Extraction and Estimation of the Amount of Nicotine in a Tobacco Leaf

ANYIAM NGOZI DONALD

Department of Industrial Chemistry, University of Ilorin, Ilorin, Nigeria

Abstract: Nicotine is found in tobacco plant, Nicotiana tabacum L. This plant comes from the nightshade family with red peppers, eggplant, tomatoes and potatoes being the other members. In this study, Nicotine was extracted from tobacco leaf using three laboratory methods- Filtration, Solvent extraction and Crystallization. The percentage yield determined after the whole extraction method was 2.47 %. This percentage yield validates the reports from literature that the amount of nicotine in tobacco leaf is between 0.3% - 5%. More so, the very small nicotine investigated depicts a significant loss of product throughout the procedure which is due to formation of emulsions and not due to washing thoroughly with ether to extract maximum yield and the multiple repetition of the filtration process. For the purposes of identification and verification of the nicotine, other physical properties were determined, such as Molecular Weight (162.23 g/mol), Melting Point (-79 ºC), Boiling Point (247 ºC), Density (1.01 gcm^{-3}) and Refractive Index (1.53). Also, the Optical Rotation $[á]_D$ of nicotine (-168.5º) at the temperature of 293.15 K was determined.

Keywords: **Nicotine, Tobacco Leaf, Filtration, Solvent Extraction, Crystallization.**

I.0 Introduction

Tobacco is a stout herbaceous plant within the genus Nicotiana of the nightshade family. It originated in the tropical America and recently being cultivated worldwide [Ren and Timko, 2001]. Nicotiana tabacum (tobacco) is a cultivated plant of the Solanaceae family which produces materials for use as perfumes, cosmetics and pharmaceuticals. Nicotine is the principal alkaloid, which accounts for approximately 95% of the total alkaloids in tobacco. In China, there is a lot of waste tobacco and the extraction of nicotine from this is a way to increase the value of this otherwise waste material [Jian-min et al., 2009]. It is nicotine in tobacco that causes the most physiological effect of tobacco. Other alkaloids in tobacco are anabasine, nornicotine, myosmine, nicotyrine, anatabine, N-methylanatabine, N-methylanabasine, nicotrlline, 2, 3-dipyridyl and antalline. The picture of tobacco leaves is as shown in Figure-1below.

Fig 1: Dried Tobacco leaves.

1.1 Literature Review

Tobacco plant known as Nicotiana tabacum L (NTL) is a source of nicotine. This plant comes from the nightshade family and has other members such as red peppers, eggplant, tomatoes and potatoes [Lawson, 2015] as well as coca leaves [Hossain and Salehudin, 2013]. The word- nicotine comes from tobacco plants, now called nicotiana tabacum, named after the French ambassador in Portugal, Jean Nicotodevillemain (1560) who sent tobacco leaves and seeds to Paris and publicized their usage in medicines. It was from Brazil by Luis de Gois that tobacco and its seeds were brought to ambassador nicot [Kocha, 2013]. Tobacco plant is a well-known cultivated commercial crop [Hu et al., 2015] [Zhang et al., 2012]. China produces and consumes 400 to 500-million tons of tobacco annually. In addition, more than 200

million tons of tobacco wastes are produced yearly in tobacco farming and cigarette manufacturing industries. These tobacco wastes, including its low quality leaves, stem, leaf vein and tobacco roots, have severe irritating odour and contribute to serious ecological pollution [Hu et al., 2015].

Globally, China is the highest cultivation and production of tobacco than other producing countries. Reports from authors reveal that China produced 2.5 million tons of tobacco leaf in 2008, compared to 2007 with 22.9% increase in production [Zhang et al., 2012]. Second to China, is Indonesia which is also one of the biggest manufacturers of tobacco, which harvests 166,262 tons yearly and out of the total production, 99% is used in manufacturing cigarettes. Previous reports of authors have shown that tobacco leaf consists of three active alkaloids namely a; D-limonene, Indole, and nicotine. In order to extract nicotine from tobacco leaf, several methods have been exploited such as binary-sustained liquid membranes, reflux abstractions, column chromatography extraction, liquid-liquid extraction using ether as solvent and ingestion [Fathi et al., 2018].

In Bangladesh, various types of tobacco shrubs are in maximum use as they are affordable and readily available. In the United States, consumption of tobacco with alcohol is one of popular drug mixtures. In the last of 19th Century, scientists commenced the recognition of the adverse effects of nicotine. The U.S. Surgeon General in 1964 presented a research findings associating smoking with lung cancer and cardiac disease [Felman, 2018].

Nicotine is an extremely poisonous nitrogen-containing compound, being appropriate to the tobacco alkaloid [De Vito et al., 2014]. It is prepared from numerous species of plants containing tobacco. It can also be prepared synthetically [Lawson, 2015]. Nicotine, 3-(1-methyl-2-pyrrolidinyl) pyridine is a colorless, slightly pale yellow, hygroscopic oily liquid existing in Nicotiana tabacum L (NTL) leaves [4]. In small quantity, nicotine stimulates the nervous system. For smoking purposes, low nicotine concentration of tobacco is used [Hopkins et al., 2017]. The Molecular formula $C_{10}H_{14}N_2$ for nicotine is derived from elemental analysis and molecular weight determination. It absorbs two molecules of CH_3 I, suggesting the tertiary nature of both nitrogen atoms. On oxidation with chromic acid, nicotine yields nicotinic acid. This depicts that alkaloid contains a pyridine nucleus with a side chain at position-3 [Hopkins et al., 2017].

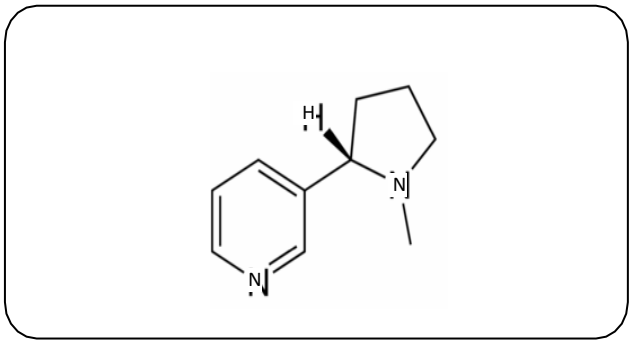
Medically, Nicotine is known to improve the health conditions of patients with schizophrenia abnormalities [Goniewcz et al., 2017] and dementia patients, dopaminergic neurons and axons, levodopa induced dyskinesia, skin mild cognitive dysfunction, [Benowitz et al., 2018]. Nicotine has antimicrobial and insecticidal functions [Heydari et al., 2017] and used as a natural pesticide with features of degradable, harmless and without environmental pollution challenges [Hu et al., 2015]. In Cigarette manufacturing, more than 20% of tobacco leaves used are disposed and removed. These discarded tobacco materials contaminate the environment and are not useful for any other purposes. Consequently, it is imperative to ascertain and exploit the discarded tobacco leaves during manufacturing. Tobacco necessary oil (TNO) unlike other necessary oils generally comprises of numerous extraordinary aromatic mixtures and a proper solvent. It is mainly used to reduce the aggressive flavor and irritancy of tobacco products. Additionally, TNO can be utilized in fragrances as well as smoking termination goods [Zhang et al., 2012]. Addiction to tobacco causes many diseases in developing countries that lead to high mortality [De Vito et al., 2014] [Khan, 2014] [Jarvik, 1991]. According to World Health Organization (WHO), 1.3 billion people smoke tobacco daily. It has been reported that smoking of Tobacco causes death of 5 million people annually. If the intake of tobacco worldwide persists at present rate, it will account for 5.4 million deaths each year [Mishra et al., 2015]. Nicotine is a powerful neurotoxin that is particularly harmful to insects [Jarvik, 1991]. Increase in heart rate, memory, alertness and reaction time have been attributed to the chemical reactions produced by nicotine in the nerve endings of humans. As an addictive drug, nicotine has been used as a depressant, as well as a stimulant. Nicotine can be taken in different ways; orally (not readily absorbed from digestive system) or snuffed (absorbed through the nasal cavity) or via smoking, Patches, Nicotine inhaler, Lozenges, and Gum [Khan, 2014].

1.2 Chemistry of Nicotine

Nicotine is the principal alkaloid in tobacco leaf that causes the most physiological effect of it. It was first isolated from the tobacco plant in 1828 by two German chemists, Posselt & Reimann. It constitutes about 0.3-5% of the tobacco plant by dry weight and biosynthesis takes place at the roots and accumulates on the leaves [Neal et al., 2009].

Nicotine is a powerful neurotoxin that is particularly harmful to insects [Ujvary, 1999]. It is a carcinogen, the compound responsible for the addictive nature of tobacco use [Munir et al., 1994]. Nicotine is also used as insecticides. The chemical structure of Nicotine is drawn as in (Fig.2) below.

Figure-2: The Chemical Structure of Nicotine



Nicotine has the IUPAC name, 3-(1-methyl-2-pyrrolindinyl) pyridine. It is a colorless liquid which is levorotary due to its single chiral centre, a nitrogen containing base built from two cyclic rings (pyridine of pka 3 and pyrolidine of pka 8). These two compounds that form nicotine can be protonated to form salts.
At pH 7, the ring is expected to be about 90% protonated. Hence, nicotine is readily soluble in water due to its ability to act as an acceptor for hydrogen bonds in water. It is also soluble in organic solvent, and forms salt with weak acids. On exposure to air, nicotine darkens, becomes more viscous and develops an offensive odour. It has a boiling point of 246.1°c, and its oxidation with $KMnO_4$ or $K_2Cr_2O_7$ yields nicotinic acid [Neal et al., 2009].

1.3 Toxicology Effect of Nicotine

The Lethal Dose, LD50 of nicotine is 50mg/kg for rats and 3mg/kg for mice. 40-60mg/kg can be lethal dosage for adult human beings. This makes it an extremely deadly poison. It is more toxic than many other alkaloids such as cocaine which has a lethal dose of 1000 mg [Pavia et al., 1976].

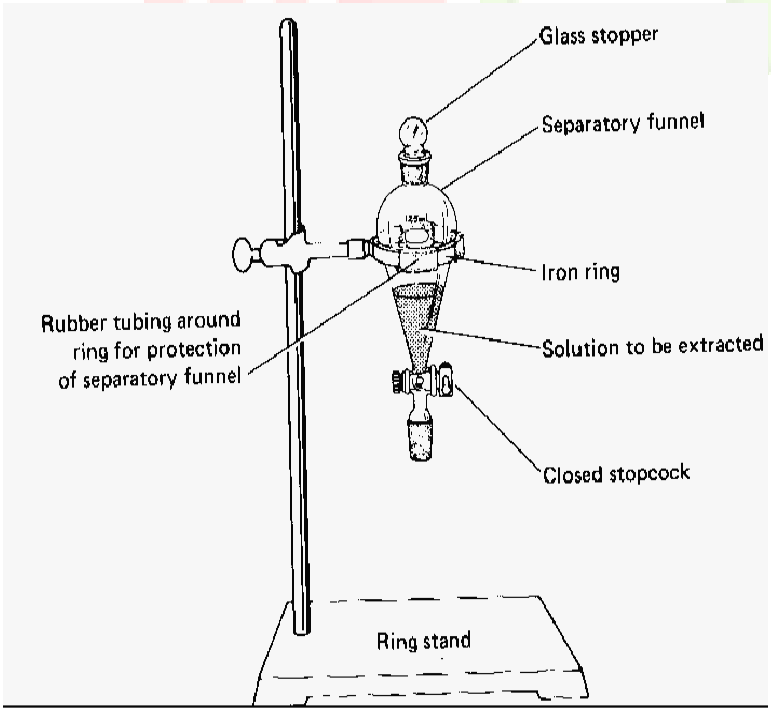
2.0 Principle of Isolation of Nicotine from Tobacco Leaves

The three main chemical processes used in the extraction of nicotine from tobacco leaf are; Filtration, Solvent Extraction and Crystallization The first and typical process is to extract nicotine into distinctively strong alkaline release environment such as NaOH solution. Although water can be used for the extraction but due to the fact that when water is added to the grinded dried tobacco leaf, the tobacco leaf absorbs all the water which results in the swelling of the tobacco leaf leaving behind tobacco jelly that is tough to deal with. This process can also be called Leaching.

The second process to be used is solvent extraction which is performed with the use of an organic solvent to remove nicotine from NaOH solution (aqueous solution) to the organic phase. The movement from the aqueous phase to the organic phase is due to the fact that like dissolves like since nicotine is an organic compound. The organic solvent to be used must be volatile to aid easy evaporation.

The third and last process involved is to convert the oily nicotine (liquid) into solid by first making the nicotine acidic to protonate the nicotine thereby converting it to nicotinium ion and then addition of picric acid leads to precipitation of yellow nicotine dipricate (as nicotine will form salt with weak acids to form dibasic complex salt). This process is carried out using the arrangement shown in figure 3 below.

Fig 3: Showing the Experimental Arrangement



3.0 Materials and Methods

Apparatus Used:

- Funnel
- Weighing balance
- Conical flasks
- Measuring cylinder
- Separating funnel
- Beakers
- Filter papers
- Mortar and pestle
- Sieve
- Lid
- Handkerchief
- Stirring rod
- Retort stand

Reagents used:

- Distilled water
- Sodium hydroxide(5%w/v)
- Methanol
- Petroleum ether
- Saturated picric acid

3.1 Experimental Procedure

Dried tobacco leaf was grinded using mortar and pestle and sieved to get a fine particle size of the leaf, after which 7.50 g was weighed and poured into the conical flask. 100 mls 0f 5% NaOH prepared was measured using a measuring cylinder and poured into the tobacco in the conical flask and stirred for 15 minutes for the extraction to take place (i.e. the removal of nicotine from the tobacco to the aqueous solution).

After 15 minutes, handkerchief was used to filter the mixture, the aqueous solution of sodium hydroxide passed through the handkerchief into the beaker while the tobacco remained as the residue. The residue was poured back into the conical flask and 30 mls of water was added into the residue to remove the remaining nicotine from the tobacco. Filtration was done thrice using handkerchief. The first, second and third filtrate were added together, which formed the aqueous phase required for the next process.

The second step is solvent extraction technique which involves partitioning between two phases (organic phase formed by organic solvent and aqueous phase formed by the aqueous solvent). The aqueous phase is formed by sodium hydroxide solution containing the nicotine extract gotten from the initial step. The organic phase is gotten by measuring 25 mls of petroleum ether

The aqueous phase was poured first into the separating funnel (with the tap locked to avoid discharge) after which 25 mls of the petroleum ether was added.

Immediately, two distinct layers were noticed. The solutions were shaken together, for extraction to take place after which the separating funnel was clamped to a retort stand for the settling of the two layers. Thereafter, the tap was opened at the bottom for the discharge of the aqueous solution while the cork at the top was opened and the organic phase (solution containing the nicotine) was poured from it. The

organic solution containing extract is poured into a beaker and covered with a lid, extraction was done thrice (using 25 mls each time) to remove nicotine from the aqueous phase.

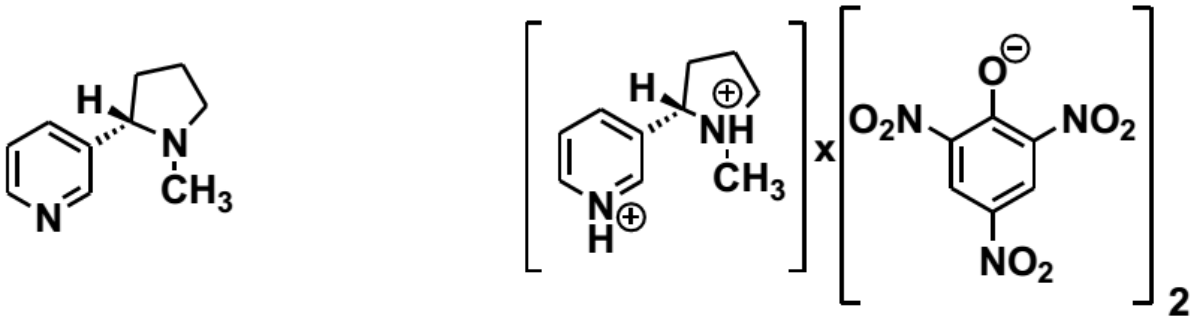
The ether extract was then sun dried to remove the solvent. Heating by water bath was not used owing to the fact that changes might occur in the chemical composition of the solution. After sun drying, a small oily liquid was gotten which was suspected to be nicotine.

The oily nicotine gotten was converted into solid for weight measurement. This was carried out by first adding 1ml of distilled water and 4 mls of methanol to the oily solution in the beaker, the solution was then filtered using a filter paper and funnel, after which the residue was washed down with 5 mls methanol to remove the remaining nicotine extract. The 10 mls filtrate containing the nicotine was poured into a separate beaker followed by the addition of 10 mls of saturated picric acid, and a yellow precipitate was formed immediately.

Filtration was performed immediately after the formation of precipitate with an already weighed filter paper (the weight of the filter paper was noted).The yellow precipitate formed is called nicotine dipicrate. The filtered precipitate was air-dried, not sun dried (to avoid chemical denaturation of the substance in the precipitate). After drying the substance, it was weighed and the mass gotten is the mass of nicotine dipicrate, not nicotine.

Fig 4: Formation and filtration of precipitate formed.

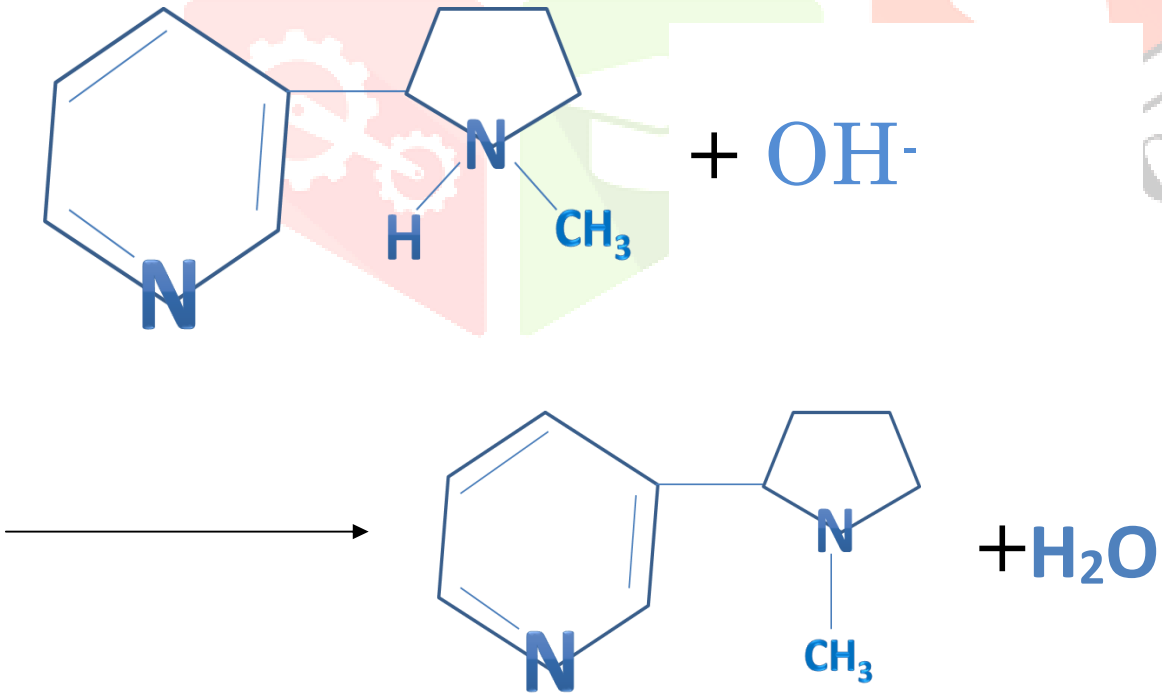
Figure 5: Showing the Structure of Nicotine and Nicotine-dipicrate (Nicotine derivative)



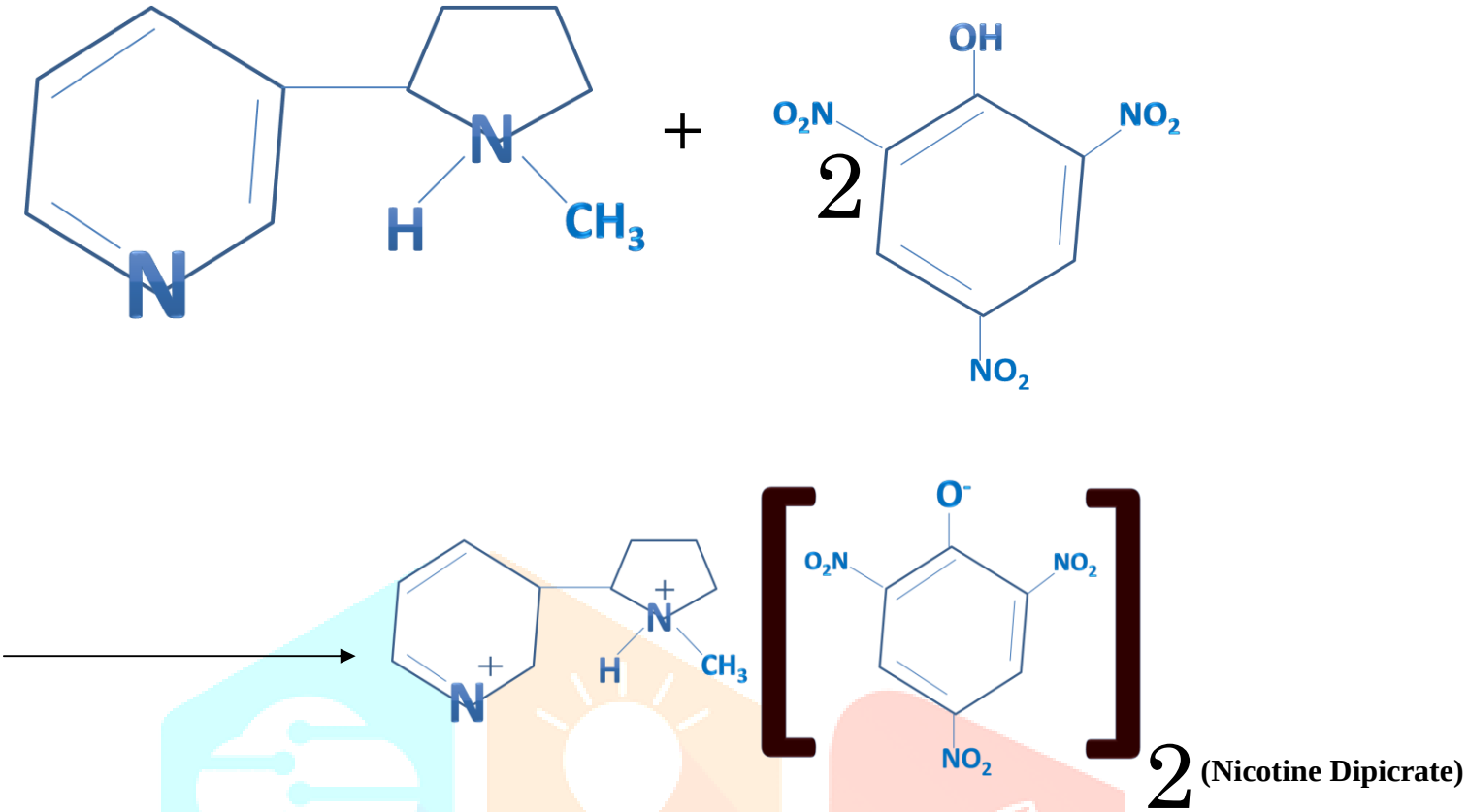
nicotine
$C_{10}H_{14}N_2$
Mol. Wt.: 162.23
Bp: 246-247°C

nicotine-dipicrate
$C_{22}H_{20}N_8O_{14}$
Mol. Wt.: 620.45
Mp: 218°C

EQUATION OF REACTION



EQUATION OF REACTION [Cont'd]



3.2 Results and Discussion

From the experiment performed
Molar mass of Nicotine = 162 g/mole
Molar mass of Nicotine dipicrate = 620.2 g/mole
Mass of Tobacco measured = 7.50 g
Mass of Nicotine dipicrate yielded = 0.710 g
Mass of Nicotine = X g

From balanced equation
1 mole of Nicotine = 1 mole of Nicotine Dipicrate
162 g of Nicotine = 620.2 g of Nicotine dipicrate
X g of Nicotine = 0.616 g of Nicotine dipricate

$$X\ g = \frac{162\ g/mole \times 0.710\ g}{620.2\ g/mole}$$

$$= 0.1855\ g \text{ of Nicotine.}$$

The amount of Nicotine in Tobacco in % is

$$= \frac{\text{Mass of Nicotine (Experimental yield)}}{\text{Mass of Tobacco Leaves (Theoretical yield)}} \times 100$$

$$= 0.1855/7.5 \times 100$$

$$= 2.47\ \%$$

The experimental yield obtained after the entire isolation method was 2.47 %. In order to verify the nicotine, different properties such as Melting Point (MP), Optical Rotation ([á]$_D$, Density, Boiling Point (BP), Molecular Weight (MW) and Refractive Index (RI) were also determined and presented in table 1.The values of the properties obtained were Boiling point (247 ^oC), Melting point (-79 ^oC), Molecular Weight (162.23 g/mol), Density (1.01 gcm^{-3}) Optical Rotation, [á]$_D$ (-168.5 ^{o}) at temperature of 293.15 K and Refractive Index [RI] (1.53).

Physical Properties	Value
Melting Point (MP)	-79ºC
Boiling Point (BP)	247 ºC
Molecular Weight (MW)	162.23 g/mol
Optical Rotation [á]$_D$	-168.5 º at 293.15 K
Density[þ]	1.01 gcm^{-3}
Refractive Index, ŋ [RI]	1.53

Table-1: Showing the values of Physical Properties of the Nicotine Isolated.

The result of the properties determined shows the extracted product as Nicotine. The calculated percent yield of 2.47% depicts that in the tobacco leaf used for this analysis, very small nicotine was present. In this extraction carried out, there is a significant loss of product throughout the procedure owing to formation of emulsions [Fauzantoro et al., 2017] and not owing to thorough washing with petroleum ether and repetitive filtration process in getting maximum yield [Ustick et al., 2018]. It is important to note that reactions of precursor with solvent pair may not be complete and as a result, 100% yield is not conceivable. Owing to much transfers in all extraction processes, this loss might occur [Xie et al., 2018].The much water added also contributed to the decrease in the concentration of nicotine obtained. These determined results verified that the isolated product was nicotine. Furthermore, the percentage yield reveals that for that particular tobacco leaf, only about 2% nicotine was present, which coincides with the theoretical value which says that the amount of nicotine in tobacco leaf is between 0.3% - 5%.

4.0 Conclusion

The nicotine contained in tobacco leaf has been extracted using Petroleum ether. From the experimental value gotten, it shows that for the particular tobacco leaf used, only about 2.47% nicotine is present, which coincides with the theoretical value which says that the amount of nicotine in tobacco leave is between (0.3-5%).

4.1 Recommendations

The Petroleum ether used in the extraction of Nicotine must not be heated directly but should be sun dried in order to preserve the Nicotine. Secondly, the laboratory personnel should ensure that the measurements of values are taken at eye level so as to avoid error due to parallax. Lastly, handkerchief should be used instead of filter paper for the first filtration owing to the fact that filter paper will swell and block the pores for filtrate to pass through.

4.2 Acknowledgement

The author's heartfelt gratitude goes to Stellar Cresco Limited Alberta, Canada for generously supplying funding to this research project for the advancement of science.

4.3 References

1) Ren, N., and Timko, M. P. (2001) AFLP analysis of genetic polymorphism and evolutionary relationships among cultivated and wild Nicotiana species, Genome, 44(4), 559-571.
2) Jian-min X., Fen-fang L. and Jiang P. (2009) Recovery and Purification of Nicotine from Waste Tobacco by Aqueous Two-Phase System/Reverse Extraction, Natural Product Communication. 4 (8): 1093-1094.
3) Lawson, N. S. (2015) from https:// www.slideshare.net/imtfuzz/introduction-aboutnicotine.
4) Hossain, A. M., and Salehuddin, S. M. (2013) Analytical determination of nicotine in tobacco leaves by gas chromatography–mass spectrometry. Arabian Journal of Chemistry, 6(3), 275-278.
5) Kocha, J. (2013) From https://www.slideshare.net/ jashankochar/nicotine-a-harmful-drug?from_ action=save.
6) Hu, R.-S., Wang, J., Li, H., Ni, H., Chen, Y. F. and Zhang, Y.-W. (2015) Simultaneous extraction of nicotine and solanesol from waste tobacco materials by the column chromatographic extraction method and their separation and purification. Separation and Purification Technology, 146, 1-7.
7) Zhang, X., Gao, H., Zhang, L., Liu, D., and Ye, X (2012). Extraction of essential oil from discarded tobacco leaves by solvent extraction and steam distillation, and identification of its chemical composition. Industrial crops and products, 39, 162-169.
8) Fathi, R. M., Fauzantoro, A., Rahman, S. F., and Gozan, M. (2018) Column chromatography isolation of nicotine from tobacco leaf extracts (Nicotiana tabaccum L.). Paper presented at the AIP Conference Proceedings.
9) Felman, A. (2018). Everything you need to know about nicotine. From https://www.medicalnewstoday.com/articles/240820.php.
10) DeVito, E. E., Herman, A. I., Waters, A. J., Valentine, G. W., and Sofuoglu, M. (2014) Subjective, physiological, and cognitive responses to intravenous nicotine: effects of sex and menstrual cycle phase. Neuropsychopharmacology, 39(6), 1431.
11) Hopkins, D. R., Ruiz-Tiben, E., Eberhard, M. L., Roy, S. L., and Weiss, A. J. (2017) Archive for the 'Guinea worm disease/Dracunculiasis' Category. Management, 500.

12) Goniewicz, M. L., Gawron, M., Smith, D. M., Peng, M., Jacob, P., and Benowitz, N. L (2017) Exposure to nicotine and selected toxicants in cigarette smokers who switched to electronic cigarettes: a longitudinal within-subjects observational study. Nicotine & Tobacco Research, 19(2), 160-167.
13) Benowitz, N. L., Pipe, A., West, R., Hays, J. T., Tonstad, S., McRae, T. and Anthenelli, R. M (2018) Cardiovascular Safety of Varenicline, Bupropion, and Nicotine Patch in Smokers: A Randomized Clinical Trial. JAMA internal medicine, 178(5): 622-631.
14) Heydari, R., Mobidi, A. M., Mohammadi, R., Forouzandeh, Z., and Rashidzadeh, S. (2017) Effect of emotional distress and academic stress on level of nicotine addiction among medical students. Nova Journal of Medical and Biological Sciences, 6(1).
15) Khan, A. (2014). From https://www.slideshare.net/AfsanaKhan1/ nicotine-presentation-41351291.
16) Jarvik, M. E. (1991). Beneficial effects of nicotine. British journal of addiction, 86(5), 571-575.
17) Mishra, A., Chaturvedi, P., Datta, S., Sinukumar, S., Joshi, P., and Garg, A. (2015) Harmful effects of nicotine. Indian journal of medical and paediatric oncology: official journal of Indian Society of Medical & Paediatric Oncology, 36(1), 24.
18) Neal L. Benowitz, Janne Hukkanen, and Peyton Jacob III (2009); Nicotine Chemistry, Metabolism, Kinetics and Biomarkers; University of California, San Francisco.
19) Ujvary, I. (1999). Nicotine and other Insecticidal Alkaloids. In Nicotinoid insecticides and the nicotinic acetylcholine receptor, Springer, Tokyo pp. 29-69.
20) Munir, C., Zaidi, M. I., and Yousaf, S. M. (1994) Zinc, cadmium and mercury as extractants of nicotine from tobacco leaves. Main Group Metal Chemistry, 17(9), 673-677.
21) Pavia, D. L., Lampman, G. M. and Kriz, G. S. Jr., (1976) Introduction to organic laboratory technique, W.B. Saunders Co., Philadelphia, p. 50-54.
22) Fauzantoro, A., Dalimunthe, A. A., and Gozan, M. (2017) Production Of Biopesticide From Tobacco Leaves (Nicotiana Tabacum) With Digestion And Reflux Extractions. Paper Presented at the 6th Indonesian Biotechnology Conference.
23) Ustick, J., Chuang, C., Muesse, D., and Russ, J. (2018) Analysis of nicotine in clay samples by solvent extraction and gas chromatography mass spectrometry. Paper presented at the Abstracts of Papers of the American Chemical Society.
24) Xie, J., Zhou, B., Zhang, T., Zeng, X., Yang, M., Wang, W., and Yang, J. (2018) Preparation of nicotine surface molecularly imprinted polymers for selective solid-phase extraction of nicotine from zero-level refill liquids of electronic cigarettes, Analytical Method.