

**METHODS DEVELOPMENT FOR THE CHARACTERIZATION OF  
MAINSTREAM BIDI CIGARETTE SMOKE AND ASSESSMENT  
OF EXPERIMENTS FOR ENVIRONMENTAL  
ANALYSIS**

by

Omobola Ajoke Oladipupo

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Graduate Faculty of Middle Tennessee State University  
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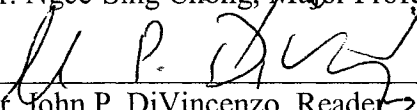
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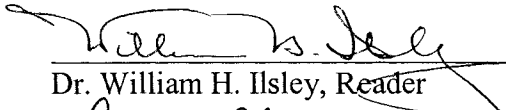
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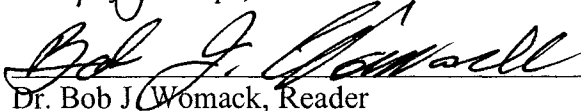
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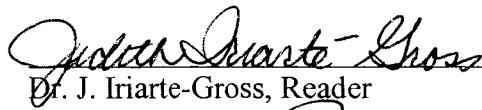
  
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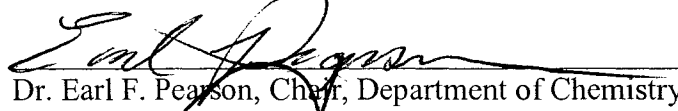
  
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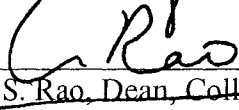
  
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\_\_\_\_\_  
Dr. J. Iriarte-Gross, Reader

  
\_\_\_\_\_  
Dr. Earl F. Pearson, Chief, Department of Chemistry

  
\_\_\_\_\_  
Dr. Abdul S. Rao, Dean, College of Graduate Studies

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## ABSTRACT

### **METHODS DEVELOPMENT FOR THE CHARACTERIZATION OF MAINSTREAM BIDI CIGARETTE SMOKE AND ASSESSMENT OF EXPERIMENTS FOR ENVIRONMENTAL ANALYSIS**

Omobola Ajoke Oladipupo

The main goal of the first part of this work is to characterize the constituents of bidi mainstream smoke using Fourier Transform infrared spectrometry (FTIR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) techniques. For the educational component, the objective is to develop and assess the effectiveness of two environmental chemistry laboratory experiments, namely, “FTIR Analysis of Mainstream Cigarette Smoke” and “Extraction of Organic Pollutants from Soil Samples for GC-MS Analysis” for undergraduate students.

The gaseous portion of bidi mainstream smoke was found to contain 310  $\mu\text{g}$ /cigarette of hydrogen cyanide via FTIR analysis while the GC-MS analysis of the sorbent tube extract indicated a higher concentration of 3-methylpyridine, cotinine, and nicotine in the mainstream smoke of bidi cigarette compared to that of the 1R5F reference cigarettes. The mainstream smoke of bidis also contained  $\alpha/\beta$ -amyryns that may have originated in the tendu leaves used to wrap the bidis, but these compounds

were absent in the mainstream smoke of 1R5F reference cigarettes. Comparative analysis of the particulate phase of mainstream cigarette smoke by GC-MS indicated a higher concentration of nicotine in the bidi smoke compared to the 1R5F reference cigarette smoke. It was found that 85% of the nicotine present in the particulate phase of bidi cigarette smoke had particle size of less than 0.25  $\mu\text{m}$ .

For the analysis of standard carbonyl-DNPH derivatives by GC-MS, an optimum injector temperature of 230 °C was ideal for C1-C3 carbonyl derivatives, while 250 °C was optimal for the C4-C6 derivatives. LC-MS was more suitable for the detection of carbonyl derivatives having greater than five carbon atoms, including aromatic aldehydes, compared to GC-MS. Both the GC-MS and LC-MS results revealed that acetaldehyde was present at the highest concentration among all the carbonyls detected in the bidi mainstream smoke.

For the educational part, assessment of the two laboratory experiments revealed that the experiments may be appropriate for enhancing students' understanding and the application of key spectroscopic and chromatographic concepts learned in class. Also, the use of real world samples and exposure to modern instruments could enhance students' enthusiasm about science and increase students' interest in research.

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**PART I**

**METHODS DEVELOPMENT FOR THE CHARACTERIZATION OF  
MAINSTREAM BIDI CIGARETTE SMOKE**

## **CHAPTER 1**

### **INTRODUCTION**

#### **Background and Significance of Study**

It was not until the nineteenth century that mass production of cigarettes became popular. Tobacco smoking is now a common practice among adults and teenagers in both developed and developing countries all over the world. Since this habit tends to start early in life, public health efforts have been geared towards discouraging this habit among teenagers due to the adverse health effects of toxicants in the cigarette smoke. Different states in the United States (U.S.) have embarked on educational campaigns to discourage teenagers and adults from getting into the habit, or to stop them if they already have an established smoking habit.

In the year 2000, smoking killed about five million people and the World Health Organization (WHO) has estimated that, by the year 2030, tobacco-related deaths will double.<sup>1</sup> The United States Surgeon General released a report<sup>2</sup> in 1989 that listed 43 carcinogenic agents found in tobacco smoke. According to a recent report by the Centers for Disease Control and Prevention,<sup>3</sup> 21.6% of U.S. adults were smokers in the year 2003. Even though there was a decline in the percent of smokers from 2002 to 2003, the rate of decline was below expectation in spite of all the campaigns against smoking.

The different forms of tobacco smoking products include cigarettes – filtered and unfiltered, bidis (Indian cigarettes), cigars, kreteks (clove cigarettes),<sup>4,5</sup> chutta (reverse smoking), and hukka (hubble-bubble). Some of the more popular brands of American

cigarettes include Marlboro, Camels Light, and American Spirits. Bidis, which originated from India, are currently attracting quite a growing number of American teenage smokers. While a lot of research has been conducted on western smoking products and tobacco generally, not much work has been done on bidis.

Cigarette smoke consists of gaseous, volatile components as well as submicron-sized solid particles. The particulate phase consists of volatile or semi-volatile compounds. More than 4,800 constituents have been identified in mainstream cigarette smoke,<sup>6</sup> while the number keeps rising as the sensitivity of tobacco smoke analysis is continuously improved. Some of these constituents include carbon monoxide, carbon dioxide, benzene, toluene, acrolein, formaldehyde, hydrogen cyanide, ammonia, and acetic acid present in the vapor phase and polycyclic aromatic hydrocarbons (PAH), tobacco specific nitrosamines (TSNA), cholesterol, quinoline, lactic acid in the particulate phase.<sup>7</sup> Others such as nicotine, phenol, and catechol are present in both the gaseous and particulate phases.<sup>7</sup> Several PAHs and aromatic amines may react with DNA in cells to form DNA adducts.<sup>8</sup> The vapor phase represents a higher weight percent (about 96%) of the cigarette mainstream smoke, with components from pyrolysis and combustion of tobacco accounting for about 13.5% of the vapor phase. Components from air represent 82% of the weight percent of these gaseous components.<sup>9</sup> Ninety percent of this 13.5% from the tobacco, still represents carbon monoxide and water<sup>9</sup> while the remaining 10% are components from tobacco. Some of these cigarette smoke constituents are carcinogens and the concentrations of some of these toxicants can be reduced by having good filter designs, use of good cigarette paper with good porosity, and the use of expanded tobacco.<sup>10</sup>



Since cigarette smoke contains a variety of harmful constituents, it should be expected that many health hazards, including cancer, are related to tobacco smoking. Shopland et al.<sup>11</sup> published a study to predict the effect of cigarette smoking on lung cancer deaths in the U.S. in 1991. They estimated that about 78% of all cancer deaths in 1991 resulting only from smoking were lung cancer deaths, while the remaining 22% were cancers from other sites different from the lung.<sup>9,11</sup> Thus, they estimated that cigarette smoke accounted for about 90% of male lung cancer deaths and 78% of female lung cancer deaths in 1991. Besides lung cancer, cancers of the esophagus, larynx, oral cavity, bladder, and pancreas have also been related to cigarette smoking. The degree of cancer development in smokers has been shown to depend on the frequency of smoking, the age at which smoking is commenced, for how long, interval between puffs, number and volume of puff, length of inhalation, type of cigarette, and length of cigarette.<sup>10</sup> Other major health effects include the birth defects of the human reproductive system and atherosclerotic diseases of the heart and blood vessels.

There are two types of cigarette smoke, namely mainstream and sidestream smoke. The mainstream smoke is the smoke that goes through the length of the cigarette before being inhaled, whereas the sidestream smoke, also referred to as the second-hand smoke or environmental tobacco smoke (ETS), is the smoke that is emitted from the tip of the cigarette. Smokers inhale the mainstream smoke primarily, while non-smokers may be exposed to the sidestream smoke where smoking is allowed. The chemical composition of both sidestream and mainstream smoke are similar, but the concentrations of the specific components are different with yields in the sidestream smoke generally greater than in mainstream smoke.<sup>12</sup>

Mainstream cigarette smoke contains benzo(a)pyrene, aromatic amines (i.e. toluidine and aniline), N-Nitrosonornicotine, formaldehyde, acetaldehyde, benzene, arsenic, and chromium that have been shown to possess the ability to induce tumors in humans or animals.<sup>7</sup> In mainstream smoke, some components such as carbon monoxide, carbon dioxide, acetone, pyridine, and hydrogen cyanide are predominant in the vapor phase, while phytosterols and linear fatty acids such as stearic acid, oleic acid, and palmitic acid are the main components in the particulate phase. The phenols and cresols are present in both the vapor and particulate phases.<sup>13</sup>

For research purposes, the analytical protocol of Federal Trade Commission (FTC), which was designed primarily to optimize reproducibility of results of different brands of cigarette,<sup>14</sup> and not necessarily mimic exactly human smoking behavior, might not give adequate results for bidis. This is because bidi smoking requires even more puff frequency and volume than is used in the FTC protocol or by smoking conventional cigarettes. The test results of the FTC method for the analysis of tobacco smoke, allow cigarettes to be classified as low-yield products and high-yield products. However, when the smoking behavior of cigarette smokers was observed, it was found that products that were classified as low yield products<sup>15</sup> were the ones which require different smoking parameters than the way they were smoked by the FTC method. As a result, enough smoke were not generated during the testing, for accurate analysis.<sup>15</sup>

Since the FTC conditions are designed primarily for reproducibility, (i.e. to have consistent measurement methods between different brands of cigarettes) the results obtained are only applicable for the conditions employed. The recommended parameters for the FTC method are a puff volume of  $35 \pm 0.5$  mL, a puff duration of  $2.0 \pm 0.2$  s, a

puff frequency of one per  $60 \pm 1$  s, as well as a butt length of 23 mm for nonfilter cigarettes, and the length of filter overwrap plus 3 mm for filtered cigarettes. Cigarettes must be conditioned at 60% relative humidity and 24 °C for at least 48 hours before smoking. It has been found that yields of nicotine, tar, and carbon monoxide in cigarette smoke are strongly affected by the puff frequency and the ventilation of the filter.<sup>10</sup>

Recent research has shown that the puff volume is usually more than 35 mL as specified by the FTC; a more realistic puff frequency is between two and three puffs per minute, compared to the recommended condition of one puff per minute.<sup>16</sup> Deeper inhalation in bidi smoking tends to result in greater exposure of smokers to constituents in the mainstream smoke. The FTC method is effective at ranking cigarettes, even though it may not completely simulate a person's smoking behavior or provide a realistic estimate of exposure to mainstream smoke. Since the gas and aerosol phases of cigarette smoke contain compounds with wide-ranging properties including polarity, vapor pressure, and sensitivity to various detectors, different analytical methods are required to fully characterize the complex cigarette smoke.

In India, bidis are known as the poor man's cigarettes. The citizens of India consume about 80% of the 500 million kilograms of tobacco produced annually. With the increase in smoking rates worldwide, bidi cigarettes are a potential threat to human health globally, because they are exported to other countries from India. Bidis contain sun-dried and finely ground tobacco wrapped in a tendu leaf obtained from *Diospyrus melanoxylon* or *Diospyrus ebenum*.<sup>17</sup> The tendu leaf makes up about 60% of the weight of a bidi cigarette,<sup>18,19</sup> whereas for any conventional cigarette, the weight of the tobacco is over 90% of the cigarette's weight. Bidis' characteristics such as tobacco weight and

resistance to airflow are not usually consistent because they are produced by cottage industries without official standards or automated machines. Bidis usually form a cylindrical shaped cigarette and are quite slimmer than the conventional cigarettes. Since the leaf is relatively non-porous, bidi smokers need to inhale deeply and puff more frequently to keep bidis lit and to avoid being self-extinguished. Bidi smokers tend to puff two to five times per minute, compared to one to two times per minute for conventional cigarettes.<sup>20</sup> The FTC recommends one puff per minute, but this is not realistic for bidi since it requires a longer puff duration and puff frequency to keep bidis lit.<sup>21</sup> Research on bidi smoking needs to be modified to accommodate this difference.

Bidis have continually been imported into the U.S. for the past forty years by a number of different importers for sale via retail stores and even on the Internet. It became popular among American teenage smokers due to the flavors added to the butts of bidis. Bidi smoking was initially popular among college students, but it is now more popular among middle and high school students<sup>22</sup> and many of them (about 44%) believe bidi smoking is safe and does not contribute to cancer.<sup>23</sup> In addition, the 1999 National Youth Tobacco Survey conducted by the American Legacy Foundation in collaboration with the Centers for Disease Control found that 2.4% of middle school students and 5.0% of high school students are bidi smokers.<sup>22</sup> This is partly because underage kids are able to buy bidis easily from the Internet.<sup>24</sup>

The bidis smoked in India are usually unfiltered and contain no additives. However, those imported into the U.S. contain flavorings such as chocolate, cherry, raspberry, mango, lime, vanilla, licorice, strawberry, cinnamon or clove. In the U.S., bidis are priced lower than the conventional American cigarettes at about two dollars for

a pack of twenty. These teens are thus more attracted to bidis due to their lower prices and candy-like flavors. Teenagers believe bidis are safer than other cigarettes because the wrapping of tendu leaf gives the false impression that bidis are herbal in origin and contain natural and unprocessed tobacco. Furthermore, teenagers assume that since each bidi cigarette contains less tobacco than is found in conventional cigarettes, it is less harmful to human health. They also feel they are sweeter, look like joints and smell better. However, in terms of smoking habits, smokers of bidis must inhale more deeply and more frequently since the tendu leaf tends to retard the free flow of air for diluting the smoke. The deeper inhalation and increased puff frequency may lead to a greater exposure of bidi smokers to toxicants.

Behera et al.<sup>25</sup> showed that bidis deliver a significant amount of carbon monoxide to the blood stream of smokers. Hoffman et al.<sup>26</sup> showed that bidi smoke contained more carbon monoxide, ammonia, hydrogen cyanide, phenol, alkyl phenols, benz(a)anthracene and benzo(a)pyrene than unfiltered American cigarette smoke. Rahman et al.<sup>27</sup> have estimated that there was an increased risk of oral cancer in bidis smokers, by giving the odds ratio as 3.1, with a 95% confidence interval of 2.0 - 5.0, while for conventional cigarette smokers, the odds ratio was 1.1, with a 95% confidence interval of 0.7 - 1.8. These data showed that bidis are potentially harmful to human health. Furthermore, the mainstream smoke of bidi cigarettes may deliver  $2.7 \pm 0.4$  mg/bidi of nicotine to the smoker.<sup>28</sup>

## **Existing Techniques for Mainstream Cigarette Smoke Analysis**

Despite the fact that bidis are consumed by millions of smokers worldwide, limited research has been performed on the characterization of bidi smoke composition and the adverse health effects of bidi smoke relative to conventional western cigarettes. Gas chromatography–mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), and liquid chromatography–mass spectrometry (LC-MS) and Fourier Transform infrared spectroscopy (FTIR) have been used to analyze different classes of compounds in ambient air samples or cigarette smoke. Common sampling procedures for cigarette smoke involve the use of a Cambridge filter pad to collect the total particulate matter (TPM) of tobacco smoke, while the gaseous components that pass through the filter are subsequently collected in gas cells, Tedlar bags, impingers, and/or sorbent tubes. Both the gaseous and TPM phases contain a wide range of compounds that require different analytical techniques for characterization.

### *Liquid Chromatography-Mass Spectrometry Technique*

For the analysis of carbonyl compounds, chemical derivatization with 2,4-dinitrophenylhydrazine (DNPH)<sup>29,30,31</sup> and N-methyl-4-hydrazin-7-nitrobenzofurazan (MNBDH)<sup>32</sup> have been used. The sample is either drawn through an impinger containing the derivatization agent, a silica gel cartridge coated with acidified DNPH,<sup>33</sup> or a passive sampling device. These derivatives are then extracted from the sampling media and analyzed on the HPLC/UV, LC-MS and sometimes on the GC-MS. Carbonyl compounds such as acetaldehyde and crotonaldehyde have been classified as possible human carcinogens while formaldehyde is classified as a probable human carcinogen.<sup>34,35</sup>

Grosjean et al.<sup>36</sup> used HPLC/UV and LC-MS with the negative ion mode of Atmospheric Pressure Chemical Ionization (APCI) to analyze and separate standard carbonyl compounds. APCI in the negative mode works best for compounds that will capture electrons and become negatively charged via vapor phase reactions. First, the solvent of the mobile phase is ionized by a corona discharge, which in turn is used to ionize the analytes through the transfer of a proton.<sup>37</sup> This is a soft ionization technique that is well-suited for the analysis of DNPH derivatives of carbonyl compounds that undergo thermal degradation. The analytical data obtained using the UV detector and the mass spectrometry detector showed good agreement with each other for the determination of formaldehyde, acetone, acrolein, butanal, and hexanal. The base peak for all the carbonyls (except two) was the M-1 ion; where M is the mass of the DNPH derivative. Sep-Pak cartridges (C<sub>18</sub>) were impregnated with DNPH and phosphoric acid before being used for sampling. The laboratory study of the atmospheric oxidation products of ozone and 4-hexen-3-one showed the presence of carbonyl compounds such as formaldehyde and glyoxal.

In addition, Zurek et al.<sup>32</sup> were able to employ LC-MS with APCI in the positive ion mode to quantify acetaldehyde in American cigarette smoke through derivatization with N-methyl-4-hydrazine-7-nitrobenzofurazan (MNBDH). The results obtained for acetaldehyde quantification through this method were in good agreement with those obtained using HPLC/UV detection. Therefore, mass spectrometry, which gives better sensitivity than the ultraviolet (UV) detector, could be advantageous for analyzing cigarette smoke.

Cigarette smoke contains a wide variety of compounds with different functional groups. Other groups of compounds that have not been investigated extensively in mainstream cigarette smoke include polynuclear aromatic hydrocarbons (PAHs), aromatic amines and nitrosamines. The tobacco-specific nitrosamines (TSNA) include N-Nitrosornicotine (NNN), 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), Nitrosoanatabine (NAT) and N-Nitrosoanabasine (NAB), which are cancer-causing agents.<sup>38,39</sup>

It has been reported that the mainstream smoke of cigarette yields 17 - 470 ng of NNK per cigarette.<sup>40</sup> The primary metabolite of NNN, NNAL, has been suspected of playing a major role in lung cancer observed among smokers in the U.S.<sup>41</sup> Analysis of N-Nitrosamines as well as tobacco-specific nitrosamines requires efficient sample preparation followed by HPLC<sup>42</sup> and GC-MS analysis.<sup>43</sup> Byrd et al.<sup>40</sup> developed a method for the analysis of NNAL in smokers' urine using LC/MS/MS with electrospray ionization and a single step solid phase extraction (SPE). The mean free NNAL concentrations obtained for the seven smokers in the study were between 101 - 256 pg/mL. The amounts of NNAL obtained from the study, when normalized to the number of cigarettes smoked, was in agreement to literature values of the amounts of NNK yields from the cigarettes.

#### *Gas Chromatography-Mass Spectrometry Technique*

Moldoveanu et al.<sup>44</sup> described the GC-MS analysis of carbonyl compounds in the 1R4F and 2R4F Kentucky reference cigarettes' mainstream smoke after derivatization with 2,4-DNPH impregnated Cambridge pads. The cigarettes were smoked using a



smoking machine and the total particulate matter (TPM) collected onto two DNPH-treated Cambridge filters. The acidity of the DNPH treatment solution was kept at 0.3:1 molar ratio of perchloric acid (70% solution) to DNPH in order to obtain the best yield of DNPH derivatives. Carbonyls such as acetaldehyde, formaldehyde, acetone, acrolein, butyraldehyde, crotonaldehyde, methyl ethyl ketone, n-valeraldehyde, 3-pentanone, cyclopentanone, furfural, and propionaldehyde were quantitated with detection limits of 1.4 to 5.6  $\mu\text{g}/\text{cigarette}$ . Methoxyacetaldehyde, which had not been previously reported in cigarette smoke, was identified and its concentration was quantitated as 1.1  $\mu\text{g}/\text{cigarette}$  in 1R4F cigarette and 1.3  $\mu\text{g}/\text{cigarette}$  in the 2R4F cigarette. Even though the feasibility of carbonyl determination using DNPH derivatization was demonstrated, the high injection port temperature of 230  $^{\circ}\text{C}$  for the GC-MS analysis compared to room temperature used during LC-MS analysis, might reduce the analytical sensitivity of thermally labile DNPH derivatives.

Shibamoto et al.<sup>45</sup> developed a method that involved the analysis of volatile carbonyl compounds in cigarette smoke by trapping them in cysteamine solution contained in a pre-evacuated separatory funnel to form thiazolidines followed by extraction and gas chromatographic analysis. Acetaldehyde was the principal compound measured in all twenty-six brands of cigarette. Shibamoto's method has the advantages of being simple and fast, but did not have a means of accurately quantitating the total volume of smoke sampled by the cysteamine solution and thus, the results are at best, semi-quantitative.

Moldoveanu et al.<sup>46</sup> developed a procedure, which involved the analysis of the aromatic amines present in the Kentucky reference cigarettes (1R4F and 2R4F)

mainstream smoke using an automated SPE. The TPM portion was collected on a Cambridge pad using a smoking machine, followed by extraction with hydrochloric acid and clean-up by dual steps of SPE with an ion exchange cartridge and a hydrophobic cartridge. The eluent from the SPE extractions was then derivatized by the addition of heptafluorobutyric anhydride (HFBA) to yield derivatives that were stable for at least one week before GC-MS analysis. Toluidine and aniline were determined to be 0.02 ng/cigarette and 1.41 ng/cigarette, respectively.

Other methods available for the analysis of nitrosamines involved the trapping of mainstream smoke in one or more impingers. Solutions containing citric acid-sodium phosphate buffer with 20 mM ascorbic acid had been used in the impingers. The solutions were subjected to liquid-liquid extraction with dichloromethane and other clean-up steps before GC analysis.<sup>39,47</sup> These methods require long sample preparation times and are undesirable for reactive compounds such as nitrosamines since their degradation or undesired reactions could cause analytical errors.

Stabbert et al.<sup>48</sup> also developed a method for the analysis of aromatic amines including *o*-toluidine, *o*-anisidine, 2-naphthylamine, and 4-aminobiphenyl in the mainstream cigarette smoke. Gas chromatography/tandem mass spectrometry (GC/MS/MS) was used to analyze the pentafluoropropionyl (PFP) derivatives of aromatic amines in two Kentucky reference cigarettes and eight American-blend cigarettes. A linear relationship was observed between the yields of aromatic amines from the mainstream smoke of the American blend cigarette and the level of tar delivery.

### *Infrared Spectrometry Technique*

Parrish et al.<sup>49</sup> developed a method based on FTIR with an extractive gas cell for puff-by-puff and intrapuff analysis of cigarette smoke. This was done in order to understand the chemical profile of mainstream cigarette smoke constituents including carbon dioxide, carbon monoxide, acetaldehyde, nitrogen oxide, hydrogen cyanide, and carbonyl sulfide.

Plunkett et al.<sup>50</sup> measured the concentrations of ammonia and ethylene in single puffs of cigarette smoke via the use of high resolution infrared tunable diode laser absorption spectroscopy (TDLAS). By choosing spectral lines which were free from interferences from other compounds, the limits of detection for both ammonia and ethylene were 2 ng and 324 ng, respectively.

### **Past Research on Bidi Cigarettes**

Bidi smokers are similar to smokers of conventional cigarettes in terms of the physiological and biochemical effects experienced by a group of smokers tested.<sup>51</sup> In terms of subjective measures, bidis were reported to be less satisfying in nicotine delivery, which could explain why bidi smokers tend to puff more frequently and for a longer duration compared to smokers of other conventional cigarettes.

Bidis and bidi smoke have also been analyzed by energy dispersive x-ray fluorescence (EDXRF), neutron activation analysis (NAA), and anodic stripping voltametry (ASV).<sup>52</sup> Using these methods, the researchers discovered that the transfer efficiencies of bromine, cobalt, chromium, iron, antimony, scandium, and zinc from the tobacco to the mainstream smoke in bidis is about 1.5 - 3.0 times higher compared to

other Indian cigarettes.<sup>52</sup> In addition, the transfer efficiencies of toxic elements from the tobacco to the smoke in non-filter cigarettes such as bidis were higher than for filter-tip cigarettes.

#### *Liquid Chromatography Methods*

Wu et al.<sup>41</sup> developed methods to assess the TSNA levels in the mainstream smoke and the tobacco filler of 14 brands of bidis smoked in the U.S. A smoking machine was used to generate the mainstream cigarette smoke and GC-MS was used for the separation and identification of the analytes in the tobacco filler, while liquid chromatography/tandem mass spectrometry (LC/MS/MS) was used for the bidi smoke analysis. In the mainstream smoke of most of the bidi cigarettes used, the NNN content was higher than the value of NNK. However, the deliveries of NNN and NNK in the mainstream bidi smoke were comparable to those of the conventional cigarettes and the delivery of these TSNA was lower in the tobacco filler than the mainstream smoke. Since the FTC method used for the study relies on a lower puff frequency than that observed in bidi smoking, the delivery levels of TSNA to bidi smokers will be higher than the estimated level obtained using the FTC method. This shows that bidi is in no way less harmful than the other conventional cigarettes and the presence of these TSNA in bidi smoke is of great concern with regard to carcinogenesis.

Malson et al.<sup>53</sup> conducted a study on the nicotine content of tobacco present in bidis and compared it to those of four U.S. commercial filtered cigarettes. Twelve brands of unfiltered bidis were used and the nicotine content in the tobacco present in bidis was 21.2 mg/g, which was a lot more than the nicotine content of the tobacco present in

filtered and unfiltered American conventional cigarettes at 16.3 mg/g and 13.5 mg/g, respectively.<sup>53</sup> Despite the lesser amount of tobacco in bidis compared to conventional cigarettes, the total nicotine content of bidis is higher and hence are potentially more likely to help initiate and sustain nicotine dependence among smokers.

#### *Gas Chromatography Methods*

Pakhale et al.<sup>20</sup> did a study on major and minor alkaloids present in mainstream and sidestream smoke of bidi, chutta, and one brand of U.S. cigarette. The analysis by gas chromatography-flame ionization detection (GC-FID) showed that bidi tobacco contained higher levels of nicotine (at 37.7 mg/g bidi tobacco to 16.23 mg/g conventional tobacco) and minor alkaloids, such as nornicotine, anabasine, and anatabine, relative to the brand of U.S. cigarette tested. In addition, bidis had a higher fraction of both TPM and nicotine delivered to the smoker through the mainstream smoke and a relatively smaller fraction of TPM and nicotine emitted as ETS.

Watson et al.<sup>28</sup> analyzed 21 brands of common bidi cigarettes and five brands of conventional cigarettes in order to determine the levels of carbon monoxide, nicotine, and tar in the mainstream smoke of filtered and unfiltered bidi cigarette, and to determine their deliveries when smoked under the same conditions. A puff interval of 15 seconds was employed for the study, as opposed to the FTC value of 60 seconds, to keep the burning bidi cigarettes from being self-extinguished. The vapor phase was collected in gas collection bags and analyzed on the FTIR. TPM was determined by weighing the filter before and after sampling. Nicotine content was determined by GC-FID after extracting the filter with isopropanol solvent. The yields of the tar, nicotine, and carbon

monoxide from the mainstream smoke of the 21 brands of bidi were, 50.90-99.28 mg tar/bidi, 1.49-4.05 mg nicotine/bidi, and 24.5-51.6 mg carbon monoxide/bidi. There was no significant difference in the amounts of nicotine, tar, and carbon monoxide present in the mainstream smoke of either a filtered or an unfiltered bidis. Bidi cigarettes delivered more tar and carbon monoxide compared to conventional cigarettes. This is in agreement with other studies that have shown that bidi mainstream smoke contain higher levels of TPM and nicotine content.<sup>54,55</sup>

### *Infrared Spectrometry Methods*

A lot of work has been done on whole cigarette smoke, but recently, work is being done on the analysis of the puff-by-puff smoke, in order to understand the chemical profile of cigarette smoke constituents. By sampling and analyzing on a puff-by-puff basis, the changes in the composition of toxicants in each puff<sup>56</sup> can be characterized in order to fully understand the smoke chemistry over the course of a burning cigarette.<sup>57</sup>

Due to the limited research on bidi smoke analysis and the complex nature of cigarette smoke, different combinations of analytical techniques are required to characterize different classes of constituents in cigarette smoke. This work was devoted to the development of methods for bidis smoke sampling and analysis using FTIR, GC-MS, HPLC/UV, and LC-MS. The chemical composition of the bidi mainstream smoke was studied and efficient analytical techniques for characterizing whole cigarette smoke and puff-by-puff analysis of bidis smoke were developed.

GC-MS and LC-MS methods were developed for the analysis of carbonyl compounds present in bidi smoke. Since GC chromatographic separation involves

separations at temperatures as high as 300 °C compared to ambient temperature of the liquid chromatographic separation, the GC-MS signals of DNPH derivatives were studied as a function of GC injection port temperatures. Furthermore, partial oxidation products, such as formaldehyde and other carbonyl compounds, were detected because of their potential toxicity and their relationships to the permeability of the tendu leaf wrapper.

For carbonyl analysis on the LC-MS, sampling of bidis cigarette was done using commercial DNPH cartridges and, after extraction, analytes were analyzed on the LC-MS with APCI in the negative ion mode. Mixtures of DNPH derivatives of carbonyls available commercially were purchased for analysis. GC-MS was used to verify the presence of carbonyls in bidi mainstream smoke by using the extracted ion chromatograms at the  $m/z$  values of 224, 238 and 250.

An FTIR technique with a sampling duration of six seconds per puff for the analysis of bidi mainstream smoke was also developed. The sampling procedure allowed the simultaneous collection of TPM onto an ASSET-32 sorbent tube and gaseous samples into an extractive gas cell for both GC-MS and FTIR analysis, respectively. Study of the types of solvents effective for extracting toxicants from the ASSET-32 sorbent tube for further analysis was performed as well as the characterization of toxicants obtained.

FTIR was used to characterize the low molecular weight constituents of bidi mainstream smoke such as carbon monoxide, hydrogen cyanide, methanol, and ethylene. The smoke samples were analyzed shortly after sampling and directly in the gas cell to avoid the adsorption or condensation of smoke constituents. A puff-by-puff sampling of the bidi smoke was investigated. In addition, the effect of smoke flow rate on the delivery level of smoke constituents was studied.

Another method was developed, based on TPM collection using the Sioutas impactor.<sup>58</sup> The Sioutas impactor is used to fractionate TPM based on its particle size distribution. The impactor consists of four impaction stages and an after-filter for the collection of the particulates and their separation into the following ranges, based on their diameters: < 0.25  $\mu\text{m}$ , 0.25-0.5  $\mu\text{m}$ , 0.5-1.0  $\mu\text{m}$ , 1.0-2.5  $\mu\text{m}$ , and 2.5-10  $\mu\text{m}$ . Thus, it efficiently separates samples into coarse, fine, and ultrafine particles. After sampling, analysis can be done chemically, gravimetrically and/or microscopically. In this work analysis of the extracts obtained from the five different filters in the impactor was performed on the GC-MS.

Analysis of bidi smoke was an important undertaking because of the popularity of bidi smoking in East Asia and its emergence in the US. There has not been much research done on the characterization of its toxicants and constituents such as carbonyls, sterols, and phenols, have not been studied adequately. Also, the puff-by-puff FTIR analysis of bidi smoke performed could help elucidate the dependence of smoke chemistry on puff characteristics (i.e. volume and duration). Since one of the main problems with mainstream smoke is the inhalation of particulates and vapor phase constituents, it was necessary to study how these constituents are distributed according to their size fractions and the results obtained using the Sioutas impactor would be valuable in this regard. It is hoped that the data obtained from this research work will increase the understanding of bidi smoke composition.



## CHAPTER 2

### MATERIALS AND METHODS

#### Instrumentation and Equipment

A Nicolet Magna 550 FTIR spectrometer and a 2.4-meter gas cell purchased from Infrared Analysis Inc. (Anaheim, CA) were used for the FTIR analysis of bidi smoke. Spectra were recorded using a Mercury Cadmium Telluride (MCT/A) detector at a resolution of  $0.5\text{ cm}^{-1}$  ( $1\text{ cm}^{-1}$  or  $2\text{ cm}^{-1}$  was used when optical throughput was too low for  $0.5\text{ cm}^{-1}$  resolution) with no zero filling and Happ-Genzel apodization. The total spectral acquisition time for co-adding 100 scans was seven minutes.

The Agilent Model 1100 benchtop liquid chromatograph equipped with a diode array detector (DAD) and a mass selective detector (MSD) was used for the analysis of carbonyl compounds present in the mainstream smoke of bidis. The column used was a Phenomenex Synergi  $4\mu$  max-RP  $80\text{Å}$ , C8 column, 250 mm x 2.0 mm (00G-4337-BO, Torrance, CA). The DAD was set at a wavelength range of 190 - 600 nm for detecting the carbonyl compounds in the form of their DNPH derivatives. The MSD was operated in the APCI negative ion mode<sup>31</sup> as previously reported for the quantitation of carbonyl-DNPH derivatives in the  $m/z$  range of 125 - 600.

The mobile phase gradient for the chromatographic separation was optimized by multiple injections of a Supelco Mix 2 solution of C1 - C8 carbonyl-DNPH standards under various mobile phase conditions. The eluent mixture was 55% acetonitrile and 45% of 9:1 water/methanol mixture. A slight gradient from 55% acetonitrile to 65%

acetonitrile was used for the separation. The conditions used for the LC-MS are shown in Table 1.

A number of parameters were also optimized using the automated flow injection analysis (FIA) mode on the LC-MS. The optimal parameters were determined to be as follows: corona current = 5  $\mu$ A, nebulizer pressure = 20 psig, vaporizer temperature = 350 °C, and capillary voltage = 1250 V.

For GC-MS analysis, an Agilent Technologies 6890N series GC interfaced to a HP 5973 mass selective detector (MSD) was used. The conditions used for the GC-MS analysis of the particulate phase of bidi constituents are shown in Table 2. A study of the effect of different GC-MS injector temperatures on carbonyl-DNPH derivatives was performed at temperatures between 190 °C and 270 °C at increments of 20 °C with a mass scan range of 35 - 450 amu. GC helium carrier flow was set at 0.9 L/min; linear flow velocity at 34.4 cm/s; GC-MS interface temperature at 300 °C; initial oven temperature at 40 °C; temperature programming rate at 20 °C/min; hold time for final oven temperature at 10 min; and the total chromatographic run time set at 22.5 min.

### **Materials and Reagents**

The MSD parameters were evaluated using a 0.5 ppm acetaldehyde-DNPH standard that was prepared from the 1000 ppm standard purchased from Supelco (47340-U, Bellefonte, PA). The solvent for dilution was a 60:40 acetonitrile/water mixture, which was the eluent concentration at the midpoint of the mobile phase gradient. The Supelco carbonyl standard for Method 1004 DNPH Mix 2, (47651-U, Bellefonte, PA) was used to optimize LC-MS conditions, and to prepare calibration standard

Table 1. Operating Conditions of the LC-MS Analysis

Parameters	Setting
Eluent flowrate	0.30 mL/min
Column temperature	25 °C
Injection volume	0.50 $\mu$ L
DAD range	190 - 600 nm
MS scan range	m/z 125 - 600
Ionization mode	APCI
Gain	5
Nebulizer pressure	20 psig
Drying gas flow	4.0 L/min
Drying gas temperature	350 °C
Vaporizer temperature	350 °C
Capillary voltage	1250 V
Fragmentor setting	50 V
Corona current	5 $\mu$ A

Table 2. Operating Conditions for GC-MS Analysis of TPM and ASSET-32 Tube Samples

Parameters	Setting
Mass Spectrometry Scan Range	35-550 amu
GC Helium Carrier Flow	0.9 mL/min
Linear Flow Velocity	34.4 cm/s
Injector Temperature	250 °C
GC-MS Interface Temperature	280 °C
Initial Oven Temperature	50 °C
Initial Hold Time	3 min
First Thermal Gradient	8 °C/min
Hold Time for First Gradient	1 min
Second Gradient	15 °C/min
Hold Time for Second Gradient	10 min
Final Gradient	20 °C/min
Final Oven Temperature	320 °C
Hold Time for Final Oven Temperature	6 min
Total Chromatographic Run Time	43.17 min

concentrations of 0.5 ppm, 1 ppm, 2 ppm, 3 ppm, 4 ppm, 6 ppm, and 8 ppm for the LC-MS and GC-MS analyses.

Three different types of the White Rhino bidi filtered cigarettes, namely vanilla, strawberry, and grape-flavored bidis (Kretek International, Moorpark, CA) were purchased from a local retail store in Brentwood, TN. An unflavored bidi type, namely Seyadu bidi, was also obtained from India. The 1R5F Kentucky reference cigarette (University of Kentucky, Kentucky Tobacco Research and Development Center, Lexington, KY) was used for the evaluation of analytical method and as a basis for comparison to data from bidi cigarettes. The 1R5F cigarette is an ultra low yield cigarette with a tar delivery of 1.67 mg/cigarette and 0.16 mg of nicotine/cigarette. The vanilla and grape bidi cigarettes were subjected to drying in the laboratory oven at 120 °C for three hours and one hour, respectively, before sampling. If the bidis were not dried, they self-extinguished during sampling. The Kentucky reference cigarettes were kept in the refrigerator and removed about 15 min before sampling, to allow equilibration to room temperature. The bidi cigarettes were also removed from the oven about 15 min before sampling, to equilibrate to room temperature.

A 0.5 µm filter, purchased from Swagelok (SS-4F-VCR-7, Memphis, TN), was used to trap the particulate matter in bidi smoke during FTIR analysis of the gaseous portion of bidi smoke. The filter helped avoid the contamination of the gas cell and minimized the need for frequent cleaning of gas cell mirrors. Two types of sampling pumps were used in this research. The pump from GAST Manufacturing Co. (Model No. 1532-V106-G557X, Benton Harbor, MI) was used for carbonyl sampling via the DNPH tubes. Supelco L<sub>p</sub>DNPH H30 Cartridges (505323, Bellefonte, PA) were used for

sampling of the carbonyls in bidi mainstream smoke. The Aircheck personal sampling pump (SKC Inc., Model No. 224-PCXR7, Eighty Four, PA) was used to sample the bidi smoke through the ASSET-32 activated charcoal tubes from Supelco (28301-U, Bellefonte, PA). The sorbent tubes were subsequently extracted with 1.5 mL methanol purchased from Burdick and Jackson (GC-MS grade, Muskegon, MI) and petroleum ether purchased from Fisher Scientific (OPTIMA grade, Fair Lawn, NJ). The DryCal DC-Lite primary flowmeter from Bios International (Model No. DCLT 12 K Rev, Pompton Plains, NJ) was used to calibrate the required flowrates prior to each sampling step.

A Sioutas cascade impactor<sup>58</sup> (SKC Inc., Cat. No. 225-370, Eighty Four, PA) was used for sampling the particulate phase in cigarette smoke onto filter pads. This impactor was designed to separate and collect particulates in five size ranges:  $>2.5 \mu\text{m}$  (Fraction A),  $1.0\text{-}2.5 \mu\text{m}$  (Fraction B),  $0.50\text{-}1.0 \mu\text{m}$  (Fraction C),  $0.25\text{-}0.50 \mu\text{m}$  (Fraction D) and  $<0.25 \mu\text{m}$  (Fraction L).

At the sampling flowrate of 9 L/min, the particulate phase traversed the impactor such that particles above the cut-point of each fraction were collected onto a  $0.5 \mu\text{m}$  PTFE (Teflon) filter of 25 mm in diameter with PTFE support (SKC Cat. No. 225-1708) successively from Fraction A to Fraction D. The particulate for the last stage (i.e.  $< 0.25 \mu\text{m}$  cut-point) was collected onto a  $2.0 \mu\text{m}$  PTFE filter of 37 mm in diameter, with a support ring (SKC Cat. No. 225-1709). After sampling, the filters were extracted with 5 mL methylene chloride (Fisher Scientific, HPLC Grade, Fair Lawn, NJ) and were sonicated using the ultrasonic bath before the remaining extract was crimped in 2 mL

vials (Agilent Cat. No. 5181-3376, Palo Alto, CA) and analyzed by GC-MS using the autosampler (7683 series, Agilent, Palo Alto, CA).

### **FTIR Sampling Methodology**

The set-up shown in Figure 1 was used for the sampling of bidi smoke for FTIR analysis. Sampling conditions involving a puff duration of 6 seconds were utilized in the experiments. First, the cigarette was dried in the oven, as described earlier, and Teflon tape was wrapped loosely around the mouthpiece to ensure a snug fit into the Teflon tubing. For the sampling, the length of tubing was minimized to avoid interferences of phthalate esters present in the tubing. All connections were wrapped with Teflon tape to ensure air-tightness. A Y-junction was connected to the cigarette so that part of the smoke was sampled into an ASSET-32 sorbent tube at 1.05 L/min while the other part went through the filter into the 2.4-meter gas cell at the different flowrates of interest. The sampling was carried out on a puff-by-puff basis for a duration of 6 seconds. An average of three puffs was taken per cigarette.

The gas cell was evacuated prior to the acquisition of the background FTIR spectra. Sample spectra were obtained and compared to the “ref 5” reference spectra, for identification of constituents, with quantitative analysis done in regions free from interference by water and carbon dioxide in the cigarette smoke. “Ref 5” is an in-house reference library of standard infrared spectra at a resolution of  $0.5\text{ cm}^{-1}$ . For sample spectra obtained at  $1\text{ cm}^{-1}$  and  $2\text{ cm}^{-1}$ , the “Ref 5” reference spectra at  $0.5\text{ cm}^{-1}$  were deresolved to the appropriate wavenumbers using the Arnold Engineering Development Center (AEDC) deresolving program before analysis.

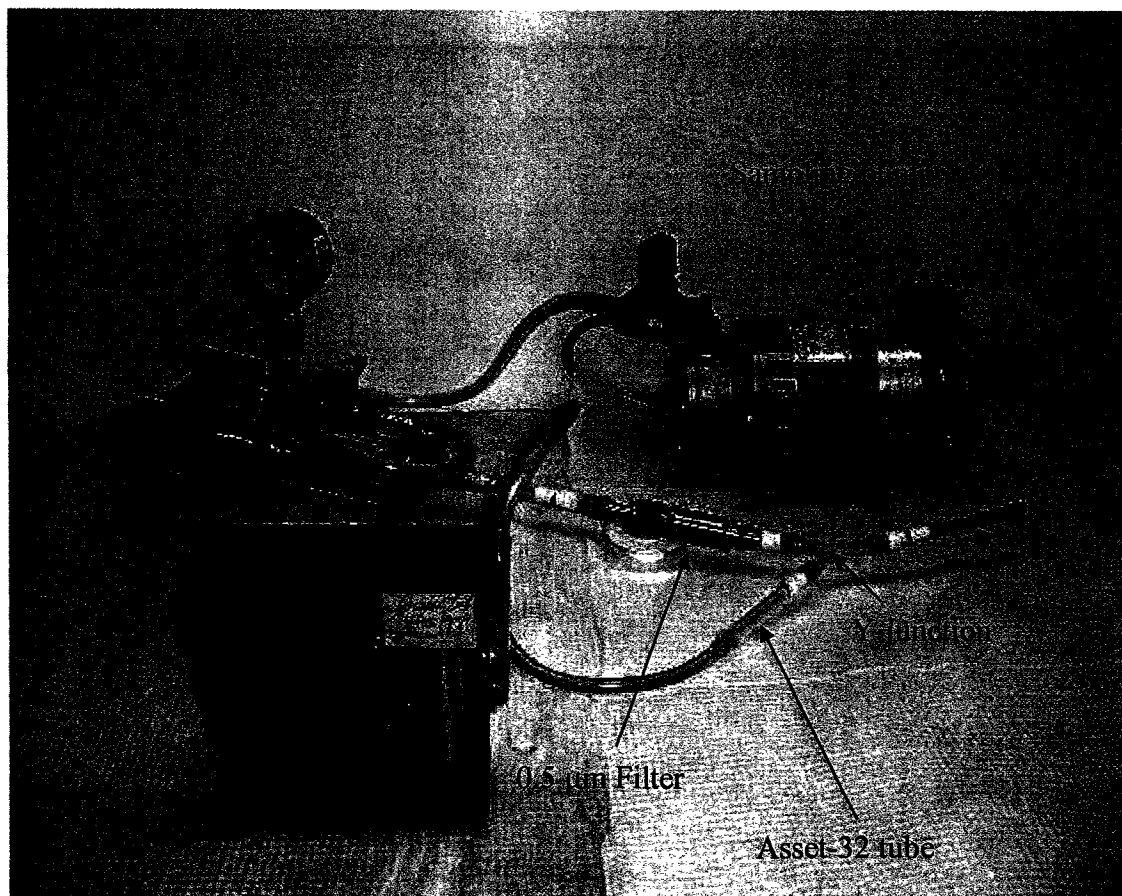


Figure 1. Set-up for sampling bidi smoke onto the activated charcoal sorbent tube and the 2.4-meter gas cell.



The analytes from the sorbent tubes were extracted using methanol and petroleum ether for GC-MS analysis. The occurrence of breakthrough was also verified by placing two ASSET-32 sorbent tubes in series and analyzing the two extracts from the tubes on the GC-MS.

### **Method for Sampling Onto the DNPH Cartridge**

For carbonyl sampling, the set up shown in Figure 2 was used with the pre-dried cigarettes. Heat shrink Teflon was used to ensure all the connections were airtight. A sampling flowrate of 1.05 L/min was calibrated with the aid of the flowmeter. Blanks were obtained for the two brands of bidis used by pulling air through the cigarette into the DNPH cartridge without lighting the attached bidis. For actual sampling, the cigarette was lit with a match and mainstream smoke was pulled through a DNPH cartridge with the aid of the GAST pump until it burned close to the 23 mm mark. The cartridge was allowed to stand for another 15 min before extraction of the constituents on the cartridge was carried out with 5 mL of acetonitrile. The extract was then pre-concentrated to 1.5 mL by blowing ultra high purity nitrogen gas gently through it. Two milligrams of  $d_4$ -acetaldehyde-2,4-dinitrophenylhydrazone derivative was spiked into the cigarettes before being burned and the standard carbonyl mix before LC-MS analysis in addition to 1.1 milligram phenyl-acetaldehyde-DNPH which served as internal standards. The samples were transferred into sealed amber vials, crimped, labeled, and kept in the refrigerator for LC-MS and/or GC-MS analysis. A commercially available carbonyl standard (Supelco Carb Method 1004 DNPH Mix 2 47651-U), containing mixtures of 13

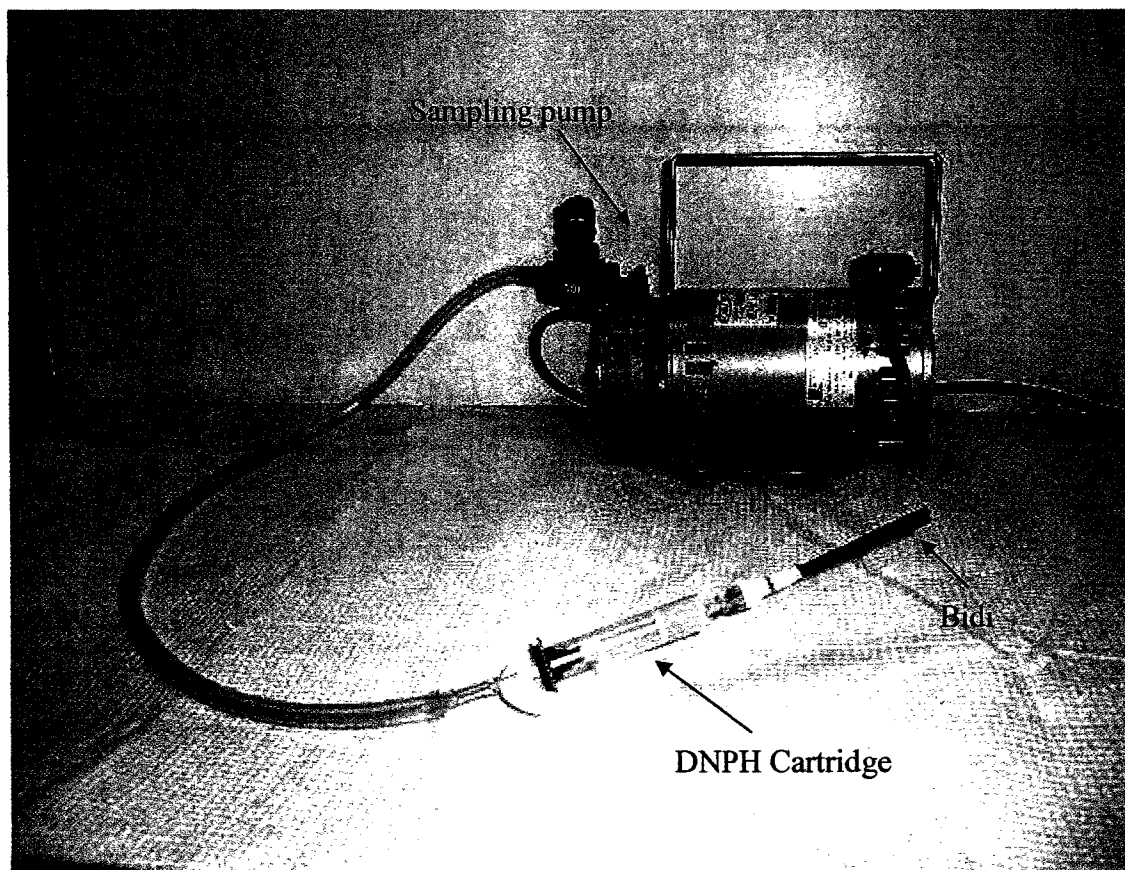


Figure 2. Set-up used for sampling cigarette smoke onto DNPH cartridge at a flowrate of 1.05 L/min.

Note: All connections were made with Teflon tubing.

different carbonyl-DNPH compounds at a concentration of 30 ppm each in acetonitrile, was used to prepare calibration standards ranging from 0.25 ppm - 8 ppm. Table 3 provides the common names and the chemical abstracts service (CAS) numbers of these carbonyl derivatives.

### **Sampling Method Using the Sioutas Impactor**

Figure 3 shows the schematic for the sampling of cigarette smoke using the Sioutas Impactor. A pump flowrate of 9 L/min was initially adjusted for the mainstream smoke with the unburned bidi cigarette attached to the inlet of the impactor.<sup>58</sup> During sampling, the flow meter was removed and the outlet of the impactor was attached directly to the pump. All connections were wrapped with Teflon tape to ensure that there were no leaks. The cigarette was then lit with a match and allowed to burn to the 23 mm mark from the end of the cigarette butt before being extinguished. Afterwards, the flowmeter was re-connected to the impactor, to verify what the flowrate was. The flowrate was usually about 8.50 L/min.

After 10 min, the impactor was disassembled in a dust-free environment. Each of the four filters and the after filter were then placed in labeled beakers for extraction with 5 mL methylene chloride for 30 min in an ultrasonic bath (Model B-22-4, Branson Company, Shelton, CT). After extraction, the samples were transferred into 2 mL amber vials and kept in the refrigerator prior to GC-MS analysis.

Table 3. Names and Identification of Carbonyl-DNPH Derivatives

DNPH-derivative	Common Name	CAS –RN <sup>a</sup>
Acetaldehyde-2,4-DNPH	Acetaldehyde-2,4-DNPH	1019-57-4
Acetone-2,4-DNPH	2-Propanone-2,4-DNPH	1567-89-1
Acrolein-2,4-DNPH	2-Propenal-2,4-DNPH	888-54-0
Benzaldehyde-2,4-DNPH	Benzaldehyde-2,4-DNPH	1157-84-2
Butyraldehyde-2,4-DNPH	Butanal-2,4-DNPH	1527-98-6
Crotonaldehyde-2,4-DNPH	2-Butenal-2,4-DNPH	1527-96-4
Formaldehyde-2,4-DNPH	Formaldehyde-2,4-DNPH	1081-15-8
Hexaldehyde-2,4-DNPH	1-Hexanal-2,4-DNPH	1527-97-5
<i>m</i> -Tolualdehyde-2,4-DNPH	3-Methylbenzaldehyde-2,4-DNPH	2880-05-9
Methacrolein-2,4-DNPH	2-Methylpropenal-2,4-DNPH	5077-73-6
Propionaldehyde-2,4-DNPH	Propanal-2,4-DNPH	725-00-8
Valeraldehyde-2,4-DNPH	<i>n</i> -Pentanal-2,4-DNPH	2057-84-3
2-Butanone-2,4-DNPH	2-Butanone-2,4-DNPH	958-60-1

<sup>a</sup>CAS-RN: Chemical Abstracts Service Registry Number.

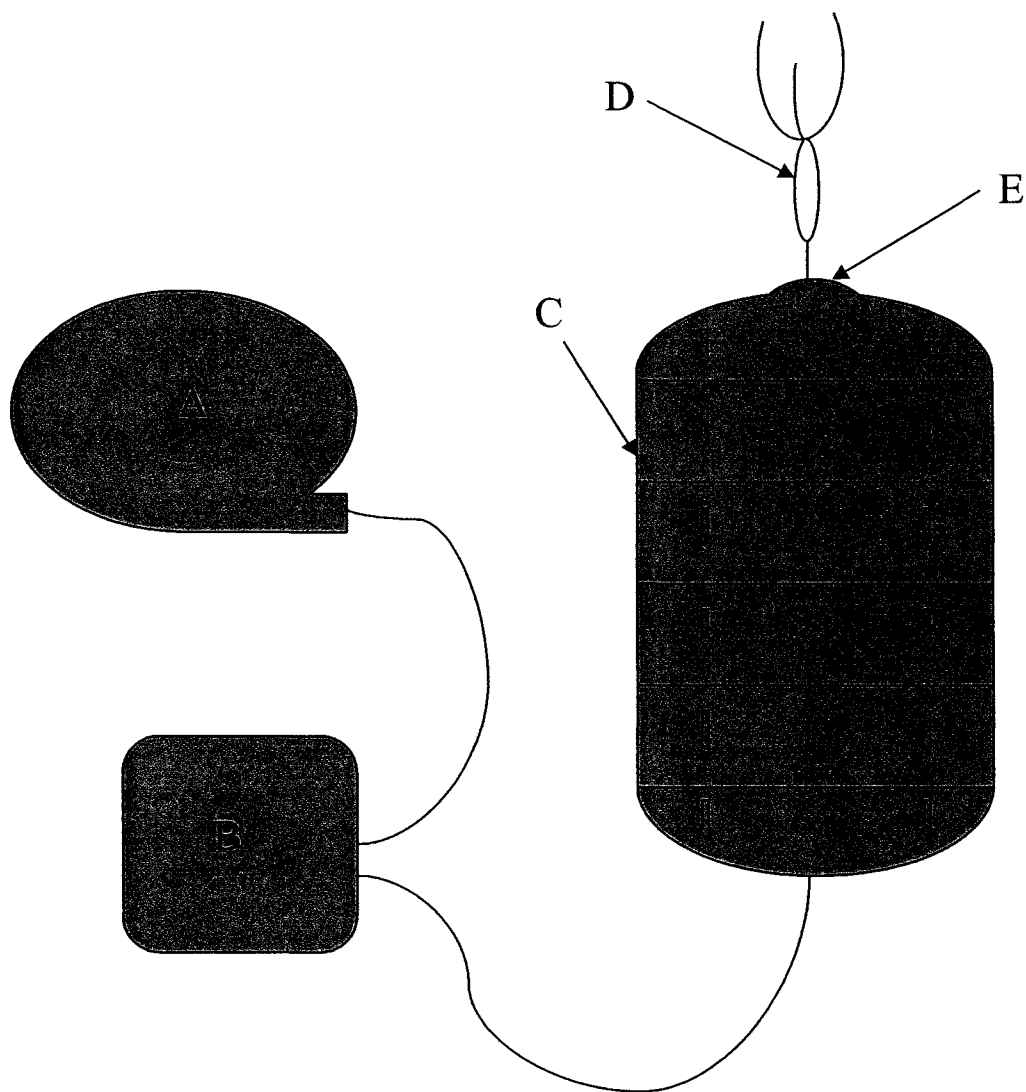


Figure 3. Schematic diagram of the set-up used for sampling bidi smoke onto the different filters in the Sioutas impactor. The cigarette (D) is attached to the impactor (C) through the inlet (E). A pump (A) attached to the Dry-Cal flow calibrator (B), is used to pull the bidi smoke through the Sioutas impactor at a flowrate of 9.0 L/min. The direction of travel of bidi smoke through the impactor is indicated by the arrows.

## CHAPTER 3

### RESULTS AND DISCUSSION

#### **Dependence of Carbonyl-DNPH Signals on the Injector Temperature of GC-MS Analysis**

GC-MS is very useful for the determination of those constituents for which commercial standards are not available. This is because the GC-MS has a standard mass spectral library that can be searched for a spectral match to the different compounds in the sample. The match indices can be used for tentative identification of compounds in a complex sample such as tobacco smoke. Relative to GC methods, HPLC and LC-MS are preferred for the analysis of thermally unstable and semivolatile compounds, because these techniques are usually performed at room temperature with minimal thermal decomposition of analytes.

GC-MS analysis of carbonyl-DNPH derivatives was performed at different injector temperatures to study how injector temperatures affect the analytical signals of these polar and thermally labile derivatives. This is because the availability of the standard search library on the GC-MS still makes this technique attractive to some researchers for the analysis of carbonyl compounds. The injector temperature for the analysis of these derivatives is very important because the carbonyl-DNPH derivatives need to be volatilized at sufficiently high temperature to reach the column for analytical separation. However, an optimum temperature must allow volatilization of these derivatives without thermally induced decomposition. Figure 4 shows the GC-MS

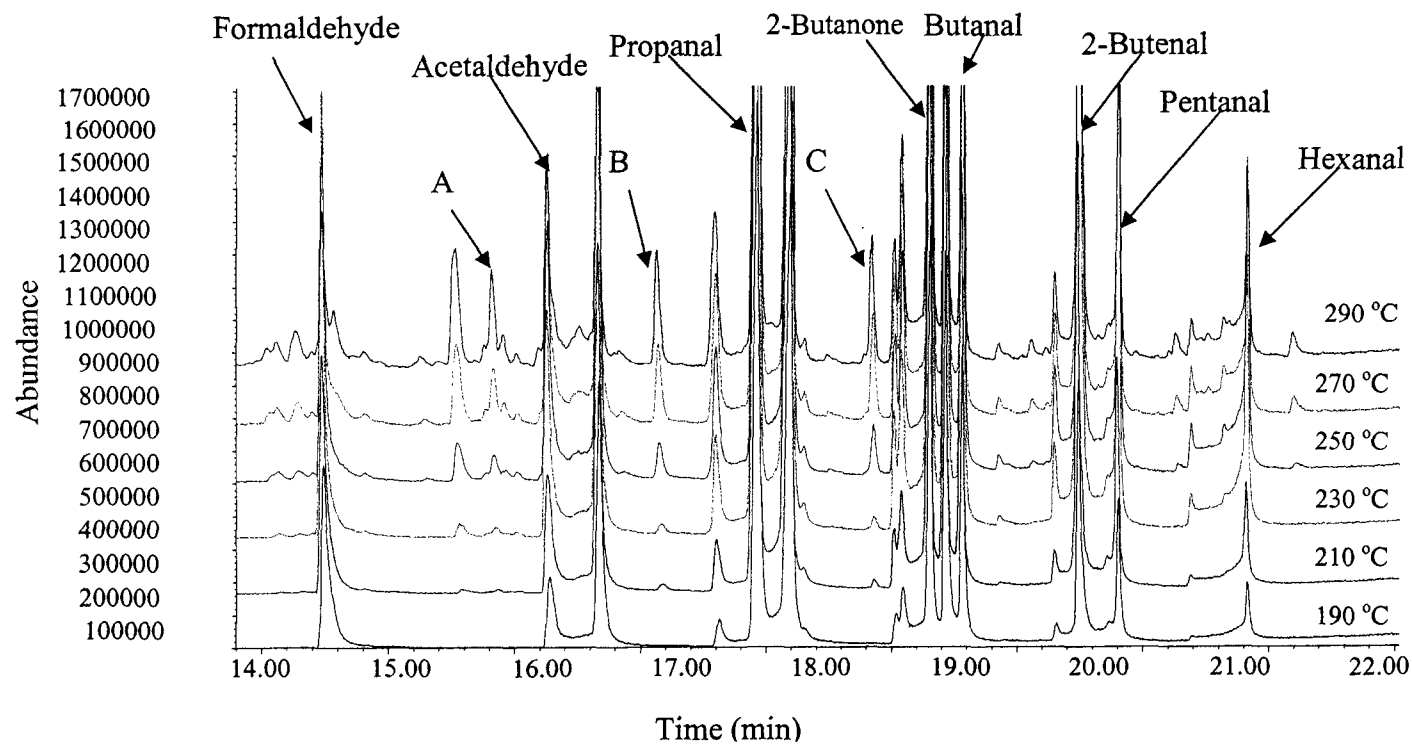


Figure 4. GC-MS chromatograms of carbonyl-DNPH derivatives and some decomposition products analyzed at different injector temperature.

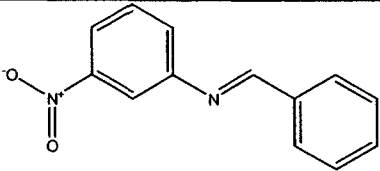
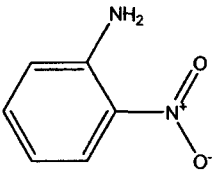
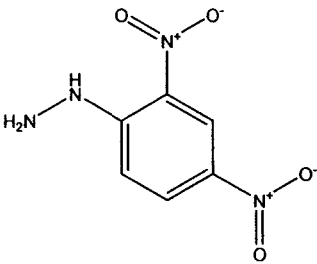
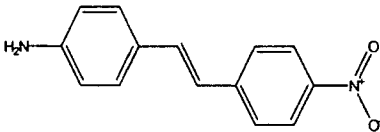
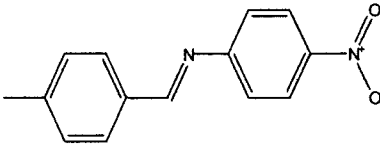
A, B, and C are decomposition products. A is cyclohexano[b] naphthalene, B is 3-nitro-N-(phenylmethylene)-benzeneamine, and C is N-[(4-methylphenyl)methylene] 4-nitro-benzeneamine.

chromatograms of these carbonyl-DNPH derivatives at a concentration of 6 ppm each as a function of injector temperature from 190 °C - 290 °C at 20 °C increments. The *anti*- and *syn*-isomers of the acetaldehyde, propanal, butanal, 2-butanone, and hexanal- 2,4-DNPH derivatives were observed at almost all the injector temperatures, which is typical of GC-MS analysis of these types of compounds. The signal for benzaldehyde-2,4-DNPH (not shown in Figure 4) was observed at a retention time of 24.62 min. The signal was relatively weak compared to C1-C5 carbonyl compounds at the same concentration for all the injector temperatures from 190 °C to 270 °C. The presence of benzaldehyde was detected at an injector temperature greater than 210 °C and its signal increased with increasing injector temperature until 250 °C before it started to decrease with increased injector temperature.

At injector temperatures of 190 °C and 210 °C, the number of DNPH-related decomposition products or impurities from the DNPH sampling tube and their intensities were less than those observed as the temperature was increased up to 290 °C. Some of the decomposition products of the carbonyl-DNPH derivatives were tentatively identified according to their match indices (MI) and retention times (RT). Decomposition products such as cyclohexano[b] naphthalene (MI = 95, RT = 15.84 min), A, 3-nitro-N-(phenylmethylene)-benzeneamine (MI = 98, RT = 17.15 min), B, and N-[(4-methylphenyl)methylene]4-nitro-benzeneamine (MI = 87, RT = 18.85 min), C, were observed at the injector temperatures greater than 250 °C. Other decomposition products observed, but which are not shown in Figure 4, include *o*-nitroaniline or *m*-nitroaniline (MI = 95 for ortho isomer and 97 for meta isomer, RT = 6.95 min). Structures of some of these decomposition products are shown in Table 4. Some of the impurities associated



Table 4. Structures of Decomposition Products and Impurities Associated with Carbonyl-DNPH Derivatives

Compound Name	Chemical Structure	Retention Time (RT)	Match Index (MI)
3-nitro-N-(phenylmethylene)-benzenamine		17.15	98
<i>o</i> -Nitroaniline		6.95	95
2,4-dinitrophenylhydrazine			
4-[2-(4-nitrophenyl)ethenyl]-benzenamine		18.83	86
N-[4-methylphenyl)methylene]-4-nitro-benzenamine		18.85	87

with sampling via DNPH tubes and identified by comparing their structures with those of breakdown fragments of carbonyl-DNPH derivative structures include 5-nitroisatoic anhydride (6.24 min), 6-ethyl-2,5-dihydroxy-1,4-naphthoquinone (RT = 15.86 min), and 7-isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one (RT = 15.82 min).

The total ion chromatogram (TIC) peak areas of selected C1 - C6 DNPH derivatives at 6 ppm were plotted as a function of GC injector temperature in Figure 5. The responses of these derivatives generally increased with increasing injector temperature starting from 190 °C. Higher molecular weight carbonyl-DNPH derivatives such as 2-butanone-DNPH, 2-butenal-DNPH, butanal-DNPH, and hexanal-DNPH show maximum responses at 250 °C, while the less volatile carbonyl-DNPH derivatives, such as formaldehyde-DNPH and acetaldehyde-DNPH, show maximum responses at 230 °C. Therefore, in order to choose the optimum injector temperature for the analysis of carbonyl compounds, the composition of the sample needs to be considered. For instance, even though an injector temperature of 230 °C may be suitable for the analysis of a sample containing formaldehyde, a higher temperature of 250 °C or greater may sometimes be necessary to yield better sensitivity for larger (i.e. C4 - C6) carbonyl compounds and to avoid the undesirable memory effects if the sample contains less volatile analytes that may contaminate the GC injector sleeve. Memory effects involve a situation in which a part of the previous sample stays in the GC injector and contributes to the signals of subsequent samples. These contaminants may confound the analysis from one sample to the others when higher injector temperatures are used, subsequently, with the contaminated injector sleeve. The GC-MS response of DNPH derivatives

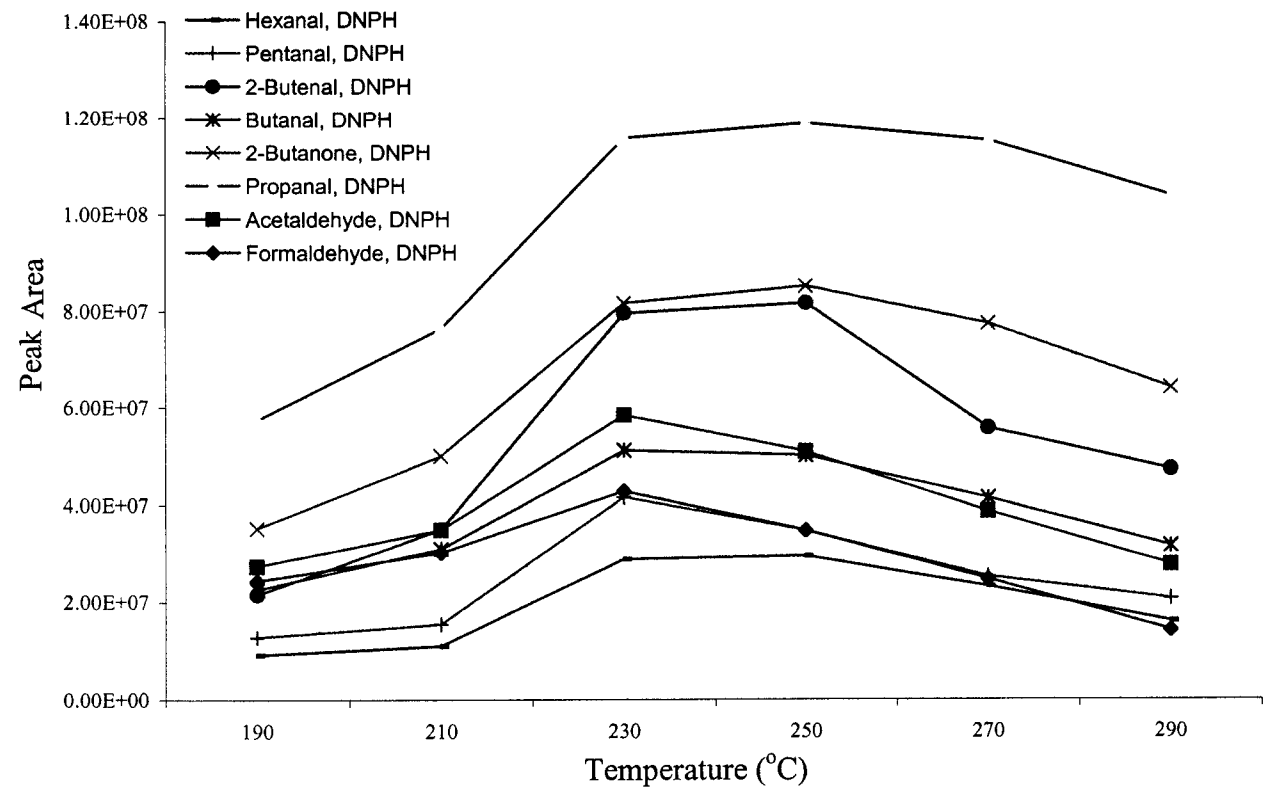


Figure 5. Plot of GC-MS peak areas of the carbonyl-DNPH derivatives at 6 ppm each as a function of injection temperature.

decreased from the optimum temperature of 230 °C to 290 °C due to the decomposition of the carbonyl derivatives. The lesser decrease observed for both propanal and 2-butanone DNPH derivatives relative to the decrease for formaldehyde and acetaldehyde derivatives was due to the greater volatilization of the larger carbonyl derivatives at higher injector temperatures. When the peak areas of two decomposition products, namely *m*-nitroaniline and 1-(2,4-dinitrophenyl)-2-methylenehydrazine, were plotted as a function of injector temperatures, as shown in Figure 6, the trend for the increased decomposition at higher temperatures was clearly shown from 250 °C to 290 °C. Between 210 °C and 230 °C, either no peaks corresponding to these decomposition products were observed in the chromatograms or the match indices were very low. The GC-MS responses of the impurities also increased as the injector temperature increased. The decomposition product 1-(2,4-dinitrophenyl)-2-methylenehydrazine has a molecular mass of 209 amu and is likely to be a fragment of acetaldehyde 2,4-DNPH, with the loss of a methyl group.

#### **FTIR Analysis of Bidi Smoke**

A method involving the simultaneous sampling of bidi smoke into an extractive gas cell for FTIR analysis and onto an Asset-32 sorbent tube for GC-MS analysis was developed. Figure 7 shows a spectrum obtained by the FTIR analysis of bidi smoke. A comparison between the spectrum of unflavored Seyadu bidi smoke and the reference spectra of methanol, ethylene, and 1,3-butadiene indicates the presence of these compounds in bidi smoke.

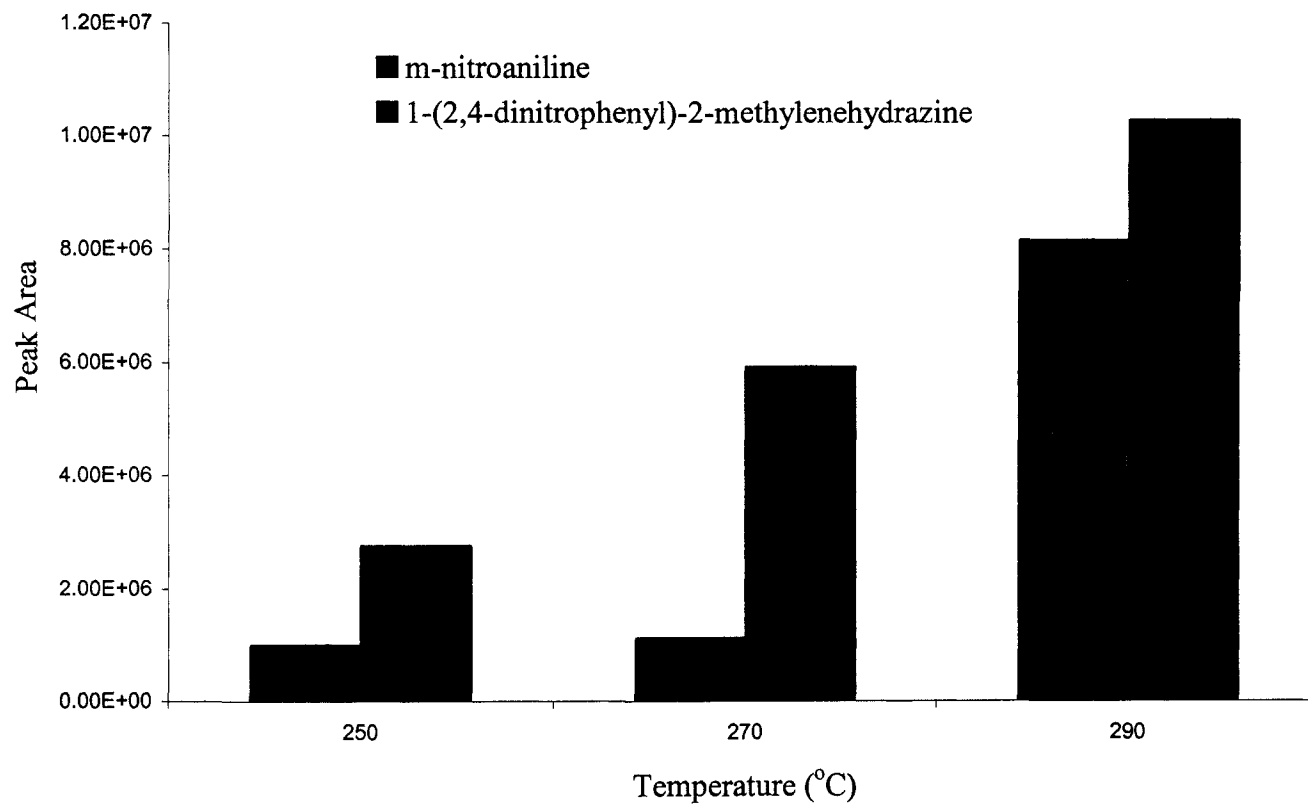


Figure 6. Plot of the GC-MS peak areas of the decomposition products of DNPH derivatives at different injector temperature.

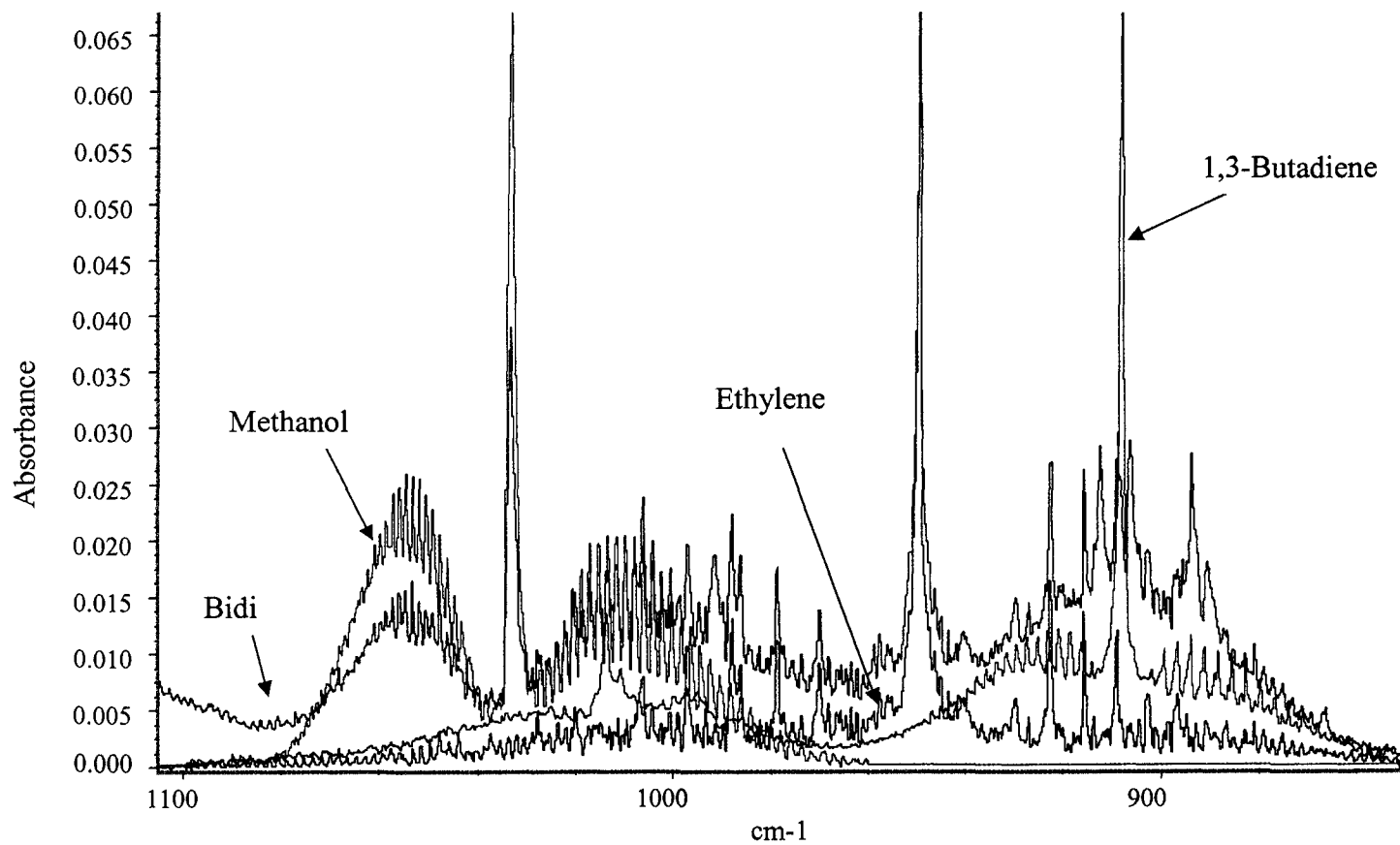


Figure 7. Comparison of the FTIR sample spectrum of bidi smoke and reference spectra of ethylene, methanol, and 1,3-Butadiene. Comparison indicates the presence of these compounds in bidi cigarette smoke.

Other components identified in whole cigarette smoke analysis of bidi smoke by FTIR at 1.05 L/min for a puff duration of six seconds include hydrogen cyanide and 1,3-butadiene. Their concentrations are presented in Table 5. From the table, the concentration of hydrogen cyanide in a six-second puff of bidi cigarette is  $780 \pm 130$  ppm, which corresponds to  $310.8 \mu\text{g}$  of hydrogen cyanide per cigarette. Previous studies of bidi smoke<sup>21,26</sup> have shown that the concentration of hydrogen cyanide in bidi smoke is in the range of 688-904  $\mu\text{g}/\text{cigarette}$ . It should be noted that the sampling conditions in terms of puff volume used by Hoffmann et al. and Pakhale et al. are different from those used in the present study. They used a puff volume of 35 mL, while the sampling volume used in this present study was 120 mL. By compensating for these differences, it is observed that the concentration of HCN obtained from this work is in agreement with that obtained from the previous work as they used a puff volume which was about three times smaller than that used in this study. For 1R4F and 1R5F reference cigarettes, the levels of hydrogen cyanide have been reported as  $125 \pm 10 \mu\text{g}/\text{cigarette}$  and  $22.8 \pm 1 \mu\text{g}/\text{cigarette}$ , respectively.<sup>59</sup>

The published concentrations of carbon monoxide, hydrogen cyanide, and 1,3-butadiene in the 1R5F reference cigarette measured using a puff volume of 35 mL, were 3.36 mg/cigarette, 22.8  $\mu\text{g}/\text{cigarette}$ , and 12.6  $\mu\text{g}/\text{cigarette}$  respectively.<sup>59</sup> Converting the concentrations of these compounds in Table 5 from ppm to mg/cigarette based on three puffs per cigarette, carbon monoxide = 4.9 mg/cigarette, hydrogen cyanide = 310  $\mu\text{g}/\text{cigarette}$ , and 1,3-butadiene = 143  $\mu\text{g}/\text{cigarette}$ . From these values, even with the use of a smaller puff volume in the previous study, it can be seen that the mainstream

Table 5. Concentrations of Selected Constituents as Measured by FTIR Spectrometry of the Mainstream Smoke of Seyadu Bidi Sampled at 1.05 L/min for a Puff Duration of 6 Seconds

Compound	Wavenumber (cm <sup>-1</sup> )	Concentration (ppm)
Carbon monoxide	2137 - 2144	12,000 ± 2180
Ethylene	920 - 972	400 ± 140
Methanol	1057 - 1069	500 ± 65
Hydrogen cyanide	703 - 772	780 ± 130
1,3-Butadiene	874 - 875	180 ± 30



smoke of bidi cigarette contains a higher concentration of carbon monoxide, hydrogen cyanide, and 1,3-butadiene.

The effect of sampling flowrate on the delivery of different components present in bidi smoke was also investigated by burning the bidi cigarettes for a period of 6 seconds for each puff of bidi smoke. Previous studies have shown that bidi smokers tend to have a greater puff frequency of 2-5 puffs<sup>19</sup> per minute, which is greater than the puff frequency for the smokers of conventional cigarettes, due to the less permeable nature of the tendu leaf used to wrap bidi cigarettes. The concentrations of different smoke constituents were plotted as a function of simulated puff flowrates, as shown in Figure 8, for carbon monoxide and carbon dioxide and in Figure 9 for ethylene.

Figure 8 shows that the concentration of carbon monoxide actually decreased in bidi smoke, sampled for each 6-second puff, as the sampling flowrate increased. This can be explained by the fact that, as sampling flowrate increases, there is more oxygen being drawn in to the cigarette such that the combustion of cigarette components is more complete. This way, the carbon monoxide that is produced by incomplete combustion is converted to carbon dioxide via reaction with the available oxygen. From Figure 8, the concentration of carbon dioxide decreased starting from the flowrate of 0.56 L/min until the flowrate of 1.25 L/min. In order to explain this trend, the effect of dilution was considered. The ratio of flowrates of 0.96 L/min to 1.25 L/min was calculated to be 0.76. Next, the ratio of carbon dioxide concentration at 1.25 L/min to its concentration at 0.96 L/min was 0.74, while the corresponding ratio for carbon monoxide was 0.63. These values suggest that, as the flowrate was increased, the effect of dilution accounts for the decrease in the concentration of carbon dioxide at the higher flowrate. However, as the

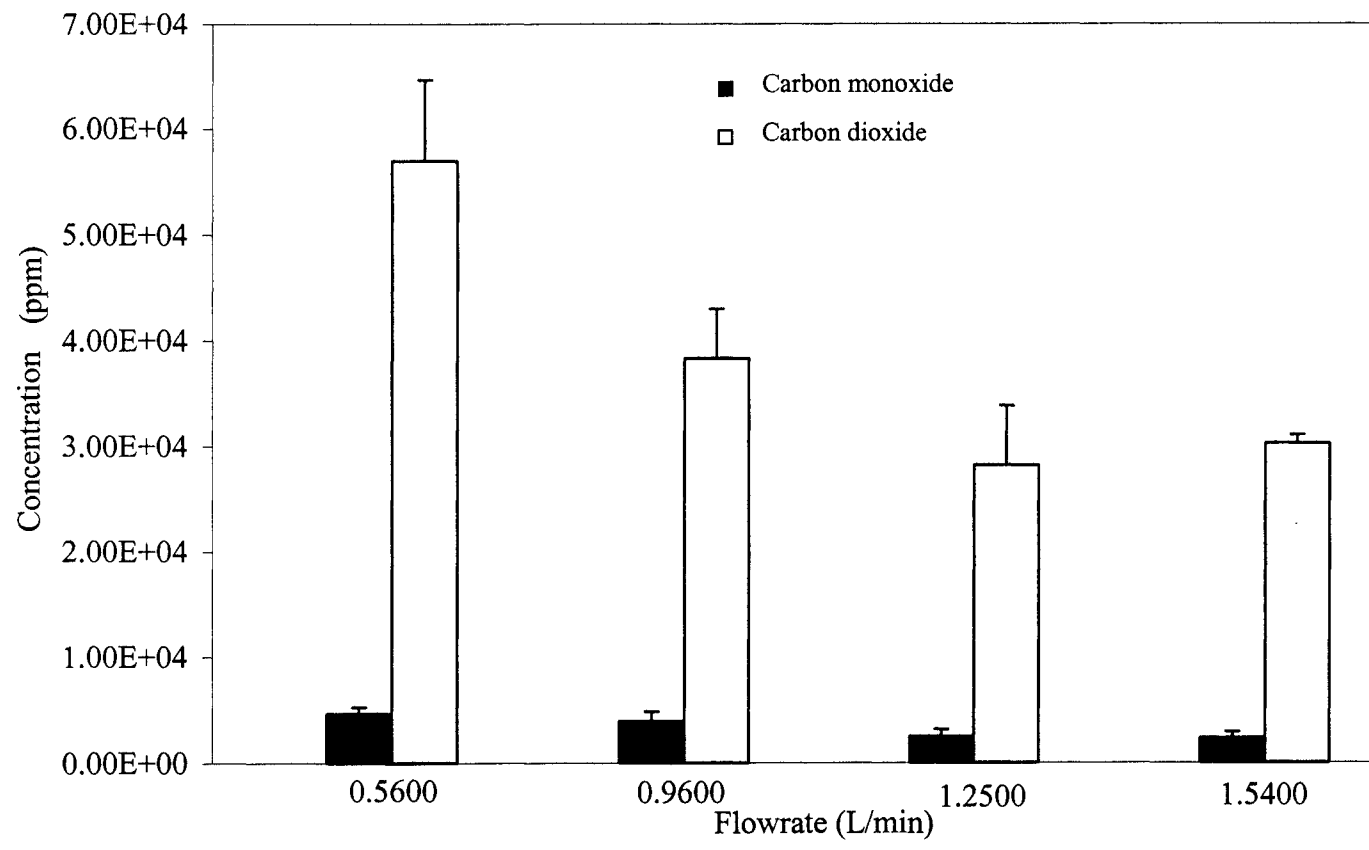


Figure 8. Plot of the concentrations of carbon monoxide and carbon dioxide in bidi smoke as a function of flowrate for a 6-second sampling period.

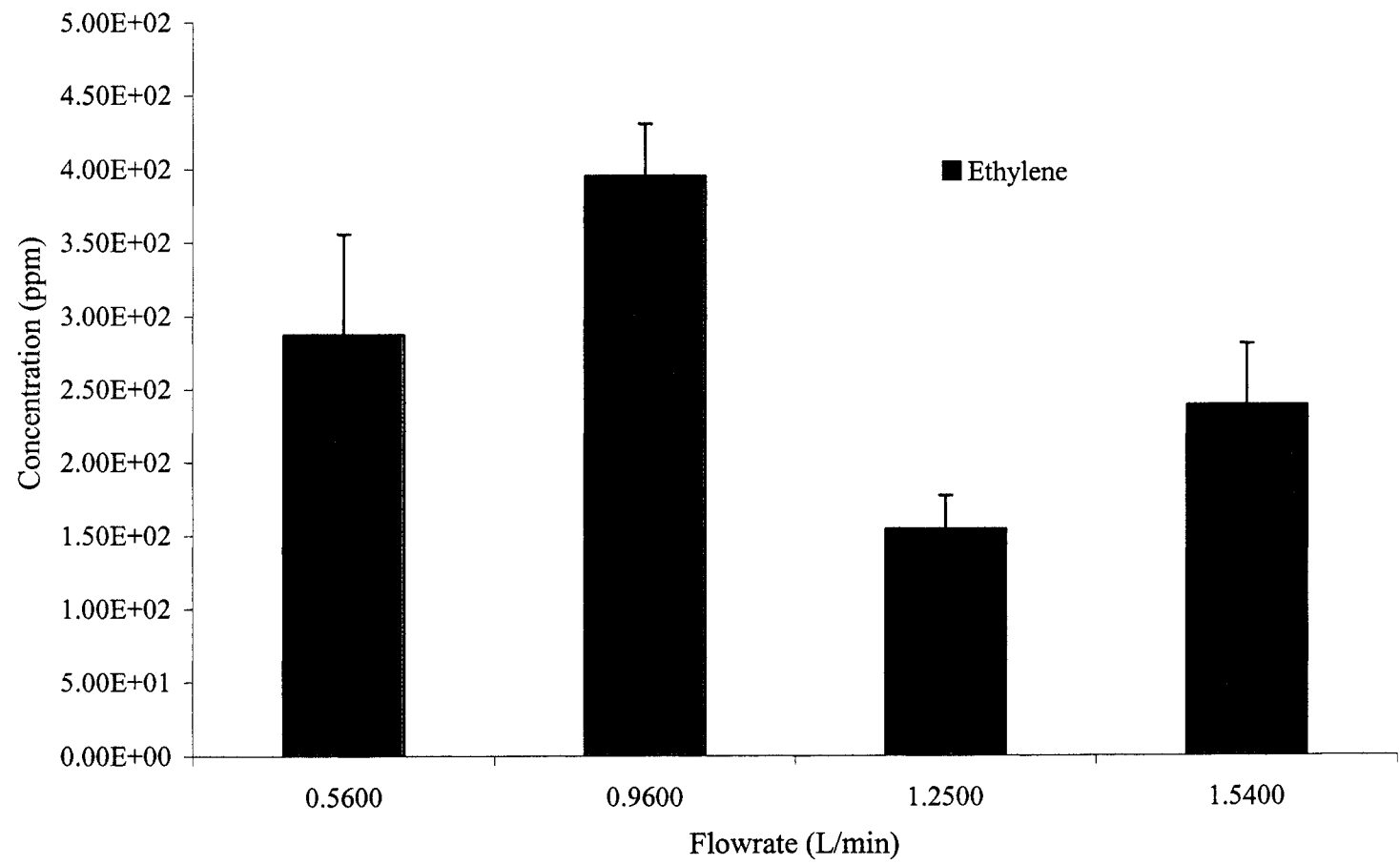


Figure 9. Plot of the concentrations of ethylene in bidi smoke as a function of flowrate for a 6-second sampling period.

sampling flowrate was increased from to 1.25 L/min to 1.54 L/min, the concentration of carbon dioxide increased slightly. This may be due to the increased rate of combustion that allowed a greater conversion of carbon monoxide to carbon dioxide. The greater availability of oxygen in the increased airflow allowed a more complete combustion process.

In Figure 9, the concentration of ethylene increased initially as the sampling flowrate increased before decreasing with increased flowrate from 0.96 L/min to 1.25 L/min. The concentrations of tobacco smoke constituents, such as ethylene, are generally determined by pyrolysis (i.e. thermal degradation of carbonaceous tobacco materials) that increases their concentrations and the combustion process that converts them to carbon monoxide and carbon dioxide. The initial increase was due to an increase in the extent of pyrolysis relative to combustion of tobacco and tendu leaf as a greater cigarette temperature was achieved via combustion with more oxygen. The pyrolysis reached a limiting rate at 0.96 L/min and, above this flowrate, a decrease in the concentration of ethylene was observed due to the increased dilution of the ethylene with air. The maximum level of ethylene was  $395 \pm 35$  ppm and it was obtained at a flowrate close to 0.96 L/min.

Figure 10 is a plot of the concentrations of methanol and methane in a six-second puff as a function of sampling flowrate. Both the concentrations of methane and methanol decreased with increased flowrates from 0.56 L/min to 1.25 L/min of the burning cigarette. Dilution effects up to a flowrate of 1.25 L/min could account for this trend. For methane, as the flowrate of 1.25 L/min was reached at a concentration of 889 ppm, there was an increase in methane concentration with increased flowrate

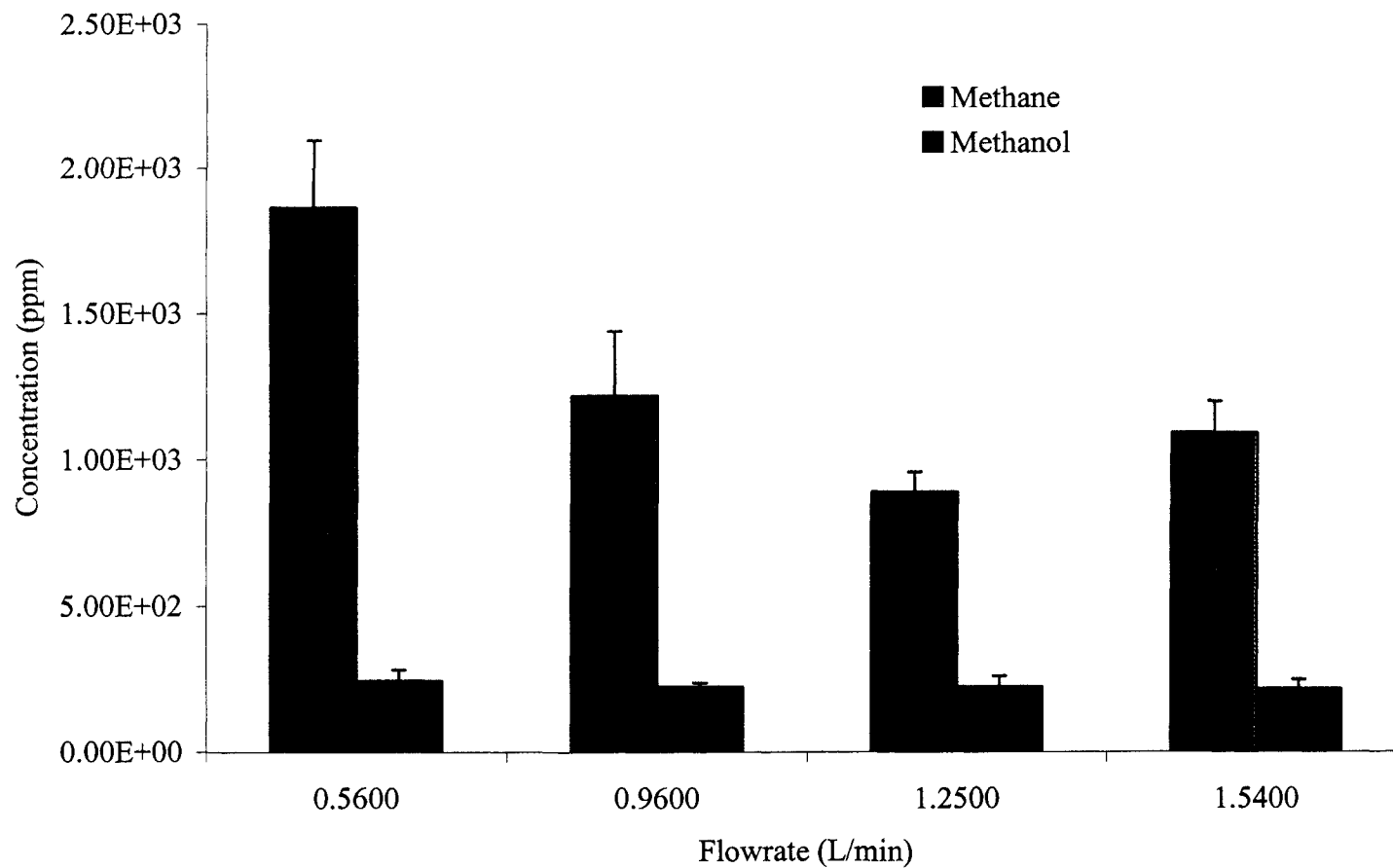


Figure 10. Plot of the concentrations of methanol and methane in bidi smoke as a function of flowrate for a 6-second sampling period.

afterwards. However, the concentration of methanol decreased further as the flowrate was increased from 1.25 L/min to 1.54 L/min.

Figure 11 shows that there were subtle differences in the signal dependence of the concentrations of ethane and ethylene on increasing flowrates of air through the burning cigarettes especially at 0.56 L/min and 0.96 L/min. These differences could be due to the fact that the concentration of ethane, which is formed by the combination of two methyl radicals via the combination of oxygen and methane,<sup>60</sup> is strongly influenced by the degree of dilution that determines the collisional frequency of these methyl radicals. Ethylene, on the other hand, follows the unimolecular decomposition kinetics of higher molecular weight hydrocarbons<sup>61,62</sup> and its abundance is mostly determined by the pyrolysis temperature.

The calculated relative standard deviations (RSD) for some of the compounds were observed to be higher than 20% and it is believed that the wrapping of the bidi cigarettes during sampling due to the cigarette shape could have allowed for some variations in measurement.

#### **Analysis of Asset-32 Sorbent Tube by GC-MS**

Figure 12 shows the chromatograms obtained by extracting the Asset-32 tube with petroleum ether and methanol. Some of the peaks that were identified when petroleum ether and methanol were used as the extraction solvent are presented in Table 6. Furfural was observed in the chromatograms obtained using either solvent, but the methanol extract gave a stronger peak for this compound compared to the petroleum ether extract. The match index was higher in methanol (MI = 91) than in petroleum ether

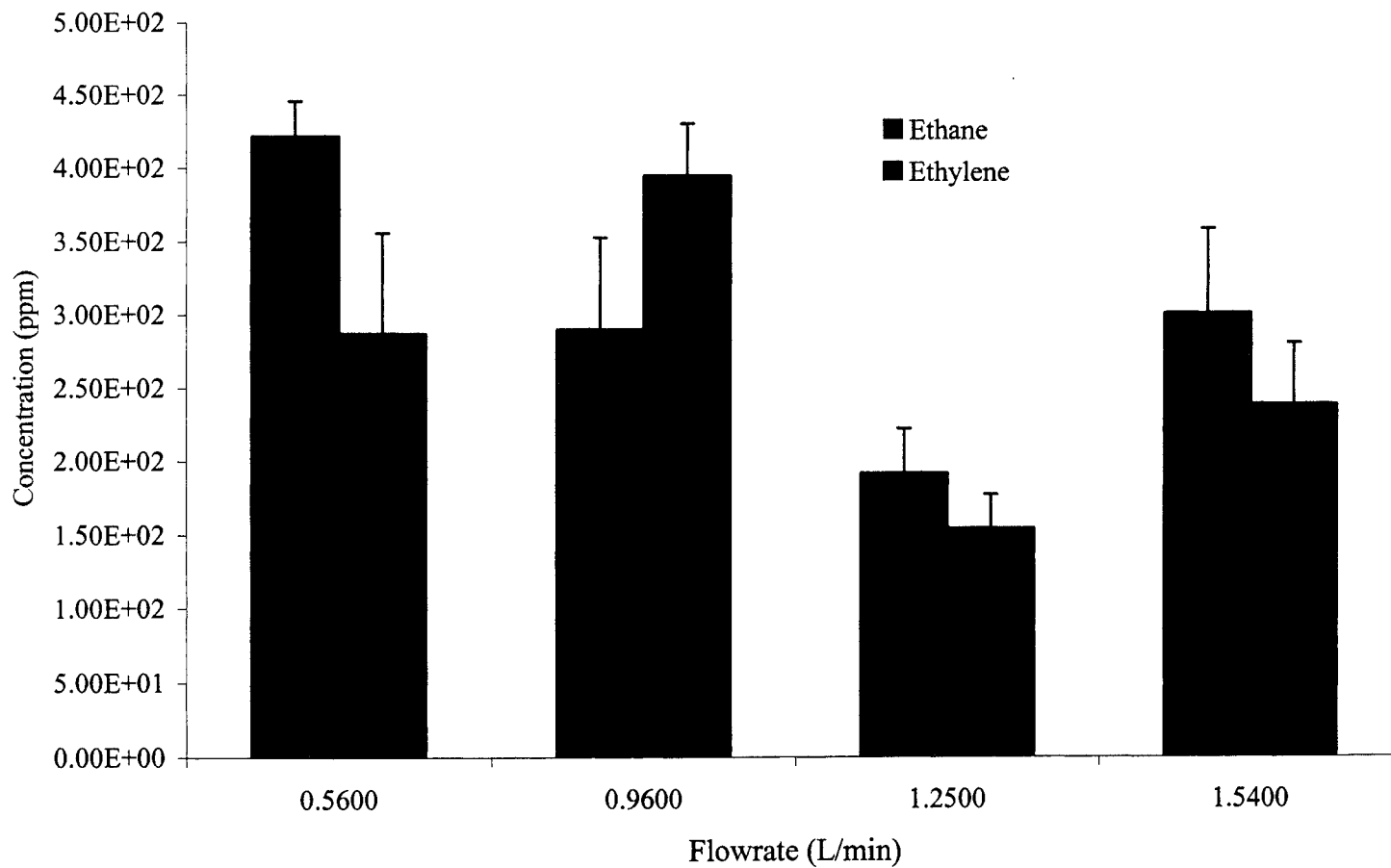


Figure 11. Plot of the concentrations of ethane and ethylene in bidi smoke as a function of flowrate for a 6-second sampling period.

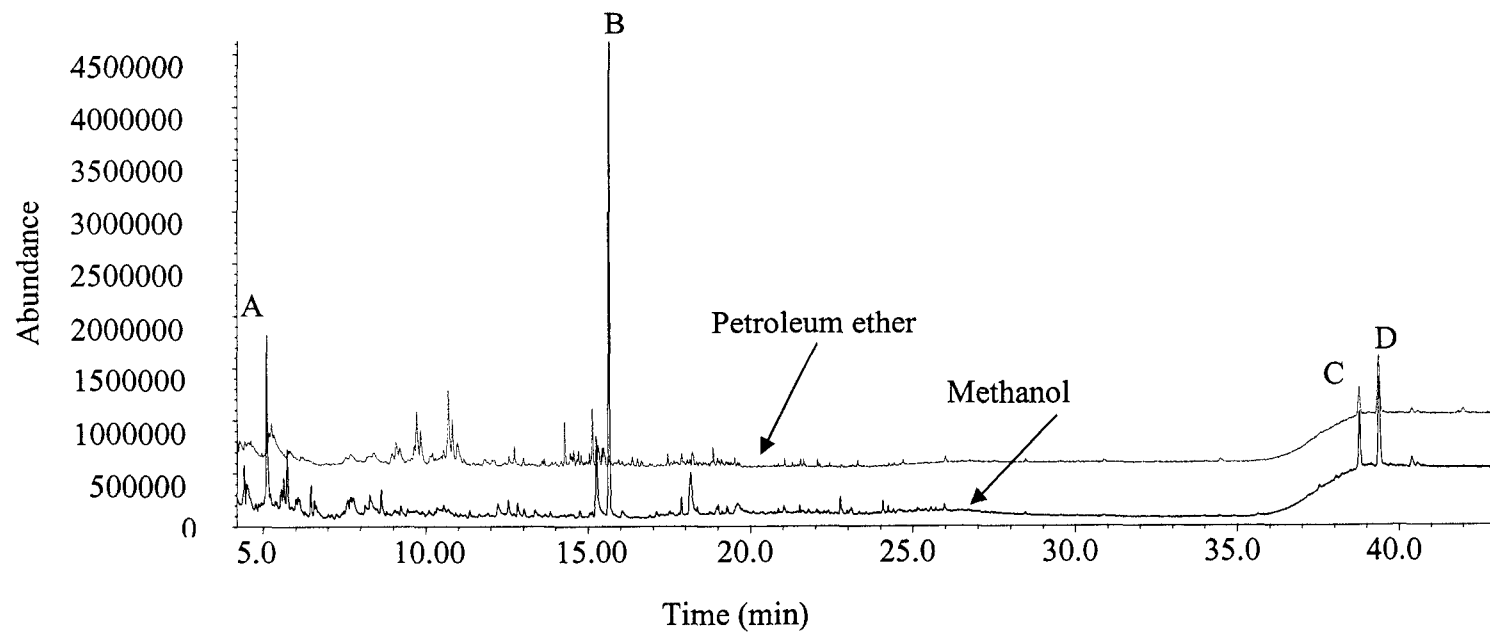


Figure 12. GC-MS chromatogram of bidi smoke sampled onto ASSET-32 activated charcoal sorbent tubes with subsequent extraction with petroleum ether and methanol. A is furfural, B is nicotine, C and D are alpha/beta amyrin.



Table 6. Compounds Identified by GC-MS Analysis of Petroleum ether and Methanol Extracts of Asset-32 Tube

<u>Petroleum Ether</u>		<u>Methanol</u>	
Name	Retention Time (min)	Name	Retention Time (min)
4-Methyloctane	5.13	2-Methylpyridine	4.87
1,2-Dimethylbenzene	5.26	2-Furanmethanol	5.54
Styrene	5.83	3-Methylpyridine	5.62
Dodecane	9.84	2-Methyl-2-cyclopenten-1-one	6.46
4-Methylundecane	10.97	Phenol	8.29
Tetradecane	12.71	Limonene	9.06
Hexadecane	14.28	2-Methylphenol	9.89
Octadecane	17.63	2-Methoxyphenol	10.30
1-Docosene	24.67	4-Methylphenol	10.35
Heptacosane	28.44	Cotinine	21.03

(MI = 76) due to co-elution with 3-furaldehyde and 1,4-dimethylpyrazole in the petroleum ether extract. Methanol was thus more suitable for the extraction and detection of different phenols and alkyl phenols, while the use of petroleum ether gave peaks that were mostly composed of hydrocarbons. Weaker peaks for the phenols were observed in the chromatogram when petroleum ether was used as the extraction solvent.

Furthermore, these phenol peaks generally had lower match indices compared to those from the methanol extracts, because of chromatographic co-elution with the less polar compounds that were extracted by petroleum ether. Due to the greater polarity of methanol relative to petroleum ether, methanol will be expected to be efficient for the extraction of the more polar compounds such as phenols, aromatic amines, and heterocyclic compounds from the ASSET-32 tube.

Figures 13 and 14 present the chromatograms obtained when strawberry bidi smoke and 1R5F reference cigarette smoke sampled onto ASSET-32 sorbent tubes were extracted with methanol and analyzed by GC-MS. From Figure 13, it can be seen that compounds such as furfural (RT = 5.09 min), 2-oxo-3-cyclopentene-1-acetaldehyde (RT=5.10 min), and 3-(1-methyl-1H-pyrrol-2-yl)pyridine (RT = 18.15 min) had a higher abundance in the bidi chromatogram compared to the reference cigarette chromatogram. Other compounds such as phenol and 2-ethyl-3-hydroxy-4H-pyran-4-one (RT = 12.84) were only observed in the bidi cigarette smoke spectra. However, 1-methyl-4-(1-methylethenyl)-cyclohexene (RT = 9.01), which is a terpenoid cigarette additive, was only identified in the chromatogram for the reference cigarette. From Figure 14, peaks corresponding to both alpha and beta amyrins were observed in bidi smoke extracted with either petroleum ether or methanol. On the contrary, there were no peaks corresponding

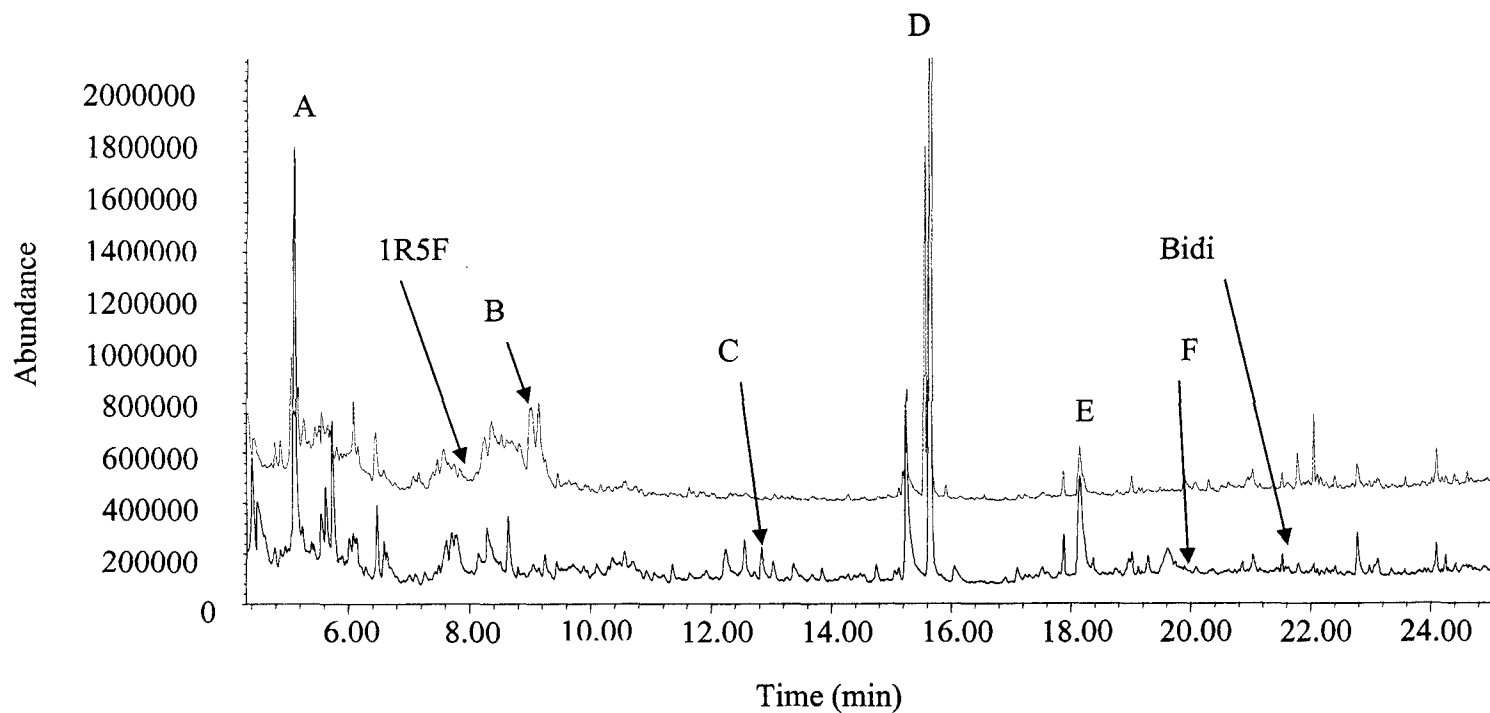


Figure 13. GC-MS chromatogram of strawberry bidi and 1R5F reference cigarette smokes sampled onto ASSET-32 sorbent tubes and extracted with methanol (early eluting compounds). A is furfural, B is limonene, C is 2-ethyl-3-hydroxy-4H-pyran-4-one, D is nicotine, E is 3-(1-methyl-1H-pyrrol-2-yl) pyridine, and F is 2,6-dimethoxy-4-(2-propenyl) phenol.

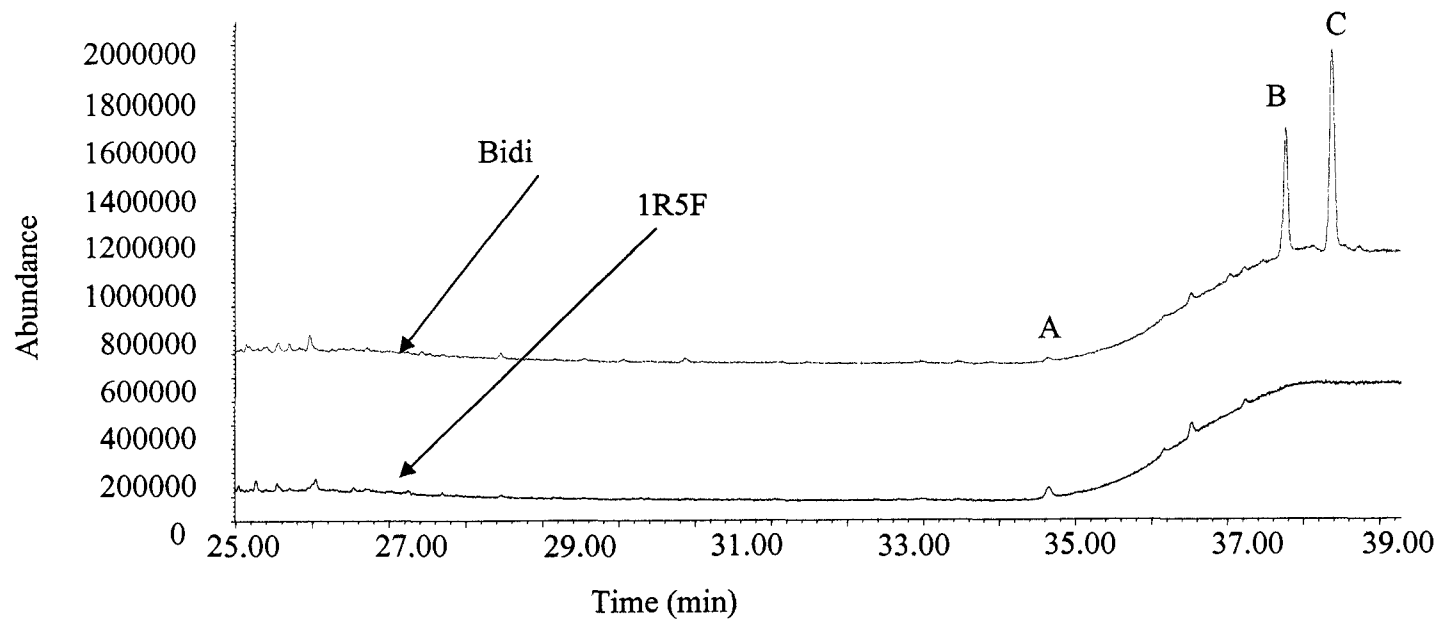


Figure 14. GC-MS chromatogram of strawberry bidi and 1R5F reference cigarette smokes sampled onto ASSET-32 sorbent tubes and subsequent extraction with methanol (late eluting compounds). A is Vitamin E, B and C are alpha/beta amyryns. No peaks corresponding to amyryns were observed in the reference cigarette smoke.

to amyrin compounds in the 1R5F reference cigarette smoke. It is believed that the amyryns have their source in the tendu leaf used to wrap the bidi tobacco, as it has been shown that both alpha and beta amyryns are present in the cuticles of leaves.<sup>63,64</sup>

Table 7 presents the comparative concentrations of 3-methyl pyridine, cotinine, nicotine, and styrene in the strawberry bidi smoke and in 1R5F cigarette smoke for a six-second puff sampling onto an ASSET-32 sorbent tube followed by extraction with methanol and GC-MS analysis. The concentrations of 3-methylpyridine, cotinine, and nicotine were higher in the bidi smoke than in the reference cigarette smoke.

Table 8 presents the concentrations and the relative standard deviation (RSD) of other polar compounds in bidi smoke obtained by the GC-MS analysis of the ASSET-32 sorbent tube extracted with methanol. The calibration curves of 2-methoxyphenol, cotinine, 4-methylphenol, and 2-methylphenol have correlation coefficients of 0.994, 0.999, 0.994 and 1.000, respectively. The RSD values were generally higher in the bidi cigarettes partly because of the variation of individual bidis that were produced in cottage industry with minimal manufacturing automation or process quality control.

#### **GC-MS Analysis of Fractionated Particulate Phase in Bidi Smoke**

The Sioutas impactor was used to fractionate the particulate phase of bidi smoke onto five different filters in the impactor according to the particle size at a flowrate of 9 L/min. The different filters have cut-off points at  $>2.5 \mu\text{m}$  (Fraction A),  $1.0\text{-}2.5 \mu\text{m}$  (Fraction B),  $0.50\text{-}1.0 \mu\text{m}$  (Fraction C),  $0.25\text{-}0.50 \mu\text{m}$  (Fraction D), and  $<0.25 \mu\text{m}$  (after Filter L). After sampling, the different filters were extracted with methylene chloride, using the ultrasonic bath, followed by the GC-MS analysis of the filter extract.

**Table 7. Comparison of Concentrations for Selected Compounds in the Mainstream Smoke of Strawberry Bidi and 1R5F Cigarettes that are Sampled onto ASSET-32 Tubes Followed by GC-MS Analysis of Their Methanol Extracts**

Compound	Strawberry bidi smoke		1R5F cigarette smoke	
	Concentration ( $\mu\text{g}/\text{puff}$ )	RSD	Concentration ( $\mu\text{g}/\text{puff}$ )	RSD
3-Methylpyridine	39.0	6.8	16.2	39.0
Styrene	7.2	16.6	10.8	3.0
Cotinine	10.0	36.4	5.5	10.3
Nicotine	496.8	37.8	146.1	1.3

Table 8. Concentrations of Other Selected Compounds in Strawberry Bidi Smoke Obtained by the GC-MS Analysis of Methanol Extracts of ASSET-32 Tubes for a 6-Second Puff Duration

Compound	Concentration ( $\mu\text{g}/\text{puff}$ )	RSD
Furfural	1.8	11.4
2-Furanmethanol	13.5	22.4
Phenol	38.5	6.8
2-Methylphenol	5.4	16.5
4-Methylphenol	15.1	9.5
2-Methoxyphenol	17.1	5.3
2-Methoxy-4-methylphenol	3.4	0.5

Nicotine was a major component observed in all size fractions. Figure 15 is a plot of the GC-MS analysis of nicotine in the mainstream smoke of grape bidi, vanilla bidi, and the 1R5F reference cigarette. The distribution of nicotine in the various size fractions for all three types of cigarettes was similar even though weights of the cigarettes were different. The average weight of each type of cigarette used in this work were vanilla bidi = 0.4995 g, grape bidi = 0.4670 g, and 1R5F reference cigarette = 0.8386 g. These values show that the bidi cigarettes have a higher nicotine content per unit weight of cigarette compared to the 1R5F reference cigarettes. The response was highest on the L filter, followed by the D filter, the A filter, the B filter, and, lastly, the C filter. Since nicotine is a semivolatile compound, its equilibrium state includes both the gaseous phase as well as the particulate phase as the mainstream smoke is being sampled by the impactor. From Figure 15, the amount of nicotine in the A filter and the last L filter was greater for the mainstream smoke of vanilla and grape bidi cigarettes compared to the ultra low yield 1R5F reference cigarette. This was consistent with earlier studies that reported bidi as containing higher nicotine content. Malson et al.<sup>53</sup> reported that the concentration of nicotine in the tobacco of bidi cigarettes was significantly higher than that in the conventional filtered cigarettes.

Nicotine can exist either in the protonated form, since it contains basic nitrogen atom, or it can exist in the non-protonated form which is referred to as the free base form.<sup>65,66</sup> When nicotine exists in the gaseous phase, it is primarily composed of the free base form, because it is the only form of nicotine that can volatilize.<sup>67</sup> The nicotine obtained in this work from the TPM portion of mainstream cigarette smoke was in the



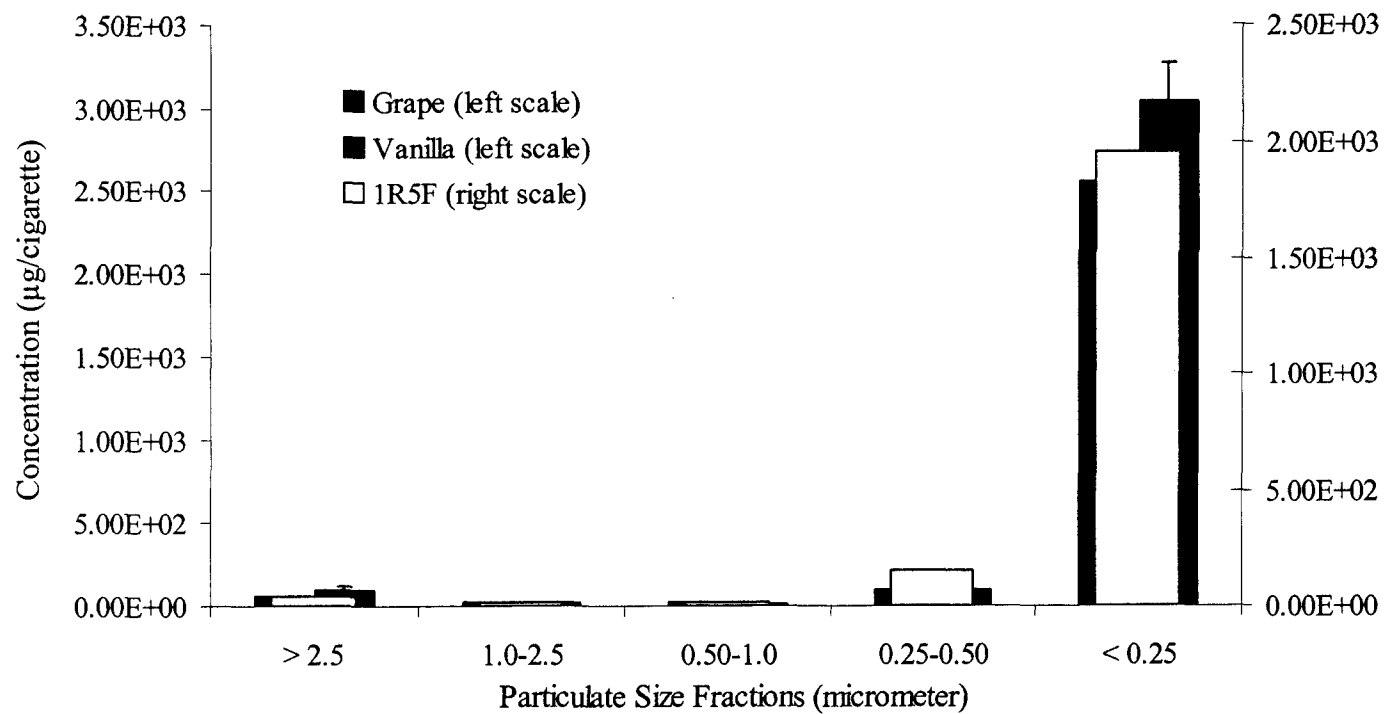


Figure 15. Plot of nicotine concentrations based on GC-MS analysis of different size fractions in the particulate phase of mainstream smokes of bidi cigarettes and 1R5F reference cigarette.

free base form, because the samples were extracted with methylene chloride, which is not known to extract protonated analytes.

Unlike the other compounds in this study, nicotine was present in all the four different filters as well as the after-filter. This is consistent with earlier evidence that nicotine is of primary importance in the particle-phase organic compounds present in cigarette smoke<sup>67</sup> and that a large amount of nicotine from cigarette smoke is in the particulate phase.<sup>66,67</sup> A large percentage of nicotine was present in size fractions of  $< 0.25 \mu\text{m}$ . These are very fine particles and, as such, they can get deposited in human lung tissues. The percent nicotine in the L filter for the three types of cigarettes are: grape bidi = 86.8%, vanilla bidi = 85.3%, and 1R5F reference cigarette = 81.5%. Therefore, the percent nicotine in the L filter for all the cigarettes was more than 80%. Nicotine delivery could occur via evaporative gas deposition (EGD) where nicotine vaporizes from the particulate phase into the gas phase and then gets deposited from the gaseous phase onto the human tissue. The remaining nicotine in the TPM could undergo delivery into the respiratory tract tissue via the particle deposition with evaporation (PDE)<sup>67</sup> mechanism in which the particle with nicotine deposits on the tissue first before vaporizing into the gas phase and then deposition from the gaseous phase. Another option would be nicotine delivery via the particle deposition with diffusion (PDD)<sup>67</sup> mechanism in the respiratory tract, which involves the nicotine from the cigarette smoke being deposited on the tissue as particle before diffusing further into the tissue.

GC-MS results of the impactor sampling showed different trends of particle size dependence for various compounds on the filters of the impactor. Figure 16 presents the chromatograms of particulate phase compounds, deposited on the L-filters, obtained

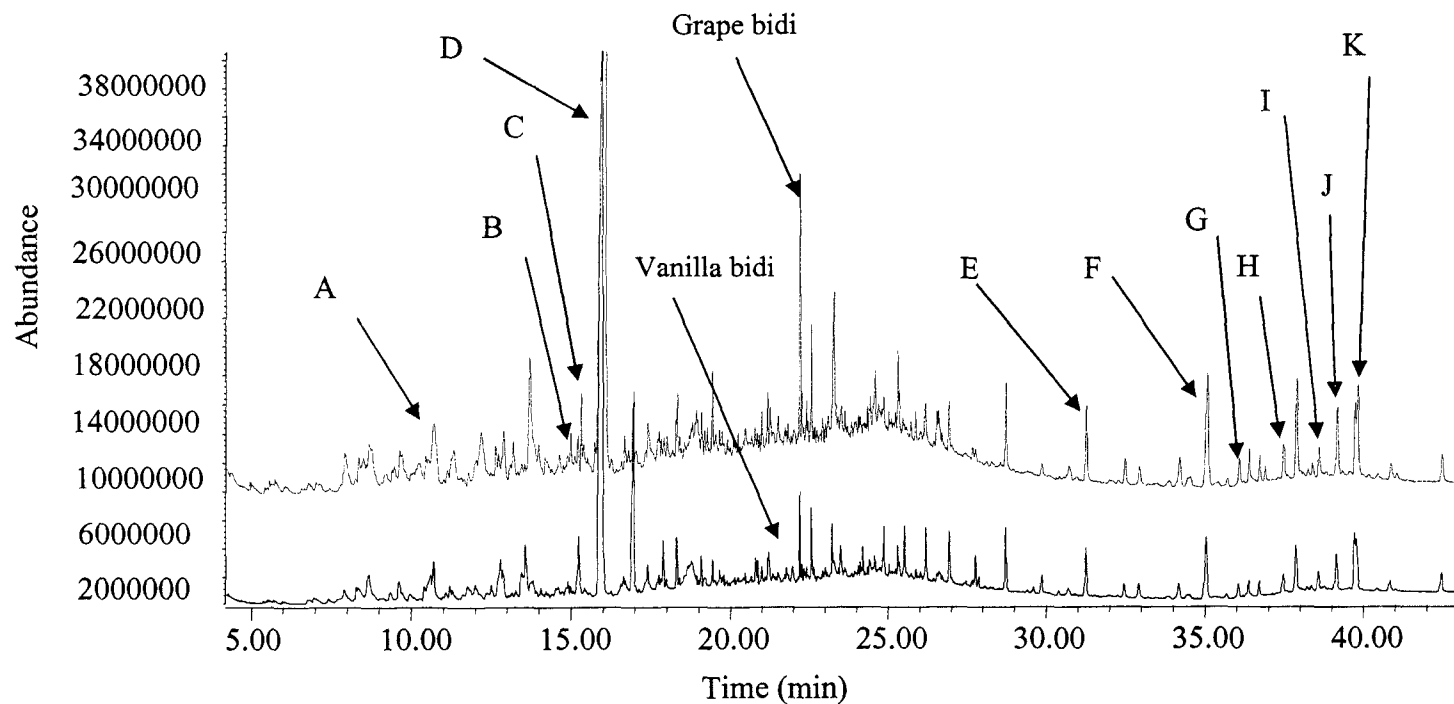


Figure 16. GC-MS chromatograms of the L-filter extracts of vanilla and grape bidi smokes sampled using the Sioutas impactor.

A is 4-methylphenol, B is indole, C is 2-methoxy-4-vinylphenol, D is nicotine, E is tetracosane, F is nonacosane, G is vitamin E, H is campesterol, I is gamma sitosterol, J/K are beta and alpha amyryn.

during the sampling of vanilla and grape bidi smokes using the impactor. The compounds identified by searching the NIST spectral library include 4-methylphenol, indole, 2-methoxy-4-vinylphenol, nicotine, tetracosane, nonacosane, vitamin E, campesterol, alpha amyirin, and beta amyirin. The figure shows that the two types of bidi smoke yielded similar compounds in their chromatograms. These data support earlier work done and reported by Watson et al.<sup>28</sup> on the determination of tar, nicotine, and carbon monoxide in bidi cigarette smoke in twenty-one brands of flavored unfiltered and filtered bidi cigarettes. The various flavored cigarettes tested by Watson et al. include chocolate, lime, raspberry, mango, cherry, grape, and vanilla flavors. It was concluded that there was no statistically significant difference in the concentrations of tar, nicotine, and carbon monoxide present in the mainstream smokes of the different types of bidi cigarettes.

Figures 17 and 18 are plots of the concentrations of selected compounds obtained by GC-MS analysis of the impactor filters for the cigarette smokes of vanilla bidi and grape bidi. Both 2-methylphenol and 4-methylphenol were found in higher levels in the L filter ( $< 0.25 \mu\text{m}$ ) than the A filter ( $> 2.5 \mu\text{m}$ ) in the grape and vanilla bidi smokes. However, the methylphenols were absent in the D filter ( $0.25 - 0.50 \mu\text{m}$ ) for the vanilla bidi. In the case of phytol and 2,3-dipyridyl, the distribution of these compounds in the filters followed the relationship that  $[L] > [D]$ . The presence of the aforementioned compounds in the smallest size fraction ( $< 0.25 \mu\text{m}$ ) makes them potentially very harmful to human health, as they can penetrate alveoli in the lungs.<sup>67</sup>

Figure 18 presents a plot of the concentration of 2-methylphenol, 4-methylphenol, myosmine, and 3-methyl-1H-indole in grape bidi smoke. These compounds were present

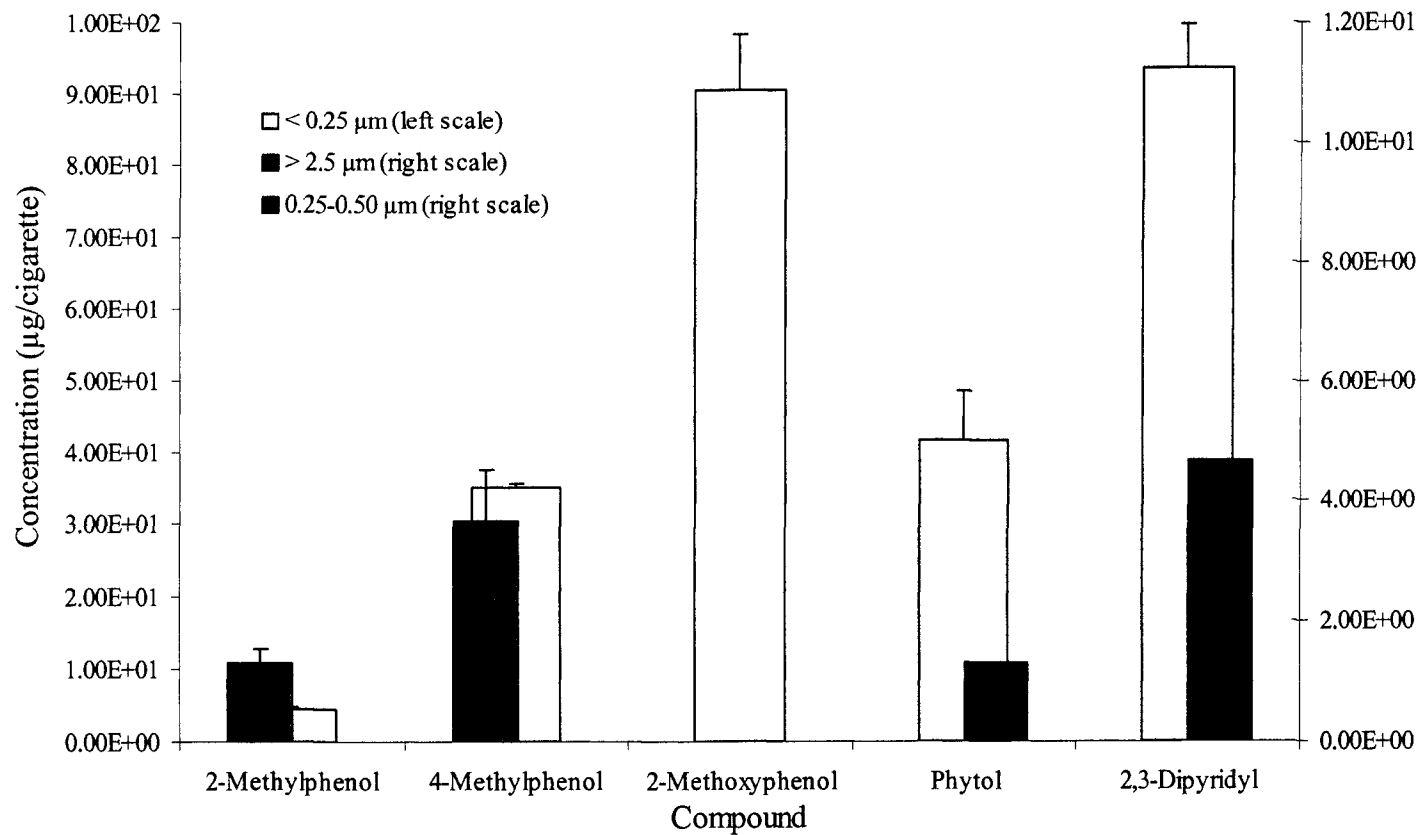


Figure 17. Plot of concentrations of selected compounds based on GC-MS analysis of different size fractions in the particulate phase of mainstream smoke of vanilla bidi.

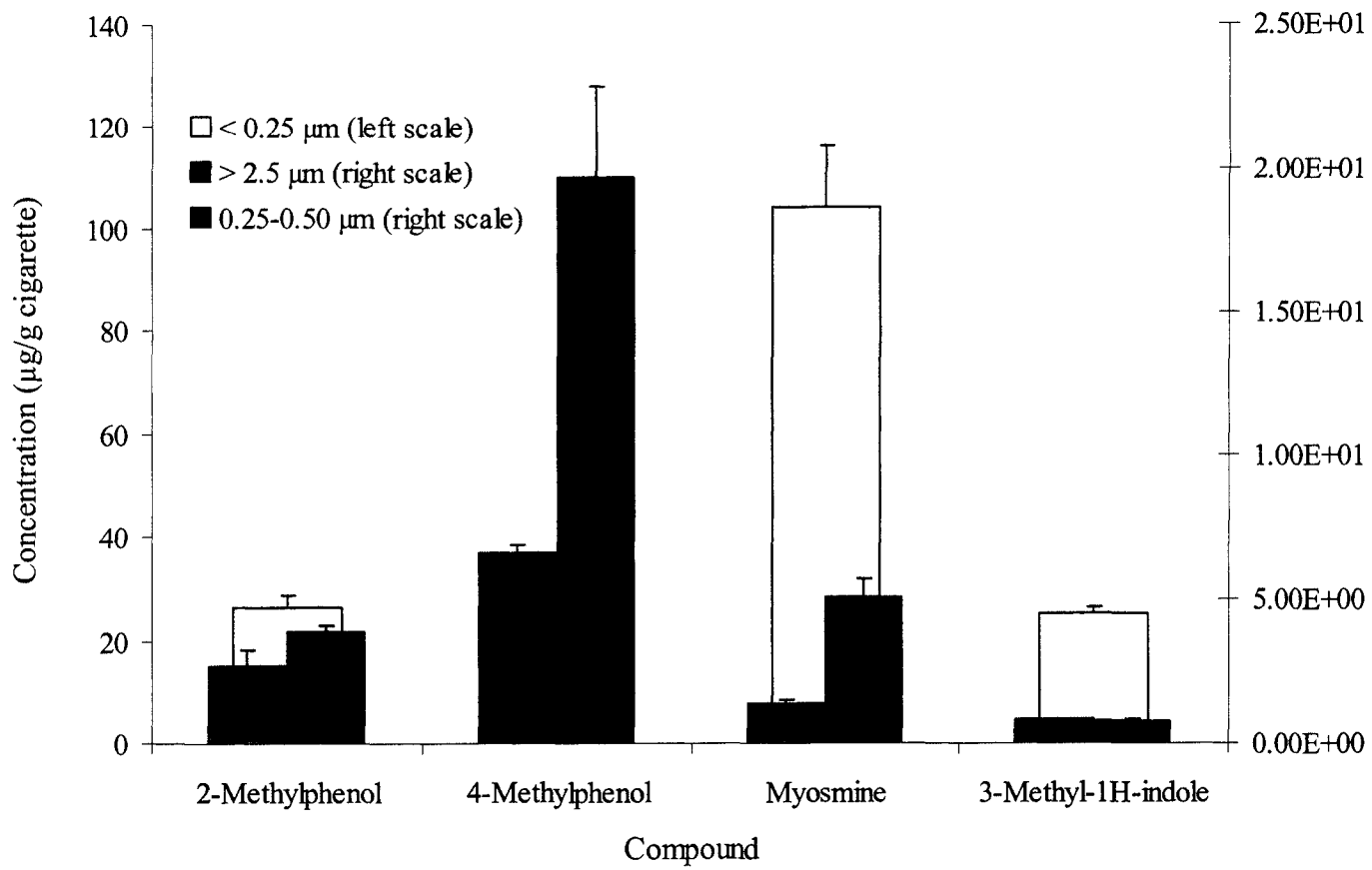


Figure 18. Plot of the concentrations of selected compounds based on GC-MS analysis of different size fractions in the particulate phase of mainstream smoke of grape bidi.

in the A, D, and L filters where their concentrations were found to be higher in smaller particle size fractions. These results followed the same trends for 2-methylphenol and 4-methylphenol in vanilla bidi. Neither of these compounds were detected in the B and C filters for particles with size fractions between 1.0 - 2.5  $\mu\text{m}$  and 0.50 - 1.0  $\mu\text{m}$ , respectively, and, as such, these filters were not represented on the plots.

Figure 19 is a plot of cotinine, vitamin E, alpha amyryn, and beta amyryn in the particulate phase of vanilla and grape bidi smoke. Cotinine and vitamin E were present in the D and L filters in both types of bidi cigarette, while both alpha and beta amyryns were observed in the A and L filters. The GC-MS analysis of the particulate phase of bidi mainstream smoke showed that all compounds studied were generally present at the highest levels in the after-filter with particle size smaller than 0.25 $\mu\text{m}$  (i.e. L filter). Relative to the phenols, the amines constitute about 60% of the particulates in the L filter. The small particle size also enhances the delivery of these particulates through human body tissues.

The concentrations and the standard deviation of selected constituents of vanilla and grape bidi mainstream smoke from GC-MS analysis of the impactor filter extracts are presented in Table 9. The concentrations of vitamin E and cotinine were higher in the L filter of the grape bidi smoke compared to those in vanilla bidi smoke. The concentration of cotinine in the L filter was the highest among the values tabulated.

Table 10 provides information about the vapor pressure and Henry's Law constants of selected compounds present in bidi smoke as well as the calculated ratio of their amounts in the gaseous phase to their concentrations in the particulate phase.

Concentrations obtained from the ASSET-32 tube analysis were used for the gaseous

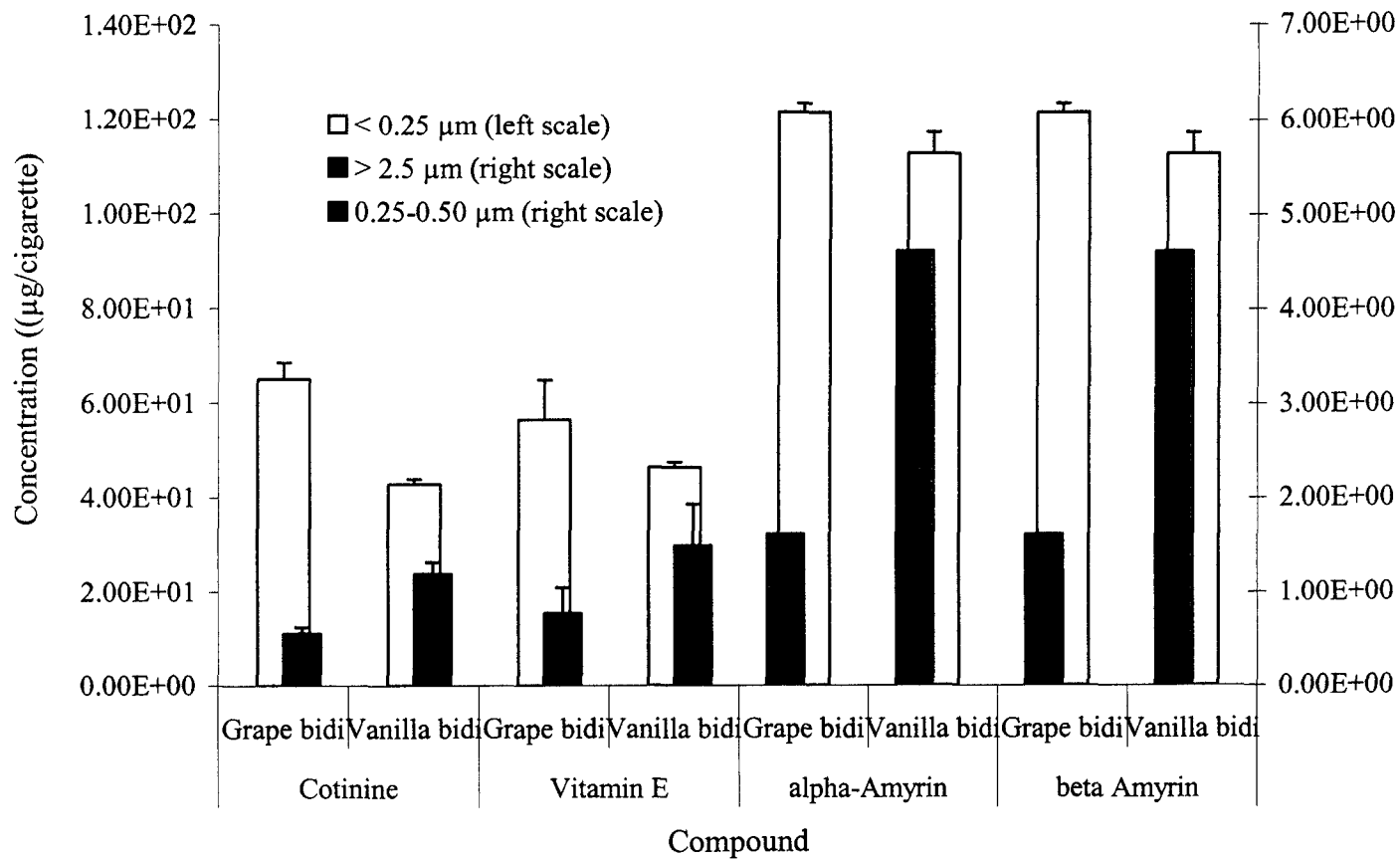


Figure 19. Plot of selected constituents of vanilla and grape bidi smoke concentrations obtained by GC-MS analysis.



Table 9. Concentrations of Selected Constituents of Bidi Mainstream Smoke Based on GC-MS Analysis of Filter Extract from the Sioutas Impactor

Compound Name	<u>Vanilla bidi (ppm)</u>			<u>Grape bidi (ppm)</u>		
	> 2.5 $\mu\text{m}$	0.25 - 0.50 $\mu\text{m}$	< 0.25 $\mu\text{m}$	> 2.5 $\mu\text{m}$	0.25 - 0.50 $\mu\text{m}$	< 0.25 $\mu\text{m}$
Indole	< MDL	< MDL	4.9 $\pm$ 0.44	< MDL	< MDL	25.8 $\pm$ 4.94
2-methylphenol	0.7 $\pm$ 0.22	< MDL	2.2 $\pm$ 0.26	1.4 $\pm$ 0.52	2.0 $\pm$ 0.17	13.3 $\pm$ 2.24
4-methylphenol	< MDL	< MDL	17.5 $\pm$ 0.58	3.3 $\pm$ 0.28	9.8 $\pm$ 3.14	15.9 $\pm$ 1.41
Vitamin E	< MDL	< MDL	23.2 $\pm$ 1.07	< MDL	< MDL	28.3 $\pm$ 8.38
2-methoxyphenol	< MDL	< MDL	45.3 $\pm$ 7.63	1.2 $\pm$ 0.35	< MDL	< MDL
Cotinine	< MDL	0.6 $\pm$ 0.12	21.5 $\pm$ 1.05	0.3 $\pm$ 0.06	< MDL	32.6 $\pm$ 3.49

< MDL: Less than minimum detection limits.

Table 10. Physical Properties and Concentrations of Selected Constituents of Bidi Smoke

Compound Name	Vapor pressure (mm Hg)	Solubility in water (g/L)	Henry's constant (atm·m <sup>3</sup> /mole)	Gas Phase concentration (ppm)	TPM concentration Σ A-L (ppm)	Ratio Gas:TPM
Cotinine	0.0000855		$3.33 \times 10^{-12}$	10.0	22.1	0.452
2-methoxyphenol	0.103	18.7	$1.20 \times 10^{-6}$	17.1	45.3	0.377
2-methylphenol	0.299	31.8	$1.20 \times 10^{-6}$	5.4	2.9	1.852
4-methylphenol	0.110	23.1	$1.00 \times 10^{-6}$	15.1	17.5	0.863

phase values while the sum of the respective compounds in the A-L filter of the impactor analysis was used for the values of the TPM concentration. Based on the tabulated vapor pressures of 2-methylphenol, 4-methylphenol, and 2-methoxyphenol, a trend was observed showing the decreasing proportion of the TPM phase as the vapor pressure of a compound increases. This is consistent with the fact that as the vapor pressure of a compound increases more of this compound will be present in the gas phase relative to the particulate phase. For polar compounds such as 2-methylphenol and 4-methylphenol, their relatively higher aqueous solubility values implies that they are more likely to be absorbed into the respiratory tract of humans. Furthermore, the higher solubility of 4-methylphenol (23.1 g/L) and 2-methylphenol (31.8 g/L) also accounts for their presence at higher levels in the A and D filters relative to less soluble compounds since the larger particles may result from the agglomeration of aqueous aerosol particles in the mainstream smoke.

A closer look at the GC-MS chromatograms obtained for the L filters of both types of bidi cigarettes revealed a list of compounds present only in the L-filter of grape bidi smoke. These are presented in Table 11. Other compounds that were detected only in the L- filter include styrene and naphthalene, which have relatively higher Henry's constants of  $2.82 \times 10^{-3} \text{ atm}\cdot\text{m}^3/\text{mol}$  and  $4.24 \times 10^{-4} \text{ atm}\cdot\text{m}^3/\text{mol}$ , respectively. This indicated that styrene and naphthalene will be present at higher levels in the gas phase than in the aerosol particle. The low aqueous solubility values of styrene and naphthalene also accounts for their presence in the L-filter with smallest particle size fraction because they do not partition readily into the larger aqueous aerosol particles. Other toxicants detected in the bidi smoke but for which definite trends were not

Table 11. Constituents Present only in the L-filter of Grape Bidi Smoke as Determined by GC-MS

Compound	Retention Time (min)	Match Index
2-Methyl, 2-cyclopenten-1-one	6.54	90
Megastigmatrieneone	20.25	93
Campesterol	37.46	95
22,23-Dihydro, stigmasterol	38.59	99

observed from one filter to another include: 3-pyridinol, benzyl nitrile, squalene, 1,2-benzenediol, 2-furanmethanol, and maltol.

### LC-MS Analysis of Carbonyl-DNPH Derivatives

A standard mixture of thirteen carbonyl-DNPH derivatives was used to prepare standard solutions of various concentrations ranging from 0.25 ppm to 8 ppm. These standards were analyzed by GC-MS as well as LC-MS. Figure 20 presents the chromatograms based on the diode array detector signals at 360 nm and 380 nm and the APCI signal of the mass spectrometer for the 0.25 ppm standard solution. From the figure, it is observed that all thirteen carbonyl-DNPH derivatives were detected at this concentration. However, for the GC-MS analysis of the standard mixture, no peak corresponding to *m*-tolualdehyde was observed. Comparing the separation obtained in this work to that in Grosjean et al.'s,<sup>36</sup> the chromatographic conditions employed in this work were capable of separating 2-butanone and methacrolein DNPH derivatives, which were not separated in Grosjean et al.'s work.

The smoke samples of the 1R5F reference cigarette and vanilla bidi were collected onto DNPH cartridge with the internal standards acetaldehyde-d<sub>4</sub>-DNPH and phenylacetaldehyde-DNPH followed by analysis on the LC-MS.

Table 12 presents the concentrations of various carbonyl compounds detected and quantitated in the mainstream smokes of the 1R5F reference and bidi cigarette and Figure 21 presents the LC-MS chromatogram of the reference cigarette and the bidi cigarette.

The peaks of both the labeled and the unlabeled acetaldehyde-DNPH derivatives both coeluted at 7.49 min as expected for this type of analysis. The left side of the peak

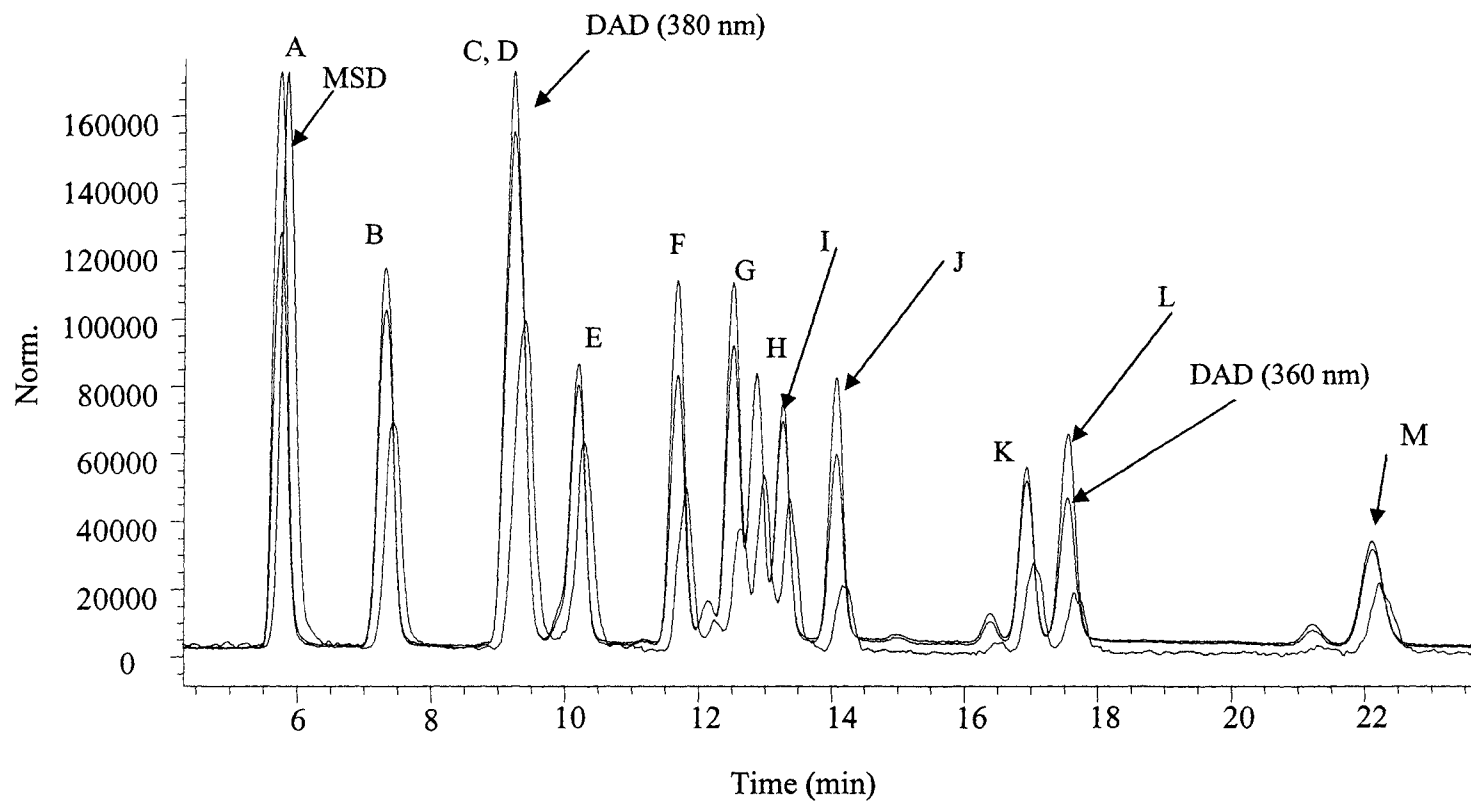


Figure 20. LC-MS chromatograms of the MS and the diode array detectors (DAD) signal at 380 nm and at 360 nm for a 0.25 ppm standard mixture of carbonyl- DNPH derivatives. A is formaldehyde, B is acetaldehyde, C and D are acrolein and acetone, E is propanal, F is crotonaldehyde, G is methacrolein, H is 2-butanone, I is butanal, J is benzaldehyde, K is pentanal, L is m-tolualdehyde, and M is hexanal.

Table 12. Concentrations of Carbonyl Compounds Detected in Reference and Bidi Cigarette Smokes

Compound Name	Base Peak	Retention Time for Stds. (min)	<u>Concentration</u>			
			<u>1R5F</u>		<u>Vanilla Bidi</u>	
			$\mu\text{g/cigarette}$	RSD (%)	$\mu\text{g/cigarette}$	RSD (%)
Formaldehyde	209	5.85	0.7	23.9	0.5	5.1
Acetaldehyde	223	7.51	258.0	9.2	263.0	2.4
Acrolein	235	9.33	9.1	2.6	16.1	2.5
Acetone	237	9.48	4.3	3.0	8.5	1.8
Propanal	237	10.34	34.6	5.4	72.8	1.8
Crotonaldehyde	249	11.83	1.8	14.2	0.8	7.8
Methacrolein	249	12.66	0.3	13.2	0.3	13.4
2-Butanone	251	13.00	0.8	28.9	1.0	5.5
Butanal	251	13.49	9.1	15.8	13.0	4.3

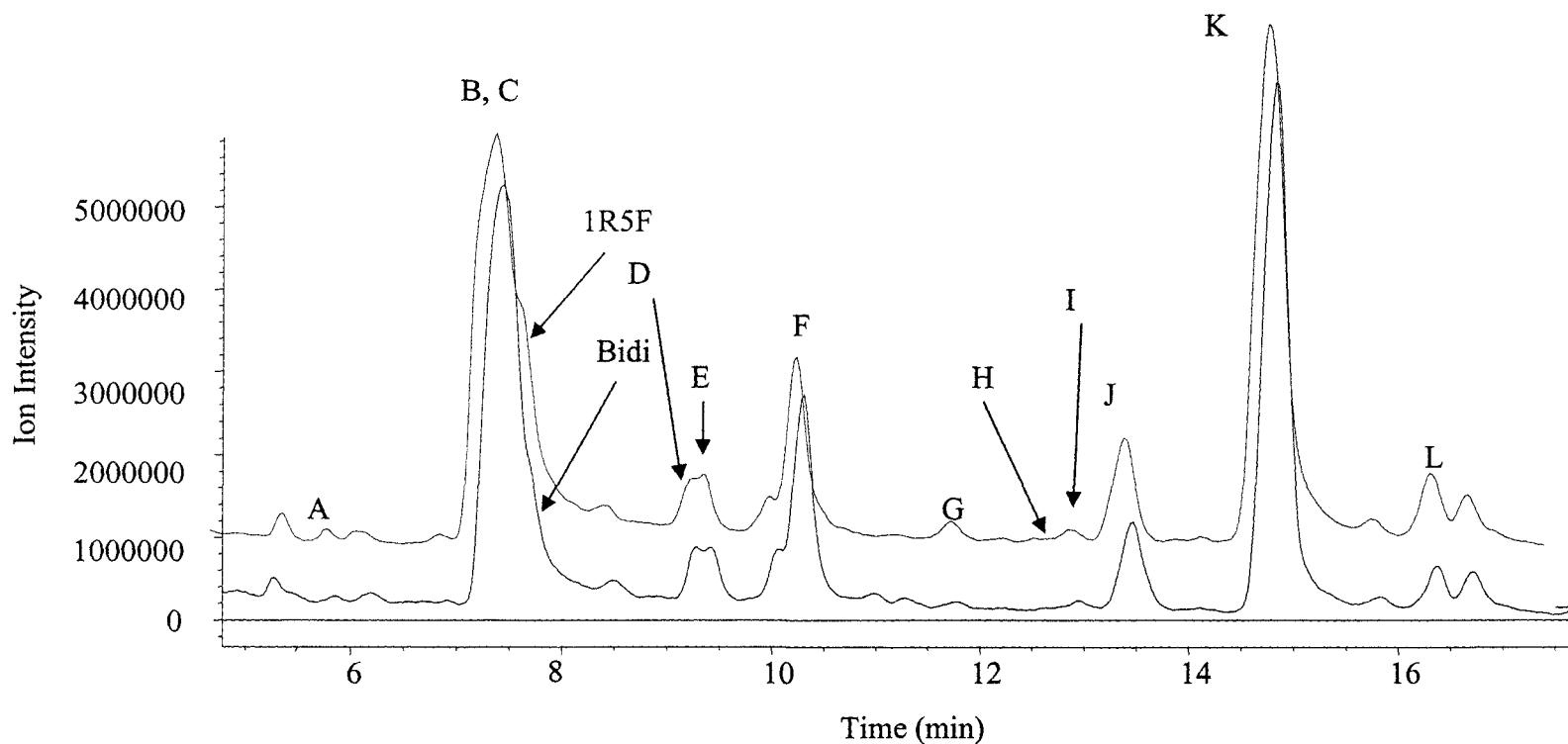


Figure 21. LC-MS chromatogram of the DNPH derivatives in reference 1R5F and vanilla bidi cigarettes. A is formaldehyde, B is acetaldehyde, C is acetaldehyde-d<sub>4</sub>, D is acrolein, E is acetone, F is propanal, G is crotonaldehyde, H is methacrolein, I is 2-butanone, J is butanal, K is phenylacetaldehyde and L is pentanal.



had more of the labeled derivative with  $m/z$  227, while the right side of the peak had more of the unlabeled derivative with  $m/z$  224. Acetaldehyde- $d_4$  is 4 mass units greater than the unlabeled acetaldehyde derivative obtained from the cigarette smoke. The two acetaldehyde derivatives were resolved for analysis by using their extracted ion chromatograms. Figure 22 presents the ratio of the labeled and unlabeled derivatives as they occurred in the peaks with  $m/z$  values of 223 and 227.

For quantitation, the response factors (RF) for both internal standards were calculated for each compound in the cigarette smoke. Phenyl acetaldehyde had a base peak of 299 at 14.87 min. The results presented in Table 12 show that most of these carbonyl compounds were present in higher concentrations in the mainstream smoke of bidi cigarette compared to the 1R5F reference cigarette except for formaldehyde, crotonaldehyde, and methacrolein. Also, acetaldehyde had the highest concentration in the cigarette smoke.

Comparing the results obtained in this work to those obtained in the literature,<sup>59</sup> the concentration of acrolein in the 1R5F reference cigarette was given as 12.6  $\mu\text{g}/\text{cigarette}$  and the concentration obtained in this work for acrolein is 9.05  $\mu\text{g}/\text{cigarette}$ . Also, the concentration reported for butanal - DNPH derivative was 9.57  $\mu\text{g}/\text{cigarette}$ , while the concentration obtained for this compound in this work was 9.06  $\mu\text{g}/\text{cigarette}$ . This is a good agreement between the two values. There is not much work reported in the literature on the quantitation of carbonyl compounds in tobacco smoke.

It has been reported that both the *syn*- and the *anti*- geometrical isomers of certain carbonyl-2,4-dinitrophenylhydrazones are produced upon the addition of an acid catalyst,

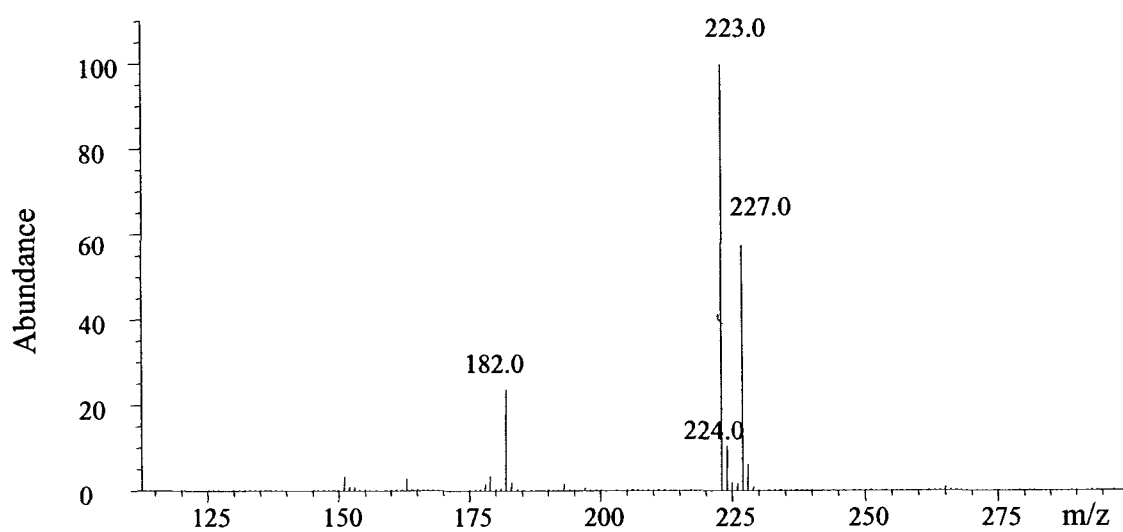


Figure 22. Mass spectrum of the base peak at 7.49 min containing both the unlabeled and labeled acetaldehyde-DNPH in bidi smoke by LC-MS.

according to a paper describing the HPLC analysis of carbonyl derivatives.<sup>30</sup> GC-MS analysis of these derivatives also yield both the syn and the anti isomers.<sup>46</sup> However, in this work, even though commercially available DNPH cartridges containing silica gel coated with acidified 2,4-dinitrophenylhydrazine was used for sampling carbonyl compounds present in cigarette smoke for LC-MS analysis, only one peak was detected for each aldehyde-DNPH derivative. GC-MS analysis of these carbonyl compounds sampled in this manner produced two peaks. Isomers of these derivatives were not detected in the LC-MS analysis because, unlike the Bonus-RP column used in Uchiyama et al.'s<sup>30</sup> work, the use of the Synergy MAX RP column did not resolve the geometrical isomers and hence yielded a composite DAD spectrum of the two isomers.

Taking a closer look at the LC-MS data obtained for the vanilla bidi, grape bidi, and the 1R5F reference cigarettes, the signal at 1.908 min gave characteristic nicotine mass fragments with  $m/z$  values of 131 and 161 on the MSD along with a maximum UV-absorption wavelength of about 260 nm on the DAD. For both the vanilla and grape bidi cigarettes, the extracted ions at  $m/z$  values of 131 and 161, were stronger than those of the 1R5F reference cigarette. This was also the trend on the GC-MS analysis. However, the nicotine signal was observed as a “shoulder” of a more prominent co-eluting peak at 1.908 min.

## **CHAPTER 4**

### **CONCLUSIONS**

Bidi cigarettes have become increasingly popular among teenagers in different parts of the world including the U.S. Due to the use of different flavoring additives that have not been characterized toxicologically, more research on the characterization of bidi mainstream smoke constituents is necessary. This work was aimed at studying the chemical composition of bidi mainstream smoke via the development of analytical techniques based on the FTIR, GC-MS and LC-MS. A combination of different analytical techniques is required to characterize different classes of compounds present in bidi smoke.

FTIR was used to identify and characterize hydrocarbons and inorganic gases present in bidi mainstream smoke; namely carbon monoxide, hydrogen cyanide, ethylene, ethane, methane, carbon dioxide, and 1,3-butadiene. A procedure with a short sampling duration of six seconds for each simulated puff of bidi smoke was developed for sampling these compounds into an extractive gas cell for FTIR analysis. Since the gaseous components were sampled directly into the gas cell, the analysis by FTIR was performed shortly after sampling to prevent the loss of volatile analytes. The FTIR results showed that the concentration of carbon monoxide in the bidi smoke decreased with the increase in the sampling flowrate. Also, the concentration of hydrogen cyanide (which is considered a toxicant in cigarette smoke) in bidi cigarette was found to be 310

$\mu\text{g}/\text{cigarette}$ , which is consistent with an earlier study that reported a concentration of 301  $\mu\text{g}/\text{cigarette}$ <sup>26</sup> for hydrogen cyanide in bidi cigarettes.

GC-MS was used to analyze the constituents of the mainstream smoke of bidi and 1R5F reference cigarettes sampled onto the ASSET-32 activated charcoal sorbent tube. The results show that the use of petroleum ether is a suitable extraction solvent for the extraction of hydrocarbons, while the more polar solvent methanol is more efficient for the detection of various phenols and alkyl phenols, some of which were quantitated. Compared to the mainstream smoke of the reference 1R5F cigarettes, bidi cigarettes contain a higher concentration of 3-methylpyridine, cotinine, and nicotine when methanol is used as the extraction solvent. Also, bidi smoke extracted with both petroleum ether and methanol contain alpha and beta amyryns, which are absent in the reference cigarette smoke. Since these compounds have been identified in the cuticle of leaves,<sup>63,64</sup> their presence in bidi smoke may have originated from the tendu leaf used to wrap the bidi cigarettes.

The TPM phase of the cigarette smoke was sampled with filter pads using the Sioutas impactor. The impactor was used to separate the TPM phase of bidi smoke onto different filters based on their size distribution. GC-MS analysis of the extracts show that nicotine was the only compound observed consistently in all filters for both bidi smoke and 1R5F reference smoke. Comparison between the concentration of nicotine obtained in bidi smoke and that obtained in the reference cigarette smoke indicates that the mainstream smoke of bidis contains a higher concentration of nicotine. This is in agreement with earlier studies that reported bidi smoke as containing a higher concentration of nicotine than other conventional cigarettes or other low tar Indian

cigarettes.<sup>53,54</sup> This is in spite of the fact that bidis contain a smaller amount of tobacco than other conventional cigarettes. About 85% of the nicotine present in the TPM phase was found in the L filter with particle size  $< 0.25 \mu\text{m}$ . Since nicotine is of primary importance in the particulate-phase of all cigarette smokes, nicotine could be delivered into human tissues via either one or the two mechanisms of particle deposition with evaporation (PDE) or the particle deposition with diffusion (PDD).

In terms of the types of constituents detected in the different types of bidis used, the GC-MS analysis of the last filter of the impactor for vanilla and grape bidi smoke reveals that they contain similar types of constituents. Furthermore, most of the compounds obtained by the GC-MS analysis of the different filters from vanilla bidi and grape bidi mainstream smoke were present mostly in the A, D, and L filters. The selected compounds of interest were not detected in the B and C filters with size fractions of 1.0-2.5  $\mu\text{m}$  and 0.50-1.0  $\mu\text{m}$ . The concentration of cotinine was highest in the last filter of grape bidi smoke relative to indole and methyl phenols as expected. Since cotinine is a metabolite of nicotine, its concentration is expected to be high. Also, in order to understand the gas/particle distribution of some of the compounds detected and analyzed in this work, the concentrations of cotinine, 2-methoxyphenol, 2-methylphenol, and 4-methylphenol in both the gas and TPM phases were compared. The results show that as the vapor pressure of the alkyl phenols increased the calculated ratio for the gas/particle distribution also increased. This was expected because the higher the vapor pressure of a compound the greater the concentration of the compound in the gaseous phase. This work shows that the Sioutas impactor may be used for the analysis of particles present in cigarette smoke in addition to ambient air analysis.

GC-MS analysis of standard carbonyl-DNPH derivatives was also performed at different injector temperatures in order to determine the optimum temperature suitable for the analysis of these compounds with minimal thermal degradation. An optimum temperature of 230 °C was obtained for the analysis of the formaldehyde and acetaldehyde-DNPH derivatives, while 250 °C was optimal for the higher molecular weight carbonyl-DNPH derivatives. Decomposition products detected include 3-nitro-N-(phenylmethylene)-benzenamine, *o*-nitroaniline, 4-[2-(4-nitrophenyl)ethenyl]-benzenamine, and N-[4-methylphenyl)methylene]-4-nitro-benzenamine. From the results, the signal intensities of these compounds increased with injector temperature from 250 °C to 290 °C.

Analysis of standard carbonyl-DNPH derivatives was performed on the LC-MS as well as the GC-MS. LC-MS analysis was capable of detecting all the derivatives present in the standard solution, but tolualdehyde-DNPH derivative was not detected in the GC-MS analysis. The 2-butanone and methacrolein DNPH derivatives, which were not separated in Grosjean et al.'s work<sup>36</sup> were separated using the optimized chromatographic conditions developed in this work. With the use of the acetaldehyde- $d_4$  and phenyl acetaldehyde-DNPH derivatives as internal standards, concentrations of various carbonyl-DNPH derivatives were quantitated and it was observed that the concentrations of acetaldehyde, acrolein, acetone, propanal, 2-butanone, and butanal DNPH derivatives were present in higher concentrations in bidi mainstream smoke compared to that of 1R5F reference cigarettes. The concentration of acetaldehyde-DNPH derivative was the highest, consistent with earlier studies on analysis of carbonyl compounds in cigarette smoke.

Future work to extend this research could include the analysis of the tendu leaf used for wrapping the bidi cigarettes in order to characterize its constituents and thus give a better overview of how it contributes to the constituents present in the mainstream smoke of bidi cigarettes. Another area of interest would be to develop a sampling technique that would allow the study of how the concentrations of the gaseous components of bidi mainstream smoke change from one puff to another. Also, the characterization of other groups of compounds, such as polycyclic aromatic hydrocarbons (PAHs) present in bidis, could be undertaken to provide a greater understanding of the composition of mainstream tobacco smoke. The Sioutas impactor could also be operated at different flowrates, such as 1.05 L/min, to study what effect the change in flowrate will have on the distribution of the particles.



## **PART II**

### **ASSESSMENT OF EXPERIMENTS FOR ENVIRONMENTAL ANALYSIS**

## CHAPTER 1

### INTRODUCTION

#### **Background**

The second part of this dissertation involved the development and assessment of two laboratory experiments directly related to environmental issues. As observed by Zoller,<sup>68</sup> a thorough understanding of the environment and the ability to make relevant decisions based on the acquired knowledge by the citizens is one of the main goals of sound education. In accordance with this philosophy, a number of colleges and universities are incorporating environmentally related courses and laboratories into their curriculum.

It is important to develop laboratory experiments for undergraduate students that will involve the use of modern instruments and also provide real-world applications to which they can relate.<sup>69-71</sup> This is because a fundamental knowledge of these instruments could be beneficial to them later in their career and using real-world samples could make the laboratory experience interesting and more meaningful for them.<sup>72-73</sup> Instruments such as the UV-vis spectrophotometer are currently being used in laboratory experiments at Middle Tennessee State University (MTSU), but students are not involved directly with the specifics of setting up the instrument.<sup>74</sup> The instruments are primarily set-up for them and all they do is to put in their sample and collect the data. Some students believe that many chemistry experiments are boring, abstract, and have little or no relevance to their life. Thus, the development of laboratory experiments that involve the use of

appropriate instrumentation, as well as projects that are relevant to their life, would be a more honest reflection of what is done by environmental scientists. These people are specialists in the analysis of pollutants in samples ranging from the ambient air to the drinking water. It could also bring a little fun to the laboratory work thereby helping students develop ability to solve problems and gain valuable experimental skills.

Laboratory activities such as this would not only engage the students' cognitive skills, but also their affective skills. They will get involved in solving "real-world" problems thereby incorporating relevancy, which is an important part of effective chemical education.<sup>75</sup> Affective domains refer to behaviors which are related to emotions, attitudes, interests, opinions, values, and feelings, while the cognitive aspect refers to everything involved in intellectual activity.<sup>76</sup>

Middle Tennessee State University (MTSU) undergraduate students are exposed to the use of some instruments on a limited basis<sup>74,77</sup> but there is the need to provide them with more in-depth opportunities to use techniques such as the Fourier transform infrared spectroscopy (FTIR), gas chromatography-mass spectrometry (GC-MS), gas chromatography-flame ionization detection (GC-FID), and to introduce them to analysis of data collected using these instruments. This provides them opportunities to apply techniques and concepts in optical spectroscopy, mass spectrometry, and chromatography. This is especially important for students going to graduate school, into industry, and other related fields.

There are very few laboratory manuals devoted to the topic of environmental analysis using analytical instruments. The style of these manuals is similar to that of a recipe and focus on outlining the procedures for obtaining analytical results that meet an

acceptable level of accuracy and precision.<sup>78</sup> The discussion of the underlying analytical principles is generally limited and the characteristics of analytical instruments are not covered sufficiently to afford a thorough understanding of operating procedures of the instruments. In summary, implementing environmental laboratory experiments has suffered from the lack of availability of suitable manuals. There are good textbooks, but limited laboratory manuals for environmental laboratories. The experimental procedures that are prescribed in the manuals give the students little room to exercise higher order cognitive skills or decision making, such as application of key concepts.<sup>78,79</sup> The laboratory manual by Shugar et. al<sup>78</sup> has different test methods on water analyses and determination of gaseous pollutants that are written in a step-by-step format with minimal explanation of the rationale behind the procedures. The questions that follow each experiment also have the answers written out underneath and hence the manual does not allow students to think about what they are doing and figure out the best way to do it. Consequently, students are not able to understand the analytical principles behind the procedures and methods development. Another laboratory manual by Csuros<sup>79</sup> does have a chapter focused on the principles of spectrophotometry without any discussion on the practical application of this technique to real samples. Furthermore, the manual does not cover sample preparation techniques and related data processing methods.

Modern instrumentation such as FTIR, GC-MS, and GC-FID are increasingly being incorporated into undergraduate curriculum for chemistry.<sup>80-87</sup> Hill<sup>82</sup> incorporated the use of infrared spectroscopy into the general chemistry laboratory and students were able to use this technique to confirm the identity of their assigned unknowns and gained an understanding of how to prepare and analyze their samples on the IR spectrometer.

However, even though the students were able to acquire the IR spectra of their samples, they were not involved in the set-up of the instrument for use as it was a general chemistry course. Bezoari<sup>85</sup> also reported the introduction of spectroscopic technique into the chemistry curriculum at Huntingdon College. Here, students in both the organic and physical chemistry courses were taught to analyze various types of samples including polymers, pharmaceutical products, lipstick, and automobile exhaust using FTIR. It was reported that these students became more interested in research projects using FTIR. Greater productivity in student research in the form of publications and conference presentations were documented. This supports the study that demonstrated that incorporating these modern analytical techniques to the analysis of real-world samples has been meaningful, interesting, and rewarding for the students.<sup>87</sup>

The laboratory experiments developed as part of this research were designed to introduce students to the use of two modern instruments for the analysis of real-world samples. This should result in enhanced interest in analysis, coupled with learning valuable skills in the operation of these instruments. These two laboratory experiments were written for students in upper level courses in environmental analysis who are primarily chemistry majors or environmental science majors. These students enrolled in the Detection of Chemical Pollutants course<sup>88</sup> offered at MTSU. Students in this course might have interests in pursuing careers in environmental research or other analytical laboratory work. Such careers would be enhanced by having experience with modern instruments that are used in most industries and research laboratories.

Newly developed laboratory experiments should be assessed for effectiveness before full introduction into coursework. These experiments were assessed to determine

if the main objectives were achieved, if reliable results were obtainable, if the response of the students was favorable, and if the time allotment was adequate. It is expected that students enrolled in Detection of Chemical Pollutants or a similar environmental analysis course have completed coursework in quantitative analysis offered by a chemistry department.<sup>89</sup> The goals of these experiments were to introduce students to sample preparation, analysis, and quantification of pollutants in real environmental samples using the FTIR and GC-MS. This research provided a preliminary assessment of the effectiveness of these laboratory experiments. Areas of improvement in design for future use were also identified.

The type of instrument used in any research determines the accuracy of the results.<sup>90</sup> The assessment instrument(s) used in any educational research influences the validity and reliability<sup>90-95</sup> of the research. Reliability is a measure of the ability of the assessment instrument or procedure to yield consistent or repeatable outcomes.<sup>90,91</sup> On the other hand, validity gives an indication of how well the assessment instrument actually determines the characteristic or phenomenon that it claims to measure.

Some options available for data collection include surveys, observations, and assessment tests. Surveys were used in this work in order to gather as much information as possible from the small number of participants. This type of data collection involved the use of questionnaires and/or open-ended questions. These quantifiable instruments were used to determine the opinions, attitudes, preference, experience, and the perceptions of the chosen sample group of interest to the researcher.<sup>90,93</sup>

Questionnaires are very good for collecting descriptive information. Participants respond to statements on the questionnaire that allowed their feelings to be inferred.

Each questionnaire was constructed to measure specific aspects of the research objectives. Questions can be closed-form, in which subjects choose predetermined responses, or open-form, in which subjects respond in their own words to the posed questions. The following are the characteristics of a good questionnaire:<sup>90,91</sup>

- (a) It deals with a topic that has clear and carefully stated significance.
- (b) It is as short as possible.
- (c) It is well organized and neatly written or printed. That is, it must have a good appearance.
- (d) Clear and complete directions are given and each item is written on only one idea in a simple way.
- (e) There are no leading suggestions.
- (f) Questions are well ordered to help respondents' thinking from general to specific ones.
- (g) It is easy to analyze.

The Likert scale, which can be a reliable and valid measure<sup>90</sup> for attitude measurement, represents one type of closed form questionnaire. On this type of questionnaire, the volunteer checks one of four or five possible responses to a statement: "strongly agree," "agree," "undecided," "disagree," or "strongly disagree." Responses are then analyzed by assigning values of 1 - 4 or 1 - 5 to the responses as appropriate, with one signifying "strongly agree" and four or five, "strongly disagree." Some questions should be written in the inverse, and should be scored in the inverse as well, in order to get the feedback that is needed. Generally, Likert scale instruments are scored so that low numerical values are desirable. Other types of attitude scales include the Thurstone-type scale and the Semantic Differential scale.<sup>90</sup> The Thurstone-type scale involves the volunteer expressing agreement or disagreement to a statement. The Semantic Differential scale is based on the view that objects could have different meanings to different people. These different meanings are known as the denotative and

connotative meanings.<sup>96,97</sup> Thus, bipolar adjectives like good-bad, successful-unsuccessful, useful-useless, are employed on the Semantic Differential scales to indirectly rate an individual's connotative meanings of objects.

Interviews are especially good when there is a need for deep understanding of the subject matter. Interviews help to dig deeply into the perceptions of the participants, even though they can be more difficult to record and analyze.<sup>93</sup> There is less guesswork as to what someone means and attitudes can be more clearly discerned without relying so heavily on inference so validity is high. In order to have good results from interviewing participants, a good rapport must first be established at the start of the interview process. Interviews and open-ended questions are hard to use with large numbers of students, but can be used for a small sample size to follow up questionnaire responses in depth or to collect enough detailed information to develop a questionnaire.

In order to analyze open-ended questions and interview data, feedback obtained can be classified into categories and analyzed by content analysis.<sup>95</sup> Content analysis uses frequency counts to give a quantitative description of the specific content data. A relationship is sought between the frequent use of specific language with a focus to observe any manifestation of the research objectives. In doing this, the data is first divided into different categories according to their commonalities and then quantified.<sup>90,92,95</sup> Instances or words are identified based on set objectives. The numbers of occurrences are counted and further analyses such as tabulations, statistics, and/or regrouping could be done. Relationships between items in the data are sought by the researcher.<sup>95</sup>



In this study, data was collected using questionnaires, open-ended survey questions, and interview. A Likert scale was used on the questionnaire and content analysis was performed on the responses to the open-ended questionnaire and survey questions.

### **Purpose of Study**

The aim of this work was to develop and assess two environmental laboratory experiments. Two experiments “FTIR Analysis of Mainstream Cigarette Smoke” and “Extraction of Organic Pollutants from Soil Samples for GC-MS Analysis” were developed for use by upper-level chemistry and environmental science students. These laboratories were designed to introduce students to procedures for environmental analysis using the FTIR and GC-MS. This study analyzed the students’ responses after performing the experiments and sought out areas of overlap between what the experiments were designed to accomplish and the students’ feedback. These results were used to determine if the experiments enhanced enthusiasm or interest in science on the part of the students. The data were also used to determine what areas of the experiments needed to be modified for future use.

### **Issues of Credibility**

Even though these experiments were designed for students taking the upper level environmental analysis course with few students enrolled, results obtained from this work are considered to be credible. This is because qualitative research requires not only collecting data, but it also involves obtaining meanings that individuals attach to their actions.<sup>98</sup> In this study, students were given open-ended questions so they could put their

views into proper perspective and express them accordingly. As a result, students' actual language were used for data analysis. There was close contact between the author and the students in the laboratories such that information from the data obtained could be related to the observed students' behaviors. Hence, the data were not analyzed solely on the use of abstract numbers. Also, results obtained from the use of different assessment instruments were used for triangulation, thus validating each other. The interview conducted validated the data obtained using the questionnaire and open-ended survey questions.

The Detection of Chemical Pollutants course is usually offered once a year, during the Spring semester and about 4-6 students generally register for this course at MTSU.

## **CHAPTER 2**

### **REVIEW OF LITERATURE**

#### **Background**

Following the approval of an undergraduate degree option in Environmental Chemistry by the American Chemical Society (ACS),<sup>99</sup> there has been recent emphasis on the development of environmentally related laboratory experiments for students. As a result, laboratory curricula have been developed and improved to prepare students for careers in the study of chemicals and chemical processes in the environment. In this chapter, literature published on the use of modern instrumental techniques, specifically FTIR and GC-MS, using such samples as cigarette smoke and soil samples will be reviewed.

#### **Effectiveness of FTIR in Laboratory Experiments**

Incorporating modern analytical techniques, such as the FTIR, with the analysis of real-life samples such as cigarette smoke or car exhaust has been meaningful, interesting, and rewarding for students.<sup>84</sup> Wagenknecht et al.<sup>100</sup> wrote a laboratory experiment that described the use of infrared spectroscopy to demonstrate the function of catalysts in catalytic converters in automobiles. The catalytic converters required about 2-3 minutes to warm up before reaching optimal performance. Thus, more air pollutants were emitted via the tailpipe in the initial period before the working temperature of the converter was reached. A catalyst made from Au/Fe<sub>2</sub>O<sub>3</sub> capable of operating at very low

temperatures was used to study the transformation of a carbon monoxide/air mixture. A gas cell, containing the product mixture of catalytic conversion, was used for the collection of the IR spectrum. Observation of the attenuated carbon monoxide bands and the increased carbon dioxide bands helped to explain the conversion of carbon monoxide to carbon dioxide in the catalytic converter to the students.

Another laboratory experiment utilizing FTIR spectroscopy for the analysis of pollution involving aerosol cans, as well as the analysis of Los Angeles air from atmospheric smog, was developed by Amey.<sup>101</sup> The toy balloon was used as the gas cell for the different gaseous samples. Using a scan time of less than thirty seconds, an introductory chemistry laboratory section with forty students was able to obtain sample spectra within the designated laboratory period. With the balloon secured in place on the IR spectrometer, the spectra of different gases were obtained. Students were able to identify the spectral peaks of carbon dioxide, water, and nitrogen dioxide and conclude that nitrogen and oxygen are not IR-active via the inquiry-based experiment.

### **Use of Cigarette Smoke as Sample Analyte**

A large number of laboratory experiments have been developed on the analysis of cigarette smoke.<sup>68,98-99</sup> Zoller<sup>68</sup> developed a study module entitled “Smoking and Cigarette Smoke” to introduce various key concepts of chemistry to students. Cigarette smoke was used to demonstrate a number of principles, concepts, and processes in chemistry. A custom-made smoking machine was used for collecting the cigarette smoke for analysis, followed by a subsequent study of its effect on human blood, using the color change it produced. Thin layer chromatography (TLC) was also introduced as a

separation method for analyzing the individual components of tar from the cigarette smoke. The results obtained were designed to make students aware of the multiple constituents of cigarette smoke, allow students to construct their own meaning, and encourage students to draw conclusions as to the effect of cigarette smoke on living cells and its composition.<sup>68</sup> The laboratory can also potentially impact students' attitudes toward smoking and their ability to make good decisions regarding starting smoking or stopping the habit if they currently smoke. A preliminary study showed that the experiment was effective in influencing those non - smoking students to not begin the smoking habit. This laboratory helped students make informed decisions about smoking.

Another laboratory experiment was developed by Mabury et al.<sup>102</sup> to quantify the amount of formaldehyde present in cigarette smoke. The main goal of the experiment was to involve students in solving health issues with “real-world” environmental concerns. Cigarette smoke was sampled using a simple apparatus that involved the trapping of formaldehyde from the cigarette smoke in an aqueous solution of either dinitrophenylhydrazine (DNPH) or cysteamine solution contained in a separatory funnel. Both derivatizing reagents were used in order to illustrate to students that there could be more than one way to analyze the same material. The formaldehyde in the smoke was trapped in the solution and underwent derivatization. Further extraction of the solution was done before analyzing the cysteamine derivatives by gas chromatography (GC) or the DNPH derivatives by high performance liquid chromatography (HPLC). Samples and blank solutions were spiked in order to determine the accuracy of the analytical method being used before analysis by GC and HPLC to validate the recovery of formaldehyde. At the end of the experiment, students found that the environmental

analysis of samples could be carried out in more than one way and they had the opportunity to learn useful extraction and analytical techniques in chemistry.<sup>102</sup> This laboratory introduced students to solid phase extraction as a sample preparation method in addition to liquid-liquid extraction method, with which they had prior experience. The time and equipment needed for this experiment was more flexible than the usual fixed three-hour laboratory time. Students reported that they enjoyed data collection and analysis and the use of chemistry to solve relevant problems.

Another chemistry laboratory experiment suitable for junior level students was developed by Finlayson-Pitts et al.<sup>103</sup> to illustrate the usefulness of HPLC as an analytical technique for complex matrices such as cigarette smoke. This laboratory also involved the use of cigarette smoke to illustrate the principles of HPLC to the students. HPLC with both fluorescence and UV-vis absorption detectors was used to analyze polycyclic aromatic hydrocarbons (PAHs) in cigarette smoke. This important analytical technique was very useful for studying various environmental problems and therefore good for students to be familiar with. Students were able to see how the sensitivity of fluorescence detection was affected by different absorption and emission wavelengths. This laboratory showed that HPLC was suitable for the analysis of PAHs and could be adapted into an experiment for an instrumental analysis laboratory for students interested in environmental chemistry.

### **Effectiveness of GC-MS in Undergraduate Laboratory Experiments**

In recent years, there have been an increasing number of articles describing the use of gas chromatography in undergraduate laboratory experiments.<sup>104-109</sup> Polyaromatic

hydrocarbons (PAHs) are a group of compounds that can be analyzed by GC-MS. Structurally, PAHs are molecules that contain two or more aromatic rings fused together. They include anthracene, benzo(a)pyrene, naphthalene, and coronene. There have been many studies performed on benzo(a)pyrene and it has been found to be a cancer-causing substance. Many of these PAHs are able to form metabolites that can react and bind with cellular DNA and eventually lead to the formation of tumors and cancer. PAHs are found in tobacco smoke, chimney soot, and even barbecued meat. The United States Environmental Protection Agency and the European community have both listed PAHs as priority pollutants. Therefore, there has been interest in raising the awareness of PAHs in the environment.

Mabury et al.<sup>110</sup> had students use GC-MS to analyze river water "samples" spiked with known amounts of different pesticides; atrazine, desethylatrazine, and simazine. Students used solid phase extraction (SPE) to preconcentrate the analytes prior to their analysis by GC-MS. The students were able to identify and quantify atrazine in the water sample and they observed that GC-MS is a valuable tool for providing quality information about analyte identification. This experiment was a valuable teaching aid on the analysis of environmental samples for the students.

Another environmental laboratory experiment was designed by Kegley et al.<sup>111</sup> for the determination of polychlorinated biphenyls (PCBs) in river and bay sediments, using capillary gas chromatography with electron capture detector (GC-ECD). Sediment samples were collected and a solvent mixture of 50% acetone and 50% hexane was used for the sonication of the sample in an ultrasonic bath, followed by filtration and concentration using a 500-mL Kuderna-Danish concentrator. After concentration, the

extract was passed through Florisil column and the adsorbed components eluted by rinsing with pure hexane; after which the extract was analyzed on the GC-ECD. The students found that the concentration of PCBs in the river was lower than the concentrations at the source via dilution effects. Students were thus able to see the relevance of environmental chemistry in a real situation.<sup>111</sup>

### **Solvent Extraction**

Usually a number of different solvents are used for the extraction of soil samples depending upon the extraction method to be employed and the organic content of the soil sample. The various extraction methods usually employed for the efficient extraction of organic pollutants from soil samples include sonication, Soxhlet extraction, KOH-digestion, mechanical shaking, supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE).

Both SFE and ASE are not readily available in undergraduate laboratories, thus the other three methods, namely Soxhlet extraction, ultrasonic extraction, and extraction by mechanical shaking, are generally utilized in undergraduate experiments. Berset et al.<sup>112</sup> showed that the methods of Soxhlet extraction and mechanical shaking were as efficient as the SFE and ASE. However, the Soxhlet extraction required large volumes of solvent when compared to the mechanical shaking method.

Wilke et al.<sup>113</sup> showed that the ultrasonic method was as efficient in extracting PAHs from moderately polluted samples as the Soxhlet extraction or the mechanical shaking method using dichloromethane/acetone mixture as the extracting solvent.



## Solid Phase Extraction

One of the goals of the second experiment designed in this work was to illustrate the principles of solid phase extraction (SPE) to students. Different SPE cartridges can be used to separate the components of the soil matrix, based on analyte polarity. Since there are different SPE cartridges available,<sup>114,115</sup> a proper choice must be made as to which one to use. SPE is commonly used in sample clean-up instead of the liquid-liquid extraction that requires a large amount of solvent for extraction.<sup>116</sup> The selection of SPE tubes is based on the polarity of adsorbent, analyte polarity, and the polarity of the solvent containing the analyte. These cartridges are also chosen based on their capacity in terms of the weight of the adsorbent in the SPE tubes. Larger weights are used for samples containing larger amounts of analytes. The three major types of extraction modes are: reverse phase, normal phase, and ion exchange.<sup>117</sup> For the normal phase mechanism, the tube packing, which is the stationary phase, is polar and the solvent, which is the mobile phase, is non-polar. As the analytes pass through the adsorbent, the more polar components are adsorbed more strongly and the non-polar compounds pass through. Reverse phase SPE tubes contain non-polar packing that retains non-polar analytes longer than polar analytes in a polar solvent.<sup>117</sup> Elution of target compounds from the adsorbent can be achieved by choosing the right packing (with appropriate polarity) and eluent in which the compounds of interest are soluble. Some of the most common adsorbents are alumina, silica, C18, and C8.<sup>118</sup> In order to effectively desorb analytes from a given adsorbent, the polarity of the eluent is important. For the silica cartridge, the elutropic series, which refers to the solvent strength in desorbing analytes from the adsorbent, have the following order: dichloromethane < acetone < methanol.<sup>119</sup>

Since silica is polar and will retain more polar analytes, an intermediate to polar solvent is required to elute the analytes from the silica stationary phase.

## CHAPTER 3

### METHODS

#### **Background**

The experiments “FTIR Analysis of Mainstream Cigarette Smoke” and “Extraction of Organic Pollutants from Soil Samples for GC-MS Analysis” were designed to introduce upper level chemistry and environmental science students to the methods of sample preparation and analysis on two modern instruments. Samples with practical relevance to students were used (see Appendices A and B).

Since human subjects were needed to assess the effectiveness of these laboratories, permission to administer and conduct this study was obtained from the MTSU Human Subject Research Review Board (see Appendix C). The data were collected in the spring semester of 2004. The target population for this research was upper division chemistry and environmental science students enrolled in the environmental analysis course in Spring 2004.

#### **Research Design**

The experiments developed were assessed by students taking the “Detection of Chemical Pollutants” course at MTSU. This course consists of a laboratory component worth 50% of the course grade and it is expected that students taking this course have already taken at least three semesters of chemistry, introducing them to basic tools used in chemistry laboratories.

Students in the Spring 2004 Detection of Chemical Pollutants course (CHEM 4630) participated in this study.

### *FTIR Analysis of Mainstream Cigarette Smoke*

The first experiment “FTIR Analysis of Mainstream Cigarette Smoke,” involved the FTIR analysis of mainstream cigarette smoke components using an extractive gas cell. FTIR offers the potential for the simultaneous, real-time measurement of a variety of gaseous species, which can be monitored simultaneously, even in complex matrices such as cigarette smoke.<sup>22</sup> This experiment was designed to introduce the students to the use of the FTIR spectrometry and data analysis, using the National Institute of Standards and Technology (NIST) and the Environmental Protection Agency (EPA) libraries for standard infrared spectra. The method of sampling cigarette smoke for analysis on the FTIR in this experiment was different from other experiments designed earlier because a Tedlar bag and a 2.4 -meter extractive gas cell, which is a White cell, are used in this experiment. The procedures for calculating the concentration for each compound were used to reinforce the students’ conceptual understanding of Beer’s law. They were thus introduced to the procedures of sampling, analysis, and quantification of cigarette smoke samples on the FTIR and not just one or the other.

Since many college students are smokers or at least know someone else that smokes, they were able to relate to the analytical sample used. Students were provided with a handout (Appendix B) including background information and the procedure for the experiment. The required materials such as the gas cell, Tedlar bag, cigarette pump, and IR were made available to the students. Students were asked to work individually since

there were four in the class. Sampling of cigarette smoke into the Tedlar bag was performed before it was transferred into the gas cell for FTIR analysis. In order for students to choose the right compounds to analyze, they were given the clues that most of the gas phase compounds were related to combustion and that compounds with more than five carbons or boiling points of less than 80 °C are more likely to be found in gas phase samples. Students were asked to apply the concept of volatility and choose from a given list of compounds using their boiling points. Since cigarette smoke is divided into the gaseous and the particulate matter phases, they were expected to choose those compounds with low boiling points for FTIR analysis. Afterwards, they were required to compare the sample spectra of compounds chosen to the reference spectra of these smoke constituents from the library, subtract them, and obtain their concentrations.

The 2.4-meter gas cell used for this experiment has a compact volume of 120 cm<sup>3</sup>, which was made possible by multiple reflections within the cell. This further helped students understand the principle of White cells which is usually used to increase the optical pathlength for FTIR analysis. A reasonably long optical pathlength is essential for the analysis of trace gases, therefore, a multi-pass gas cell such as the 2.4-meter gas cell used in this experiment was ideal for cigarette smoke analysis. Students should recognize how the phenomenon of the multiple reflection of infrared beam at the two sets of end mirrors in the gas cell is used to increase the effective pathlength for absorption. Students should learn that according to Beer Lambert's law, the increased pathlength leads to increased absorbance for a given analyte concentration. Hence, long pathlength cells provide the greater sensitivity required for the analysis of trace pollutants.

In the procedures for quantitative FTIR analysis, students were introduced to the different databases of IR reference spectra. These databases include libraries from the National Institute of Science and Technology (NIST) and the Environmental Protection Agency (EPA) library. The NIST library has reference spectra of about 21 volatile organic compounds available at resolutions of  $0.125\text{ cm}^{-1}$ ,  $0.25\text{ cm}^{-1}$ ,  $0.5\text{ cm}^{-1}$ ,  $1.0\text{ cm}^{-1}$ , and  $2.0\text{ cm}^{-1}$  using five apodization functions. The spectral library from the EPA has reference spectra of over 100 common air pollutants available only at a resolution of  $0.25\text{ cm}^{-1}$  and most of these EPA spectra are recorded for at least two concentrations of each compound. For quantitation, students used the univariate method involving the use of just one wavelength region per component for the analysis of spectral data.

#### *Extraction of Organic Pollutants from Soil Samples for GC-MS Analysis*

The second experiment “Extraction of Organic Pollutants from Soil Samples for GC-MS Analysis” involved the analysis of polycyclic aromatic hydrocarbons (PAHs) present in soil samples collected from Superfund sites at Hickman County in Tennessee. Students were introduced to the methods of sample preparation, which involves extraction, clean up, and subsequent analysis by the GC-MS. Students were able to compare the effectiveness of various extraction solvents and sample clean-up methods, as they were introduced to the principles of solid phase extraction (SPE). This experiment demonstrated to students the differences between different cartridges available and the mode of selection.

Real soil samples in different airtight jars were provided and the students were once again asked to work individually. They had to extract the PAHs from the soil

samples, using appropriate solvent, followed by filtration. Sample clean up was done using SPE tubes, before GC-MS analysis. They then performed spectral matching using the NIST library database. Students used different solvents in order to make individual judgments as to whether any of the solvents is better than others. This way, they were able to utilize critical thinking about the results obtained and the experiment also emphasized the conceptual understanding of Beer's law, White cells, and spectrophotometry.

Students investigated the effect of using different solvents in extraction and sample preparation on the extraction efficiency of the PAHs from the soil samples. They were also introduced to the use and choice of internal standards in analysis. In this case, it was not likely that bromobenzene and bromohexadecane would be present in the real soil samples, so these compounds were chosen as internal standards. Internal standards are expected to have similar physical and chemical properties as the expected analytes so that variation due to analyte extraction efficiency and volatilization losses can be minimized.

In order to assess the effectiveness of these experiments, students enrolled in the environmental analysis course in Spring 2004, had to perform these two experiments as part of the eight experiments they were expected to perform during the semester. After performing these two experiments, participation in the assessment of the experiments was requested from volunteers. After the experiments were performed, the main purpose of the research was discussed with them and then volunteers were asked to fill out consent forms, a questionnaire, and a survey for each of the two experiments containing open-ended questions. The surveys and questionnaires required them to reflect and provide

feedback on the experiments they had performed. Anonymity was maintained by assigning each student a particular number to put on the survey and questionnaire.

The questionnaire was comprised of ten questions probing the students' attitude about the experiments, the format of the presentation of laboratory results, and time required, as well as questions asking about the content of the experiments. There were also two free response questions on the questionnaire. A Likert scale of 1 - 4, was used ranging from "strongly agree" to "strongly disagree." A choice of "neutral" or "undecided" was avoided in this case to force a choice from the participants about their assessment of the experiments rather than just choosing "neutral."

The open-ended questions in the questionnaire and the survey questions were also analyzed by content analysis. The questions on the questionnaire were grouped into three categories based on their main themes. Questions one, five, six, and ten related to the attitudes of the students regarding the experiments, questions two, three, four, and seven related to the content of the laboratory experiment, while questions eight and nine related to the format of the laboratories.

Responses to the questionnaire items were analyzed by calculating the mean and median of the Likert scale responses for each question separately, and also for the different categories grouped together. Frequency counts for responses relating to each group of questions and percentages were also calculated for the three groups of questions.

Content analysis was performed on the two questionnaire open response questions together. Four categories namely "content," "format," "clarity," and "computer program use" were identified and frequency counts from the answers to all items were performed and tabulated. The category "content" referred to comments based on the content of the



experiments, use of instrument, style, and real world application. “Format” referred to the way the laboratory was set up and “clarity” refers to how clear instructions were, while “computer program use” referred to the difficulty or ease of the instruments’ computer program use.

Open-ended surveys were used to probe the students more deeply. In order to better explain the answers given in the questionnaire, students filled out one survey each for the two experiments after completing the questionnaire. Students are used to taking surveys and they usually feel comfortable expressing their opinions and ideas about the topic of interest through this means, once they know their confidentiality was maintained. The surveys used in this research required the students to fill in their assigned number, age, and year in school before answering the open-ended questions (Appendix A). The survey questions were designed to assess background knowledge of concepts applied in the experiment, the usefulness of the experiments, and what they learned therein. Questions such as the recommendation of the laboratory experiments to other students and other general suggestions for improvement were also included in the surveys. These surveys were particularly important, in order to obtain as much information as possible from willing participants.

Content analysis was performed on the survey questions and major themes in the participants’ comments were identified and grouped together in different categories. Similarities and differences in the sets of data were sought and categorized accordingly. These similarities and differences gave good information about what the students learned from the experiments. Statements and experiences that were similar with respect to the laboratory experience were aggregated together. Frequency counts for each category

were performed by counting the numbers of occurrences of words relating to each category and the percent of each category in all the categories together were calculated.

Comparisons were made between the two experiments.

One student was also interviewed one-on-one in order to probe the survey responses and confirm that the questions were clear. Copies of the consent form, questionnaire, and surveys are provided in Appendix A.

## **CHAPTER 4**

### **RESULTS AND DISCUSSION**

All four students enrolled in the Detection of Chemical Pollutants class during the Spring 2004 semester volunteered to participate in this study. All of them were seniors and they were all above the age of eighteen.

After performing the two experiments, “FTIR Analysis of Mainstream Cigarette Smoke” and “Extraction of Organic Pollutants from Soil Samples for GC-MS Analysis,” as part of the requirement for the environmental course they were all enrolled in, all volunteered to participate in this study and completed the questionnaire and surveys.

#### **Questionnaire Item Responses**

The ten questionnaire items had a four-point Likert scale response option ranging from “strongly agree” to “strongly disagree.” Values of one to four were assigned to the responses with one indicating “strongly agree” and four indicating “strongly disagree.” For questions framed negatively, a value of four was assigned to the response “strongly agree,” while a value of one was assigned to “strongly disagree.” This indicated that a low value is favorable for both types of questions. As mentioned earlier, the subjects were not presented with a choice of “neutral” or “not applicable.”

After the questionnaires were completed, the questions were grouped into two types, those phrased positively and those phrased negatively. Questions one through eight were phrased positively, while only Questions nine and ten were phrased

negatively. The mean values for these two groups (i.e. questions framed positively and negatively on the questionnaire) were calculated to be 1.81 and 3.00, respectively.

From these values, the students agreed with most of the questions framed positively, while the majority disagreed with the negatively framed questions. These mean values of the responses indicated that the students had some problem with the amount of time required for the experiments since those were the questions framed negatively. However for the positively framed questions, which dealt with the other aspects of the laboratory, the mean was 1.81, indicating that they generally agree with these questions.

Table 13 presents the different mean, median, and standard deviation for the responses to all the questionnaire items. The majority of the questions have mean responses that were less than two, indicating that the students agreed with the main ideas of the questionnaire items. This is because a value of two indicates they “agreed” with the question and a value of one indicates “strongly agree.” The exceptions were items three, nine, and ten. Question three dealt with prior background knowledge of FTIR and GC-MS before doing the experiments. The mean score of 3.50 for this item indicated that all of them had just minimal or no prior knowledge of these instruments before performing these laboratory experiments. However, relating this to the mean score of 1.50 for item four, they all agreed that these experiments have introduced them to these new analytical techniques and procedures.

This shows that one of the goals of this research, which was to introduce students to the methods of analysis on these modern instruments, was achieved.

Table 13. Responses to the Different Questions on the Questionnaire

Questions	Mean	Median	Standard deviation
1	1.50	1.50	0.57
2	2.00	2.00	0.00
3	3.50	3.50	0.57
4	1.50	1.50	0.57
5	1.00	1.00	0.00
6	1.50	1.50	0.57
7	1.75	2.00	0.50
8	1.75	1.50	0.95
9	3.75	4.00	0.50
10	2.25	2.00	1.25

Questions nine and ten dealt with the amount of time required to complete the experiments. Question nine asked the students to indicate if it took them a longer time for each experiment than was expected for the laboratory session that counted as one credit hour. Question ten requested students to indicate if they would be willing to do short experiments that will not take more than three hours, even if they would not learn as much as they will in open-ended laboratories. From the students' responses, even though they do not want to spend more time on these experiments than is necessary, they were still willing to compromise by putting in more time, if it increased the amount of experience or knowledge acquired from these experiments.

Responses to the questionnaire items were grouped into three categories: attitude, content, and format of the laboratories. Questions one, five, six, and ten related to attitude. Questions two, three, four, and seven related to the content of the laboratory experiments, while Questions eight and nine related to the format of the laboratories. Table 14 gives the mean, median, and standard deviation for these three categories of questions.

A closer look at Table 14 indicated that the mean response for the questions relating to the students' attitude was more favorable, followed by the mean response for the questions relating to the content of the laboratory experiments, and then the responses relating to the format of the laboratories. The mean response for the format of the experiments with respect to presenting their results rather than writing reports and the time requirement was above the value of two, indicating that the way in which the results are reported could be a potential area of improvement. Responses to questions measuring the students' attitudes with respect to the experiment are tabulated separately in Table 15.

Table 14. Responses to Questions Relating to the Three Different Categories on the Questionnaire

Category	Mean	Median	Standard deviation
Attitude	1.56	1.00	0.81
Content	1.62	1.50	0.45
Format	2.75	3.00	1.28

Table 15. Responses Relating to the Five Questions about Attitudes on the Questionnaire

Response Type	Counts	Percent (%)
Strongly Agree	9	56.2
Agree	4	25.0
Disagree	2	12.5
Strongly disagree	1	6.3



A large percent of students either agreed or strongly agreed with questions indicating that they enjoyed the opportunity to use these modern instruments, loved the challenge of interpreting the IR and mass spectra, and were interested in learning from these experiments. As shown in Table 15, it can be seen that thirteen out of sixteen responses to questions measuring students' attitudes were "strongly agree" or "agree." Therefore, generally, the students expressed a good attitude toward the two experiments and were pleased with the material included in the experiments.

The responses to Questions two, three, four, and seven related to the content of the experiments, specifically background material provided, relevance of the experiments to real world applications that they can relate to, and the availability of resources needed for successful performance of these experiments. Table 16 lists the frequency count of each response type and the percent.

Table 16 shows that all the students either agreed or strongly agreed with the questions regarding the coverage of adequate content required for the experiments. These responses were very positive. The students also agreed that they were introduced to valuable real world analytical techniques and procedures. They indicated that the spectral libraries provided for the analysis of the results were valuable resources for the experiments.

With regard to the two questions relating to the time demand and the format of reporting the results, a majority of the students still indicated a preference for presenting their results the way it was done, that is, preparing PowerPoint slides to use in presenting their results to the whole class, rather than writing reports for each laboratory. However, they expected that a laboratory session that counted as one credit hour should require a

Table 16. Responses Relating to the Three Questions about Content on the Questionnaire

Response Type	Counts	Percent (%)
Strongly Agree	5	31.3
Agree	11	68.7
Disagree	0	0.0
Strongly disagree	0	0.0

shorter time period than was demanded by these experiments. Table 17 presents these results. It can be said that these students were generally satisfied with the material covered and the general set-up of the laboratory experiments.

### **Questionnaire Open-Ended Responses**

The questionnaire contained two questions that were open-ended in nature. These questions asked the students to identify different aspect(s) of the experiment that they really liked and to explain their rationale. Also, they were asked to identify areas of improvement for the experiments. All the students responded to these questions.

Table 18 presents the frequency counts which were obtained by counting how many comments made by the students related to each category, and the percent of responses from the content analysis of the two open-ended questions on the questionnaire.

The responses were grouped into five categories namely “content,” “format,” “clarity,” “laboratory set-up,” and “computer program use.” In this case, “clarity” referred to how clear the instructions were. Comments grouped in the category “format” referred to the flexibility of the time required to perform the experiments. Some of these comments were:

“I like the flexibility of the laboratories.”

“I like the independence in the lab.”

The category “laboratory set-up” referred to comments that related to how the laboratory experiments were carried out. Representative comments were:

“One problem I had was chasing chemicals, equipment”

Table 17. Responses Relating to the Two Questions about the Laboratory Format on the Questionnaire

Response Type	Counts	Percent (%)
Strongly Agree	5	62.5
Agree	2	25.0
Disagree	1	12.5
Strongly Disagree	0	0.0

Table 18. Content Analysis of Responses to the Two Open-Ended Questions on the Questionnaire

Category	Counts	Percent (%)
Clarity	6	26.1
Computer Program Use	2	8.7
Content	10	43.5
Format	2	8.7
Laboratory Set Up	3	13.0

“Soil sample preparation might be improved if the samples are cleaned of unusable particles (rocks, twigs).”

The category “clarity” included suggestions to give better operational instructions and examples of these are:

“More detailed analytical instructions.”

“Students need to know they will lose the volatile components, even if they cover the test tubes.”

The category “computer program use” referred to how the computer programs used for data analysis on the instruments could be made easier. Representative comments included:

“Have clearer step-by-step instruction for each computer program used.”

“Keeping up with how to use the computer programs was difficult.”

“The library for the FTIR can be improved.”

The category “content” referred to comments relating to the material covered in the experiments and generally included positive comments on the hands-on use of the instruments, relevance of experiments to life, and the use of real samples. The comments here indicated that they were impressed with the opportunity to actually use and understand modern analytical instruments and the opportunity to learn about troubleshooting, limitations, and procedures on these instruments. Examples of these comments are:

“ I have not had much opportunity to use actual modern chemical instruments, until I accomplished these two experiments.”

“The main thing I enjoyed was the real world applicability of the experiments.”

“I found the use of modern instruments interesting.”

From Table 18, almost half of the comments related to the content of the experiments. Therefore, the tally of comments within this subcategory of “content” is shown in Table 19.

These data indicated that comments relating to hands-on use of these instruments were more than half of the content related comments, followed by relevance of the experiments to their everyday lives.

### **Analysis of Surveys**

Content analysis was also performed on the surveys the students completed. The students' responses to the open-ended questions were read many times and then placed in different categories based on similar themes and comments. These data are shown in Table 20.

For the FTIR experiment, the comments relating to the category “attitude” of the students stood out. These comments included:

“Experiment is interesting.”

“Experiment is good.”

“I enjoyed the experiment.”

“Laboratory experiment taught me new skills.”

“The good use of hands-on instrument experience.”

Both experiments included comments in the category “conceptual understanding.” It was the highest percent of responses in the FTIR experiment. Students expressed this through comments like:

**Table 19. Breaking Down Comments on the Content of Experiments into Specific Sub-Categories**

Sub-Category	Counts	Percent (%)
Hands-on use of instrument.	7	63.6
Relevance to life	4	36.4



Table 20. Survey Results of the FTIR and GC-MS Experiments

<u>Categories</u>	<u>FTIR</u>		<u>GC-MS</u>	
	Counts	Percent (%)	Counts	Percent (%)
Attitude	5	15.2	3	11.1
Conceptual Understanding	9	27.3	6	22.2
Future Benefit	9	27.3	2	7.4
Techniques	3	9.0	7	26.0
Relevance of samples	7	21.2	9	33.3

“Importance of polarity and the use of SPE.”

“Sample preparation is critical.”

“Use Beer's law to calculate concentration from known pathlength of cell, absorbance, and reference sample concentration.”

“The laboratory experiment taught me new and better skills.”

“This experiment expanded my knowledge of spectroscopy, especially IR spectroscopy.”

“Organic chemistry laboratory also used spectrophotometer, but did not allow a first-hand operation of the equipment.”

“It develops laboratory skills with experiment set-up and efficiency.”

It should be noted that there were more comments in the category “techniques” relating to troubleshooting and difficulty of the experiment in the GC-MS experiment than the FTIR experiment. Some of these comments included:

“Important to see difficulty of using real soil samples.”

“Too time consuming.”

“Difficult to find unknowns.”

“To me solid phase extraction cartridges were a pain to use.”

“Interesting exploration.”

This can be explained by the fact that the GC-MS experiment required more computer skills for data analysis and interpretation. The students might have found it more difficult. Also, the second experiment requires more time for sample preparation, compared to the first one, thus explaining why there are more comments in this sub-category.

“Relevance of samples” was a category that was important to students in both experiments. The students valued the relevance of the samples which was supported with comments like:

“This experiment involves analyzing something in which most college students can relate to and one in which they are probably in contact with everyday.”

“I would choose to use the solid phase extraction cartridge and spend the extra time.”

“Important to see everything involved in sample preparation and see difficulty of using real soil samples. Spiked samples lack the adventure.”

“Interesting to study something that affects us or people we know.”

These comments indicated that students were interested in working with real samples. For both experiments, there were comments about transferring the experience they have gained in the laboratory to what they can use in their future careers. These are grouped in the category “Future benefit.” Comments representative of this category included:

“Workplace requires real samples and not spiked ones. Need to learn true analytical process.”

“Familiarity with the equipment and technology will transfer anywhere I go.”

It has been demonstrated that these experiments benefited the students because they have acquired valuable skills, introduced them to general operational procedure, and analysis on these modern instruments.

Out of the four students who participated in this study, three of them took the course because of personal interest. Only one person took the course because it was a

requirement for graduation. They recommended the first experiment “FTIR Analysis of Mainstream Cigarette Smoke” for use with future students, because it was interesting and different from other laboratory experiments for technical experience and because they could relate to it. However, for the second experiment, “Extraction of Organic Pollutants from Soil Samples for GC-MS Analysis,” only those who took it for interest purposes (three out of four) said they would recommend it for future students. The one student who did not recommend the experiment was also the one taking the course as a requirement. He believed he was required to spend more time on the GC-MS experiment than he thought was reasonable for the amount of credit earned.

### **Interview**

The one student who was interviewed took the course because he wanted to learn analytical techniques which would be useful in his prospective job in the chemical industry. He was excited to know the identity of some of the toxicants present in cigarette smoke, because he had a close personal friend who smokes. This experience initiated an interest in him for further research work in this area and he started thinking of doing future work on the analysis of toxicants present in smokers’ exhaled breath. This supports the fact that these experiments would have a positive benefit for students in the future.

In terms of the content of the experiments, this student said the FTIR experiment helped him understand Beer's law more and he could see how it could be applied in industry for quantitating different compounds. He preferred the format of reporting the

laboratory reports, as was done for the experiments, where he presented the results to the whole class orally using PowerPoint slides, rather than just written laboratory reports.

## **CHAPTER 5**

### **CONCLUSIONS**

It is clear from results obtained that students were excited to work on problems they could relate to. In the GC-MS experiment, students indicated they were willing to use real samples instead of spiked ones and in the FTIR experiment, they were excited to analyze cigarette smoke. This is consistent with earlier studies that showed that students benefit by using real world samples, which they could relate to, and introduced them to analytical techniques using instruments. The students also felt that these laboratory experiments were more hands-on than some other laboratories that they have been involved in. By hands-on, they mean they had to operate the instruments themselves and thus troubleshoot and interact with the equipment for a reasonable amount of time. This was more than what they had in other laboratories where they only ran their samples quickly because of the number of students in the class waiting to use the instrument. They indicated they were happy to have a more authentic experience with the FTIR and GC-MS. Therefore, two main aspects of the experiments the students liked were the opportunity to have hands-on experience with modern instruments and the use of relevant samples. The laboratory experience was interesting to the students when they could relate to what was being done and really get to analyze the samples themselves.

With little or no prior knowledge of these instruments, students benefited immensely from doing these experiments. They were able to use and understand the FTIR and GC-MS to analyze their samples and also saw the application of Beer's law and

polarity. These experiments are, therefore, quite appropriate for introducing undergraduate students to methods of analysis on modern instruments, namely IR and GC-MS as indicated by this set of students. After performing these experiments, the students all confirmed they had been introduced to methods of analysis and troubleshooting on these modern instruments. These students were able to effectively use these instruments without damaging them, which is consistent with the literature. At the same time, they were introduced to key analytical procedures and techniques.

These experiments were designed to introduce students to the application of key concepts, such as Beer's law in analysis. Participants in this project indicated they were able to apply Beer's law to calculate concentrations of toxicants in cigarette smoke by relating concentration and absorbance in the FTIR experiment. In the GC-MS experiment, they focused more on sample preparation, but they were able to understand why different SPE cartridges were used, based on the concept of polarity. Thus, the application of key concepts such as the Beer's law and polarity were achieved and the experimental procedures were successful in introducing students to the principles of sample preparation in the GC-MS experiment. These laboratories are potentially effective for encouraging students to apply key concepts they have learned in the classroom to laboratory work. Therefore, extensive interaction with these instruments has the potential to enhance the understanding of key concepts such as spectrophotometry.

From the outcome of the interview with one student, it can be said that using real-world samples in the laboratory can help in developing students' interest in research. It brings their real world experience into relation with intellectual skill acquired as they

gain new knowledge. Students can create their own value and enhance their appreciation of environmental issues by using these samples in the laboratory. These experiments proved valuable to the students and effectively enhanced their enthusiasm about science, which included enriched interest in research.

These are worthwhile experiments for use in upper level environmental or chemistry laboratories. They aid students in the understanding of IR and GC-MS analyses. They are interesting and rewarding and could be used to attain higher cognitive skills, such as application of concepts taught in class, and the evaluation of analytical data. These skills are expected of upper level students and were achieved via these experiments by the application of key concepts to analyze real samples. These experiments could also be effective in enhancing the understanding of key spectroscopic and chromatographic concepts to analysis. These experiments supported earlier research that showed that cigarette smoke is a good sample to use for teaching important analytical principles.<sup>68,98,99</sup>

More detailed operational instructions, better computer operational procedures, and more background information on the basic concepts should be provided for future use. The amount of time required for these experiments, especially the GC-MS experiment, should also be revisited for future use of these experiments in the laboratory. Another area of improvement for the experiments could be the inclusion of discussion questions in the background information on the handouts, to help students put their thoughts together before beginning the experiments.

Overall, students had a positive attitude towards these laboratory experiments and the content of the experiments was appropriate for this level of students in achieving the



stated objectives. The experiments were meaningful and worthwhile for the students, because of the use of real samples and the opportunity to work on modern instruments. One student indicated a direct connection of this laboratory experience to an increased interest in pursuing research. This indicated that research interests could be enhanced by exposing students to the use of real world samples and analysis using modern instruments such as the FTIR and GC-MS.

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**APPENDIX A**  
**ASSESSMENT HANDOUTS**

Consent form, questionnaire, and the surveys that were handed out to the students after performing the two experiments, “FTIR Analysis of Mainstream Cigarette Smoke” and “Extraction of Organic Pollutants from Soil Samples for GC-MS Analysis” are included in this appendix.

## CONSENT AND RELEASE FORM

Dear Student,

I am currently working towards obtaining my doctorate degree in chemistry at MTSU. As part of my research, I have developed two experiments that I hope will be useful in environmental chemistry laboratories. In order to assess their effectiveness, I would like to ask you to fill out a survey for each of the experiments, FTIR analysis of mainstream cigarette smoke and extraction of organic pollutants from soil samples for GC-MS analysis.

This survey does not count as a grade in this course, participation is voluntary, and you could decide not to participate or withdraw at any time. The two surveys should not take you more than thirty minutes.

### IF YOU CHOOSE TO PARTICIPATE:

During this laboratory period, you will be given two surveys. One on each of the following experiments:

- (a) FTIR analysis of mainstream cigarette smoke.
- (b) Extraction of organic pollutants from soil samples for GC-MS analysis.

In each survey, you will be given a few questions about each experiment to answer, in order to ascertain the effectiveness of these experiments in achieving their goals. Results obtained from this study will be analyzed and summarized to be included in my dissertation.

Please note that this consent form and the surveys will be kept confidential. Your name or any personal information will not be included in any permanent record. Only results obtained from the analysis of this survey will be included in my dissertation and any journal or presentation.

### IF YOU CHOOSE NOT TO PARTICIPATE:

Participation in this study is voluntary and if you choose not to participate, your grade will not be affected in any way and your information will not be included in this study.

Please indicate your consent or otherwise below.

\_\_\_\_\_ I give my consent to participate in this study. I have read and understand the purpose of this research and I am doing this voluntarily. I understand that I can withdraw at any time and my grade in this class will not be affected in any way.

\_\_\_\_\_ I choose not to participate in this study and I understand that my grade will not be affected in any way.

Name: \_\_\_\_\_  
 Signature: \_\_\_\_\_  
 Date: \_\_\_\_\_

**General Directions - Please read.**

Please answer the questions on the attached sheets and try to give the best answer as accurately as possible to each question as it applies to you.

You have been assigned a number, which you should please indicate at the top of your surveys.

If you have any comments, please contact me (Bola Oladipupo). My email address is [oo2b@mtsu.edu](mailto:oo2b@mtsu.edu)

## CIGARETTE SMOKE AND SOIL EXPERIMENTS

### QUESTIONNAIRE

Please answer each question as accurately as it applies to you.

1 Strongly agree      2 Agree      3 Disagree      4 Strongly disagree

- |   |   |   |   |   |
|---|---|---|---|---|
| 1. I enjoyed the two experiments.   | 1 | 2 | 3 | 4 |
| 2. The background material provided was accurate.   | 1 | 2 | 3 | 4 |
| 3. I had a good understanding of FTIR and GC-MS before doing these experiments.   | 1 | 2 | 3 | 4 |
| 4. These experiments introduced me to valuable real world analytical techniques and procedures.   | 1 | 2 | 3 | 4 |
| 5. I enjoyed having the opportunity to use the FTIR and GC-MS on my own.  | 1 | 2 | 3 | 4 |
| 6. I like the challenge of interpreting IR and mass spectra.  | 1 | 2 | 3 | 4 |
| 7. The IR and MS spectral libraries (i.e. NIST, Wiley, Ref5) were valuable resources for the FTIR and GC-MS experiments.  | 1 | 2 | 3 | 4 |
| 8. I like the idea of presenting my results, better than writing reports for each lab.  | 1 | 2 | 3 | 4 |
| 9. It took me a longer time for each experiment than is expected for the laboratory session that counted as 1 credit hour.  | 1 | 2 | 3 | 4 |
| 10. Considering other things I have to do, such as work and other courses with laboratory components, I prefer to do short experiments, that will not take more than 3 hours, even if I will not learn as much as I will, in open-ended laboratories. | 1 | 2 | 3 | 4 |

11. What aspect(s) of these experiments do you really like? Why?

12. What aspect(s) of these experiments do you think should be improved? How?

**FTIR ANALYSIS OF MAINSTREAM CIGARETTE SMOKE****SURVEY**

Assigned number: \_\_\_\_\_

Age: \_\_\_\_\_

Year/Level at school (junior, senior): \_\_\_\_\_

Please give short answers to the following questions:

- (1) Have you studied the concept of Beer's law in any of your courses? If so, when?
  
- (2) Do you think you have applied the knowledge of Beer's law in this experiment?
  
- (3) What aspect of Beer's law has this experiment reinforced for you?
  
- (4) What can you say about the "relevancy" of this experiment?
  
- (5) Do you feel this experiment has added to your skill of using modern instruments for environmental analysis?
  
- (6) How would you compare your understanding of spectroscopy through this experiment, to the understanding you got from the quantitative analysis laboratory experiment you did on the determination of orthophosphate ion, using the spectrophotometer?

- (7) Would you say this experiment has been interesting and rewarding? Explain briefly.
  
- (8) Do you think this experiment could help you, if you find yourself working in an analytical/environmental laboratory in the future? Please explain.
  
- (9) Why did you take this course?
  
- (10) Would you recommend this experiment for use by future students? Why or why not?
  
- (11) Any other comment or suggestions about this experiment?



**EXTRACTION OF ORGANIC POLLUTANTS FROM SOIL SAMPLES  
FOR GC-MS ANALYSIS**

**SURVEY**

Assigned number: \_\_\_\_\_  
Age: \_\_\_\_\_  
Year/Level at school (junior, senior): \_\_\_\_\_

Please give short answers to the following questions:

- (1) How would you consider the analysis of real soil samples compared to spiked samples?
  
  
  
  
  
  
  
  
  
  
- (2) What are the key concepts that this experiment has taught you or reinforced for you?
  
  
  
  
  
  
  
  
  
  
- (3) Specifically, what did you learn from using the various SPE cartridges for sample clean up?
  
  
  
  
  
  
  
  
  
  
- (4) If you have a choice, would you be willing to spend the extra time for SPE clean up of real soil samples as you did in this experiment, or you will prefer to work with synthetic samples requiring no clean up procedures? Explain.
  
  
  
  
  
  
  
  
  
  
- (5) Has this experiment taught you anything about analysis on the GC-MS?

- (6) Would you say this experiment has been interesting and rewarding in terms of what you learn from it? Explain briefly?
  
- (7) Would you recommend this experiment for use by future students? Why or why not?
  
- (8) Any other comment or suggestions about this experiment?

**APPENDIX B**  
**LABORATORY HANDOUTS**

This appendix contains the handouts for the two experiments, “FTIR Analysis of Mainstream Cigarette Smoke” and “Extraction of Organic Pollutants from Soil Samples for GC-MS Analysis”.

## FOURIER TRANSFORM INFRARED SPECTROMETRIC (FTIR) ANALYSIS OF MAINSTREAM CIGARETTE SMOKE

### Objectives:

- To introduce students to the instrumentation and application of the FTIR spectrometry.
- To introduce students to procedures for analyzing gaseous environmental samples such as cigarette smoke.
- To introduce students to the use of standard reference spectra in the National Institute of Standards and Technology (NIST) and the Environmental Protection Agency (EPA) spectral libraries.
- To introduce students to procedures for calculating analyte concentrations in gaseous samples using the univariate method of Beer's Law.

### Introduction:

There are two types of cigarette smoke, namely the mainstream smoke and the sidestream smoke. The mainstream smoke is the smoke that goes through the length of the cigarette that the smoker inhales, whereas the sidestream smoke is the smoke that goes out from the tip of the cigarette to the environment and which is also referred to as the environmental tobacco smoke (ETS). These two types of cigarette smoke have similar constituents that are present in different concentrations. However, both types are very harmful.

Furthermore, cigarette smoke contains constituents that could be present either in the gaseous or the particulate matter phases. Gaseous components are present in the gaseous phase, while the higher molecular weight compounds are present in the particulate phase. These gaseous components include such compounds as carbon monoxide, methane, hydrogen cyanide and the particulate matter contains components such as tar. Some gaseous components are present in trace levels in the cigarette smoke and hence, a long optical pathlength is essential for their IR spectrometric analysis. Multiple reflections of the IR beam in White cells are usually used to increase the optical pathlength for FTIR analysis. According to Beer-Lambert's law, the increased pathlength leads to increased absorbance for a given analyte concentration. Hence, long pathlength cells provide greater sensitivity required for the analysis of trace pollutants.

Gaseous components are sampled into the gas cell, their spectra are obtained using the FTIR, and data analysis is performed on the data obtained. Different databases of IR reference spectra are available for the comparison of sample spectra and reference spectra, in order to calculate the concentration of the target analyte.

These databases include libraries from the National Institute of Science and Technology (NIST), the Environmental Protection Agency (EPA) library, and "QASoft" developed by Infrared Analysis, Inc. The NIST library has reference spectra of about 21 volatile organic compounds available at resolutions of  $0.125\text{ cm}^{-1}$ ,  $0.25\text{ cm}^{-1}$ ,  $0.5\text{ cm}^{-1}$ ,

1.0  $\text{cm}^{-1}$ , and 2.0  $\text{cm}^{-1}$  using five apodization functions. The spectral library from EPA has reference spectra of over 100 common air pollutants available only at a resolution of 0.25  $\text{cm}^{-1}$  and most of these EPA spectra are recorded for at least two concentrations for each compound. The commercial IR spectral “QASoft” library contains reference spectra of about 269 compounds at resolutions of 0.25  $\text{cm}^{-1}$ , 0.5  $\text{cm}^{-1}$ , 1.0  $\text{cm}^{-1}$  and 2.0  $\text{cm}^{-1}$ .

In this experiment, the NIST and the EPA databases will be used. For this experiment, students will use a 2.4-meter gas cell that is available commercially. It is a multi-pass gas cell, with a pathlength of 2.4 meters folded into a compact volume of 120  $\text{cm}^3$ .

**Procedure:**

- Obtain liquid nitrogen from the NMR room behind the computer lab and pour into the designated chamber on the Magna 550 FTIR, to cool its MCT detector.
- Turn on the Magna 550 spectrometer and open the OMNIC software for FTIR data acquisition.
- Obtain the 2.4-meter gas cell and with the aid of the pump, evacuate the gas cell to 30 mm Hg of vacuum. Collect the background spectra of the evacuated gas cell on the spectrometer, using the conditions given on the table below in the “Collect Setup” menu and name the file as “24M050xy”. “24” refers to the pathlength of the gas cell, “M” refers to Magna FTIR, “050” refers to the spectrometer resolution, and “xy” will be your initials.

• Instrument	• Magna 550
• Number of scans	• 100
• Resolution	• 0.5 $\text{cm}^{-1}$
• Final Format	• Absorbance
• Correction	• None
• Zero filling	• None
• Apodization	• Happ-Genzel

- Obtain a new Tedlar bag and set it up in the “suitcase” for sample collection. Connect the sampling pump to the vacuum port of the “suitcase” and obtain an American Spirit cigarette and mark it with a pencil 23 mm from the mouth end of the cigarette. Wrap some Teflon tape around the mouth end of the cigarette, so as to fit firmly into the sample port and attach it to the sample port of the “suitcase.” With the aid of a calibrator, calibrate the flow rate to 1.05 L/min. Use Teflon tape at every connection to ensure the set-up is airtight. Open the valve on the Tedlar bag in the “suitcase” and close the “suitcase” completely. Start the pump and light the cigarette. Allow the cigarette to burn, up to the 23 mm mark and stop the pump, as well as close the valve on the bag.

Make sure the valve on the bag is closed before detaching the pump and cigarette connections at the end of sample collection.

- Allow the bag to sit for about five minutes to allow the settling of any particulate matter present in the cigarette smoke so that it will not contaminate the cell during subsequent transfer of gas sample. After five minutes, attach the evacuated 2.4-meter gas cell to the sample inlet end of the suitcase and open the Tedlar bag valve to transfer some cigarette smoke into the gas cell.
- Take the gas cell to the spectrometer and analyze the smoke sample using the same conditions as you have in the table above. After the sample spectra are obtained, save them using filenames of “IRxy”, with xy still referring to your initials.
- Compare your sample spectrum with the reference spectrum from NIST for the identification and quantitation of constituents in the smoke sample. Use the NIST reference spectrum at  $0.5\text{ cm}^{-1}$ .
- For the quantitative analysis of cigarette smoke, one has to choose the compounds that are more likely to exist in the gas phase at ambient conditions. Most of the gas phase compounds are related to combustion and compounds with less than five carbons or boiling points of less than  $80^{\circ}\text{C}$  are usually present in gas phase samples. Please see the table given below. Apply the concept of volatility and choose from the given list of compounds with their boiling points.
- Compare the sample spectra and reference spectra in the “fingerprinting” spectral regions of the compounds chosen and apply Beer-Lambert’s Law for quantitative determination.

Compound	Boiling Point ( $^{\circ}\text{C}$ )	Melting Point ( $^{\circ}\text{C}$ )
Carbon monoxide	-191.5	-205.05
Methane	-161.45	-182.47
1,3 butadiene	-4.4	-108.9
Catechol	245	104

## EXTRACTION OF ORGANIC POLLUTANTS FROM SOIL SAMPLES FOR GC-MS ANALYSIS

### Objectives:

- To introduce students to the methods for sample preparation, extraction, and clean-up for GC-MS analysis.
- To compare the effectiveness of various extraction solvents for use with the soil samples.
- To become familiar with the differences between various extraction methods, such as the mechanical shaking and the ultrasonic bath methods, used for the extraction of PAHs from soil samples.
- To introduce students to the principle of solid phase extraction (SPE) as a sample clean-up method and to the use of internal standards in analysis.

### Introduction:

Different soil samples have different compositions of sand, silt, and clay. It is expected that clay will adsorb pollutants more strongly than silt and sand. There are however, different ways in which soil can become contaminated. One group of compounds that can be present in contaminated soil is called polycyclic aromatic hydrocarbons (PAHs).

Structurally, PAHs are molecules that contain two or more aromatic rings that are fused together. They include anthracene, 1,2-benzo(a)pyrene, naphthalene, and coronene. There have been many studies related to benzo(a)pyrene and it has been found to be a cancer-causing substance. Many of these PAHs are able to form metabolites that can react and bind with cellular DNA and eventually lead to the formation of tumors and cancer. PAHs are found in tobacco smoke, chimney soot, and even barbecued meat. Therefore, there have been interests in reducing the levels of PAHs in the environment. The United States Environmental Protection Agency and the European Community have both listed PAHs as priority pollutants.

In order to extract PAHs from soil samples, a number of different solvents are used and they depend on the extraction method employed. The various extraction methods used for the efficient extraction of organic pollutants from soil samples include sonication, Soxhlet extraction, KOH-digestion, mechanical shaking, supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE).

After extracting the PAHs from the soil sample with the appropriate method, a choice has to be made as to the right clean-up procedure to use. One of these methods is the use of solid phase extraction (SPE). Different SPE cartridges can be used to separate the components of the soil matrix based on analyte polarity. SPE is commonly used in sample clean-up and it has replaced the liquid-liquid extraction that requires a large amount of solvent for extraction. The selection of SPE tubes is based on the polarity of adsorbent, analyte polarity, and the polarity of the solvent containing the analyte. These cartridges are also chosen based on their capacity in terms of the weight of adsorbent.

Larger weights are used for samples containing larger amounts of analyte. The three major types of extraction modes are: reversed phase, normal phase, and ion exchange. For the normal phase mechanism, the tube packing, which is the stationary phase, is polar and the solvent, which is the mobile phase, is non-polar. As the analytes pass through the adsorbent, the more polar components are adsorbed more strongly and the non-polar compounds pass through more readily. Reversed phase SPE tubes contain non-polar packing that retains non-polar analytes longer than polar analytes in a polar solvent. Choosing the right packing type and using the appropriate eluent, in which the compounds of interest are soluble and of the right polarity, elution of target compounds from the adsorbent is achieved. Some of the most common adsorbents are alumina, silica, C18, and C8. In order to effectively desorb analytes from a given adsorbent, the polarity of the eluent is important. For the silica cartridge, the elutropic series, which refers to the solvent strength in desorbing analytes from the adsorbent, have the following order: dichloromethane < acetone < methanol. Since silica is polar and will retain more polar analytes, moderately polar solvent is required to elute the analytes from it.

In this experiment, you will be provided with soil samples from one of the Superfund sites at Hickman County. These samples will be provided in airtight jars and you will be required to work individually.

#### **Procedure:**

- Weigh out about 1-2 g of each of the four soil samples into separate Erlenmeyer flask.
- Pipette 5 mL of your assigned solvent(s), which is either methanol, acetone or methylene chloride, into the soil samples.
- For adequate solvent extraction, divide your samples into two sets. Put one set on a mechanical shaker and the other set in an ultrasonic bath for an hour. After one hour, spike each soil sample with 20  $\mu$ L each of bromobenzene and bromohexadecane.
- Carefully filter the liquid extracts in the Erlenmeyer flasks and transfer the filtrates into centrifuge tubes, with each tube carefully labeled with the sample identification, in order to avoid mix up.
- Centrifuge the filtrates for about five minutes and transfer the supernatant fluid into a clean test-tube for further clean up.
- Set up the solid phase extraction (SPE) tubes for sample extraction. For your first sample set, you will use the LC-Si SPE cartridge and Sep-Pak cartridge will be used for the other set.
- Condition your SPE tubes first with 5 mL of methanol, followed by 5 mL of your assigned solvent that was used for your extraction. Obtain a blank for each type of SPE tube you use, by collecting the eluant from the tube when your solvent was passed through. Concentrate this to 1.5 mL by bubbling high purity nitrogen gas through it and



transfer the extract to a properly labeled amber vial, followed by crimping, for GC-MS analysis.

- Set up your SPE tubes on the SPE manifold and condition them with 5 mL distilled water and then your solvent. Draw your samples in the test tubes into 10 mL syringes and attach to the top of the SPE tubes, with the help of adapters. Discard any liquid collected.
- Elute your SPE tube with 2 mL of your assigned solvent and transfer the eluate into well-labeled 2 mL amber vials, for GC-MS analysis.
- A splitless injection volume of 0.5  $\mu\text{L}$  of sample should be injected into the GC-MS and analysis should be performed at a helium flowrate of 0.8 mL/min. The initial temperature should be set at 50  $^{\circ}\text{C}$  and the final temperature at 280  $^{\circ}\text{C}$ . The GC oven-heating rate should be 20  $^{\circ}\text{C}/\text{min}$ . Other conditions will be pre-set for you.
- Once you obtain your data, spectral matching using the NIST library database with the sample solutions should be performed, and afterwards report those components with match index above 80%.

## **APPENDIX C**


### **PERMISSION FROM THE INSTITUTIONAL REVIEW BOARD**

This appendix contains a copy of the letter obtained from the Institutional Review Board at MTSU, giving the permission to administer the research using human subjects.



Dr. Robert B. Blair, CPS/CAP, MOUS, NBCT  
Business/Aerospace Building N438  
(615) 898-5284 (O)  
(615) 898-5438 (F)  
rbblair@mtsu.edu

P.O. Box 40  
1301 East Main Street  
Middle Tennessee State University  
Murfreesboro, TN 37132

**TO:** Omobola Oladipupo  
**FROM:** Robert B. Blair   
**DATE:** April 23, 2004  
**RE:** Fourier Transform Infrared Spectrometry Analysis of Cigarette Smoke and Extraction of Organic Pollutants for Gas Chromatography-Mass Spectrometry Analysis (Protocol No. 04-210)

The Middle Tennessee State University Institutional Review Board (IRB), or a representative of the IRB, has reviewed your research proposal identified above. It has determined that the study poses minimal risk to subjects and qualifies for an expedited review under 45 CFR 46.110 and 21 CFR 56.110. Any change to the protocol must be submitted to the IRB before implementing the change.

Please note that any unanticipated harms to subjects or adverse events must be reported to the Office of Sponsored Programs at 615.898.5005.

Approval is granted for 10 subjects based on the maximum number submitted in the protocol. Final approval is for one (1) year from the date of this memorandum. You will be required to submit an end-of-project report to the Office of Research and Sponsored Programs upon completion of your project.

C: Office of Sponsored Programs  
Dr. Ngee-Sing Chong

**Final Approval: April 23, 2004**

**Jennings A. Jones College of Business**

MTSU, a Tennessee Board of Regents institution, is an equal opportunity, non-racially identifiable, educational institution that does not discriminate against individuals with disabilities.