AMERICAN UNIVERSITY OF BEIRUT

Tobacco Smoking: Phenols and Cyanide Comprising The Borderline Between Narghile and Cigarettes

by
RASHA ABDUL HALIM

A thesis
submitted in partial fulfillment of the requirements
for the degree of Master of Science
to the Department of Chemistry
of the Faculty of Arts and Sciences
at the American University of Beirut

Beirut, Lebanon
July 2010

AMERICAN UNIVERSITY OF BEIRUT

Tobacco Smoking: Phenols and Cyanide Comprising The Borderline Between Narghile and Cigarettes

by

RASHA ABDUL HALIM

Approved by:

____

Dr. Najat A. Saliba, Associate Professor — Advisor
Chemistry

____

Dr. Alan Shehadeh, Associate Professor — Member of Committee
Mechanical Engineering

____

Dr. Digambara Patra, Assistant Professor — Member of Committee
Chemistry

Date of thesis defense: July 20, 2010

AMERICAN UNIVERSITY OF BEIRUT

THESIS RELEASE FORM

I, Rasha Abdul Halim

☐ authorize the American University of Beirut to supply copies of my thesis to libraries or individuals upon request.

☐ do not authorize the American University of Beirut to supply copies of my thesis to libraries or individuals for a period of two years starting with the date of the thesis defense.

Signature

Date

// AN ABSTRACT OF THE THESIS OF

Rasha AbdulHalim for Master of Science
Major : Chemistry

Title : Tobacco Smoking : Phenols and Cyanide Comprising The Borderline Between Narghile and Cigarettes

Narghile is becoming one of the most prevailing trends for tobacco smoking especially in the Middle East. Although little studies have been conducted on narghile, we expect to find hazardous chemicals similar to those in cigarettes. However, due to their different structures and mode of smoking and heating, this study is interested in identifying the chemicals, particularly phenols and hydrogen cyanide, which draw the line between cigarettes and narghile.

The source of heating the tobacco inside the narghile head, charcoal, and the high levels of sugar in the tobacco constitute the major difference between narghile and cigarettes. Although charcoal's temperature ranges between 700°C-900°C similar to the tobacco inside the cigarettes, this indirect heating of the mo'assel will cause the temperature of the tobacco inside the narghile head to drop to 450°C[43]. McGrath et al.[27] showed that phenols, hydroquinone, resorcinol, catechol and cresols are produced in higest amounts at pyrolysis temperatures ranging between 350°C and 450°C[27].

Phenols will be extracted[21] from narghile filters collected at the Aerosol Lab, separated from other matrices using PS-DVB SPE cartridges[30], derivatized[30] and analyzed, after derivatization, using GC-MS[27].

As for HCN, it's pyrolyzed at temperatures ranging between 700°C-900°C[19] which implies that low amount of HCN will be detected. HCN in narghile filters will be complexed with pyridine-pyrazolone solution and analyzed using UV-Vis spectrophotometry[26].

In this study, results showed that HCN was either not present in the smoke or present below our detection limits. As for phenols, we were able to identify phenol, catechol and hydroquinone from particle phase narghile sample, but we were able to quantify only phenol with a concentration of 36 μg/narghile session.

Contents

Abstract **vi**

Contents **vii**

List Of Figures **ix**

List Of Tables **x**

1 INTRODUCTION **1**

2 HCN **7**
2.1 Historical Background . . . 7
2.2 HCN Sources . . . 8
2.3 HCN Chemical and Physical Properties . . . 10
2.4 Health Hazards of HCN . . . 11
2.5 Mechanisms of HCN Formation . . . 13
2.5.1 HCN coming from burned tobacco . . . 13
2.5.2 HCN coming from Charcoal Pyrolysis . . . 17
2.6 Standard Analytical Methods: Overview . . . 20
2.7 Materials and Methods . . . 21
2.7.1 Apparatus . . . 21
2.7.2 Materials and Reagents . . . 22
2.7.3 Method of color development . . . 23
2.7.4 Calibration curve . . . 23
2.7.5 Tobacco Smoke Preparation and Sampling . . . 25
2.7.6 Sampling Mainstream (Sidestream) Charcoal . . . 27
2.7.7 Sampling mainstream and sidestream charcoal for artifact verifications . . . 28
2.8 Results . . . 29
2.8.1 Cigarettes . . . 29
2.8.2 Narghile . . . 30
2.9 Quality control . . . 30
2.9.1 Validation of experimental and analytical procedures . . . 31
2.9.2 Assessing saturation of the basic solution and adequacy of the residence time . . . 31
2.9.3 Assessing saturation of filter pads . . . 32

2.9.4 Assessing effect of pH on the stability of the obtained blue color 32
2.9.5 Minimizing interferences 33
2.10 Discussion of Results 33
2.10.1 Sources of HCN and Temperature Effect 33
2.10.2 Artifact effect 35
2.10.3 Effect of glycerol on trapping HCN 38

3 Phenols 39
3.1 Introduction 39
3.2 Sources of Phenols 41
3.3 Chemical and Physical Properties 42
3.4 Health Hazards 44
3.5 Mechanisms of Phenol Formation 44
3.5.1 Formation of phenols and nitrophenols during daytime 46
3.5.2 Formation of phenol and nitrophenol during the night 48
3.6 Method Development and Measurement of Phenols in Narghile Smoke 51
3.6.1 Materials and Methods 55
3.6.2 Extraction, Cleaning and Detection of Phenols from Narghile Smoke 58
3.6.3 Polystyrene Divinylbenzene SPE cartridges 68
3.6.4 Derivatization 69
3.6.5 Tobacco Smoke Preparation and Sampling 72
3.7 Quality Control 74
3.7.1 Determination of percent recovery (%R) 74
3.7.2 Blank 76
3.7.3 Validation of experimental and analytical procedure 77
3.8 Results 79
3.8.1 Comparing Phenols between Cigarettes and Nargileh Smoke . 82

4 Conclusion and Future Work 83

References 94

List of Figures

1.1 The burning cigarette. 3

2.1 Hydrolysis of amygdalin. 9
2.2 Cyanogenic glycosides in major edible plants (JECFA, 1993) Amygdalin occurs in (among others) almonds, dhurrin in sorghum, linamarin in cassava, lotaustralin in cassava and lima beans, prunasin in stone fruits, and taxiphyllin in bamboo shoots. 10
2.3 Cyanide binds the terminal enzyme of the cytochrome oxidase enzyme system. The enzyme system is located within the inner lamina of the mitochondria. The blockade interrupts the electron flow through the cytochrome oxidase system, thereby disrupting ATP production and both mitochondrial and cytoplasmic ionic balance. 12
2.4 Suggested pyrolysis reactions for 2,5-piperazinedione 14
2.5 Decomposition mechanism of (1) pyrrolidine and (2) N-methy pyrrolidine 15
2.6 Decomposition mechanism of (1) 2-azetidinone and (2) 2,4-diazetidinone 16
2.7 Decomposition mechanism of (A) β-aminobutyric acids and (B) γ-aminobutyric acids 16
2.8 Formation of HCN from glutamic acid 17
2.9 Miller's mechanism for HCN formation 18
2.10 The pyrolysis of coal-N leads to release of all nitrogen functionalities found in coal. Reaction A leads to pyridine, B to amine/quaternary nitrogen, C to 2-pyridone and D to pyrrole 19
2.11 Pyrolysis of pyrrole and pyridine[19] 19
2.12 Complexation mechanism, formation of glyoxal. 21
2.13 Complexation mechanism and formation of final complex. 21
2.14 Hydrogen cyanide calibration curve using a range of standards from 12.75 to 102.05ppb. 25
2.15 Mainstream smoke captured in a bubbler containing 0.1M NaOH. . . 27
2.16 Formation of cyanohydrin 36
2.17 Structures of Lactonitrile, isobutyraldehyde, and acrolein cyanohydrins respectively 36

3.1 Chemical structures of phenolic compounds. 40
3.2 Resonanace structures of phenoxide ion. 43
3.3 Formation mechanism from benzene/benzene derivatives 47

3.4 Thermal decomposition of N_2O_5; M being a third body mainly air, $O_2$and N_2 acts to collisionally stabalizie the association complex . . . 47
3.5 Formation mechanism of nitrophenol from phenol 48
3.6 Formation mechanism of nitrophenol from Toluene 48
3.7 Atkinson's mechanism for nitrophenol formation 50
3.8 Bolzacchini's mechanism for formation of nitrophenol 51
3.9 Formation of nitrophenol from o-cresol 51
3.10 Structural formula of two polyphenols, quercetin dihydrate and chlorogenic acid hemihydrate 52
3.11 GC-MS full scan chromatogram of a standard at a concentration of 3.125ppm 60
3.12 GC-MS full scan chromatogram of narghile filters extracted using ethylacetate 61
3.13 GC-MS full scan chromatogram of cigarette filters extracted using ethylacetate 62
3.14 GC-MS selected ion current profile for phenol from a standard at 3.125ppm 62
3.15 GC-MS selected ion current profile for phenol from narghileh sample . 63
3.16 GC-MS selected ion current profile for phenol from a cigarette sample 63
3.17 GC-MS selected ion current profile for catechol (rt 10.26) and resorcinol (rt 11.71) from a standard at 3.125ppm 64
3.18 GC-MS selected ion current profile for catechol (rt 10.28) and resorcinol (rt 11.71)from narghileh samples 64
3.19 Selected ion current profile for catechol (rt 10.28) and resorcinol (rt 11.72) with MS of catechol, from a cigarette sample 65
3.20 Selected ion current profile for catechol (rt 10.28) and resorcinol (rt 11.72) with MS of resorcinol, from a cigarette sample 65
3.21 Effect of acidity on extracton efficiency of internal standard phenol-d6 66
3.22 Variation of Area with volume of extraction as observed for 4 trials . 67
3.23 Formation mechanism of trimethylsilylether of phenols upon BSTFA addition to give glyoxal 72
3.24 Narghile set up 73
3.25 Filter set-up 74

List of Tables

2.1 Volume (μl) required for the preparation of working stock solutions. 24
2.2 Mass of HCN (μg/g) in the gas and particle phase for mainstream smoke cigarette 30
2.3 Average yield of HCN (μg/g) in gas and particle phase for six runs compared to reported yields of HCN in mainstream cigarette. 31
2.4 Mass of HCN (μg/g) in mainstream cigarette smoke 32
2.5 Variation of absorbance with pH 33
2.6 Mass of HCN (μg/g) collected in the gas phase from both mainstream and sidestream smoke 34

3.1 Dissociation constants,K_a, for phenol, p-cresol and p-nitrophenol . . . 43
3.2 Wavelength program for HPLC fluorescence 54
3.3 Thermo Trace GC-Ultra with AI 3000 autoinjector and ITQ-900 ion trap MS 57
3.4 Volume of acid needed to prepare three solutions, of 100ml final volume, at different pH 66
3.5 The average %recovery based on 3 trials of 5 different concentrations spiked on glass filters using the developed analytical method 76
3.6 Mass(ppm) of phenolic compounds obtained from cigarette smoke reported from three sessions. 78
3.7 Mass(ppm) of phenolic compounds obtained from cigarette smoke conducted by our lab compared to other two studies. (a) David Ashley; (b) Count et al 79
3.8 The average R^2 of the Phenols for direct and extracted calibration curve. 80
3.9 Phenol concentration in eleven sessions expressed in ppm and normalized to mg/session 81
3.10 Continue Table3.9 81
3.11 Continue Table3.10 81
3.12 Mass(ppm) of phenolic compounds obtained from narghile smoke and compared to current study cigarette and the other two studies. (a) David Ashley; (b) Count et al. Results reported as μg/narghile or μg/cigarette 82

Chapter 1

INTRODUCTION

Tobacco leaves were discovered by Christopher Columbus in 1492. Initially, these leaves have been recommended by European doctors as cure for toothache, worms, and other ailments. Queen Catherine de Medici used tobacco leaves to cure her migraines after they were introduced in France by the French ambassador in Portugal, Jean Nicot, in 1561.In 1753, the Swedish botanist Carolus Linnaeus named the tobacco plant genus Nicotiana in honor of the French ambassador[49].

Tobacco as a plant material is a very complex biomass matrix which consists of over 2500 chemical compounds. These compounds could be biopolymers, non-polymeric and inorganic compounds[16].

In the 18th century, snuff and pipe smoking were the most prevailing forms of tobacco use, whereas the age of cigar started in the 19th century. Cigarette smoking was introduced to the English speaking world during the Crimean War when the

British soldiers started emulating the Ottoman Turkish comrades in rolling tobacco in newsprint paper. In the 1850s machines were used to manufacture cigarettes hence opening the way for mass production and the development of modern forms of cigarettes in the 20th century[49]. Since 1950s, many attempts have been made to selectively remove or reduce the chemicals in smoke that are associated with adverse health effects in order to produce potentially less-hazardous cigarettes.

Cigarette smoke contains more than 4,800 identified substances which explain the complexity of the smoke[22]. When a cigarette is smoked, combustion takes place in two ways: during a puff, air is drawn into a cigarette and mainstream smoke is formed and inhaled by the smoker while between the puffs, cigarette smolders and sidestream smoke is released into the environment from the lit end of the cigarette. Generally, both sidestream and mainstream smoke contains same components, yet they vary in %yield depending on cigarette construction and on the smoke component under study; in addition, sidestream smoke is generated at lower temperatures. Smoke constituents are distributed between gas and particle phase. Mainstream smoke particles are larger than sidestream particles. There are two main regions inside a burning cigarette: a combustion zone and a pyrolysis-distillation-pyrosynthesis[43] (Fig1.1).

Inside the combustion zone, oxygen reacts with carbonized tobacco producing CO_2, CO and H_2 where temperatures as high as 9500 °C are generated during a puff. The cooler pyrolysis-distillation-pyrosynthesis zone is located downstream

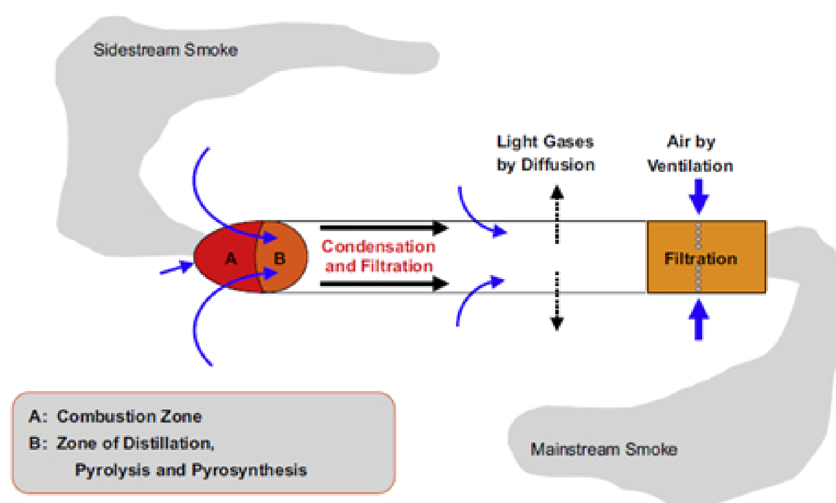


Figure 1.1: The burning cigarette.

from the combustion zone where the bulk of more than 4700 chemicals in smoke are generated. The super-saturated vapor rapidly cools in the tobacco rod and condenses into aerosol particles that make up the smoke. Cigarette smoke is an aerosol of liquid droplets, called the particulate phase, suspended in a mixture of gas and semi-volatile compounds. Particulate phase is the fraction retained by glass fiber filter (Cambridge filter). Cambridge filters are preferred over any other type, for they effectively retain particles at room temperature, they are non-hygroscopic, easily fashioned into filter of uniform efficiency, requires minimum user preparation and inexpensive[17]. However, gas phase pass through the filter and requires specific trapping solutions to retain them. A number of compounds such as polyaromatic hydrocarbons (PAH), tobacco-specific nitrosamines (TSNAs), phytosterols and the metals are found practically in the particulate phase only. Some compounds such as phenol and cresols are partitioned between the particulate and gaseous phases and

are termed semi-volatiles[49].

Tobacco smoke became a controversial issue due to the growing evidence of health risks as well as its increasing popularity with a worldwide consumption of 5.6×10^{12} cigarettes as reported in 2000 by the American Cancer Society. The chemical composition of cigarette smoke has been intensively researched in order to decrease the health risks associated with smoking. These risks include various types of cancer (larynx, esophagus, pancreas, lung and urinary bladder), as well as strokes, heart failure, and pulmonary diseases. In 1989 more than 81.8% of all deaths from chronic obstructive pulmonary disease were attributed to cigarette smoking in the United States; moreover, more than 30% of 514,000 cancer deaths were attributed to cigarette smoking as reported in 1991[22].

These substances, particularly those listed as Hofmann analytes, are known to have toxic properties such as carcinogenicity and cytotoxicity. These analytes include nicotine which causes tobacco dependence, as well as CO, HCN and tar which promote cardiovascular diseases. Moreover, HCN, volatile aldehydes and nitrogen oxides cause chronic obstructive lung disease, PAH and NNK cause lung and Larynx cancer, NNK and NNN cause oral cavity cancer, and NNK and NNAL cause pancreas cancer[22].

Water-pipe, another smoking phenomenon, is increasing sharply in its popularity in regions where it's culturally rooted as well as in Europe and North America

where it's drawing new and young smokers. It started by indigenous people of Africa and Asia for more than four centuries. It is believed that water-pipe was invented by an Indian physician, Hakim Abdul Fath, during the reign of Emperor Akbar as a less harmful way of consuming tobacco. He believed that when smoke passes through water, the smoke is rendered harmless. Unfortunately, this concept is still believed by water-pipe users up to this date, which calls for a deep research concerning the toxicity of the produced smoke. Contrary to ancient lore and popular belief, the smoke that emerges from narghile smoke contains numerous toxicants known to cause lung cancer, heart disease, and other diseases[34].
Intensive studies on the chemical composition, toxicity and carcinogenicity of generated cigarette smoke is accomplished to understand its health effects which is complemented by in-vivo and epidemiological studies of smoking, and provide even better understanding of the toxicity and carcinogenicity of cigarette smoke.

On the other hand, despite of water-pipe's popularity, little research has been conducted on the chemical composition of the generated leaving the public with dearth information about its potential hazards (PAH, CO and tar). Moreover, it's not possible to extrapolate this information from cigarette, for the narghile differs from cigarette smoke in various ways including smoke delivery, the smoke aerosol generation, heating source, the tobacco being hydrated and heavily flavored; in addition, it has puff volumes of an order of magnitude greater and with tobacco burning at several hundreds of degrees Celsius lower[44]. All of these differences call for developing research methods and smoke composition data specific to the narghile

water-pipe.

Previous studies have shown that the mainstream smoke of a narghile session contains 3.8 and 11.5 times the amounts of CO and nicotine found in the mainstream of a single cigarette, respectively[44]. Current studies conducted at the Analytical Lab of the Chemistry Department, and the Aerosol Lab of the Mechanical Engineering Department at the American University of Beirut (AUB), showed that mainstream narghile smoke contains carcinogenic heavy metals, such as arsenic, beryllium, nickel, cobalt, chromium and lead with concentrations ranging between 65ng for beryllium and 6870ng for lead[16]

.

Moreover, it contains 3- to 6-membered ring polycyclic aromatic hydrocarbons (PAH) in the mainstream smoke[44]. A single narghile session delivers around 50 times the quantities of carcinogenic 4- and 5-memebered ring PAHs as a single 1R4F cigarette smoked using FTC protocol[42]. Furthermore, it contains high levels of aldehydes such as formaldehyde, acetaldehyde, acrolein, propionaldehyde and methacrolein, with concentrations ranging between 106 μg/smoking session for methacrolein and 2520 μg/smoking session for acetaldehyde[2]. Other compounds that are expected to be present in narghile smoke such as HCN and phenols will be discussed in this study. Due to the differences between the narghile and cigarette systems, NPDES method# 335.2 for HCN and EPA Method-528 for phenols would be optimized to accommodate the complex matrix extracted from the narghile sessions.

Chapter 2

HCN

2.1 Historical Background

Hydrogen cyanide, also known as hydrocyanic acid, prussic acid and formonitrile, is a colorless liquid with a bitter almond odor[5]. Its ionic form, cyanide, acts as an intermediate in the synthesis of organic compounds such as nitriles, carboxylic acids, amides, esters and amines, and in the manufacturing of chelating agents[47]. Furthermore, it is used as a fumigant in ships, railroad cars, and large buildings as well as in the fumigation of peas and seeds in vacuum chambers[47]. Moreover, it's incorporated in electroplating, mining, and in the production of synthetic fibers, plastics, dyes and pesticides[1]. Cyanide has been mainly utilized as a poison for thousands of years. It is present in plants such as bitter almonds, cherry laurel leaves, peach pits and cassava and has been employed by ancient Egyptians as lethal poisons. Although these plants have been characterized as poisonous, cyanide, which is the primary toxic agent, was not identified until 1782 by Carl Wilhelm Scheele, a

Swedish pharmacist and chemist. He isolated cyanide by heating the dye Prussian Blue (Blue Berlin) with dilute sulfuric acid, obtaining a flammable gas, now known as hydrogen cyanide, which was water soluble and acidic. Scheele called his new compound Berlin blue acid, which later on became prussic acid, and today is known as cyanide originating from the Greek word “Kyanos” meaning blue [8]. During World War I, cyanide was exclusively produced for the purpose of killing. France started the large-scale use of cyanide as a chemical weapon and later enhanced it by producing cyanogen chloride which is more effective at lower concentrations, has lower volatility compared to HCN and had a cumulative effect on its victims. Moreover, cyanide had been the typical agent used in “gas chambers” to execute murderers and still is in different states [8].

2.2 HCN Sources

Humans are exposed to cyanide from natural and anthropogenic sources. Anthropogenic sources of cyanide release to the environment are diverse. It is released from chemical manufacturing and processing industries, such as metal plating and extraction of gold and silver from low grade ores. Additional sources include volatilization from cyanide wastes disposed of in landfills and waste ponds, emissions from municipal solid waste incinerators, biomass burning, fossil fuel combustion and the production of coke or other coal carbonization procedures. Furthermore, HCN is formed during the incomplete combustion of nitrogen-containing polymers, such as plastics, silk and wool[47]. On the other hand over 2000 plant species, includ-

ing fruit and vegetables, such as cassava, sorghum, sweet potatoes, yams, bamboo, limes, apples and prunes[8], constitute natural sources of cyanides. These plants contain cyanogenic glycosides, which rapidly release cyanide, by hydrolysis upon ingestion,as illustrated in Fig2.1, when the plant cell structure is disrupted[8].

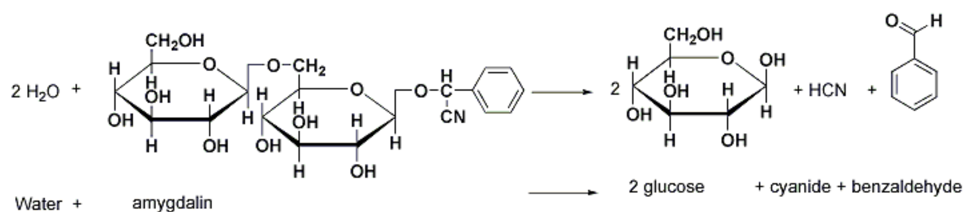


Figure 2.1: Hydrolysis of amygdalin.

Common cyanogenic glycosides, shown in Fig2.2, in plants include amygdalin, linamarin, prunasin, dhurrin, lotaustralin, and taxiphyllin.

Furthermore, they are released from natural biogenic processes, volcanoes, higher plants, bacteria and fungi[8].

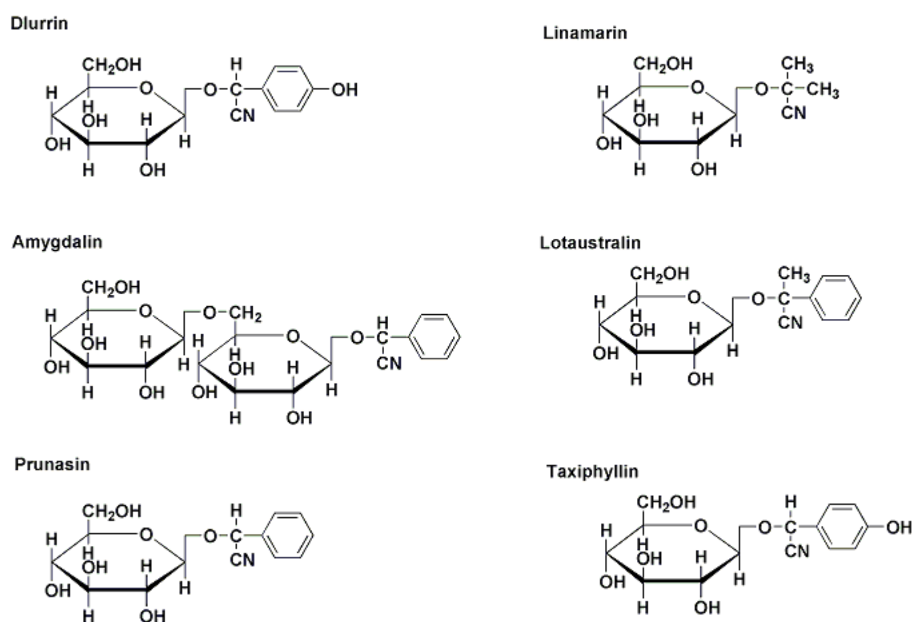


Figure 2.2: Cyanogenic glycosides in major edible plants (JECFA, 1993) Amygdalin occurs in (among others) almonds, dhurrin in sorghum, linamarin in cassava, lotaustralin in cassava and lima beans, prunasin in stone fruits, and taxiphyllin in bamboo shoots.

2.3 HCN Chemical and Physical Properties

HCN is a colorless or pale blue liquid or gas with a bitter-almond odor. The odor is detectable at 2-10ppm, and the perception of the odor is a genetic trait where 20%-40% of the population are unable of detecting it. It's also known as hydrocyanic acid and prussic acid. It is a very weak acid with pka value of 9.2 at 25 °C. Hydrogen cyanide is lighter than air with a high vapor pressure of 740mm Hg at 27.2 °C and a low octanol/water partitioning coefficient (log kow) of 0.66 thus indicating that hydrogen cyanide exists mainly in gaseous phase[47]. It boils at 25.7 °C close to room temperature. Hydrogen cyanide is unstable and phosphoric acid is usually

added to its solution to prevent decomposition and explosion[5]. It can react with amines, oxidizers, and ammonia. Moreover, it is completely miscible in water at 25 °C, alcohols and almost all organic solvents[5].

2.4 Health Hazards of HCN

Cyanides are known for their high acute toxicity and chronic toxicity. Although mild effects occur at inhalation exposure levels of 20-40mg HCN/m^3 and 50-60mg/m^3 can be tolerated without immediate intervention, 120-150mg/m^3 may lead to death within an hour[47]. The permissible limit of exposure PEL reported by OSHA[5] does not exceed 10ppm averaged over 15 minutes. Cyanide is known to bind and inactivate several enzymes especially those containing iron in the ferric state (Fe^{3+}) and cobalt[8]. It exerts its lethal effect of histotoxic anoxia, cessation of oxidative metabolisms, by binding to the active site of cytochrome c oxidase which is the terminal protein in the electron transport chain located within mitochondrial membranes. This binding can occur in minutes. Thus cyanides prevent the transfer of electrons to molecular oxygen as shown in Fig.2.3. Although oxygen is present in the blood, it cannot be utilized toward adenosine triphosphate (ATP) generation. First, cells attempt to replenish the ATP energy source through glycolysis, which leads to the production of lactic acid and may produce severe acid-base imbalance. Furthermore, this source of replenishment is short lived particularly in the metabolically active heart and brain[8].

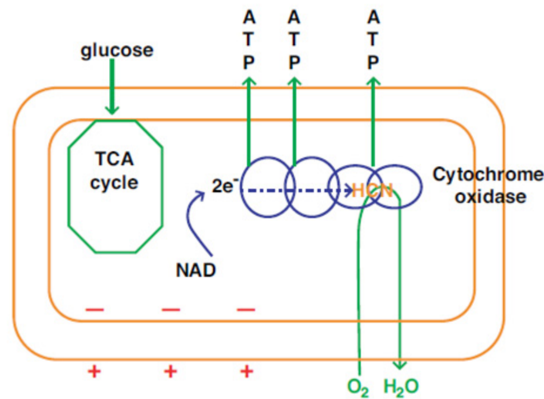


Figure 2.3: Cyanide binds the terminal enzyme of the cytochrome oxidase enzyme system. The enzyme system is located within the inner lamina of the mitochondria. The blockade interrupts the electron flow through the cytochrome oxidase system, thereby disrupting ATP production and both mitochondrial and cytoplasmic ionic balance.

Moreover, a more rapid effect is observed with neuronal transmission. Cyanide can inhibit carbonic anhydrase which converts carbon dioxide in the blood to carbonic acid and bicarbonate as it is transported to the lungs and converts it back to CO_2 when it reaches the lungs to be exhaled. This interaction may prove to be an important contributor to the well documented metabolic acidosis resulting from significant cyanide intoxication[8].

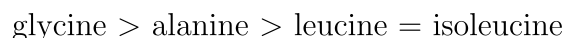
2.5 Mechanisms of HCN Formation

HCN can be formed either from burned tobacco or charcoal.

2.5.1 HCN coming from burned tobacco

HCN coming from tobacco results mainly from the pyrolysis of amino acids and nitrogenated compounds such as isomeric aminobutyric acids, dicarboxylic acids, and amines[23]. Johnson and Kang[23] suggested different mechanisms for HCN formation, at temperatures greater than 700 °C. They can result from thermal reactions such as deamination, decarboxylation or 2,5-piperazinedione (I) formation. However, the fact that HCN yield was affected by substituents indicated that the third mechanism, shown in Fig.2.4 is the most probable route since the first two are not influenced by substituents. Johnson's general mechanism for HCN formation includes dehydrogenation of methylenimine (CH_2=NH) or its diradical ·CH_2NH· produced upon ring cleavage. Therefore, Methylenimine formation requires cyclization of the reactant.

This mechanism depends on the ease by which amino acid can cyclize, and on its tendency to break and give HCN. Following that reasoning, the yield of HCN from amino acids will be


glycine $>$ alanine $>$ leucine $=$ isoleucine

HCN coming from nitrogen heterocycles will be formed through the same mechanism. Similarly structural influence will affect the yield of HCN. Johnson and Kang investigated the effect of the following variables on %HCN formed. They

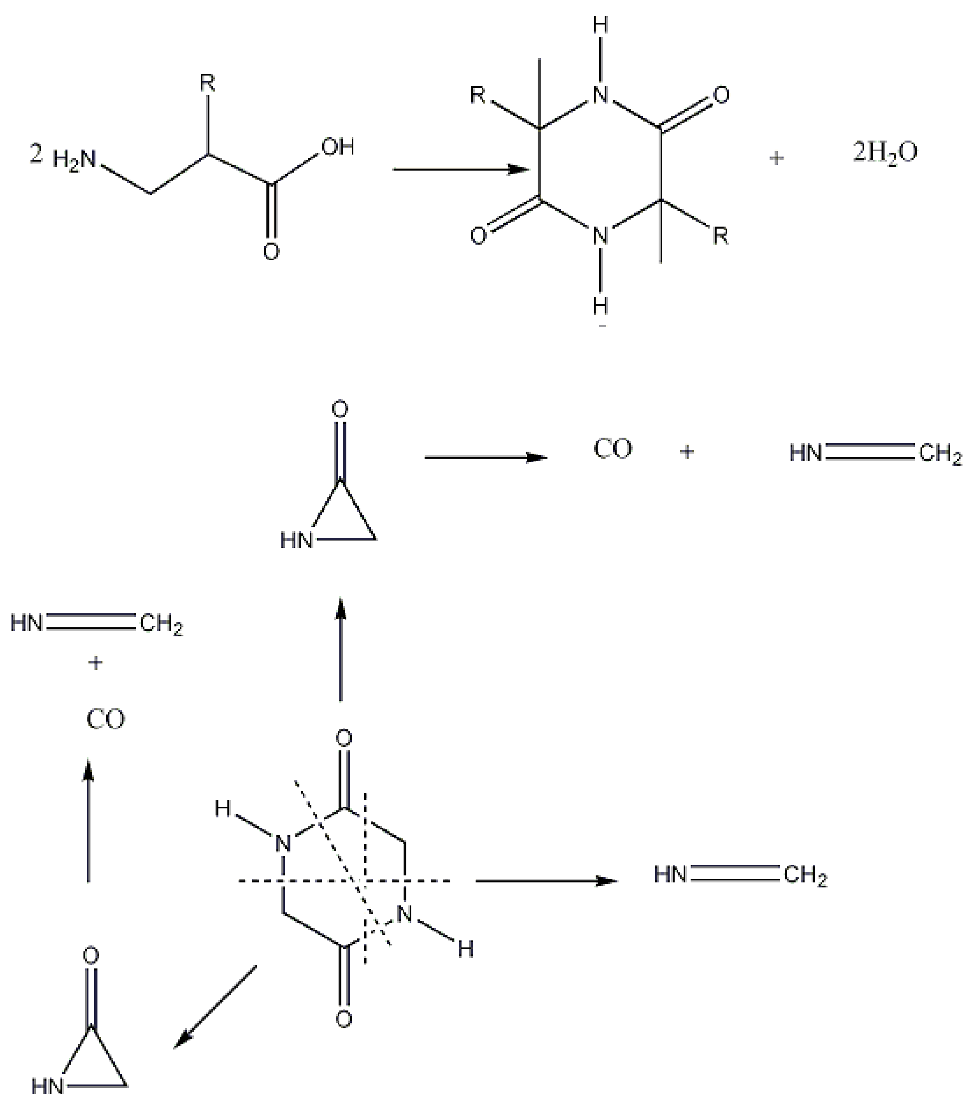


Figure 2.4: Suggested pyrolysis reactions for 2,5-piperazinedione

first studied the effect of the ring size on the amount of HCN formed. A comparison between 2-pyrrolidine, 2,5-piperazinedione and 2-oxohexamethylenimine showed that five-membered rings are favored for HCN formation since 2-pyrrolidine showed highest degree of decomposition. Then they investigated the effect of the stability of the formed rings on the decomposition rate. This was accomplished by comparing rings with variable degrees of saturation such as pyrrole, 3-pyrroline , and pyrroli-

dine with pyrrolidine giving highest %HCN, showing that unsaturation stabalizes the ring making its cleavage harder, thus leading to a decrease in %HCN.

Moreover, substituting hydrogen, attached to nitrogen, with a methyl group reduces HCN yield by more than 50%. This is because another decomposition route is followed for N-CH_3 derivatives, as shown in Fig.2.5, where cleavage of N-methyl group is favored over ring cleavage.

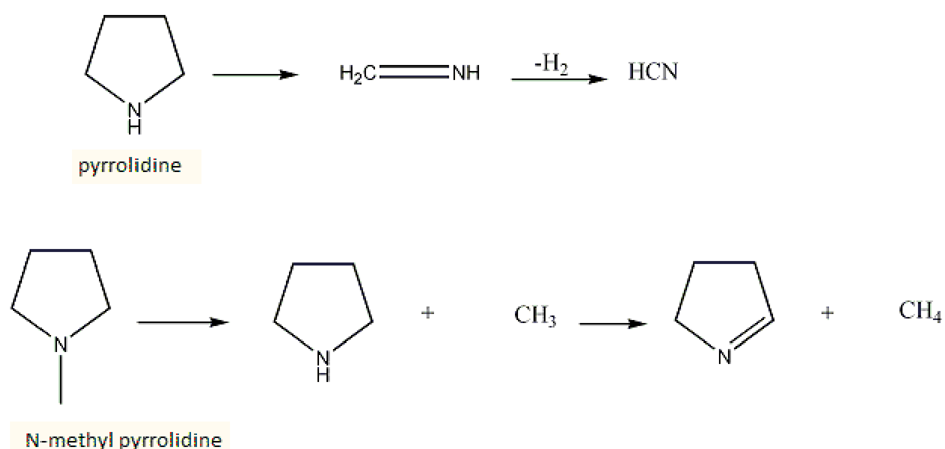


Figure 2.5: Decomposition mechanism of (1) pyrrolidine and (2) N-methy pyrrolidine

In addition, they inspected the substituent effect on neighboring carbon atoms at 800 °C. A mono substitution of a hydrogen with a carbonyl group showed no significant effect; whereas, the presence of two adjacent carbonyls had profound effect on %HCN. Consequently, succinimide, in comparison with other substituted cyclic compounds, gave the least amount of HCN. Fig.2.6 shows that in case of adjacent carbonyls, a different mechanism for HCN formation is employed causing a substantial drop in the %HCN.

As for linear nitrogenated compounds, isomeric aminobutyric acids, dicarboxylic

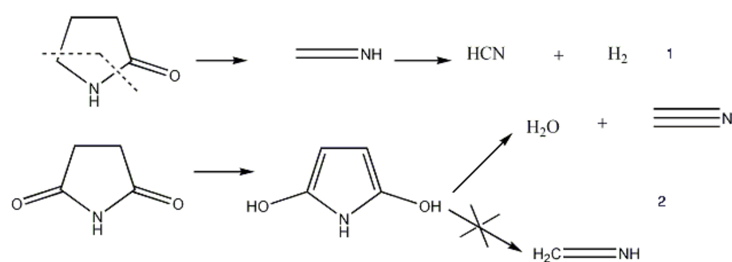


Figure 2.6: Decomposition mechanism of (1) 2-azetidinone and (2) 2,4-diazetidinone

acids, and amines were reviewed at temperatures greater than 700 °C. For isomeric aminobutyric acids α-, β-, and δ-derivatives, the %HCN was highest for δ- aminobutyric acids and lowest for α-aminobutyric acids with an intermediate value for β-aminobutyric. This is consistent with the cyclization mechanism since δ-aminobutyric acids have higher tendency for intramolecular ring formation than β-, and δ-aminobutyric acids, as shown in Fig2.7.

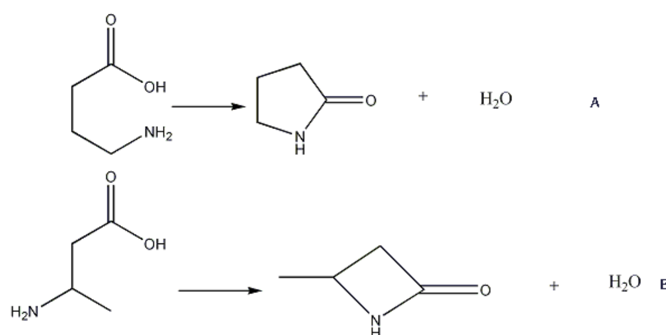


Figure 2.7: Decomposition mechanism of (A) β-aminobutyric acids and (B) γ-aminobutyric acids

The cyclization mechanism was also verified for dicarboxylic acids, Fig2.8, such as glutamic acid and ascorbic acid, glutamine and asparagine and for amines such as 1,4-diaminobutane.

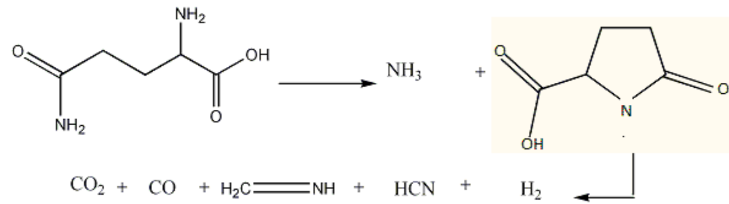


Figure 2.8: Formation of HCN from glutamic acid

2.5.2 HCN coming from Charcoal Pyrolysis

The nitrogen present in coal (coal-N) is emitted as NOx and N2O which upon reduction gives HCN[51]. Coal-N is distributed between volatile-N and char-N with volatile-N being formed during primary pyrolysis and the rest retained as char-N[31]. The distribution of volatile-N components depends on coal type and pyrolysis conditions[28]. It is represented by HCN, NH_3, oil-N and tar-N with hydrogen cyanide and ammonia produced from secondary pyrolysis of tar decomposition[31]. Tar being primarily made up of cyclic nitrogenated compounds[28]. At low heating rates and high temperatures, HCN and NH_3 would be produced with tar decomposition. Miller[28] proposed a mechanism, which represents atmospheric processes, for conversion of volatile-N to HCN starting from NO or HNCO as shown in Fig2.9.

Moreover, hydrogen cyanide can result from the decomposition of nitrogenated compounds coming from the tar. Biomass pyrolysis can be treated as the superposition of cellulose, hemicelluloses, lignin and protein[51]. Similarly, pyrolysis of solid fuel, such as coal, can be treated as a superposition of the fuel's different composition. Coal consists of aromatic clusters bound together by weak bonds which break into

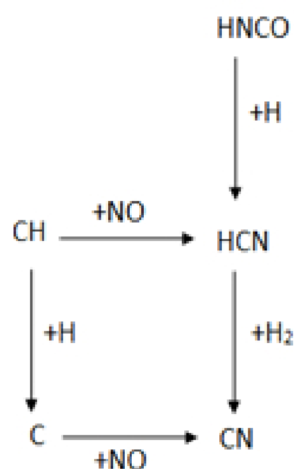


Figure 2.9: Miller's mechanism for HCN formation

fragments released as tar. Thus, the pyrolysis of coal tar can be treated as the superposition of the clusters' different chemical functionalities assuming that the nitrogenous composition of coal tar is similar to that of parent coal though no clear separation of the constituents can be made[51].

Hansson et al modeled an experimental study, conducted by Ladesma, for the pyrolysis of coal at 600 °C. His model was based on the assumption that tar has the same nitrogen composition as the parent coal. Thus the pyrolysis of tar nitrogen can be treated as the superposition of pyrrole, amine/quaternary nitrogen, 2-pyridone and pyridine. Fig2.10 represents tar decomposition into different nitrogenated functionalities. Nitrogen atom can be embedded in a pyrrolic form such as pyrrole, indole, carbazole or larger ring clusters. Their thermal decomposition depends on the exact structure of the pyrrolic molecule where similar compounds such as pyrrole and indole will have similar decomposition rates. On the other hand, larger clusters will be subjected to larger differences in their decomposition rates. The decomposition

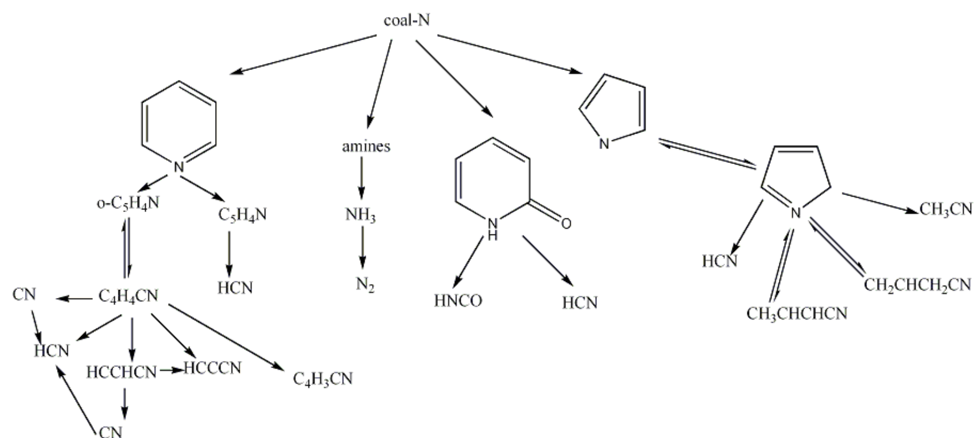


Figure 2.10: The pyrolysis of coal-N leads to release of all nitrogen functionalities found in coal. Reaction A leads to pyridine, B to amine/quaternary nitrogen, C to 2-pyridone and D to pyrrole

of Pyridine, amine, 2-pyridone and pyrrole would generate temperature-dependent compounds such as NH3, HCN and HNCO.

Both pyridine and pyrrole generates HCN, at T > 800 °C as illustrated in Fig2.11.

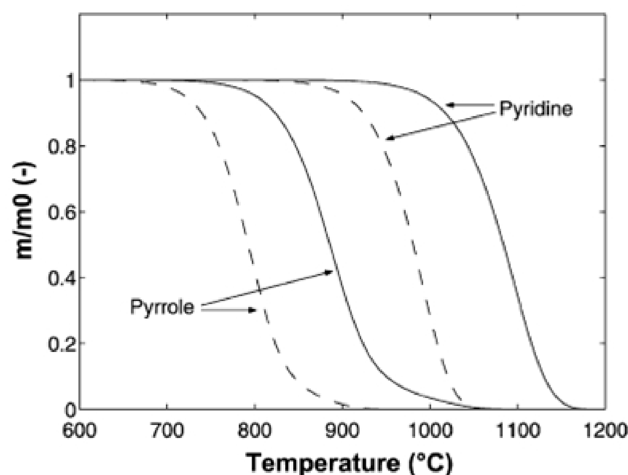


Figure 2.11: Pyrolysis of pyrrole and pyridine[19]

2.6 Standard Analytical Methods: Overview

Few methods, consisting of preparative and determination steps, have been reviewed in the literature for the analysis of cyanide n cigarette smoke. They can be analyzed using spectrophotmetry, titrimetry, ion chromatography (IC), high performance liquid chromatography (HPLC), gas chromatography (GC) and ion selective electrode (ISE) [55] . Spectrophotometric methods are based on preparing reagents that develop a color upon reacting with cyanide anion. Three reagents have been proposed, phenolphthalein which has low sensitivity, as well as pyridine-benzidine and pyridine-pyrazolone reagents which have higher sensitivity with the latter forming the most stable color with cyanide[26]. Ion chromatography employs anionic exchange and separation is achieved using a gradient solvent system[48]. Gas chromatography uses gas samples of smoke and constituents are separated following direct injection from the bags used for collection[55]. Most of the methods reported are deemed unsuitable either due to carcinogenicity and cytotoxicity of the used chemicals and reagents or to the presence of interfering ions. Low sensitivity is another factor that hinders analysis and detection[55]. Moreover, the determination of cyanide with high sensitivity requires a preparative step which is usually complicated and time-consuming thus requiring an automated analysis. The employed method, NPDES Method#335.2, in this study is the pyridine-pyrazolone spectrophotometric method[**?**, 12], mechanism illustrated in Fig2.12-2.13.

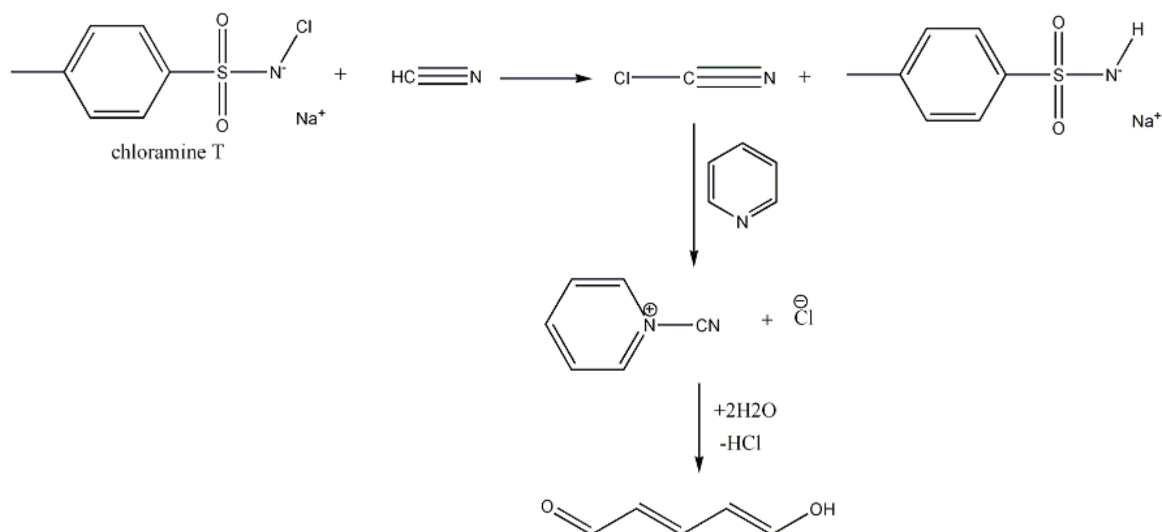


Figure 2.12: Complexation mechanism, formation of glyoxal.

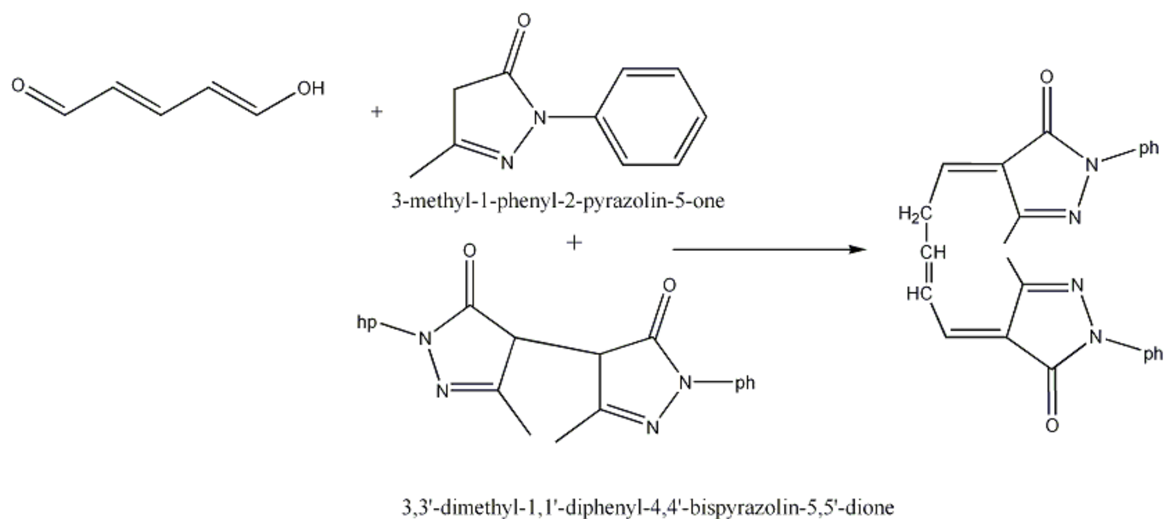


Figure 2.13: Complexation mechanism and formation of final complex.

2.7 Materials and Methods

2.7.1 Apparatus

1-cm Quartz cuvettes were used for absorbance measurements using a JASCO, V-570 UV/VIS/NIR Spectrophotometer with VWTS-581 color analysis software. The

pH measurements were made on an Orion 3-Star Plus Benchtop Dissolved Oxygen Meter pH meter. 47 mm glass fiber filters (type A/E PALL) were used to collect the particle phase of the smoke, whereas 29/42 impingers with coarse fritted glass, obtained from Kontes, were used to collect the gas phase.

2.7.2 Materials and Reagents

Spectrophotometeric Method

Potassium cyanide 97% ACS is used for the preparation of standards and potassium hydroxide 98% used to adjust the pH of standards. A 1% solution of chloramine-T trihydrate 98% was used to oxidize the cyanide to cyanogen chloride[38]. The aqueous pyrazolone solution is prepared by dissolving 0.25g of crystallized 3-methyl-1-phenyl-2-pyrazolin-5-one in 50 ml of distilled water heated with stirring to 60 °C and then allowed to cool to room temperature[18]. The bis-pyrazolone is prepared by dissolving 0.01g of 3, 3´-dimethyl-1,1´-diphenyl-[4,4´-bi-2-pyrazoline]-5,5´dione in 10ml pyridine 99% for spectroscopy. Then aqueous pyrazolone solution is filtered through nylon filter followed by bispyrazolone to remove any undissolved particles[18]. The solution is kept at 4 °C and freshly prepared for analysis. The pH of each sample is adjusted to 7 using 1M KH_2PO_4 99%. All chemicals were obtained from Medilife.

Smoking system

Tobacco mixture named Nakhla Tobacco (Egypt) flavored "two apples' was obtained from local retail outlets, as were the Three Kings (Holland) brand quick-light charcoal disks used in this study. The cigarettes used were from Marlboro brand.

2.7.3 Method of color development

About 0.5 ml of 1% chloramine-T is added to a known volume of sample, 50ml in case of standards, after adjusting pH of sample to 7, and then flask is stoppered and shaken for 2 minutes. Then 5ml of the pyridine-pyrazolone reagent are added, sample diluted to 100ml and allowed to stand for 30 minutes until a blue color has fully developed. Immediately afterwards the absorbance of the solution is measured at λ=620nm[18].

2.7.4 Calibration curve

Standard cyanide solutions, whose concentrations range between 12.75 and 102.05μg/l, are prepared from a standard stock solution cyanide, and used to trace a calibration curve of cyanide.

Preparation of Potassium Cyanide Stock Solution

Cyanide stock solution is prepared in a 1000ml volumetric flask by dissolving 2.51g of KCN and 2g KOH in 900ml distilled water.

Preparation of Potassium Cyanide Intermediate Stock Solution

A known volume, as illustrated in Table2.1, of the stock solution is diluted in a 1000ml volumetric flask with distilled water.

Table 2.1: Volume (μl) required for the preparation of working stock solutions.

Standard	Volume (μl)	concentration of working stock solution (ppb)
1	25	12.75
2	50	25.51
3	100	51.02
4	150	76.54
5	200	102.05

Working standard solutions are prepared daily by delivering 500ml of the intermediate stock solution to a 1000ml volumetric flask and then adding 50ml of 1.25M NaOH.

Obtained Calibration Curve

Following Beer's law, the following relationship was obtained

A=0.0022C -0.033

Determining the concentration of hydrogen cyanide (μg/cigarette) is done following

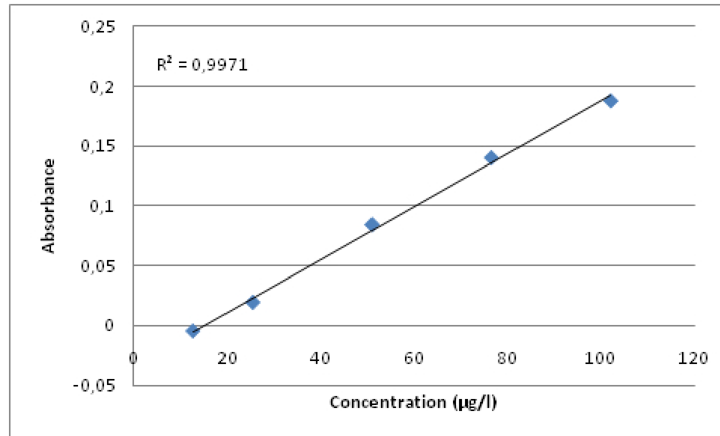


Figure 2.14: Hydrogen cyanide calibration curve using a range of standards from 12.75 to 102.05ppb.

the equation given below:

$$Hydrogencyanide \mu g/l = \frac{C \times V \times D}{N} \tag{2.1}$$

Where: C=concentration (μg per ml) of hydrogen cyanide determined from the calibration curve

V= volume (ml) of smoke extracts (190ml, 15ml, 5ml) N= number of cigarettes smoked

D=dilution factor

2.7.5 Tobacco Smoke Preparation and Sampling

Preparation of Narghile Set-up

The head is filled with 10g of fruit-flavored tobacco mixture, molasses, then covered with aluminum foil which is perforated randomly, providing eighteen holes that allow

air to flow through the narghile system. At the beginning of the session a quick-light piece of charcoal (5.5-6 g) is placed at the top of the head. A vacuum pump, simulating the human lung, connected to the narghile creates vacuum in the narghile with each taken puff thus allowing air to be drawn through the narghile head where it is heated, the water bowl, the hose, and finally to what should have been the lungs of the smoker. Upon exiting the hose of the narghile, the pumped air is loaded with the products of tobacco and charcoal combustion[43]. The smoking machine operates by a specially designed software[43], and the smoking parameters employed in this study are taken from Shihadeh et al. (2004), and set at 171-puff smoking session, 2.6 s puff duration, 17 s inter-puff interval and 12 LPM flow-rate[44].

After setting the Narghile, the impinger is calibrated using the smoking machine while the charcoal is placed unlit on the head. Then the charcoal is lit and smoking starts. The two filters placed in parallel are changed at regular intervals of 40, 60, 80, 95, 110, 125, 140 and 171 puffs. Another half charcoal is added at the 105 puff. The impinger placed contains 190ml NaOH (0.1M) and the solution is not changed for the whole session[44].

Sampling Mainstream Narghile Smoke

Hydrogen cyanide is collected in the gas and particle phase. The particle phase is trapped on glass fiber filter pads placed upstream of the impinger; whereas, gas phase HCN is trapped in a 0.1M NaOH solution using a bubbler with coarse fritted glass as shown in Fig2.15.

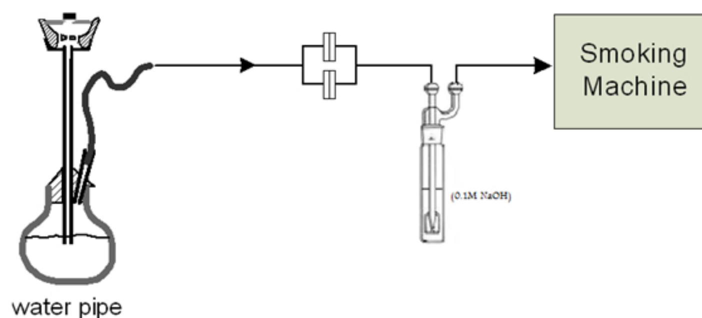


Figure 2.15: Mainstream smoke captured in a bubbler containing 0.1M NaOH.

Sampling Sidestream Narghile Smoke

The narghile head is placed in a Teflon-coated box in order to prevent the escape of sidestream smoke constituents and their deposition on the walls of the small box. A HEPA filter is placed to ensure the cleanliness of the pumped air. The particulate phase is collected on glass filters, whereas, gaseous cyanide is collected in a 0.1M NaOH solution using a coarse fritted glass for dispersion as shown in the figure below. The flow rate is kept at 1.5LPM. Note that filter pads are always placed upstream from the impingers to prevent the clogging of the fritted glass and to avoid dissolving aerosols in the solution which might increase interferences.

2.7.6 Sampling Mainstream (Sidestream) Charcoal

A similar setup as that developed in Section2.7.6 (Section**??**) is constructed with only one variation. No tobacco is placed inside the head.

2.7.7 Sampling mainstream and sidestream charcoal for artifact verifications

A similar set up as in Section2.7.6 and?? is used. Now instead of placing one impinger for the collection of smoke, two impingers are placed in parallel downstream from the filter. One impinger will contain 0.1M NaOH and the second one will contain 0.1M formaldehyde. The aim of using formaldehyde is to check if the released HCN is reacting with formaldehyde resulting in cyanohydrin.

Cigarette Set-up

The Marlboro brand cigarettes used were 85mm in length packed with the original American blend. Cigarettes were smoked using the same machine developed for narghile smoking, following the conditions set by the Massachusetts Department of Public Health (MDPH) with a slight deviation where the vents are kept open. The average number of puffs per cigarette is 16 puffs with a puff duration of 2 s and interpuff interval of 13 s[15]. The cigarette is extinguished when its bud is 25mm long. Three runs were carried out, each run constituting of 5 cigarettes. The trapping solution was not changed throughout the 15 smoked cigarettes to ensure maximum collection of hydrogen cyanide, whereas, a new filter was placed by the end of each run. A cigarette is attached upstream the filter holder using polystyrene tubing. The filter holder is followed by a 29/42 impinger which contains 190ml NaOH (0.1M). The impinger is connected to a pump where the flow rate is kept at 1.402 L.min^{-1}, and then the cigarette is lit[12].

Extraction and sample preparation

Filter pads are folded in half and in half again with the clean side facing out using tweezers. They are placed in 24ml vials containing 15ml 0.1M NaOH solution. Then the vials are tightly sealed and sonicated for 30 minutes. The impinger solution is directly used without any pretreatment[12]. 1ml of the filtrate is delivered into a 100ml volumetric flask for analysis. The pH of the sample is adjusted to 7 using KH_2PO_4, and then 0.5ml of chloramine-t is added to the sample to change cyanide ion into cyanogens chloride. This is followed by the addition of 5ml of the pyridine-pyrazolone solution where a pink color appears instantaneously. Absorbance is measured at λ=620nm after 40 minutes allowing the blue color to fully develop. A similar procedure is adopted for the analysis of gas phase cyanide trapped in the basic solution except that the volume of the aliquot used is 5ml instead of 2ml.

2.8 Results

2.8.1 Cigarettes

A set of 15 runs have been done for the collection of hydrogen cyanide in the particle phase. The reproducibility of the results was assessed by calculating %RSD, %RSD calculated as shown below. High reproducibility was obtained with a %RSD of 13% and an average of 84.1μg/g. As for the collection of HCN in the gas phase a total of 6 samples were collected with an average yield of 140.87μg/g and a %RSD of 9.4%

summarized in Table2.2.

$$\%RSD = \frac{\sqrt{SD} \times 100}{\sqrt{average}} \tag{2.2}$$

Table 2.2: Mass of HCN (μg/g) in the gas and particle phase for mainstream smoke cigarette

	Trial1	Trial2	Trial3	Trial4	Trial5	Trial6	Average	%RSD
Gas phase	139.7	166.5	174.4	114.4	108.8	NA	140.7	9.4
Particle phase	84.8	85.4	86.5	68.61	101.5	77.40	84.1	13

2.8.2 Narghile

Both gas and particle phases were collected using an impinger and filter pads respectively. The experiment has been repeated 6 times. Two narghile sessions were conducted using same trapping solution to maximize yield. Thus a total of 12 narghile sessions was done and we did not detect HCN in any of those runs.

2.9 Quality control

Quality control experiments were conducted on cigarette sessions which would allow us to compare our results to those reported in the literature, since no studies have been done on narghile yet.

2.9.1 Validation of experimental and analytical procedures

The experimental set up and procedures used in this study were assessed and validated through 6 cigarette runs. These values are summarized in Table2.2. When compared to literature for cigarettes,Table2.3, our results are in good agreement. This shows that the experimental and analytical procedures employed in this study for the quantification of cyanides in narghile smoke are valid. Our values differ from those reported by Count et al, for Marlboro brand, by 4.7 and 4.8% for gas and particle phase, respectively.

Table 2.3: Average yield of HCN (μg/g) in gas and particle phase for six runs compared to reported yields of HCN in mainstream cigarette.

	Current Study	Count et al	%error
Gas phase	140.7	147.6	4.7
Particle phase	84.1	88.4	4.8

2.9.2 Assessing saturation of the basic solution and adequacy of the residence time

This is obtained by placing two impingers in series containing 0.1M NaOH. A 29/42 impinger filled with 190ml of NaOH followed by a 40ml impinger containing 15ml NaOH. Three runs were carried out, each of 5 cigarettes where trapping solution of 40ml impinger was changed after each run, whereas, the 29/42 impinger was kept unchanged through the three runs. Results showed no traces of HCN in the second

impinger which implies that both the quantity of NaOH solution and the residence time employed are adequate.

2.9.3 Assessing saturation of filter pads

This is accomplished by varying the number of cigarettes collected on one filter. During a run of 5 cigarettes, 2 cigarettes were collected on one filter, followed by another 2 collected on another filter and then the fifth cigarette collected on a separate filter. Table2.4 shows that 2 cigarettes can be collected on one filter pad without causing any saturation. Results were obtained with 12.5%RSD which reflects the reproducibility of the sampling and the analytical method.

Table 2.4: Mass of HCN (μg/g) in mainstream cigarette smoke

# of cigarettes/filter	Trial1	Trial2	Trial3	Trial4	Trial5	Trial6
2 cigarettes/filter	88.55	88	75.96	81.05	83	83.98
2 cigarettes/filter	63.72	91.41	107.92	74.63	86.72	87.70
1 cigarette/filter	102.16	76.98	75.81	62.39	36.13	60.54
Average	84.81	85.46	86.56	72.69	68.61	77.407

2.9.4 Assessing effect of pH on the stability of the obtained blue color

A standard solution of 51.0748ppb was prepared at different pH conditions to assess the stability of the complexing reagent assessed by the stability of the blue color.

Samples are acidified using 99.8% acetic acid. Table2.5 illustrates the variation of absorbance for the same concentration at different pH values showing that optimal conditions correspond to pH values of 6.85 to 7.58. A pH of 7 was chosen for this study because pH must be kept below 8 for complexation to take place.

Table 2.5: Variation of absorbance with pH

Theoratical value pH	$V_{aceticacid}$	Experimental value pH	Absorbance
4	14.508	4.3	-0.033
5	3.42	5.97	0.066
6	2.31	6.85	0.122
7	2.2	7.58	0.135

2.9.5 Minimizing interferences

Small sampling volumes were enough to minimize interferences. On the other hand, larger volumes lead to turbid solutions providing inaccurate quantitative assessment.

2.10 Discussion of Results

2.10.1 Sources of HCN and Temperature Effect

Reviewing the literature showed that hydrogen cyanide can be formed either from pyrolysis of proteins where hydrogen cyanide would be coming from tobacco[51, 23], or upon pyrolysis of pyridine, pyrrole and 2-pyridone and in this case HCN would be coming from charcoal[19]. Although different mechanisms are employed for the

emission of HCN, both require elevated temperatures above 700 °C as illustrated in Section2.5. Since the temperature of tobacco in the narghile system ranges between 50 and 450 °C [43], then this could explain the absence of HCN in both mainstream and sidestream smoke of narghile.

However, it is expected that charcoal burns at temperatures as high as 900 °C; therefore, we should be able to detect HCN. To verify that charcoal is giving HCN, the system described in Section2.7.6 was produced. Table2.6 shows mass of HCN, in the gas phase, collected in mainstream and sidestream smoke. HCN was not detected in the particle phase. A total of 5 sessions were collected for the mainstream smoke with an average of 1.28μg/g HCN and %RSD of 2%. A total of 6 sessions were collected for the sidestream smoke with an average of 4.92μg/g and %RSD of 4%.

Table 2.6: Mass of HCN (μg/g) collected in the gas phase from both mainstream and sidestream smoke

	Trial1	Trial2	Trial3	Trial4	Trial5	Trial6	%RSD
Mainstream	1.12	1.24	1.55	1.28	1.24	NA	2
Sidestream	5.39	5.34	4.75	4.82	4.17	5.04	4

Moreover, the effect of temperature was illustrated through a study conducted by Moir et al [29]). The aim of this study was to assess smoking constituents of marijuana and tobacco cigarette smoke under two smoking conditions. The first set was conducted following the ISO standards with a puff volume of 35ml, a puff duration of 2 s and a 60 s interval; whereas, the second one was carried under extreme conditions with puff volume of 70ml, a puff duration of 2 s and a 30 s

interval. By increasing the puff volume along with decreasing the interval, tobacco is expected to reach higher temperatures and for longer periods of time, and thus HCN is expected to be present in higher yields. Moreover, this should also be manifested by comparing mainstream smoke to sidestream smoke where the constituents of the latter are generated at lower temperatures. Results showed that HCN coming from mainstream smoke increased almost by 53.85% when extreme conditions were applied which validate the theory that HCN requires higher temperatures to be generated. Furthermore, temperature effect was reflected by comparing mass of HCN from mainstream smoke to sidestream smoke. Sidestream smoke operates at lower temperatures than mainstream smoke and so lower yield of HCN expected and was actually the case.

2.10.2 Artifact effect

Good and co-workers[17] illustrate a major drawback in the trapping systems. The gas phase of filtered smoke, using Cambridge pad, contains few nitrogenous compounds other than nitriles. When the pad was removed, larger number of volatile components which were not usually observed in the gas phase was detected. This is due to the condensation of water on the Cambridge pad. Since many volatile nitrogen-containing compounds are soluble in water, then they are effectively removed from smoke.

One type of produced artifact during smoke collection is formation of cyanohydrins. It is well known that smoke contains both hydrogen cyanide and volatile carbonyl compounds which are capable of forming cyanohydrins, as shown in Fig2.16

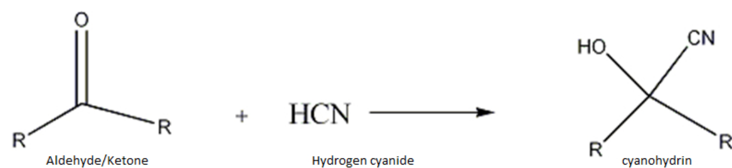


Figure 2.16: Formation of cyanohydrin

Dube and Green identified the formation of the following cyanohydrins such as lactonitrile, isobutyraldehyde, and acrolein cyanohydrins, shown in Fig2.17.

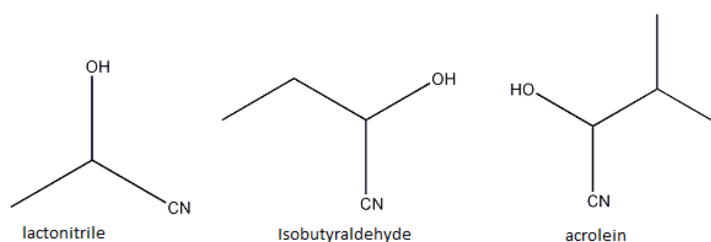


Figure 2.17: Structures of Lactonitrile, isobutyraldehyde, and acrolein cyanohydrins respectively

Schwartz[41] reported that in any region with mixed production of hydrogen cyanide and formaldehyde, the actual species reaching the surface would tend to be the combined form of the two compounds. That is the cyanohydrin of formaldehyde, glyconitrile. This reaction is kinetically fast with an equilibrium constant of 4.6×10^5.

Moreover, the formation of an artifact was also verified in a study carried by Torikaiu et al[51]. The aim of the study was to assess the correlation between tobacco components and smoke constituents. Torikaiu noticed that adding protein to burley tobacco increased the yield of HCN significantly and decreased the amount of aldehydes obtained[51]. However, the same additive was added to another type

of tobacco, namely flue-cured tobacco, where only a slight increase in HCN was obtained. Torikaiu concluded that although protein has the potential to produce HCN, other components are present in the smoke acting as inhibitors for the generation of HCN. One way to explain what's happening is by comparing the types of tobacco used. First burley tobacco is a high nitrogen content tobacco with very low sugar content, compared to flue-cured which has a high carbohydrate, both sugar and starch, content[49]. This implies that more aldehydes are generated by the flue-cured than the burley tobacco. Thus these aldehydes would be able to scavenge HCN produced to a high extent and show less increase in the HCN yield upon addition of protein. On the other hand, burley tobacco has minimal concentration of aldehydes, and as shown in Torikaiu paper, aldehydes concentration actually decreased tremendously with the signicant increase in HCN.

Now following the same mode of thought, we tried to verify experimentally that cyanohydrins are formed once HCN is trapped in the basic solution. A set-up, arranged as described in Section2.7.7, was employed to divide the smoke equally between the two impingers. Equal amounts of HCN are expected to be present if equal flow rate is set. Therefore, one of the impingers contained 0.1M NaOH and the other 0.1mM aldehyde. Samples collected in aldehyde solution showed no traces of HCN; whereas, same amount of HCN collected in the basic solution as reported in Table2.6. Therefore, we concluded that what small fraction of HCN being produced from the charcoal is scavenged by aldehydes released from smoke especially because recent results reported by M. Al Rachidi[2] showed that narghile contains very high levels of aldehydes ranging from 2520μg/narghile for acetaldehyde to 106μg/g for

methacrolein.

2.10.3 Effect of glycerol on trapping HCN

One might think that glycerol, due to its polarity, can act as an adequate trapping solution of HCN, and that could be another reason for why HCN was not detected. A similar set-up to that used in Section2.7.7 was constructed with the difference in the solution placed in second impinger. In this case, instead of formaldehyde, a 0.1M glycerol was placed in the impinger. Results actually show that HCN can be trapped in a glycerol solution. Therefore, some of the produced HCN, coming from charcoal, might be trapped in the high content glycerol tobacco.

Chapter 3

Phenols

3.1 Introduction

Phenols are a class of aromatic organic compounds consisting of one or more hydroxyl group attached to a benzene ring. They act primarily as precursors during the manufacturing of phenolic resins, human made polymers, such as of phenol, aniline, bisphenol A, and captrolactam[6, 14]. Bisphenol A is used in the manufacturing of polycarbonate plastics, epoxy resins and non-polymer additives. Caprolactam is utilized in nylon 6 and other synthetic fibers[14]. Other uses include the production of explosives, fertilizers, pharmaceutical products, dyes and indicators[3].

The simplest member, phenol, has a molecular formula of C_6H_5OH, with one hydroxyl group attached to a benzene ring; phenolic compounds are listed in Figure 3.1.

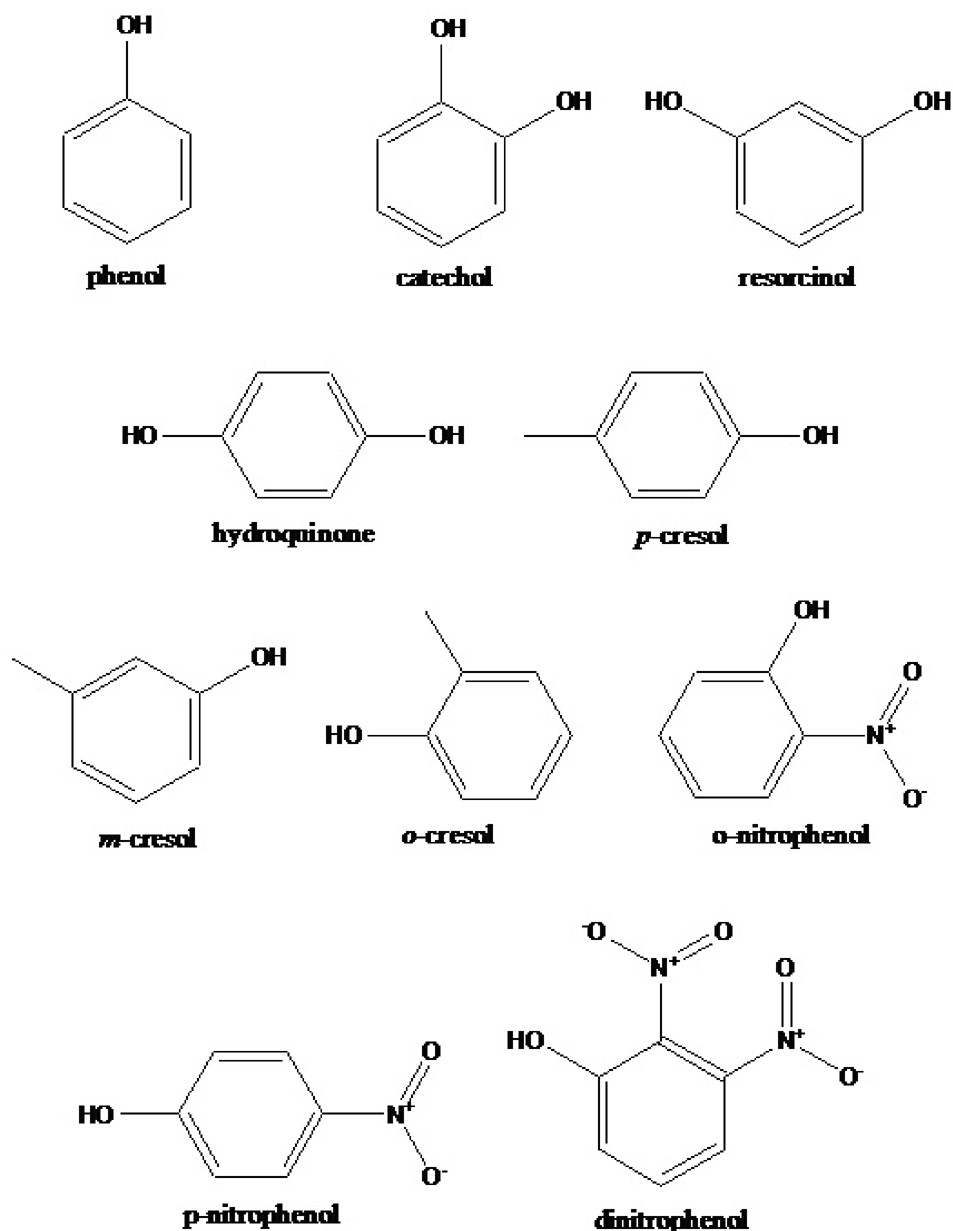


Figure 3.1: Chemical structures of phenolic compounds.

It has been first isolated by Runge, a German Chemist, in 1834, and was named karbolsaure (coal-oil acid or carbolic acid), though its composition was only known in 1841. Its source remained natural for almost half a century until Wichelhaus realized the value of Faraday's reaction by which aromatic sulfonic acids could

be fused with alkali to yield the hydroxyl-compounds. This method was applied for β-naphthol, an important precursor for dye industry [7, 24].

Phenol is one of the most widely used organic compounds standing as a basic structural unit for various synthetic organic compounds including agricultural chemicals and pesticides. It is ranked in the top 50 chemical volumes produced in the United States with the housing and construction accounting for about half of the consumed amount [24].

The determination of these compounds is of interest in many fields, such as environmental control, neurochemistry, and pharmaceuticals [36]. Moreover, some of them are formed during the pyrolysis during tobacco smoking which contributes to another source of pollution especially in closed areas. In this study, we would be focusing on the identification and quantification 7 phenolic compounds, phenol, o-cresol, p-cresol, m-cresol, catechol. resorcinol and hydroquinone in the particle phase of mainstream narghile smoking session.

3.2 Sources of Phenols

Phenol is produced naturally in the environment or synthesized as a manufactured chemical. It is a constituent of coal tar formed during the natural decomposition of organic materials [11] and a by-product of human and animal wastes. Food such as tomatoes, apples, bananas, peanuts and milk, and non-food such as salicylate produced by plants contain phenols [14]. The majority of phenol in the atmosphere however is from anthropogenic activities. Residential wood burning, exhaust gasses

and photochemical degradation of benzene are all potential anthropogenic sources [11]. Moreover, smoked food products and cigarette smoke release a variety of phenolic compounds. In 1986, IARC reported a mass of phenol emitted from cigarettes that ranged between $60 - 140\mu g$ for 1 non-filtered cigarette, $19 - 35\mu g$ for a filter-tipped cigarette and $20 - 107\mu g$ for cigars [30].

3.3 Chemical and Physical Properties

Phenol is translucent, colorless, crystalline mass of hygroscopic properties, white powder or thick syrupy liquid at room temperature[6]. It turns pink to red if exposed to air and light. Pure phenols have sweet, tar like odor that is readily detected at concentrations as low as 0.05 ppm in air [6]. It's also known as carbolic acid, benzophenol, and hydroxybenzene. Phenols are acidic with a variable degree of acidity depending on the substituents on the ring. Their pka value can range from 0.3 for 2,4,6-trinitrophenol to 9.92 for phenol [11]. Electron withdrawing group enhance the acidity tremendously because they stabilize the phenoxide ion by dispersing the negative charge through resonance, Figure 3.2, while electron donating groups destabilize the conjugate base.

Therefore, p-nitrophenol, is expected to have higher ka value than phenol and p-cresol. Table 3.1 confirm our speculations since nitro-group is an electron withdrawing group, whereas, methyl group in p-cresol is electron donating group that destabilizes the conjugate base by enhancing the negative charge on the phenoxide ion. Moreover, the distribution of negative charge over benzene ring is what

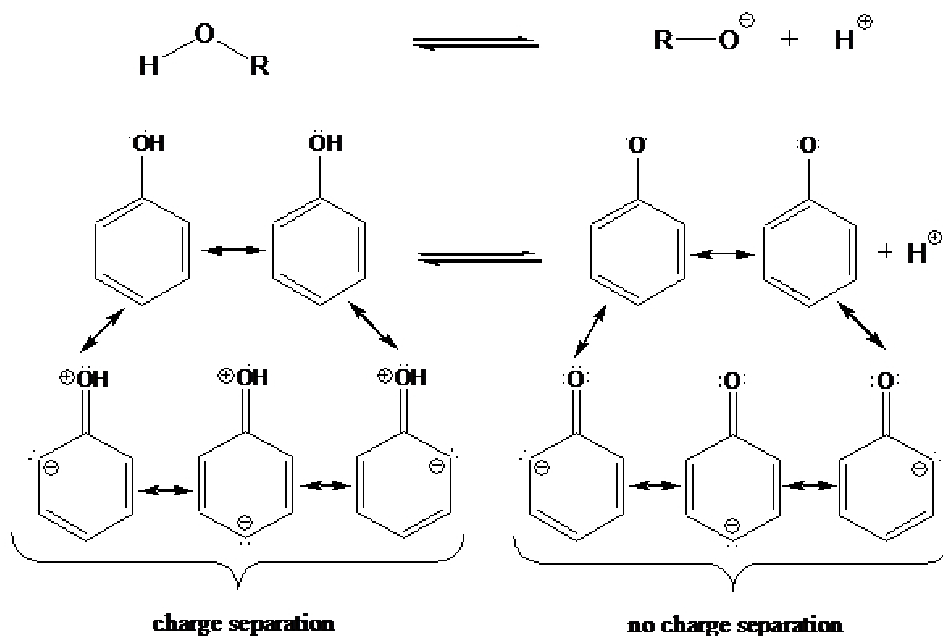


Figure 3.2: Resonanace structures of phenoxide ion.

Table 3.1: Dissociation constants,K_a, for phenol, p-cresol and p-nitrophenol

Compound	Phenol	p-cresol	p-nitrophenol
K_a	1.1×10^{-10}	0.67×10^{-10}	690×10^{-10}

makes phenols more acidic than any other alkyl alcohol.

Other properties include their high reactivity with oxidizing agents, calcium hypochlorite, aluminum chloride and acids [16]. Phenol vapor is heavier than air with a very low vapor pressure of 0.35 mmHg at 20 °C (1), and an octanol/water partitioning coefficient ($logP_{ow}$) of 1.46 indicating that phenol can exist in gas and particulate phase. It has a high boiling point [6] of 182 °C. In addition, it is soluble in organic solvents such as alcohol, glycerol, petroleum, and has limited solubility in water[6, 3] of 6.7g/100ml, 9%, at 25 °C.

3.4 Health Hazards

Phenol is readily absorbed following inhalation, ingestion, and skin contact. However, exposure to phenol through inhalation is a less probable route than dermal or oral. This is because its heavier than air and has low vapor pressure thus limiting inhalation hazards [6, 7].

The permissible skin exposure limit (PEL) set by OSHA is 5ppm averaged over 8-hour work shift. Fortunately, the odor threshold for phenol is about 100 times lower than that value which provides adequate warning of hazardous concentrations [6].

Phenol, a corrosive substance, denatures proteins and generally acts as a protoplasmic poison. They are known as tumor promoters, though EPA classified them as Group D, thats is non-human carcinogens [14, 7]. Systematic poisoning can occur after inhalation, skin contact, eye contact, or ingestion. Acute exposure causes chronic damage to the central nervous system (CNS) and eventually to death. Milder symptoms following phenol poisoning are dizziness, seizures, sudden blood pressure elevation followed by progressive severe low blood pressure, or irritation of the respiratory tract. Moreover, prolonged skin contact can cause severe burns even with low concentrations (1% to 2%)[6, 7].

3.5 Mechanisms of Phenol Formation

Aromatic hydrocarbons constitute one of the most important classes of volatile organic compounds (VOCs) emitted to the troposphere. They represent an important portion of the reactive organics emitted into polluted urban atmospheres. This is

reflected by their significant contribution to the formation of ozone, photooxidants, and secondary organic aerosols (SOA) in urban air. Therefore, understanding the atmospheric chemistry of aromatic compounds is crucial for understanding the chemistry governing air pollution.

Benzene and the alkyl-substituted benzenes such as toluene, ethylbenzene, xylenes and trimethyl-benzenes are major atmospheric pollutants. Benzene represents the simplest aromatic compound emitted into the atmosphere as a result of anthropogenic processes[53]. It's of major concern for it had been classified by the US Environmental Protection Agency as Group- A human carcinogen[10]. Benzene is exclusively scavenged from the atmosphere upon reacting with OH and NO_3 radicals[3] yielding OH-aromatic adducts such as OH-benzene, OH-toluene and OH-trimethylbenzene. OH radical-initiated reactions dominate during daytime; whereas, NO_3 radical-initiated reactions dominate over nighttime[20]. These adducts constitutes precursors for formation of other pollutants such as OH-benzene giving phenol, OH-toluene given cresol and xylene giving dimethylphenol. These products can undergo further transformations resulting in nitrophenols and dinitrophenols. As mentioned above, the formation mechanism of phenol and its derivatives vary between daytime and night time because different precursors exist at different times.

3.5.1 Formation of phenols and nitrophenols during daytime

OH radical reaction proceeds via H-atom abstraction from C-H bonds of alkyl-substituent group or from the C-H bonds of aromatic ring in case of benzene. Then OH radical addition to the aromatic ring to form hydroxycyclohexadienyl or alkyl-substituted hydroxycyclohexadienyl radical (OH-aromatic adduct). The dominant reaction of adduct is with O_2.

Phenols[50]

•OH attacks a benzene ring, or toluene, to create an OH/benzene, OH/toluene, adduct. Then the OH-benzene derivative adduct can react with O_2 from air to give phenol or phenol derivatives.

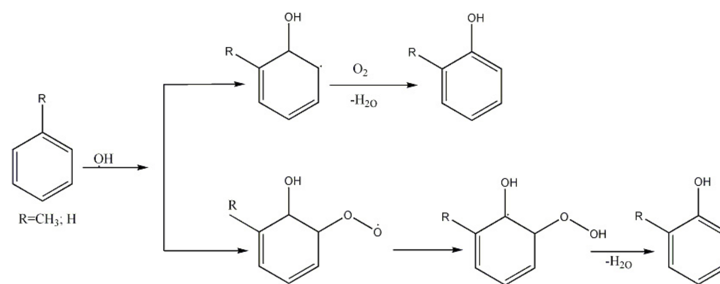


Figure 3.3: Formation mechanism from benzene/benzene derivatives

Nitrophenol

Nitrophenol is a by-product of phenol. Both OH and NO_2 radicals are major precursor for nitrophenol formation. NO_2 can be formed upon decomposition of N_2O_5[29]

Figure 3.4: Thermal decomposition of N_2O_5; M being a third body mainly air, $O_2$and N_2 acts to collisionally stabalizie the association complex

The first step includes formation of phenoxy radial upon OH radical attack on phenol ring, then NO_2 radical attacks the ring resulting in o-nitrophenol. This mechanism also presents the formation of catechol, being major product, upon oxidation of phenol.

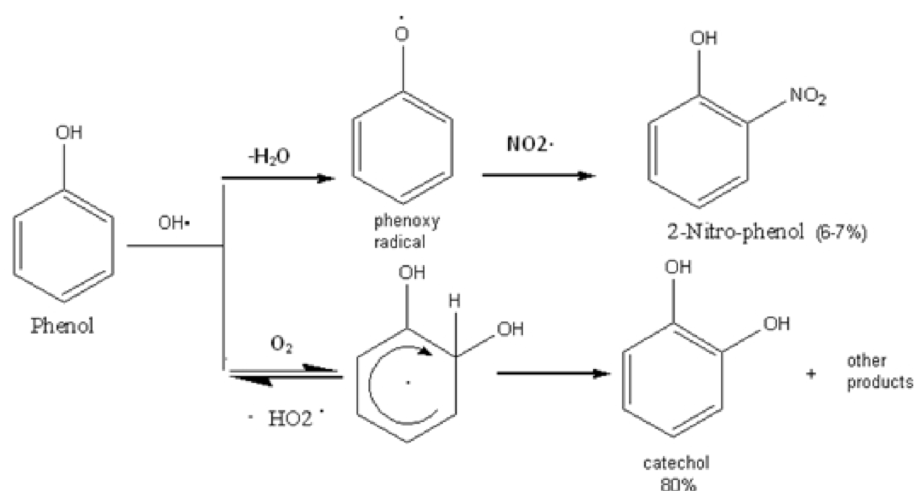


Figure 3.5: Formation mechanism of nitrophenol from phenol

Similar mechanistic path is followed in case cresol is present in the atmosphere and reacting with OH and NO_2 radicals[20]

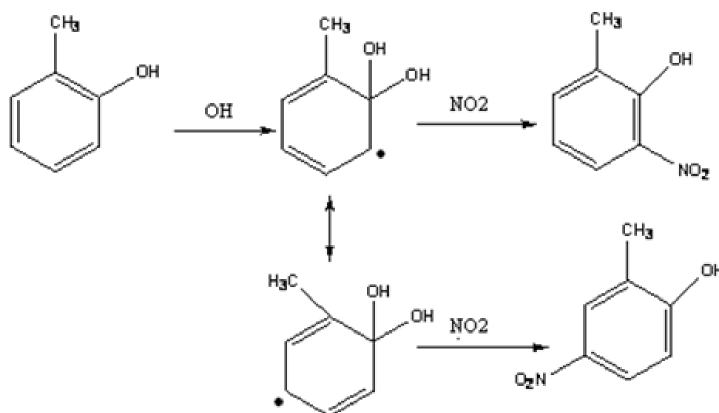


Figure 3.6: Formation mechanism of nitrophenol from Toluene

3.5.2 Formation of phenol and nitrophenol during the night

NO_3 is the major precursor for nitrophenol formation. It is generated either upon thermal decomposition of N_2O_5, as shown in Fig3.4, or in-situ through the following

mechanism[4] $NO_2 + O_3 \rightarrow NO_3 + O_2$. Two mechanisms have been reported on nitrophenol formation. One suggested by Atkinson, mechanism2.8 where first step includes formation of phenoxy-radical upon NO_3 radical addition to the phenol ring on a carbon atom adjacent to the OH-moeity forming a six-membered ring upon loss of HNO_3. Then NO_2 radical attacks ring from two different positions to form the isomers o- and p- nitrophenol.

Another mechanism has been suggested by Bolzacchini[4] who assumed that the reaction proceeds via the addition of NO_3 radical to the ipso carbon to form $\bullet NO_3$-aromatic adduct of a cyclohexadienyl structure. This adduct is stabilized by intramolecular hydrogen bond. Then the method proceeds similarly to Atkinson where nitrophenol is formed upon HNO_3 cleavage and NO_2 radical addition, mechanism2.9.

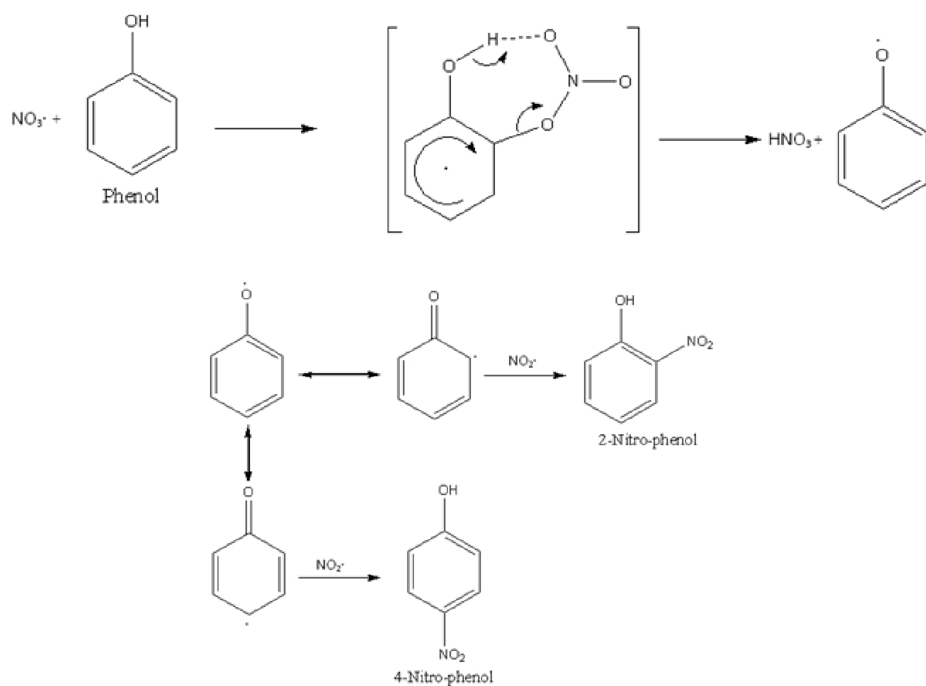


Figure 3.7: Atkinson's mechanism for nitrophenol formation

Similarly nitro-cresol is formed upon NO_3 addition to cresol and then lose of HNO_3 upon NO_2 addition would lead to nitro-cresol[28].

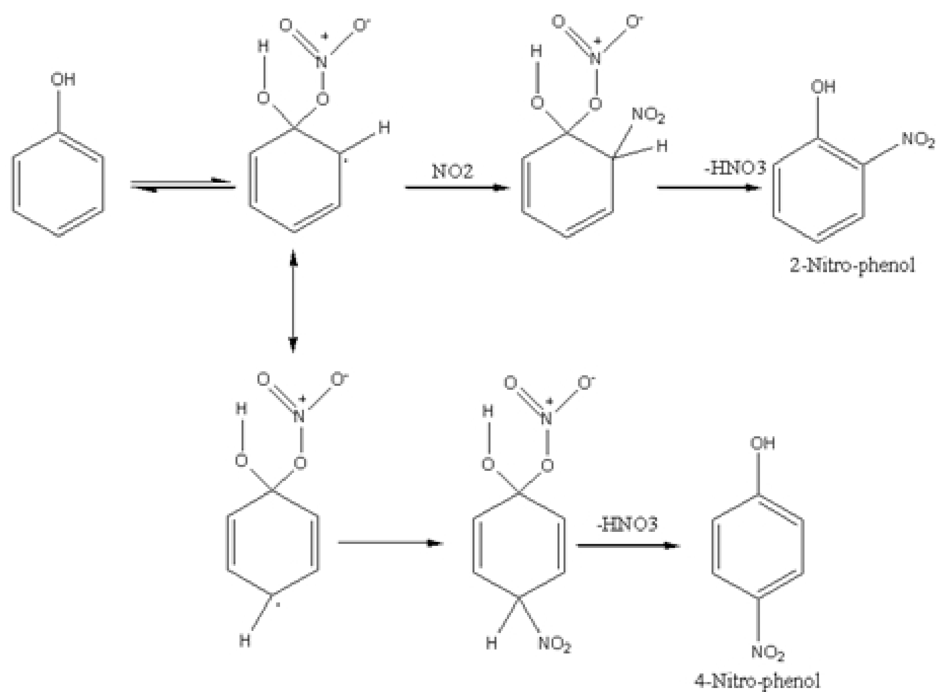


Figure 3.8: Bolzacchini's mechanism for formation of nitrophenol

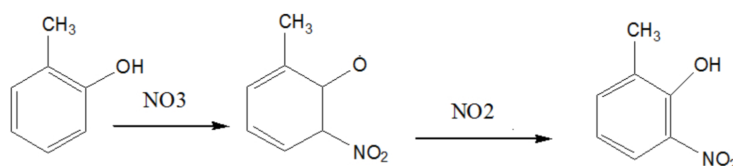


Figure 3.9: Formation of nitrophenol from o-cresol

3.6 Method Development and Measurement of Phenols in Narghile Smoke

Phenols present in cigarette smoke contribute to its sensory properties, flavor and aroma[35]; they are widespread environmental pollutants which are formed during the pyrolysis of tobacco constituents such as cellulose and polyphenols, chlorogenic

acid and quercetin dihydrate, acting as major precursors for catechol and phenol[16]. Fig3.10 represents two polyphenols, Quercetin dihydrate and Chlorogenic acid hemihydrates, which release catechol upon C-C bond cleavage at T > 800 °C.

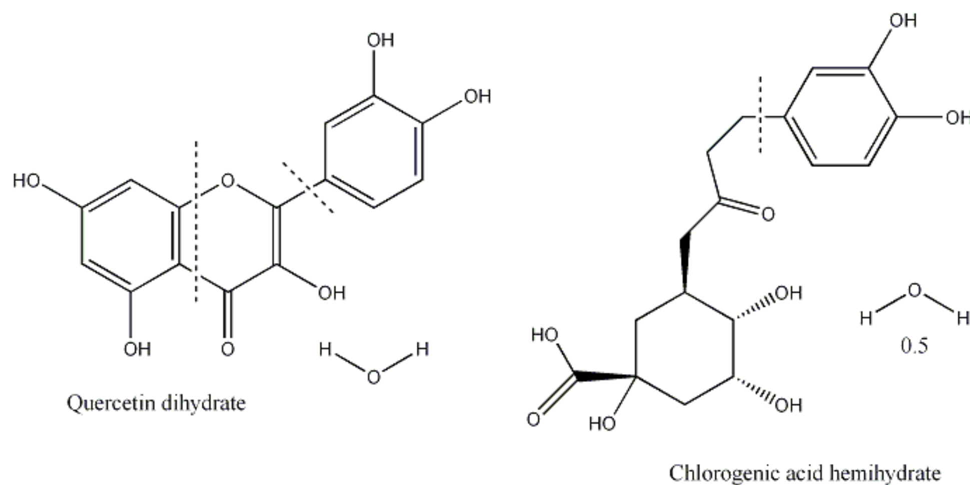


Figure 3.10: Structural formula of two polyphenols, quercetin dihydrate and chlorogenic acid hemihydrate

Phenol and its derivatives is the basic structural unit for a wide variety of synthetic organics including many pesticides. Phenol emissions include numerous sources such as automobiles[32], pesticides, accidental spills, or unintentional release associated with manufacturing processes and waste disposal[54]. They had been listed as a priority pollutant in the US Environmental Protection Agency (EPA)[54] due to their high toxicity at very low concentrations[56]. The ingestion of such contaminated water in the human body causes protein degeneration, tissue erosion and paralysis of the nervous system, and it also damages the kidney, liver and pancreas[56]. Moreover, anthropogenic phenols are of specific environmental con-

cern because of the ecological risk associated with their high toxicity and relatively high mobility in soil and groundwater environment. Phenols and nitrophenols have been given much attention because of their dangerous impact on humans and plants; it is believed that they are responsible for forest declination in Central and North Europe as well as in other parts of the world.

Studies have shown that hydroxybenzenes, such as catechols and hydroquinones, are important co-carcinogens of tobacco smoke and are capable of blocking lymphocyte proliferation and increasing lung cancer metastasis[52]. Cigarette smoke condensate (CSC) contains mono- and dihydroxybenzene and their alkylated derivatives derived from both natural and man-made sources[35]. Catechol can inhibit DNA synthesis, while hydroquinone free radical system present in tar can cause DNA damage[52]. Their high toxicity has called for the development of a sensitive analytical method for the determination of these compounds. Many analytical approaches have been used for the trace analysis of phenols, mainly using high performance liquid chromatography (HPLC) or capillary gas chromatography[32] (GC).

For HPLC, a fluorescent detector is preferred over UV because it provides better sensitivity and selectivity. A gradient solvent of water and 1% acetic acid is used at a flow rate of 1.4 l.min-1 and the program for λ_{em} and λ_{ex} set as shown in Table3.2[30]

As for GC procedures, they require some type of prior chromatographic separation or purification to produce a purified phenolic fraction, derivatization and a pre-concentration step. This technique depends solely on the volatility of the molecule. Glass capillary columns are preferred over packed columns because they

Table 3.2: Wavelength program for HPLC fluorescence

Time (min)	0	3.5	13.2
λ_{em} (nm)	338	298	310
λ_{ex} (nm)	304	274	232

provide higher resolution. Furthermore, derivatization agents are employed to produce the trimethylsilyloxy derivatives to lower their volatility and thus obtain better separation[35]. For phenol analysis, it's preferable to derivatize them prior to analysis because it provides better resolution even at low concentrations.

This study will concentrate on finding a reliable analytical method that will be adopted to identify and quantify the seven biologically active phenols, as listed in EPA, released from mainstream particulate matter collected from a narghile smoking session. This entails the determination and optimization of the extraction of phenols from particles, cleaning the samples, as well as the identification and quantification of the emitted phenols. Although many analytical applications have been used for the trace-level analysis of phenols mainly using HPLC, GC is often preffered, offering unrivalled high resolution and easy coupling with sensitive and selective detectors, thus our decision to use GC-MS over HPLC.

Therefore, we are utilizing gas chromatography as our analytical technique for the analysis of 7 phenols including phenol, o-cresol, p-cresol, m-cresol, catechol, resorcinol, and hydroquinone in narghile and cigarette smoke mainstream particle phase.

3.6.1 Materials and Methods

Chemicals

Acetonitrile (HPLC grade) and ethylacetate pure were obtained from Acros, methanol (LC-MS, chromasolv), ascorbic acid powder (99%), dichloromethane (DCM, HPLC¿99.9%) and dimethylformamide anhydrous (DMF, 99.8%) were obtained from Sigma-Aldrich. Hydrochlodric acid (37% HCl) from AnalaR-BDH, acetic acid (100% extra pure) from Riedel-de-Haen. A 1000ppm standard of phenolic mixture (99% pure) was supplied from Absolute Standards INC. A 1000ppm internal standard of p-cresol-d8 (98.6% pure) and phenol-d6 (98.9% pure) obtained from Absolute Standards INC. 5ml BSTFA for GC with 1% TMCS obtained from sigma Aldrich.

Apparatus and Analysis

12×32mm Crimp Style standard mouth amber vials with 11mm Aluminum Blue cap of PTFE/Butyl Septa 40mil obtained from discovery sciences. 24ml and 12ml Wheaton type vials and 47mm glass fiber filters (type A/E PALL). Polystyrene divinylbenzene (PS-DVB) SPE cartridges (200mg, 3ml) Easy-Chrom from Sorbent Technology. A Genius 3 Vortex with an orbital shaker from IKA. A TECHNE sample concentrator and heater used to concentrate samples under N_2 gas and also used as a heater. All measurements were performed with a Thermo Trace GC-Ultra equipped with ITQ-900 ion trap MS and AI-3000 auto-injector. TR-5 ms column (30m, 0,25mm ID, 0,25 μm film thickness).

Optimized Conditions of Phenol Detection on GC-MS

Calibration standards were obtained by diluting the 7 phenolic standard mixture solution in ethylacetate. Standard solutions helped in optimizing the separation of the 7 phenolic standard mixture in the adopted chromatographic methods (Table3.3) (GC-MS) in order to determine their retention times as well as their limit of detection. The lowest concentration that can be measured on the GC-MS with acceptable accuracy and precision was found to be 75 ng/ml.

Table 3.3: Thermo Trace GC-Ultra with AI 3000 autoinjector and ITQ-900 ion trap MS

Gas Chromatography	
Column	TR-5ms (30m, 0.25mm ID, 0.25 μm film thickness)
Carrier gas	Helium
Injection volume	1 μl splitless
Temperature Program	
Initial column temperature	40 °C
Initial hold time	1min
Program	15 °C/min to 133 °C and hold for 3min
	10 °C/min to 140 °Cand hold for 2min
	10 °C/min to 160 °Cand hold for 2min
	10 °C/min to 183 °Cand hold for 1min
	15 °C/min to 270 °Cand hold for 3min
Mass Spectrometer	
Transfer line temperature	280 °C
Ionization mode	EI
Mass range	100 to 300 amu, full range data aquiition (SCAN) mode

3.6.2 Extraction, Cleaning and Detection of Phenols from Narghile Smoke

Extraction of Phenols

The first step in the determination of phenols from filter pads is extraction, which can be performed by mechanical shaking using a vortex or a shaker followed by cleaning and detection. The extraction efficiency of phenols depends on the solvent used for extraction as well as on the sample matrix. Based on the literature review, the following method of extraction has been adopted. Filter pads are extracted on a vortex, through mechanical shaking using acidified water and 0.1%ascorbic acid. Ascorbic acid is added to prevent any potential oxidation of phenols[30].

Samples were prepared by soaking one filter in 20ml of acidified water in 24ml vial and placed on the mechanical shaker for 2h at room temperature. Acidified water is prepared by diluting 8.71ml of HCl (37%) and 1g of ascorbic acid in a 1000ml volumetric flask. Then the total extraction is loaded on PS-DVB SPE cartridges using a vacuum pump. After loading, samples are washed 3 times with 3ml acetic acid (1%). Phenols would be retained from the smoke extract and the washing will not remove the phenols from the cartridge. Next, the cartridge is left to dry under vacuum. After 2h, the sample is eluted using 6ml ethylacetate. Then the volume is reduced to 200μl under nitrogen. 100μl of the ethylacetate solution was taking from the eluant and placed in a GC-vial, and 60μl of BSTFA with 1%TMCS was added. The vial is caped and heated at 60 °C for 20min to obtain the trimethylsilyl (TMS) derivatives of phenols that are to be analyzed by GC-MS.

For narghile extracts, the GC-MS total ion chromatogram (TIC) shows that phenols are masked by other compounds that are present at much higher concentrations (Fig3.11-3.13). Hence, quantification of the pre-concentrated sample was done using GC-MS selected ion current profile in order to increase the sensitivity and selectivity of the analysis. Chromatograms showed the intensities of each m/z being monitored as a function of time (EPA Method-528). The selected ion current profile applied to the full scan chromatogram for each of the 7 Phenolic compounds, led to the isolation of these compounds with relatively higher resolution and so quantification of smaller concentrations became possible.

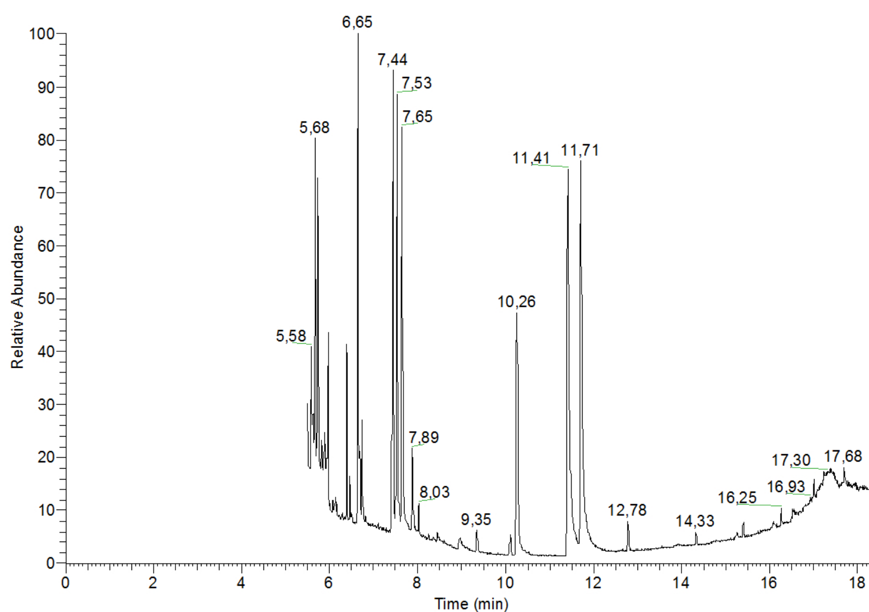


Figure 3.11: GC-MS full scan chromatogram of a standard at a concentration of 3.125ppm

The GC-MS selected ion current profile of the extract discerned some phenols, such as phenol, catechol and resorcinol that are present at high concentrations. Other phenols are present at very low concentrations, like o-, p- and m-cresols, that showed unresolved peaks and thus cannot be assessed quantitatively even with the selected ion current profile. Fig3.14-3.20 are examples of the isolation of phenol, catechol, and resorcinol in narghile and cigarette samples as well as in standards.

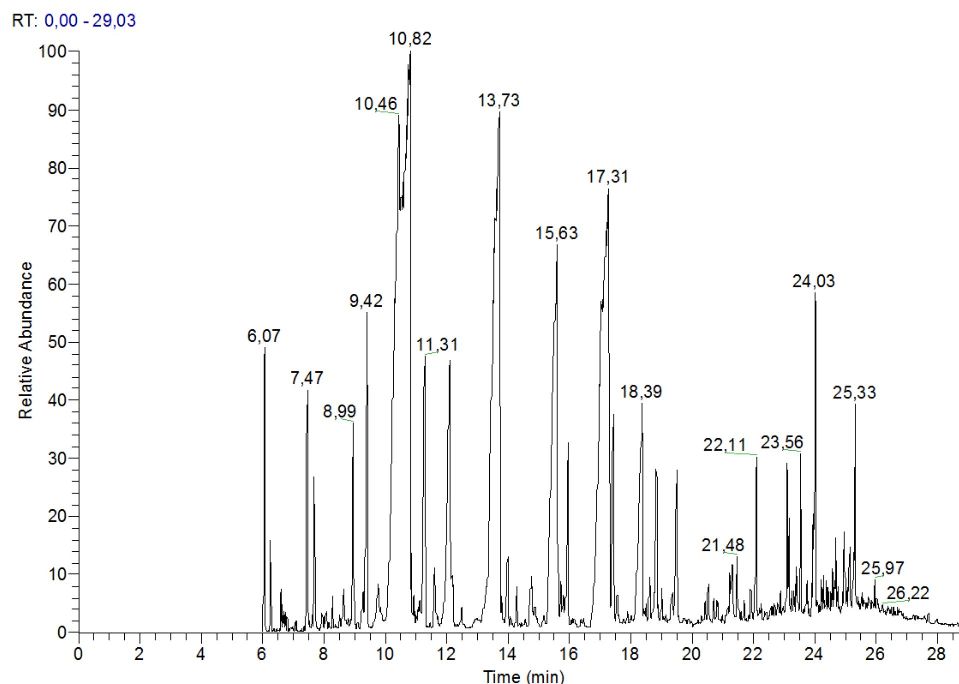


Figure 3.12: GC-MS full scan chromatogram of narghile filters extracted using ethylacetate

Effect of Acidity on Extraction Efficiency

The pH of water is directly proportional with the extraction efficiency. It affects the matrix as well as the sorbent efficiency in the SPE cartridge. pH values should be adjusted to 2-3 to minimizes ionization of phenols since at neutral pH even most acidic phenol is largely deprotonated. At pH 2 or lower, the ionized form becomes insignificant, thus the amount of analyte extracted by the fiber increases[39]. Moreover, an acidic pH provides higher stability for the PS-DVB sorbent and diminishes interferences coming from nicotine and related weak bases[13]. The pH is assessed by preparing 3 samples containing internal standard under 3 different acidic conditions. Narghile filters were spiked with 40μl of 1ppm Istd (p-cresol-d_8 and phenol-d_6), and

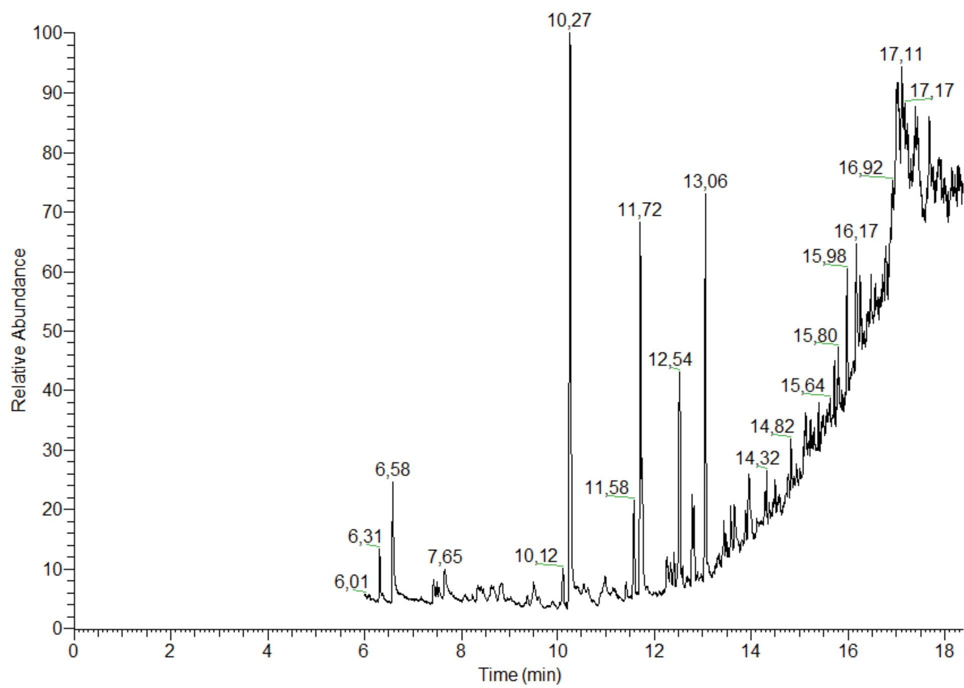


Figure 3.13: GC-MS full scan chromatogram of cigarette filters extracted using ethylacetate

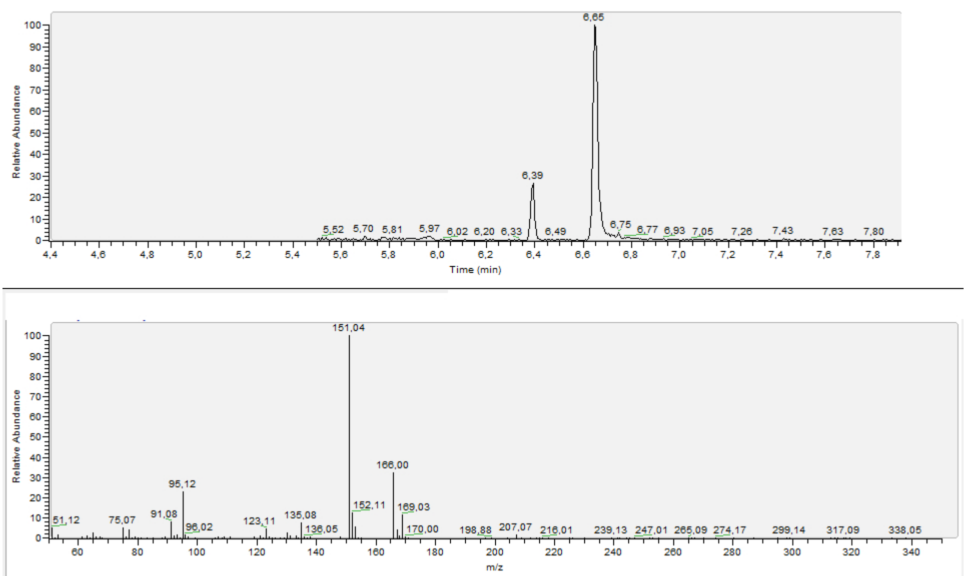


Figure 3.14: GC-MS selected ion current profile for phenol from a standard at 3.125ppm

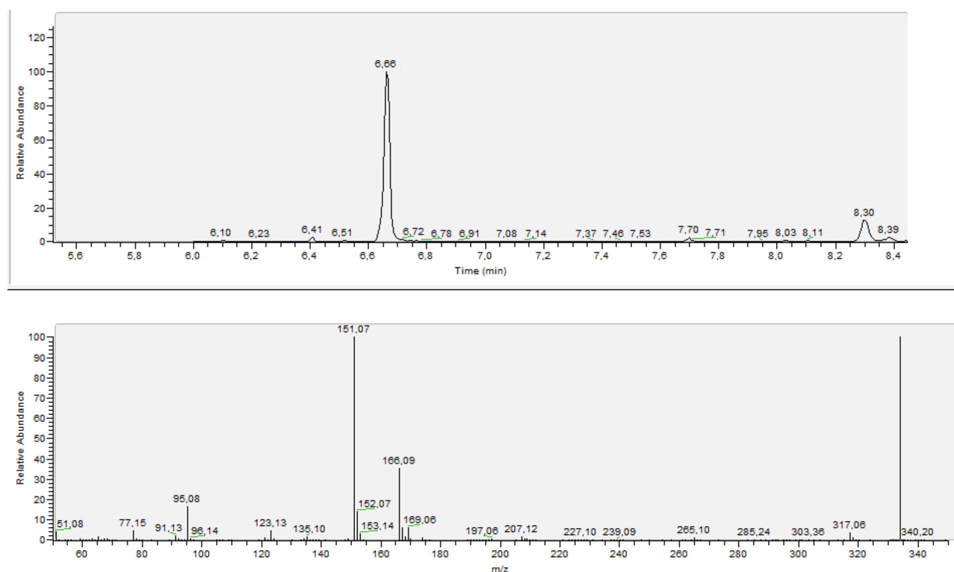


Figure 3.15: GC-MS selected ion current profile for phenol from narghileh sample

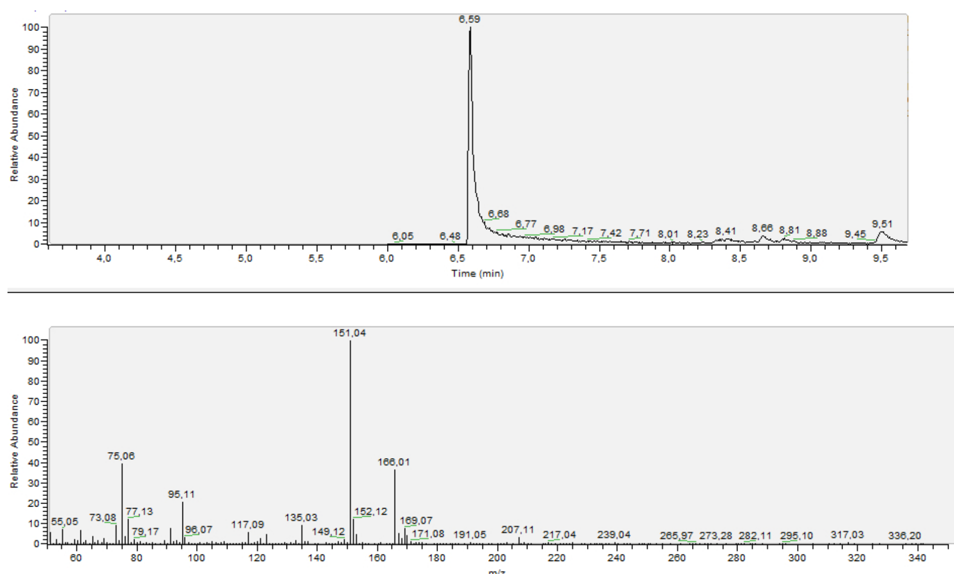


Figure 3.16: GC-MS selected ion current profile for phenol from a cigarette sample

then soaked with 20ml acidified water containing 0.1% ascorbic acid. The three pH's were prepared as shown in Table3.4.

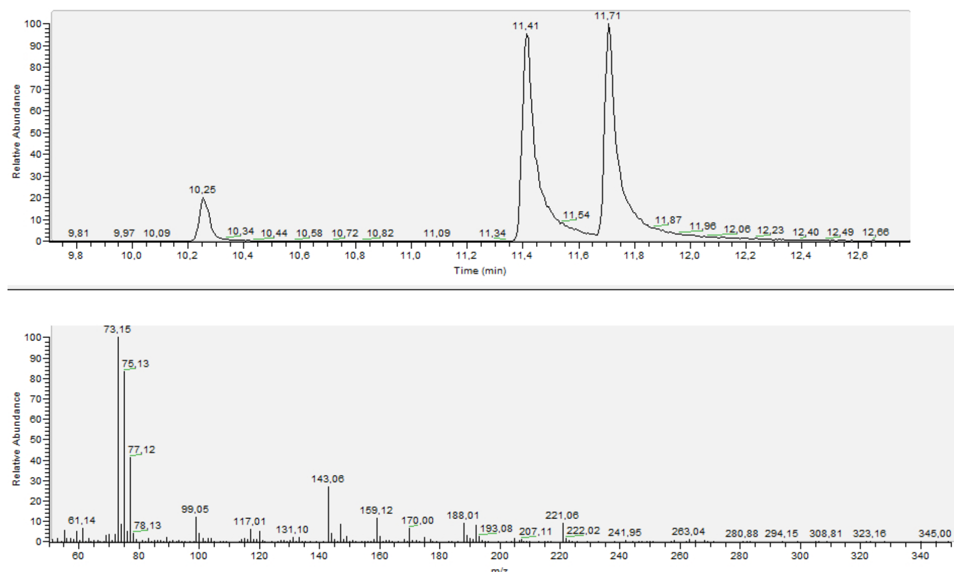


Figure 3.17: GC-MS selected ion current profile for catechol (rt 10.26) and resorcinol (rt 11.71) from a standard at 3.125ppm

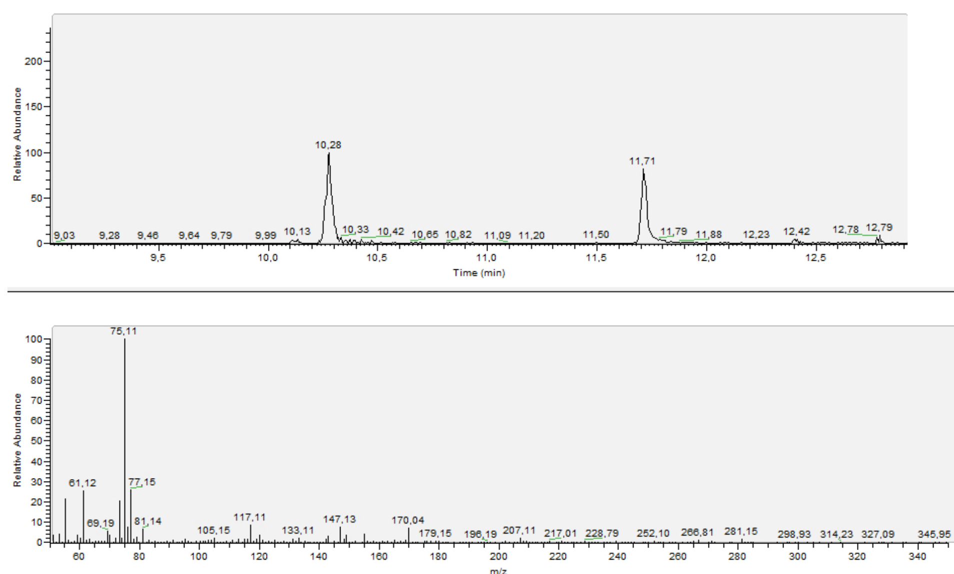


Figure 3.18: GC-MS selected ion current profile for catechol (rt 10.28) and resorcinol (rt 11.71)from narghileh samples

Three replicates are prepared, and the best recovery was obtained when filters were soaked with 20ml SampleA solution. Fig3.21 shows the variation of

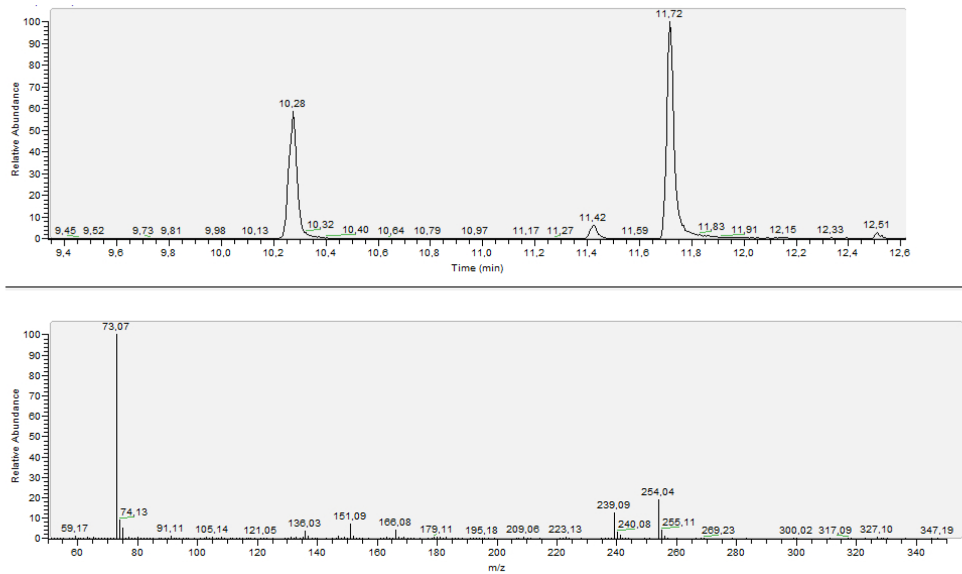


Figure 3.19: Selected ion current profile for catechol (rt 10.28) and resorcinol (rt 11.72) with MS of catechol, from a cigarette sample

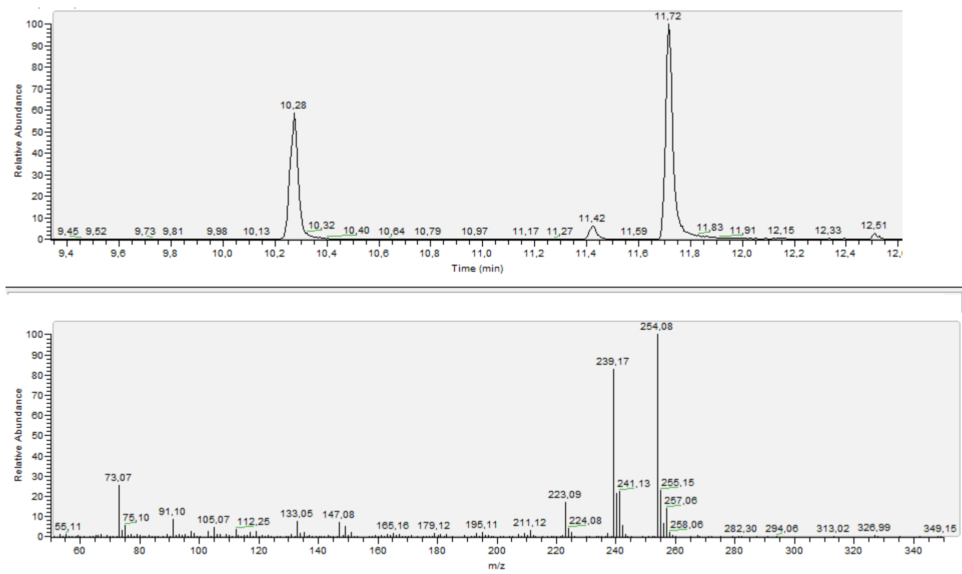


Figure 3.20: Selected ion current profile for catechol (rt 10.28) and resorcinol (rt 11.72) with MS of resorcinol, from a cigarette sample

Area with pH

Table 3.4: Volume of acid needed to prepare three solutions, of 100ml final volume, at different pH

	$pH_{experimental}$	V_{acid}
SampleA	0.95	1ml HCl (37%)
SampleB	3.02	1ml of SampleA
SampleC	5.42	1ml of SampleB

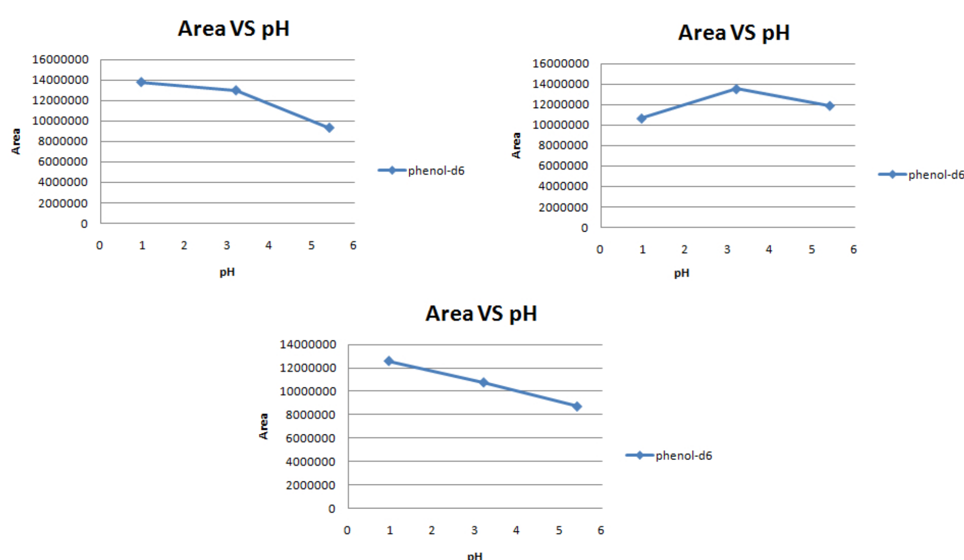


Figure 3.21: Effect of acidity on extracton efficiency of internal standard phenol-d6

Optimizing volume of extraction

An important step in extraction is the volume of solvent required to extract phenolic compounds. This was achieved by extracting samples with variable solvent volume. Samples are prepared following the same procedure described Section3.6.2, then they are soaked in 10, 20 and 30ml of acidified water (pH= 0.85). After 4 replicates,

results illustrated in Fig3.22, show that extraction efficiency increased from 10ml to maximize at 20ml and then drop at 30ml.

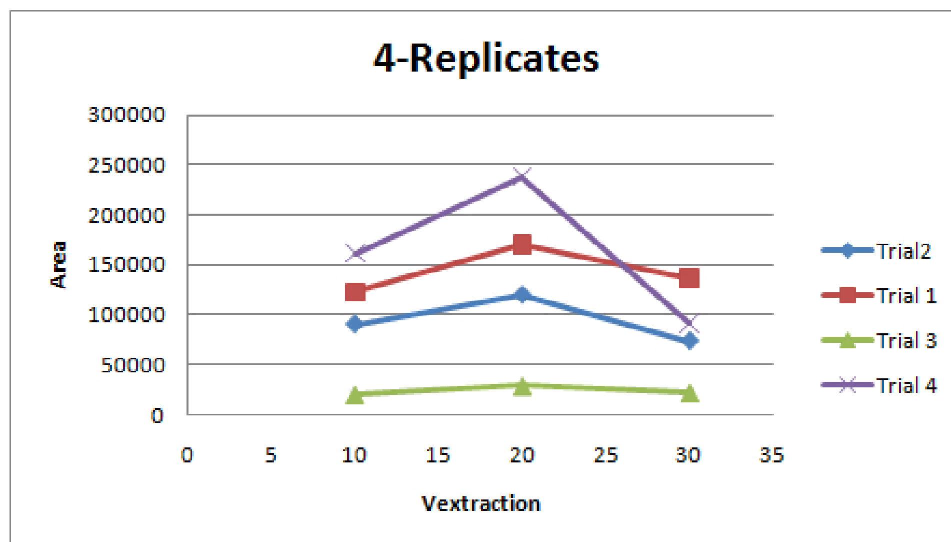


Figure 3.22: Variation of Area with volume of extraction as observed for 4 trials

Assessing Eluting Solvent and Volume of Elution

Reviewing the literature showed that different solvents such as anhydrous dimethylformamide (DMF), acetonitrile (ACN), methanol, acetone, iso-propanol, or ethylacetate, have been used to desorb phenols from PS-DVB SPE cartridges. As for the eluting volume, it depends on kind of solvent, amount of sorbent, SPE cartridges, and polarity of each phenol. In our work, we studied the elution selectivity and efficiency of DMF, ACN, isopropanol and ethylacetate and volume of elution.

4 sets of samples have been prepared and repeated three times. Samples are prepared by spiking narghile filters with 40μl of 1ppm Istd and soaking them with 20ml solvent. Ethylacetate showed highest selectivity for phenols; whereas, ACN and DMF chromatograms showed a lot of interferences that would prevent quan-

titative assessment. As for isopropanol, prior to heating, a brown color developed which prevented us from analyzing it. Therefore, the solvent with highest selectivity and that would be used for all analysis is ethylacetate.

Volume of elution was assessed, for ethylacetate, by eluting SPE at different intervals and analyzing each one aside. SPE cartridge was eluted with 6ml ethylacetate 3 times with each 6ml collected individually. Reduced volume of set 1 was 150μl and 80μl of BSTFA was added, set 2 and 3 had a 100ul reduced volume and 20μl BSTFA was added to set 2. No phenols were detected in set 2 which implies that 6ml ethylacetate is enough to elute all phenols from cartridge.

3.6.3 Polystyrene Divinylbenzene SPE cartridges

Solid phase extraction (SPE) is preferred for the separation and enrichment of polar environmental from sample solution to improve the detection limit and decrease the concentration level for the determination of various phenols[25]. Solid phase extraction is used to concentrate higher sample volumes with quantitative recoveries (high breakthrough volumes), and eluting retained volumes with minimum amounts of organic solvents since phenols are usually extracted using aqueous solutions which cannot be injected in GC-MS[39].

Several types of SPE sorbents have been developed for selected phenols such as Amberlite XAD-4, cylohexyl-bonded phases, graphitized carbon black[33]and polymeric resins. Phenols are usually trapped on by a C18 material through Van der Waals interactions between analyte and sorbent[39]. More advanced sorbents such as polymeric sorbents have been developed by modification of earlier used XAD resins, and

introduced to SPE cartridges. They comprise a polystyrene-divinylbenzene (PS-DVB) hydrophobic structure of variable particle sizes, areas and crosslinked grades. It has higher capacity for polar analytes, due to higher carbon content and surface-area exhibited by polymers. It has high selectivity for phenolic compounds and it excludes humic substances which might interfere with phenols in later analytical steps.

First step to concentrate phenols using PS-DVB SPE cartridges is sorbent activation which is achieved by conditioning the sorbent which also remove potential interferences from basic and non-polar species retained in cartridge. In our method, the conditioning criteria is the following, first the cartridge is washed by 9ml dichloromethane (DCM), followed by 9ml methanol, then activated with 9ml HCl (0.01) (Agilent[37]). Phenols are retained on PS-DVB sorbents through reversed phase mechanism and π-π interactions among electrons from the aromatic ring in the sorbent and phenol molecules.

3.6.4 Derivatization

Derivatization is a chemical process for modification of compounds in order to generate new products with better chromatographic characteristics. It is mainly imployed to improve the thermal stability of compounds, mainly compounds with polar functional groups ameliorating compounds' volatility. For GC analysis, molecules containing HO-, SH- and NH- can form intermolecular hydrogen bond leading to weak volatility, insufficient thermal stability, or may induce interactions of the compounds with the column packing resulting in lowering detection limit.

Derivatization comprises the substitution of a polar functional group where most reactions are alkylation, acylation and silylation. Alkylation reagents reduce the polarity by replacing labile hydrogens with aliphatic/aromatic moieties. In acylation, compounds containing labile hydrogen are transformed into estrs,thioesters and amides through the action of carboxylic acid/derivatives. This method requires a purification step prior to GC-injection because of the presence of a residual acid.

As for silylation reactions, labile hydrogen from acids, alcohols, thiols, amines, amides or enolizable ketones and aldehydes is replaced by a trimethylsilyl group. Reaction occurs through nucleophilic attack (SN_2), and the presence of a strong leavinggroup often improves the reaction yield, yielding more thermally stable and volatile products. Silylation includes direct injection to GC opposite to acylation[40].
Silylation is the most prevalent technique, and common reagents are trimethylchlorosilane (TMCS), trimethylsilylimidazole (TMSI), N-methyl-bis-(trifluoroacetamide) (MBTFA), N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA), and N-(t-butyldimethylsilyl)-N-methyl-trifluoroacetamide (MTBSTFA), whereof the two last ones are most frequently used, particularly when analyzing phenols, sterols and sugars. Choosing one of the two depends on the steric hindrance and molecular mass of the compound under study. Compounds with sterically hindered sites are better derivatized using BSTFA, whereas, compounds with high molecular mass are better derivatized using MTBSTFA.

Derivatization of phenols is often recommended even if not necessary because phenols, and specifically nitrophenols, tend to give broad, tailed peaks in gas chromatography due to their high polarity. This tailing becomes more pronounced with increasing age of the column, especially if highly polluted samples are analyzed[33]. In our study, we are derivatizing non-hindered phenols with relatively moderate molecular weight which implies that BSTFA can be employed.

BSTFA is an effective trimethylsilyl donor with donor strength. One of the particular advantages of BSTFA over other silylating reagents is the volatility of its by-products. Mono-(trimethylsilyl)trifluoro-acetamide and trifluoroacetamide. Low boiling compounds, TMS-amino acids and TMS-Krebs cycle acids, are co-eluted with by-products from most TMS derivatization reagents. Good chromatographic separations can be obtained with BSTFA, as the by-products from this reagent usually elute with the solvent front. The reactivity of BSTFA can be enhancement upon TMCS addition. This substitution is particularly appropriate when the peaks of interest have relatively low retention times and tend to be obscured by the derivatization reagent or by the primary reaction products from the derivatization reagent[40]. The cited method relies upon conversion of the phenolics to trimethylsilyloxy ethers[13], mechanism[46] is shown in Fig3.23. An assessment study has been done to check the time needed to heat the sample to get complete derivatization. This study was accomplished by complexing 1ppm internal standard with BSTFA, and 1μl was injected into GC after heating for 10 minutes. Study was conducted over 3h. Results showed that heating for 10min at 60 °C is enough to obtain complete derivatization.

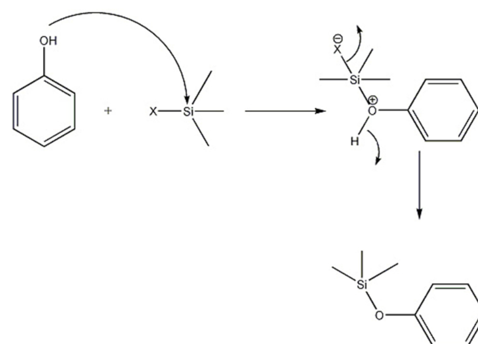


Figure 3.23: Formation mechanism of trimethylsilylether of phenols upon BSTFA addition to give glyoxal

3.6.5 Tobacco Smoke Preparation and Sampling

Narghile

Preparation of narghile system is similar to the preparations carried out in Section2.7.5. As for the set-up, same connections are made as described in Section 2.7.5 except that now the bubbler is removed, Fig3.24. Only particulate phase of mainstream smoke is collected for analysis. The smoking machine protocol is set as described in Shehadeh et al (2004)[45].

Cigarette set-up

All connections are made as described in Section2.7.7 except that the bubbler is removed. Smoking conditions follow the ISO standards with 35ml as puff volume,2s puff duration and 58s inter-puff duration with 1 puff per minute. 2 cigarettes are collected per filter.

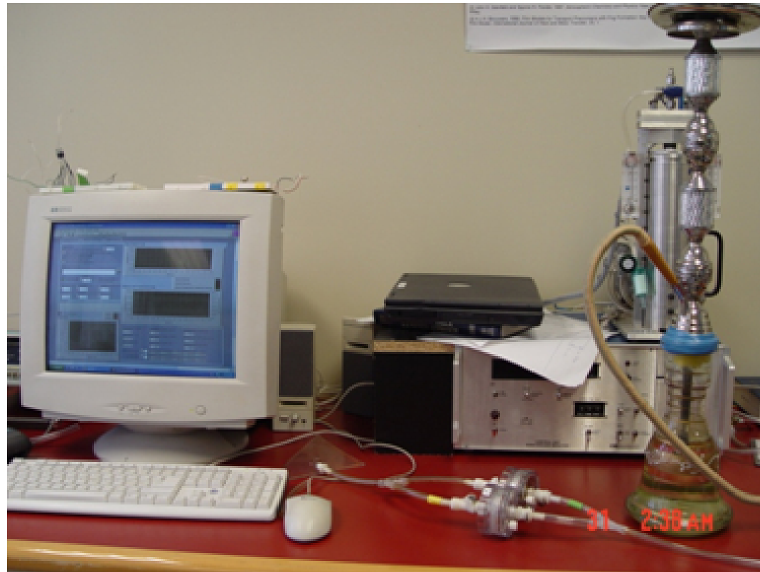


Figure 3.24: Narghile set up

TPM

Total particulate matters emitted from a complete argileh smoking session using tobacco and coal, were collected on 16 glass fibre filters. The complete argileh session burns on average 4.9 g of tobacco and around 9.3 g of charcoal to generate 171 puffs which were collected on 4-pairs of filters sequentially as illustrated in Fig3.25. The average mass of total particulate matter (TPM) collected on filters is 100mg. Each line of filters is changed at 60, 95, 125, and at 171 puffs. Four sets from each run were taken to be extracted separately: each set has a filter from one of the branches (Fig. 2.10), i.e. (F_1, F_5, F_9, F_{13} or F_2, F_6, F_{10}, F_{14}).

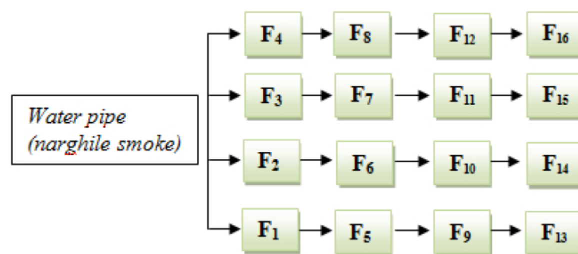


Figure 3.25: Filter set-up

3.7 Quality Control

Quality control experiments were conducted to assess the validity, reproducibility and reliability of the established analytical method. This was accomplished by calculating the percent recovery, accounting for phenols in the blank and analyzing cigarette filters, using the established method, to assess the collection procedure and the efficiency of the analytical method by comparing obtained results with literature values.

3.7.1 Determination of percent recovery (%R)

Since the extraction of Phenols required several steps, the recovery after finishing the extraction was assessed. Recovery is important for the validation and the precision assessment of the analytical method. This was achieved by preparing phenolic standard mixture at 5 different concentrations and dividing them in to two sets. The first set used for direct injection, prepared by complexing 100μl of a standard with 60μl BSTFA and heated at 60 °C for 15min. As for the second set, glass filter pads are spiked with 100μl these standards, and placed in a 24ml vial containing 20ml water, containing 0.1%ascorbic acid, at pH 1. Solutions were extracted using

a mechanical shaker for 2h at room temperature. The extracted solution is then loaded, under vacuum, on PS-DVB SPE cartridges already conditioned with 9ml DCM, 9ml methanol and 9ml HCl (0.05M). Samples are washed 3 times with 3ml acetic acid (1%) to remove unwanted residues and then left to dry for 2h. 6ml ethylacetate is used to collect the phenolic mixture from SPE cartridges, and volume is reduced to 100μl under dry nitrogen. Then 60μl BSTFA is added to sample and heated at 60 °C for 15min, samples are transferred to a 200μl insert placed in GC vials for analysis. Recovery is obtained by dividing the area of the standard obtained from extraction, by that obtained from direct injection and then multiplied by 100. Results are presented in Table 3.5

$$\%R = \frac{Areaofextraction \times 100}{Areafromdirectinjection} \tag{3.1}$$

Table3.5 shows that the average percent recovery does not follow any trend. For example, % recovery (%R) for phenol, o-, p-, and m-cresol increased from C_1 increased with concentration till C_4, but decreased at C_5. %R of hydroquinone increased with concentration from C_1 to C_5. On the other hand, catechol and resorcinol did not show any trend. In general, an increase in concentration should lead to higher recovery because molecules tend to adsorbed to solvent and would take longer time to evaporate, thus less solute is lost[15].

Table 3.5: The average %recovery based on 3 trials of 5 different concentrations spiked on glass filters using the developed analytical method

Phenols	0.125ppm	0.625ppm	3.125ppm	5ppm	7.5ppm
Phenol	61	51.9	61.8	71.8	64.4
o-cresol	72.5	67.9	68.8	78.0	68.7
p-cresol	68.8	68.2	67.5	79.3	68.5
m-cresol	47.4	62.0	64.1	73.9	64.9
catechol	52.5	30.8	24.3	31.8	32,8
resorcinol	21	20.01	19,9	23.5	24,5
hydroquinone	NA	0.9	4.0	5.8	6,9

3.7.2 Blank

Extraction, cleaning and injection procedures were applied to blank filters which do not contain any smoke particulates. Phenol, p- and m-cresol, and hydroquinone were detected on blank filters with considerable amounts which were accounted for during calculations.

3.7.3 Validation of experimental and analytical procedure

In order to test the validity of the experimental and analytical procedure employed in this study, we need to compare our results with other studies that employed the same sampling and analytical procedure. However, no studies have dealt with quantification of phenols in water-pipe smoke which we can refer to. The other choice would be to conduct cigarette samples and compare those to one reported in the literature. The total number of sessions is 5 sessions, with a total of 10 cigarettes, with each filter collected containing the smoke of 2 cigarettes. However, 2 sessions were disregarded due to errors employed during sample preparations and whats reported is the values of the other 3 sessions. These values are summarized in Table3.6.

Table 3.6: Mass(ppm) of phenolic compounds obtained from cigarette smoke reported from three sessions.

Phenols	Session 1	Session 2	Session3	Average	SD	%RSD
Phenol	0.0248	0.0256	0.0210	0.0238	0.002	10.4
o-cresol	0.0024	0.0023	0.0022	0.0023	7.5×10^{-5}	3.2
p-cresol	0.0029	0.0040	0.0025	0.0031	0.0007	24.7
m-cresol	0.0141	0.0199	0.0133	0.015	0.004	22.7
Catechol	0.577	0.678	0.574	0.609	0.06	9.8
Resorcinol	0.008	0.009	0.008	0.0086	0.0008	9.8
Hydroquinone	0.0212	0.0225	0.0191	0.0209	0.002	8.0

When compared to literature, Table3.7, our results are either within the reported values or higher which implies the validity of the experimental and analytical procedures employed for this study. However, we noticed that both catechol and resorcinol are migh higher than any reported value. This could be attributed to the fact that sample concentrations were higher than the calibration curve concentrations prepared which implies that further optimization is called for.

Table 3.7: Mass(ppm) of phenolic compounds obtained from cigarette smoke conducted by our lab compared to other two studies. (a) David Ashley; (b) Count et al

Phenols	Current study($\mu g/g$)	$Cigarette^a$	$Cigarette^b$
Phenol	23.8	16.3	11.3-21.9
o-cresol	2.3	4.9	3.54-4.29
p-cresol	3.1	3.5	8.3-13.1 (p+m-cresol)
m-cresol	15.8	9.1	
Catechol	609.8	49.5	41.1-59.7
Resorcinol	8.7	1.7	0.88-1.12
Hydroquinone	20.9	44	42.15-72.2

3.8 Results

Quantization of Phenols by GC-MS is based on comparing the area of a specific molecular ion peak in the sample to the area of the same specific molecular ion peak determined using the Phenol standards. The areas were obtained from Thermo Galactic Grams/AI (7.01) software to avoid any errors that might come during manual integration.

Calibration curves are plotted using the concentrations 0.125, 0.625, 3.125, 5 and 7.5 ppm. Table3.8 shows the average, over three trials, regression analysis R^2 of the Phenols calibration curves.

Table 3.8: The average R^2 of the Phenols for direct and extracted calibration curve.

Phenols	R^2_{direct}	$R^2_{extracted}$
Phenol	0.9894	0.9832
o-cresol	0.9899	0.9809
p-cresol	0.9898	0.9801
m-cresol	0.9875	0.9795
Catechol	0.9691	0.9739
resorcinol	0.9706	0.9786
Hydroquinone	0.9771	0.9814

The amount of identified and quantified phenol is expressed in ppm as shown in Tables3.9-3.11. The validated method has been successful in identifying and quantifying phenol in nargileh smoke aerosols collected on glass fiber filters. Concentrations of phenol emitted from eleven smoking sessions, standard deviation, and the percent relative standard deviation (%RSD) between the three trials are detailed in Tables3.9-3.11. The results of each nargileh session are based on the extraction of four sets of filters with each set one filter, and each filter represents the four filters that are connected in parallel.

As shown in Table3.9-3.11, we were able to quantify phenol; whereas for cigarette smoke all 7 compounds were identified and quantified.

Table 3.9: Phenol concentration in eleven sessions expressed in ppm and normalized to mg/session

Phenols	Session1	Session2	Session3	Session4	Session5	Session6	Session7
Phenol	0.0122	0,0281	0.0398	0.0273	0.0232	0,0668	0.0521

Table 3.10: Continue Table3.9

Phenols	Session8	Session9	Session10	Session11
Phenol	0,0464	0.0467	0.0338	0.02543

Table 3.11: Continue Table3.10

Phenols	Average	SD	%RSD
Phenol	0.0365	0.0156	42.6

3.8.1 Comparing Phenols between Cigarettes and Nargileh Smoke

Although both narghile and cigarette are based on tobacco burning, the two entities have essentially different matrices and different yield is expected for the emission of molecules from both modes of smoking. Table3.12, shows the differences of %yield of phenol between narghile and cigarette.

Table3.12 shows how phenol varies between cigarette samples collected in our study to those done by David Ashley and Count et al, they are close to the upper range reported by Count et al; whereas, narghile seems to be different.

Table 3.12: Mass(ppm) of phenolic compounds obtained from narghile smoke and compared to current study cigarette and the other two studies. (a) David Ashley; (b) Count et al. Results reported as μg/narghile or μg/cigarette

Phenols	Current study narghile	Current study cigarette	Cigarette[a]	Cigarette[b]
Phenol	36	23.8	16.3	11.3-21.9
+/-SD	15.6	2	NA	0.8-2.6

Chapter 4

Conclusion and Future Work

From the widespread smoking habit of argileh water pipe emerged the importance to study the chemical composition of gases and particulates emitted during an argileh smoking session. My work is divided in to two major parts. The first part includes identification and quantification of HCN in both gas and particle phase narghile smoke. The second part includes identification and quantification of 7 phenolic compounds which are known for their toxicity and some of which act as tumor promoters.

The nargileh water pipe smoke is generated by the combustion of a quick-light charcoal disk heated along with 10 g of mo'assal tobacco. Argileh smoke was generated using steady periodic smoking model that consists of 171-puffs, each of 0.53 L volume, 2.6s duration and interpuff interval of 17s. Glass fiber filters system were used to collect the total particulate matter (TPM) and analyze their HCN and phenolic content. Impingers, with coarse fritted glass, were used to collect gas

phase HCN. The analytical work was developed on the basis of collecting HCN from gas and particle phase, both of which showed no traces of HCN. HCN in smoke is affected by temperature, produced artifacts and glycerol which is present in high content in molasses. For each effect present in the literature, an experiment was carried to validate it. In the temperature case, charcoal burning at higher temperature did produce HCN; for artifact, when smoke emitted from burned charcoal was collected in aldehyde solution, no traces of HCN were detected which verifies that cyanohydrins are produced. As for glycerol, placing glycerol as trapping solution showed that it can trap HCN since it is an organic and polar solvent. Therefore, we can conclude that either the amount of HCN produced from charcoal was trapped in the glycerol found in HCN or reacted with the produced aldehydes which are present in high concentrations[2].

The second part of my work includes the identification and quantification of 7 phenolic compounds. The analytical work was developed on the basis of high recovery of phenols, minimal interferences and high quantification resolution. The filter extraction was accomplished by mechanical shaking using water at pH 1 and ascorbic acid to prevent oxidation of phenols. Water at pH1 showed higher extraction efficiency and recovery for phenols from nargileh smoke matrix than water at pH 3 or 5. The PS-DVB SPE cartridges was determined to be more selective in retaining phenolic compounds minimizing most interferences and the preconcentration of solutions was done using a nitrogen flow. Derivatization of phenols is an important procedure since phenols are compounds with high volatility which implies they can

be eluted along with a lot of noise from the background. On the other hand, the ether form of phenols is less volatile and can be eluted at a later stage allowing well resolved peaks. Samples were injected on GC-MS to identify and quantify phenol, where selected ion current profile was used to increase the sensitivity and selectivity of the analysis in the crowded chromatograms. Standard smoking 171-puffs nargileh sessions were done and phenol, catechol and hydroquinone were identified, but we were able to quantify only phenol which implies that more work need to be done to quantify the other phenols. On the other hand, when cigarette smoke was collected, all 7 compounds were identified and quantified; however, the amount of catechol collected is much higher than any reported value which implies that catechol is overlapping with other compounds and further purification or dilutions are needed to separate it.

The presence of phenolic compounds has severe consequencies on the atmosphere. As shown in the formation of phenol section, we notice that phenolic compounds can further react with nitrate radicals to nitrophenols. They are widespread pollutants in the atmosphere being present in both gas and particle phase, as well as in fog water, rainwater and snow[20]. They are known for their phytotoxic properties as uncoupling agents for oxidation phosphorylation; combined with their ability to penetrate into plant tissues. Thus, nitrophenols could give a substantial contribution to forest decline in highly polluted areas. The nitrophenols formed from nitration of phenols are o-nitrophenol and p-nitrophenol; o-nitrophenol can be rapidly photolyzed at a rate of 2.9×10^{-5} s^{-1} to give HONO[9], thus increasing

the acidity in the atmosphere. Moreover, HONO is responsible for 60% of OH radicals in the atmosphere, which are major precursors for formation of most pollutants in the atmosphere. Therefore, increasing the emitted levels of HONOs means more OH radicals would be emitted in the atmosphere and more pollutants formed. As for p-nitrophenols, they can react further with OH radicals, to give multi-hydroxyl compounds or benzoquinone with more than 98% of p-nitrophenols removed in 12 min[57].

Therefore, although we were not able to detect HCN, the produced cyanohydrins are highly toxic because they are capable of releasing HCN if heated. Phenols are toxic compounds that are present in high amount in water-pipe smoke. Other phenolic compounds such as catechol and hydroquinone which are considered tumor promoters were identified which adds to the toxicity of the narghile smoke. Further studies are needed to assess the severity of catechol and hydroquinone present in the smoke based on their concentration range.

References

[1] Hydrogen cyanide (formonitrile; hydrocyanic acid; prussic acid). *Chronic Toxicity Summary, CAS Registry Number: 74-90-8.*

[2] A.; Saliba N.A. Al Rashidi, M.; Shihadeh. Volatile aldehydes in the mainstream smoke of the narghile waterpipe. *Food and Chemical Toxicology*, 46:3546–3549, 2008.

[3] R Atkinson. Gas-phase degradation of organic compounds in the troposphere. *Pure and Appli. Chem*, 70:1327–1334, 1998.

[4] S.M; Arey J Atkinson, R; Aschmann. Reactions of oh and no3 radicals with phenol, cresols, and 2-nitrophenol at 296 +/- 2 k. *Environ. Sci. Technol*, 26:1379–1403, 1992.

[5] ATSDR. Hydrogen cyanide (hcn). *CAS 74-90-8; UN 1051.*

[6] ATSDR. Phenol. *CAS 108-95-2.*

[7] A; Thangavelu V Basha, K. M; Rajendran. Recent advances in the biodegradation of phenol: A review. *Asian J. Exp. Biol. Sci*, 1:219–234, 2010.

[8] J.B; Maliner B.I; Rockwood G.A; Zoltani C Baskin, S.; Kelly. Cyanide poisoning. *Medical Aspects of Chemical Warfare*, pages 371–410.

[9] Y; Barnes I; Benter T; Bohn B; Wiesen P; Kleffmann J Bejan, I.; Aal. The photolysis of ortho-nitrophenols : a new gas phase source of hono. *Physical Chemistry Chemical Physics*, 8:2028–2035, 2006.

[10] O Berndt, T; Boge. Formation of phenol and carbonyls from the atmospheric reaction of oh radicals with benzene. *Physical Chemistry Chemical Physics*, 8:1205–1214, 2006.

[11] S Bull. Phenol, toxicological overview. *Health Protection Agency; Chapd HQ, HPA*, 2007.

[12] Health Canada. Determination of hydrogen cyanide in mainstream tobaco smoke. 1999.

[13] J.E Clark, T.J; Bunch. Quantitative determination of phenols in mainstream smoke with solid-phase microextraction-gas chromatography-selected ion monitoring mass spectrometry. *Journal of Chromatographic Science*, 34:272–275, 1996.

[14] BCERC COTC. Breast cancer & the environment research centers early life exposure to phenols and breast cancer risk in later years. *Fact Sheet*, 2007.

[15] M.J; Laffoon S.W; Cox R.H; Lipowicz P.J Counts, M.E; Morton. Smoke composition and predicting relationships for international commercial cigarettes

smoked with three machine-smoking conditions. *Regulatory Toxicology and Pharmacology*, 41:185–227, 2005.

[16] M.; Varhegyi G.; Jakab E.; Liu Ch.; Nappi L. Czegeny, Z.; Blazso. Formation of selected toxicants from tobacco under different pyrolysis conditions. *Journal of Analytical and Applied Pyrolysis*, 85:47–53, 2009.

[17] C.R Dube, M.F; Green. Methods of collection of smoke for analytical purposes. *R . J . Reynolds Tobacco Company. Winston-Salem, North Carolina 27102.*

[18] Approved for NPDES. Cyanide, total (titrimetric; spectrophotometric). *Method no. 335.2*, Technical Revision 1980.

[19] J; Amand L.E; Tullin C Hansson, K.M; Samuelsson. The temperature's influence on the selectivity between hnco and hcn from pyrolysis of 2,5-diketopiperazine and 2-pyridone. *Fuel*, 82:2163–2172, 2008.

[20] S; Borghesi D; Vione D; Arsene C; Olariu R.I Harrison, M.A.J; Barra. Nitrated phenols in the atmosphere: a review. *Atmospheric Environment*, 39:231–248, 2005.

[21] H.J Heberer, T.; Stan. Detection of more than 50 substituted phenols as their t-butyldimethylsilyl derivatives using gas chromatography-mass spectrometry. *Analytical Chimica Acta*, 341:21–34, 1997.

[22] I.; El-Bayoumy K. Hoffmann, D.; Hoffmann. The less harmful cigarette: A controversial issue. a tribute to ernst l. wynder. *Chemical Research in Toxicology*, 14:768–785, 2001.

[23] J.C Johnson, W.R; Kang. Mechanisms of hydrogen cyanide formation from the pyrolysis of amino acids and related compounds. *Journal of Organic Chemistry*, 36:189–192, 971.

[24] N Kenyon, R.L; Boehmer. Phenol by sulfonation. *Industrial and Engineering Chemistry*, 42:1446–1455, 1950.

[25] A; Sommer-L Kostrhounova, R; Hrdlicka. Solid phase extraction of phenol and chlorophenols on octadecylsilica and amberlite xad 2 sorbents in the presence of cationic surfactant. *Michrochim Acta*, 142:95–99, 2003.

[26] M.G Kruse, J.M; Mellon. Colorimetric determination of cyanide and thiocyanate. *Analytical Chemistry*, 25:446–450, 1953.

[27] A.P.; Meruva-N.K.; Chan W.G. McGrath, T.E.; Brown. Phenolic compound formation from the low temperature pyrolysis of tobacco. *Journal of Analytical and Applied Pyrolysis*, 84:170–178, 2009.

[28] C.T Miller, J.A; Bowman. Mechanism and modeling of nitrogen chemistry in combustion. *Prog. Energy Combust. Sci*, 15:287–338, 1989.

[29] W.S; Levasseur-G; Larose Y; Maertens R; White P; Desjardins S Moir, D; Rickert. A comparison of mainstream and sidestream marijuana and tobacco cigarette smoke produced under two machine smoking conditions. *Chemical Research in Toxicology*, 21:494–502, 2008.

[30] M. Moldoveanu, S.C; Kiser. Gas chromatography/mass spectrometry versus liq-

uid chromatography/fluorescence detection in the analysis of phenols in mainstream cigarette smoke. *Journal of Chromatography A*, 1141:90–97, 2007.

[31] K; Ohtsuka-Y Mori, H; Asami. Role of iron catalyst in fate of fuel nitrogen during coal pyrolysis. *Energy and Fuels*, 10:1022–1027, 1996.

[32] A; Mirabel-P; Millet M Morville, S; Scheyer. A multiresidue method for the analysis of phenols and nitrophenols in the atmosphere. *J. Environ. Monit*, 6:963–966, 2004.

[33] K; Radeck-W Mubmann, P; Levsen. Gas-chromatographic determination of phenols in aqueous samples after solid phase extraction. *Fresenius J Anal Chem*, 348:654–659, 1994.

[34] World Health Organization. Waterpipe tobacco smoking: Health effects, research needs and recommended actions y regulators. *World Health Organization*, 2005.

[35] J.W Park. Analyses of phenolics in cigarette smoke by gc-ms with the multiple ion selection technique. *Arch. Pharm*, 5:71–77, 1982.

[36] M.S.D; Centurion M.E; Palomeque M.E; Lista A.G; Band BSF Pistonesi, M. F; Nezio. Determination of phenol, resorcinol and hydroquinone in air samples by synchronous fluorescence using partial least-squares (pls). *Talanta*, 69:1265–1268, 2006.

[37] A.A Reese. Solid phase extraction of phenol and gas chromatography/mass

spectrometry analysis of selected phenols application. *Agilent Technologies*, 2002.

[38] P.B Rickert, W.S; Stockwell. Automated determination of hydrogen cyanide acrolein and total aldehydes in the gas phase of tobacco smoke. *The Journal of Automatic Chemistry*, 1:152–154, 1979.

[39] M.P; Cela R Rodriguez, I; Llompart. Solid-phase extraction of phenols. *Journal of Chromatography A*, 885:291–304, 2000.

[40] O; Appenzeller B.M.R; Wennig R; Millet M Schummer, C; Delhomme. Comparison of mtbstfa and bstfa in derivatization reactions of polar compounds prior to gc/ms analysis. *Talanta*, 77:1473–1482, 2009.

[41] A.W Schwartz. Chemical evolution: The first stages. *Die Naturwissenschaften*, 70:373–377, 1983.

[42] A.; Saliba N.A. Sepetdjian, E.; Shihadeh. Measurement of 16 polycyclic aromatic hydrocarbons in narghile waterpipe tobacco smoke. *Food and Chemical Toxicology*, 46:1582–1590, 2008.

[43] A. Shihadeh. Investigation of mainstream smoke aerosol of the argileh water pipe. *Food and Chemical Toxicology*, 41:143–152, 2003.

[44] R Shihadeh, A.; Saleh. Polycyclic aromatic hydrocarbons, carbon monoxide, tar, and nicotine in the mainstream smoke aerosol of the narghile water pipe. *Food and Chemical Toxicology*, 43, 2005.

[45] S.; Antonios C.; Haddad A Shihadeh, A.; Azar. Towards a topographical model of narghile water-pipe cafe´ smoking: a pilot study in a high socioeconomic status neighborhood of beirut, lebanon. *Pharmacol. Biochem. Behav*, 79:75–82, 2004.

[46] sigma aldrich. Bstfa+tmcs. *Supelco, CAS Number: 75-77-4*, 1997.

[47] L. Simeonova, F.P; Fishbien. Hydrogen cyanide and cyanides: Human health aspects. *World Health Organization, Concise International Chemical Assessment Document 61*, 2004.

[48] K SV. Anion-exchange chromatography of metal cyanide complexes with gradient separation and direct uv detection. *Journal of Chromatography A*, 956:229–235, 2002.

[49] H.; Muller L. Thielen, A.; Klus. Tobacco smoke: Unraveling a controversial subject. *Experimental and Toxicological Pathology*, 60:141–156, 2008.

[50] S; Takahashi H Torikai, K; Yoshida. Effects of temperature, atmosphere and ph on the generation of smoke compounds during tobacco pyrolysis. *Food and Chemical Toxicology*, 42:1409–1417, 2004.

[51] Y; Nakamori T; Tarora W.; Takahashi H Torikai, K;Uwano. Study on tobacco components involved in the pyrolytic generation of selected smoke constituents. *Food and Chemical Toxicology*, 43:559–568, 2005.

[52] S.B; Polzin G.M; Ashley D.L; Watson C.H Vaughan, C; Stanfill. Automated de-

termination of seven phenolic compounds in mainstream tobacco smoke. *Nicotine and Tobacco Research*, 10:1261–1268, 2008.

[53] V; Minero C; Lucchiari M; Pelizzetti E Vione, D; Maurino. Nitration and hydroxylation of benzene in the presence of nitrite/nitrous acid in aqueous solution. *Chemosphere*, 56:1049–1059, 2004.

[54] W.B; Dudas M.J Xing, B; Mcgill. Sorption of phenol by selected biopolymers: Isotherms, energetics, and polarity. *Environ. Sci. Technol*, 28:466–473, 1994.

[55] H.; Yan X.; Du S.; Yao Z.; Liu S Xu, J.; Tong. Sensitive determination of cyanide in cigarette smoke by capillary gc with a microecd. *Chromatographia*, 64:609–612, 2006.

[56] G Yan, J; Quan. Equilibrium and kinetic studies of phenol sorption by chitosan coated montmorillonite. *J. Chil. Chem. Soc*, 54:73–76, 2009.

[57] X; An T; Song Z; Fu J; Sheng G; Cui M Zhang, W; Xaio. *J Chem Technol Biotechnol*, 78:788–794, 2003.