Investigation of electronic cigarette refillable solutions and vapors

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ABSTRACT

- The potential risks and benefits of electronic cigarettes (e-cigarette) are of intense debate in the medical, public health and tobacco industries. E-cigarettes have been introduced as a smoking cessation method to reduce exposures to harmful byproducts during smoking such as tar and carcinogens.
- This study aims in the characterization of e-cigarette vapors (e-vapors) and liquids (e-liquids) composition by harnessing the unique molecular fingerprints and multivariate spectral analysis tools. E-vapors were generated using a novel vaping machine and samples were collected on appropriate media. We applied nuclear magnetic resonance spectroscopy (NMR) to estimate the prevalence of nicotine and identify the molecular structure of chemicals in e-liquids and e-vapors.

INTRODUCTION AND OBJECTIVES

- An e-cigarette is a battery powered electronic NICOTINE delivery system (ENDS), that has been designed to work like cigarettes to deliver vaporized e-liquids to user via inhalation without combustion
- There are different categories of e-cigarettes and all functions in similar manner. An e-cigarette is composed of different parts as seen in figure 1.

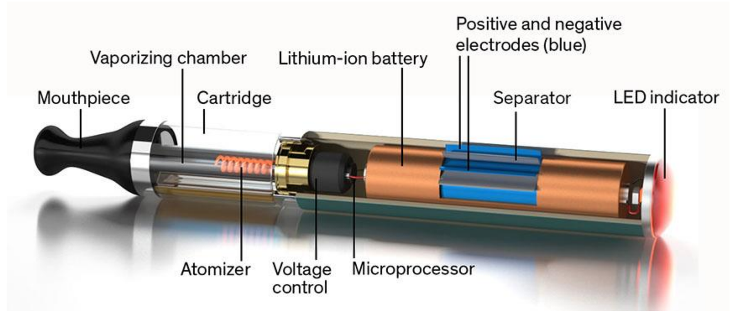

Figure 1: Parts of an e-cigarette

- E-liquids are available in different flavors and contain nicotine (up to 10%), propylene glycol (~50%), glycerin (30%), water (10%) and chemicals (less than 5%) to maintain the flavor, integrity and stability of the liquid.
- E-liquids have unknown quantities of unidentified chemicals used as solvents, flavors and preservatives. While some of them are classified as "generally regards as safe" chemicals when used as food additives, their effects when inhaled are largely unknown.
- Due to extraordinary variability in e-liquids, coupled with the highly inconsistent user patterns and conditions, e-vapors may contain more chemicals formed during the e-liquid heating including known carcinogens such as formaldehyde and aromatic hydrocarbons.
- The specific objectives of this study are:
  - I. Characterize the molecular composition of e-liquids including unknown chemicals and their toxicological properties;
  - II.Estimate the prevalence of e-liquid chemicals in e-vapors and the dependence of user conditions
  - III. Identify e-vapor chemicals formed during the e-liquid heating and their dependence to parent chemical and user conditions

CONCLUSIONS

- A methodology using NMR spectroscopy to obtain qualitative and quantitative information about the content of an e-liquid has been developed.
- 2D NMR has been used to identify some compounds.
- The major ingredients of e-liquid and chemical species used as flavors and preservatives have been identified and quantified in commercially available liquids: Propylene glycol, glycerin and nicotine were abundant in both e-liquids and e-vapors.
- Aromatic volatile organic compounds such as benzene and toluene were only detected in e-vapors due to the thermally-induced reactions of propylene glycol.
- The percent nicotine content may modify the NMR spectra due to the effect of pH on chemical shift

FUTURE STUDIES

- Categorize e-liquid flavors into groups based on compounds found in the NMR analysis, specifically using omics based method of stratification.
- Use NMR to determine e-vapor composition by collecting vapors through filters and Determine the size distribution of e-vapor generated
- Investigate Specific flavors prone to give potential inhalation toxicity

METHODS AND MATERIALS

NMR SPECTROSCOPY

NMR spectroscopy consists of three steps:

- The sample is placed in a static magnetic field
- Nuclei in the sample are excited by the incoming radio frequency pulse
- Measure the frequency of the signals emitted by the sample

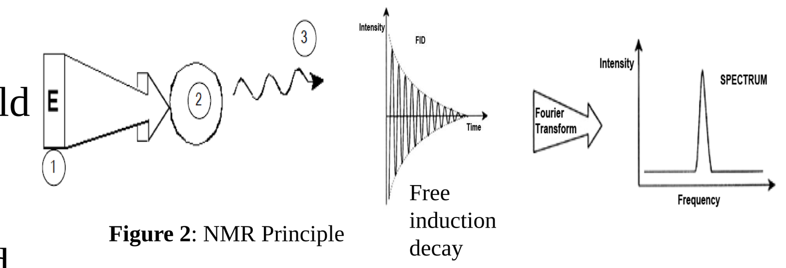

Figure 2: NMR Principle

- 1H NMR spectra were qualitatively analyzed to determine the types of unknown chemicals. 2D homonuclear 1H-1H COSY and heteronuclear 1H-13C HSQC and HMBC experiments were done to delineate the relationships between peaks and determine the molecular structure of unidentified compounds.
- NMR Equipment: Bruker Avance III-HD 600 and 850 Hz ultrashield spectrometer, processed on TopSpin 3.5pl6.
- Samples: 43 e-liquids were analyzed for the 1H NMR and 23 e-liquids for 2D H NMR. These e-liquids represent 96% of the US retail market based on popular flavor and nicotine content.
- Sample preparations: 50µl of each e-liquid was mixed with 350µl of distilled water and 200µl of NMR buffer.

SPECTROMETRIC METHOD

A two-tiered spectrometric method was developed and validated to identify and quantify chemicals present in 3 e-liquid flavors: menthol, tobacco and fruit.

- For the e-vapor generation, a single port e-cigarette exposure generating system, adapted from Zhao et. Al. (2016) was used.

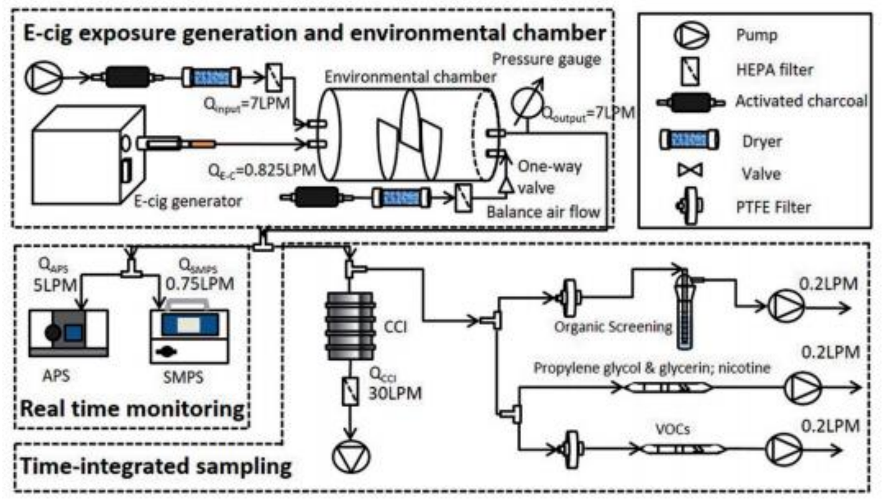

Figure 3: E-cigarette Exposure Generating System

- Each flavor used, had 10mg/ml of nicotine concentration (v/v). The puff protocol includes 4 sec duration puffs every 30sec with a total puff volume of 55ml. Disposable and cigarette vape pen were used for the experiment.
- For validation purposes, nicotine was analyzed with gas chromatography system equipped with flame thermionic detector and glycols were analyzed with gas chromatography equipped with flame ionization detector using NIOSH-approved methods.

RESULTS AND DISCUSSION

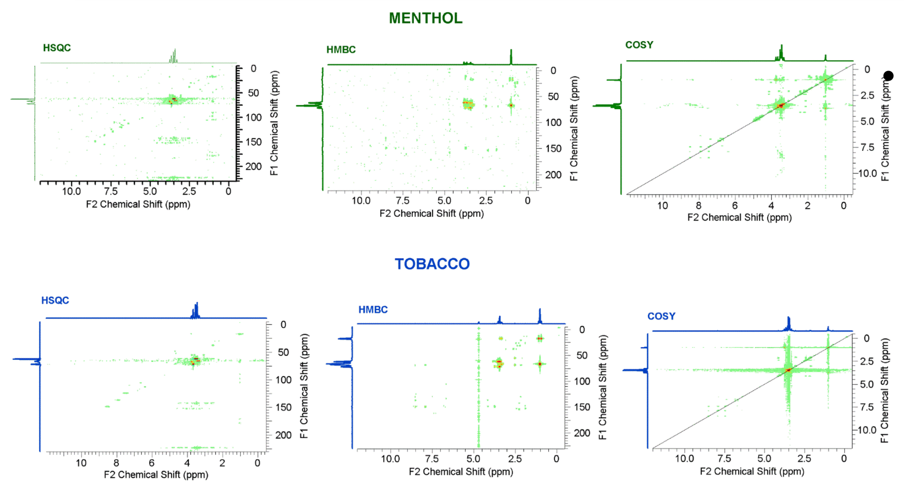

Figure 4: 2D NMR Spectra (COSY, HSQC and HMBC) for Menthol and Tobacco

- Figure 4 showed menthol and tobacco flavored e-liquid with 1.2% nicotine content. The magnetic fields show sharp and highly convoluted resonances of known major compounds for all three procedures..

- There are several resonances in the low and high-field regions, this is due to the presence of compounds with aliphatic and aromatic groups, respectively.
- Suspected compound identified are menthol and vanillin based on referenced NMR spectrum in HMDB. These compounds were also identified in e-vapors.

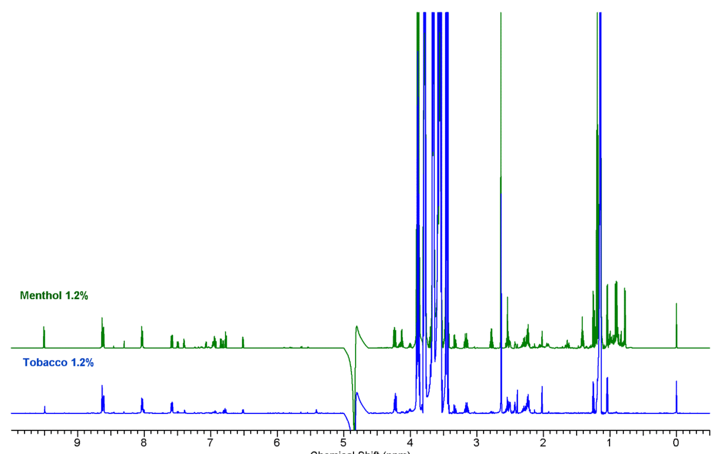

Figure 5: 1H NMR spectra for Menthol and Tobacco

- From figure 5, we see spectral profiles of propylene glycol, glycerin and nicotine mixtures showed distinct and non-overlapping fingerprints allowing for their explicit identification and quantification.

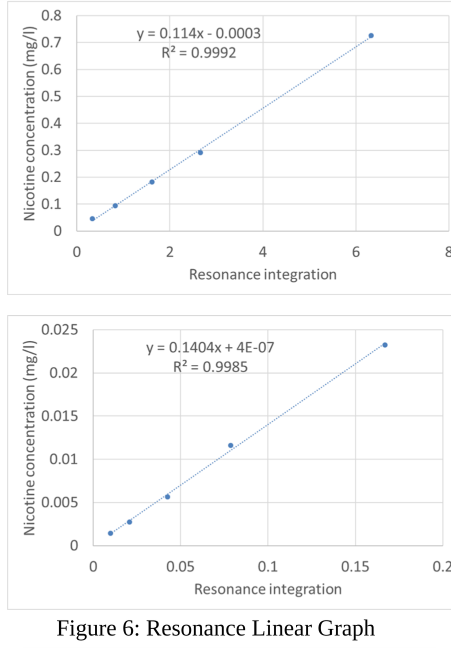

Figure 6: Resonance Linear Graph

- The linearity graph in figure 6 was calculated using nicotine concentration (10 µg/ml to 0.70 mg/ml) in each samples and the integration of resonances identified. We see a strong linear response of R2 = 0.99 for e-liquids and R2 = 0.99 for e-vapor.

- To validate the linearity for figure 6, another linearity was calculated based on the internal standard of the solution, figure 7. This shows an excellent agreement between internal standard and calibrated nicotine concentration.

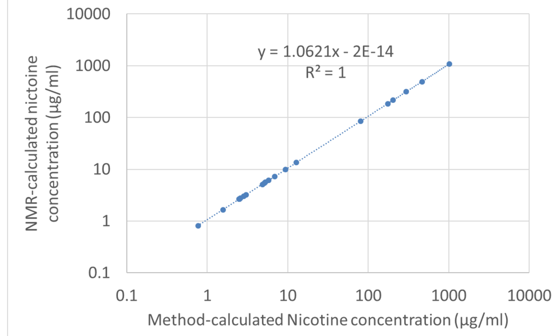

Figure 7: Internal Standard Linear Graph

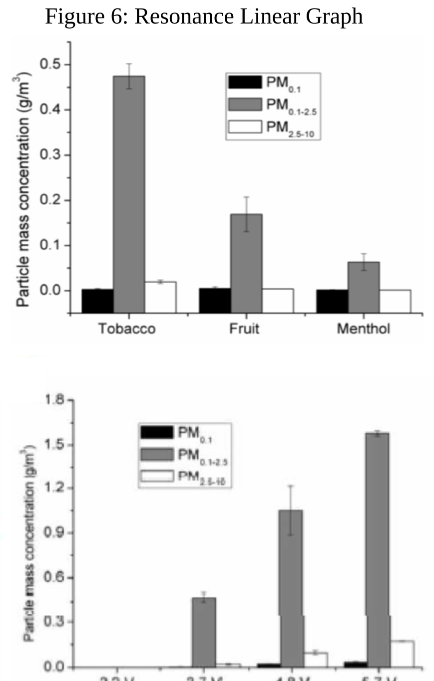

Figure 8: Particle Mass Concentration of Flavors and Voltage

- For the e-vapor particle generation: PM 0.1 – 2.5 µm were mostly generated, with tobacco having PM in the fine range as seen in figure 8.
- Particle mass concentration increased as the voltage/temperature increased.
- Benzene and toluene were detected, menthol having the highest for both compounds.
- Benzene and toluene concentration increased as the voltage/temperature increased as seen in figure 9.

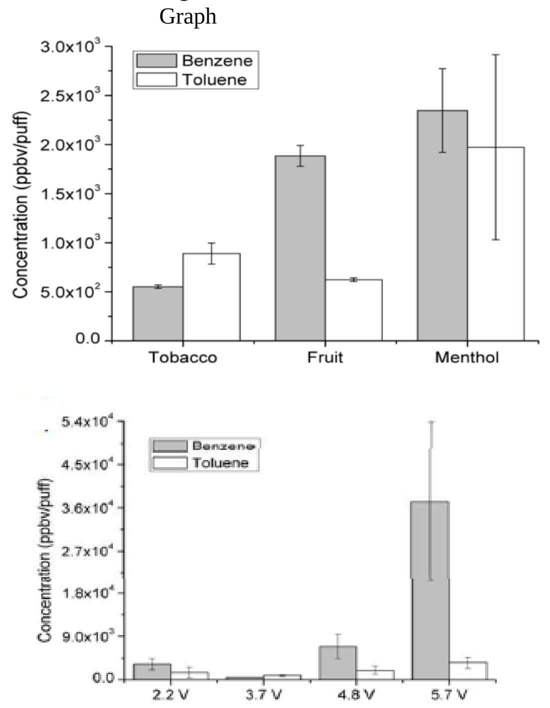

Figure 9: Particle Concentration of Aromatic VOCs

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