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Analysis of the Aerosol Composition, Its Deposition and Uptake in Electronic Cigarette Users

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ANALYSIS OF THE AEROSOL COMPOSITION, ITS DEPOSITION AND UPTAKE IN ELECTRONIC CIGARETTE USERS

by

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ABSTRACT

Electronic cigarettes (e-cigarettes) generate aerosol (particles in a gas matrix) by vaporizing the e-liquid, consisting of propylene glycol (PG), glycerol (GL), nicotine, and other additives. Aerosol enters the respiratory tract (RT) of the user, where part of it is deposited, while the rest is exhaled. Compounds in the gas and particle phase have different deposition mechanisms in the RT, thus determining the location of deposition. The location of deposition and amount of a compound will influence the biological response in the body. Identifying the composition of each constituent in the gas phase and particle phase would enhance the understanding of their deposition in the RT, thus absorption into the body.

Study 1: assess the composition difference in aerosol particle phase (PP) from its e-liquid, and identify the mechanism associated with the composition difference. Gas chromatography analysis was conducted on e-liquid and aerosol PP for PG, GL, and nicotine. Experiments were designed to isolate the mechanism for composition difference by testing evaporation, aging, vaporization, and condensation. **Study 2:** streamline a methodology to study impact of user-specific parameters on aerosol deposition in e-cigarette users. A pilot study was conducted with participation of e-cigarette consumers to test the usability of exhale breath collection device. Feasibility of quantifying yield, exhale breath, puff and respiratory topographies were evaluated. Salivary cotinine boost was quantified to measure nicotine uptake.

PG in aerosol PP showed a significant reduction from the e-liquid. Nicotine composition difference between e-liquid and the PP for non-acidified e-liquid was greater from acidified e-liquids. Study data is in agreement with the condensation model to explain the composition change of aerosol PP vs the initial e-liquid. The pilot study successfully quantified exhale breath composition, deposition efficiencies, salivary cotinine boost, puff and respiratory topographies. Findings confirmed the composition of aerosol PP to be different from its e-liquid and identified the mechanism for the difference. Pilot study successfully demonstrated the proof of concept to study the impact of user-specific parameters in e-cigarette users.

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NOMENCLATURE

Aerosol PP – aerosol particle phase

cTPM – concentration of total particulate matter (aerosol mass concentration)

E-cigarette – electronic cigarette

EBC – exhale breath collection

ENDS – electronic nicotine delivery system

EVALI – e-cigarette, or vaping, product use associated lung injury

FDA – Food and Drug Administration

GC – gas chromatography

GC-FID – gas chromatography flame ionization detector

GL – glycerol

HPHC – harmful and potentially harmful constituent

IRB – Institutional Review Board

$NicH^+$ - protonated nicotine

PCU – power control unit

PG – propylene glycol

PMTA – premarket tobacco product application

PPT – participant

RIT – Rochester Institute of Technology

RT – respiratory tract

RTL – Respiratory Technologies Lab

TAP – topography analysis program

THC – Tetrahydrocannabinol

TPM – total particulate matter

Chapter 1 INTRODUCTION TO ELECTRONIC CIGARETTES

1.1 Background on electronic cigarettes

Electronic cigarette (e-cigarette), also known as the electronic nicotine delivery system (ENDS) was first introduced in 2003 by Hon Lik as a low health risk alternative to tobacco cigarettes.[1] Since then, it has become more popular, and the complexity of the device has increased. E-cigarettes have become more popular than tobacco cigarettes, especially among the young population of middle school, high school, and those in their early 20s.[2] This could be due to diverse product features and degree of customization. The popularity had surged the global e-cigarette and vaping market value to USD 22.45 billion in 2022, and it is expected to reach USD 182.84 billion by the year 2030.[3]

E-cigarettes were first introduced as smoking cessation devices. The main reason for considering e-cigarettes to be healthier than tobacco cigarettes is that, unlike tobacco cigarettes, combustion, which produces substantial toxic compounds, does not occur in e-cigarettes. The goal of introducing e-cigarettes was reduction or complete abstinence from tobacco cigarettes in the adult population.[4] A transition to e-cigarettes as an alternative to tobacco cigarettes can be beneficial to certain vulnerable populations, such as smokers with chronic respiratory or cardiovascular illnesses or mental health conditions. However, e-cigarettes still produce toxic substances, including fine particulate matter, metals, and nicotine, even though they do not produce combustible toxic products. For example, nicotine present in e-cigarettes causes adverse effects on child development, which suggests their use during pregnancy can be harmful to the fetus.[5] Furthermore, there are concerns that switching to e-cigarettes might discourage current tobacco cigarette users from quitting smoking. The long-term adverse effects of e-cigarette usage are not yet clear due to the lack of epidemiological studies and clinical trials.

Health complications related to e-cigarette use have been observed in recent years. A major example from recent history is the outbreak of e-cigarette, or vaping, product use-

associated lung injury (EVALI). Reported hospitalized EVALI cases as of February 18, 2020, was 2,807, while confirmed number of deaths was 68 since its first case in December 2019.[6] Product sample testing and patient reports showed tetrahydrocannabinol (THC) containing e-cigarettes or vaping products are associated with EVALI. Vitamin E acetate, which has been reported as the primary cause of EVALI, is a diluent of THC found in tested product samples and patient lung fluid samples.[7] In addition to EVALI, several studies have linked e-cigarette consumption with health risks such as asthma, breast cancer development, and lung metastasis.[8,9] There is no adequate research to identify pathways causing these health risks in e-cigarette users.

The study of e-cigarette toxicity on users is challenging due to the presence of diverse device types in the market, different compositions in e-liquid, changes in puff behavior and inhalation behavior. Regulations are much needed on the e-cigarette industry to control rapid product changes and related toxicity. The U.S. Food and Drug Administration (FDA) has enforced regulations on flavored e-cigarette products and premarketing of tobacco products.[10,11] Premarket tobacco product application (PMTA) has to be submitted for any tobacco product to gain FDA marketing order. PMTA should be supported by scientific evidence that the product is suitable for public health. PMTAs have been granted for 23 e-cigarette devices and products by NJOY LLC, R.J. Reynolds Vapor Company, and Logic Technology Development LLC manufacturers.[12] However, more than 200 tobacco-related products including two Vuse brand menthol e-cigarette products faced PMTA denial.[13] FDA received nearly 6.7 million PMTA applications for tobacco related products by the September 9, 2020 deadline, which indicates the flux of products introduced to market.[14] Thus, there's a dire need for scientific studies to regulate the e-cigarette market and to identify and minimize vaping-associated health risks.

1.2 Types of electronic cigarette devices

All e-cigarettes are based on the same thermal aerosolization process, but they evolved over time in terms of design, function, and performance. E-cigarette devices can be categorized into four generations according to the time they were first released (Figure

1.1). First generation device contained a small lithium battery and a cartomizer, which was designed to mimic the look and feel of combustible cigarettes. Cartomizer consists of a polyester material soaked in e-liquid.[15] First generation devices do not contain e-liquid filled tanks or cartridges. The first-generation devices didn't provide much control to users over device power or design choices. Second generation devices resembled large pens in shape. They consisted of a rechargeable lithium battery and a refillable tank filled with e-liquid. Tank design allowed it to store more e-liquid compared to the first-generation devices. Third generation devices are called "mods" because they allow users to adjust multiple parameters such as voltage, wattage and power. The newer coils in the 3rd generation devices have lower resistance, which boosts the vapor production.[1] They contain e-liquid filled tanks and large capacity lithium batteries.[16] Fourth generation devices, known as "pod mods" or "pods" contain prefilled or refillable pods that come in different designs. Differences in battery, coil, and designs could vary the amount of aerosol produced. JUUL® brand pod mods introduced in 2015 became the most popular e-cigarette brand in USA accounting for 76% of retail e-cigarette market at the end of year 2018.[17] JUUL became popular among both young adults and adolescents due to the appealing design of the device, and the more satisfying experience provided by its e-liquid.[18] Popularity of JUUL products influenced other manufacturers to produce similar products.

(A) First Generation (B) Second Generation (C) Third Generation (D) Fourth Generation

Figure 1.1 E-cigarette devices of each generation

Different e-cigarette devices came to the retail market very rapidly with modifications to design, e-liquid, or flavors. The number of unique products quadrupled from year 2021 to

2022, going from 453 to 2023 products.[19] In the study by Hensel et. al. (2021), thirteen unique e-cigarette products were studied showcasing the different characteristics in product size and shape, battery capacity, coil resistance, and e-liquid composition.[20] The considered thirteen products included devices with presence/absence of rechargeable power control unit (PCU), refillable reservoir, adjustable power, and different reservoir capacity, different battery capacity and more features, showcasing the diverse design features available in the current e-cigarette market. It is difficult to study the predominant e-cigarette device due to the high volume of novel products introduced to the market, and the diversified modifications in devices. Understanding the fundamental mechanism of how the device behaves benefits in studying the risks associated with exposure to vaping, and potential controllers for harm reduction.

1.3 Operation mechanism of an electronic cigarette

Main components of an e-cigarette are the battery, the heating coil, and the cartridge which contains the e-liquid (Figure **1.2**). The heating element in the device can be activated by two different methods based on the device type. One method is by the user manually pressing a button to activate the heating element. In the second method, the user draws upon the e-cigarette and an airflow sensor automatically activates the heating element by detecting the pressure changes. The activated heating element vaporizes the e-liquid in the cartridge. When the vapor travels away from the heating element, nucleation occurs forming aerosol particles due to the temperature drop.[21] Therefore, emissions from the e-cigarette are particles consisting of both a vapor phase and a particle phase. E-liquid components that condense stay as particles and the rest is in the vapor phase. Emissions from e-cigarette also contain byproducts of PG and GL due to thermal degradation.[22,23] When the aerosol enters into the respiratory tract, species from both gas and particle phases deposit, and the rest is exhaled. Deposition varies depending on many factors such as particle size, vapor pressure of the species, and temperature.[24] A number of compounds, including propylene glycol, glycerol, and nicotine, could possibly be in the gas phase and particle phase depending on their volatility. Species in the aerosol gas and particle phases uptake into the body through

different mechanisms.[25] Therefore, the composition in the gas and particle phases plays an important role in understanding adverse health effects due to e-cigarette consumption.

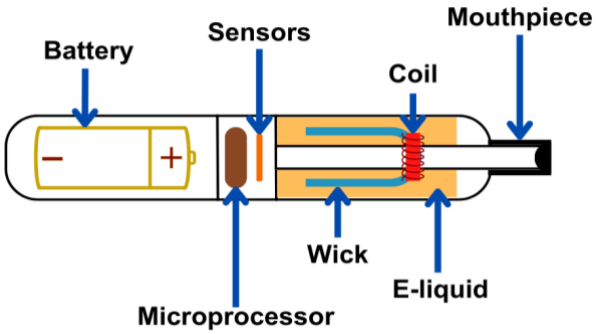


Figure 1.2 Schematic diagram of an electronic cigarette

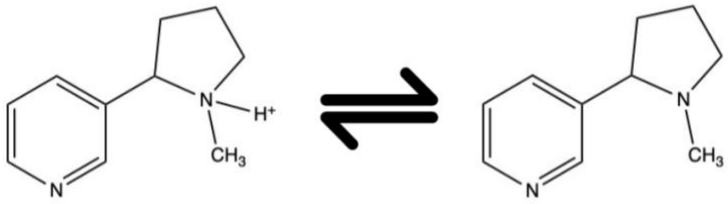
1.4 E-liquid composition

1.4.1 Propylene glycol and glycerol

Composition in the e-liquid varies among manufacturers. The main constituents of the e-liquid are propylene glycol (PG), glycerol (GL) also referred to as “vegetable glycerin”, and nicotine. Propylene glycol and glycerol are humectants used as carriers for nicotine. These humectants produce a cloud effect that mimics tobacco cigarette smoke. The ratio of propylene glycol to glycerol can be different in each of the products depending on the manufacturer. PG:GL ratio of e-liquids available in the market could vary between 100% PG to 100% GL. A study by Hensel *et. al.* (2021), discusses PG:GL mass ratio variation from 20:80 to 74:26 among thirteen products. Among the thirteen products, all except for four products had different PG:GL compositions. PG:GL ratio alters the visual appearance of the exhaled vapor and sensitivity to flavorings.[26] E-cigarette users have reported that PG produces better “throat hit” and carries flavors better than glycerol. However, glycerol base e-liquids have been reported to be much smoother than PG base ones.[26–28] A better “throat hit” can be appealing to experienced tobacco cigarette users, while the smoother vaping experience can be preferred by new users. PG:GL ratio difference is another changing factor that contributes to user exposure.

1.4.2 Nicotine

Nicotine has been identified as a harmful and potentially harmful constituent (HPHC) by the FDA which causes addiction and toxicity in reproduction and development.[29] Nicotine content in the e-liquid can vary between 12-50 mg/mL depending on the manufacturer.[20,29] However, when the nicotine concentration is high it causes a harshness in the throat during consumption. In the 1960s, Philip Morris discovered the sensory effects caused by nicotine can be manipulated by changing the form of nicotine.[30] There are two forms of nicotine based on the solution pH, (1) unprotonated nicotine known as “free-base nicotine”, and (2) protonated nicotine referred to as “nicotine salt” by the e-liquid manufacturers (Figure **1.3**). Philip Morris used ammonia to increase unprotonated nicotine in Marlboro cigarettes which produced a powerful nicotine kick compared to the cigarettes in the US market. In 2015 PAX Labs, Inc., the original developers of JUUL brand e-cigarettes obtained a patent for a nicotine salt formulation created by adding an acid to the e-liquid.[31] Since then JUUL and many other e-liquid products in the market has been supplemented with an acid to produce protonated nicotine, allowing manufacturers to increase the nicotine content in the e-liquid without causing an aversiveness. Benzoic acid and lactic acid are commonly used to protonate nicotine in these e-liquids.[32,33] The protonated nicotine avoids alkaline activation of protective mechanisms in the airways making inhalation less harsh. Another reason for less aversiveness could be the low percentage of nicotine in the gas phase of produced aerosol by acidified e-liquid compared to that of the non-acidified e-liquid. Previous studies have shown that compounds in the gas phase tend to deposit more compared to the particle phase.[25] Presence/ absence of acid could change the composition of nicotine forms in e-liquids across the market which is a challenge when assessing risks associated with e-cigarettes. In addition, higher nicotine amounts in e-liquid could increase health risks associated with nicotine consumption.



Protonated Nicotine ($NicH^+$)

Non-protonated Nicotine (Nic)

Figure 1.3 Chemical structures of protonated and non-protonated nicotine forms

1.4.3 Flavors and non-identified compounds

In addition to the main constituents PG, GL, nicotine, other compounds can be present in e-liquid. Different flavored e-liquids made e-cigarettes popular among both young and adult users. Flavors are able to mask the harsh taste of tobacco, which initiated vaping among new users, and progression among current users.[34] However, United States Food and Drug Administration (FDA) announced a flavor enforcement policy on January 2020 and implemented it on February 2020.[7] The policy was enforced on cartridge based e-cigarette products except for tobacco or menthol flavors. Current market still has a variety of flavored e-liquids attracting the vaping populations, which highlights the necessity of more regulations. In some studies, 60 to 110 identified and non-identified compounds were detected.[35,36] There can be additional compounds in the produced aerosol than in the respective e-liquid, because the vaporization process generates byproducts such as formaldehyde, acetaldehyde, acrolein by thermal degradation.[37] Consumers are directly exposed to the produced aerosol which is determined by the composition of the e-liquid. Therefore, e-liquid composition is an important factor to consider in e-cigarette exposure studies.

1.5 Deposition of e-cigarette aerosol in respiratory tract (RT)

E-cigarette aerosols first drawn to the mouth cavity travel through trachea and intrathoracic region of the RT to the lower respiratory airways. Constituents in the aerosol

get deposited into the body via both gas and particle phases of the aerosol. The deposition mechanisms of gas and particle phases are dissimilar due to their dynamic behaviors.

Particle phase deposition region and the amount are influenced by the particle size and flow rate. However, particle size changes after entering the RT due to the mechanisms of coagulation and hygroscopic growth. Coagulation occurs when the particles collide due to their random motions forming larger particles, while the high relative humidity environment in the RT causes the growth of particles by uptaking water vapor which is referred to as hygroscopic growth of the particles. Particle deposition in the RT mainly occurs via inertial impaction, gravitational sedimentation, and Brownian diffusion.[38] Airflow in the RT moves particles along the RT. However, it is challenging for the particles to follow the curvature of the RT due to their finite mass, making them impact or stick to the surface of the RT. Sedimentation occurs due to the gravitational forces on particles, which would cause particles to settle on the surface of the lungs. The third mechanism of particle deposition is diffusion, which is applicable to smaller particles. Smaller particles go through vigorous movements causing it to cross the air streamlines and deposit on the lung walls.[39]

Location of the particle deposition determines the absorption and response from the body. Particles deposited in the trachea are cleared by the natural defense mechanism known as mucociliary clearance. Mucociliary clearance is performed by the ciliated epithelial cells and airway surface layer.[40] Cilia are organelles specialized in providing the necessary force to transport foreign particles from RT towards the mouth, where the particles would be swallowed or coughed. The airway surface layer consists of a mucus layer which entraps the inhaled foreign particles, and a low viscosity periciliary layer which helps lubricate airway surfaces and facilitates mucus clearance. All these components coordinate together in clearing the foreign particles deposited in the trachea region of the respiratory tract. However, the very fine particles would travel to the alveoli area of the lungs and absorb to blood by crossing the blood-air barrier.

Gas phase molecules are able to transport and deposit in the alveolar region of the lungs. Molecules in the gas phase directly deposit on the RT tissue.[25] Deposited gas phase molecules could absorb into the blood by crossing blood-air barrier. Deposition of gas and particle phase molecules follow discrete mechanisms which determine the natural body response to the deposited molecules and their absorption into blood. Therefore, understanding the mechanism of gas-particle phase partitioning and the fraction of each molecule in gas and particle phases is necessary for unraveling health risks associated with e-cigarette consumption.

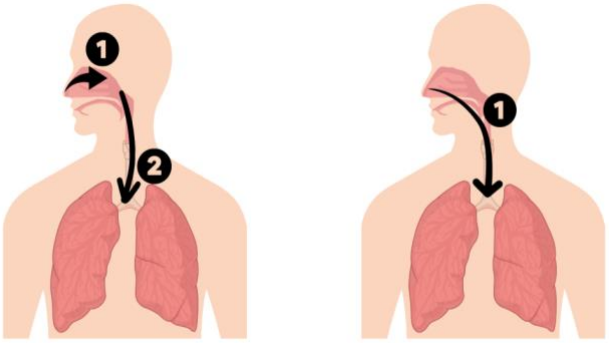
1.6 Effect of puff topography on deposition

Puffing behavior of the user such as puff volume, flow rate, and duration are known as puff topography. Puff topography can influence aerosol intake by consumers.[41] A good understanding of puff topography helps in clinical, experimental, and computational model deposition efficiency studies. Puff topography has been extensively studied related to tobacco cigarettes, but not many studies on the impact of puff topography on deposition efficiency. Lopez *et. al.* (2016) studied 16 e-cigarettes where participants used second generation e-cigarettes with different e-liquid nicotine concentrations.[42] They observed that puff volume and puff duration tend to decrease with increasing nicotine concentration, but there was no clear correlation with puff flow rate. Dawkins *et. al.* (2016) observed in their study that experienced e-cigarette users took fewer and shorter puffs when a high nicotine concentration was used, compared to low nicotine concentration.[43] These studies show that puff topography can vary among users and devices affecting the produced amount of aerosol which enters user's respiratory tract.

1.7 Effect of user topography on deposition

When e-cigarette users take a puff from the device, the puff can be inhaled in two different methods. The first method is the mouth-to-lung method, and the second method is the direct lung method (Figure **1.4**). In the mouth-to-lung method, the user takes a puff slowly from the e-cigarette device to the mouth and keeps the puff in the mouth for a certain time

with the mouth closed. After a couple of seconds, by relaxing the soft palate in the mouth, the puff is breathed into the lungs and quickly exhaled. The mouth to lung method is known to be a much closer experience to using a tobacco cigarette. In the direct lung method, the user directly inhales the puff without holding it in the mouth. Inhalation flow rate, which is the flow rate for inhaling the puff from mouth to lungs, can be user dependent. This inhalation flow rate directly impacts on the aerosol deposition efficiency in the respiratory tract.



(A) Mouth to lung method (B) Direct lung method

Figure 1.4 Diagram of two puff inhalation methods in e-cigarette users. (A) Mouth to lung method and (B) direct lung method.

In mouth to lung method, the amount of time the puff is kept in the mouth is known as "breath-hold duration". In the study by Haghnegahdar *et al.* (2018) they found that increase of breath-hold durations results in higher deposition efficiencies.[44] When e-cigarette aerosol is kept in the mouth, the particles go through physical changes such as coagulation and condensation processes which changes the particle size. Therefore, breath hold duration also has a direct impact on aerosol deposition efficiency of e-cigarette users.

1.8 Significance of the present study

Differences in device type, e-liquid composition, puff topography, and inhalation topography discussed in this chapter have an overall effect on the deposition of constituents in the respiratory tract which leads to the adverse health effects related to e-

cigarette consumption. Current study consists of two main projects focusing on the parameters yield, deposition, and uptake (Figure **1.5**). In the first project (Chapter 2), composition of the aerosol delivered to the user (yield) will be studied. In the second project (Chapter 3), a pilot human subject study will be conducted to streamline a methodology to understand effect of user specific characteristics on nicotine uptake in e-cigarette users, focusing on yield, deposition, and uptake.

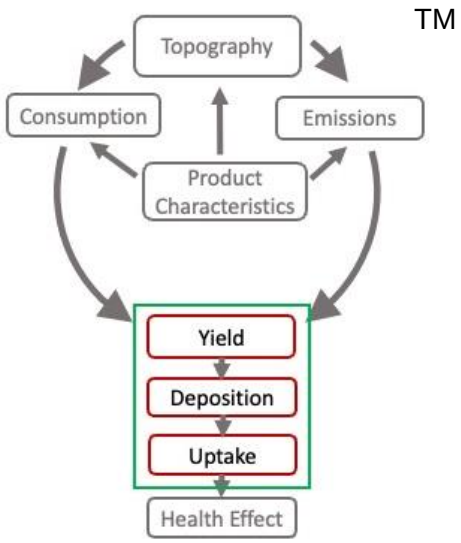


Figure 1.5 Diagram of factors relating to e-cigarette associated health effects. Current study will focus on the parameters yield, deposition, and uptake.[45]

Chapter 2 ASSESSING THE MECHANISM FOR THE COMPOSITION DIFFERENCE BETWEEN E-LIQUID AND ITS AEROSOL PARTICLE PHASE

2.1 Introduction

E-cigarettes are operated by vaporizing the e-liquid to generate aerosol, which is delivered to users. E-cigarette aerosol contains main e-liquid constituents propylene glycol (PG), glycerol (GL), nicotine, and other additives. In addition, thermal degradation by-products such as formaldehyde, acetaldehyde, acrolein, and metals can also be present in trace amounts.[46,47] In most studies, e-cigarette user's exposure to Harmful and Potentially Harmful Constituents (HPHC) is discussed based on the e-liquid composition. However, some studies have reported the composition of aerosol particle phase (PP) to be different from its original e-liquid, in which e-liquid composition is not the ideal measurement of HPHC exposure. Therefore, the study of e-liquid aerosol composition is necessary for understanding HPHC exposure which leads to health risks associated with e-cigarettes.

2.1.1 Potential mechanisms and current evidence on composition change between e-liquid and its aerosol particle phase

In the e-liquid aerosolization process, device heating power vaporizes the e-liquid bringing all its components to the vapor phase. When the vapor moves away from the heating element, nucleation occurs, condensing vapor due to the temperature drop (Figure **2.1**). Therefore, e-cigarette emissions (*i.e.,* aerosols) consist of both a particle phase and a gas phase. In the aerosolization process, two potential circumstances for composition change can be identified, (1) during the vaporization of the e-liquid and (2) during the condensation of the vapor. A literature review was conducted to understand the current knowledge of vaporization, condensation, and the differences in aerosol composition. There are multiple studies focusing on the composition of PG, GL, and Nicotine in aerosols. However, there is a scarcity in studies comparing aerosol with e-liquid composition.

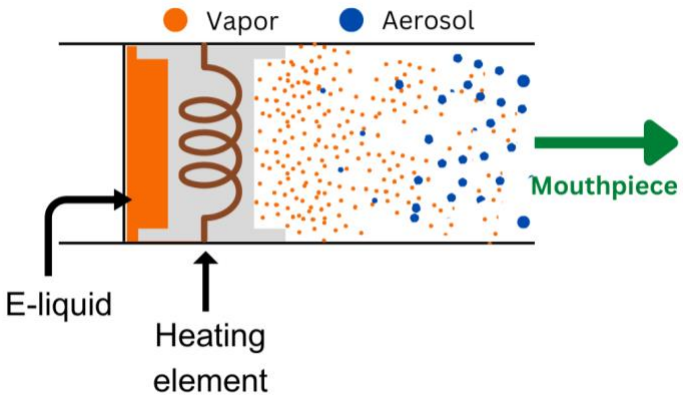


Figure 2.1 A schematic diagram of aerosol formation in an e-cigarette

PG and GL composition

Literature on the composition difference between e-liquid and its aerosol is limited. However, few studies reported evidence of observing a higher PG gas phase fraction compared to that of GL.[48–50] Another observation in the studies is that the PG-based e-liquids produce more aerosol yield compared to GL-based e-liquids.[51] Above observations for PG and GL have been rationalized by different mechanisms in the literature.

In the study by David *et.al.*, (2020), they investigated changes in an aerosol particle by optical trapping a single e-cigarette aerosol particle *in situ*.[49] They observed the initial PG concentration in the aerosol particle to be very similar to the e-liquid and a decrease in the PG concentration of more than a factor of three within the next ~20 s. They mentioned that the PG concentration drop is due to post-emission evaporation from the droplet. Their experiment was conducted by isolating a single particle of e-cigarette aerosol and flowing clean air over it to observe the composition changes over time. These conditions are different from the real-world environment, and time durations are much longer in the experiment making their claims irrelevant to the actual e-cigarette aerosol composition changes. They also claimed the standard technique for sampling e-cigarette emissions by capturing aerosol particle phase in filter pads could cause composition changes

without providing any experimental evidence. Aerosol study by trapping particle phase in filter pads is a technique use in our experiments and many other labs.

PG and GL composition change due to their vapor pressure has been discussed in couple of studies. Both PG and GL are semi-volatile compounds, PG having a higher volatility than GL due to differences in their vapor pressure values (Table **2.1**). Li *et. al.* (2021) studied e-cigarette aerosol as a function of PG:GL ratio.[48] They found PG to be almost entirely in the gas phase, while GL stayed in the particle phase. Alderman et. al. (2014), in their study on the particle size distribution and Cambridge filter pad collection efficiency also reported PG fraction in the gas phase to be higher than that of the GL.[50] Both studies rationalize their observations by physical characteristics of chemical components such as vapor pressure. However, they do not discuss the mechanism of composition change due to the vapor pressure of components.

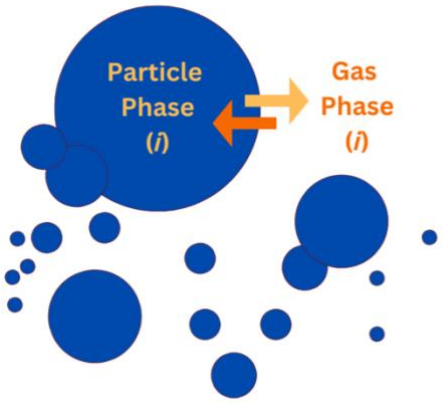


Figure 2.2 Schematic diagram of gas-particle partition of an analyte "*i*" in aerosol.

A theoretical model on PG and GL gas-particle partitioning in e-cigarette aerosol has been proposed by Pankow (2017), which predicts the distribution of each constituent in particle vs. gas phase.[52] Volatile or semi-volatile chemicals in aerosols can be seen in both gas phase and particle phase (Figure **2.3**), which applies to the e-cigarette aerosol as well.

According to the model, gas/particle coefficient ($m^3/\mu g$) for a volatile analyte of interest "*i*" when partitioning into liquid particles is defined as below.

$$K_{p,i}\left(\frac{m^3}{g}\right) = \frac{RT}{10^6 \underline{MW} \varsigma_i P^o_{L,i}} \quad 2.1$$

R = gas constant (8.2×10^{-5} m^3.atm/mol.K)

T = temperature (K)

$\underline{MW}$(g/mol) = mole average molecular weight of the absorbing liquid phase

ς_i (dimensionless) = mole fraction scale activity coefficient in the liquid phase

$P^o_{L,i}$ = vapor pressure (atm) of pure liquid *i* at temperature

The model also calculated the fraction of *i* in the gas phase ($f_{g,i}$) and the fraction of *i* in the particle phase ($f_{p,i}$).

$$f_{g,i} = \frac{1}{1 + K_{p,i} TPM} \quad 2.2$$

$$f_{p,i} = \frac{K_{p,i} TPM}{1 + K_{p,i} TPM} \quad 2.3$$

TPM (Total Particulate Matter, $\mu g/m^3$) = total mass of suspended particulate matter per m^3 in the gas+particle aerosol system.

Equation 2.1 shows that the gas-particle coefficient $K_{p,i}$ is governed by the vapor pressure of the liquid. According to equations 2.2 and 2.3, fraction in the gas/particle phase depends on the $K_{p,i}$ (i.e., vapor pressure) and the mass concentration of total particulate matter (TPM) (Figure **2.3**). Gas-particle partition theory showcases that the increasing vapor pressure and decreasing TPM favor the gas phase. Gas-particle phase partition theory is also supported by previous applications on atmospheric secondary organic aerosols.[53,54]

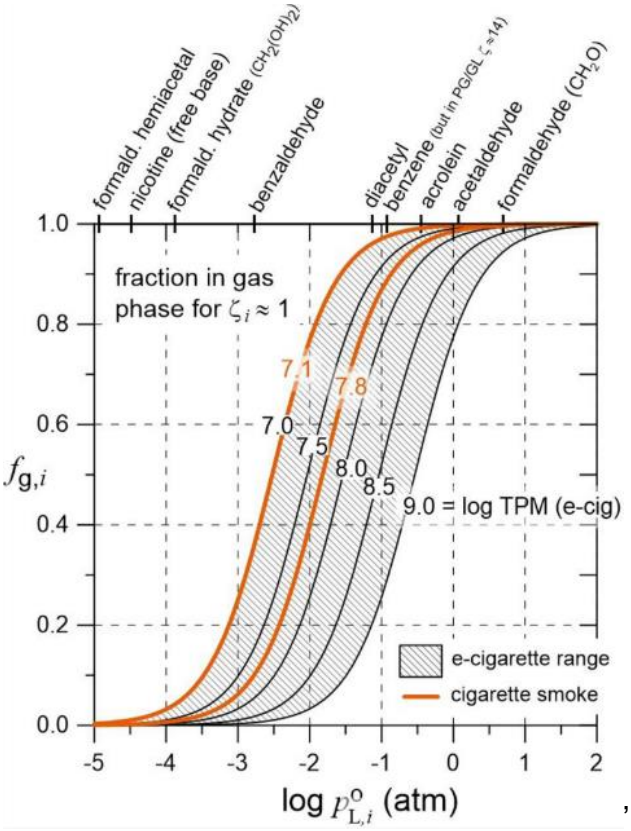
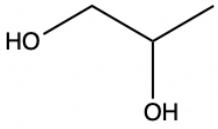
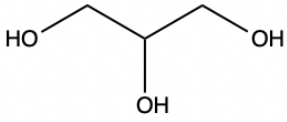
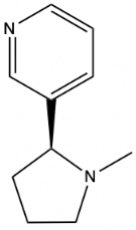


Figure 2.3 Fraction in the gas phase ($f_{g,i}$) of an e-cigarette or tobacco smoke aerosol as a function of the liquid vapor pressure of a pure compound ($P^{o}_{L,i}$) for varying values of TPM. The figure is adapted from Pankow, J.F., (2017).[52]

Table 2.1 Chemical structure, molecular weight, and vapor pressure of the main constituents in the e-liquid PG, GL, and Nicotine.

	PG	GL	Nicotine
Chemical structure			
Molecular weight (g/mol)	76.09	92.09	162.23
Vapor pressure at 20°C (Pa)	10.6	0.01	6.0
Vapor pressure at 20°C (atm)	1.05×10^{-4}	9.87×10^{-8}	5.92×10^{-5}

Nicotine composition

Nicotine in aerosol particle phase can exist in two forms. Some commercial e-liquids in the market include an acid, typically benzoic or lactic acid, whereas some don't. Presence of an acid in the e-liquid protonates a fraction of Nicotine resulting in protonated nicotine ($NicH^+$). Non-protonated fraction is referred to as free-base Nicotine (Nic). Upon aerosol formation from an acidified e-liquid, nicotine in vapor is in its non-protonated form. When the vapor condenses, the particle phase will contain the acid, and thus a fraction of Nic protonates based on the pH.

Nicotine composition difference between e-liquid and aerosol particle phase has been discussed in few studies.[29,55–57] The gas-particle partition theory by Pankow discussed above related to PG composition applies to Nicotine as well due to its semi-volatile nature. Gas-particle partition theory brings out the effect of vapor pressure and TPM on nicotine compositions in gas and particle phases.

Talih et. al., (2020) examined effect of nicotine form on yield of nicotine, where they did not detect a change in nicotine yield based on the Nicotine form.[29] Findings from Talih et. al., (2020)'s study disagrees with the aerosol PP composition study results by David et.al., (2020), where they observed the e-liquid nicotine composition of ~5% to change in its aerosol phase upon changing pH of the e-liquid (i.e. acidified e-liquid).[49] E-liquids of a higher pH 9.9 demonstrated a decrease in nicotine concentration in the aerosol PP over time, whereas lower pH 6.5 e-liquids showed a reverse behavior of increasing nicotine concentration. Both studies rationalized the observations by differences in volatility in the protonated and non-protonated forms of nicotine. However, the study by David et.al. was conducted by isolating a single droplet of e-cigarette aerosol particle and longer time of ~40 s, which deviates the conditions from actual vaping. Also, the underlying mechanism of composition change due to pH was not addressed in the study.

2.1.2 Research gaps

In the literature, there's definite evidence of aerosol PP composition to be different from its e-liquid. It's been discussed in relevance to PG, GL, and Nicotine. However, there are discrepancies among the studies about the underlying reason and mechanism for the composition difference. The exact mechanism of the composition difference has not been systematically studied yet. In addition, there are claims about currently established analysis methods resulting in aerosol PP composition changes, thus, it will be addressed in the current study.

Four potential mechanisms for aerosol PP composition change have been identified based on literature and the aerosolization process. Those four mechanisms will be addressed in the experimental design of the current study.

Potential mechanisms of aerosol composition change

1. Vaporization: In aerosolization, heat provided by the device vaporizes the e-liquid. Composition change could happen due to selective vaporization of the e-liquid.

2. Condensation: When the e-liquid vapor moves away from the heating source, nucleation occurs followed by condensation of vapor due to the temperature drop. Fractional condensation could cause the composition change.

3. Evaporation: In the analysis of aerosol PP, the aerosol is produced by a custom emissions system. It is tested if the composition could change by evaporation when air flows through the particles collected during emissions testing.

4. Aging: When analyzing aerosol, the particle phase is collected in a filter pad and sealed in an amber jar. Composition change could happen due to aging while sitting in the jar if analysis gets delayed.

2.1.3 Hypothesis and aims

In the current study, we address multiple test conditions that could lead to the composition changes. These conditions include vaporization of the e-liquid, condensation in aerosol formation, evaporation of the e-liquid during machine puffing, and aging of the filter pads containing aerosol PP. We hypothesize that the composition change in aerosol PP from its original e-liquid occurs during condensation in aerosol formation due to the chemical and physical properties of the e-liquid constituents.

Aim 1: Evaluate the mechanism for PG:GL ratio change in the aerosol particle phase from its original e-liquid.

Quantify PG and GL in the e-liquid, aerosol PP, and the stated test conditions using a Gas Chromatography Flame Ionization Detector (GC-FID) method and calculate PG:GL ratio. Do a comparison of PG:GL ratio of aerosol PP and each test condition to that of the original e-liquid.

Aim 2: Evaluate the mechanism for Nicotine:GL ratio change in the aerosol particle phase from its original e-liquid.

Quantify Nicotine and GL in the e-liquid, aerosol PP, and the stated test conditions using the GC-FID method and calculate Nicotine:GL ratio. Do a comparison of Nicotine:GL ratio of aerosol PP and each test condition to that of the original e-liquid.

Aim 3: Evaluate the effect on nicotine yield by the presence of acid in e-liquid.

Quantify Nicotine and GL in the e-liquid, aerosol PP, and the stated test conditions using the GC-FID method and calculate Nicotine:GL ratio. Do a comparison of Nicotine:GL ratio of aerosol PP and each test condition to that of the original e-liquid. Analyze and compare data for both acidified and non-acidified e-liquids to understand the influence on nicotine yield by the presence of an acid.

2.2 Materials and Methods

The composition change between the e-liquid and its aerosol PP could arise from different reasons. Vaporization of the e-liquid, condensation during aerosol formation, evaporation from filter pads, and sampling were identified as possible theorems for the observed composition change. This section discusses the experiment design and methodology to test each of these scenarios.

2.2.1 Preparation of solutions

Test e-liquids

Experiments were carried out using two lab-synthesized e-liquids and two commercial e-liquids. The base of both lab-synthesized e-liquids was 50% PG and 50% GL by mass. The PG:GL = 50:50 solution was prepared by weighing the equal mass of PG and GL and stirring it for 30 minutes at low speed. “Lab_NonProtonate”, non-acidified e-liquid, was prepared by adding nicotine to the base liquid so that the final solution was 5% Nicotine and 95% PG:GL = 50:50 by mass. “Lab_Protonate”, acidified e-liquid, was prepared by adding nicotine and benzoic acid to the base liquid to make a final solution to be 5% Nicotine, 5% Benzoic Acid and 90% of the PG:GL = 50:50 solution by mass. Two commercial e-liquids, JUUL and Coastal Cloud were purchased. Manufacturer-reported composition for each e-liquid is as follows. JUUL e-liquid consists of 5% Nicotine, Benzoic Acid and PG:GL ratio of 30:70. Coastal Cloud e-liquid consists of Nicotine 0.6%, and PG:GL = 30:70. The composition of all solutions was verified by the GC-FID method as discussed in Chapter 2.2.2.

Calibration series

Internal standard method was used to quantify the constituents of all samples (e-liquid and aerosol PP) to eliminate uncertainty caused by the instrument. 1,2-butanediol was used as the internal standard for PG and GL. Quinoline was used as the internal standard for nicotine and benzoic acid. Internal standard stock solution was made of 12.5 mg/mL

1,2-butanediol and 5 mg/mL quinoline. Quinoline 1.250 g and 1,2-butanediol 3.125 g were weighed in a 250.00 mL volumetric flask using the analytical balance. It was diluted to the line with methanol and mixed well. A constant volume of internal standard solution was used for all the test solutions and in the calibration series.

Table 2.2 Concentrations of PG, GL, nicotine and benzoic acid in each standard solution of the high concentration calibration series.

Standard Solution	PG Concentration (mg/mL)	GL Concentration (mg/mL)	Nicotine Concentration (mg/mL)	Benzoic Acid Concentration (mg/mL)
L1	0.06	0.06	0.01	0.01
L2	0.12	0.12	0.02	0.02
L3	0.30	0.30	0.05	0.05
L4	1.20	1.20	0.20	0.20
L5	3.00	3.00	0.50	0.50
L6	6.00	6.00	1.00	1.00

Table 2.3 Concentrations of PG, GL, nicotine and benzoic acid in each standard solution of the low concentration calibration series.

Standard Solution	PG Concentration (mg/mL)	GL Concentration (mg/mL)	Nicotine Concentration (mg/mL)	Benzoic Acid Concentration (mg/mL)
L1	0.01	0.01	0.002	0.002
L2	0.02	0.02	0.004	0.004
L3	0.03	0.03	0.005	0.005
L4	0.06	0.06	0.01	0.01
L5	0.12	0.12	0.02	0.02
L6	0.25	0.25	0.04	0.04

Different calibration series were prepared with each solution including the main chemicals PG, GL, nicotine, and benzoic acid. Expected results for the main chemical components were calculated for each experiment prior to preparing calibration series to decide on the required calibration range. L1-L6 high concentration solutions in Table **2.2** were used when analyzing e-liquids and particulate phase related to Chapter 2. For e-liquid, expected results for each component were calculated based on minimum 30 mg and maximum 80 mg of e-liquid, which is the range of approximate e-liquid amount used in analysis. For the aerosol PP, expected results were calculated based on the observed

total particulate matter (i.e., mass increase in the filter pad collecting aerosol PP) in previous experiments. Low concentration series of L1-L6 mentioned in Table **2.3** was used to analyze exhale breath samples for Chapter 3. Expected exhale amount for each component was calculated by considering the deposition percentages in literature.[58] All calibration series were tested by GC-FID method discussed in Chapter 2.2.2 for a linear curve with a higher R^2 value prior to analyzing the samples.

To make the calibration series, two stock solutions (Stock 1 and Stock 2) were prepared of 6.0 mg/mL PG, 6.0 mg/mL GL, 1.0 mg/mL nicotine, and 1.0 mg/mL benzoic acid. Standard solutions L1-L6 were made using the two stock solutions alternatively. Purpose of alternating two stock solutions was to minimize the error that comes from solution preparation. In addition, a stock internal standard solution was prepared with the two internal standards 1,2-butanediol and quinoline, bringing their final concentrations to 12.5 mg/mL 1,2-butanediol, and 5 mg/mL quinoline.

Table 2.4 L1 to L5 standard solutions preparation measurements.

Standard Solution	Stock Solution	Stock Solution Volume (mL)	Final Volume (mL)
L1	Stock 1	1.00	100.00
L2	Stock 2	1.00	50.00
L3	Stock 1	5.00	50.00
L4	Stock 2	10.00	50.00
L5	Stock 1	25.00	50.00

For each standard, a known volume of stock solution was transferred using a volumetric pipette into a volumetric flask and was diluted to the line with methanol. L6 standard was made with 25.00 mL of the Stock 2 solution mixed with 1.00 mL of the internal standard solution. Rest of the L1 to L5 standards were prepared according to the Table **2.4** measurements followed by 25.00 mL of each solution mixing with 1.00 mL of the stock internal standard solution, resulting in the identical concentration of each internal standard in all standards and sample solutions. The nominal concentration of internal standards in the final test solution were: 4.81×10^{-1} mg/mL 1,2-butanediol, and 1.92×10^{-1} mg/mL quinoline.

2.2.2 Chromatographic conditions of GC-FID and data processing

Chemical composition of e-liquids, aerosol PP, and samples from Experiment 1-3 along with the standards were analyzed by GC-FID (Shimadzu GC-2010 Plus with an AOC-20s autosampler) equipped with a fused-silica capillary column Rtx-BAC Plus 1 (30 m length, 0.32 mm inner diameter, 1.8 µm film thickness, Restek). The carrier gas He had a column flow of 1.5 mL/min at 65.7 kPa pressure. The GC method was programmed to inject 5 µL of samples in the split mode at 300 ^{o}C inlet temperature. The temperature program was set to start at 100 ^{o}C and hold for for 1 min, from 100 ^{o}C to 300 ^{o}C ramp at 15 ^{o}C/min, from 130 ^{o}C to 220 ^{o}C at 40 ^{o}C/min and hold at 220 ^{o}C for 5 min, from 220 ^{o}C to 250 ^{o}C at 40 ^{o}C/min and hold at 250 ^{o}C for 2 min. The oven was then cooled to the initial temperature to prepare for the next sample injection. Signals from the analytes were detected by FID, operated at 275 ^{o}C. Sampling was at a rate of 40 msec, over a period of 10 min. Shimadzu LabSolutions software was used for instrument operation and to obtain data.

Table 2.5 Retention times of interested chemical components in GC-FID chromatograms.

Chemical Component	Retention Time (minutes)
PG	3.4
1,2-butanediol	4.3
GL	5.4
Benzoic Acid	7.0
Quinoline	8.2
Nicotine	9.4

All the standards were run in triplicate and samples in duplicate to increase the accuracy of results. GC chromatograms were obtained from the software (Figure **2.4**), and area under the curve for each interested analyte was recorded based on their retention times (Table **2.5**).

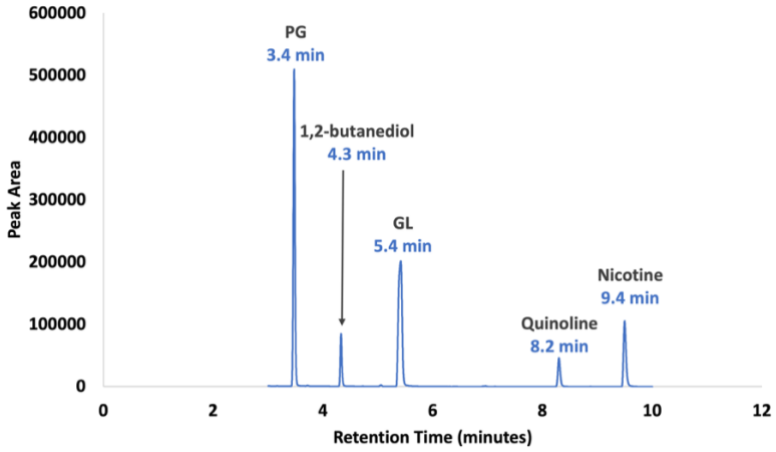


Figure 2.4 A sample chromatogram showcasing main chemical e-liquid constituents PG, GL, Nicotine, and the two internal standards 1,2-butanediol and Quinoline.

Internal standard method was used to minimize the uncertainty due to sample workup and instrumentation drift. For each sample, the area ratio of analyte to internal standard was obtained from the chromatogram. Quinoline was used as the internal standard for PG and GL. 1,2-butanediol was used as the internal standard for nicotine and benzoic acid. Calibration curve for each analyte was plotted as the area ratio of the analyte vs. concentration (Figure **2.5**).

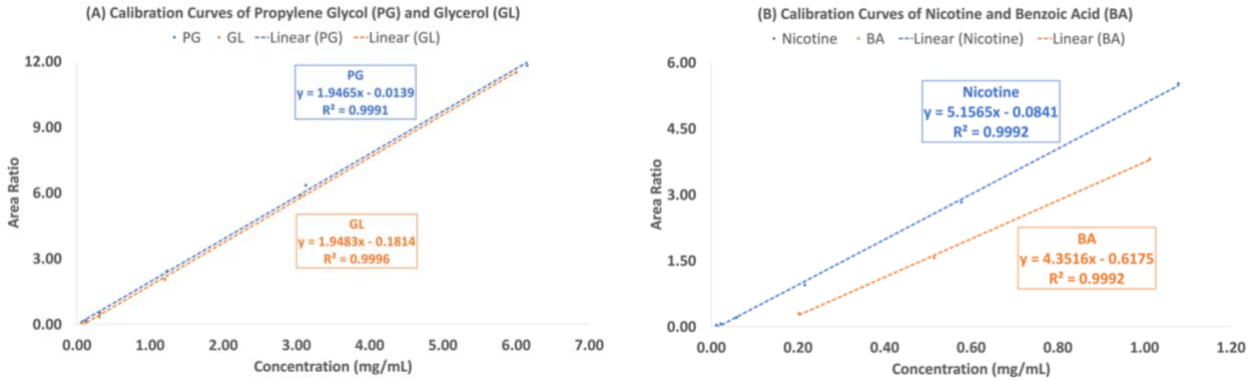


Figure 2.5 Representative calibration curves of PG and GL (A), Nicotine and BA (B).

2.2.3 Generation of emissions by PES-1™

Emissions were generated using the PES-1™ computer-controlled custom emissions system designed by the Respiratory Technologies Laboratory at the Rochester Institute

of Technology.[59] A full description of PES-1 is outside of the scope of this document. The capabilities and validation of PES-1 is discussed in Hensel et al. (2018).[59] In brief, PES-1 allows for an e-cigarette to be puffed at precise topographies, fixed or variable, with high reproducibility. A fixed puffing profile of 35 mL/s flow rate, 3.5 s puff duration, 23.8 s inter-puff duration, and 25 puffs was used for all the emissions trials in this study. Emissions from each e-liquid were captured in filter pads.

2.2.4 E-liquid characterization

E-liquid characterization is an important role in this study for both qualitative and quantitative analysis of each analyte. Test solutions were prepared according to Section 2.2.1 using a known mass of e-liquid.

2.2.5 Mass analysis of aerosol particle phase

The aerosols generated from each e-liquid were analyzed to quantify total particulate matter (TPM), and the main constituents PG, GL, and Nicotine. The two lab-synthesized e-liquids and Coastal Cloud e-liquid were filled into Blankz pods for emissions tests. Commercially purchased JUUL pre-filled pods were used. E-liquid filled pods along with JUUL Power Control Unit (PCU) were used to perform machine generated aerosolization by PES-1^{TM} according to the parameters discussed in section 2.2.3. Filter pads were weighed before and after the emission session to quantify generated TPM. After weighing, each filter pad was placed in an amber jar for storage until chemical analysis of the composition of the particulate matter was performed. In addition, the amount of e-liquid consumed was determined by taking the mass difference of the e-liquid pod before and after an emissions trial. All mass measurements were taken in Mettler Toledo Model Number AE240-1 S/N J65956 analytical balance with a manufacturer reported readability of 0.1 (mg), approximate accuracy of 0.4 (mg), and full-scale range of 200 (grams) with a linearity of $\pm$ 0.02 (mg). Aerosol PP of all four e-liquids was analyzed using this method.

2.2.6 Experimental design

Experiments were designed to test out each of the theorems that could cause the composition change between e-liquid and its aerosol PP. All samples were prepared and analyzed following the protocol discussed in Section 2.2.7.

Experiment 1 - Evaporation

Objective of this experiment was to identify any selective evaporation that occurs while producing emissions by PES-1^{TM}. A known mass of e-liquid was spotted on a filter pad. Emission tests were conducted under the conditions discussed in Section 2.2.3. using a dead battery PCU and an empty Blankz pod, passing the air through the spotted filter pad. Filter pad was weighed using an analytical balance before and after each emissions trial. Six trials were performed on each e-liquid following the same protocol.

Experiment 2 - Aging

This experiment was designed to identify possibility of composition change with time when the aerosol particles trapped filter pads are in sealed jars. We examined the difference that could happen within a week. A known mass of e-liquid was spotted on a filter pad and kept in a sealed jar for a week. After one week the filter pad was submerged in a mix of 25.00 mL methanol and 1.00 mL of internal standard solution and shaken to break the pad. The methanol extract was filtered through a micropipette filter tip and then a 0.5 µm filter. Six repeated trials were conducted.

Experiment 3 - Vaporization

Experiment 3 was designed to test the theory that composition change could arise by selective vaporization of the e-liquid. It is expected to see a different pattern of composition change in the e-liquid left in a pod after emissions trials compared to that of aerosol PP if the composition change is due to selective vaporization. Emissions were

produced by PES-1^{TM} under the conditions discussed in 2.2.3 section using a completely full e-liquid pod until only a small amount of e-liquid is left. Initial mass of the completely full pod and the end mass of the pod with a small amount of e-liquid were recorded using an analytical balance.

2.2.7 Chemical composition analysis of e-liquid and emitted particles

There were five different types of samples obtained including initial (1) e-liquid, (2) captured aerosol PP, (3) e-liquid spotted on a pad and air puffed through it (evaporation), (4) e-liquid spotted on a pad and left for a week (aging), and (5) the e-liquid left in the pod after emissions tests. E-liquid samples and filter pad samples were prepared following two different methods for GC-FID analysis.

E-liquid sample preparation

For e-liquid samples, a known amount of e-liquid was mixed with 25.00 mL methanol and 1.00 mL stock internal standard solution. Mixed samples were transferred to GC vials for analysis.

Filter pad analysis

The three types of samples containing filter pads were stored in amber vials to make the sample handling identical. Each filter pad was submerged in a mix of 25.00 mL methanol and 1.00 mL of internal standard solution and shaken to break the pad. The methanol extract was filtered through a micropipette filter tip and then a 0.45 µm filter. Filtered samples were transferred to GC vials for analysis.

Composition analysis

GC-FID analysis was performed using an internal standard method following the protocol discussed in Chapter 2.2.2. Area ratio was calculated for PG, GL, and nicotine with their respective internal standards for both samples and standards. Calibration curves for each

analyte were graphed separately. Using the slope and intercept values of the calibration curves, mass concentration of each analyte was obtained, and then mass (mg) of each analyte in the initial 25.00 mL of the test solution was calculated.

PG and Nicotine analyte compositions were reported as a ratio per GL (Equation 2.4). GL has a very low vapor pressure compared to PG and Nicotine which lead to the assumption that almost all GL stays in the particle phase unlike PG or Nicotine. Reporting the values as a ratio to GL ties all data to a single component minimizing the effect from other constituents present in the test solutions.

$$\text{Analyte GL ratio} = \frac{\text{Analyte mass in the sample}}{\text{GL mass in the sample}} \qquad 2.4$$

2.2.8 Data Analysis

The composition difference of the aerosol PP and the samples of three experiments in respect to e-liquid was calculated as a percentage for PG and Nicotine (analyte) using Equation 5 (X = analyte).

$$\%\text{Difference} = \frac{(\text{E} - \text{liquid average X: GL ratio}) - (\text{Experiment average X: GL ratio})}{\text{E} - \text{liquid average X: GL ratio}} \qquad 2.5$$

X = analyte

2.2.9 Statistical Analysis

In the sample analysis all calibration standards were run in triplicate and samples in duplicate. For the calibration standards, average and standard deviation values were calculated for analyte to internal standard area ratio for each analyte (PG, GL, Nicotine). For the samples, average and standard deviation values of concentrations were calculated for each analyte.

PG/GL ratio and Nicotine/GL ratio of e-liquid was compared with that of aerosol PP and three experiments. Significance of difference between the two parameters were also estimated by calculating the P value. P values were calculated by hypothesis test for two means in JMP software.

2.3 Results and discussion

Objective of this study is to confirm the change of composition in aerosol PP from its original e-liquid, and to identify the mechanism for this change. Experiments were performed on two lab synthesized e-liquids of known composition and two commercial e-liquids. Results from the experiments will be discussed under two sections: PG composition change, and nicotine composition change.

2.3.1 PG composition change

PG composition data is presented as a ratio per GL in Table **2.6**. PG/GL ratio was calculated for the original e-liquid, its aerosol PP, and the three experiments under each of the four e-liquids using Equation 2.6. PG composition of aerosol PP and each experiment is compared with that of the original e-liquid. An increase of PG/GL ratio means PG composition in the experimental sample has increased compared to GL, from that of the original e-liquid. A decrease in the PG/GL implies the PG composition has dropped compared to GL from that of the original e-liquid.

$$\text{PG: GL ratio} = \frac{\text{PG mass in the sample}}{\text{GL mass in the sample}} \qquad 2.6$$

PG/GL ratio for all experiments across the four e-liquids are graphically represented in Figure **2.6**. In each graphical figure the PG/GL ratio of the aerosol PP, and the three experiments are compared with that of the original e-liquid. In all four sets of experiments the PG/GL ratio of the generated aerosol PP is statistically lower than the original e-liquid. For the Lab_NonProtonated e-liquid, PG/GL ratio in the aerosol PP is significantly different from the e-liquid $p<0.0001$ (Table **2.7**). None of the other experimental conditions

(evaporation, aging, vaporization) show a similar reduction in the PG/GL ratio with the e-liquid (Figure **2.6**). The significant difference of PG composition between the e-liquid and aerosol PP is common across all four e-liquids (Table 2.7). Difference of PG/GL ratio between each experimental condition with their original e-liquid was calculated as a percentage (Table 2.8). Aerosol PP shows the highest %difference value with the e-liquid compared to the three experiments, which is common across all four e-liquids.

Table 2.6 PG/GL ratio by mass average ± standard deviation for different e-liquids in each experiment

E-liquid	**Average PG/GL ratio by mass in e-liquid**			
	Lab_NonProtonated	**Lab_Protonated**	**Coastal Cloud**	**JUUL**
E-liquid	1.04±0.010	1.06±0.006	0.31±0.006	0.46±0.002
Aerosol PP	0.87±0.034	0.89±0.047	0.27±0.007	0.39±0.015
Evaporation	1.02±0.018	1.02±0.013	0.29±0.004	0.43±0.006
Aging	1.04±0.014	1.06±0.008	0.31±0.003	0.44±0.438
Vaporization	1.02±0.015	1.03±0.015	0.31±0.009	0.45±0.002

The four e-liquids used in this experiment include a non-acidified lab-synthesized e-liquid (Lab_NonProtonated), non-acidified commercial e-liquid (Coastal Cloud), acidified lab-synthesized e-liquid (Lab_Protonated), and an acidified commercial e-liquid (JUUL). Reduction of PG from all e-liquids is the same, thus it does not appear to be affected by the presence of an acid (i.e. change in pH). Considering the %difference results in Table 2, both lab-synthesized e-liquids show approximately 16% difference, and the two commercial e-liquids show an approximate difference of 14%. Differences in the percentages between lab synthesized and the commercial e-liquids could be due to the dissimilarities in the composition. Both lab-synthesized e-liquids have a PG:GL ratio of 50:50, whereas both commercial e-liquids have a PG:GL ratio of 30:70. Having a low amount of PG in the commercial e-liquid could result in a low %difference compared to that of the lab synthesized e-liquids. Higher PG composition in the lab-synthesized e-liquids results in a higher PG gas phase loss compared to the commercial e-liquids. Therefore, lab-synthesized e-liquid particle phase PG composition is low relative to the commercial e-liquids, giving a higher %difference with its e-liquid.

Table 2.7 P value calculated by hypothesis testing between two means at a 0.05 confidence level for the PG/GL of each condition with the e-liquid`

E-liquid	%Difference of PG/GL ratio with the e-liquid			
	Lab_NonProtonated	**Lab_Protonated**	**Coastal Cloud**	**JUUL**
Aerosol PP	<.0001	<.0001	<.0001	<.0001
Evaporation	0.0528	<.0001	<.0001	0.0002
Aging	0.9126	0.7647	0.0039	0.3391
Vaporization	0.0684	0.0077	0.0003	0.5960

Table 2.8 %Difference of PG/GL of each condition with the original e-liquid.

E-liquid	%Difference of PG/GL ratio with the e-liquid			
	Lab_NonProtonated	**Lab_Protonated**	**Coastal Cloud**	**JUUL**
Aerosol PP	16.62%	16.24%	13.72%	14.71%
Evaporation	1.79%	3.94%	5.12%	6.95%
Aging	0.08%	0.11%	0.83%	4.08%
Vaporization	1.59%	2.19%	0.81%	1.85%

While the generated aerosol PP showed a difference in the PG/GL from the e-liquid, none of the other experiments showed a consistent difference from the initial e-liquid. This implies that the difference does not derive from evaporation or aging of the aerosol particles, or vaporization of constituents from the aerosol particles on the collection filter pad. In the process of aerosolization, e-liquid vaporizes and then condenses into aerosol particles. This study does not have an experiment to specifically test condensation. However, experimental data eliminates the other possible mechanisms considered, confirming our hypothesis that PG composition difference could occur during the condensation process of the e-liquid.

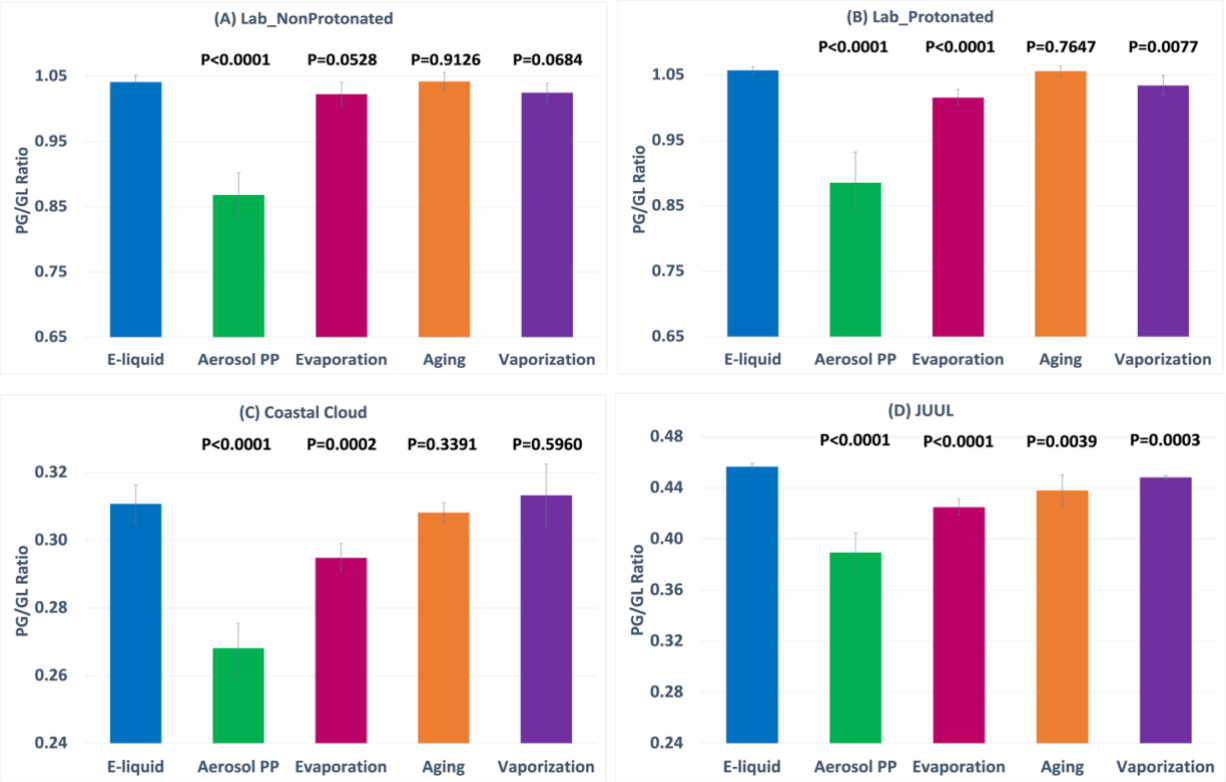


Figure 2.6 PG/GL ratio of the e-liquid, aerosol PP, and the experiments to test evaporation, aging, vaporization for (A) Lab_NonProtonated e-liquid (B) Lab_Protonated e-liquid (C) Coastal Could e-liquid (D) JUUL e-liquid.

There is also evidence in the literature about PG and GL generating byproducts such as formaldehyde during thermal degradation of the e-liquid.[22,23,37,46,47,60,61] Results from the literature suggest that approximately 2% of byproducts from the e-liquid are generated, which is a significantly lower amount compared to our experimental %difference of 14-17%.[22,47,48] Therefore, thermal degradation is not a valid explanation for the observed PG composition difference between e-liquid and aerosol PP. Our experimental results are also supported by the gas-particle phase distribution model proposed for e-liquid aerosol by James F. Pankow (Chapter 2.1.1) on the distribution of chemical constituents in gas-particle phases based on their chemical and physical characteristics.[52]

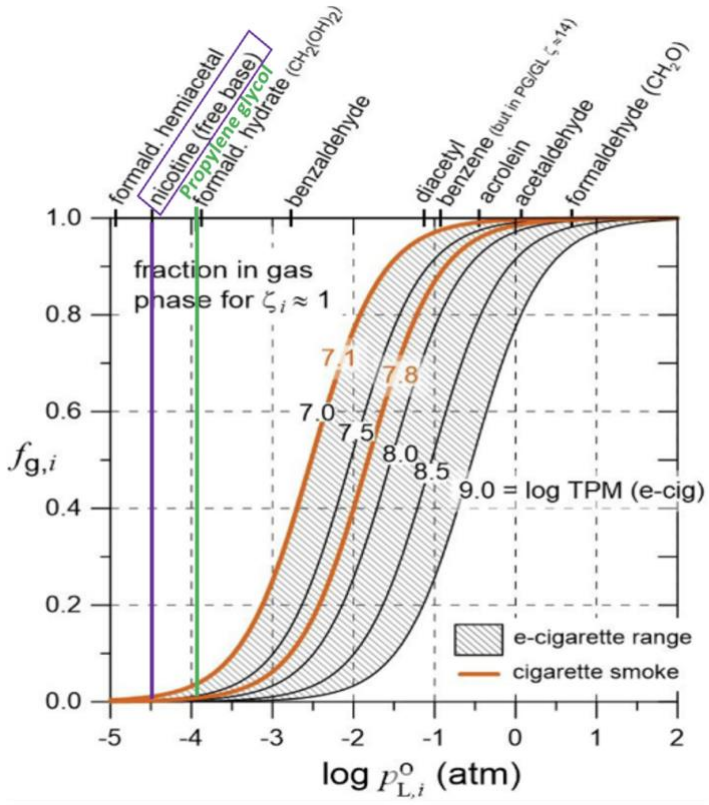


Figure 2.7 Fraction in the gas phase ($f_{g,i}$) of an e-cigarette or tobacco smoke aerosol as a fraction of the liquid vapor pressure of a pure compound ($P^{o}_{L,i}$) for varying values of TPM. The figure in part is adapted from Pankow, J.F., (2017).[52]

The gas-particle phase distribution model predicts the distribution of each constituent in particle vs. gas phase. Model shows that the aerosol phase distribution depends on molecular properties and aerosol mass concentration (cTPM). A governing molecular property mentioned in the model is vapor pressure of the molecules. The model indicates increasing vapor pressure and decreasing cTPM of constituents favor the gas phase. PG has a very high vapor pressure compared to GL and nicotine (Table 2.1). Therefore, according to the model, the fraction in the gas phase for PG is expected to be higher compared to the GL and nicotine. Figure **2.7** shows the variation in gas phase fraction base on the vapor pressure and mass concentration. Log(cTPM) values for the e-liquids used in our experiments fall in the range of 7.3-7.7 (Table 2.9). Within this range the PG fraction in the gas phase is approximately 0.02. If the %difference observed in our data between e-liquid and the aerosol PP (Table 2.7) is due to the PG present in the gas phase,

the fraction of gas phase PG for Lab_NonProtonated e-liquid is 0.17. The experiment value for PG gas phase fraction is higher than the theoretical value from the model. However, the gas-particle phase distribution model supports the concept that PG/GL ratio drop from e-liquid to aerosol PP is due to the fraction of PG partitioned to gas phase, which we couldn't quantify experimentally. Gas-particle phase partitioning occurs during the condensation process of aerosol formation. Therefore, from our experimental data it was identified that the mechanism behind PG composition difference is condensation in aerosol formation, and it is also supported by the gas-particle phase distribution model.

Table 2.9 Calculated log(cTPM) values for each e-liquid emissions under the current study parameters.

E-liquid	Log(cTPM)
Lab_NonProtonated	7.7
Lab_Protonated	7.6
Coastal Cloud	7.5
JUUL	7.3

2.3.2 Nicotine composition change

Table 2.10 Nicotine/GL ratio by mass in different e-liquids for each experiment

E-liquid	Average Nic/GL ratio by mass in e-liquid			
	Lab_NonProtonated	Lab_Protonated	Coastal Cloud	JUUL
E-liquid	0.11±0.005	0.11±0.003	0.01±0.001	0.08±0.005
Aerosol PP	0.08±0.004	0.10±0.002	0.01±0.001	0.08±0.003
Evaporation	0.10±0.002	0.11±0.002	0.02±0.002	0.09±0.007
Aging	0.10±0.005	0.10±0.001	0.01±0.001	0.09±0.007
Vaporization	0.10±0.003	0.10±0.005	0.01±0.001	0.08±0.001

Nicotine (Nic) composition data is presented as a ratio per GL. Nic/GL ratio results for the original e-liquid, its aerosol PP, and the three experiments for each of the four e-liquids can be found in Table 2.10. Nic/GL ratio for all experiments across the four e-liquids are

graphically represented in Figure **2.8**. The difference of Nic/GL ratio for aerosol PP and each experiment with their original e-liquid was calculated as a percentage (Table 2.11).

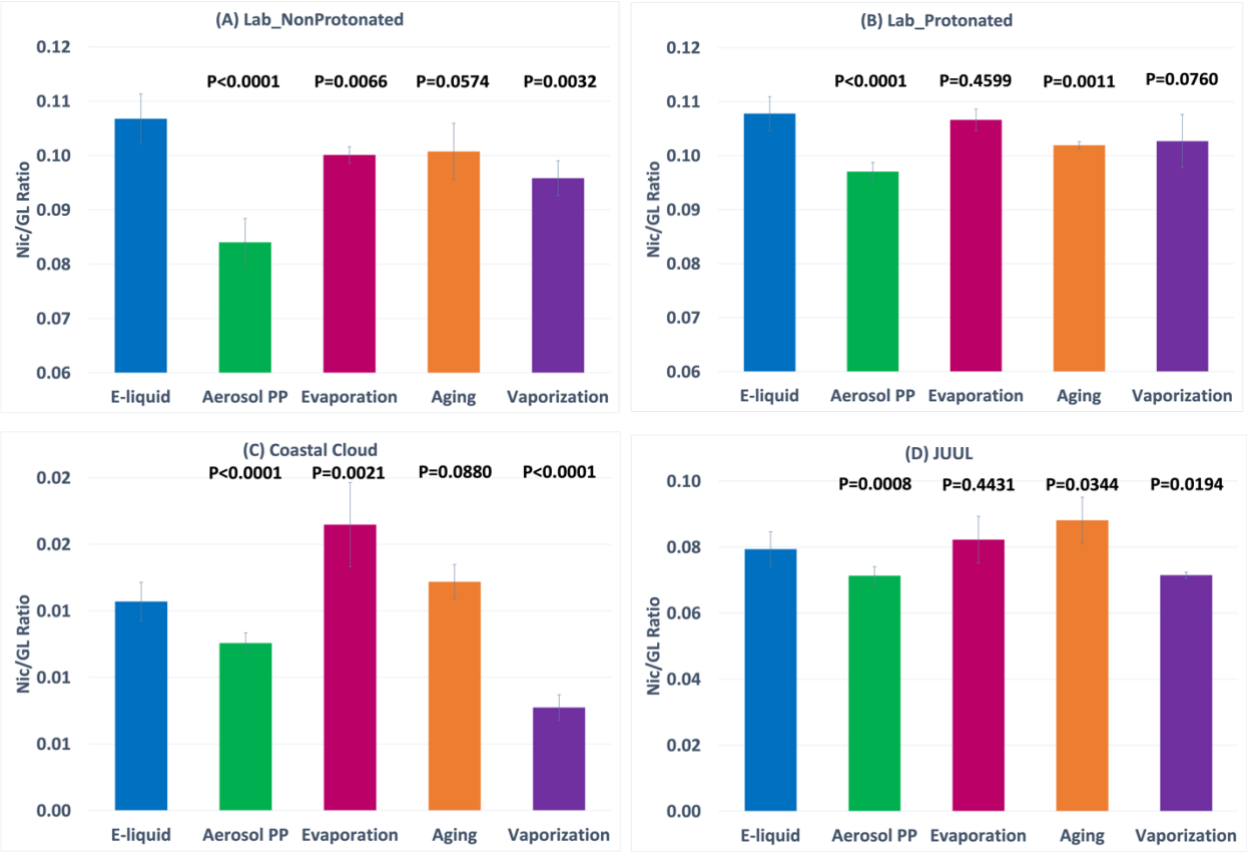


Figure 2.8 Nic/GL ratio of the e-liquid, aerosol PP, and the experiments to test evaporation, aging, vaporization for (A) Lab_NonProtonated e-liquid (B) Lab_Protonated e-liquid (C) Coastal Could e-liquid (D) JUUL e-liquid.

Similar to the PG composition, nicotine also shows a significant difference in the Nic/GL ratio in the aerosol PP from its original e-liquid (Table 2.12). Considering the lab-synthesized e-liquids, the %difference values of the aerosol PP with its e-liquid is not similar to experiments for evaporation, aging, and vaporization (Table 2.11). Therefore, condensation in aerosolization could be the mechanism causing the nicotine composition difference. This can be explained by the effect of vapor pressure in constituent gas-particle phase distribution. Nicotine has a vapor pressure of 5.92×10^{-5} atm which is lower than PG, but considerably higher than GL (Table 2.1). Due to the vapor pressure, a higher fraction of nicotine could stay in the gas phase compared to GL, resulting in the lower

composition of Nic/GL in the particle phase to that of the e-liquid. Similar results to lab synthesized e-liquids were expected in the commercial e-liquids as well. Both commercial e-liquids show a decrease in Nic/GL ratio in the aerosol PP from its e-liquid. However, the three experiments for Coastal Cloud results were unusual to the expected even after a set of repeated experiments on fresh e-liquid. This could be due to the low nicotine composition of 0.6% in Coastal Cloud compared to the other three e-liquids, reflecting a low nicotine amount in the aerosol PP. This can be a future direction to explore more on the effect of low nicotine in e-liquid on its aerosol PP composition.

Table 2.11 %Difference of Nic/GL for each condition with the original e-liquid

E-liquid	**%Difference of Nic/GL ratio with the e-liquid**			
	Lab_NonProtonated	**Lab_Protonated**	**Coastal Cloud**	**JUUL**
Aerosol PP	21.31%	9.96%	13.92%	9.64%
Evaporation	6.24%	1.06%	25.86%	3.44%
Aging	5.64%	5.43%	6.66%	10.48%
Vaporization	10.25%	4.71%	35.59%	9.48%

Table 2.12 P value calculated by hypothesis testing between two means at a 0.05 confidence level for the Nic/GL of each condition with the e-liquid.

E-liquid	**%Difference of PG/GL ratio with the e-liquid**			
	Lab_NonProtonated	**Lab_Protonated**	**Coastal Cloud**	**JUUL**
Aerosol PP	<.0001	<.0001	0.0008	<.0001
Evaporation	0.0066	0.4599	0.4431	0.0021
Aging	0.0574	0.0011	0.0344	0.0880
Vaporization	0.0032	0.0760	0.0194	<.0001

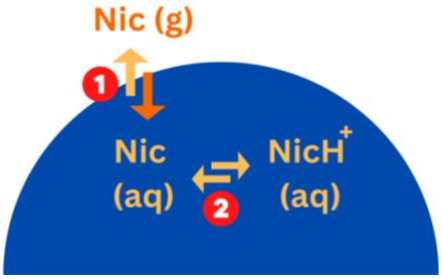


Figure 2.9 Schematic diagram of the equilibriums between Nicotine forms in e-liquid aerosol gas/particle phases.

A significant nicotine composition difference between the non-acidified and acidified e-liquids was observed. Considering the lab synthesized e-liquids, the Lab_NonProtonated aerosol PP shows a 21.31% Nic/GL difference with its e-liquid, whereas the Lab_Protonated is only 9.96%. Irrespective to the similar PG, GL, and Nicotine (PG:GL = 50:50, nicotine 5%) composition in both lab synthesized e-liquids, the two %difference values indicate a significant change. Results from the two commercial e-liquids also show a similar observation. The %difference value between the e-liquid and its aerosol PP for the acidified e-liquid JUUL (9.64%) is lower than that for the non-acidified e-liquid Coastal Cloud. The two acidified e-liquids Lab_Protonated and JUUL show similar %difference values of 9.96% and 9.64% respectively. However, the non-acidified commercial e-liquid Coastal Cloud shows a lower %difference of 13.92% compared to the 21.31% of Lab_NonProtonated e-liquid which could be due to their nicotine composition dissimilarities. Presence of an acid in the e-liquid protonates a fraction of nicotine. When acidified e-liquid aerosolizes, nicotine exists only in its non-protonated form in the gas phase. However, in the particle phase, both protonated and non-protonated forms of nicotine exist in equilibrium. Therefore, in the aerosol of an acidified e-liquid, the particle phase non-protonated nicotine is in two equilibriums with the particle phase protonated nicotine and the gas phase non-protonated nicotine (Figure 2.9). Due to the additional equilibrium, more nicotine is pulled to the particle phase in an acidified e-liquid aerosol compared to the non-acidified, which results in a low composition difference with the original e-liquid. Our experimental results show that the composition change from e-liquid

to its aerosol PP could happen during the condensation phase, and the presence of an acid in the e-liquid affects the composition in the aerosol PP.

2.3.3 Summary of outcomes and implications

Objective of the study was to identify composition changes in aerosol particle phase from the e-liquid and to understand the mechanism behind it. PG content in the aerosol PP was lower compared to that of the e-liquid across all four tested e-liquids. Variation in the PG content was not influenced by presence of an acid. In addition to PG, nicotine also showed a lower composition in the aerosol PP from the e-liquid. A significant difference in nicotine content was observed between acidified and non-acidified e-liquids, non-acidified e-liquids showing a greater reduction of nicotine in the aerosol PP. Based on aerosolization process and literature, evaporation, aging, vaporization, and condensation were identified as potential mechanisms for composition change. Experimental results eliminated evaporation, aging, and vaporization mechanisms, demonstrating condensation as the cause for composition differences between e-liquid and aerosol particle phase. Aerosol is a suspension of condensed particles in vapor pressure. Fraction in gas and particle phases depend on chemical and physical properties of chemical compounds, influencing the composition changes between e-liquid and aerosol particle phase.

Outcomes from the study confirmed current established aerosol PP analysis methods of generating aerosols using computer-controlled emissions systems, and capturing particle phase in filter pads could not result variations in chemical composition. In addition, results confirmed that sampling methods do not influence changes in composition. Findings from this study ensures the outcomes from above mentioned existing aerosol PP analysis methods do not cause composition changes in the aerosol PP.

Most studies discuss e-cigarette consumers' exposure to HPHC relevance to composition of the e-liquid. Results from this study verified PG and nicotine composition changes in aerosol which have been previously reported in literature and deduced that variations occur during condensation of aerosolization. Therefore, findings from this study

recommends using aerosol composition as a measurement to evaluate HPHC exposure in e-cigarette users.

Chapter 3 QUANTIFICATION OF AEROSOL DEPOSITION AND NICOTINE UPTAKE IN ELECTRONIC CIGARETTE USERS (A PILOT STUDY).

3.1 Introduction

Multiple health complications have been reported in e-cigarette users in the literature.[9,62–65] However, the direct impact of vaping on health risks is not properly understood yet. Health risks can be influenced by the e-cigarette product characteristics, and user specific parameters such as puff and respiratory topographies (Figure **1.5**). E-cigarette product characteristics have been extensively studied. However, there's only limited literature on aerosol composition delivered to the users (yield), deposition in the respiratory tract (RT), and their uptake in the human body. When e-cigarette aerosol enters the RT, part of it is deposited in the upper RT (mouth, larynx, trachea) through both gas and particle phases, while the rest travels to the lungs and gets deposited. Deposited chemicals are then absorbed through the membrane cells and into the bloodstream. Upon exhaling the fraction that is not deposited is removed from the body. Deposition and uptake in the RT can be influenced by user-specific parameters such as puff topography, respiratory topography, and metabolism, as discussed in Chapter 1. The total amount of deposition and how much of it is uptake into the body has not been fully studied yet.

3.1.1 Aerosol deposition in the respiratory tract

Literature on deposition in e-cigarette users is limited, with few studies reporting total deposition in the RT, while some provide results only on limited compounds (Table 3.1). Reported studies are either experimental, clinical, or computational, with total deposition values varying between a wide range of 15-90%. The inconsistency in the results could be due to the diversity of e-cigarette and e-liquid products used and the limited number of participants (≤15) in human subject studies. In addition, computational studies require experimental data on aerosol particle size and puff topography in their models, which might not be highly accurate due to the lack of studies on e-cigarettes. Exhale breath analysis and Positron Emission Tomography/ Computed Tomography (PET/CT) techniques have been used in previous studies as well.[58,66,67]

Table 3.1 Aerosol deposition efficiencies reported in e-cigarette users.

Experimental Method	Total Aerosol Deposition Efficiency (%)	PG Deposition Efficiency (%)	GL Deposition Efficiency (%)	Nicotine Deposition Efficiency (%)	Citation
Human subject PET/CT study	-	-	-	31-36	[66]
Computational model	45	-	-	-	[68]
Human subject study	-	98.3	94.8	99.6	[58]
Computational model	15-45	-	-	-	[69]
Computational model	>90	-	-	-	[70]
Computational model	-	-	37.90	-	[71]

Inconsistency in reported literature implies the myriad of unknown factors in affecting deposition in the RT. Deposition is governed by multiple factors as discussed in the previous paragraph, especially user specific parameters. Understanding the influence of each parameter such as puff topography, respiratory topography on deposition would unravel more factors relating to the impact of vaping.

3.1.2 Nicotine uptake in e-cigarette users

Nicotine uptake in the body can be quantified either by taking nicotine or cotinine measurements.[72] Cotinine is the major metabolite of nicotine. An average of 70-80% of absorbed nicotine is converted into cotinine in human body.[73] Cotinine has a longer elimination half-life of 12-16 hours compared to 2-2.5 hours of nicotine, and cotinine shows a linear relationship with the nicotine dose (Figure 3.1).[74–76] Cotinine has been identified as a better biomarker for nicotine uptake than nicotine itself in the extensive

literature on tobacco cigarette users, due to cotinine's longer half-life. However, there's a scarcity in studies on cotinine levels in e-cigarette users, specifically quantification analysis for time stamps of post vaping. Cotinine can be detected in human blood, saliva, and urine. Cotinine concentrations in blood and saliva are highly correlated, but in urine cotinine concentration tends to be higher.[77,78] Sampling saliva is a non-invasive method, which makes it a more suitable method than blood or urine sampling to quantify cotinine in humans.

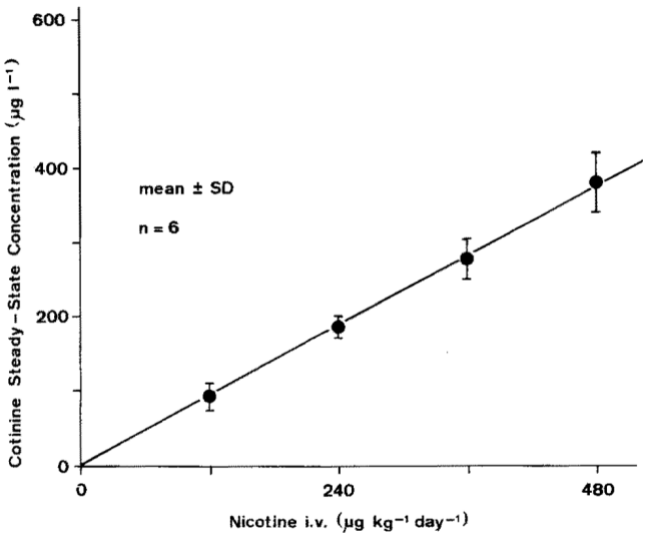


Figure 3.1 Steady-state plasma concentration of cotinine vs dose of nicotine.[74]

3.1.3 Study overview

This was a pilot human subject study designed to streamline a methodology to quantify the deposition of e-liquid constituents in the RT, and the uptake of nicotine in the users, and to identify the influence of different user-specific parameters on deposition. The study was conducted with the participation of a small set of human subjects to evaluate the feasibility of data collection methods and achieving quantifiable outcome measurements. Participants attended in-lab sessions where they vape ad-lib and answer questionnaires about their vaping behaviors. Primary measurements collected in each lab session are the e-liquid consumed, exhale breath particles, puff topography, respiratory topography, and pre and post-vaping saliva samples. The methodology for data collection and

processing is discussed thoroughly in Section 3.2. In summary, exhale breath was collected during the vaping session using a lab-constructed device, puff topography data by the wPUM^{TM} monitor, and respiratory topography data using Hexoskin Smart Garment.

3.1.4 Hypothesis and aims

The primary outcome measures from the study are yield produced from the e-cigarette, deposition efficiency in the respiratory tract and nicotine uptake. In this study, we tested the hypothesis that there will be a stronger correlation between deposition efficiency of nicotine and nicotine uptake in the body than the correlation between nicotine yield and nicotine uptake in e-cigarette users. In addition, influence of secondary outcome measures on deposition efficiency was studied. The main objective of the pilot study was to evaluate the proof of concept to test the hypothesis.

Aim 1: Assess the feasibility of collecting quantifiable amount of exhale breath, and applicable secondary outcome measures.

Capture exhale breath using a lab-constructed breath collection device, and quantify exhaled PG, GL, and Nicotine amounts by GC-FID methods. Collect and analyze the puff topography data and respiratory topography data using technologies developed by the Respiratory Technologies Lab (RTL).

Aim 2: Evaluate correlations of nicotine uptake with yield produced from the e-cigarette and deposition efficiency.

Use established mass balance methods to quantify PG, GL, and Nicotine yield produced from the e-cigarette. Collect exhale breath and quantify exhale PG, GL, and Nicotine by GC-FID technique. Calculate deposition efficiency for each participant from data of yield and exhaled mass. Use salivary cotinine method to quantify the nicotine uptake in the body. Report cotinine boost vs. nicotine deposition efficiency, and cotinine boost vs. yield to compare the two correlations.

Aim 3: Evaluate correlations of deposition efficiency with secondary outcome measures.

Calculate deposition efficiency for each participant from data of yield and exhaled mass. Report correlations between puff and respiratory topographies with deposition efficiencies of nicotine, PG and GL.

3.2 Methodology

Study was designed to understand the correlations between the yield of constituents delivered to the users, deposition in the respiratory tract, and the uptake to the user's blood system. This was a pilot study with the participation of two e-cigarette users to streamline the methodology and show proof of concept of our overall breath collection device and method. Study protocol was reviewed and approved by the Rochester Institution of Technology (RIT) Human Subjects Research Office and the Institutional Review Board (IRB). This study consists of two sessions. In Session 1, participants used JUUL product along with the JUUL wPUM^{TM} monitor to collect puff topography data. In Session 2 they used their current personal e-cigarette brand while wearing the Hexoskin Smart Garment with the Hexoskin Smart Device to collect respiratory topography. Protocol was similar in both sessions.

3.2.1 Participant recruitment

Eligibility criteria for this study is current e-cigarette users of age 21 years and older, not pregnant at the time. Recruitment process was via email by sending out study details to the previous participants of Respiratory Technology Lab human subject studies.

3.2.2 Protocol for the lab visit

Participants were asked to abstain from vaping from the night before until the lab visit. During the lab visit, participants were given a candidate ID, and demographic information was collected, followed by informed consent for the study. Eligibility for the study was

verified by date of birth being age of 21 or above, a negative pregnancy test result if applicable, and an expired carbon monoxide (CO) level of <6 ppm. CO biomarker level was collected using a Micro^{+} ™ ^{basic} Smokerlyzer®, a standard CO monitor to confirm abstinence from using combustible cigarettes. Eligible participants were enrolled in the study and a participant ID was provided.

Prior to the vaping session, pre-vape saliva sample was taken using a Salimetrics' passive drool collection kit, which was used to get pre-vape salivary cotinine level. Participants were given a maximum of 30 minutes time frame for ad-lib vaping. However, they were informed to vape only until they feel satisfied. During the vaping session, the first breath after using the e-cigarette was exhaled through the mouth into a custom Exhale Breath Collection (EBC) device. The participant was instructed to exhale only through the mouth, and this was demonstrated and practiced prior to the session. When the participant exhales into the mouthpiece of the device, the particle phase of the breath is captured to the filter pad inside the EBC device. 15 minutes after the vaping session post-vape saliva sample was taken. All saliva samples were stored in the freezer immediately after collection. Participants answered questionnaires about their vaping behavior, craving, and emotional state prior to vaping in Session 1 and during the 15-minute wait in Session 2. In Session 1, a short interview was conducted about the EBC device usage during the post-vape 15-minute wait time.

E-cigarette device and the filter pad inside the EBC device were weighed before and after the vaping session to obtain their Initial and End Mass. All the mass values were taken using Mettler Toledo Model Number AE240-1 S/N J65956 analytical balance with a manufacturer-reported readability of 0.1 (mg). The difference between the End Mass and the Initial Mass of the device is a measure of the Total Particulate Matter (TPM) delivered to the e-cigarette user.

3.2.3 Sample analysis

Samples collected during each lab session are the filter pad in the EBC device, pre-vape saliva sample, and post-vape saliva sample. In addition, e-liquids used by each participant were characterized.

E-liquid analysis

JUUL e-liquid used in Session 1 and its aerosol PP were well characterized before the start of the human subject study using the methods described in Chapter 2.2. In Session 2, both participants used Hyde brand e-cigarette devices in two different flavors. Both e-liquids were extracted after the session and analyzed by the method discussed in Chapter 2.2.

Exhale breath sample analysis

Both the filter pad and the EBC device mouthpiece area were separately analyzed for the exhale breath particulate phase. Filter pad was submerged in methanol 10.00 mL in an amber jar and was spiked with internal standard stock solution 200 µL (Chapter 2.2.1). Jar was closed, sealed with parafilm and shaken to initially break up the filter pad. Jars were placed on a shaker table at 180 rpm to completely break up the pad. Samples were filtered through 0.45 µm filters and transferred to gas chromatography (GC) vials. The mouthpiece section of the EBC device was rinsed with 10.00 mL of methanol. Methanol extract was spiked with the internal standard stock solution 200 µL and mixed well. EBC device extract was transferred to a GC vial. Filter pad samples and EBC device extracts were analyzed in GC-FID along with the calibration series following the method described in Chapter 2.2.2.

Saliva sample analysis for cotinine

Saliva samples were assayed at the Salimetrics' SalivaLab (Carlbad, CA) using the Salimetrics Salivary Cotinine Assay Kit (Cat. No. 1-2002), without modifications to the manufacturers' protocol. Provided method from the laboratory is as follows. Samples were thawed to room temperature, vortexed and centrifuged for 15 minutes at approximately 3000 RPM. Saliva sample test volume of 20 µL was tested for cotinine using a high sensitivity enzyme immunoassay (Cat. No. 1-2002). The assay has a lower limit of sensitivity of 0.15 ng/mL, a standard curve range from 0.8-200 ng/mL, and an average intra-assay coefficient of variation of 6.38%, which meets the manufacturers' criteria for accuracy and repeatability.

Topography data analysis

Inhalation data collected from wPUM™ monitor and respiration data collected from Hexoskin Smart Garment were analyzed by the TAP™ program to obtain puff topography profiles. wPUM™ monitor and Hexoskin Smart Garment have been used in previous studies to collect data on user puff and respiratory topographies.[79,80]

3.2.4 Data analysis

Mass percentages of PG, GL, and Nicotine in each e-liquid were calculated from the e-liquid characterization data.

$$\text{Analyte mass\%} = \frac{\text{Analyte mass (mg)}}{\text{E} - \text{liquid mass (mg)}} \times 100 \qquad 3.1$$

TPM delivered to the user is calculated by the e-liquid mass decrease from pre-vape to post-vape. Mass measurements are taken by weighing e-cigarette deceive with the e-liquid.

$$\text{TPM delivered (mg)} = [\text{Pre vape mass (mg)}] - [\text{Post vape mass (mg)}] \qquad 3.2$$

Each analyte delivered to the user (Analyte yield) was calculated using analyte mass% and the TPM delivered.

$$\text{Analyte yield (mg)} = \text{TPM delivered (mg)} \times \text{Analyte mass\%} \quad 3.3$$

Exhaled amount for each analyte was obtained from the GC-FID data for EBC filter pad and device extract. Using the yield and exhaled amount, deposition in the respiratory tract for each analyte was calculated.

$$\text{Deposition (mg)} = \text{Yield (mg)} - \text{Exhaled amount (mg)} \quad 3.4$$

Deposition as a percentage of yield (i.e., deposition efficiency) was calculated.

$$\text{Deposition efficiency\%} = \frac{\text{Deposition (mg)}}{\text{Yield (mg)}} \times 100 \quad 3.5$$

Cotinine boost is the increase of cotinine level during the vaping session calculated by the difference between salivary cotinine levels before (Pre-Vape) and after (Post-Vape) the vaping session.

$$\text{Cotinine boost} = \text{Post vape cotinine (mg)} - \text{Pre vape cotinine (mg)} \quad 3.6$$

Correlations of each analyte deposition efficiency with salivary cotinine level, puff topography data, and inhalation topography data were studied.

3.2.5 Devices utilized for user topography data collection.

Exhale breath collection (EBC) device

EBC device (Figure **3.2**) was constructed base on literature to capture exhale breath from PPTs.[67] A replaceable mouthpiece is attached to the breath intake inlet, and a filter pad

is there inside the holder to capture the exhale breath. After each trial the mouthpiece is removed, and the filter pad is taken out for analysis. EBC devices used in previous studies were assisted with a vacuum pump to make breathing easier. However, when testing the device constructed for the current study, it was evident that vacuum assistance was unnecessary. This is the first time of using the EBC device constructed by Respiratory Technologies Lab (RTL) in the field. Therefore, streamlining the device usage is a main objective of the study.

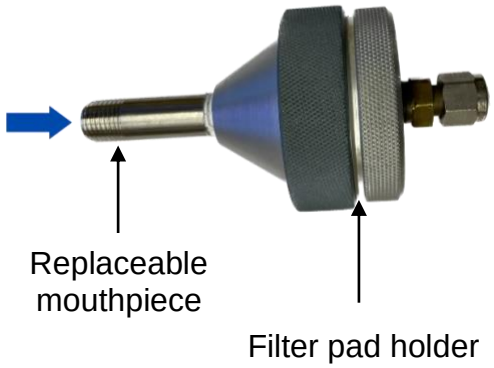


Figure 3.2 Exhale breath collection device constructed in the RTL, RIT.

Topography data collection

Figure 3.3 (A) wPUM^{TM} portable monitor for puff topography data collection. (B) Hexoskin Garment Shirt for respiratory topography data collection.[22]

Puff topography data and respiratory topography data are collected in this study. Puff topography data will be collected using wPUM^{TM} (Figure **3.3** - A), a portable monitor built by RTL that's been used in previous studies.[41,79] Data from wPUM^{TM} is analyzed with the

RIT Topography Analysis Program (TAP), providing puff flow rate, volume, and duration.[41]

Respiratory data is collected by the Hexoskin Wearable Garment (Figure **3.3** - B) that's been tested and used in previous RTL studies.[80,81] Collected data is analyzed with the RIT TAP program, providing information on respiratory volume, flow rate and duration.

3.3 Results and discussion

A pilot human subject study was carried out to understand the influence of user specific parameters in deposition and uptake of e-liquid components in human respiratory tract (RT), and to assess the capability of different devices in providing data in the field. This section includes results of the human subject study and a discussion on feasibility of utilizing technologies collect data.

3.3.1 Main outcome measures from the study

Table 3.2 Summary of participant and product data of the two lab sessions ("√" = yes, "x" = no)

	Candidate ID	Participant ID	Abstained from vaping	E-cigarette device	E-liquid Flavor	EBC device	wPUM Monitor	Hexoskin Smart Garment
Session 1	SV7-C01	SV7-01	√	JUUL pod with JUUL PCU	Virginia Tobacco	√	√	x
	SV7-C02	SV7-02	√	JUUL pod with JUUL PCU	Virginia Tobacco	√	√	x
Session 2	SV7-C01	SV7-03	√	Hyde Edge Rechargeable	Blue Ruzz Ice	√	x	√
	SV7-C02	SV7-04	-	Hyde Edge Disposable	Strawberry Ice Cream	√	x	√

Two participants (PPTs) were recruited for the human subject study, each participating in two sessions. PPT details and devices used in each session are summarized in Table **3.2**. In Session 1, PPTs used given JUUL brand e-cigarettes with Virginia Tobacco

flavored e-liquid. In Session 2, PPTs used their preferred brand of e-cigarette, which was Hyde for both, however, each used a different flavor. Main outcome measures of the study are the salivary cotinine boost, yield, exhale mass of individual constituents, and deposition efficiency. In some samples PG and Nicotine levels were too small to detect in Gas Chromatography (GC) chromatogram, where the exhale amount was assumed to be equal to or less than the concentration of the lowest standard. Secondary outcome measures from the study are the puff topography data from wPUM^{TM} monitor, and respiratory topography data from Hexoskin Smart Garment.

Deposition efficiency

Deposition efficiency of each constituent was dissimilar for both PPTs in the two sessions. PG shows the highest deposition efficiency, followed by Nicotine and GL (Table **3.3**). Across the four PPTs, average deposition efficiencies for PG, GL, and Nicotine were 98.47%±1.20, 87.05%±9.33, 96.41%±2.96 respectively. GL shows the highest variation among the PPTs, while inconsistencies in PG and Nicotine are low. GL in all four exhale breath samples were within the limit of detection, but PG for SV7-01 and Nicotine for SV7-01 and SV7-04 were below the limit. Therefore, it can be considered that reported overall GL values are more similar to actual values than PG and Nicotine.

Deposition efficiency data shows a correlation to vapor pressure of each constituent which influences the gas-particle partitioning, as discussed and demonstrated in Chapter 2. Vapor pressure decreases from PG to Nicotine to GL, so the gas phase fraction is expected to increase from GL to Nicotine to PG (Table **2.1**). Previous studies have shown gas phase particles tend to deposit more compared to the particle phase.[25,44,66,70] Therefore, assuming 100% deposition of the gas phase, deposition efficiency is expected to increase from GL to Nicotine to PG, which is consistent with results from the present study. Gas-particle phase partitioning is also influenced by the temperature, which has been shown in the literature. Pankow et. al. has modeled the gas phase fraction of e-cigarette constituents at room temperature of 20 ^{o}C, and the body temperature of 37 ^{o}C.[52,82] In the model, increase in the temperature demonstrated a higher gas phase

fraction. Findings from our study align with the previous studies, indicating the effect of constituent vapor pressure on gas-particle phase distribution, contributing to deposition patterns in the RT.

Table 3.3 Summary of main outcome measures for PG, GL and nicotine. SV7-C indicates Candidate ID, and SV7- Participant ID)

Constituent	Measurement	Session 1		Session 2	
		SV7-C01	SV7-C02	SV7-C01	SV7-C02
		SV7-01	SV7-02	SV7-03	SV7-04
PG	Yield (mg)	12.41	9.45	16.31	53.93
	Exhale amount (mg)	*0.12	0.19	0.48	0.11
	Deposition (mg)	*12.29	9.26	15.83	53.82
	Deposition Efficiency %	*99.03	97.99	97.06	99.80
GL	Yield (mg)	27.18	20.71	16.36	45.85
	Exhale amount (mg)	1.44	2.87	4.22	3.14
	Deposition (mg)	25.74	17.83	12.14	42.71
	Deposition Efficiency %	94.70	86.14	74.21	93.15
Nicotine	Yield (mg)	2.26	1.72	1.78	3.97
	Exhale amount (mg)	*0.05	0.05	0.14	*0.05
	Deposition (mg)	*2.21	1.67	1.64	*3.92
	Deposition Efficiency %	*97.96	96.82	92.13	*98.74

*Analyte was below the limit of detection. It was assumed that the exhale amount is equal to the LOD (concentration of the lowest standard in the calibration series). Therefore, values are estimated.

Salivary cotinine measurements

Salivary cotinine samples were collected from each PPT before (Pre-vape) and after (Post-vape) each vaping session. Difference between two measurements was calculated as the cotinine boost. Both PPTs in all sessions showed a significant boost in cotinine 15 min after the vaping session. Therefore, a 15 min post-vape window can be considered as a sufficient amount of time to measure cotinine boost. Cotinine boost in 15 minutes time span was also reported in the literature for plasma cotinine concentrations by Galeazzi et. al. (1985).[74]

Table 3.4 Salivary cotinine results of the participants

	Candidate ID	Participant ID	Salivary cotinine (ng/mL)		
			Pre-vape	Post-vape	Cotinine Boost
Session 1	SV7-C01	SV7-01	202.16	317.14	114.98
	SV7-C02	SV7-02	68.46	86.64	18.18
Session 2	SV7-C01	SV7-03	418.82	562.15	143.33
	SV7-C02	SV7-04	248.26	260.69	12.43

Participant SV7-C01 showed a much greater cotinine boost in both sessions than SV7-C02. Results from the study shows cotinine boost is tied specifically to the user, but two data sets from two PPTs is not enough to draw a conclusion. In Session 2, even with a lower yield (amount delivered to the respiratory tract) SV7-C01 showed a higher cotinine boost. From the nicotine dependence survey questions (Table **3.5**) SV7-C01 PPT was identified as a frequent user of e-cigarettes (higher addiction) compared to SV7-C02. Our study does not have enough data to do a statistical analysis between frequency of e-cigarette use and salivary cotinine levels. However, preliminary results from the two PPTs agree with findings from previous studies on a positive correlation between frequency of vaping and cotinine level.[5,6]

Table 3.5 Responses for the nicotine dependence survey questions

	Session 1		Session 2	
	SV7-C01	**SV7-C02**	**SV7-C01**	**SV7-C02**
	SV7-01	**SV7-02**	**SV7-03**	**SV7-04**
How many times per day do you usually use your electronic cigarette?	35	6-10	15	10
On days that you can use your electronic cigarette freely, how soon after you wake up do you first use your electronic cigarette?	as soon as I wake up	immediately	within 5 mins	immediately
Do you sometimes awaken at night to use your electronic cigarette?	Yes	No	No	Yes
If yes, how many nights per week do you typically awaken to do so?	3	0	0	2
Do you use an electronic cigarette now because it is really hard to quit?	Yes	Yes	Yes	Yes
Do you ever have strong cravings to use an electronic cigarette?	Yes	Yes	Yes	Yes
Over the past week, how strong have the urges to use an electronic cigarette been?	Very Strong	Moderate	Very Strong	Moderate
Is it hard to keep from using an electronic cigarette in places where you are not supposed to?	No	No	No	No
When you have not used an electronic cigarette for a while, OR when you tried to stop using one: Did you feel more irritable because you couldn't use an electronic cigarette?	Yes	No	Yes	No
When you have not used an electronic cigarette for a while, OR when you tried to stop using one: Did you feel nervous, restless or anxious because you couldn't use an electronic cigarette?	No	Yes	No	Yes
I find myself reaching for my e-cigarette without thinking about it.	Almost Always	Sometimes	Often	Often
I drop everything to go out and buy e-cigarettes or e-juice.	Sometimes	Rarely	Rarely	Sometimes
I vape more before going into a situation where vaping is not allowed.	Often	Never	Sometimes	Never
When I haven't been able to vape for a few hours, the craving gets intolerable.	Rarely	Sometimes	Almost Always	Often

SV7-02 PPT shows a significant difference of high yield, higher deposition efficiency, and a lower cotinine boost in Session 2 compared to Session 1. And there's also a discrepancy in survey answers of both PPTs between the two sessions (Table **3.5**), specifically for the questions "*How many times per day do you usually use your electronic cigarette?*", "*Do you sometimes awaken at night to use your electronic cigarette?*", and "*I drop everything to go out and buy e-cigarettes or e-juice.*". For the SV7-C02, answers for the above questions changed from Session 1 to Session 2 suggesting an increase in craving for e-cigarettes, which also correlates with the enhanced yield and deposition in Session 2.

For both PPTs the Pre-Vape cotinine measurement was higher in Session 2 than in Session 1. However, SV7-C02 PPT's Session 2 Pre-Vape cotinine level is about three times to that of the Session 1. Prior to the study all PPTs were asked to abstain from vaping since the night before the lab visit to avoid any interference with cotinine measurements. SV7-C02 PPT didn't abstain from vaping in the Session 2, which is most likely the reason for higher Pre-Vape cotinine level. Among all four sessions, salivary cotinine Pre-Vape levels range between 68.46-418.82 ng/mL which agrees with the cotinine levels of e-cigarette users reported in the literature.[7–9] Reason for the broad range of cotinine concentration could be the effect of multiple factors influencing nicotine metabolism such as age, gender, diet, race, ethnicity, etc..[10] There's not enough data in the pilot study to analyze effect of each factor on cotinine level, however collecting PPT data on demographics, diet, and medications is recommended for future studies.

Cotinine boost can depend on both yield and deposition efficiency. It could be assumed that deposition efficiency for SV7-C01 is higher due to the higher cotinine boost in both sessions. However, data from the pilot study does not completely support this assumption. Wide discrepancies in the patterns among yield, deposition efficiency, and cotinine boost suggest requirement of further studies. Also, calculated values can be different from actual due to exhale nicotine amounts of some PPTs being too small to quantify, and differences in cotinine boost by not abstaining from vaping before the lab visit.

3.3.2 Secondary outcome measurements

Puff topography data

Pilot study method development incorporates the puff and respiratory topography analysis technologies developed by RTL to enhance the study results. Method development of the study is a work in process. In the first session where PPTs used JUUL, a wPUM™ compatible with JUUL products, developed and tested by RTL was used to collect puff topography data. Puff topography profiles of each PPT are summarized in. While one session is not enough to do a statistical analysis, few deductions can be made from the results. Comparing the flow rate data, SV7-01 puffed in a slower flow rate. It has been documented in the literature that e-cigarette aerosol yield is inversely proportional to the flow rate. As stated above, SV7-01 is an experienced user, and the slower flow rate and higher yield demonstrate PPT's ability to use the e-cigarette device efficiently. Adequate data is needed to determine the association between puff topography and deposition efficiency.

Table 3.6 Summary of Session 1 puff topography data collected from wPUM™

Candidate ID	Participant ID	Number of Puffs	Puff Flow Rate (mL/s)			Puff Duration (s)			Puff Volume (mL)		
			Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
SV7-C01	SV7-01	28	16.18	30.08	21.32	1.20	5.18	3.72	24.54	100.55	77.82
SV7-C02	SV7-02	16	30.08	53.09	44.76	1.45	9.88	6.88	54.38	524.31	315.10

Respiratory topography data

During Session 1, each PPT mentioned tobacco flavored JUUL product gave a strong hit and expressed their preference on personally preferred products which are different to JUUL. Considering the comments from PPTs, Session 2 of the study was designed to understand variations in results when their preferred product is used. Both PPTs used Hyde products but in two different flavors. One complication in using Hyde for method development was that RTL has not developed a wPUM compatible with Hyde devices.

Therefore, in session two puff topographies were not collected. Also, results from the Session 1 showed incorporating respiratory topographies would maximize the data collection, thus providing a deeper understanding about the user behavior patterns.

At RTL, Dr. Shehan Jayasekera developed a technology to collect respiratory behavioral data utilizing Hexoskin Garment Shirt, both while PPTs are using e-cigarette device and in between puffs. Results can inform us on whether the user's inhalation behavior is direct lung or mouth-to-lung. Direct lung is similar to the behavior of using a waterpipe or hookah, while mouth-to -lung is more associated with use of conventional cigarettes by taking a puff into the mouth followed by a deep inhalation of clean air. In addition, breath hold data and puff count can be obtained. Example respiratory data for SV7-04 can be seen in

Figure **3.4**. In the figure, “Clipped Volume” indicates results from the Hexoskin Smart Garment, while “Trapezoid Volume” is volume calculated from the model. During the lab visit visual puff number was counted using a stopwatch, which is referred as “Puff”. Visually taken times match with most of the broader peaks, indicating broader peaks to be puff-associated breathing cycles. Further analysis of respiratory cycle volume would provide more information on correlation between Puff Associated Respiration (PAR) and Tidal Breathing (Non-PAR) which is beneficial in understanding respiratory topography in e-cigarette users.

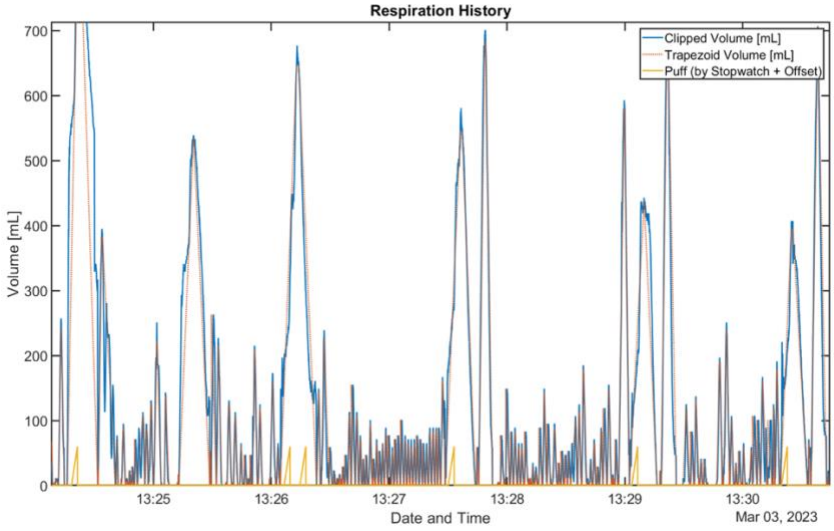


Figure 3.4 Respiration topography data base on the Hexoskin Smart Garment for the participant SV7-04

This is the first human subject study to use the EBC device constructed by RIT Respiratory Technologies Lab. Data and results from the study provide proof that the EBC device is capable of effectively collecting exhale breath from PPTs. Feedback from PPTs about the EBC device usability was positive, affirming its suitability for in-lab studies. However, in the pilot study, only the immediate exhale breath was collected after each puff. Assuming the following exhales include some particles, then the deposition efficiency values would decrease from the reported values in this study. Therefore, the reported depositions in this study are upper-bound values.

In this study, Exhale Breath Collection (EBC) device, wPUM^{TM} monitor and Hexoskin Smart Garment were used to collect data. In the current study, wPUM^{TM} was used in Session 1 and Hexoskin Smart Garment in Session 2. However, having the capacity to use all three devices in the same session would provide puff topography, respiratory topography, and exhale amount data for each PPT maximizing outcomes of future studies.

Chapter 4 IMPLICATIONS AND FUTURE WORK

E-cigarette consumption directly exposes users to the aerosol generated by the e-cigarette device. Aerosols entering the user's respiratory tract (RT) travel through the mouth cavity, trachea, and intrathoracic region of the lung to the alveoli region in lower respiratory airways. Aerosols consist of a gas phase and a particle phase, where volatile molecules are partitioned between both phases. Molecules in gas and particle phases deposit in the RT and get absorbed (i.e., uptake) into the blood while a fraction of the aerosol is exhaled. The gas phase molecules are transported to the alveoli region of the lungs, whereas only small particles are able to travel into the deep alveoli region. Molecules deposited in the alveoli are absorbed into the blood by crossing the blood-air barrier. Therefore, it can be assumed that most of the gas phase molecules get absorbed. Hence knowing the gas and particle fractions of the molecules and the exact mechanisms for partitioning between two phases would provide a better understanding of aerosol deposition in e-cigarette users.

4.1 Assessing the mechanism for the composition difference between e-liquid and its aerosol particle phase

The first study discussed in Chapter 2 focused on identifying the exact mechanism for the composition difference between e-liquid and its aerosol particle phase (PP). Four potential mechanisms were identified and tested for both acidified e-liquids and non-acidified e-liquids. During aerosolization, e-liquids vaporize at high temperatures and initiate condensation due to the temperature drop when the vapor moves away from the heating element. In the present study, condensation during aerosolization was identified as the mechanism causing composition differences between e-liquid and aerosol PP, eliminating literature statements on the effects of sampling techniques on composition differences.

A significant decrease in PG composition was observed in the aerosol PP compared to the e-liquid, which was explained by the much higher vapor pressure of PG compared to

GL. When the vapor pressure of a molecule is high, its gas phase fraction tends to be higher compared to a molecule with lower vapor pressure. Rationalizing PG composition difference by the higher vapor pressure was also supported by theoretical models in the literature.

Nicotine showed a composition difference in the aerosol PP compared to the e-liquid. However, the difference for the non-acidified e-liquids was higher than the acidified e-liquids, i.e., aerosol PP of acidified e-liquids contained a higher nicotine amount than the non-acidified e-liquids. Nicotine composition differences between the two e-liquids were explained by the presence of both protonated and non-protonated forms of nicotine in the aerosol PP of the acidified e-liquid.

Nicotine is a stimulant that causes addiction to e-cigarettes. Gas and particle phase nicotine amounts are influenced by the presence of an acid in the e-liquid and also the nicotine quantity in the e-liquid. The aerosol gas phase fraction of acidified e-liquid is low compared to the non-acidified e-liquid. However, by adding acid, manufacturers are able to increase the nicotine content in the e-liquid without creating a harshness during vaping. Therefore, when quantifying the gas-particle phase nicotine, the presence of an acid and also the nicotine amount in the e-liquid should be considered.

A future development on the current study would be to attempt mass balance experiments where gas phase is also collected in addition to the particle phase, and characterize for PG, GL, and nicotine in the gas phase. It would experimentally confirm the rationale provided in the current study for the composition difference between e-liquid and the aerosol particle phase. Thermal degradation products are also produced during the aerosolization of the e-liquid. Another future work is to analyze gas phase decomposition products, mainly aldehydes from PG and GL.

The compositions of gas-particle phases identified in this study are for the room temperature during aerosol formation followed by entering into the RT. Gas-particle partitioning is also affected by temperature. Body temperature is high than the room

temperature; thus, the fraction in the gas phase would change in the body from the amounts observed at the room temperature.

4.2 Quantification of aerosol deposition and nicotine uptake in electronic cigarette users (a pilot study)

The second study discussed in Chapter 3 focused on developing a methodology to quantify deposition and exhaled amounts of PG, GL, and nicotine in e-cigarette users. In this study, the exhale breath collection (EBC) device constructed in the Respiratory Technologies Lab (RTL) was tested for the very first time in a human subject study. EBC device was able to capture quantifiable amounts of PG, GL, and nicotine particles. Yield, the amount of e-liquid consumed by the user, was calculated by established mass balance methods. Deposition for each participant was calculated for PG, GL, and nicotine using the yield and exhaled values. It was observed that deposition varied between the two participants, and even in the same participant between two sessions. More data is required to do statistical analysis in order to draw conclusions about inconsistencies in deposition.

During the pilot study participants were asked for their feedback on using the EBC device while vaping. Both participants mentioned the use of the EBC device didn't deviate them from their natural vaping behaviors. However, using a lighter material to construct the EBC device would make it convenient to utilize in future studies. In the current study, only the immediate exhale after a puff was collected in the EBC device. In the current study, most likely, all particles were captured. However, collection of multiple exhales following a puff would confirm presence of particles in exhales following the immediate breath.

Nicotine uptake was determined by using the salivary cotinine biomarker. A significant cotinine boost was observed for all participants after 15 min of vaping. There was only one study in the literature where they studied cotinine boost over time, where the highest cotinine level was given after 15 minutes in plasma. Our study confirms the 15-minute time frame to be sufficient to provide a significant cotinine boost in saliva samples.

The amount of the cotinine boost did not show correlations with yield or deposition but rather a higher correlation with the user. This study collected only limited data, so conclusions cannot be made. However, observations from the study bring up questions for the future. It was clear that a 15-minute time was enough to see a boost in cotinine level in saliva, but the question remains if the observed level was the maximum for the participant. It is possible that nicotine metabolism would have different rates in each participant, and it might have been faster in SV7-C01 than SV7-C02. This would have a large effect on the outcomes. It is recommended to look at the rate of nicotine metabolism and the time taken for maximum cotinine level to be detected in saliva.

Puff and respiratory topographies were also measured in the pilot study by using a wPUM^{TM} portable monitor and Hexoskin Smart Garment respectively. wPUM^{TM} monitor provided results on mean puff flow rate, puff duration, and puff volume. Hexoskin Smart Garment provided data on respiratory volumes for puff-associated respiration and tidal breathing patterns (breathing in between puffs). Limited data collected on puff and respiratory topographies were not adequate enough to draw conclusions on the effects of user-specific topographies on deposition patterns.

Observations from the pilot study indicated potential avenues for future studies with respect to correlations between the vapor pressure of constituents and their deposition, the correlation of salivary cotinine boost with the specific user, and the ability of experienced consumers to use e-cigarette devices more efficiently. It is necessary to collect more data to conduct statistical analysis in order to draw conclusions about the stated observations. It is recommended to use the EBC device, wPUM monitor and Hexoskin Smart Garment to maximize the data collection in future studies. In the pilot study, participants stated they prefer their usual e-cigarette product over the JUUL product provided by the lab. There were inconsistencies in data for the same participant when using JUUL vs their preferred product. It is hard to conclude that the inconsistencies were due to the product difference. However, allowing participants to use their preferred product would provide data similar to their natural vaping.

The pilot study successfully streamlined a method to quantify exhale amounts, deposition, and topographies specific to the users. In future studies, collection of more data would a better understanding of depositions in e-cigarette users and how the user-specific parameters would contribute to deposition. In addition, the current understanding of gas-particle phase distribution of e-liquid constituents combined with deposition data would unravel the contribution of gas and particle phase constituents to deposition in the respiratory tract of e-cigarette users.

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