THE GAS/PARTICLE PARTITIONING OF AMMONIA AND NICOTINE IN MAINSTREAM TOBACCO SMOKE AND ITS IMPLICATIONS FOR ACID/BASE CHEMISTRY OF TOBACCO SMOKE

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This dissertation "The Gas/Particle Partitioning of Ammonia and Nicotine in Mainstream Tobacco Smoke And Its Implications for Acid/Base Chemistry of Tobacco Smoke" by Cai Chen has been examined and approved by the following Examination Committee:

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ABSTRACT

The Gas/Particle Partitioning of Ammonia and Nicotine in Mainstream Tobacco Smoke
And Its Implications for Acid/Base Chemistry of Tobacco Smoke
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Nicotine is recognized as the tobacco component that is responsible for most if not all of the addictive nature of tobacco. In mainstream tobacco smoke (MTS), the amount of nicotine that is in the free-base form is generally believed to be well correlated with physical "impact", "strength", and "harshness" of such smoke. There are also reasons to expect that the amount of free-base nicotine may be related to the addictiveness of tobacco smoke. Evidence from previously secret tobacco industry documents indicates that ammonia-producing compounds have been added to cigarette tobacco as "impact boosters". Knowledge of the acid/base chemistry of tobacco smoke is required for a proper understanding of the effect of ammonia additives on nicotine chemistry in MTS. The goal of this work was to improve our understanding of this chemistry by studying the interdependent gas/particle (G/P) partitioning of nicotine and ammonia in MTS, including the study of components in MTS that are related to the chemistries of nicotine and ammonia (organic amides).

In the theoretical portion of this work, equations describing the interdependent G/P partitioning of ammonia and nicotine in MTS were derived using established acid/base theory, together with G/P partitioning theory. The G/P partitioning coefficient of free-base ammonia ($K^{a}_{p,fb}$) and the activity coefficient of ammonia were estimated using existing data, including values of the G/P partitioning coefficient of free-base nicotine ($K^{n}_{p,fb}$). It was predicted that $\log K^{a}_{p}$ and $\log K^{n}_{p}$ will tend to be linearly correlated for typical MTS samples.

In the experimental portion of this work, samples from eleven brands of cigarettes and two brands of cigar-like products were machine-smoked according to a standard protocol and the MTS was collected. The levels of ammonia and nicotine in both the gas phase and particulate matter (PM) of MTS were measured along with other parameters, including the levels of water and other acid-base relevant components in the PM. The analytical methods used were specially developed for this work. For example, extraction of MTS PM for determination of ammonia was carried out using 2-propanol to avoid hydrolysis of labile, ammonia-containing smoke compounds such as amides. Generally, the cigar-like products delivered higher per-puff amounts of ammonia than did the cigarettes; the delivered levels of nicotine were similar across all products tested. The measured $\log K_p^n$ were found to be negatively correlated with total ammonia delivery, suggesting that increasing ammonia levels in MTS can increase the percentage of the nicotine that is found in the gas phase.

During the consideration of the advantages of 2-propanol as the extraction solvent for ammonia from MTS PM, it was confirmed that the use of water (with or without added acid) could trigger the ammonia-releasing reactions during extraction. Several lines of evidence were obtained that strongly suggest that these reactions are hydrolytic in nature. For example, the release rate increases with the acidity of the aqueous extraction solvent. Possible tobacco smoke reactants for the hydrolysis reactions include amides, nitriles and other neutral nitrogen-containing compounds. The presence of two specific amides was confirmed by two-dimensional gas chromatography (GCxGC) gas chromatography coupled to a time of flight mass spectrometer (ToFMS). Since the formation of an amide by combination of ammonia with an organic acid will serve to neutralize the acid, the presence of amides in tobacco smoke is highly relevant to the study of the acid/base dependent G/P portioning of both ammonia and nicotine.

The experimentally observed correlation of $\log K_{\mathrm{p}}^{\mathrm{a}}$ *vs.* $\log K_{\mathrm{p}}^{\mathrm{n}}$ is highly consistent with theoretical predictions made here. This confirms the importance of acid/base chemistry in controlling the G/P partitioning of both ammonia and nicotine in MTS. This conclusion also indicates that the theories and assumptions developed here are generally appropriate for the study of acid/base chemistry of MTS.

CHAPTER 1
INTRODUCTION

1.1 Background

1.1.1 A brief history: the epidemic of tobacco use

There are more than 90 known tobacco species in the genus Nicotiana, which belongs to the Solanaceae family of plants [D'Acry, 1991]. The majority of the known tobacco species were found in the continents of Americas [Winter, 2000]. The earliest tobacco consumers were American Indians, who used both wild and cultivated tobacco for religious and medicinal purpose, but rarely for relaxation [Elferink, 1983; Spinden, 1950]. Archaeological evidence suggested that the cultivation of tobacco by American Indians can be dated to around 6000 B.C. [WHO, 2002]. The discovery of the New World by Columbus started the spread of tobacco from the Americas to Europe, first to Spain, then to England, Portugal and France. European traders helped disperse tobacco throughout Asia and Africa. It took about 150 years for the "strange leaves" to be distributed worldwide [WHO, 2002].

In the following centuries, more and more people adopted tobacco use. With the invention of the cigarette rolling machine, the introduction of blends and curing process that allowed easier inhalation of tobacco smoke, the invention of the safety match, the improvements in mass production, transportation that permitted widespread distribution of cigarettes, and the use of mass media advertising to promote cigarettes, the consumption of tobacco products boomed in the early 20th century. The cigarette became the predominant tobacco products by the 1920s [CDC, 1999; Kluger, 1997]. Other smoking tobacco products include the cigar, biddi, chutta, and kretek [IARC, 2004]. Worldwide, cigarette consumption grew from only a few billion per year in 1900 to approximately 5.5 trillion by 2000. In the United States, annual per capita cigarette

consumption increased from 54 cigarettes in 1900 to 4345 cigarettes in 1963, then decreased to 2261 in 1998 [CDC, 1999].

In the early stage of its spread in Europe, tobacco was considered as a plant with curative powers. In his letter to the Queen of France, Jean Nicot described tobacco as a panacea. However, with its further adoption and popularity among Europeans in the 1600s, this new hobby did not reveal any marvelous cures, but rather its adverse and addictive effects. In 1610, Sir Francis Bacon noticed the increasing tobacco use in England and the addictive nature of such use. In the following three centuries, many health hazards, ranging form headache, to indigestion, to cancer, to vascular disease were linked to tobacco use [Lane, 1845; Doll, 1998; Proctor, 2004]. However, because many of those claims were based on minimal scientific evidence, it's hard to say those claims had significant impact on the attitude on the use of tobacco. At the same time, the use of tobacco was banned in many area of the world, mainly because of moral concerns. However, in most cases, those tobacco bans were quickly lifted for economic reasons.

The major breakthrough in recognizing the health impact of tobacco use occurred with the causal link between tobacco smoking and lung cancer being well established in the 1950s [Doll, 1998; Proctor, 2004]. Epidemiological studies, animal experiments, and studies demonstrating pathologic changes in lung tissues at autopsy were three pivotal sources of evidence [Proctor, 2004]. Milestones for those scientific findings include the studies by Ernest Wynder and Evarts A. Graham [1950] in the United States and by Richard Doll and A. Bradford Hill [1950] in England. Another milestone was the publication of the first Surgeon General's Report on smoking and health in 1964 [USDHHS, 1964]. On the basis of around 7000 articles relating to smoking and disease, this report concluded that cigarette smoking was a cause of lung and laryngeal cancer in men, a probable cause of lung cancer in women, and the most important cause of chronic bronchitis in both sexes. It was regarded as an important factor contributing to the decreasing prevalence of tobacco smoking since the 1960s. After the first Surgeon General's Report on tobacco smoking, more evidence emerged. The publication of other Surgeon General's Reports on tobacco smoking in following years recognized the link between cigarette smoking and more disease on human health [USDHHS, 1989]. The

latest Surgeon General's Report on this topic concluded that tobacco smoking is harmful for almost all organs in human body [USDHHS, 2004].

Today, despite the established and well-publicized links between tobacco use and adverse health effects, tobacco use is still one of the major epidemics threatening public health of human beings, and the tobacco industry is still one of the world's largest industries, with cigarettes accounting for 96 percent of total sales [WTO, 2002]. In the year of 2000, world tobacco production was 5,883,324 tons, and 5,573 billion of cigarettes were consumed worldwide [USDA, 2001]. For the year 2000, it was estimated that there were more than 1.2 billion smoker worldwide, including 1.0 billion males and 0.2 billion females, amounting to 47.5% and 10.3% of the population aged 15 years and older in each category [Guindon, 2003]. Tobacco causes one-third of all cancer deaths in developed countries [Peto J, 2001]. Tobacco use is projected to cause nearly 450 million deaths worldwide in next 50 years [Peto R, 2001]. A 40-year study indicates that lifelong smokers have a two-three times greater risk of dying prematurely than those who never smoked [Doll, 1994]. Based on 10 more years of study, Doll et al. [2004] confirmed their previous conclusion, and found that cessation at age 50 halved the hazard, and cessation at age 30 avoided almost all of it. In the United States, approximately 21.6% of adults were smokers in 2003 [CDC, 2005]. A study showed that the leading cause of death in 2000 was tobacco use. It counted 18.1% of total US deaths. This was about 10 times higher than motor vehicle accidents [Mokdad, 2004]. During 1995-1999, smoking caused approximately 440,000 premature deaths in the United States annually and approximately $157 billion in annual health-related economic losses [CDC, 2002].

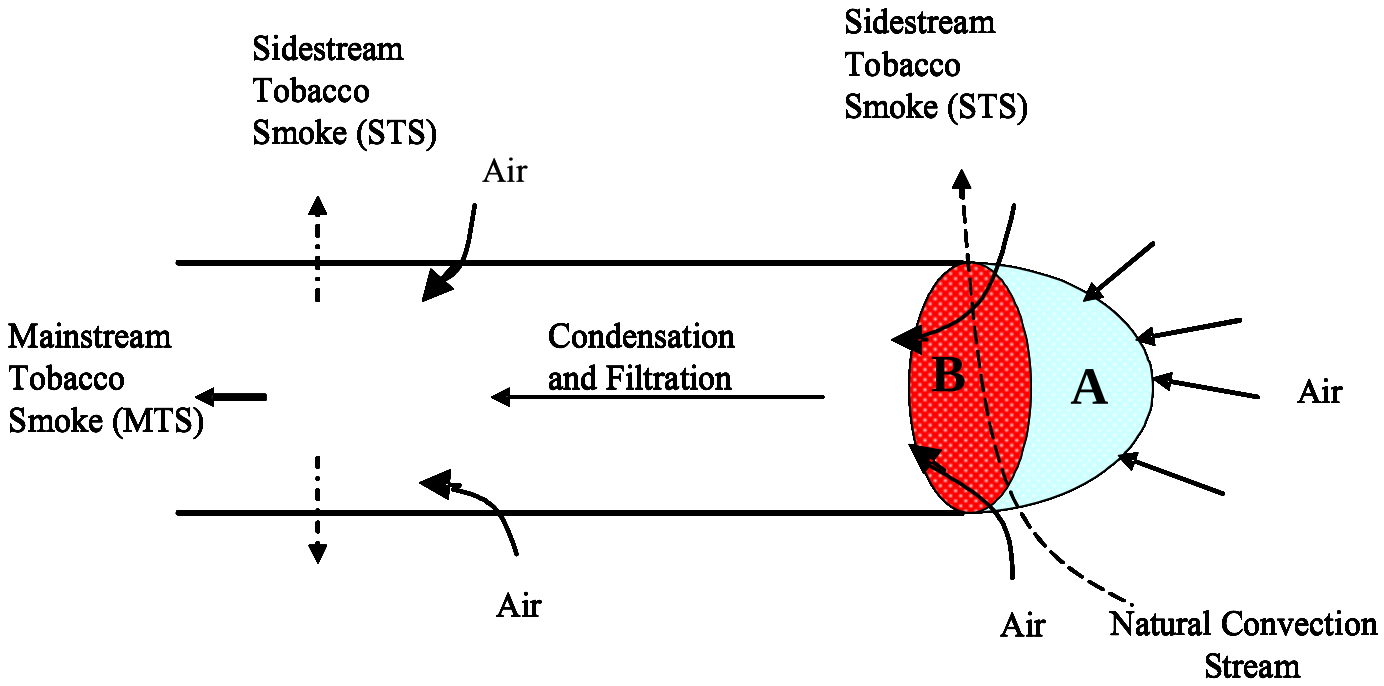
1.1.2 Overview: tobacco smoke and its chemical composition

Although there are various ways to use tobacco, the major practice is the smoking of tobacco, with cigarettes as its dominant form. When a cigarette is lighted and smoked, two types of burning take place: puffing and natural smoldering between puffs. When a cigarette is being smoked, two oxygen-deficient burning zones form: the combustion zone with temperatures from 700 to 900 ºC, and the pyrolysis/distillation zone with temperature from 200 to 600 ºC (Figure 1). Major products in the combustion zone are

carbon monoxide, carbon dioxide, and water. Most of the components in smoke are generated in the pyrolysis/distillation zone [Baker, 1987]. During puffing, the highly concentrated gas mixture is drawn into and through the rest of the cigarette rod, which includes the tobacco filler, the wrapping paper, and often a filter. Because of dramatically dropping temperature, less volatile components in the smoke stream can condense onto these surfaces. They can also interact with condensation nuclei and form aerosol particles. The aerosol stream passing through the cigarette rod interacts with air that penetrates into the cigarette through the cigarette wrapping paper, and some components of the aerosol stream may diffuse out of the cigarette wrapping paper [Baker, 1987].

Mainstream tobacco smoke (MTS) is the portion of smoke drawn out of the mouth or "butt" end of the cigarette when a cigarette is puffed. Sidestream tobacco smoke (STS) is formed mostly in the intervals between puffs, and is the portion of smoke released from the burning cone or cigarette wrapping paper. Similar lists of compounds are found in MTS and STS, but the concentrations of each component can be very different. The third type of smoke, environmental tobacco smoke (ETS), or secondhand smoke, forms when STS and exhaled MTS are released and diluted into ambient air. While the primary health concern from MTS involves smokers, the primary health concern from STS and ETS involves involuntary smokers [CaEPA, 1997; Jenkins, 2000; IARC, 2004].

Cigarette smoke is one of the most highly concentrated aerosols known. One mL of fresh MTS can contain about 10^{10} particles with diameter between 0.1 and 1.0 μm (mean diameter 0.2 μm) [USDHHS 1989]. Like other pyrolysis products, cigarette smoke is a complex mixture of chemical compounds. The number of identified compounds in smoke has increased with advances in analytical methodologies [Green, 1996]. More than 4000 compounds have been identified, and these compounds account for more than 95% of the weight of MTS [Dube and Green, 1982; Green, 1996]. It has been estimated that total number of species in tobacco smoke could be as high as 100,000 [Jenkins, 2000]. To demonstrate the complexity of tobacco smoke, Dube & Green [1982] estimated the numbers of identified organic compounds in cigarette smoke in 15 groups (Table 1-1). It should be noted here that some compounds contain multiple functional groups, so that



A: Combustion zone (700 ℃ - 900 ℃); Pyrolysis/distillation zone (200 ℃ - 600 ℃)

Figure 1-1 Conceptual scheme of a burning cigarette (Adapted from Baker [1987])

Table 1-1 Approximate numbers of identified tobacco smoke organic compounds in some major compound classes*

Class	Number
Alcohols	379
Aldehydes	108
Amides, Imides, Lactams	237
Amines	196
Anhydrides	11
Carbonhydrates	42
Carboxylic Acids	227
Esters	474
Ethers	311
Hydrocarbons	755
Ketones	521
Lactones	150
Nitriles	106
N-Heterocycles	921
Phenols	282

*Adapted from Dube and Green [1982]

some compounds are included in more than one group. Inorganic and metal related compounds are also found in tobacco smoke.

Cigarette smoke is composed of two phases: gas and particulate matter (PM). Compounds in smoke could exist in both phases. The total particulate matter (TPM) in tobacco smoke has been operationally defined as that portion of the smoke collected on a conventional "Cambridge" filter pad. Material passing through the "Cambridge" filter has been operationally defined as the gas phase of cigarette smoke [Baker, 1999]. The term "tar" is often used to represent TPM minus nicotine and water. To get comparable results from different laboratories, several standardized smoking protocols have been established for the collection of MTS samples [Bradford, 1936; IARC, 2004]. The description and key parameters for the smoking protocols are listed in Table 1-2. Although these protocols, especially the FTC method, have been widely applied in the survey of the yields of chemical components in MTS, and thus the human being exposure level to those components, machine smoking protocols can never exactly reflect individual smoking behavior. Nevertheless, quantitative data of some typical components in MTS are listed in Table 1-3. Those components which are more than 50 percent appear in the gas phase

of fresh MS are considered volatile smoke constituents; all others are particulate phase components [USDHHS, 1989].

Table 1-2 Machine-smoking protocols for measuring smoke yields of tobacco products [#]

Protocol	Puff duration (sec)	Puff Interval (sec)	Puff volume (mL)	Butt length (mm)	Filter ventilation holes
Tobacco Research Council	2	60	35	25	NA
Federal Trade Commission (FTC)	2	60	35	23	Open
International Standards Organization (ISO)	2	60	35	23	Open
Massachusetts	2	30	45	23	50% blocked
Health Canada 1998-99	2	26	56	23	Fully Blocked
Health Canada 2000	2	30	55	23	Fully blocked
International Committee for Cigar Smoke Study	1.5	40	20	33	Open

[#] Adapted from the reference IARC [2004].

Given its complex composition, tobacco smoke is a challenge for analytical chemists. Traditionally, for the identification and quantification of the components, smoke samples generated using large number of cigarettes have been divided into several groups and sub-groups with multi-step fractionation processes, mostly involving liquid-liquid extraction (LLE). Each group of compounds was analyzed with specific analytical techniques [Tennessee Eastman, 1956; Swain, 1969; Hecht, 1981; Snook, 1981, 1984]. Varieties of analytical technologies have been used in analysis of tobacco smoke [Tennessee Eastman, 1956; Green, 1996; Rustemeier, 2002; Chen, 2003; Gregg, 2004]. In last few decades, each breakthrough in analytical technology led to a dramatic increase in the number of identified chemicals in smoke [Green, 1996]. Gas chromatography (GC), high performance liquid chromatography (HPLC), and mass spectrometry (MS), with its identification power, have been the most powerful tools for the analysis of tobacco smoke.

1.1.3 Nicotine in tobacco and tobacco smoke

The importance of nicotine in tobacco and tobacco smoke is apparent, especially for scientists in tobacco industry. For example, in a report to the board of Phillip Morris, the world's largest tobacco company, the Vice President of Research and Development once stated that, "In the past we at R & D have said that we're not in the cigarette business, we're in the smoke business. It might be more pointed to observe that the cigarette is the vehicle of smoke, smoke is the vehicle of nicotine, and nicotine is the agent of a pleasurable body response." [Philip Morris, 1969]. In a meeting about nicotine research, Charles Ellis, long time scientific adviser to the board of British American Tobacco company (BAT) stated that, "We are in a nicotine rather than tobacco industry." [Johnson, 1971]. In a research conference, William L. Dunn of Philip Morris wrote in his paper, "The majority of the conferees would go even further and accept the proposition that nicotine is the active constituent of cigarette smoke. Without nicotine, the argument goes, there would be no smoking." [Dunn, 1972]. In the same paper, Dunn continued, "The cigarette should be conceived not as a package. The product is nicotine. The cigarette is but one of many package layers. There is the carton, which contains the pack, which contains the cigarette, which contains the smoke. The smoke is the final package. The smoker must strip off all these package layers to get to that which he seeks."

Nicotine was first isolated from tobacco by French scientist Louis-Nicolas Vanquelin in 1809. He noted that one constitute of tobacco leave was "a peculiar acrid, volatile, colorless substance, soluble in water and alcohol, and which appears different from any thing known in the vegetable kingdom. It is this principle which gives to prepared tobacco its peculiar character, and it is perhaps not to be found in any other species of plant. Its medicinal activity is supposed to reside in this volatile portion, which is the "essential oil" [Lane, 1845]. Later studies found that "tobacco essential oil" contains at least 20 different, but related, pyridine alkaloids. Of these the dominant alkaloid is nicotine, making up nearly 88% of the total alkaloid content of some tobaccos, and it has been reported that the average nicotine content of tobacco is 1.5% by weight [Fowles, 2001].

Table 1-3 Major components of the mainstream smoke of non-filter cigarette [a]

Vapor Phase		Particulate Phase	
Compound	Mass/cigarette	Compound	Mass/cigarette
Nitrogen	280-320 mg	Nicotine	1.0-3.0 mg
Oxygen	50-70 mg	Nornicotine	50-150 μg
Carbon dioxide	45-65 mg	Anatabine	5-15 μg
Carbon monoxide	14-23 mg	Anabasine	5-12 μg
Water	7-12 mg	Other tobacco alkanoids (17)	NA[c]
Argon	5 mg	Bipyridyls (4)	10 μg
Hydrogen	0.5-1.0 mg	n-Hentriacontane (n-H31H64)	100 μg
Ammonia	10-130 μg	Total nonvolatile hydrocarbons (45)	300-400 μg
Nitrogen oxides (NO_x)	10-600 μg	Naphthalene	2-4 μg
Hydrogen cyanide	400-500 μg	Other naphthalenes (23)	3-6 μg
Hydrogen sulfide	20-90 μg	Phenanthrenes (7)	0.2-0.4 μg
Methane	1.2-2.0 mg	Anthracenes (5)	0.05-0.1 μg
Other volatile alkanes (20)[b]	1.0-1.6 mg	Fluorenes (7)	0.6-1.0 μg
Volatile alkenes (16)	0.4-0.5 mg	Pyrenes (6)	0.3-0.5 μg
Isoprene	0.2-0.4 mg	Fluoranthenes (5)	0.3-0.45 μg
Butadiene	20-40 μg	Carcinogenic polynuclear aromatic hydrocarbons (11)	0.1-0.25 μg
Acetylene	20-35 μg		

Table 1-3 (Cont'd)

Benzene	12-50 µg	Phenol	80-160 µg
Toluene	20-60 µg	Other phenols (45)	60-180 µg
Styrene	10 µg	Catechol	200-400 µg
Other volatile aromatic hydrocarbons (29)	15-30 µg	Other catechols (4)	100-200 µg
Formic acid	200-600 µg	Other dihydroxybenzenes (10)	200-400 µg
Acetic acid	0.3-1.7 mg	Scopoletin	15-30 µg
Propionic acid	100-300 µg	Other polyphenols (8)	NA
Methyl formate	20-30 µg	Cyclotenes (10)	40-70 µg
Other volatile acids (6)	5-10 µg	Quinones (7)	0.5 µg
Formaldehyde	20-100 µg	Solanesol	0.6-1.0 mg
Acetaldehyde	0.4-1.4 mg	Neophytadienes (4)	200-350 µg
Acrolein	60-140 µg	Limonene	30-60 µg
Other volatile aldehydes (6)	80-140 µg	Other terpenes (200-250)	NA
Acetone	100-650 µg	Palmitic acid	100-150 µg
Other volatile ketones (3)	50-100 µg	Stearic acid	50-75 µg
Methanol	80-180 µg	Oleic acid	40-110 µg
Other volatile alcohols (7)	10-30 µg	Linoleic acid	60-150 µg
Acetonitrile	100-150 µg	Linolenic acid	150-250 µg
Other volatile nitriles (10)	50-80 µg	Lactic acid	60-80 µg

Table 1-3 (Cont'd)

Furan	20-40 µg	Indole	10-15 µg
Other volatile furans (4)	45-125 µg	Skatole	12-16 µg
Pyridine	20-200 µg	Other indoles (13)	NA
Picolines (3)	15-80 µg	Quinolines (7)	2-4 µg
3-vilylpyridine	10-30 µg	Other N-heterocyclic hydrocarbons (55)	NA
Other volatile pyridines (25)	20-50 µg	Benzofurans (4)	200-300 µg
Pyrrole	0.1-10 µg	Other o-heterocyclic hydrocarbons (42)	NA
Pyrrolidine	10-18 µg	Stigmaterol	40-70 µg
N-methylpyrrolidine	2.0-3.0 µg	Sitoseterol	30-40 µg
Volatile pyrazines (18)	3.0-8.0	Campesterol	20-30 µg
Methylamine	4-10 µg	Cholesterol	10-20 µg
Other aliphatic amines (32)	3-10 µg	Aniline	0.36 µg
		Toluidines	0.23 µg
		Other aromatic amines (12)	0.25 µg
		Tobacco specific N-nitrosamines (4)	0.34-2.7 µg
		Glycerol	120 µg

[a]Adapted from US DHHS [1989]
[b]Numbers in parentheses represent individual compounds identified in a given group
[c]NA: not available

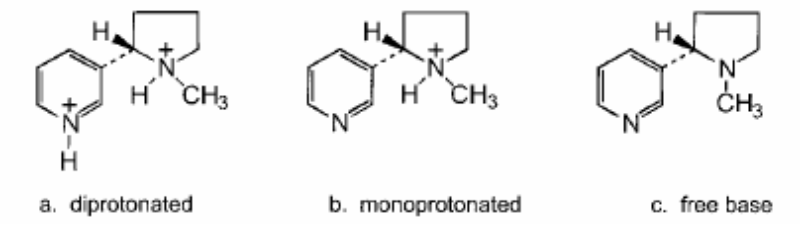


Figure 1-2 Three forms of nicotine

Nicotine is one of the most intensively studied chemical compounds [Larson, 1961, 1968, 1971, 1975]. As shown in Figure 1-2, one nicotine molecule contains two nitrogen atoms, and can combine one or two protons. It has been stated that nearly all thenicotine in tobacco exists as nicotine salts of organic acids [Perfetti, 2000]. During the burning process, nicotine in tobacco is transferred into tobacco smoke, both in gas phase and particulate phase. Different types of tobacco can yield various amounts of nicotine in MTS (Table 1-4). An FTC report showed that the range of machine-smoking nicotine yield from cigarettes sold in the US in 1988 was 0.1 to 2.0 mg/cig [FTC, 2000]. The average nicotine yield has dropped from 1.35 mg/cig in 1968 to 0.88 mg/cig in 1988. However, it should be noted that this trend for nicotine deliveries measured by machine-smoking protocols does not necessarily mean the uptake of nicotine by the typical smoker is also dropping.

Table 1-4 Nicotine in tobacco smoke *

	Flue Cured	Burley	Turkish	American Blended
TPM (μg /puff)	1300	1520	800	1654
Nicotine (μg /puff)	139	205	46	112
Nicotine/TPM (ng/μg)	107	135	58	68

* Data summarized from Ingebrethsen [2001].

1.1.4 Addictiveness of nicotine

As nicotine enters the body, it quickly gets distributed through the bloodstream. In small doses nicotine has a stimulating effect, increasing the heart rate and blood pressure, while reducing the appetite. In large doses it may cause vomiting and nausea [USDHHS, 1988]. Although the toxic properties of nicotine have long been realized, and nicotine has

even been applied as an insecticide, reports of acute deaths from tobacco use are rare [Elferlink, 1983]. Most of the human toxicity of cigarette smoke is considered to be from other compounds, including CO, HCN, and polynuclear aromatic hydrocarbons (PAHs) [IARC, 1986]. The concern about nicotine is mostly a result of its addictive properties. It is the combination of the addictive nicotine with the other toxic componetns that makes cigarette smoking a deadly practice for smokers [Hecht, 1999].

Although many internal documents of the tobacco industry have discussed that nicotine is the addictive agent in smoke [BAT, 1962, 1967, 1979; B & W, 1963, 1973; Philip Morris, 1984, 1992] , until recent years, tobacco companies openly denied the facts [The Tobacco Institute, 1988; Glanz, 1996]. Though admissions of the addictive nature of tobacco smoking are now forthcoming, a new strategy that tobacco industry has used is to dispute the definition of addiction [Carlisle, 1998; Proctor, 1998]. Given the wide scope of characteristics and variety of disciplines that it rests on, drug-addiction is a concept that requires careful definition [Shaffer, 1997]. One definition of addiction that has been given is: "*A state, psychic and sometimes also physical, resulting from the interaction between a living organism and a drug, characterised by behavioural and other responses that always include a compulsion to take the drug on a continuous or periodic basis in order to experience its psychic effects, and sometimes to avoid the discomfort of its absence. Tolerance may or may not be present.*" [WHO, 1969]. According to this definition, the addictiveness of nicotine and tobacco smoking can be clearly demonstrated with at least two aspects: the existence of withdrawal symptoms from smoking, and the fact of low cessation rates. Withdrawal symptoms include irritability, difficulty in concentrating, anxiety, restlessness, increased hunger, depression, and a pronounced craving for tobacco [Henningfield, 1998; Jarvis, 2004]. These symptoms may last for months or even years [Benowitz, 1992], although they peak at around 24-72 hours [Hughes, 1994; West, 1994]. Despite a high proportion of smokers that say they would like to quit, cessation rates are low, and relapse rates are high [Benowitz, 1992]. It has been consistently found that withdrawal symptoms can be relieved by nicotine replacement (patches, gum, etc.), but not by a placebo (patches, gum, etc. that do not contain nicotine). Nicotine replacement therapy approximately doubles the chance of successful cessation [Silagy, 2004].

Studies have shown that, compared with other forms of tobacco use, smoking cigarettes is particularly addictive [Cohen, 1991; Le Houezec, 2003]. Cigarette smoke provides a vehicle that maximizes the addiction potential of nicotine by delivering nicotine directly into the lungs, and then the brain within 10-20 seconds [Balfour, 2002; Le Houezec, 2003]. It has been widely accepted that the more rapidly drugs of abuse reach the brain, the greater their potential for addiction [Samaha, 2005]. This might be one reason why cocaine and nicotine are more addictive when they are smoked than when they are administered by other routes. A cigarette is a precisely designed tool for nicotine delivery. By changing the chemical and physical parameters of a cigarette, the delivery patterns of nicotine to smokers vary. Changing the design of a cigarette could also affect the addiction potential of nicotine in smoke [Hoffmann, 1997].

As discussed previously, nicotine can exist in smoke as its free-base form and its protonated forms. It has been argued that free-base nicotine in MTS is more toxic and absorbed faster into body tissue and blood stream than its ionized forms [Harold, 1930; Ellisor, 1936; Richardson, 1940; Staufer, 1977; USDHHS, 1988; Tomar, 1997]. The effect of chemical forms of nicotine on its addiction potential has also been considered by the tobacco industry. For example, the minutes of a conference mentioned that, “Smoking is an addictive habit attributable to nicotine and the form of nicotine affects the rate of absorption by the smoker.” [BAT, 1967] In latter parts of this chapter, chemical factors that affect the addictiveness of smoke will be discussed in more detail.

1.2 The “impact” of cigarette smoke

In tobacco documents, the physical effect of nicotine on smokers has been described with different terms, such as “strength” [BAT, 1965; Lekovic, 1990; Lorillard, 1980, 1982], and “impact” [PME, 1974; Schori, 1974, 1979; Teague, 1973]. As early as the 1930s, it was considered that it is not the amount of nicotine in the smoke, but rather the amount of free-base nicotine in the smoke determines the degree of smoke “impact”. Shmuk used the “nicotine number” as an objective indicator of the strength of tobacco smoke. The “nicotine number” was defined as the ratio of the total amount of nicotine toa measure of free-base nicotine in smoke called “extractable nicotine” [Schori, 1979]. In

1959, Shakhovskii also argued that smoke strength is related to the amount of free-base nicotine in the smoke [Schori, 1979]. Since the 1960s, numerous studies have been done to investigate the correlation between the "impact" of smoke and the content of free-base nicotine [Backhurst, 1966; Hirji, 1973, 1976]. However, even with the current level of analytical technology, it is still difficult to directly measure the amount and percent of free-base nicotine in tobacco or smoke.

Generally, the "extractable nicotine" in tobacco or tobacco smoke TPM was determined using extraction into followed by extraction with the same volume of chloroform. Since free-base nicotine is very soluble in chloroform, free-base nicotine is preferentially extracted from aqueous solution by chloroform, whilst the protonated nicotine is not. The content of nicotine in chloroform was defined as the "extractable nicotine", and the percentage of "extractable nicotine" was defined as the ratio of the nicotine content in chloroform to the total amount of nicotine both in chloroform and aqueous phase. However, the pH of the water phase will not exactly reflect the specific acid/base balance in tobacco or tobacco smoke PM, and the extraction with chloroform will also change the acid/base balance of nicotine.

One study done by BAT [Backhurst, 1966] found that the "strength" or "impact" of smoke had no correlation either to the nicotine content of tobacco, or to the nicotine content in smoke. However, when the cigarettes were arranged in the order of "extractable smoke nicotine", smoke panel members tended to rate the cigarettes with the "greatest" amounts of "extractable nicotine" as the "strongest" cigarette. The study also showed that the tobacco with higher weight of "extractable nicotine" could generate smoke with higher "extractable nicotine". Another notable point of the study was that the range of the weight of "extractable nicotine" in smoke was very narrow, and suggested that the smokers were very sensitive to changes in "extractable nicotine".

Hirji and Wood [1976] derived equations relating impact with "extractable nicotine" and other cigarette variables. The best equation was:

$$\text{Impact} = 1.8 + 59.7\,[\text{ext. nic.}] + 9.7\,[\text{non-ext. nic.}] - 0.115\,[\text{P.D.}] \quad (1\text{-}1)$$

where [ext. nic.] is the amount of "extracted nicotine" in PM with units of mg/puff; [non-ext. nic.] is the amount of "non-extracted nicotine" in PM with units of mg/puff; and [P.D.] is pressure drop in cm. The above equation confirmed the correlation between the nicotine content in smoke and its impact potential on smokers.

1.3 Maintaining the impact potential of cigarette smoke

Within the past decades, especially with the introduction and marketing of so-called "light", or "ultra-light" cigarettes, TPM and tar values measured with machine smoking protocols have declined [Hoffmann, 1997]. With the declining TPM delivery, it has been viewed that there are two possible ways to maintain the "impact" potency of smoke: maintaining higher nicotine content in TPM [Dunn, 1975; Robinson, 1990; Philip Morris, 1998]; or obtaining a higher ratio of free-base nicotine in smoke by causing a higher "smoke pH" [Teague, 1982; Brown & Williamson, 1991]. To achieve these two goals, many studies have been carried out by tobacco industry. Examples of the studies included adding nicotine into tobacco materials [Patents, 1994], cultivating high nicotine tobacco plants [Fisher, 1988; Lewan, 1998], changing the design of cigarettes [Thornton, 1969; Ball, 1970; BAT, 1972; Reynolds, 1973; McMurtrie, 1980; Sudholt, 1985], and introducing chemical additives into tobacco products [McBride, 1992; Cantrell, 1981; Browne, 1987; Stewart, 1988]. Baskevitch [1987] discussed two examples of how to change delivery of TPM and nicotine by combining these practices. In one example, the TPM was reduced from 25.0 to 14.3 mg/cigarette, whereas nicotine was slightly reduced from 1.2 to 1.0 mg/cigarette. The ratio of nicotine to TPM was thus increased from 0.048 to 0.070. In the other example, the delivery of tar was reduced from 22 to 15 mg/cig, whilst the nicotine delivery changed only slightly from 1.0 to 0.95 mg/cigarette.

Available tobacco documents indicate that the most common interest in the manipulation of nicotine availability include strategies to change the physical parameters of cigarettes, and change the composition of cigarettes by adding additives.

Physical parameters

In the design of a cigarette, the physical parameters that can be used to affect the

delivery of nicotine and TPM include: cigarette length, circumference, pressure drop, paper porosity, ventilation of filter, cuts per inch of tobacco, and packing density [BAT, 1972, 1976; Reynolds, 1973; McMurtrie, 1980; Anonymous A, 1986]. The effects of these parameters are summarized in Table 1-5, which shows that, among the physical parameters, the ventilation of filter and the porosity of tobacco paper could be the most important. Kiefer [1978] observed that ventilated filters could reduce the delivery of dry TPM and nicotine as the degree of ventilation increases from 0 to 76%, but the increase of nicotine content in TPM could be up to 34 % at the same time. Keith [1980] concluded that, "By an appropriate combination of filtration, ventilation, and combustion, it is possible to design a cigarette with almost any level of delivery of tar, nicotine, and gaseous components." Not only increasing the nicotine to tar ratio, air dilution can also increase the "smoke pH" [Minnemeyer, 1976]. Skladaowski [1977] found that a total air dilution cigarette, without a filter plug, could increase the nicotine/tar ratio by 64%, while increase the "alkalinity of smoke" by 1.1 "smoke pH" units. Klus et al. [1981] found that among the tested cigarettes, which only vary in the degree of tip ventilation, there was a clear dependence of the "smoke pH" on the degree of ventilation. For the fifth puff of the tested cigarettes, as the degree of tip ventilation increased from zero to 70%, the "smoke pH" changed from 6.48 to 7.17. The authors estimated that, with such an increase of "smoke pH", the fraction of free-base nicotine in smoke could increase from only 4.07% to 17.2%.

Composition of cigarettes

Changing the composition of cigarettes is another important way to affect either the delivery of nicotine to smoke or the percent of free-base nicotine in smoke. The most obvious of this category is to add nicotine into tobacco materials [Nicotine Augmentation Project, Lorillard, 1976], or to use a tobacco blend with high nicotine content. It was found that small amounts of supplemental nicotine could "lead to a satisfactory low tar cigarette" [Minnemeyer, 1976]. Baskevitch and Ferrer [1982] tested three types of reconstituted tobacco A, B, and C with nicotine content of 0.20%, 0.74%, and 4.77%. The above three reconstituted tobaccos were all blended with a tobacco having 1.55% nicotine content. The blending ratio was 15:85. Smoke from cigarettes using reconstituted tobacco

Table 1-5 The effects of physical parameters on the delivery of TPM and Nicotine

	Length of cigarette	Circumference of cigarette	Cuts (higher cuts means finer tobacco)	Packing density	Pressure drop	Paper porosity	Ventilation of filter
TPM	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Nicotine	Pos.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
N/T	No effect	Weakly neg.	No effect, or weakly pos.	No effect	Unknown	Sig. pos.	Sig. pos.

Note: pos., positive correlation; neg., negative correlation; Sig., significantly; N/T, ratio of nicotine to TPM.

C had significantly higher nicotine to tar ratio across the range of filter ventilation.

Applying additives into tobacco products is another way to change the composition of cigarettes by affecting the "impact" potency of smoke. Additives can be natural or synthetic, including artificial tobacco substitutes, extracts of tobacco and other plants, exogenous enzymes, powdered cocoa, and synthetic substances [WHO, 2000].Tobacco companies have used about 1400 additive materials in cigarettes [Manus, 1989]. In the United Kingdom, there are 600 allowed additives in cigarettes [Bates, 1999]. Among these additives, a group of compounds have been of concern due to their ability to affect the delivery of nicotine to smoke and change the acid/base balance in the smoke. This group includes ammonia and compounds that can release ammonia by heating or burning. In 1995, an article published in the Wall Street Journal (WSJ) revealed that leading U.S. tobacco companies had a long history of using ammonia additives [WSJ, Oct. 18, 1995]. One of the two Brown & Williamson Tobacco Corporation documents cited in this WSJ article was a handbook with the title of "Root Technology, A Handbook for Leaf Blenders and Product Developers" [B&W, 1991]. This handbook summarized the forms of ammonia technology developed and used in major tobacco companies, including Philip Morris, RJR, B&W, BAT, and Lorillard. One of the four important functions of ammonia described in this handbook is that ammonia works as an "impact booster", which was explained in the handbook as, "Ammonia, when added to a tobacco blend, reacts with the indigenous nicotine salts and liberates free nicotine. As a result of such change, the ratio of extractable nicotine to bound nicotine in the smoke may be altered in favor of extractable nicotine. As we know, extractable nicotine contributes to impact in cigarette smoke and this is how ammonia can act as an 'impact booster'. " It was estimated that the US cigarette industry used about 10 million pounds of ammonia-containing compounds a year. This corresponds to about 10 mg per cigarette [Johnson, 1989].

As shown in Table 1-6, when heated, free-base nicotine is vaporized at lower temperature as compared with nicotine various salt forms. Although free-base nicotine and nicotine salts may decompose similarly in the combustion zone (Figure 1-1) [Seeman, 1999], free-base nicotine transfer to smoke may be more favorable in the pyrolysis/distillation zone. As a base, ammonia has the ability to convert a portion of a

nicotine salt to free-base nicotine. Increasing the proportion of free-base nicotine in tobacco may increase the availability of nicotine for transfer into smoke [Norman, 1981]. The highest transfer efficiency of free-base nicotine, shown in Table 1-6, is consistent with above discussion. Using ammonia additives in cigarettes can also significantly increase the concentration of ammonia in smoke [Coleman, 1991], causing more basing smoke [Brown & Williamson, unknown date A]. The result can be not only higher delivery of nicotine into smoke, but also higher fraction of free-base nicotine in the smoke.

Table 1-6 The peak transfer temperature and transfer efficiency of nicotine

Nicotine salt	Base : Acid	Peak transfer temperature	Nicotine transfer efficiency (%)
Free-base nicotine		138 [a]; 110-125 [b]	20.0 [c]
Acetate	1:1	NA	14.1 [c]
	1:3	110-125 [b]	NA
	1:9	NA	15.0 [c]
Oxalate	1:1	258 [a]	9.0 [c]
Malate	1:1	110-210 [b]	9.3 [c]
	1:2	165-210 [b]	NA
Hydrogen Tartrate	1:2	195-210 [b]	6.8 [c]
Citrate	1:1	NA	7.4 [c]
Pectin	1:1	165 [a]	NA
	1:10	367 [a]	NA

[a] Data from Matkin [1984];
[b] Data from Seeman [1999];
[c] Data from Brown & Williamson [unknown date B].

Other tobacco company documents have suggested that use of ammonia additives in tobacco filler can either increase the delivery of nicotine into smoke or the fraction of free-base nicotine in smoke [Newton, 1970; Routh, 1977; Johnson, 1989; Watson, 1991]. Interestingly, a relationship between transfer efficiency of nicotine and the market share

of cigarettes has been discussed, with higher nicotine transfer efficiency seemly correlated with higher market share of the brand [Anonymous B, 1994].

1.4 Acid/base chemistry in tobacco smoke: pH, nicotine and ammonia

The roles of ammonia and ammonia additives in the chemistry of tobacco smoke have been debated both in tobacco trials [Hurt, 1998] and in the scientific research [Henningfield, 2004]. One focus of the debate is the ability of ammonia to change the acid/base balance in smoke, and therefore change the fraction of free-base nicotine and the "impact" potential of smoke. One way to resolve the debate is by means of an improved understanding of the acid/base chemistry in smoke. The most direct way to unveil the acid/base balance in smoke is to measure the pH of the PM phase. In past decades, many methods have been developed for the determination of "tobacco smoke pH". The "smoke pH" values measured with those methods have been widely used in the study of nicotine in tobacco smoke [Philip Morris, 1994; Rodgeman, 2000]. It has been widely stated that the "smoke pH" of MTS from blended cigarettes or from cigarettes made from flue-cured tobacco ranges from 5.5 to 6.2 [IRAC, 1986; USDHHS, 1989]. In above range, the fraction of free-base nicotine was estimated to be lower than 3%, and so assumed that nicotine exists dominantly in its protonated form in MTS [Morie, 1972]. Ellis et al. [1999] reported that, although ammonia in MTS increased as a result of adding ammonia-producing compounds, the addition of ammonia-producing compounds did not increase the "smoke pH" and nicotine yield, and there was no correlation between the ammonia content of MTS and the "smoke pH". Based on "smoke pH" values measured by Labstat [1997], Dixon [1999] concluded that there was no correlation between the ammonia content of tobacco and nicotine or ammonia yields of MTS, and that ammonia yields of MTS and "smoke pH" are not positively related. Cochran et al. [1999] applied a diffusion-denuder method to investigate the effects of "smoke pH" on vapor-phase nicotine yields from different types of cigarettes, including cigarettes treated with ammonium carbonate or urea. The authors stated that the "smoke pH" of the treated cigarettes was higher than those of non-treated cigarettes. However, the authors also concluded that the lack of a correlation between the percent of initial vapor-phase

nicotine and "smoke pH" indicated that "smoke pH" was not an indicator of the release of nicotine into the vapor phase.

In recent years, some researchers have realized that although the "smoke pH" values that have been measured might be used for measuring the relative acidity/alkalinity of tobacco smoke, they were not the true pH of the tobacco smoke PM phase. With current levels of science and technology, it is very difficult to directly measre the pH of this phase [Rodgeman, 2000; Henningfield, 2004]. Pankow [2001] suggested that an alternative approach for understanding the acid/base balance of PM is to determine α_{fb}, the fraction of free-base nicotine, and effective pH of the PM. Pankow et al. [2003] reported that the percentage of free-base nicotine for some brands could be as high as 38%, and calculated that effective pH could be in the range of 5.80 to 7.81.

Table 1-7 Fractions of gas phase nicotine of treated and untreated cigarettes [a]

Cigarette type	% dry weight of NH_3 and KOH	"Filler pH" [b]	% of total nicotine initially in gas phase	
			Average	Standard deviation
Reference cigarette	0	5.30	1.60	0.54
NH_4OH-amended	0.55	6.15	1.83	0.41
DAP-amended	1.13	6.40	2.03	0.23
KOH-amended	2.0	7.20	2.47	0.47

[a] Data derived from Braem [1997];
[b] Measured on 0.5 g of tobacco stirred during 1-hour in 50 mL de-ionized water.

Work done by Pankow and co-workers [2001, 2003] also suggested that the direct dependence of the partitioning properties of basic and acidic compounds on the acid/base balance in the PM phase provides an alternative and indirect approach to understand the acid/base chemistry of tobacco smoke. With relatively constant partitioning coefficient of free-base nicotine, the fraction of nicotine in gas phase is an important indicator of acid/base balance and the fraction of free-base nicotine in PM. Although many tobacco documents claimed that there were no correlation between "smoke pH" and the application of ammonia and other basic additives in cigarette, others did show positive correlations between ammonia additives and percentage of gas phase nicotine in smoke.

For example, Braem [1997] found that, by adding basic additives, the filler "pH" of cigarette could be elevated from 5.30 to 7.20 (Table 1-7). More importantly, the percentage of total nicotine in gas phase could be increased from 1.60 to 2.47. Pankow and co-workers [1997; 2003] also found that, by exposing environmental tobacco smoke (ETS) or MTS particles to ammonia gas, the concentration of nicotine in the gas phase could be increased by more than 100 times, which indicated the potential of ammonia to convert protonated nicotine in PM to its free base form.

1.5 Objectives and overview

Besides serving as "impact booster", ammonia has been described as "ameliorant", "taste enhancer", and "flavor promoter" in smoke [Brown & Williamson, 1991]. These multifunctional aspects of ammonia suggest that it may be involved in reactions during the tobacco smoking process that could be very complicated. In other words, ammonia additives may serve as precursor for nitrogen-containing compounds produced in the smoking process. It can also be product of reactions involving those compounds. Schmeltz and Hoffmann [1977] estimated that about 30% of all compounds in tobacco leaf and smoke contain nitrogen. As discussed above, researchers have had conflicting views concerning the effects of ammonia on the delivery of nicotine in smoke and the "smoke pH". The complications of the smoking process, the complex composition of smoke, and the difficulties of chemical analysis all contribute to the confusion. To resolve the debate, much research is needed [Henningfield, 2004].

This study is one step of our efforts to further understand the acid/base chemistry in smoke. The work was started with a study of nicotine and ammonia. As a stong base and volatile compound, ammonia is an analog to nicotine regarding its response to change in the acid/base balance of tobacco smoke. The study of ammonia, and its partitioning properties in smoke, can thus help us understand the parameters that affect the acid/base chemistry of tobacco smoke and the tobacco some chemistry of nicotine. The study could also help us test current theories and develop new theories regarding the acid/base chemistry in tobacco smoke.

In Chapter Two, the theories of partitioning of nicotine and ammonia are introduced and discussed. Possible correlations between ammonia and nicotine are also discussed. In Chapter Three, the methods for collecting and analyzing nicotine and ammonia, both in the gas phase and PM are introduced; data quality control and quality analysis are also discussed. In Chapter Four, the data of nicotine and ammonia in smoke from eleven brands of cigarettes and two brands of small cigars are reported. In Chapter Five, the ammonia-releasing process of PM samples by hydrolysis in different solvents is discussed. In Chapter Six, the methods for analyzing some amides in smoke with conventional GC-MS and a state-of-art GC×GC-time of flight mass spectrometer (ToFMS) are introduced. The results from the two methods are compared. Chapter Seven is a summary of the study.

CHAPTER 2

THE THEORY OF ACID/BASE CHEMISTRY AND GAS/PARTICLE PARTITIONING FOR NICOTINE AND AMMONIA IN MAINSTREAM TOBACCO SMOKE

2.1 Introduction

As discussed in Chapter 1, nicotine can exist in free-base as well as protonated forms (Figure 1-2). Considering that MTS is an aerosol with two phases, there are two interrelated chemistries: acid/base chemistry in the PM phase and gas/particle partitioning between the gas phase and the PM phase (Figure 2-1). It has been suumed that for most tobacco smoke PM phase, the fraction of the di-protonated nicotine is negligible; the dominant forms are free-base nicotine and mono-protoanted nicotine. Because of the low vapor pressure of protonated nicotine, only free-base nicotine can exist in the gas phase of smoke. Pankow et al. [1997, 2001, 2003] used the partitioning coefficient of free-base nicotine ($K^{n}_{p,fb}$) to describe the partitioning properties of free-base nicotine in smoke, and found that $K^{n}_{p,fb}$ was relatively constant for different samples of smoke PM. With a constant $K^{n}_{p,fb}$, the acid/base balance in the PM phase is the major factor determining the fraction of protonated and free-base nicotine in PM, and the position of the equilibrium of nicotine between the gas phase and the PM phase. In this thesisreport, mainstream tobacco smoke PM is denoted as PM_{MTS}; the fraction of the nicotine in PM_{MTS} that is in the free-base form is denoted α^{n}_{fb} [Pankow, 2001, 2003; Henningfield, 2004]. The superscript "n" is added here because α^{a}_{fb}, the fraction of ammonia in its free-

Acid/base chemistry in PM phase:

$$NicH^+ \longleftrightarrow Nic + H^+ \quad K_a^n = \frac{\{H^+\}\{Nic\}}{\{NicH^+\}}$$

Gas/Particle partitioning chemistry:

$$Nic(p) \longleftrightarrow Nic(g) \quad K_{p,fb}^n = \frac{[Nic(p)]}{[Nic(g)]}$$

Figure 2-1 Equilibria of nicotine in mainstream tobacco smoke

Note: $NicH^+$, protonated nicotine; Nic, free-base nicotine. $Nic(p)$: free-base nicotine in PM; $Nic(g)$: nicotine in gas phase. K_a^n, acid dissociation constant of $NicH^+$; $K_{p,fb}^n$, partitioning coefficient of free-base nicotine in MTS.

base form will also be discussed. Only the free-base form of nicotine is directly volatile from tobacco smoke PM, and so the rate and physiologic location of nicotine uptake from inhaled PM_{MTS} will be strongly influenced by α_{fb}^n [Pankow, 2001]; the connection between nicotine delivery rate and addiction potential has been discussed by Henningfield et al. [2004]. The importance of the acid/base chemistry of tobacco smoke in controlling α_{fb}^n is well established [Pankow, 1997, 2001]; Pankow et al. [1997] have confirmed that PM_{MTS} equilibrated with increasing levels of the base ammonia exhibit increasing values of α_{fb}^n. The available range is $0 < \alpha_{fb}^n < 1$. For fresh PM_{MTS} from commercial brands of cigarettes, Pankow et al. [2003] have reported α_{fb}^n values in the range 0.010 up to 0.38.

As discussed in Chapter 1, a variety of nitrogen-containing compounds (ammonia, ammonium salts, urea, etc.) have been added to the tobacco materials used in cigarettes [B & W, 1989, 1990, 1991; RJ Reynolds, 1990; Johnson, 1996]. One reason for adding ammonia-related compounds that has been discussed in industry documents involves improving the physical strength of the "reconstituted tobacco sheet" ("RTS") that has been widely used as a way to make use of tobacco "fines" and leaf stems, and to carry various other additives into the final cigarette blend [Hind, 1966]. However, another prominently mentioned reason for adding ammonia-related compounds involves adjusting smoke potency characteristics. The facility with which ammonia can deprotonate protnated nicotine to form free-base nicotine is related to the relative strengths, as bases, of NH_3 and nicotine (Figure 2-2). These strengths are directly related to $(K_a^a)^{-1}$ and $(K_a^n)^{-1}$, which are the inverses of the acidity constants for the species NH_4^+

and $NicH^+$, respectively. In particular, in water-containing systems (which would include PM_{MTS}), one measure of the strength of a nitrogen base B (e.g., NH_3 or nicotine) is its ability to abstract a proton from water according to

$$B + H_2O = BH^+ + OH^- \qquad K_b = \frac{\{BH^+\}\{OH^-\}}{\{B\}} \tag{2-3}$$

where K_b is the basicity contant. The acid dissociation reaction for BH^+ is

$$BH^+ = B + H^+ \qquad K_a = \frac{\{B\}\{H^+\}}{\{BH^+\}} \tag{2-4}$$

Since water dissociates according to

$$H_2O = H^+ + OH^- \qquad K_w = \{H^+\}\{OH^-\} \tag{2-5}$$

By combining eqs 2-4 and 2-5, it may yield

$$K_b = K_w (K_a)^{-1} \tag{2-6}$$

In water at room temperature, $(K_a^a)^{-1} \approx 20(K_a^n)^{-1}$ and NH_3 is about 20 times stronger as a base than is free-base nicotine (Nic). In PM_{MTS}, relative base strengths are not as well understood, though NH_3 is likely to be a stronger base than nicotine.

2.2 pH and effective pH

The acid dissociation equilibria for protonated nicotine ($NicH^+$) and ammonium ion (NH_4^+) are described in Figure 2-2. With brackets representing concentration, for nicotine in PM_{MTS}, it has been defined that [Pankow, 1991]:

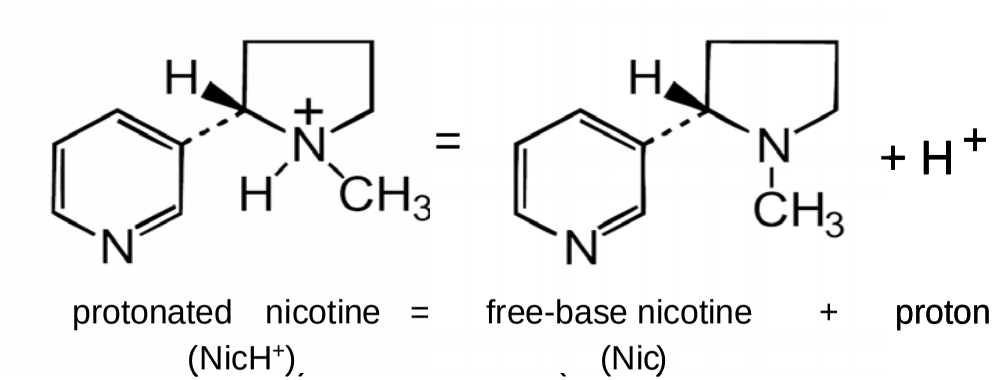
$$\alpha_{fb}^n = \frac{[Nic]}{[Nic] + [NicH^+]} \tag{2-7}$$

The value of any given α value is independent of the scale used for concentration: molal, molar, and ng/µg can all be used, though use of ng/µg requires that $[NicH^+]$ be calculated as the equivalent ng/µg concentration of the free-base Nic form.

The fraction of the free base ammonia in PM_{MTS} is defined

$$\alpha_{fb}^a = \frac{[NH_3]}{[NH_3] + [NH_4^+]} \tag{2-8}$$

The chemical activity of H^+ in a solution is given by $\{H^+\} = [H^+]\gamma_{H^+}$ and the definition



$$K_a^n = \frac{\{Nic\}\{H^+\}}{\{NicH^+\}} \qquad (2-1a)$$

$$= \frac{[Nic]\,\gamma_{Nic}[H^+]\,\gamma_{H^+}}{[NicH^+]\,\gamma_{NicH^+}} \qquad (2-1b)$$

$$K_a^a = \frac{\{NH_3\}\{H^+\}}{\{NH_4^+\}} \qquad (2-2a)$$

$$= \frac{[NH_3]\,\gamma_{NH_3}[H^+]\,\gamma_{H^+}}{[NH_4^+]\,\gamma_{NH_4^+}} \qquad (2-2b)$$

Figure 2-2. The analogous acid/base reactions for nicotine and ammonia.

K_a^n and K_a^a are the equilibrium acid dissociation constants for protonated nicotine and for protonated ammonia, respectively. The chemical equilibria involve the chemical activities of the involved species, e.g. {H+} measures the chemical activity level of the proton. Interactions in a solution can make a chemical act differently for what would be expected based on concentration alone. Thus, each chemical activity $\{i\} = [i]\gamma i$ where $[i]$ = molal concentration of i and γi = activity coefficient of i.

of pH in any solution is

$$pH \equiv -\log\{H^+\} = -\log[H^+] - \log\gamma_{H^+} \quad (2\text{-}9)$$

In PM_{MTS}, the parameters $[H^+]$, γ_{H^+}, and γ_{NicH^+} are all exceedingly difficult to measure. Given that they all appear together with γ_{Nic} in eq 1b, Pankow [2001] has proposed collecting all of these quantities in the group $[H^+]\gamma_{H^+}(\gamma_{Nic}/\gamma_{NicH^+})$. With the superscript n denoting nicotine, Pankow [2001] thus defines the effective pH of PM_{MTS} for nicotine according to

$$pH^n_{eff} \equiv -\log\frac{[H^+]\gamma_{H^+}\gamma_{Nic}}{\gamma_{NicH^+}} \quad (2\text{-}10)$$

$$= -\log[H^+] - \lg\gamma_{H^+} + \log\frac{\gamma_{NicH^+}}{\gamma_{Nic}} \quad (2\text{-}11)$$

$$= pH + \log\frac{\gamma_{NicH^+}}{\gamma_{Nic}} \quad (2\text{-}12)$$

so that

$$10^{-pH^n_{eff}} = \frac{[H^+]\gamma_{H^+}\gamma_{Nic}}{\gamma_{NicH^+}} \quad (2\text{-}13)$$

Substitution of eq 2-13 into eqs 2-1b and 2-7 allows the complexity of eqs 2-9 to 2-12 to be reduced to

$$\alpha^n_{fb} = \frac{10^{-pK^n_a}}{10^{-pK^n_a} + 10^{-pH^n_{eff}}} \quad (2\text{-}14)$$

and equivalently

$$pH^n_{eff} = pK^n_a + \frac{\alpha^n_{fb}}{1-\alpha^n_{fb}} \quad (2\text{-}15)$$

pK^n_a has been measured to be 8.01 at 25 ^oC in water [Fowler, 1954]. In dilute water, all γ values equal unity so that $pH^n_{eff} = pH$.

In dilute water, all γ in eqs 2-10 to 2-13 equal 1, but in PM_{MTS} all $\gamma \neq 1$. pH^n_{eff} thus allows one to discuss an observed α^n_{fb} for PM_{MTS} in terms of the equivalent pH

conditions required in dilute water to yield that α_{fb}^{n}. For example, when $\alpha_{fb}^{n} \approx 0.50$ in PM_{MTS}, then $pH_{eff}^{n} \approx 8.0$ in that solution, just as $\alpha_{fb}^{n} \approx 0.50$ in dilute water when pH ≈ 8.0 (Figure 2-3).

For ammonia, by analogy with eqs 2-10 to 2-15,

$$pH_{eff}^{a} \equiv -\log \frac{[H^+]\gamma_{H^+}\gamma_{NH_3}}{\gamma_{NH_4^+}} \quad (2\text{-}16)$$

$$= -\log[H^+] - \log\gamma_{H^+} + \log \frac{\gamma_{NH_4^+}}{\gamma_{NH_3}} \quad (2\text{-}17)$$

$$= pH + \log \frac{\gamma_{NH_4^+}}{\gamma_{NH_3}} \quad (2\text{-}18)$$

so that

$$10^{-pH_{eff}^{a}} = \frac{[H^+]\gamma_{H^+}\gamma_{NH_3}}{\gamma_{NH_4^+}} \quad (2\text{-}19)$$

Substitution of eq 2-19 into eqs 2-2b and 2-8 yields

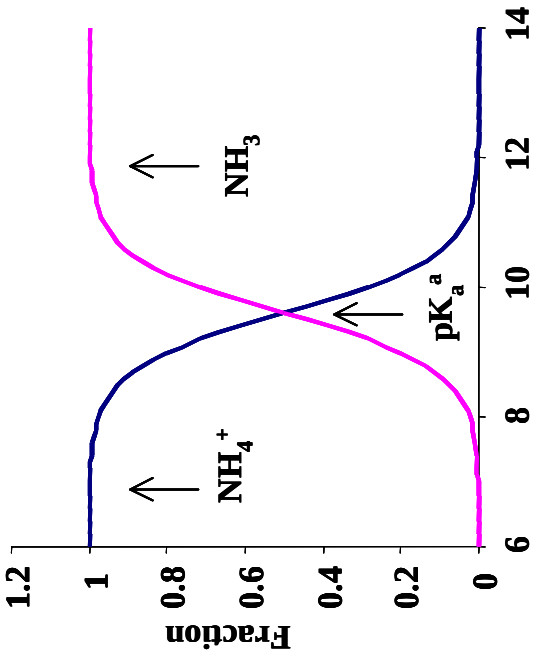
$$\alpha_{fb}^{a} = \frac{10^{-pK_a^a}}{10^{-pK_a^a} + 10^{-pH_{eff}^{a}}} \quad (2\text{-}20)$$

$$pH_{eff}^{a} = pK_a^a + \frac{\alpha_{fb}^{a}}{1-\alpha_{fb}^{a}} \quad (2\text{-}21)$$

pK_a^a has been measured to be 9.40 at 20 °C in water [Subcommittee on Ammonia, National Research Council, 1978]. By analogy with pH_{eff}^{n}, we can use pH_{eff}^{a} to discuss the extent of protonation of ammonia in PM_{MTS} in terms that relate an observed α_{fb}^{a} value to the equivalent pH conditions that would be present in dilute water to yield that α_{fb}^{a}. For example, when $\alpha_{fb}^{a} \approx 0.50$ in PM_{MTS}, at 20 °C, then we know that $pH_{eff}^{a} \approx 9.40$ (Figure 2-3).

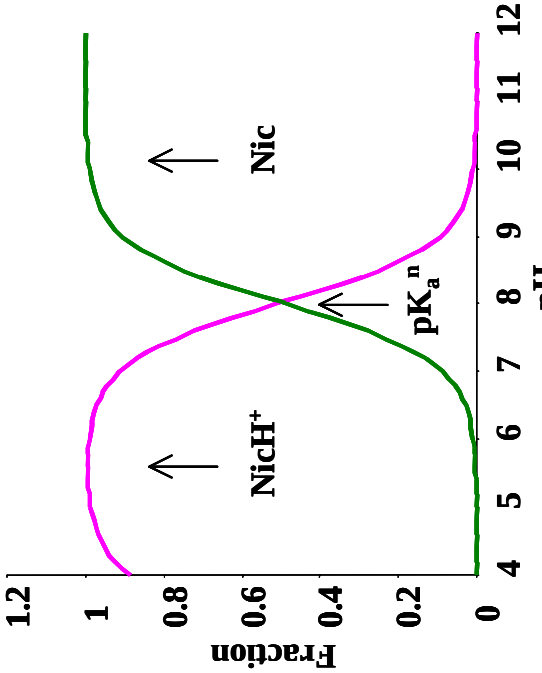
Subtracting eq 2-12 from eq 2-18 yields

$$pH_{eff}^{a} = pH_{eff}^{n} + \log \frac{\gamma_{NH_4^+}}{\gamma_{NH_3}} - \log \frac{\gamma_{NicH^+}}{\gamma_{Nic}} \quad (2\text{-}22)$$



$$NH_4^+ = NH_3 + H^+$$

$$K_a^a = \frac{\{H^+\}\{NH_3\}}{\{NH_4^+\}} = \frac{[H^+][NH_3]}{[NH_4^+]} = 9.40$$



$$NicH^+ = Nic + H^+$$

$$K_a^n = \frac{\{H^+\}\{Nic\}}{\{NicH^+\}} = \frac{[H^+][Nic]}{[NicH^+]} = 8.01$$

Figure 2-3 Fractions of nicotine and ammonia in dilute aqueous solution at 20℃

however, $(\gamma_{NH_4^+}/\gamma_{NH_3}) \neq (\gamma_{NicH^+}/\gamma_{Nic})$ so that $pH_{eff}^a \neq pH_{eff}^n \neq pH$, and there will be an

offset between pH^{a}_{eff} and pH^{n}_{eff}; the nature of the offset will depend on the directions and magnitudes of the effects on the ratios $(\gamma_{NH_4^+} / \gamma_{NH_3})$ and $(\gamma_{NicH^+} / \gamma_{Nic})$.

The NH_3 molecule is quite polar, and NH_4^+ carries ionic charge. Both of these species can be viewed as "comfortable" in water. Since PM_{MTS} is undoubtedly less polar than water, both NH_3 and NH_4^+ will be less comfortable in that type of phase. The ion $NicH^+$ will also likely be less comfortable in PM_{MTS} than in water. However, the magnitude of the effect on $NicH^+$ when going from dilute water to PM_{MTS} will likely not be as great as on NH_4^+ because the organic nature of the $NicH^+$ structure will be relatively well accommodated in typical PM_{MTS}. Table 1 provides rough estimates of the extent to which the γ values of these three species could rise above 1 when dissolved in typical PM_{MTS}. The case of nicotine is special. Since this organic molecule will be more comfortable in typical PM_{MTS} than in dilute water, its γ value can be expected to less than 1 in typical PM_{MTS}, perhaps ~0.25. These considerations, summarized in Table 1, lead to the estimate for typical PM_{MTS} that $(\gamma_{NH_4^+} / \gamma_{NH_3}) \approx 0.1\,(\gamma_{NicH^+} / \gamma_{Nic})$ so that

$$pH^{a}_{eff} \approx pH^{n}_{eff} - 1.0 \qquad \text{(rough estimate)} \qquad (2\text{-}23)$$

In an example application of eq 2-23, when $pH^{n}_{eff} \approx 8.0$ so that $\alpha^{n}_{fb} \approx 0.50$, then $pH^{a}_{eff} \approx 7.0$ so that $\alpha^{a}_{fb} \approx 0.005$.

2.3 Gas/Particle (G/P) Partitioning of Nicotine and Ammonia

In tobacco smoke, Pankow et al. [1997, 2001] have discussed that only free-base nicotine is volatile, in direct chemical exchange with the gas phase, and subject to G/P partitioning with an equilibrium constant that is given by

$$K^{n}_{p,fb} = \frac{c^{n}_{p,fb}}{c^{n}_{g}} \qquad (2\text{-}24)$$

where $K^{n}_{p,fb}$ is the equilibrium G/P partitioning coefficient of free-base nicotine, $c^{n}_{p,fb}$ (ng μg^{-1}) is the concentration of free-base nicotine in PM_{MTS}, and c^{n}_{g} (ng m^{-3}) is the concentration of nicotine in the gas phase (only Nic, no $NicH^{+}$). For partitioning between the nicotine in the gas phase and the total nicotine in the PM-phase [Pankow, 1997, 2001],

$$K^{n}_{p} = \frac{c^{n}_{p}}{c^{n}_{g}} \qquad (2\text{-}25)$$

where c^{n}_{p} (ng μg^{-1}) is the total concentration of nicotine in the PM phase (i.e., Nic + $NicH^{+}$; mass of $NicH^{+}$ is calculated as equivalent ng of free-base nicotine).
Since $c^{n}_{p,fb} = \alpha^{n}_{fb} c^{n}_{p}$, then

$$K^{n}_{p} = \frac{K^{n}_{p,fb}}{\alpha^{n}_{fb}} \qquad (2\text{-}26)$$

As with nicotine, only the free-base form of ammonia is volatile. By analogy with nicotine,

$$K^{a}_{p,fb} = \frac{c^{a}_{p,fb}}{c^{a}_{g}} \qquad (2\text{-}27)$$

$$K^{a}_{p} = \frac{c^{a}_{p}}{c^{a}_{g}} \qquad (2\text{-}28)$$

where $K^{a}_{p,fb}$ is the equilibrium G/P partitioning coefficient of free-base ammonia, $c^{a}_{p,fb}$ (ng μg^{-1}) is the concentration of the free-base ammonia in PM_{MTS}, c^{a}_{g} (ng m^{-3}) is the concentration of ammonia in the gas phase (only NH_3, no NH_4^+), K^{a}_{p} is the equilibrium G/P constant for ammonia partitioning between the gas phase and the total (*i.e.*, NH_3 + NH_4^+) PM-phase ammonia concentration c^{a}_{p} (ng μg^{-1}, calculated with assuming all ammonia in the NH_3 form). As with nicotine, for ammonia we have $c^{a}_{p,fb} = \alpha^{a}_{fb} c^{a}_{p}$ so that

$$K^{a}_{p} = \frac{K^{a}_{p,fb}}{\alpha^{a}_{fb}} \qquad (2\text{-}29)$$

$K^{n}_{p,fb}$ values for PM_{MTS} from 11 brands of commercial cigarettes were measured by Pankow et al. [2003] to be in the range $10^{-5.18}$ to $10^{-4.83}$ m^3 μg^{-1} at 20 °C. These results

agree well with the predicted range of $10^{-5.24}$ to $10^{-4.91}$ m^3 μg^{-1} estimated by Pankow [2001] using G/P partitioning theory and reasonable assumptions concerning the molecular properties of typical PM_{MTS}.

For $K^a_{p,fb}$, while we are unaware of any determinations for PM_{MTS}, the Henry's Gas Law constant $K^a_{H,fb}$ (molal/atm) for partitioning of free-base ammonia between dilute water and air is well known [Jaeschke, 1998; Lammel, 1992]. Over the range, T = 273 K to 313 K, Clegg and Brimblecombe [1989] give the temperature dependence of this partitioning as

$$\ln K^a_{H,fb} = -8.09694 + 3917.507/T - 0.00314T \qquad (2\text{-}30)$$

At T = 293.15 K (20 °C), eq 2-30 gives $K^a_{H,fb} = 10^{1.89}$ (m/atm). Unit conversions between $K^a_{p,fb}$ and $K^a_{H,fb}$ yield

$$K^a_{p,fb} = K^a_{H,fb} RT \times 10^{-9} \qquad (2\text{-}31)$$

where R is the ideal gas constant (8.2×10^{-5} m^3 atm/mol-K). Eq 2-31 therefore gives

$$K^a_{p,fb} = 10^{-8.73}\ m^{-3}\ \mu g^{-1} \quad NH_3 \text{ partitioning to water, 20 } ^oC \qquad (2\text{-}32)$$

The equation governing the G/P partitioning of a neutral compound (including K_p values for both free-base ammonia and free-base nicotine) is [Pankow, 1994]

$$K_p = \frac{760\ RT}{10^6\ \overline{MW}\ \zeta\ p^o_L} \qquad (2\text{-}33)$$

where $\overline{MW}$ is the mean molecular weight of the solution phase into which the partitioning is occurring. The parameter p^o_L is the T-dependent liquid vapor pressure (Torr) of the partitioning compound. Like γ, the parameter ζ is an activity coefficient "comfort factor" for dissolved constituents; ζ and γ are measured relative to different "reference states", just as the height of a building can be measured relative to the sidewalk in front of the building, or relative to mean sea level. Dilute water is the reference state for measurement of all γ values (as in Table 2-1), and so every γ = 1 in dilute water. In dilute water γ_{NH_3} = 1, but in pure liquid ammonia $\gamma_{NH_3} \neq 1$. Pure liquid i is the reference state for all ζ_i, so in pure liquid ammonia ζ_{NH_3} = 1 and in dilute water

$\zeta_{NH_3} \neq 1$. For a dilute water solution of ammonia at 20 °C, by substituting $\overline{MW}_w$ = 18.0 g/mol, $K^a_{p,fb}$ = $10^{-8.73}$ m^{-3} μg^{-1} (Eq. 2-32), and $p^o_L = 10^{3.79}$ Torr [Subcommittee on Ammonia, National Research Council, 1978] into Eq. 2-33, the activity coefficient of ammonia in water can be estimated as ζ_{NH_3} = 0.088. This value is lower than 1, and reflects the favorably low energy level (high comfort) that ammonia has when dissolved in water.

Because of the great importance of ammonia as a chemical, $K^a_{H,fb}$ values have been measured for dissolution in a wide variety of solvents besides water. Table 2 provides a summary of some of these values along with corresponding solvent dielectric constant ε values as well as calculated $K^a_{p,fb}$ and ζ_{NH_3} values. Each dimensionless ε value provides a measure of solvent polarity; as shown in Figure 2-4, the Table 2-2 values of log ζ_{NH_3} are well correlated with log ε. The $K^a_{p,fb}$ and ζ_{NH_3} values in Table 2-2 provide insight regarding probable values for these two parameters for ammonia dissolved in typical PM_{MTS}. At 20 °C, in water which is extremely polar, ε = 80.1 and ζ_{NH_3} = 0.088. In *n*-hexane, which is extremely non-polar, ε = 1.9 and ζ_{NH_3} = 4.8. PM_{MTS} is a mixture of nicotine, other alkaloids, organic acids, a variety of less-polar constituents (e.g., solanesol), and some water [USDHHS, 1989]. The polarity of typical PM_{MTS} will thus be inside the range demarcated by water and *n*-hexane. Based on the information in Table 2-2, the range $0.2 < \zeta_{NH_3} < 2$ is proposed for typical PM_{MTS}. This range is consistent with the fact that $\zeta_{Nic} \approx 1$ in PM_{MTS} [Pankow, 1997, 2003]. Assuming $60 < \overline{MW}_{PM} < 129$ g/mol for conventional PM_{MTS} [Pankow, 2003], we then estimate that $12 < \overline{MW}_{PM}\ \zeta_{NH_3} < 258$. Taking p^o_L for ammonia at 20 °C to be $10^{3.79}$ Torr, then by Eq. 2-33 this range for $\overline{MW}_{PM}\ \zeta_{NH_3}$ suggests that $K^a_{p,fb}$ values for most conventional PM_{MTS} samples will fall within the range ~$10^{-9.6}$ m^3 μg^{-1} (upper bound) to $10^{-10.9}$ $m^3/\mu g$ (lower bound) (see Table 2-3).

Table 2-1. Estimated effects on γ activity coefficient values for NH_4^+, NH_3, $NicH^+$, and Nic, when going from dilute water to PM_{MTS}, and corresponding estimates for the offset between pH_{eff}^a and pH_{eff}^n.

species	in very dilute water			in typical PM_{MTS}			
	γ	γ ratio	pH_{eff}^a in relation to pH_{eff}^n by eq 2 -22	less or more comfortable compared to being in dilute water?	γ estimate (rough)	γ ratio estimate (rough)	pH_{eff}^a in relation to pH_{eff}^n by eq 2-22 (roughly)
NH_4^+	$\gamma = 1$	$\gamma_{NH_4^+} / \gamma_{NH_3} = 1$	$pH_{eff}^a = pH_{eff}^n + 0$ (= pH)	much less	$\gamma \approx 20$	$\gamma_{NH_4^+} / \gamma_{NH_3} \approx 4$	$pH_{eff}^a \approx pH_{eff}^n - 1$
NH_3	$\gamma = 1$			less	$\gamma \approx 5$		
$NicH^+$	$\gamma = 1$	$\gamma_{NicH^+} / \gamma_{Nic} = 1$		less	$\gamma \approx 10$	$\gamma_{NicH^+} / \gamma_{Nic} \approx 40$	
Nic	$\gamma = 1$			more	$\gamma \approx 0.25$		

Table 2-2. $K^{a}_{H,fb}$ and computed values of $K^{a}_{p,fb}$ and $\zeta_{ammonia}$ for ammonia dissolved in solvents with varying dielectric constant ε (dimensionless)

Solvent	Dielectric constant[a]	MW (g/mol)	$\log K^{a}_{H,fb}$ (*m*/atm, 20 °C)	$\log K^{a}_{p,fb}$ ($m^3/\mu g$, 20 °C)	ζ_{NH_3} (20 °C)
water	80.1	18.0	1.89[a]	-8.73	0.088
methanol	33.0	32.0	0.85[b]	-9.67	0.46
ethanol	25.3	46.1	0.70[c]	-9.83	0.48
1-propanol	20.8	60.1	0.67[c]	-9.85	0.41
1,2-dichloroethane	10.4	99.0	0.04[c]	-10.68	1.45
1,2,3-propanetriol triacetate	7.1	218.2	0.08[d]	-10.61	0.63
chloromethyl-benzene	6.9	126.6	-0.30[c]	-10.95	2.10
chlorobenzene	5.7	112.6	-0.36[c]	-11.03	2.76
bromobenzene	5.5	157.0	-0.47[c]	-11.27	3.45
chloroform	4.8	119.4	0.47[c]	-10.32	0.59
1-methylnaphthalene	2.9	142.2	-0.52[e]	-11.15	2.91
toluene	2.4	92.1	-0.52[c]	-11.07	3.74
benzene	2.3	78.1	-0.35[c]	-10.92	3.07
carbon tetrachloride	2.2	154.4	-0.55[c]	-11.35	4.17
cyclohexane	2.0	84.2	-0.99[c]	-11.50	10.67
n-hexane	1.9	86.2	-0.72[e]	-11.16	4.78
1,1'-bicyclohexyl	N/A	166.3	-1.16[e]	-11.72	9.07

[a] Clegg and Brimblecombe [1989];
[b] Based on data in Kertes [1985] on $NH_{3(g)}$ solubility in the range 273.2-301.6 K;
[c] Based on data in Kertes [1985] on $NH_{3(g)}$ solubility at 293.2 K;
[d] Based on equation given in Kertes [1985] on $NH_{3(g)}$ solubility as $f(T)$;
[e] Based on extrapolation of data in Kertes [1985] on $NH_{3(g)}$ solubility in the range 300 - 475 K.

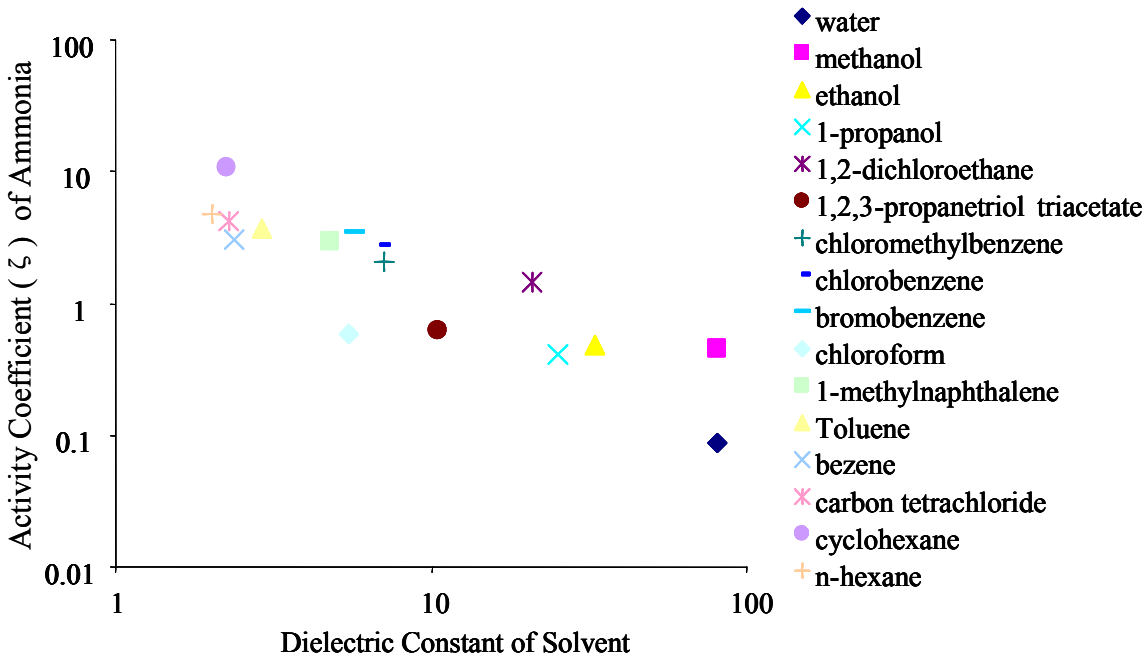


Figure 2-4 Activity coefficients (ζ) of ammonia vs. dielectric constants

Table 2-3. Estimated bounds for ζ_{NH_3}, $\overline{MW}_{PM}$, the product $\zeta_{NH_3} \times \overline{MW}_{PM}$, and for $\log K^{a}_{p,fb}$ at 20 °C. (Values of $\log K^{a}_{p,fb}$ (20 °C) calculated by eq. 2-32 assuming p^{o}_{L} for ammonia at 20 °C to be $10^{3.79}$ Torr.

ζ_{NH_3} (20 °C)	$\overline{MW}_{PM}$	$\zeta_{NH_3} \times \overline{MW}_{PM}$	$\log K^{a}_{p,fb}$ (20 °C)
estimated lower bounds →			corresponding upper bound
0.2	60	12	-9.6
estimated upper bounds →			corresponding lower bound
2	129	258	-10.9

CHAPTER 3
THE GNERATION OF MAINSTREAM TOBACCO SMOKE SAMPLES AND THE MEASUREMENTS OF NICOTINE AND AMMONIA

3.1 The generation of MTS samples

MTS samples were generated with a procedure similar to that was described by Pankow et al. [2003]. As shown in Figure 3-1, the major components of the smoking apparatus were a four-liter capacity chamber and a smoking bag. The smoking bag is inside the chamber, and connected with cigarettes via a modified 3-way glass/TFE stopcock, a ¼ in i.d. TFE Teflon Swagelok union, and a 2-arm glass cigarette holder. The 2-arm glass cigarette holder can be substituted with a single holder or 4-arm cigarette holder. The chamber and the lid were sealed with four adjustable C clamps. Via a brass or a stainless steel tube, air in the chamber could be removed with a Gast pump (model 1031-102A-351, Benton Harbor, MI) and an Asco 8262G solenoid valve. The opening and closing of the pumping system was controlled by an in-house built timing control system. The flow speed of the pumping system was controlled with a needle valve.

Smoking bags were fabricated from 0.005 in thick FEP Teflon sheet (Saint-Gobain Performance Plastics, Wayne, NJ). The rolled Teflon was cut into rectangular sheets. The sheets were washed once each with de-ionized (DI) water and methanol, then dried in a pre-warmed oven at 50 ^{o}C for 24 hours. To make the smoking bag, clean and dry Teflon sheet was folded onto itself, and three sides of the folded sheet were sealed with an AIE-305 Heat Sealer (American International Electric, Whittier, CA). As shown in Figure 3-2, the fourth side was cut to allow for the 3 cm port at the top. Both sides of that port and the rest of the fourth side were sealed. Bags of two sizes were made. The small size has a capacity of about 450 ml, was for collecting smoke from the first 3 puffs. The large size bag, which has a capacity from 1000 to

1200 ml, was for collecting smoke from the remaining puffs. Smoking bags were mounted onto stopcocks by inserting vertical arm of the 3-way stopcock into the 3 cm port, and sealed with ¼ in plastic hose clamps.

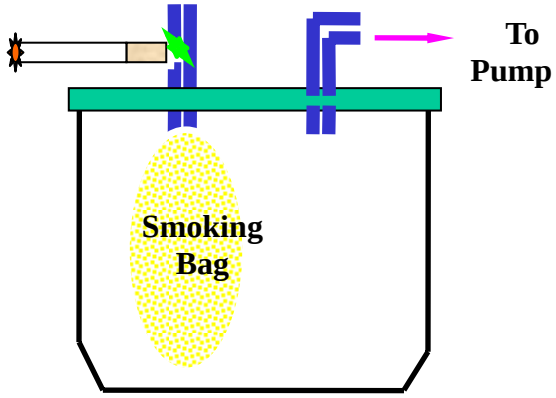


Figure 3-1 Smoking Apparatus

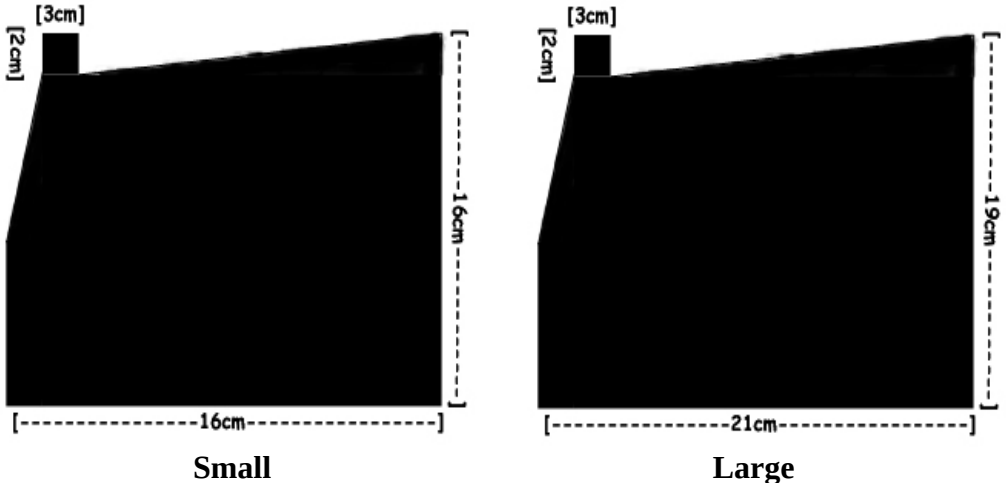


Figure 3-2 Shape and dimensions of smoking bags

Before smoking, relative humidity (RH) in the sampled cigarette pack was measured with a RH probe. The RH probe was calibrated beforehand with the headspace air of saturated water solutions of $CaCl_2$ (29% RH), NaBr (58% RH) and KBr (82% RH). After the measurement of RH, cigarettes were used for smoking without any further conditioning. The mouth end of each cigarettes was wrapped with a small amount of clean ¼ inch Teflon tape to obtain a seal between the cigarette and the holder. Filter ventilation holes were 100% open in this study. Two cigarettes were

lighted simultaneously, and the pump system started removing air from the chamber. The negative pressure in the chamber drew MTS into the smoking bag. For all samples, a 90 mL (45 mL per cigarette) puff of 2 seconds duration in every 30 seconds was employed. The first three puffs and the remaining puffs in each smoking event were collected as two samples. After the MTS of the 1st three puffs had been collected, the stopcock was turned to close the smoking bag. The burning cigarettes, together with the cigarette holder, were then removed from the chamber for the 1st three puffs, and connected immediately to the second chamber to collect MTS of the remaining puffs. Cigarettes were smoked to a 23 mm "butt length". Puff numbers for the remaining puff samples are counted.

3.2 Sampling process

Gas phase nicotine

Nicotine in the gas phase MTS was sampled and analyzed according to the protocol described by Pankow et al. [2003]. Glass cartridges from Supelco (Bellefonte, PA) were used to sample nicotine in gas phase. Each cartridge was packed with ~0.1 g of Tenax-TA (35/60 mesh, Buchem BV, Holland), and plugged with silane treated glass wool (Alltech Associates, part # 4037) in both ends. To clean the Tenax-packed cartridges, a solution of 1:1 hexane/acetone was passed through at a flow rate of 8 mL/min for 45 minutes. Residual solvent in cartridges was purged with N_2 for fifteen minutes. The cartridges were then placed in a conditioning oven and purged with a stream of He at 120 mL/min. The initial temperature was 25 ^oC for thirty minutes, then 250 ^oC for one hour. Each conditioned cartridges was capped with brass Swagelok endcaps and Teflon ferrules, and stored in a clean glass culture tube. The endcaps and ferrules were pre-cleaned by rinsing in methanol and baking for 90 min at 90 ^oC. Before taking samples, the cleaned cartridges were prepared by injecting 4 μL of an internal standard solution which contained 2.5 ng/μL nicotine-*d3* and 5 ng/μL naphthalene-*d8*. The solvent of the internal standard solution was removed by purging the cartridges with N_2 at 50 ml/min for 10 minutes.

15 to 20 minutes after the smoking process had been finished, the cigarette holder was replaced with a TFE Teflon filter holder. In the filter holder, 7 mm diameter mini-filters were used to separate PM from gas phase samples. The mini-

filters were cut from Zeflour supported PTFE 47mm filters with 0.5 μm pore size (Pall Life Science, Providence, RI, Part # P5PQ047). Before being put into the filter holder, the mini-filters were sonicated in 20 mL MeOH in a Teflon beaker for five minutes. The sonication process was performed three successive times. After the sonication, the filters were placed in a pre-warmed oven (50 ^{o}C) to dry. A 30 mL capacity gas-tight syringe was used for withdrawing gas phase through the mini-filters and into the cartridges loaded with internal standard. The sampling rate was below 5 mL/minute.

Before sampling with the Tenax-TA cartridges, the mini-filters were saturated with nicotine by withdrawing 30 ml of gas phase MTS through the filters and a dummy cartridge. For each smoking bag, three gas phase nicotine samples were generated with Tenax-TA cartridges. For each cartridge, and depending on brand or type of cigarettes, the sampling volume was in the range of 5 to 20 ml. Immediately after the sample being taken, the cartridge was purged with N_2 to sweep all sampled nicotine into adsorbent bed, and remove part of volatile compounds from the cartridge. Each sample-taken cartridge was then capped, and sealed in a screw cap culture tube. In most cases, the cartridges were analyzed on the same day. Otherwise, the cartridges were kept in freezer and analyzed on the second day.

Gas phase ammonia

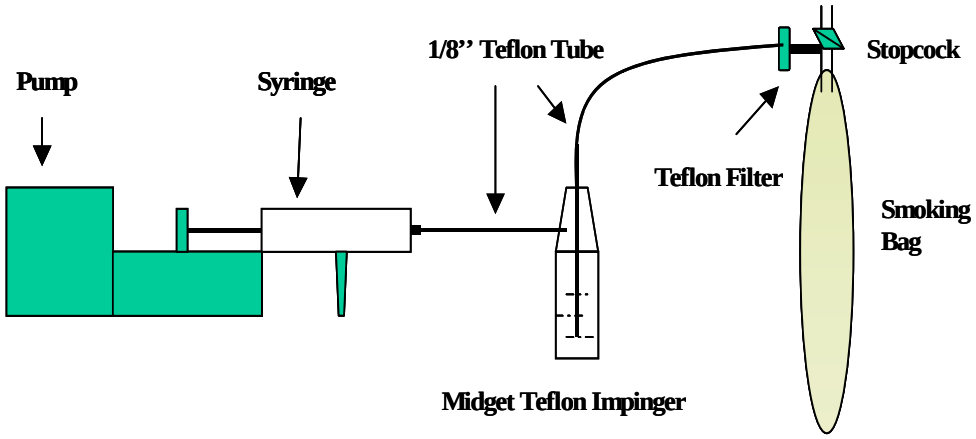


Figure 3-3 Sampling gas phase ammonia

A diagram of the sampling process for ammonia in gas phase MTS is given in

Figure 3-4. After the gas phase nicotine samples had been taken, the mini-filter used for sampling nicotine was replaced with a clean mini-filter. Gas phase was withdrawn through a 22 mL Teflon midget impinger by a syringe pump. 5 to 20 mL of 0.3 mM H_2SO_4 in the impinger was used to trap ammonia from gas phase MTS. The rate of sampling was about 12 mL/min. Samples were analyzed on the same day.

PM sample

After gas phase samples had been taken, the remaining volume was measured. The evacuated bag with particulate matter was weighed. 1000 ng/μL nicotine-*d3* (100 μL for samples of the 1st 3 puffs, and 200 μL for samples of the remaining puffs) was spiked into the smoking bag. Low-water 2-propanol was spiked into the smoking bag as extracting solvent for particulate matter in the bag. Nicotine-d_3 was spiked into the extracted solution as an internal standard for nicotine analysis. After agitation, the 2-propanol solution was transferred from the bag into a 40 mL amber glass vial and stored in a freezer.

3.3 Analysis and quantification

Gas phase nicotine

Tenax-TA cartridges containing sampled nicotine were analyzed with an automatic thermal desorption (ATD, Perkin-Elmer ATD 4000) and GC (Hewlett-Packard model 5890, Palo Alto, CA)/MS (Finnigan 4000, Sunnyvale, CA) system. The cartridges were desorbed in a backflush mode at 200° C for 10 minutes, purging at 50 mL min^{-1} with ultrapure He. A Tenax trap at 5° C was used as a second trap to focus the analytes prior to thermal transfer to GC. After the primary desorption, the focusing trap was heated at 40°C/s to 250°C and then held for 4 minutes to desorb the trapped analytes with a flow of He at 5 mL/min. The overall split ratio was 4:1. In the GC oven, a DB-5 (J&W) GC column, 30m x 0.25 mm ID, 0.25 μm film thickness, was used. Carrier gas was He at 18.5 psi head pressure. The temperature program was: hold for one minute at 50 °C, then 10 °C/min to 100°C, then 18°C/min to 280°C, then hold for four minutes. The GC/MS interface temperature was 210 °C. Ionization mode of the MS was electron impact at 70 eV. MS scan range was 50 to 300 amu, and the scan rate was 380 ms/scan. Source temperature was 220 °C, and manifold temperature

was 115 ^{o}C. The MS calibration reagent was FC43 (perfluorotributylmine). MS data were acquired and processed with a GALAmbY MS data system (LGC, San Jose, CA).

For the quantification of gas phase nicotine, six external standards, containing 1, 2, 3, 5, 10, and 20 ng/μL of nicotine and nicotine-d_3, were prepared in 2-propanol solution. All standards contained 5 ng/μL naphthalene-d_8. With the same procedure of preparing gas phase nicotine sampling cartridges, 4 μL of each standard solution were loaded onto Tenax-TA cartridges. The standard cartridges were analyzed together with sample cartridges. With MS spectra of standards and samples, the quantification process utilized the following steps:

1) Peak areas of quantification ions were integrated. For nicotine, the quantification ions were m/z 84 and 162. For nicotine-d_3, the quantification ions were m/z 87 and 165. For naphthalene-d_8, the quantification ion was 136.
2) For each nicotine standard, a response factor (RF^n) was calculated for quantification ion 84 of nicotine, by using quantification ion 87 of nicotine-d_3 as internal standard, according to

$$RF^n = \frac{A_{STD}^n \times M_{STD}^{n-d_3}}{A_{STD}^{n-d_3} \times M_{STD}^n} \qquad (3\text{-}1)$$

where, A_{STD}^n and $A_{STD}^{n-d_3}$ were the peak areas of quantification ion 84 and 87 for nicotine and nicotine-d_3 , M_{STD}^n and $M_{STD}^{n-d_3}$ were the mass amounts of nicotine and nicotine-d_3 in the standard.

3) An average response factor (RF_{Ave}^n) was calculated for all the standards.
4) The mass of nicotine in the samples was calculated according to:

$$M_g^n = \frac{A_g^n \times M_g^{n-d_3}}{A_g^{n-d_3} \times RF_{Ave}^n} \qquad (3\text{-}2)$$

5) The concentration of nicotine in gas phase samples was calculated as

$$C_g^n = \frac{M_g^n \times 10^6}{V_g^n} \qquad (3\text{-}3)$$

where, C_g^n was the concentration of gas phase nicotine with unit of ng/m^3, M_g^n was mass of nicotine in the sample with unit of ng, and V_g^n was the volume of the gas phase sample with unit of mL.

Particulate phase nicotine

Before each analysis of PM nicotine, the 2-propanol solution of PM samples was taken out from the freezer and allowed to come to room temperature. 100 μL of sample solution was combined with 890 μL of 2-propanol and 10 μL of 500 ng/μL Napthalene-d_8. The standards for the analysis of particulate phase nicotine were the same as the ones used for the analysis of gas phase nicotine. Standards and the diluted samples were analyzed by GC/MS with the following profile. The injection temperature was 220 ^{o}C. Initial oven temperature was 60 ^{o}C for 1 minute, then 15 ^{o}C/min to 300 ^{o}C, then hold for 2 minutes. The Injection volume was 0.5 μL. Mass of nicotine and nicotine-*d3* in particulate phase samples were calculated with the same process for gas phase samples. Concentration of nicotine in particulate phase MTS was calculated according to:

$$C_p^n = \frac{M_p^n}{M_{TPM}} \qquad (3\text{-}4)$$

where, C_p^n was the concentration of nicotine in particulate phase MTS with the unit of ng/μg, M_{Nic}^p was the mass of nicotine in particulate phase MTS with the unit of ng, and M_{TPM} was the weight of PM.

Gas phase ammonia

Gas phase ammonia samples were analyzed with a HPLC system comprised of an ISCO, Model 2350 pump, coupled with an Alcott 708 autosampler, and an Attech 550 conductivity detector. An Alltech Universal Cation column (100 mm in length and 4.6 mm in diameter) and MF guard cartridge (7.5 mm in length and 4.6 mm in diameter) were used for this system. The mobile phase was 0.3 mM H_2SO_4, no gradient. Flow rate of the mobile phase solution was 1mL/min. Volume of the injection loop was 100 μL. Temperature of conductivity detector was 35 ^{o}C. Back pressure on column was in the range of 1230 to 1370 psi. To minimize the interference from Na^+, containers made from plastic or Teflon were used for standard solution, sample solution and mobile phase solution. Volume of the small vial for autosampler was 1 mL. Volume of standard solution, gas phase sample solution and filter sample solution in auto sampler vials was 830 μL. Caps without filters were

used for these vials. The chromatographic data were acquired and processed with a PeakSimple data system. For the quantification of ammonia, standard solutions containing 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 0.8, 1.0, 2.0, and 5.0 μg/mL of NH_4^+ (as N) were prepared. Retention time of peaks in chromatogram was used to identify target analytes.

The quantification of ammonia by IC involved the following steps:

1) Peak areas of NH_4^+ in chromatograms from standards and samples were integrated.
2) Theoretically, there is a linear relation between the concentration (or mass) of a specific ion in sample solution and the response of a conductivity detector:

$$G = \frac{\Lambda C}{K \times 1000} \qquad (3\text{-}5)$$

where G is conductance measured by the detector; Λ is the equivalent conductance with unit of mhos cm^{-2} $equiv^{-1}$ (constant for a specific ion); K is the cell constant, and equals to "L/A", with "L" as the distance between two electrodes in cm, and A as the area of the electrodes in cm^{-2}. C is the concentration in equivalents per 1000 cm^3. In this study, linear calibration curves were plotted with the peak areas vs. concentration of NH_4^+ in a series of standards. Figure 3-4 showed a typical calibration curves for ammonia.

3) The slopes and intercepts of the calibration curves were calculated according to:

$$A^a_{STD} = a^a \times C^a_{STD} + b^a \qquad (3\text{-}6)$$

where, A^a_{STD} represents the peak area of NH_4^+ in a standard, C^a_{STD} represents the concentration of NH_4^+ in standard solution, a^a and b^a are the slope and intercept of the regression line, respectively.

4) The values of a^a and b^a were used to calculate concentration of NH_4^+ in sample solution:

$$C^a_{SAMP} = \frac{A^a_{SAMP} - b^a}{a^a} \qquad (3\text{-}7)$$

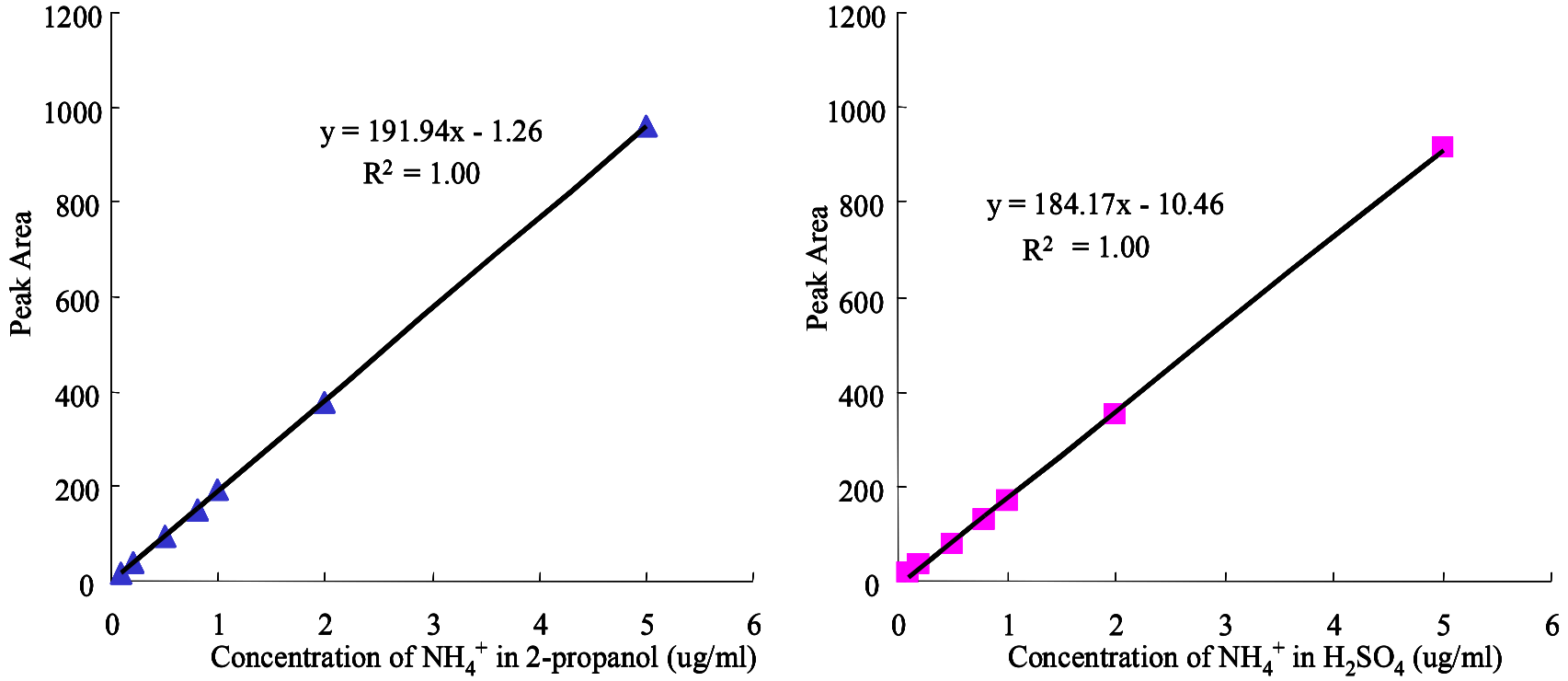


Figure 3-4 Calibration curves for ammonia analysis

(chart on the left-hand side: standards in 2-propanol; chart on the right-hand side: standards in H2SO4)

where, C^a_{SAMP} represents the concentration of NH_4^+ in the sample solution, and A^a_{SAMP} represents the peak area of NH_4^+ in the sample solution.

5) Concentration of ammonia in gas phase was calculated as

$$C^a_g = \frac{C^a_{SAMP} \times V^a_{SAMP} \times 10^9}{V^a_g} \qquad (3\text{-}8)$$

where, C^a_g represents the concentration of ammonia in the gas phase with units of ng/m^3, C^a_{SAMP} is the calculated concentration of ammonia in the sample solution (see step 3) with units of μg/ml. V^a_{SAMp} is the volume of sample solution with units of mL, and V^a_g is the volume of gas phase smoke for the sample with units of mL.

Particulate phase ammonia

Before the analysis of ammonia in PM samples, the 2-propanol solutions were taken out from the freezer and allowed to come to room temperature. If needed, PM samples were diluted with 2-propanol to reduce the concentration of ammonia to a proper level. 810 μΛ of original or diluted sample solution was transferred into a 1 mL auto-sampler vial, and the vials were capped with cap with filter. The protocol of instrumental analysis was the same as that for gas phase ammonia. For quantification, standard solutions, which contained 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 0.8, 1.0, 2.0, and 5.0 μg/mL of ammonia, were prepared in 2-propanol. A typical calibration curve for standards in 2-propanol was shown in Figure 3-5. The quantification process was similar to that of gas phase ammonia. At the final step, the concentration of ammonia in particulate phase are calculated as

$$C^a_p = \frac{C^a_{SAMP} \times V^a_{SAMP}}{M_{TPM}} \qquad (3\text{-}9)$$

where, C^a_p is the concentration of ammonia in particulate phase with units of ng/μg, C^a_{SAMP} is the concentration of ammonia in particulate phase sample solution with units

of μg/mL, V^{a}_{SAMP} is the volume of particulate phase sample solution with units of mL, and M_{TPM} is the weight of particulate phase with units of mg.

3.4 Data quality analysis and quality control

Gas phase nicotine

Nicotine-d_3 that was spiked onto sampling cartridges before sampling was used to evaluate the sampling efficiency for nicotine. The mass of nicotine-d_3 on the sampling cartridges was calculated with following steps:

1) Calculate response factor for nicotine-d_3 by using naphthalene-*d8* as internal standard for each standard:

$$RF^{n-d_3} = \frac{A^{n-d_3}_{STD}}{M^{n-d_3}_{STD} \times A^{nap-d_8}} \quad (3\text{-}10)$$

where, $A^{n-d_3}_{STD}$ and A^{nap-d_8} are peak areas of quantification ion 87 and 136 for nicotine-d_3 and naphthalene-d_8, $M^{n-d_3}_{STD}$ is the mass of nicotine-d_3 in the standard.

2) Calculate the average response factor $RF^{n-d_3}_{Ave}$ for all the standards.

3) Based on the peak area of quantification ions for nicotine-d_3 and naphthalene-d_8, $A^{n-d_3}_{SAMP}$ and A^{nap-d_8}, the mass of nicotine-d_3 in the sample cartridges, $M^{n-d_3}_{SAMP}$, was calculated as:

$$M^{n-d_3}_{SAMP} = \frac{A^{n-d_3}_{SAMP}}{RF^{n-d_3}_{Ave} \times A^{nap-d_8}} \quad (3\text{-}11)$$

4) The sampling efficiency was calculated as

$$E^{n-d_3} = \frac{M^{n-d_3}_{SAMP}}{M^{n-d_3}_{spike}} \times 100 \quad (3\text{-}12)$$

where, E^{n-d_3} is sampling efficiency (%), and $M^{n-d_3}_{spike}$ is spiked mass of nicotine-d_3.

The sampling efficiency was measured for gas phase nicotine for about 250 sampling events. For most sampling events, the sampling efficiency of gas phase

nicotine was in the range of 80 to 120%. Only a small number of samples were out of that range. Since the concentration of nicotine in gas phase smoke was calculated by using nicotine-d_3 as internal standard, the effect of sampling efficiency on the results was included in the calculations.

Gas phase ammonia

One concern for the study was the ability to detect and measure ammonia in the gas phase tobacco smoke samples. As shown in Figure 3-5, the linear calibration range of ammonia standard could be extended down to as low as 0.01 μg/mL. A method detection limit (MDL) estimation was conducted by measuring concentration of ammonia in a standard solution of 0.02 μg/mL of ammonia seven times. The concentration of ammonia in the seven standards was calculated with the calibration curve in Figure 3-5. The standard deviation of the seven calculated concentrations was calculated as 0.005 μg/mL ammonia as N. For ammonia in 200 mL of gas phase MTS that was absorbed with 5 mL solvent, the corresponding detecting limit was calculated to be 3.75 ng/m^3 of ammonia. As we can see in latter part of this thesis, the concentration of gas-phase ammonia in all smoke samples was higher than this level.

To evaluate the sampling efficiency of collecting ammonia in the gas phase smoke with the impinger system, ammonia gas standards were prepared by diluting known amount of pure ammonia gas with dry N_2 in Teflon bags prepared as with bags for smoking. The ammonia gas standards were sampled and analyzed in a manner similar to that used for sampling gas phase ammonia in MTS. The concentration of ammonia in the gas standard was calculated as the total mass of ammonia spiked into the bag divided by the total volume of the gas. The sampling efficiency was calculated as the ratio of the concentration of ammonia calculated from the samples collected with the impinger system to the concentration of ammonia in the bag. For ammonia gas standards with concentrations of ammonia in range of 0.34×10^6 ng/m^3 to 50×10^6 ng/m^3, the sampling efficiency was in the range of 80% to 110% (Figure 3-6) (For a per cigarette smoke volume of ~400 mL, each mg/m^3 unit of NH_3 in the gas phase contributes ~0.4 μg of NH_3). The smaller frame in Figure 3-6 was enlarged for ammonia concentration in the range of 0 to 5 mg/m^3. The low MDL and the results in Figure 3-6 indicate that the protocol developed in this study could efficiently collect and determine ammonia in the gas phase of MTS efficiently.

PM phase ammonia

For the determination of ammonia in PM samples, the issue of most concern was interference from other compounds, considering the complex composition of PM. As shown in Figure 3-7, to verify the ability of the method to determine ammonia in PM, results from standard addition experiments were compared with results obtained with the method described in Secton 3.3. Results for three typical PM samples, with code name A, B, and C, are listed in Table 3-1. Ratio of results from standard addition experiments to those from calibration curves showed consistent results, and indicated that interference from other compounds on the measurement of ammonia in PM was small.

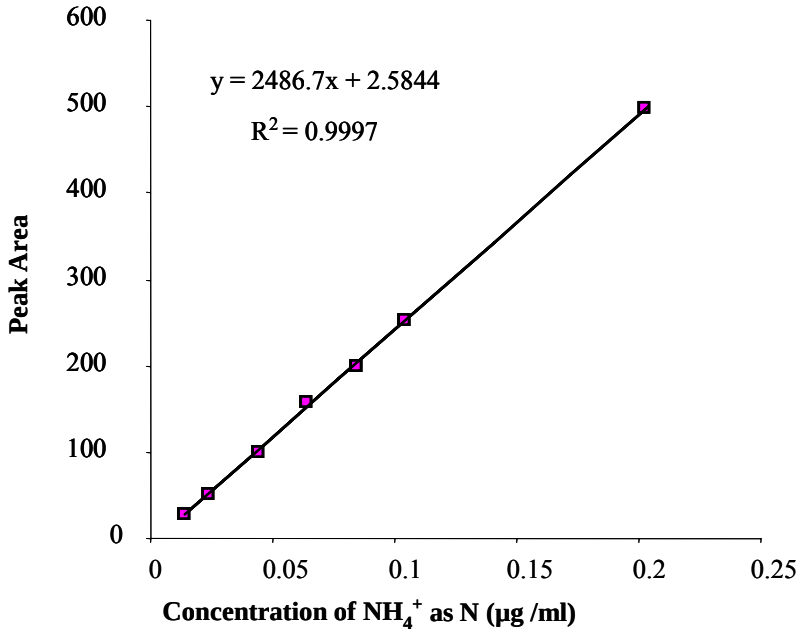


Figure 3-5 Calibration curve for low level ammonia

Table 3-1 Efficiency of measuring ammonia in PM

PM Sample	Concentration of NH3 in PM (ng/ug)		
	Calibration Curves	Standard Addition	***Ratio***
A	0.593	0.576	***1.03***
B	0.682	0.695	***0.98***
C	0.255	0.266	***0.96***

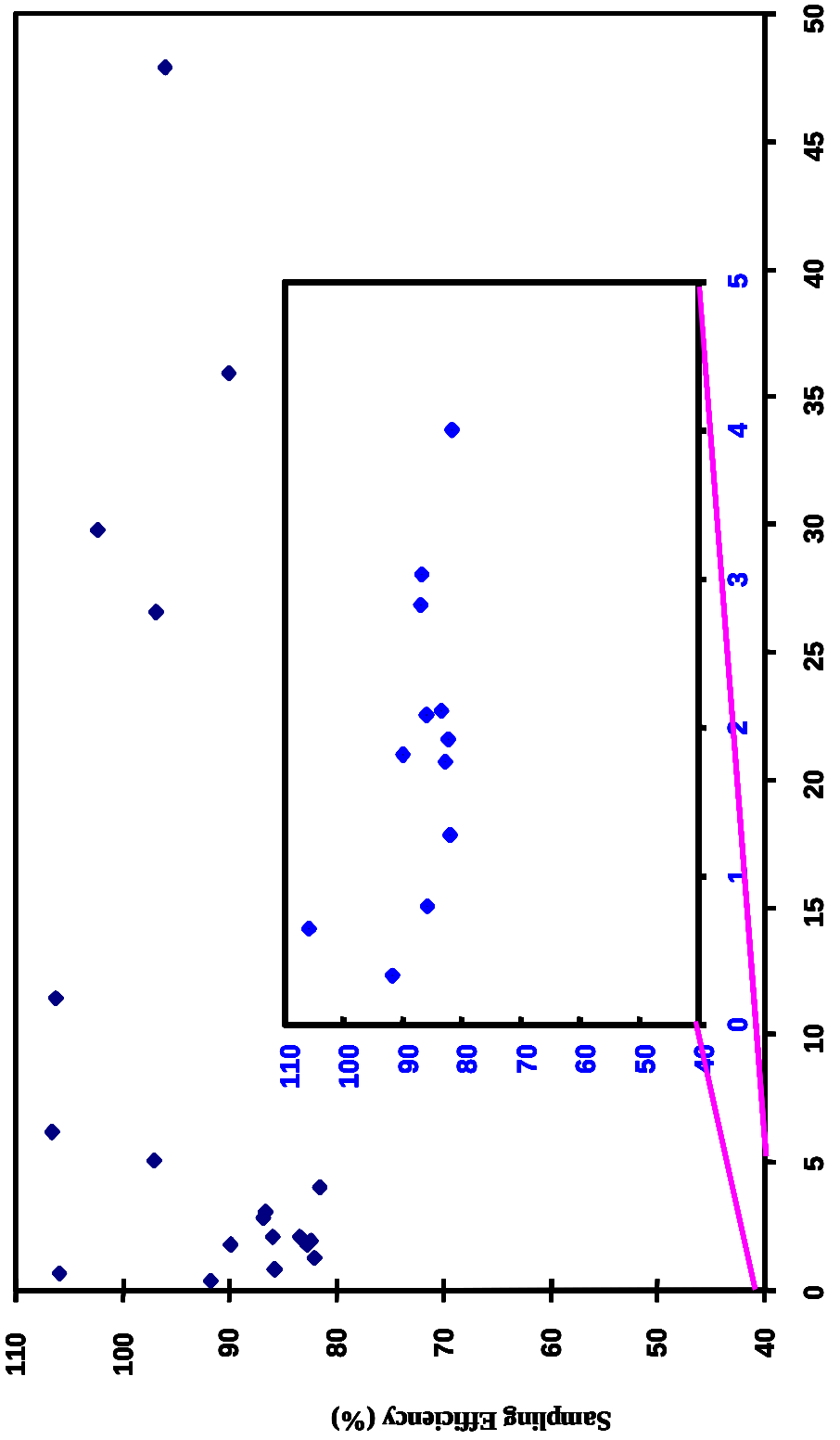


Figure 3-6 Sampling efficiency for gas phase ammonia

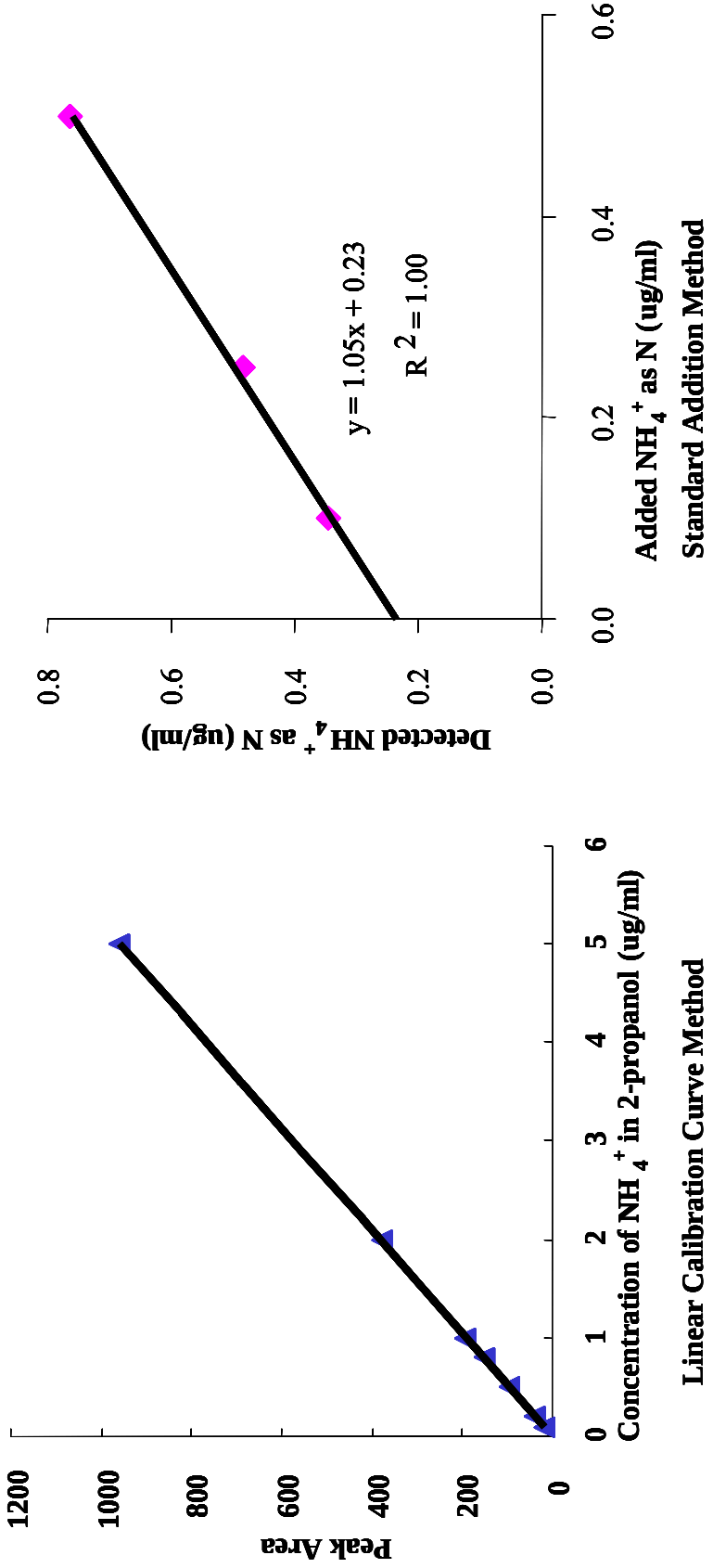


Figure 3-7 Calibration curves for measuring ammonia in PM

CHAPTER 4
THE PARTITIONING OF NICOTINE AND AMMONIA IN MAINSTREAM TOBACCO SMOKE

4.1 Introduction

Previous studies as introduced in Chapter 1 and the theories discussed in Chapter 2 suggest that the acid/base balance in MTS is important for the delivery of nicotine by tobacco smoke and the "impact" potential of tobacco smoke. Internal documents of the tobacco industry also suggest that ammonia-producing additives have been studied as "impact boosters". The dependence of the partitioning properties of basic components of smoke, such as nicotine and ammonia, on the acid/base balance in smoke can be used in the study of the acid/base chemistry in smoke. Discussions in Chapter 2 also indicate that the partitioning properties of nicotine and ammonia will be well correlated. To test the assumptions and theories discussed in Chapter 2, MTS samples from a variety of cigarettes and cigar-like products were studied. In this chapter, the results related to the partitioning properties of nicotine and ammonia in MTS were reported and discussed.

4.2 Experimental

In this study, eleven brands of commercial cigarettes and two brands of commercial little cigars were smoked and sampled according to protocols in Chapter 3. All brands of cigarettes and cigarette-like products were purchased in the US market during March 2003 to May 2003, and June 2005. Descriptions are given in Table 4-1. The MTS samples were analyzed with protocols introduced in Chapter 3. For each MTS sample, either from the 1st three puffs and or from the remaining puffs, the major

parameters determined in this study included: the volume of the smoke, the puff counts, the weight of TPM, the concentration of nicotine and ammonia in both phases.

Table 4-1 Description of cigarettes smoked in the study

Brand Code	Type and Additional Description [a]
A	FF, F, 100's, HP
B	Lights, F, 100's, HP
C	F, KS, SP
D	F, KS, HP
E	FF, F, 100's, HP
F	F, KS, HP
G	F, 100's, HP; ground tobacco wrapped with brown paper.
H	F, KS, HP
I	F, KS, SP
J	FF, F, KS, HP
K	NF, KS, SP
L	NF; ground tobacco wrapped with tobacco sheets
M	NF; ground tobacco wrapped with tobacco sheets

[a] FF, full flavor; KS, king size; SP, soft pack; HP, hard pack; F, Filtered; NF, nonfiltered.

4.3 Results and discussion

Table 4-2 and Table 4-3 are the master data tables for ammonia and nicotine. For each MTS sample, the concentration of ammonia and nicotine in both phases, and the logarithm of partitioning coefficients of ammonia and nicotine were calculated. The delivery of ammonia and nicotine are defined as the mass of ammonia and nicotine in

each puff of one cigarette. These values were calculated by dividing the total mass of ammonia and nicotine in an MTS sample by numbers of puffs, then dividing by 2, since each MTS sample was generated from 2 cigarettes. The percentage of ammonia and nicotine in gas phase was also calculated for each sample. The total mass of ammonia was calculated by adding up the mass of ammonia in the 1st 3 puffs and remaining puffs from the same two cigarettes, then dividing to 2. For those brands with duplicate or more samples, the results are the average values plus or minus one standard deviation.

Total Ammonia

The total ammonia values obtained here are summarized by brand in Table 4-2. Eight of the eleven cigarettes examined exhibited average measured total ammonia between 4 and 11 μg cig^{-1}. The exception was brand K (35.2 ± 11.8 μg/cig), which is unfiltered, and may contain significant amount of "burley" tobacco; burley is the conventional tobacco choice for cigars, and tends to yield high levels of ammonia in MTS [National Cancer Institute, 1998]. In the "cigar-like" category, the products examined included brand G (39.2 μg/cig), brand L (432.6 ± 298.18 μg cig^{-1}) and brand M (488.9 ± 133.3 μg cig^{-1}). Brand G is marketed as a "little cigar", but has a brown paper wrapping and is constructed like a filtered cigarette. Brand L and brand M are made with what appears to be a tobacco leaf wrapping, but contain cut tobacco rather than rolled tobacco as in a conventional cigar. The high ammonia values for brand L and brand M are consistent with previously reported data for cigar-like products. For example, Brunnemann and Hoffmann [1975] found that two types of little cigars generated 288 and 148 μg cig^{-1} of ammonia.

G/P partitioning of ammonia and nicotine: K_p^a and K_p^n values

MTS values of c_p and c_g were determined for ammonia and nicotine for each of the 13 brands examined. Corresponding average values of $\log K_p^a$ and $\log K_p^n$ were calculated according to eqs 2-28 and 2-25 are given in Tables 4-2 and 4-3 for 20 ^oC. Pankow et al. [2003] measured K_p^n values at 20 ^oC for MTS from eleven brands of commercial cigarettes. Four of those brands were also studied here; their $\log K_p^n$ values as

Table 4-2 Ammonia in mainstream smoke of tobacco cigarettes and cigars [a]

Brand Code	Puffs	C_p^a (ng/μg)	C_g^a (10^6ng/m^3)	log K_p^a (m^3/μg)	Del. of NH_3 (μg/puff/cig)	NH_3 in Gas (%)	Total NH_3 (μg/cig)
A	1st 3	0.148±0.017	0.721±0.197	-6.68±0.07	0.49±0.20	10.58±1.62	8.38±2.80
	Remaining	0.277±0.041	0.746±0.272	-6.42±0.15	1.77±0.37	2.88±0.98	
B	1st 3	0.185±0.040	0.793±0.125	-6.64±0.03	0.20±0.01	19.83±2.02	3.85±0.11
	Remaining	0.192±0.002	1.27±0.030	-6.82±0.01	0.41±0.02	16.38±0.99	
C	1st 3	0.160±0.052	1.01±0.070	-6.81±0.15	0.31±0.09	15.19±4.23	9.14±4.07
	Remaining	0.282±0.116	0.833±0.094	-6.49±0.21	1.00±0.36	4.33±1.40	
D	1st 3	0.103±0.021	0.865±0.064	-6.93±0.08	0.23±0.08	17.64±1.65	6.06±2.59
	Remaining	0.221±0.068	1.25±0.135	-6.76±0.09	0.60±0.31	11.10±4.82	
E	1st 3	0.142±0.041	1.04±0.413	-6.85±0.17	0.30±0.09	13.40±6.69	8.67±1.19
	Remaining	0.245±0.042	1.70±0.316	-6.84±0.01	0.97±0.12	8.79±1.51	
F	1st 3	0.233±0.060	0.776±0.106	-6.53±0.15	0.33±0.06	10.44±0.67	6.64±1.32
	Remaining	0.218±0.039	1.18±0.220	-6.73±0.12	0.66±0.18	7.20±2.16	
G	1st 3	0.272	2.75	-7.01	0.33	30.98	39.22
	Remaining	3.028	10.4	-6.54	2.89	14.26	

Table 4 -2 (Cont'd)

H	1st 3	0.128	1.55	-7.08	0.25	28.81	10.69
	Remaining	0.393±0.038	1.58±0.223	-6.60±0.02	1.11±0.39	6.11±1.28	
I	1st 3	0.056±0.010	0.711±0.195	-7.10±0.19	0.13±0.02	25.73±7.66	4.10±0.91
	Remaining	0.113±0.016	0.532±0.126	-6.67±0.16	0.36±0.08	7.93±3.19	
J	1st 3	ND	ND	N/A	N/A	N/A	N/A
	Remaining	0.036±0.001	0.58±0.03	-7.21±0.04	0.058±0.015	56.37±7.24	
K	1st 3	0.168±0.005	4.74±0.514	-7.45±0.05	0.71±0.09	25.32±3.39	35.24±11.80
	Remaining	1.214±0.451	12.2±5.33	-6.99±0.06	6.63±2.37	8.06±0.62	
L	1st 3	0.60±0.02	4.40±1.21	-6.86±0.15	1.56±0.29	11.84±3.32	432.6±298.18
	Remaining	7.51±3.00	327.1±243.	-7.60±0.11	47.55±33.03	36.89±2.26	
M	1st 3	0.59±0.07	2.65±0.35	6.65±.0.07	1.16±0.29	12.93±2.90	488.9±133.3
	Remaining	9.60±1.45	254.4±56.6	7.42±0.06	45.76±13.37	33.81±4.83	

[a] First entry, first three puffs; second entry, remaining puffs. Values are means ± 1s for brands with duplicate or more measurements; no standard deviation for single measurement.

Table 4-3 Nicotine in mainstream smoke of tobacco cigarettes and cigars [a]

Brand Code	Puffs	C_p^n (ng/μg)	C_g^n (10^5ng/m^3)	$\log K_p^n$ (m^3/μg))	Del. of Nicotine (μg/puff/cig)	Nicotine in gas ($\times 10^{-5}$)
A	1st 3	57.18±2.79	1.28±0.182	-3.35±0.04	169.01±56.9	5.50±0.75
	Remaining	50.92±8.18	1.06±0.147	-3.32±0.12	316.7±73.3	2.33±0.51
B	1st 3	52.07±1.22	1.94±0.666	-3.56±0.14	46.7±8.5	21.4±8.18
	Remaining	56.48±1.94	2.73±0.258	-3.68±0.03	100.0±0.33	14.2±0.31
C	1st 3	59.60±5.54	1.98±0.611	-3.51±0.13	98.6±18.5	8.95±2.63
	Remaining	59.12±5.40	2.36±0.740	-3.59±0.12	201.6±16.0	5.47±0.91
D	1st 3	58.20±13.83	1.38±0.296	-3.38±0.01	103.0±12.2	5.95±0.54
	Remaining	62.14±1.42	3.73±2.64	-3.72±0.33	144.5±38.7	13.8±12.3
E	1st 3	60.71±4.47	3.60±1.03	-3.76±0.09	109.3±9.5	11.7±2.93
	Remaining	58.01±3.73	2.40±0.861	-3.60±0.14	211.3±27.1	5.59±1.36
F	1st 3	77.89±26.57	1.96±0.394	-3.41±0.18	97.6±13.3	9.07±2.55
	Remaining	66.50±4.39	3.33±0.501	-3.70±0.06	186.8±39.1	7.06±1.82
G	1st 3	35.25	1.71	-3.69	29.2	21.5
	Remaining	44.68	3.79	-3.93	36.6	40.9

Table 4-3 (Cont'd)

H	1st 3	62.29	1.52	-3.39	86.7	8.18
	Remaining	58.48±3.49	1.59±0.426	-3.43±0.14	154.8±50.3	4.68±2.65
I	1st 3	86.91±3.75	6.28±0.984	-3.86±0.09	154.1±19.5	19.7±4.10
	Remaining	91.41±1.10	2.14±1.29	-3.32±0.24	264.1±28.6	4.21±2.73
J	1st 3	87.63±7.67	7.14±1.96	-3.90±0.10	54.97±14.44	73.48±23.49
	Remaining	92.76±10.09	12.81±2.61	-4.14±0.10	69.84±39.17	121.6±57.1
K	1st 3	53.30±5.93	12.2±5.62	-4.32±0.18	168.4±40.2	25.7±6.08
	Remaining	57.55±1.04	7.42±1.63	-4.10±0.09	291.0±47.6	11.6±3.16
L	1st 3	24.86±2.03	1.53±1.02	-3.07±0.36	56.92±8.51	12.62±10.07
	Remaining	49.24±9.06	18.47±10.72	-4.53±0.17	186.18±81.72	54.16±4.74
M	1st 3	43.06±11.47	3.00±1.51	-3.81±0.32	75.33±32.08	25.32±14.92
	Remaining	63.56±7.06	34.16±9.52	-4.72±0.12	199.17±51.31	99.54±2.98

[a] First entry, first three puffs; second entry, remaining puffs. Values are means ± 1*s* for brands with duplicate or more measurements; no standard deviation for single measurement.

reported here are in good general agreement with the values of Pankow et al. [2003]. $\log K^{n}_{p,fb}$ and α^{n}_{fb} values could not be measured here by the method of Pankow et al. [2003], as doing so would have required addition of ammonia to the sampled smoke PM_{MTS}, and that would have prevented measurement of c^{a}_{p} and thus calculation of K^{a}_{p}.

Among all brands and for both sample types (i.e. "first three puffs" and "remaining puffs"), the largest K^{n}_{p} was $10^{-3.32}$ $m^3/\mu g$ (GPC 100s, "remaining puffs"); the smallest was $10^{-4.72}$ $m^3/\mu g$ (Brand M, "remaining puffs"). For the cigarette brands examined here, the largest K^{n}_{p} was $10^{-3.32}$ $m^3/\mu g$ (brand A, "remaining puffs"); the smallest was $10^{-4.32}$ m^3 μg^{-1} (brand K, "1st 3 puffs"). For the cigar-like products, the results for K^{n}_{p} for "1st 3 puffs" samples were within the range observed for the cigarette samples. However, for the "remaining puffs" samples, the cigar-like products gave markedly lower $\log K^{n}_{p}$ values (-4.72 to –4.53) than did the cigarettes.

Among all brands and for both sample types (i.e. "first three puffs" and "remaining puffs"), the largest K^{a}_{p} was $10^{-6.42}$ m^3 μg^{-1} (brand A, "remaining puffs"); the smallest was $10^{-7.60}$ m^3 μg^{-1} (brand L, "remaining puffs"). For the cigarette brands considered, the largest average K^{a}_{p} measured was $10^{-6.42}$ m^3 μg^{-1} (brand A, "remaining puffs"). The lowest K^{a}_{p} was $10^{-7.45}$ m^3 μg^{-1} (brand K, "1st 3 puffs"). For the "1st 3 puffs" samples, the cigar-like products gave K^{a}_{p} values that were within the range observed for the cigarette brands considered. However, for the "remaining puffs" samples, the cigar-like products gave K^{a}_{p} values that were significantly smaller than values observed for the cigarettes.

log $\mathbf{K^{a}_{p}}$ *vs. log* $\mathbf{K^{n}_{p}}$

Within a given PM_{MTS} phase of interest, both $\log K^{n}_{p}$ and $\log K^{a}_{p}$ will tend to increase with increasing alkalinity in the PM_{MTS}. For $\log K^{n}_{p}$, the dependence is through α^{n}_{fb} (eq 2-26) and thus through pH^{n}_{eff} (eq 2-14); for $\log K^{a}_{p}$, the dependence is through α^{a}_{fb} (eq 2-29) and thus through pH^{a}_{eff} (eq. 2-20). Since there is a relationship between pH^{a}_{eff} and pH^{n}_{eff}, the value of $\log K^{a}_{p}$ can be related to the value of $\log K^{n}_{p}$.

However, making specific calculations regarding that relationship in a given PM_{MTS} requires information about $\log K^{n}_{p,fb}$ and $\log K^{a}_{p,fb}$ for that PM_{MTS}, and about the offset represented by $(pH^{a}_{eff} - pH^{n}_{eff})$.

Table 4-4 assumes a temperature of 20 °C, that $\log K^{n}_{p,fb}$ = -5.0 and $\log K^{a}_{p,fb}$ = -10.3 (see Table 2-3), and that the offset $(pH^{a}_{eff} - pH^{n}_{eff})$ = -1.0 (see eq. 2-23). A plot of $\log K^{n}_{p}$ vs. $\log K^{a}_{p}$ based on Table 4-4 is given in Figure 4-1. When $pH^{n}_{eff} < pK^{n}_{a}$ (= 8.01 in water at 20 °C), $\log K^{n}_{p}$ decreases essentially linearly with increasing pH^{n}_{eff} (slope ≈ -1); then, as pH^{n}_{eff} increases above 8.01, $\log K^{n}_{p}$ asymptotically approaches the assumed value of $\log K^{n}_{p,fb}$ (= -5.0). In a completely analogous manner, when $pH^{a}_{eff} < pK^{a}_{a}$ (= 9.41 in water at 20 °C), $\log K^{a}_{p}$ decreases essentially linearly with increasing pH^{a}_{eff} (slope ≈ -1); then as pH^{a}_{eff} increases above 9.41, $\log K^{a}_{p}$ asymptotically approaches the assumed value of $\log K^{a}_{p,fb}$ (= -10.3). Bold type is used in Table 4-4 to mark the extents of the nearly linear ranges for $\log K^{n}_{p}$ vs. pH^{n}_{eff} and for $\log K^{a}_{p}$ vs. pH^{a}_{eff}. The ranges spanned by the experimental values obtained here for $\log K^{n}_{p}$ and $\log K^{a}_{p}$ are marked with boxes. Because: 1) most of the experimentally observed range for $\log K^{n}_{p}$ falls within the linear range for $\log K^{n}_{p}$ vs. pH^{n}_{eff}; and 2) all of the experimentally observed range for $\log K^{a}_{p}$ falls within the linear range for $\log K^{a}_{p}$ vs. pH^{a}_{eff}, then a log-log plot of the experimentally-observed values may also be roughly linear (slope ≈ +1). The degree of the linearity will depend on the extent to which all of the PM_{MTS} samples considered share similar $\log K^{a}_{p,fb}$ values as well as similar $\log K^{n}_{p,fb}$ values.

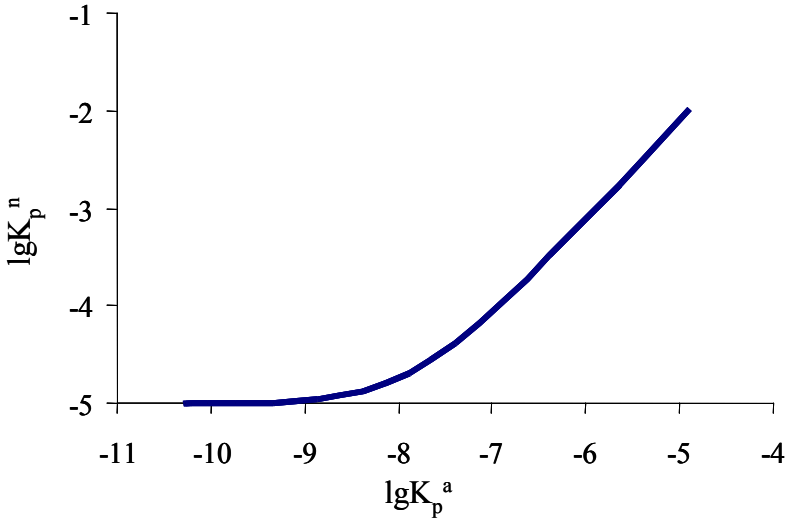


Figure 4-1 Estimated $\log K_p^n$ vs. $\log K_p^a$ based on Table 4-4

Plots of $\log K_p^n$ vs. $\log K_p^a$ for the data obtained here are provided in Figure 4-2.a (1st three puffs) and Figure 4-2.b (remaining puffs). While the amount of scatter in Figure 4-2.a is significant, the points in Figure 4-2.b are much more highly correlated and moreover closely arranged near the $\log K_p^a$ vs. $\log K_p^n$ line based on the values in Table 4-4. Difficulties with measuring samples based only on the first three puffs are a suspected source of the scatter in Figure 4-2a. Also, prior evidence [Pankow, 2003] indicates that the various brands of cigarettes examined here will not be characterized by the exact same values of $\log K_{p,fb}^n$, and the same can be inferred to be the case for $\log K_{p,fb}^a$. Assuming as in Table 4-4 that the offset $(pH_{eff}^a - pH_{eff}^n)$ = -1.0, and $\log K_{p,fb}^n$ = -5.0, the data points are all located between the lines for $\log K_{p,fb}^a$ = -10.9 and –9.6, the estimated lower and higher bound for $\log K_{p,fb}^a$ (see Table 2-3). The line for $\log K_{p,fb}^a$ = -10.3 corresponds to the data reasonably well.

Table 4-4. Values at 20 °C of α_{fb}^{n}, $\log K_{p}^{n}$, α_{fb}^{a}, and $\log K_{p}^{a}$ as functions of pH_{eff}^{n}.

col. A	col. B	col. C	col. D	col. E	col. F
	assuming $pK_{a}^{n} = 8.01$ then:	assuming $\log K_{p,fb}^{n} = -5.00$ then:	assuming $pH_{eff}^{a} - pH_{eff}^{n} = -1.0$ then:	assuming $pK_{a}^{a} = 9.41$ then:	assuming $\log K_{p,fb}^{a} = -10.30$ then:
pH_{eff}^{n}	α_{fb}^{n}	$\log K_{p}^{n}$	pH_{eff}^{a}	α_{fb}^{a}	$\log K_{p}^{a}$
5.0	**0.00098**	**-1.99**	**4.0**	**0.0000039**	**-4.89**
5.5	**0.0031**	**-2.49**	**4.5**	**0.000012**	**-5.39**
6.0	**0.0097**	**-3.00**	**5.0**	**0.000039**	**-5.89**
6.5	**0.030**	exptl. **-3.49**	**5.5**	**0.00012**	exptl. **-6.39**
7.0	**0.089**	data **-3.96**	**6.0**	**0.00039**	data **-6.89**
7.5	**0.24**	range **-4.38**	**6.5**	**0.00012**	range **-7.39**
8.0	0.49	-4.70	**7.0**	**0.0039**	**-7.89**
8.5	0.76	-4.88	**7.5**	**0.012**	**-8.38**
9.0	0.91	-4.96	**8.0**	**0.037**	**-8.87**
9.5	0.97	-4.99	**8.5**	**0.11**	**-9.34**
10.0	0.99	-4.996	**9.0**	**0.28**	**-9.75**
10.5	0.997	-4.999	9.5	0.55	-10.04
11.0	0.999	-4.9996	10.0	0.80	-10.20
11.5	0.9997	-4.9999	10.5	0.92	-10.27
12.0	0.9999	-4.99996	11.0	0.97	-10.29
		↓			↓
		-5.0			-10.30
		$= \log K_{p,fb}^{n}$			$= \log K_{p,fb}^{n}$

It is assumed that $pH_{eff}^{a} - pH_{eff}^{n} = -1.0$. Entries in cols. A-C are emboldened for the range wherein $\log K_{p}^{n}$ vs. pH_{eff}^{n} is nearly linear, and in cols. D-E for the range wherein $\log K_{p}^{a}$ vs. pH_{eff}^{a} is nearly linear. Other column-dependent assumptions are as indicated.

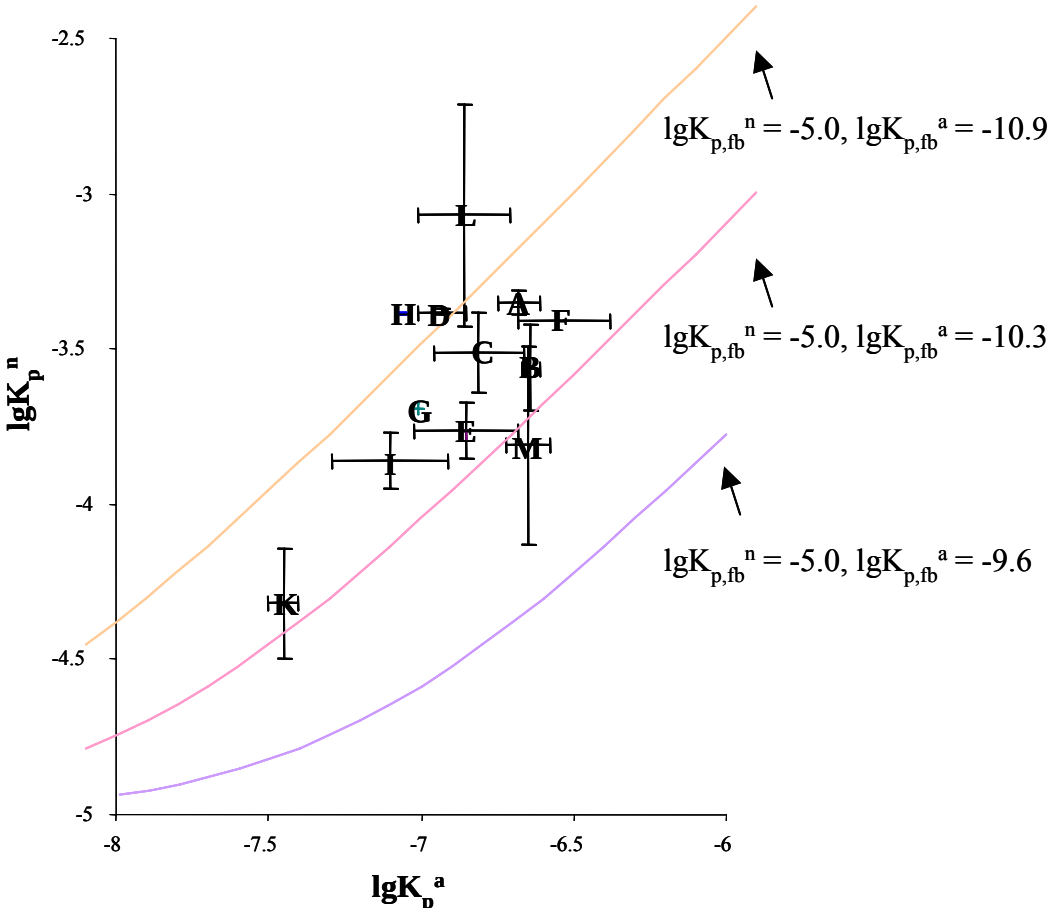


Figure 4-2a. $\log K_p^n$ vs. $\log K_p^a$ at 20 °C for the 1st three puffs

Letters in the chart represent brands listed in Tables 4-1; coordinates of the letters are ($\log K_p^n$,$\log K_p^a$); for brands with replicate samples, the values are average; error bars are +/- standard deviation, no standard deviation for single measurement. Three lines in the chart are estimated correlation between $\log K_p^n$ and $\log K_p^a$, assuming $pK_a^n = 8.01$, $pK_a^a = 9.41$, ($pH_{eff}^a - pH_{eff}^n$) = -1.0, $\log K_{p,fb}^n$ = -5.0, and $\log K_{p,fb}^a$ = -9.6, -10.3, -10.9.

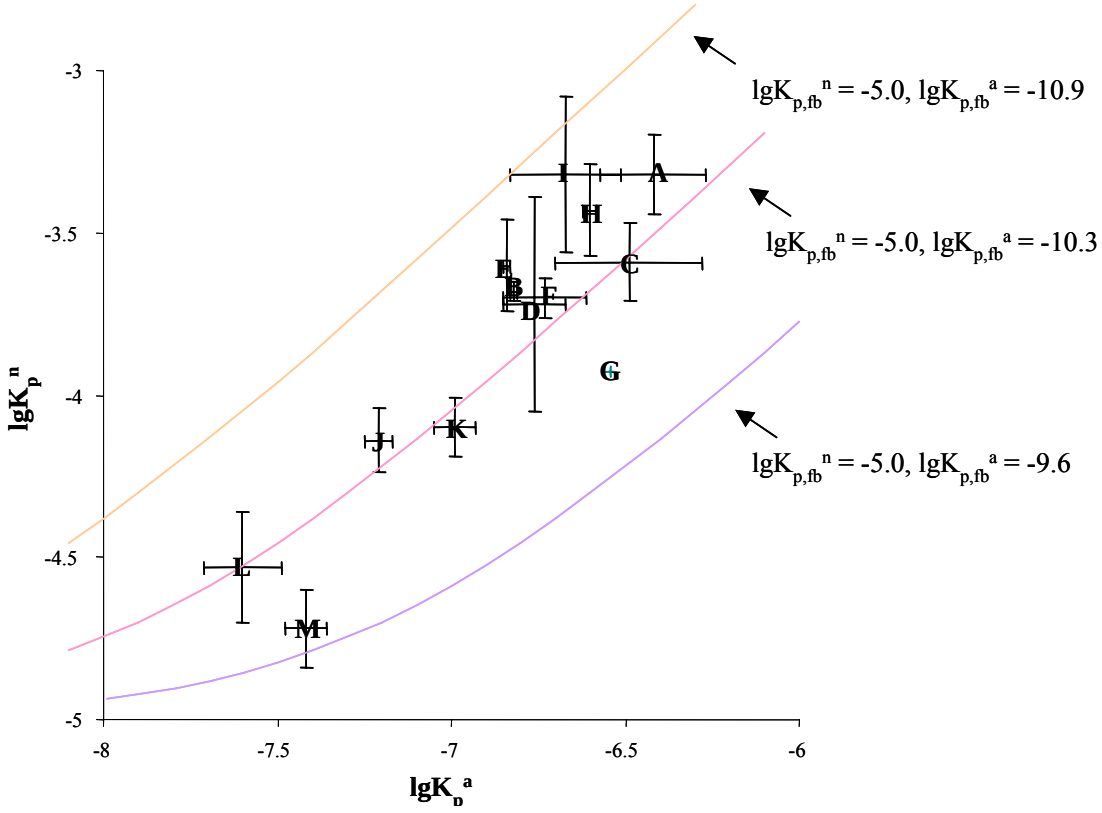


Figure 4-2b. $\log K_p^n$ vs. $\log K_p^a$ at 20 °C for the remaining puffs

Letters in the chart represent brands listed in Tables 4-1; coordinates of the letters are ($\log K_p^n$,$\log K_p^a$); for brands with replicate samples, the values are average; error bars are +/- standard deviation, no standard deviation for single measurement. Three lines in the chart are estimated correlation between $\log K_p^n$ and $\log K_p^a$, assuming pK_a^n = 8.01, pK_a^a = 9.41, ($pH_{eff}^a - pH_{eff}^n$) = -1.0, $\log K_{p,fb}^n$ = -5.0, and $\log K_{p,fb}^a$ = -9.6, -10.3, -10.9.

$\log K_p^n$ vs. log ($NH_{3,TOT}$ / Nic_{TOT})

As discussed above, the extent to which nicotine chemistry (and in particular K_p^n values) is related to smoke ammonia levels has been of significant interest [Pankow, 2001]. $NH_{3,TOT}$ and Nic_{TOT} are defined as the amount of ammonia and nicotine in the smoke sample, both the gas phase and PM. Values of ($NH_{3,TOT}$ / Nic_{TOT}) as the molar ratio were thus computed for the data obtained here, and Figure 4-3a provides a plot of $\log K_p^n$ vs. log ($NH_{3,TOT}$ / Nic_{TOT}) for the 1st three puffs data; Figure 4-3b provides the analogous plot for the remaining puffs data. Although data points in Figure 4-3a for the 1st three puffs are not well correlated, the data points in Figure 4-3b for the remaining puffs are better correlated. The trend is that those samples with higher level of ammonia, relative to nicotine, also have lower values of $\log K_p^n$, which means higher pH_{eff}^n for these samples.

4.4 Conclusions

Results of the study support the hypothesis that the acid/base balance in PM is the major factor affecting the distribution and partitioning of ammonia and nicotine in MTS. The composition of MTS could be important as well. The consistency between the predicted and measured correlation of $\log K_p^n$ vs. $\log K_p^a$ suggests that the assumptions and theories proposed in this study are appropriate and reasonable for the study of ammonia and nicotine in MTS, and the acid/base chemistry in MTS.

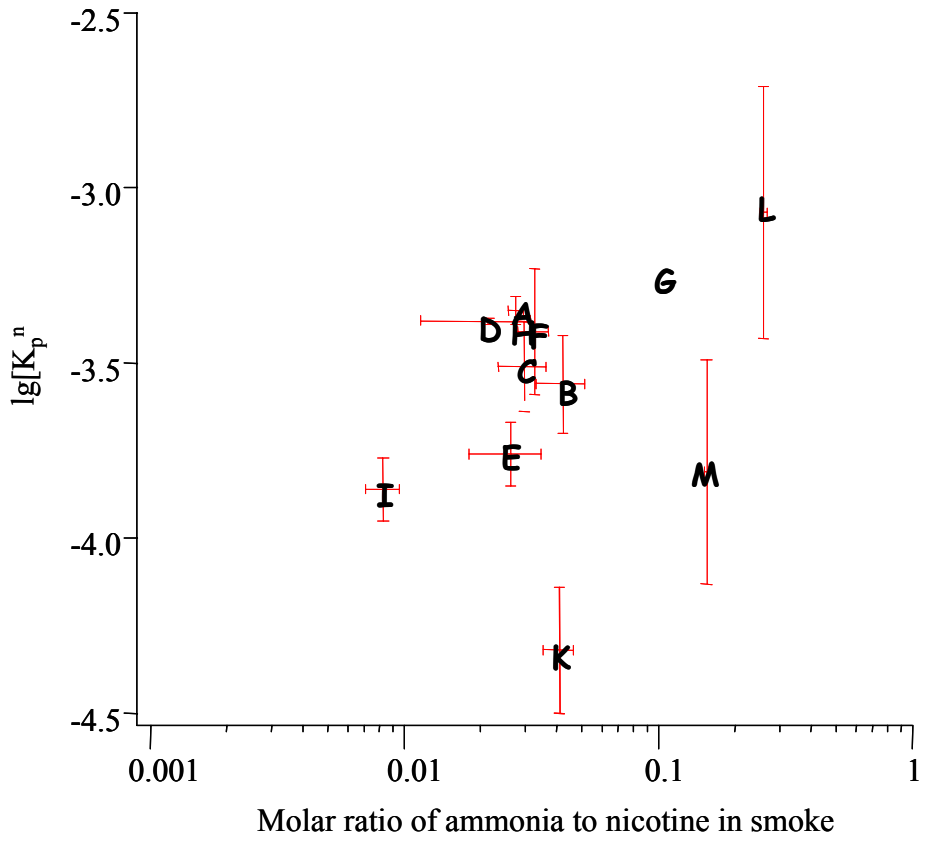


Figure 4-3a $\log K_p^n$ vs. $\log (NH_{3,TOT} / Nic_{TOT})$ for the 1st three puffs

Letters in the chart represent brands listed in Tables 4-1; coordinates of the letters are ($\log K_p^n$, $\log (NH_{3,TOT} / Nic_{TOT})$ as molar ratio); for brands with replicate samples, the values are average; error bars are +/- standard deviation.

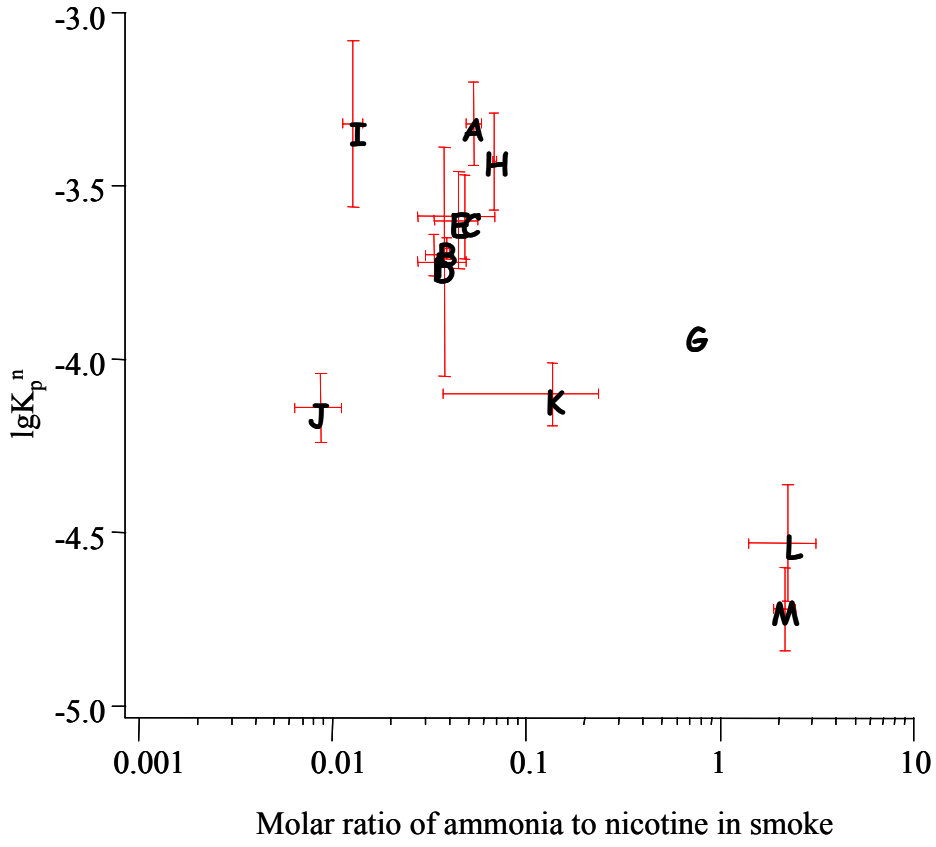


Figure 4-3b $\log K_p^n$ vs. log ($NH_{3,TOT}$ / Nic_{TOT}) for the remaining puffs

Letters in the chart represent brands listed in Tables 4-1; coordinates of the letters are ($\log K_p^n$, log ($NH_{3,TOT}$ / Nic_{TOT}) as molar ratio); for brands with replicate samples, the values are average; error bars are +/- standard deviation.

CHAPTER 5

THE STABILITY AND HYDROLYSIS OF AMMONIA SAMPLES FOR PARTICULATE MATTER OF MAINSTRAM TOBACCO SMOKE

5.1 Introduction

A variety of approaches been developed for the measurement of ammonia in tobacco smoke. these include methods involving colorimetric techniques [Harrell, 1974; Labstat, 1997], ion specific electrode [Sloan and Morie, 1974], laser spectroscopy [Parrish, 1987], gas chromatography (GC) [Ayers, 1969; Brunnemann and Hoffmann, 1975], and ion chromatography (IC) [Ellis, 1999; Vilcins, 1987; Nanni, 1990; Huang, 2003]. To generate and prepare ammonia samples for instrumental analysis, researchers have developed different protocols. Ayers [1969] and Brunnemann and Hoffmann [1975] collected smoke ammonia in bubbler traps containing 0.1 *N* H_2SO_4; the H_2SO_4 solutions were then concentrated by evaporation. Sloan and Morrie [1974] collected smoke in 0.1 *M* HCl, added NaOH to each sample, then concentrated the ammonia by steam-distillation. Battelle [1997], Labstat [1998, 2000], and Huang et al. [2003] collected PM of MTS (PM_{MTS}) on Cambridge filters and gas phase ammonia in impingers containing acidic solutions, such as H_2SO_4, HCl and malic acid, in the range of 0.005 to 0.2 M. The PM and gas phase samples were combined by extracting the Cambridge filters with the acidic solutions in the impingers.

Harrell [1974] suggested that harsh sample processing methods such as those used by Ayers [1969], Brunnemann and Hoffmann [1975], and Sloan and Morrie [1974] had the potential to produce significant ammonia in tobacco smoke samples by "degradation of amides, nitriles, and other nitrogen-compounds" that were also present. Similarly, Brunnemann and Hoffmann [1975] mentioned problems with production of ammonia by hydrolysis of amides during steam distillation of ammonia

from smoke extracts prior to determination by GC. A study by Battelle [1997] found that the concentration of ammonium in the sample did not plateau even after 72 hours following sample collection. To produce reproducible results, the samples were analyzed exactly 15 minutes after sample collection. Otherwise, the samples were discarded.

A comparison of ammonia levels in PM_{MTS} from samples of the 1R4F Kentucky Reference cigarette when smoked by the same Federal Trade Commission (FTC) protocol [IARC, 2004] but analyzed by different methods are of interest. In particular, Huang et al. [2003] used acidic solutions such as 0.01 M H_2SO_4 to extract PM_{MTS}, and reported ammonia levels of 15- 20 μg/cig; Nanni et al. [1990] used a methanol extraction procedure and reported ammonia levels of ~5 μg/cig. Although differences between the batches and/or packs and/or ages of the Kentucky Reference cigarettes used in the two studies are unknown, these results along with the other considerations cited above suggest that measurement of the actual ammonia level in PM_{MTS} may require fairly gentle extraction conditions.

For the data discussed in Chapter 4, the concentration of ammonia in PM_{MTS} samples were measured by extracting PM with 2-propanol. In this chapter, the effects of different solvents (including 2-propanol and water-based solutions) on the determination of ammonia are compared. The implications of the results for acid/base chemistry in smoke and the delivery of nicotine are discussed.

5.2 Experimental

One major task of this portion of the study is to investigate and compare the influence of different solvents on measurement of ammonia in PM_{MTS}. Since it is hard to generate reproducible smoke samples, even with cigarettes from the same pack, a single PM sample in 2-propanol was diluted with different solvent as solutions. PM samples were generated by a procedure similar to that described by Pankow et al. [2003] (see Chapter 2). Experiments in this study included two parts: a) the same original PM sample in 2-propanol was diluted with 2-propanol, water, and H_2SO_4 to different levels of dilution, and the concentration of ammonia in the diluted solution was determined; and, b) the changing concentration of ammonia in diluted solutions in a span of time was observed. Two brands of cigarettes, X and Y, were selected for

this study. These two brands represent two types of cigarette in terms of studying ammonia in MTS. Brand X is a filter tipped, regular cigarette with high market share, and is known for the application of ammonia technology in it [Teague, 1973; B&W, 1991]. Brand Y is a non-filter regular cigarette, and contains significant "burley" tobacco. Burley is the conventional tobacco choice for cigar, and tends to yield high level of ammonia in MTS [National Cancer Institute, 1998]. The cigarettes were purchased from US market. The PM samples were collected according to the protocols described in Chapter 3.

5.3 Results and Discussion

Figure 5-1 provides the results for part (a) of the experiments when PM samples were diluted with different solvents. For brand X, four PM samples were studied. For brand Y, three PM samples were studied. Each sample was diluted with 2-propanol, water, 0.3 mM H_2SO_4 to 1/2, 1/5, and 1/10 of original concentration. For convenience of comparison, the concentration of ammonia in the diluted solution was converted to ammonia in PM with units of ng/μg. For each brand, the height of columns in Figure 5-1 represents the average value of all samples (four samples for brand X, and three samples for brand Y). The length of the error bars is +/- one standard deviation.

Although for each category (of solvent), the level of ammonia in PM samples from brand Y was about two times higher than that from brand X, the trends shown in Figure 5-1 are similar: the ammonia level calculated from samples diluted with 2-propanol was lower than those diluted with water or H_2SO_4 solution. In the same level of dilution, solutions in water and H_2SO_4 generated much higher ammonia levels than the 2-propanol solutions. The level of dilution with 2-propanol had little effect on the final results. This indicates that dilution itself had little effect on the determination of ammonia. Dilution with H_2SO_4 solutions, however, resulted in hgher calculated concentration of ammonia in PM. These results suggest a process of releasing ammonia, triggered by using water or H_2SO_4. For both brands, the use of acid could increase the concentration of ammonia by more than two-fold. Higher calculated concentration of ammonia in PM with higher level of dilution with H_2SO_4 solution also suggests that this ammonia releasing process is favored under acidic conditions.

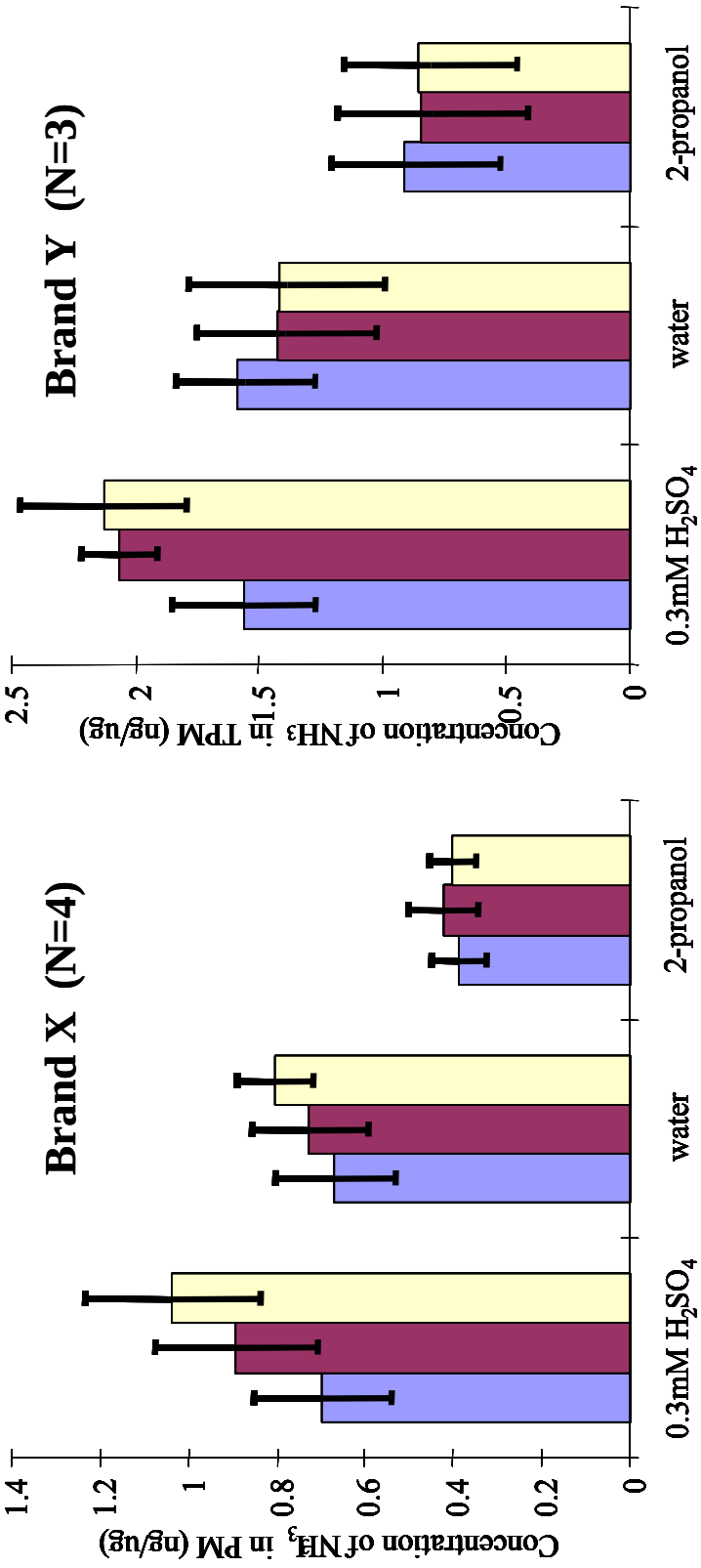


Figure 5-1 Calculated concentration of ammonia in PM when diluted with different solvents

To investigate the effect of acid on the ammonia releasing process, further research in experiments as part (b) was done by diluting one original PM sample (in 2-propanol) with 2-propanol, water, 0.01 mM, 0.1 mM, 1.0 mM H2SO4, 0.01 mM, 0.1 mM, and 1.0 mM KOH. The dilution ratio of the PM sample in 2-propanol to the diluent solution was 1:9 in each case. After mixing, the concentration of ammonia in the diluted solution was measured as soon as possible (the time delay between mixing and injecting for ion chromatographic analysis (IC) was shorter than 5 minutes). The concentration of ammonia in the mixture solution was also measured 1, 2, 3, 4, 5, and 7 hours after mixing. The measured concentration of ammonia in the diluted solution was converted to ammonia in PM with units of ng/μg. The trend lines in Figure 5-2 showed that during the time of monitoring, concentration of ammonia in 2-propanol was stable and only small variabilities were observed. This is consistent with the conclusion from part (a) of the experiments: the dilution with 2-propanol has little effect on the concentration of ammonia in solution. However, for acidic solutions and water (in left-side charts), and basic solutions (in right-side charts), the concentration of ammonia tended to increase with time. The rates of ammonia increase in the initial hour varied among different solvents. The highest initial rate was observed in the solution of 1.0 mM H_2SO_4. Generally for both brands, the rank order based on the initial rate was 1.0 mM H_2SO_4 >> 0.1 mM H_2SO_4> 0.01 mM H_2SO_4 ≥ water ≥ 0.01 mM KOH ≥ 0.1mM KOH > 1.0 mM KOH. This order is consistent with the previous conclusion: the release of ammonia is favored under acidic conditions

The highest level of acidity used in this study was still much lower than those used by other researchers. Although either more acidic or more basic solutions were not evaluated in this study due to experimental difficulties, it is still reasonable to predict that the rate of ammonia release in more acidic solutions could be much faster than those data shown in Figure 5-2. Compared with 2-propanol solution and others, the concentration of ammonia in 1.0 mM H_2SO_4 at 0 hour, i.e. less than five minutes after mixing, was already significantly higher than that in 2-propanol seven hours after mixing. This indicates that the method developed by Battelle [1997] that analyzed smoke samples in 15 minutes after collection may be affected by the release of ammonia. Both parts (a) and (b) of the experiments in this effort suggest that organic solutions, such as 2-propanol, could be more appropriate than water or acidic solvent for the measurement of unbound ammonia in tobacco smoke PM.

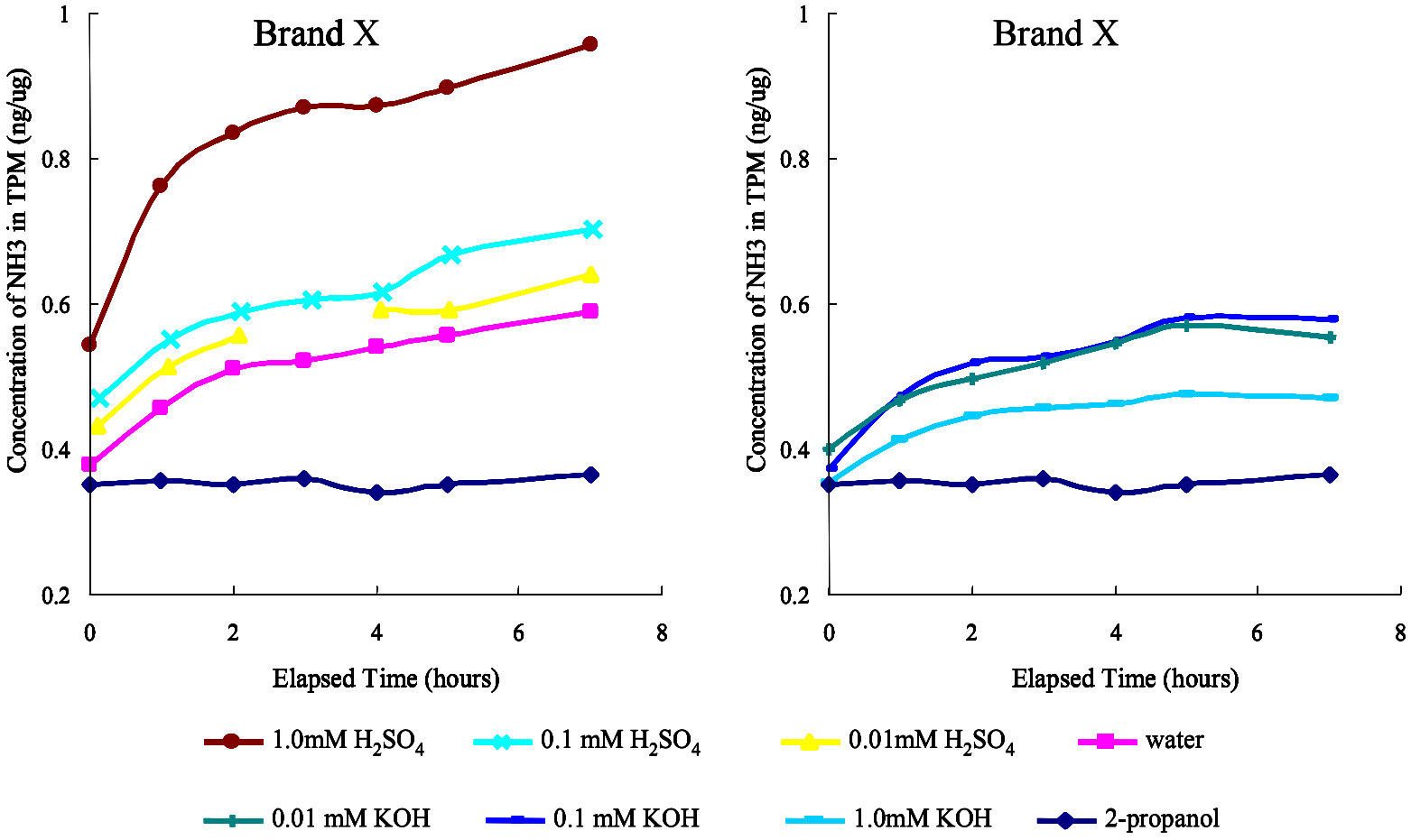


Figure 5-2a Changing concentration of ammonia with time for PM sample diluted in different solutions

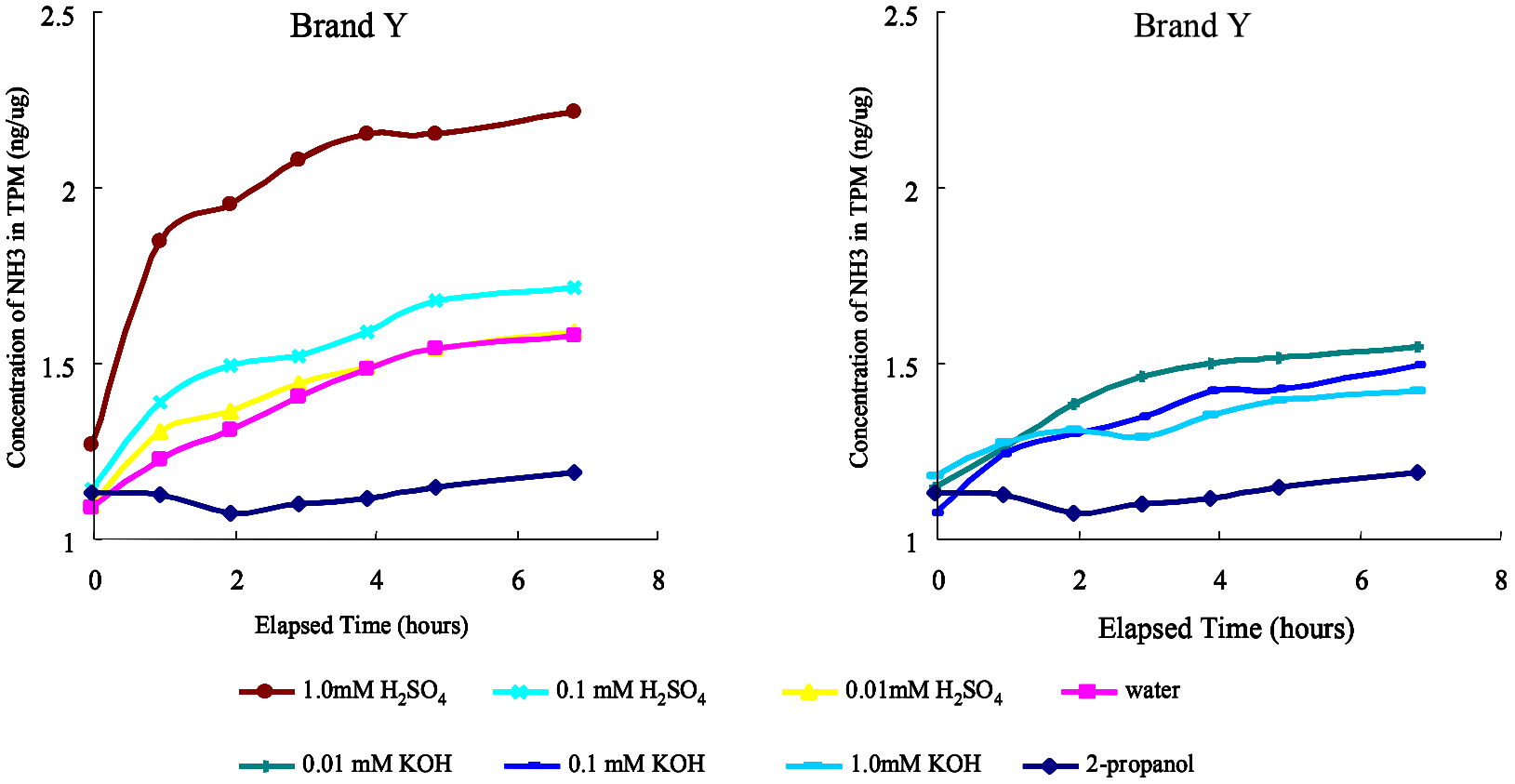


Figure 5-2b Changing concentration of ammonia with time for PM sample diluted in different solutions

The slow rate of ammonia release in the solution considered here suggest that the source of ammonia was unlikely of a simple ammonium salt in the PM. Schmeltz and Hoffmann [1977] estimated that about 30% of all compounds in tobacco leaf and smoke are nitrogen-containing compounds. Among the hundreds of nitrogen-containing compounds, as suggested by Harrell et al. [1974], neutral compounds that can release ammonia upon hydrolysis include HCN, nitriles and amides. Johnson et al. [1973] measured three aliphatic amides, namely formamide, acetamide and propionamide, in PM_{MTS}, and found total concentration of the amides ranged from 0.123% of the PM_{MTS} of the bright cigarette to 0.37% of the burley particulate. They believed that the amides are synthesized during the smoking process in that the un-smoked tobaccos yielded no such amides when subjected to analysis. A study cited by Brunnemann and Hoffmann [1982] incorporated ^{15}N-nitrate of potassium, sodium or calcium, or ^{15}N-glycine into tobacco of the Kentucky reference cigarette 1R1. 30-50% of the ^{15}N was recovered as ammonia in the sidestream smoke and <1% in the mainstream smoke. Additional significant amounts of the ^{15}N were recovered in mainstream plus sidestream smoke of the cigarettes as formamide, acetamide, and propionamide. The authors hypothesized that $^{15}NO_3$ and /or the resulting nitrogen oxides are reduced during combustion to $^{15}NH_3$ and that part of it reacts with suitable intermediates to produce amides.

As discussed in previous chapters, ammonia and ammonia additives could be applied to tobacco as "impact booster" by enhancing the delivery of nicotine and increasing the fraction of free-base nicotine in smoke. A theory behind the "impact booster" concept is that most of nicotine in tobacco and tobacco smoke exists as salts of organic acids; being burned with tobacco, the ammonia and ammonia additives can produce ammonia; ammonia can react with the nicotine salts of organic acids to liberate nicotine to its free base form, and ammonia itself becomes ammonia salts of organic acids [B&W, 1991]. However, some of the ammonia may react to form amides.

The process of heating ammonium salts of corresponding acids is one of the major reactions for preparing amides [Smith; Vogel's Textbook]. Amides can also be prepared by heating organic acids or ammonium salts of organic acids with urea, an important ammonia additive used in tobacco products, at 120 ºC. Amides could also

be converted to nitriles via dehydration [Vogel's Textbook]. The thermodynamics [Mitchell and Reid, 1931] and kinetics [Morawetz and Otaki, 1963] of such reactions suggest that the temperature and other conditions of a burning cigarette, especially the distillation zone (200-600ºC) [Baker, 1987], might be favorable for the above reactions. The conceptual scheme summarized in Figure 5-3 shows that, when ammonia reacts with nicotine salts of organic acids, one mole of ammonia will liberate one mole of protonated nicotine to its free base form. A portion of the consumed ammonia may end up as ammonium salts and/or other relevant compounds in smoke. Another portion of the organic acids may end up in the form of amides or nitriles, instead of salts. In either case, the acid is neutralized and not available for the acid/base reactions in smoke any more, unless the amides and/or nitriles are hydrolyzed to ammonia and organic acids. This hypothesis is consistent with the results of a study done by Coleman and Crellin [1991]. Data in Table 5-1 suggest that, as ammoniated tobacco is added into a cigarette, the amount of ammonia and other compounds, such as HCN and acetamide, does increase.

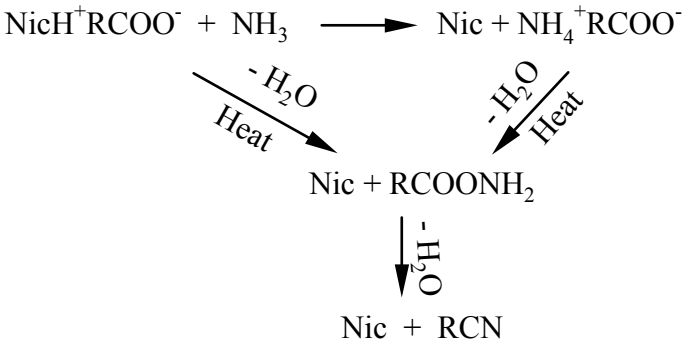


Figure 5-3 Conceptual scheme of reactions between ammonia and nicotine

Recently, tobacco industry researchers have claimed that ammonia or ammonia-producing additives in tobacco only affect the flavor of tobacco products, and have no correlation with the delivery or transfer of nicotine to smoke and to smokers [Cochran, 1999; Dixon, 1999; Ellis, 1999]. Results from our study indicate that to understand the relation between ammonia or ammonia additives in tobacco and the chemistry of nicotine in smoke, the study of ammonia in mainstream tobacco smoke might not be enough. All of the reactions in Figure 5-3 must be considered.

Table 5-1 Changing levels of some nitrogen-containing compounds in tobacco smoke as the results of using ammoniated tobacco*

Cigarette	NH_3	HCN	Acetamide	Total Nitrogen
A1:Venezuelan blend with 25% DEER	1	1	1	1
A2: Venezuelan blend with 25% half ammoniated DEER	1.38	1.39	1.18	1.04
A3: Venezuelan blend with 25% fully ammoniated DEER	1.46	1.52	1.47	1.00
B1: German blend without Emerge	1	1	1	1
B2: German blend with normal level of EMERGE	1.17	0.80	1.40	0.88
B3: German blend with 3 times normal level of EMERGE	1.33	0.93	1.60	0.99
C1: UK Virginia blend	1	1	1	1
C2: UK Virginia blend with 2% EMERGE on stem	1.00	1.14	1.17	1.05
C3: UK Virginia blend with 9% CPCL-9	3.80	1.43	1.50	1.34
C4: UK Virginia blend with 9% fully ammoniated DEER	2.20	1.43	1.33	1.34

* Data in this table are derived from Table 7 in Coleman and Crellin [1991]; all data are normalized to un-ammoniated tobacco, A1, B1, and C1, in each of the three groups; “fully ammoniated” refers to the ammoniation process of the DEER which in this case is carried out by ammonia solution and diammonium hydrogen phosphate (DAP); "half ammoniated" DEER is ammoniated by DAP only. EMERGE is based on the reaction of mixed ammonium salts of organic acids with pectin, and was added to the stem as a casing; CPCL-9 is a reconstituted tobacco developed by B & W.

Under suitable conditions, such as the experiments carried out in this study, compounds (like amides and nitriles) that may be generated by the reaction between ammonia and organic acids could release ammonia. The extent and rate of the ammonia releasing process will depend on the specific conditions. Based on denuder experiments and modeling calculation, researchers on behalf of tobacco companies have suggested that ammonia will “off gas” from PM soon after tobacco smoke gets into respiratory system and so be depleted before the PM reaches lung [Ingebrethsen, 2001; Seeman, 2004]. The work suggest that bound ammonia would continue to neutralize associated organic acids even if all ammonia volatilized immediately.

CHAPTER 6
THE DETERMINATION OF AMIDES IN MTS WITH GAS CHROMATOGRAPHY (GC)/TIME OF FLIGHT MASS SPECTROMETER (TOFMS) AND TWO DIMENTIONAL GC/TOFMS

6.1 Introduction

In previous chapters, the acid/base chemistries of nicotine and ammonia in mainstream tobacco smoke have been introduced and discussed. Theories and experimental data showed that the study of components other than nicotine and ammonia is necessary for further understanding of the acid/base chemistry of smoke, and for understanding the effect of ammonia and ammonia-producing additives in tobacco products on the delivery of nicotine to smoke and smokers. A conceptual scheme proposed in Chapter 5 is that amides and nitriles might be generated during the smoking process as the result of reaction between ammonia and organic acids. According to the discussion in Chapter 5, amides might be important product compounds. To test the proposed reaction model, it is necessary to determine these neutral nitrogen-containing compounds in tobacco smoke.

Dube & Green [1982] identified hundreds of smoke components of interest in the above regard (see Table 1-1). However, only a few amides have been measured and reported for tobacco smoke. Most available studies concern acetamide and other aliphatic amides. Data from several studies are summarized and compiled in Table 6-1. For each amide in Table 6-1, there are two columns of data, one for the delivery of amides with the unit of μg per cigarette, the other one for the concentration of amides in TPM with the unit of μg/mg when values of TPM are available. Among the studies, only Johnson et al. [1973] measured aliphatic amides. The other studies only measured acetamide. The delivery values for acetamide range from 0.6 to 111 μg/cig. Even the same type of single blend cigarettes generated different levels of acetamide. The differences can be attributed to a variety of factors, such physical parameters of

Table 6-1 Amides in mainstream tobacco smoke

		Formamide		Acetamide		Propionamide	
	Cigarettes	µg/cig	µg/mg (in TPM)	µg/cig	µg/mg (in TPM)	µg/cig	µg/mg (in TPM)
Johnson et al. 1973	Burley	12	0.60	56	2.80	6	0.30
	Bright	4	0.12	38	1.12	7	0.21
	Turkish	4	0.13	46	1.44	7	0.22
	Ky. Ref 1R1	27	0.75	51	1.42	25	0.69
Sakuma et al. 1984	Burley			70			
	Bright			105			
	Turkish			107			
	Domestic			111			
White et al. 1990	Ky. Ref. 1R4F			3.71~5.08			
Coleman and Crellin, 1991	Venezuelan blend with 25% DEER			4.90	0.23		
	Venezuelan blend with 25% fully ammoniated DEER			7.20	0.29		
	Venezuelan blend with 25% half ammoniated DEER			5.80	0.23		
	German blend without EMERGE			1.00	0.12		

Table 6-1 (Cont'd)

Coleman and Crellin, 1991	German blend with normal level of EMERGE		1.40	0.16
	German blend with 3 times normal level of EMERGE		1.60	0.19
	UK Virginia blend		0.60	0.07
	UK Virginia blend with 2% EMERGE on stem		0.70	0.08
	UK Virginia blend with 9% CPCL-9		0.90	0.08
Few, 1992	Marlboro Lights	Australia	23.25	1.59
		France	18.19	1.68
		USA	23.25	1.70
		Japan	23.21	1.59
		Hong Kong	22.23	1.66
		Germany	9.11	1.42
		Saudi	13.15	1.45
		Taiwan	22.22	1.67
	Flue-cured standard		9.00	0.49

cigarettes, chemical components in tobacco, and sampling protocol. Another important factor could be analysis protocol. Possible hydrolysis of amides may occur as suggested in Chapter 5.

Prominent advancements for the analysis of tobacco smoke in recent years include the application of solid phase extraction (SPE) for sample preparation [Gmeiner, 1997; Smith, 2003, 2004], the application of multi-dimensional chromatography [White, 1990; Dalluege, 2002; Lu, 2003, 2004a, 2004b] and new MS technique [Dalluege, 2002; Lu, 2003, 2004a, 2004b] and new ion sources [Mitschke, 2005; Adam, 2005]. The advancements in these analytical technologies have allowed led the analyses of tobacco smoke with decreasing amount of sample pretreatment [Mitschke, 2005; Adam, 2005; Takanami, 2003].

This chapter discusses the measurement of amides in smoke without any sample pre-treatment. Several amides were measured both with gas chromatography time of flight mass spectrometry (GC-ToFMS) and comprehensive two-dimensional gas chromatography time of flight mass spectrometry (GCxGC-ToFMS). The results from both techniques are compared and discussed.

6.2 Experimental

Table 6-2 Amides samples

Sample	Description
A	Brand I, 2 cigarettes, 69.51mg TPM in 15 ml 2-propanol;
B	Brand II, 4 cigarettes, 108.67mg TPM in 15 ml 2-propanol;
C	500ul Sample #B + 1ul 1mg/ml amides standard; the concentration of spiked amides (ng/μL), Formaide, 2.34, Acetamide, 2.188, Propionamide, 2.141;
D	500ul Sample #B + 10ul 1mg/ml amides standard; the concentration of spiked amides (ng/μL), Formaide, 22.96, Acetamide, 21.49, Propionamide, 21.035.

In a Fresh PM_{MTS} sample, most of the amides exist in PM phase, thus, only PM was examined here. Two brands, the un-filtered brand I and full-flavor, king-size and filtered brand II were smoked, and PM samples were collected in 2-propanol, according to protocols described in Chapter 3. To measure the efficiency of

instrumental analysis, two samples were spiked with d by spiking different amounts of amides.. The sample information is summarized in Table 6-2. All samples were sent to LECO (Las Vegas, LV) for analysis. The instrument conditions for analysis were summarized in Table 6-3 for both the GC-ToFMS and GCxGC-ToFMS. The results and discussion in this chapter are based on the analysis report from LECO.

6.3 Results and discussions

The GC chromatogram of sample A is shown in Figure 6-1a. The number of identified peaks with a signal/noise (S/N) greater than 100 is 328. For samples B, C and D, the numbers of identified peaks with a S/N greater than 100 are 391, 335 and 376. Compared with GC, as shown in Figure 6-1b, the number of identified peaks with a S/N greater than 100 is more than 4000 in the two-dimensional GC. With similar chromatographic methods and run time, the sensitivity, resolution and available peak capacity dramatically increase with the use of two-dimensional GC. As shown in Figure 6-1c, many co-eluted components in one-dimensional GC or the first dimension of the two-dimensional GC were further separated in the second dimension of the two-dimensional GC. The two-dimensional gas chromatograms of for the spiked samples showed that the three amides that we are interested in were separated well from the rest of the analytes.

Quantitative studies of the amide standards were performed over a specific calibration range. The calibration curve for acetamide is shown in Figure 6-2. Information on the quantitative calibration for the other two amides is listed in Table 6-4. The linear calibration ranges for all three amides are wide. Based on the calibration information listed in Table 6-4, the concentrations of the three amides in samples A and B were calculated and listed in Table 6-5. For further comparison with different data sets, the concentration of each target compound was calculated on the basis of extract solution, mass of PM, and per cigarette. In both samples, formamide was below the detection limit, and the concentration of acetamide was about five times higher than that of propionamide. Both acetamide and propionamide in sample A are twice as much as those in sample B. The results are not surprising, since cigarette brand I is non-filtered, and cigarette brand II is filtered.

Table 6-3 Instrument conditions for the analysis of amides

	GC-ToFMS	GCxGC-ToFMS
GC	Agilent 6890	Agilent 6890 equipped with a LECO Thermal Modulator
Column	DB-5, 30x0.25x0.25	Primary: DB-1, 30x0.25x0.25 Seondary: Rtx-200, 1x0.18x0.18
Carrier gas	Helium, 1.5 mL/min (36 cm/s)	Helium, 1.5 mL/min (36 cm/s)
Injection size (μL)	0.5	0.5
Inlet temperature (ºC)	250	250
Split ratio	Splitless	Splitless
MS	LECO Pegasus ToFMS	LECO Pegasus ToFMS
Ionization	EI at 70 ev	EI at 70 ev
Mass range	40 to 350	40 to 350
Acquisition rate	5 spectra/second	100 spectra/second
Source temperature (ºC)	200	200
Transfer line temperature (ºC)	280	280
Solvent delay	120 s	120 s
Operating conditions	Initial, 50 ºC (1.0 min); Ramp, 10 ºC/min; Final, 320 ºC (5.0 min)	Main oven: Initial, 50 ºC (1.0 min); Ramp, 10 ºC/min; Final, 320 ºC (5.0 min); Secondary oven: Initial, 55 ºC (1.0 min); Ramp, 10 ºC/min; Final, 325 ºC (5.0 min).
Modulator temperature		20 ºC offset
Modulator period		3.0 s
Hot pulse duration		0.5 s

Compared with the available prior data listed in Table 6-1, the level of acetamide and propionamide found in the smoke from brand I are similar to those of Johnson et al. [1973], and about half that reported by Sakuma et al. [1984]. The level of acetamide in the smoke from brand I is much higher than those reported by White et al. [1990], Coleman and Crellin [1991], and Few [1992]. Details regarding the use of filters by Johnson et al. [1973] and Sakuma et al. [1984] are not clear. Since those studies were carried out in the 1970s and 1980s, it is reasonable to assume that they may have utilized un-filtered cigarettes. The level of acetamide in the smoke of brand II in this study similar to that for Marlboro Lights studied by Few [1992], and much higher than data reported by White et al. [1990], and by Coleman and Crellin [1991].

The standard-spiked sample C and D were designed to evaluate the efficiency and accuracy of the methods for measuring amides. The recovery is calculated as:

$$RV = \frac{C_M - C_B}{C_{Spike}} \times 100 \qquad (6\text{-}1)$$

where RV is the recovery of a given spiked compound, C_M is the measured concentration of the compound in a spiked samples, C_B is measured concentration of the compound in sample B, and C_{Spike} is the intended spiked concentration of the compound. Results in Table 6-6 indicate that the efficiency and accuracy of the method to determine all three amides in the level of 2 ng/μL was low for this preliminary effort, but much better for all three amides at the 20 ng/μL level. Further study, such as changing the parameters of the GC temperature program and MS conditions, could enhance the efficiency and accuracy of determining the amides. Considering the complexity of the smoke samples and three amides studied here, even the recoveries of amides in low-level spiked sample indicate generally promising results.

In conclusion, GCxGC-ToFMS is a technique that is well-suited for the analysis of tobacco smoke. Data in this study showed that the resolution in both dimensions was excellent and the available peak capacity allowed for easy identification of various structures of interest as well as homologous series within the samples. Acquiring spectra over a wide mass range allows the analyst to view the acquired data by unique mass whereby removing any matrix interference that may be imposed when viewing the total ion count (TIC). Quantitative information is easily obtained by processing the data

against analytical calibration curves created using the ChromaTOF software. In addition to the work presented here, the ChromaTOF software has the ability to use ion ratios, comparative spectra to identify compounds by groups. All of these features would be very useful for further exploration of tobacco smoke.

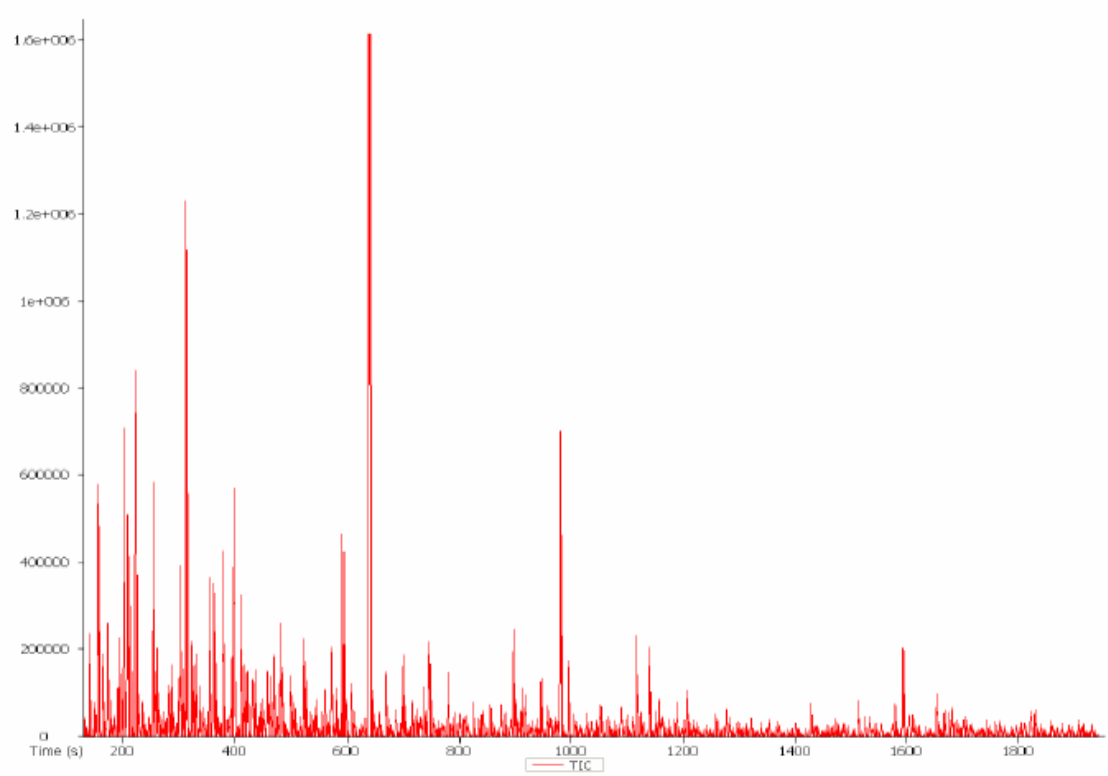


Figure 6-1a GC chromatogram of sample A.

Displayed signal is Total Ion Count (TIC). 328 peaks were identified with a S/N greater than 100.

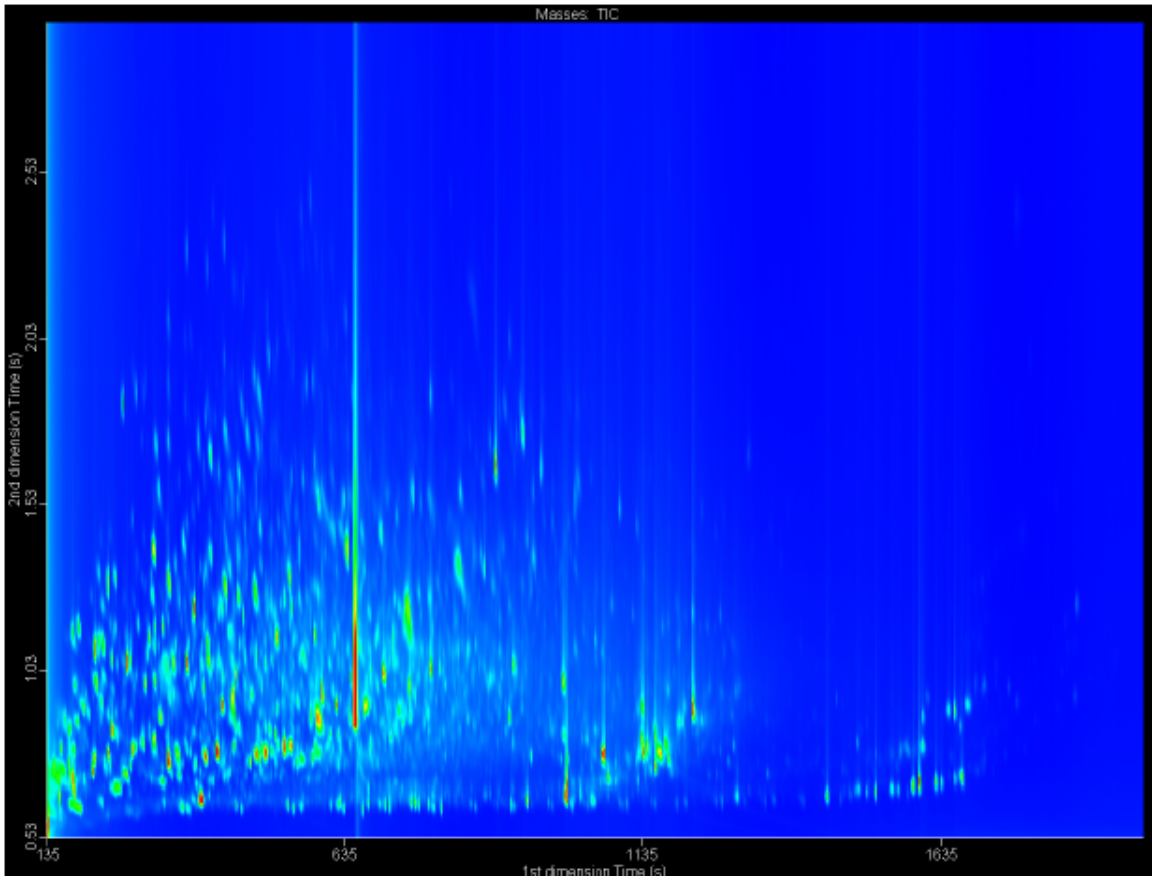


Figure 6-1b GCxGC chromatogram of sample A.

Displayed signal is Total Ion Count (TIC). Over 4000 peaks were identified with a S/N greater than 100.

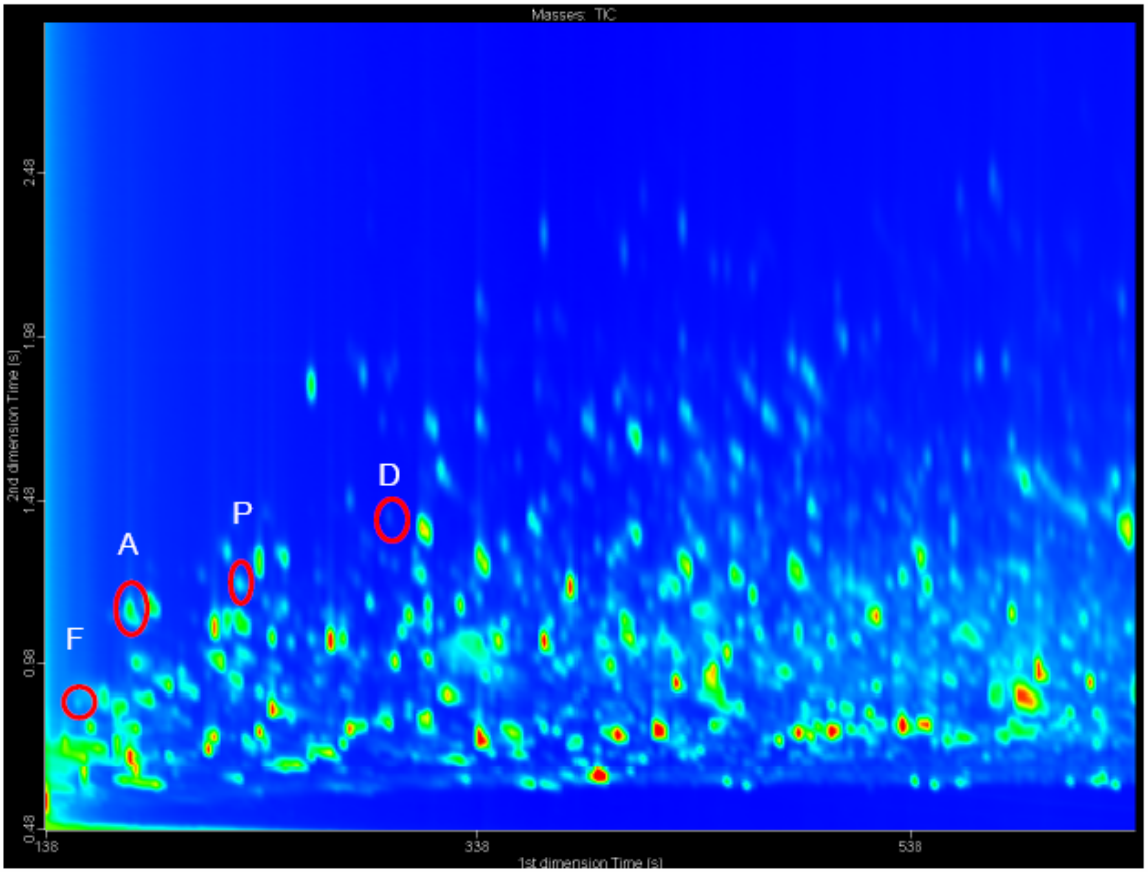


Figure 6-1c Amides identified in GCxGC chromatogram of sample A

Note the resolution in the second dimension for analytes of interest: **F**, formamide, **A**, acetamide, **P**, propionamide.

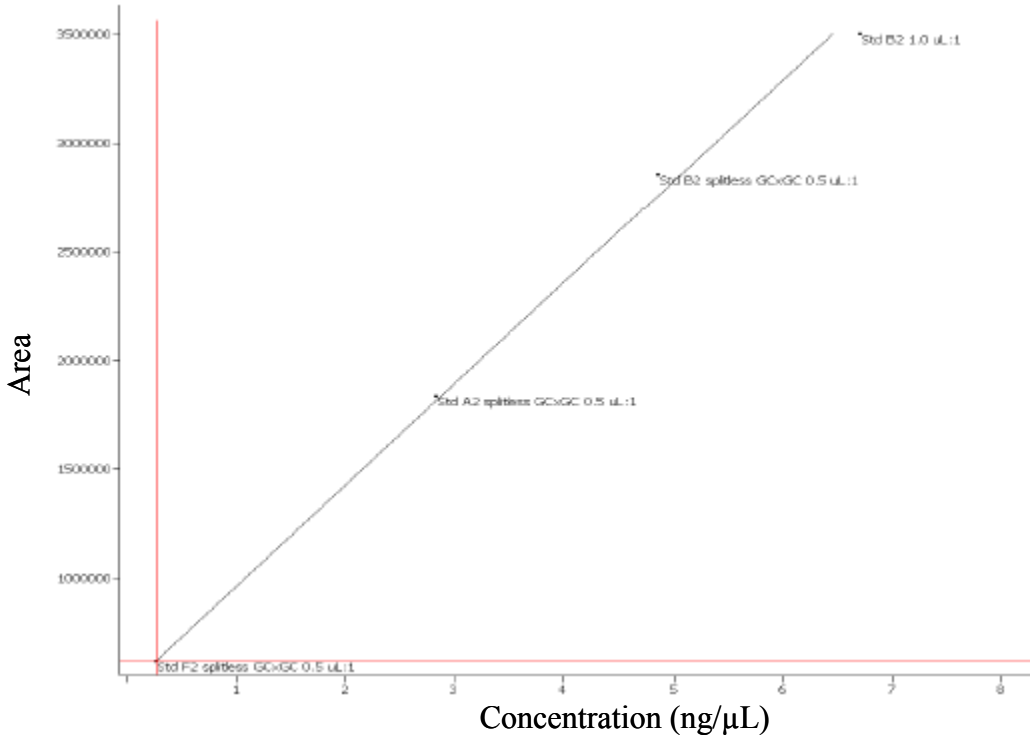


Figure 6-2 Calibration curve for acetamide covering approximately 1-7 ng/µL

Table 6-4 Calibration standards for quantitative calculation

Compound	RT (s)	Unique Mass	Quant Mass	Mass Range (ng/µL)	Correlation Coefficients
Formamide	153, 0.90	54	45	0.226-4.528	0.98899
Acetamide	177, 1.16	59	59	0.268-9.706	0.99781
Propionamide	228, 1.23	98	73	0.100-2.012	0.99543

Table 6-5 Concentration of amides in PM

Sample	Compound	Concentration		
		ng/μL in solution	μg/mg in PM	μg/cig
A	Formaide	less than 0.01	N/A	N/A
	Acetamide	7.71	1.66	57.83
	Propionamide	1.62	0.35	12.15
B	Formaide	less than 0.01	N/A	N/A
	Acetamide	6.84	0.94	25.65
	Propionamide	1.2	0.17	4.50

Table 6-6 Recovery of spiked amides in PM samples

Sample	Compound	Concentration (ng/μL)			RV (%)
		C_M	C_{Spike}	$C_M - C_B$	
C	Formaide	0.69	2.34	0.69	29.5
	Acetamide	7.63	2.19	0.79	36.1
	Propionamide	4.8	2.14	3.60	168.1
D	Formaide	14.44	22.96	14.44	62.9
	Acetamide	24.36	21.49	17.52	81.5
	Propionamide	19.2	21.04	18.00	85.6

CHAPTER 7
SUMMARY

This thesis began with an overview of the chemistry of tobacco smoke, focusing on the factors that can affect the delivery and "impact" potential of nicotine in mainstream tobacco smoke (MTS). Evidence reviewed in Chapter 1 showed that one important way to manipulate the potential effect of nicotine may be the application of ammonia additives in cigarettes. The understanding of acid/base chemistry in MTS is important for determining the effect and mechanism of ammonia additives. MTS is a complex mixture of tens of thousands compounds. With such complexity, it is very difficult to study the acid/base balance in MTS directly, such as measuring the pH value of MTS. As a weakly basic and semi-volatile compound, nicotine can exist both in the gas and particulate phase of MTS. Studies by Pankow et al. [1997, 2001, 2003] suggest that gas/particle (G/P) partitioning of nicotine provides an indirect, and yet effective tool for the study of acid/base chemistry in MTS, because of the close correlation between the acid/base balance in MTS and the G/P partitioning of nicotine.

Theories developed in Chapter 2 consider that ammonia is more basic and more volatile than nicotine. The G/P partitioning of ammonia in MTS is therefore different from that of nicotine. In spite of this difference, the coherent acid/base chemistry in MTS links the two compounds. Through such a linkage, the behaviors of ammonia and nicotine in MTS are correlated, by parameters such as G/P partitioning coefficient, K_p. Based on existing data, the G/P partitioning coefficient of free-base ammonia, $K^{a}_{p,fb}$, was estimated to be in the range of $10^{-10.9}$ to $10^{-9.6}$ $m^3/\mu g$.

To test above theories, eleven brands of commercial cigarettes and two brands of cigar-like products were investigated according to methods that were developed in this study and described in Chapter 3. The results presented in Chapter 4 include ammonia, nicotine and water in both phases of MTS from the investigated cigarettes and cigar-like

products. In contrast to the narrow range of nicotine delivery and the fact that there is no significant difference between the delivery of nicotine from cigarette or from cigar-like products, the delivery of ammonia varies over a much wider range, and cigar-like products and certain unfiltered cigarettes have a significantly higher delivery of ammonia than the rest of the cigarettes. The logarithm of partitioning coefficient of nicotine, $\log K_p^n$, is well and negatively correlated with total ammonia in smoke, especially for MTS from the remaining puffs (other than the 1st three puffs). Above results suggest that more ammonia in smoke could affect the delivery of nicotine from smoke, and change the acid/base balance in smoke to be more basic, thus increase the fraction of free-base nicotine. The data points in the chart of $\log K_p^a$ *vs.* $\log K_p^n$, the logarithm of the partitioning coefficients of ammonia and nicotine, are between the two theoretically predicted lines for $K_{p,fb}^a$ as $10^{-10.9}$ and $10^{-9.6}$ $m^3/\mu g$. The data points of the remaining puffs correspond to the predicted line for $K_{p,fb}^a$ as $10^{-10.3}$ $m^3/\mu g$.

Data in Chapter 5 showed that MTS samples collected water-based solution are not stable for the analysis of ammonia. The use of organic solvent, such as 2-propanol, could generate more stable MTS samples for the analysis of ammonia. The acid-favorable hydrolysis could increase the measured concentration of ammonia to more than two-fold, compared to samples in 2-propanol. Based on previous studies and the kinetics of the hydrolysis process studied in this work, a mechanism was proposed to explain the reactions between organic acids and ammonia and produces neutral nitrogen-containing compounds, like amides and nitriles. With the proposed mechanism, one mole of ammonia can neutralize one mole of protonated nicotine to free-base nicotine, but not necessarily end up as one mole of ammonium salts in smoke. To test the proposed mechanism, methods were developed to measure three amides in MTS, by one-dimensional and two-dimensional gas chromatography (GC). Results showed that two-dimensional GC is much powerful than one-dimensional GC, and can efficiently separate the target compounds in MTS samples without any pre-treatment. The concentrations of acetamide and propionamide in MTS of two brands of cigarettes were comparable to data found in previous studies. The relatively high yield of amides in MTS from Marlboro

implied the correlation between amides in smoke and the known use of “ammonia technology” in Marlboro products.

The consistence between the predicted and measured G/P partitioning of nicotine and ammonia suggests that, with reasonable assumptions and despite the complexity of tobacco smoke, classical acid/base chemistry of aqueous solution and the theories about G/P partitioning in atmospheric aerosols can be very useful for the understanding of the acid/base chemistry in tobacco smoke. However, the chemistry of tobacco smoke involving ammonia is very complicated, thus the theories need to be revised with new experimental data. With the advancements in analytical methodologies, more powerful tools will be available for further studies in this field.

Reference

Adam T, Ferge T, Mitschke S, Streibel T, Baker RR, Zimmermann R, 2005. Discrimination of three tobacco types (Burley, Virginia and Oriental) by pyrolysis single-photon ionisation-time-of-flight mass spectrometry and advanced statistical methods. *Analytical and Bioanalytical Chemistry* **381** (2):487-99.

Anonymous A, 1986. The influence of cigarette design on the nicotine availability in smoke. Bates Number: 2028435336/5345. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/hhk24e00

Anonymous B, 1994. Nicotine transfer efficiency (NTE) share of Philip Morris sales (percent SOPM). Bates Number: 2074450126/0132. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/mri52c00

Ayers CW. 1969. Determination of ammonia in tobacco and smoke. *Talanta* **16:** 1085-1088.

Backhurst JD, 1966. Further work on "extractable" nicotine report No. RD.437-R. Bates Number: 650009836/9905. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/zky14f00

Baker RR, 1987. A review pyrolysis studies to unravel reaction step in burning tobacco. *Journal of analytical and Applied Pyrolysis* **11**: 555-573.

Balfour DJ, 2002. The neurobiology of tobacco dependence: a commentary. *Respiration* **69**(1): 7-11.

Ball LL, 1970. Relationships of density of tar, nicotine, puffs and cigarette pressure drop. Bates Number: 655035736/5741. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/bip99e00

Baskevitch N, 1987. Modern cigarette design. Bates Number: 2053686369/6376. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/eko96e00

Bates C, Javis DM, Connolly G, 1999. Tobacco additives. Cigarette engineering and nicotine addiction. ASH, London.

Battelle, 1997. Analytical methodology for the determination of specific chemical constituents in cigarette smoke volume 1: mainstream smoke". Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/hfl85c00

Benowitz NL, 1992. Cigarette smoking and nicotine addition. *Medical Clinics of North America* **76** (2): 415-37.

British American Tobacco, 1965a. The treatment of flue-cured tobacco with ammonia. Bates Number: 570340701/0720. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/vud51f00

British American Tobacco, 1965b. The Effect of additives on smoke chemistry: action of ammonia on flue-cured tobacco. Bates Number: 620513602/3616. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/rns60f00

British American Tobacco, 1967. BAT: R & D Conference – Montreal. Bates Number: 500014079/4085. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/qct20f00

British American Tobacco, 1965. A relation between the "strength" of a cigarette and the "extractable nicotine" in the smoke. Bates Number: 620364222. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/kgt83f00

British American Tobacco, 1972. Cigarette design handbook: TPM and nicotine, 2nd edition. Bates Number: 670149085/9124. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/diw79e00

British American Tobacco, 1979. Key areas – product innovation over the next ten years for long-term development. Bates Number: 2077864172/4173. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/hgw62c00

British American Tobacco, 1962. Research conference, Southhampton, 1962 smoking and health – policy on research. Bates Number: TIOK0034806/4863. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/jws91f00

Brown & Williamson, 1989. R&D-B032-89. Ammonia technology conference minutes, 890518-890519. Bates Number: 570234362/4513. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/utb33f00

Brown & Williamson, 1963. Implications of Battelle HIPPO I & II and the Griffith filter. Bates Number: 2046754905/4909. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/knz67e00

Brown & Williamson, 1973. Secondary source digest. Bates Number: 683025739/5754. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/xqf13f00

Brown & Williamson, 1990. Ammonia technology. Bates Number: 583201978/1985. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/oof13f00

Brown & Williamson, 1991. Root technology a handbook for leaf blenders and product developers. Bates Number: 2082113761/3794. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/jdm46c00

Brown & Williamson, unknown date a. Ammonia disposition in Marlboro; smoke pH profile; sensory characteristics of commercial nonmenthol cigarettes; irritation vs. tar/puff; blend, smoke, ammonia & NTE; alkanoids in recons; analytical separation scheme; results; paper & band cast recons; Marlboro paper recon changes in urea level with age; expanded tobacco; ammonia disposition; reverse engineering; role of DAP & ammonia in dark recon; urea application sites; smoke sensory; & conclusion. Bates Number: 599000153/0180. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/bya30f00

Brown & Williamson, unknown date b. Physical properties; miscibility of nicotine & water; transfer efficiencies; mainstream nicotine vs. sidestream; tobacco rod filtration; collision frequency z; aerosols & vapour pressure; cigarette circumference effects on nicotine; filtration vs. cytrel rod & tobacco rod; paper permeability; effect of pH on cigarette smoke filtration; & sensory properties. Bates Number: 599006869/6907. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/dbb30f00

Browne C and Celanese. Additives to change the tar/nicotine delivery properties of acetate filter tips. Bates Number: 617000233/0255. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/mih93f00

Brunnemann KD and Hoffmann D, 1975. Chemical studies on tobacco smoke XXXIV. Gas chromatographic determination of ammonia in cigarette and cigar smoke. *Journal of Chromatographic Science* **13**: 159-163.

Brunnemann KD and Hoffmann D, 1982. Pyrolytic origins of major gas phase constituents of cigarette smoke regarding: ammonia; hydrogen cyanide & acetonitrile; volatile N-nitrosamines (VNA). Presented at the 36th Tobacco Chemists Research Conference. Bates #503236565/6569. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/keb01c00

California Environmental Protection Agency, Office on Environmental Health Hazard Assessment, 1997. *Health effects of exposure to environmental tobacco smoke.* Sacramento: California Environmental Protection Agency.

Cantrell D, 1981. Nicotine study (effect of chemical additives on nicotine delivery)/324. Bates Number: 620302012/2026. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/edx83f00

Carlisle J (of the TMA (UK)), 1998. Quoted in an interview in Punch magazine, April 11th.

Centers for Disease Control (CDC), 2002. Annual smoking-attributable mortality, years of potential life lost, and economic costs-United States, 1995-1999. *The Journal of the American medical Association (JAMA)* **287** (18): 2355-2358.

Centers for Disease Control (CDC), 2005. Cigarette smoking among adults – United States, 2003. *Morbidity and Mortality Weekly Report (MMWR)* **54**: 509-513.

Centers for Disease Control (CDC), 1999. Tobacco use- United States, 1900-1999. *The Journal of the American medical Association (JAMA)* **282** (3): 2202-2204.

Chen PX and Moldoveanu SC, 2003. Mainstream smoke chemical analyses for 2R4F Kentucky Reference Cigarette. *Contributions to Tobacco Research* **20** (7): 448-458.

Clegg SL and Brimblecombe P, 1989. Solubility of ammonia in pure aqueous and multicomponent solution. *Journal of Physical Chemistry* **93:**7237-7248.

Cochran EW, Joseph MJ, Stinson SL, and Summers SS, 2003. Application of a diffusion-denuder method for the investigation of effects of "smoke pH" on vapor-phase nicotine yields from different types of cigarettes. *Contributions to Tobacco Research* **20** (6): 365-372.

Cohen C, Pickworth WB, and Henningfield JE, 1991. Cigarette smoking and addiction. *Clin. Chest. Med.* **12** (4): 701-710.

Coleman M, Crellin R, 1991. Root technology – decomposition products report No: RD.2203 911126. Bates Number: 570264615/4667. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/vvs20f00

Crouse WE, 1980. Smoke pH/free and bound smoke nicotine of B3FR burley cigarettes a simple regression analysis with smoke strength. Bates Number: 00114590/4592. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/aho99d00

Crouse WE, 1982. An investigation of the interrelationship between leaf nicotine and total organic acids and its effect upon the smoke strength of selected domestic and offshore burley tobaccos. Bates Number: 00266636/6652. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/hfw61e00

D'Acry WG, 1991. "The Solanaceae since 1976, with a Review of Its Biogeography", in "Solanaceae III: Taxonomy, Chemistry, Evolution", edited by Hawkes JG, et al., pp. 75-138. Richmond, UK: Royal Botanic Gardens, Kew.

Dalluge J, van Stee LL, Xu X, Williams J, Beens J, Vreuls RJ, and Brinkman UA, 2002. Unravelling the composition of very complex samples by comprehensive gas chromatography coupled to time-of-flight mass spectrometry. Cigarette smoke. *Journal of Chromatography A* **974** (1-2):169-84.

Dixon M, 1999. Ammonia, smoke pH, and nicotine: TMA presentation to the UK Dept. of Health. Bates #2075174550/574. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/qiy56c00

Dixon M, LaXing K, and Seeman JI, 2000. Mini-review: on the transfer of nicotine from tobacco to the smoker. A brief review of ammonia and "pH" factors. *Contributions to tobacco research*, **19** (2): 103-113.

Doll R, 1998. Uncovering the effects of smoking: historical perspective. *Statistical Methods in Medical Research* **7** (2):87-117.

Doll R and Hill AB, 1950. Smoking and carcinoma of the lung: preliminary report. *British Medical Journal (BMJ)* **2** (4682):739-48.

Doll R, Peto R, Wheatley K, Gray R, and Sutherland I, 1994. Mortality in relation to smoking:" 40 years' observation on male British doctors, *British Medical Journal (BMJ)* **309** (6959): 901-911.

Doll R, Peto R, Boreham J, and Sutherland I, 2004. Mortality in relation to smoking: 50 years' observations on male British doctors. *British Medical Journal (BMJ)* **328**(7455): 1519.

Dunn WL, 1972. Motives and incentives in cigarette smoking. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/jcl78e00

Dunn WL Jr, Houck W, Jones B, and Meyer LF, 1975. 1600 – Smoker psychology low delivery cigarettes and increased nicotine / tar ratio, III (POL-1606). Bates Number: 2056140434/0449. This document is available at wepage: http://legacy.library.ucsf.edu/tid/tdr72e00

Elferlink GR, 1983. The narcotic and hallucinogenic use of tobacco in pre-Columbian central America. *Journal of Ethnopharmacology* **7** :111-222.

Ellis CL, Cox RH, Callicutt CH, Laffoon SW, Podraza KF, and Seeman JI, 1999. The effect of ingredients, including ammonia compounds, on the FTC nicotine yield and "smoke pH" a commercial Marlboro Lights cigarette. Bates Number: 2078525627/638. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/epn70c00

Ellisor LO, 1936. A toxicological investigation of nicotine on the goldfish and the cockroach. *Iowa State College Journal of Science* **11**: 51-53.

Evans CR and Jorgenson JW, 2004. Multidimensional LC-LC and LC-CE for high-resolution separations of biological molecules. *Analytical and Bioanalytical Chemistry* **378**(8):1952-61.

Federal Trade Commission (FTC), 2000. "Tar," nicotine, and carbon monoxide of the smoke of 1294 varieties of domestic cigarettes for the Year 1988. Retrieved July 12, 2006, from http://www.ftc.gov/reports/tobacco/1998tar&nicotinereport.pdf

Fisher PR, 1988. High nicotine tobacco/Brazil/000. Bates Number: 620102731. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/jrm90f00

Fowler RT, 1954. A determination of the ionization constants of nicotine. *Journal of Applied Chemistry* **4**: 449-452.

Fowles J, 2001. Chemical factors influencing the addictiveness and attractiveness of cigarettes in New Zealand. Prepared as part of a New Zealand Ministry of Health Contract for Science Service. Retrieved July 12, 2006, from http://www.ndp.govt.nz/tobacco/documents/cigaretteaddictiveness.PDF

Giddings JC, 1995. Sample dimensionality: a predictor of order-disorder in component peak distribution in multidimensional separation. *Journal of Chromatography A* **703**(1-2): 3-15.

Glantz SA, Slade J, Bero LA, Hanauer P, and Barnes DE, editors, 1996 *The Cigarette Papers.* Berkeley: University of California Press, pp100. http://ark.cdlib.org/ark:/13030/ft8489p25j/

Gmeiner G, Stehlik G, and Tausch H, 1997. Determination of seventeen polycyclic aromatic hydrocarbons in tobacco smoke condensate. *Journal of Chromatography A* **767**: 163-169.

Green CR and Rodgeman A, 1996. The tobacco chemists' research conference: a half century forum for advances in analytical methodology of tobacco and its products. *Recent Advances in Tobacco Science* **22**: 131-304.

Gregg E, Hill C, Hollywood M, Kearney M, McAdam K, McLaughlin D, Purkis S, Williams M, 2004. The UK smoke constituents testing study. Summary of results and comparison with other studies. *Contributions to Tobacco Research* **21** (2): 117-138.

Guindon, 2003. Past, current and future trends in tobacco use. Retrieved July 12, 2006, from http://www1.worldbank.org/tobacco/pdf/Guindon-Past,%20current-%20whole.pdf

Harold H, 1930. The effect of hydrogen-ion concentration on the toxicity of nicotine, pyridine and mehtylpyrrolidine to mosquito lavae. *Journal of Agricultural Research (Washington, D. C.)* **41**: 337-48.

Harrell TG, Rush KL and Sensabaugh JR AAJ. 1974. Colorimetric method for the determination of ammonia in tobacco smoke. *Tobacco Science* **18:** 145-147.

Heckman, 1976. Research proposal. The role of sugar in reducing nicotine harshness. Bates Number: 502987309/7310. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/ibt68d00

Hecht SS, Carmella S, Mori H, and Hoffmann D, 1981. A study of tobacco carcinogenesis XX: Role of catechol as a major cocarcinogen in the weakly acidic fraction of smoke condensate. *Journal of National Cancer Institute* **66**(1): 163-9.

Hecht SS, 1999. Tobacco smoke carcinogens and lung cancer. *Journal of National Cancer Institute* **91**: 1194-1210.

Henningfield JE, Benowitz NL, Slade J, Houston TP, Davis RM, and Deitchman SD, 1998. Reducing the addictiveness of cigarettes. Council on Scientific Affairs, American Medical Association. *Tobacco Control* **7** (3): 281-93.

Henningfield JE, Pankow JF, and Garrett BE, 2004. Ammonia and other chemical base tobacco additives and cigarette nicotine delivery: Issues and research needs. *Nicotine & Tobacco Research* **6** (2): 199-205.

Hicks WG and Randolph HR. 1990. A procedure for the simultaneous determination of total ammonia in mainstream and sidestream smoke for cigarettes. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/osw71f00.

Hind J, Seligman R, and Philip Morris Inc., 1966. United States patent No. 3,411,515 - method of preparing a reconstituted tobacco sheet employing a pectin adhesive. Bates Number: 599008366/8374. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/jqi41f00

Hirji T and Wood DJ, 1973. Impact: its relationship with extractable nicotine and with other cigarette variables (Report No. RD. 1052-R). Bates Number: 650318009/8038. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/wgm00f00

Hirji T and Wood DJ, 1974. Equations relating impact with extractable nicotine and other cigarette variables (Report No. RD.1337). Bates Number: 570311988/2015. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/iav03f00

Huang CB, Field RB, Dabney B, and Hsu F, 2003. Determination of total ammonia in mainstream smoke. *Contributions to tobacco Research* **20 (6):** 389 –393.

Hughes JR, Higgins ST, and Bickel WK, 1994. Nicotine withdrawal versus other drug withdrawal syndromes: similarities and dissimilarities. *Addiction* **89** (11):1461-70.

Hurt RD, Robertson CR, 1998. Prying open the door to the tobacco industry's secrets about nicotine: the Minnesota Tobacco Trial. *The Journal of the American Medical Association (JAMA)* **280** (13):1173-81.

IARC, 1986. Monographs on the evaluation of carcinogenic risks to humans, Volume 38: tobacco smoking, pp. 84.

IARC, 2004. Monographs on the evaluation of carcinogenic risks to humans, Volume 83: tobacco smoke and involuntary smoking, pp. 56.

Ingebrethsen BJ, Lyman CS, Risner CH, Martin P, and Gordon BM, 2001. Particle-gas equilibria of ammonia and nicotine in mainstream cigarette smoke. *Aerosol Science and Technology* **35**: 874-886.

Jaeschke W, Dierssen JP, Gunther A, and Schumann M. 1998. Phase partitioning of ammonia and ammonium in a multiplephase system studied using a new vertical wet denuder technique. *Atmospheric Environment* **33**: 365-371.

Jarvis MJ, 2004. Why people smoke. *British Medical Journal (BMJ)* **328**: 277-279.

Jenkins RA, Gurerin MR, and Tomkins BA, 2000. The chemistry of environmental tobacco smoke: composition and measurement, CRC Press, Boca Raton, Florida.

Johnson JM, 1996. Summary of the effects of ammonium carbonate, ammonium bicarbonate, urea and diammonium phosphate on smoke pH, smoke data and leaf chemistry Q 449 analytical support. Bates Number: 83171424/1436. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/jhg94c00

Johnson RR, 1971. Comments on nicotine, notes of a meeting held on 30 June, 1971. Bates Number: 2083458824/8826. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/whs92c00

Johnson RR. 1989. R&D-B032-89. Ammonia technology conference minutes. 890518-890519. Bates numbers: 570234362/4513. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/utb33f00

Johnson WR, Hale RW and Nedlock JW, 1973. Aliphatic amides in cigarette smoke. *Tobacco Science* **17**: 73.

Keith CH, 1980. Physical methods for the modification of tobacco smoke. Bates Number: 680407557/7570. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/ypl21c00

Kiefwe JE, 1978. Filters and their effect on smoke composition. Bates Number: 81052501/2516. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/zwm40e00

Kertes AS (Editor-in-chief), 1985. Ammonia, amine, phosphine, arsine, stibine, silane, grmane and stannane in organic solvents Solubility data series Vol 21, Pergamon Press.

Kluger R, 1997. Ashes to Ashes: America's hundred-year cigarette war, the public health, and the unabashed triumph of Philip Morris, Landon House Inc., New York.

Klus H, 1981. The effect of filter-ventilation on the pH of the mainstream smoke. Bates Number: 2028560522/0536. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/bvx14e00

Labstat, 1997. Partial characterization of 10 "common" brands of American cigarettes. Bates Number: 2074451866/903. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/ydr66c00

Labstat, 1998. Ammonia contents, smoke pH and yields of "tar" and nicotine: "ammonia cigarette study". Bate Number: 2063132092/2120. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/oct22d00

Labstat, 2000. RV3 ammonia content of cigarette filler and mainstream smoke from selected brands of commercial available cigarettes. Bates Number: 2084323998/4082. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/jor19c00

Lammel G, Pohlmann G. 1992. Phase behaviour of ammonia, nitric acid, and particular ammonium nitrate: the influence of the aerosol characteristics. *Journal of Aerosol Science* **23** Suppl. 1: 5941-5944.

Larson PS, Haag HB, Silvette H. 1961. Tobacco experimental and clinical studies: a comprehensive account of the world literature. Baltimore: Williams and Wilkins. pp 1–932.

Larson PS, Silvette H. 1968. Tobacco experimental and clinical studies: a comprehensive account of the world literature. Suppl 1. Baltimore: Williams and Wilkins. pp 1–803.

Larson PS, Silvette H. 1971. Tobacco experimental and clinical studies: a comprehensive account of the world literature. Suppl 2. Baltimore: Williams and Wilkins. pp 1–563.

Larson PS, Silvette H. 1971. Tobacco experimental and clinical studies: a comprehensive account of the world literature. Suppl 2. Baltimore: Williams and Wilkins. pp 1–798.

Le Houezec J, 2003. Role of nicotine pharmacokinetics in nicotine addiction and nicotine replacement therapy: a review. *The International Journal of Tuberculosis and Lung Disease* **7** (9): 809-810.

Lekovic O, Nikolic M, Provolic N, 1990. Influence of nicotine content in the neutral fraction of CSC on completeness and strength of smoke taste. Bates Number: 2021552123/2131. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/uhe58e00

Lewan T, 1998. B&W is still using high-nicotine tobacco. Bates Number: 106043398/3399. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/edj02d00

Lloyd RA, 1979. Quarterly section research report. Bates Number: 502804871/4896. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/zkd78d00

Lorillard, 1976. Nicotine Augmentation Project (NAP), Bates #00044537/4544. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/rmf20e00

Lorillard, 1980. Smoke pH/free and bounded smoke nicotine of B3FR burley cigarettes a simple regression analysis with smoke strength. Bates Number: 00114590/4592. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/aho99d00

Lorillard, 1982. An investigation of the interrelationship between leaf nicotine and total organic acids and its effect upon the smoke strength of selected domestic and offshore burley tobaccos. Bates Number: 00266636/6652. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/hfw61e00

Lu X, Cai J, Kong H, Wu M, Hua R, Zhao M, Liu J, Xu G. 2003. Analysis of cigarette smoke condensates by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry I acidic fraction. *Analytical Chemistry* **75** (17):4441-51.

Lu X, Zhao M, Kong H, Cai J, Wu J, Wu M, Hua R, Liu J, Xu G. 2004a. Characterization of cigarette smoke condensates by comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry (GC x GC/TOFMS). Part 2: basic fraction. *Journal of Separation Science* **27** (1-2): 101-9.

Lu X, Zhao M, Kong H, Cai J, Wu J, Wu M, Hua R, Liu J, Xu G. 2004b. Characterization of complex hydrocarbons in cigarette smoke condensate by gas chromatography-mass spectrometry and comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. *Journal of Chromatography A* **1043** (2): 265-73.

Manus P, 1989. Flavorings: The right combination. Tobacco Reporter 1989, April: 26. This document is cited in reference: WHO, 2000

Marriott PJ, Haglund P, Ong RC. 2003. A review of environmental toxicant analysis by using multidimensional gas chromatography and comprehensive GC. *Clinica Chimica Acta* **328** (1-2): 1-19.

Matkin DA, 1984. Project SHIP pectin-nicotine interaction. Bates Number: 510001455/1459. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/kes24f00

McBride C, 1992. A review of the effects of cigarette paper burn additives on puff number & mainstream deliveries of tar, nicotine & carbon monoxide. Bates Number: 570265430/5489. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/hsf51f00

McMurtrie A, 1980. Cigarette paper effects on tar/nicotine and CO/tar ratios/322. Bates Number: 620075917/5926. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/led93f00

Mitchell JA and Reid EE, 1931. The preparation of aliphatic amides. *Journal of the American Chemical Society* **53:** 1879-1883.

Morawetz H and Otaki PS, 1963. Kinetics and equilibria of amide formation in aqueous media. *Journal of the American Chemical Society* **85:** 463-468.

Minnemeyer HJ, 1976. Progress report on nicotine augmentation project. Bates Number: 00044525/4528. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/bbu99d00

Mitschke S, Adam T, Streibel T, Baker RR, and Zimmermann R, 2005. Application of time-of-flight mass spectrometry with laser-based photoionization methods for time-resolved on-line analysis of mainstream cigarette smoke. *Analytical Chemistry* **77** (8): 2288-96.

Mokdad AH, Marks JS, Srroup DF, and Gerberding JL, 2004. Actual causes of death in the United States, 2000. *The Journal of the American Medical Association (JAMA)* **291** (10): 1238-1245.

Morie GP, 1972. Fraction of protonated ad unprotonated nicotine in tobacco smoke at various pH values. *Tobacco Science* **16**:167.

Nanni EJ, Lovette ME, Hicks RD, Fowlers KW, and Borgerding KW. 1990. Separation and Quantitation of monovalent anionic and cationic species in mainstream cigarette smoke aerosols by high-performance ion chromatography. *Journal of Chromatographic Science* **28:** 432-436.

National Cancer Institute, 1998. Smoking and Tobacco Control Monographs, Monograph 9: Cigars: Health Effects and Trends. Retrieved July 12, 2006, from http://cancercontrol.cancer.gov/tcrb/monographs/9/

Newton R, 1970. Alkaline Tobacco Smoke: Effect of Urea and Urea/Urease on Smoke Chemistry. Bates Number: 566416141. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/ink51f00

Norman V, 1981. Nicotine transfer efficiency of cigarette. Bates Number: 80006223/6226. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/dku31e00

Pankow JF. 1991. Aquatic Chemistry Concepts: Chapter 5. Lewis Publishers. Chelsea, Michigan.

Pankow JF. 1994. An absorption model of gas/particle partitioning of organic compounds in the atmosphere. *Atmospheric Environment* **28A**: 185-188.

Pankow JF, Mader BT, Isabelle LM, Luo W, Pavlick A, and Liang C. 1997. Conversion of nicotine in tobacco smoke to its volatile and available free-base form through the action of gaseous ammonia. *Environmental Science and Technology* **31**(8): 2428-2433.

Pankow JF, 2001. A consideration of the role of gas/particle partitioning in the deposition of nicotine and other tobacco smoke compounds in the respiratory tract. *Chemical Research in Toxicology* **14:** 1465-1481.

Pankow JF, Tavakoli AD, and Luo W, 2003. Percent free-base nicotine in the tobacco smoke particulate matter of selected commercial and reference cigarettes. *Chemical Research in Toxicology* **16:** 1014-1018.

Pankow JF, Luo W, Tavakoli AD, Chen C, Isabelle LM, 2004. Delivery levels and behavior of 1,3-butadiene, acrylonitrile, benzene, and other toxic volatile organic compounds in mainstream tobacco smoke from two brands of commercial cigarettes. *Chemical Research in Toxicology* **17**: 805-813

Parrish M. 1987. The determination of mainstream and sidestream gaseous ammonia by TDL for the blend component cigarettes evaluated by the north complex smoking panel. Bates #2023363067/3131. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/vkp98e00

Patents, 1994. Increase nicotine content by adding nicotine to tobacco rod. Bates Number: 2056172324/2343. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/das72e00

Perfetti TA, Norman AB, Gordon BM, Coleman WM, Morgan WT, Dull GM, and Miller CW, 2000. The transfer of nicotine from nicotine salts to mainstream smoke. *Contributions to Tobacco Research* **19** (3): 141-158.

Peto J, 2001. Cancer epidemiology in the last century and the next decade. *Nature* **411**: 390-395.

Peto R and Lopez AD. Future worldwide health effects of current smoking patterns. In: Koop CD, Pearson C, Schwarz MR, eds. Critical issues in global health. New York, NY: Jossey-Bass; 2001,

Philip Morris, 1969. Why one smokes. Bates Number: 1003287836/7848. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/pds74e00

Philip Morris, 1984. The cigarette consumer. Bates Number: 2063541403/1419. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/fkl67d00

Philip Morris, 1992. Table competitive analysis 1st draft organization 1st draft production – to follow -. Bates Number: 2021118664/8680. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/mzf12a00

Philip Morris, 1994. pH research chronology internal reports. Bates Number: 2050878805/8820. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/und74a00

Philip Morris, 1998. Nicotine / tar ratio for ultra low delivery brands (6mg tar or less) from 950000 FTC report. Bates Number: 2051993747/3750. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/ahc74a00

PME, 1974. Smoke impact. Bates Number: 1003724400/4401. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/hpp08e00

Proctor C, BAT Industries - Smoking Gun? Statement in *the Observer*, 1998, 1 March, pp.13

Proctor RN, 2004. The global smoking epidemic: a history and status report. *Clinical Lung Cancer* **5** (6): 371-6.

Regnier F and Huang G, 1996. Future potential of targeted component analysis by multidimensional liquid chromatography-mass spectrometry. *Journal of Chromatography A* **750** (1-2): 3-10.

Rev. Benjamin I. Lane, 1845, 1846, 1851. The Mysteries of Tobacco, New York: Wiley and Putnam. Web link: http://medicolegal.tripod.com/lane1845.htm#effectonbody

Reynolds ML, 1973. Review of factors affecting tar/nicotine ratios/323/73-23. Bates Number: 621018821/8827. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/ghs21f00

Reynolds RJ, 1973. Implication and activities arising from correlation of smoke pH with nicotine impact, other smoke qualities, and cigarette sales. Bates Number: 83847718/7748. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/rus34c00

Reynolds RJ, 1984. G7A. Bates Number: 512173594. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/xxc43d00

Reynolds RJ, 1990. "Ammoniation Review". Objective: to catalog methods which R&D Staff have used to incorporate "ammonia" into tobacco/tobacco smoke and the resultant impact on smoke characteristics (sensory). Bates Number: 508108585/8596. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/lzo04d00

Richardson CH and Ellisor LO, 1940. Effect of pH on the toxicity of nicotine injected into the cockroach, Periplaneta americana (L). *Iowa State College Journal of Science* **14**: 305-16.

Robinson JH, 1990. Lower "tar": nicotine ratio cigarettes. Bates Number: 515998761/8763. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/aqv03a00

Rodgman A, 2000. "Smoke pH": a review. *Contributions to Tobacco Smoke* **19** (3): 117-235.

Routh WE, 1977. Ammonia treatment of tobacco. Bates #00044858/4879. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/jtm99d00

Rustemeier K, Stabbert R, Haussmann HJ, Roemer E, and Carmines EL. 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: chemical composition of mainstream smoke. *Food and Chemical Toxicology* **40** (1): 93-104.

Samaha A and Robinson TE, 2005. Why does the rapid delivery of drugs to the brain promote addiction? *Trends in Pharmacological Sciences* **26**: 82-87.

Schmeltz I, Hoffmann D, 1977. Nitrogen containing compounds in tobacco and tobacco smoke. *Chemical Review* **77** (3): 295-311.

Schori TR, 1979. Free nicotine: its implication on smoke impact. Bates Number: 542001986/1996. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/mzh24f00

Schori TR, 1974. Smoke impact the influence of tobacco type and nicotine a proposal. Bates Number: 2062951373/1375. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/urt49e00

Seeman JI, Fournier JA, Paine JB, III, and Waymack BE, 1999. The form of nicotine in tobacco. Thermal transfer of nicotine and nicotine acid salts to nicotine in the gas phase. *Journal of Agricultural and Food Chemistry* **47** (12): 5133-5145.

Seeman JI, Lipowicz PJ, Piade JJ, Poget L, Sanders EB, Snyder JP, and Trowbridge CG, 2004. On the deposition of volatiles and semivolatiles from cigarette smoke aerosols: relative rates of transfer of nicotine and ammonia from particles to the gas phase. *Chemical Research in Toxicology* **17**(8): 1020-1037.

Shaffer HJ, 1997. The most important unresolved issue in the addictions: conceptual chaos. *Substance Use & Misuse* **32** (11): 1573-1580.

Shellie R, Marriott P, Morrison P, 2004. Comprehensive two-dimensional gas chromatography with flame ionization and time-of-flight mass spectrometry detection: qualitative and quantitative analysis of West Australian sandalwood oil. *Journal of Chromatographic Science* **42** (8): 417-22.

Silagy C, Lancaster T, Stead L, Mant D, Fowler G, 2004. Nicotine replacement therapy for smoking cessation. *Cochrane Database of Systematic Reviews.* 2004;(3):CD000146

Skladaowski MA, 1977. Vapor phase removal and nicotine augmentation with all air dilution cigarettes. Bates number: 00399961/9975. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/tat61e00

Sloan CH and Morie GP. 1974. Determination of ammonia in tobacco and tobacco smoke with an ammonia electrode**.** *Analytica Chimica Acta* **69**(1)**:** 243-247.

Smith CJ, Dooly GL, Moldoveanu SC, 2003. New technique using solid-phase extraction for the analysis of aromatic amines in mainstream cigarette smoke. *Journal of Chromatography A* **991**: 99-107.

Smith CJ, Qian X, Zha Q, Moldoveanu SC, 2004. Analysis of α- and β-carbolines in mainstream smoke of reference cigarettes by gas chromatography-mass spectrometry. *Journal of Chromatography A* **1046**: 211-216.

Smith PAS, *Open-chain nitrogen compounds*, Volume I. W. A. Benjamin, Inc., New York, New York. pp. 152.

Snook ME, Fortson PJ, and Chortyk OT, 1981. Isolation and identification of aza-arenes of tobacco smoke. *Contributions to Tobacco Research* **11** (2): 67-78.

Snook ME, Chortyk OT, and Arrendale RF, 1984, Isolation and ldentification of N '-acylalkaloids of cigarette smoke. *Contributions to Tobacco Research* **12** (5): 227-243.

Spinden HJ, 1950. Tobacco is American; the story of tobacco before the coming of the white man. New York Public Library, New York.

Stauffer HP and Riedwyl H, 1977. Interaction and pH dependence of effects of nicotine and carbon monoxide in cigarette smoke inhalation experiments with rats. *Agents and Actions* **7** (5-6): 579-88.

Stewart CA and Lawrence BM, 1988. Effects of levulinic acid, tobacco essence and nicotine salts on smoke pH. Bates Number: 507862872/2880. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/zyk14d00

Subcommittee on Ammonia, National Research Council. 1978. *Ammonia, pp. 1*. University Park Press, Baltimore, Maryland.

Sudholt MA, 1985. Design of 1 mg tar cigarette with 40percet more nicotine, 33 percent less CO, and one more puff per cigarette than a Carlton 85. Bates Number: 80642632/2636. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/ldb41e00

Swain AP, Cooper JE, Stedman RL, Bock FG, 1969. Composition studies on tobacco XL: large scale fractionation of the neutrals of cigarette smoke condensate using adsorption chromatography and solvent partitioning. *Contributions to Tobacco Research* **5** (3): 109-114.

Takanami Y, Chida M, Hasebe H, Sone Y, and Suhara S, 2003. Analysis of cigarette smoke by an online thermal desorption system and multidimensional GC-MS. *Journal of Chromatographic Science* **41** (6): 317-22.

Teague CE Jr, 1973. Implications and activities arising from correlation of smoke pH with nicotine impact, other smoke qualities, and cigarette sales. Bates Number: 2077984432/4463. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/adz75c00

Tennessee Eastman, 1956. Composition of tobacco smoke. Bates Number: 650341505/1821. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/yfl00f00

The Tobacco Institute, 1988. Claims that cigarettes are addictive contradict common sense. Bates Number: 950229454. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/uuq31a00

Thornton RE, 1969. Handbook of cigarette design (TPM and nicotine). Bates Number: 650339012/9042. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/efw14f00

Tomar SL and Henningfield JE, 1997. Review of the evidence that pH is a determinant of nicotine dosage from oral use of smokeless tobacco. *Tobacco Control* **6** (3): 219-25.

Tranchida PQ, Dugo P, Dugo G, and Mondello L. 2004. Comprehensive two-dimensional chromatography in food analysis. *Journal of Chromatography A* **1054** (1-2): 3-16.

U.S. Department of Agriculture, 2001. Tobacco: world markets and trade. Retrieved July 12, 2006, from http://www.fas.usda.gov/tobacco/circular/2001/0109/sep.pdf

U.S. Department of Health, Education, and Welfare, 1964. *Smoking and Health. Report of the Advisory Committee to the Surgeon General of the Public Health Service.* U.S. Department of Health, Education, and Welfare, Public Health Service, Communicable Disease Center.

U.S. Department of Health and Human Services. The health consequence of smoking: nicotine addiction. A report of the Surgeon General, 1988. U.S. Department of Health and Human Services. Public Health Service. Centers for Disease Control. Center for Health Promotion and Education. Office on Smoking and Health. DHHS Puhltcation No. (CDC) 88-8406.

U.S. Department of Health and Human Services, 1989. *Reducing the Health Consequences of Smoking: 25 Years of Progress. A Report of the Surgeon General*. Rockville (MD): U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

U.S. Department of Health and Human Services, 2004. *The Health Consequences of Smoking: A Report of the Surgeon General*. [Atlanta, Ga.]: Dept. of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; Washington, D.C.

Vilcins G, Grantham PF, and Baker G, 1987. Optical spectroscopy of tobacco and smoke. Bates #2051201132/179. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/brs82e00

Vogel's Textbook of Practical Organic Chemistry, 4th edition, pp515

Watson DC, 1991. Gas phase nicotine – status (reference: D.C. Watson to R. Ferguson, 'Gas Phase Nicotine', 910903). Experiments designed to determine the potential of open tube denuders for separation of vapor phase / particulate phase nicotine. Bates Number: 2023569357/9362. Retrieved July 12, 2006, from http://legacy.library.ucsf.edu/tid/mri24e00

West R and Gossop M, 1994. Overview: A comparison of withdrawal symptoms from different drug classes. *Addiction* **89** (11):1483-1489.

White EL, Uhrig MS, Johnson TJ, Gordon BM, Hicks RD, Borgerding MF, Coleman WM 3rd, Elder JF Jr, 1990. Quantitative determination of selected compounds in a Kentucky 1R4F reference cigarette smoke by multidimensional gas chromatography and selected ion monitoring-mass spectrometry. *Journal of Chromatographic Science* **28** (8): 393-9.

Winter JC, 2000. Tobacco use by Native North Americans : sacred smoke and silent killer. University of Oklahoma Press, Norman.

World Health Organization, 1969. WHO Technical Report Series no. 407, Geneva, 1969.

World Health Organization, 2000. Monograph: Advancing knowledge on regulating tobacco products. Retrieved July 12, 2006, from http://www.who.int/tobacco/media/OsloMonograph.pdf

World Health Organization, 2002. The Tobacco Atlas. Retrieved July 12, 2006, from http://www.who.int/tobacco/resources/publications/tobacco_atlas/en/index.html

Wynder EL and Graham E, 1950. Tobacco smoking as a possible etiologic factor in bronchiogenic carcinoma: a study of 684 proven cases. *The Journal of the American medical Association (JAMA)* **143**: 329-336.

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Publications:

Pankow JF, Luo W, Bender DA, Isabelle LM, Hollingsworth JS, Chen C, Asher WE, and Zogorski JS, 2003. Concentrations and co-occurrence correlations of 88 volatile organic compounds (VOCs) in the ambient air of 13 semi-rural to urban locations in the United States. *Atmospheric Environment* **37** (36): 5023-5046.

Pankow JF, Luo W, Tavakoli AT, Chen C, and Isabelle LM, 2004. Delivery levels and behavior of 1,3-butadiene, acrylonitrile, benzene, and other toxic volatile organic compounds in mainstream tobacco smoke from two brand of commercial cigarettes, *Chemical Research in Toxicology* **17**: 805-813.