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CHEMOSELECTIVE REAGENTS FOR DERIVATIZATION OF TRACE-LEVEL
VOLATILE CARBONYL COMPOUNDS USING A MICROREACTOR APPROACH

By

Mumiye A. Ogunwale

B. Tech (*Hons.*), Federal University of Technology Akure, 2003
M.S., Tennessee State University, 2011

A Dissertation
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College of Arts & Sciences of the University of Louisville
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University of Louisville
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December 2017

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DEDICATION

To:

1. The love of my life, Omowunmi.
2. To those who showed me light when I sat helplessly in the dark.

ACKNOWLEDGMENTS

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ABSTRACT

CHEMOSELECTIVE REAGENTS FOR CAPTURE AND DERIVATIZATION OF TRACE-LEVEL VOLATILE ALDEHYDES AND KETONES USING A MICROREACTOR APPROACH

Mumiye A. Ogunwale

November 6, 2017

Detection and analysis of trace level volatile aldehydes and ketones has become a significant research frontier because of the applicability for environmental monitoring and assessment, noninvasive diseases diagnosis, and in food safety assessment for the US Food and Drug Administration. The number of derivatization reagents for detection of aldehydes and ketones has increased considerably over the last decade. However, the majority of these derivatization reagents are not efficient in derivatizing unsaturated carbonyl compounds due to the presence of electron withdrawing groups adjacent to the reactive functional moieties making them insufficiently nucleophilic.

The analysis of trace-level carbonyl compounds challenges existing analytical instrumentation because their concentrations are below current instrument limits of detection.

This study shows for the first time the application of an innovative silicon-based microreactor for preconcentration of carbonyl compounds in electronic cigarette aerosols. The microreactor is coated with an aminooxy reagent, typically 4-(2-aminooxyethyl)-morpholin-4-ium chloride (AMAH) or 2-(aminooxy)-N, N, N-trimethylethanammonium iodide (ATM). The aminooxy functional group chemoselectively traps trace aldehydes and ketones generated by aerosolization of electronic liquids by means of oximation reactions. The aminooxy-carbonyl adducts and unreacted aminooxy reagent are eluted from the microreactor using 150 μ L of methanol followed by addition of an internal standard (for quantification) and then analyzed by Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry (MS) or gas chromatography mass spectrometry (GC-MS), depending on the capture reagent used.

Chapter 1 describes different methods of detection and analysis of volatile organic aldehydes and ketones in gaseous samples such as exhaled breath and electronic cigarette aerosols. Chapter 2 presents the analysis and quantification of carbonyl compounds in electronic liquid aerosols. Chapter 3 describes the kinetic studies of oximation reactions of the aminooxy reagents AMAH, ADMH and ATM. It also outlines the synthesis of a cationic hydrazine-based reagent for derivatization of aldehydes and ketones. Chapter 4 describes the quantification of nicotine in e-cig liquids and derived aerosols using both FT-ICR-MS and GC-MS. Chapter 5 provides the overall summary and future direction.

TABLE OF CONTENTS

DEDICATION	iii
ACKNOWLEDGMENTS	iv
ABSTRACT	vi
LIST OF FIGURES	viii
LIST OF TABLES	xix
LIST OF SCHEMES	xx
CHAPTER 1. DETECTION AND ANALYSIS OF VOLATILE ORGANIC COMPOUNDS	1
1.1. HYPOTHESIS AND CONTRIBUTION OF THIS DISSERTATION	2
1.2. INTRODUCTION	4
1.3. PRECONCENTRATION TECHNIQUES	6
1.3.1. Physical adsorption	7
1.3.2. Solid phase microextraction	8
1.3.3. Microfluidic chip	10
1.4 SURVEY OF DERIVATIZATION REAGENTS FOR CARBONYL COMPOUNDS	12
1.5. ANALYTICAL DETECTION TECHNIQUES	14

1.5.1. Gas chromatography mass spectrometry	15
1.5.2. Liquid chromatography–tandem mass spectrometry (LC–MS/MS).....	15
1.5.3. Ion mobility mass spectrometer (IMS)	16
1.5.4. Electronic nose.....	16
1.5.5. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR- MS)....	17
1.6. DISSERTATION ORGANIZATION	18
CHAPTER 2. CARBONYL COMPOUNDS IN ELECTRONIC LIQUIDS AND AEROSOLS.....	19
2.1. ELECTRONIC CIGARETTES	20
2.2. SIGNIFICANCE OF CARBONYLS IN ELECTRONIC CIGARETTES.....	21
2.2.1. Pyrolysis of propylene glycol	23
2.2.2. Pyrolysis of glycerol	24
2.2.3. Hemiacetal formation in aerosols of e-liquids	25
2.3. E-CIGARETTE AEROSOL GENERATION; CAPTURE, DERIVATIZATION AND ANALYSIS OF CARBONYL COMPOUNDS.....	26
2.4. RESULTS AND DISCUSSION	28
2.4.1. Carbonyls in aerosols of commercial e-liquids with flavorants.....	28
2.4.2. Generation of carbonyls from e-cigarettes aerosols: effects of battery power output	33
2.5. CONCLUSION.....	39
2.6. EXPERIMENTAL.....	40

2.6.1. FT-ICR-MS.....	41
2.6.2. NMR analysis of hemiacetals	43
2.6.3. GC-MS analysis of carbonyl adducts	43
CHAPTER 3. KINETIC STUDIES OF OXIMATION REACTION AND SYNTHESIS	
OF A CATIONIC HYDRAZINE REAGENT	45
3.1. INTRODUCTION OF OXIMATION REACTIONS.....	46
3.2. KINETIC STUDIES OF REACTION BETWEEN AMINOOXIES AND	
CARBONYLS	47
3.3. RESULTS AND DISCUSSION	48
3.4. HYDRAZINE SYNTHESIS.....	58
3.4.1. Synthetic Route to HTM.....	58
3.4.2. Capture efficiency	65
3.4.3. Cigarette smoke and exhaled breath analysis	66
3.5. CONCLUSION.....	70
3.6. EXPERIMENTAL SECTION	72
3.6.1. Materials and methods	72
3.6.2. Measurement of reaction kinetics	72
3.6.3. FT-ICR-MS analysis.....	73
3.6.4. 2-Hydroxy-N, N, N-trimethylethan-1-ammonium iodide	73
3.6.5. 2-Chloro-N, N, N-trimethylethan-1-ammonium iodide.....	74
3.6.6. 2-Hydrazinyl-N, N, N-trimethylethan-1-ammonium iodide (HTM)	74
3.6.7. Exhaled breath analysis.....	75

3.6.8. Cigarette smoke analysis.....	75
CHAPTER 4. NICOTINE IN E-CIG LIQUIDS AND DERIVED AEROSOLS.....	77
4.1. INTRODUCTION	78
4.1.1. Properties of nicotine	79
4.1.2. Nicotine biosynthesis	79
4.2. STANDARD METHOD FOR NICOTINE COLLECTION AND QUANTIFICATION.....	81
4.3. EXPERIMENTAL DESIGN FOR NICOTINE SAMPLE COLLECTION AND ANALYSIS.....	83
4.4. RESULTS AND DISCUSSION	85
4.4.1. Measurement of nicotine kinetics of protonation by NMR spectroscopy	85
4.4.2. Calibration curve of protonated nicotine by FT-ICR-MS.....	90
4.4.3. Calibration curve of nicotine by GC-MS.....	92
4.4.4. Nicotine levels in e-liquids	93
4.4.5. Nicotine levels in e-cigarette aerosols	94
4.5. CONCLUSION.....	97
4.6. EXPERIMENTAL SECTION	98
4.6.1. Experimental materials	98
4.6.2. FT-ICR-MS.....	99
4.6.3. GC-MS analysis of nicotine.....	99
4.6.4. Measurement of nicotine NMR spectroscopy.....	99
4.6.5. Kinetics measurement of nicotine protonation	100

4.6.6. Analysis of nicotine in e-liquids	101
4.6.7. Collection and analysis of nicotine in e-cigarette aerosols	101
CHAPTER 5. SUMMARY AND FUTURE WORK	104
5.1. Summary	104
5.2. Future directions	106
REFERENCES	110
CURRICULUM VITAE.....	122

LIST OF FIGURES

Figure 1.0.	The structures of ATM, AMAH, HTM and nicotinium chloride salt	4
Figure 1.1.	SPME procedure for GC and LC	9
Figure 1.2.	2-(Aminooxy) ethyl-N, N, N-trimethylammonium iodide (ATM).....	11
Figure 1.3.	Photographs of the microreactor	11
Figure 1.4.	Reagent for derivatization of carbonyl compounds	13
Figure 1.5.	DNPH cartridge for derivatization of carbonyl compounds	14
Figure 2.1.	Anatomy of an electronic cigarette	21
Figure 2.2.	Cigarette-smoking robot system for mechanical generation of aerosols from e-cigarettes	27
Figure 2.3.	Schematic diagram of the preconcentration set-up for capture of carbonyl compounds in e-cig. aerosols	27
Figure 2.4.	The FT-ICR-MS of the aerosols of 100% PG, 100% VG and PG/VG in 50/50 ratio	31
Figure 2.5.	Amounts of formaldehyde, acetaldehyde, acrolein and acetone as a function of the percentage of propylene glycol (PG) in the mixtures of vegetable glycerin (VG) and propylene glycol.	31
Figure 2.6.	Amounts of formaldehyde, acetaldehyde, acrolein and acetone as a generated while vaping a set of e-cigarettes as compared with neat PG and neat VG.	32

Figure 2.7. Amounts of formaldehyde, acetaldehyde, acrolein and acetone generated while vaping another set of e-cigarettes as compared with neat PG and neat VG.....	32
Figure 2.8. Representative GC-MS chromatograms of aldehydes generated from Halo Mentol Ice at a battery power output of 11.7 W, 14.7 W, and 16.6W	34
Figure 2.9. ^1H NMR spectra ($\text{DMSO}-d_6$) for detection of hemiacetal for.....	36
Figure 2.10. ^1H NMR spectra ($\text{DMSO}-d_6$) of hemiacetals for EL05.....	37
Figure 2.11. The relationship between the amounts of formaldehyde-hemiacetal in 10 puffs of aerosols collected from e-liquid EL05	38
Figure 2.12. The calibration curves of standard AMAH-carbonyl compounds.....	42
Figure 3.1. The plot of $1/C$ against time for oximation reaction between AMAH and acetone at -21°C , 0°C , 21°C	50
Figure 3.2. FT-ICR-MS spectra overlay of oximation reaction between AMAH and acetone at 21°C at times $t=60, 30, 600, 900$, and 1200 seconds.....	51
Figure 3.3. The plot of $1/C$ against time for oximation reaction between ADMH and acetone at -21°C , 0°C , 21°C	51
Figure 3.4. Spectra overlay of oximation reaction between ADMH and acrolein at 21°C at times $t=60, 30, 600, 900, 1200$ seconds.....	52
Figure 3.5. The graph of the natural logarithms of the reaction rate constants of oximation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and acetone.	52

Figure 3.6. The graph of the natural logarithms of the rate constants of oximation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and propanal.....	55
Figure 3.7. The graph of the dependence of the natural logarithms of the rate constants of oximation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and acrolein.....	55
Figure 3.8. The graph of the dependence of the natural logarithms of the rate constants of oximation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and crotonaldehyde.....	56
Figure 3.9. The graph of the dependence of the natural logarithms of the rate constants of oximation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and 2-heptanone.....	56
Figure 3.10. The graph of the dependence of the natural logarithms of the rate constants of oximation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and methyl isobutyl ketone (MIBK).....	57
Figure 3.11. 2-Hydrazinyl- <i>N, N, N</i> - trimethylethan-1-ammonium iodide (HTM).....	58
Figure 3.12. The ^1H NMR spectrum of alcohol 2 in $\text{DMSO}-d_6$	59
Figure 3.13. The ^1H NMR spectrum of chloride 3 in $\text{DMSO}-d_6$	60
Figure 3.14. The high-resolution mass spectrometry spectra overlay of alcohol 2 and chloride 3.	60
Figure 3.15. The ^1H NMR spectrum of HTM in $\text{DMSO}-d_6$	61
Figure 3.16. The ^{13}C NMR spectrum of HTM in $\text{DMSO}-d_6$	61

Figure 3.17. The FT-ICR-MS of the HTM and HTM-acetone- d_6 adduct (internal standard).....	62
Figure 3.18. The graph of 1/C against time for hydrazone formation reaction between HTM and acrolein at -21 °C, 0 °C, and 21 °C.	63
Figure 3.19. The graph of the dependence of the natural logarithms of the rate constants of hydrazone formation reaction as a function of the reciprocal of temperature between HTM and propanal, acetone, acrolein, and crotonaldehyde.....	63
Figure 3.20. The graph of the dependence of the natural logarithms of the rate constants of hydrazone formation reaction as a function of the reciprocal of temperature between ATM and propanal, acetone, acrolein, and crotonaldehyde.....	64
Figure 3.21. The experimental set-up showing the preconcentration of carbonyls in the microchip.	66
Figure 3.22. The capture efficiency graph of HTM and ATM with acrolein and crotonaldehyde.....	66
Figure 3.23. The calibration curves of HTM-carbonyl adducts using HTM-acetone- d_6 adduct as internal standard.....	67
Figure 3.24. The calibration curves of ATM-carbonyl adducts using ATM-acetone- d_6 adduct as internal standard.....	68
Figure 3.25. The FT-ICR-MS spectra overlay of HTM-carbonyl compounds detected in exhaled breath of a smoker and a non-smoker subjects.....	69
Figure 4.0. The structure of nicotine	78

Figure 4.1. XAD-4 sorbent tube for nicotine sampling.....	82
Figure 4.2. The schematic diagram of the inExpose Scireq smoking robot.....	84
Figure 4.3. Impinger optimization: capture efficiencies of 5 impingers connected in series	85
Figure 4.4. The plot of $n(\text{nicotine})/n(\text{benzene})$ vs. $H(\text{nicotine})/H(\text{benzene})$, serving as a calibration curve for ^1H NMR measurement of nicotine protonation.....	86
Figure 4.5. ^1H NMR spectra ($\text{DMSO}-d_6$) of neutral nicotine and ^1H NMR spectra ($\text{DMSO}-d_6$) of extracted sample after 30 minutes of protonation in water and HCl mixture	87
Figure 4.6. Percent conversion of nicotine to its nicotinium salt at different temperatures over time	88
Figure 4.7. Dependence of $\ln [\text{nicotine}]$ on the reaction temperature T ($^{\circ}\text{C}$)	89
Figure 4.8. The relationship between $\ln k$ and $1/T$ for protonation of nicotine in HCl solution.....	89
Figure 4.9. The calibration curve of nicotine by plotting the ratio of intensity of nicotine-to-nicotine- d_3 ($I\text{-Nic}/I\text{-Nic-}d_3$) against the ratio of the amounts (mole) of nicotine-to-nicotine- d_3 ($M\text{-Nic}/M\text{-Nic-}d_3$)	91
Figure 4.10. Comparison of FT-ICR-MS spectra of standard calibration curve working solutions, each spiked with 7.78 nmol nicotinium- d_3 as an internal standard.	91
Figure 4.11. The calibration curve of nicotine built by plotting ratio of mole of nicotine-to-quinoline against the ratio of peak area of nicotine-to-quinoline.....	93

Figure 4.12. Comparison of GC chromatogram of standard calibration curve working solutions	93
Figure 4.13. Nicotine delivery profile of e-cigarette cartridges and e-liquids with different nicotine levels.....	96
Figure 4.14. The relationship between nicotine aerosol and nicotine levels in e-liquids at constant puff numbers.....	97
Figure 5.0. ATM, ADMH, AMAH, HTM and nicotinium chloride salt.....	104

LIST OF TABLES

Table 1.0.	Adsorbent materials and their composition	8
Table 2.1.	Physical properties of propylene glycol (PG) and vegetable glycerol (VG)	30
Table 2.2.	Effect of varying battery power output on generation of aldehydes.....	35
Table 2.3.	Characteristics of e-cigarette cartridges and e-liquids used in this study	36
Table 3.1.	The activation energies and frequency factor of AMAH, ADMH, and ATM reactions with acetone, propanal, 2-heptanone, MIBK, acrolein and crotonaldehyde.....	57
Table 3.2.	Activation energies of HTM and ATM adducts of carbonyl compounds ...	65
Table 3.3.	Carbonyl compounds quantified from 3R4F cigarette smoke	68
Table 3.4.	The average and standard deviation of carbonyl concentrations of gaseous breath samples.....	69
Table 4.0.	Results of nicotine analysis from selected commercial e-liquids	94
Table 4.1.	Comparison of measurements of nicotine in aerosol samples collected by sorbent tube and impinger methods	97

LIST OF SCHEMES

Scheme 1.0. Formation of hydrazones and oximes by reaction of alpha nucleophiles with carbonyl compounds	2
Scheme 1.1. Condensation reactions of aminoxy reagent ATM and hydrazine reagent HTM with aldehydes and ketones.....	3
Scheme 1.2. Derivatization of carbonyl compounds with Girard's reagent T and AMAH	13
Scheme 2.0. The homogeneous oxidation routes of propylene glycol	23
Scheme 2.1. Possible reactions occurring in glycerol pyrolysis.....	25
Scheme 2.2. Reversible formtion of hemiacetal.....	26
Scheme 2.3. Proposed pyrolysis of vegetable glycerol (VG) and propylene glycol (PG) humectants inelectronic cigarette aerosols.....	30
Scheme 2.4. Microreactor oximation of carbonyl compounds by AMAH	41
Scheme 3.0. Imine-, hydrazone-, and oxime-bond formation	47
Scheme 3.1. Quaternary ammonium aminoxy reagents and oximation of aldehydes or ketones	48

Scheme 3.2. Synthesis of ADMH reagents and conditions	49
Scheme 3.3. Synthesis of HTM, reagents and conditions	59
Scheme 3.4. Resonance structure of acrolein	71
Scheme 4.0. Biosynthetic pathway of nicotine.....	81
Scheme 4.1. Regioselective protonation of nicotine to form the nicotinium salt.....	83
Scheme 5.0. Oxidative N-nitrosation of nicotine to form NNK and NNA	107
Scheme 5.1. Decomposition of 2-methylbutyraldehyde to generate acrolein and propionaldehyde.....	108

CHAPTER 1

DETECTION AND ANALYSIS OF VOLATILE ORGANIC COMPOUNDS

- 1.1. HYPOTHESIS AND CONTRIBUTION OF THIS DISSERTATION
- 1.2. INTRODUCTION
- 1.3. PRECONCENTRATION TECHNIQUES
- 1.4. SURVEY OF REAGENTS FOR DERIVATIZATION OF CARBONYL COMPOUNDS
- 1.5. ANALYTICAL TECHNIQUES
- 1.6. DISSERTATION ORGANIZATION

1.1. HYPOTHESIS AND CONTRIBUTION OF THIS DISSERTATION

Because many of available carbonyl-selective reagents including 2,4-dinitrophenylhydrazine (2,4-DNP)¹⁻² do not react efficiently with α,β -unsaturated carbonyl compounds (many of which have been identified either as causative agents or promising markers of diseases), there is a need to develop more effective derivatization reagents for analysis of α,β -unsaturated carbonyl compounds. Aminoxy and hydrazine moieties are more reactive toward aldehydes and ketones than standard amines due principally to the α -effect.³⁻⁴ The α -effect refers to high nucleophilicity induced by the presence of a lone electron pair on an atom immediately adjacent (i.e., “alpha”) to the nucleophilic atom (scheme 1.0). Consequently, aminoxy- and hydrazine-based reagents have been used widely to chemoselectively react with carbonyl compounds

Scheme 1.0. Formation of hydrazones and oximes by reaction of alpha nucleophiles with carbonyl compounds

The condensation reactions of 2-(aminoxy) ethyl-*N, N, N*-trimethylammonium iodide (ATM) however, with α,β -unsaturated carbonyl compounds, are notably less efficient so much so that we sought to employ analogous reagent 2-hydrazinyl-*N, N, N*-trimethylethan-1-ammonium iodide (HTM, Scheme 1.1) as a means to improve the capture efficiency for this class of compounds. Since oxygen is more electronegative than nitrogen, hydrazine is expected to be more nucleophilic than aminoxy and should have

higher reactivity towards carbonyl compounds, especially unsaturated species. We therefore proposed investigating 2-hydrazinyl-*N,N,N*-trimethylethan-1-ammonium iodide abbreviated HTM (Figure 1.0).

We also sought the utility of 2-(aminooxy) ethyl-*N,N,N*-trimethylammonium iodide (ATM) and 4-(2-aminooxyethyl)-morpholin-4-ium chloride (AMAH) as carbonyl capture reagents for electronic cigarette aerosol analysis (Figure 1.0). While the hydrazine and aminooxy functionalities will enable chemoselective derivatization of carbonyl compounds in the analytical samples, the quaternary ammonium functional group, a permanent positive charge on ATM and HTM, and a titratable positive charge on AMAH, enables a microchip preconcentration approach (through electrostatic bonding with micropillars of the microreactor) of the volatile organic compounds in electronic cigarettes, tobacco cigarettes and exhaled breath. The quaternary ammonium functional group also improved the ionization efficiency of the adduct in direct infusion electrospray ionization.⁵

Scheme 1.1. Condensation reactions of aminooxy reagent ATM and hydrazine reagent HTM with aldehydes and ketones.

The pyrolysis of propylene glycol and glycerol generates carbonyl compounds. Since an electronic liquid humectant is principally propylene glycol or glycerol or a

mixture of both, with our aminoxy or hydrazine-coated, silicon-based microreactor, we could capture and derivatize the carbonyl compounds emitted during aerosolization of e-liquids. We were therefore motivated to determine the contribution of the propylene glycol and glycerol to the generation of carbonyl compounds and to both detect and accurately measure aldehydes from aerosols of e-cigarettes using our silicon-based microreactor

This project also sought to quantify nicotine in e-cig liquids and derived aerosol. Direct protonation of pyrrolidine nitrogen of nicotine with an organic acid afforded quaternary ammonium functional group (Figure 1.0) which would also enhanced the ionization efficiency of the nicotinium chloride by FT-ICR-MS.

Figure 1.0. The structures of ATM, AMAH, and HTM showing aminoxy and hydrazine functional groups to chemoselectively react with aldehydes and ketones. The positive end as well as that of the nicotinium salt will enhance electrospray ionization efficiency.

1.2. INTRODUCTION

Detection and analysis of trace carbonyl compounds, especially aldehydes and ketones, are of high importance. Some of these carbonyls especially acrolein, acetaldehyde, and formaldehyde, are considered the most significant cardiovascular and pulmonary toxins in tobacco smoke.⁶ For instance, many studies have shown that acute exposure to low levels of acrolein can induce dyslipidemia,⁷ vascular injury,⁸ endothelial

dysfunction,⁹ and platelet activation,¹⁰ whereas chronic exposures accelerate cardiovascular disease (CVD).¹¹⁻¹⁵

Carbonyl compounds can be found in the atmosphere. A trace amount of formaldehyde, derived from photochemical oxidation (hydroxyl radicals) of hydrocarbons, is present in the air.¹⁶⁻¹⁷ Automobile exhaust in urban centers are a significant source of important aldehydes in air both via direct emission of aldehydes and via emission of hydrocarbons, which in turn are converted to aldehydes through photochemical oxidation reactions. Because of the rise in the use of alternate and reformulated fuels, the number of carbonyl compounds has increased. Increased amounts of formaldehyde or acetaldehyde are now emitted in automobile exhaust depending on the type of oxygenated additives (ethanol, methanol, or methyl tert -butyl ether) added to the automotive fuels.¹⁸⁻²⁰ Using Los Angeles as an example, The most abundant carbonyls expressed as percentages of Los Angeles air carbonyl content on a parts per billion basis are formaldehyde (24%), acetaldehyde (18%), glyoxal/methylglyoxal (8%), acetone (7%), and acrolein (5%).²¹⁻²²

Also, a large number of toxic compounds have been reported in cigarette smoke, including over 3000 organic chemicals.²³ These toxic compounds also include polynuclear aromatic hydrocarbons, N-nitrosamines²⁴⁻²⁵, dioxins²⁶ acrylamide²⁷ and very importantly reactive carbonyl compounds such as acrolein, glyoxal, methylglyoxal, and malonaldehyde (MA).²⁸ These toxic aldehydes in cigarette smoke are of great importance because tobacco smoke is one of the major sources of toxic aldehydes contamination in indoor air.

Besides, carbonyl compounds have been used as chemical markers for disease screening and diagnosis.^{29, 30-31} For example, the detection of 4-hydroxyhexenal (4HHE) and 4-hydroxy-2-nonenal (HNE) in expired breath has been correlated with lung cancer.^{32,33} Lipoperoxidative production of reactive aldehyde species, such as malondialdehyde (MDA), HNE, 4-hydroxy-2-hexenal (HHE), and acrolein, appears to be the source of many of the α,β -unsaturated carbonyl compound markers associated with disease.³⁴

The VOCs concentrations are typically low in some of the analytical samples. These concentrations range from a few parts per million (ppm) to a few parts per trillion (ppt).³⁵⁻³⁶ Therefore, preconcentration of VOCs is necessary before analysis.

1.3. PRECONCENTRATION TECHNIQUES

Various preconcentration methods for detection and analysis of carbonyl compounds are now available. These techniques can be used for carbonyl compounds in tobacco cigarette, electronic cigarette and exhaled breath analysis. Among them are physical adsorption, solid phase microextraction, and microelectromechanical systems (MEMS) preconcentration devices. For example, analyte adsorptions and subsequent desorption and determination using gas- chromatography mass spectrometry (GC/MS) is a popular preconcentration approach. Adsorbents in the collection traps must be carefully selected to prevent carryover and breakthrough effects. Activated charcoal, different types of graphitized carbon, molecular sieves, and organic polymers have been used for enrichment of VOCs in analytical samples.

1.3.1. Physical adsorption

Table 1.0 shows different adsorbent materials and their composition. The sorbents used for the adsorptive enrichment in combination with thermal desorption should generally meet the following conditions to ensure accurate determination of VOCs:

1. Total enrichment of target analytes
2. Total and fast desorption of analytes
3. The surface must be inert and homogeneous to prevent artifact formation, irreversible desorption and catalytic result during sampling, storage of the loaded adsorbent tubes and desorption
4. Low adsorption capacity for other inorganic constituents of air, for example, nitrogen oxide, sulfur dioxide, carbon dioxide or ozone
5. High thermal and mechanical stability
6. Unreactive towards species like ozone
7. Lack of affinity to water to prevent displacement and hydrolysis and to reduce interferences with gas chromatographic analysis such retention time shift
8. Multiple usability.

Table 1.0. Adsorbent materials and their composition.³⁷

Adsorbent	Sampling range	T Max (°C)	Elemental Composition						Density (g mL ⁻¹)	Specific surface area (m ² g ⁻¹)
			C	H	N	S	O	Cl		
Carbon molecular sieves										
Carboxen 563	C2-C5	>400	81.4	2.6	-	10.6	4.7	0.1	0.53	510
Carboxen 564	C2-C5	>400	88.7	0.8	-	5.8	2.4	0.6	0.60	400
Carboxen 569	C2-C5	>400	93.7	0.3	-	4.0	1.1	0.4	0.58	485
Carboxen 1000	C2-C5	>400	91.9	0.9	-	0.2	4.0	1.3	0.44	1200
Carboxen 1001	C2-C5	>400	93.2	0.6	-	3.3	1.3	1.2	0.61	500
Carboxen 1003	C2-C5	>400	95.8	0.28	-	2.41	0.9	0.6	0.46	1000
Carbosieve SIII	C2-C5	>400	95.7	0.8	-	-	2.0	1.1	0.61	820
Carbosphere	-	400	90.5	0.5	-	0.1	2.1	0.2	-	1000
Graphitized carbon blacks										
Carbotrap F	>C20	>400	99.8	0.1	-	-	0.1	0.1	0.66	5
Carbotrap C	C12-C 20	>400	99.7	0.1	-	-	-	0.2	0.72	10
Carbotrap GCB	C5-C12	>400	99.9	0.1	-	-	-	-	0.36	100
Carbotrap Y GCB	C12-C20	>400	99.6	0.2	-	-	-	0.3	0.42	25
Carbotrap X GCB	C3-C5	>400	99.2	0.1	-	0.1	-	0.1	0.41	250
Carbograph 5 GCB	C3-C5	>400	100	-	-	-	-	-	-	560
Porous organic polymers										
Chromosorb106 (Styrene-divinylbenzene-copolymer)	Small molecule	250	92.1	7.8	-	0.2	0.7	0.3	-	750

1.3.2. Solid phase microextraction

The most laborious and time-consuming parts of chemical analysis are sample preconcentration and preparation. Currently sample preparation methods have received great attention. The requirement for their miniaturization, avoidance of toxic solvents, and the ease of coupling with analytical methods have been put to the fore.³⁸ Solid Phase Microextraction (SPME) was developed by by Arthur and Pawliszyn in the early 90s as a rapid extraction and preconcentration method for analyzing volatile and semi-volatile compounds from a variety of matrices.³⁹ It integrates sampling, extraction, concentration and sample introduction into a single solvent-free step. Analytes in the sample are

directly extracted and concentrated on an extraction fiber.⁴⁰ Its advantages include miniaturization ability; reduction of sample preparation time; reduction or even complete elimination of toxic solvents; high concentration factors; low cost; simplicity of coupling with instrumental methods of analysis; and possibility of automation.⁴¹

Figure 1.1. SPME procedure for GC and LC. Reproduced from Vas, G.; Vékey, K., J. Mass Spectrom. 2004; 39:233-254 Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis.⁴⁰

SPME coatings are made with organic and organosilicon polymers or hybrid organomineral adsorbents. The most commonly used materials for preconcentrating nonpolar and weakly polar compounds are polymeric coatings of polydimethylsiloxane (PDMS), polyacrylate (PAC), polydivinylbenzene (DVB).⁴² The sampling process is illustrated in Figure 1.0. The sample is placed in a vial and sealed with a Mininert valve. Partitioning between the sample matrix and the stationary phase takes place when the SPME needle pierces the septum and the fiber is extended through the needle into the

sample. If the fiber is exposed to only vapor phase above a gaseous, liquid or solid sample, the process is headspace, (HS-SPME). On the other hand, if the fiber is directly immersed in liquid samples, then the process is direct-immersion, (DI-SPME). After extraction, the fiber is withdrawn back into the needle assembly and removed from the valve and inserted immediately into the GC injection port. SPME can be used with GC, GC-MS HPLC and HPLC-MS.^{40,43-44}

1.3.3. Microfluidic chip

The use of microfluidic system as preconcentration devices has attracted considerable attention and has experienced rapid growth in the past two decades because of the promise of miniaturization, real-time analysis and power efficiency, and integration and automation.⁴⁵ A typical preconcentration device fabricated on a silicon wafer by Alfeeli and Agah⁴⁶ consists of embedded high aspect-ratio three-dimensional (3-D) micro pillars coated with an adsorbent polymer. Their work was applied to handheld point-of-care breath analysis instrumentation by preconcentrating VOCs, like n-decane (C_{10}), n-dodecane (C_{12}), 2,6-diisopropylphenol (Propofol), n-tetradecane (C_{14}), and n-hexadecane (C_{16}), in exhaled breath of patients undergoing anesthesia. Fu *et al*⁴⁷ also fabricated a silicon-based microreactor containing thousands of micropillars in channels for capture of trace-level volatile aldehydes and ketones. By chemically functionalizing each micropillar surface, gaseous aldehydes and ketones in exhaled breath samples are chemoselectively preconcentrated thereby enabling ultra-trace, rapid analysis by direct-infusion Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometry (MS). The micropillar surfaces ((Figure 1.2) are coated with quaternary

ammonium aminooxy salt 2-(aminooxy) ethyl-N, N, N-trimethylammonium iodide (ATM) (Figure 1.1) for capturing trace carbonyl VOCs by means of an oximation reaction. This silicon-based microreactor system was used for sample preconcentrations for the purpose of this dissertation.

Figure 1.2. 2-(aminooxy) ethyl-N, N, N-trimethylammonium iodide (ATM)

Figure 1.3. Photographs of the microreactor: (a) Optical micrograph of the microreactor before bonded with a glass wafer. (b) SEM micrograph of the micropillar array in the microreactor. (c) The microreactor connected to two fused silica tubes. A dime was placed near the microreactor to indicate the size of the microreactor, (Photo adapted from Fu *et al.*³¹).

1.4 SURVEY OF DERIVATIZATION REAGENTS FOR CARBONYL COMPOUNDS

The analysis and quantification of low molecular weight aldehydes and ketones is particularly difficult because many of the carbonyl compounds are volatile, unstable, reactive and have low abundance.⁴⁸ The conventional way to mitigate this problem is to derivatize these carbonyl compounds with a suitable reagent. Although the number of derivatization reagents for aldehydes and ketones have increased considerably over the last decade, a majority of these derivatization reagents are not efficient in derivatizing unsaturated carbonyl compounds. Among the existing derivatization reagents for aldehyde and ketones are aryl hydrazines, such as 2,4-dinitrophenylhydrazine (2,4-DNP).^{1,2,49,50,51} Other reagents include 4-hydrazino-4-oxobutyl-[tris(2,4,6-trimethoxyphenyl)- phosphonium bromide (TMPP-PrG), 1-(Carboxymethyl)pyridinium chloride hydrazide (Girard's reagent P), (Carboxymethyl)trimethylammonium chloride hydrazide, (Girard's reagent T), and pentafluorophenylhydrazine (PFH)⁵²⁻⁵³ and the newly developed aminooxy-based 2-(aminooxy)-*N,N,N*-trimethylethan-1-ammonium iodide (ATM)^{54,31, 47} and 4-(2-(aminooxy)ethyl)-morpholin-4-ium chloride (AMAH)⁵⁵ reagents (Figure 1.3). A typical derivatization reaction of carbonyl compounds with Girard's reagent T leads to the formation of an acyl hydrazone while AMAH will undergo the same reaction to form an oxime ether as shown in scheme 1.2.⁵⁶

The derivatives of the carbonyl compounds are usually analyzed with a suitable analytical detection instrument. For the purpose of this study, cationic aminooxy and hydrazine -based reagents were used because of their high reactivity and easy adaptability to FT-ICR-MS.

Figure 1.4. Reagents for derivatization of carbonyl compounds.

Scheme 1.2. Derivatization of carbonyl compounds with Girard's reagent T and AMAH.

Volatile carbonyl compounds are conventionally derivatized with 2,4-dinitrophenylhydrazine (DNPH). The DNPH are usually acidified with a mineral acid like hydrochloric acid and impregnated on silica gel.^{23, 57} They are sold in pre-packed cartridges. After derivatization, the DNPH-carbonyl adducts are washed off from the cartridge with acetonitrile and an aliquot of the adducts are analyzed and quantified with HPLC (Figure 1.4). DNPH can also be acidified and used in an impinger trap containing

a known amount of acetonitrile to trap and derivatize the carbonyl compounds followed by analysis with HPLC. These techniques usually involve the use of an acid to drive the derivatization reaction. They also are cumbersome, involve the use of large volume of solvents during HPLC analysis and have fewer prospects for miniaturization. Besides, the DNPH cartridge is usually not re-useable.

Figure 1.5. DNPH cartridge for derivatization of carbonyl compounds.

1.5. ANALYTICAL DETECTION TECHNIQUES

Conventional spectroscopic methods such as infrared, fluorescent and nuclear magnetic resonance spectroscopic methods have been employed in the analysis of VOCs, especially carbonyl compounds. However these methods suffer from inadequate specificity, sensitivity, and inability to detect many classes of compounds.⁵⁸ Thus, there is a need for analytical methods that can provide high sensitivity, specificity, and adequate mass resolving power. Gas chromatography mass spectrometry (GC-MS), liquid chromatography–tandem mass spectrometry (LC–MS/MS), ion mobility mass

spectrometer (IMS), electronic nose, and fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) are instrument of choice for a analysis of VOCs.

1.5.1. Gas chromatography mass spectrometry

Gas chromatography coupled with mass spectrometry (GC-MS) is the most commonly used analytical technique for trace-level VOCs due to its high sensitivity and reliability in analyte identification. Also, GC-MS has been used for identification and analysis of certain metabolic products like acetone, ethanol and isoprene, and other VOCs in normal human expired breath. It has also been used for analyzing carbonyl compounds in electronic cigarette aerosols.⁵⁹ More detailed analytical information and analyte identity can be derived from GC- MS than the proton transfer reaction mass spectrometry (PTR-MS). Multidimensional gas chromatography introduced few decades ago, especially two-dimensional gc typically coupled with a time-of-flight or quadrupole mass analyzer mass spectrometer, has also been used to analyze volatomes in exhaled breath to provide a more effective way of enhancing the resolving power.⁶⁰⁻⁶¹ Even though the use of GC-MS for analysis of preconcentrated VOCs is both sensitive and reliable, and relatively cheap, it has the downside of laborious sample preparation and the whole process is cumbersome.

1.5.2. Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Liquid Chromatography coupled with tandem mass spectrometry is a powerful technique for analysis of biological samples without extensive sample preparation. It has also found application in the analysis of carbonyl compounds in e-cigarette aerosols.⁶²

Accuracy and precision at very low analyte concentrations however are a challenge when using this technique.

1.5.3. Ion mobility mass spectrometer (IMS)

Another analytical detection technique is ion mobility spectrometry (IMS). It is not yet very popular in the study of VOCs of cancer markers. Westhoff *et al*⁶³ carried out the first study of VOCs with IMS. IMS has low selectivity hence complex mixtures are usually analyzed with a pre-preparation method like multi-capillary column (MCC). It is however a comparatively cheap detection technique with high promise for miniaturization.⁶⁴

1.5.4. Electronic nose

An electronic nose contains an array of nonspecific, gas sensitive, chemical sensors as artificial odor receptors. An electronic nose sensor includes components such as a quartz microbalance, carbon-polymer array, and colorimetric sensor array.⁶⁵⁻⁶⁶ In an electronic nose (E-Nose), the VOCs adsorb onto a sensor where a change in conductivity, color or oscillation of a crystal is detected by the sensing system of the E-nose. The E-nose responds to only a mixture of compounds in the sample. The pattern recognition system (computer) interprets and detects the high levels of VOCs. The drawbacks include the extensive preparation of the breath samples, lack of quantitative data and calibration.⁶⁷

1.5.5. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS)

The advent of ultrahigh resolution mass spectrometers has created opportunities for exploration of high-throughput analysis of trace volatile carbonyl compounds in breath, electronic cigarettes and environment, especially in conjugation with novel chemoselective (CS) probes designed for these instruments.

Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) can analyze several compounds in seconds via extreme mass resolution combined with ultra-high mass accuracy mass-resolving power.⁶⁸⁻⁶⁹ The resolution of FT-ICR-MS is very high that it can fit several of unique chemical ions, including all stable isotopes, in a single analysis, eliminating the time-consuming chromatography that has plagued analysis of highly complex mixtures by all other types of MS. These characteristics make it particularly suitable for analysis of volatile aldehydes and ketones in electronic cigarette aerosols and exhaled breath. FT-ICR-MS was therefore an analytical detection instrument of choice for my graduate research.

With the use of a silicon-based microreactor on which a highly reactive aminoxy or a hydrazine reagent is coated as a preconcentration method of choice and high resolution FT-ICR-MS as analytical detection tool, the problems associated traditional DNPH-silica gel cartridge method, including cumbersomeness, the use of large volume of solvent nonreusability, and lack of prospect for miniaturization can be avoided.

However, despite the advantages offered by FT-ICR-MS, it is difficult to distinguish between two compounds having the same mass to charge ratio in full scan mode except by tandem mass spectrometry (MS/MS) and quantification of such compounds when they were present became difficult.⁷⁰ Because of difficulties

experienced with the MS/MS we therefore turned to gas chromatography mass (GC-MS) spectrometry to separate functional isomers like acetone and propanal, butanone and butanal.

1.6. DISSERTATION ORGANIZATION

This dissertation contains five chapters. Chapter II presents the results of studies using the existing 4-(2-aminooxyethyl)-morpholin-4-ium chloride (AMAH) derivatization reagent in the analysis of carbonyl compounds in electronic cigarette aerosols using the prefabricated microreactor technology enabled with gas chromatography-mass spectrometry (GC-MS) and Fourier Transform Ion Cyclotron Mass Spectrometry FT-ICR-MS.

Chapter III describes a kinetics study on the oximation reactions of selected aminooxy and carbonyl compounds. It also shows the synthesis, characterization and utility of novel reagent 2-hydrazinyl-*N, N, N*-trimethylethan-1-ammonium iodide (HTM) in analysis of carbonyl compounds, especially α,β -unsaturated species in exhaled breath.

Chapter IV gives a detailed work on the development of a new method for nicotine quantification in electronic cigarettes liquids and aerosols. Chapter V concludes the dissertation and outlines the future work.

CHAPTER 2

CARBONYL COMPOUNDS IN ELECTRONIC LIQUIDS AND AEROSOLS

- 2.1. ELECTRONIC CIGARETTES
- 2.2. SIGNIFICANCE OF CARBONYLS IN ELECTRONIC CIGARETTES
- 2.3. E-CIGARETTE AEROSOL GENERATIONS; CAPTURE, DERIVATIZATION AND ANALYSIS OF CARBONYL COMPOUNDS
- 2.4. RESULTS AND DISCUSSION
- 2.5. CONCLUSION
- 2.6. EXPERIMENTAL SECTION

2.1. ELECTRONIC CIGARETTES

Electronic cigarettes are battery-powered, tobacco-free nicotine delivery devices that aerosolize a nicotine-containing solution known as e-liquid or e-juice without combustion or smoke.⁷¹⁻⁷³ The electronic cigarette was invented by Chinese pharmacist Hon Lik in the early 2000s to function as a substitute for quitting smoking.⁷⁴⁻⁷⁶ It is designed to look like and provide the feel of a traditional cigarette.⁷⁷ E-juices are usually a mixture of propylene glycol or glycerin, or both, and distilled water laced with nicotine and other food grade additives that may or may not be approved for use by the US Food and Drug Administration (FDA).⁷⁸ The electronic cigarette (e-cigarette) device is electrically driven by a battery -usually a lithium battery -and contains an atomizer in which the e-juice is housed and then aerosolized by application of electrical energy that generates resistance heat encircling a wick (Figure 2.1).⁷⁹⁻⁸⁰

Electronic cigarettes come either as disposable or refillable. The disposable unit must be discarded once the electronic liquid is exhausted while the refillable unit, which usually is a tank type, may be used indefinitely because it can be replenished once the liquid is exhausted.⁸¹ The electronic juice in electronic cigarettes is laced with several flavors, such as chocolate, vanilla, menthol, tobacco or fruit flavors.⁸²

E-cigarette usage has surged since being introduced to the US market in 2007 as a safer alternative to traditional cigarettes. There are currently over 40 million smokers in the U.S. and about 20% of them have tried e-cigarettes.⁸³ The growing popularity of e-cigarette users has raised serious public health concerns of potential harm to e-cigarette users.^{15, 23, 76, 84-86} The exponential increase of e-cigarette users among young people may also cause nicotine addiction and transition to tobacco cigarettes.^{76, 85} One way of

evaluating the safety of electronic cigarettes as an alternative to traditional cigarettes is by measuring the concentration of carbonyl compounds that can be emitted during vaping.

Figure 2.1. Anatomy of an Electronic Cigarette.⁸⁷

2.2. SIGNIFICANCE OF CARBONYLS IN ELECTRONIC CIGARETTES

Certain low molecular weight aldehydes like acetaldehyde, acrolein and formaldehyde which are considered very harmful constituents of tobacco smoke⁸⁸ are found in high concentrations in cigarette smoke between 700-800 $\mu\text{g}/\text{cigarette}$ in mainstream smoke⁸⁹⁻⁹⁰, cigars, and waterpipes (hookah, narghile, bidis, smokeless tobacco products (snus and snuff), and are also present in e-cigarettes aerosols.⁹¹⁻⁹⁵

A link between e-cigarette use and risk stems from the presence of harmful and potentially harmful constituents identified in aerosols of e-cigs, including metals, particles, carbonyls and flavoring compounds.^{71, 79, 96-100} However, the presence of toxic carbonyl compounds in e-cigarette aerosols derived from humectants and/or flavoring chemicals used in e-liquid formulations is raising health concerns.^{98, 101} In order to

address these concerns, we set out to measure the generation of carbonyl compounds from the humectants propylene glycol and glycerol, as well as from commercial e-liquids containing flavoring chemicals in “tank type” refillable e-cigarette devices.

The e-cig device, e-liquid composition and puff topography are the principal factors that determine the extent of toxic carbonyl compound generation in the aerosol. The temperature of the heating coil for aerosolization of an e-liquid is a function of the battery power output determined by the battery voltage output and the coil resistance. The temperature of the heating coil is also affected by the amount of heat transferred to vapor or aerosol as determined by the puff volume or air flow rate, puff duration and the puff frequency, and a theoretical model has been developed to predict the coil temperature of the atomizer.¹⁰² The battery power output is the critical factor for determining the coil temperature, and several publications have indicated that the battery power output dramatically affects generation of aldehydes in aerosols.^{71, 97, 103} Moreover, high battery power output could cause wick starvation and lead to a “dry puff” wick that exponentially increases the generation of formaldehyde, acetaldehyde and acrolein.^{71, 104}

Thousands of e-liquids are commercially available. In addition to small amounts of water, nicotine and flavoring compounds, the two main components of e-liquids are humectants, predominately propylene glycol (PG) and vegetable glycerin (VG, glycerol). Our study compared carbonyl compounds generated from varying mixtures of humectants PG and VG with corresponding carbonyl compounds in aerosols of selected popular e-liquids.

2.2.1. Pyrolysis of propylene glycol

Propylene glycol (propane-1, 2-diol, PG) is a hygroscopic organic compound. It is used as pharmaceutical formulation solvent, as a humectant in electronic liquid formulation and in food additives as a plasticizer. It has also found application as a moisturizer in medicine, cosmetics, food, and the tobacco industry.

Propylene glycol has been known to react with O_2 between 227-427 °C using silver (Ag) catalyst to produce acetol (hydroxyacetone), which subsequently undergoes oxidation to form methylglyoxal.¹⁰⁵⁻¹⁰⁶ The homogeneous oxidation of PG between 127-327 °C has also been shown to produce acetone, acetaldehyde, formaldehyde, and acetol while acetaldehyde can undergo aldol condensation reaction to form crotonaldehyde (Scheme 2.0).

Scheme 2.0. The homogeneous oxidation routes of Propylene glycol as proposed by Diaz *et al.*¹⁰⁷

2.2.2. Pyrolysis of glycerol

The composition of the product mixture formed during the thermal decomposition of glycerol is temperature dependent. For instance, dehydration of glycerol to acrolein is increased at low temperatures as a result of the ionic mechanism and acrolein is obtained as the main product.¹⁰⁸ The thermal decomposition of glycerol is highly endothermic, and demands a great deal of heat input. This results in steep thermal gradient, and produces various non-equilibrium products.¹⁰⁹ The two major pathways for thermal decomposition glycerol involve dehydration and dehydrogenation of glycerol. Hydroxyacetone and 3-hydroxypropanal are produced during dehydration process (equation 1), the latter being acrolein precursor (equation 2) while glyceraldehydes and dihydroxyacetone are produced during dehydrogenation (equation 3). These compounds can be converted into various intermediates, such as acetaldehyde, and decomposed further into syngas at a high temperature.¹¹⁰ Scheme 2.1 shows the possible reactions occurring in glycerol pyrolysis.

Scheme 2.1. Possible reactions occurring in glycerol pyrolysis (adapted from Yu-Chuan Lin¹¹¹).

2.2.3. Hemiacetal formation in aerosols of e-liquids

Hemiacetals are formed when alcohols, such as propylene glycol and glycerol in e-liquids, add reversibly to the carbonyl functional group of aldehydes¹¹²⁻¹¹³, as shown in Scheme 2.2. The reaction between formaldehyde and propylene glycol or glycerin of e-cigarette liquids during vaporization is therefore thought to form measurable formaldehyde-hemiacetal, as detected by 1H NMR spectroscopy. A recent report suggested that emission of formaldehyde in e-cigarette aerosols is higher than a direct measurement of formaldehyde because a portion of formaldehyde is sequestered in the

form of a hemiacetal (the so called “hidden formaldehyde”), which prompted more health concerns over using e-cigarettes.⁷¹ Unfortunately, this illuminating work on measurement of formaldehyde-hemiacetal did not measure free or unreacted formaldehyde or any other aldehydes in the aerosols of the e-cigarettes. We addressed this deficiency by measuring both free formaldehyde as well as formaldehyde-hemiacetal produced during vaping.

Scheme 2.2. Reversible formation of hemiacetal by reaction of an aldehyde and an alcohol.

2.3. E-CIGARETTE AEROSOL GENERATION; CAPTURE, DERIVATIZATION AND ANALYSIS OF CARBONYL COMPOUNDS

In order to accurately quantify the carbonyl compounds in electronic cigarettes aerosols, we use cigarette-smoking robot to puff electronic cigarette and the aerosol samples were collected in a 5 or 10-litre Tedlar bag (Figure 2.2). An aliquot of the aerosol can be transferred to a 1 L tedlar bag for analysis. We use our powerful silicon microreactor coated with an aminooxy reagent AMAH as a derivatization reagent (coating) reagent and connected to a vacuum pump to pull the aerosols through the microreactor for carbonyl derivatization until the tedlar bag is empty (Figure 2.3).

Figure 2.2. Cigarette-smoking robot system for mechanical generation of aerosols from e-cigarettes.

After the preconcentration, the microreactor was eluted with methanol followed by the addition of internal standard. The FT-ICR-MS spectra of the samples were obtained and the quantification of carbonyl compounds achieved with the calibration curves.

Figure 2.3. Schematic diagram of the preconcentration set-up for capture of carbonyl compounds in e-cig. Aerosols.

2.4. RESULTS AND DISCUSSION

To understand the contributions of thermal decomposition of PG or VG (physical properties in Table 2.1) to carbonyl generation and for comparison with carbonyl generation from commercial e-liquids, we measured carbonyl compounds in aerosols made from neat PG (100%), neat VG (100%), and mixtures, e.g., 50:50. The amounts of acetaldehyde, acetone, acrolein and formaldehyde were measured as a function of the percentage of PG in the mixtures (Figures 2.4 and 2.5). Fluid consumption varied from 6.0 to 11.5 mg/puff at the puff volume of 91 mL. Neat PG generated the highest level of acetaldehyde (1.01 ± 0.34 $\mu\text{g/puff}$), then formaldehyde (0.25 ± 0.12 $\mu\text{g/puff}$), acetone (0.11 ± 0.007 $\mu\text{g/puff}$) and low levels of crotonaldehyde yet above LoD ($0.25 \pm 0.12 \text{ ng/puff}$). The acrolein level was below the limit of detection (LoD, estimated <0.03 ng/puff). Neat VG produced acetaldehyde (0.70 ± 0.03 $\mu\text{g/puff}$), formaldehyde (0.59 ± 0.11 $\mu\text{g/puff}$), acetone (0.11 ± 0.01 $\mu\text{g/puff}$), acrolein (0.08 ± 0.002 $\mu\text{g/puff}$) but not crotonaldehyde.

2.4.1. Carbonyls in aerosols of commercial e-liquids with flavorants

This work measured generation of carbonyl compounds from commercial e-liquids with flavoring chemicals using “tank type” refillable e-cigarette devices to generate aerosols. Acetaldehyde and formaldehyde levels in aerosols of Classic Tobacco flavor e-liquid were lower than both neat PG and VG, while acrolein was below the LoD. Formaldehyde levels in aerosols of the other three Set I e-liquids (Magnificent Menthol, Vivid Vanilla, and Cherry Crush e-cigs (blu®)) were in the range of formaldehyde generated from neat PG to neat VG, while acetaldehyde levels in aerosols of the other

three e-liquids were lower than the levels generated from neat PG and VG (Figure 2.6). Acrolein in aerosols from the other 3 e-liquids was higher than that in aerosols from pure VG (Figure 2.4). Aldehydes also were measured in aerosols from three Set II e-liquids (Menthol Ice, Mocha Café and Southern Classic). Menthol and Southern Classic e-liquids generated much higher levels of formaldehyde, acetaldehyde and acrolein in aerosols than did pure PG or VG or any PG:VG mixtures (Figure 2.7). Formaldehyde in aerosols from Menthol e-liquid was 8.1-times greater than that produced from pure PG and 3.4-times greater than that of pure VG, while formaldehyde in aerosols from Southern Classic e-liquid was 8.7 times that of pure PG and 3.7 times that of pure VG. The 3-8 fold increases of acetaldehyde, formaldehyde, acrolein and levels in the derived aerosols from Set II (Menthol and Southern Classic) e-liquids in comparison with pure PG and VG likely were induced by decomposition of the flavoring chemicals. GC-MS analysis indicated that the level of propionaldehyde was much lower ($<1/20$; give value) than the levels of acetone, formaldehyde and acetaldehyde, thus propionaldehyde. Hydroxyl-acetone also was detected in aerosol of both neat PG and VG (Figure 2.4). As can be expected based on molecular structure (Scheme 2.3), the thermally induced degradation of PG generated a higher level of acetaldehyde whereas that of VG generated a higher level of formaldehyde. The levels of these carbonyl compounds in aerosols were at their lowest points when generated from the mixture at 25 wt% of PG compared with all other mixtures. These lowest levels may be induced by a combination of mass transfer and heat transfer related the mixture of 25 wt.% of PG. As the percentage of PG increased from 25 to 100 wt.%, the levels of acetone, acetaldehyde and formaldehyde also increased, while acrolein levels decreased to below the LoD). The detection of hydroxyl-acetone provides

experimental data to support the theoretical mechanisms (Scheme 2.3). No carbonyl compounds were detected in unvaped neat propylene glycol and glycerol and this proved that the carbonyl compounds detected in the aerosols were formed as a result of the thermal decomposition of the humectants.

Table 2.1. Physical properties of propylene glycol (PG) and vegetable glycerol (VG).

	Chemical Formula	Mw (g/mol)	Density (g/cm ³)	B.P. (°C)	Viscosity (Pa·s) at 25 °C	Vapor Pressure at 200 °C
PG	C ₃ H ₈ O ₂	76.10	1.036	188.2	0.042	1030 mmHg
VG	C ₃ H ₈ O ₃	92.09	1.261	290	1.412	46 mmHg

Scheme 2.3. Proposed pyrolysis of vegetable glycerol (VG) and propylene glycol (PG) humectants in electronic cigarette aerosols.

Figure 2.4. The FT-ICR-MS of the aerosols of 100% PG, 100% VG and PG/VG in 50/50 ratio showing different carbonyl compounds formed.

Figure 2.5. Amounts of formaldehyde, acetaldehyde, acrolein and acetone as a function of the percentage of propylene glycol (PG) in the mixtures of vegetable glycerin (VG) and propylene glycol. Error bars represent one standard deviation of triplicate measurements (N=3).

Figure 2.6. Amounts of formaldehyde, acetaldehyde, acrolein and acetone as a generated while vaping a set (I) of e-cigarettes as compared with neat PG and neat VG. Error bars represent one standard deviation of triplicate measurements (N=3).

Figure 2.7. Amounts of formaldehyde, acetaldehyde, acrolein and acetone as a generated while vaping another set (II) of e-cigarettes as compared with neat PG and neat VG. Error bars represent one standard deviation of triplicate measurements (N=3).

2.4.2. Generation of carbonyls from e-cigarettes aerosols: effects of battery power output

Aerosols generated from 10 puffs (puff duration of 4 sec, puff volume of 91 mL/puff, puff frequency of 2 puffs/min) were collected in Tedlar bags using a software-controlled (FlexiWare) cigarette-smoking robot (CSR) (Sci-Req, Montreal, CAN) as described earlier. The puff duration, puff volume and puff frequency in this study are within the ranges used by e-cigarette users.¹¹⁴⁻¹¹⁵ To study the effect of puffing topography on emission of aldehydes in aerosols, polypropylene syringes with a 60 mL capacity were also used to collect aerosols of e-cigarettes by manually varying puff duration and puff volume. While the first generation e-cigarette has a fixed battery voltage of 3.7 V (power: 4.6 W), the battery power of the newer generation e-cigarette was tested at 9.1 W (3.7 V), 11.7 W (4.2 V), 14.7 W (4.7 V) and 16.6 W (5.0 V) for vaporization of e-liquids. For aerosols collected from e-liquids EL04-EL-06 (Table 2.3) at the battery power output of 14.7 W and 16.6 W, the aerosol samples were diluted 50 times with N₂ and then drawn through the microreactors because of much higher levels of generated aldehydes.

Because increasing battery power output of newer e-cigarette devices increases the atomizer coil temperature that results in emissions of more aldehydes in aerosols of e-liquids,^{97,2636} we investigated the effect of the battery power output of the newer e-cigarette devices on generation of carbonyl compounds in aerosols of e-liquids. EL04-EL04 and EL05 generated much more carbonyl compounds at a power output of 11.7 W and above. Figure 2.8 shows GC-MS chromatograms of AMAH and its adducts from the aerosol samples collected from Halo Menthol Ice at vaporization power of 11.7 W (4.2 V), 14.7 W (4.7 V) and 16.6 W (5.0 V). Acetaldehyde, acrolein, formaldehyde,

propionaldehyde and butyraldehyde were detected. Increasing the power from 11.7 W to 16.6 W resulted in dramatic increases of the levels of these aldehydes (Table 2.2).

Figure 2.8. Representative GC-MS chromatograms of aldehydes. The newer e-cigarette device (iTaste) was used to vaporize e-liquid Halo Mentol Ice at a battery power output of 11.7 W (4.2 V), 14.7 W (4.7 V), and 16.6W (5.0V).

Similar results of dramatic increases of acetaldehyde, acrolein and formaldehyde with increasing vaping power output to 9 W and above have been reported.¹¹⁶ Higher power results in overheating of the coil and leads to excessive aldehyde generation by thermal decomposition of humectants (“dry puff” condition).¹¹⁶

Table 2.2. Effect of varying battery power output on generation of aldehydes in the aerosols from e-liquid Halo Menthol Ice.*

Voltage (W)	Acetaldehyde μg (ppm)	Acrolein μg (ppm)	Formaldehyde, μg (ppm)	Acetone μg (ppm)	Propionalde hyde μg, (ppm)	Butyrald ehyde,μg (ppm)
11.7 W	22.7±3.4 (35.9±5)	1.2±0.8 (1.5±1.0)	129.6±9.7 (300.8±22.4)	11.46±0.50 (13.75±0.60)	0.6±0.4 (0.7±0.5)	0.5±0.3 (0.5±0.3)
14.7 W	134.30±7.8 (212.33±12.3)	3.2±0.7 (3.9±0.9)	386.8±11.0 (898.1±26)	984.92±50.10 (1181.54±60.10)	3.4±1.5 (4.0±1.8)	5.0±8.5 (4.8±8.2)
16.6 W	532.1±60.2 (841.3±95.2)	16.2±0.3 (20.3±0.4)	819.81±76.8 (1902.8±178.3)	808.72±72.6 (970.17±87.1)	17.9±0.9 (21.4±10)	13.6±0.5 (13.1±0.5)

* A total of 10 puffs of aerosol was collected at the puff volume of 91 mL, puff duration of 4s. Each experiment was performed in triplicate and the data are expressed as the average [±SD] of the measured values.

To quantify the fraction of aldehydes that reacted with propylene glycol and/or glycerin to form hemiacetals in the aerosols of e-cigarettes, we collected aerosolized e-liquids in NMR tubes containing DMSO- d_6 using the newer e-cigarette device. We initially attempted to detect the formation of a formaldehyde-derived hemiacetal in aerosols of all e-cigarettes in table 2.3. No formaldehyde hemiacetal signal was detected in any of the aerosols generated from the first generation blu e-cigarettes with all tested puff volume and puff duration scenarios (data not shown). No hemiacetal was detectable in the aerosols of EL01, EL02 and EL03 at all battery power output from 9.1 W to 16.6 W as shown in figure 2.9 (a). In a positive control experiment, formaldehyde gas was introduced into EL01 e-liquid and under this condition a triplet signal at δ 6.18 ppm and a doublet signal at δ 4.61 ppm were observed, confirming formation of a formaldehyde hemiacetal (Fig. 2.9(b)). Thus, the lack of detectable formaldehyde hemiacetal in aerosols of the first generation e-cigarette and e-liquids EL01 to EL03 was likely related to the

generally low amount of formaldehyde present in these aerosols, even when at higher battery power output.

Table 2.3. Characteristics of e-cigarette cartridges and e-liquids used in this study.

Product code	Brand name	Type	Nicotine content (label)	Manufacturer
EC01	blu Tobacco	Classic Cartridge	16 mg	Imperial Tobacco
EC02	blu Magnificent Menthol	Cartridge	16 mg	Imperial Tobacco
EC03	blu Vanilla	Vivid Cartridge	16 mg	Imperial Tobacco
EC04	Blu Crush	Cherry Cartridge	16 mg	Imperial Tobacco
EL01	eVo Black diamond	e-liquid	6 mg/mL	Nicopure Lab USA
EL02	Smooththol	e-liquid	6 mg/mL	NicQuid
EL03	Perfected Vape/Clearwater	e-liquid	6 mg/mL	Delaware Vapor USA
EL04	Halo Mocha	Café e-liquid	6 mg/mL	Halo USA
EL05	Halo Ice	Menthol e-liquid	6 mg/mL	Halo USA
EL06	Halo Southern Classic	e-liquid	6 mg/mL	Halo USA

Figure 2.9. ^1H NMR spectra (DMSO- d_6) for detection of hemiacetals, (a) e-liquid EL01 vaporization at the battery power output of 16.6 W (no hemiacetal detected); (b) unvaporized EL01 spiked with formaldehyde.

Figure 2.10. ^1H NMR spectra ($\text{DMSO}-d_6$) of hemiacetals, (a) unvaporized e-liquid EL05; the tank type e-cigarette (iTaste) was used to vaporize e-liquid EL05 at a battery power output of (b) 11.7 W (4.2 V), (c) 14.7 W (4.7V), and (d) 16.6 W (5.0V).

Formaldehyde hemiacetal in aerosols of EL05 was detected at the battery power output from 11.7W to 16.6 W by NMR. No other aldehyde hemiacetal such as acetaldehyde hemiacetal and acrolein hemiacetal was detected. Figure 2.10 shows that there was an increase in peak intensity of the hemiacetal as the e-cigarette battery power output was increased from 11.7 W (4.2V) to 16.6 W (5.0V). At a battery power output below 11.7 W, formaldehyde hemiacetal was below the limit of detection. The amounts of formaldehyde hemiacetal in aerosol increased as the power was increased and the calculated amounts (mean $\pm$ SD) of hemiacetal were based on the internal standard (Figure 2.11).

Figure 2.11. The relationship between the amounts of formaldehyde-hemiacetal in 10 puffs of aerosols collected from e-liquid EL05 and the battery power output of 11.7 W, 14.7 W and 16.6 W.

At a battery power output of 11.7 W, $78.6 \pm 23.8 \mu\text{g}/10$ puffs of formaldehyde hemiacetal was measured, whereas at 16.6 W, $250.4 \pm 56.1 \mu\text{g}/10$ puffs of the hemiacetal was measured. We were able to estimate the amount of the bound formaldehyde as $22.2 \pm 6.7 \mu\text{g}$ and $70.7 \pm 15.8 \mu\text{g}$ from the measured formaldehyde hemiacetal at 11.7 W and 16.6 W, respectively. These amounts of formaldehyde could be released from reversible reaction of formaldehyde hemiacetal. Much higher formaldehyde of 380 (puff volume of 50 mL for 10 puffs) was reported from formaldehyde-hemiacetal at the e-cigarette voltage output of 5V for vaporization of the e-liquid EL04.⁷¹ Given the puff volume of 35 mL, we could estimate that the formaldehyde in formaldehyde-hemiacetal could be approximately 44.6% of free formaldehyde at the power output of 11.7 W and approximately 22.4% of free formaldehyde at the power output of 16.6 W. Higher formaldehyde levels of 380 (puff volume of 50 mL for 10 puff s) was reported from

formaldehyde–hemiacetal at the e-cigarette voltage output of 5 V for vaporization of EL04 e-liquid.

2.5. CONCLUSION

Our experimental results have shown that aldehydes are formed from heating of humectants and flavorants in e-cigarette liquids. Mechanisms to form these carbonyl compounds have been proposed previously.¹¹⁷⁻¹¹⁸ The detection of hydroxyl-carbonyl intermediates (e.g., acetol, hydroxyl-acetaldehyde, 3-hydroxy-propanal) in this study provides experimental data in support of the theoretical mechanisms.¹¹⁸⁻¹¹⁹ Regardless of the paths to aldehyde generation, our research indicates that the percentages of PG and VG as well as added flavorants to e-liquids affect formation of these aldehydes in aerosols. As can be expected based on molecular structure, VG generated higher levels of formaldehyde and acrolein, whereas PG generated a higher level of acetaldehyde. Lower levels of carbonyls were generated from mixtures of the humectants, notably 25% PG: 75% VG, likely due to multiple factors including mass transfer and heat transfer as well as rate-determining dehydration kinetics associated with the mixture composition. In order to understand the specific contribution of flavorants to generation of carbonyl compounds and formaldehyde-hemiacetal, further studies involving aerosolization of humectants laced with each flavorant and subsequent analysis of the aerosols for carbonyl compounds will be necessary. The chemistry of additives with e-liquids when subjected to a high temperature needs investigation. Also, formaldehyde-hemiacetals was generated during aerosolization of some e-liquid brands. The potential toxicity of the hemiacetal is not yet understood. The ability of the hemiacetal to convert to formaldehyde after

inhalation by humans therefore needs to be investigated. Heavy metals like cadmium, chromium, lead, manganese, and, nickel have been reported in electronic liquid and derived aerosols.¹²⁰⁻¹²¹ Some of these are transition metals are used as catalysts in organic reactions. Therefore, effects of different metals on generation on generation of carbonyl compounds in electronic cigarette aerosolization also need to be investigated.

2.6. EXPERIMENTAL

A customized e-cigarette holder was regulated by a software-controlled (FlexiWare) system (Sci-Req, Montreal, CAN) for generation of aerosols. The puffing protocol consisted of a 4 s puff duration, a 91 mL puff volume, and a 56 s puff interval to mirror typical e-cigarette user puffing topography.³¹⁻³² Neat PG (100%), neat VG and PG and VG mixtures (PG: VG, 25:75; 50:50, 75:25 wt%) were used for aerosol generation. For comparison, commercial e-liquids (described above) were used in e-cigarette aerosol generation. The e-cigarette battery (bluPLUS+) power output was 7.6 watts (3.7 V) in all experiments. Batteries were fully charged overnight before use.

Details of the microreactor have been published elsewhere.^{47, 54} Micropillar surfaces in the microreactor were functionalized by infusion of a solution of AMAH (1×10^{-6} mol; 4-(2-aminooxyethyl)-morpholin-4-ium chloride (AMAH⁵⁵) in methanol followed by evaporation of the solvent in a vacuum oven at 40 °C. Fused silica capillary tubes (350 μ m o.d., 250 μ m i.d.) were connected to the inlet and outlet ports of the microreactor, respectively, with a silica-based bonding agent.

To capture carbonyl compounds for analysis, aerosol samples were collected in Tedlar bags and evacuated through the microreactors by a vacuum (3.5 mL/min). After

the evacuation process, the microreactors were eluted with 150 μL MeOH followed by addition of an internal reference (IR). For FT-ICR-MS analyses, the AMAH-deuterated acetone adduct was added as the IR. For GC-MS analysis, AMAH-cyclohexanone (1x10⁻⁷ mol) was chosen because it is symmetrical (i.e., no geometrical isomers for the AMAH-cyclohexanone adduct) and because cyclohexanone was undetected in e-cigarette aerosols. GC-MS was used to quantitate isomeric compounds reacted with AMAH, e.g., acetone and propionaldehyde. Calibration curves of internal standards for all detected carbonyls were established for quantitative measurements in both FT-ICR-MS and GC-MS. For GS-MS analyses, poly-4-vinylpyridine (PVP, 5 mg) was added to the eluted solutions to convert positively charged AMAH adducts to neutral AMA adducts (Scheme 2.4).⁵⁵ The suspension was vortex-mixed for 30 s and allowed to stand for 30 min for the sedimentation of PVP particles, after which a 20 μL aliquot solution was used for GC-MS analysis.

Scheme 2.4. Microreactor oximation of carbonyl compounds by AMAH and neutralization of adducts with PVP prior to GC-MS analyses.

2.6.1. FT-ICR-MS

An FT-ICR-MS instrument (Finnigan LTQ-FT, Thermo Electron, Bremen, Germany) equipped with a TriVersa NanoMate ion source (Advion BioSciences, Ithaca,

NY) fitted with an electrospray chip (nozzle inner diameter 5.5 μm) was used for all mass spectral analyses. The TriVersa NanoMate was operated in positive ion mode by applying 2.0 kV with no head pressure. Initially, low-resolution MS scans were acquired for 1 min to ensure the stability of ionization, after which high mass accuracy data were collected using the FT-ICR analyzer, where MS scans were acquired for 5 min and at the target mass resolution of 100,000 at 200 m/z .

Using AMAH-deuterated acetone adduct as internal reference (IR), the linearities of standard AMAH-carbonyls were first established by calibration curves built by plotting the ratio of intensity of AMAH-carbonyl to intensity IR against of peak area of analytes to IS ($I_{\text{carbonyl}}/I_{\text{IR}}$) versus the ratio of the amount (mole) of the AMAH-carbonyl to amount of IR ($M_{\text{carbonyl}}/M_{\text{IR}}$) (Figure 2.12). Linear regression was used to determine the slope, intercept, and coefficient of determination (R^2). The calibrations curved were used to quantify the carbonyl compounds of interest generated during aerosolization.

Figure 2.12. The calibration curves of standard AMAH-carbonyl compounds. The ration of Intensity of carbonlys to Intensity of Internal Reference (IR) was plotted against the ratio of amount of carbonlys to amount of IR.

2.6.2. NMR analysis of hemiacetals

Ten puffs (puff volume: 35 mL; puff duration: 4 s, puff frequency of 1 puff/min) of the aerosols generated by the first generation e-cigarette and the “tank type” e-cigarette were collected using a 60 mL capacity polypropylene syringe with a very short rubber tube to connect the e-cigarette. After collection, the rubber tube and e-cigarette were immediately removed, and then the syringe was fitted with a long stainless needle to transfer the aerosolized liquid into an NMR test tube in an ice bath. During the transfer, most of the aerosol condensed and was collected as liquid. 400 μ L of deuterated DMSO was added to the NMR tube followed by the addition of a known amount of benzene (1.72×10^{-6} mol) as an internal standard. Then, ^1H -NMR spectra (referenced to TMS) were immediately taken at 400 MHz. To verify formation of formaldehyde-hemiacetal in e-liquid, formaldehyde was generated by heating 1,3,5-trioxane and 8N sulfuric acid at 95 $^{\circ}\text{C}$ ¹²² and then introduced as a gas into e-liquids. Formaldehyde-hemiacetal was quantified by relative integration against the known amount of benzene added as an internal standard.

2.6.3. GC-MS analysis of carbonyl adducts

A Thermo Scientific GC-MS instrument equipped with an AI 1310 automatic sampler, a TRACE 1310 GC with a split/splitless injector and an ITQ 1100 series ion trap MS was used for analysis. The GC had an Agilent J&W DB-17ms column (60 m $\times$ 0.25 mm $\times$ 0.25 μ m film thickness). Carrier gas helium flow rate was 1.5 mL/min. Column temperature was 50 $^{\circ}\text{C}$ for 1 min, then increased by 10 $^{\circ}\text{C}/\text{min}$ up to 160 $^{\circ}\text{C}$, and then to 200 $^{\circ}\text{C}$ by 2 $^{\circ}\text{C}/\text{min}$. After that, the temperature was increased by 12 $^{\circ}\text{C}/\text{min}$ up to 280 $^{\circ}\text{C}$

and was held at 280 °C for 5 min. The total running time was 41 min.

The samples were split injected with split flow of 15 mL/min and a slit ratio of 10.

CHAPTER 3

KINETIC STUDIES OF OXIMATION REACTION AND SYNTHESIS OF A CATIONIC HYDRAZINE REAGENT

- 3.1. INTRODUCTION OF OXIMATION REACTIONS
- 3.2. KINETIC STUDIES OF REACTION BETWEEN AMINOOXIES AND
CARBONYLS
- 3.3. RESULTS AND DISCUSSION
- 3.4. HYDRAZINE SYNTHESIS
- 3.4. CONCLUSION
- 3.5. EXPERIMENTAL SECTION

3.1. INTRODUCTION OF OXIMATION REACTIONS

The reaction between an aminoxy moiety (RONH_2) and the carbonyl group of an aldehyde or ketone- known as an oximation reaction- is a versatile click chemistry¹²³⁻¹²⁴ coupling that generates a robust oxime ether linkage. Carbonyl-selective derivatizing aminoxy agents have been used for detection of oxidized cellular metabolites.¹²⁵⁻¹²⁶ Moreover, oxime based chemistries have been used for efficient bioconjugation of proteins and polysaccharides for the preparation of conjugate vaccines,¹²⁷ to generate homogeneously glycosylated proteins, to recombinantly produce protein-bearing tailored glycans at specific sites.¹²⁸ The reaction is also used to selectively capture aldehyde and ketone metabolites directly from air, such as exhaled breath which has been used for noninvasive detection of lung cancer,^{31, 47, 129} Furthermore, it is used to ligate linker molecules to fluorophores and gold nanoparticles,¹³⁰ to analyze ketones in crude oil and coal tar¹³¹ and to selectively ligate carbonyls for labeling glycoconjugates for microscopy.¹³²

In addition, biocompatible click reactions have shown promise for in situ ligations and applications in living organisms. Carbonyl condensation reactions such as imine-, hydrazone-, and oxime-bond formation (Scheme 3.0), Staudinger and Diels-Alder reactions as well as azide-alkyne cycloadditions are used for situ ligation. The oxime ligation is of special interest because of its efficiency and chemoselectivity in aqueous systems under mild acidic conditions. It is compatible with most biomolecule functionalities and water is the only side-product formed in this process.¹³³

Scheme 3.0. Imine-, hydrazone-, and oxime-bond formation. E and Z isomers may be present depending on the nature of substituents and conditions.

Atmospheric carbonyl compounds are either derived from direct emissions or produced as reaction intermediates from oxidation of hydrocarbons initiated by OH radicals and ozone.¹³⁴⁻¹³⁵ Carbonyl compounds also play a central role in atmospheric chemistry close to the tropopause, and this is directly relevant to issues such as the assessment of the impact of air traffic and ozone depletion.¹³⁶ It is thus crucial to understand the reactions and kinetics of carbonyl compounds particularly with respect to derivative formation for purposes of monitoring or analyses.

3.2. KINETIC STUDIES OF REACTION BETWEEN AMINOOXIES AND CARBONYLS

In spite of the importance of oximation reactions in the analysis of trace carbonyl compounds in air, exhaled breath and bio-liquids, very little has been done to study the reaction kinetics of aminoxy compounds with aldehydes and ketones. The oximation reaction kinetics of aminoxy compounds is very important for quantitative analysis of trace carbonyl compounds. In this thesis, we determined the reaction kinetics between quaternary ammonium aminoxy salts 2-(aminoxy)-N, N, N-trimethylethan-1-

ammonium iodide (ATM), 2-(aminoxy)-*N*, *N*-dimethylethan-1-aminium chloride (ADMH), and 4-(2-(aminoxy)ethyl)-morpholin-4-ium chloride (AMAH) (Scheme 3.1) and some selected carbonyl aldehydes and ketones. We used FT-ICR-MS to study the kinetics of oximation because of its ability measure fast reaction rates that are otherwise difficult or impossible to monitor using other spectrometric techniques.

Scheme 3.1. Quaternary ammonium aminoxy reagents and oximation of aldehydes or ketones (Z = ammonium moiety; R, R' = alkyl group).

3.3. RESULTS AND DISCUSSION

The synthesis 2-(aminoxy)-*N*, *N*-dimethylethan-1-aminium chloride (ADMH) (3) as depicted in scheme (Scheme 3.2) was accomplished in three steps: (1) reaction of ethanolamine (5) with *N*-hydroxyphthalimide (NHP) under standard Mitsunobu conditions (equimolar amounts of NHP/PPh₃/DIAD) to obtain phthaloyloxy amine 6 in good yield; (2) hydrazinolysis by treatment of 6 with methylhydrazine at 0 °C in dry dichloromethane followed by Kugelrohr distillation of the liberated aminoxy product; and (3) acidification using aqueous hydrochloric acid under forcing conditions to hydrolyze atmospheric oxime ether adducts formed during handling of the Kugelrohr distillate. Recrystallization of the crude salt from isopropyl alcohol afforded ADMH as a white solid.

Scheme 3.2. Synthesis of ADMH. Reagents and conditions: *a.* *N* hydroxyphthalimide, PPh₃, DIAD, THF, 0 °C to rt, 12h; *b.* CH₃NHNH₂, CH₂Cl₂, 0 °C, 4.5h; *c.* 6M HCl, reflux, 22h.

The reaction kinetics of aminooxy reagent reacting with carbonyl compounds is initially assumed to be second order, irreversible reaction. The reaction rate is given by the following equation for the same molar amount of aminooxy reagent and carbonyl compound:

$$r = \frac{dC_{\text{carbonyl}}}{dt} = -kC_{\text{Aminooxy}}C_{\text{carbonyl}} = -kC_{\text{carbonyl}}^2 \quad (1)$$

where *k* is the specific reaction rate, *C* is the concentration of the reactants, and *t* is the reaction time. The following equation can be obtained by integration of equation (1) from time zero to *t* for the concentration of carbonyl compounds from initial *C*₀ to *C* (*t*)

$$\frac{1}{C(t)} - \frac{1}{C_0} = kt \quad (2)$$

In order to verify that the reaction is irreversible elementary second order reaction for both reactants, plots of 1/*C* vs. time were made for all reactions. Figure 3.1 shows a representative plot 1/*C* vs. *t* for AMAH reacted with acetone at -21, 0, and 21 °C. Fig. 3.2 shows time dependent FT-ICR-MS spectra of AMAH-acetone. Figure 3.3 shows the plot of 1/*C* vs. *t* for ADMH reacted with acrolein at -21, 0, and 21 °C. Figure. 3.4 shows time dependent FT-ICR-MS spectra of ADMH-acetone. The linear plots with high *R*² were obtained for all three aminooxy compounds reacted with carbonyl compounds. Therefore, the oximations reactions were found to be irreversible second order reactions. The

corresponding values of the specific reaction rate k were obtained from the slope of the linear plot of $1/C$ vs. t .

Figure 3.1. The plot of $1/C$ against time for oxidation reaction between AMAH and acetone at -21°C, 0 °C, 21°C.

Figure 3.2. FT-ICR-MS Spectra overlay of oximation reaction between AMAH and acetone at 21 °C at times $t=60, 30, 600, 900,$ and 1200 seconds corresponding with figure 3.2.

Figure 3.3. The plot of $1/C$ against time for oximation reaction between ADMH and acetone at $-21^{\circ}\text{C}, 0^{\circ}\text{C}, 21^{\circ}\text{C}$.

Figure 3.4. Spectra overlay of oximation reaction between ADMH and acrolein at 21 °C at times $t=60, 30, 600, 900, 1200$ seconds corresponding with figure 3.4.

Figure 3.5. The graph of the natural logarithms of the reaction rate constants of oximation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and acetone.

After the specific reaction factor k was determined for at least three different temperatures, the activation energy and frequency factor were determined from the *Arrhenius* equation,

$$\ln k = -\left(\frac{E_a}{R}\right) \cdot \left(\frac{1}{T}\right) + \ln k_0, \quad (3)$$

the activation energy E_a and frequency factor k_0 were determined from linear regression of the plot of the natural logarithm of k ($\ln k$) vs. the reciprocal of temperature $1/T$.¹³⁷ Figure. 3.5 shows the plots of $\ln k$ vs. $1/T$ for ADMH, AMAH, ATM reaction with acetone. The specific reaction rate k_0 of ATM reaction with acetone is higher than that of AMAH and ADMH reaction with acetone.

Figures. 3.6 to 3.10 show the linear regression plots of the natural logarithm of reaction constant $\ln k$ between ADMH, AMAH and ATM and carbonyl compounds. These plots are all linear with negative slope from which the activation energies of the oxime ethers were computed. Table 3.1 shows the activation energy E_a and frequency factor k_0 for the reactions ADMH, AMAH and ATM with different aldehydes and ketones including α , β -unsaturated aldehydes, acrolein and crotonaldehyde. Generally, activation energies of ADMH are lowest of all the three aminoxies reacting with all the carbonyl compounds followed by AMAH. We suspect that this could be because it is the smallest of the three molecules. The smaller the molecule, the faster it moves and the higher kinetic energy, which results in collision and formation of products. AMAH is however bulkier than ATM but has smaller activation energy and this can be attributed to the presence of titratable acidic proton which can catalyze the reaction by activating the carbonyl carbon of the aldehydes and ketones. The E_a are also seen to increase with increasing carbon number of the ketones. The E_a of acetone is higher than propanal even

though they both have 3 carbon atoms because aldehydes are more reactive than ketones. The k_0 , which shows the frequency of collision increase down the table for all the carbonyl compounds except for MIBK with some branching. This behavior can also be attributed to the presence of titratable acidic proton, which catalyzes the reaction by lowering the activation energy. Because of low activation energies of ADMH and AMAH reactions with carbonyls, the contribution of k_0 to the specific reaction rate k is significant. Therefore, even if the activation energy for ADMH and AMAH reaction with carbonyls increase, the increase of k_0 for this reaction results in higher specific reaction rate.

Figure. 3.7 and Figure 3.8 show the plots of $\ln k$ vs. $1/T$ for ADMH, AMAH, ATM reaction with acrolein and crotonaldehyde, the α , β -unsaturated species. The activation energy E_a of the three aminooxies are generally higher than those of the saturated aldehyde and ketones because of the π electron density of the double bond, which slows down the nucleophilic attack by the aminooxy reagent on the carbonyl carbon. Also the energy of activation of ADMH and AMAH with these α,β -unsaturated aldehydes are closer and lower than that of ATM. They are therefore more reactive towards acrolein and crotonaldehyde than ATM. The higher frequency factor k_0 for the reactions of ATM with acrolein and crotonaldehyde did not necessarily translate to higher reactivity. In fact, the E_a of ATM with crotonaldehyde is 1.5 times higher than that of ADMH.

Figure 3.6. The graph of the natural logarithms of the rate constants of oximation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and propanal.

Figure 3.7. The graph of the dependence of the natural logarithms of the rate constants of oximation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and acrolein.

Figure 3.8. The graph of the dependence of the natural logarithms of the rate constants of oxidation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and crotonaldehyde.

Figure 3.9. The graph of the dependence of the natural logarithms of the rate constants of oxidation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and 2-heptanone.

Figure 3.10. The graph of the dependence of the natural logarithms of the rate constants of oximation reaction as a function of the reciprocal of temperature between ADMH, AMAH, ATM and methyl isobutyl ketone (MIBK).

Table 3.1. The Activation Energies and frequency factor of AMAH, ADMH, and ATM reactions with acetone (C3), propanal (C3), 2-heptanone (C7), MIBK (C6) acrolein (C3) and crotonaldehyde (C4).

ADMH						
E_a (kJmol ⁻¹)	32.21	29.20	33.16	33.1	45.66	49.81
K_o (s ⁻¹)	1.92x10 ⁹	3.27x10 ⁸	4.28x10 ⁸	8.18x10 ⁸	1.29x10 ¹²	3.10x10 ¹²
AMAH						
E_a (kJmol ⁻¹)	34.30	34.54	37.33	34.38	48.2	54.21
K_o (s ⁻¹)	8.94x10 ⁹	1.14x10 ⁹	2.57x10 ¹⁰	1.82E x10 ¹⁰	2.33Ex10 ¹²	2.43x10 ¹³
ATM						
E_a (kJmol ⁻¹)	36.80	30.72	37.20	37.02	51.94	74.02
K_o (s ⁻¹)	9.75 x10 ¹⁰	2.46Ex10 ⁹	2.81x10 ¹⁰	2.86 x10 ⁹	1.46 x10 ¹³	3.51 x10 ¹⁶

3.4. HYDRAZINE SYNTHESIS

We proposed to synthesize 2-hydrazinyl-*N, N, N*-trimethylethan-1-ammonium iodide abbreviated HTM (Figure 3.11) because it is expected to be more nucleophilic and reactive towards carbonyl compounds than ATM. The reactivities of the two reagents (HTM and ATM) were also studied and compared.

Figure 3.11. 2-Hydrazinyl-*N, N, N*-trimethylethan-1-ammonium iodide (HTM).

3.4.1. Synthetic Route to HTM

The synthesis of 2-hydrazinyl-*N, N, N*-trimethylethan-1-ammonium iodide (HTM) is depicted in Scheme 3.3 and was accomplished in three steps beginning with quaternization of commercially available 2-(*N, N*-dimethylamino) ethanol with methyl iodide.¹³⁸⁻¹³⁹ The resultant quaternary ammonium alcohol **2** was converted to chloride **3** by refluxing in excess thionyl chloride. Figures 3.12 and 3.13 show ¹H NMR spectra of the alcohol **2** and chloride **3** that highlight the disappearance of hydroxyl proton (triplet, δ 5.25) as well as the downfield shifts in the methylene proton of the chloride **3**. The product formation was also confirmed by high-resolution mass spectrometry (Figure 3.14). Hydrazine functionality was installed by refluxing chloride **3** in a solution of excess hydrazine monohydrate in tetrahydrofuran. Excess hydrazine monohydrate was distilled off and the residue crystallized from ethanol to give the pure HTM. Figures 3.15 and 3.16 show ¹H NMR and ¹³C NMR of purified HTM.

Scheme 3.3. Synthesis of HTM. Reagents and conditions: a. CH_3I , CH_2Cl_2 , sealed tube, 50 °C, 12 h, 90%; b. SOCl_2 , 80 °C, 4 h, 98%; c. $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ (xs), THF, reflux, 6 h, 43%.

It is noteworthy that the FT-ICR-MS spectrum of pure HTM showed that this compound complexes with iodide to form a dimer, but the dimer readily reacts with acetone d_6 to form the corresponding hydrazone adduct (Figure 3.17). This formation of dimer complex with iodine and a silver nitrate test that gave a pale yellow coloration confirmed that indeed the counter-ion of the HTM is iodide.

Figure 3.12. The ^1H NMR spectrum of alcohol 2 in $\text{DMSO-}d_6$.

Figure 3.13. The ¹H NMR spectrum of chloride **3** in DMSO-*d*₆.

Figure 3.14. The high-resolution mass spectrometry spectra overlay of alcohol **2** and chloride **3**.

Figure 3.15. The ¹H NMR spectrum of HTM in DMSO-*d*₆

Figure 3.16. The ¹³C NMR spectrum of HTM in DMSO-*d*₆.

Figure 3.17. The FT-ICR-MS of the HTM and HTM-acetone- d_6 adduct (internal standard). The FT-ICR-MS spectrum of HTM-acetone d_6 (bottom) was acquired after the addition of an equimolar amount acetone d_6 to HTM sample and allowing it to stand for 1 hour.

In order to compare the reactivity of the existing aminoxy reagent (ATM) with HTM, we performed a kinetic study of these reagents reacting with carbonyl compounds. We selected two saturated and two unsaturated carbonyl compounds: propanal, acrolein, acetone and crotonaldehyde. The reaction kinetics of HTM and ATM with these carbonyl compounds was studied using FT-ICR-MS. The reaction kinetics of aminoxy and hydrazine reagents reacting with carbonyl compounds is initially assumed to be an elementary second order, irreversible reaction. By applying equation 2, a plot of $1/C$ against t gave straight-line graphs from where k values were obtained. Figure 3.18 shows a plot of $1/C$ against t for the reaction between HTM and acrolein at -21°C , 0°C , and 21°C . Also, application of *Arrhenius* equation 3 and a plot of $\ln k$ against $1/T$ afforded straight-line graphs with negative slope from where the activation energies for selected

carbonyl compounds were computed. Figure 3.19 shows the plot of $\ln k$ against $1/T$ for HTM-propanal, HTM-acetone, HTM-acrolein and HTM-crotonaldehyde while figure 3.20 shows the plot of $\ln k$ against $1/T$ for ATM-propanal, ATM-acetone, ATM-acrolein and ATM-crotonaldehyde.

Figure 3.18. The graph of $1/C$ against time for hydrazone formation reaction between HTM and acrolein at -21°C , 0°C , and 21°C .

Figure 3.19. The graph of the dependence of the natural logarithms of the rate constants of hydrazone formation reaction as a function of the reciprocal of temperature between HTM and propanal, acetone, acrolein, and crotonaldehyde.

Figure 3.20. The graph of the dependence of the natural logarithms of the rate constants of hydrazone formation reaction as a function of the reciprocal of temperature between ATM and propanal, acetone, acrolein, and crotonaldehyde.

The activation energies for reactions of HTM with volatile ketones and aldehydes were compared to those measured for ATM – the aminoxy analogue. The results showed that HTM has lower activation energy for α,β -unsaturated compounds than ATM implying that HTM reacts the faster with the unsaturated substrates because the energy barrier that must be overcome for the reaction to occur is low. On the other hand, the aminoxy reagents have lower activation energies for saturated carbonyl compounds thereby reacting faster with saturated carbonyl substrates than HTM (Table 3.2).

Table 3.2. Activation energies of HTM and ATM adducts of selected carbonyl compounds.

Entry	Carbonyl	Ea HTM adduct	Ea ATM adduct
1	Propanal	37.39	36.80
2	Acrolein	30.30	51.96
3	Acetone	43.18	34.54
4	Crotonaldehyde	31.51	74.02

E_a in KJmol⁻¹

3.4.2. Capture efficiency

To compare reactivities of aminooxy reagent ATM and hydrazine HTM with α,β -unsaturated aldehydes in a silicon microreactor, we determined the percentages of unsaturated carbonyl compounds that were captured by the HTM- and ATM-coated chips. Acrolein and crotonaldehyde were selected for this study. A known amount of acrolein or crotonaldehyde in methanol was injected into 1 L of air in a Tedlar bag. The loaded air sample then passed through an ATM- or HTM-coated chip (Figure 3.21). The percentage of the captured carbonyl was calculated as capture efficiency. Figure 3.22 shows the capture efficiency of HTM and ATM with acrolein and crotonaldehyde. The capture efficiency of HTM is about 2 times higher than ATM in trapping acrolein or crotonaldehyde.

Figure 3.21. The experimental set-up showing the preconcentration of carbonyls in the microchip.

Figure 3.22. The capture efficiency graph of HTM and ATM with acrolein and crotonaldehyde. The graph shows that HTM captures the unsaturated carbonyls with efficiency twice as high as ATM.

3.4.3. Cigarette smoke and exhaled breath analysis

To derivatize of the carbonyl compounds generated in tobacco cigarette smoke, a Kentucky reference cigarette 3R4F smoke was collected using a 100 mL capacity syringe fitted with rubber tubing. 60 mL of smoke was collected per puff and a total of 9 puffs were collected into a 10-liter Tedlar bag. A 2 mL aliquot was taken out and injected into a 500 mL tedlar bag containing 498 mL of pure air for derivatization and analysis.

In order to quantify carbonyl compounds, calibration curves were built using FT-ICR-MS by plotting the ratio of the relative abundance of carbonyl compounds to an internal reference (y), against the mole ratio of the carbonyl compounds to the internal reference (x). HTM and ATM internal standards were prepared by reacting and equimolar amount deuterated acetone- d_6 to HTM and ATM and left overnight to form HTM and ATM acetone- d_6 adduct. 4 nmol of the internal standard was added to each of the serially diluted HTM- or ATM-carbonyl compound adduct solution. The calibration curve showed an excellent linearity between the intensity ratio of carbonyl adduct to internal reference ($I_{\text{Carbonyl}}/I_{\text{IR}}$) and the molar ratio of carbonyl adduct to reference ($M_{\text{Carbonyl}}/M_{\text{IR}}$) with 0.02 nmol to 7 nmol dynamic range. The concentrations of detected carbonyl compounds were calculated from the calibration curves for different carbonyl adducts (Figures 3.23. and 3.24).

Figure 3.23. The calibration curves of HTM-carbonyl adducts using HTM-acetone- d_6 adduct as internal standard.

Figure 3.24. The calibration curves of ATM-carbonyl adducts using ATM-acetone- d_6 adduct as internal standard.

Table 3.3 shows the amounts of carbonyl compounds, in $\mu\text{g}/\text{cigarette}$, captured by HTM and ATM. The amount of unsaturated acrolein and crotonaldehyde derivatized by the HTM was higher than those of the ATM whereas the ATM captured more of the saturated aldehydes and ketones.

Table 3.3.¹⁴⁰ Carbonyl compounds quantified from 3R4F cigarette smoke.^{a, b}

	Formaldehyde	Acetaldehyde	Acrolein	Acetone	2-butanone	crotonaldehyde
ATM	90.50 $\pm$ 6.18	906.40 $\pm$ 97.51	29.96 $\pm$ 2.96	699.22 $\pm$ 53.07	15.69 $\pm$ 3.97	10.74 $\pm$ 0.93
HTM	72.53 $\pm$ 7.88	729.39 $\pm$ 69.45	49.36 $\pm$ 8.94	560.04 $\pm$ 64.82	10.1 $\pm$ 3.90	19.32 $\pm$ 4.25
CORESTA	21.5 $\pm$ 7.8	540.3 $\pm$ 135.30	49.0 $\pm$ 14.10	256.70 $\pm$ 51.40	47.2 $\pm$ 14.60	13.2 $\pm$ 5.20

^aAmount in $\mu\text{g}/\text{cig}$

^bEach experiment was performed in triplicate, and the data are expressed as the average ($\pm$ SD) of the measured values.

Carbonyl compounds in exhaled breath of a smoker and non-smoker were also analyzed and the results compared. The exhaled breath of smokers was collected 20 minutes after smoking. The carbonyl profiles as determined using HTM are shown in FT-ICR-MS spectra (Figures 3.25). Table 3.4 provides the concentration of carbonyls

captured by each derivatization reagent. While ATM was more effective in derivatizing formaldehyde acetone and 2-butanone than HTM, HTM was able to unmask acrolein and crotonaldehyde that could not be detected by ATM in exhaled breath of non-smokers. The amounts of unsaturated carbonyls detected by HTM were also higher than those of ATM in exhaled breath of smokers. Generally, smokers have a higher concentration of carbonyls than non-smokers except for acetone where exhaled breath of non-smoker is higher than that of smokers.

Table 3.4. The average and standard deviation of carbonyl concentrations of gaseous breath samples of a smoker and a non-smoker subject with HTM and ATM.

Carbonyl VOCs	HTM		ATM	
	Non-smoker	Smoker	Non-smoker	Smoker
formaldehyde	0.20±0.03	0.49±0.08	1.03±0.44	0.67±0.09
acetaldehyde	0.16±0.013	1.37±0.14	0.55±0.13	0.61±0.08
acetone	4.68±1.10	4.48±0.57	5.75±1.03	5.00±2.31
2-butanone	ND	0.39±0.37	1.81±0.08	0.31±0.16
acrolein	0.10±0.005	2.02±0.20	ND	0.96±0.26
crotonaldehyde	ND	1.73±0.30	ND	0.99±0.03

Values in nmolL⁻¹

ND= Not Detected

Figure 3.25. The FT-ICR-MS spectra overlay of HTM-carbonyl compounds detected in exhaled breath of a smoker and a non-smoker subjects.

3.5. CONCLUSION

The kinetics of oximation reactions was studied. The reactions between ketones and aldehydes with short alkyl chain have lower activation energy, compared with carbonyl compounds with long alkyl chain. This is because the short chain carbonyl compounds have higher mixing kinetic energy than the long chain carbonyl compounds. ADMH and AMAH generally have lower activation energy than ATM although the frequency factor of ATM is generally higher than those of ADMH and AMAH. The activation energies of these reagents with acrolein and crotonaldehyde which are α,β -unsaturated aldehydes were higher than those of the saturated substrate because the α,β -unsaturated are less electrophilic due to the presence of π electrons of terminal alkene. These experimental results can be used as a guide to the choice aminoxy compounds as the coating materials for preconcentration of specific carbonyl compounds.

We have also developed a new hydrazine reagent, 2-hydrazinyl-*N,N,N*-trimethylethan-1-ammonium iodide (HTM). HTM is a hydrazine analog of ATM designed to target and chemoselectively react with α,β -unsaturated carbonyls, which have hitherto been difficult to derivatize using aminoxy reagents. Both kinetic and capture efficiency studies show that derivatization of α,β -unsaturated acrolein and crotonaldehyde occurs twice as efficiently with HTM than when using ATM.

The comparative analysis of carbonyl compounds in exhaled breath and tobacco cigarettes shows that the novel hydrazine reagent captures more α,β -unsaturated carbonyl species than ATM. HTM could capture the low abundant acrolein and crotonaldehyde present in exhaled breath more than ATM could.

The reactions of an aminoxy and hydrazine reagents with α,β -unsaturated carbonyls are usually slow because alkene π -electrons deactivates the carbonyl carbon via resonance. However, we expected the new hydrazine reagent to be more reactive to both saturated and unsaturated carbonyls than aminoxy because of its stronger nucleophilic character. In a conjugated carbonyl system, there is an extra resonance structure that also shows electrophilic character at the terminal alkene carbon (Scheme 3.4). The resonance structures of acrolein are shown in scheme. Therefore, α,β -unsaturated aldehydes and ketones can potentially react with nucleophiles at two sites: directly at the carbonyl carbon or the end of the conjugated system (1,4-addition). In direct addition, the nucleophile attacks "directly" at the carbonyl (C=O) (1,2-addition). These are two competing reactions. However, we did not detect the 1,4-addition product. The electron density that could be supplied to the carbonyl carbon by the terminal π -electrons likely lowered the 1,2-addition reaction. However, the reason for higher reactivity of aminoxy ATM towards saturated carbonyl compounds is not known and needs to be investigated.

Scheme 3.4. Resonance structure of acrolein.

3.6. EXPERIMENTAL SECTION

3.6.1. Materials and methods

All reagents and solvents, including deuterated acetone (acetone- d_6) (99.9%), acetone (99%), propanal (99%), 2-pentanone, propanal, acrolein ($\geq 99.5\%$), crotonaldehyde ($\geq 99.5\%$, mixture of *cis* and *trans*), 2-dimethylaminoethanol ($\geq 99.5\%$), thionyl chloride, hydrazine monohydrate (98%), and methanol (99.9%), were purchased from Sigma-Aldrich. Acrolein (99.5%) was purchased from Fluka Analytical and methyl iodide was purchased from Alfa Aesar. Reagents ATM (**1**),¹⁴¹ and AMAH (**2**)⁵⁵ (Figure 3.3.0) were prepared according to literature procedures. The Kentucky reference cigarette 3R4F was purchased from the University of Kentucky College of Agriculture Reference Cigarette Program. The silicon microreactors were fabricated from 4"-silicon wafers using standard microelectromechanical systems techniques. Details of the microreactor design and fabrication have been published elsewhere.^{31, 47, 54}

3.6.2. Measurement of reaction kinetics

The corresponding acetone- d_6 adducts of ADMH, AMAH, ATM and HTM (5.0×10^{-6} mol) were added (to serve as isotopically labeled internal standards) to 200 μ L spectroscopic grade methanol containing the respective quaternary ammonium aminoxy reagent (5.0×10^{-7} mol) before reaction with a carbonyl compound. Acetone (5.0×10^{-7} mol), or other carbonyl compound, then was added to the reaction solution at either room temperature (21 °C), 0 °C, or -21 °C. A mixture of 90% ethylene glycol and 10% ethanol¹⁴² was used to achieve -21°C and are stable for about 45 min. Aliquots of the reaction mixture (15 μ L) were analyzed by FT-ICR-MS at different time intervals. The

unreacted carbonyl compound concentration $C(t)$ was calculated by subtraction of the reacted carbonyl compound concentration from the original concentration C_0 . Other kinetic studies were done following this procedure.

3.6.3. FT-ICR-MS analysis

The methanol solutions of the aminoxy reagent-carbonyl mixtures were analyzed on a hybrid linear ion trap FT-ICR-MS instrument (Finnigan LTQ-FT, Thermo Electron, Bremen, Germany) equipped with a TriVersa NanoMate ion source (Advion BioSciences, Ithaca, NY) with an electrospray chip (nozzle inner diameter 5.5 μm). The TriVersa NanoMate was operated in positive ion mode by applying 2.0 kV with no head pressure. Initially, low-resolution MS scans were acquired for 1 min to ensure the stability of ionization, after which high mass accuracy data were collected using the FT-ICR analyzer where MS scans were acquired for 8.5 min and at the target mass resolution of 100,000 at 800 m/z . The quaternary aminoxy compound and its adducts were assigned on the basis of their accurate mass by first applying a small (typically <0.0005) linear correction based on the observed mass of the internal standard.

3.6.4. 2-hydroxy-*N,N,N*-trimethylethan-1-ammonium iodide

To a solution of 2-(*N,N*-dimethylamino) ethanol (**1**) (2.00 g, 22.44 mmol) in CH_2CH_2 (60 mL) in a pressure tube was added in one portion methyl iodide (2.10 mL, 33.66 mmol). The tube was sealed and the reaction mixture was heated at 50 $^\circ\text{C}$ for 12 h. On cooling, the reaction mixture was concentrated under reduced pressure in a fume hood to afford iodide salt **2** (4.67 g, 90%) as a white solid that was used directly in the next

step; mp 272-274 °C (272-274 °C¹⁴³); ¹H NMR (400 MHz, DMSO-d₆) δ 5.32 – 5.12 (t, 1H), 3.82 (d, J = 4.8 Hz, 2H), 3.40 (dd, J = 9.9, 4.7 Hz, 2H), 3.11 (d, J = 4.1 Hz, 9H) ppm; ¹³C NMR (100 MHz, DMSO-d₆) δ 66.87, 55.11 53.15 ppm; HRMS (m/z): cal.104.1070; obt. 104.1070.

3.6.5. 2-Chloro-N, N, N-trimethylethan-1-ammonium iodide

Alcohol **2** (2.00 g, 8.66 mmol) was dried under vacuum at 100 °C for 1 h immediately prior to addition of thionyl chloride (9.48 mL, 129.8 mmol). The mixture was heated at 80 °C for 4 h. After cooling to room temperature, unreacted thionyl chloride was removed by distillation. The residue was dissolved in methanol (*ca.* 20 mL) followed by concentration using a rotary evaporator. This procedure was repeated two more times. The solid obtained then was dried under high vacuum to give **3** (2.11 g, 98%) as a yellow crystalline solid which was used without further purification; mp 230-231 °C (228-230 °C¹⁴³); ¹H NMR (400 MHz, DMSO-d₆) δ 4.11 – 4.02 (t, J = 6.8 Hz, 2H), 3.77 – 3.67 (t, J = 6.9 Hz, 2H), 3.12 (s, 9H); ¹³C NMR (100 MHz, DMSO-d₆) δ 64.99, 52.77, 36.33; HRMS (m/z): cal.122.0731; obt. 122.0732.

3.6.6. 2-hydrazinyl-N, N, N-trimethylethan-1-ammonium iodide (HTM)

To a stirred solution of hydrazine monohydrate (3.31 mL, 61.00 mmol) in THF (35 mL) at room temperature was added iodide **3** (1.50g g, 6.12 mmol) in 3 portions over an hour. After complete addition, the reaction mixture was stirred for 30 minutes and then refluxed for 4h. The solvent was removed by rotary evaporation. Excess hydrazine monohydrate was distilled off and the residue crystallized by dissolving in 5 mL of

boiling ethanol and allowing it to stand overnight in refrigerator to form the crystal of desired HTM. (4) (White hygroscopic solid, 0.63g, and 43%) mp 120-121°C ¹H NMR (400 MHz DMSO-d₆) 3.71 (2 H, m), 3.16 (9 H, s), 3.05 (2 H, dd, J 10.2, 6.6); ¹³C NMR (100 MHz, DMSO-d₆); 62.40, 52.95, 46.95; HRMS (m/z): cal.118.1339; obt. 118.1341.

3.6.7. Exhaled breath analysis

The exhaled breath of a smoker and a non-smoker was collected. That of a smoker was collected 20 minutes after smoking. The preconcentration and derivatization of exhaled breath samples from a smoker and a non-smoker were achieved by the microreactors coated with 0.5 μmol of HTM or ATM. Exhaled breath sample was pulled through the microreactor and evacuated under vacuum at a flow rate of 3.5 mL/min. The eluted solutions were analyzed by FT-ICR-MS. 4 nmol of internal standard was added to each sample and quantification achieved using calibration curves.

3.6.8. Cigarette smoke analysis

The method of derivatization of the carbonyl compounds generated from cigarette smoke is similar to that of the exhaled breath. A Kentucky reference cigarette 3R4F was burnt and the smoke collected with a 100 mL syringe fitted with rubber tubing. 60 mL of smoke was collected per puff and a total of 9 puffs were collected into a 10 Liter tedlar bag. 2 mL aliquot was taken out and injected into a 500 mL tedlar bag and made up to 500 mL with 498 mL pure air with a cylinder. The Tedlar bag was connected to the microchip loaded into which 6.25×10^{-7} mol of the HTM or ATM, evacuated, and analyzed as described on electronic cigarette aerosol evacuation with an optimal flow rate

of 3 mL/min. After evacuation, the chip is eluted into a vial having an insert with 200 μ L MeOH followed by the addition of 4 nmol internal standard. The spectra are obtained with FTICRMS and quantification of carbonyls achieved with calibration curves.

CHAPTER 4

NICOTINE IN E-CIG LIQUIDS AND DERIVED AEROSOLS

- 4.1. INTRODUCTION
- 4.2. STANDARD METHOD FOR NICOTINE COLLECTION AND QUANTIFICATION
- 4.3. EXPERIMENTAL DESIGN FOR NICOTINE SAMPLE COLLECTION AND ANALYSIS
- 4.4. RESULTS AND DISCUSSION
- 4.5. CONCLUSION
- 4.6. EXPERIMENTAL SECTION

4.1. INTRODUCTION

Nicotine is a toxic, potent alkaloid that is quickly absorbed through the skin and mucous membranes in its neutral form.¹⁴⁴ Nicotine has two nitrogen atoms capable of accepting a proton. The pyrrolidine nitrogen is more basic than the pyridine nitrogen (Figure 4.0).¹⁴⁵

Figure 4.0. The structure of nicotine (* = stereogenic center).

Nicotine plays a significant role in the development of cardiovascular disease.¹⁴⁶ Nicotine is known to constrict blood vessels and reduce the flow of blood to the hands and feet. In addition to its central nervous system effects, nicotine also inhibits the release of prostacyclin, a vasodilation prostaglandin, from vascular tissue and induces hormonal changes associated with hypothalamic pituitary axis (HPA).¹⁴⁷ The health effects, which are of major concern, include coronary artery and peripheral vascular disease, hypertension, peptic ulcer disease, and reproductive disorders.¹⁴⁸ Nicotine has been implicated in stimulating neuroendocrine tumor cell line proliferation,¹⁴⁹ a factor in the pathogenesis of lung cancer¹⁵⁰ and apoptosis prevention.¹⁵¹ A lethal dose of nicotine in humans is 30-60 mg.¹⁵²

Nicotine levels in e-liquids are intentionally formulated to create target strengths, yet measured levels may not match the manufacturer's claim. There are a variety of e-liquids, including flavored e-liquids, with different formula and

nicotine strength (typically from 0 to 36 mg/mL). The efficacy of nicotine delivery by e-cigarettes is not well understood. Consequently, there is a great public health concern regarding exposure to harmful chemicals in aerosols of e-cigarettes.^{88,153,117} Therefore, there is a need for efficient measurement of nicotine in e-liquids and in the derived aerosols of e-cigarettes as well as better evaluation of the efficacy of nicotine delivery by electronic nicotine delivery devices (ENDs).

4.1.1 Properties of nicotine

Nicotine is a colorless liquid, which turns to a yellow-brown oily liquid over time or on exposure to sunlight. It is hygroscopic and miscible with water in its basic form. It has a flash point of 95 °C and an autoignition temperature of 224 °C with a vapor pressure of 5.5 Pa at 25 °C. Nicotine is optically active and exists as two enantiomers. The naturally occurring form of nicotine has (*S*) configuration⁸⁶ and is levorotatory with a specific rotation of $[\alpha]_D = -166.4^\circ$ ((-)-nicotine). The dextrorotatory form, (+)-nicotine, is physiologically less active than (-)-nicotine. (-)-Nicotine is more toxic than (+)-nicotine. The salts of (+)-nicotine are usually dextrorotatory. The hydrochloride and sulphate salts become optically inactive if heated in a closed vessel above 180 °C. Nicotine can occurs naturally in the leaves of *Nicotina rustica* and tobacco plants.¹⁵⁴

4.1.2. Nicotine biosynthesis

Nicotine is synthesized by condensation of an intermediate in the nicotinamide adenine dinucleotide (NAD) salvage pathway and the methylpyrrolinium cation derived from ornithine via putrescine. This cation is also used for biosynthesis of tropane

alkaloids, such as hyoscyamine and scopolamine. Enzymes involved in nicotine synthesis include: ODC; ornithine decarboxylase, PMT; putrescine N-methyltransferase, DAO; diamine oxidase, AO; aspartate oxidase, QS; quinolinate synthase, and QPT; quinolinate phospho-ribosyltransferase (Scheme 4.0).

The nicotine pyrrolidine ring is derived from *N*-methylpyrrolinium cation, which is a spontaneous cyclization product of the oxidative deamination reaction from *N*-methylputrescine catalyzed by diamine oxidase (DAO). *N*-Methylputrescine is produced from putrescine by putrescine *N*-methyltransferase (PMT).¹⁵⁵ The nicotine pyridine ring is formed from the NAD biosynthetic pathway,¹⁵⁶⁻¹⁵⁷ however it is not clear whether nicotinic acid itself or a metabolite derivative is the direct precursor of nicotine. The amino acid sequence of PMT is highly homologous to the sequence of spermidine synthase (SPDS), which transfers the amino-propyl moiety of decarboxylated *S*-adenosylmethionine (dSAM) to putrescine. PMT catalyzes a transfer of the methyl moiety of *S*-adenosylmethionine (SAM) to putrescine. It is assumed that PMT evolved from SPDS after restricted alterations of critical dSAM binding amino acid residues. Tobacco DAO may have been evolved from a DAO widespread in nature by optimization of substrate specificity. The DAOs involved in nicotine and tropane alkaloid biosynthesis have higher affinity for *N*-methylputrescine than for putrescine and other symmetrical diamines.¹⁵⁸

styrene divinylbenzene polymer having a pore diameter of 50 Angstroms. It is suitable for low molecular weight compounds including nicotine. The method has some issues, such as nicotine partially escaping from the sorbent tube during sample collection process and inefficient desorption of nicotine from the sorbent. The process is also time-consuming and cumbersome. The analysis of nicotine in e-liquids by GC requires extraction of nicotine from the e-liquids using solvents such as ethyl acetate and toluene.^{23, 82, 159} Incomplete extraction will cause significant measurement errors. Recently, other methods were reported for collection of nicotine in the aerosols of e-cigarettes including flowing aerosols through filters or cold solvents.^{82, 160} We therefore sought a new method for rapid analysis and quantification of nicotine in electronic cigarettes and electronic cigarette aerosols by converting nicotine to nicotine salt (Figure 1.5) followed by rapid analysis with direct infusion FT-ICR-MS.

Figure 4.1. XAD-4 sorbent tube for nicotine sampling.

4.3. EXPERIMENTAL DESIGN FOR NICOTINE SAMPLE COLLECTION AND ANALYSIS

Equipped with a powerful analytical tool, FT-ICR-MS, and our knowledge of nicotine pkb, we reasoned that by protonating the pyrrolidine nitrogen with an aqueous inorganic acid, such as hydrochloric acid, to form the quaternary ammonium salt nicotinium chloride (Scheme 4.1), we could quantify the salt in e-cig liquids and aerosols. This approach would circumvent most of the problems associated with the conventional standard nicotine method of analysis. Protonated nicotine in both e-liquids and as collected from derived aerosols facilitates effective and quantitative analysis by FT-ICR-MS.

Scheme 4.1. Regioselective protonation of nicotine to form the nicotinium salt.

The standard nicotine sample collection method is usually suitable for nicotine samples existing in trace levels but is not suitable for aerosol sample collection. Most sorbent tubes have limited capacity (300 µg for XAD-4) and, if exceeded, may result in breakthrough. To increase the sample collection capacity in electronic cigarette aerosol generation where several puffs will generate several micrograms to milligrams of nicotine, depending on the nicotine concentration in the e-liquid formulation, we conceived the idea of having a cold trap (an impinger) containing acidified methanol into which the aerosol would be continually delivered from a smoking robot (Figure 4.2). At the end of the puffing regime, an aliquot of the solution could then be analysed for nicotine quantification. Impinger optimization was done by connecting five impingers in

series to determine the number of impingers that would be required for efficient aerosol collection with no nicotine loss. With electronic cigarette ECC02, having nicotine concentration of 15.56 mg/mL, 10 puffs (puff number lower limit) and 40 puffs (puff number upper limit) were used for the study. They were delivered separately into 5 impingers connected in series and an aliquot sample from each impinger analysed for nicotine. The capture efficiency of each impinger was calculated. The first impinger trapped 74% and 78% of the total nicotine delivered with 10 and 40 puffs respectively. The second impinger trapped between 19% and 23% while third impingers trapped 3% and 5% respectively (figure 4.3). No nicotine was delivered into impingers 4 and 5 even when the nicotine concentration in e-liquid was higher. We therefore proceeded with the use of three impingers connected in series for this study.

To test this concept for nicotine analysis, we first studied the rate of protonation of the pyrrolidine ring, since we were interested in cutting down the analysis time. In order to do this we set to measure the activation energy E_a in KJ/mol of nicotine protonation at the pyrrolidine site.

Figure 4.2. The schematic diagram of the inExpose Scireq smoking robot connected to three impingers connected in series for trapping nicotine in aerosol of e-cigarettes.

Figure 4.3. Impinger optimization: capture efficiencies of 5 impingers connected in series.

4.4. RESULTS AND DISCUSSION

4.4.1. Measurement of nicotine kinetics of protonation by NMR spectroscopy

Based on pK_b considerations, El Hellani *et al*⁶¹ reported that nicotine is predominantly present in free base form in both e-liquids and aerosols of electronic cigarettes. Nicotine in e-liquids was extracted using toluene and amenable to analysis by GC-MS. The hypothesis of this thesis work is that protonation of nicotine in e-liquids using strong acid, such as hydrochloric acid, will form a nicotinium ion (Scheme 5.3) that can be readily measured by direct infusion FT-ICR-MS. We were gratified to learn that this approach is effective and the nicotinium cation was readily quantified using FT-ICR-MS.

To determine the rate of protonation of the pyrrolidine nitrogen of the nicotine molecule, we used nuclear magnetic resonance spectroscopy. For more efficient extraction of free nicotine for NMR analysis, we chose ethyl acetate rather than toluene. To prepare a calibration curve for measurement of nicotine using ^1H NMR spectroscopy, six solutions containing different amounts of nicotine dissolved in 400 μL $\text{DMSO-}d_6$ were prepared. The concentrations of nicotine in these standards ranged from 0.78, to 39 $\mu\text{mol/mL}$. A known amount of benzene was added to each standard solution as an internal reference. The integration of the benzene hydrogen signal at δ 7.37 ppm was set at a constant 6, while the integrations of the nicotine protons between δ 8.45-8.49 ppm were recorded. A linear calibration curve (Fig. 4.4) shows a good linear dependence of $n(\text{nicotine})/n(\text{benzene})$ on $H(\text{nicotine})/H(\text{benzene})$, where n = moles and H = corresponding proton integration of NMR spectra. We used this plot as an NMR calibration curve to measure nicotine concentration in our kinetics study.

Figure 4.4. The plot of $n(\text{nicotine})/n(\text{benzene})$ vs. $H(\text{nicotine})/H(\text{benzene})$, serving as a calibration curve for ^1H NMR measurement of nicotine protonation.

The ^1H NMR spectra of 5 μL pure nicotine added to 400 μL $\text{DMSO-}d_6$ was compared to the spectra from the ethyl acetate extract in 400 μL $\text{DMSO-}d_6$ obtained after reaction of 5 μL nicotine with HCl in water for 30 min (Figure 4.5). More than 98% of nicotine was protonated and extracted into the aqueous phase based on the NMR data of this experiment.

Figure 4.5. (a) ^1H NMR spectra ($\text{DMSO-}d_6$) of neutral nicotine and (b) ^1H NMR spectra ($\text{DMSO-}d_6$) of extracted sample after 30 min of protonation in water and HCl mixture (less than 2% of free base remains in the sample).

A kinetics study on the protonation of nicotine (Scheme 5.3) was conducted using ^1H NMR by measuring the amount of nicotine at different reaction times and reaction temperatures of 0, 22, 40, and 60 $^{\circ}\text{C}$. Unprotonated nicotine was extracted with ethyl acetate from acidified (protonated) nicotine solution in water at a given temperature. With the addition of benzene as an internal standard, the amount of basic nicotine that was not protonated was measured. Complete conversion of nicotine to nicotine was achieved at 22 $^{\circ}\text{C}$ in 60 minutes (Figure 4.6).

Figure 4.6. Percent conversion of nicotine to its nicotinium salt at different temperatures over time.

The first order reaction kinetics for nicotine protonation under the experimental conditions can be written as in equation 1, where $[\text{nicotine}]$ indicates the concentration of nicotine at t minutes and k is the apparent first order reaction rate coefficient.

$$d[\text{nicotine}]/dt = -k [\text{nicotine}] \quad (1)$$

Integration of both sides of equation 1 and rearrangement affords equation 2,

$$\ln [\text{nicotine}] = -kt + \ln [\text{nicotine}]_0 \quad (2)$$

where $[\text{nicotine}]_0$ denotes the initial concentration of nicotine. Taking the natural logarithm of Arrhenius' equation yields equation 3,

$$\ln k = -E_a/RT + \ln A \quad (3)$$

where E_a is the activation energy, R is the universal gas constant, T is the reaction temperature, and A is the pre-exponential factor.

The plotted results of $\ln[\text{nicotine}]$ vs. time t at 0, 22, 40, and 60 °C demonstrated a good linear relationship in agreement with equation 2, and validated the first order reaction kinetics (Figure 4.7). Figure 4.8 shows the slope of the plot of $\ln k$ vs. the reciprocal of T and the intercept ($\ln A$). When applying equation 3, A is obtained as $2.46 \times 10^5 \text{ min}^{-1}$ and the activation energy (E_a) for nicotine protonation is $30.05 \text{ kJ mol}^{-1}$.

Figure 4.7. Dependence of $\ln [\text{nicotine}]$ on the reaction temperature T (°C).

Figure 4.8. The relationship between $\ln k$ and $1/T$ for protonation of nicotine in HCl solution.

4.4.2. Calibration curve of protonated nicotine by FT-ICR-MS

Having studied rates of nicotine protonation, we proceeded to measure nicotine in e-liquids and aerosols of electronic cigarettes using FT-ICR-MS. A nicotine calibration curve using FT-ICR-MS for quantitative measurements was obtained by plotting the ratio of the relative abundance of nicotine to an internal standard (y) against the mole ratio of the analyte to the internal standard (x). Tetraethylammonium bromide was initially selected as the internal standard because of its stability and lack of volatility, but our inability to obtain reproducible results led us to select deuterated nicotinium (nicotinium- d_3) chloride as the internal standard, which gave reproducible measurements. In addition, the deuterated nicotinium ion, $[\text{nicotine-}d_3\text{-H}]^+$, gave a strong and stable signal ion in FT-ICR-MS at $m/z = 164.1418$. In this study, a fixed amount of 7.78 nmol of protonated-deuterated nicotine was added to serially diluted protonated nicotine solutions as an internal reference to obtain a calibration curve. Figure 5.10 shows the FT-ICR-MS spectra overlay of the calibration samples at different protonated nicotine concentrations. Figure 4.9 shows the calibration curve of nicotine measured by FT-ICR-MS that was used to determine the amount of nicotine in all e-liquids as well as the collected aerosols for puff-by-puff nicotine delivery measurements. The calibration curve showed linearity between the intensity ratio of nicotine-to-nicotine- d_3 (I-Nic/I-Nic- d_3) and the molar ratio of nicotine-to-nicotine- d_3 (M-Nic/M-Nic- d_3) in the working mole ratio range of nicotine-to-nicotine- d_3 from 1 to 13 ($y = 1.1323x - 0.3315$, $R^2 = 0.99388$). The insert in Figure 4.9 shows the linearity between the intensity ratio of nicotine-to-nicotine- d_3 and the molar ratio of nicotine-to-nicotine- d_3 . Figure 4.10 shows the FT-ICR-MS spectra of standard calibration curve working solutions, each spiked with 7.78 nmol nicotinium- d_3 .

as an internal standard. The nicotine peak grows relative to the standard peak as the nicotine concentration increases. The calibration curve was used to quantify nicotine in e-liquid and their derived aerosols. To determine the limit of detection (LOD) for nicotine, a series of low concentration nicotine samples were prepared and analyzed by FT-ICR-MS. The LOD of nicotine defined as nicotine signal-to-noise ratio of 3 (S/N=3) was obtained as 1×10^{-12} mol/L.

Figure 4.9. The calibration curve of nicotine by plotting the ratio of intensity of nicotine-to-nicotine- d_3 (I-Nic/I-Nic- d_3) against the ratio of the amounts (mole) of nicotine-to-nicotine- d_3 (M-Nic/M-Nic- d_3).

Figure 4.10. Comparison of FT-ICR-MS spectra of standard calibration curve working solutions, each spiked with 7.78 nmol nicotine- d_3 as an internal standard. The nicotine peak increases relative to the standard peak as the nicotine concentration increases.

4.4.3. Calibration curve of nicotine by GC-MS

To compare our novel method of nicotine analysis with standard method for collection and analysis of nicotine in air (National Institute for Occupational Safety and Health, NIOSH 2551) using GC-MS, a calibration curve was required. Quinoline was used as internal standard and Method 2551 was modified for nicotine analysis.¹⁶² 9.9 μ L of nicotine was diluted to 10 mL ethyl acetate solution of triethylamine (desorbing solvent) to give 100mg/mL. A modified ethyl acetate solution is ethyl acetate containing 0.01% triethylamine. 100 μ L of the nicotine solution was made up to 10 mL with the desorbing solution. A serial dilution of the solution was made with a working range of containing 3.9 nmol to 0.12 μ mol of nicotine. A nicotine calibration curve using GC-MS for quantitative measurement was obtained by plotting the mole ratio of nicotine to an internal standard (y) against the peak area of the analyte to the internal standard (x). Figure 4.11 shows the calibration curve of nicotine measured by GC-MS that was used to determine the amount of nicotine in some e-liquids as well as the collected aerosols for puff-by-puff nicotine delivery measurements. The calibration curve showed an excellent linearity between the mole ratio of nicotine-to-quinoline (Amt of Nicotine/Amt IR) and peak area ratio of nicotine-to-quinoline (Area of nicotine/Area of IR) ($y=0.5641x+0.2083$, $R^2=0.99192$). Figure 4.12 shows the GC chromatogram overlay of selected calibration working samples at different nicotine concentrations.

Figure 4.11. The calibration curve of nicotine built by plotting ratio of mole of nicotine–to–quinoline against the ratio of peak area of nicotine–to–quinoline.

Figure 4.12. Comparison of GC chromatogram of standard calibration curve working solutions, each spiked with 40 nmol quinoline as an internal standard. The nicotine peak grows relative to the standard peak as the nicotine concentration increases.

4.4.4. Nicotine levels in e-liquids

To validate the method of protonation of nicotine in e-liquids and analysis by FT-ICR-MS, a known amount of nicotine was spiked into a PG/VG (50/50) mixture (zero nicotine e-liquid). One aliquot of the mixture was used for protonation of nicotine described in e-liquids and analyzed by FT-ICR-MS. The other aliquot was used for extraction of nicotine from the PG/VG mixture to ethyl

acetate and analyzed by GC-MS. The results indicated that the FT-ICR-MS analysis was within 2% difference from the spiked amount of nicotine, while the GC-MS analysis could be 10 to 15% less than the spiked amount of nicotine due to the inefficiency of ethyl acetate extraction. After validation of the method of protonation of nicotine in e-liquids, popular brands of e-liquids (shown in Table 5.4) were examined for nicotine content. The measured nicotine values for all e-liquid brands in Table 5.4 had standard deviations less than 7%. Of the 10 brands tested, we found that the range difference between the measured nicotine content and the manufacturer-specified content ranged from -2.9% to 25.2%.

Table 4.0. Results of nicotine analysis from selected commercial e-liquids. Each sample was analyzed in triplicate and the data are expressed as the average [$\pm$ SD] of the measured values.

EC code	Brand name	Model/Flavor	Country	Source of product	Vendor's Claim nicotine (mg/mL)	Nicotine (mg/mL) measured	% Difference
EL01	EVO	Blackdiamond	USA	Kiosk	6.00	6.28 $\pm$ 0.17	4.67
EL02	NicQuid	Smooththol	USA	Kiosk	6.00	6.54 $\pm$ 0.41	9.00
EL03	Perfected Vapes	Clearwater	USA	Kiosk	6.00	7.04 $\pm$ 0.40	17.33
EL04	Halo	Menthol Ice	USA	Online	6.00	6.53 $\pm$ 0.20	8.83
EL 05	Halo	Mocha Café	USA	Online	6.00	6.42 $\pm$ 0.05	7.00
EL06	Halo	Southern Cafe	USA	Online	6.00	6.26 $\pm$ 0.05	4.33
EL07	NJOY	Classic Tobacco	USA	Online	10.00	12.25 $\pm$ 0.53	25.20
EL08	NJOY	Classic Tobacco	USA	Online	15.00	16.22 $\pm$ 0.72	8.13
EL09	VaporFi	Classic Tobacco	USA	Online	18.00	17.47 $\pm$ 0.45	-2.94
EL10	VaporFi	Classic Tobacco	USA	Online	36.00	37.22 $\pm$ 1.26	3.39
ECC01	Blu	Magnificent	USA	Online	13-16	14.14 $\pm$ 0.73	8.76 to 11.63
ECC02	Blu	Menthol Classic Tobacco	USA	Online	13-16	15.67 $\pm$ 1.01	20.53 to 2.06)

4.4.5. Nicotine levels in e-cigarette aerosols

The smoking robot was used to generate aerosols from blu plus® e-cigarette filled with a number of e-liquids. The generated aerosol flowed through three

traps (impingers) connected in series. Each experiment was performed in triplicate and the data were reported as the average [$\pm$ SD] of the measured values. Figure 4.13 shows the plots of nicotine in aerosol vs. the puff number for five different e-liquids with measured actual nicotine level from 6.28 mg/mL to 37.22 mg/L shown in Table 5.4. There is a good linear relationship between the amount of nicotine in the aerosols and the puff number in the studied puff number range. The measurements indicate the difference of nicotine in aerosols even for 10 puffs for the blu plus® magnificent methanol (measured nicotine 14.14 mg/mL) and classic tobacco cartridges (measured nicotine 15.67 mg/mL) even though both cartridges were labeled as 13-16 mg/mL of nicotine level. Table 5.5 shows a comparison of measured nicotine in aerosols of e-cigarettes collected by this impinger method and the NIOSH 2551 sorbent method. The results indicate that nicotine collected by the sorbent tubes were consistently lower than that collected by the impinger method (Table 5.5). Figure 4.14 shows plots of measured nicotine concentration in the e-liquids vs. the amount of nicotine in aerosols at constant puff numbers of 20, 30, and 40. Again, there is a good linear relationship between measured nicotine concentrations in these e-liquids vs. measured nicotine amount in aerosols at the same total puff numbers. These results show that the amount of nicotine in aerosols depends on both its level in e-liquids and number of puffs.

The average nicotine levels in aerosols for a single puff at the puff volume of 91 mL/puff were from 18 μ g (eVo e-liquid, 6.28 mg/mL nicotine) 72 μ g (blu plus® classic, 15.67 mg/mL nicotine). Previous publications indicate nicotine levels from a single puff volume of 70 mL in e-liquids was between 1.7 and 51.3

μg.¹⁷ However, both the nicotine levels in e-liquids and cartridges and the puff volume are different. Therefore, it is hard to make a meaningful comparison. A dose inhaled from one conventional cigarette smoke was measured from 1.54 to 2.0 mg.²³ If assuming a series of 10 puffs of e-cigarettes is equivalent to smoking one tobacco cigarette, the e-liquids might deliver 0.18 mg to 0.72 mg nicotine, which is much lower than one tobacco cigarette. Our results confirm previously reported findings.¹⁶³ However, if the e-liquid with the highest nicotine level of 37.22 mg/mL is used, the e-cigarette may deliver 2.3 mg for 10 puffs of aerosols, which is comparable with nicotine inhaled from the mainstream smoke of one tobacco cigarette. A survey of e-liquid market indicates that some e-liquid manufactures provide the highest strength of 36 to 42 mg/mL of nicotine in e-liquids.

Figure 4.13. Nicotine delivery profile of e-cigarette cartridges and e-liquids with different nicotine levels.

Figure 4.14. The relationship between nicotine aerosol and nicotine levels in e-liquids at constant puff numbers.

Table 4.1. Comparison of measurements of nicotine in aerosol samples collected by sorbent tube and impinger methods.

E-liquids		EL01 (mg)	ECC01 (mg)	EL10 (mg)
10 puffs	Sorbent	0.104	0.184	2.07
	Impinger	0.214	0.422	2.51
20 puffs	Sorbent	0.214	0.406	2.86
	Impinger	0.488	1.03	3.29

4.5. CONCLUSION

We have developed a new method for collection and analysis of nicotine in the electronic liquids and derived aerosols. The method involves protonation of nicotine to exploit use of FT-ICR-MS for nicotine quantification. The kinetics of nicotine protonation was determined to be a first order reaction. The activation energy (E_a) for nicotine protonation was found to be 30.05 kJmol^{-1} . The measured nicotine levels of commercial e-liquids were within a difference range of -2.94% to 25.20% from the manufacturer specified values. Nicotine in aerosols linearly increased as the number of

puffs increased. Nicotine in aerosols also linearly increases with the nicotine concentration in e-liquids at the same puff number.

Unlike NIOSH 2551 GC-MS method, nicotine analysis in e-liquids using FT-ICR-MS does not require solvent extraction. Aerosol sample collection involving connection of three impingers in series is more efficient than NIOSH 2551 sorbent tubes. Also, analysis is of both the e-liquids and derived aerosol is easier, faster, and overall this method is more suitable for e-liquid aerosols than NIOSH 2551 method.

4.6. EXPERIMENTAL SECTION

4.6.1. Experimental materials

One popular electronic cigarette, blu plus® battery with two different flavored blu plus® cartridges (Classic Tobacco, Magnificent Menthol), was selected for this study. Ten popular e-liquids in the US market: three different flavored Halo e-liquids, two NJOY e-liquids, two VaporFi e-liquids with different levels of nicotine, an eVo Black diamond e-liquid, a Perfected Vapes, Clearwater e-liquid, and a Smooththol Nic Quid e-liquid, were also purchased. Table 1 lists detailed information of these e-liquids and cartridges. 37% Hydrochloric acid, nicotine, and methanol were acquired from Sigma Aldrich. Deuterated nicotine was purchased from CDN Isotopes Inc. XRD-4 sorbent tubes for trapping nicotine were purchased from SKC company. The ultra-pure water used in this study was prepared using a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA).

4.6.2. FT-ICR-MS

An FT-ICR-MS instrument (Finnigan LTQ-FT, Thermo Electron, Bremen, Germany) equipped with a TriVersa NanoMate ion source (Advion BioSciences, Ithaca, NY) fitted with an electrospray chip (nozzle inner diameter 5.5 μm) was used for all mass spectral analyses. The TriVersa NanoMate was operated in positive ion mode by applying 2.0 kV with no head pressure. Initially, low-resolution MS scans were acquired for 1 min to ensure the stability of ionization, after which high mass accuracy data were collected using the FT-ICR analyzer, where MS scans were acquired for 5 min and at the target mass resolution of 100,000 at 200 m/z.

4.6.3. GC-MS analysis of nicotine

An Agilent Technologies GC instrument equipped with a G4513A automatic sampler, Agilent 5975 series Mass Selective Detector in Electron Impact a TRACE 1310 GC with a split/splitless injector The GC had an Agilent 190915-433 column (30 m $\times$ 0.25 mm $\times$ 0.25 μm film thickness) Carrier gas helium flow rate was 1.5 mL/min. Column temperature was 55 $^{\circ}\text{C}$ for 4 min, then increased by 55 $^{\circ}\text{C}/\text{min}$ up to 220 $^{\circ}\text{C}$ and was held at 220 $^{\circ}\text{C}$ for 3 min. The total running time was 10 min. The samples were split injected with split flow of 100 mL/min and a split ratio of 10 to 1.

4.6.4. Measurement of nicotine NMR spectroscopy

To prepare a calibration curve for measurement of nicotine using ^1H NMR spectroscopy, six solutions containing different amounts of nicotine dissolved in 400 μL DMSO- d_6 were prepared. The concentrations of nicotine from solution 1

to solution 6 were set at 0.78, 1.56, 3.11, 7.78, 15.55, and 38.88 $\mu\text{mol/mL}$, respectively. Benzene was added to each solution as an internal reference at a concentration of 56.11 $\mu\text{mol/mL}$. ^1H NMR spectra were obtained using a Varian 7600-AS instrument (400 MHz for ^1H). The integration of the benzene hydrogen signal at δ 7.37 ppm was set at a constant 6, while the integrations of the nicotine protons between δ 8.45-8.49 ppm were recorded. A linear calibration curve was built and Fig. 3.2 shows a good linear dependence of $n(\text{nicotine})/n(\text{benzene})$ on $H(\text{nicotine})/H(\text{benzene})$, where n = moles and H = corresponding proton integration of NMR spectra. We used this plot as an NMR calibration curve to measure nicotine concentration in the following kinetics study of nicotine protonation

4.6.5. Kinetics measurement of nicotine protonation

A kinetics study on the protonation of nicotine was conducted by measuring the amount of nicotine at different reaction times at reaction temperatures of 0, 22, 40, and 60 $^{\circ}\text{C}$ using ^1H NMR. To a solution of nicotine (5.0 μL , 31.1 μmol) in water (291 μL) at a given temperature, 37% HCl (3.8 μL , 37.4 μmol) was added. The mixture was magnetically stirred for a specified time. The reaction was terminated at a set time by addition of ethyl acetate (400 μL) to extract any unprotonated nicotine. The organic layer then was separated from the aqueous layer with the aid of a pasture pipette and evaporated under vacuum. DMSO- d_6 (400 μL) was added to the extracted neutral nicotine and the solution was analyzed by ^1H NMR using benzene as the internal reference for quantification.

4.6.6 Analysis of nicotine in e-liquids

Typically, 10 μL of each e-liquid was added to a solution of 37% HCl (5.0 μL , 49.2 μmol) in a mixture of MeOH (457.5 μL) and water (37.5 μL) at room temperature. The reaction mixture was stirred for a minimum of 30 minutes to protonate nicotine. Then, 10 μL of the reaction solution was removed and directly analyzed, in triplicate, by FT-ICR-MS. The measured nicotine was expressed as the average [$\pm\text{SD}$] values.

4.6.7. Collection and analysis of nicotine in e-cigarette aerosols

A software-controlled (FlexiWare) cigarette-smoking robot (CSR) (Sci-Req, Montreal, CAN) was used to generate aerosols from blu plus® electronic cigarette battery (fixed voltage 3.7V) with e-liquids in the refillable mystic® cartridges. The puffing topography of e-cigarette users has been intensively studied.¹¹⁴⁻¹¹⁵ The mean puff duration, puff flow rate, and puff volume varied significantly among the subjects. The puffing protocol in this work consisted of 4 seconds of puff durations, 91.1 mL of puff volumes, and 26 seconds of puff intervals to closely mirror typical puffing topography of e-cigarette users.¹¹⁴⁻¹¹⁵ Aerosols generated by the smoking robot flowed through a series of three impingers as shown in Figure 1 of the schematic diagram. Each impinger was charged with a solvent mixture of MeOH (91.5 mL), water (7.5 mL), and 37% HCl (1 mL). Initial experiments for collection of the aerosols with a series of four impingers indicated no nicotine in the fourth impinger. These experiments verified that the use of three impingers was sufficient for collection of all the aerosolized nicotine. After collection, 3 mL nicotinium-d₃ was added to 500 mL of the solution from each impinger as an internal standard. The solution was then analyzed using FT-ICR-MS. To compare

this collection of nicotine in aerosols with standard sorbent adsorption method (NIOSH 2551), nicotine in e-cigarette aerosols was also collected using XRD-4 sorbent tubes. NIOSH 2551 instruction was followed for collection of nicotine by the sorbent tubes, desorbing nicotine from the sorbent in ethyl acetate solution and analysis by GC-MS.

CHAPTER 5

SUMMARY AND FUTURE WORK

- 5.1. SUMMARY
- 5.2. FUTURE DIRECTION

5.0. SUMMARY AND FUTURE WORK

This research focused on using a silicon-based microreactor coated with derivatization reagents combined with either FT-ICR-MS or GC-MS for chemoselective capture, derivatization and analysis of trace level aldehydes and ketones. Sample sources included electronic cigarette aerosols, tobacco cigarettes and human expired breath. The quantification of nicotine in e-cig liquids and derived aerosols was also studied using an impinger set up.

5.1. Summary

We have used our powerful silicon microreactor coated with the aminoxy reagent AMAH as a derivatization reagent (coating) to capture and derivatize carbonyl compounds generated during aerosolization of electronic liquids. The oximation reaction of the functional cationic aminoxy compounds shown below with gaseous carbonyl species is a significant part of this dissertation.

Figure 5.0. ATM, ADMH, AMAH, HTM and nicotinium chloride salt

The installation of quaternary ammonium functional group on aminoxy (ADMH, ATM, and AMAH) and hydrazine (HTM) reagents, as well as nicotine analyte (Figure 5.0), enabled electrospray ionization efficiency of FT-ICR-MS. The aminoxy and hydrazine functional groups enabled chemoselective derivatization of the carbonyl compounds. We

have used AMAH extensively to analyze carbonyl compounds in tobacco and electronic cigarettes.

We have demonstrated that the aerosolization of neat propylene glycol (PG) generated the highest level of acetaldehyde followed by formaldehyde and acetone. Hydroxyacetone was also produced. A low level of crotonaldehyde was also generated and this may have been produced from the aldol condensation of two molecules of acetaldehyde. This is the first time crotonaldehyde is reported in aerosol of neat propylene glycol. No acrolein was detected in neat PG. On the other hand neat glycerol (VG) also produced highest level of acetaldehyde followed by formaldehyde, acetone, and acrolein. No crotonaldehyde was produced. The use of a silicon-based microreactor for preconcentration of these trace-level carbonyl compounds in e-liquid aerosols and subsequent analysis with FT-ICM-MS represent a major contribution to the on-going research in analysis of carbonyl compounds in electronic cigarettes.

The carbonyl compounds produced during vaping of e-liquids increased as the vaping power increased. Consequently, the amount of carbonyl compounds detected in electronic cigarette aerosols increased as the vaping power (which is a function of coil resistance and vaping voltage) increased.

We also developed HTM to derivatize carbonyl compounds in expired breath using the silicon-based microreactor. This hydrazine salt has higher capture efficiency for α,β -unsaturated aldehydes than its aminoxy analog ATM. Consistent with this observation, HTM activation energies are also lower with α,β -unsaturated aldehydes than those of ATM.

We have also regioselectively protonated the pyrrolidine nitrogen of nicotine with

an aqueous inorganic acid to form nicotinium chloride, which then was quantified with FT-ICR-MS. This approach solved most of the problems associated with the conventional standard nicotine method of analysis. Protonation of nicotine in both e-liquids and their derived aerosols facilitates effective and quantitative analysis by FT-ICR-MS.

NIOSH method utilizes a sorbent tube for nicotine sample collection and GC for analysis using quinoline as internal standard. The sorbent tube is not particularly suitable for collecting nicotine in e-liquid aerosols because these tubes have limited sample capacity. This limitation makes collection of aerosol samples where over 40 puffs need to be delivered, difficult. The use of impinger traps in this research mitigated this problem. The results of this research indicated that nicotine collected by the sorbent tube method were consistently lower than that collected by the impinger method. The lower values of the NIOSH method were induced by the escape of nicotine from the sorbent tube and incomplete desorption of nicotine from the sorbent. Also, NIOSH method involved extracting nicotine several times and this resulted in long sample preparation and consumption of high volume of solvent. Our new method did not involve nicotine extraction but direct infusion mass spectrometry. Therefore solvent volume and analysis time were significantly reduced. This impinger method of nicotine collection in e-liquid aerosols and analysis by FT-ICR-MS is therefore a remarkable novel contribution to nicotine analysis research.

5.2. Future directions

Our initial results supported the formation of carbonyl compounds from thermal decomposition of humectants. The contribution of thermal decomposition of other e-liquid additives to the generation of these carbonyl compounds during aerosolization

Scheme 5.1. Decomposition of 2-methylbutyraldehyde to generate acrolein and propionaldehyde.

α,β -Unsaturated aldehydes, such as 4-hydroxyhexenal (4HHE) and 4-hydroxyhexenal (4HNE), are known lung cancer biomarkers. Our research group has hitherto used ATM to analyze these unsaturated aldehydes for lung cancer diagnosis. Since all experimental results so far in this work have indicated that HTM is more reactive towards α,β -unsaturated aldehydes than the other hydrazine reagents, the use of this novel hydrazine reagent to analyze 4HHE and 4HNE needs to be explored. This idea will require collaboration with medical personnel for collection and experimentation on expired breath samples of lung cancer patients.

A significant limitation in this research is the use of internal standard. We introduced an internal standard at the last step after the elution of the microreactor, prior to analysis using FT-ICR-MS or GC. This is because previous studies showed that more than 98% the acetone *d*-₆ internal standard introduced to the tedlar bag was recovered at the end of evacuation of the Tedlar bag.^{54, 129} Yet, an internal standard is best added at the very beginning of any analytical quantification studies including our own. This is to compensate for variations in sample preparation and instrument variation. Since we added it at the very end, it only compensated for instrument variation. Also, this research

can be greatly improved by using isotopically labeled version of each molecule to be quantified in a multiplex quantification. That means each analyte should have its own isotopically labeled internal standard for excellent quantification. Also, the recovery of an analyte in an analytical sample should be performed. It is the detector response obtained from an amount of the analyte added to and extracted from the matrix, compared to the detector response for the true concentration of the pure authentic standard (seized materials). Although this has been established in form of capture efficiency of the system but this should be periodically reassessed. Recovery of the analyte in a sample needs not to be 100%. However, recovery extent of both the internal standard and the analyte needs to be consistent and reproducible.

Another limitation of this research is that an aminoxy or a hydrazine-coated microchip can only identify/detect the carbonyl compounds components of analytical samples with accurate chemical formula, it cannot identify other components of the sample like dienes, furans, alcohols, ether, thiols etc. This limitation can be circumvented by incorporation of other chemoselective reagents into the microreactor. For example, a very powerful dienophile can derivatize cis-1, 3-dienes.

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CURRICULUM VITAE

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ACADEMIC & WORK EXPERIENCE

University of Louisville, Louisville, KY
Graduate Research Assistant

August 2012 -Present

- Method development, derivatization, analysis and quantification of trace-level volatile organic compounds (VOCs) using Fourier transform ion cyclotron mass spectrometry, GC-MS, NMR, and FT-IR
- Wafer processing including photolithography, alignment, deep reactive ion etching, wet etching and thermal oxidation
- Multi-step organic synthesis of chemoselective reagents for the capture of trace volatile aldehydes and ketones in complex mixtures, such as breath, cigarette and e-cigarette aerosols
- Maintain laboratory instruments/equipment; enforce University chemical lab safety practices
- Coordinate and manage group research activities; develop and execute laboratory work plans/schedules
- Maintain high quality laboratory documentation

Tennessee State University, Nashville, TN
August 2009- December 2011
Graduate Teaching Assistant

- Supervision of undergraduate laboratory courses in general Chemistry
- Synthesis, characterization, and analysis of novel compounds having biological activity

Coates Brothers (WA) Ltd, Lagos Nigeria
Coatings Chemist

March 2005- August 2009

- Color matching and formulations of polyester-based coatings
- Support scale-up from lab to production scale, including process development
- Quality control, rheology of polyester-based coatings and varnishes, measurements of dispersion, viscosity, gloss, corrosion resistance, scratch test, fastness and bleeding of coating inks
- Certifying production output to make sure that all the quality specifications were met for packaging
- Technical services involving troubleshooting for coating products
- Writing detailed reports and maintaining high quality laboratory documentation

SKILLS

Computer & Technology Skills

- ChemDraw, Microsoft Office
- Databases: SciFinder Scholar, Reaxys

Instrumentation

- Spectroscopy: GC-MS, NMR, FT-IR, FT-ICR-MS
- Microfabrication: Photolithography, Front and back optical alignment, Deep Reactive Ion Etching (DRIE), Wet etching
- Metrology: Dektak surface profilometer, Ellipsometry

EDUCATION & RESEARCH

University of Louisville, KY

August 2012- November 2017

- Philosophy in Chemistry
Dissertation (in progress): Chemoselective reagents for capture of trace-level volatile aldehydes and ketones in breath using a microchip approach
Advisors: Michael H. Nantz, PhD (Chemistry) & Xiao-An Fu, PhD (Chemical Engineering)

Tennessee State University, Nashville, TN

August 2009 – December 2011

- MS Chemistry
Thesis: Synthesis of new fluorinated hexahydroquinoline derivatives as potential calcium channel modulators
- **Advisor:** Cosmas O. Okoro, PhD

Federal University of Technology, Akure, Nigeria

February 1999-December 2003

- B. Tech. (Hons) Industrial Chemistry
Project: The Chemical composition and mineral content of sorghum bicolor L stem used as a color additive in food
Advisor: Abayomi Adetuyi, PhD

PUBLICATIONS

- Mumiye A. Ogunwale, Mingxiao Li, Mandapati V. Ramakrishnam Raju, Yizheng Chen, Michael H. Nantz, Daniel J. Conklin and Xiao-An Fu. Aldehyde detection in electronic cigarette aerosols. *ACS Omega* **2017**, 2, 1207-1214.
- Mumiye A. Ogunwale, Yizheng Chen, Whitney S. Theis, Michael H. Nantz, Daniel J. Conklin and Xiao-An Fu. A novel method of nicotine quantification in electronic cigarette liquids and aerosols. *Anal. Methods* **2017**, 9, 4261-4266.
- Mumiye A. Ogunwale, Daniel J. Conklin, Yizheng Chen, Michael H. Nantz, Xiao-An Fu, Daniel W. Riggs, Pawel Lorkiewicz, Aruni Bhatnagar, and Sanjay Srivastava. Electronic cigarette-generated aldehydes: The contribution of e-liquid components to their formation and the use of urinary aldehyde metabolites as biomarkers of exposure. *Aerosol Science & Technology* (Submitted)

- Mumiye A. Ogunwale, Xiao-An Fu and Michael H. Nantz. 2 Hydrazinyl N, N, N-trimethylethan ammonium iodide: a chemoselective reagent for isolation of volatile aldehydes and ketones using a microreactor approach. (Manuscript in preparation for Analyst).
- Mumiye A. Ogunwale, Ralph J. Knipp, Michael H. Nantz, and Xiao-An Fu. Reaction kinetics of quaternary ammonium aminoxy compounds with carbonyl compounds. (*Manuscript in preparation for ACS Physical Chemistry A*)
- Cosmas O. Okoro, Mumiye A. Ogunwale and Tasneem Siddiquee. Synthesis of Some New Fluorinated Hexahydroquinoline and Acridinedione Derivatives in Trifluoroethanol. *Appl. Sci.* **2012**, 2(2), 368- 374.

CONFERENCE PRESENTATIONS

- Mumiye A. Ogunwale; Xiao-An Fu; Michael H. Nantz. “2-Hydrazinyl-N, N, N-trimethylethan-1- ammonium iodide: A reagent for chemoselective isolation of volatile aldehydes and ketones from exhaled breath” 253 ACS National Meeting & Exposition, San Francisco, CA, (Oral, final paper number: ANYL 468), April 2017
- Mumiye A. Ogunwale; Yizheng Chen; Whitney S. Theis; Michael H. Nantz; Daniel J. Conklin; Xiao-An Fu. “Nicotine in Electronic Liquids and aerosols” NIH-FDA TCORS Grantee Meeting, Bethesda, MD, November, 2016 (Poster)
- Mumiye A. Ogunwale; Mingxiao Li; Mandapati V. Ramakrishnam Raju; Michael H. Nantz; Daniel J. Conklin; Xiao-An Fu “Aldehyde Detection in Electronic Cigarette Aerosols” ACS 2016 Central Regional Meeting (CERM), Covington, KY, May 2016 (Poster)
- Mumiye A. Ogunwale; Mingxiao Li; Mandapati V. Ramakrishnam Raju; Michael H. Nantz; Daniel J. Conklin; Xiao-An Fu. “Detection of carbonyl Compounds in E-cigarettes Vapor” NOBCCChE Annual Conference, September 2015 (Poster)
- Mumiye A. Ogunwale and Cosmas O. Okoro. “Synthesis of Novel Trifluoromethylated Hexahydroquinoline Derivatives as Potential Calcium Channel Modulators” 41st ACS National Meeting & Exposition, Anaheim, CA, United States, MEDI-142 (Poster), March 2011

AWARDS

- Institute for Molecular Diversity & Drug Design (IMD3), University of Louisville Travel Award (April, 2017)
- Graduate Research Assistantship (August 2014 to date)
- NOBCCChE Advancing Science Travel Grant (August 2015)
- University of Louisville Pre doctoral Fellowship award (August 2012- July 2014)
- RCCG Agape House, Nashville, Tennessee Initiative for Academic Excellence Scholarship Award (2009)
- Federal Government (Nigeria) Academic Excellence Scholarship Award (2001/2002 Academic session)

CERTIFICATION

- Six Sigma Green Belt (Institute of Industrial and Systems Engineers) -August 2017

PROFESSIONAL ASSOCIATION

- American Chemical Society (member)
- Society for Research on Nicotine and Tobacco (member)
- National Organization for Professional Advancement of Black Chemists and Chemical Engineer NOBCChE (Member)

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