definition
ACI	Andersen Cascade Impactor
BAL	British anti-lewisite
DF	Deposition Fraction
DPI	Dry powder inhaler
DTPA	Diethylenetriaminepentaacetic acid
EDTA	Disodium ethylenediaminetetraacetic acid
FDA	U. S. Food and Drug Administration
FPF	Fine particle fraction
GSD	Geometric standard deviation
IR	Infrared spectrometer
MDI	Metered dose inhaler
MMAD	Mass median aerodynamic diameter
NGI	Next Generation Pharmaceutical Impactor
PIM	Particle Instrument Manager (software)
pMDI	Pressurized metered dose inhaler
PSD	Particle size distribution
RV	Residual volume
SLPM	Standard liter per minute
STD	Standard deviation
TLC	Total lung capacity
TOF	Time-of-flight
USP	United States Pharmacopeia
UV	Ultraviolet
VC	Vital capacity

1 Chapter: Introduction

1.1 Thesis statement

The Chernobyl disaster and Fukushima nuclear disaster in 1986 and 2011, respectively, were two of the most catastrophic nuclear events in history, both classified as level 7 events by the International Nuclear Event Scale. Due to equipment failure and structural damage in the nuclear plants, radioactive materials or particulate matters were leaked into the environment, polluting the air, water and soil. Sadly, the scope of nuclear disasters is not limited to accidental incidents such as nuclear meltdowns. Some hypothetical events may include the use of small scale dirty bombs, which when detonated, would lead to an intentional spread of radioactive contamination over a large area.

With advances in nuclear technology, mitigation planning of nuclear fallout has become a widely discussed topic (Sorkhabi, 2011). When it comes to nuclear catastrophes, planning is crucial, since these events not only impact the current state of the environment and the health of those in the fallout region, but also future generations to come. It is also essential to provide proper treatment to individuals who were exposed to radiation.

As a FDA-approved (U.S. Food and Drug Administration) decorporation agent for radioactive internal contamination, Zn-DTPA (diethylenetriaminepentaacetic acid) has been widely administered during the medical emergencies response after incidents. The common administration methods are intravenous injection, medication inhalation, or oral administration. Inhalation therapy of aerosols can directly deliver medication to the affective area with a lower required dosage than the injection or oral administration. It is also a non-invasive method which gives less discomfort to patients.

This thesis will study the feasibility of aerosolizing Zn-DTPA solution using commercial nebulizers (inhalation devices) that are normally used in the treatment of asthma and bronchitis. Whether the particles are inhalable is dictated by the particle size distribution (typically, from 1 to 5 microns in diameter). The particle size distribution of Zn-DTPA aerosols produced from two different nebulizers are measured. The mass medium aerodynamic diameter (MMAD) and geometric standard deviation (GSD) are used to evaluate the particle size distribution. Several factors affecting the particle size distribution have been researched.

1.2 Demand for elimination of internal radioactive contamination

Internal contamination with radioactive materials in particulate form such as plutonium, americium, or curium can occur through a variety of routes including ingestion, inhalation, or direct contact through wounds. These materials are toxic to humans and, even though small amounts of radioactive materials are released, the invisible radiation can sufficiently spread and contaminate a wide area. The effects of radiation on human body are dependent on the radiation dosage. The increased radiation levels will lead to life threatening outcomes (Craig & Jungerman, 1990). Details are shown in Table 1.1.

If someone is accidentally exposed to such hazardous materials, the radioactive components will start to decay inside the body. Since only the metal lead can significantly block different kinds of radiation, tissues and organs easily absorb all the radiation during internal contamination. The absorption of radiation will stop cell division, damage the DNA structure and can induce cancer. If the internal radioactive contamination is not eliminated in time, the contamination will incorporate to tissues, organs or even marrows (Loveland, Morrissey & Seaborg, 2006). Hence,

patients will receive chronic radiation exposure inside their bodies. Any contact with radioactive contaminants through inhalation into the lungs, will require immediate treatments.

Table 1.1 Effects of radiation levels on the human body

Dose/ Sv	Effects
0.05-0.2	Possible late effects; possible chromosomal damage.
0.2-1	Temporary reduction in white blood cells.
1-2	Mild radiation sickness within a few hours: vomiting, diarrhea, fatigue; reduction in resistance to infection.
2-3	Serious radiation sickness effects as in 1-2 Sv and hemorrhage; exposure is a Lethal Dose (LD) to 10-35% of the population after 30 days (LD 10-35/30).
3-4	Serious radiation sickness; also marrow and intestine destruction; LD 50-70/30.
4-10	Acute illness, early death; LD 60-95/30.
10-50	Acute illness, early death in days; LD 100/10.

Retrieved from Craig & Jungerman, 1990.

1.3 Introduction to the respiratory system

The human respiratory system (see Figure 1.1) consists of different regions, including the trachea, bronchi, bronchioles, lungs, and diaphragm. Its main function is providing oxygen and simultaneously eliminating carbon dioxide from the internal organs in order to provide most of the energy for metabolism in the body. The exchange of oxygen and carbon dioxide between blood and the external environment takes place in the alveoli in the lungs by diffusion. The respiratory system can be divided into two parts, the conduction zone and the respiratory zone, according to the function of gas transportation or gas exchange.

The conduction zone, where the outer air or oxygen is conducted into the respiratory exchange zone, consists of the extrathoracic region and trachea-bronchial region. These two regions are connected with each other. As the entrance of the respiratory tract, the extrathoracic region includes the mouth, the nose, the pharynx, and the larynx. “Throat” is usually referred to the combination of the pharynx and the larynx. In the trachea-bronchial region, there is the trachea at

the start, followed by the bronchi and the bronchioles as the terminal path for air delivery from the larynx into the lungs. The air is also filtered, humidified and laminarized (no turbulence) in this region. Normally, an airway will bifurcate (branch) into two smaller airways (sometimes more than two), making up the respiratory tree with different generations. After the trachea, the biggest airway in the respiratory tract, the bronchi branch off into two airways along the first three generations, called the main bronchi, to supply air into the right and left lungs.

Distal to the trachea-bronchial region is the alveolar region which is also called to be the respiratory zone or the pulmonary region. The “lungs” become the combination of the trachea-bronchial region and the alveolar region. The alveolar region refers to all parts of the lung with alveoli, which consists of about 150 million tiny air sacs inside the lungs where the gas exchanges between oxygen and carbon dioxide in blood occur. The respiratory zone starts at the respiratory bronchioles and the respiratory bronchioles are the first generation of daughter tubes branching off the terminal bronchioles. More alveoli appear on increasingly deeper alveolar ducts, fully covered with alveoli. The alveoli sacs are the smallest daughter generation in the alveoli. To describe the overall geometry, models of the lungs normally use 24 generations from the trachea to the alveoli (Weibel, 1963).

Figure 1.1 Geometry of the respiratory system (adapted from Finlay, 2001)

1.3.1 Lung capacity

There are some definitions related to the lung capacity. The total lung capacity (TLC) is the total volume for all the airspace in the lung with the maximum inflation. Normally the TLC for a typical adult is 6 L and it can be defined as the sum of vital capacity (VC) and residual volume (RV). Tidal volume (V_t) is the average inhaling and exhaling volume in a breathing cycle. This inhalation and exhalation is known as a respiratory rate or breathing frequency (f). Generally, the breathing frequency for adults (i.e., number of breaths per minute) is 12. Tidal volume only occupies a small percentage in the total lung capacity. For adults, the tidal volume is approximately 0.75 L. Age, gender, height, weight, activity, race and even diseases influence the lung volume among different subjects. For example, females tend to have a 10-12% lower capacity than males (Bellemare, Jeanneret & Couture, 2003). Tall people are going to have a larger TLC than short people. Professional athletes will have a larger TLC than the general

public as athletes receive much more cardiopulmonary training. Since pulmonary diseases, such as asthma and lung cancer can alter the physical geometry of the lung, for example, limiting the diameter of certain bronchi. Heavy smokers have a significantly lower TLC than non-smokers (Awan & Alphonso, 2007). For nebulizers, knowledge of the tidal volumes becomes important since they operate under a normal breathing pattern, which is the tidal breathing. Similar to other lung volumes, tidal volumes, flow rates and breathing frequencies are also affected by age, gender, height, weight and activity, etc. In addition, because of the attachment of mouthpieces or facemasks, the normal breathing pattern may be affected during normal nebulizer usage.

1.3.2 Mechanism of aerosol deposition

Particle deposition is caused by sedimentation, inertial impaction, Brownian diffusion (shown in Figure 1.2), and turbulent dispersion in the respiratory tract. Sedimentation, also called gravitational settling, dominantly affects particles smaller than 2 microns. The geometry of the specific region in the lung and the fluid velocity through the ducts affects the sedimentation in the alveolar airways (Khajeh-Hosseini-Palasm & Longest, 2015). Deep in the lung, sedimentation is the prominent method of deposition while impaction becomes less apparent. Impaction occurs with large particles (larger than 3 microns) moving in high velocity. The possibility of the impaction of particles with the same size in a particular generation in the respiratory tract is linearly dependent on the flow rate that carries the aerosols. Generally speaking, Brownian diffusion is not a dominant mechanism of deposition of aerosol particles since only particles smaller than 1 micron will be affected by diffusion. Turbulence in the flow will force particles towards adjacent walls, increasing particle deposition. In forced inhalation (deep and rapid inhalation), the flow is turbulent in the mouth-throat region.

Figure 1.2 Mechanisms of lung deposition (adapted from Muchão & Filho, 2010)

1.3.3 Flow rate and respirable particle size

The flow rate of tidal breathing for adults is around 18 L/min. In a single tidal breathing cycle, the inhalation duration is shorter than the exhalation duration. Therefore, the flow rate for inhalation is higher than exhalation. Flow rate is an important factor for the deposition of aerosol particles in the respiratory tract. A higher flow rate will increase the possibility of mouth-throat deposition while the total dose delivered to the lung will decrease. When the flow rate is high enough, indicating that impaction becomes dominant in trachea-bronchial region, further increasing the flow rate results in more trachea-bronchial deposition. Aerosols surely inclines to deposit in trachea-bronchial region when they pass through mouth=throat region. On the contrary, decrease of the inhaling flow rate leads to a shift in deposition from the mouth-throat region towards the lung, owing to the lack of impaction. When the flow rate is low enough, the particles tend to follow the air flow and increase the chance of deposition in the lower respiratory airways. Such deposition depends on the particle size. For instant, approximately half of the aerosols

particles that are larger than 10 microns will be deposited in the mouth-throat region when the inhalation flow rate is 18 L/min while deposition under 60 L/min is more than 90%.

Another dominant factor for the deposition in the respiratory tract is the particle size. Note that it will be impossible to precisely target the deposition areas where the air flow carries the aerosol to the lung but broad targeting is feasible. Patients cannot be expected to precisely control their breathing flow rate to a steady level. The variation between individuals and the gradual change of factors affecting the deposition from region to region in the lung account for the broad targeting. The factors influencing the deposition will change in terms of different regions in the lung so it will be difficult to select a specific region for deposition.

Respirable particles are small particles that can be inhaled into the terminal bronchioles and alveoli. In some *in vitro* studies, particle size is the most commonly used approach in predicting the broad deposition in the respiratory tract (Heyder, 2004; Newman & Chan, 2008; Poli & Lipworth, 2015). Increasing the particle size will increase the chance of mouth-throat deposition and then reduce the medications dosage entering the lung. The percentage of small size particles that can penetrate further than the mouth-throat region is referred as the fine particle fraction (FPF) of aerosols. The particle mass below 5 microns is normally regarded as the fine particle mass. According to the data from experiments, particles between 1 to 5 microns is the optimal size for pharmaceutical aerosols to reach the lower respiratory airways whereas particles exceeding 5 microns tend to impact in the upper respiratory airways, being swallowed later (Capstick & Clifton, 2012; Finlay, 2001; Hess *et al.*, 1996; Tena & Clarà, 2012; Tsapis, 2014; Zhou *et al.*, 2007). This is an empirical principle of particle size to determine whether the aerosol particles can be deposited in the lung. Details about the particle size and deposition site are shown in Table 1.2. Particles below 1 micron can rarely deposit in the lung and they will travel

along with the air flow, exiting the respiratory tract through the exhalation cycle. This is because assuming the density is constant for all particles. Particles with small diameter carry small mass, which decreases the sedimentation and impaction in the trachea-bronchial region (Tsapis, 2014).

Table 1.2 Site of deposition of particles as a function of their aerodynamic diameter (adapted from Tsapis, 2014)

Site of deposition	Aerodynamic diameter/ μm
Mouth-Throat-Trachea	10-30
Bronchi, Bronchioles	3-10
Alveoli	< 3

Geometric diameter and aerodynamic diameter are two common approaches in describing the size of the aerosol particles. Geometric diameter is the actual diameter for each particle in the aerosols. Aerodynamic diameter of an irregular particle is defined as the equivalent diameter of a spherical water particle settling with the same terminal velocity in the same fluid as the particle to be measured. Because the shape of small particles is usually irregular rather than perfectly spherical, the aerodynamic diameter is an alternative to simulate idealized particles with identical behaviour to the actual particles. Density of the aerosol particles, which plays a role in geometric diameter, also affect the deposition as impaction and sedimentation are affected by density. The relationship between geometric diameter (d) and aerodynamic diameter (d_{ae}) is described below (Carvalho, Peters & Williams III, 2010; Finlay, 2001).

$$d_{ae} = \sqrt{\frac{\rho_{particle}}{\rho_{water}}} d \quad (1.1)$$

For pharmaceutical aerosols, aerodynamic diameter is more accurately to characterize and predict the particle behaviour in fluids and it can be used to compare various aerosol particles

regardless of the different morphology and density (Hess *et al.*, 1996; O’Callaghan & Barry, 1997).

1.3.4 Particle size distribution

There are different ways of characterizing the particle size distribution (PSD) in the pharmaceutical aerosols. Most of the particles in the pharmaceutical aerosol field are polydisperse with a relatively wide distribution in size. Experimental results have shown that aerosols generated from inhalation devices generally contain particles with different sizes.

(1) Log-normal distribution

Particle size distributions can be based on different weightings, count, surface and volume. The count distribution is a function between the number of particles in the aerosol and the diameter of particles. The number concentration is normalized by the distribution function. The diameter is generally demonstrated using the logarithmic scale (thus the name of log-normal distribution), which is the best empirical fit for aerosols generated from the same inhalation devices.

The log-normal count distribution $f(x)$ for pharmaceutical aerosols is defined as:

$$f(x) = \frac{1}{x\sqrt{2\pi} \ln \sigma_g} \exp \left[\frac{-(\ln x - \ln x_g)^2}{2(\ln \sigma_g)^2} \right] \quad (1.2)$$

Two important parameters, the count median diameter (x_g) and the geometric standard deviation (σ_g , abbreviated to GSD), are used to statistically describe the log-normal distribution. The value of x_g is defined as the median value of $f(x)$, satisfying

$$\int_0^{x_g} f(x) dx = 1/2 \quad (1.3)$$

$$\ln x_g = \int_0^{\infty} \ln x f(x) dx \quad (1.4)$$

The value of σ_g is defined as the standard deviation of the logarithm of particle diameter, satisfying

$$\ln \sigma_g = \left[\int_0^\infty (\ln x - \ln x_g)^2 f(x) dx \right]^{1/2} \quad (1.5)$$

The GSD describes the distance between the size of each particle and the mean diameter in the distribution. Lower GSD value refers to monodisperse ($GSD \leq 1.25$), which usually has a narrow size distribution whereas higher GSD value refers to polydisperse ($GSD > 1.25$), which usually has a wide size distribution (TSI, 2012).

Although count distributions are useful and straightforward to present the PSD in aerosols, in order to better evaluate the therapeutic effect by mass of the drugs delivered into the lungs, mass distributions will be more appropriate for medication deposition than the count distributions.

Mass distribution is defined as the mass of particles $m(x) dx$ having the diameters between x and $x + dx$. The mass normalized distribution $m_{normalized}(x)$ is the fraction of the aerosols mass contained in particles having the diameters between x and $x + dx$. Similar to the log-normal distribution of count distribution, the $m_{normalized}(x)$ can be defined as

$$m_{normalized}(x) = m(x) / (\text{total mass of aerosol})$$

$$m_{normalized}(x) = \frac{1}{x\sqrt{2\pi} \ln \sigma_g} \exp \left[\frac{-(\ln x - \ln MMAD)^2}{2(\ln \sigma_g)^2} \right] \quad (1.6)$$

Where MMAD is the abbreviation of mass medium aerodynamic diameter, which shows that half of the aerosol mass is contained in particles with diameter less or equal to the MMAD. It is one of the parameters of great importance in assessing the aerosol deposition in the lungs.

The normalized mass distribution $m_{normalized}(x)$ is defined as the fraction of aerosol mass contained in particles with diameters from x to $x + dx$. The definition of normalized volume $v_{normalized}(x)$ is similar to the normalized mass distribution. Supposing that the particles in the

pharmaceutical aerosols are spherical and all the particles in an aerosol have the same density, the normalized volume distribution $v_{normalized}(x)$ will be identical to the normalized mass distribution $m_{normalized}(x)$. The volume of a spherical particle can be expressed as $\pi x^3/6$ with the diameter x so that the fraction of volume occupied by spherical particles of diameter x can be simplified to

$$v_{normalized}(x) = \frac{f(x)x^3}{\int_0^\infty f(x)x^3 dx} \quad (1.7)$$

Therefore, equation (1.7) shows the relationship between mass distribution and count distribution.

(2) Cumulative size distributions

Besides defining the PSD according to the number or mass of particle of a certain diameter, there is another way to define the PSD by considering the number or mass of particles that are smaller than a specific diameter. Such distribution is known as the cumulative size distributions.

Cumulative count distribution $F(d)$, it refers to the total count of particles, F , having the diameter smaller than d .

$$F(d) = \int_0^d f(x) dx \quad (1.8)$$

Similarly, the cumulative mass distribution $M(d)$ will be

$$M(d) = \int_0^d m(x) dx \quad (1.9)$$

While the normalized cumulative mass distribution $M_{normalized}(d)$ will be

$$M_{normalized}(d) = \int_0^d m_{normalized}(x) dx \quad (1.10)$$

Specifically, the MMAD will be $M_{normalized}(d) = 1/2$ according to its definition mentioned before.

The GSD can be obtained from the log-normal distribution. Statistically, the values spread symmetrically and are centered on the mean. 68.26% of the values in a normal distribution lies in the range of $mean \pm standard deviation$. Since the normalized mass distribution for aerosol particles, demonstrates that 68.26 % of the particles contain the mass between $MMAD/\sigma_g$ and $MMAD \times \sigma_g$ implies that 34.13% of aerosol mass falls between $MMAD/\sigma_g$ and $MMAD$. Thus, $50 \% + 34.13 \% = 84.13 \%$ of the aerosol mass are within diameters $x = 0$ and $x = MMAD \times \sigma_g$. It is also valid for $50\% - 34.13\% = 15.87\%$ of the aerosol mass are within diameters $x = 0$ and $x = MMAD/\sigma_g$. The following correlations can be written,

$$\sigma_g = d_{84.13}/MMAD \quad (1.11)$$

$$\sigma_g = MMAD/d_{15.87} \quad (1.12)$$

Where $d_{84.13}$ is the diameter at which 84.13% of the aerosol masses are contained in diameter less than this diameter. Same for $d_{15.87}$. Therefore,

$$\sigma_g^2 = d_{84.13}/d_{15.87} \quad (1.13)$$

$$\sigma_g = \sqrt{d_{84.13}/d_{15.87}} \quad (1.14)$$

For log-normal distribution of spherical particles with constant density, the MMAD can be obtained from the geometric mean diameter (the same as count median diameter, x_g) and geometric standard deviation (GSD, σ_g).

$$MMAD = x_g \exp \left[3(\ln \sigma_g)^2 \right] \quad (1.15)$$

Regarding pharmaceutical aerosol deposition in the lungs, MMAD is a straightforward measure to predict how deep the aerosol can reach into the lungs and help to determine the dosage of

medication. For the comparison between multiple pharmaceutical aerosols with identical MMAD, GSD can be also useful to demonstrate the percentage of particles in the aerosols falling into the fraction of respiratory particle size since small values of GSD indicate narrow distributions around the MMAD and vice-versa. Considering that all particles smaller than 5 microns can be inhaled into the respiratory system, a larger GSD along with the same MMAD could cause fewer particles to be inhaled when the MMAD is smaller than 5 microns (Zhou et al., 2007). Therefore, MMAD and GSD are both functioned as the criteria in the evaluation of particle size distribution.

1.4 Commercial alternatives for decorporation therapy of internal radioactive contamination

Decorporation therapy is based on the actinide ion binding (or chelating) properties of decorporation agents or chelators that can prevent radioactive contaminants from being re-absorbed in the body as well as increase the rate of elimination or excretion of absorbed, inhaled, or ingested radionuclides (Guilmette & Muggenburg, 1993; Ansoborlo *et al.*, 2007). The effectiveness of these drug products can be only tested on animals due to the ethical considerations. Different radioactive contaminants require different decorporation agents in the treatment. Not all medications can receive FDA approval (CDER, 2006).

1.4.1 Ca-DTPA and Zn-DTPA

Both Ca-DTPA and Zn-DTPA are recognized as medications to treat internal contamination caused by transuranium elements (plutonium, americium or curium). More details about these two medications will be given in Section 2.1.

1.4.2 Prussian blue

Prussian blue capsules (Radiogardase®, Heyl, Berlin, Germany) are used to remove internal contamination of cesium, thallium, and rubidium, especially for ^{137}Cs . It can significantly decrease the biological half-life of ^{137}Cs from approximately 110 days to 30 days. Each capsule contains 500 mg of Prussian blue and the treatment can last 3 weeks or longer as required.

Prussian blue pills can work safely with majority of adults including pregnant women, as well as children. However, individuals with constipation, blockages in the intestines, or certain stomach problems need to consult physicians before taking the treatment (Marcus, 2004 and Ohmachi, 2015).

1.4.3 British Anti-Lewisite (BAL) and its derivatives (DMPS, DMSA)

British Anti-Lewisite (BAL-in Oil®, Akorn Pharmaceuticals, Inc., Lake Forest, IL) is 2,3-dimercaptopropanol, which can eliminate heavy metal mercury, arsenic and gold from the blood, receiving the FDA approval in 1946. When used together with disodium ethylenediaminetetraacetic acid (EDTA), BAL is effective for acute lead intoxication. The treatment is administered by injection into muscle. Some researches show that BAL can also decorporate polonium by accelerating the excretion of ^{210}Po and reducing the mean retention time of ^{210}Po . Nevertheless, researchers also found that BAL had a chance of redistributing ^{210}Po into the brain. In order to solve this problem, derivatives of BAL, DMPS (Dimaval®, Heyl, Berlin, Germany) and DMSA (Succimer, Chemet®, Ovation Pharmaceuticals, Inc., Lincolnshire, UK) can keep the polonium from moving to patients' brain. DMSA, as an oral capsule (obtained orphan drug designation from FDA in 1984), is a lead removal medication for children. It is also used in the prevention of cysteine kidney stone formation in those homozygous for cystinuria

after mercury intoxication. DMPS, currently without FDA approval, can deal with the absorption of mercury, arsenic and lead and increase the excretion of polonium from tissue except for kidneys. DMSA has a modest efficiency for ^{210}Po decorporation with less toxicity compared with BAL (Cassatt *et al.*, 2008; Ohmachi, 2015).

1.4.4 Penicillamine and trientine

Penicillamine (Cuprimine®, Aton Pharma, Inc., Lawrenceville, NJ) and trientine (Syprineare®, Aton Pharma Inc., Lawrenceville, NJ) are chelators for ^{60}Co and ^{210}Po . The thiol penicillamine and polyamine trientine (triethylenetetraamine) are both FDA-approved to utilize for the Wilson's Disease treatment which is an autosomal recessive genetic disorder of copper metabolism. The thiol penicillamine can cure rheumatoid arthritis and cystinuria besides decorporating heavy metal such as copper, iron, mercury, lead and gold. It is demonstrated from scientific researches that the thiol penicillamine is more effective in the elimination of ^{60}Co contaminations in rats than EDTA and DTPA. For those who have resistance against penicillamine, polyamine trientine is a compensator as a decorporation agent of copper, mercury, nickel, bismuth and palladium with relatively high efficacy in addition to zinc, cadmium, lead and cobalt with relatively low efficacy (Cassatt *et al.*, 2008; Ohmachi, 2015).

2 Chapter: Background and Literature Review

2.1 Complex binding to radioactive transuranium elements

2.1.1 Properties of DTPA

Diethylenetriaminepentaacetic acid (DTPA), also known as pentetate acid, is commonly used as a chelating agent. Its structure is illustrated in Figure 2.1. This fine white powder can bind to most of the metal ions with strong affinity. DTPA can envelop a metal ion by forming coordination bonds with strong attraction. Ultimately, some transuranium elements, such as plutonium, americium, or curium can bind to this organic ligand. Scientists have taken advantage of this important property to integrate the compound into treatments of nuclear medicine. An important property of DTPA is its slight solubility in water. The salt form of DTPA can increase the solubility of this chelating agent, which helps to develop an adequate dosage for treatment.

Figure 2.1 Structure of DTPA

Calcium-DTPA (Ca-DTPA) and Zinc-DTPA (Zn-DTPA) are two common salt forms of DTPA. Their structures are shown in Figure 2.2. The calcium or zinc ion is readily displaced by more highly charged cations such as the ions of the transuranium elements. Prescription drugs of Ca-

DTPA and Zn-DTPA for intravenous injection (Hameln Pharmaceuticals Ltd., Gloucester, UK 1000 mg in 5 mL ampoule bottle, see Figure 2.3) and oral administration (tablets) are commercially available in the current market.

Figure 2.2 Structure of Ca-DTPA (left), Zn-DTPA (right) and the decorporation equation

Figure 2.3 Commercially available Zn-DTPA (left) and Ca-DTPA (right) Injection Products

(Photo credit: Retrieved from <http://orise.orau.gov/reacts/resources/package-inserts.aspx>)

Ca-DTPA and Zn-DTPA can accelerate the natural elimination rate of the internal radioactive contamination in blood stream, thereby reducing the radiation retained in the body (Takada & Fujita, 1973). Scientists have been researching these two drug products for over 4 decades. In 1974, two German researchers, Volf and Seidel, injected Ca-DTPA and Zn-DTPA into rats and hamsters to test the efficiency of eliminating internal radioactive contamination (Volf & Seidel, 1974). Since then, an accumulating number of scientific findings have come out to detail this function of radiopharmaceuticals. Researchers found that they could accelerate the excretion of the transuranium elements plutonium, americium, and curium from the body through urine. As a result, they decrease body absorption and retention time of metal radioactive nuclides. Both of these products can be safe and effective to work as decorporation agents (Volf, 1984). However, Ca-DTPA and Zn-DTPA will not treat contamination with radioactive iodine, uranium and neptunium, or the complications resulting from radiation exposure (e.g., bone marrow suppression). If these conditions are suspected, other treatments are required.

In order to track the effectiveness of DTPA excretion from the body, scientists injected DTPA labeled with ^{14}C , a radioactive nuclide, to measure the DTPA distribution in urine and plasma. As displayed in Figure 2.4, most of the ^{14}C -DTPA will distribute to plasma in the first 7 hours of ingestion and finally accumulate in subject's urine 24 hours after the injection. Since there is not much Zn-DTPA absorbed in the digestive system, the injected DTPA would be naturally excreted by the renal system. Zn-DTPA is cleared from the plasma to urinary excretion by glomerular filtration. For inhalation therapy, around one fifth of the inhaled Ca- ^{14}C -DTPA would be absorbed in patient's lung. The absorption percentage for oral administration is merely 5%, much lower than inhalation (FDA, 2004).

Figure 2.4 Percent of ^{14}C -DTPA distribution after injection (adapt from FDA, 2004)

After internal contamination, the effectiveness of chelation reduces with time because the transuranium elements become incorporated into the tissues. As such, chelation treatment should be provided as soon as possible after known or suspected internal contamination with transuranium elements has occurred.

Bailey *et al.* tested the efficiency of decorporation therapy after a puncture was contaminated with plutonium and americium. The therapy included the surface decontamination on the wound part and inhalation of Ca-DTPA immediately after discovering the wound. Zn-DTPA was also administered for 5 consecutive days. Based on the data from the urinary excretion, this decorporation therapy with Ca-DTPA or Zn-DTPA could significantly reduce the retention of plutonium and americium in the patient's body (Bailey, 2003). Through rat tests, Stather *et al.* found that aerosol inhalation was more effective in eliminating ^{238}Pu and ^{241}Am contamination in the lungs while intravenous injection was more effective in liver and skeletal clearance (Stather *et al.*, 1985).

In 2004, the U.S. Food and Drug Administration (FDA) approved Ca-DTPA and Zn-DTPA as drugs to treat internal radioactive contamination of plutonium, americium, and curium. The treatment goal of Ca-DTPA and Zn-DTPA is to enhance the removal of these radioactive contaminants. Therefore, the risk of potential future biological effects including the development of certain cancers, which may occur years after exposure, will be reduced (FDA, 2004).

2.1.2 Side effects of Ca-DTPA and Zn-DTPA

The main side effect of Ca-DTPA is the loss of certain essential nutritional metals, such as zinc, from the body. Nevertheless, the loss of zinc can be compensated by taking oral zinc supplements. Although Zn-DTPA may also lower the levels of certain nutritional metals, the effect (which can be countered by taking mineral supplements) is less than with Ca-DTPA. Chelation therapy administered by nebulized inhalation might cause breathing difficulties in some individuals. In addition, Ca-DTPA should be used with caution in patients suffering from a severe form of a disease known as hemochromatosis. The toxicity of Zn-DTPA coming from the chelation of zinc can cause nausea, vomiting, chills, diarrhea, fever, pruritus (skin itching), muscle cramps and anosmia (smell sense loss) (Shankar *et al.*, 2014).

2.1.3 Methods of delivery and corresponding dosage

DTPA can be administrated into the body by intravenous injection, aerosol inhalation and oral administration, as mentioned before. Non-invasive administration such as inhalation or oral administration of either Ca-DTPA or Zn-DTPA would be an appropriate and simple way to

eliminate internal contamination after the accidental release of plutonium and americium into the public environment, which will affect numerous people.

Ca-DTPA and Zn-DTPA are usually administered into the blood stream through injection.

However, when radioactive contamination is inhaled into respiratory tract, the prompt administration of nebulized Ca-DTPA or Zn-DTPA aerosols is required. If a patient is contaminated through route of entry, for example, by contact as well as inhalation of the radioactive dust, intravenous injection will be more favourable in the decorporation treatment (Phan *et al.*, 2006).

Conventional nebulizers used in asthma treatment can be used to aerosolize Ca-DTPA and Zn-DTPA (Kostial *et al.* 1987). In general, nebulizers, either jet or ultrasonic, are sustainably running while medication is being administered. Nebulizers can convert the medication solution into an aerosol which is a mixture of fine solid particles or small liquid droplets, at the micron scale, accompanied by a gas coflow. A single nebulization treatment usually lasts less than half an hour. The size of the particles resulting from the nebulization determines the deposition region in the respiratory system. The bigger particles, the more chance they deposit in the mouth-throat area. The smaller particles, the more chance they deposit in the alveoli and bronchioles. The required treatment dosage of Ca-DTPA or Zn-DTPA varies depending on the administration route selected to deliver the medication (shown in Table 2.1). According to Ménétrier *et al.*, the maximum daily dosage cannot exceed 1 g for long term treatment. Inhalation therapy requires the smallest dosage of all three administration routes (Ménétrier *et al.*, 2005).

Table 2.1 Required treatment dosages for different administered routes (adapted from Ménétrier *et al.*, 2005)

Administered Route	Intravenous Injection	Nebulized Inhalation	Oral Administration
Required Dosage/ $\mu\text{mol}\cdot\text{kg}^{-1}$	30	2	95

2.1.4 Recommended sequences of decorporation therapy

Ca-DTPA and Zn-DTPA should not be administered at the same time. If both products are available, Ca-DTPA should be used as the first dose. If additional treatment is required, it should be Zn-DTPA. This treatment sequence is recommended since Ca-DTPA is more effective than Zn-DTPA during the first 24 hours. After the initial 24 hours, according to tests in rats and hamsters, Zn-DTPA has a comparable effectiveness to Ca-DTPA in tightly binding the transuranium elements in rat and hamsters with less toxicity. The animal tests showed that daily Zn-DTPA treatment following emergency therapy can increase the average survival time. At comparable doses, Zn-DTPA had less toxicity (e.g., less depletion of trace metals, lower rate of mortality, absence of kidney and liver vacuolization, and absence of small bowel hemorrhagic lesions) (FDA, 2004). In addition, Zn-DTPA treatment is recommended for pregnant women with internal radioactive contamination (FDA, 2015; Shankar *et al.*, 2004).

When it comes to operation procedures, physicians need to first inform patients about the details. Secondly, decorporation therapy should be initiated through early inhalation of DTPA medication combined with a follow-up intravenous injection. For the sake of external absorption through a wound, solutions of DTPA medications can be applied to wash the wound along with the treatment. Thirdly, the retention of radioactive material in urinary and plasma excretion should be monitored to assess the intake. Additionally, a physical examination might be needed as indicated (Ménétrier *et al.*, 2005).

2.2 Different types of nebulizers

Nebulizers are routinely used to aerosolize medications for lung delivery. They are considered to be the first inhalation devices to deliver pharmaceutical aerosols into patients' lung. They were

first introduced as clinical treatment around the 19th century. The working principle of nebulizers is to break down a liquid by utilizing an external energy source, converting the liquid into a mist of small droplets that are easy for inhalation. Nebulizers can continuously deliver small droplets at a relatively constant flow rate with a specific range of diameters through tidal breathing. Pharmaceutical aerosols are delivered for a period of minutes. For very young children, the elderly, or those patients with severe broncho-obstruction, conventional nebulizers should be the top option for them. Additionally, the nebulizers are appropriate for medication that is unsuitable to apply in pressurized metered dose inhalers (pMDIs) and dry power inhalers (DPIs), for example, Arformoterol (long-acting bronchodilator), Dornase Alpha (mucoactive drugs) and Aztreonam (antibiotics) (Tiddens *et al.*, 2014). However, nebulizers still have significant limitations of specific requirements for drugs, long treatment times and poor portability. Medications also have to be in solution or suspension forms, which constrain the choice of pharmaceuticals. In addition, the long treatment times may lead to poor patient compliance and the instability of medication due to hydrolysis and evaporation (Finlay, 2001; Gardenhire *et al.*, 2013).

Nowadays, there are three designs of nebulizers that are widely used in clinical treatment. They can be categorized into jet nebulizer, ultrasonic nebulizer and mesh nebulizer. The nebulizers are mainly chosen by the properties of the medication to be administered. All of the nebulizers will be connected with a mouthpiece or a face mask to help patients inhale aerosols.

2.2.1 Jet nebulizer

The first pressurized inhaler was invented by Sales-Girons in 19th century, using pressure to atomize the liquid medication (Nerbrink, 2003). It is still the most frequently used nebulizer in clinical treatment. All jet nebulizers have a chamber to contain liquid medication and utilize

pressurized air generated from a compressor which comes out from a narrow Venturi nozzle inside the nebulizer as displayed in Figure 2.5 (A). The compressed air is forced through an orifice for expansion. This creates a decrease in pressure and a large increase in velocity. Owing to its high kinetic energy, the pressurized air elevates and breaks up the liquid medication in the nebulizer reservoir through the liquid feeding tube. The droplets produced above the nozzle hit the first baffle then split into primary droplets.

The primary droplets flying with the high-velocity air will hit the secondary baffle and split into even smaller droplets. Those particles escaping the baffles will either leave the nebulizer or fall back into the medication chamber by gravity. The smaller droplets will be inhaled by the patient. The particle size is proportional to the nozzle diameter and the output flow rate of the compressor. The recommended output flow rate for the jet nebulizer is 6 to 8 L/min.

Jet nebulizers can work with various medications in water or organic solvents. It is one of the most affordable atomization devices to convert medications into aerosols with simple operation. Since high velocity compressed air or oxygen is needed to generate the aerosols, compressors or the medical gas supply systems in hospitals are required along with the jet nebulizer, making the device less portable. Jet nebulizers have less efficiency than other inhalation devices as most of the medication will be wasted, either being trapped in the baffles and nebulizer reservoir, or being exhaled, swallowed or deposited in the oropharynx area. Moreover, during the jet nebulization, the medication liquid in the nebulizer reservoir will be cooled down and its concentration will be condensed in the residue volume as a result of solvent evaporation, accompanying the aerosol output.

Figure 2.5 Schematic illustrations of a typical jet nebulizer (A) and ultrasonic nebulizer (B) (adapted from Yeo *et al.*, 2010)

2.2.2 Ultrasonic nebulizer

The ultrasonic nebulizer utilizes acoustic waves as the energy source, usually at a frequency of 2.4 MHz, for liquid nebulization. As displayed in Figure 2.5 (B), the ultrasonic nebulizer works with a transducer (piezoelectric crystal) producing periodic mechanical vibration to break the liquid into small droplets. When the oscillatory pressure is transferred to the liquid medium, an instant oscillation takes place in the fluid, which results in aerosol droplet generation owing to the interfacial capillary waves (a wave affected by surface tension moving between the boundary of two fluids). On the whole, a fountain of liquid is created at the surface of air-fluid interface. Similar to the jet nebulizer, there are baffles to break down droplets. Large droplets are recycled, falling back to the reservoir, while small droplets are ready for inhalation.

Generally speaking, ultrasonic nebulizers have a higher output of aerosol compared with jet nebulizers, which shortens the treatment time (Gessler *et al.*, 2001). Since the ultrasonic nebulizer utilizes a silent ultrasonic vibration, instead of relatively bulky and noisy compressors

to generate aerosols, it is quieter more portable than the jet nebulizer. It only works with the aqueous liquid with low viscosity. Viscous liquid is not appropriate for ultrasonic nebulization as high viscosity is a barrier for aerosol formation (McCallion *et al.*, 1995). It is also unable to aerosolize suspension. Because the solution in the reservoir will be heated up by the piezoelectric crystal, the ultrasonic nebulizer cannot deal with heat-broken medication, particularly for proteins (Muchão & Filho, 2010).

2.2.3 Mesh nebulizer

The vibrating-mesh nebulizer has become commercially available recently. It employs a sophisticated plate with tiny holes for vibration to generate aerosols. According to the types of vibration push, the vibrating-mesh nebulizer can be categorized into “active” or “passive”.

Schematic diagrams of the meshes of Aeronex Pro Nebulizer, an active mesh nebulizer, and Omron Micro Air NE-U22, a passive mesh nebulizer are shown in Figure 2.6 and 2.7. A micro pump system is employed in the active mesh nebulizer to force the liquid to pass through the mesh plates with tiny holes. The mesh, a combination of the aperture plate and piezoceramic vibrational element, moves upward and downward to generate aerosol. The passive mesh nebulizer uses a piezoelectric crystal attached to a horn transducer that induces mesh vibrations to produce aerosols.

Figure 2.6 Schematic diagrams for Aeroneb Pro Nebulizer (adapted from Ghazanfari *et al.*, 2007)

Figure 2.7 Schematic diagrams for OMRON MICROAIR NE-U22 (adapted from Ghazanfari *et al.*, 2007)

The mesh nebulizer can overcome a lot of drawbacks of both jet nebulizers and ultrasonic nebulizers. High efficiency compared with other kinds of nebulizers, low residue volume, muted and easy operation, light weight and short treatment time are the advantages of mesh nebulizers. They can also optimize the particle sizes for different medications by adjusting the output rate of nebulizer or the sizes of the reservoir and chamber. In spite of the advantages mentioned above, there are still some obstacles associated with mesh nebulizers. First of all, the price of high

output is the potential of overdose. Since the mesh nebulizers have at least double of the output than other nebulizers, the dosage of medication needs to be altered to avoid overdose (Ari *et al.*, 2010). This requires additional monitoring of the side effects when providing the treatment. Moreover, mesh nebulizers have inability with suspensions or liquid which will be crystallized after drying owing to the porous design of mesh. Cleaning the mesh nebulizer is another challenge for the general application. Last but not least, mesh nebulizers are unaffordable to some patients because they are usually much more expensive than jet nebulizers and ultrasonic nebulizers.

2.3 Techniques of Measuring Particle Size Distribution

Measuring the particle size distribution (PSD) is the main focus of *in vitro* studies of pharmaceutical aerosol deposition. As discussed before, aerodynamic diameter is a key parameter for inhaled aerosols that will affect the medication deposition in the respiratory tract. There are different techniques to measure particle size in the pharmaceutical field, for instance, inertia techniques using cascade impactors, laser diffraction, and particle time of flight (TOF).

2.3.1 Impactor

Cascade impactors (Illustrated in Figure 2.9 and 2.10) with multiple stages are commonly used in the inertia techniques. It separates an aerosol sample on the basis of impaction inertia, which is related to the velocity and the aerodynamic particle size. The flow rate is controlled by a vacuum pump and normally stays steady throughout the duration of the measurement.

At the entrance of the cascade impactor, there is an inverted L-shaped induction port, bending the air flow and mimicking the mouth-throat region. In the Rudolf model (Rudolf, Köbrich; Stahlhofen, 1990), the possibility of impactional deposition (P_i) in mouth-throat region can be expressed in empirical equation:

$$P_i = 1 - \frac{1}{1.1 \times 10^{-4}(d_{ae}Q^{0.6}V_t^{-0.2})^{1.4} + 1} \quad (2.1)$$

Where d_{ae} is the aerodynamic diameter of aerosol particles, Q is the flow rate of inhalation and V_t is the tidal volume. The equation (2.1) demonstrates that larger particles will have more chance to deposit in the mouth-throat region, as the flow rate remains constant in the same cascade impaction test. In this elbow piece, large particles mostly deposit inside the wall because of inertial impaction. They will leave the streamline rather than going into the cascade impactor. Therefore, the induction port can filter out the large particles that normally do not enter the respiratory tract.

The cascade impactor consists of a series of stages with different size and number of nozzles on them, corresponding to different cut-off diameters of each stage associates with the flow rate and the size of the nozzles. It is usually assembled sequentially from large to small nozzle sizes.

Figure 2.8 shows how cascade impactor classifies aerosol particles according to their size. After the aerosol particles pass through the nozzles, they either remain entrained in the air stream and escape the impingement on the collective plates through a specific angle by following the curved streamlines, or deviated from the flow line, thus impacting on the collective plate. The driving force behind this phenomenon is the inertia. Those particles with adequate inertia will be collected on the plates while the rest with insufficient inertia will continue travelling to the next stage until gaining enough inertia to impact on the plates. Hence, particle within a particular size

range will be captured and those below the size range will pass through. The collection of increasingly small particles comes with the decrease of nozzle size or increase in flow velocity.

Figure 2.8 Mechanism of particle deposition in ACI (adapted from USP 29-NF 24, General Chapters #601)

After the medication is collected on the collection plates, the deposition on each plate can be determined by chemical or biochemical assay, typically by high pressure liquid chromatography or ultraviolet-visible spectroscopy. Analyzing the amount of deposition through each stage, for example, using suitable solvent to wash away the contents on each collection plate for assays, can demonstrate the PSD. The MMAD and GSD of the PSD can be estimated by plotting the cumulative percentage of mass of medications less than a stated aerodynamic diameter versus the aerodynamic diameter in logarithmic scale, which is stated in Sub-Section 1.3.4. Therefore, the

results from a cascade impactor can be compared amongst different types of medications. The introduction of chemical assay contributes to time consuming procedures in the measurement of PSD by cascade impactor, generally speaking, merely one batch completed within a day.

The cascade impactor captures the entire dosage of a treatment rather than part of or only a snapshot of the dosage in the laser diffraction or time-of-flight measurements, which help to completely characterize the particle size distribution in the test. The recommended total mass of deposition will be between 75 to 125% of the minimum dosage of the inhalation device requirements.

The cascade impactor is usually made out of a metal material such as aluminum, 316 stainless steel or titanium. The application of metal materials can avoid the unwanted electrostatics effects inside the cascade impactor with promising service life, long-term accuracy and high cost effectiveness balance.

Nowadays, the most widely used cascade impactors are: 1) Andersen Cascade Impactors (ACI), and 2) Next Generation Pharmaceutical Impactor (NGI). Both of them are recommended by the USP (United States Pharmacopoeia) in the analysis of particle size distribution. The former one is a vertical standing instrument, imitating different generations in the lung geometry whilst the latter one is a horizontal standing.

ACI is considered to be the first commonly used cascade impactor in the pharmaceutical industry since the 1990s. A total of 8 individual stages provides accurate particle size distribution between 0.4 to 9 microns under 3 standard flow rates, 28.3 L/min, 60 L/min and 90 L/min ($\pm 5\%$), in terms of different types of inhaled devices.

When assembling different models of the ACI, the choice of stages and collection plates whether there is a central hole or not, is in accordance with the flow rate. The size of the nozzles on each

stage and the operation flow rate are important factors influencing the deposition of particles with different aerodynamic diameter. The cut-off diameters of different stages will be changed and can be calibrated by an empirical equation. The manufactured nozzle diameters and calibrated cut-off diameters of each stage under different standard flow rates for the ACI is shown in Table 2.2.

Table 2.2 Manufacture diameters and cut-off diameters of ACI

Manufacturing Specification Diameters/ mm				Cut-off Diameters/ μm		
Stage	Nozzles #	Nominal Value	Tolerance (TOL)	28.3 L/min	60 L/min	90 L/min
Stage -2	95	5.50	0.025	N/A	N/A	8.0
Stage -1	96	4.50	0.025	N/A	8.6	6.5
Stage -0	96	2.55	0.025	N/A	6.5	5.3
Stage 0	96	2.55	0.025	9.0	N/A	N/A
Stage 1	96	1.89	0.025	5.8	4.4	3.5
Stage 2	400	0.914	0.0127	4.7	3.2	2.6
Stage 3	400	0.711	0.0127	3.3	1.9	1.7
Stage 4	400	0.533	0.0127	2.1	1.2	1.0
Stage 5	400	0.343	0.0127	1.1	0.55	0.22
Stage 6	400	0.254	0.0127	0.7	0.26	N/A
Stage 7	201	0.254	0.0127	0.4	N/A	N/A

Retrieved from Copley, 2015; Ph. Eur. Chapter 2.9.18; USP 29-NF 24, General Chapters #601, and Nichols, Mitchell, Shelton & Roberts, 2013

Once the assembled stack is in position, the clamping action by stretching coil clamps of the base stage onto the entrance attached together with the silicon O-rings on each stage can easily reach the sealing requirement and prevent gas leakage during the test.

ACI is a well-established instrument and serve the industry for longevity, but it still suffers from the criticism of incapability of automation as well as the complicated assembling procedures when the need of different flow rates occurs. In order to solve these problems, the NGI, first launched in 2000 and subsequently accepted into the Pharmacopoeia in 2005, is designed for easy automation with excellent stage efficiency, accuracy and reproducibility. It covers a wider particle size range from 0.24 to 11.7 microns under flow rate of 15 L/min, 30 L/min, 60 L/min and 100 L/min ($\pm 5\%$) with the same series of stages.

There are three main components in the NGI (shown in Figure 2.9 & 2.10), the cup tray capturing medication deposition, the bottom frame supporting the cup tray and the lid holding removable nozzles. The seal body is part of the lid. Similar to the ACI, all of the components are clamped tightly above a tear-shaped cup into a single seal body and the silicon O-rings are also used in the NGI.

Figure 2.9 Layout of interstage passageways of NGI (adapted from USP 29-NF 24, General Chapters #601)

Figure 2.10 Component of NGI (adapted from Copley, 2015)

Various flow rates share the same series of the removable nozzles and cup trays. The cup-off diameters of each cup tray are influenced by the operation flow rate. The manufactured nozzle diameters and calibrated cut-off diameters of each stage under different standard flow rates for the NGI are shown in Table 2.3. Figure 2.11 shows the configuration of the manufacture nozzles.

Table 2.3 Manufacture diameters and cut-off diameters of NGI

Stage	Nozzles #	Manufacturing Specification Diameters/ mm		Cut-off Diameters/ μm			
		Nominal Value	Tolerance (TOL)	15 L/min	30 L/min	60 L/min	100 L/min
Stage 1	1	14.30	0.05	14.10	11.72	8.06	6.12
Stage 2	6	4.880	0.04	8.61	6.40	4.46	3.42
Stage 3	24	2.185	0.02	5.39	3.99	2.82	2.18
Stage 4	52	1.207	0.01	3.30	2.30	1.66	1.31
Stage 5	152	0.608	0.01	2.08	1.36	0.94	0.72
Stage 6	396	0.323	0.01	1.36	0.83	0.55	0.40
Stage 7	630	0.206	0.01	0.98	0.54	0.34	0.24
MOC	4032	≈ 0.070	-	0.70	0.36	0.14	0.07

Retrieved from Copley, 2015; Ph. Eur. Chapter 2.9.18 and USP 29-NF 24, General Chapters #601

Figure 2.11 Nozzle configuration of NGI (adapted from Copley, 2015)

2.3.2 Laser diffraction analyzer

Laser Diffraction Analyzer adopts a wide dynamic range of rapid detection with instant feedback from submicron to millimeter without the necessity of calibration. Laser diffraction measures the PSD from 0.02 to 2000 microns by measuring the angular variation in intensity of light scattered, also called diffraction, as a laser beam passes through particles samples dispersed in either air or liquid media. The particles must be well dispersed in a homogeneous stream when laser passes through the dispersion medium. There are two light sources with different wavelength in the laser beam, blue laser measuring small particles and red laser detecting large particles. Large particles scatter light at small angles relative to the laser beam while small particles scatter light at large angles, as illustrated in Figure 2.12. Signal of angular scattering intensity from the particles are thus captured by the detectors and transformed into particle size, using the light scattering to calculate the particle size distribution. The particle size is reported as a volume equivalent sphere diameter with high reproducibility (Eshel *et al.*, 2004).

Figure 2.12 Models for determination of particle size distribution

Some limitations for laser diffraction in particle size measurements still need to be overcome. Firstly, it cannot deal with suspensions as the sample needs to be evenly dispersed in the media. Secondly, the laser diffraction analyzer detects the volume equivalent spherical diameter of the particle rather than the mass equivalent diameter. The assumption that both of them are equal to each other is based on the constant density of the particles. If part of the particles has high density, sedimentation probably turns up which results in that the whole sample will not be measured. Thirdly, the laser diffraction analyzer only measures part of the aerosol cloud instead of the whole dosage. Whether the results can be representative for the product remain unknown. Lastly, optical detection is unable to distinguish the active pharmaceutical ingredients in the aerosols, which is a drawback in the PSD determination of mixed medications.

In contrast to the cascade impactor, laser diffraction analyzer can handle hundreds of measurements per day. It is also a well-established technique which is covered by ISO 13320

(ISO, 2009). Apart from providing useful data for fast measurement of aerosol particle size, it also provides other dynamic data like the velocity of the particles and the number of the detected particles.

2.3.3 Time-of-flight analyzer

Most of the time-of-flight analyzer uses a single-particle-counting techniques to determine aerodynamic particle size through time-of-flight and time-in-beam analysis. Figure 2.13 shows the laser beams and detectors in the time-of-flight analyzer. When particles of various mass are introduced into an expanding gas jet, they will undergo different amounts of acceleration. Based on this principle, aerodynamic time-of-flight (TOF) particle size measurement utilizes two overlapping laser beams to measure the particle velocity in the acceleration air stream created by an acceleration nozzle. Particles will accelerate in the air stream according to their size since small particles accelerate more quickly than large particles with greater inertia (Fields, 2002). Particles passing through the first laser diode lead to scattered light which is used to detect the presence of particles and activate a timer circuit. As soon as particles reach the second laser diode, the timer circuit stops so the time of flight of each particle is recorded. As the time of flight is correlational to the mass of the particle, supposing the particles are spherical with constant density, the size of particle can be directly converted from the time of flight by a pre-set calibration table and particle density known in advance.

Figure 2.13 Schematic for laser beams and detectors for time-of-flight particle size analyzer (adapted from Mitchell, 1999, CPS, n.d., and TSI, 2004)

The aerodynamic time-of-flight particle size measurement supplies number-weighting aerodynamic particle size distribution for aerosols. With the help of software such as the Particle Instrument Manager (PIM), the number-weighting particle size distribution can be converted into mass-weighting particle size distribution. The aerodynamic time-of-flight particle size analyzer is capable for measurement of both geometric diameter and aerodynamic diameter. It offers fast analysis (usually within a minute) with relatively high resolution and sensitivity for a wide dynamic scale, from approximately 0.2 to 1000 microns. In addition, it requires no sample preparation and small sample amount, which makes the operation simple. It can work with aerosols from dry powders and solution.

However, alike the laser diffraction technique, the aerodynamic time-of-flight particle size analysis needs to overcome the limitation of the absence of chemical assay detecting the chemical composition in the aerosols generated from mixed medications. This problem can be solved by coupling with other analytical chemistry instrument such as mass spectrometers. It is incapable with measuring liquid suspensions of particles or particles less than 0.2 micron. After all, such technique is ideally suitable for research application as well as pharmaceutical industry (CPS, n.d.).

Despite the fact that various techniques of particle sizing determination are available, the impactor approach is the only one to receive regulatory approval by the US and European Pharmacopeias during the medication development cycle and quality control. Newly developed pharmaceutical aerosols products need to be detected via cascade impactor during an approval process. Thus, the impactor approach is still one of the most widely used techniques in the measurement of particle size distributions. Although an increasing number of research show that other measurement may be superior to the impaction approach for convenience and speed, comparing the particle size distribution from other alternative techniques with the impaction will be important for correlation. Laser diffraction techniques and time-of-flight approach can provide rapid measurement as supplemental alternatives for the impaction approach.

2.4 Research gap

The majority of research on the evaluation of nebulizer performance is conducted by measurement with conventional asthma medication or saline solution. Under these circumstances, researchers have found out a few factors affecting the particle size of aerosols from nebulizers. Factors such as the design of jet nebulizer (Hess *et al.*, 1996), the flow rate of

jet air from compressor (Ari *et al.*, 2010) and the total running time of compressors and nebulizers (Awad, Willians & Berlinski, 2014) will affect the medication performance. The particle size distribution of aerosols generated by various jet nebulizers could have significant influence by different detection methods (Song *et al.*, 2015; Ziegler & Wachtel, 2005).

O’Callaghan *et al.* researched the effects of the aerosolization efficiency and particle size distributions of salbutamol by switching the carrier gas from air into heliox (mixture of helium and oxygen). They used the percentage of particles that are smaller than 5 microns as the criteria to evaluate the medication performance of a nebulizer. They found that by changing the carrier gas into a lighter gas, the percentage increased (O’Callaghan *et al.*, 2007). However, most of the researchers concentrated on the mechanical factors of a nebulizer as well as focused on the medication properties in the nebulizer. Within the current literature survey, there seems to be a lack of information in the literature regarding the main factors influencing the PSD of Zn-DTPA aerosol. This study is trying to measure how properties of medication can affect the PSD of Zn-DTPA aerosols generated from two nebulizers.

3 Chapter: Methodology

Two techniques of determining the particle size distribution of Zn-DTPA aerosols generated from two types of nebulizers, a jet nebulizer as well as an ultrasonic nebulizer were performed using the TSI Particle Size Distribution Analyzer 3603 (PSD 3603) and Copley Andersen Cascade Impactor (ACI). In this chapter, both devices used in the particle size distribution (PSD) experiments are explained.

3.1 Experimental description

Experiments were performed to provide the relationships between the PSD and some of the parameters in the Zn-DTPA aerosolization, such as the concentration and filling volume of the Zn-DTPA solution, as well as temperature and humidity in the measurement. The particle size distribution of Zn-DTPA aerosols were described by the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). The main objectives of the experiments were to understand how these factors would affect the particle size distribution. From these investigations, it was possible to establish a proper method to deliver Zn-DTPA into lungs to treat internal radioactive contamination, with the intent of providing valuable information for clinical treatment.

3.1.1 Chemicals

DTPA ($C_{14}H_{23}N_3O_{10}$, Fluka, Sigma-Aldrich Corporation, St Louis, MO), zinc acetate ($Zn(CH_3COO)_2 \cdot 2H_2O$, Fisher Scientific, Ottawa, ON) or zinc chloride ($ZnCl_2$, Sigma-Aldrich

Corporation, St Louis, MO) and sodium hydroxide (NaOH) are weighted in different amounts in order to synthesize the Zn-DTPA solutions for experiments.

3.1.2 Equipment

The equipment and chemicals used in the experiments are listed in the following section. The theory and description are also explained.

(1) Nebulizers

Two types of nebulizers were used in the experimentation for comparison of the particle size distribution of Zn-DTPA aerosols generated from commercially available nebulizers. A jet nebulizer (PARI LC® Star Reusable Nebulizer) and an ultrasonic nebulizer (MagicFly MF001) were applied (shown in Figure 3.1). The compressed air for jet nebulizer was supplied by the PARI Proneb Ultra Compressor (PARI GmbH, Stamberg, Germany). The theory of how nebulizers convert liquids into aerosols or small droplets has been discussed in Sub-Section 2.2.1.

Figure 3.1 PARI LC® Star Reusable Nebulizer (left) and MagicFly MF001 Ultrasonic Nebulizer (right)

Figure 3.2 PARI Proneb Ultra Compressor

The output flow rate for the PARI Proneb Ultra Compressor (shown in Figure 3.2) was about 10 L/min and the output for the PARI LC® Star Reusable Nebulizer (PARI GmbH, Stamberg, Germany) is approximately 7.5 L/min. The filling volume in the nebulizer cup should be between 2 to 8 mL in accordance with the instruction manual.

For the MagicFly MF001 Ultrasonic Nebulizer (MagicFly, Los Angeles, CA), two working modes with different output flow rate can be switched by pressing the Mode button. The first mode with a larger output is high power with an output flow rate of 0.5 L/min and an automatic timer of 10 min, while the second mode with a smaller output is low power with an output flow rate of 0.35 L/min and an automatic timer of 20 minutes. The maximum filling volume in the vessel is 10 mL and only water or water-based liquid can be added into the vessel.

(2) Equipment for characterization

(a) Ultraviolet-visible spectroscopy system

The Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA) was used to measure the absorbance. It is a single beam spectrophotometer with wavelength from 190 to 1100 nm and two types of lamps are used to achieve the wide coverage of wavelength. A deuterium arc lamp covering the UV range from 190 to 800 nm and a tungsten lamp emitting light over 370 to 1100 nm wavelength range is used as the light sources. Zhao *et al.* and Dong *et al.* found that the UV absorption of Zn-DTPA can be detected in 200 nm (Zhao *et al.*, 2001 and Dong *et al.*, 2013), which means the UV-visible spectrophotometer can be used to determine the deposition of Zn-DTPA in the cascade impactor. The optical system for the Agilent 8453 spectrophotometer is shown in Figure 3.3. A collimated light passes through the source lens, which minimizes the light dispersion, and then it is directed towards the sample (8 cuvette compartment, allowing 8 samples). A stray-light correction filter with half blocking at 420 nm will filter out the negative effects of stray light for short wavelengths lower than 190 nm. For the reading below 400 nm, stray light is the only light used in the measurement so the intensity of stray-light is subtracted from the intensity spectrum over the whole wavelength range from 190 - 1100 nm to provide a stray-light corrected spectrum. The bias limit of the spectrophotometer is 2%.

Figure 3.3 Optical system of Agilent 8453 Spectrophotometer (adapted from Agilent, 2000)

After passing through the samples, the light beam is refocused and collimated by the spectrograph lens before reaching the photo diode array detector. Located at the focus of the spectrograph lens, the slit which is a narrow aperture in a plate, with the exact size of one of the photodiodes in the array, ensures that only the proper photodiode is projected onto by the band of wavelengths. A concave holographic grating that disperses the light onto the diode array at an angle linearly proportional to wavelength is used to combine the dispersion and spectral imaging. The light finally arrives at the photo diode array detector after passing through the gating. The detector consists of 1024 separated photodiodes controlling circuits etched onto a semiconductor chip, which gives the reading of the intensity of the light. The sampling interval is 0.9 nm for the photo diode array detector.

The spectrum of different chemicals is determined by its composition. Functional group in the chemicals will absorb certain wavelength of light and the absorbance is proportional to the amount in the solution in terms of the Beer-Lambert law. By comparing the intensity of light before and after it passes through the sample, the absorbance in the samples can be calculated.

The Beer-Lambert law is used to build up the calibration curve, showing the relationship between the absorbance and the concentration of sample. At the point of the maximum absorption wavelength, the sensitivity and accuracy for the measurement is the greatest and meanwhile the impact from impurities in the sample can be largely minimized.

The Beer-Lambert law describe the linear relationship between concentration of a solution and absorbance in the same path length. Generally, the law is written as:

$$Abs = \epsilon bc \quad (3.1)$$

Where Abs is the absorbance, ε is the molar absorptivity coefficient, dependent on wavelength (λ) with units of $L \cdot mol^{-1} cm^{-1}$, b is the path length with units of cm and c is the analyte concentration with units of $mol \cdot L^{-1}$.

In modern absorption instruments, the usual experimental measurements are transmittance (T), defining as the ratio of initial light intensity (I_0) and light intensity after passing through the sample (I). The relationship between absorbance and transmittance is defined as:

$$Abs = -\log T = -\log \frac{I}{I_0} \quad (3.2)$$

Samples are normally tested in the 1 cm thick quartz cuvette so the absorbance is linearly proportional to the concentration. For an unknown solution, the concentration of analyte can be determined by the amount of light intensity before and after passing through the sample and applying the Beer-Lambert law. As long as the analyte and the measuring wavelength are unchanged, the coefficient will remain the same. If the absorptivity coefficient is unknown, the sample concentrations can be determined by a calibration curve of concentrations of standard solutions versus their absorbance.

(b) Infrared spectroscopy system

Infrared Spectroscopy (IR Spectroscopy) is a useful tool specialized in structure determination and verification for compounds involving the electromagnetic (EM) radiation in the infrared region with the frequencies from 14,000 to 10 cm^{-1} in wavenumber. The fact that the vibrations of chemical bonds, especially for the covalent bonds in organic compounds, absorb certain EM radiation, lead to the structure characterization theory by IR spectroscopy.

The frequency of vibrations is relevant to particular motion modes and bond type. Usually only dipole molecules are IR active because the symmetrical vibrations hardly absorb IR radiation.

Chemical bonds in different environments will absorb IR radiation with varying wavenumbers and varying intensities. The IR spectrum presents the absorption information, the intensities associated with the amount of chemical bonds by the Beer-Lambert Law and the wavenumbers where the absorptions of IR radiation can be correlated directly to specific chemical bonds. Thus, IR techniques can be applied in both qualitative and quantitative research.

The wavenumber of absorbed IR radiation from the chemical bond is determined by the structure of the bond:

$$\nu = \frac{1}{2\pi c} \sqrt{\frac{k}{\mu}} \quad (3.3)$$

Where c is the light speed, k is the spring constant for the bond, and μ is the reduced mass of the A-B system:

$$\mu = \frac{m_A m_B}{m_A + m_B}. \quad (3.4)$$

Here m_i is the mass of atom i .

Fourier transform infrared spectrometer (FT-IR) is one of the most frequently used laboratory instruments. It measures the percentage of infrared light transmission through the samples and the raw data is preceded by Fourier transform techniques. The Fourier transformation improves the quality of infrared spectra and minimizes the time required to obtain data (Stuart, 2004). The infrared light output in the spectrum is in a form of wavenumber, which is equivalent to the infrared wavelength. The spectrums of the sample are comparable to reference spectrums.

There are various preparation techniques for solid samples. One common method is mixing and grinding certain amount of sample with a special fine purified salt, usually potassium bromide (KBr), and then pressing the powder mixture in a mechanical press to develop a translucent pellet where the infrared beam can pass.

The ABB Bomem MB 100 Fourier Transform Infrared Lab Analyzer (FTLA) was utilized to measure the IR radiations of Zn-DTPA and DTPA with a spectral range from 4000 to 600 cm^{-1} and the IR spectra were collected by the GRAMS software. The IR beam of this instrument is within mid-infrared region from 6500 to 350 cm^{-1} . The translucent pellet is inserted on the Arid-Zone sample station between the IR beam and detector. (ABB, 2002)

(3) Equipment for PSD measurement

(a) Andersen Cascade Impactor

A Copley Andersen Cascade Impactor (Copley Scientific Limited, Nottingham, UK) was used in the experiments to measure the PSD of Zn-DTPA aerosols produced by two types of nebulizers, which is shown in Figure 3.4. The theory of cascade impactor has been described in detailed in Sub-Section 2.3.1.

Figure 3.4 Andersen Cascade Impactor

(b) Particle size distribution analyzer

The theory of the TSI Particle Size Distribution Analyzer Model 3603 (TSI Incorporated, Shoreview, MN) has been described in Sub-Section 2.3.3. In this analyzer, small particles (below 20 microns) accelerate quickly and produce a time-of-flight signal which has two peaks. Medium sized particles (between 20 and 100 microns) produce a less defined time-of-flight signal so are analyzed using both time-of-flight and time-in-beam measurements. Large particles (above 100 microns) accelerate slowly and are large enough to span the distance between the beams and as such can only be analyzed using time-in-beam measurements. Picture of the TSI Particle Size Distribution Analyzer Model 3603 (PSD 3603) is shown in Figure 3.5.

Figure 3.5 TSI Particle Size Distribution Analyzer Model 3603

The PSD 3603 is an integrated system for particle size measurement which provides fast, reliable results, and the bias limit of the measurement is 2%. The PSD 3603 works with the Particle Instrument Manager (PIM) software that provides an intuitive user interface and allows for efficient data analysis and regulatory compliance. When data collection is started, the run properties dialog box opens and different windows of Size Graph, Size Table, Cumulative Table

and Statistics Table are available to show particle statistics, including the size range, aerodynamic mean value and geometric standard deviation. By monitoring the size graph, cumulative table and statistics table, the MMAD of specific aerosol particles can be easily calculated.

(c) The mass flow meter

A Brooks Mass Flow Meter ® 5863S (Brooks Instrument, LLC., Hatfield, PA) was utilized in the experiments to control the flow rate. With an thermal sensitive sensor, the flow meter can interpret the temperature change that takes place when a gas flows over a heated element (8 °C), producing an electrical signal that is recorded by the National Instruments™ Data Acquisition USB-6009 Device (National Instruments Corporation, Austin, TX). The detect limit for flow rate can be up to 1000 L/min and the output electrical signal can vary between 0-5 V (DC). The bias error of the flow meter is 0.2% and the accuracy of flow rate is $\pm 0.7\%$ under the circumstance of factory set calibrations. The maximum flow rate of this mass flow meter is 150 SLPM referring to the standard liters per minute of air at 0 °C and 1 atm. Ahead of using the flow meter, the factory calibration parameters were logged to LabView™, which is the program used to obtain the flow rate during each experiment, so that the voltage output can be related to the flow rate.

(d) Temperature and humidity sensor

A DHT22 temperature and humidity sensor was applied in the particle size distribution measurement with ACI so as to clearly monitor the temperature and humidity in the chamber boxes. The DHT22 is a reliable and stable device outputting calibrated digital signal by digital signal collecting techniques and humidity sensing technology. The temperature reading is

compensated and calibrated in an accurate calibration chamber. The relatively humidity is calculated based on the temperature reading, which can be defined in the program. When the sensor is detecting, the calibration coefficients saved in the OTP memory will be retrieved from the memory. The circuit diagram for wiring of the sensor is displayed in Figure 3.6. Technical Specification is shown in Table 3.1 and the code for Arduino can be found in Appendix C.

Figure 3.6 Circuit diagram for wiring of DHT22

Table 3.1 Technical specification of DHT22

Model	DHT22
Power Supply	3.3-5.5 V DC
Output signal	Digital signal via 1-wire bus
Sensing element	Polymer humidity capacitor
Operating range	Humidity: 0~100% RH; temperature: -40~80°C
Accuracy	Humidity: $\pm 2\%$ RH (Max $\pm 5\%$ RH); temperature: $\pm 0.5^\circ\text{C}$
Resolution or sensitivity	Humidity: 0.1% RH; temperature: 0.1°C
Repeatability	Humidity: $\pm 1\%$ RH; temperature: $\pm 0.2\%$ °C

(e) Vacuum pump

A Welch ® Dry Vacuum Pump Model 2585B (Welch-Illvac, Niles, IL) was used to adjust the flow rate in the experiments. The flow rate can be up to 200 L/min passing through the system, capable to handle the required flow rate for the ACI test and air refreshment in the chamber box.

(4) Scanning electron microscope

Scanning electron microscope (SEM) can provide high-resolution surface images of solid samples. The microscope scans samples with a focused beam of high-energy electrons, which interact with the atoms in the sample and produce a variety of signals about the morphology of sample's surface. Vega II XMU (Tescan, Czech) was used to study the morphology of Zn-DTPA aerosol particles.

3.2 Experimental apparatus

The PSD of Zn-DTPA aerosols from PARI LC Star nebulizer and MagicFly MF001 ultrasonic nebulizer were measured by PSD 3603 and ACI. The equipment was chosen and arranged in terms of the schematic graphs displayed below.

3.2.1 PSD measurement by PSD3603

In order to determine the PSD, a setup was adapted to accommodate the PSD 3603. A designed plastic chamber box diluted the aerosol to allow for more accurate measurements. The chamber box also reduced the amount of environmental contaminants which may alter experimental results and reduced the amount of Zn-DTPA exposure received by surrounding subjects. The chamber box was altered by adding a small outlet at the bottom that can connect tightly with the

inlet to the PSD 3603 so this inlet could be introduced into the isolated environment inside the chamber. In order to maintain the atmospheric balance of chamber box during the experiments, a Bacterial/Viral Respirgard II™ Filter (Vital Signs, Inc., Englewood, CO) was incorporated on the chamber box. A mouthpiece was attached to the nebulizer in order to direct the aerosol into the inlet of the distribution analyzer through the chamber box. The compressor was then attached to the nebulizer as the source of compressed air. The jet nebulizer was held vertically in place by a clamp to ensure consistent measurements. The jet nebulizer, chamber box and inlet of the particle size analyzer were assembled together as shown in the experimental apparatus schematic shown in Figure 3.7.

Figure 3.7 Jet nebulizer experimental setup

When it came to the measurement of ultrasonic nebulizer, the only modification was to add another hole to adjust the top cover of the MagicFly MF001 Ultrasonic Nebulizer (see Figure 3.8). Other parts of the set-up remained unchanged as the previous set-up with the jet nebulizer.

Figure 3.8 Ultrasonic nebulizer experimental setup

3.2.2 PSD measurement by ACI

Temperature and humidity are important factors in the PSD test with ACI. The ambient temperature and humidity increases the chance of evaporation of the droplets resulting in the shrinkage of aerosols particle size (Finlay & Stapleton, 1999, and Zhou *et al.*, 2005). Hence, when using the ACI to measure the PSD, some modifications to the original experimental apparatus were required.

In order to lower the temperature to 10 °C and raise the humidity to over 90%, the ACI would be chilled in a refrigerator overnight until the experiment began. During the experiment, the ACI was immersed in a mixture of ice and water. Another identical chamber box, approximately one fourth filled with ice-water mixture, was stacked upon the original one, also approximately one fourth filled with ice-water mixture, as a humidifier, which introduced humid flow into the chamber box connecting to the nebulizer. The two chamber boxes were connected by the Respirgard II Filter to make sure that the humid flow entering the bottom chamber box was

clean of ambient particles. Both chamber boxes were completely sealed by silicon glue and covered by 1 cm thick foams to preserve the low temperature. An additional MagicFly MF001 Ultrasonic Nebulizer was connected to the top chamber box and provides water spray to increase the humidity. The DHT22 sensor displaying the real-time temperature and humidity was attached inside the top chamber box.

The PARI Proneb Ultra Compressor was connected to the PARI LC Star nebulizer that was vertically held by a clamp to adapt a proper height making it easier to connect to the chamber box and ensuring consistent measurements. On the opposite side, induction port of the ACI is connected to the chamber box, administering the aerosol into the ACI, which was driven by the Welch Dry Vacuum Pump 2585B. There were a valve and mass flow meter between the ACI and the vacuum pump to control the flow rate in the whole system.

Figure 3.9 Jet nebulizer experimental setup with the Andersen Cascade Impactor

All the components for the tests were connected with each other as the schematic shown in Figure 3.9. Except for the vacuum pump, all of them were positioned in-line to develop a full flow across a workbench. Separating the vacuum pump and putting it on another workbench helped to reduce the interference of vibrations from the running vacuum pump and to protect the data acquisition devices. All the connections between the instruments and rubber tubes were wrapped around with Parafilm M® film (Bemis Company, Inc., Oshkosh, WI) and Teflon tubing tapes as well as secured by ring clamps in order to avoid gas leakage, which would affect the measured flow rate.

3.3 Experimental procedures

3.3.1 Preparation for Zn-DTPA solutions

(1) Zn-DTPA solutions for TSI particle size distribution analyzer tests

To make the Zn-DTPA solutions with different concentration, DTPA powders, zinc acetate crystals and sodium hydroxide were weighted according to the amount listed in the Table 3.1 with a RADWAG AS 82/220, R2 Analytical Balance (RADWAG Balances and Scales, Radom, Poland) and placed them into 10 mL volumetric flasks. Then some double distilled water was added and put into flasks in a Branson® Ultrasonic Bath 2510 (Branson Ultrasonics Corporation, Danbury, CT) for approximately 10 minutes until all DTPA powder and zinc acetate crystals were dissolved. After all the solids were dissolved, double distilled water was added into the flasks until the water surface reached the volume limit on the volumetric flasks. With the help of sodium hydroxide and ultrasound, 6 Zn-DTPA solutions were prepared with different concentration levels, 2.5 mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL, 30 mg/mL and 40 mg/mL according to Table 3.2.

Table 3.2 Solution preparation

Conc./ mg·mL ⁻¹	DTPA/ mg	Zinc acetate/ mg	Sodium hydroxide/ mg
2.5	18.82	10.50	9.57
5	37.63	21.00	19.13
10	75.25	41.99	38.26
20	150.51	83.99	76.53
30	225.76	125.98	114.79
40	301.02	167.98	153.05

(2) Zn-DTPA solutions for ACI tests

The acetate groups of zinc acetate have the same structure as the carboxylate group of Zn-DTPA, which is the only functional group to absorb UV light within the range of UV-visible wavelength for the Agilent 8435 Spectrophotometer. The acetate of zinc acetate interferes with the Zn-DTPA absorption test by the UV-visible spectrophotometer. Thus, zinc acetate was replaced with zinc chloride in the ACI experiment. The PSD of aerosols generated from the 40 mg/mL Zn-DTPA solution was measured. The required amount of zinc chloride for synthesizing Zn-DTPA was adopted to 104.32 mg according to its molecular weight. Other operations remained the same as those with zinc acetate.

3.3.2 PSD tests by PSD 3603

Prior to each experiment with the PSD 3603, a vacuum pump would be running for approximately 30 minutes to intake clean air through the filter to eliminate the internal contamination inside the chamber box which helps to lower the background. After that, instruments were assembled as the experimental apparatus. The prepared Zn-DTPA solutions were introduced into the nebulizer reservoir.

Before each measurement of the sample, a blank sample, referring to a batch without introducing any aerosols into the chamber box, was measured and the count per second of the blank sample was determined by the PSD 3603. This value was used to assess the cleanness of the chamber box. The batch would not start until the count per second was lower than 10.

For tests with the PARI LC Star nebulizer, 4 different filling volumes (2 mL, 4 mL, 6 mL and 8 mL) of 5 mg/mL Zn-DTPA solutions and 6 different concentration levels varied from 2.5 mg/mL to 40 mg/mL were tested. Once correctly positioned, the compressor was switched on and the aerosol was allowed to develop for a short amount of time. The distribution analyzer was set to detect the aerosol simultaneously and it allowed aerosol sampling for 30 seconds. The size distribution graphs were produced using the Particle Instrument Manager (PIM) Software package provided with the distribution analyzer. PSD was demonstrated under the numeric distribution weighting. The presenting size boundary was set between 0.1 to 10 microns as the function of induction port of the ACI. The mean, standard deviation of the particle size distribution obtained from the PIM software can be applied in the calculation of MMAD by equation (1.15). MMAD and GSD were used to evaluate the particle size distribution. A student's t-test is conducted to show the statistics significance between values. After each measurement, the chamber box was cleaned with soap and water and the air inside the chamber box was filtered by running the vacuum pump.

The operation for the MagicFly MF001 Ultrasonic Nebulizer was similar with the PARI LC Star nebulizer. Both of the high power mode (mode 1) and low power mode (mode 2) were tested separately for the 6 different concentration levels while in the aspect of filling volume, only 5 mL and 10 mL. Previous to start the experiment, water was filled into the water reservoir to the level of latex ring. The Zn-DTPA solution was added into the medication cup to no more than

the 10 mL mid line in the cup. The medication cup was placed into the reservoir and reassembled into the ultrasonic nebulizer by re-installing the rubber gasket and top cover. After replacing the nebulizer to the system, the transformer was plugged into the power supply. A LED green light indicated that the device was in standby condition. The light would turn red after press on the Mode button once, indicating the high power mode. After pressing an additional time, the flashing red and green light implied the low power mode. The particle size distribution of Zn-DTPA aerosols of both high and low power modes were tested in the experiments.

Each sample measurement would be repeated 5 times with the particle size analyzer for averaging and 3 Zn-DTPA solutions with the same concentration level would be prepared for separate experiments.

3.3.3 Characterization of Zn-DTPA

In order to perform the IR test, dry Zn-DTPA powder prepared with zinc chloride was needed. The powder of Zn-DTPA was collected from all the deposition plates in ACI and oven-dried overnight. Approximately 1.5 mg dry powder of Zn-DTPA was mixed with oven-dry KBr powder throughout in mortar with pestle, cleaned with acetone in advance. The powder mixture was pressed in the mechanical press to form the translucent pellet for tests. The operation for DTPA powder was identical to the Zn-DTPA powder. By comparing the structure between the product Zn-DTPA and the reactant DTPA, whether the DTPA is incorporated with the zinc ion can be confirmed.

3.3.4 PSD tests by ACI

(1) Assembling apparatus

All the ACI components were assembled in terms of manual. The 8 stages of the ACI based on the stage sequences. Deposition plates were inserted between different stages. At the stage F, the last stage above the base of ACI, a piece of Kimwipes (Kimberly-Clark Professional, Roswell, GA) was placed under the O-ring as a filter, to prevent that the aerosol particles would enter the flow meter. After ensuring that all inter-stage seals were correctly aligned to avoid gas leaks, all the stages were attached together with three spring clamps on the base of the ACI. The induction port was also attached to the ACI to achieve an airtight seal. Meanwhile, a rubber tube was used to connect the outlet nozzle of ACI to the flow meter with Teflon tapes and fastened ring clamps. The ACI was kept frozen in the refrigerator for at least 3 hours.

Ice and water mixtures were poured into both the top and bottom chamber boxes. All the connections between instruments and the chamber boxes were sealed with Parafilm and Teflon tapes to keep the gas tightness. After inserting the DHT22 sensor to the top chamber box and connecting the sensor to the desktop for temperature and humidity monitoring, the ultrasonic nebulizer was turned on to supply mist in order to accelerate the decrease of temperature and the increase of humidity in the chambers. Not until the temperature was lower than 10 °C and the humidity was higher than 90% was the ultrasonic nebulizer turned off and taken off from the system. The rubber tube connecting the top chamber box and ultrasonic nebulizer was sealed with Parafilm. Meanwhile, the PARI LC Star nebulizer was filled with 40 mg/mL Zn-DTPA solution that was previously prepared. The nebulizer and mouthpiece were joined together before connecting the compressor to the nebulizer. The nebulizer was held vertically with a clamp to a proper height to connect to the chamber box.

The assembled ACI was taken out from the refrigerator and connected its outlet with the mass flow meter with tube, which was wrapped with Teflon tape and ring clamp in the connection for

water proof and gas leakage prevention. After completing the connection, the assembled ACI was then immersed into a tank filled with ice and water. This procedure would operate within 5 minutes so as to retain the low temperature of the ACI.

Lastly, once all the components were ready, the compressor was switched on for 5 minutes to fill the bottom chamber box with Zn-DTPA aerosols. The vacuum pump was turned on and the valve was quickly adjusted to attain 60 L/min ($\pm 5\%$) flow rate, which was monitored by software on the desktop computer that was connected to the mass flow meter. The total running time for the vacuum pump was set to be 1 minute. A timer was used.

(2) Washing procedures

After finishing the tests with the ACI, the ACI was disassembled for analysis. All 8 deposition plates were placed in the plastic petri dishes individually, without touching each other. The deposition plates were washed separately with 20 mL distilled water, which volume was measured using a 10 mL transfer pipette.

(3) Deposition measurements

The absorbance of each washing solution from different deposition plates was measured by the Agilent 8435 UV-visible Spectrophotometer and read in the Agilent ChemStation software.

Distilled water was used as the blank sample for the absorption tests and it would be tested before each experiment for subtraction. Each sample solution would be measured three times with three quartz cuvettes and the average absorbance was used to calculate the deposition amount of Zn-DTPA. Before each measurement, a small amount of sample solution was filled into and rinsed over inside the whole quartz cuvette for three times to keep the concentration of

solution in the quartz cuvette to be the same as the sample solution. Quartz cuvettes were used to hold the solution during the absorbance measurement. Before and after use, each cuvette was rinsed three times with distilled water. The outer surface of cuvettes needed to be crystal clear to avoid interference for the reading so it was wiped and cleaned using Kimwipes and glasses cloth. Full scans were firstly performed to acquire the full scan spectrum of a Zn-DTPA solution to find out the maximum absorption wavelength within the range of UV-visible wavelength for the Agilent 8435 Spectrophotometer. A fixed wavelength mode at the maximum absorption wavelength for Zn-DTPA was used for all readings. All the UV absorbance of Zn-DTPA are measured under the maximum absorption wavelength, obtained from the full scans.

(4) Calibration

The standard solutions for the calibration curve in the UV-visible spectroscopy tests were synthesized by DTPA powder, zinc chloride and sodium hydroxide. 1 mmol/L Zn-DTPA solution was prepared in a 50 mL volumetric flask as the stock solution, which should contain 19.67 mg DTPA, 6.82 mg zinc chloride and 10 mg sodium hydroxide. 10 standard solutions were prepared by the diluting stock solution into 10 concentration levels. Certain volume of stock solution was transferred by adjustable pipette into volumetric flasks and diluted with distilled water into different proportions as the standard solutions. The dilute ratio is shown in Table 3.3. In advance to the particle size distribution test by the ACI, the UV absorbance of each standard solution was measured. The calibration curve was developed by plotting the concentration of each standard solution to its absorbance. Based on the curve, the linear range, relationship between the concentration and absorbance and the correlation coefficient could be obtained. By this curve, the amount of Zn-DTPA impacted on each plate can be determined.

Table 3.3 Dilute ratio for standard solutions

Standard. Solution Conc./ $\mu\text{mol}\cdot\text{L}^{-1}$	Dilute Ratio	Stock Solution Volume/ μL	Flask Volume/ mL
5	1/200	50	10
10	1/100	100	10
20	1/50	200	10
50	1/20	500	10
75	3/40	750	10
100	1/10	1000	10
150	3/20	1500	10
200	1/5	3000	15
250	1/4	2500	10
300	3/10	4500	15

(5) MMAD and GSD calculation

Based on the calibration curve and the absorbance of the washing solutions of each deposition plate, the mass fraction of the deposition on each stage can be determined. According to the calculation from USP 29 (U.S. Pharmacopeia), the MMAD and GSD can be calculated from the plot of the cumulative percentage of mass less than the cut-off aerodynamic diameter of each stage on a probability scale versus aerodynamic diameter on a logarithmic scale (shown in Figure 3.10). The MMAD and GSD calculation procedures were described in Sub-Section 1.3.4 and the MMAD and GSD can be calculated from the cumulative mass distribution by Matlab R2014b (MathWork®, Natick, MA).

$$GSD = \sqrt{d_{84.13}/d_{15.87}}$$

Figure 3.10 MMAD and GSD calculation from cumulative deposition fraction

(6) Cleaning procedures

After all the experiment were accomplished, all the stages, deposition plates, and petri dishes were washed with soap and warm water, as well as rinsed with distilled water three times. Parts were let to dry overnight.

(7) Error and uncertainty for deposition fraction

Errors of measurement are inevitable and they usually have multiple sources such as calibrations, data acquisition, data reduction and other external factors. Predicting the uncertainty of the

deposition fraction of each plate can help to determine the main source of errors from the experimental set-up design. The method used for the uncertainty analysis in this section is based on the American National Standards Institute/American Society of Mechanical Engineers (ANSI/ASME) Measurement Uncertainty Standard (1985).

There are two types of error sources, the bias errors, also called systematic errors, caused by the inability to give out true results from the measuring instruments and precision errors, also called random errors, from unexpected variation in the measurements. For one thing, the bias errors are constant for the duration of the test and they can never be eliminated. The total bias error is usually an accumulation of each bias error so it will be important to identify all the bias errors. For another, the precision errors can be observed in repeated tests. The standard deviation of repeated tests reflects the distribution of precision errors. The precision errors are reducible by using the average of multiple measurements instead of a single measurement.

(a) Bias errors

In real practice, it will be impossible to measure the actual bias error of any measuring instruments. The bias error for a particular instrument has to be estimated. The bias limit can be propagated by individual bias limit. The total bias limit is

$$B = \sqrt{\sum_{i=1}^i B_i^2} \quad (3.5)$$

Where i is the index for the bias limit and B is the bias limit for the instrument.

For the ACI, the nozzles on each stage affect the deposition fraction and the effective diameter varies from the theoretical diameter. The mean and standard deviations (STD) of nozzle diameter of each stage (shown in Table 3.4) can be obtained from the stage mensuration certificate from

the manufacture. All the diameters of nozzles are measured by Mitutoyo QV404 Pro Vision Inspection System.

Table 3.4 Stage mensuration for ACI

Stage	-1	-0	1	2	3	4	5	6
Mean/ mm	4.492	2.546	1.886	0.9115	0.7194	0.5342	0.3488	0.2539
STD	0.001	0.001	0.002	0.0006	0.0004	0.0006	0.0006	0.0005
B/ %	0.022	0.039	0.11	0.066	0.056	0.11	0.17	0.20

The deposition fraction (DF) of one stage is defined as:

$$DF = \frac{m_k}{m_T} \quad (3.6)$$

$$m_T = \sum_{k=-1}^k m_k \quad (3.7)$$

Where m_k is the deposition mass of each plate and m_T is the total deposition mass.

The bias limit for each stage can be defined as

$$\begin{aligned}
\left(\frac{B_{DF-1}}{DF_{-1}}\right)^2 &= \left(\frac{1}{DF} \frac{\partial DF}{\partial m_{-1}} B_{-1}\right)^2 + \left(\frac{1}{DF} \frac{\partial DF}{\partial m_{-0}} B_{-0}\right)^2 + \left(\frac{1}{DF} \frac{\partial DF}{\partial m_1} B_1\right)^2 + \left(\frac{1}{DF} \frac{\partial DF}{\partial m_2} B_2\right)^2 \\
&+ \left(\frac{1}{DF} \frac{\partial DF}{\partial m_3} B_3\right)^2 + \left(\frac{1}{DF} \frac{\partial DF}{\partial m_4} B_4\right)^2 + \left(\frac{1}{DF} \frac{\partial DF}{\partial m_5} B_5\right)^2 + \left(\frac{1}{DF} \frac{\partial DF}{\partial m_6} B_6\right)^2 \\
&= \left(\frac{m_T - m_{-1}}{m_T m_{-1}} B_{-1}\right)^2 + \left(-\frac{B_0}{m_T}\right)^2 + \left(-\frac{B_1}{m_T}\right)^2 + \left(-\frac{B_2}{m_T}\right)^2 + \left(-\frac{B_3}{m_T}\right)^2 \\
&+ \left(-\frac{B_4}{m_T}\right)^2 + \left(-\frac{B_5}{m_T}\right)^2 + \left(-\frac{B_6}{m_T}\right)^2 \quad (3.8)
\end{aligned}$$

$$\begin{aligned}
\left(\frac{B_{DF-0}}{DF_{-0}}\right)^2 &= \left(\frac{m_T - m_{-0}}{m_T m_{-0}} B_0\right)^2 + \left(-\frac{B_{-1}}{m_T}\right)^2 + \left(-\frac{B_1}{m_T}\right)^2 + \left(-\frac{B_2}{m_T}\right)^2 + \left(-\frac{B_3}{m_T}\right)^2 + \left(-\frac{B_4}{m_T}\right)^2 \\
&+ \left(-\frac{B_5}{m_T}\right)^2 + \left(-\frac{B_6}{m_T}\right)^2 \quad (3.9)
\end{aligned}$$

$$\begin{aligned} \left(\frac{B_{DF_1}}{DF_1}\right)^2 &= \left(\frac{m_T - m_1}{m_T m_1} B_1\right)^2 + \left(-\frac{B_{-1}}{m_T}\right)^2 + \left(-\frac{B_{-0}}{m_T}\right)^2 + \left(-\frac{B_2}{m_T}\right)^2 + \left(-\frac{B_3}{m_T}\right)^2 + \left(-\frac{B_4}{m_T}\right)^2 \\ &\quad + \left(-\frac{B_5}{m_T}\right)^2 + \left(-\frac{B_6}{m_T}\right)^2 \end{aligned} \quad (3.10)$$

$$\begin{aligned} \left(\frac{B_{DF_2}}{DF_1}\right)^2 &= \left(\frac{m_T - m_2}{m_T m_2} B_2\right)^2 + \left(-\frac{B_{-1}}{m_T}\right)^2 + \left(-\frac{B_{-0}}{m_T}\right)^2 + \left(-\frac{B_1}{m_T}\right)^2 + \left(-\frac{B_3}{m_T}\right)^2 + \left(-\frac{B_4}{m_T}\right)^2 \\ &\quad + \left(-\frac{B_5}{m_T}\right)^2 + \left(-\frac{B_6}{m_T}\right)^2 \end{aligned} \quad (3.11)$$

$$\begin{aligned} \left(\frac{B_{DF_3}}{DF_3}\right)^2 &= \left(\frac{m_T - m_3}{m_T m_3} B_3\right)^2 + \left(-\frac{B_{-1}}{m_T}\right)^2 + \left(-\frac{B_{-0}}{m_T}\right)^2 + \left(-\frac{B_1}{m_T}\right)^2 + \left(-\frac{B_2}{m_T}\right)^2 + \left(-\frac{B_4}{m_T}\right)^2 \\ &\quad + \left(-\frac{B_5}{m_T}\right)^2 + \left(-\frac{B_6}{m_T}\right)^2 \end{aligned} \quad (3.12)$$

$$\begin{aligned} \left(\frac{B_{DF_4}}{DF_4}\right)^2 &= \left(\frac{m_T - m_4}{m_T m_4} B_4\right)^2 + \left(-\frac{B_{-1}}{m_T}\right)^2 + \left(-\frac{B_{-0}}{m_T}\right)^2 + \left(-\frac{B_1}{m_T}\right)^2 + \left(-\frac{B_2}{m_T}\right)^2 + \left(-\frac{B_3}{m_T}\right)^2 \\ &\quad + \left(-\frac{B_5}{m_T}\right)^2 + \left(-\frac{B_6}{m_T}\right)^2 \end{aligned} \quad (3.13)$$

$$\begin{aligned} \left(\frac{B_{DF_5}}{DF_5}\right)^2 &= \left(\frac{m_T - m_5}{m_T m_5} B_5\right)^2 + \left(-\frac{B_{-1}}{m_T}\right)^2 + \left(-\frac{B_{-0}}{m_T}\right)^2 + \left(-\frac{B_1}{m_T}\right)^2 + \left(-\frac{B_2}{m_T}\right)^2 + \left(-\frac{B_3}{m_T}\right)^2 \\ &\quad + \left(-\frac{B_4}{m_T}\right)^2 + \left(-\frac{B_6}{m_T}\right)^2 \end{aligned} \quad (3.14)$$

$$\begin{aligned} \left(\frac{B_{DF_6}}{DF_6}\right)^2 &= \left(\frac{m_T - m_6}{m_T m_6} B_6\right)^2 + \left(-\frac{B_{-1}}{m_T}\right)^2 + \left(-\frac{B_{-0}}{m_T}\right)^2 + \left(-\frac{B_1}{m_T}\right)^2 + \left(-\frac{B_2}{m_T}\right)^2 + \left(-\frac{B_3}{m_T}\right)^2 \\ &\quad + \left(-\frac{B_4}{m_T}\right)^2 + \left(-\frac{B_5}{m_T}\right)^2 \end{aligned} \quad (3.15)$$

The total bias for the experiments involves the bias limits of deposition fraction of each stage as well as the bias limits for all measuring instrument. The bias limit for the spectrophotometer is estimated to be about 2% and 0.5% for the 10 mL transfer pipette. For the mass flow meter, the

bias limit has been stated to be 0.7%. Hence, the total bias error of each stage can be calculated.

$$Bias\ error = \sqrt{\left(\frac{B_{DF_k}}{DF_k}\right)^2 + \left(\frac{B_Q}{Q}\right)^2 + \left(\frac{B_{Vol}}{Vol}\right)^2 + \left(\frac{B_{Abs}}{Abs}\right)^2} \quad (3.16)$$

Where Q is the volume flow rate, Vol is the washing volume and Abs is the absorbance.

$$\frac{B_Q}{Q} = \frac{0.7\%}{60} = 0.012\% \quad (3.17)$$

$$\frac{B_{Vol}}{Vol} = \frac{0.5\%}{20} = 0.025\% \quad (3.18)$$

$$\frac{B_{Abs}}{Abs} = 2\% \quad (3.19)$$

(b) Precision errors

Precision errors will change as a scatter of the experiment results. The random standard uncertainty, $S_{\bar{x}}$, needs to be found in estimating the precision error. It is defined as

$$S_{\bar{x}} = \frac{S_x}{\sqrt{n}} \quad (3.20)$$

Where S_x is the standard deviation of experiments results and n is the number of trials (Figliola & Beasley, 2011).

The precision limit (P) can be calculated by the equation $P = tS_{\bar{x}}$ where t is the critical value of 95% Confidence Interval for the two-tailed Student's t distribution. In this case, the t value is 2.776 with a degree of freedom $df = n - 1 = 4$.

(c) Total uncertainty

The *root-sum-squares* method is a widely recognized approach to combine all individual measurement errors into total uncertainty. The total uncertainty is the root sum of square of the

bias error and precision error. It can be found by using the following equation.

$$U_{RSS} = \sqrt{B^2 + P^2} = \sqrt{B^2 + (tS_{\bar{x}})^2} \quad (3.21)$$

4 Chapter: Results and Discussion

The results of the study are presented in this chapter. The experiments results are displayed at the first part and the uncertainty analysis to verify the significance of the results follows afterward.

The purpose of these experiments results is to find out the relationship between particle size distribution (PSD) of Zn-DTPA aerosols from both nebulizers which is tested from the PSD 3603 as well as the ACI and factors of filling volume in the nebulizer, concentration of the Zn-DTPA solution and the temperature and humidity during the tests, which will be discussed in the second part of this section.

4.1 Experimental results

4.1.1 Characterization of Zn-DTPA

The IR analysis was performed with the KBr method. IR spectrums (see Appendix A) can provide structural information of Zn-DTPA and DTPA. Transmission percentages of peaks are shown in Table 4.1 and Table 4.2. The wavenumber range of the IR spectroscopy was from 4000 cm^{-1} to 600 cm^{-1} .

Table 4.1 IR peak information for Zn-DTPA

Wavenumber/ cm^{-1}	Transmission/ %	Wavenumber/ cm^{-1}	Transmission/ %
3432.2	22.388	1326.1	43.000
2926.9	60.740	1098.0	57.522
1607.6	2.790	925.07	56.764
1400.1	12.948	725.83	52.568

Table 4.2 IR peak information for DTPA

Wavenumber/ cm^{-1}	Transmission/ %	Wavenumber/ cm^{-1}	Transmission/ %
3422.7	50.172	1202.5	8.1395
3075.2	26.306	1145.9	13.641
3020.3	22.825	1091.4	13.874
2544.9	50.500	991.06	14.929
1733.0	3.7929	961.28	12.100
1699.0	4.8475	907.29	13.361
1634.4	5.3563	801.26	17.142
1397.3	20.835	692.16	22.235
1242.0	7.9090	647.79	30.780

Comparing the IR spectrums of Zn-DTPA and DTPA can demonstrate whether the DTPA were used to coordinate with the zinc ion in the preparation procedures. As the nitrogen and oxygen were the coordination sites between ligand and metal ion, the coordination was expected to weaken the stretching vibration of COO group and C-N bonds, leading to a shift in the wavenumber. Theoretically, the uncoordinated stretching band for COO group occurs at 1750-1700 cm^{-1} whereas the coordinated COO stretching band depends on the nature of the metal: 1610-1590 cm^{-1} for metals such as Zn (II) (Nakamoto, 2009). After the complexation with in the site of C-N bonds, the bonds become weakened, resulting in a shift in the wavenumber (Grima *et al.*, 2006). The observed bathochromic shifts in both vibrations (see Table 4.3). Both of the spectrums were also similar to the previous findings from other researchers (Zhang *et al.*, 2015; Silva *et al.*, 2007).

Table 4.3 Infrared wavenumber (cm^{-1}) of selected vibrations between DTPA and Zn-DTPA

IR Vibration	DTPA	Zn-DTPA
$\nu_{\text{C=O}}$	1634.4, 1699, 1733	1607.6
$\nu_{\text{C-N}}$	1202.5, 1242	1098

4.1.2 Filling volume and PSD

Experimental results from the PSD 3603 are presented in this section. The aerodynamic mean diameters and geometric standard deviation (GSD) were measured by the PSD 3603. Applying the value of aerodynamic mean diameter and GSD with size boundaries between 0.1 to 10 microns into the equation (1.14) for mass median aerodynamic diameter (MMAD) calculation, the MMAD of each measurement is obtained. Raw data for the PSD 3603 measurements for all experiments are present in Appendix B.1.

(1) Jet nebulizer measurement

For the PARI LC Star nebulizer used in the experiment, the minimum and maximum filling volume were 2 mL and 8 mL according to the manufacture. In order to find out if the filling volume in the reservoir would affect the PSD, the reservoir was filled with 5 mg/mL Zn-DTPA solution with 4 different volumes: 2 mL, 4 mL, 6 mL, and 8 mL. The shape of particle size distribution of these 4 distinct filling volumes (see Figure 4.1) resembled with each other, having a size peak at around 1 micron and the presence of a second peak located at over 10 microns, this second peak growing gradually along with increasing the filling volume. The result of average MMAD and GSD is listed in Table 4.4.

Figure 4.1 PSD graphs for 4 filling volumes of 5 mg/mL Zn-DTPA solution

(a) 8 mL; (b) 6 mL; (c) 4 mL; (d) 2 mL.

Table 4.4 MMAD and GSD results of 4 filling volumes of 5 mg/mL Zn-DTPA solution

Filling Volume/ mL	Aerodynamic Mean/ μm	Average GSD	STD _{GSD}	Average MMAD/ μm	STD _{MMAD}
8	0.966	1.69	0.079	2.24	0.24
6	1.00	1.74	0.10	2.56	0.47
4	1.04	1.81	0.084	3.10	0.80
2	1.12	1.96	0.27	3.70	1.1

To find out how filling volume in the nebulizer will affect the PSD, the average MMAD and GSD were plotted versus the filling volume in Figure 4.2 and Figure 4.3. The results were presented in the form of (mean $\pm$ standard deviation). The mean and standard deviation MMAD will decrease with the increasing of the filling volume in the nebulizer and so did the GSD. From 8 mL to 2 mL, the MMAD increased by 65.2%. The trend of results implies that the more solution volume is placed in the nebulizer cup, the smaller are the particles that the PARI LC Star nebulizer can generate. Filling the jet nebulizer with the maximum volume of Zn-DTPA solution could have more stable results as well as smaller MMAD than any other filling volumes.

Therefore, 8 mL of Zn-DTPA solution was adopted as the filling volume for all of the rest of experiments.

Figure 4.2 Relationships between MMAD and filling volume of Zn-DTPA solution in the jet nebulizer

Figure 4.3 Relationships between GSD and filling volume of Zn-DTPA solution in the jet nebulizer

As described in Sub-section 2.2.1, when the compressed air passed through the nozzle, primary droplets were generated above the jet. Increasing the filling volume in the reservoir from 2 mL to 8 mL resulted in more solution in the feeding tube, which shortened the distance between the interface and the jet and made the liquid medication easier to be elevated. It can be conjectured that more droplets were produced above the nozzle and they had more chance to hit the baffles and split into small droplets. According to the experiment result, altering the filling volume in the jet nebulizer can change the particle size distribution.

In the article of Hess *et al.*, they researched the deposition mass and aerosol particle size of albuterol (asthma medication) to evaluate the medication performance of different brands of nebulizers. They found that increasing the filling volume decreased the amount of albuterol trapped in the dead volume, the volume of solution remaining in the nebulizer cup after the nebulization, and increased the amount delivered to the patient (Hess *et al.*, 1996). It will be reasonable that the growing percentage of smaller size of aerosol particles that can be inhaled into and deposited in the terminal bronchiole or alveolus in the lung contributes to the rising of deposition mass in human lungs.

(2) Ultrasonic nebulizer measurement

For the measurement of MagicFly MF001 Ultrasonic Nebulizer, as there are two running modes existing, both of them were tested separately. The maximum filling volume in the ultrasonic nebulizer here is 10 mL and no volume marks are shown on the medication cup. The mechanism of droplet formation can be regarded as vibrating the solution in the medication cup and initiating the destabilization of interfacial capillary waves by periodical ultrasonic vibration, which break the liquid into small droplets (Yeo *et al.*, 2010). It can be assumed that the amount

of solution in the medication cup will not affect the formation of droplets in the ultrasonic nebulizer in terms of the mechanism. If the particle size distribution of half-filled volume, i.e., 5 mL, were similar with that of fully-filled volume, i.e., 10 mL, the assumption will be correct. Hence, only 5 mL, and 10 mL of 5 mg/mL Zn-DTPA solution was measured. Aerodynamic mean diameters and GSD were obtained by the PSD 3603. As shown in Figure 4.4, the shape of particle size distribution of 2 different filling volumes was similar. Significant peaks at about 1 micron as well as tiny peaks at about 13 microns were presented in all the PSD graphs. Besides, the peaks of running mode 1 were narrower than those of running mode 2. Applied the values of the aerodynamic mean diameter and GSD with size boundaries between 0.1 to 10 microns into the equation (1.14) for MMAD calculation and the MMAD of each measurement could be obtained. The result of average MMAD and GSD was listed in Table 4.5.

Figure 4.4 PSD graphs for 2 filling volumes of 5 mg/mL Zn-DTPA solution

(a) 5 mL in mode 1; (b) 5 mL in mode 2; (c) 10 mL in mode 1; (d) 10 mL in mode 2.

Table 4.5 MMAD and GSD results of 2 filling volumes of 5 mg/mL Zn-DTPA solution

Mode	Filling Volume/ mL	Aerodynamic Mean/ μm	Average GSD	STD _{GSD}	Average MMAD/ μm	STD _{MMAD}
1	5	1.07	1.60	0.073	2.14	0.29
	10	1.04	1.59	0.028	1.96	0.09
2	5	1.06	1.80	0.094	3.05	0.45
	10	1.04	1.76	0.096	2.79	0.49

The MMAD and GSD versus two filling volume, 5 mL and 10 mL, were plotted in Figure 4.5 and Figure 4.6, respectively. Unlike the jet nebulizer, the difference between half-filled and fully-filled under these two running modes is not obvious. Overall, the values of MMAD of mode 1 were all smaller than those of mode 2 (21.6% increase for 10 mL and 42.5 % increase for 5 mL). GSD of mode 1 was slightly less than that of mode 2. Based on the bar charts demonstrating the relationship between filling volume and MMAD as well as filling volume and GSD, 10 mL as the filling volume under mode 1 provided the most stable result since the

standard deviations of both MMAD and GSD are the lowest among all the data. So 10 mL of Zn-DTPA solutions would be filled into the medication cup in the remainder of experiments.

Figure 4.5 MMAD for the ultrasonic nebulizer with different filling volumes

Figure 4.6 GSD for the ultrasonic nebulizer with different filling volumes

4.1.3 Zn-DTPA concentration and PSD

The graphs of particle size distribution for different concentration levels Zn-DTPA solutions aerosolized from the PARI LC Star nebulizer were shown in Figure 4.7. PSD of all concentration levels were similar in shape with a big peak located from 1 to 1.5 microns range, except for a growing peak at about 13 microns along with the rising concentrations within the size limits between 0.1 to 10 microns. MMAD and the standard deviation (STD) were obtained from the aerodynamic mean diameter and average. The results of average MMAD and GSD was listed in Table 4.6.

Figure 4.7 PSD graphs for 6 concentration levels of Zn-DTPA solution

(a) 2.5 mg/mL; (b) 5 mg/mL; (c) 10 mg/mL; (d) 20 mg/mL; (e) 30 mg/mL; (f) 40 mg/mL

Table 4.6 MMAD and GSD results of different concentration of Zn-DTPA solution

Conc. / mg·mL ⁻¹	Aerodynamic Mean/ μm	Average GSD	STD _{GSD}	Average MMAD/ μm	STD _{MMAD}
2.5	0.931	1.68	0.038	2.08	0.20
5	0.966	1.69	0.079	2.24	0.24
10	1.05	1.82	0.026	3.10	0.20
20	1.11	1.94	0.022	4.13	0.22
30	1.14	1.99	0.011	4.74	0.23
40	1.18	2.03	0.013	5.28	0.066

When plotting the average MMAD versus the concentration of the Zn-DTPA solution, the mean and standard deviation of MMAD increased with the increasing of the concentration of the Zn-DTPA solution. The standard deviation of the results also dropped as the rising of the concentration. Higher concentration was easier to reach the required dosage of Zn-DTPA so as to shorten time for treatment. From the bar charts illustrated the relationship between concentration and MMAD as well as concentration and GSD, the difference between last 5 concentration levels from 5 mg/mL to 40 mg/mL is mutually significant. The values of MMAD increased 38.4%, 33.2%, 14.8% and 11.4% from 5 mg/mL to 10 mg/mL, 10 mg/mL to 20 mg/mL, 20 mg/mL to 30

mg/mL and 30 mg/mL to 40 mg/mL, respectively. The values of GSD increased 7.69%, 6.59%, 2.58% and 2.01% from 5 mg/mL to 10 mg/mL, 10 mg/mL to 20 mg/mL, 20 mg/mL to 30 mg/mL and 30 mg/mL to 40 mg/mL, respectively.

Figure 4.8 MMAD for jet nebulizer with different concentration

Figure 4.9 GSD for jet nebulizer with different concentration

In the aspect of MagicFly MF001 Ultrasonic Nebulizer, 6 different concentration levels were also tested under two running modes also using the PSD 3603. The graphs of particle size distribution are illustrated in Figure 4.10. Calculated MMAD and GSD were listed in Table 4.7. Similar peaks (one between 1 to 1.5 micron and another small peak at approximately 13 microns) were also observed.

(e)

(f)

(g)

(h)

(i)

(j)

Figure 4.10 PSD graphs for 6 concentration levels of Zn-DTPA solution

(a) 2.5 mg/mL in mode 1; (b) 2.5 mg/mL in mode 2; (c) 5 mg/mL in mode 1;
 (d) 5 mg/mL in mode 2; (e) 10 mg/mL in mode 1; (f) 10 mg/mL in mode 2;
 (g) 20 mg/mL in mode 1; (h) 20 mg/mL in mode 2; (i) 30 mg/mL in mode 1;
 (j) 30 mg/mL in mode 2; (k) 40 mg/mL in mode 1; (l) 40 mg/mL in mode 2.

Table 4.7 MMAD and GSD results of different concentration of Zn-DTPA solution

Mode	Conc. / mg·mL ⁻¹	Aerodynamic Mean/ μm	Average GSD	STD _{GSD}	Average MMAD/ μm	STD _{MMAD}
1	2.5	0.986	1.65	0.039	2.12	0.15
	5	1.04	1.59	0.028	1.96	0.094
	10	1.14	1.56	0.024	2.07	0.065
	20	1.24	1.54	0.016	2.18	0.050
	30	1.34	1.54	0.016	2.36	0.11
	40	1.40	1.56	0.0046	2.53	0.0061
2	2.5	0.967	1.72	0.065	2.39	0.22
	5	1.03	1.76	0.096	2.79	0.49
	10	1.14	1.69	0.032	2.65	0.16
	20	1.27	1.70	0.070	2.82	0.33
	30	1.34	1.63	0.013	2.76	0.08
	40	1.34	1.75	0.031	3.42	0.13

The PSD results from the ultrasonic nebulizer were categorized into two running modes. By plotting the MMAD and GSD versus the Zn-DTPA concentration in both modes, it is straightforward to obtain the relationship between concentration and PSD. In mode 1, the trend of MMAD looked like a smooth valley with a minimum occurring at 5 mg/mL and the maximum MMAD appeared at 40 mg/mL with the smallest standard deviation. The MMAD decreased 7.55% from 2.5 mg/mL to 5 mg/mL while increased 5.61%, 5.31%, 8.26% and 7.20% 5 mg/mL to 10 mg/mL, 10 mg/mL to 20 mg/mL, 20 mg/mL to 30 mg/mL and 30 mg/mL to 40 mg/mL, respectively. In mode 2, the MMAD fluctuated when the concentration was. Figure 4.11 and 4.12 show the relationships between different concentration levels and MMAD in mode 1 and mode 2.

Figure 4.11 MMAD for the ultrasonic nebulizer with different concentration in mode 1

Figure 4.12 MMAD for the ultrasonic nebulizer with different concentration in mode 2

The trend of GSD in mode 1 was that dropping down from 2.5 mg/mL to 20 mg/mL and gradually rising again. The GSD at 40 mg/mL was relatively the same as at 10 mg/mL in mode 1. Also, the least standard deviation at 40 mg/mL referred to the chance of stability in high concentration measurement. The GSD dropped 3.64%, 1.89% and 1.28% from 2.5 mg/mL to 5 mg/mL, 5 mg/mL to 10 mg/mL and 10 mg/mL to 20 mg/mL while rose 1.30% from 30 mg/mL to 40 mg/mL. There is no trend for the GSD in mode 2 as they overlap each other and fluctuate a lot. Figure 4.13 and 4.14 show the relationships between different concentration levels and GSD in mode 1 and mode 2.

Figure 4.13 GSD for the ultrasonic nebulizer with different concentration in mode 1

Figure 4.14 GSD for the ultrasonic nebulizer with different concentration in mode 2

4.1.4 PSD from ACI

(1) Calibration curve

A full scan from 190 nm to 1100 nm for the Zn-DTPA solution prepared for the deposition experiment was firstly performed to figure out the maximum absorption wavelength of Zn-DTPA in the Agilent 8435 UV-visible Spectrophotometer. The retrieved UV spectrum is illustrated in Figure 4.15. From the UV spectrum, within the wavelength in the spectrophotometer, the peak for greatest absorption in the spectrum for Zn-DTPA was in 190 nm. Since the sensitivity of greatest absorption will be the highest, experiments will be operated at 190 nm for detection and quantification.

Figure 4.15 UV spectrum of Zn-DTPA

In order to quantify the amount of deposition of Zn-DTPA on different deposition plates in the ACI, it was essential to determine the calibration function between the concentration of Zn-DTPA and the absorbance. To prepare the 1 mmol/L Zn-DTPA solution, the exact weighting

mass of DTPA powder, zinc chloride and sodium hydroxide was 19.45 mg, 7.81 mg and 10.23 mg, were performed, respectively. Therefore, the concentration of stock solution of Zn-DTPA will be

$$c(\text{Zn} - \text{DTPA}) = \frac{n(\text{DTPA})}{V \times M(\text{DTPA})} = 0.9889 \text{ mmol/L}$$

$$= \frac{m(\text{DTPA}) \times M(\text{Zn} - \text{DTPA})}{V \times M(\text{DTPA})} = 0.5169 \text{ mg/mL} \quad (4.1)$$

The standard solutions were diluted from the stock solution in distilled water by different ratio so the concentration of each standard solution was known beforehand. The absorbance readings of different standard solutions were taken at a fixed wavelength of 190 nm and the results are presented in Table 4.8.

Table 4.8 Absorbance of standard solutions

Standard. Solution Conc./ $\mu\text{mol} \cdot \text{L}^{-1}$	Standard. Solution Conc./ $\text{mg} \cdot \text{mL}^{-1}$	Absorbance			
		1	2	3	Average
4.944	2.584e-3	0.050203	0.059570	0.058949	0.056241
9.889	5.169e-3	0.072644	0.074347	0.075363	0.074118
19.78	0.01034	0.14231	0.15060	0.15405	0.14899
49.44(*)	0.02584	0.34572	0.35013	0.34661	0.34749
74.17(*)	0.03877	0.48195	0.48429	0.48472	0.48365
98.89	0.05169	0.60447	0.60433	0.60438	0.60439
148.3	0.07754	0.86965	0.87119	0.87247	0.87110
197.8	0.1034	1.1594	1.1603	1.1600	1.1599
247.2(*)	0.1292	1.3817	1.3822	1.3884	1.3841
296.7(*)	0.1551	1.5859	1.5909	1.5937	1.5902

*: excluded for a better linear fit

Figure 4.16 Calibration curve

From the average absorbance of three measurements, the calibration curve was found and the equation for it with the best linear fit is given by

$$Abs = 10.953c + 0.0281 \quad (4.2)$$

Where the unit of concentration was in mg/mL and the absorbance was taken at 190 nm. The calibration curve also supplies the linear range for the quantification of Zn-DTPA solution, helping to judge the volume of distilled water in the washing procedures.

The correlation coefficient was also determined as

$$R^2 = 0.99971 \quad (4.3)$$

This coefficient implied that the calibration results closely follow the line of best fit with little error and deviation.

(2) Deposition measurements

Deposition measurements were used to demonstrate the PSD of the Zn-DTPA aerosols generated from the PARI LC Star nebulizer. Based on the calibration curve, the deposition mass of Zn-

DTPA on each plate can be determined. The average deposition percentages are summarized in Table 4.9. The error bars shown in the particle size distribution are the standard deviation of 5 trials. Detailed absorbance results of each trial are presented in Appendix B.2.

It can be seen from Figure 4.17 that the peak of the distribution locates at stage 5 with a cut-off diameter between 1.20 to 1.90 microns. From stage 3 to stage F, the particle size distribution is approximately normal distribution with a small tail spreading to upper stages (i.e., bigger cut-off diameters). More particles deposit on stage 0, the first stage of the ACI, compared with those on stage 1. Some large particles still following the streamline rather than impacting inside the induction port may account for this finding of more particles deposition on stage 0 than stage 1.

Table 4.9 Deposition fraction of each deposition plate

d(μ m) DF(%)	8.60+ (Stage 0)	6.50 - 8.60 (Stage 1)	4.40 - 6.50 (Stage 2)	3.20 - 4.40 (Stage 3)	1.90 - 3.20 (Stage 4)	1.20 - 1.90 (Stage 5)	0.55 - 1.20 (Stage 6)	0.26 - 0.55 (Stage F)
Trial 1	6.002	3.875	4.736	7.555	20.30	28.22	20.59	8.717
Trial 2	4.315	4.211	4.897	10.25	23.18	28.44	17.89	6.821
Trial 3	3.432	1.721	3.615	8.653	26.96	30.83	19.08	5.713
Trial 4	3.534	2.856	5.673	9.932	26.62	27.37	17.49	6.518
Trial 5	4.106	3.412	2.790	8.762	22.56	29.42	20.27	8.676
Average	4.278	3.215	4.342	9.030	23.92	28.86	19.06	7.289
STD	1.033	0.9782	1.137	1.083	2.828	1.324	1.380	1.347

Figure 4.17 Particle size distribution measured by ACI

In order to estimate the MMAD and GSD from the particle size distribution, the average deposition fractions are converted to the cumulative distribution shown in Table 4.10. After plotting the cumulative distribution to the aerodynamic diameter which is in logarithmic scale in Matlab, the MMAD is found to be 1.76 microns and the GSD is 2.07. The codes written in Matlab can be found in Appendix D.

Table 4.10 Cumulative deposition fraction

d (μm)	Cumulative Deposition Fraction (%)	d (μm)	Cumulative Deposition Fraction (%)
0.26	0	3.2	79.13
0.55	7.289	4.4	88.60
1.2	26.35	6.5	92.95
1.9	55.21	8.6	96.16

4.1.5 Error and uncertainty

The deposition fractions of all stages have been summarized in Table 4.9 in Sub-Section 4.1.4. In order to increase the reliability of the experimental results, understanding the uncertainty of the results is necessary. The bias (B) error, precision (P) error and total uncertainty (U) of each stage can be calculated according to equation (3.8) to (3.21) in Sub-Section 3.3.4. The deposition fractions and experimental uncertainties are shown in Table 4.11.

Table 4.11 Experimental results and uncertainty

d (μm)	8.60+ (Stage 0)	6.50 - 8.60 (Stage 1)	4.40 - 6.50 (Stage 2)	3.20 - 4.40 (Stage 3)	1.90 - 3.20 (Stage 4)	1.20 - 1.90 (Stage 5)	0.55 - 1.20 (Stage 6)	0.26 - 0.55 (Stage F)
DF	0.04278	0.03215	0.04342	0.09030	0.2392	0.2886	0.1906	0.07289
B	0.02183	0.02221	0.02238	0.02158	0.02147	0.02148	0.02154	0.02226
P	0.01283	0.01214	0.01412	0.01344	0.03512	0.01643	0.01713	0.01673
U	0.02532	0.02531	0.02646	0.02543	0.04116	0.02704	0.02752	0.02784

4.2 Discussion

4.2.1 Concentration boundary

The concentrations used in this study are constrained from 2.5 mg/mL to 40 mg/mL. The reason why the concentration tested here should lower than 40 mg/mL is because that the concentration of sodium ion in the solution must not exceed the blood sodium level. As it is known that the concentration of sodium ion in 0.9% saline solution is 9 mg/mL, meaning that the concentration of NaOH used here should be

$$c(\text{NaOH}) \leq \frac{c(\text{Na}^+) \times M(\text{NaOH})}{M(\text{Na}^+)} = \frac{9 \times 40.00}{22.99} = 15.66 \text{ mg/mL}. \quad (4.4)$$

Judging from the ratios of reactants in the Zn-DTPA preparation procedures, the concentration of Zn-DTPA also needs to be

$$c(Zn - DTPA) \leq \frac{c(NaOH) \times M(Zn - DTPA)}{5 \times M(NaOH)} = \frac{15.66 \times 522.69}{5 \times 40.00} = 40.92 \text{ mg/mL}. \quad (4.5)$$

Generally speaking, for a nebulized therapy, the usual inhalation time will be within half an hour, regardless of the type of nebulizer (Gardenhire *et al.*, 2013). As is mentioned in Sub-Section 2.1.4, required dosage of Zn-DTPA for inhalation therapy is 2 $\mu\text{mol/kg}$. Supposing the average weight for an adult is 70 kg, then the required mass of Zn-DTPA for a single treatment will be

$$m(Zn - DTPA) = \text{Dosage} \times M(Zn - DTPA) \times m = \frac{2 \times 522.69 \times 70}{1000} = 73.18 \text{ mg}. \quad (4.6)$$

Since the maximum daily dosage of Zn-DTPA is 1 g, administering 73.18 mg of Zn-DTPA is safe to individuals.

The output flow rate of the PARI LC Star nebulizer, roughly 0.15 mL/min, is lower than that of the MagicFly MF001 Ultrasonic Nebulizer. If the treatment will be repeated three times a day, then the concentration of Zn-DTPA in this situation is

$$c(Zn - DTPA) = \frac{m(Zn - DTPA)}{3 \times t \times Q} = \frac{73.18 \text{ mg}}{3 \times 30 \times 0.15} = 5.42 \text{ mg/mL}. \quad (4.7)$$

The inhalation time and times of treatment can be adjusted on the basis of requirement. Hence, the concentration boundary was set to be within 2.5 to 40 mg/mL.

4.2.2 Size boundary

In the PIM software, data is displayed between the selected boundaries and all the statistics are computed based on the range of data between the boundaries. The upper and lower boundaries of the data's size range can be changed. According to the manufacturer manual, the size boundary can be set manually from 0.1 to 1000 microns (TSI, 2004). The induction port from the ACI is

able to filter out particles larger than 10 microns. Limiting the size boundary into 10 microns in the PSD 3603 can make result from both instruments comparable.

Small peaks at approximate 13 microns occur in vast parts of the particle size distribution graphs from the PSD 3603. They are physically formed in the nebulizing system. It might be caused by size change of particles condensation in the chamber box (Finlay, 2001). Additionally, Zn-DTPA has both hydrophilic and hydrophobic groups. It could act as a surfactant in the aqueous solution. The surface tension of the solution decreases as the ion strength (or concentration in this circumstance) of surfactant increases. Sander *et al.* investigated the interfacial tension of mercury electrode in presence of different amount of Cr-DTPA complex. They found that increasing the concentration of Cr-DTPA can significantly decrease the surface tension (Sander *et al.*, 2002). In addition, a decreasing the surface tension by adding ethanol, in the explanation from Sirr *et al.*, increases the volatility and formation of ^{99m}Tc -DTPA aerosol droplets (Sirr *et al.*, 1985). Zn-DTPA has a similar structure as Cr-DTPA and ^{99m}Tc -DTPA. Therefore, higher concentration level of Zn-DTPA solution might lead to generating more aerosol particles, indicating more aerosol particles being trapped in the chamber box which increases the probability of particles condensing to each other. Hence, the increasing concentration level results in producing larger aerosol particles and forming a peak at about 13 microns in the particle size distribution. The interference from these peaks are exclusive in the MMAD and GSD calculation after setting the size boundary.

4.2.3 Student's t-test results

After plotting the concentration or filling volume versus the MMAD and GSD in bar charts, some error bars overlap with each other, a student's t-test was conducted to demonstrate the

statistical significance of all the reported results, assuming each aerosol sample was measured independently from each other in the PSD 3603 and the values were sample from a normal distribution. The null hypothesis in this analysis was that the mean difference between different variables (filling volume, concentration or power mode) was zero. Based on the calculated t-value, the two-tailed probability (p) can be obtained to evaluate the probability to reject the null hypothesis. This t-test is used to determine whether experimental results collected from the PSD 3603 with various variables were truly different.

In the experiments for the filling volume of jet nebulizer, as the error bars of MMAD of 4 filling volumes (see Figure 4.2) overlap with each other and so does the error bars of GSD (see Figure 4.3), t-values and p between different combination of the 4 filling volumes are calculated and presented in Table 4.12. The GSD is found to be approximately constant between the adjacent filling volumes while the MMAD has a trend of growing as the filling volume decreases, which is the reason for adopting 8mL as the filling volume for all of the remaining experiment for the jet nebulizer.

Table 4.12 Difference between different filling volumes for jet nebulizer

Difference between	MMAD		GSD	
	t-value	Two-tailed probability (p)	t-value	Two-tailed probability (p)
8 mL & 6 mL	2.348	0.034	1.519	0.15
8 mL & 4 mL	3.988	0.013	4.030	0.0012
8 mL & 2 mL	5.022	0.00019	3.717	0.0023
6 mL & 4 mL	1.878	0.081	2.076	0.057
6 mL & 2 mL	3.691	0.0024	2.959	0.010
4 mL & 2 mL	1.708	0.11	2.054	0.059

In the filling volume experiments of the ultrasonic nebulizer, it is obvious that between mode 1 and mode 2, the MMAD and GSD is different when the filling volume is the same. However,

there is no difference between 5 mL and 10 mL under the same power mode, as the t-value and p shown in Table 4.13.

Table 4.13 Difference between fully filled and half filled for ultrasonic nebulizer

Difference between		MMAD		GSD	
		t-value	Two-tailed probability (p)	t-value	Two-tailed probability (p)
Mode 1	5 mL & 10 mL	2.296	0.038	0.4418	0.63
Mode 2	5 mL & 10 mL	1.514	0.15	1.153	0.27

In the concentration experiments using jet nebulizer, there is an overlap between 2.5 mg/mL and 5 mg/mL. The t-values for the MMAD and GSD are 1.984 and 0.4418, respectively. The corresponding p for them is 0.067 and 0.66, indicating that, the MMAD and GSD may remain constant in concentration lower than 5 mg/mL while in high concentration, the MMAD and GSD increase with the concentration.

In the concentration experiments using ultrasonic nebulizer, the difference between mode 1 and mode 2 can also be confirmed by different concentration levels (see Table 4.14).

Table 4.14 Difference between mode 1 and mode 2

Difference between		MMAD		GSD	
		t-value	Two-tailed probability (p)	t-value	Two-tailed probability (p)
2.5 mg/mL	mode 1 & mode 2	3.927	0.0015	3.576	0.0030
5 mg/mL	mode 1 & mode 2	6.443	0.000015	6.584	0.000012
10 mg/mL	mode 1 & mode 2	13.00	0	12.59	0
20 mg/mL	mode 1 & mode 2	7.426	0.0000032	8.630	0.00000056
30 mg/mL	mode 1 & mode 2	11.39	0	16.91	0
40 mg/mL	mode 1 & mode 2	26.48	0	23.48	0

In mode 1, the difference between different concentration levels are shown in Table 4.15. All the adjacent concentration levels have significantly different MMAD except for 2.5 mg/mL and 10

mg/mL. As the MMAD first slightly decreases from 2.5 mg/mL to 5 mg/mL and increases from 5 mg/mL to 40 mg/mL. It is inevitable that the MMAD of 2.5 mg/mL and 10 mg/mL is no difference in a parabola. The GSD of 2.5 mg/mL is significantly different from other concentration levels. Although the MMAD of different concentration levels are different from each, the values of them are still within a narrow range. Considering that GSD of 2.5 mg/mL is the biggest, the distribution is wider than others, which increases the delivery efficiency of aerosols particles within 1 to 5 microns.

Table 4.15 Difference between concentration in mode 1

Difference between	MMAD		GSD	
	t-value	Two-tailed probability (p)	t-value	Two-tailed probability (p)
2.5 mg/mL & 5 mg/mL	-3.501	0.0035	-4.840	0.00026
2.5 mg/mL & 10 mg/mL	-1.184	0.26	7.612	0.0000024
2.5 mg/mL & 20 mg/mL	5.692	0.000056	10.11	0
2.5 mg/mL & 30 mg/mL	4.997	0.00020	10.11	0
2.5 mg/mL & 40 mg/mL	10.53	0	8.876	0.00000040
5 mg/mL & 10 mg/mL	3.728	0.0022	3.151	0.0071
5 mg/mL & 20 mg/mL	8.003	0.0000014	6.005	0.000032
5 mg/mL & 30 mg/mL	10.71	0	6.005	0.000032
5 mg/mL & 40 mg/mL	53.05	0	4.095	0.0011
10 mg/mL & 20 mg/mL	20.12	0	2.685	0.018
10 mg/mL & 30 mg/mL	8.790	0	2.685	0.018
10 mg/mL & 40 mg/mL	27.29	0	0	1.0
20 mg/mL & 30 mg/mL	5.769	0.000049	0	1.0
20 mg/mL & 40 mg/mL	26.91	0	4.653	0.00037
30 mg/mL & 40 mg/mL	5.976	0.000034	4.653	0.00037

In mode 2, the fluctuation of MMAD and GSD is too big, implying this running mode might be unreliable. This is because it has to press the buttons twice to run the ultrasonic nebulizer in

mode 2. This operation introduces interference of residue aerosol particles from either mode 1 or time delays.

4.2.4 UV absorption measurement at 190 nm in distilled water

Because all the Zn-DTPA solution were aqueous, distilled water should work as the blank sample. The absorbance of distilled water would be measured before each test. Figure 4.18 showed the UV spectrum of distilled water used for the preparation of standard solution. The absorbance for blank sample in 190 nm was -3.33786×10^{-6} but the absorbance of the standard solution with the lowest concentration (see Table 4.8) had an order of magnitude of 10^{-2} , indicating the absorbance of samples was at least three orders of magnitude higher than that of the blank. The influence from water in 190 nm was negligible.

The objective of the UV absorption test was to find out the deposition fraction on each plate so as to obtain the particle size distribution. Even though it will introduce minor errors from measuring the deposition fraction in this extreme wavelength, the particle size distribution will not be affected as long as the instrumental condition remained constant for all the standard and sample solutions. Therefore, measuring UV absorption at 190 nm in distilled water had no significant interference to the particle size distribution results.

Figure 4.18 UV spectrum of distilled water (blank)

4.2.5 Concentration of Zn-DTPA solution for the ACI test

Owing to the limit of time, only aerosols of 40 mg/mL of Zn-DTPA solution generated by jet nebulizer was tested in the ACI experiments. For one thing, the ACI experiments were designed to examine the feasibility of detecting Zn-DTPA aerosols in the ACI other than finding out the relationships between different factors and MMAD or GSD, which was demonstrated by the PSD 3603. For another, more literatures were published about detecting aerosols from jet nebulizer with the ACI than ultrasonic nebulizer. Choosing the jet nebulizer makes it easier to design the experiment setups.

From the experimental results of both jet nebulizer and ultrasonic nebulizer, the results of 40 mg/mL has the least standard deviation among all the concentration levels, indicating the results became stable at high concentration levels. Moreover, it exhibited larger MMAD and GSD than any other concentration level from the results of PSD 3603. In the mechanism of the production of aerosol droplets, bubbles creation exists in both jet nebulizer and ultrasonic nebulizer. It can be postulated that high concentration helps to stabilize the formation of bubbles. Based on the consideration mentioned above, 40 mg/mL of Zn-DTPA solution was adopted as the representative to be tested in the ACI experiments.

4.2.6 Influence of flow rate, temperature and relative humidity

Zhou *et al.* found that the jet nebulizer produces cool aerosols and the temperature was 10 °C lower at the exit of the jet nebulizer (Zhou *et al.*, 2005). Moreover, the relative humidity (RH) inside the buildings controlled by central air-conditioning is comparative low. As the pharmaceutical formulations aerosolized in the jet nebulizer are usually water-based solution with low concentration of salts or medications so the nebulized droplets will rapidly evaporate

when exposed to ambient temperature and humidity. Along the way from the exit to the jet nebulizer to the entrance to the impactor, the heating and dryness of the ambient air usually cause a significant decrease (typically up to 50%) of the MMAD from the jet nebulizer. Finlay and Stapleton have confirmed the importance to make sure the air carrying aerosols into and through the impactor is in equilibrium with the droplets at the entrance to the impactor in case of the interference of particle size shrinkage (Finlay & Stapleton, 1999). Berg *et al.* suggested that putting the impactor in the refrigerator more than 90 minutes and starting the experiment within 5 min after removing out of the refrigerator can increase the chance of affecting the original particle size distribution of the nebulizational delivery (Berg, Svensson & Asking, 2007). Therefore, temperature and humidity control to avoid the particle size shrinkage is one of the main purposes in the design of experiment setups with the ACI. The top chamber box works as a humidifier providing cool and humid air to carry the aerosols into the impactor. The ice inside both chamber boxes and immersing the impactor under ice bath also ensure the low temperature for measurement.

Many researchers used a T-junction to connect the nebulizer, humidifier and impactor in the experiment setup of PSD evaluation of jet nebulizers with the ACI (Zhou *et al.*, 2005). However, since the path lines of airflows coming from the humidifier and the aerosols producing from the nebulizer do not overlap each other, the turbulence within the fluids is unavoidable when the vacuum pump is running. The behavior of nebulizer in this situation might be different from the normal operation. By previously mixing the aerosols and humid air in the chamber boxes, this interference could be eliminated.

The operation flow rate for PSD assessment of jet nebulizer in the ACI is 28.3 L/min, which is recommended by the manufacture (Copley, 2015). Subtracting the output of jet nebulizer, the

flow rate entering the ACI at this flow rate is more than 20 L/min of room air vacuumed along with the aerosols produced from the nebulizer. A higher flow rate indicates more room air being introduced into the ACI. In the research finding from Zhou *et al.*, a high flow rate will accelerate the evaporation of the solvent inside the droplets, which will also result in the particle size shrinkage. Comparing the PSD from different operation flow rates, the higher flow rate, the smaller MMAD and GSD were, implying smaller inhaled dose of drugs than they are expected to intake (Zhou *et al.*, 2007). Therefore, it can be predicted that higher flow rate for the same medication in the impaction experiment will decrease the MMAD of the aerosols.

Generally speaking, the flow rate for tidal breathing for adults is 18 L/min. For deep breath, the inhalation flow rate will be 60 L/min. This flow rate is commonly used in the impaction measurement of pressurized metered dose inhaler (usually abbreviated pMDI) that requires deep breathing in inhaling the pharmaceutical aerosols. It is indicated that the inhalation flow rate for patients will not exceed 60 L/min with the jet nebulizer. The results of the ACI with 60 L/min refer to an extreme example of the limit of the MMAD.

4.2.7 Comparison with SEM analysis

The trend between MMAD of Zn-DTPA aerosol particles and concentration of Zn-DTPA solution matches the result from the SEM analysis. From the SEM image shown in Figure 4.19, the morphology of the Zn-DTPA aerosol particles are approximate spherical. The SEM images from top to bottom represent Zn-DTPA aerosols generated from 5 mg/mL, 10 mg/mL and 20 mg/mL Zn-DTPA solution by PARI LC® Star Reusable Nebulizer. This results also support the growing trends between the concentration of the Zn-DTPA solution and the MMAD of aerosols.

(a)

(b)

(c)

Figure 4.19 SEM images of Zn-DTPA aerosols

(a) 5 mg/mL; (b) 10 mg/mL; (c) 20 mg/mL

5 Chapter: Conclusions and Future Work

5.1 Conclusions

This thesis aimed at developing a procedure to aerosolize Zn-DTPA with commercially available nebulizers. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) are used to describe the particle size distribution. The jet nebulizer and ultrasonic nebulizer are proved to be appropriate to generate inhalable aerosols from Zn-DTPA solution and the Zn-DTPA aerosols are inhalable into the lung as their MMAD are within 1 to 5 μm . Different filling volumes and concentrations were examined in the experiments. A new experimental set-up with the Andersen Cascade Impactor (ACI) was utilized to measure the particle size distribution of 40 mg/mL Zn-DTPA solution. The overall experiment results indicate that:

- (1) For different filling volumes in the jet nebulizer, there is a decrease in MMAD and GSD from when the filling volume of the Zn-DTPA solution increases from minimum to maximum filling volume. The MMAD of the maximum filling volume was 65.2% higher than that of the minimum filling volume.
- (2) The MMAD and GSD will increase along with the increase of the Zn-DTPA solution concentration in the jet nebulizer. The values of MMAD increased 38.4%, 33.2%, 14.8% and 11.4% from 5 mg/mL to 10 mg/mL, 10 mg/mL to 20 mg/mL, 20 mg/mL to 30 mg/mL and 30 mg/mL to 40 mg/mL while the values of GSD increased 7.69%, 6.59%, 2.58% and 2.01% from 5 mg/mL to 10 mg/mL, 10 mg/mL to 20 mg/mL, 20 mg/mL to 30 mg/mL and 30 mg/mL to 40 mg/mL, respectively.

- (3) For the ultrasonic nebulizer, comparing the MMAD and GSD of aerosols generated from the same filling volume and concentration solution in both modes, on the whole, the MMAD and GSD of high power mode is smaller than that of low power mode.
- (4) The difference between fully filled and half filled with Zn-DTPA solution is not significant in both high power mode and low power mode.
- (5) The trend between concentration and MMAD or GSD in high power mode does not resemble with low power mode. In high power mode, the MMAD will increase with the rising concentration except the lowest concentration tested in the experiments. The MMAD decreased 7.55% from 2.5 mg/mL to 5 mg/mL while increased 5.61%, 5.31%, 8.26% and 7.20% 5 mg/mL to 10 mg/mL, 10 mg/mL to 20 mg/mL, 20 mg/mL to 30 mg/mL and 30 mg/mL to 40 mg/mL, respectively. The GSD is found to be parabolic after increasing the concentration. The GSD dropped 3.64%, 1.89% and 1.28% from 2.5 mg/mL to 5 mg/mL, 5 mg/mL to 10 mg/mL and 10 mg/mL to 20 mg/mL but rose 1.30% from 30 mg/mL to 40 mg/mL. In low power mode, both MMAD and GSD fluctuate with the concentration and no obvious trend can be observed.
- (6) The deposition fractions of different stages of the ACI demonstrate the particle size distribution of aerosols. The results suggest that the Zn-DTPA aerosols are detectable in the ACI at a flow rate of 60 L/min.

5.2 Future work

In spite of the findings from the present work, further experiments can be implemented for improving the experimental results of this thesis. The recommendations are as follows:

(1) Only one model of jet nebulizer and ultrasonic nebulizer were tested in the experiment. In order to verify the findings with other models of different types of nebulizers, more nebulizers can be used in additional experiments.

(2) After purchase of new hardware, additional experiment using the ACI at a recommended flow rate of 28.3 L/min can be performed, which is lower than the current flow rate (60 L/min) in the experiments.

(3) Alternative techniques for particle size measurement such as laser diffraction techniques can be considered to assess the particle size distribution of Zn-DTPA aerosols.

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Appendix

Appendix A IR Spectra

IR spectrum of Zn-DTPA and DTPA are shown in Figure A.1 and Figure A.2.

Transmission / Wavenumber (cm-1)

File # 1 = INSTRUMENTLAB3.TRANSMITTANCE

Paged X-Zoom CURSOR

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Figure A.1 IR spectrum of Zn-DTPA

Transmission / Wavenumber (cm-1)

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Figure A.2 IR spectrum of DTPA

Appendix B Experiment Results

B.1 PSD 3603 results

B.1.1 Jet nebulizer

(1) Filling volume

Table B.1 Results of 2 mL

Solution	Trial	Mean/ μm	GSD	MMAD/ μm
1	1	1.05	1.93	3.84
	2	1.06	1.88	3.50
	3	1.06	1.93	3.88
	4	1.08	2.00	4.56
	5	1.09	1.99	4.51
2	1	1.19	2.02	5.24
	2	1.11	1.99	4.59
	3	1.12	1.99	4.64
	4	1.12	1.94	4.18
	5	1.13	1.96	4.40
3	1	1.04	1.65	2.21
	2	1.06	1.71	2.21
	3	1.06	1.67	2.51
	4	1.06	1.69	2.33
	5	1.10	1.73	2.71

Table B.2 Results of 4 mL

Solution	Trial	Mean/ μm	GSD	MMAD/ μm
1	1	1.01	1.84	3.08
	2	1.05	1.89	3.54
	3	0.974	1.83	2.91
	4	1.00	1.88	3.30
	5	1.05	1.95	4.00
2	1	1.05	1.86	3.34
	2	1.06	1.93	3.88
	3	1.09	1.92	3.91
	4	1.07	1.95	4.08
	5	1.05	1.87	3.40
3	1	1.02	1.68	2.29
	2	1.03	1.67	2.27
	3	1.03	1.63	2.11
	4	1.02	1.64	2.12
	5	1.03	1.65	2.18

Table B.3 Results of 6 mL

Solution	Trial	Mean/ μm	GSD	MMAD/ μm
1	1	0.978	1.76	2.55
	2	0.974	1.71	2.31
	3	1.01	1.82	2.96
	4	0.980	1.75	2.51
	5	0.973	1.76	2.54
2	1	0.994	1.83	2.97
	2	1.02	1.85	3.17
	3	0.987	1.84	3.01
	4	1.00	1.82	2.93
	5	1.01	1.83	3.02
3	1	1.01	1.62	2.03
	2	1.02	1.61	2.01
	3	0.999	1.68	2.24
	4	1.01	1.63	2.07
	5	1.03	1.61	2.03

Table B.4 Results of 8 mL

Solution	Trial	Mean/ μm	GSD	MMAD/ μm
1	1	0.948	1.68	2.12
	2	0.947	1.70	2.20
	3	0.959	1.69	2.19
	4	0.973	1.72	2.35
	5	0.930	1.73	2.29
2	1	0.929	1.77	2.47
	2	0.930	1.76	2.42
	3	0.951	1.76	2.48
	4	0.938	1.76	2.45
	5	0.930	1.79	2.57
3	1	1.02	1.61	2.01
	2	1.01	1.59	1.92
	3	1.02	1.57	1.88
	4	1.01	1.64	2.10
	5	0.997	1.64	2.08

(2) Concentration**Table B.5 Results of 2.5 mg/mL**

Solution	Trial	Mean/ μm	GSD	MMAD/ μm
1	1	0.954	1.73	2.35
	2	0.959	1.70	2.23
	3	0.960	1.73	2.36
	4	0.957	1.73	2.36
	5	0.951	1.71	2.26

2	1	0.934	1.68	2.09
	2	0.931	1.63	1.90
	3	0.922	1.67	2.03
	4	0.927	1.64	1.93
	5	0.920	1.69	2.10
3	1	0.899	1.67	1.98
	2	0.907	1.67	2.00
	3	0.913	1.63	1.87
	4	0.921	1.63	1.88
	5	0.912	1.64	1.90

Table B.6 Results of 5 mg/mL

Solution	Trial	Mean/ μm	GSD	MMAD/ μm
1	1	0.948	1.68	2.12
	2	0.947	1.70	2.20
	3	0.959	1.69	2.19
	4	0.973	1.72	2.35
	5	0.930	1.73	2.29
2	1	0.929	1.77	2.47
	2	0.930	1.76	2.42
	3	0.951	1.76	2.48
	4	0.938	1.76	2.45
	5	0.930	1.79	2.57
3	1	1.02	1.61	2.01
	2	1.01	1.59	1.92
	3	1.02	1.57	1.88
	4	1.01	1.64	2.10
	5	0.997	1.64	2.08

Table B.7 Results of 10 mg/mL

Solution	Trial	Mean/ μm	GSD	MMAD/ μm
1	1	1.04	1.82	3.05
	2	1.05	1.80	2.96
	3	1.06	1.80	2.99
	4	1.07	1.79	2.96
	5	1.04	1.80	2.93
2	1	1.05	1.79	2.90
	2	1.04	1.79	2.88
	3	1.04	1.81	2.99
	4	1.06	1.82	3.11
	5	1.03	1.83	3.08
3	1	1.06	1.81	3.05
	2	1.09	1.83	3.26
	3	1.06	1.83	3.17
	4	1.06	1.86	3.36
	5	1.07	1.92	3.84

Table B.8 Results of 20 mg/mL

Solution	Trial	Mean/ μm	GSD	MMAD/ μm
1	1	1.12	1.96	4.36
	2	1.10	1.96	4.28
	3	1.15	1.92	4.12
	4	1.12	1.90	3.85
	5	1.13	1.94	4.22
2	1	1.09	1.95	4.15
	2	1.11	1.92	3.98
	3	1.11	1.95	4.23
	4	1.12	2.01	4.83
	5	1.12	1.97	4.45
3	1	1.08	1.94	4.03
	2	1.11	1.97	4.41
	3	1.09	1.94	4.07
	4	1.09	1.92	3.91
	5	1.08	1.81	3.10

Table B.9 Results of 30 mg/mL

Solution	Trial	Mean/ μm	GSD	MMAD/ μm
1	1	1.09	1.97	4.33
	2	1.11	2.01	4.79
	3	1.12	2.01	4.83
	4	1.09	1.99	4.51
	5	1.09	2.00	4.61
2	1	1.16	2.00	4.90
	2	1.22	2.07	5.97
	3	1.17	1.97	4.64
	4	1.14	1.94	4.26
	5	1.17	2.03	5.26
3	1	1.15	1.96	4.47
	2	1.15	1.98	4.66
	3	1.13	1.98	4.58
	4	1.13	2.00	4.78
	5	1.12	1.98	4.54

Table B.10 Results of 40 mg/mL

Solution	Trial	Mean/ μm	GSD	MMAD/ μm
1	1	1.17	1.95	4.46
	2	1.14	2.02	5.02
	3	1.14	2.01	4.92
	4	1.17	2.08	5.85
	5	1.16	2.05	5.44

2	1	1.19	1.98	4.85
	2	1.21	2.04	5.56
	3	1.19	2.04	5.47
	4	1.19	2	5.03
	5	1.19	2.01	5.14
3	1	1.19	2.03	5.35
	2	1.19	2.03	5.35
	3	1.17	2.02	5.16
	4	1.19	2.01	5.14
	5	1.22	2.04	5.60

B.1.2 Ultrasonic nebulizer

(1) Filling volume

Table B.11 Results of half-filled

Solution	Trial	Mode	Mean/ μm	GSD	MMAD/ μm	Mode	Mean/ μm	GSD	MMAD/ μm
1	1	1	1.03	1.59	1.96	2	1.04	1.76	2.71
	2		1.06	1.52	1.79		1.06	1.69	2.42
	3		1.08	1.58	2.02		1.02	1.86	3.24
	4		1.04	1.59	1.98		1.04	1.9	3.58
	5		1.07	1.30	1.32		1.04	1.96	4.05
2	1	1	1.16	1.61	2.29	2	1.09	1.69	2.49
	2		1.16	1.65	2.46		1.12	1.66	2.42
	3		1.11	1.77	2.95		1.11	1.69	2.54
	4		1.09	1.60	2.11		1.10	1.70	2.56
	5		1.07	1.62	2.15		1.13	1.71	2.68
3	1	1	1.03	1.51	1.71	2	1.02	1.96	3.97
	2		1.03	1.57	1.90		1.06	1.87	3.43
	3		1.02	1.86	3.24		1.03	1.95	3.92
	4		1.04	1.68	2.33		1.03	1.78	2.79
	5		1.04	1.54	1.82		1.07	1.78	2.90

Table B.12 Results of full-filled

Solution	Trial	Mode	Mean/ μm	GSD	MMAD/ μm	Mode	Mean/ μm	GSD	MMAD/ μm
1	1	1	1.03	1.60	1.98	2	1.04	1.89	3.51
	2		1.02	1.64	2.12		1.02	1.91	3.58
	3		1.06	1.57	1.95		1.03	1.91	3.62
	4		1.04	1.70	2.42		1.04	1.78	2.93
	5		1.01	1.57	1.86		1.02	1.80	2.88
2	1	1	1.19	1.54	2.08	2	0.984	1.64	2.05
	2		1.04	1.62	2.09		1.03	1.74	2.58
	3		1.03	1.53	1.77		1.06	1.71	2.51
	4		1.02	1.53	1.75		1.09	1.60	2.11
	5		1.03	1.59	1.96		1.08	1.64	2.25

3	1	1	1.03	1.55	1.83	2	1.05	1.61	2.07
	2		1.04	1.56	1.88		1.04	1.61	2.05
	3		0.971	1.59	1.85		1.05	1.92	3.76
	4		1.02	1.6	1.98		1.05	1.87	3.40
	5		1.04	1.56	1.88		1.05	1.73	2.58

(2) Concentration

Table B.13 Results of 2.5 mg/mL

Solution	Trial	Mode	Mean/ μm	GSD	MMAD/ μm	Mode	Mean/ μm	GSD	MMAD/ μm
1	1	1	0.986	1.65	2.09	2	0.955	1.79	2.64
	2		0.971	1.58	1.82		0.949	1.79	2.62
	3		0.97	1.63	1.98		0.939	1.74	2.36
	4		0.939	1.77	2.50		0.936	1.77	2.49
	5		1.00	1.62	2.01		0.958	1.76	2.50
2	1	1	1.03	1.75	1.83	2	1.01	1.77	2.68
	2		0.981	1.7	2.64		1.00	1.81	2.88
	3		0.995	1.69	2.28		0.967	1.73	2.38
	4		0.995	1.69	2.27		0.959	1.64	2.00
	5		0.968	1.63	1.98		0.971	1.78	2.63
3	1	1	0.963	1.52	1.63	2	0.973	1.59	1.85
	2		1.05	1.70	2.44		0.969	1.61	1.91
	3		0.958	1.63	1.96		0.992	1.74	2.49
	4		1.01	1.62	2.03		0.977	1.68	2.19
	5		0.969	1.60	1.88		0.957	1.70	2.23

Table B.14 Results of 5 mg/mL

Solution	Trial	Mode	Mean/ μm	GSD	MMAD/ μm	Mode	Mean/ μm	GSD	MMAD/ μm
1	1	1	1.03	1.60	1.98	2	1.04	1.89	3.51
	2		1.02	1.64	2.12		1.02	1.91	3.58
	3		1.06	1.57	1.95		1.03	1.91	3.62
	4		1.04	1.70	2.42		1.04	1.78	2.93
	5		1.01	1.57	1.86		1.02	1.80	2.88
2	1	1	1.19	1.54	2.08	2	0.984	1.64	2.05
	2		1.04	1.62	2.09		1.03	1.74	2.58
	3		1.03	1.53	1.77		1.06	1.71	2.51
	4		1.02	1.53	1.75		1.09	1.60	2.11
	5		1.03	1.59	1.96		1.08	1.64	2.25
3	1	1	1.03	1.55	1.83	2	1.05	1.61	2.07
	2		1.04	1.56	1.88		1.04	1.61	2.05
	3		0.971	1.59	1.85		1.05	1.92	3.76
	4		1.02	1.6	1.98		1.05	1.87	3.40
	5		1.04	1.56	1.88		1.05	1.73	2.58

Table B.15 Results of 10 mg/mL

Solution	Trial	Mode	Mean/ μm	GSD	MMAD/ μm	Mode	Mean/ μm	GSD	MMAD/ μm
1	1	1	1.16	1.48	1.84	2	1.15	1.78	3.12
	2		1.14	1.52	1.93		1.15	1.69	2.63
	3		1.15	1.58	2.15		1.12	1.8	3.16
	4		1.16	1.54	2.03		1.14	1.64	2.38
	5		1.14	1.55	2.03		1.15	1.62	2.31
2	1	1	1.13	1.51	1.88	2	1.14	1.74	2.86
	2		1.12	1.65	2.38		1.13	1.69	2.58
	3		1.12	1.55	1.99		1.15	1.71	2.73
	4		1.13	1.62	2.27		1.13	1.74	2.84
	5		1.11	1.56	2.01		1.17	1.72	2.83
3	1	1	1.17	1.55	2.08	2	1.15	1.72	2.78
	2		1.13	1.63	2.31		1.14	1.57	2.10
	3		1.14	1.6	2.21		1.16	1.69	2.65
	4		1.14	1.52	1.93		1.11	1.64	2.31
	5		1.1	1.57	2.02		1.14	1.67	2.51

Table B.16 Results of 20 mg/mL

Solution	Trial	Mode	Mean/ μm	GSD	MMAD/ μm	Mode	Mean/ μm	GSD	MMAD/ μm
1	1	1	1.25	1.54	2.19	2	1.23	1.65	2.61
	2		1.26	1.48	2.00		1.25	1.63	2.56
	3		1.22	1.54	2.13		1.23	1.62	2.47
	4		1.23	1.57	2.26		1.25	1.72	3.02
	5		1.27	1.51	2.11		1.26	1.70	2.93
2	1	1	1.2	1.55	2.14	2	1.18	1.8	3.33
	2		1.25	1.51	2.08		1.2	1.73	2.96
	3		1.24	1.54	2.17		1.2	1.74	3.01
	4		1.32	1.54	2.31		1.18	1.75	3.02
	5		1.25	1.54	2.19		1.16	1.86	3.68
3	1	1	1.23	1.57	2.26	2	1.21	1.63	2.48
	2		1.25	1.53	2.15		1.21	1.69	2.76
	3		1.25	1.53	2.15		1.2	1.64	2.50
	4		1.24	1.59	2.36		1.21	1.66	2.61
	5		1.23	1.57	2.26		1.23	1.61	2.43

Table B.17 Results of 30 mg/mL

Solution	Trial	Mode	Mean/ μm	GSD	MMAD/ μm	Mode	Mean/ μm	GSD	MMAD/ μm
1	1	1	1.3	1.51	2.16	2	1.33	1.63	2.72
	2		1.31	1.49	2.11		1.33	1.66	2.87
	3		1.33	1.51	2.21		1.33	1.58	2.49
	4		1.34	1.59	2.55		1.3	1.61	2.57
	5		1.33	1.59	2.54		1.34	1.65	2.84

2	1	1	1.27	1.54	2.22	2	1.35	1.61	2.66
	2		1.31	1.51	2.18		1.36	1.67	2.99
	3		1.32	1.52	2.23		1.36	1.59	2.59
	4		1.38	1.52	2.34		1.33	1.6	2.58
	5		1.33	1.56	2.41		1.32	1.66	2.85
3	1	1	1.34	1.56	2.42	2	1.30	1.70	3.02
	2		1.39	1.53	2.39		1.36	1.63	2.78
	3		1.39	1.57	2.56		1.35	1.64	2.81
	4		1.36	1.6	2.64		1.37	1.62	2.75
	5		1.37	1.54	2.40		1.37	1.65	2.91

Table B.18 Results of 40 mg/mL

Solution	Trial	Mode	Mean/ μm	GSD	MMAD/ μm	Mode	Mean/ μm	GSD	MMAD/ μm
1	1	1	1.43	1.57	2.63	2	1.34	1.76	3.50
	2		1.33	1.55	2.36		1.38	1.74	3.46
	3		1.36	1.59	2.59		1.35	1.75	3.45
	4		1.48	1.54	2.59		1.39	1.72	3.36
	5		1.45	1.52	2.45		1.36	1.71	3.22
2	1	1	1.36	1.53	2.34	2	1.31	1.79	3.62
	2		1.39	1.59	2.65		1.31	1.82	3.84
	3		1.36	1.54	2.38		1.3	1.77	3.46
	4		1.39	1.61	2.74		1.31	1.77	3.48
	5		1.43	1.54	2.50		1.3	1.76	3.39
3	1	1	1.4	1.55	2.49	2	1.34	1.65	2.84
	2		1.38	1.55	2.46		1.41	1.68	3.16
	3		1.35	1.55	2.40		1.37	1.76	3.57
	4		1.4	1.61	2.76		1.34	1.72	3.24
	5		1.44	1.55	2.56		1.31	1.80	3.69

B.2 Deposition results

Results for the deposition of each plate are presented below.

Table B.19 Deposition results for Trial 1

Stage	-1	-0	1	2	3	4	5	6
Abs ₁	0.15689	0.10853	0.1196	0.19797	0.46267	0.64513	0.45524	0.21946
Abs ₂	0.13777	0.090388	0.12902	0.17442	0.46408	0.63055	0.4774	0.18649
Abs ₃	0.18189	0.13866	0.14519	0.20569	0.48463	0.6529	0.49754	0.24807
Abs	0.15885	0.11253	0.13127	0.19269	0.47046	0.64286	0.47673	0.21801
m/ mg	0.2387	0.1542	0.1884	0.3005	0.8077	1.122	0.8192	0.3468
DF/ %	6.002	3.875	4.736	7.555	20.30	28.22	20.59	8.717

Table B.20 Deposition results for Trial 2

Stage	-1	-0	1	2	3	4	5	6
Abs ₁	0.11032	0.11127	0.12618	0.24548	0.49909	0.64003	0.43339	0.17434
Abs ₂	0.13667	0.10495	0.12958	0.25064	0.55415	0.61558	0.39458	0.18216
Abs ₃	0.11119	0.13534	0.13934	0.23875	0.50207	0.63363	0.39212	0.16074
Abs	0.11939	0.11719	0.13170	0.24496	0.51844	0.62975	0.40670	0.17241
m/ mg	0.1667	0.1627	0.1892	0.3960	0.8953	1.099	0.69131	0.2635
DF/ %	4.315	4.211	4.897	10.25	23.18	28.44	17.89	6.821

Table B.21 Deposition results for Trial 3

Stage	-1	-0	1	2	3	4	5	6
Abs ₁	0.11999	0.081439	0.093594	0.26217	0.67349	0.79092	0.50955	0.17183
Abs ₂	0.12408	0.077432	0.12078	0.23882	0.68423	0.77622	0.49225	0.18145
Abs ₃	0.089251	0.05029	0.13226	0.21114	0.68268	0.75407	0.46688	0.14555
Abs	0.11111	0.06972	0.11554	0.23738	0.68013	0.77374	0.48956	0.16628
m/ mg	0.1516	0.07600	0.1597	0.3821	1.191	1.362	0.8426	0.2523
DF/ %	3.432	1.721	3.615	8.653	26.96	30.83	19.08	5.713

Table B.22 Deposition results for Trial 4

Stage	-1	-0	1	2	3	4	5	6
Abs ₁	0.12679	0.10191	0.20455	0.32199	0.75705	0.79701	0.51176	0.19951
Abs ₂	0.13339	0.097904	0.17536	0.31194	0.76598	0.7922	0.52351	0.20754
Abs ₃	0.11676	0.12097	0.17414	0.27272	0.76549	0.76125	0.49756	0.21695
Abs	0.12565	0.10693	0.18468	0.30222	0.76284	0.78349	0.51094	0.20800
m/ mg	0.1781	0.1439	0.2859	0.5005	1.342	1.379	0.8817	0.3285
DF/ %	3.534	2.856	5.673	9.932	26.62	27.37	17.49	6.518

Table B.23 Deposition results for Trial 5

Stage	-1	-0	1	2	3	4	5	6
Abs ₁	0.15721	0.11344	0.11741	0.25133	0.61427	0.79873	0.5529	0.24854
Abs ₂	0.14745	0.11433	0.108	0.26718	0.64367	0.83673	0.58591	0.25603
Abs ₃	0.1119	0.1327	0.084643	0.2749	0.65209	0.83021	0.58572	0.28189
Abs	0.13885	0.12016	0.10335	0.26447	0.63668	0.82189	0.57484	0.26215
m/ mg	0.2022	0.1681	0.1374	0.4316	1.111	1.449	0.9983	0.4274
DF/ %	4.106	3.412	2.790	8.762	22.56	29.43	20.27	8.676

Appendix C Arduino Code

```
// Example testing sketch for various DHT humidity/temperature sensors
// Written by ladyada, public domain

#include "DHT.h"

#define DHTPIN 5    // what digital pin we're connected to

// Uncomment whatever type you're using!
// #define DHTTYPE DHT11 // DHT 11
#define DHTTYPE DHT22 // DHT 22 (AM2302), AM2321
// #define DHTTYPE DHT21 // DHT 21 (AM2301)

// Connect pin 1 (on the left) of the sensor to +5V
// NOTE: If using a board with 3.3V logic like an Arduino Due connect pin 1
// to 3.3V instead of 5V!
// Connect pin 2 of the sensor to whatever your DHTPIN is
// Connect pin 4 (on the right) of the sensor to GROUND
// Connect a 1K resistor from pin 2 (data) to pin 1 (power) of the sensor

// Initialize DHT sensor.
// Note that older versions of this library took an optional third parameter to
// tweak the timings for faster processors. This parameter is no longer needed
// as the current DHT reading algorithm adjusts itself to work on faster procs.
DHT dht(DHTPIN, DHTTYPE);

void setup() {
  Serial.begin(9600);
  Serial.println("DHTxx test!");

  dht.begin();
}

void loop() {
  // Wait a few seconds between measurements.
  delay(2000);

  // Reading temperature or humidity takes about 250 milliseconds!
  // Sensor readings may also be up to 2 seconds 'old' (its a very slow sensor)
  float h = dht.readHumidity();
  // Read temperature as Celsius (the default)
  float t = dht.readTemperature();
  // Read temperature as Fahrenheit (isFahrenheit = true)
  float f = dht.readTemperature(true);
```

```

// Check if any reads failed and exit early (to try again).
if (isnan(h) || isnan(t) || isnan(f)) {
    Serial.println("Failed to read from DHT sensor!");
    return;
}

// Compute heat index in Fahrenheit (the default)
float hif = dht.computeHeatIndex(f, h);
// Compute heat index in Celsius (isFahreheit = false)
float hic = dht.computeHeatIndex(t, h, false);

Serial.print("Humidity: ");
Serial.print(h);
Serial.print(" %\t");
Serial.print("Temperature: ");
Serial.print(t);
Serial.print(" *C ");
Serial.print(f);
Serial.print(" *F\t");
Serial.print("Heat index: ");
Serial.print(hic);
Serial.print(" *C ");
Serial.print(hif);
Serial.println(" *F");
}

```

Appendix D Matlab Code

D.1 Code for Average Deposition Fraction

```
diameter = [0.26 0.55 1.2 1.9 3.2 4.4 6.5 8.6];
depofrac = [0 7.289097331 26.35480858 55.21062725 79.13475721 88.60448259 92.94662027
96.16170663];
xx = linspace(min(diameter),max(diameter),1000);
yy = spline(diameter,depofrac,xx);

figure(1)
semilogx(diameter,depofrac,'x')
hold on
semilogx(xx,yy)
hold off

y15 = 15.87;
x15 = interp1(yy,xx,y15)

y50 = 50;
x50 = interp1(yy,xx,y50)

y84 = 84.13;
x84 = interp1(yy,xx,y84)

x15 =

    0.8777

x50 =

    1.7645

x84 =

    3.7659
```

D.2 Code for Deposition Fraction of Trial 1

```
diameter = [0.26 0.55 1.2 1.9 3.2 4.4 6.5 8.6];
depofrac = [0 8.716942344 29.309441044 57.527657754 77.832508994 85.387539168
90.123165133 93.998419158];
```

```
xx = linspace(min(diameter),max(diameter),1000);
yy = spline(diameter,depofrac,xx);
```

```
figure(1)
semilogx(diameter,depofrac,'x')
hold on
semilogx(xx,yy)
hold off
```

```
y15 = 15.87;
x15 = interp1(yy,xx,y15)
```

```
y50 = 50;
x50 = interp1(yy,xx,y50)
```

```
y84 = 84.13;
x84 = interp1(yy,xx,y84)
```

```
x15 =
```

```
0.7957
```

```
x50 =
```

```
1.6994
```

```
x84 =
```

```
4.1655
```

D.3 Code for Deposition Fraction of Trial 2

```
diameter = [0.26 0.55 1.2 1.9 3.2 4.4 6.5 8.6];
depofrac = [0 6.82097117 24.71534648 53.15218231 76.32794985 86.57768241 91.474337465
95.685019672];
xx = linspace(min(diameter),max(diameter),1000);
yy = spline(diameter,depofrac,xx);
```

```
figure(1)
semilogx(diameter,depofrac,'x')
hold on
semilogx(xx,yy)
hold off
```

```
y15 = 15.87;  
x15 = interp1(yy,xx,y15)
```

```
y50 = 50;  
x50 = interp1(yy,xx,y50)
```

```
y84 = 84.13;  
x84 = interp1(yy,xx,y84)
```

```
x15 =
```

```
0.9189
```

```
x50 =
```

```
1.8152
```

```
x84 =
```

```
4.0645
```

D.4 Code for Deposition Fraction of Trial 3

```
diameter = [0.26 0.55 1.2 1.9 3.2 4.4 6.5 8.6];  
depofrac = [0 5.712954002 24.792150352 55.620712672 82.579218262 91.231822202  
94.847247081 96.568051726];  
xx = linspace(min(diameter),max(diameter),1000);  
yy = spline(diameter,depofrac,xx);
```

```
figure(1)  
semilogx(diameter,depofrac,'x')  
hold on  
semilogx(xx,yy)  
hold off
```

```
y15 = 15.87;  
x15 = interp1(yy,xx,y15)
```

```
y50 = 50;  
x50 = interp1(yy,xx,y50)
```

```
y84 = 84.13;
```

```
x84 = interp1(yy,xx,y84)
```

```
x15 =
```

```
0.9450
```

```
x50 =
```

```
1.7649
```

```
x84 =
```

```
3.3625
```

D.5 Code for Deposition Fraction of Trial 4

```
diameter = [0.26 0.55 1.2 1.9 3.2 4.4 6.5 8.6];  
depofrac = [0 6.518246622 24.012921032 51.382551872 78.004100082 87.936062486  
93.609485407 96.465629624];  
xx = linspace(min(diameter),max(diameter),1000);  
yy = spline(diameter,depofrac,xx);
```

```
figure(1)  
semilogx(diameter,depofrac,'x')  
hold on  
semilogx(xx,yy)  
hold off
```

```
y15 = 15.87;  
x15 = interp1(yy,xx,y15)
```

```
y50 = 50;  
x50 = interp1(yy,xx,y50)
```

```
y84 = 84.13;  
x84 = interp1(yy,xx,y84)
```

```
x15 =
```

```
0.9355
```

```
x50 =
```

1.8618

x84 =

3.8407

D.6 Code for Deposition Fraction of Trial 5

```
>> diameter = [0.26 0.55 1.2 1.9 3.2 4.4 6.5 8.6];  
depofrac = [0 8.676372516 28.944183976 58.370031676 80.930008876 89.692260378  
92.481819915 95.894366625];  
xx = linspace(min(diameter),max(diameter),1000);  
yy = spline(diameter,depofrac,xx);
```

```
figure(1)  
semilogx(diameter,depofrac,'x')  
hold on  
semilogx(xx,yy)  
hold off
```

```
y15 = 15.87;  
x15 = interp1(yy,xx,y15)
```

```
y50 = 50;  
x50 = interp1(yy,xx,y50)
```

```
y84 = 84.13;  
x84 = interp1(yy,xx,y84)
```

x15 =

0.8038

x50 =

1.6901

x84 =

3.5922