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Molecular Characterization of Nicotinein Mainstream Cigarette Smoking

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Abstract

The most studied plant in the plant kingdom owing to its use in form of cigarettes and consequently its poisonous nature is tobacco. This paper therefore investigates one of the most addictive alkaloids (nicotine) in tobacco believed not only to be carcinogenic but also a precursor for other health problems bedeviling smokers. In this work, two commercial cigarette brands, SM1 and ES1 are explored for evolution of nicotine over a modest temperature range of 200 – 700˚C at 1 atmosphere under conditions representative of real world cigarette smoking. 1µL of cigarette pyrolysate collected in methanol was injected into a gas-chromatograph hyphenated to a mass selective detector (MSD). The peak area for nicotine for all the pyrolysis temperatures was recorded and consequently, product distribution curves of nicotine in each cigarette brand were reported. It is evident from the results that ES1 cigarette yields high levels of nicotine over the entire pyrolysis temperature range. At 400˚C, the concentration of nicotine from ES1 was 7.90 x 10^8 GC-area counts while that of SM1 was 6.39 x 10^7 GC-area counts. Nonetheless, the concentration of nicotine for SM1 cigarette brand peaked at about 500˚C and decreased exponentially to 8.8 x 10^7 GC-area counts at 700˚C. Based on these results alone, it can be deduced that ES1 cigarette is more toxic than SM1 cigarette. The toxicity indices for nicotine and its corresponding nicotinyl radical were determined using Quantitative Structural Activity Relation (QSAR) in HyperChem computational program and found to be 0.22 and 0.74 respectively. These toxicity values are referenced to the partition coefficient between octanol and water. The consequences of nicotine exposure have also been discussed in this paper.

Keywords: Addiction, cigarette, pyrolysis, toxicity.

Introduction

Cigarette smoking is an old age addictive habit that has attracted serious condemnation because of the clinical implications associated with it[1-4]. Tobacco is a complex plant comprising of 6-15% cellulose, 10-15% pectin, about 2% lignin, and many other components, the true composition being dependent on the tobacco type and growing environments[5]. Tobacco consists of more than 2500 chemical constituents, including biopolymers, non-polymeric and inorganic compounds [6]. For Instance, sugars such as cellulose, pectin, alginates, laminarin and ethyl cellulose are natural tobacco components which are also often added to tobacco during the manufacturing process[3,4,7]. Nicotine from cigarette smoking may pose significant environmental risks and cognitiveproblems for developing fetus[4,8]. Nicotine in mainstream cigarette smoke exists in the particulate phase[8-10].

Nicotine mimics certain actions of the neurotransmitter acetylcholine and it produces its discriminative stimulus effect in the hippocampus portion of the brain[10]. The addictive properties of nicotine stem from its binding to nicotinic acetylcholine receptors[11]. Nonetheless, nicotine is a primary substance in cigarette smoke that strongly affects brain development[13,14]. Research on animals support biological evidence for increased motor action, neurobehavioral, learning and memory deterioration, and change in neurotransmitter function due nicotine uptake[4,14,15].

Considering the chemical and the intricate nature of cigarette smoke, with over 7,000 well-known constituents, and its biological variety characterized by the presence of mutagens and poisonous compounds, any effort to identify a particular compound which cause lung cancer in smokers is arduous [12,16]. Inhalation studies of cigarette smoke have proposed that laboratory animals avoid as much as possible inhaling cigarette smoke[16,17]. The concept of tobacco development, composition, and toxicity is an important area of study due to potential dangers associated with it[15]. In recent years, a lot of effort has been devoted to the evaluation of the by-products of tobacco burning and the potency of cigarette smoking[18]. However, the shortfalls in elucidating the toxic compound formation mechanism during tobacco burning and challenges of developing model compounds which can burn under conditions that mimic actual cigarette smoking compromise this undertaking[19]. Figure-1 shows the structures of nicotine. The blue atoms represent nitrogen in molecular nicotine.

The attack on lung cells byge-notoxic toxins in cigarette smoke has led to disastrous cancerous effects that have been demonstrated in numerous studies[8,13,17]. Accordingly, cigarette smoking materializes as the greatest voluntary human exposure to a mixture of chemical mutagens with horrendous outcomes in terms of lung cancer mortality all over the world[13,17].

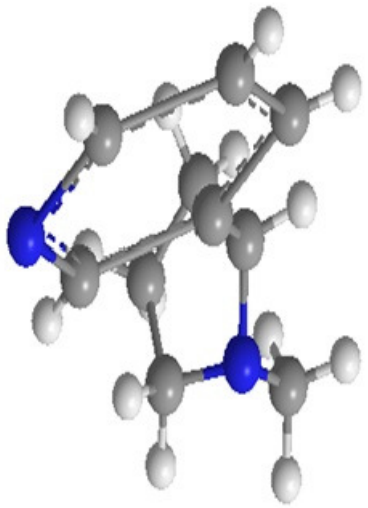


Figure-1
The molecularstructure of nicotine

Material and Methods

Commercial cigarettes SM1 and ES1 were purchased from local retail outlets and used without further treatment. Due to the confidentiality and illnesses occasioned by cigarette smoking, the cigarette brands investigated in this work have been assigned neutral names (SM1 and ES1).

Sample Preparation: 50 mg of tobacco from commercial cigarettes brands SM1 and ES1 were accurately weight to the nearest mg and packed in a quartz reactor of dimensions: i.d 1 cm x 2 cm (volume ≈ 1.6 cm^3). The sample in the quartz reactor was placed in an electrical heater whose temperature can be varied between 20 ˚C to 1000 ˚C. The heater (muffle furnace) was purchased from Thermo Scientific Inc., USA. SM1 or ES1 Were burned in flowing nitrogen in a quartz reactor and the smoke effluent was allowed to pass through a transfer column. The pyrolysis employed in this study was conventional pyrolysis in which new tobacco sample was used at every pyrolysis temperature. The details of this method are discussed elsewhere[20]. The pyrolysate was collected in 1mL methanol in crimp top vials for a total pyrolysis time of 3 minutes.

GC-MS Characterization of Nicotine in Cigarette Smoke: Agilent 6890 GC hyphenated to a mass selective detector (MSD) was used to determine quantitatively the yields of nicotine at various combustion temperatures. 1 µL of pyrolysate dissolved in methanol was injected into a GC column (HP-5MS, 30m x 250µm x 0.5µm). The temperature of the injection port was set at 200^0C and temperature programming was 25^0C for 10 minutes, holding for 2 minute at 250^0C, followed by a heating rate of 10^0C for 5 minutes, and holding for 5 minutes at 300^0C. The principal focus of this experiment was to determine the release of nicotine between 200 and 700 ˚C. The mass selective detector (MSD) was operated on the total ion current mode (TIC) and the ion source was set at 70eV. Nicotine was identified using the National Institute of Science and Technology software (NIST), USA. To ensure that nicotine was positively identified, the shape of nicotine peak was compared with that of nicotine in the instrument's database. Owing to its classified nature as a banned drug, standards for this compound (nicotine) were not available. Nevertheless, the GC-area counts were recorded as concentration. Moreover, it is well known in tobacco research that the peak with the highest concentration is usually nicotine; therefore identification of nicotine in this work corroborates literature surveys.

Results and Discussion

The evolution of nicotine was investigated using GC-MS and the results presented in Figure-2. It is clear that the yields of nicotine peaked at about 400^0C for ES1 while that of SM1 peaked at about 500^0C. Evidently, at low temperatures (200-400^0C), the yields of nicotine from SMI cigarette was very low but rose sharply between 400 and 500^0C. On the other hand, ES1 generally yielded high levels of nicotine in the whole pyrolysis temperature range. Above 500^0C nicotine yields were lower for ES1 while that of SM1 rose steadily before decreasing exponentially to about 8.8 x 10^7 GC-area counts at 700^0C. Remarkably, the monitoring of the yields of nicotine with change in temperature is one of the fundamental results of this investigation. Few studies if any have attempted to examine the release of nicotine from cigarettes as the temperature is varied. Consequently, this work is a masterpiece of the product distribution of nicotine with smoking temperatures. This study is informed by the fact that a burning cigarette is in itself a reactor and reaches a maximum temperature $>900^0$C[1,21].

This study reveals that the lethal temperature region for cigarette smokers could be as low as 100^0C for ES1, judging from the results presented in figure-2 above. SM1 however does not give significant yields of nicotine between 200– 400^0C. Nonetheless, at high temperatures $> 600^0$C, toxic polycyclic aromatic hydrocarbons (PAHs) such as benzo [a] pyrene are pyrosynthesized[22,23]. These PAHs are well established carcinogens[22,24] and thus the development of toxic free cigarettes is a challenge researchers have to grapple with for many years to come. This is because when one compound is targeted for elimination, a new more toxic compound is formed and the whole subject of toxin reduction in cigarette smoking is ever complicated. Perhaps, the only way out of this crisis is for smokers to quit tobacco use altogether. Although this study has focused primarily on nicotine, tobacco smoke contains overnumerous (≈ 7000) compounds, 10% of which are either cancerous or deadly in their own right[12].

Figure-3 depicts the evolution of nicotine at a retention time of 12.46 minutes for SM1 cigarette brand at 500 ˚C pyrolysis temperature. Itis clear from the chromatograms generated in this study that nicotine was the predominant pyrolysis product of mainstream cigarette smoke.

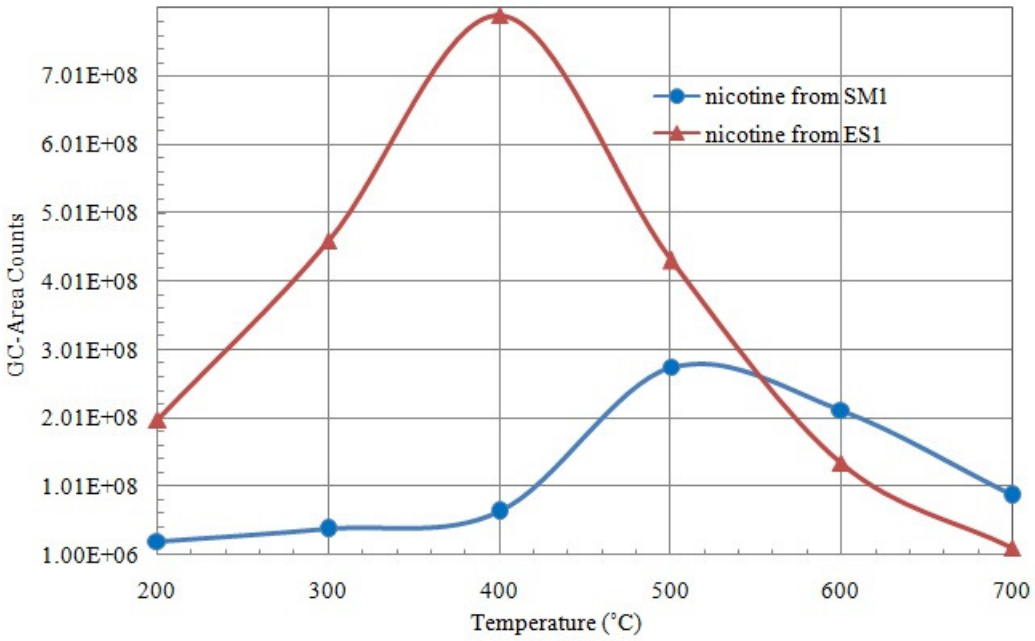


Figure-2
Yields (GC-Area counts) of nicotine from the combustion of ES1 and SM1 cigarette brands at various smoking temperatures

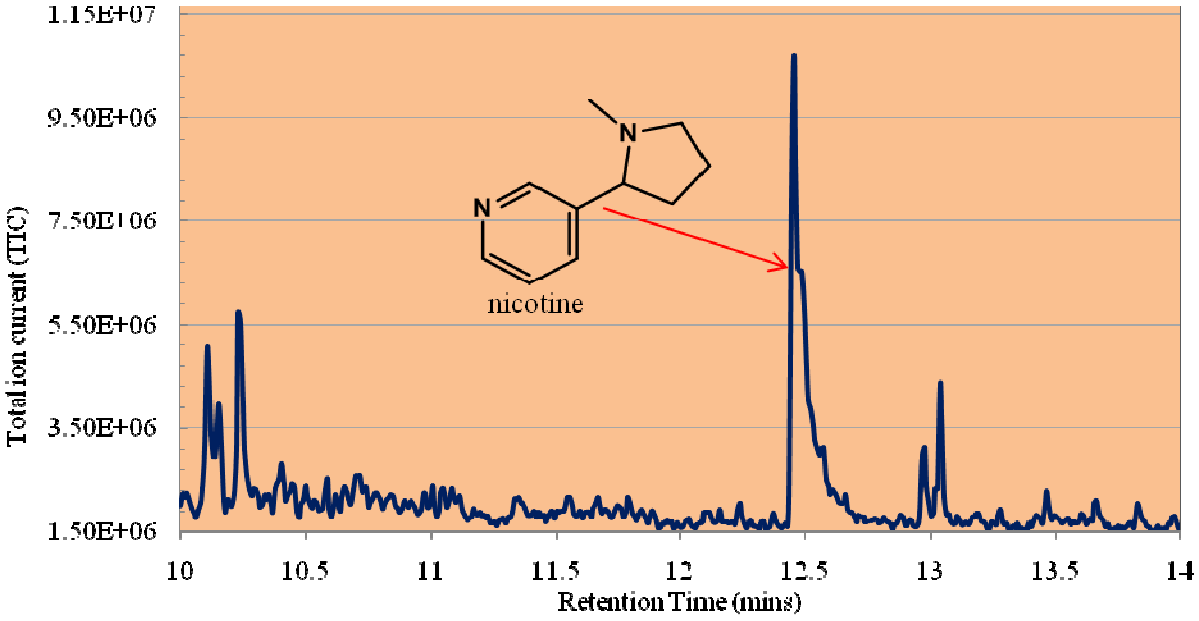
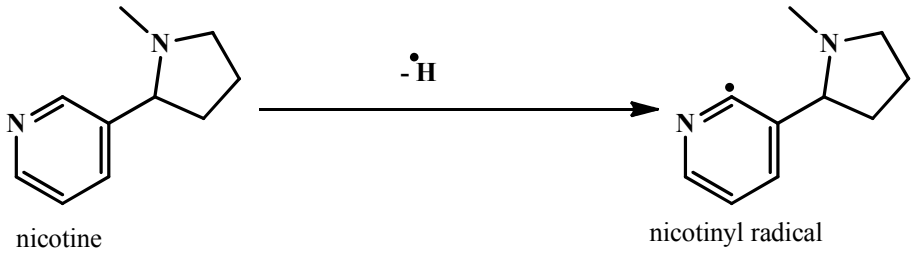


Figure-3
The chromatogram for nicotine from combustion of SM1 cigarette at 500 ˚C

Abstraction of hydrogen atom during pyrolysis of tobacco is a common event in radical formation. Consequently, the formation of nicotinyl radical is proposed to proceed according to the reaction mechanism presented in scheme-1. Radical species are well-known reactive species which can trigger reactive oxygen species in biological systems such as DNA, lipids, and proteins[25]. Free radicals are any species having one or additional unpaired electrons, can be formed from various classes of chemicals, and are largely considered asvery reactive[26]. Moreover, free radicals are transient species with short-life times although some radical species can persist in the environment for longthus causing serious biological and environmental damage[27].



Scheme-1
The proposed formation of nicotinyl radical from molecular nicotine

Toxicity Indices: The toxicity values for nicotine and nicotinyl radical were estimated using Hyper Chem computational program[28]. Quantitative structural activity relationship (QSAR) is a very important command found in Hyper Chem computational code mainly used to calculate the relative toxicity indices of a compound and determine its toxicological implications in humans and environmental ecosystems. The estimated toxicity indices for nicotine and its corresponding free radical were 0.22 and 0.74 respectively. These values suggest that nicotine and its respective radical are lyophilic. Lyophilicity compares with the extent of toxicity of a compound and therefore nicotine is not only a relaxant as widely reported in literature but also toxic. Lipophilicity, as measured by the base 10 logarithm of the octanol-water partition coefficient (P) and represented as $\log P$, is included as a likely contributory factor to the toxicity of a compound[29]. $\log P$ correlates with various biological activities including *in vitro* mutagenicity and carcinogenicity in animals[30]. Lipophilic compounds such as nicotine can cross biological barriers containing lipids, for example, cell or microsomal membranes and skin layer[24,25]. Therefore, $\log P$ influences metabolic fate, key biological activities and the transport properties of chemicals in biological environments[24]. Nicotine has a high degree of dissolving in blood plasma thereby executing pleasurable effects among smokers. This 'nice' feeling caused by nicotine is what impedes the campaign to quit cigarette smoking.

Conclusion

Toxicology data has proven that nicotine is highly soluble in biological fluids and falsely acts as a neuron transmitter, and thus capable of mimicking neuron transmitters such as acetylcholine, and dopamine which are well-known natural relaxants in the human brain. From this work, it was noted that most of the nicotine was evolved between 300˚C and 500˚C especially for ES1 cigarettes. Although the yield of nicotine for SM1 cigarettes was comparatively low in this temperature region, it was nonetheless high above 500 ˚C. Therefore, in order to design modern cigarettes, efforts should be towards developing cigarettes that can be smoked at optimum temperatures (below 400 ˚C) which do not favour the formation of high levels of nicotine. Nonetheless, it should be noted that nicotine cannot be eliminated completely even at low smoking temperatures and therefore the safest way is to quit cigarette smoking completely. This appears a herculean task due the addictive nature of nicotine but efforts must be to sensitize the general public on the hazards of cigarette smoking.

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