AMERICAN UNIVERSITY OF BEIRUT

ASSESSMENT OF TOXIC EMISSIONS FROM ELECTRONIC CIGARETTE LIQUIDS AND SUCRALOSE ADDITIVE

by
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Title: Assessment of Toxic Emissions from Electronic Cigarette Liquids and Sucralose Additive

Electronic cigarettes (ECIGs) have always been advertised as a "safer" alternative to combustible tobacco cigarettes. However, according to previous studies, ECIGs produce carcinogens (such as benzene and formaldehyde) and induce inflammation and infections in airway epithelial cells. Oxidants may be one of the culprits of these effects. This is why it is essential to quantitate the reactive oxygen species (ROS) in ECIG emissions.

The fluorescent probe, 2',7'-Dichlorofluorescein (DCFH) was used to analyze ROS emissions. Effects of power, basic liquid composition, and number of coils in the supra- and sub- ohm devices on ROS generation were assessed. This data was compared to the combustible cigarette and to the IQOS device. Results showed that ECIGs intrinsically emit ROS, and depending on the combination of chemical and physical parameters they can emit high levels of ROS comparable to combustible cigarette.

The ROS study focused on the basic liquid components (propylene glycol (PG), vegetable glycerin (VG), and nicotine), while a complimentary study focused on one additive, sucralose. The sweetener sucralose is being used in liquids of ECIGs to improve taste. Sucralose under thermal degradation has proven to release hydrochloric acid, which lead to the formation of chloropropanols in the presence of VG.

In this study, Nuclear magnetic resonance (NMR), thermal gravimetric analysis coupled with IR (TGA-IR), Fourier Transform Infrared (FTIR), and pH experiments were conducted to assess the gas phase emissions of a sub-ohm ECIG device when sucralose was added to the ECIG liquid. Results showed that the HCl produced catalyzed the reaction of PG and acetaldehyde to produce propylene acetal in the gas phase.

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ABBREVIATIONS

ECIG	Electronic Cigarette
P	Power
V	Voltage
R	Resistance
SOD	Sub-ohm Device
ROS	Reactive Oxygen Species
LOQ	Limit of Quantification
LOD	Limit of Detection
P/SA	Power per Surface Area
PG	Propylene Glycol
VG	Vegetable Glycerin
IQOS	I Quite Ordinary Smoking
NMR	Nuclear Magnetic Resonance
FTIR	Fourier Transform Infrared
TGA	Thermogravimetric Analysis
MCP	1-Monochloro-2-propanol
Sol A	Propylene Glycol
Sol B	1% wt/wt sucralose in Propylene Glycol
Sol C	1% wt/wt sucralose-1%wt/wt nicotine in Propylene Glycol
Sol D	1% Nicotine in Propylene Glycol
Sol A’	Propylene Glycol / Vegetable Glycerin (30/70)

Sol B’	1% wt/wt sucralose in Propylene Glycol / Vegetable Glycerin (30/70)
Sol C’	1% wt/wt sucralose-1%wt/wt nicotine in Propylene Glycol / Vegetable Glycerin (30/70)
Sol D’	1% Nicotine in Propylene Glycol / Vegetable Glycerin (30/70)
FDA	Food and Drug Administration

CHAPTER I

INTRODUCTION

A. Electronic cigarettes

Electronic cigarettes (ECIGs) were introduced to the market in 2004. Ever since, they have been advertised as the “safe” alternative to cigarette smoking. ECIG use is not restricted to current or former smokers, but also non-smokers, including adults and youth, are using these devices. To enhance the perception that ECIG is safer than combustible tobacco cigarettes, the word vaping replaced smoking to describe their use. It was reported that ECIG sales have increased from 2012 till 2013 to reach 636.2 million USD and is expected to keep on increasing.[1] This means that the number of people vaping is increasing. Nowadays, more than 400 brands and 7,000 flavors exist on the market shelves.[2] As the popularity of ECIGs continues to increase among users, scientists are urged to gain interest in these devices and understand their operating mechanisms in delivering nicotine and generating other toxicants.

B. ECIG anatomy and operation

All ECIGs share the same concept of activation: a solution in propylene glycol (PG) and/or vegetable glycerol (VG) that may contain nicotine and flavorings, is heated on a metallic coil powered by a Li-ion battery. The design of ECIG has tremendously evolved in the last 15 years from cig-a-like disposable designs to exotic looking and highly customizable advanced vaping devices. The user now has access to modify the liquid content, the power input and recently the coil metal type and design. Figure 1 illustrates the main ECIG compartments including a battery, a coil, a wick, and liquid reservoir. A cartomizer is the compartment where the liquid, the metal coil wire, and the wick are present. The liquid travels through the wick by capillarity (this is known as wicking). The coil is wrapped around this wick. Upon the activation of the battery manually or by inhaling (through a pressure sensor), the coil heats up making the liquid vaporize from the wick. This process causes the aerosols to be generated from the mouthpiece of the ECIG and thus the action of vaping.

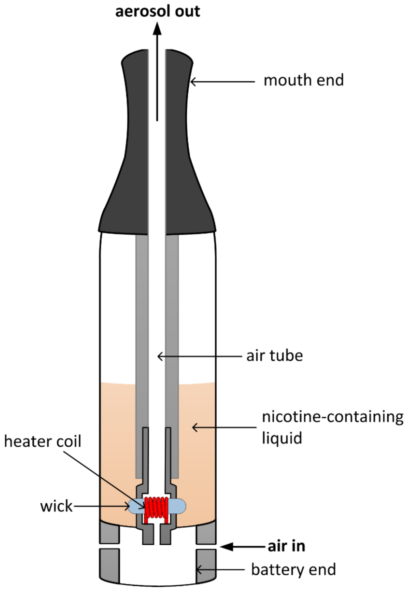


Figure 1: Electronic cigarette detailed anatomy (Baasiri et al. (2016)

The first generation of ECIG was the disposable ECIG, called Cig-a-like. The second generation of ECIG includes a rechargeable battery and a prefilled or refillable cartomizer. The rechargeable battery can be set at a specific voltage or power by the manufacturer or can be altered according to the preference of the user, depending on the design of the ECIG. The power is dependent on the battery voltage output and the resistance of the coil, according to the equation $P=V^2/R$. These devices proved to be efficient in delivering nicotine to the users [3-4] since they were able to reach higher power, thus higher temperature on the coil.[5-6] The third generation of ECIGs includes the devices operating at high powers reaching up to 200W. This can be achieved by a coil of resistance of less than 1 ohm, and this is why they are known as "sub-ohm" devices (the other ECIGs with resistance $> 1\ \Omega$ are referred to as supra-ohm devices). Both, the second and third generation devices allow users to refill liquid for multiple vaping sessions and to alter the power at which the device functions according to their satisfaction. The fourth generation of ECIG contains, on top of the third generation ECIG features, a temperature sensor that allows users to operate their devices on temperature-controlled regimes.

Figure 2: The different ECIG generations and their characteristics

C. Heat-not-burn nicotine delivery systems

Besides ECIGs, an old approach for smoking harm reduction was revived. It relies on heat-not-burn tobacco leaves or extracts. A new arrival in this approach was the I-Quit-Ordinary-Smoking “IQOS” recently introduced by Philip Morris International. Figure 3 shows the IQOS device in which a tobacco stick called “Heet” is inserted in a slim coil head connected to a battery. The coil head consists of a sheet like metal that goes inside the Heet stick. The user has to activate the battery with an on/off button and then inhale the generated aerosol from the heated tobacco. In this dissertation, supra- and sub-ohm ECIGs will be compared to IQOS and combustible cigarettes.

Figure 3: IQOS device

D. Toxicants from ECIG

Similar to its parent counterpart, the combustible cigarette, research on ECIG is still progressing. However, the ECIG system, the products formed, and their mechanisms of formation have not been fully understood yet. Due to the ECIG popularity, it is of great importance to assess the safety of this device. Studies aid in the formulation of guidelines and regulations for the safety of users.[7]

The main constituents of the ECIG liquid, including solvents, nicotine and flavors are recognized as safe food additives according to the Food and Drug Administration (FDA).[8] However, in the process of vaping, thermal degradation and other decomposition reactions of the liquid constituents take place, especially at high powers.[9] According to the literature, many harmful products were proven to be present in the aerosols of ECIGs, such as carbonyls and mainly formaldehyde, which is classified as group 1 human carcinogen, furanic compounds, aromatic compounds such as benzene, which is classified as group 2B carcinogen.[10-16]

Early reports on ECIGs concentrated on the detection of toxicants in their liquids.[17] These toxicants if present will be distilled to the vapor that will be eventually inhaled by the user. Studies reported the detection of tobacco specific nitrosamines (TSNAs) that were extracted together with nicotine from the tobacco leaves during the processing of ECIG liquids.[18] Trace amounts of carbonyls and alcohols were detected in the liquids using different chromatographic techniques.[19] In addition, metal leachates were detected in the liquids and their aerosols.[20]

Later on, researchers concentrated on the toxicants that could be generated during vaping. These mainly come from the chemical transformation or degradation of the liquid main ingredients. Carbonyls were the most studied family in this aspect, with reports claiming higher levels of carbonyls emitted from ECIG in comparison to combustible cigarettes.[21] They are thought to come from oxidation and decomposition of PG and VG molecules on the hot surface of the coil.[22] They are usually analyzed by gas phase derivatization with 2,4-dinitrophenylhydrazine (DNPH) prior to HPLC analysis. Other reports showed the detection of volatile organic compounds (VOCs) in the aerosols of ECIG including benzene, toluene, xylene and smaller compounds.[19] These compounds were detected using thermal desorption and head-space techniques coupled to GC-MS systems. A recent report analyzed the emissions of ECIG by NMR and reported the detection of propylene oxide and glycidol (epoxides) in addition to carbonyls.[23] They also detected allylic alcohols for the first time in the literature.[23] Other reports showed that only trace amounts of polycyclic aromatic hydrocarbons are present in the aerosols of ECIGs.[24]

In addition to the assessment of liquid parameters that may affect toxicant generation including PG/VG ratio, nicotine content and flavors, reports showed that the physical parameters of the ECIG can affect its toxicant emissions. Several studies have been reported on the effect of power, and its correlation with coil temperature, on the extent of degradation of the liquid. A recent report showed that ventilation also affects the performance of ECIG, although this was previously shown to be of marginal effect.[25] One very important hypothesis of toxicant generation is the so called "dry-puff" phenomenon, in which liquid vaporization from the wick due to quick heating, is faster than its wicking.[26] Dry-puff is usually attributed to operating the ECIG at very high powers, but the detection of toxicants at low powers challenge this hypothesis.[26]

In addition to the physical and chemical parameters that may affect the mechanisms of toxicant generation in the ECIG, user behavior is also crucial. The puff duration, frequency and inter-puff intervals are important aspects to be considered in the assessment of the health risk that may be associated with ECIG use.[27]

Although detected at lowers levels than combustible cigarettes, toxicants from ECIGs contribute to the health concern about ECIG use. Recent reports shed light on the possible correlation of ECIG use with cardiovascular diseases, toxicity of airway epithelial cells, and chronic inflammations.[28-30] One important family to toxicants that was detected in the aerosol of ECIGs is reactive oxygen species (ROS), which plays a major role in cytotoxicity and raise many health concerns.[30] These species are formed from thermal degradation of ECIG liquids.[31]

E. Reactive Oxygen Species

Reactive oxygen species are oxygen based compounds including radicals such as hydroxyl radicals, superoxide anions, singlet oxygen, alkoxyl, and alkylperoxy radicals.[32] ROS can be formed endogenously by the cell or exogenously by pollutants, UV radiation, cigarette smoke, and most importantly ECIGs.[33,32] Endogenous generation of ROS is critical for the cell's function. For example, they are generated as a defense mechanism against antigens in the immune system.[34] ROS are produced from the electron transport chain in the process of cellular respiration and play a role in signal transduction pathways.[34] Many other organelles in the cell, such as lysosomes, also generate ROS.[35] All these endogenous formations are regulated by the cells via various anti-oxidants. At any time unwanted oxidants are present, antioxidants can terminate them. Normally, there is a balance of antioxidants and oxidants in the body. However, if ROS levels increase in the body, this balance will be deteriorated. The imbalance will lead to the further generation of antioxidants from the cells. Once there is an excess of ROS, inflammation, cell malfunction, cell damage, and cell death can be triggered.[36] Due to their high reactivity, they immediately react with whatever is around, meaning it will destroy anything in their path. Oxidants at high levels in the body cause oxidative stress, and chronic oxidative stress can lead to chronic diseases, such as cardiovascular or neurological diseases.[32, 37] Previous studies have reported the detection of ROS in ECIG emissions using cellular[30, 38-40] and acellular methods.[31, 38, 41] A cellular study has indicated that ROS emitted from ECIGs causes DNA damage and reduced cell viability.[30] Another study reported oxidative and inflammatory responses when ECIG emissions were exposed to lung tissue[38]; elevated cytotoxicity, inhibition of cell multiplication, and change in cell morphology were also observed.[39]

In one study, acellular ROS was measured in the emissions of a rechargeable Blu ECIG (tobacco flavor) and two brands of combustible tobacco cigarettes. The authors concluded that the levels of ROS in the rechargeable ECIG and the two brands of conventional tobacco cigarettes were similar.[41] It was not taken into account that the ECIG and the combustible cigarettes have different puff topographies. Also, the emissions of the ECIG and the tobacco cigarette were exposed to the fluorescent probe, Dichlorofluorescein, for different intervals of time.

A second study measured acellular ROS in a disposable ECIG using two flavors, tobacco (16 mg nicotine) and mentol (0 mg of nicotine) and in a refillable ECIG using two commercial e-liquid of different nicotine concentration (0 and 24mg), PG or VG alone, and heating element alone. The fluorescent probe, Dichlorofluorescein was used to quantitate ROS. It was reported that as nicotine concentration increases, ROS decreases. However, the compared sets of data in the disposable ECIG were of different flavors and in the refillable ECIG of unidentified liquid composition.[38]

A third recent acellular study (2018), addressing ROS, measured ROS levels in the emissions of two brands of supra-ohm devices, two different flavors, three sets of powers, and two puffing protocols. As the power increased, there was an increase in ROS. Changing the puff protocol, flavors, and brand, the levels of ROS differed.[31] ROS were trapped in one impinger containing an antioxidant, trolox. Sub-ohm devices were not studied.

Furthermore, electron paramagnetic resonance studies have proved the presence of radicals in the emissions of ECIG.[31, 41-42] Lerner et al. proved the presence of carbon centered radicals, and Zhoa et al. showed, in a recent study, the presence of hydroxyl radical.[31, 41] In this thesis, a systematic study was conducted using supra- and sub-ohm devices to assess the parameters that affect ROS emissions, like power, liquid composition, and number of coils in the ECIG. The different ratios or concentrations of liquid used for this ROS study included PG, VG, and nicotine. No flavor mixtures were included in this study in order to eliminate the possibility of other compounds interfering or being part of the ROS emissions. This is the core of chapter two including optimization of detection method in addition to the assessment of the different operating conditions of ECIG that may affect ROS emissions.

F. Sucralose as an additive

The contribution of additives and flavors to the general toxicant profile of ECIG is recently gaining consideration. Flavors and additives are added to increase appeal among targeted groups of users, like youth, or to enhance the perceptions of safety of the device among all users. However, these additives are also prone to thermal degradation and thus increasing quantities and variety of toxicant emissions. One study claimed that flavors are the main contributors in the emissions of toxic aldehydes.[43] It was reported that adding flavorants to the liquid increases the amount of aldehydes emitted, and these levels exceed the standards of occupational safety.[43] Another study tested the presence of aldehydes in several flavored disposable ECIGs.[44] In 60% of the samples, diacetyl, a respiratory hazard, and acetoin were the most prevalent in the vapors of ECIGs.[44] Moldoveanu et al. proved the presence of diacetyl and acetylpropionyl in aerosols of ECIGs containing flavors.[45] In fruity flavors, benzaldehyde is the key ingredient in the aerosols generated from these liquids.[46] Kosmider et al. measured the level of benzaldehyde in vapors of smoked fruity liquids. Most of the flavors contained benzaldehyde, with the cherry flavor having the highest levels of benzaldehyde.[46] Upon long-term exposure, health risk might emerge.[46] Another study by Pankow et al. detected the presence of benzene in vapors of ECIG upon smoking the flavor chemical benzaldehyde.[11] Soussy et al. detected 5-hydroxymethylfurfural and furfural upon vaping sucrose and fructose at different concentration, puff duration, and power input.[12] Moreover, testing the unvaporized liquid, a study by Fagan et al. showed the presence of sugars, glucose, sucrose, and fructose, and aldehydes, acetaldehyde, formaldehyde, and acroelin, in flavored liquids across brands.[47] These studies confirm the importance of studying the effects of flavorants and additives on ECIG users. In line with these efforts we decided to systematically approach the flavor question. One of the additives that has not been assessed in ECIGs is the sweetener sucralose.

The sweetener sucralose is one of the additives that enhance the sweet flavor in ECIGs.[48] The non-nutritive sweetener is reported to be six hundred times sweeter than sucrose.[49-51] Sucralose is used in liquids of ECIGs.[52-53] This sweetener has been approved as a food additive.[54] Sucralose was first synthesized in 1976.[50] In vitro and in vivo studies showed no carcinogenicity.[49] Also, as the concentration of sucralose intake increases no carcinogen effects were observed.[55]

Since sucralose is a food additive, recent reports have addressed its thermal stability. Several reports showed that sucralose start to decompose at temperatures just above the boiling point of water.[56] The main decomposition product of sucralose, the chlorinated analogue of sucrose, is hydrogen chloride gas. This was confirmed by pH measurements and IR characterization.[55-56]

The thermal stability of sucralose in the presence of glycerol in food was recently addressed.[57] The authors hypothesized that HCl emitted from sucralose upon heating will substitute hydroxyl groups in glycerol. They found that chloropropanols, some of which are classified as human possible carcinogens, resulted from their experiment.[57]

This study is particularly interesting since the ECIG liquid glycerin is one of the main liquid constituents. So, upon the addition of sucralose to the liquid of ECIG, will chloropropanols or any other new products form?

The assessment of sucralose stability in the liquid vaped in sub-ohm ECIG device is the core of chapter 3. Nuclear magnetic resonance (NMR), thermal gravimetric analysis (TGA), FTIR, and pH experiments were done to assess the emissions of a sub-ohm ECIG when sucralose was added to the ECIG liquid.

CHAPTER II

REACTIVE OXYGEN SPECIES EMISSIONS FROM SUPRA- AND SUB-OHM ELECTRONIC CIGARETTES

A. Introduction

Electronic cigarette (ECIG) use has become an epidemic worldwide, especially among youth.[58-59] ECIG use prevalence among cigarette smokers, former smokers, and previously nicotine-naïve groups alike has increased tremendously in the last decade.[60-61] While it is often claimed that ECIGs are good smoking cessation tools [62], the issue is still controversial and empirical data to resolve it is sparse.[63] On the other hand, ECIGs may re-normalize smoking among users and bystanders [64-65] and may initiate nicotine dependence among young users, potentially constituting a gateway to cigarette smoking.[66-70] Toxicants detected in ECIG aerosols are either present in the liquid solutions even prior to heating [71-72] or are produced via the thermal decomposition of the liquid constituents on the hot surface of the heating coil. [27, 43, 73] The most studied toxicants formed in situ are carbonyl compounds that result from the dehydration and oxidation of the alcohol functional groups on a metal surface.[22] Other toxicants include furanic and aromatic compounds, which have been identified when additives such as sugar and fruit flavors are present in the mix.[11, 74] In addition to carbonyls, thermal breakdown of chemical bonds in ECIG liquids may lead to the formation of reactive oxygen species (ROS), a class of chemicals, which induce oxidative stress in cells.

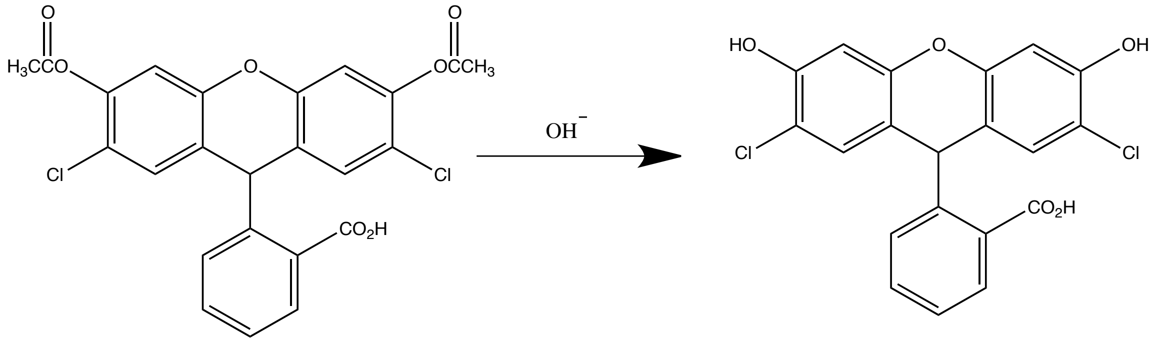
It has been well established that oxidative stress from cigarette smoke exposure leads to pulmonary diseases.[75-78] A growing number of studies have linked ROS emissions from ECIG to cytotoxicity in pulmonary tissues.[79-81] Several studies in the literature have reported ROS emissions in ECIG aerosols using cellular and acellular assays.[30, 41, 82-85] In addition, electron paramagnetic resonance (EPR) studies have revealed the presence of radical species in ECIG aerosols, [31, 86] and a recent report by Zhao et al. assessed the effect of various parameters, including brand, flavor, power, and users' puffing regimens, on the generation of ROS.[31]

In order to assess and quantitate the ROS emissions as a function of the different parameters, an acellular ROS detection technique will be used.

When detecting ROS, some studies aim to scavenge specific species of ROS, such as hydroxyl radical or hydrogen peroxide,[87-88] and others aim to detect ROS in general. Herein, detecting unspecific ROS is the target.

Scoping the literature, many probes and spectroscopic methods were utilized to detect total ROS.[89-91] Examples of these probes are 2',7'-dichlorofluorescien diacetate ($DCFH_2$-DA), plasmid assay (DNA unwinding), salicylic acid, depletion of antioxidants assay, cytochrome-c, lipid peroxidation.[90-91] Various techniques are used to detect ROS using these probes. For instance, lipid peroxidation assay utilizes high performance liquid chromatography or fluorescence spectroscopy. Cytochrom-c requires the use of a spectrophotometry. The antioxidant depletion assay utilizes high performance liquid chromatography or spectrophotometry. The plasmid assay uses gel-electrophoresis. Fluorescence spectroscopy is used for the DCFH assay.[90-91]

Reviewing the different spectroscopic methods, fluorescence spectroscopy has high sensitivity.[90] High performance liquid chromatography is also a sensitive technique; however, it is time consuming. Fluorescence remains the most commonly used technique. Many fluorescence probes have been reported in the literature.[90] When choosing a probe to rely on, its stability and selectivity must be taken into consideration. Looking through, $DCFH_2$-DA has shown promising results. This probe was first introduced and synthesized in 1965.[92] Initially, it was used for cell-free systems.[92] One of its advantages is that it is a positive fluorogenic probe, meaning a non-fluorescent compound yields a highly fluorescent product.[90] The $DCFH_2$-DA starting material is a stable non-fluorescent reagent.[92] After oxidation, the probe turns into a fluorescent compound, dichlorofluorescein (DCF). This probe has shown to detect micro quantities of ROS due to its sensitivity.[90] Researchers have relied on it for the past 40 years; it is easy to prepare, simple for analysis, and a fast and robust method.[93] The first step of the reaction requires DCFH to be deacytelated using a strong base, such as NaOH (figure 4).[93]

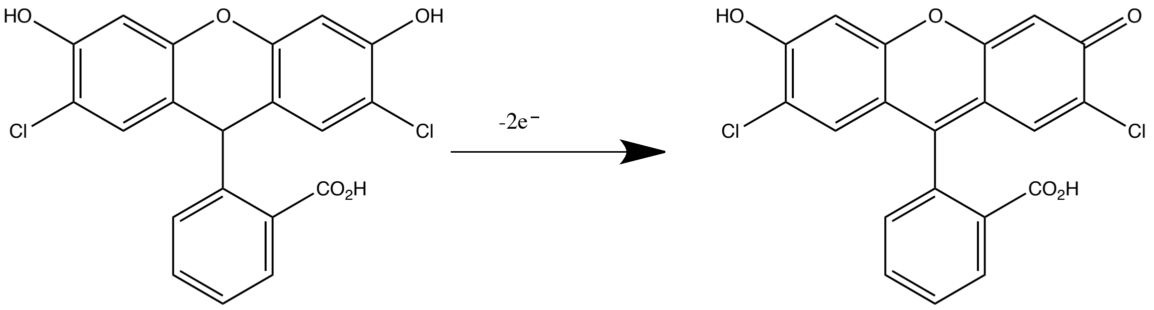


$DCFH_2$-DA

$DCFH_2$

Figure 4: Deacytelation of $DCFH_2$-DA

In the second step, $DCFH_2$ undergoes a two-electron oxidation to form the DCF fluorescent product (Figure 5).[94] However, this second step reaction is pH dependent.[93] The pH should be greater than 5 so that oxidation reactions can take place. Buffers of pH 7 reportedly gave a good linear correlation when tested with hydrogen peroxide standards.[95] Sodium phosphate or potassium phosphate buffer can be used.[95]



DCFH$_2$

DCF
pH>5 fluorescence

Figure 5: The two-electron oxidation of DCFH$_2$ into DCF at pH>5

The DCF product is monitored at 490 nm excitation wavelength and at 510 nm emission wavelength using a fluorometer or a microplate reader.[32]

Like any other probe, along with its advantages, DCFH has some disadvantages. As previously mentioned, the 2',7'-dichlorofluorescein diacetate starting material is stable; however, upon deacetylation the probe starts auto-oxidizing and photo-oxidizing. To minimize or prevent this, the solutions must be prepared in the dark or in light protected vessel and kept in ice.[90] Also, it is advised to prepare fresh solution daily.[90]

In this work, we used an optimized 2',7'-dichlorofluorescin (DCFH) probe solution in order to measure ROS emissions from conventional tank and sub-ohm ECIG devices (SODs) as a function of power, coil head geometry, and ECIG liquid composition (PG/VG ratio, nicotine content). ROS emissions were compared across conditions and to ROS emissions from combustible cigarette, and plotted versus power per coil surface area, which we have recently shown is the relevant predictor of ECIG toxicant emissions that are formed in situ.[96]

B. Materials and Methods

1. Materials

PG (99.5%), VG (99–101%), ethanol, and deionized water (DI) were procured from Sigma-Aldrich. Pure nicotine, horseradish peroxidase (HRP) (52 units/mg), potassium phosphate monobasic, and dibasic were purchased from Sigma-Aldrich. 2’,7’-dichlorofluorescin diacetate (DCFH-DA) was purchased from Molecular Probes (product code D399). Quartz filters (ADVENTEC, QR-100.47 mm) were procured from Whatman International.

2. Preparation of DCFH probe solution

DCFH-DA was dissolved in ethanol in order to prepare a 125 µM solution. The DCFH-DA solution (10 mL) was deacetylated with 40 ml of 0.01 M NaOH aqueous solution. The activated DCFH solution was wrapped in aluminum foil and kept in the dark for 30 min. A phosphate buffer (pH=7.1), prepared by mixing monobasic and dibasic potassium phosphate to attain a 0.25 mM concentration (200 mL), was added to 50 mL of DCFH solution. Horseradish peroxidase (0.5 units/mL) was added (2.4 mg) to amplify the fluorescence signal. The final 250 ml working solution had a concentration of 5 µM of DCFH. A linear calibration curve (1×10^{-7} to 10^{-6} M) was constructed using hydrogen peroxide (H_2O_2) to express ROS equivalents. The limit of detection was 0.14×10^{-7} M, and the limit of quantification was 0.48×10^{-7} M of H_2O_2. The calibration curve showed linearity with $R^2 = 0.989$, and the percent relative standard deviation was calculated to be less than 20%.

3. Probe solution

The optimal experimental conditions of the DCFH solution were determined so that the photo- and auto-oxidation of the probe solution were minimized.[93] Several combinations of DCFH concentration, storage temperature and duration, and mixing time were tested in order to achieve this goal (Figure 6).

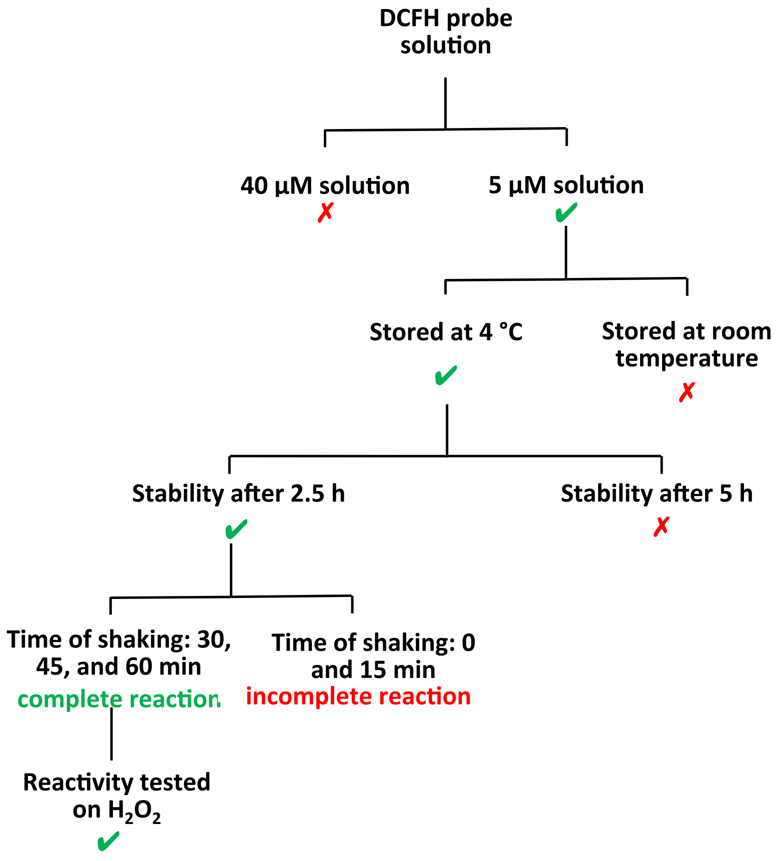


Figure 6: The optimization process of the DCFH experimental setup that led to minimized photo- and auto-oxidation conditions

The final probe was a 5 μM DCFH solution, stored at 4 °C and mixed for 30 min with the samples.[32, 97] This solution provided a >98% calibration R^2 with less than 6% bias error due to auto-oxidation at the maximum allowed solution storage time of 2.5 h. All storage and reacting samples were wrapped in aluminum foil to prevent photo-oxidation.

4. *Aerosol generation*

The American University of Beirut's aerosol lab vaping instrument (ALVIN) [98] was used to generate ECIG aerosols. Puff duration, inter-puff interval, and flow rate were selected to represent the pattern of an "experience" ECIG user (4 s puff duration, 10 s inter-puff interval, 1 L/min flow rate).[98] A vaping session constituted from five puffs on a supra-ohm ECIG and two puffs on a sub-ohm ECIG, both sessions having a 4-s puff duration, a 10-s puff interval, and a volume of 67 mL/puff. In the case of the conventional cigarette, 10 puffs were executed using ISO protocol puffing parameters (2-s puff duration, 60-s inter-puff interval, 35 mL/puff), as for the IQOS, 14 puffs were generated also using the ISO protocol. For both the ECIG and combustible cigarette conditions, the aerosol was drawn through a particulate filter trap as described in Zhao 2012.[32]

5. *Study design and sampling*

ROS emissions in the total particulate matter (TPM) of ECIG aerosols and the smoke of the tobacco cigarettes were assessed, as it was previously found that ROS concentrations in the particle phase are much greater than in the gas phase.[32] TPM was trapped on a 47-mm quartz filter installed at the mouth end of the ECIG and the tobacco cigarettes and then directly immersed in 20 mL of a freshly prepared DCFH probe solution. Fluorescence was read on a SpectraMax M5 microplate reader acting as a fluorimeter. Liquids containing 50/50 PG/VG solution with 12 mg/mL of nicotine were vaped in a VaporFi platinum tank at two different powers (5 W and 11 W) and in a sub-ohm SmokTFV8 device equipped with a V8-T8 coil head (8 coils) at five different powers (50 W, 75 W, 100 W, 150 W, and 200 W). Keeping the power and liquid constant at 50 W and using a 50/50 PG/VG solution with 12 mg/mL of nicotine, the effect of different coil heads was assessed using the sub-ohm device equipped with V8-Q4 (4 coils), V8-T8 (8 coils), V8-T10 (10 coils), and TF-Q4 (4 coils) (Figure 7). Three PG/VG ratios were prepared from standard liquid PG and VG (100/0, 50/50, and 0/100 PG/VG ratios), and three different nicotine loads in a 50/50 PG/VG solution were tested (0, 6, and 12 mg/mL nicotine concentrations). These solutions were vaped at two different powers for each device (5 and 11 W for supra- and 50 and 150 W for sub-ohm device). Each condition was repeated in triplicate, and the results are reported as the mean of three measurements after blank subtraction.

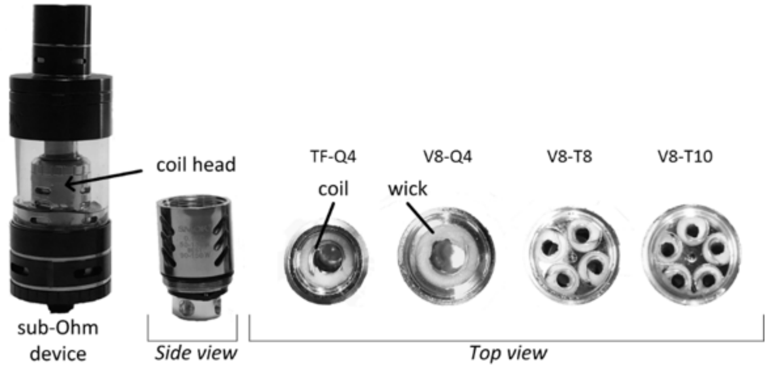


Figure 7: The sub-ohm SmokTFV8 device with illustration of the different coil heads (retrieved from Talih et al. 2017)

6. TPM, surface area, and ROS flux

The amount of TPM was determined gravimetrically by weighing the filter pad and its holder before and after each sampling session. The total surface area of the coil was calculated based on the coil wire diameter (measured using calipers), the length of coil wire, and the number of coils.[96] ROS emissions are reported as the number of moles of H_2O_2 equivalent per second of vaping/smoking in order to facilitate comparison between different puffing regimens.

7. Statistical Analysis

T-test was used to estimate the statistical significance of the difference between powers relative to the lowest power for each ECIG and relative to the combustible cigarette level. It was also used to assess the effect of liquid composition (PG/VG ratio and nicotine content) on ROS emission.

C. Results

1. Effect of power and power per unit coil surface area

ROS emission rates as a function of power are shown in Table 1 for Vapor Fi Platinum and Smok TFV8 SOD. In the supra-ohm device, the ROS flux in the aerosols generated using 11 W was three times higher than that of 5 W ($P < 0.1$). In the sub-ohm device, the ROS flux showed a significant increase between 50 and 200 W ($P < 0.1$). ROS emissions at the highest power tested (200 W) in SOD device were comparable to those of conventional cigarettes. IQOS emissions showed a significant difference from that of tobacco cigarette ($P < 0.05$) (Table 1).

Table 1: ROS flux as a function of power and coil head in Vapor Fi Platinum and Smok TFV8 SOD devices in comparison to a conventional cigarette. Statistical significance is shown in comparison with the conventional cigarette

ECIG	Coil Head	Power (W)	ROS Flux (nmole/s)
VaporFi	Single coil	5	0.238 ± 0.253 **
		11	0.696 ± 0.096 *
Smok TFV8 SOD	V8-T8	50	0.114 ± 0.034 **
		75	0.109 ± 0.042 **
		100	0.167 ± 0.117 **
		150	0.241 ± 0.029 *
		200	1.143 ± 0.606
	V8-Q4	50	0.066 ± 0.030 **
	V8-T10	50	0.045 ± 0.019 **
	TF-Q4	50	0.049 ± 0.016 **
IQOS			0.760 ± 0.070 *
Tobacco cigarette			1.240 ± 0.210

Significant difference from tobacco cigarette level: *: $P < 0.05$, **: $P < 0.01$.

Holding power and liquid composition constant, there was significant difference only between the coil head V8-T8 and both V8-T10 and TF-Q4 ($P < 0.1$). ROS emission was weakly correlated with power across devices ($R^2 = 0.26$); however, a significant correlation was found when the ROS flux was plotted as a function of power per surface area, P/SA ($R^2 = 0.78$), as shown in Figure 8. The two data points of the supra-ohm device seem to fit well within this correlation.

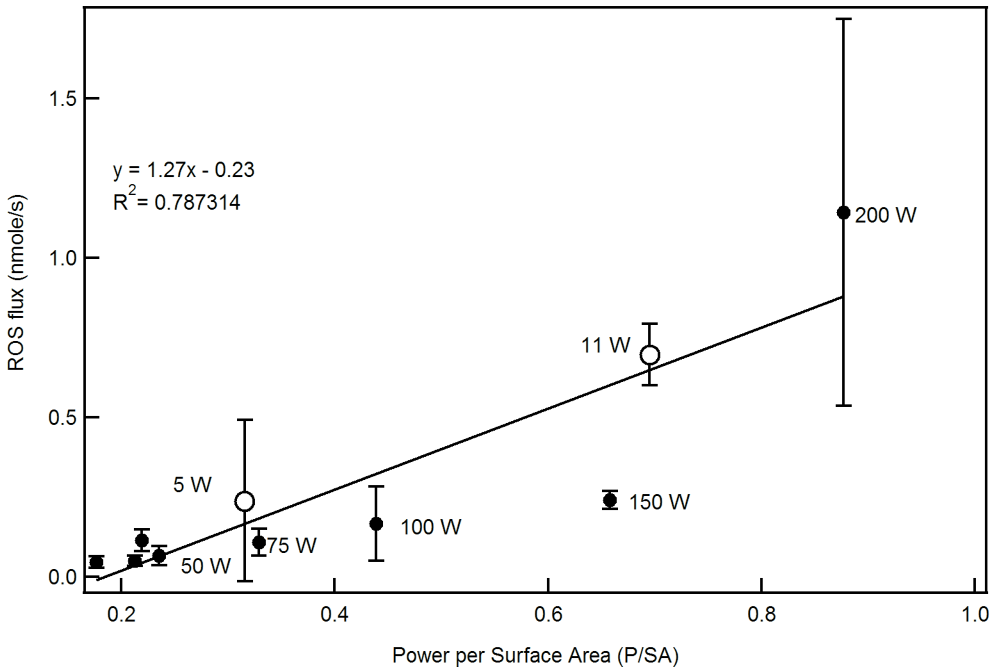


Figure 8: ROS flux as a function of power per surface area of the coil (N=3)

2. *Effect of liquid composition*

The effect of the PG/VG ratio on ROS emissions from ECIG is shown in Figures 9A and B for the VaporFi and the SMOK TFV8 SOD. In both devices, ROS flux trended downward with increasing VG content and attained significance difference between pure PG and VG liquids ($P < 0.05$). On the other hand nicotine concentration did not have any effect on ROS emissions (Figures 9C and D).

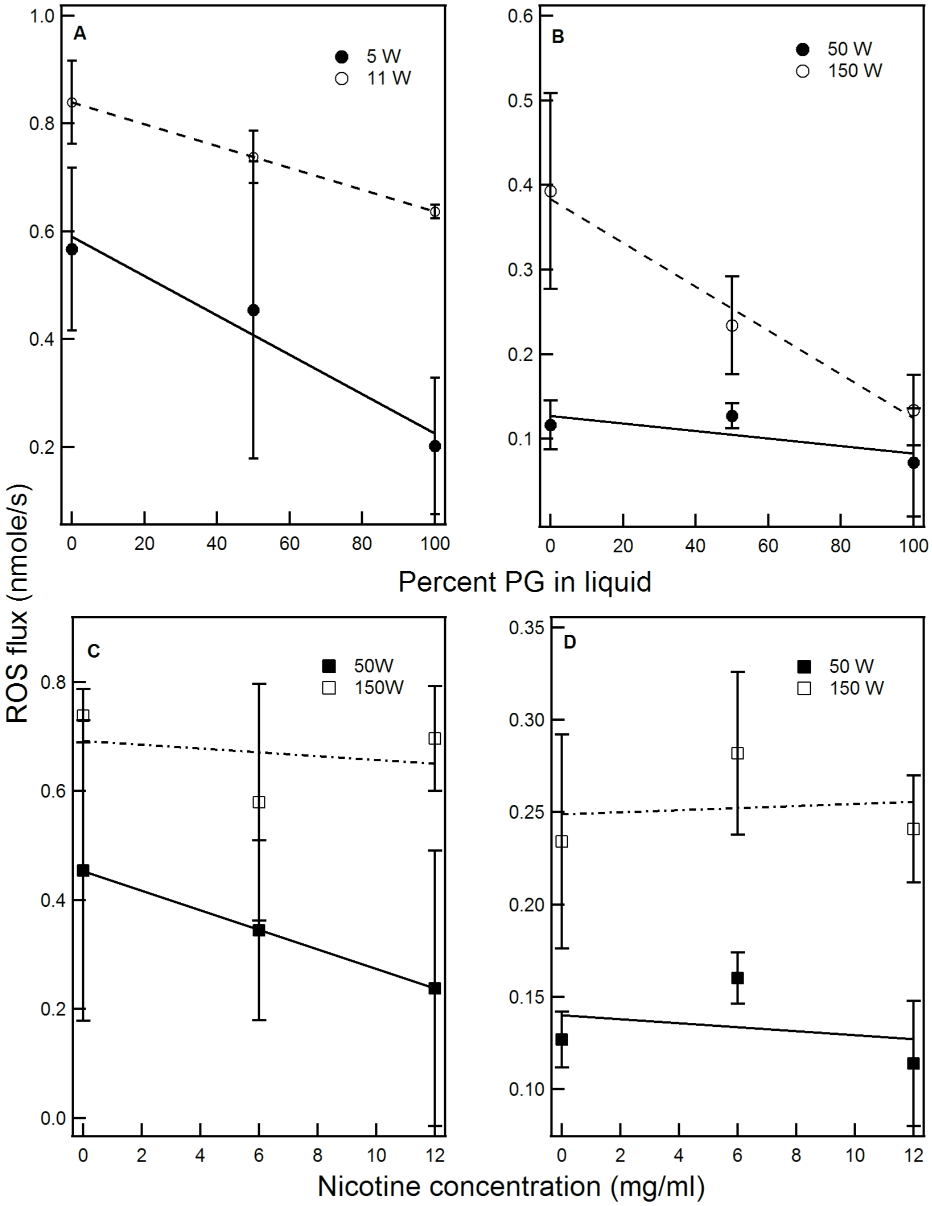


Figure 9: ROS flux as a function of the PG/VG ratio in the liquid vaped on the supra- (A) and sub-ohm device (B). ROS flux versus nicotine content in the vaped liquid on the supra- (C) and sub-ohm device (D) (N=3)

D. Discussion

Our results showed that ROS flux in tank and SOD ECIGs increases with power within the same device design. At high powers, ROS emissions from both ECIGs, especially the SOD, can reach levels that are similar to those of tobacco cigarettes. Higher powers have been associated with elevated temperatures on the coil surface causing an increase in the TPM emitted and/or a higher probability of the degradation of the chemical bonds in the molecules of the vaped liquids.[31] ROS emission is not always significantly affected by the coil head design. In this study, we showed that the ROS flux is significantly correlated (78%) with P/SA, supporting the theory that P/SA is a better predictor of toxicant emissions in general than the power or the number of coils.[96] The surge of the ROS flux level at 200 W is not linked to an increase in TPM (Figure 10), and therefore high ROS flux in this particular case can be ascribed to a spike in temperature caused by the "dry puff" phenomenon.

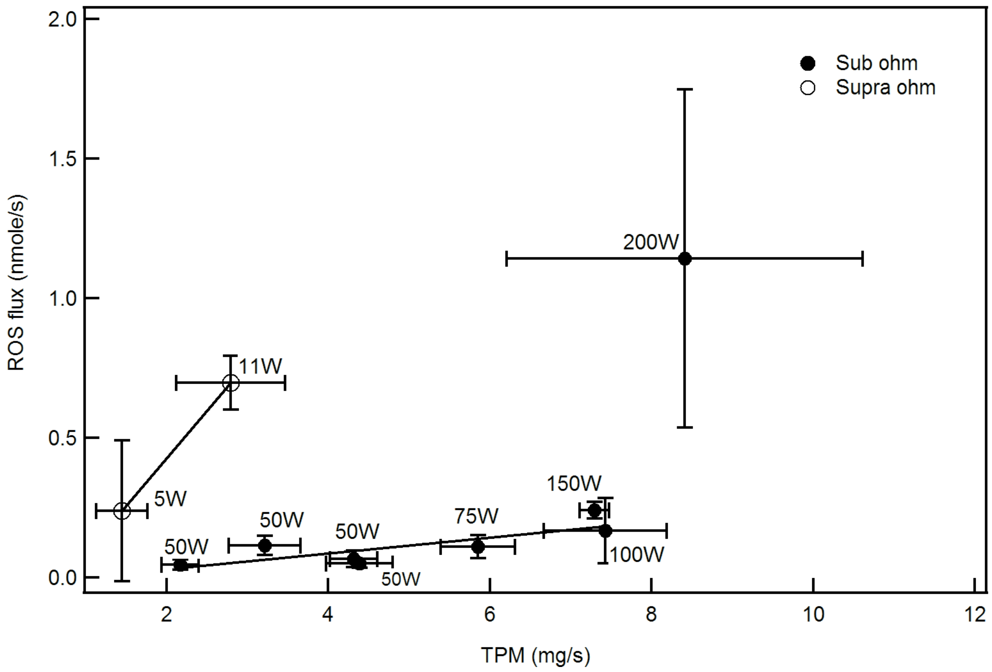


Figure 10: ROS flux (nmole/s) versus TPM flux (mg/s) in both devices at different powers and with different coil heads in the case of the sub-ohm device (N=3)

The chemical degradation of the ECIG liquids—PG, VG, and nicotine—are thought to play a determinant role in ROS emissions. In this study, we showed that an increase in the VG percentage in the liquid yielded higher ROS flux, and this may be due to the emission of higher ROS from VG molecules or to a slower wicking and consequently higher probabilities of the “dry-puff” phenomenon, particularly at high powers. This is in disagreement with a recent paper in which a higher PG/VG ratio in the liquid was correlated with higher radical emissions from ECIGs [99]. Our study also showed that nicotine does not affect ROS emissions, which are mainly a function of the chemical nature of the solvent and the P/SA of the coil.

E. Conclusion

Our results showed that ECIGs intrinsically emit ROS even in the absence of flavorants. ROS levels from conventional tank ECIGs and SODs at high powers could reach tobacco cigarette-like levels. P/SA was a better predictor of ROS emissions than power. In addition, ROS emission was not affected by liquid composition. Toxicant flux is an easy tool to compare results across different puffing regimens and among studies. IQOS and ECIGs at moderate powers are safer than the conventional tobacco cigarette.

CHAPTER III

THE SWEETENER, SUCRALOSE IN ECIG LIQUIDS

A. Introduction

Flavors are the main source of attraction for ECIG users, especially for non-smokers and youth. Their variety has exceeded 7000 flavors.[2] The effect of flavorings on the chemical composition and toxicity of ECIG emissions is not fully understood. One of the flavorings or additives found in liquids of ECIG is Sucralose.[52] Sucralose is used as a sweetener in ECIG liquids.[48, 52-53] Sucralose has the same structure of Sucrose with three chlorine atoms substituting three hydroxyl groups (Figure 11). It is six hundred times sweeter than sucrose.[49-51] Its ingestion at high concentrations showed no carcinogenicity.[49,55]

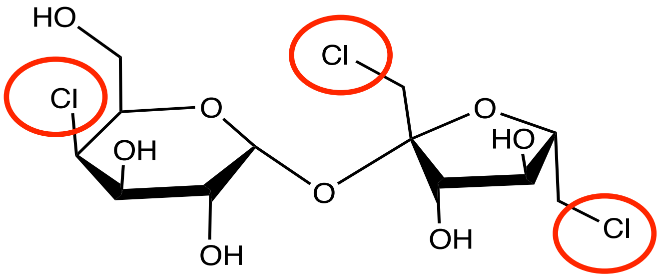
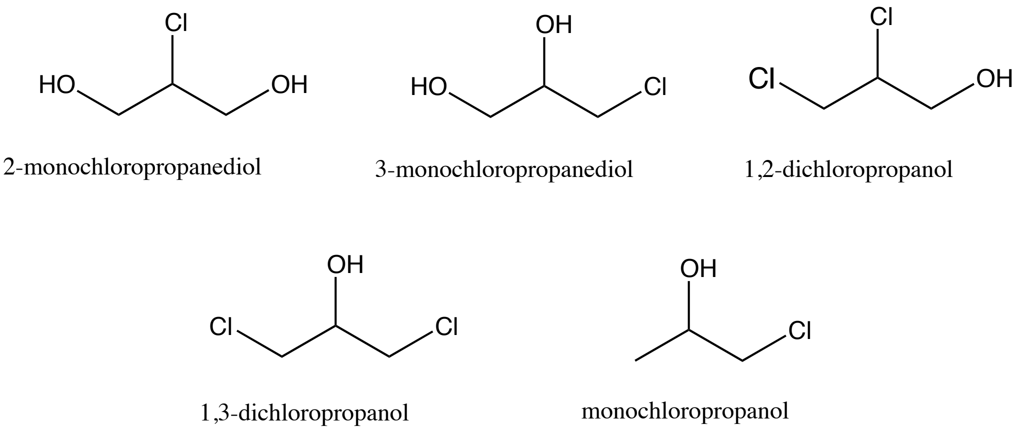


Figure 11: Sucralose structure

The safety of sucralose upon exposing it to high temperature was questioned. Since sucralose is a food additive, its thermal stability in cooking was assessed. Bannach et al. (2009) studied the thermal stability of sucralose using thermogravimetric and differential thermal analysis (DTA-TGA). Between 119 °C and 132 °C, an endothermic peak was observed indicating the thermal decomposition of sucralose, which lead to the release of water and hydrochloric acid (HCl).[55] To confirm the HCl release when sucralose was heated, the pH of the condensed vapor was equal to 1, and an $AgNO_3/HNO_3$ test proved the presence of chloride ions.[55] Furthermore, De Oliveira et al. assessed the thermal degradation of sucralose using thermogravimetric analysis coupled with infrared spectroscopy (TGA-IR). The IR spectrum revealed the presence of HCl along with other minor compounds.[56] A study by Hutchinson et al. showed that the amount of chloride produced increased as the temperature increase, this was confirmed with the decrease in pH upon heating from 4.95 to 1.68.[51]

A food chemistry study assessed the products generated upon heating sucralose with glycerin at 250°C.[57] The generated HCl, upon heating sucralose, is expected to chlorinate the glycerol giving off chloroporopanols.[57] These compounds are viewed as potentially toxic.[57] 3-monochloropropnaediol, 1,2-dichloropropanol, and 1,3-dichloropropanol were detected in this study (Figure 12).[57] The first two chloropropanols are classified as group 2B, possible carcinogens.[16] This study is particularly interesting since the ECIG liquid glycerin is one of the main liquid constituents.



2-monochloropropanediol 3-monochloropropanediol 1,2-dichloropropanol

1,3-dichloropropanol monochloropropanol

Figure 12: Possible chloropropanol formed

In fact, the addition of Sucralose in ECIG liquids was recently studied by Rosbrook et al. They reported that the sweetness perceived by the consumer was primarily from the smell of the liquid, more than the taste of the aerosols.[48] The transmitted taste of sucralose to the consumer differed from one device to another.[48]

So, upon the addition of sucralose to the liquid of ECIG, will chloropropanols or any other new products form? Figure 12 shows the different chloropropanols that might form when reacted with PG and VG. Figure 13 sums up the steps of formation of chloropropanols.

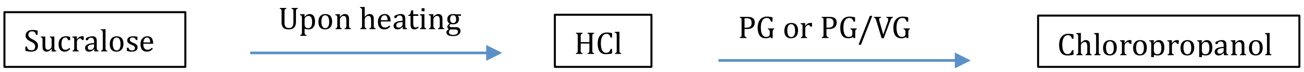


Figure 13: HCl released and chloropropanols formation

In this study, Nuclear magnetic resonance (NMR), thermal gravimetric analysis coupled with IR (TGA-IR), Fourier Transform Infrared (FTIR), and pH experiments will be conducted to assess the emissions of a sub-ohm ECIG device when sucralose is added to the PG-VG-nicotine solution.

B. Materials and methods

1. Materials

PG (99.5%), VG (99.5%), pure nicotine, 1-monochloro-2-propanol (MCP) (70%) (30% of 2-monochloro-1-propanol), and sucralose (98%) were purchased from Sigma-Aldrich. Glass fiber filters (ADVENTEC, QR-100.47 mm) were procured from Pall Corporation. A VGOD ProDrip dual-coil sub-ohm ECIG was used. Stainless steel wire was purchased from RBA depot. A coil master builder was used to make the coils, which were of 10 wraps and a 3mm diameter. For the wick, Japanese cotton was used. A zero air gas tank was used. An Avatar 360 FTIR with a long path gas cell, a Bruker NMR 500 MHz, a Hach sensION+ PH3 basic pH benchtop meter, and TG 209 F1 libra Netzsch thermal analysis (TGA) were used.

2. Solution preparation

Three solutions were prepared, PG alone (sol A), 1% wt/wt sucralose in PG (sol B), 1% wt/wt nicotine-1%sucralose in PG (sol C), and 1% wt/wt nicotine in PG (sol D). To prepare sol B, 0.1040g of sucralose wad weighed and added to 10ml of PG. To prepare sol

C, 51.3 µL of nicotine was added to sol B, to give a total of 10 ml. These solutions were vortexed and sonicated. Another set of these solutions were prepared but with PG/VG 30/70, instead of PG, giving sol A', sol B', sol C', and sol D'. The prepared solutions are summarized in Table 2. Also, 1% wt/wt of MCP in PG was prepared.

Table 2: Solution prepared and their abbreviations

Solution abbreviation	Solution composition	ECIG power (W) for FTIR
Sol A	PG	125
Sol B	1% wt/wt sucralose in PG	75, 100, 125, 150
Sol C	1% wt/wt sucralose-1%wt/wt nicotine in PG	125
Sol D	1% Nicotine in PG	125
Sol A'	PG/VG 30/70	-
Sol B'	1% wt/wt sucralose in PG/VG 30/70	75, 100, 125
Sol C'	1% wt/wt sucralose-1%wt/wt nicotine in PG/VG 30/70	125
Sol D'	1% Nicotine in PG/VG 30/70	-

3. *Aerosol generation into an FTIR set-up*

Figure 14 describes the FTIR sampling process. First, a gas tank of zero air was connected to a flow meter, which in turn was connected to a compartment encapsulating the aerosols of the ECIG. This positive flow generated from the tank (indicated by the arrows on Figure 14) ensures an atmosphere free of CO and H_2O that have high interferences in the IR spectrum. To start experimentation, the vacuum pump was used to create vacuum in the gas cell. Then, the generated aerosols from the ECIG upon turning on the battery were directed towards filter pad to trap the particles and let the gas pass through a mass controller in its way to the long path gas cell of the FTIR. The mass controller, at 1L/min, controls the flow of gas entering the gas cell. Once the gas is trapped in the cell, the FTIR machine generates a spectrum.

Four solutions, sol A, sol B, sol C, and sol D, were vaped and their gas phase samples were analyzed by FTIR. Sol B was tested at different powers: 75W, 100W, 125W, and 150W. Sol B’ and sol C’ were vaped. Sol B’ was tested at different powers, 75W, 100W, and 125W (Table 2).

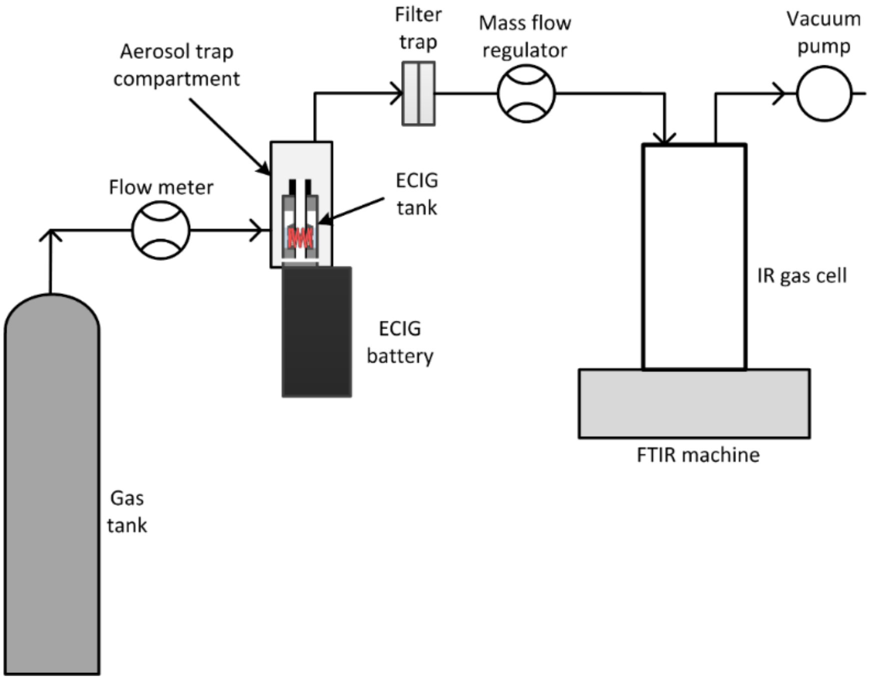


Figure 14: FTIR setup

4. NMR setup and sampling

Figure 15 describes the setup of the NMR experiments. A positive flow is initiated by a compressor, which is connected to a mass flow regulator fixed at 0.5 L/min. The flow permits the ECIG to generate aerosols and channels them into the filter to the GC-vial, sampling only the gas phase. All solutions presented in table 1 (sol A-D, sol A'-D') were vaped at 125W. The gas phase was bubbled in NMR solvent, 1 ml of $CDCl_3$, and tested using ^1H NMR.

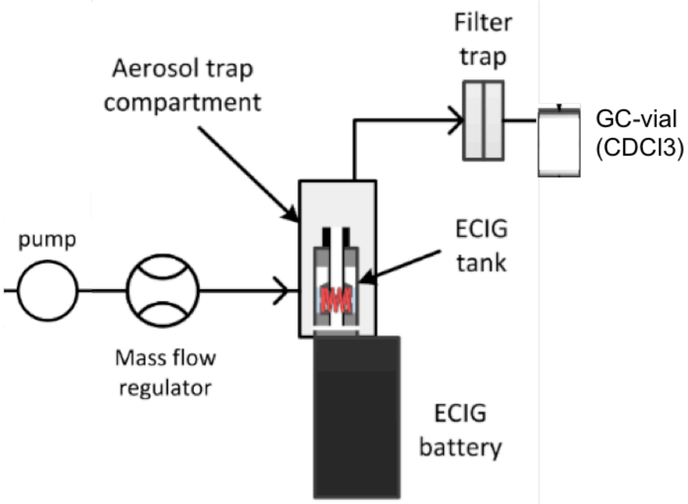


Figure 15: NMR setup

5. pH setup and sampling

The same setup of the NMR was used, but the filter pad and the GC vial were replaced by an impinger filled with 20 mL of deionized water (Figure 16). Solutions of sol A, sol B, sol C, sol D were vaped.

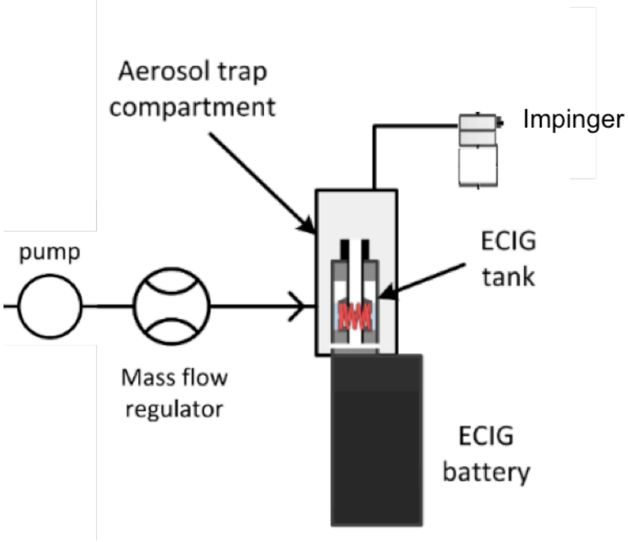


Figure 16: pH setup

C. Results and Discussion

1. TGA coupled with IR

Sucralose stability upon thermal degradation was tested using a TGA coupled with IR. Pure sucralose was placed on a microbalance and heating of the sample begins. Upon heating the sucralose solid, a spectrum was recorded from the vapors that enter into the gas cell of the IR. Figure 17 shows the spectrum recorded at 140°C. In the range of 3100 till 2600 cm^{-1}, HCl was identified due to its unique vibrational and rotational patterns.

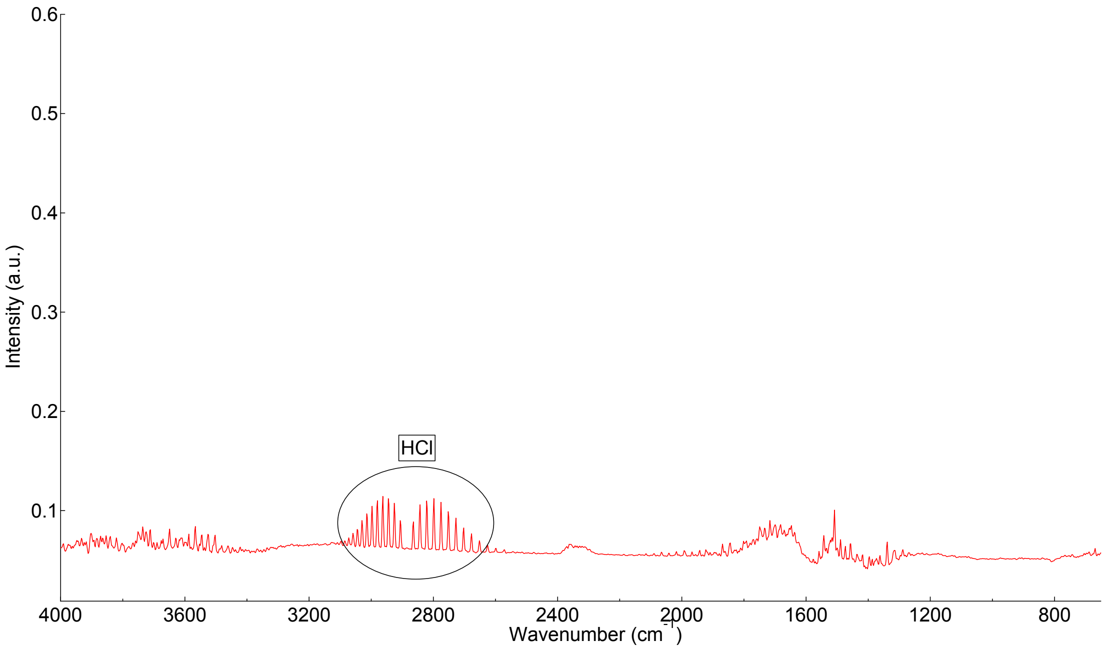


Figure 17: TGA-IR of pure sucralose powder in aluminum crucibles

2. FTIR experiments

Sol B was vaped at different powers, 75, 100, 125, and 150W. Figure 18 demonstrates the FTIR spectra obtained. Blanks were obtained throughout the experiments and subtracted from the sample spectra in order to remove any background. A standard of MCP was bubbled into the FTIR setup to obtain a reference spectrum for comparison. This spectrum was superimposed on top of sol B spectra obtained at different powers (Figure 18). The –OH stretch at around 3600 cm^{-1} in MCP is not present in the sol B at all powers. Also, MCP standard and the sol B show different spectra. This confirms that MCP is not present in the gas phase of sol B.

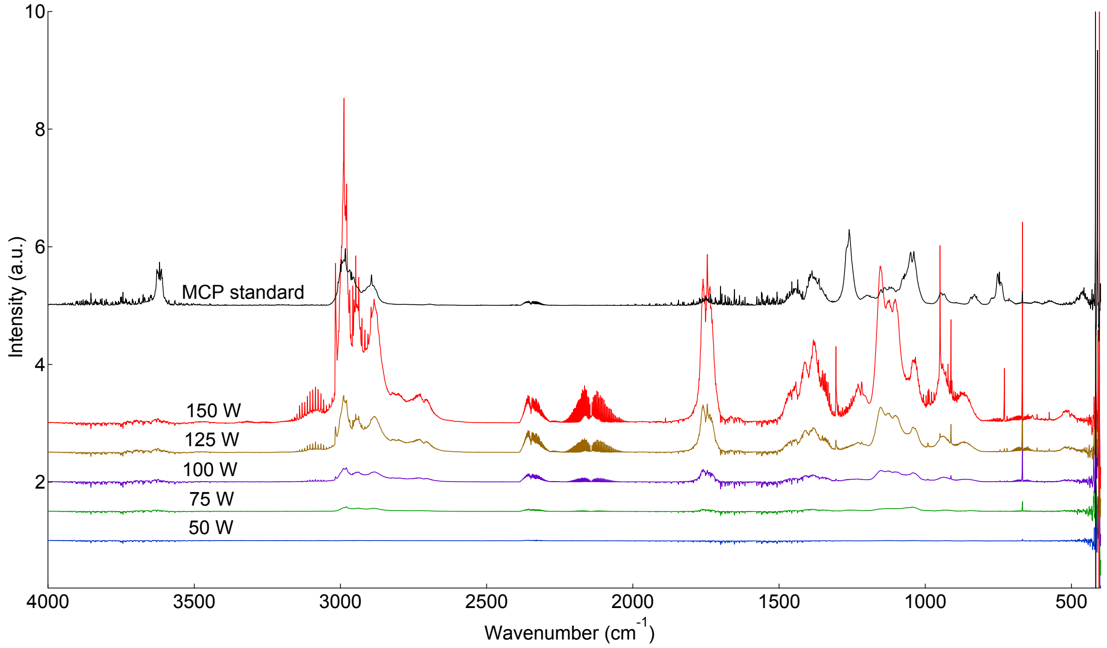


Figure 18: FTIR of 1% Sucralose in PG (sol B) at different powers compared with MCP

Furthermore, sol B was vaped versus sol A at 125W (Figure 19). Comparing the two spectra, the sol B spectra has all the peaks present in sol A along with 2 extra unique peaks, a peak at 1200-1000 cm^{-1} corresponds to a C-O-C bond, and an sp^3 C-H shift at around 3000 cm^{-1}.

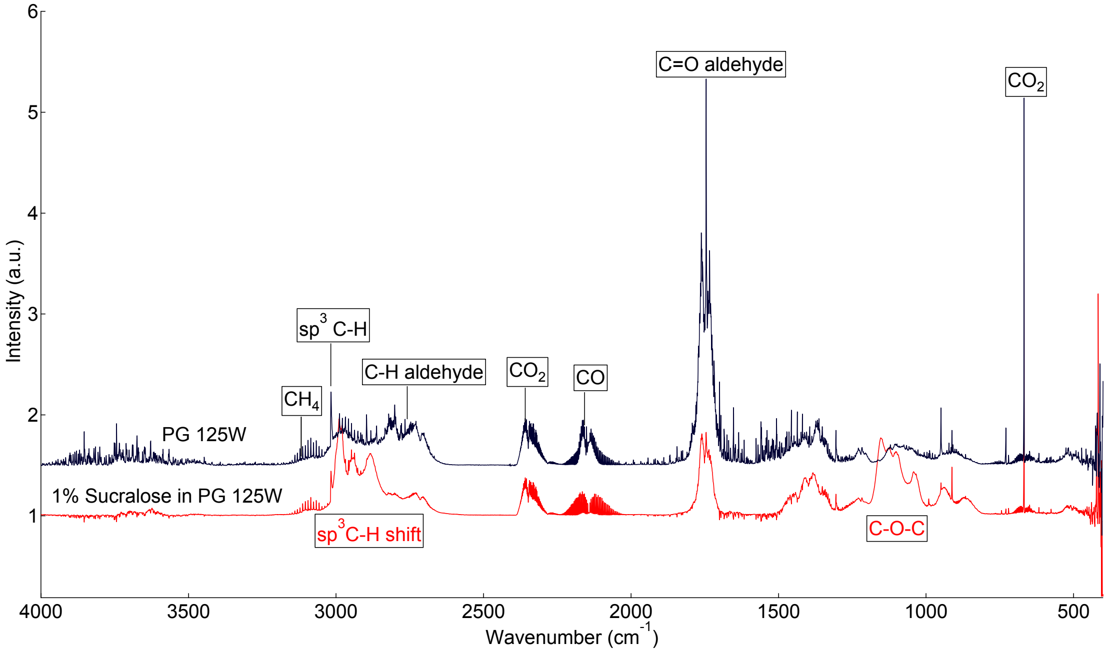


Figure 19: FTIR of 1% sucralose in PG (sol B) vs. PG (sol A) at 125W

Next, 1% MCP in PG was vaped at 150W. Figure 20 shows a comparison between 1% MCP in PG vaped, standard MCP, and sol B vaped at 150W. It was noticed that the 1% MCP in PG and the sol B vaped showed similar spectra. This indicates that sucralose and MCP induced the same modification on the profile of liquid degradation products.

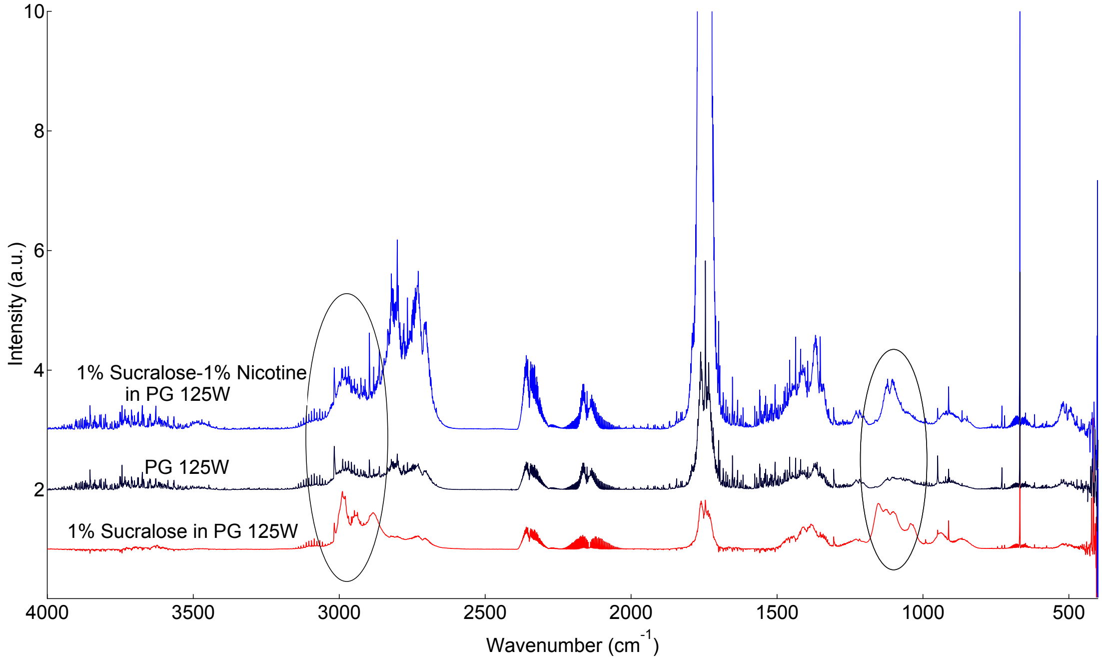


Figure 20: FTIR of 1% MCP in PG compared to 1% Sucralose in PG (sol B) and MCP standard

The fact that no MCP was detected in the gas phase pushed us to assess if it got stuck on the filter. MCP standard was bubbled into the FTIR setup, but this time we had the filter soaked in PG solution. A blank spectrum was obtained, indicating that MCP dissolved in PG and did not enter the gas phase.

The above results did not exclude the formation of MCP in the aerosol of ECIG. In order to assess if MCP is formed or not, we thought about trapping the HCl, which is the precursor of MCP, generated from sucralose break down before it reacts with PG.

Next, nicotine was tested upon its addition to 1% sucralose in PG, sol C. This was compared to sol B (Figure 21).

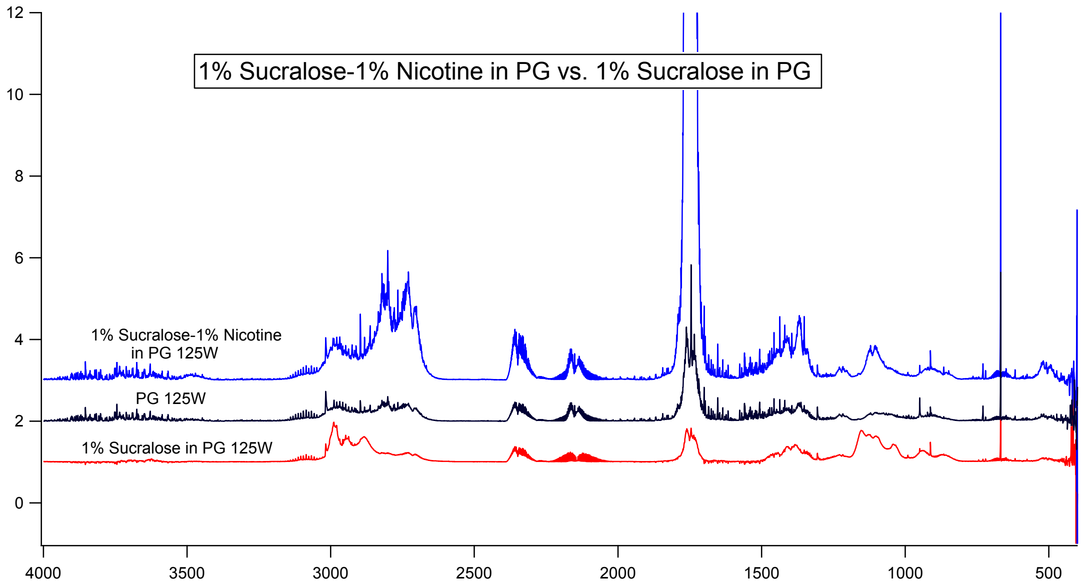


Figure 21: FTIR of 1% sucralose in PG without (sol B) and with nicotine (sol C)

It is shown that addition of nicotine gave the same spectrum as PG alone (sol A). This means that nicotine intercepted the mechanism of formation of the new peaks that we identified in the previous sections.

The same combinations of additives were tested in PG/VG solutions, and the same trends were observed in the IR spectra. Briefly, the C-O-C peak was observed along with the sp^3 C-H shift. MCP was not present in the gas phase when vaping sol B'.

3. ^{1}H NMR experiments

In the aim of identifying the C-O-C product formed (deduced from FTIR results), NMR experiments were conducted. Running through the NMR sol A, sol B, sol C, and sol D at 125W, all spectra contained aldehydes, such as acetaldehyde (9.8 ppm). However, the sol B had unique peaks between 3.3 and 5.3 ppm (Figure 22). With the help of ^{13}C NMR and cosy, this product was identified to be propylene acetal (Figure 23 and 24).

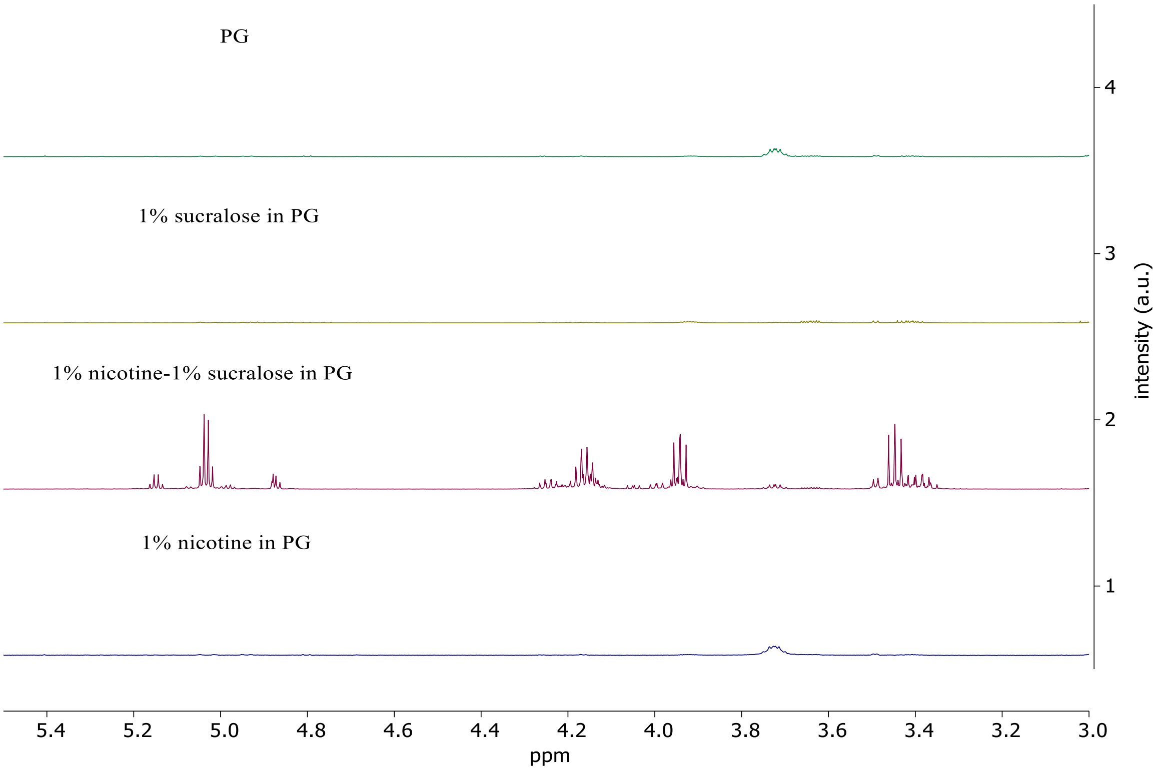


Figure 22: Comparison of PG alone (sol A), 1%sucralose in PG (sol B), 1%sucralose-1%nicotine in PG (sol C), and 1%nicotine in PG (sol D)

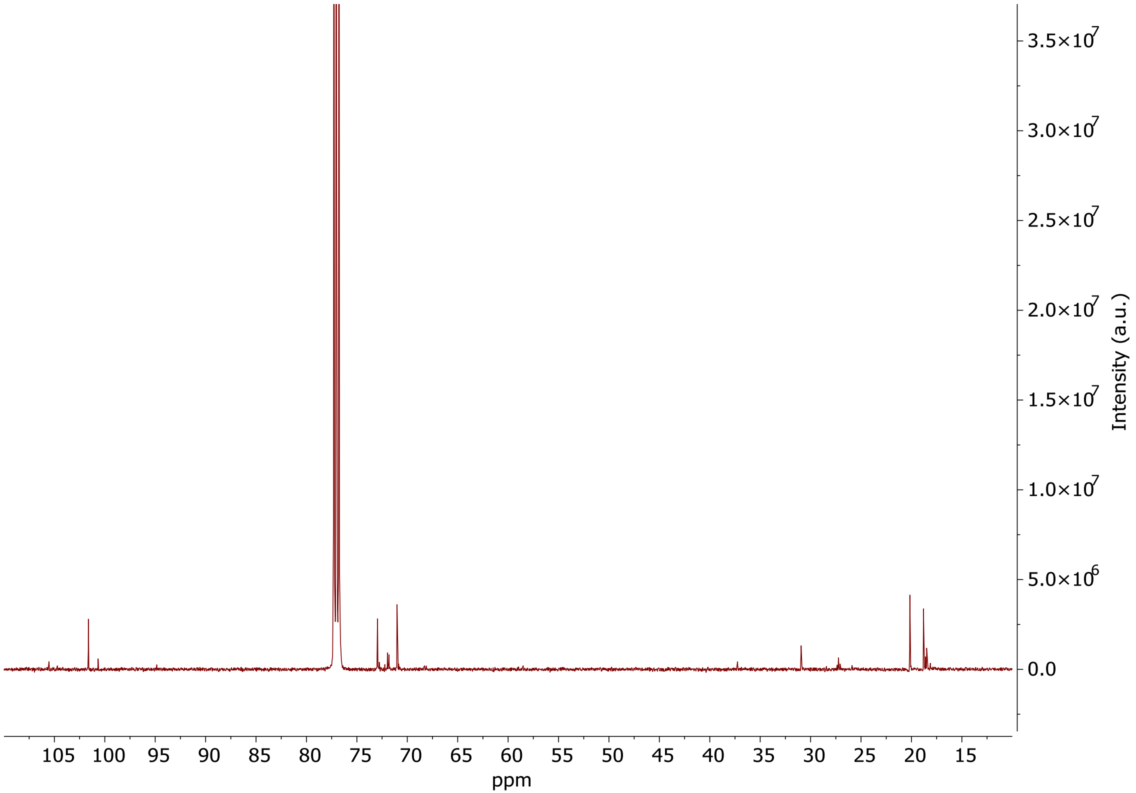


Figure 23: ^{13}C NMR of 1% sucralose in PG (sol B) at 125W

Figure 23 shows a peak at around 102 ppm, which corresponds to the O-C-O carbon as per the NMR reference tables.

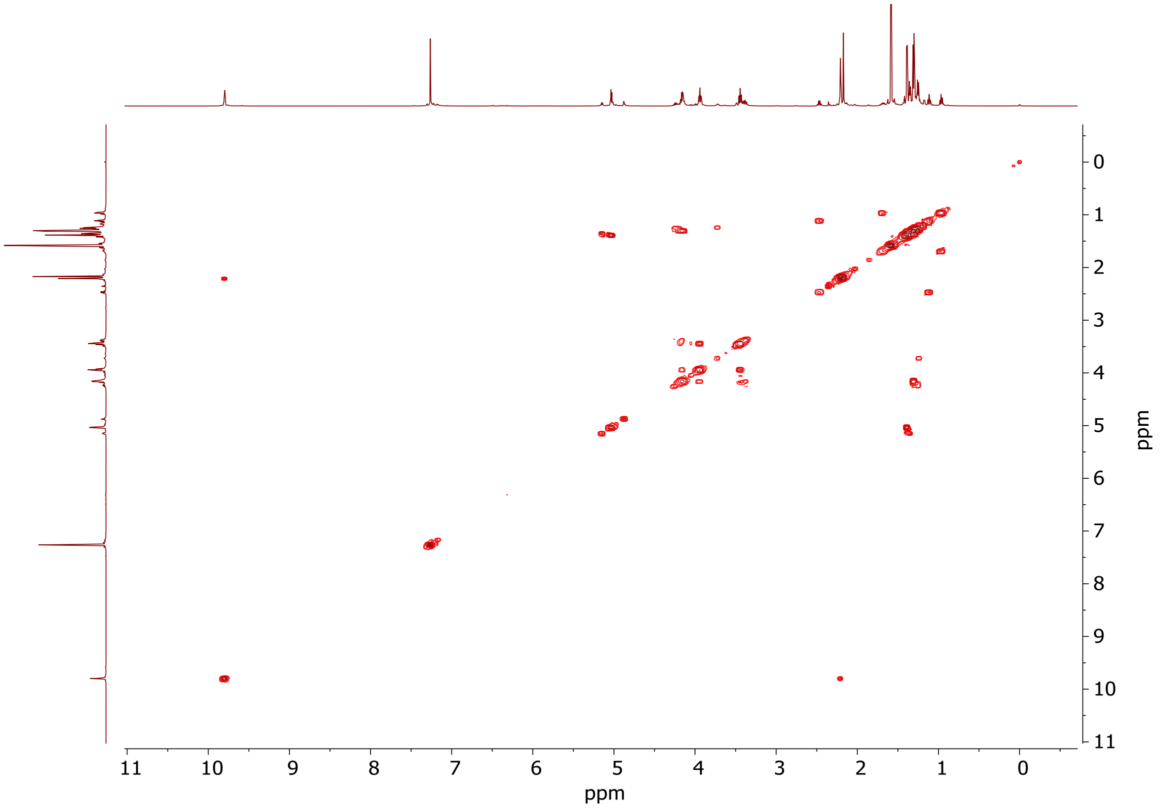


Figure 24: Cosy of 1% sucralose in PG (sol B) at 125W

From Figure 24, the peaks at 1.2, 3.4 ppm, at 3.9 ppm, at 4.1, and at 5.0 are coupled to each other. This confirms that these peaks belong to the same molecule, which was found to be propylene acetal.

It is known that acetals form in acidic media. Since it was already established, through literature and experimentation that HCl forms upon heating sucralose it is hypothesized that this acid catalyzed the formation of propylene acetal from the two initial reactants found, acetaldehyde and PG (Figure 25).

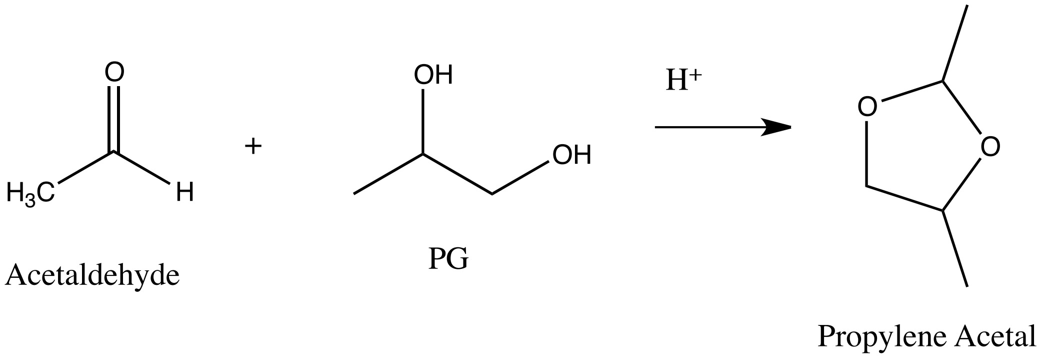


Figure 25: Proposed mechanism of formation of propylene acetal

Figure 12 shows that when vaping sol C, acetal was not formed. This may be due to nicotine reacting with the acid (HCl) through an acid-base reaction and preventing the acid from forming the acetal (Figure 26).

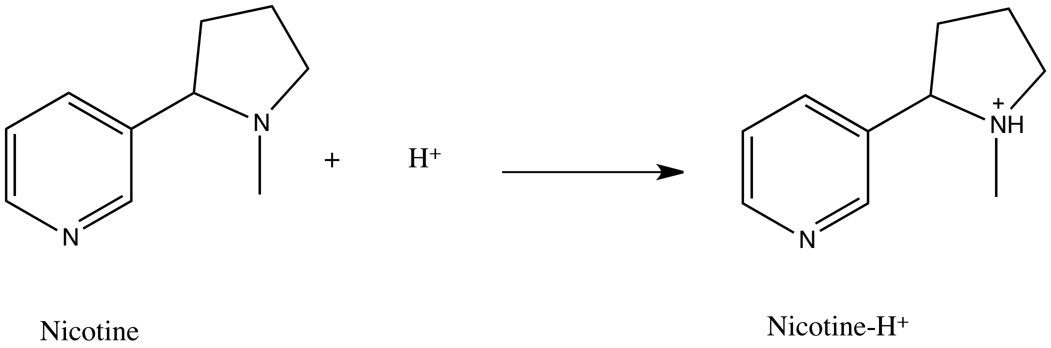


Figure 26: Reaction of nicotine with HCl

Repeating the same experiments using sol A’, sol B’, sol C’, and sol D’, propylene acetal only formed when vaping sol B’ and not with the rest. Similar to sol B, in sol B’ nicotine may have reacted with HCl, going through an acid-base reaction. This reaction is faster and seems to be preferred over the formation of the acetal in the presence of nicotine.

4. pH experiments

The pH experiments were conducted where two solutions of sol C and sol D were vaped at 125W, and their aerosols were bubbled into an impinger. Triplicates were done for each solution. After smoking, the pH was measured using a pH meter. Initial pH was recorded by preparing 100 ppm of the two solutions in water. The concentration 100 ppm was chosen due to the estimation of the total particulate matter that will be bubbled into the impinger.

Table 3: pH of vaped 1%sucralose-1%nicotine in PG (sol C) and 1%nicotine in PG (sol C)

		Liquid		Aerosol	
	Solution vaped	pH	%NicH$^+$ calculated	pH	%NicH$^+$ calculated
Sol C	1%Nic-1%Sucralose-PG	8.87 ±0.24	12.4	**7.83** ±0.05	60.8
Sol D	1%Nic-PG	9.05 ±0.06	8.5	**8.68** ±0.17	18

From Table 3, the pH of sol C solution decreased by 1.04 units; however, the sol D decreased by 0.37 units. Since in the presence of nicotine there was a larger decrease in pH that means nicotine is going from its basic to its acidic form. This observation supports the claim that nicotine is reacting with the acid, forming nicotine-H^+.

In Table 3, the amount of nicotine-H^+ calculated before and after smoking. This was done using the Henderson-Hasselbalch equation, the pKa of the pyrrolidine ring of nicotine (pKa=8.02)[100] and the pH recorded. In the presence of sucralose, there was an increase in the amount of nicotine-H^+ from 12.5 to 60%; however, in the absence of sucralose the increase was only 18%. Sol C and sol D pH values recorded after smoking were statistically significant ($P<0.05$).

D. Conclusion

In conclusion, heating sucralose emits HCl. Upon the addition of sucralose to the ECIG liquid, PG or PG/VG, propylene acetal is formed by a reaction catalyzed by HCl. When nicotine was added, the acetal was not formed due to the abstraction of the acid by nicotine, undergoing an acid-base reaction. In addition, since chloropropanols might be present in the particle phase, the filters will be further assessed in future studies. Figure 27 sums up the main points.

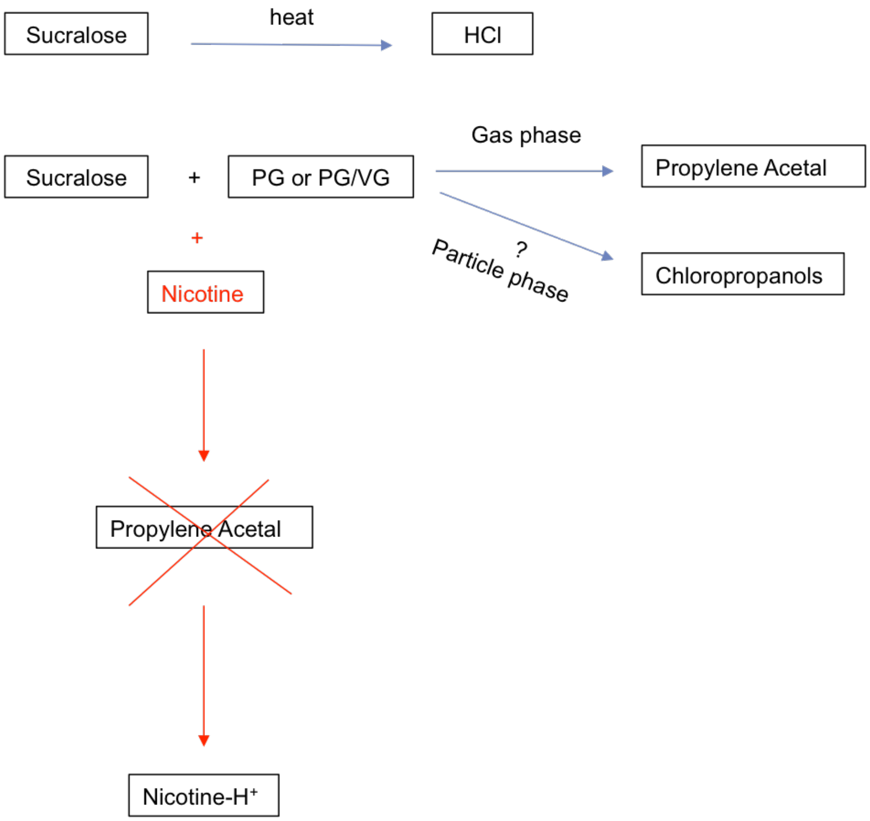


Figure 27: Concluding Scheme of sucralose degradation in gas phase of ECIG

CHAPTER IV

CONCLUSION

The effects of physical and chemical parameters on ROS emission were assessed in supra- and sub-ohm devices in comparison to tobacco cigarette and the IQOS device. The parameters studied were power, coil head design, PG/VG ratio, and nicotine concentration. ECIGs proved to intrinsically emit ROS, with levels from supra- and sub-ohm devices at high powers reaching tobacco cigarette-like levels. Power per surface area was found to be a better predictor of ROS emissions than power. Liquid composition did not affect ROS emissions. ROS flux is an easy tool to compare results across different puffing regimens and among studies.

In the second half of this thesis, one of the additives in ECIG liquids, which is the sweetener sucralose, was studied. TGA-IR results show that heating sucralose emits HCl. Upon the addition of sucralose to the ECIG liquid, PG or PG/VG, propylene acetal is formed by a reaction catalyzed by HCl. When nicotine was added, the acetal was not formed due to the abstraction of the acid by the scavenger, nicotine, undergoing an acid-base reaction. These results were confirmed by NMR, IR, and pH experiments. In addition, since the possible carcinogen, chloropropanols might be present in the particle phase; the filters will be further assessed in future studies.

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