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Characterization of Perphenazine and Scopolamine Aerosols Generated Using the Capillary Aerosol Generator

Xihao Li
Virginia Commonwealth University

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CHARACTERIZATION OF PERPHENAZINE AND SCOPOLAMINE AEROSOLS
GENERATED USING THE CAPILLARY AEROSOL GENERATOR

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

by

XIHAO LI
B.S., Beijing Medical University, China, 1998
M.S., Peking Union Medical College, China, 2001

Directors: MICHAEL HINDLE, ASSOCIATE PROFESSOR
FRANK E. BLONDINO, ASSOCIATE PROFESSOR
PETER R. BYRON, PROFESSOR
DEPARTMENT OF PHARMACEUTICS, SCHOOL OF PHARMACY

Virginia Commonwealth University
Richmond, Virginia
May, 2006
This thesis is dedicated to
my precious parents and my dear husband.
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<thead>
<tr>
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<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>APCI</td>
<td>Atmospheric pressure chemical ionization</td>
</tr>
<tr>
<td>CAG</td>
<td>Capillary aerosol generator</td>
</tr>
<tr>
<td>CID</td>
<td>Collision induced dissociation</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>D</td>
<td>Capillary (tube) diameter</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimeter</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>DFN</td>
<td>Difference from nominal</td>
</tr>
<tr>
<td>FPF</td>
<td>Fine particle fraction</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>GSD</td>
<td>Geometric standard deviation (dimensionless)</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>ID</td>
<td>Internal diameter</td>
</tr>
<tr>
<td>L</td>
<td>Liter</td>
</tr>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid Chromatography-Mass Spectrometry</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mL/min</td>
<td>Milliliter per minute</td>
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<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>MMAD</td>
<td>Mass Median Aerodynamic Diameter</td>
</tr>
<tr>
<td>MOUDI</td>
<td>Micro Orifice Uniform Deposit (Cascade) Impactor</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>N_Re</td>
<td>Reynolds number</td>
</tr>
<tr>
<td>p</td>
<td>Partial pressure</td>
</tr>
<tr>
<td>p_s</td>
<td>Saturation vapor pressure</td>
</tr>
<tr>
<td>PG</td>
<td>Propylene glycol</td>
</tr>
<tr>
<td>R_0</td>
<td>Resistance at 0°C</td>
</tr>
<tr>
<td>Symbol</td>
<td>Term</td>
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<tr>
<td>--------</td>
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<tr>
<td>R&lt;sub&gt;T&lt;/sub&gt;</td>
<td>Resistance at temperature T</td>
</tr>
<tr>
<td>Rt</td>
<td>Retention time</td>
</tr>
<tr>
<td>RH</td>
<td>Relative humidity</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>S/N</td>
<td>Signal to noise ratio</td>
</tr>
<tr>
<td>SSP</td>
<td>Steady state power</td>
</tr>
<tr>
<td>t</td>
<td>time</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>V</td>
<td>Voltage</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume to volume</td>
</tr>
<tr>
<td>µg/mL</td>
<td>Microgram per milliliter</td>
</tr>
<tr>
<td>µm</td>
<td>Micrometer</td>
</tr>
<tr>
<td>η</td>
<td>Viscosity</td>
</tr>
<tr>
<td>ρ</td>
<td>Density</td>
</tr>
<tr>
<td>°C</td>
<td>Temperature degree centigrade</td>
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\textit{<Mathematical Symbols>}

<table>
<thead>
<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>(%)</td>
<td>percentage</td>
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<tr>
<td>(=)</td>
<td>equal to</td>
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<tr>
<td>(&gt;)</td>
<td>greater than</td>
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<tr>
<td>(\geq)</td>
<td>greater than or equal to</td>
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<td>(&lt;)</td>
<td>less than</td>
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<tr>
<td>(\leq)</td>
<td>less than or equal to</td>
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</table>
Abstract

CHARACTERIZATION OF PERPHENAZINE AND SCOPOLAMINE AEROSOLS
GENERATED USING THE CAPILLARY AEROSOL GENERATOR

BY Xihao Li, M.S.

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

Virginia Commonwealth University, 2006

Major Directors:  Michael Hindle, Ph.D., Associate Professor
                Frank E. Blondino, Ph.D., Associate Professor
                Peter R. Byron, Ph.D., Professor
                Department of Pharmaceutics

The characterization of perphenazine and scopolamine aerosols generated using
the capillary aerosol generator (CAG) was reported. Variables including steady state
power, the formulation vehicle, the drug concentration and the formulation flow rate
were studied for their effects on the chemical stability and particle size of these drug
aerosols.

Stability-indicating HPLC and LC-MS assays were developed and validated for
perphenazine and scopolamine, respectively. The chemical stability of each compound
was investigated under a variety of stress conditions and the structure of degradation products was proposed.

Perphenazine aerosols were generated from propylene glycol (PG) formulations with concentrations of 9, 48 and 90mM at formulation flow rates of 2.5 and 5.0 µL/s at a series of steady state powers. At higher aerosolization powers, the low concentration formulation (9mM) degraded with dehalogenation being the major pathway. The size of perphenazine aerosols was between 0.4 to 0.6 µm. Changing the solute concentration produced only small changes (~0.2 µm) in perphenazine aerosol particle size. The formulation flow rate did not significantly affect the aerosol size.

Scopolamine degraded significantly when aerosolized in PG formulations. It was possible to generate chemically stable scopolamine aerosols from ethanol formulations. Significant amounts of degradation products were formed only at or above 4.6 W at 5.0 µL/s. Hydrolysis and dehydration appeared to be the major degradation pathways at higher powers and low formulation flow rate. The MMAD of scopolamine aerosols was between 0.5 and 2.0 µm from 8, 20 and 40 mM formulations at 5.0 and 10.0 µL/s. The size of scopolamine aerosols increased as a function of increasing the solute concentration. Increasing the formulation flow rate increased the linear velocity of the spray, thus the Reynolds number was increased and smaller particles were generated.
CHAPTER I

INTRODUCTION

Antiemetics that merit rapid onset of action may be used as candidates for pulmonary administration. Efficient systemic delivery of these compounds can be achieved by delivering aerosols with aerodynamic diameters less than 5μm, which are essential for deep lung penetration. Two antiemetic agents: perphenazine and scopolamine were chosen as model compounds for this study.

The capillary aerosol generator (CAG) is a bench top aerosol generation device that is able to generate condensation aerosols or soft mist aerosols. The size of these aerosols ranges from 0.4 to 2μm, which are considered to be suitable for pulmonary administration (Hindle et al., 1998; Hong et al., 2002; Gupta et al., 2003; Shen et al., 2004).

Propylene glycol (PG) and ethanol have been used to formulate drug compounds for aerosolization by the CAG (Gupta et al., 2003; Hong, 2003; Shen, 2003; Hindle et al., 2004). Both vehicles have been used in pharmaceutical applications. Specifically for inhalation drug delivery, both vehicles have been used as either co-solvents or solvents in nebulizer or metered dose inhaler solutions to deliver drug compounds by inhalation. In comparing the two processes with the vehicles, with the CAG operation, PG can form condensation aerosols and total vaporization of the solution formulation is

1
required to efficiently form condensation aerosols. The PG solution formulation bears temperature exposure up to 500°C during aerosolization (Shen et al., 2004) and pyrolysis may be involved in the process. With ethanol as the vehicle, soft mist aerosols can be produced (Hindle et al., 2004). The temperature exposure should be lower than the case of PG due to the low boiling point of ethanol, and pyrolysis or thermal degradation of drug formulation could be reduced or avoided.

The purpose of this thesis is to investigate the effects of formulation variables and operational parameters on the stability and characteristics of drug aerosols generated by the CAG. In one study, drug aerosols are generated using a condensation process, where perphenazine (Figure 1.1.a), an oxidation prone compound was formulated with PG. In the other study, scopolamine (Figure 1.1.b), a thermally labile compound, was formulated with PG and ethanol, respectively, to assess the drug stability during aerosolization. Attempts were also made to optimize the formulation and operational parameters to control the drug particle size.

In this chapter, we will first briefly describe the operation of the CAG, then the mechanisms of aerosol generation will be discussed followed by the review of stability and assays of perphenazine and scopolamine.
Figure 1.1. Structures of (a) perphenazine and (b) scopolamine.
I.A. THE CAPILLARY AEROSOL GENERATOR

The capillary aerosol generator (CAG) was invented by Howell and Sweeney (Howell and Sweeney, 1998) and further developed by investigators at Virginia Commonwealth University (Hindle et al., 1998; Hong et al., 2002; Gupta et al., 2003; Shen et al., 2004; Hindle et al., 2004).

The device generates fine particle aerosols by pumping liquid formulations through a heated capillary tube. In its present manifestation, the electrical energy used to heat the capillary and vaporize the formulation passing through the stainless steel capillary is precisely controlled by computer maintenance of the capillary’s electrical resistance. Direct current is passed through the capillary, at the onset of each experiment, to achieve a preselected target resistance, $R_T$, that is proportional to the mean steady state temperature of the capillary (Hindle et al., 1998; Hong et al., 2002; Gupta et al., 2003; Shen et al., 2004). Thus, as larger values are selected for $R_T$, larger amounts of energy are passed through the capillary. In this manner, the physical characteristics of the aerosols can be changed (Hindle et al., 1998; Gupta et al., 2003; Shen et al., 2004) and temperatures to which formulations are exposed can be increased (Gupta et al., 2003; Shen et al., 2004).

During aerosol generation, the software measures total energy and steady state power, which can be used as a measure of the CAG performance for each experiment. The total applied energy is the cumulative energy supplied to the circuit during aerosolization, which is determined every 0.1s during the run. The steady state power, the energy provided per unit time to maintain a chosen resistance target (while the
liquid formulation is pumped at a fixed rate through the capillary), was determined and
defined throughout this thesis by Equation 1.1,

\[ SSP = \frac{(E_{90} - E_{50})}{(t_{90} - t_{50})} \]  

Equation 1.1

where \( E_{90} \) and \( E_{50} \) are the energies at 90% and 50% of the run time, respectively. \( t_{90} \) and
\( t_{50} \) are the time points at 90% and 50% of total run time. Steady state power is a
measurement of the power consumption of the CAG during a period of constant aerosol
production known as “the steady state of aerosol generation”. In this thesis, steady state
power (also called power) will be presented as one of the descriptions of the
experimental conditions.

1.B. CONDENSATION AEROSOLS

Condensation aerosols usually are monodispersed aerosols generated from
condensation aerosol generators (Sinclair and La Mer, 1949; Muir, 1965). The size of
these condensation aerosols often ranges from 0.05 to 10\( \mu \)m and they have been used in
aerosol instrument calibration and metal particle generation (Lesniewski and Koch,
1998). The CAG is able to generate fine particle condensation aerosols from PG
formulations or other glycol solvents, such as ethylene glycol, dipropylene glycol,
diethylene glycol, triethylene glycol, and tetraethylene glycol (Hong et al., 2002; Gupta
et al., 2003; Shen et al., 2004). A review on condensation aerosol generation and
specifically, condensation aerosols generated by the CAG will be presented.
I.B.1. Theory of Condensation Aerosol

The formation of condensation aerosols usually requires a supersaturated vapor and is initiated by the presence of nuclei that serve as sites for particle formation and growth. Saturation vapor pressure, $p_s$, is the equilibrium vapor pressure for a plane liquid surface at given temperature. The vapor is supersaturated if the saturation ratio (ratio of the partial pressure, $p$, of the vapor and the saturation vapor pressure) is greater than 1 (Hinds, 1999).

The relationship between the supersaturation ratio and the equilibrium droplet size has been shown by Kelvin equation (Equation I.2), where $\gamma$ is the surface tension,

$$\frac{p}{p_s} = \exp[4\gamma M / \rho RTd^*] \quad \text{Equation I.2.}$$

$M$ is the molecular weight, $\rho$ is the density of the liquid and $d^*$ is the Kelvin diameter. If a droplet size is smaller than $d^*$, its surface vapor pressure is higher than the surrounding vapor pressure therefore it will evaporate. If the droplet size is larger than $d^*$, its surface vapor pressure is lower than the surrounding and it will serve as nuclei to grow. When a droplet has the size equal to $d^*$ it is at equilibrium and will not grow nor evaporate at the saturation ratio $p/p_s$.

Homogeneous nucleation may occur when the saturation ratio is very high (Lesniewski and Koch, 1998). Due to the attractive forces between molecules the molecules collide to form clusters but disintegrate soon. When the vapor is supersaturated, the number concentration of clusters increases to a point at which they collide with the others more frequently. Once the size of the cluster is larger than the
Kelvin diameter, the cluster becomes stable and grows by condensation to form a large particle or droplet (Hinds, 1999).

Heterogeneous nucleation could occur at relatively low saturation ratios. In the case of particles or ions presented in the vapor, such foreign nuclei may serve as sites for condensation to take place (Hinds, 1999). The particle size and dispersity of the heterogeneous condensation aerosols can be controlled by the aerosolization conditions, such as the number and composition of the nuclei, and the flow rate of gas (Swift, 1967; Ristovski et al., 1998). Swift found that at low nuclei concentration ($<4 \times 10^4$/cm$^3$), the size of final particles formed from heterogeneous nucleation was independent of that concentration. At moderate nuclei concentration of $10^5$-10$^7$/cm$^3$, the size of particles and the concentration showed a relationship of $d \propto n^{-0.23}$. At high nuclei concentration ($>10^7$/cm$^3$), the diameter of particles formed from heterogeneous nucleation could be calculated by equation 1.3,

$$d_p = \left( \frac{6c_0}{\pi \rho_p n} \right)^{1/3}$$  

Equation 1.3.

where $d_p$ is the particle diameter, $c_0$ is the vapor mass concentration, $\rho_p$ is the density of the condensate and $n$ is the number concentration of nuclei (Swift, 1967).

The growth of particles in the cloud could then be condensation or coagulation dominated, depending on the number concentration of particles formed by nucleation (Lesniewski and Koch, 1998). At low number concentration, condensation growth dominates. The rate of condensation growth can be affected by the saturation ratio, particle size, particle size relative to gas mean free path, surface area, and temperature
(Hinds, 1999). At high number concentration coagulation mainly controls particle growth (Lesniewski and Koch, 1998; Kousaka et al., 2000).

Condensation aerosols can also experience evaporation as described by the Kelvin equation 1.1. Due to the high surface vapor pressure of particles smaller than the Kelvin diameter more molecules will evaporate than condense on the surface of such a particle and the result is size shrinkage (Hodgson, 1984).

1.B.2. Characteristics of Condensation Aerosols Generated by the CAG

1.B.2.a. Effect of energy on the condensation aerosol generated by the CAG

Shen has investigated the effect of energy on the generation of condensation aerosols from pure PG solutions by the CAG (Shen et al., 2004). Total vaporization of PG is required to form condensation aerosols. It was observed that the optimum energy range to aerosolize 35mg PG by the CAG is in agreement with the theoretical minimum energy requirement to heat the same amount of PG from room temperature to its boiling point and convert it into vapor. The aerosol formation under those conditions favored a homogeneous nucleation process due to the high supersaturation of PG. The size of PG aerosols was dependent on the energy used and showed minimum MMAD at an optimum energy range defined in that paper (Shen et al., 2004). The change of PG aerosol particle size at different energy ranges could be attributed to the change of the relative nucleation rate at those conditions (Shen et al., 2004).

The capillary wall temperature at fixed positions increased as a function of the increasing energy supply. The observed highest temperatures on the capillary wall were
between 320 and 540°C. Due to such high temperatures, thermal degradation of drug compounds pumped in solution through the CAG might well be involved in the aerosolization process (Shen et al., 2004).

1.8.2.b. Effect of solute volatility on CAG aerosols

In the operation of the CAG to form condensation aerosols from drug PG solutions, where the aerosol formation process could be heterogeneous nucleation dominant, the size of total and drug aerosols has been shown to depend on the physicochemical properties of the solute present in PG, such as the volatility. Aerosols formed by the CAG can be characterized with respect to the size of both the total aerosol and the size of the solute aerosol formed. The two size distributions are not necessarily the same. Three model drugs were investigated by Shen et al. in the CAG. These were benzil (BZ), caffeine and deoxycorticosterone (DOC) (Shen, 2003). The order of volatility of these three compounds are: BZ>caffeine>DOC. At the same solute concentrations of 12mM in PG, both the total aerosol and the aerosol size with respect to the solute, BZ had the largest MMADs. Both the total aerosol and the solute DOC aerosol sizes were suppressed significantly when compared with aerosolized solutions of BZ and caffeine, probably due to the low volatility of DOC (Shen, 2003). On addition of a solute with low volatility into PG solutions being aerosolized by the CAG, vaporized solute can form condensation nuclei in the vapor. These solute nuclei should promote heterogeneous condensation of PG vapor and the solute where homogeneous nucleation of PG was suppressed. Heterogeneous nucleation process became
predominant when the volatility of the solute was much lower than the solvent because presumably the saturation ratio required to nucleate low volatility solutes is larger (Nicolaon and Kerker, 1973), resulting in greater solute nucleation rates. Therefore, the size of both total aerosol and drug aerosol can decrease when low volatility drugs were employed.

1. B. 2.c. Effect of solute concentration on CAG aerosols

The effect of solute concentration has also been investigated (Gupta et al., 2003; Shen, 2003). Generally, at very low solute concentrations, the size of total aerosol and drug aerosol was not significantly different than seen with pure PG, probably because the heterogeneous nucleation process was not significant and homogeneous nucleation of PG aerosols was predominant in the cases studied. Solute dominant heterogeneous nucleation became significant at high solute concentrations. The size of total aerosol and the solute BZ aerosol decreased significantly at a BZ concentration of 0.5% (w/w) (Gupta et al., 2003). In the case of DOC in PG solutions, where the volatility of DOC is much lower than either BZ or PG, the reduction of total aerosol size was even more significant as solute concentration increased. Both the size of the total and the DOC aerosol were decreased and a difference between these two aerosol sizes started to show. It was assumed that larger numbers of solute nuclei were formed in the vapor when solute concentration was increased. The effect of DOC concentration in PG formulations can also be partly explained using equation 1.3. The particle size of DOC aerosols was found to be bimodal distributed at concentrations of 0.125 and 0.25%
(Gupta et al., 2003). The drug aerosols exhibited in two modes: a fraction less than 0.172µm and the other fraction depositing along with PG droplets. The particle size distribution of DOC aerosols returned to mono modality as the solute concentration increased to 0.5% and above. In general, depending on the volatility and concentration of the solute in the vehicle (or solvent) used in the CAG, the condensation aerosols generated by this technique may be mono or bimodal distributed.

1.B.2.d.  
**Effect of reservoir chambers on CAG aerosols**

It has been shown that the size of BZ aerosols generated by the CAG can be controlled by the varying the coagulation process (Hong et al., 2002). By using reservoir chambers with decreasing volumes, the coagulation of CAG generated aerosols could be promoted and the size of the aerosols increased overtime, due to the effects of initially high number concentrations.

1.B.2.e.  
**Effects of formulation flow rate and nozzle diameter on CAG aerosols**

The effects of formulation flow rate and nozzle diameter on the size of PG condensation aerosols have also been investigated (Cox et al., 2002). In their studies, the formulation flow rates ranged from 0.8 to 5.2mg/s. With increasing flow rate, the MMAD of PG aerosols first decreased and then leveled off to an almost constant value. At all formulation flow rates studied, the MMAD decreased as the nozzle diameter was reduced from 0.37 to 0.15mm. Hong has also investigated the effect of formulation flow rate on the size of BZ aerosols generated by the CAG (Hong, 2003). The BZ MMADs
were shown to decrease as the formulation flow rate increased from 2.6 to 3.9 and 5.2mg/s. When the mass flow rate was increased to 7.8mg/s, the MMADs showed no change compared to 5.2mg/s (Hong, 2003); these results were consistent with those described by Cox et al. (2002).

I.B.2.f.  Effect of relative humidity on the CAG condensation aerosols

Due to the hygroscopic characteristics of PG, the size of PG condensation aerosols could be affected by the relative humidity (RH) at certain temperatures. Hong has investigated the size change shown by caffeine in PG aerosols at controlled RH and temperature environments. The size growth of those aerosols at RH 96% compared RH 40% was a 20-40% larger MMAD seen by cascade impaction (Hong, 2003).

The final particle size of condensation aerosols from the CAG is a net result of nucleation, condensation, coagulation, and evaporation processes. Notably however, MMADs of CAG aerosols generated from PG formulations were in the range of 0.4-2μm, which are all considered suitable for respiratory drug delivery.

I.C.  AEROSOLS GENERATED BY SPRAY DRYING

During aerosolization of ethanol formulations by the CAG, the drug solution is heated and passes the capillary through a small orifice (ID=60μm) that can be considered to be a nozzle. The heating process causes the volume expansion of the solution inside the capillary, which increases the pressure inside the capillary during
aerosolization. To a certain extent, due to the increased pressure within the capillary, the solution is atomized through the orifice and sprays are produced upon the exit of the heated formulation from the capillary tip. The exiting spray entrains the ambient air and the evaporation of the vehicle (ethanol) is the dominant process (Hindle et al., 2004). The generation of soft mist aerosols from ethanol or ethanol-water mixtures by the CAG has been reported, for compounds such as cromolyn sodium, budesonide and insulin. The sizes of these drug aerosols were 0.04-2.6μm (Brown et al., 2003; Hindle et al., 2004) and generally, the aerosol generation from ethanol formulations by the CAG can be viewed as a process similar to spray drying. This section describes the procedure involved in spray drying as a background to this type of aerosol generation using a non-condensing vehicle.

Spray drying is extensively used in food and pharmaceutical industries to produce powders, agglomerates or granulates (Masters, 1991). Spray dried particles have been used for the production of pharmaceutical aerosols (Chew and Chan, 2002; Sham et al., 2004; Tarara et al., 2004). Spray drying involves atomization of liquid into spray followed by the drying of the spray in the drying medium. This process is suitable for the drying of heat-sensitive materials due to the short drying time and low droplet temperature often seen to occur (Masters, 1991).

I.C.1. **Atomization**

Atomization is the break up of bulk liquid into droplets. Pressure energy, centrifugal energy and gaseous energy (also called kinetic energy, Masters, 1991) are
the common forms of energy employed in atomization (Dombrowski, 1968). The aerosol generation process from ethanol formulations by the CAG in which the solutions are also heated during passage down the capillary, involves liquid atomization by pressure energy along with the evaporation of the solvent.

Droplets are formed from the disintegration of liquids by increasing the surface area of the liquid until it's unstable. The classic theory of liquid jet break up was established by Rayleigh (Rayleigh, 1894). He showed that a jet break up is the consequence of hydrodynamic instability (Rayleigh, 1879; Lin and Reitz, 1998) and developed a theory that stated that a circular cylindrical liquid jet was unstable if the disturbance of wavelength along its length was greater than the jet diameter. For a nonviscous liquid, the jet instability is at maximum when the wavelength of disturbance is 4.5 times the jet diameter (Marshall, 1954; Dombrowski, 1968). According to Rayleigh’s theory, for straight jets, the primary droplet size would be of the order of a few times the diameter of the undisturbed jet, assuming that no satellite drops were formed (Raleigh, 1879; Wong et al., 2004). Weber considered the effect of viscosity of the liquid and proposed an equation to correlate the optimum wavelength for jet disintegration with jet diameter, viscosity, surface tension and density of the liquid (Weber, 1931). A series of droplets with uniform diameter can be produced from a uniform thread but these are separated by a small fraction of satellite droplets. However, in a realistic atomization process, non-uniform threads will be produced and this results in a wide range of droplet size (Dombrowski, 1968).
The viscosity and surface tension of the liquid affect the disintegration of liquids during spray drying. The viscosity of the liquid resists the formation of droplets and the stability of the sheet was increased due to increased viscosity as shown by Dombrowski in a study using a fan spray nozzle (Dombrowski, 1968). The surface tension of the liquid resists the deformation of a sheet, but assists in droplet formation after the sheet has broken. The density of liquid has little influence on atomization and its effect can be neglected (Dombrowski, 1968). The viscosities of PG and ethanol at 25°C are 40.4 and 1.09cP, respectively (Gomez and Solimo, 2002). Compared with PG, ethanol has a much lower viscosity and low surface tension of 22.4mN/m at 20°C, therefore under the same operational conditions the formation of droplets is easier with ethanol than it is with PG formulations.

In a pressure atomizer, the liquid is passed through an orifice under pressure. When liquids pass through a swirl-type pressure nozzle, a conical sheet can also be produced. The form of the conical sheet depends on the working pressure. The pressure at which the various conical forms take place depends on the nozzle design and physical properties of the liquids, especially viscosity and surface tension (Dombrowski, 1968). The cone angle can be affected by the divergence of the spreading surface, liquid viscosity and the orifice diameter (Dombrowski, 1968).

The size distribution of droplets formed by spray driers is dependent on the specific spray nozzle design and the prediction of droplet size is often based on empirical studies. Duffie has investigated the effects of nozzle diameter, feed rate and viscosity on the size distribution of droplets generated from a stainless-steel, small-bore
capillary tube in a vertical, cylindrical spray dryer (Duffie and Marshall, 1953). An equation (Equation 1.4) to correlate the geometric mean drop size with the nozzle diameter and Reynolds number was deduced from the experimental data, where $D_G$ is the geometric mean drop size,

$$D_G = 36D_0^{0.56}N_{Re}^{-0.1}$$  

Equation 1.4.

$D_0$ is the nozzle diameter (both in $\mu$m) and $N_{Re}$ the Reynolds number of the fluid. Similar equations have been proposed although they differ in the exponents of the nozzle diameter and the Reynolds number. If $N_{Re}$ of 3000 is assumed for the ethanol sprays generated at CAG operational conditions, using the nozzle diameter of 60$\mu$m, the geometric mean diameter of the initial droplets of approximately 160$\mu$m can be calculated from Equation 1.4.

1.C.2. **Drying of droplets and sprays**

Drying of droplets involves vaporization of the liquid and its diffusion from the droplet surface. It is a process with heat and mass transfer between the droplet and its surrounding drying medium, in most cases the air. Considering a boundary layer at the liquid-vapor interface, the driving force is the concentration and temperature difference for vapor and heat transfer, respectively. The rate of vapor/heat transfer depends on the mass/heat transfer coefficient, the concentration/temperature gradient and the transfer surface area (Dombrowski, 1968).

The rate of evaporation and contact time of the droplet with surrounding air affect the amount of drying of a droplet (Dombrowski, 1968). For a commercial spray
dryer, the contact time with the drying medium is dependent on the falling velocity of the droplet and the length of the flow path in the drier. In the case of CAG operation, it is assumed that at optimum operational conditions, the solvent evaporates very fast and completely, as soon as the droplet is in contact with the surrounding air.

During the evaporation process, as soon as the solvent is leaving the droplet, deposition or crystallization of solute may start (Dombrowski, 1968). A crust may eventually form once the solubility of the solute is reached and as the net concentration of solute increases. This can increase the resistance to solvent mass transfer and retard the evaporation process. Subsequent evaporation of the crust can cause the dried particles to present in different physical forms, depending on the porosity and rheological properties of the crust (Crosby and Marshall, 1957; Charlesworth and Marshall, 1960; Dombrowski, 1968).

Drying of sprays is a much more complex situation because of the interactions between the droplets, the size of the sprays, the movement between the sprays and the drying medium. Basic theories of droplet drying may still be applied to sprays and approaches have been made to investigate the drying of sprays considering the size distribution and the interactions between droplets (Marshall, 1954; Manning and Gauvin, 1960).

Theoretically, the size of the dried particles can be related to the size of the droplets by the mass balance of the solid content before and after drying process. Equation 1.5 could be used to correlate the initial droplet (dp₁) and final droplet size (dv₂).
\[
\frac{dv}{dp} = \frac{\rho_l(1+W_2)}{\rho_2(1+W_1)} \left(1 + \frac{1}{W_1}\right) \left(1 + \frac{1}{W_2}\right)^{1/3}
\]

Equation IV.5.

where \(\rho_l\) is the density of the liquid droplet, \(\rho_2\) is the final particle density, \(W\) is the water or liquid content. Therefore, \(1/(1+W_1)\) and \(1/(1+W_2)\) are the initial and final solids content, respectively. When evaporation is complete, \(1+W_2\) is close to 1 due to the low final liquid contents. Nonetheless, calculation by this equation requires more accurate data on primary particle size and effective density (Duffie and Marshall, 1953). A study investigating the initial droplet and final particle size of spray dried lactose particles showed an almost linear relationship between the initial droplet and final particle size (Elversson, et al., 2003).

The nozzle design, feed rate, liquid viscosity, solute concentration, and drying temperature can affect the atomization and drying procedure and therefore affect the final size of particles generated by spray drying. It was found that in general, for solutions as the solute concentration decreased, the dry particle size decreased (Crosby and Marshall, 1958; Fell and Newton, 1971). In most cases, increasing the inlet temperature increases the dried particle size but exceptions have been observed (Crosby and Marshall, 1958; Fell and Newton, 1971; Broadhead et al., 1993). Liquid feed rate can also affect the final particle size for certain nozzles (Fell and Newton, 1971; Broadhead et al., 1993).

I.D. STABILITY AND ANALYSIS OF TWO ANTIEMETIC AGENTS-
PERPHENAZINE AND SCOPOLAMINE
Antiemetic agents are compounds used in the treatment of nausea, vomiting and motion sickness. They are currently available for administration by oral, parental, rectal and transdermal. However, oral antiemetic therapy often fails and injections are often deemed to be undesirable. A rapid onset of systemic action associated with pulmonary delivery is often considered desirable for this class of compounds.

Perphenazine and scopolamine (Figure I.1.a and b), being antidopaminergics and anticholinergics, respectively; are strong antiemetic agents. In this study, perphenazine and scopolamine were chosen as model compounds to investigate the feasibility of aerosol generation of antiemetics using the CAG.

As described previously, the aerosol generation by the CAG is achieved by heating the solution formulations. The stability of drugs during this heating and aerosolization process is thus a matter of concern. One of the objectives of this project is to investigate the stability of the drugs during aerosolization therefore, a review on the present state of knowledge on the stability and assays of both compounds are presented below.

I.D.1. **Stability of perphenazine and other phenothiazine derivatives**

Perphenazine (Figure 1.1.a), 4-[3-2(-Chloro-10H-phenothiazin-10-yl)propyl]-1-piperazineethanol, is a phenothiazine derivative. Phenothiazine derivatives generally possess a phenothiazine ring and either a two-carbon or three-carbon side chain connecting to the nitrogen of the phenothiazine ring. This class of compounds has been used as antipsychotics, antiemetics, and sedatives for over fifty years.
Previous work has shown that phenothiazine derivatives are oxidation prone compounds (Wunderlich and Stark, 1970; Bornschein et al., 1972; Roseboom and Perrin, 1977a; Underberg, 1978a and b). When phenothiazine was stored in an oxygen-saturated ethanol solution at 80°C for four days, several oxidation products were formed. Oxidation occurred at 5-S, 3-C and 7-C on the phenothiazine ring (Roseboom and Fresen, 1975). It was also reported that trifluoperazine degraded completely to trifluoperazine sulfoxide in 0.67% hydrogen peroxide solution after being treated for 30 minutes in a boiling water bath (El-Gindy et al., 2002). The oxidation degradation pathway appeared to be first order with respect to the parent compound (Roseboom and Perrin, 1977a; Underberg, 1978c; El-Gindy et al., 2002). The rate of oxidation could be affected by pH, oxygen, the presence of metal ions, and antioxidants (Underberg, 1978c).

The stability of phenothiazine derivatives at elevated temperatures has been investigated mostly along with oxidation reactions. The temperatures of the reactions were generally between 65 and 100°C (Roseboom and Fresen, 1975; Underberg, 1978b; Roseboom and Forch, 1979; El-Gindy et al., 2002). Oxidation on the sulphur and the other positions on the phenothiazine ring were observed. Roseboom investigated the degradation kinetics of 10-methylphenothiazine and 10-acetylphenothiazine in acidic medium at 85°C in the presence of saturated oxygen for 5 and 3 days, respectively. Under all circumstances the degradation appeared to be first order with respect of the parent compound. It appeared that the introduction of methyl and acetyl group reduced the oxidation rate constants as compared with phenothiazine, which was probably due
to the steric effect of the groups introduced at the nitrogen of the phenothiazine ring or the electron withdrawing properties of acetyl group (Roseboom and Perrin, 1977a; Roseboom and Forch, 1979). In another study on the kinetics of oxidation degradation of a series of phenothiazine derivatives with different C-2 substituents at 90°C during a period of 12 days, it was found that the reactions were apparently first order and there was a relationship between the rate constant and the substituents. It was concluded that the C-2 substituents such as Cl, CF₃, and SO₂N(CH₃)₂ decrease the reactivity of the molecule compared with the unsubstituted compound, while the SC₂H₅ and COCH₂CH₂CH₃ groups enhance the compound reactivity (Pawelczyk et al., 1975).

Photochemical degradation of phenothiazines has been studied extensively (Felmeister and Discher, 1964; Huang and Sands, 1964; Schley, 1986; Abdel-Moety et al., 1996). When chlorpromazine hydrochloride aqueous solution was irradiated at 253.5nm with UV light, semiquinone free radicals were formed. Chlorpromazine sulfoxide was produced from the free radicals in both absence and presence of dissolved oxygen. Phenolic acid and several other unidentified compounds were also formed and appeared to be the degradation products of chlorpromazine sulfoxide. This photoreaction appeared to be a zero-order process (Felmeister and Discher, 1964). In another study on the photostability of chlorpromazine hydrochloride, upon irradiation with UV light for three days, several degradation products were formed. Both the N-oxide and S-oxide were separated from the mixture (Huang and Sands, 1964). More recently, in a photostability study of trifluoperazine in bulk form and pharmaceutical formulations under both normal lab conditions (fluorescent and daylight) and UV light
exposures, several photodegradation products were formed including trifluoperazine sulfoxide and another degradant characterized by mass spectrometry (Abdel-Moety et al., 1996).

The degradation mechanism of phenothiazines following oxidation and light exposure has been investigated previously (Felmeister and Discher, 1964; Roseboom and Perrin, 1977b; Underberg, 1978b). The proposed degradation scheme is shown in Figure I.2. Under certain conditions, a reversible reaction of losing an electron from the parent compound (I) to give a semiquinone free radical occurs. The free radical is stable only at strong acidic conditions and it can lose another electron to become a phenothionium ion (II), which disproportionate to give the parent compound (I) and compound III. Compound III is hydrolyzed to give the sulfoxide (IV).

Perphenazine was therefore chosen as an oxidation-prone molecule to investigate whether it could be successfully aerosolized using a traditional condensation method from PG (Gupta et al., 2003; Shen et al., 2004). Potentially, the molecule’s breakdown could occur both during the heating and vaporization process as well as by interactions with air during condensation aerosol formation. The assessment of breakdown product and the degree to which this occurs was believed to be a useful benchmarking study for evaluating the feasibility of delivery of drug aerosols by the CAG.
Figure 1.2. Degradation pathways of phenothiazines (I) parent compound, (II) free radical phenothionium, (III) phenothiazium, (IV) sulfoxide of the parent compound.
I.D.2. Analysis of phenothiazines

I.D.2.a. HPLC methods

Phenothiazine derivatives have strong UV absorption due to the presence of the phenothiazine ring. Several HPLC methods have been developed for the assay of perphenazine, other phenothiazine derivatives, their degradants and metabolites. An HPLC method for the determination of perphenazine and its sulfoxide has been published (Beaulieu and Lovering, 1986). However, the ability of this method to separate perphenazine from its photo degradation products was not evaluated. Mandal et al. reported an HPLC/UV method using a reversed phase column to determine perphenazine in rabbit plasma (Mandal and Ace, 1993). The method was rapid and sensitive (LOQ 0.05μg/ml) although its capability of resolving perphenazine from its metabolites was not shown. Foglia et al. developed an HPLC assay using a coulometric detector for the quantitative determination of perphenazine and its metabolites in human plasma (Foglia et al., 1995). The method was able to quantitate perphenazine and its major known metabolites. Stability indicating HPLC assays have been reported for the determination of trifluoperazine with its photo degradants and oxidation products (Abdel-Moety et al., 1996; El-Gindy et al., 2002).

I.D.2.b. LC-MS methods

LC-MS and LC-MS/MS methods have been reported for the study of phenothiazines in plasma and urine (Pieniaszek et al., 1989; Kumazawa et al., 2000). Pieniaszek et al. developed an LC-MS method with thermal spray interface to
quantitatively analyze unlabeled and labeled moricizine utilizing selected-ion monitoring (SIM). The method was validated and found to be appropriate for the assay of moricizine in human plasma (Pieniaszek et al., 1989). In a study of 11 phenothiazine derivatives including perphenazine in human body fluids, LC-MS/MS with electro spray ionization (ESI) in a positive mode was used (Kumazawa et al., 2000). It was found that all compounds showed base peaks of [M+H]^+ molecular ions. The MS of the compounds and their product ions were also reported. The base peaks of the product ions appeared to be due to the side chains liberated from the phenothiazine ring for all compounds except flupentixol. Selected reaction monitoring (SRM) using the base peaks of the product ions allowed the quantification of these compounds in plasma and urine at very low concentrations.

Other analytical methods such as GC and GC-MS have also been used for the determination of phenothiazines (Larsen and Naestoft, 1975; McKay et al., 1982; Ventura et al., 2002). Generally, these methods required extensive sample preparation and sometimes derivatization of the samples prior to the assays was needed.

1.D.3. **Stability of scopolamine and other tropane alkaloids**

Scopolamine (Figure 1.6) belongs to a class of tropane alkaloids that was first isolated from plant sources. It has been used therapeutically for over 150 years as an antispasmodic, antiemetic and antivertigo agent.
Due to the presence of an ester bond scopolamine is easily hydrolyzed. It may degrade in basic or acidic solutions to become tropic acid and (dl) scopoline (Holmes and Manske, 1950). It was also reported that scopolamine hydrobromide was stable in acidic solutions for a few days but degraded almost completely in basic solutions within half an hour. Under heating conditions, dehydration may take place to form aposcopolamine and atropic acid as degradation products (Holmes and Manske, 1950; Windheuser et al., 1972). When atropic acid is heated in contact with air or boiled in water solutions, isatropic acid can be produced due to dimerization reactions (Fittig and Wurster, 1879).

The structure of atropine (Figure I.3), also a tropane alkaloid, is different to scopolamine in the absence of the epoxide group. The degradation pathways of atropine have been reported (Holmes and Manske, 1950; Lund and Hansen, 1978; Jira and Fabini, 1982). The degradation scheme is shown in Figure I.3. Under acidic/basic and heating conditions, competitive and consecutive reactions of hydrolysis, dehydration, and dimerization are involved in the degradation process. Due to the similarity of structures of atropine and scopolamine, under similar conditions, these reactions could occur for scopolamine.
Figure 1.3. Degradation pathways of atropine
Scopolamine was therefore chosen as a thermally labile molecule to investigate whether it could be successfully aerosolized using condensation method from PG (Gupta et al., 2003; Shen et al., 2004) and soft mist aerosol generation method from ethanol (Hindle et al., 2004). Scopolamine could degrade during the heating and vaporization process of condensation aerosol formation. Previous work done in our lab has shown scopolamine degradation during aerosolization from PG solutions (Poklis et al., unpublished data). The breakdown of the molecule could also occur during the heating and atomization process of soft mist aerosol generation from ethanol solutions. The assessment of breakdown product and the degree to which this occurs using both vehicles was believed to be a useful bench marking study for evaluating the feasibility of aerosol delivery for thermally labile compounds by the CAG.

I.D.4. Analysis of scopolamine and tropane alkaloids

I.D.4.a. HPLC Methods

Tropane alkaloids usually have low UV absorptivity. HPLC UV methods have been developed for the determination of scopolamine, other tropane alkaloids and their degradant mixtures. Due to the presence of both acids and bases in the degradation products, ion-pairing agents were usually used in the mobile phase for better resolution of structurally similar tropane alkaloids and their degradation products (Brown and Sleeman, 1978; Richard and Andermann, 1984; Oshima et al., 1989). The ion-pairing agents used in these studies were mostly non-volatile therefore the HPLC methods could not be readily used for LC-MS assays that require volatile mobile phase
constituents. HPLC methods using sodium acetate buffer, phosphate buffer with methanol or acetonitrile mixtures have also been successfully developed to determine tropane alkaloids and the degradation products with UV detectors (Lund and Hansen, 1978; Pennington and Schmidt, 1981; Fliniaux et al., 1993). An HPLC method with a photo-diode array (PDA) detector at UV 230nm has been developed and validated for the determination of scopolamine hydrobromide and atropine sulfate in pharmaceutical formulations. However, the author mentioned this method was not appropriate for stability indicating purposes (Ceyhan et al., 2001).

I.D.4.b. LC-MS Methods

LC-MS methods for analyzing tropane alkaloids have been published previously (Auriola et al., 1991; Steenkamp et al., 2004). Auriola et al. reported that the thermo spray mass spectra of both scopolamine and atropine showed abundant [M+H]^+ ions. The fragmentation of both alkaloids could be increased by increasing the capillary temperature or by collision induced dissociation with ion repeller potentials. The major fragmentation peaks of both compounds were discussed (Auriola et al., 1991). An LC-MS (ESI+) method has been used in the determination of scopolamine and atropine in a toxic plant (Steenkamp et al., 2004). The LOD of scopolamine was decreased to 100pg/ml with ZMD MS detector compared to 1µg/ml with a PDA detector. Both analytes showed [M+H]^+ ions at cone voltage of 25V. By increasing the cone voltage from 25 to 50V, in-source collision-induced dissociation could be induced, which resulted in characteristic fragmentation pattern of each alkaloid serving as one more
confirmation of the analyte. Both LC-MS methods used basic ammonium acetate buffer and acetonitrile as mobile phase and a reversed phase column. Their abilities to determine the metabolites or degradation products were not reported.

I.D.4.c. GC and GC-MS Methods

GC methods have been used to determine tropane alkaloids (Solomon et al., 1969; Windheuser et al., 1972). GC-MS and GC-MS/MS methods have been reported in the determination of tropane alkaloids in plasma (Saady and Poklis, 1989; Deutsch et al., 1990; Oretel et al., 1996; Namera et al., 2002). Generally, there are two problems associated with sensitive GC methods. The derivatization of the analytes usually is needed before analysis and decomposition of the actives under GC conditions are likely to occur.

This research project was designed to investigate the effects of formulation and operational variables that may affect the stability and aerosol characteristics of these two specific compounds, perphenazine and scopolamine; during aerosolization by the CAG. These variables were the vehicles, the steady state power input, the solute concentrations, and the formulation flow rate/run time. Stability indicating HPLC assays were developed and validated for each compound and then, the drug stability under a range of typical aerosolization conditions was investigated. In addition, the particle sizes of the drug aerosols were characterized under selected “drug-stable” aerosolization conditions.
CHAPTER II

HYPOTHESES

The aim of this research project was to investigate the formulation and operating parameters that may affect the chemical stability of the perphenazine and scopolamine CAG aerosols. In addition, their effects on the aerodynamic particle size distribution of the drug aerosols was reported. The following hypotheses were tested:

Hypothesis 1.

The mechanism of aerosol generation with CAG will be dependent on the physicochemical properties of the vehicle. With PG as the vehicle, aerosol formation should be controlled by vaporization, nucleation and condensation processes. With ethanol as the vehicle, drug aerosols may be formed by simultaneous and/or consecutive atomization and evaporation of solutions. Modifying the vehicle will alter the aerosolization mechanism thus enable the control of chemical stability of the drug and the size of drug aerosols. The temperature at which these two processes take place will differ due to the boiling points of the vehicles and will contribute to the chemical stability and drug degradation profiles.

Hypothesis 2.
At a fixed formulation flow rate, increasing the steady state power increases the temperature of the aerosolization process and may increase the possibility of thermal drug degradation.

**Hypothesis 3.**

The formulation flow rate may affect the chemical stability of the drug aerosols. Varying the formulation flow rate will affect the transit time through the capillary and therefore the temperature exposure. The lower formulation mass flow rates will have relatively longer heat exposure and may be expected to show greater thermal degradation.

**Hypothesis 4.**

Both solute concentration and formulation flow rate may affect the particle size of drug aerosols. Solute concentration affects both the nucleation process for condensation aerosols and the evaporation process in the generation of soft mist aerosols; thus both may affect the final particle size. Formulation flow rate affects the linear velocity of the vapor jet or spray and may also change the final particle size.
CHAPTER III

DEVELOPMENT OF METHODS TO ASSESS THE CHEMICAL STABILITY OF PERPHENAZINE IN PG AEROSOLS

III.A. INTRODUCTION

The heating process of CAG aerosol generation poses a challenge during drug product development, and aerosolization conditions must be selected to minimize solute decomposition due to overheating. Perphenazine, a phenothiazine derivative with strong antiemetic properties, was believed to represent vulnerable molecules for aerosolization with the CAG, previous findings had shown that similar phenothiazines were easily oxidized and were light sensitive (Felmeister and Discher, 1964; Roseboom and Fresen, 1975; Abdel-Moety et al., 1996; El-Gindy et al., 2002).

In order to assess the feasibility of aerosol generation of perphenazine in PG formulations by the CAG, in this chapter, the development and validation of a stability-indicating HPLC method and an LC-MS method for perphenazine was described.
Forced degradation of perphenazine under different conditions was investigated to produce potential degradation products. Potential degradation products were evaluated by mass spectrometry and their structures proposed.

III.B. MATERIALS AND INSTRUMENTATION

III.B.1. Chemicals

Perphenazine, USP grade, was purchased from Spectrum Chemical Co. (Gardena, CA). Propylene glycol (PG), USP grade, was purchased from Fisher Scientific Co. (Fairlawn, NJ). HPLC grade methanol, acetonitrile and ammonium acetate were purchased from Fisher Scientific Co. USP/FCC grade acetic acid was also purchased from Fisher Scientific Co. 10% and 30% palladium-carbon was purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Silica gel preparative TLC plates were also purchased from Sigma-Aldrich, Inc.

III.B.2. Instrumentation

III.B.2.a. HPLC System

The final HPLC system consisted of a Waters 2695 separation module and a 2995 photodiode array (PDA) detector (Waters Corp., Milford, MA). UV absorbance was monitored at 256nm. The chromatographic separations were performed on a Waters
Nova-Pak C-8 column (3.9×150mm, 4μm). The mobile phase was 0.01M ammonium acetate/acetic acid buffer pH 3.0 and acetonitrile (52:48, v/v). The LC system was operated isocratically at a flow rate of 1.0mL/min at room temperature. The run time was 16 minutes.

III.B.2.b. LC-MS System

The developed LC-MS method was performed on a Waters 2695 separation module as above, with a Waters 996 PDA inline to a Micromass ZMD 4000 single quadruple mass spectrometer (Waters Corp., Milford, MA). The HPLC conditions were the same as described in section III.B.2.a. The electrospray ionization (ESI) MS studies were carried out in positive ionization mode. For each injection, two scans were performed at cone voltages of 35 and 60V. Full scan data was obtained over a mass range of m/z 100-500 and analyzed by Waters Masslynx Software (Version 3.5, Waters Corp., Milford, MA).

III.C. METHODS

III.C.1. Standard Solution and HPLC Analysis

A stock solution of perphenazine, 268.0μg/mL, was diluted with mobile phase to make a working solution of 26.8μg/mL. The working standard was diluted serially to
prepare the standard curve, with concentrations ranging 0.01-26.80μg/mL in mobile phase. Triplicate injections of 60μL were made and the response ($A_{256nm}$) plotted versus concentration. The limit of detection (LOD) and limit of quantitation (LOQ) were determined based upon the relative standard deviation (RSD) of the peak area at the lowest concentrations. The injection repeatability was tested at a concentration of 5.36μg/mL over six replicate injections. The precision and accuracy was tested using perphenazine in mobile phase solution with a concentration of 16.0μg/mL. The solution was injected three times per day on three different days. The “inter-day” precision was calculated as the RSD of the peak area. The accuracy was expressed as the percentage difference from the nominal value (%DFN) by comparing the measured concentrations with the nominal value. The unknown concentration of the active in the sample was determined using the calibration curve by comparing the peak area of the unknown with the standard at 256nm.

III.C.2. Mass Spectrometry Conditions

To identify the appropriate ionization conditions for perphenazine, standards were prepared in mobile phase solutions and were infused via the ESI or APCI probe into the mass spectrometer using a syringe pump (Model 44; Harvard Apparatus, Holliston, MA) at a flow rate of 20μL/min. Both APCI and ESI probes were
investigated in positive ionization mode during the optimization. Cone voltages were changed over a range of 10 to 100V (Table III.1). Mass spectra were acquired using the test ionization conditions following 1 min equilibration. The data was collected over a mass range of 100 to 500 Daltons in the MCA mode (the accumulation of ion intensities of each scan) for 1 minute.

III.C.3  Forced Degradation Studies of Perphenazine

III.C.3.a  Perphenazine stored in acidic and basic solutions

Perphenazine was stored in acidic and basic solutions to monitor possible acid or base catalyzed reactions. Due to its poor solubility in water, perphenazine was first dissolved in a small volume of methanol, then diluted with 0.1N HCl or 0.1N NaOH (nominal perphenazine concentrations were 15.86±0.54μg/mL, 8% methanol by volume, n=3). The samples were stored at room temperature (24±2°C) and in an oven (50±2°C) for 3 days. Aliquots were withdrawn over a period of 3 days, neutralized and assayed using the HPLC method described above.
Table III.1. Ionization conditions used to optimize the ionization of perphenazine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>APCI</th>
<th>Settings</th>
<th>Parameter</th>
<th>ESI</th>
<th>Settings</th>
</tr>
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<td>Capillary Voltage (kV)</td>
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<td></td>
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<tr>
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<td></td>
<td>Cone voltage (V)</td>
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<tr>
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<td></td>
<td>Extraction Voltage (V)</td>
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<tr>
<td>RF lens Voltage (V)</td>
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<td></td>
<td>RF lens Voltage (V)</td>
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<tr>
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<td>Source Block, Temperature (°C)</td>
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<tr>
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<td>Nitrogen Gas Flow Rate (L/hr)</td>
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<td></td>
<td>Nitrogen Gas Flow Rate (L/hr)</td>
<td>450</td>
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</tr>
</tbody>
</table>
III.C.3.b Perphenazine stored in hydrogen peroxide solution

A methanolic perphenazine solution was diluted with 0.5% \( \text{H}_2\text{O}_2 \) aqueous solution to perform the oxidation study (nominal perphenazine concentration 25.6\( \mu \)g/mL, 1% methanol by volume) and stored at room temperature (24±2°C) in the dark. Aliquots were withdrawn over a period of five hours and analyzed using the HPLC method above.

III.C.3.c Perphenazine in PG solutions stored in the dark

Perphenazine in PG solutions (nominal perphenazine concentrations were 4.88±0.23mg/mL, \( n=3 \)) was stored at room temperature (24±2°C) for two months in the dark to evaluate formulation stability in sealed containers under likely aerosol inhaler reservoir conditions. At the time of assay, approximately 9mg aliquots of solution were accurately weighed, diluted with 5.0mL mobile phase, and assayed using the HPLC method described above.

III.C.3.d Perphenazine in PG solutions stored under fluorescent light

At room temperature (24±2°C), perphenazine in PG solutions (nominal perphenazine concentrations were 4.99±0.14mg/mL, \( n=3 \)) was exposed to lights (Panasonic cool white light, F15T8/CW, Japan; Sylvania cool white light, F15T8/CW,
GTE, USA) for 27 hours in sealed clear glass containers. The light strength at the surface of the solutions was estimated to be approximately 3200Lux measured by a light meter (VWR Traceable light meter, Ridgeport, NJ). Aliquots were weighed accurately at different time points so that approximately 9mg of each solution was diluted with 5.0mL mobile phase, and analyzed by the HPLC method described above.

III.C.3.e. Perphenazine stored at elevated temperature conditions

Perphenazine stability at elevated temperature conditions was tested using a differential scanning calorimeter (DSC) (DSC-7, Perkin-Elmer Inc., Wellesley, MA) to heat the powder sample. Approximately 1.0mg perphenazine powder was placed in an aluminum pan and sealed. The pan and the contents were heated in the DSC. The initial temperature was 25°C. The temperature increased from 25°C to 50°C at 100°C/min and was held at 50°C for one minute. The temperature was then increased to 400°C at a rate of 100°C/min. The total heating time was approximately 4.5 minute. Following heating, the residue was dissolved into 10.0mL methanol, diluted and analyzed by the HPLC method described above.
III.C.4. **Dose Capture Experiments Following Perphenazine in PG**

**Aerosolization**

Perphenazine in PG solutions with a concentration of 12.4 mM was aerosolized using a 28 gauge, 44 mm long stainless steel capillary. The details on the operation of CAG aerosol generation will be described in Chapter IV.C.3. The formulation flow rate was 5.0 μL/s and the run time was 10 s, resulting in a nominal 50 μL delivered volume. Sham experiments (n=3) were performed by collecting the formulation as it was pumped from the capillary tip, in the absence of heating, to determine the actual delivered dose. Different target resistances were employed to provide a range of steady state powers to aerosolize the formulation. Experiments were performed in quintuplicate (n=5) and the steady state power consumed was recorded at each target resistance. Dose capture experiments were performed to determine the recovery of the perphenazine after aerosolization as described previously (Blondino et al., 2002). Briefly, a glass trap (Research Glass, Richmond, VA) was filled with 10.0 mL collection solvent (HPLC mobile phase in this case), and housed in an ice-water bath to capture the generated aerosol (Figure III.1). An airflow of 0.5 L/min was drawn over the capillary tip, to condense the aerosol and pull it through the collection solvent. The trap was connected to an air line with an additional back-up filter inline (SKC Inc., Eighty Four, PA) in each case. After each sample collection, the collection solvents (mobile
phase in this case) were used to wash the whole apparatus thoroughly and were analyzed by the HPLC method described above.

**III.C.5. Synthesis of 2-[4-(3-Phenothiazin-10yl-propyl)-piperazin9]-ethanol (Figure III.2.c)**

Perphenazine (1.6g) was hydrogenated in acetic acid (50mL) in the presence of anhydrous sodium acetate (Kishimoto and Uyeo, 1964). In addition, increasing quantities of palladium-carbon were added and the mixture hydrogenated over an 11-day period (additions were made as follows, to the filtrate, after filtration of the mixture: 0.13g of 10%, 0.13g of 30%, 0.9g of 30% and 1.0g of 10% palladium-carbon at time zero, 72, 120 and 192 hours). At 264 hours, the mixture was filtered and the filtrate evaporated to dryness under reduced pressure. A solution of 0.1N HCl (50mL) was added to dissolve the residue and the solution heated under reflux for 12hrs. The solution was evaporated to dryness under reduced pressure and about 200mg of the residue loaded on several silica gel preparative TLC plates (20×20cm, GF 254) and chromatographed using CH₂Cl₂:MeOH 95:5. The fraction with the lowest Rf value (Rf=0.15) was scraped from the plates, pooled and eluted from the gel using CH₂Cl₂:MeOH 90:10. The eluent was evaporated and 20mg of the (approximately
Figure III.1. Schematic set up of dose capture experiments
Figure III.2. Structures of (a) perphenazine (b) perphenazine sulfoxide (c) 2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol and (d) prochlorperazine
50mg) residue was dissolved in 1.5mL water with pH adjusted to 12 with concentrated NaOH solution. This solution was then extracted three times into diethyl ether, the ether phase pooled and evaporated under reduced pressure to give approximately 13mg (0.035mmol) 2-[4-(3-phenothenazin-10-yl-propyl)-piperazino]-ethanol (Figure III.2.c).

III.D. RESULTS AND DISCUSSION

III.D.1. Stability-indicating HPLC Method

The HPLC method was developed from a previously established HPLC method for prochlorperazine analysis (Figure III.2.d, X. Li et al., unpublished data). The HPLC method for prochlorperazine was developed from a USP method (USP IXX, 1999), which used a C-18 column and a mobile phase of 0.01M ammonium acetate (pH 4.5 adjusted by acetic acid), acetonitrile and methanol (48:39:13, v/v/v). During method development, the C-18 column was changed to a C-8 column in order to reduce the retention time of prochlorperazine. The mobile phase components were simplified to ammonium acetate buffer and acetonitrile. The pH of ammonium acetate buffer was decreased from 4.5 to 3.0 to reduce the tailing of the peak. By testing the prochlorperazine samples generated at high temperatures, the ratio of organic and aqueous phase was varied to achieve separation. The HPLC analysis for prochlorperazine used a mobile phase of 0.01M ammonium acetate (pH 3.0 adjusted by
acetic acid) and acetonitrile (60:40, v/v) operated at a flow rate of 1.0mL/min. This method was able to provide separations of prochlorperazine and its degradation products formed at high temperatures up to 400°C using the DSC.

Due to the similarity of the structures between perphenazine and prochlorperazine, changes in the ration of organic and aqueous phase of the mobile phase were made for perphenazine study. The final HPLC method for perphenazine analysis used a mobile phase of 0.01M ammonium acetate buffer pH 3.0 and acetonitrile (52:48, v/v) on a C-8 column. The retention time of perphenazine was 3.8±0.1 min. The validation of the stability-indicating properties of this method will be discussed in section III.D.3.

The HPLC assay method for perphenazine was found to be linear in the range 0.03-26.8μg/mL. The correlation coefficient of the regression line was 0.9999 (9 concentrations measured in triplicate). The calibration curve is shown in Figure III.3. The LOD for perphenazine was 0.01μg/mL and the LOQ was 0.03μg/mL (the RSD of the peak area at these two concentrations was 6.3% and 3.0%, respectively). The injection repeatability (RSD, n=6) was found to be 0.25%. The “inter-day” precision (RSD, n=9) was 0.5%. The accuracy (%DFN, n=9) for perphenazine analysis was found to be 2.9%.
Figure III.3. Calibration curve of perphenazine (n=3, error bars are SD)
III.D.2. Optimization of Mass Spectrometry Conditions

Generally, for most compounds, using LC-MS analysis with an ESI or APCI probe, the parent ion \([M+H]^+\) will be visible in the mass spectra (Watson, 1997). The \([M+H]^+\) ion is useful for the identification of unknown compound because the molecular weight of the analyte can be easily obtained. Fragment ions are also useful for identification purposes as the characteristic pattern of fragments may provide structural information (Niessen, 1999).

In this thesis, the mass spectra of the degradation products were used to deduce possible structures. However, the ionization conditions for perphenazine need to be chosen where \([M+H]^+\) and fragment ions can be obtained with relatively high ion intensities. For a single quadrupole mass spectrometer, the commonly used method to generate fragment ions is to increase the cone voltage to promote collision-induced dissociation. In this study, cone voltages were varied using both APCI and ESI ionization probes to determine the optimum conditions.

Figure III.4 (a-d) shows the mass spectra following perphenazine infusion at different cone voltages using APCI probe in positive ion mode. At cone voltages of 10, 30 and 50V, the parent ion \([M+H]^+\) of 404 was present (Figure III.4.a-c). The ion intensities of the parent ion were shown to increase as a function of increasing the cone voltage from 10 to 50V. The \([M+H]^+\) ion of 404 was shown to be the base peak at 50V.
(Figure III.4.c). As the cone voltage was increased to 100V, the parent ion [M+H]^+ intensity was significantly reduced (Figure III.4.d) and extensive fragmentation of perphenazine parent ion was observed. The major fragment ions of m/z 143, 171, and 246 were produced at cone voltages above 10V and the intensities increased as the cone voltage increased over the range of 10 to 100V (Figure III.4.b-d). The fragmentation pattern of perphenazine will be fully described in section III.D.3.

Figure III.5. (a-f) shows the mass spectra of perphenazine using ESI probe at different cone voltages from 10 to 80V. The parent ion [M+H]^+ of 404 was present under all the investigated conditions. Its ion intensity was shown to increase as a function of increasing the cone voltage over a range of 10 to 35V. The parent ion intensity was then decreased as a function of further increasing the cone voltage from 35V to 80V. The major fragment ions of m/z 143, 171 and 246 were produced at cone voltages above 50V and their intensities were shown to increase as the cone voltage increased, which was due to the collision-induced dissociation of perphenazine at high cone voltages. The [M+H]^+ ions were the base peaks at cone voltage of 35V and 50V (Figure III.5.c and d). The fragment ions of m/z 171 and 143 were the base peaks at cone voltage of 60V and 80V, respectively (Figure III.5.e and f).

Observing the mass spectra of perphenazine under APCI and ESI conditions, it was found that the parent ion was generated using both ionization methods. However,
the major fragmentation ions of 143, 171 and 246 were observed at higher intensities using the ESI probe at cone voltages above 50V. Therefore, the ESI probe in positive ion mode was chosen for the LC-MS study. Using the ionization conditions described above, at cone voltage of 35V and 60V base peaks of the parent ion and fragment ions of perphenazine could be produced, respectively. These two cone voltages were used in LC-MS studies in order to obtain structural data from both the parent ion and the fragment ions. However, it should be noted that the ionization conditions employed were those optimized for perphenazine. Therefore, these conditions may not necessarily be the optimized conditions for each individual degradation product.

Figure III.6 shows the mass spectra of perphenazine standard solution under LC-MS (ESI) conditions. Similar to the mass spectra of directly infused perphenazine, the parent ion of m/z 404 was the base peak at cone voltage of 35V, corresponding to the protonated molecular ion (MW=403) (Figure III.6.a). The ion with an m/z of 406 had an ion intensity of approximately 1/3 of the parent ion. This was due to the isotope effect of chlorine (Watson, 1997). This chlorine isotope effect would prove useful when investigating the structural characteristics of the degradation product. The ions of m/z 420 and m/z 436 were of low intensities at 35V (<10%), and were probably due to the oxygen adducts of the parent ion formed under LC-MS conditions. At 60V, its predominant fragment ions were m/z 143 and 171, corresponding to the piperazine side
Figure III.4. Mass spectra obtained using APCI following perphenazine infusion in mobile phase at cone voltages of (a) 10V, (b) 30V, (c) 50V, and (d) 100V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
Figure III.5. Mass spectra obtained using ESI following perphenazine infusion in mobile phase at cone voltages of (a) 10V, (b) 20V, (c) 35V, (d) 50V, (e) 60V, and (f) 80V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
Figure III.5 continued. Mass spectra obtained using ESI following perphenazine infusion in mobile phase at cone voltages of (a) 10V, (b) 20V, (c) 35V, (d) 50V, (e) 60V, and (f) 80V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
Figure III.6. Mass spectra of perphenazine standard (Rt=3.8min) under LC-MS conditions at cone voltages of (a) 35V and (b) 60V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
chain. Another fragment ion at m/z 246 also showed the chlorine isotope pattern, corresponding to the tricyclic ring. The parent ion and fragmentation pattern of perphenazine was in agreement with the literature data (Kumazawa et al., 2000).

III.D.3. Products of Degradation and Validation of Stability-indicating Assay for Perphenazine

Perphenazine degradation products could be produced under oxidation, photo irradiation, and elevated temperatures. In the studies throughout this thesis, it was assumed that the extinction coefficients of the degradation products were the same as the parent compound. This assumption may not be true for some of the cases, however, it is generally accepted when extinction coefficient of degradation product is not known. The concentrations of degradation products were expressed as the percentage of its peak area to the total peak area of each chromatogram. In this thesis, a degradation product was defined as a major degradation product when the percentage of its peak area is higher than 0.5% of the total peak area.

III.D.3.a. Perphenazine stored in acidic and basic solutions

Following exposure to acidic and basic conditions for 3 days in dark conditions, the apparent concentrations of the active were expressed relative to their values at time
zero. The mean recoveries (SD) (% remaining) in acidic solutions were 100.25 (1.79) and 99.06 (2.72) at 24±2°C and 50±2°C, respectively. In basic solutions the corresponding mean (SD) (% remaining) were 96.75 (2.21) and 97.29 (1.91), respectively. No chromophoric degradation products with areas larger than 0.5% of the total were detected at all the conditions described above. No co-elution of degradation products with perphenazine was found by LC-MS studies. Perphenazine was stable in acidic or basic solutions in the dark for three days.

III.D.3.b. Perphenazine stored in hydrogen peroxide solution

Figure III.7 shows the UV and total ion chromatogram obtained after 30min exposure in 0.5% H₂O₂ solution. Two major peaks were observed besides the solvent front. Perphenazine was present in the sample with a retention time of 3.9min. The mass spectra of this peak are shown in Figure III.8. Comparing the mass spectra of this peak with the mass spectra of perphenazine standard (Figure III.6), it was found that the two were not significantly different. This indicated that there was no co-elution of degradation product with perphenazine in H₂O₂ solution. The major degradation product (compound A) had a retention time of 1.5min and its mass spectra are shown in Figure III.9. There was a base peak of m/z 420 at cone voltage at 35V. The peak of m/z 442 might be due to sodium adducts. At the cone voltage of 60V, predominant
fragmentation ions were at 143, 171 and 246, similar to perphenazine. Compound A was confirmed to be perphenazine sulfoxide (Figure III.2.b) by comparing chromatograms and mass spectra with those of (±) perphenazine sulfoxide from USP (Rockville, MD; Table III.2). One other minor degradation product (compound B, <0.5% of total area at all sample times) was observed (retention time 2.6min). Its mass spectra are shown in Figure III.10 but it was not identified. The degradation of perphenazine followed an apparent first-order process (k=0.063min⁻¹, Figure III.11), with kinetics similar to the degradation of phenothiazine and promethazine in oxygen saturated media (Roseboom and Perrin, 1977a; Underberg, 1978).

**III.D.3.c. Perphenazine in PG solutions stored in the dark**

The apparent concentrations of perphenazine after 60 days dark storage in PG at room temperature were expressed relative to their values at time zero. The mean recovery (SD) (% remaining) was 102.10 (2.84). No chromophoric degradation products with areas larger than 0.5% of the total were detected. The LC-MS studies showed no co-elution of degradation products with perphenazine in the samples. Perphenazine was stable when formulations suitable for aerosolization in the CAG (solutions in PG) were stored in the dark.
Figure III.7. The UV and total ion chromatogram of perphenazine in 0.5% H₂O₂ solution after 30min (a) UV chromatogram at 256nm, (b) total ion chromatogram at cone voltage of 35V, and (c) single ion chromatogram at m/z 420.
Figure III.8. Mass spectra of perphenazine (Rt=3.9min) in 0.5% H₂O₂ solution at cone voltages of (a) 35V (b) 60V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
Figure III.9. Mass spectra and structure of compound A (Rt=1.5 min) formed in 0.5% H₂O₂ solution at cone voltages of (a) 35V (b) 60V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
Figure III.10. Mass spectra of compound B (Rt=2.6min) formed in 0.5% H₂O₂ solution at cone voltages of (a) 35V (b) 60V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
Figure III.11. Perphenazine degradation profile in 0.5% H₂O₂ solution (n=1)
III.D.3.d. *Perphenazine in PG solutions stored under fluorescent light*

Figure III.12 shows the UV and total ion chromatograms of perphenazine stored in PG solutions under fluorescent lights after 48hr. Two major degradation products (Compounds C and D) were formed. The HPLC method provided baseline separation of the active from these two major degradation products.

Compound C had a retention time of 2.5min. Its mass spectra are shown in Figure III.13. At a cone voltage of 35V, compound C showed a base peak of 370, which is 34 mass units lower than perphenazine (Figure III.13.a). The lack of the chlorine isotope pattern, observed for the base peak ion, indicated the loss of chlorine from the aromatic ring upon photo irradiation. At increased cone voltage, the predominant fragment ions were m/z 143 and 171, corresponding to the piperazine side chain; and m/z 212, corresponding to the tricyclic ring without chlorine. The cleavage of the carbon-chlorine bond has been reported in the literature for chloroaromatic compounds under photolytic conditions (Pinhey and Rigby, 1969). In addition, the dehalogenation reaction of phenothiazine derivatives has been induced during the FAB-MS process (Edom et al., 1991). It has also been reported that dechlorinated metabolites appeared in plasma following administration of chlorpromazine (Sgaragli et al., 1986).
Figure III. 12. The UV and total ion chromatogram of perphenazine in PG under fluorescent light after 48hr. (a) UV chromatogram at 256nm, (b) total ion chromatogram at cone voltage of 35V, (c) single ion chromatogram at m/z 370, (d) single ion chromatogram at m/z 444.
Figure III.13. Mass spectra and structure of compound C (Rt=2.5 min) formed in PG solutions under fluorescent lights at cone voltages of (a) 35V (b) 60V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
The dechlorinated compound C was proposed to be 2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol (Figure III.2.c). In order to test the hypothesis, chemical synthesis of the proposed molecule was performed. The melting point of synthesized product was 93-95°C (Literature m.p. 98-99°C, GB patent, 1960). \(^1\)H-NMR and \(^1^3\)C-NMR spectra were shown in Figure III.14 &15, respectively. \(^1\)H-NMR (CDCl\(_3\)) \(\delta\): 1.96 (m, 2H, H-2'), 2.85 (m, 12H), 3.61 (t, J=5.4Hz, 2H, H-8'), 3.94 (t, J=6.9Hz, 2-H, H-9'), 6.92 (m, 4H, aromatic), 7.14, (m, 4H, aromatic). \(^1^3\)C-NMR (CDCl\(_3\)) \(\delta\): 24.0 (C-2'), 44.8 (C-1'), 52.4 (C-5'), 52.9 (C-6'), 55.2 (C-3'), 57.3 (C-8'), 58.7 (C-9'), 115.0 (C-1, C-9), 121.9 (C-4, C-6), 124.6 (C-2, C-8), 126.7 (C-3, C-7), 127.0 (C-4a, C-5a), 144.8 (C-9a, C-10a). The m.p., \(^1\)H-NMR and \(^1^3\)C-NMR spectra (Figure III.14) confirmed the synthesis of 2-[4- (3-phenothiazin-10-yl-propyl)-piperazino]-ethanol (GB patent, 1960; Post et al., 1980; Abadi et al., 1999). Analysis of the product by the LC-MS method indicated that it had the same retention time, UV spectrum and mass spectra as the photodegradation product C (Table III.2). Compound C was confirmed to be 2-[4-(3-[phenothiazin-10-yl -propyl]-piperazino)-ethanol.

Compound D had a retention time of 1.8min and its mass spectra are shown in Figure III.15. It had a base peak of 444 at cone voltage of 35V; once more there was no chlorine isotope pattern observed. At an increased cone voltage of 60V, fragment ions
Figure III.14. (a) $^1$H-NMR and (b) $^{13}$C-NMR of 2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol
Figure III.15. Mass spectra and proposed structure of compound D (Rt=1.8min) formed in PG solutions under fluorescent lights. Cone voltage was at (a) 35V (b) 60V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
Figure III.16. Perphenazine degradation profile in PG solution stored under fluorescent light (n=3, error bars are SD)
of m/z 143, 171 and 286 were observed. Again, m/z 143 and 171 corresponded to the liberation of the side chain as seen with perphenazine and compound C. A fragment ion of m/z 286 may correspond to the tricyclic ring. Compared with compound C, the m/z of both the parent ion and fragment ion of the tricyclic ring for compound D was 74 mass units higher, that suggested the nucleophilic substitution of propylene glycol to the tricyclic ring during photo irradiation. Although the position of the substitution was not determined it is probably on the C-2 or C-3 position, as the activation of the site (C-2) meta to the electron withdrawing group on an aromatic system under photo conditions has been reported (Horsepool, 1976), and for phenothiazines, C-3 and C-7 have the highest electron density enabling their reaction with a nucleophile (Roseboom and Perrin, 1977b).

The degradation of perphenazine in PG solutions under fluorescent lights followed an apparent zero-order process (k=82.34µg/mL•hr; Figure III.16) with kinetics similar to those reported for chlorpromazine hydrochloride under UV irradiation (Felmeister and Discher, 1964).

III.D.3.e. Perphenazine stored at elevated temperature conditions

Perphenazine stability in solid state at elevated temperatures was investigated as a worst-case scenario for perphenazine under high temperature exposure. Significant
amounts of degradation products of perphenazine were formed under the investigated high temperature conditions. There were approximately 60% of the active remained in the samples. Compounds A and C as described above were present in the DSC samples. Other major degradation products—compounds E, F, G, H, I, and J were detected both by HPLC and LC-MS method as shown in Figure III.17. Their retention times, $\text{UV}_{\text{max}}$, base peaks and fragment ions in mass spectra are shown in Table III.2. The HPLC method permitted the separation of perphenazine with most of the degradation products (Figure III.17.a). No attempts were made to achieve baseline separation of the active from degradation products—compound F and G because they were not formed in the dose capture samples even at high power levels as described in Section III.D.4.

Compound E (Rt 3.5min) showed a base peak of m/z 155 at cone voltage of 35V (Figure III.18.a). Its parent ion probably belonged to m/z 388 with chlorine isotope pattern observed. At cone voltage of 60V, fragment ion of m/z 127 became the base peak (Figure III.18.b). The structure of compound E was not proposed based on its mass spectra.

Compound F (Rt 3.7min), had a base peak of m/z 372 at cone voltage of 35V with chlorine isotope pattern present (Figure III.19.a), indicating that dechlorination had not taken place. At an increased cone voltage of 60V, fragment ion of m/z 214 with chlorine isotope pattern was observed, which may correspond to the tricyclic ring
Table III.2. The retention time, UV maximum absorbance, and characteristics of mass spectra of the potential degradation products and standards

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>UV&lt;sub&gt;max&lt;/sub&gt; (nm)</th>
<th>Base peak&lt;sup&gt;a&lt;/sup&gt; (m/z)</th>
<th>Fragment Ions&lt;sup&gt;b&lt;/sup&gt; (m/z) (relative intensities)</th>
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<tr>
<td>A</td>
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<td>238, 275, 300, 343</td>
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<td>171 (52), 143 (48), 246 (25)</td>
</tr>
<tr>
<td>B</td>
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<td>420</td>
<td>143 (100), 246 (85), 171 (70)</td>
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<tr>
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<td>256</td>
<td>155</td>
<td>127 (100), 155 (82)</td>
</tr>
<tr>
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<td>238, 262, 298</td>
<td>372</td>
<td>214 (98)</td>
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<tr>
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<td>127 (100), 155 (76)</td>
</tr>
<tr>
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<td>233</td>
<td>198 (100), 248 (45), 233 (45)</td>
</tr>
<tr>
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<td>145</td>
<td>145 (100), 162 (5), 186 (3)</td>
</tr>
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<td>171 (93), 143 (81), 246 (25)</td>
</tr>
<tr>
<td>2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol</td>
<td>2.7</td>
<td>254</td>
<td>370</td>
<td>171 (100), 143 (98), 212 (29)</td>
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<tr>
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<td>256</td>
<td>374</td>
<td>141 (100), 113 (98), 246 (22)</td>
</tr>
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</table>

Data collected at cone voltage of<sup>a</sup>35V and<sup>b</sup>60V.
Figure III.17. Perphenazine at elevated temperatures up to 400°C. (a) UV chromatogram at 256nm, (b) total ion chromatogram at cone voltage of 35V, single ion chromatogram at (c) m/z 233, (d) m/z 360, (e) m/z 370, to be continued.
Figure III.17. Continued. Perphenazine at elevated temperatures up to 400°C. Single ion chromatogram at (f) m/z 372, (g) m/z 374, (h) m/z 388, (i) m/z 420
Figure III.18. Mass spectra of compound E (Rt=3.5 min) formed at elevated temperatures up to 400°C. Cone voltage was at (a) 35 V (b) 60 V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
Figure III.19. Mass spectra and proposed structure of compound F (Rt=3.7 min) formed at elevated temperatures up to 400°C. Cone voltage was at (a) 35 V (b) 60 V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
(Figure III.19.b). The ions of m/z 157 and m/z 207 could not be assigned. Compared with perphenazine, both the base peak at 372 and fragment peak at 214 were 32 units less than the base peak and fragment ion due to tricyclic ring of perphenazine, which suggested the loss of sulfur on the tricyclic ring. Desulfurization of organosulfur compounds under thermal conditions has been reported previously (Cava et al., 1960; Nicolaou et al., 1980). The proposed structure of compound F was shown in Figure III.19.

Compound G, had a base peak of m/z 360 and m/z 127 at cone voltage of 35 and 60 V, respectively (Figure III.20). The ion of m/z 360 might be the parent ion of F and was 44 mass units lower than the parent ion of perphenazine. The ions of m/z 372 and m/z 388 might be the oxygen adducts under the LC-MS conditions, which had also been observed for the perphenazine standard (Figure III.6). The fragment ion of m/z 127 was also 44 mass units less than m/z 171, which was the fragment ion of the side chain liberated from perphenazine. This may indicate the loss of the hydroxyethyl group of the piperazine ring at high temperatures compared to perphenazine. Hopkinson et al. has reported the elimination of methyl groups attached to heteroatoms, such as N and P, at high temperatures (Hopkinson et al., 1976). The proposed structure of compound G is shown in Figure III.20.
Compound H, had a base peak of 374 at cone voltage of 35V with chlorine isotope pattern observed and it was probably the parent ion of compound H (Figure III.21.a). Similarly, ions at m/z 390 and 406 might be due to the oxygen adducts. At cone voltage of 60V, fragment ions of 113, 141 and 246 were present (Figure III.21.b). The fragment ion at m/z of 246 was considered to be due to the tricyclic ring, which was the same as perphenazine, suggesting that the phenothiazine ring was intact. The fragment ions at m/z of 113 and 141 probably corresponded to the side chain. They were 30 mass units less than the m/z 143 and 171, which were formed by the side chain liberation of perphenazine, respectively. This may indicate the loss of the hydroxymethyl group from the piperazine ring resulting in the known compound prochlorperazine. The mass spectra, retention time and UV spectrum of compound H was in agreement with prochlorperazine (Figure III.2.d, and Table III.2).

Compound I had a retention time of 7.2min. Its mass spectra showed a base peaks at m/z 388 at cone voltage of 35V (Figure III.22.a). At cone voltage of 60V it showed fragment ions of m/z 127 and m/z 155 (Figure III.22.b). Compound J (Rt 13.4min) had a base peak at m/z 233 at cone voltage of 35V. Ions of m/z 198, 243 and 248 were observed in the mass spectra collected at cone voltage of 60V (Figure III.23). The structures of these two compounds were not proposed based on the LC-MS results.
Figure III.20. Mass spectra and proposed structure of compound G (Rt=4.8 min) formed at elevated temperatures up to 400°C. Cone voltage was at (a) 35V (b) 60V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
Figure III.21. Mass spectra and structure of compound H (Rt=5.9min) formed at elevated temperatures up to 400°C. Cone voltage was at (a) 35V (b) 60V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
Figure III.22. Mass spectra of compound 1 (Rt=7.2 min) formed at elevated temperatures up to 400°C. Cone voltage was at (a) 35V (b) 60V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
Figure III.23. Mass spectra of compound J (Rt=13.4 min) formed at elevated temperatures. Cone voltage was at (a) 35V (b) 60V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
III.D.4. Degradation of Perphenazine in CAG Condensation Aerosols

The degradation of perphenazine during aerosolization was investigated by collecting the condensation aerosols using dose capture apparatus. The mass of perphenazine recovered after aerosolization was expressed relative to the mass recovered from sham experiments performed in the absence of heating. The dose capture results are shown as % recoveries in Table III.3 at different powers. More studies on perphenazine recoveries following aerosolization will be shown and discussed in Chapter IV.

Two degradation peaks (compounds C and K) could be detected in dose capture samples at powers around 8.1W while a third degradation peak (Compound A) was detected when aerosol samples were generated at powers of approximately 9.8W. Compound C was increased at 9.8W compared with samples generated at lower powers. At the highest tested power of 12.6W, 3 degradation peaks (Compounds A, C and K) could be detected in all five dose capture samples where the mean peak areas for A, C and K accounted for 0.13±0.02, 0.28±0.03, 0.15±0.01% of the total peak area, respectively. The LC-MS method was employed to evaluate these degradation products, their retention times, UV maximal, and mass spectra (Table III.2). The other degradation products formed at elevated temperature conditions as described in section III.D.3.b were not detected in the dose capture samples. The HPLC method was able to
provide baseline separation of perphenazine with its degradation products formed during aerosolization at high powers. Compounds A and C were found to be perphenazine sulfoxide and 2-[4- (3-phenothiazin-10-yl-propyl)-piperazino]-ethanol, respectively, by comparing with the standards described above. Figure III.24 shows the mass spectra of compound K. It had a base peak of m/z 145 (35V cone voltage) and additional peaks of m/z 162 and 186 may have been due to ammonia and acetonitrile adduct ions, respectively. At a cone voltage of 60V, the intensities of m/z 162 and 186 decreased, while m/z 145 remained the most abundant. The chlorine isotope pattern was observed for each of the three major peaks and this compound was not identified based on the LC- MS results. Overall, oxidation and dehalogenation appeared to be the main degradation pathways at high power operation conditions following aerosolization.
Table III.3. Perphenazine recoveries of dose capture experiments from 12.4mM perphenazine in PG solutions

<table>
<thead>
<tr>
<th>Steady State Power (W)</th>
<th>Perphenazine Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.10 (0.12)</td>
<td>100.81 (1.46)</td>
</tr>
<tr>
<td>8.60 (0.07)</td>
<td>100.84 (2.47)</td>
</tr>
<tr>
<td>9.10 (0.14)</td>
<td>98.89 (0.86)</td>
</tr>
<tr>
<td>9.76 (0.12)</td>
<td>95.27 (2.04)</td>
</tr>
<tr>
<td>11.02 (0.07)</td>
<td>92.57 (2.15)</td>
</tr>
<tr>
<td>12.64 (0.21)</td>
<td>93.53 (0.91)</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD). n=5
Figure III.24. Mass spectra of compound K (Rt=1.7min) formed in dose capture samples. Cone voltage was at (a) 35V (b) 60V. Y-axis unit is the relative ion intensity normalized to the base peak intensity of 100%.
III.E. CONCLUSIONS

Stability-indicating HPLC and LC-MS methods for the analysis of perphenazine were developed and validated. The methods were capable of resolving perphenazine from its major degradation products formed under a wide variety of conditions. In solution, perphenazine sulfoxide was the major oxidation product formed in dilute hydrogen peroxide and two degradation products were formed when dissolved perphenazine was exposed to light in PG. In the solid state, perphenazine degraded to several degradation products at elevated temperatures. These degradants were tentatively identified using LC-MS and one of their structures was confirmed by the synthesis of 2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol. When the CAG was operated at higher power levels (>9.8W), minor degradation products could be detected in collected aerosols. These were largely consistent with those seen to result from oxidation and photolysis of perphenazine in solution, showing that oxidation and dehalogenation appeared to be the main degradation pathways.
CHAPTER IV

CHARACTERIZATION OF PERPHENAZINE IN
PROPYLENE GLYCOL AEROSOLS

IV.A. INTRODUCTION

In the operation of the CAG, the delivered drug dose can be controlled by
varying the drug concentration, the formulation flow rate and the aerosol generation
time. It is possible to achieve the appropriate dose for pulmonary delivery for each
specific compound by the manipulation of these variables.

In this chapter we describe studies of perphenazine stability during
aerosolization and characterize the particle size of these aerosols under different
aerosolization conditions. The objectives were to investigate the effects of power, solute
concentration and formulation flow rate on the chemical stability of perphenazine
during aerosolization. Studies investigating the effects of solute concentration and
formulation flow rate on the particle size distribution of perphenazine aerosols are also
presented. It was hypothesized that increasing the applied power during aerosolization
would increase the possibility of perphenazine degradation due to the elevated

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temperatures. The effect of solute concentration on perphenazine stability may depend on the chemical kinetics of major degradation pathways. Varying the solute concentration and the formulation flow rate may affect the condensation aerosol formation process, thus changing the aerosol particle size.

IV.B. MATERIALS

Perphenazine, USP grade, was purchased from Spectrum Chemical Co. (Gardena, CA). Propylene glycol (PG), USP grade, was purchased from Fisher Scientific Co. (Fairlawn, NJ). HPLC grade methanol, acetonitrile and ammonium acetate were purchased from Fisher Scientific Co. USP/FCC grade acetic acid was also purchased from Fisher Scientific Co.

IV.C. METHODS

IV.C.1. Solubility of Perphenazine in PG

The solubility of perphenazine in PG at room temperature (24±2°C) was determined using a method described by Shen (Shen, 2003). Excess amounts of perphenazine were added to 5.0ml of PG in a volumetric flask. The flask was closed with a stopper, sealed with parafilm and wrapped by aluminum foil to avoid direct light exposure. The flask was then continuously shaken by a Wrist Action Shaker (Model 75,
Burrell Corp., Pittsburgh, PA). After 48 hours, an aliquot of the perphenazine in PG solution was sampled into a syringe and was passed through a filter (0.45 μm, Millex-HV, Millipore, Bedford, MA). Approximately 3 mg of the filtered solution was weighed and diluted with 10 mL mobile phase solution (Section III.B.2.a). The sample was analyzed by the stability-indicating HPLC method described in Chapter III and the concentration of perphenazine was determined. This concentration was used to determine the perphenazine concentration in PG, and hence the solubility. Samples from the saturated perphenazine in PG solutions were taken also at 72 and 96 hours to ensure perphenazine concentration did not change significantly.

IV.C.2. Preparation of Perphenazine in PG Formulations

Perphenazine was dissolved in PG to prepare nominal concentrations of 9.4, 47 and 94 mM solutions. Perphenazine was accurately weighed, placed in a glass vial and the required volume of PG was then added to the vial. The vial was sealed and wrapped with aluminum foil to avoid direct light exposure, and then sonicated for 15 to 20 minutes until the drug was dissolved. Following dissolution, aliquots of the formulations were assayed to determine the drug concentration.

IV.C.3. The Operation of the Capillary Aerosol Generator (CAG)
Two types of capillary units were used in this thesis: CAG 28Ga 44mm and CAG 32Ga 35mm. The first one was used in the studies using PG as the vehicle and the second one for ethanol formulation studies. The capillary heater unit is housed in a plastic chamber as shown in Figure IV.1. Two wire leads are soldered on the wall of the capillary at both ends. The area between the two wires is the heating zone of the capillary (Figure IV.2). The wire leads are connected to an adjustable direct current (DC) power supply, which is controlled by a computer through an electronic input/output (I/O) controller. During CAG operation, a closed circuit system is formed between the capillary and the power supply.

Prior to the CAG operation, solution formulation is loaded on a syringe, which is placed on a syringe pump (Harvard apparatus, Holliston, MA). The syringe is connected to the capillary tube through an internal union. The syringe pump is also controlled by the computer through the I/O controller (Figure IV.1). Operational parameters such as the pump flow rate (formulation flow rate), run time, and target resistance are put into the computer software supplied by Chrysalis Technologies, a Division of Philip Morris U.S.A. (Richmond, VA). Upon aerosolization, solution formulation is pumped through the capillary at a fixed formulation flow rate and simultaneously the capillary is heated by the electronic power supply.
For a stainless steel capillary, the relationship between the resistance of the capillary tube and the temperature is shown by Equation IV.1, where $R_T$ is the resistance at temperature $T$, $\alpha$ is the temperature coefficient of the material at $0^\circ$C,

$$R_T = R_0 + R_0 \alpha T \quad \text{Equation IV.1}$$

$R_0$ is the resistance at $0^\circ$C (Tuma, 1983). In CAG operation, a voltage is supplied to the circuit system and a current is formed. Due to the high resistance of the capillary tube, the temperature is increased. Thus, in turn, it increased the resistance of the capillary. Target resistance is used to control the heating of the capillary. It is a pre-selected value where the software controls the resistance to reach this value and maintain it during aerosolization by adjusting the power supply through a feedback loop. Electrical current in a form of 1 millisecond pulse is applied to the circuit and the resistance is measured. Additional pulse is supplied if the resistance is lower than the target resistance and no pulse is supplied if the resistance is higher than the target. The cycle is repeated and the resistance is maintained at the value of the target resistance during the aerosolization process. Thus based on equation IV.1, by keeping the resistance at a constant during aerosolization, the temperature of the capillary tube is controlled and remains at a steady state level. The larger the target resistance, the larger the power consumed and the higher the temperature is of the capillary.
IV.C.4. Temperature Determination of the Capillary Wall

In order to investigate the temperature exposure of the solution formulation during aerosol generation the temperatures of the capillary wall were measured at 10, 30, 40 and 43mm along the capillary length (Figure IV.2). The temperature acquisition system and the 28 gauge 44mm long stainless steel capillary were provided by Chrysalis Technologies. Thermocouples (K type, #5SC-TT-K-36-36) purchased from Omega Engineering Inc. (Stamford, CT) were positioned in contact with outside of the capillary wall at fixed positions as shown in Figure IV.2. PG was aerosolized at a series of target resistance to achieve different steady state power levels at formulation flow rates of 2.5 and 5.0µL/s, respectively (Section IV.C.3; Gupta et al., 2003; Hong, 2003).

IV.C.5. Aerosolization Conditions for Perphenazine in PG Formulations

For all the aerosol generation studies with PG formulations in this thesis, a stainless steel capillary that was 28 gauge with a 44mm long heating zone was employed. At formulation flow rates of 2.5 and 5.0µL/s, the run time were 10 and 5s, respectively, both resulting in a delivered volume of 25µL.
Figure IV.1. Schematic set up of the CAG
Figure IV 2. Positions on the capillary for temperature measurements
Sham experiments and dose captures were performed to determine the delivered solute dose and the mass of drug recovered after aerosolization, respectively, as described in Chapter III. It has been shown in Chapter III that perphenazine degradation was observed to increase for the 12mM drug concentration when aerosolized at 5.0μL/s at mean (SD) power above 9.8 (0.1)W. In order to further investigate the effect of power on perphenazine stability during aerosolization, target resistances were chosen to achieve a power range of 9.6 (0.2)-12.0 (0.3)W at 5.0μL/s. As the formulation flow rate decreased to 2.5μL/s, reduced amounts of power were required for aerosolization (Hong, 2003). At a formulation flow rate of 2.5μL/s, similar target resistances produced a power range of 4.5 (0.2)-5.7 (0.2)W, which were approximately half the power used in the 5.0μL/s studies. Perphenazine in PG solutions with nominal concentrations of 9.4, 47.7 and 92.4mM were used in dose capture studies.

The MOUDI™ cascade impactor (MSP Corp., Minneapolis, MN) was used to determine the particle size distribution of perphenazine aerosols for three perphenazine concentrations. An airflow of 30±2L/min through a USP stainless steel induction port was used to sample the aerosols (Marple et al., 1991). The aluminum foil on each stage was coated with silicone (Dow Corning Corp., Midland, MI) before sample collection. After aerosol sampling, the impactor was disassembled, perphenazine was washed from the USP induction port and each MOUDI™ stage impaction surface by adding a
defined volume of solvent (mobile phase). The resulting solutions were analyzed by HPLC. Interstage drug deposition ("wall losses") were pooled and determined by wiping the walls and nozzle arrays of each stage of the impactor with a solvent-moistened tissue and analyzed for perphenazine.

In MOUDI experiments, perphenazine in PG solutions with nominal concentrations of 9.8mM, 47.7mM and 92.2mM were aerosolized with steady state power of 5.1 (0.2)W and 10.3 (0.2)W at formulation flow rates of 2.5 and 5.0μL/s, respectively.

IV.D. RESULTS AND DISCUSSION

IV.D.1. Perphenazine Solubility in PG

Table IV.1 shows the mean (SD) perphenazine concentration measured to determine the equilibrium solubility in PG. The perphenazine concentration did not change significantly up to 96 hours, indicating equilibrium solubility had been achieved. The mean (SD) solubility of perphenazine in PG was determined to be 40.20 (1.01) mg/mL, corresponding to approximately 100mM.

IV.D.2. Perphenazine Stability During Aerosol Generation
Perphenazine recovered following aerosolization was expressed relative to the mass recovered from sham experiments performed in the absence of heating.

Table IV.2 shows the effect of increasing power during aerosolization of 9, 47 and 90mM perphenazine in PG solutions at a formulation flow rate of 2.5μL/s. As a function of increasing power, there was no measurable difference in perphenazine recoveries at each concentration (p>0.05, ANOVA). However, for the 9mM solution, as the power increased there was a measurable change in degradation product. Table IV.3 reveals that compound C (2-[4-(3-phenothiazin-10-yl-propyl]-piperazino]-ethanol) was detected at 0.48% (0.19) and 0.45% (0.15) of the total area in the samples generated at 5.4 (0.2) and 5.7 (0.2)W, respectively. The peak area of compound C was below LOQ at powers below 5.4W.

Table IV.4 shows the perphenazine recoveries following aerosolization at 5.0μL/s at three concentrations. Similarly as in the studies at 2.5μL/s, as the power increased, no measurable changes in the recoveries were found for each of the concentration (p>0.05, ANOVA). Compound C accounted for 0.52 (0.19)% in the samples generated at 11.8 (0.2)W from 9mM solutions.

The studies show that the recoveries were less than 100% under all conditions. In the dose capture experiments the loss of drug can be due to the vapor diffusion loss, the loss of uncondensed vapor to the SKC filter, and the degradation products (Shen,
2003). In this study, the concentrations of observed degradation products and perphenazine following aerosolization could not account for 100% mass balance. As discussed in Chapter III, the degradation product was expressed as the percentage of its peak area to the total peak area of the chromatogram by assuming their extinction coefficients are the same. However, this assumption may not be true as the extinction coefficient may change with the structure of individual degradation product. Some degradation products may not have a chromophore and can not be detected by the UV detector. Also, the concentrations of some degradation products may be below LOD. These factors could account for the errors in mass balance.

In these studies, the perphenazine recoveries were not sensitive to the change of degradation product and may not be a good indicator for the stability study as shown above, therefore only the degradation product was discussed below to investigate the various effects on perphenazine stability during aerosolization.

Limit of detection (LOD) is the lowest amount of an analyte in a sample that can be detected but not necessarily quantitated as an exact value (FDA, 1995). LOD can be calculated as 3 times signal to noise ratio (3S/N) or determined experimentally as described in Chapter III. Limit of identification can be used to describe the presence of an analyte and equals to 6S/N, therefore 2LOD. Limit of quantitation (LOQ) is the lowest amount of an analyte that can be quantitatively determined with suitable
Table IV.1. Mean (SD) perphenazine concentrations measured to determine perphenazine solubility in PG

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Perphenazine Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>39.53 (0.99)</td>
</tr>
<tr>
<td>72</td>
<td>40.41 (0.88)</td>
</tr>
<tr>
<td>96</td>
<td>40.65 (0.94)</td>
</tr>
<tr>
<td>Average Concentration (mg/mL)</td>
<td>40.20 (1.01)</td>
</tr>
</tbody>
</table>

Data were expressed as mean (SD), n=3.
Table IV.2. Perphenazine recoveries from the dose capture experiments generated from perphenazine in PG formulations with measured concentrations of 9.3, 47.0 and 89.9mM at a formulation flow rate of 2.5μL/s with run time of 10s

<table>
<thead>
<tr>
<th>Steady State Power (W)</th>
<th>Recovery (%)</th>
<th>Steady State Power (W)</th>
<th>Recovery (%)</th>
<th>Steady State Power (W)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6 (0.2)</td>
<td>95.48 (1.25)</td>
<td>4.6 (0.2)</td>
<td>97.18 (1.67)</td>
<td>4.5 (0.2)</td>
<td>96.86 (0.93)</td>
</tr>
<tr>
<td>4.8 (0.1)</td>
<td>96.96 (1.46)</td>
<td>4.8 (0.2)</td>
<td>96.25 (1.43)</td>
<td>4.8 (0.2)</td>
<td>97.57 (1.49)</td>
</tr>
<tr>
<td>5.1 (0.2)</td>
<td>95.38 (1.49)</td>
<td>5.2 (0.2)</td>
<td>96.75 (1.04)</td>
<td>5.1 (0.1)</td>
<td>96.31 (1.19)</td>
</tr>
<tr>
<td>5.4 (0.2)</td>
<td>94.71 (0.87)</td>
<td>5.3 (0.2)</td>
<td>95.81 (0.96)</td>
<td>5.3 (0.2)</td>
<td>97.14 (3.00)</td>
</tr>
<tr>
<td>5.7 (0.2)</td>
<td>94.74 (0.87)</td>
<td>5.6 (0.2)</td>
<td>94.49 (1.19)</td>
<td>5.7 (0.2)</td>
<td>98.94 (2.39)</td>
</tr>
</tbody>
</table>

n=5, data were expressed as mean (SD).
Table IV.3. Summary of the degradation products in the aerosolized dose capture samples under all investigated conditions

<table>
<thead>
<tr>
<th>Perphenazine Concentration</th>
<th>Compound A</th>
<th>Compound C 2.5μL/s</th>
<th>Compound C 5.0μL/s</th>
<th>Compound K</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.3 and 9.2mM</td>
<td>&lt;LOD</td>
<td>0.48 (0.19)% at 5.4 (0.2)W</td>
<td>0.52 (0.19)% at 11.8 (0.2)W</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.45 (0.15)% at 5.7 (0.2)W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47.0 and 47.1mM</td>
<td>&lt;LOD</td>
<td>&lt;LOI</td>
<td>&lt;LOI at 11.8 (0.2)W</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>89.9 and 82.2mM</td>
<td>&lt;LOD</td>
<td>&lt;LOI</td>
<td></td>
<td>&lt;LOD</td>
</tr>
</tbody>
</table>
Table IV.4. Perphenazine recoveries from the dose capture experiments from perphenazine in PG formulations with measured concentrations of 9.2, 47.1 and 88.2 mM at a formulation flow rate of 5.0 μL/s with run time of 5s.

<table>
<thead>
<tr>
<th>Steady State Power (W)</th>
<th>Recovery (%)</th>
<th>Steady State Power (W)</th>
<th>Recovery (%)</th>
<th>Steady State Power (W)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.6 (0.1)</td>
<td>94.55 (1.40)</td>
<td>9.6 (0.2)</td>
<td>95.90 (2.51)</td>
<td>9.7 (0.2)</td>
<td>96.81 (1.58)</td>
</tr>
<tr>
<td>10.2 (0.2)</td>
<td>95.81 (1.41)</td>
<td>10.2 (0.2)</td>
<td>94.46 (2.47)</td>
<td>10.2 (0.2)</td>
<td>96.71 (1.49)</td>
</tr>
<tr>
<td>10.7 (0.2)</td>
<td>94.04 (2.46)</td>
<td>10.8 (0.2)</td>
<td>94.90 (2.25)</td>
<td>10.7 (0.2)</td>
<td>96.10 (1.16)</td>
</tr>
<tr>
<td>11.2 (0.3)</td>
<td>93.93 (1.96)</td>
<td>11.3 (0.2)</td>
<td>95.42 (1.62)</td>
<td>11.2 (0.2)</td>
<td>98.06 (2.68)</td>
</tr>
<tr>
<td>11.8 (0.2)</td>
<td>93.22 (1.75)</td>
<td>11.8 (0.3)</td>
<td>92.61 (3.44)</td>
<td>12.0 (0.3)</td>
<td>97.57 (0.87)</td>
</tr>
</tbody>
</table>

n=5, data were expressed as mean (SD).
precision and accuracy. LOQ can be calculated as 10S/N or determined by experiments and other methods (FDA, 1995). In this thesis, the concentrations of degradation products were discussed using LOD, LOI and LOQ as shown in Table IV.3.

At both formulation flow rates, perphenazine degradation in the 9mM formulation was found to increase at the elevated power levels, which was probably due to the increased temperatures at higher powers, although total recoveries were not significantly different. In temperature measurement the most significant changes in temperatures were observed at the position of 30 and 40mm. At 2.5μL/s, the average temperature at 30 and 40mm increased from to 155 to 282°C, and 300 to 340°C, respectively, when the power increased from 4.7 to 5.3W. At 5.0μL/s, the average temperature at these two positions increased from 157 to 200°C, and 360 to 440°C, respectively, when the power increased from 9.6 to 11.8W. The increased temperature exposure at both formulation flow rates probably caused the increased degradation product as a function of increasing the power. This increased temperature at higher power may also account for the concentration change of compound C generated from 47mM solutions at 5.0L/s (Table IV.3).

No changes in degradation product levels for perphenazine concentration at 90mM were observed (Table IV.3), suggesting no significant degradation as a function of increasing the power.
The dose capture samples generated from 47mM and 90mM solutions were diluted 5 and 10 times, respectively, prior to HPLC analysis. Thus, the concentrations of both perphenazine and degradation products in the dose capture samples were diluted, which might explain the lower concentration of compound C in the samples generated from these two concentrations compared to those generated from 9mM solutions (Table IV.3). However, in general, the lowest concentration formulation (9mM) appeared to be more susceptible to the heating process during the aerosolization at higher power conditions.

Within the investigated power ranges, at a formulation flow rate of 5.0μL/s, the temperatures of the capillary wall at 40mm were generally 60 to 100°C higher than they were at 2.5μL/s. However, the presence of degradation products was similar at both formulation flow rates (Table IV.3). It appeared that the higher temperatures at 5.0μL/s did not cause significant change in perphenazine degradation compared to 2.5μL/s. This was probably due to the reduced transit time of the solution formulation at these high temperatures. For 1μL of solution, the transit time to pass through the capillary is 0.4s and 0.2s, at 2.5μL/s and 5.0μL/s, respectively. Doubling the formulation flow rate reduced the transit time through the capillary by half, which compensated the high temperature exposure.
IV.D.3.  The Particle Size Distribution of Perphenazine in PG Aerosols

To investigate the effects of solute concentration and the formulation flow rate on the particle size distribution of perphenazine aerosols, target resistances were chosen to achieve steady state power of approximately 5.1W and 10.3W. The aerodynamic diameter of perphenazine aerosols at formulation flow rates of 2.5 and 5.0μL/s was determined using the MOUDITM cascade impactor.

Tables IV.5 and 6 show the mean perphenazine recoveries, the mass median aerodynamic diameter (MMAD), fine particle fractions (FPF) and fraction of perphenazine less than 0.172μm at formulation flow rates of 2.5 and 5.0μL/s, respectively. The total perphenazine recovered mass included the mass recovered from the USP induction port and the cascade impactor. The recoveries were expressed as percentages relative to total sham recovered mass. The MMAD was defined as the particle size at 50 percentile of a cumulative percent mass recovered in the impactor and was calculated using linear interpolation. The fine particle fraction was defined as the mass fraction of particles less than 5.6μm and was expressed as percentage of the total recovered mass. The induction port deposition and wall loss were the percentage of mass recovered from the induction port, and impactor wall and nozzles, respectively, to the total recovered mass. The fraction of less than 0.172μm was calculated as
percentage of mass of aerodynamic diameters less than 0.172μm recovered from the MOUDI cascade impactor.

At all investigated conditions, the perphenazine recoveries were 94 to 98% from MOUDI experiments and were consistent with the dose capture results in section IV.D.2. The FPFs were between 84 and 98% of total recovered mass. These results were consistent with previous findings on drug aerosols generated from PG formulations by the CAG (Hong, 2003; Shen, 2003). The FPFs of perphenazine aerosols were much higher than the FPFs reported for conventional MDI and DPI (Srichana et al., 1998; Kamiya et al., 2004) and indicated potentially higher pulmonary delivery efficiency. Mean induction port deposition was small and less than 10% in all cases. Mean wall losses were less than 5% in all cases, which were in agreement with the recommendation of USP (USP, 1999).

The MMAD was between 0.4 and 0.6μm under all investigated conditions. Together with the high fine particle fractions, these perphenazine aerosols appeared to be appropriate for effective systemic drug delivery through inhalation. However, there were significant fractions (14%-28%) of particles less than 0.172μm (Table IV.5 and 6). The particle size distribution appeared to be bimodal distributed (Figure IV.3-5), therefore the MMADs calculated from linear interpolation may not describe the size accurately (Gupta et al., 2003). Two modes are visible from the figures, the first mode
being particles less than 0.172\(\mu\)m, and a second mode with particles of the size around 0.5-1.0\(\mu\)m.

In order to investigate the most appropriate method of data analysis for these bimodal aerosols, two methods of MMAD calculation were employed. Firstly, the traditional MMAD described above, calculated by linear interpolation from a cumulative percentage distribution plot, and is a commonly used parameter for description of particle size of aerosols (Hinds, 1999). Secondly, the MMAD of each mode calculated using a bimodal distribution equation (Gupta et al., 2003). However, it should be noted that due to the nature of the experiments, such as aerosol collection and drug quantification, several processes after aerosol generation occurred. The processes of coagulation, PG evaporation and Oswald ripening could take place prior to aerosol collection and PG evaporation can also happen during aerosol collection procedure within the cascade impactor. These all could affect the final particle size of drug aerosols. The deductions made about the microsecond time scale of events at the CAG nozzle could not be drawn in any absolute sense.
Table IV.5. Particle size distribution of perphenazine aerosols generated from PG formulations with measured concentrations of 9.5, 48.9, and 90.0 mM at a formulation flow rate of 2.5 μL/s with run time of 10s

<table>
<thead>
<tr>
<th>Perphenazine Concentration (mM)</th>
<th>Recovery (%)</th>
<th>MMAD$^a$ (μm)</th>
<th>Fine Particle Fraction$^b$ (%)</th>
<th>Induction Port Deposition (%)</th>
<th>Wall loss (%)</th>
<th>Fraction less than 0.172 μm$^c$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.5 (0.02)</td>
<td>5.08 (0.08)</td>
<td>94.71 (2.56)</td>
<td>0.55 (0.04)</td>
<td>93.54 (1.52)</td>
<td>1.54 (0.69)</td>
<td>4.81 (0.84)</td>
</tr>
<tr>
<td>48.9 (0.41)</td>
<td>5.12 (0.05)</td>
<td>96.36 (2.14)</td>
<td>0.39 (0.01)</td>
<td>95.96 (1.01)</td>
<td>0.94 (0.62)</td>
<td>2.73 (0.22)</td>
</tr>
<tr>
<td>90.0 (0.56)</td>
<td>5.01 (0.07)</td>
<td>97.89 (2.18)</td>
<td>0.46 (0.04)</td>
<td>92.83 (2.68)</td>
<td>4.85 (2.33)</td>
<td>1.21 (0.62)</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD). n=5. $^a$MMAD was calculated by linear interpolation. $^b$Fine particle fraction was calculated as percentages of particles less than 5.6 μm of total recovered dose. $^c$Fraction of less than 0.172 μm was calculated as percentages of particles less than 0.172 μm of total recovered dose in MOUDI.
Table IV.6. Particle size distribution of perphenazine aerosols generated from PG formulations with measured concentrations of 9.2, 48.3, and 90.0mM at a formulation flow rate of 5.0µL/s with run time of 5s

<table>
<thead>
<tr>
<th>Perphenazine Concentration (mM)</th>
<th>SSP (W)</th>
<th>Recovery (%)</th>
<th>MMAD(^a) (µm)</th>
<th>Fine Particle Fraction(^b) (%)</th>
<th>Induction Port Deposition (%)</th>
<th>Wall loss (%)</th>
<th>Fraction less than 0.172µm(^c) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.2 (0.04)</td>
<td>10.38 (0.08)</td>
<td>92.24 (3.98)</td>
<td>0.46 (0.02)</td>
<td>84.81 (3.86)</td>
<td>8.89 (3.70)</td>
<td>4.73 (1.04)</td>
<td>28.52 (2.34)</td>
</tr>
<tr>
<td>48.3 (0.98)</td>
<td>10.24 (0.09)</td>
<td>94.30 (4.55)</td>
<td>0.38 (0.01)</td>
<td>93.83 (2.83)</td>
<td>1.91 (1.45)</td>
<td>3.71 (1.24)</td>
<td>22.21 (3.64)</td>
</tr>
<tr>
<td>90.0 (0.56)</td>
<td>10.27 (0.06)</td>
<td>96.14 (3.08)</td>
<td>0.39 (0.00)</td>
<td>96.87 (0.55)</td>
<td>1.04 (0.50)</td>
<td>1.74 (0.16)</td>
<td>14.63 (1.16)</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD). n=5. \(^a\)MMAD was calculated by linear interpolation. \(^b\)Fine particle fraction was calculated as percentages of particles less than 5.6µm of total recovered dose. \(^c\)Fraction of less than 0.172µm was calculated as percentages of particles less than 0.172µm of total recovered dose in MOUDI.
Figure IV.3. Particle size distribution of perphenazine aerosols generated from (a) 9.2mM at 5.08 (0.08)W, (b) 9.5mM at 10.38 (0.08)W.
Figure IV.4. Particle size distribution of perphenazine aerosols generated from (a) 48.9mM at 5.12 (0.05)W, (b) 48.3mM at 10.24 (0.09)W.
Figure IV.5. Particle size distribution of perphenazine aerosols generated from (a) 90.0mM at 5.01 (0.07)W, (b) 90.0mM at 10.27 (0.06)W.
IV.D.3.a. *Perphenazine Particle Size Treated by Linear Interpolation Method*

At a formulation flow rate of 2.5μL/s, for all three solute concentration formulations the total perphenazine recoveries were similar (p>0.05, ANOVA). As the solute concentration increased, the MMADs were changed significantly (p<0.05, ANOVA with Turkey). However, no clear trend of the concentration effect on the particle size was observed. The largest mean (SD) MMAD of perphenazine aerosols at 0.6 (0.04)μm was generated from 9mM formulations. The mean (SD) size decreased to 0.4 (0.01)μm as the solute concentration increased to 48mM. It then increased to 0.5 (0.04)μm at a concentration of 90mM. At a formulation flow rate of 5.0μL/s, total perphenazine recoveries were also similar for all three concentrations (p>0.05, ANOVA). The MMADs of 9mM were significantly larger than they were of 48 and 90mM (p<0.05, ANOVA with turkey). The MMADs of 48mM and 90mM were similar.

Significant amounts of fraction less than 0.172μm were found at all conditions. The fraction less than 0.172μm at a formulation flow rate of 2.5μL/s is listed in Table IV.5. The fraction less than 0.172μm was significantly different as the solute concentration increased (p<0.05, ANOVA). However, the trend of the change was not clear. For the 9mM perphenazine in PG solutions, mean (SD) fractions of particles less than 0.172μm was 23.01% (1.60). Increasing the concentration to 48mM increased the mean (SD) fraction significantly to 26.13% (0.55). When the solute concentration
increased further to 90mM, the fractions less than 0.172μm decreased significantly to 18.30% (1.56). Table IV.6 shows the mean (SD) fraction less than 0.172μm from three concentrations at 5.0μL/s. The fraction less than 0.172μm was shown to decrease as a function of increasing solute concentration (p<0.05, ANOVA).

Perphenazine is a low volatility compound. It has a melting point of 94-100°C and boiling point of 278-281°C at 1mm Hg pressure (The Merck Index, 1996). Due to its low volatility, perphenazine could have been supersaturated well before PG (Gupta et al., 2003; Shen, 2003). It is hypothesized that perphenazine may nucleate and serve as sites for perphenazine condensation to take place (homogeneous nucleation) to produce a fraction of particles less than 0.172μm. These perphenazine nuclei could also serve as sites for PG to condense (heterogeneous nucleation) and form perphenazine in PG droplets, which was the main component of perphenazine aerosols.

Swift has investigated the effect of number concentration of nuclei on the final particle size of aerosols formed by heterogeneous condensation (Swift, 1967). It was found that at low number concentration (n<4×10⁴/cm³), the particle size was independent of concentration. At moderate nuclei concentration (10⁵-10⁷/cm³), the particle size d was proportional to n⁻⁰.²³. At nuclei concentration above 10⁷ /cm³, the relationship was d ∝ n⁻⁰.³³ (Equation 1.3). In the case of CAG operation number concentration of nuclei are considered to be higher than 10⁷/cm³ and equation 1.3 can be
applied. It is hypothesized that for a given solute, higher solute concentration may result in increased number of nuclei, which would produce smaller particles (Swift, 1967; Gupta et al., 2003; Shen, 2003). The effect of solute concentration on the MMADs of aerosols from three concentrations in our studies was not completely consistent with previous findings. The MMAD generated for 9mM perphenazine formulations was the largest at both flow rates. The change in the MMADs between 48 and 90mM was not clear, probably because the concentration change between these two was not large enough. However, by varying the solute concentration, the difference between MMAD was less than 0.2µm, which was a small value and can be considered as practically insignificant.

The fraction less than 0.172µm is hypothesized to be formed predominantly by homogeneous nucleation of the solute (Gupta et al., 2003; Shen, 2003). The growth of homogeneously formed aerosols could be coagulation controlled (Lesniewski and Koch, 1998; Hinds, 1999). Kousaka et al. has studied the homogeneous nucleation process of the generation of titanium dioxide particles in the vapor phase. They found that the number concentration of self nucleated particles (in our studies, the perphenazine nuclei formed by homogeneous nucleation) increased almost linearly with the concentration of precursor monomers (in our studies, the concentration of perphenazine in the vapor). At low number concentration of nuclei (approximately less than 6×10⁹/cm³), where
coagulation was negligible, the size of the nucleated particles was independent of the concentration of the precursor monomers. At high number concentrations, the size of the nucleated particles increased as the concentration of precursor monomers increased which could be contributed by coagulation (Kousaka et al., 2000).

Coagulation controlled particle growth is hypothesized to be involved in the formation of perphenazine aerosols less than 0.172μm. For the highest concentration solution (90mM perphenazine in PG), larger number drug nuclei concentrations were available in the vapor and coagulation controlled particle size growth took place. The size of particles increased, which was shown as a decrease of the fractions less than 0.172μm. As the solute concentration decreased to 9mM, the number of drug nuclei was decreased, particle growth caused by coagulation was reduced and the fraction less than 0.172μm in 9mM was shown to increase. An exception was found for 48mM at 2.5L/s and this can not be explained by the coagulation controlled growth hypothesis. More studies with different concentrations are needed to further investigate the effect of solute concentration on the fraction of particles less than 0.172μm.

Comparisons were also made for each solution formulation at 2.5 and 5.0μL/s to investigate the effect of formulation flow rate. It has been shown increasing the vapor jet velocity decreases the size of aerosols (Langmuir, 1942). Changing the mass flow rate (therefore the vapor jet velocity) of the CAG from 2.6 to 5.2mg/s decreased the size
of benzil aerosols (Hong, 2003). In our studies, increasing the formulation flow rate from 2.5 to 5.0μL/s decreased the MMADs of perphenazine aerosols generated from both 9mM and 90mM perphenazine in PG solutions (p<0.05, t-test). The size change, however, was less than 0.1μm. The small change in the size was practically insignificant. The MMADs of perphenazine aerosols generated from 48mM were not changed as a function of the formulation flow rate (p>0.05, t-test). The formulation flow rate did not affect the size of perphenazine aerosols in a significant way.

IV.D.3.b. Particle Size Distribution Treated By Bimodal Distribution Equation

There appeared to be two modes in the distribution: the first mode in the size range of 0-0.172μm and the second mode of size being 0.5-1.0μm. A bimodal distribution equation was used to perform the curve fit of the data using Sigma Plot (Version 9.0, Jandel Corp., San Rafael, CA). It used a least square, nonlinear regression method to fit the experimental data to a curve (Gupta et al., 2003). Here, x and y are the aerodynamic diameter and mass fraction/μm calculated from the original data points, respectively. The equation and inputs of parameters used for the software are shown in Appendix A. To start, an initial estimate of MMAD₁ (size of particles in the first mode), MMAD₂ (size of particles in the second mode), GSD₁ (geometric standard deviation of the first mode), GSD₂ (geometric standard deviation of the second mode), and p (the
fraction of mass in the first mode) are needed. AUC was calculated using the trapezoidal method. The MMAD\textsubscript{1} and MMAD\textsubscript{2} were estimated from AUC\textsubscript{1} and AUC\textsubscript{2}, respectively. The ratio of AUC\textsubscript{1}/AUC\textsubscript{total} was used to estimate p. An initial estimation of MMAD\textsubscript{1} of 0.1, MMAD\textsubscript{2} of 0.5, GSD\textsubscript{1} of 1.5, GSD\textsubscript{2} of 1.5 and p of 0.5 was used. The MMAD\textsubscript{1}, MMAD\textsubscript{2}, GSD\textsubscript{1}, GSD\textsubscript{2}, p, and \( r^2 \) obtained from non-linear curve fit of each experiment were reported in Table IV.7-12. Figure IV.6-11 shows the fit curve and the original data points generated from 9, 46 and 91\,mM perphenazine in PG solutions at both flow rates. The goodness of fit was evaluated by \( r^2 \), standard error of each parameters and the actual fitted curve. In all the cases, \( r^2 \) was close to 1, the standard error of each parameters were small and the actual fit curve matched well with the original data points. These indicated that this bimodal curve fitting approach was appropriate to describe the particle size distribution.

The calculated MMAD\textsubscript{1} was approximately 0.04\,\mu m under all conditions (Table IV.13 and 14). The usefulness of this estimation of MMAD\textsubscript{1} is questionable because MMAD\textsubscript{1} was determined only by three data points. This parameter might be insensitive to the actual changes in the fraction of mass in the first mode of perphenazine aerosols.

Table IV.13 and 14 list the mean (SD) MMAD\textsubscript{2} generated from 9\,mM, 48\,mM and 90\,mM formulations at 2.5\,\mu L/s and 5.0\,\mu L/s, respectively. At both flow rates, the
MMAD$_2$ was the largest at 9mM formulations. No significant difference was found between MMAD$_2$ of 48 and 90mM.

Heterogeneous condensation process is hypothesized to be predominantly involved in the formation of the second mode of perphenazine aerosols. As discussed in section IV.D.3.a, increasing the solute concentration increased the nuclei concentration thus reducing the MMAD of perphenazine aerosols. The low concentration formulation (9mM) had the largest MMAD$_2$ and the results were consistent with previous findings (Gupta et al., 2003; Shen, 2003). The difference of solute concentration between 48 and 90mM may not be large enough to significantly affect the number concentration of nuclei in the vapor, thus the particle size was not changed significantly. The change of mean MMAD$_2$ by varying the solute concentration was at most 0.2µm. The size changes were probably too small to have significant effect practically.

Comparing the MMAD$_2$ for each formulation at two formulation flow rates, the MMAD$_2$ was smaller at 5.0µL/s than 2.5µL/s ($p<0.05$, t-test). However, the differences were all less than 0.1µm, which were generally small and they were practically not useful. The formulation flow rate had no significant effect on the MMAD$_2$ of perphenazine aerosols generated by the CAG.
Table IV.7. Parameters estimated using bimodal distribution equation for individual experiments of perphenazine aerosols generated from 9.5mM perphenazine in PG formulations at a formulation flow rate of 2.5μL/s with run time of 10s

<table>
<thead>
<tr>
<th>SSP (W)</th>
<th>MMAD$_1$ (μm)</th>
<th>GSD$_1$</th>
<th>MMAD$_2$ (μm)</th>
<th>GSD$_2$</th>
<th>p</th>
<th>R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.19</td>
<td>0.04 (6.4×10$^{-5}$)</td>
<td>1.31 (0.006)</td>
<td>0.67 (0.03)</td>
<td>1.63 (0.050)</td>
<td>0.57 (0.029)</td>
<td>0.9997 (0.030)</td>
</tr>
<tr>
<td>5.13</td>
<td>0.04 (6.8×10$^{-5}$)</td>
<td>1.32 (0.006)</td>
<td>0.64 (0.02)</td>
<td>1.53 (0.053)</td>
<td>0.60 (0.029)</td>
<td>0.9997 (0.035)</td>
</tr>
<tr>
<td>5.01</td>
<td>0.04 (7.8×10$^{-5}$)</td>
<td>1.31 (0.008)</td>
<td>0.73 (0.04)</td>
<td>1.63 (0.059)</td>
<td>0.55 (0.036)</td>
<td>0.9995 (0.033)</td>
</tr>
<tr>
<td>5.05</td>
<td>0.04 (1.0×10$^{-4}$)</td>
<td>1.31 (0.006)</td>
<td>0.60 (0.02)</td>
<td>1.44 (0.077)</td>
<td>0.64 (0.036)</td>
<td>0.9991 (0.053)</td>
</tr>
<tr>
<td>5.00</td>
<td>0.04 (7.4×10$^{-5}$)</td>
<td>1.31 (0.006)</td>
<td>0.65 (0.03)</td>
<td>1.59 (0.052)</td>
<td>0.58 (0.031)</td>
<td>0.9995 (0.034)</td>
</tr>
</tbody>
</table>

Data were expressed as mean (SE).
Table IV.8. Parameters estimated using bimodal distribution equation for individual experiments of perphenazine aerosols generated from 48.9 mM perphenazine in PG formulations at a formulation flow rate of 2.5 μL/s with run time of 10s.

<table>
<thead>
<tr>
<th>SSP (W)</th>
<th>MMAD$_1$ (μm)</th>
<th>GSD$_1$</th>
<th>MMAD$_2$ (μm)</th>
<th>GSD$_2$</th>
<th>p</th>
<th>R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.13</td>
<td>0.04 (4.8×10$^{-5}$)</td>
<td>1.33 (0.004)</td>
<td>0.45 (0.01)</td>
<td>1.58 (0.025)</td>
<td>0.60 (0.015)</td>
<td>0.9998 (0.023)</td>
</tr>
<tr>
<td>5.06</td>
<td>0.04 (4.9×10$^{-5}$)</td>
<td>1.33 (0.004)</td>
<td>0.49 (0.01)</td>
<td>1.63 (0.023)</td>
<td>0.57 (0.015)</td>
<td>0.9998 (0.021)</td>
</tr>
<tr>
<td>5.18</td>
<td>0.04 (6.9×10$^{-5}$)</td>
<td>1.33 (0.006)</td>
<td>0.48 (0.02)</td>
<td>1.64 (0.041)</td>
<td>0.58 (0.024)</td>
<td>0.9997 (0.035)</td>
</tr>
<tr>
<td>5.13</td>
<td>0.04 (1.6×10$^{-5}$)</td>
<td>1.33 (0.001)</td>
<td>0.51 (0.00)</td>
<td>1.60 (0.007)</td>
<td>0.58 (0.005)</td>
<td>0.9999 (0.007)</td>
</tr>
<tr>
<td>5.09</td>
<td>0.04 (2.2×10$^{-5}$)</td>
<td>1.33 (0.002)</td>
<td>0.48 (0.00)</td>
<td>1.53 (0.009)</td>
<td>0.59 (0.007)</td>
<td>0.9999 (0.011)</td>
</tr>
</tbody>
</table>

Data were expressed as mean (SE).
Table IV.9. Parameters estimated using bimodal distribution equation for individual experiments of perphenazine aerosols generated from 90.0mM perphenazine in PG formulations at a formulation flow rate of 2.5μL/s with run time of 10s

<table>
<thead>
<tr>
<th>SSP (W)</th>
<th>MMAD₁ (μm)</th>
<th>GSD₁</th>
<th>MMAD₂ (μm)</th>
<th>GSD₂</th>
<th>p</th>
<th>R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.11</td>
<td>0.04 (3.6×10⁻⁵)</td>
<td>1.29 (0.002)</td>
<td>0.48 (0.01)</td>
<td>1.59 (0.016)</td>
<td>0.57 (0.011)</td>
<td>0.9998 (0.016)</td>
</tr>
<tr>
<td>4.97</td>
<td>0.04 (2.8×10⁻⁵)</td>
<td>1.30 (0.002)</td>
<td>0.46 (0.00)</td>
<td>1.54 (0.010)</td>
<td>0.54 (0.008)</td>
<td>0.9999 (0.012)</td>
</tr>
<tr>
<td>5.02</td>
<td>0.04 (1.0×10⁻⁴)</td>
<td>1.29 (0.005)</td>
<td>0.55 (0.02)</td>
<td>1.44 (0.039)</td>
<td>0.58 (0.030)</td>
<td>0.9981 (0.049)</td>
</tr>
<tr>
<td>4.92</td>
<td>0.04 (5.4×10⁻⁵)</td>
<td>1.28 (0.002)</td>
<td>0.50 (0.01)</td>
<td>1.45 (0.014)</td>
<td>0.58 (0.014)</td>
<td>0.9996 (0.023)</td>
</tr>
<tr>
<td>5.01</td>
<td>0.04 (9.0×10⁻⁵)</td>
<td>1.27 (0.003)</td>
<td>0.54 (0.02)</td>
<td>1.50 (0.032)</td>
<td>0.60 (0.024)</td>
<td>0.9984 (0.038)</td>
</tr>
</tbody>
</table>

Data were expressed as mean (SE).
Table IV.10. Parameters estimated using bimodal distribution equation for individual experiments of perphenazine aerosols generated from 9.2mM perphenazine in PG formulations at a formulation flow rate of 5.0μL/s with run time of 5s

<table>
<thead>
<tr>
<th>SSP (W)</th>
<th>MMAD₁ (μm)</th>
<th>GSD₁</th>
<th>MMAD₂ (μm)</th>
<th>GSD₂</th>
<th>p</th>
<th>R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.36</td>
<td>0.04 (2.7x10⁻⁵)</td>
<td>1.32 (0.002)</td>
<td>0.61 (0.01)</td>
<td>1.62 (0.021)</td>
<td>0.60 (0.012)</td>
<td>0.9999 (0.014)</td>
</tr>
<tr>
<td>10.36</td>
<td>0.04 (3.0x10⁻⁵)</td>
<td>1.33 (0.003)</td>
<td>0.60 (0.01)</td>
<td>1.56 (0.020)</td>
<td>0.60 (0.012)</td>
<td>0.9999 (0.015)</td>
</tr>
<tr>
<td>10.49</td>
<td>0.04 (4.6x10⁻⁵)</td>
<td>1.34 (0.005)</td>
<td>0.60 (0.02)</td>
<td>1.51 (0.039)</td>
<td>0.66 (0.020)</td>
<td>0.9999 (0.027)</td>
</tr>
<tr>
<td>10.28</td>
<td>0.04 (2.3x10⁻⁵)</td>
<td>1.34 (0.002)</td>
<td>0.58 (0.01)</td>
<td>1.56 (0.015)</td>
<td>0.61 (0.009)</td>
<td>0.9999 (0.013)</td>
</tr>
<tr>
<td>10.43</td>
<td>0.04 (2.2x10⁻⁶)</td>
<td>1.35 (3.0x10⁻⁴)</td>
<td>0.59 (0.00)</td>
<td>1.60 (0.002)</td>
<td>0.60 (0.001)</td>
<td>0.9999 (0.001)</td>
</tr>
</tbody>
</table>

Data were expressed as mean (SE).
Table IV.11 Parameters estimated using bimodal distribution equation for individual experiments of perphenazine aerosols generated from 48.3mM perphenazine in PG formulations at a formulation flow rate of 5.0μL/s with run time of 5s

<table>
<thead>
<tr>
<th>SSP (W)</th>
<th>MMAD$_1$ (μm)</th>
<th>GSD$_1$</th>
<th>MMAD$_2$ (μm)</th>
<th>GSD$_2$</th>
<th>p</th>
<th>R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.34</td>
<td>0.04 (7.9×10$^{-3}$)</td>
<td>1.31 (0.004)</td>
<td>0.42 (0.01)</td>
<td>1.56 (0.031)</td>
<td>0.55 (0.022)</td>
<td>0.9993 (0.036)</td>
</tr>
<tr>
<td>10.19</td>
<td>0.04 (7.9×10$^{-3}$)</td>
<td>1.31 (0.004)</td>
<td>0.42 (0.01)</td>
<td>1.56 (0.031)</td>
<td>0.55 (0.022)</td>
<td>0.9994 (0.036)</td>
</tr>
<tr>
<td>10.12</td>
<td>0.04 (7.3×10$^{-3}$)</td>
<td>1.30 (0.004)</td>
<td>0.44 (0.01)</td>
<td>1.58 (0.029)</td>
<td>0.55 (0.020)</td>
<td>0.9994 (0.031)</td>
</tr>
<tr>
<td>10.24</td>
<td>0.04 (3.0×10$^{-3}$)</td>
<td>1.31 (0.002)</td>
<td>0.44 (0.00)</td>
<td>1.54 (0.015)</td>
<td>0.56 (0.008)</td>
<td>0.9999 (0.013)</td>
</tr>
<tr>
<td>10.32</td>
<td>0.04 (6.5×10$^{-3}$)</td>
<td>1.30 (0.003)</td>
<td>0.43 (0.01)</td>
<td>1.53 (0.022)</td>
<td>0.53 (0.016)</td>
<td>0.9995 (0.027)</td>
</tr>
</tbody>
</table>

Data were expressed as mean (SE).
Table IV.12. Parameters estimated using bimodal distribution equation for individual experiments of perphenazine aerosols generated from 90.0mM perphenazine in PG formulations at a formulation flow rate of 5.0μL/s with run time of 5s

<table>
<thead>
<tr>
<th>SSP (W)</th>
<th>MMAD₁ (μm)</th>
<th>GSD₁</th>
<th>MMAD₂ (μm)</th>
<th>GSD₂</th>
<th>p</th>
<th>R square</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.35</td>
<td>0.04 (5.2×10⁻⁵)</td>
<td>1.28 (0.002)</td>
<td>0.42 (0.01)</td>
<td>1.52 (0.015)</td>
<td>0.52 (0.012)</td>
<td>0.9996 (0.020)</td>
</tr>
<tr>
<td>10.20</td>
<td>0.04 (8.7×10⁻⁵)</td>
<td>1.28 (0.003)</td>
<td>0.41 (0.01)</td>
<td>1.54 (0.027)</td>
<td>0.52 (0.020)</td>
<td>0.9988 (0.034)</td>
</tr>
<tr>
<td>10.21</td>
<td>0.04 (4.0×10⁻⁵)</td>
<td>1.27 (0.001)</td>
<td>0.41 (0.00)</td>
<td>1.50 (0.010)</td>
<td>0.50 (0.008)</td>
<td>0.9997 (0.014)</td>
</tr>
<tr>
<td>10.30</td>
<td>0.04 (1.2×10⁻⁵)</td>
<td>1.28 (0.001)</td>
<td>0.43 (0.00)</td>
<td>1.52 (0.003)</td>
<td>0.52 (0.003)</td>
<td>0.9999 (0.005)</td>
</tr>
<tr>
<td>10.29</td>
<td>0.04 (2.2×10⁻⁵)</td>
<td>1.28 (0.001)</td>
<td>0.42 (0.00)</td>
<td>1.49 (0.006)</td>
<td>0.54 (0.005)</td>
<td>0.9999 (0.008)</td>
</tr>
</tbody>
</table>

Data were expressed as mean (SE).
Table IV.13. Summary of MMADs estimated using bimodal curve fitting method for perphenazine in PG at a formulation flow rate of 2.5μL/s

<table>
<thead>
<tr>
<th>Perphenazine Concentration (mM)</th>
<th>SSP (W)</th>
<th>MMAD$_1$ (μm)</th>
<th>MMAD$_2$ (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.5 (0.02)</td>
<td>5.08 (0.08)</td>
<td>0.04 (0.00)</td>
<td>0.66 (0.05)</td>
</tr>
<tr>
<td>48.9 (0.41)</td>
<td>5.12 (0.05)</td>
<td>0.04 (0.00)</td>
<td>0.48 (0.02)</td>
</tr>
<tr>
<td>90.0 (0.56)</td>
<td>5.01 (0.07)</td>
<td>0.04 (0.00)</td>
<td>0.50 (0.04)</td>
</tr>
</tbody>
</table>

n=5, data are expressed as mean±SD.
Table IV.14. Summary of MMADs estimated using bimodal curve fitting method for perphenazine in PG at a formulation flow rate of 5.0μL/s

<table>
<thead>
<tr>
<th>Perphenazine Concentration (mM)</th>
<th>SSP (W)</th>
<th>MMAD$_1$ (μm)</th>
<th>MMAD$_2$ (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.2 (0.04)</td>
<td>10.38 (0.08)</td>
<td>0.04 (0.00)</td>
<td>0.60 (0.01)</td>
</tr>
<tr>
<td>48.3 (0.98)</td>
<td>10.24 (0.09)</td>
<td>0.04 (0.00)</td>
<td>0.44 (0.02)</td>
</tr>
<tr>
<td>90.0 (0.56)</td>
<td>10.27 (0.06)</td>
<td>0.04 (0.00)</td>
<td>0.42 (0.01)</td>
</tr>
</tbody>
</table>

n=5, data are expressed as mean±SD.
Figure IV.6. Individual fit curve of perphenazine aerosols generated from 9.3mM perphenazine in PG formulation at 5.19W at a formulation flow rate of 2.5μL/s with run time of 10s.
Figure IV.7. Individual fit curve of perphenazine aerosols generated from 48.9mM perphenazine in PG formulation at 5.18W at a formulation flow rate of 2.5μL/s with run time of 10s.
Figure IV.8. Individual fit curve of perphenazine aerosols generated from 90.0mM perphenazine in PG formulation at 5.02W at a formulation flow rate of 2.5μL/s with run time of 10s.
Figure IV.9. Individual fit curve of perphenazine aerosols generated from 9.2mM perphenazine in PG formulations at 10.36W at a formulation flow rate of 5.0μL/s with run time of 5s.
Figure IV.10. Individual fit curve of perphenazine aerosols generated from 48.3mM perphenazine in PG formulations at 10.19W at a formulation flow rate of 5.0μL/s with run time of 5s.
Figure IV.11. Individual fit curve of perphenazine aerosols generated from 90.0mM perphenazine in PG formulations at 10.20W at a formulation flow rate of 5.0μL/s with run time of 5s.
IV.E. CONCLUSIONS

The study of perphenazine stability under various aerosolization conditions from three solute concentration formulations was described. At both formulation flow rates, levels of one degradation product increased from the lowest concentration formulations (9mM) at increased power levels. Dehalogenation appeared to be the major degradation pathway. The formulation flow rate did not affect perphenazine stability significantly during aerosolization.

Fine particle perphenazine aerosols can be produced from perphenazine in PG solutions. The MMAD were in the range of 0.4-0.6μm under all conditions and significant amounts (14-28%) of fraction less than 0.172μm was observed. The fine particle fractions were above 84%. The small size and high FPFs of these aerosols indicated potentially high pulmonary delivery efficiency. Perphenazine aerosols appeared to be bimodal distributed, which was hypothesized to be a result of homogeneous and heterogeneous nucleation process during aerosolization. A bimodal distribution equation was used to describe the bimodality and MMAD$_2$ was used to describe the size of the second mode of the aerosols, which was hypothesized to be predominantly formed by heterogeneous condensation. Both the MMAD and MMAD$_2$ from 9mM formulations were found to be the largest due to the reduced nuclei available in the vapor. However, the change of the size by varying the concentration was
relatively small and practically not useful. It was hypothesized the fraction less than 0.172μm was formed predominantly by homogeneous nucleation. Increasing the solute concentration decreased the fraction less than 0.172μm of perphenazine aerosols in most cases probably due to coagulation controlled growth. The effect of formulation flow rate on the size of perphenazine aerosols was practically insignificant.
CHAPTER V
STABILITY AND CHARACTERIZATION OF
SCOPOLAMINE AEROSOLS FROM ETHANOL
FORMULATIONS

V.A. INTRODUCTION

Scopolamine is a strong antiemetic agent to treat nausea and motion sickness. Stability studies showed that it was easily hydrolyzed and dehydrated in acidic and basic solutions, and also at high temperatures (Holmes and Manske, 1950; Windheuser et al., 1972; Jira and Pohloudek-Fabini, 1983). It was chosen as a thermally labile model compound for this study. In this chapter, the development and validation of a stability-indicating HPLC assay and an LC-MS method for scopolamine was described. Scopolamine degradation under stressed conditions was investigated to produce potential degradation products. The LC-MS method was used to evaluate the structures of these degradation products.

To assess the feasibility of generating chemically stable scopolamine aerosols by the CAG, scopolamine hydrobromide trihydrate (scopolamine in the rest of the chapter)
was first formulated in PG solutions. Similar to perphenazine studies, the power was chosen at 4.7W and 9.8W, at formulation flow rates of 2.5 and 5.0μL/s, respectively. At all conditions investigated, scopolamine recoveries from dose capture experiments were between 30 and 60% with significant amounts of degradation products detected by both HPLC and LC-MS methods. Due to the relatively high temperature accompanied with the aerosolization process using PG as the vehicle, the degradation of scopolamine during aerosol generation was expected.

In order to reduce or prevent scopolamine degradation during aerosolization, ethanol formulations were used to generate aerosols using the CAG. Ethanol has a molecular weight of 46 Daltons, a boiling point of 78°C at 760mmHg, which is much lower than PG (188°C at 760mm Hg) (Merck, 1996). During aerosolization the scopolamine ethanol solution is heated and a liquid spray is generated. Upon exiting the capillary tip, evaporation of ethanol takes place and scopolamine particles are produced. Unlike PG, the aerosolization of ethanol solutions is not a process to generate condensation aerosols. Therefore, complete vaporization of ethanol is not required. The temperature exposure during aerosolization should be significantly reduced compared with PG studies. Thus, chemical stability of scopolamine during aerosol generation may be maintained.
The aerosolization of scopolamine in ethanol formulations was investigated. This chapter describes the studies on scopolamine stability during aerosolization within certain power ranges for scopolamine in ethanol formulations at different concentrations. The effects of drug concentration and formulation flow rate on the particle size distribution of scopolamine aerosols were also investigated.

V.B. MATERIALS AND INSTRUMENTATION

V.B.1. Chemicals

Scopolamine hydrobromide trihydrate, USP grade, was purchased from Sigma-Aldrich (St. Louis, MO). Ethyl alcohol (200 proof) was also purchased from Sigma-Aldrich. Methanol, HPLC grade, was purchased from Fisher Scientific Co. (Fairlawn, NJ). Heptafluorobutyric acid (HFBA) and tropic acid (98% purity) was purchased from Sigma-Aldrich (St. Louis, MO). N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) was purchased from Alltech (Deerfield, IL).

V.B.2. Instrumentation

The HPLC system and LC-MS system was as described in Chapter III. The GC-MS system was an Agilent GC 6890 series GC system, 7683 series injector and auto
sampler and 5973 network mass selective detector (Agilent Technologies, Palo Alto, CA).

V.C. METHODS
V.C.1. HPLC Analysis

The HPLC chromatographic separations were performed using a Spectrum Discovery C-18 column (4.0×150mm, 5μm; Sigma-Aldrich, St. Louis, MO) with detection at UV 258nm. The mobile phase was 0.03M ammonium formate with 0.2% HFBA buffer (pH 3.5 adjusted by formic acid) and methanol (68:32, v/v). The LC system was operated isocratically at a flow rate of 1.0mL/min for a run time of 28 minutes.

A scopolamine stock solution of 127.20μg/mL was prepared and it was diluted volumetrically to prepare the calibration curve, with scopolamine concentrations ranging 1.02-127.20μg/mL. Triplicate injections of 100μL were made and the response (A_{258nm}) plotted versus concentration. The LOD and LOQ were determined based on the RSD of the peak area of scopolamine. The injection repeatability was tested at a concentration of 50.88μg/mL over six replicate injections. The precision and accuracy was tested using scopolamine standard solution of 62.0μg/mL. The solution was injected three times per day on three different days. The "inter-day" precision was
calculated as the RSD of the peak area. The accuracy was expressed as the percentage difference from the nominal value (\%DFN) by comparing the measured concentrations with the nominal value. The unknown concentration of scopolamine in the sample was determined by comparing the peak area of the active with the standard at 258\text{nm}.

V.C.2. LC-MS Analysis

LC-MS assay was performed using LC conditions described above, post column, a flow splitter (Upchurch Scientific, Inc., Oak Harbor, WA) was used to reduce the flow rate to the mass spectrometer to approximately 0.4\text{mL/min}. ESI-MS positive ionization mode was used and two scans were performed at cone voltages of 30 and 50\text{V}. Full scan data was obtained over a mass range of m/z 100-600 and analyzed by Waters Masslynx software (Version 3.5, Waters Corp., Milford, MA). Full details of the ionization conditions are shown in Table V.1.

V.C.3. GC-MS Analysis

The GC-MS method used an Agilent J&W DB-5 column (15\text{m}×0.25\text{mm}, 0.1\mu\text{m}; Agilent Technologies, Palo Alto, CA). The temperature during the assay was controlled as following: the temperature was set at 80\text{°C} for 1\text{min}, ramped to 290\text{°C} at a rate of 30\text{°C/min} and held at 290\text{°C} for 1\text{min} (Oretel et al., 1996). The electron
ionization (EI) mass spectrometry was collected at 70eV in a mass range from 50 to 500. Prior to GC-MS analysis, the samples were dissolved into 100μL acetonitrile, and 100μL MSTFA was added to prepare trimethylsilyl derivatives. The mixture was heated at 70°C for approximately 15min. 1μL of the mixture was injected into the GC system.

V.C.4. Solubility of Scopolamine in Ethanol

The solubility of scopolamine in ethanol was tested at room temperature (23±2°C). Excess amounts of scopolamine were added into 5.0mL ethanol in a volumetric flask. The flask was closed with a stopper and sealed with parafilm. The flask was then continuously shaken by a Wrist Action Shaker (Model 75, Burrell Corp., Pittsburgh, PA). After 24 hours, an aliquot of the solution was sampled and passed through a filter (0.45μm, Millex-HV, Millipore, Bedford, MA). Approximately 0.02mL of the filtrate was taken (accurately measured) and diluted with 5mL methanol water solution. The sample was analyzed by the HPLC method and the concentration of scopolamine in the sample was determined, hence the solubility of scopolamine. The mixture of scopolamine in ethanol was also taken at 48 and 120 hours until the solute concentration in ethanol did not change significantly. The solubility of scopolamine hydrobromide trihydrate in ethanol at room temperature was determined to be 20.59±0.70mg/mL (n=3), which is approximately 47mM.
Table V.1. The MS conditions for the evaluation of scopolamine ionization

<table>
<thead>
<tr>
<th>Parameters</th>
<th>APCI Settings</th>
<th>ESI Parameters</th>
<th>ESI Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corona Voltage</td>
<td>3kV</td>
<td>Capillary Voltage</td>
<td>3kV</td>
</tr>
<tr>
<td>Cone Voltage</td>
<td>30, 60V</td>
<td>Cone voltage</td>
<td>25, 30, 50, 55V</td>
</tr>
<tr>
<td>Extraction Voltage</td>
<td>3V</td>
<td>Extraction Voltage</td>
<td>3V</td>
</tr>
<tr>
<td>Rf lens Voltage</td>
<td>0.5V</td>
<td>Rf lens Voltage</td>
<td>0.5V</td>
</tr>
<tr>
<td>Source Block Temp.</td>
<td>100°C</td>
<td>Source Block Temp.</td>
<td>100°C</td>
</tr>
<tr>
<td>APCI Heater Temp.</td>
<td>250°C</td>
<td>Desolvation Temp.</td>
<td>120°C</td>
</tr>
<tr>
<td>Nitrogen Gas Flow</td>
<td>450L/hr</td>
<td>Nitrogen Gas Flow</td>
<td>450L/hr</td>
</tr>
<tr>
<td>Rate</td>
<td></td>
<td>Rate</td>
<td></td>
</tr>
</tbody>
</table>
V.C.5. **Forced Degradation of Scopolamine Under Stressed Conditions**

*V.C.5.a. Scopolamine in acidic and basic solutions*

Scopolamine was dissolved in 0.1N HCl to give nominal concentrations of 120.5, 119.5 and 124.5μg/mL. The solutions were stored at 23±2°C and 50±2°C for three days. Aliquots were taken over three days and assayed for scopolamine using the HPLC method.

Scopolamine was dissolved in 0.1N NaOH to give nominal concentrations of 125.0, 126.0 and 130.0μg/mL. The samples were stored at 23±2°C for 30 minutes. Aliquots were taken at 30 minutes, neutralized with 0.1N HCl and analyzed using the HPLC method.

*V.C.5.b. Scopolamine in hydrogen peroxide solution*

Scopolamine was dissolved in 3% H₂O₂ solution to give a nominal concentration of 87.2μg/mL. The solution was stored at 23±2°C for 24hr. Aliquot was taken at 24hr and analyzed by the HPLC method.

*V.C.5.c. Scopolamine in ethanol solution in dark and light*

Scopolamine was dissolved in ethanol solution to give nominal concentrations of 8.95, 9.03 and 9.13mg/mL. Each solution was divided into two sealed vials. One was
stored at 23±2°C in the dark for 7 days and the other was exposed to fluorescent lights for 3 days. The light strength at the surface of the solution was approximately 3000Lux.

At the time of assay, 0.1mL of the solution was taken, diluted with 10.0mL MeOH:H₂O (2:8) mixture and analyzed by the HPLC method described above.

**V.C.5.d. Scopolamine at elevated temperature conditions**

Scopolamine stability at elevated temperatures was tested using the DSC (DSC-7, Perkin-Elmer Inc., Wellesley, MA). Approximately 1.0mg scopolamine was placed in an aluminum pan and sealed. The pan and the contents were heated by controlling the temperature of the DSC. The initial temperature was 25°C. The temperature increased to 50°C at 100°C/min and was held at 50°C for one minute. The temperature was then increased to 250°C at a rate of 100°C/min. The total heating time was 3min. The residue was dissolved into 10.0mL methanol water mixture, diluted and analyzed by the HPLC method described above.

**V.C.5.e. Synthesis of the potential degradation product—atropic acid**

Barium hydroxide octahydrate (28g) was mixed with 60mL deionized water in a round bottomed flask (Raper, 1923). The mixture was boiled to dissolve the barium hydroxide. Tropic acid (10g) was dissolved into 40mL warm water. The tropic acid
solution was then slowly added to the barium hydroxide solution. The mixture was boiled and refluxed overnight. After 19hrs, the reflux was stopped. The reaction solution was acidified with concentrated HCl to pH 1 and a white precipitate was formed. The precipitate was then filtered, washed with 25mL 0.1N HCl and then air-dried overnight. A total 5.5g of the product was obtained. 1g of the product was taken and recrystalized from benzene.

V.C.6. **Aerosolization of Scopolamine in Ethanol Formulations**

*V.C.6.a. Aerosolization conditions of scopolamine in ethanol formulations*

Scopolamine in ethanol solutions was aerosolized using a 32 gauge, 35mm long stainless steel capillary with a formed tip. Scopolamine concentrations of 8, 20 and 40mM in ethanol were employed. The formulation flow rates were 5.0 and 10.0µL/s with aerosol generation times of 10 and 5s, respectively. Appropriate target resistances were chosen to achieve a range of different steady state values for power at each flow rate.

*V.C.6.b. Temperature determination of the capillary wall during aerosolization*

The temperature of the capillary wall was measured at 5, 20, 30 and 35mm along the capillary in a similar way as described in Section IV.C.3 (Figure V.1). Ethanol
was aerosolized and appropriate target resistances were chosen to achieve a range of powers at formulation flow rates of 5.0 and 10.0μL/s, respectively.

V.C.6.c. Single stage filter deposition experiments

Single stage filter deposition experiments were performed to determine the recovery of scopolamine following aerosolization and to assess the aerosolization efficiency (Shen, 2003). Figure V.2 shows the schematic set up for these experiments. The USP induction port was attached to a glass fiber filter stage and the aerosols were collected through the induction port at an airflow rate of 30L/min. After sample collection, the induction port and the filter were washed with a defined volume of methanol/water and were assayed using the HPLC method.
Figure V.1. Positions (mm from zero) on the capillary for temperature measurements
Figure V.2. Schematic set up of single stage filter experiments

[Diagram of USP Induction port, CAG, Glass fiber filter stage, and 30 L/min]
V.C.6.d.  Determination of particle size distribution of scopolamine aerosols

The MOUDI™ cascade impactor was used to determine the aerodynamic diameter of these aerosols at a flow rate of 30±2L/min with the USP stainless steel induction port. The experimental procedure was similar to the method described in Chapter IV.C.5.b. The aluminum foil collection substrates were coated with silicone spray before sample collection. After sampling, the impactor was disassembled, the induction port and MOUDI stages were washed with a defined volume of solvent. The resulting solutions were analyzed by the HPLC assay. “Wall loss” was determined by wiping the walls and nozzle arrays of each stage with a solvent-moistened tissue and analyzed for scopolamine.

V.C.6.e.  Image analysis of scopolamine aerosols using scanning electron microscope (SEM)

The images of scopolamine aerosols were taken using a Jeol JSM-820 scanning electron microscope (SEM) (Japan). A glass cover slip was taped to the aluminum foil below MOUDI stages of #6 and #10 before sample collection. Scopolamine aerosols were then generated from 8.4mM ethanol solutions at 5.5W at a formulation flow rate of 10.0μL/s. The glass cover slips were removed, attached to SEM stubs and coated with gold using EMS 550x sputter coater prior to SEM analysis.
V.D. RESULTS AND DISCUSSION
V.D.1. Ionization Conditions

As described in Section III.D.2, the ionization conditions for scopolamine were optimized to produce parent ions and fragment ions with relatively high intensities. Both APCI and ESI probes in positive ion mode were tested at different cone voltages. Table V.1 lists the tested ionization conditions using both APCI and ESI probes.

Figure V.3 shows the mass spectra of scopolamine using APCI probe at cone voltages of 30 and 60V. The parent ion $[M+H]^+$ of m/z 304 was the base peak at cone voltage of 30V. The fragment ion m/z 138 ([M+H-tropic acid]$^+$) became the base peak at cone voltage of 60V.

Figure V.4 (a-d) shows the mass spectra of scopolamine using the ESI probe at different cone voltages from 25 to 55V. The parent ion $[M+H]^+$ of 304 was present under all the conditions investigated. It was the base peak at cone voltage of 25 and 30V(Figure V.4.a and b). As the cone voltage increased to 50-55V, the ion intensity of the $[M+H]^+$ peak was decreased due to the collision-induced dissociation. The predominant fragment ions of m/z 138 and 156 were produced at all the investigated conditions. The fragment ion m/z 138 ([M+H-tropic acid]$^+$) and m/z 156 corresponded to the basic moiety of scopolamine structure (Auriola and Martinsen, 1991; Steenkamp
et al., 2004). The ion intensities of m/z 138 were shown to increase as the cone voltage increased and it was the base peak at cone voltage of 50V-55V (Figure V.4.c and d).

The parent ion of scopolamine can be generated using both APCI and ESI probes. However, the major fragmentation ions of 138 and 156 were of higher intensities under ESI ionization conditions. Therefore, ESI probe in positive ion mode was chosen for the LC-MS study. At cone voltage of 30V and 50V base peaks of the parent ion and fragment ions of scopolamine could be produced, respectively. These two cone voltages were used in LC-MS studies. However, it should be noted that these mass spectrometry conditions were optimized for scopolamine analysis and may not be the optimum ionization conditions for the degradation products.
Figure V.3. Scopolamine mass spectra using APCI probes at cone voltages of (a) 30V (b) 60V.
Figure V.4. Scopolamine mass spectra using ESI probes at cone voltages of (a) 25V, (b) 30V, (c) 50V, and (d) 55V.
V.D.2. Development of Stability-indicating HPLC Methods for Scopolamine

Brown and Sleeman reported an HPLC method using 65% acidic solution (pH=3.4) of an ion pairing agent (0.01M 1-heptane sulfonic acid) and 35% acetonitrile as mobile phase and a C-18 column for the determination of atropine sulfate and tropic acid (Brown and Sleeman, 1978). Richard and Andermann reported a similar method using 50% sodium heptane sulfonic acid solution with 50% methanol as the mobile phase for the analysis of atropine sulfate and tropic acid (Richard and Andermann, 1984). Due to the structure similarity between atropine and scopolamine, we developed an HPLC method based on these two literature reported methods. A Discovery C18 (4.0×150mm, 5μm; Sigma-Aldrich Co., St. Louis, MO) column was used and operated at a flow rate of 1.0mL/min. 0.01M sodium heptane sulfonate solution was prepared and pH was adjusted to 3.5 with 0.01M ammonium acetate and acetic acid buffer. The ratio of aqueous to organic phase was varied to achieve separation of scopolamine with potential degradation products. The mobile phase was a mixture of 60.5% 0.01M sodium 1-heptanesulfonate pH 3.5 and 39.5% methanol. The chromatogram at UV 258nm of a scopolamine DSC sample showed that the method was able to provide baseline separation of scopolamine with its potential degradation products generated under elevated temperature conditions (Figure V.5). However, this method used a non-
volatile component: sodium 1-heptanesulfonate and it cannot be used on the LC-MS system.

Therefore, another HPLC method using a volatile ion-pairing agent was developed to allow the coupling of the HPLC system to the MS detector. Heptafluorobutyric acid (HFBA) is a commonly used volatile ion-pairing agent in LC-MS studies (Castro et al., 1999; Zhu et al., 2000) and was chosen for this study. The amount of HFBA was increased from 0.05% to 0.2% (v/v) during method development. With 0.05% and 0.1% HFBA in the mobile phase there were only a few peaks observed in the UV chromatogram when the ratio of aqueous phase changed from 60.5% to 75%. Co-elution of degradation peaks with scopolamine was found under those conditions. The amount of HFBA was then increased to 0.2% and pH of the aqueous phase was adjusted to 3.5 with ammonium formate and formic acid. The ratio of aqueous phase was varied in the range of 62% to 75% to achieve separation. More peaks were observed under these conditions and the separation of scopolamine with its potential degradation products was achieved at a ratio of 68:32 (aqueous: organic). The run time was varied from 28 to 45 minutes to ensure no peaks were eluted after 28 minutes. The final mobile phase was a mixture of 0.03M ammonium formate and 0.2% HFBA buffer solution with pH 3.5 with methanol at a ratio of 68:32 (v/v) operated on the Discovery
C18 column (4.0×150mm, 5μm; Sigma-Aldrich Co., St. Louis, MO) at 1.0mL/min. The validation of its stability indicating properties will be discussed below in detail.

Scopolamine has a retention time of 8.2min on the UV chromatogram (Figure V.6). The linearity range of the method was 4.07-127.2μg/mL with a correlation coefficient of the regression line of 0.9998 (7 concentrations measured in triplicate). Figure V.7 shows the graph of the calibration curve. The LOD of scopolamine was 2.04μg/mL and the LOQ was 4.07μg/mL (the RSD of peak area at these two concentrations was 6.0% and 2.2%, respectively). The injection repeatability was 0.64%. The “inter-day” precision (RSD, n=9) was found to be 0.29%. The accuracy (%DFN, n=9) for scopolamine assay was −0.73%.

V.D.3. Products of Degradation and Validation of Stability Indicating Assay

V.D.3.a. Degradation of Scopolamine under stressed conditions

Scopolamine degraded almost completely when stored in 0.1N NaOH solutions for 30min. Figure V.8 shows the UV (258nm) and total ion chromatogram of scopolamine in basic solutions after 30min. Scopolamine (Rt=8.2min) in the UV chromatogram could be detected but was below LOI (Figure V.8.a).
Figure V.5. UV chromatogram ($\lambda=258$nm) of scopolamine DSC samples using 60.5% 0.01M sodium 1-heptanesulfonate pH 3.5 and 39.5% methanol.
Figure V.6. UV chromatogram ($\lambda=258$nm) of scopolamine standard solution (Rt=8.2min)
Figure V.7. Calibration curve of scopolamine analysis (n=3, error bars are standard deviations and are covered by the size of the plotted symbol on the graph.)
Figure V.9 shows the mass spectra of the scopolamine standard from LC-MS studies. Similar to the mass spectra of directly infused scopolamine sample, at a cone voltage of 30V the parent ion of m/z 304 was the base peak (Figure V.9.a). At cone voltage of 50V, the predominant fragment ions were at m/z 138 and 156 and m/z of 138 was the base peak (Figure V.9.b). The mass spectra of scopolamine in 0.1N NaOH solutions (Figure V.9.c and d) were not significantly different with the mass spectra of scopolamine standard (Figure V.9.a and b) under LC-MS conditions, indicating no co-elution of ionized degradation product with scopolamine in the samples.

The major degradation product (compound A) had a retention time of 5.0min on the UV chromatogram and was not ionized under the LC-MS conditions (Figure V.8). It was found to be tropic acid by comparison with the standard. Several peaks were found on the total ion chromatogram but were undetectable on the UV chromatogram at 258nm probably due to their low concentrations or weak UV absorbance at 258nm. There was a peak in the total ion chromatogram at 2.6 minutes (compound B). Its mass spectra are shown in Figure V.10. It had a base peak at m/z 156, which is the basic moiety of scopolamine. Compound B is proposed to be scopine or scopoline as shown in Figure V.10. Both had been reported as hydrolysis degradation products (Holmes and Manske, 1950; Windheuser et al., 1972). Compound C had a retention time of 5.5min on the total ion chromatogram. Figure V.11 shows the mass spectra of compound C. It
had a base peak of m/z 125 at both cone voltages and its structure was not proposed. Compound D had a retention time of 26.8 min in the total ion chromatogram. Its mass spectra are shown in Figure V.12. The base peak at cone voltage of 30V was m/z 286 and was 18 mass units less than the parent ion of scopolamine. The fragment ions of m/z 138 became the base peak at cone voltage of 50V due to collision-induced dissociation. Dehydration of atropine in acidic or basic solutions had been reported previously (Holmes and Manske, 1950; Jira and Pohloudek-Fabini, 1982). Compound D was proposed to be aposcopolamine and its structure is shown in Figure V.12.

The structures (proposed or confirmed) of degradation products that were discussed in this chapter were summarized in Table V.2.

The recoveries of scopolamine after storage in acidic solutions for 3 days were expressed relative to their values at time zero. The mean recoveries (SD) (% remaining) of scopolamine at 23±2°C and 50±2°C were 98.73 (1.70) and 99.15 (1.49), respectively. No chromatographic degradation product with areas larger than 0.5% of the total area was detected. No peak besides scopolamine was observed in the total ion chromatogram. Scopolamine was stable when stored in 0.1N HCl solutions for three days at 23±2°C and 50±2°C.
Figure V.8. Scopolamine in 0.1N NaOH solution after 30min (a) UV chromatogram ($\lambda=258$nm), (b) total ion chromatograms at cone voltage of 30V, (c) single ion chromatogram at m/z 125, (d) single ion chromatogram at m/z 156, (e) single ion chromatogram at m/z 286.
Figure V.9. Mass spectra of scopolamine (Rt=8.2min) (a) standard at cone voltage of 30V, (b) standard at cone voltage of 50V, (c) in 0.1N NaOH solution after 30min at cone voltage of 30V, (d) in 0.1N NaOH solution after 30min at cone voltage of 50V
Figure V.10. Mass spectra and proposed structure(s) of compound B (Rt=2.6 min) formed in 0.1 N NaOH at cone voltage of (a) 30 V (b) 50 V.
Figure V.11. Mass spectra of compound C (Rt=5.5 min) formed in 0.1N NaOH solutions at cone voltage of (a) 30V (b) 50V
Figure V.12. Mass spectra and proposed structure of compound D (Rt=26.8 min) formed in 0.1N NaOH solutions at cone voltage of (a) 30V (b) 50V.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Proposed or confirmed structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound A</td>
<td>Tropic acid</td>
</tr>
<tr>
<td>Compound B</td>
<td>Scopoline, Scopine</td>
</tr>
<tr>
<td>Compound C</td>
<td>Unknown</td>
</tr>
<tr>
<td>Compound D</td>
<td>Aposcopolamine</td>
</tr>
<tr>
<td>Compounds E, F, G</td>
<td>Unknown</td>
</tr>
<tr>
<td>Compound H</td>
<td></td>
</tr>
<tr>
<td>Compounds I, J, K, L, M</td>
<td>Unknown</td>
</tr>
<tr>
<td>Compound N</td>
<td>Atropic acid</td>
</tr>
<tr>
<td>Compounds O, P, Q, R</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
Several degradation products (Compounds E-H) were formed when scopolamine was stored in 3% hydrogen peroxide solutions after 24hr. The HPLC method permitted separation of the active with the major degradation products (Figure V.13.a). Compounds E, F and G had retention times of 3.4, 4.2 and 5.6min, respectively. Their mass spectra (Figure V.14 and 15) all showed a base peak of 320 at cone voltage of 30V, which are 16 mass units higher than the parent ion of scopolamine. The fragment ions of m/z 138 and 156 were of high intensities at cone voltage of 50V. However, the structures of these compounds could not be reasonably hypothesized. Figure V.16 shows the mass spectra of compound H with retention time of 7.0min on the total ion chromatogram (Figure V.13.b). Similar to compounds E-G, it also had a base peak of m/z 320 at cone voltage of 30V. However, its predominant fragmentation ions were m/z 154 and 172, which were 16 mass units higher than the fragment ions at m/z 138 and 156 of scopolamine, respectively. This suggested the oxidation on the basic moiety of scopolamine. The proposed structure of compound H is shown in Figure V.16 with the oxidation taking place on the nitrogen.

Figure V.17 shows the UV (258nm) and total ion chromatogram of scopolamine after exposure at elevated temperatures up to 250°C for 3min using the DSC. The major degradation product was tropic acid (compound A) as shown on the UV chromatogram (Figure V.17.a). Additional degradation products on the UV chromatogram included
peaks at 5.8min (compound I), 7.2mm (compound J) and 26.2min. Compounds I and J were not ionized under the LC-MS conditions and their UV$_{max}$ were approximately 248 and 240nm, respectively. The peak at 26.2min was found to be compound D, which was also formed in basic solution as described previously. Several peaks (compounds K, L, M) were observed on the total ion chromatogram with retention time of 9.1, 12.8 and 15.0min, respectively, but were not detected on the UV chromatogram at 258nm. The structures of these degradation products could not be proposed based on their mass spectra. Table V.3 summarizes the degradation products formed at elevated temperatures up to 250°C.

V.D.3.b. Stability of scopolamine in vehicles for aerosol delivery

Scopolamine stability in formulations suitable for aerosolization in CAG (solutions in ethanol in both dark and light conditions) was tested. The apparent concentrations of the active were expressed relative to their values at time zero. The mean recovery (SD) (% remaining) of scopolamine in ethanol in dark and light conditions was 102.41 (2.62) and 102.53 (1.89), respectively. No chromophoric degradation products with areas larger than 0.5% of the total were detected. The LC-MS results were also studied and no co-elution of potential degradation product with
Figure V.13. Scopolamine in 3% H₂O₂ after 24hr (a) UV chromatogram at 258nm, (b) total ion chromatogram at scan 1 of cone voltage 30V, (c) single ion chromatogram at m/z 156, (d) single ion chromatogram at m/z 320.
Figure V.14. Mass spectra of compound E (Rt=3.4min), compound F (Rt=4.2min) formed in 3% H₂O₂ solutions (a) compound E at cone voltage of 30V, (b) compound E at cone voltage of 50V, (c) compound F at cone voltage of 30V, (d) compound F at cone voltage of 50V.
Figure V.15. Mass spectra of compound G (Rt=5.6min) formed in 3% H₂O₂ solutions at cone voltage of (a) 30V (b) 50V.
Figure V.16. Mass spectra and proposed structure of compound H (Rt=7.0 min) formed in 3% H₂O₂ solutions at cone voltages of (a) 30V and (b) 50V.
Figure V.17. UV and total ion chromatogram of scopolamine DSC sample. (a) UV chromatogram at 258nm. (b) total ion chromatogram at cone voltage of 30V.
Table V.3. The retention time, UVmax, and mass spectra characteristics of degradation products formed at elevated temperatures up to 250°C.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>UV$_{max}$ (nm)</th>
<th>Base peak$^a$</th>
<th>Fragment Ions$^b$ m/z (relative intensities)</th>
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</thead>
<tbody>
<tr>
<td>B</td>
<td>2.6</td>
<td>na</td>
<td>156</td>
<td>110 (100)</td>
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<td></td>
<td></td>
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<td></td>
<td>124 (18)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>138 (95)</td>
</tr>
<tr>
<td>A</td>
<td>5.0</td>
<td>258</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>na</td>
</tr>
<tr>
<td>I</td>
<td>5.8</td>
<td>248</td>
<td>na</td>
<td>na</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>na</td>
</tr>
<tr>
<td>J</td>
<td>7.2</td>
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<td>na</td>
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<td>K</td>
<td>9.1</td>
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<td>142</td>
<td>124 (100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>142 (88)</td>
</tr>
<tr>
<td>L</td>
<td>12.9</td>
<td>na</td>
<td>306</td>
<td>140 (100)</td>
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<tr>
<td></td>
<td></td>
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<td>122 (10)</td>
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<tr>
<td>M</td>
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<td>140 (100)</td>
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<td></td>
<td>122 (10)</td>
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<tr>
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<td>110 (48)</td>
</tr>
</tbody>
</table>

Data collected at cone voltage of $^a$30V and $^b$50V.
scopolamine was observed. Scopolamine was stable in ethanol formulations when stored in the dark for 7 days and under light for at least 3 days.

The forced degradation studies of scopolamine under stressed conditions showed that the HPLC method was able to separate scopolamine from its potential degradation products. The LC-MS method could provide useful information on the structure of the degradation products.

V.D.4. Aerosolization of Scopolamine in Ethanol Formulations

V.D.4.a. Stability of scopolamine during aerosolization in ethanol formulations

A series of power values were chosen at each formulation flow rate and single stage filter experiments were performed to assess scopolamine stability following aerosolization under those conditions.

Scopolamine recovery after aerosolization was expressed relative to the mass recovered from sham experiments performed in the absence of heating. Table V.4 lists the scopolamine recoveries from three solute concentration formulations at a formulation flow rate of 5.0 μL/s in a power range of 1.3-5.0 W. At powers ≤4.0 W, the mean recoveries of scopolamine were higher than 90% at all concentrations studied. No chromophoric degradation product larger than 0.5% of total area was observed. Thus,
although a thermally labile compound, scopolamine stability during aerosolization was maintained using ethanol as the vehicle. At powers of 4.6W and above, the recoveries decreased significantly and large amounts of degradation products were formed in the samples.

Table V.5 shows the scopolamine recoveries from three scopolamine formulations with different concentrations at a formulation flow rate of 10.0μL/s. The investigated power range was between 2.8 and 8.5W. The mean recoveries were higher than 90% under all investigated conditions. No chromophoric degradation product larger than 0.5% of total area was observed in any samples. Scopolamine was stable during aerosolization at powers of 8.5W and below at 10.0μL/s.

The filter deposition was expressed as the percentage of mass recovered from the glass fiber filter stage to the sham. The filter deposition may be indicative of the aerosolization efficiency. The larger the filter deposition, the higher is the aerosolization efficiency (Shen, 2003). Figure V.18.a shows the filter deposition at a formulation flow rate of 5.0μL/s for three scopolamine solution concentrations. The filter deposition was the lowest at the lowest powers, indicating that ethanol solutions were sprayed and deposited mainly in the USP induction port. As the power increased from 2.8 to 4.0W, high filter deposition values >85% were achieved suggesting good aerosolization. As the power increased above 4.0W, filter deposition decreased significantly, accompanied
by the degradation of scopolamine. Figure V.18.b shows the filter deposition at a formulation flow rate of 10.0µL/s. A relatively broad plateau was achieved between 5.9W and 8.5W with high filter deposition of above 80%. Therefore, the range of 2.8 (0.1) to 4.0 (0.1)W, and 5.8 (0.1) to 8.5 (0.0)W is considered the optimum power for aerosol generation from scopolamine ethanol formulations at formulation flow rates of 5.0 and 10.0µL/s, respectively.

The aerosol generation from ethanol formulations using the CAG is a thermal mechanical process (Hindle et al., 2004). It is hypothesized the pressure inside the capillary and the heating process of drug solution caused the atomization of the solution formulation and ethanol sprays are generated. The predominant process after the spray exits the capillary into the ambient air is solvent evaporation. In the CAG operation, the pressure inside the capillary during aerosolization is caused by the volume expansion of the heated solution thus is predominantly dependent on the temperature of the solution. As the power increases, the temperature of the solution should be increased and the pressure inside the capillary increases. The power needs to be high enough to heat and atomize the ethanol solution and enable the complete evaporation of ethanol sprays with or without the solute. The measured temperatures of the capillary wall at both flow rates were shown in Figure V.19. At both flow rates, the highest measured temperature was observed at the position of 30mm and it was above 80°C in the optimum power ranges.
Table V.4. Scopolamine recoveries from the single stage filter experiments at a formulation flow rate of 5.0μL/s

<table>
<thead>
<tr>
<th></th>
<th>8.2mM</th>
<th>20.2mM</th>
<th>40.4mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Steady State</td>
<td>Steady State</td>
<td>Steady State</td>
</tr>
<tr>
<td>Power (W)</td>
<td>Recovery (%)</td>
<td>Power (W)</td>
<td>Recovery (%)</td>
</tr>
<tr>
<td>1.4 (0.1)</td>
<td>99.37 (5.35)</td>
<td>2.4 (0.1)</td>
<td>90.43 (1.99)</td>
</tr>
<tr>
<td>2.2 (0.1)</td>
<td>94.65 (4.93)</td>
<td>2.9 (0.2)</td>
<td>99.53 (5.63)</td>
</tr>
<tr>
<td>2.9 (0.0)</td>
<td>99.38 (2.44)</td>
<td>3.5 (0.1)</td>
<td>96.97 (3.74)</td>
</tr>
<tr>
<td>4.0 (0.0)</td>
<td>102.23 (3.01)</td>
<td>4.0 (0.1)</td>
<td>99.23 (7.37)</td>
</tr>
<tr>
<td>4.9 (0.1)</td>
<td>71.00 (15.85)</td>
<td>4.6 (0.1)</td>
<td>76.27 (9.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0 (0.0)</td>
<td>84.49 (4.31)</td>
</tr>
</tbody>
</table>

n=5, data were expressed as mean (SD).
Table V.5. Scopolamine recoveries from the single stage filter experiments at a formulation flow rate of 10.0μL/s

<table>
<thead>
<tr>
<th></th>
<th>8.2mM</th>
<th>20.2mM</th>
<th>40.4mM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Steady State</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Power (W)</td>
<td>Recovery (%)</td>
<td>Power (W)</td>
<td>Recovery (%)</td>
</tr>
<tr>
<td>2.8 (0.3)</td>
<td>103.35 (2.37)</td>
<td>3.0 (0.1)</td>
<td>98.01 (1.69)</td>
</tr>
<tr>
<td>3.9 (0.1)</td>
<td>100.41 (5.19)</td>
<td>3.9 (0.1)</td>
<td>96.74 (2.29)</td>
</tr>
<tr>
<td>6.2 (0.0)</td>
<td>101.12 (4.61)</td>
<td>5.5 (0.1)</td>
<td>95.93 (4.74)</td>
</tr>
<tr>
<td>8.5 (0.0)</td>
<td>100.93 (2.05)</td>
<td>5.8 (0.1)</td>
<td>94.64 (3.42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.5 (0.2)</td>
<td>97.05 (3.19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.4 (0.1)</td>
<td>100.96 (2.94)</td>
</tr>
</tbody>
</table>

n=5, data were expressed as mean (SD).
Figure V.18. Filter deposition of scopolamine from single filter stage experiments at formulation flow rates of (a) 5.0μL/s, (b) 10.0μL/s.
found in filter deposition studies. The highest measured temperature at the same position was 110 (19) and 152 (32)°C, respectively, at 5.0 and 10.0μL/s. The sprays generated at different powers at 10.0μL/s are shown in Figure V.20. At low powers, the sprays were more visible and probably had larger size due to the low pressure generated at lower temperature. These sprays mainly deposited in the induction port as shown in Figure V.18.b. As the power was increased, the sprays became less visible probably due to 1) vaporization of the solution formulation and 2) faster and complete evaporation of ethanol.

It has been shown in Figure V.19 that the capillary wall temperatures at 10.0μL/s were much higher than they were at 5.0μL/s. However, despite the higher temperatures scopolamine remained stable at high flow rate conditions. This might be due to the reduced transit time of the solution formulation passing through the capillary at higher flow rate. For example, for 1μL solution, the transit time through the capillary tube at flow rates of 5.0 and 10.0μL/s was 200ms and 100ms, respectively.
Figure V.19. Temperature of the capillary wall at formulation flow rates of (a) 5.0μL/s (b) 10.0μL/s (n=3. Error bars were standard deviation. Data were plotted mean+SD.)
Figure V.20. Pictures of scopolamine aerosol generation at (a) 3.1W, (b) 5.7W, and (c) 9.6W at formulation flow rate of 10.0μL/s
V.D.4.b.  Scopolamine degradation products formed during aerosolization

Figure V. 21 shows the UV and total ion chromatogram of scopolamine samples collected on the filter from one single filter stage experiment at 4.9W at 5.0μL/s. The recovery of scopolamine from this sample was 57.9%. On the UV chromatogram at 258nm, four major degradation products were observed (Figure V.21.a). Their retention times were 5.0, 7.3, 16.9, and 26.3min. Only the peak at 26.3min was ionized under LC-MS conditions. It was proposed to be aposcopolamine (compound D), which was also formed in basic solutions and elevated temperatures up to 250°C as described previously. The peak at 5.0min was found to be tropic acid (compound A) by comparing the chromatographic profile with a standard of tropic acid. The peak at 7.3min (compound J) was also formed at elevated temperatures as described previously. Compound N had a retention time of 16.9min and its UV max was at 247nm. Attempts were made to collect and isolate the two peaks at 7.3 and 16.9min during HPLC analysis by fractionation. The nitrogen-dried samples of the eluents were reconstituted in small volume of solvents, such as acidified or basified methanol and acetonitrile, and were infused into mass spectrometer using both APCI and ESI probes in positive and negative ion modes to attempt to ionize. However, no useful mass spectrometry information was obtained.
Atropic acid is a potential degradation product of tropic acid and has $\text{UV}_{\text{max}}$ of 248nm (Holmes and Manske, 1950). In order to investigate whether atropic acid was one of the unknown products, atropic acid was synthesized from tropic acid (Raper, 1923). The synthesized product had an m.p. of 103-104°C (Literature 106-107°C). The $^1\text{H}$-NMR and $^{13}\text{C}$-NMR of the synthesized product were shown in Figure V.22 and were consistent with the structure of atropic acid. $^1\text{H}$-NMR (CDCl$_3$) $\delta$: 5.84 (d, 1H, $\equiv\text{CH}_2$), 6.02 (d, 1H, $\equiv\text{CH}_2$), 7.28 (m, 5H, aromatic). $^{13}\text{C}$-NMR (CDCl$_3$) $\delta$: 126.6 (aromatic), 128.6 (aromatic), 137.3 (RHC=), 142.1 (=$\text{CH}_2$), 168.4 (COOH). The mass spectrum collected by GC-MS is shown in Figure V.23. The mass spectrum of the synthesized atropic acid showed a peak of 221, which is considered to be the $[\text{M}]^+$ ion of the trimethylsilylized atropic acid. Analysis of the product by HPLC and GC-MS indicated that it had the same retention time, UV spectrum and mass spectrum as degradation product N. Compound N was confirmed to be atropic acid.

Fractionation collection of the peak eluted at 7.3min was performed during HPLC assay and the fractionation was repeated several times. The eluent was then dried under constant flow of nitrogen at room temperature and was reconstituted in 100μL acetonitrile and 100μL MSFTA, heated at 70°C for 15min prior to GC-MS assay. It had a retention time of 2.9min on the GC chromatogram. The mass spectrum revealed that it
had an ion of 147 as the base peak (Figure V.24). The major ions were present at m/z 190, m/z 100 and m/z 75. Its structure, however, could not be reasonably hypothesized.

There were several peaks observed in the total ion chromatogram. Some of these peaks (compounds K and M) were also formed at temperatures up to 250°C as described previously. Compounds O, P, Q and R showed retention time of 10.0, 11.6, 13.3 and 17.5 min on the total ion chromatogram (Figure V.21.b). The structures of these peaks could not be proposed based on their mass spectra. A group of peaks from 2.5 to 4.5 min was shown on the total ion chromatogram and had base peaks of m/z 381 on the mass spectrum at 30V (Figure V.21.h). The structures of these peaks were not clear. The retention time, \( \text{UV}_{\text{max}} \) and mass spectra of degradation products formed during aerosolization are summarized in Table V.6.

Significant amounts of degradation products could be formed during aerosolization at higher power levels for the 5.0 \( \mu \text{L/s} \) flow rate. Hydrolysis and dehydration of scopolamine appeared to be the major degradation pathways, which was consistent with previous findings as shown in Figure I.2. Secondary degradation, such as the formation of atropic acid, was involved in the process. It appeared that the high concentration formulations (20.2 mM and 40.4 mM) had relatively higher recoveries and less degradation in the samples generated at 5.0 W. However, large variability in recovery was associated with scopolamine recoveries from the lowest concentration
Figure V.21. Scopolamine single stage filter sample generated at 4.9W, (a) UV chromatogram at 258nm, (b) Total ion chromatogram at cone voltage of 30V, to be continued.
Figure V.21. Continued. Scopolamine single stage filter sample generated at 4.9W, (c) single ion chromatogram at m/z 156, (d) single ion chromatogram at m/z 286, (e) single ion chromatogram at m/z 288, to be continued.
Figure V.21. Continued. Scopolamine single stage filter sample generated at 4.9W, (f) single ion chromatogram at m/z 290, (g) single ion chromatogram at m/z 306, (h) single ion chromatogram at m/z 381, (i) single ion chromatogram at m/z 384.
Figure V.22. (a) $^1$H-NMR and (b) $^{13}$C-NMR of synthesized product—atropic acid
Figure V.23. Mass spectrum of synthesized atropic acid collected using GC-MS
Figure V.24. Mass spectrum of fractionated compound J collected using GC-MS
Table V.6. The retention time, UVmax, and mass spectra characteristics of degradation products formed during aerosolization

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>UVmax (nm)</th>
<th>Base peak&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fragment Ions&lt;sup&gt;b&lt;/sup&gt; m/z (relative intensities)</th>
<th>GC-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>2.6</td>
<td>na</td>
<td>156</td>
<td>110 (100) 124 (18) 138 (95)</td>
<td>na</td>
</tr>
<tr>
<td>A</td>
<td>5.0</td>
<td>258</td>
<td>na</td>
<td>na</td>
<td>73 (100) 147 (80) 280 (40)</td>
</tr>
<tr>
<td>I</td>
<td>5.8</td>
<td>248</td>
<td>na</td>
<td>na</td>
<td>147 (100) 190 (45) 75 (44)</td>
</tr>
<tr>
<td>J</td>
<td>7.3</td>
<td>240</td>
<td>na</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>9.2</td>
<td>256</td>
<td>290</td>
<td>142 (100) 124 (86)</td>
<td>na</td>
</tr>
<tr>
<td>O</td>
<td>10.0</td>
<td>230</td>
<td>306</td>
<td>140 (43)</td>
<td>na</td>
</tr>
<tr>
<td>P</td>
<td>11.6</td>
<td>258</td>
<td>384</td>
<td>218 (31)</td>
<td>na</td>
</tr>
<tr>
<td>Q</td>
<td>13.3</td>
<td>na</td>
<td>288</td>
<td>122 (100) 140 (28)</td>
<td>na</td>
</tr>
<tr>
<td>M</td>
<td>15.4</td>
<td>na</td>
<td>306</td>
<td>140 (55)</td>
<td>na</td>
</tr>
<tr>
<td>N</td>
<td>16.9</td>
<td>247</td>
<td>na</td>
<td>na</td>
<td>205 (100) 211 (38) 103 (30)</td>
</tr>
<tr>
<td>R</td>
<td>17.5</td>
<td>na</td>
<td>384</td>
<td>218 (32)</td>
<td>na</td>
</tr>
<tr>
<td>D</td>
<td>26.3</td>
<td>247</td>
<td>286</td>
<td>138 (100) 110 (20)</td>
<td>na</td>
</tr>
</tbody>
</table>

Data collected at cone voltage of<sup>a</sup>30V and<sup>b</sup>50V.
solutions (8.2mM). These were probably caused by the complexity of the degradation pathways.

**V.D.4.c. Particle Size Distribution of Scopolamine Aerosols Generated from Ethanol Formulations**

Target resistances were chosen to achieve steady state powers of 2.9 and 6.0W, respectively, at formulation flow rates of 5.0 and 10.0μL/s to determine the aerodynamic diameter of scopolamine aerosols using MOUDI™ cascade impactor.

Tables V.7 and 8 show the mean recoveries of scopolamine, MMAD, GSD, FPF, USP induction port deposition, and wall loss at formulation flow rate of 5.0 and 10.0μL/s, respectively. Similarly as described in Section IV.D.3, the total recovered scopolamine included the mass recovered from the USP induction port and the cascade impactor. The total recoveries were expressed as percentages relative to the recovered mass from sham. The MMAD and GSD were calculated by linear regression. FPF was the percentage mass of particles less than 5.6μm to the total recovered mass. Induction port deposition and wall loss were expressed as percentages of mass recovered from the induction port, the nozzle arrays and the wall of the impactor stage to the total recovered mass, respectively.
At a formulation flow rate of 5.0μL/s, scopolamine mean recoveries were higher than 86%. The MMAD ranged from 1.4 to 2.2μm and the GSD was between 2.2 and 3.4. The mean FPF was found to be between 72% and 82%. Both the mean induction port deposition and wall loss were less than 10%.

At a formulation flow rate of 10.0μL/s, the mean recoveries of scopolamine were between 84 and 94%. The MMAD was between 0.5 and 1.4μm and the GSD was in the range of 2.0 to 2.7. The mean FPF was between 85 and 97%. The mean induction port deposition was below 10% and the mean wall loss was less than 5%.

The MMAD and the high fine particle fraction of these scopolamine aerosols suggested highly respirable particles and are suitable for pulmonary administration to achieve systemic effects. Figures V.25-27 shows the particle size distribution of scopolamine aerosols generated from 8, 20 and 40mM formulations at 5.0 and 10.0μL/s, respectively. Figure V.28 shows the cumulative particle size distribution of these aerosols.

At both formulation flow rates, the MMAD increased as a function of increasing the solute concentration. The effect of solute concentration on the particle size was consistent with the previous findings on spray-dried particles (Crosby and Marshall, 1958; Fell and Newton, 1971). It was found that increasing the solute concentration the particle size was increased. This effect may be better understood using Equation 1.4 and
5. As described in chapter I, equation I.4 was derived empirically when liquid droplets were atomized from a capillary tube by pressure. As discussed previously, the aerosol generation from ethanol formulation was hypothesized to involve the atomization of solution by pressure and equation I.4 can be used to estimate the initial droplet size. According to this equation, it is reasonable to assume that under similar aerosolization conditions the initial droplet size were similar for different solute concentration

\[ D_G = 36D_0^{0.56}N_{Re}^{-0.1} \quad \text{Equation I.4} \]

formulations since the Reynolds number was not likely to be affected by the solute concentration. If we assumed that the ethanol evaporated completely at optimum aerosolization conditions, then the solvent content in the final particles is 0 and equation I.5 becomes equation V.1,

\[ \frac{d\nu_2}{dp_i} = \frac{\rho_L(1)(1+W_2)}{\rho_2(1+W_1)(1)} \]  \quad \text{Equation I.5.} \]

\[ \frac{d\nu_2}{dp_i} = \left[\frac{\rho_L(1+W_2)}{\rho_2(1+W_1)}\right]^{1/3} \quad \text{Equation V.1} \]

where \( d\nu_2 \) is the final size, \( dp_i \) is the initial droplet size, \( \rho_L \) and \( \rho_2 \) are the density of the initial and final particles, and \( W_1 \) is the initial solvent content. The size of the final particles is dependent on the size of initial droplets, the density of initial and final droplets and the solvent content in the initial droplet. In this study, the density of initial droplets can be assumed to be same for different solute concentration solutions. Therefore, for different solute concentration formulations, both the density of the final
Table V.7. Particle size distribution summary of scopolamine in ethanol formulations at a formulation flow rate of 5.0μL/s

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Recovery SSP (W) (%)</th>
<th>Recovery MMAD (μm)</th>
<th>GSD 3.35 (1.50)</th>
<th>Fine Particle Fraction (%)</th>
<th>Induction Port Deposition (%)</th>
<th>Wall loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2</td>
<td>2.8 (0.1)</td>
<td>86.46 (7.06)</td>
<td>1.37 (0.18)</td>
<td>82.39 (4.78)</td>
<td>8.62 (3.44)</td>
<td>8.99 (1.37)</td>
</tr>
<tr>
<td>20.8</td>
<td>3.0 (0.1)</td>
<td>88.67 (6.35)</td>
<td>1.67 (0.06)</td>
<td>79.95 (5.21)</td>
<td>9.96 (6.28)</td>
<td>6.08 (1.00)</td>
</tr>
<tr>
<td>40.5</td>
<td>2.8 (0.0)</td>
<td>91.64 (2.37)</td>
<td>2.24 (0.09)</td>
<td>71.62 (2.09)</td>
<td>9.25 (1.64)</td>
<td>8.92 (0.90)</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD). n=5. MMAD and GSD were calculated by linear interpolation. Fine particle fraction was calculated as percentages of particles less than 5.6μm of total recovered dose.
<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>SSP (W)</th>
<th>Recovery (%)</th>
<th>MMAD (µm)</th>
<th>GSD</th>
<th>Fine Particle Fraction (%)</th>
<th>Induction Port Deposition (%)</th>
<th>Wall loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.4</td>
<td>6.2 (0.1)</td>
<td>84.43 (4.71)</td>
<td>0.50 (0.00)</td>
<td>1.96 (0.02)</td>
<td>96.94 (4.42)</td>
<td>0.53 (1.19)</td>
<td>2.53 (3.49)</td>
</tr>
<tr>
<td>19.8</td>
<td>5.9 (0.2)</td>
<td>92.90 (4.27)</td>
<td>0.81 (0.08)</td>
<td>2.33 (0.23)</td>
<td>92.24 (1.90)</td>
<td>3.44 (0.71)</td>
<td>4.32 (1.32)</td>
</tr>
<tr>
<td>39.2</td>
<td>5.9 (0.1)</td>
<td>94.40 (2.02)</td>
<td>1.43 (0.12)</td>
<td>2.72 (0.12)</td>
<td>85.07 (4.76)</td>
<td>8.19 (4.37)</td>
<td>3.17 (0.35)</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD). n=5. MMAD was calculated by linear interpolation. Fine particle fraction was calculated as percentages of particles less than 5.6 µm of total recovered dose.
Figure V.25. Particle size distribution of scopolamine aerosols generated from 8mM ethanol formulations at (a) 2.8 (0.1)W, 5.0μL/s and (b) 6.2 (0.1)W, 10.0μL/s (n=5, error bars are SD).
Figure V.26. Particle size distribution of scopolamine aerosols generated from 20mM ethanol formulations at (a) 3.0 (0.1)W, 5.0μL/s and (b) 5.9 (0.2)W, 10.0μL/s (n=5, error bars are SD).
Figure V.27. Particle size distribution of scopolamine aerosols generated from 40mM ethanol formulations at (a) 2.8 (0.0)W, 5.0µL/s and (b) 5.9 (0.1)W, 10.0µL/s (n=5, error bars are SD).
Figure V.28. Mean cumulative % mass undersize distribution for scopolamine aerosols generated from ethanol formulations at different concentrations at formulation flow rates of (a) 5.0μL/s, (b) 10.0μL/s. Error bars are SD.
particles and initial solvent content (thus the drug concentration in this case) affect the size of the final particles. The density of the final particles in our studies was not known. It has been reported that the density of spray-dried particles decreased as particle size increased (Crosby and Marshall, 1958).

It was also found that increasing the formulation flow rate, the particle size was decreased at all three concentrations \((p<0.05, \text{ t-test})\). Reynolds number is directly proportional to the linear velocity of the spray as shown in equation V.2,

\[ N_{Re}=D \cdot V \cdot \rho / \eta \]  
Equation V.2

where \(D\) is the diameter of the capillary, \(V\) is the velocity of the spray, \(\rho\) is the density of the fluid and \(\eta\) is the viscosity. For a capillary tube, increasing the formulation flow rate increases the linear velocity of the spray and the Reynolds number was increased. According to equation I.4, the size of the initial droplets was decreased. Since the final particle size is directly related to the initial droplet size (Crosby and Marshall, 1958; Elversson et al., 2003), the size of the sprayed droplets must have been reduced significantly at higher flow rate. Moreover, the fact that aerosols produced using the CAG, from these ethanolic solutions, were generally less than 5μm in size, following evaporation, this implied an initial droplet size of the order of 10 to 20μm, with an approximate numbers concentration of around \(10^6\). This implied that droplet formation in the CAG was much more efficient than would have occurred if the mechanism was
simply spray formation at the tip of the nozzle, as described by Duffie and Marshall (Duffie and Marshall, 1953). It is thus likely that droplet formation begins within the capillary during the heating and pumping process, and that this continues with spray atomization at the exit.

SEM was taken when scopolamine aerosols were generated from 8mM at 5.5W at 10.0μL/s. Figure V.29 shows the SEM of these scopolamine aerosols collected on stage 6 and stage 10 of MOUDI. From the SEM pictures, it was found that the particles were spherical, and discretely distributed. No crystal properties of the particles were observed.
Figure V.29. SEM of scopolamine aerosols collected on (a) stage 6, (b) stage 10 of MOUDI cascade impactor
V.E. CONCLUSIONS

A stability-indicating HPLC method for the analysis of scopolamine was developed and validated. The method was capable of resolving scopolamine from its major degradation products formed under a wide variety of stressed conditions. Scopolamine degraded almost completely in 30 min in basic solutions and gave tropic acid as the major degradation product. Several oxidation products were formed when scopolamine stored in hydrogen peroxide solutions. In the solid state, scopolamine degraded to several degradation products at elevated temperatures. The structural information of the degradants was obtained using LC-MS.

Chemically stable scopolamine aerosols can be generated from ethanol formulations by optimizing the aerosolization conditions. The optimum power ranges were 2.8 (0.1) to 4.0 (0.1) W, and 5.8 (0.1) to 8.5 (0.0) W at 5.0 and 10.0 μL/s, respectively. At flow rate of 5.0 μL/s, scopolamine degraded significantly at powers of 4.6 W and above. Tropic acid and synthesized atropic acid were found to be the major degradation products. Hydrolysis and dehydration appeared to be the major degradation pathways of scopolamine during aerosolization.

Scopolamine aerosols of size ranging from 0.5 to 2.2 μm could be generated at optimum powers. Increasing the solute concentration increased the particle size of scopolamine aerosols. Increasing the formulation flow rate reduced the size of these aerosols. The size of scopolamine aerosols generated from ethanol formulations thus can be controlled by altering formulation and operational variables.
CHAPTER VI

OVERALL SUMMARY AND CONCLUSIONS

The capillary aerosol generator (CAG) can generate fine particle aerosols by heating a solution formulation as it passes through a capillary tube. Depending on the nature of the excipient, it can either condense to produce a droplet condensation aerosol, or evaporate to produce a soft mist aerosol. Because the formulations are exposed to elevated temperatures for short periods of time during passage through the capillary, the thermal stability of the drug must be assured. Pulmonary delivery of antiemetic agents may have the advantages of fast onset of action, increased bioavailability and reduced side effects. Two strong antiemetics, perphenazine and scopolamine were chosen as oxidation prone and thermally labile model compounds, respectively, for the study.

In this thesis, the chemical stability of perphenazine and scopolamine under various aerosolization conditions was investigated. Factors such as vehicle, drug concentration, steady state power and formulation flow rate were varied to study their effects on drug stability during aerosolization. The effects of drug concentration and formulation flow rate on the particle size of drug aerosols were also investigated.

Stability-indicating HPLC and LC-MS methods were developed and validated for perphenazine. Solution and solid-state degradation products of perphenazine were
generated to enable validation of the assay methods and to elucidate possible aerosol degradation products. Structures of these degradants were proposed based on their mass spectra and, where possible, identification was confirmed by chemical synthesis or use of a chemical standard.

Perphenazine stability during aerosolization was investigated at drug concentrations of 9, 48 and 90mM in PG solutions. The CAG generates condensation aerosols from PG formulations by vaporizing the solutions. Between the power ranges of 4.5(0.2)-5.7(0.2)W and 9.6(0.2)-12.0(0.3)W at formulation flow rates of 2.5 and 5.0µL/s, respectively, there was no statistically significant difference in the aerosol recovery of perphenazine, indicating no change in chemical stability at each concentration. At both flow rates, increased amounts of the degradation product (2-[4-(3-phenothiazin-10-yl-propyl)-piperazino]-ethanol) were observed for the 9mM solution when aerosolized at the high power levels, probably due to the increased temperatures experienced by the drug at those powers. There were no changes in the amounts of degradation products generated from the 48 and 90mM formulations. The low concentration formulation (9mM) appeared to be more susceptible to degradation under similar aerosolization conditions. Dehalogenation appeared to be the major degradation pathway for perphenazine during aerosolization and the reaction was hypothesized to be a zero order process. Although the temperature exposure at 5µL/s formulation flow rate was much higher than at 2.5µL/s, perphenazine stability during aerosolization was not affected by rate, this probably due to the reduced transit time within the capillary of the solution at higher flow rate.
The particle size distribution of perphenazine aerosols generated under optimum power conditions were shown to be bimodally distributed: the first mode being particles less than 0.172μm, and the second mode being particles with aerodynamic diameters of 0.5-1.0μm. A bimodal distribution equation was used to describe the bimodality of perphenazine aerosols. Both homogeneous and heterogeneous nucleation processes, for the drug and the drug and PG respectively, were believed to be involved in the formation of perphenazine aerosols and this could have resulted in bimodal distribution. Because of the nature of the experiments however, where aerosols were collected, and drug was quantified, a number of events after aerosol generation had occurred, deductions made about the microsecond time scale of events at the CAG nozzle could not be drawn in any absolute sense; this because the processes of coagulation, PG evaporation and Ostwald ripening could all be happening prior to aerosol collection, while PG evaporation can occur at collection sites within the cascade impactor. Following collection, drug-based aerosol MMAD’s were between 0.4 and 0.6μm. Varying the drug concentration affected MMAD in a relatively small way ~0.2μm. Varying the drug concentration affected the aerosols in different manners perhaps depending on the nucleation mechanism. Increasing the drug concentration probably increased the available number of drug nuclei in the vapor, therefore promoting possible coagulation growth of homogeneously nucleated drug particles, increasing the aerosol size of the first (submicrometers) mode. In general however, increasing the drug concentration in the formulation and the vapor, decreased the magnitude of the submicrometer aerosol fraction. Sub-micron MMAD’s as well as fine particle fractions
were higher than 84% in all cases which indicated potentially high pulmonary delivery efficiency of these aerosols. Increasing the formulation flow rate did not significantly affect the particle size of perphenazine aerosols.

Stability-indicating HPLC and LC-MS methods were also developed and validated for scopolamine hydrobromide trihydrate. Degradation products were formed in basic solutions, hydrogen peroxide solutions and in the solid state, at elevated temperatures. The structures of these degradation products were proposed based on their mass spectra or confirmed by comparing with a standard or by chemical synthesis where appropriate.

Scopolamine degraded significantly when aerosolized by the CAG in PG formulations due to the relatively high temperatures combined with its thermal vulnerability. Scopolamine was then formulated in ethanol solutions at concentrations of 8, 20 and 40nM and was aerosolized over the power ranges of 1.3 (0.1) to 5.0 (0.0)W and 2.8 (0.3) to 8.5 (0.0)W, respectively, at formulation flow rates of 5.0 and 10.0µL/s. The chemical stability of scopolamine could be maintained during aerosolization at or below 4.0 (0.1)W and 8.5 (0.0)W, at 5.0 and 10.0µL/s, respectively. The peak capillary temperature during aerosolization of ethanol solutions was at or below 160°C in all cases. Degradation of scopolamine was observed at applied powers at or above 4.6 (0.0)W at 5.0µL/s. The major degradation products were found to be tropic acid and atropic acid. Another major degradation product was not identified. Hydrolysis and dehydration of scopolamine appeared to be the major degradation pathways during aerosolization and it is possible that use of scopolamine trihydrate salt
may be an important determinant of these observed degradation pathways. Due to the complexity of the degradation pathways, the effect of drug concentration on scopolamine stability during aerosolization was not clear. Similar to the findings in the perphenazine study, at the higher formulation flow rate the temperature during aerosolization was higher. However, this feature did not affect scopolamine stability during aerosolization probably because of the reduced transit time through the capillary and reduced exposure seen by the drug at the higher formulation flow rate.

The MMAD’s of scopolamine aerosols generated from ethanol formulations with concentrations of 8, 20 and 40mM at 5.0 and 10.0μL/s were between 0.5 and 2.2μm when generated under optimum conditions where sufficient power was supplied to raise the temperature of and evaporate the ethanol in the formulation. The aerodynamic particle size distributions were dependent on both drug concentration and formulation flow rate. The drug aerosol size increased as a function of increasing the drug concentration and decreasing the formulation flow rate through the CAG. The aerosol generation from ethanol formulations was considered to be a process similar to spray drying although the efficiency with which new surface was created in the CAG using heating, was far greater than that seen with spraying alone. Ethanol sprays were generated in part by the pressure inside the capillary tube but the coincident evaporation of ethanol during passage along and upon exiting the capillary enabled much smaller initial droplets to be formed when this system was compared to a conventional droplet spray process. Increasing the drug concentration in this situation decreased the solvent content of initial droplets and probably contributed somewhat to the increased size of
final particles. Increasing the formulation flow rate increased the linear velocity of the spray, turbulence within the CAG and thus the Reynolds number at the exit, all of which reduced the size of initial droplets. The SEM of scopolamine aerosols collected on MOUDI stages revealed that the powders were spherical and discretely distributed.

Overall, during aerosolization by the CAG, chemical stability of the drugs can be maintained by varying formulation and operationing parameters. In addition, these studies have shown the possibility of using these same variables to control the aerosol particle size within the range that is most suitable for drug delivery by inhalation.
Literature Cited
References


APPENDIX
Appendix I

LOG-NORMAL CURVE FITTING IN SIGMAPLOT (BI-MODAL DISTRIBUTION)

[Parameters]
\( \text{MMAD}_1 = 0.1 \)
\( GSD_1 = 1.5 \)
\( \text{MMAD}_2 = 0.5 \)
\( GSD_2 = 1.5 \)
\( p = 0.5 \)

[Variables]
\( x = \text{col}(1) \)
\( y = \text{col}(2) \)

[Equations]
\[ f = p \cdot \left( \frac{1}{x \cdot \ln(GSD_1) \cdot \sqrt{2\pi}} \right) \cdot \exp\left(-\frac{(\ln(x) - \ln(\text{MMAD}_1))^2}{2 \cdot (\ln(GSD_1))^2}\right) + (1-p) \cdot \left( \frac{1}{x \cdot \ln(GSD_2) \cdot \sqrt{2\pi}} \right) \cdot \exp\left(-\frac{(\ln(x) - \ln(\text{MMAD}_2))^2}{2 \cdot (\ln(GSD_2))^2}\right) \]
fit \( f \) to \( y \)

[Constraints]

[Options]
iterations = 10000
stepsize = 1
tolerance = 0.00001
VITA

Xihao Li was born in Luohe, China on Nov.9, 1977 and is a Chinese citizen. She received a BS in Pharmacy from Beijing Medical University (now Peking University) in 1998 and MS in Medicinal Chemistry from Peking Union Medical College & Chinese Academy of Medical Science in 2001. In Spring 2002, she entered the graduate program at Virginia Commonwealth University to pursue her Ph.D. degree in Pharmaceutics under the guidance of Drs. Frank E. Blondino, Michael Hindle and Peter R. Byron. During the course of her Ph.D. studies, she has published one research article and four abstracts, currently she has one research paper in preparation. Xihao is a member of AAPS and AAAS.