



## Nicotine Detection Kit

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### ABSTRACT

**Aim:** To develop and demonstrate the effectiveness of a Nicotine Detection Kit for detecting nicotine presence in saliva of the tobacco users.

**Method:** We propose a qualitative experiment to demonstrate the presence of nicotine in the saliva of the tobacco users and highlight how it forms compounds with metal. This experiment involves extracting nicotine from tobacco and then creating a reaction between nicotine and Co(II).

**Results:** Our novel kit delivers precise and reliable nicotine detection using saliva, making it a user-friendly, non-invasive tool for tracking nicotine exposure. This advancement holds great promise for applications in healthcare and public health.

**Keywords:** Nicotine, Tobacco users in India, Detection kit, Inexpensive kit, Narcotics detection method.

### INTRODUCTION

Nicotine is naturally found in the plants belonging to the Solanaceae family (Tobacco, tomato, potato). The majority of tobacco users are addicted to nicotine delivered by tobacco product. Nicotine is a stimulant drug that acts as an agonist at nicotinic acetylcholine receptors. Nicotine consumed with tobacco (various form like smoking and non-smoking form) is probably the second most used drug in the world after caffeine from coffee and tea<sup>1</sup>. All forms of tobacco carry the same level of harm. Tobacco or nicotine consumption is a common practice observed in numerous countries, cultures, and diverse religious traditions worldwide. Cigarette smoking is the most common form of tobacco use worldwide. Other tobacco products include waterpipe tobacco, cigars, cigarillos, heated tobacco, roll-your-own tobacco, pipe tobacco, bidis and kreteks, and smokeless tobacco products. Nearly 2.5 million were nonsmokers who died from heart disease or lung cancer caused by exposure to second hand smoke<sup>1</sup>. Nicotine is widely recognized for its substantial systemic impact, and its potent addictive qualities are just one facet of its influence. This compound exerts detrimental effects on various bodily systems, including the cardiovascular, reproductive, pulmonary, and renal systems, among others.

Nicotine is an unusual alkaloid in that it has two nitrogen-containing heterocycles, pyridine and pyrrolidine. It is, of course, the tobacco component that makes smoking highly addictive, leading to the consequence that long-term smoking causes cancer

Nicotine appears as a colorless to light yellow or brown liquid. Combustible. Toxic by inhalation and by skin absorption. Produces toxic oxides of nitrogen during

combustion. Nicotine is primarily metabolized in the body by the cytochrome P450 (CYP) enzyme family, specifically the CYP2A6 isoform. This enzyme converts nicotine into cotinine, and further metabolizes cotinine into trans-3'-hydroxycotinine. Other metabolites of nicotine include cotinine-N-oxide, nornicotine, norcotinine, 4-oxo-4-(3-pyridyl)-butanoic acid, 4-hydroxy-4-(3-pyridyl)-butanoic acid, and nicotine-N'-oxide<sup>6</sup>.

### Statistics data about tobacco user (worldwide)

Tobacco kills more than 8 million people each year, including 1.3 million non-smokers who are exposed to second-hand smoke. In 2020, 22.3% of the world's population used tobacco: 36.7% of men and 7.8% of women. More than 80% of all smokers now live in countries with low or middle incomes. China produced and consumed more than 30% of the cigarettes in the world. In many developing countries, tobacco use is notably prevalent. For instance, China stands out with a striking contrast in smoking prevalence between genders, as 74% of males are smokers, while only 8% of females use tobacco. Every day, almost 2,500 children under 18 years of age try their first cigarette, and more than 400 of them will become new, regular daily smokers<sup>2</sup>. Individuals who initiate smoking during their formative years face a higher risk of developing a strong nicotine addiction compared to those who commence smoking later in life. Since 2014, electronic cigarettes (e-cigarettes) have consistently held the title of the most frequently used tobacco product among young people (below age 22). Nicotine pouch sales have seen a rapid increase in the U.S.



## Statistics Data About Tobacco User in India

Tobacco use is widespread in India, with varying patterns across the country. Of the total estimated 28.6% tobacco consumption, only 10.7% is from cigarettes and bidis (traditional leaf-wrapped tobacco), while the majority, 21.4%, is in the form of smokeless tobacco products<sup>3</sup>. Tobacco consumption varies significantly across different regions of India. According to data from the GATS 2016–17 surveys, certain northeastern states, including Tripura, Mizoram, Manipur, Assam, Meghalaya, Odisha, Arunachal Pradesh, West Bengal, Nagaland, and Chhattisgarh, exhibit a notably high prevalence of tobacco use.

Conversely, in regions such as Goa, Puducherry, and Kerala, the prevalence of tobacco usage is notably lower. Smokeless tobacco products are extensively consumed in the following states: Tripura, Manipur, Odisha, Assam, Arunachal Pradesh, Nagaland, Chhattisgarh, Jharkhand, Uttar Pradesh, Karnataka, Gujarat, Bihar, and Maharashtra. According to global adults tobacco survey 10.3% using smoking tobacco, 21.45% using non-smoking tobacco and 3.4% using both smoking and non-smoking tobacco

## Nicotine Content in Indian Tobacco Products

### Smoking Tobacco

Tobacco products in India display a wide range of nicotine content, with cigarettes typically containing about 14.5 milligrams of nicotine per cigarette, while bidis have notably higher levels, averaging around 26.9 milligrams of nicotine per bidi. In comparison to cigarettes, bidis indeed contain significantly higher levels of nicotine. Additionally, when compared to many international tobacco products, Indian tobacco products tend to have higher nicotine concentrations<sup>4</sup>.

### Non-Smoking tobacco

In India, various smokeless tobacco products like chewing tobacco, snuff, and snus are accessible, all of which contain lower nicotine levels (e.g., 3.4mg/gm, 3.6mg/gm, and 3.2mg/gm) compared to smoking tobacco. Nevertheless, it's crucial to understand that even though these smokeless alternatives have less nicotine, they still pose significant health risks and potential harm to individuals who use them.

## METHODOLOGY

1. Nicotine Extraction
2. Artificial Saliva Preparation
3. Sample Preparation
4. Preliminary Test Paper
5. Nicotine Detection Solution

## 1. Nicotine Extraction

### 1.1. The core concept behind isolating nicotine from tobacco leaves<sup>5</sup>

- In the extraction of nicotine from tobacco leaves, two primary chemical processes are employed: Filtration and Solvent Extraction. The initial and conventional step involves extracting nicotine within a highly alkaline solution, typically using sodium hydroxide (NaOH)
- The second step involves solvent extraction, where an organic solvent is employed to extract nicotine from a sodium hydroxide (NaOH) aqueous solution. This transition from the aqueous phase to the organic phase occurs because similar substances tend to dissolve in one another, and nicotine being an organic compound, it readily dissolves in the organic solvent. It's essential to select a volatile organic solvent for this process to facilitate convenient evaporation.

### 1.2. Experimental procedure for isolating of nicotine from tobacco leaves

- Dried tobacco leaves were finely ground and 10 grams were put into a conical flask. Then, 100 mL of a 5% sodium hydroxide solution was added using a measuring cylinder, and the mixture was stirred for 15 minutes to extract nicotine into the solution.
- After a 30-minute wait, a filter cloth was used to filter the mixture. The sodium hydroxide solution passed through, while the tobacco remained as residue. The residue was returned to the flask, mixed with 30 mL of water, and this filtration was done twice to create the aqueous phase for the next step.
- In the second step, we use a solvent extraction method to split the mixture into two separate parts: one is the organic phase made with an organic solvent, and the other is the aqueous phase formed by the sodium hydroxide solution containing nicotine extract obtained in the first step. To create the organic phase, we measure and add 25 mL of petroleum ether.
- The process started by gently transferring the aqueous phase into a separating funnel, with extra care to secure the tap to prevent any spillage. Following this, 25 mL of petroleum ether was introduced into the funnel, leading to an instant separation into two distinct layers. These layers were then vigorously mixed to enhance the extraction process.
- Afterward, the separating funnel was clamped to a retort stand, allowing the two layers to settle. Following the settling period, the tap at the bottom was opened to allow the discharge of the aqueous solution, while the cork at the top was opened to pour out the organic phase, which contained the nicotine extract. This organic solution was carefully transferred into a beaker and covered with a lid. This extraction process was repeated three times, each time using 25 mL of petroleum ether,



to ensure the complete removal of nicotine from the aqueous phase.

- The ether extract was allowed to air dry in the sun to eliminate the solvent, as we avoided using a water bath to prevent any potential alterations in the solution's chemical makeup. Following sun drying, we obtained a small oily liquid, which raised suspicions of it being nicotine.



**Figure 1:** Extraction of nicotine from tobacco leaves using the partition coefficient technique

### 1.3. Qualitative analysis of Nicotine

Thin-Layer Chromatography (TLC) was conducted using TLC plates measuring 10x20 cm in dimensions, and these plates were coated with a thin layer of silica gel 60 F254, with a thickness of 0.25 mm. The sheets were created within glass chambers lined with paper and filled with 10 ml of a dichloromethane-methanol solution (90-10 ratio), which had been allowed to equilibrate for a minimum of 15 minutes beforehand. Nicotine visualization was accomplished by applying a solution of ninhydrin (0.2% in ethanol) onto the sheets through spraying. A pale pink coloration tends to appear against the background. The presence of a blue-pink color is attributed to the formation of Ruhemann's purple. To determine the distance to the center of the spot (referred to as "dspot") and the distance from the spot line to the solvent front (referred to as "dsolv"), measurements are taken using a ruler, and The Rf (retention factor) for the spot is calculated as  $R_f = \text{dspot} / \text{dsolv}$ . The TLC developing solvent effectively separates nicotine within the range of Rf values from 0.20 to 0.25.



**Figure 2:** TLC is treated with ninhydrin, the presence of nicotine should yield a vivid purple coloration, indicating a positive result.

## 2. Artificial Saliva preparation







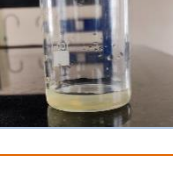
An artificial saliva solution was formulated using a composition comprising 99% water and 1% of various components, including methyl paraben, methyl cellulose, potassium chloride, potassium phosphate, sodium chloride, sodium fluoride, and dextrose.




## 3. Sample preparation

We have prepared a set of 10 samples, each designated as follows:

- Samples 1, 2, 3, 4, and 5 contain artificial saliva. Samples 6, 7, 8, 9, and 10 contain a mixture of artificial saliva with a controlled addition of nicotine extract.

**Table 1:** Samples

No of samples	Content	Visual Example
Sample-1	Artificial Saliva	
Sample-2	Artificial Saliva	
Sample-3	Artificial Saliva	
Sample-4	Artificial Saliva	
Sample-5	Artificial Saliva	
Sample-6	Artificial Saliva + Nicotine Extraction	
Sample-7	Artificial Saliva + Nicotine Extraction	

Sample-8	Artificial Saliva + Nicotine Extraction	
Sample-9	Artificial Saliva + Nicotine Extraction	
Sample-10	Artificial Saliva + Nicotine Extraction	

#### 4. Preliminary Test Paper

##### 4.1 The core concept behind in Preliminary test paper formulation (Hager's Test)

- The Hager's test is based on the principle of colorimetry. This method relies on the fact that nicotine, when reacted with Hager's reagent (a mixture of potassium hydroxide and picric acid), produces a distinctive and measurable change in color. The intensity of this color change is directly proportional to the concentration of nicotine in the sample.

*Hager's reagent strip + Nicotine* → *Deep Blue Colour*

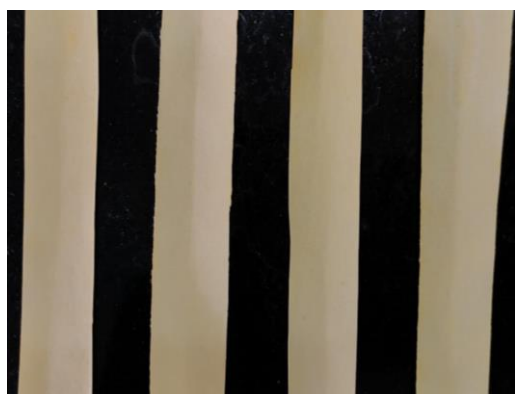


Figure 3: Preliminary test paper

##### 4.2 The experimental procedure to formulate the preliminary test paper

- To create the preliminary test paper, we utilized Whatman filter paper and cut it into rectangular strips using a pair of scissors.
- The next step is to carefully dip the rectangular strip of paper into Hager's reagent. The paper will absorb the reagent, resulting in a conversion to a yellow color. Afterward, it is essential to allow the strip of paper to dry for a few hours. This process ensures the stability and accuracy of the test paper for further experiments.

#### 5. Nicotine Detection solution

##### 5.1. The core concept behind in nicotine detection solution<sup>5</sup>

When nicotine reacts with copper(II) nitrate, it forms a complex called nicotine Cu(II) complex. This complex is known for its green color. The green color is a result of the interaction between the nicotine molecule and the copper(II) ion. The nicotine molecule acts as a ligand, donating a pair of electrons to form a bond with the copper(II) ion. This bonding creates a stable complex with a characteristic green color.

Copper Nitrate Solution + Nicotine → Green Colour Complex  $[Cu(NIC)2NO3]^+$

##### 5.2 The experimental procedure to prepare nicotine detection solution

- The nicotine detection solution was created using 100 mL of a Copper Nitrate Solution with a concentration of 10%. This indicates that within every 100 mL of this solution, there are 10 grams of dissolved copper nitrate
- To make the solution, you would need to measure out 10 grams of copper nitrate and dissolve it in 100ml of distilled water. Stir the solution well to ensure complete dissolution of the copper nitrate
- This will serve as the base solution for the nicotine detection
- Once the base solution is prepared, it can be used directly for nicotine detection.



Figure 4: Nicotine detection solution

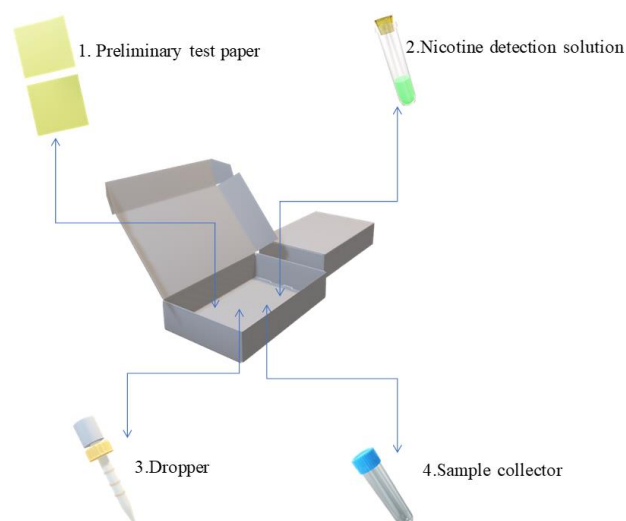















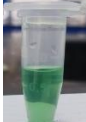

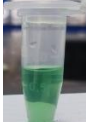

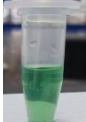

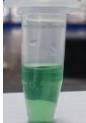
Figure 5: Nicotine detection kit

### 3D Model Design of Nicotine Detection Kit

The nicotine detection kit comprises essential components, including preliminary test paper, nicotine detection solution, a sample collector, and a precision dropper. Each element plays a vital role in the accurate and efficient detection of nicotine presence, making the kit a comprehensive tool for such analyses. Together, these components ensure a professional and reliable nicotine detection process for various applications.

### RESULTS AND DISCUSSION

Table 2: Test Results

Samples	Preliminary test	Nicotine detection test
Sample-1		
Sample-2		
Sample-3		
sample-4		
Sample-5		
Sample-6		
Sample-7		
Sample-8		
Sample-9		
Sample-10		

✚ Samples 1, 2, 3, 4, and 5 for initial and nicotine detection tests. These samples turned out negative because they only had artificial saliva, with no nicotine. **To check for nicotine, we used both the preliminary test paper and nicotine detection solution, but there was no change in color, indicating the absence of nicotine.**

✚ Samples 6, 7, 8, 9, and 10 were readied for initial testing and nicotine detection. These samples showed positive outcomes due to the inclusion of artificial saliva with a small amount of nicotine. **Nicotine's presence was verified through a preliminary test paper, turning it blue, and also with a nicotine detection solution, changing the color from blue to green, indicating the presence of nicotine.**

### CONCLUSION

- The development of the nicotine detection kit is an important milestone in our research work. Our team created a new method to detect nicotine in the saliva qualitatively.
- The kit is very cheap and affordable for everyone.
- Through this research, we have successfully established a reliable method for determining whether nicotine is present or not. The nicotine detection kit serves as a valuable tool for both researchers and professionals in related fields.
- We suggested Implementing the use of nicotine detection kits in all schools and colleges. I think It's a positive initiative that can have long-term benefits for the students and the entire educational community
- We suggested to incorporating these nicotine detection kits into a sports academy to ensure a healthy and clean environment for athletes and other sports players.
- This research contributes to the growing field of point-of-care diagnostics.
- The kit is designed to be user-friendly, allowing individuals to perform the test without the need for specialized training. The kit's portability allows users to carry it easily, making it convenient for use on the go.
- Nicotine act as a valuable tool for individuals aiming to quit tobacco use by tracking their progress.
- The kit could contribute to international efforts to reduce the prevalence of tobacco-related diseases.
- The impact of the kit may lead to increased support for organizations working towards tobacco control and prevention.
- Our future objective is to transform the qualitative analysis kit into a quantitative kit by incorporating advanced sensors. This enhancement aims to provide precise measurements of nicotine levels in saliva, offering a more accurate representation of the percentage of nicotine present.

- We have planned to integrated into mobile apps or wearable devices for real-time monitoring of nicotine levels
- The research has immediate applications in clinical and forensic settings

**Table 3:** Test Observations

Test	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Sample-6	Sample-7	Sample-8	Sample-9	Sample-10
Preliminary test	+	+	+	+	+	-	-	-	-	-
Nicotine detection kit	+	+	+	+	+	-	-	-	-	-

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