Forensic Analysis of Salivary Nicotine by Calix[4]Arene Customized Gold Nanoparticles in Water and Soil

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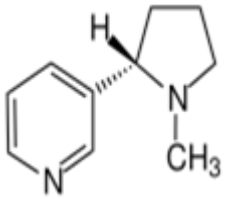
Abstract: Nicotine, a potent stimulant found in tobacco products, has significant effects on oral health and overall well-being. Saliva samples showing the presence of nicotine can be very helpful as evidence in a variety of situations. Salivary nicotine levels can reveal smoking habits. It offers proof of whether a person has recently used tobacco goods. Saliva testing can be used, for instance, to support or contradict smoking behaviours in court cases involving custody disputes, child endangerment, or employment regulations. Saliva nicotine testing can be used in criminal investigations to determine a suspect's smoking history. If saliva includes nicotine traces, it can be used to identify the person to a place or occasion, particularly at the crime scene which may reveal discarded cigarette butts or other tobacco-related evidence. Also, exposure to nicotine impacts general health. Saliva tests can reveal whether or not a person uses tobacco, which is important information for determining health risks. For example, nicotine testing can be used by medical practitioners to assess a patient's willingness to stop smoking or to track their progress while participating in smoking cessation programmes. Moreover, some insurance providers take nicotine usage into account when setting insurance premiums or providing coverage. Saliva test results can impact judgments about disability claims, health insurance, and life insurance. However, it's crucial to properly interpret the findings of nicotine tests. Due to the variety of benefits associated with the identification of salivary nicotine, this article aims to develop an affordable technique that can facilitate the in-situ, real-time detection of nicotine in saliva samples.

Keywords: Forensic Analysis, Nicotine, Gold Nanoparticles, Water And Soil

1. INTRODUCTION

The main and minor salivary glands' acinar cells secrete saliva, a complex biological fluid. It serves as a marker for different components of plasma.[i] Its use as a forensic and diagnostic tool has been studied more and more in recent years upon. Saliva includes oral cavity epithelial cells, which make it a potential source of DNA for forensic examination.[ii] Saliva may also be utilised to identify the presence of specific illnesses and to detect medications and poisons. It may be gathered from bite marks on the skin and in food, from surface stains, and from a variety of objects such as straws, phones, stamps, cigarette butts, and cutlery.[iii]

Nicotine is a common stimulant of the central nervous system that can be inhaled through cigarettes, cigars, pipes, beedis, or by sniffing it. It may be found in tobacco smoke and is also used as an insecticide. Adults who use nicotine orally must consume between 40 and 60 mg to be killed.[iv] Biological samples including blood, urine, hair, and saliva can all contain nicotine.[v] Nicotine can be used as a marker for tobacco usage in forensic science.[vi] In situations of nicotine poisoning, it can also be utilised to identify the cause of death. Since saliva includes oral cavity epithelial cells, it can be employed as a source of nicotine for forensic analysis.



In forensic science, nicotine can serve as a marker for tobacco usage. In situations of nicotine toxicity, it can also be utilised to ascertain the reason for demise. Saliva includes oral cavity epithelial cells, which make it a viable source of nicotine for forensic examination.

Many methods pertaining to Gas Chromatography, LCMS, and HPLC have been used in modern times. Nevertheless, these methods need costly equipment, highly skilled professionals, and time-consuming, laborious analysis. We have created a straightforward, affordable, and in-situ technique that makes it simple to detect nicotine in real-time.

Analytes may be easily seen with the naked eye due to colorimetric detection. Numerous noble metal nanoparticles based on colorimetric sensors have been created because noble metal nanoparticles, such as gold and silver, allow for exceptional Plasmon assimilation. By modifying noble metal nanoparticles with suitable organic molecules as ligands, colorimetric sensors may be efficiently designed. When organic compounds are added to metal nanoparticles, host-guest interaction occurs. Additionally, if channeling structures are placed between the metal nanoparticles and analytes, the analytes and noble metal nanoparticles aggregate, changing the colour and UV-vis spectrum results.

Sulphonato calix[4]arene is water soluble and extremely hydrophilic because (SO_3^-) heads occupy the top rims of the calixarene moiety. Calix[4]arene is a supramolecule with an interior cavity of dimension 3 A^ and is generally hydrophobic in nature. This moiety's hydrophilicity facilitates its combination with the insecticide that has been dissolved in water. On the other hand, significant precise monitoring of the host molecule by metal nanoparticles aids in the effective and specific colorimetric detection of nicotine by producing an apparent colour shift. The goal of this work is to employ gold nanoparticles modified with para-sulphonato calix[4]arene to detect nicotine that is divided in soil and water.

The creation of Para Sulphonato Calix[4]arene modified Au Nanoparticles (pSC4 – Au Nps) and their usage as colorimetric sensors for salivary nicotine detection—which is often employed for chewing and sniffing—are reported in this research.

2. EXPERIMENT

2.1 Materials:

Samples were prepared as per the protocol for soil sampling, soil and water analysis from ICAR. The protocol includes tools required for soil sampling, collection of soil samples, and precautions to be taken while collecting the sample. The protocol does not specifically mention nicotine, but it can be used as a general guideline for soil sampling.[vii]

2.2 Preparation of Para – sulphonato calix[4]arene

Sulphonation of Para – H – calix[4]arene v=can be achieved in various ways, but we selected the simplest way, i.e. direct sulphonation of Para – H – calix[4]arene as first described by Shinkai. This is the most common route for the preparation of Para – Sulphonato Calix[4]arene, where the strong acid (Conc. H_2SO_4) is mixed with the base molecule and heated to 100^0C, for the reaction to occur. After cooling the precipitates were recovered by filtration and the product was obtained after evaporation of water. The product was dried overnight. The product can be used for the reaction after proper dilution in required concentration.

2.3 Microwave-assisted synthesis of Au Nanoparticles

Before being used, all of the glassware was carefully cleaned with recently made 3:1 (HCl: HNO3) and then washed with double distilled water. The process was conducted in a sealed jar to create microwave-assisted Au nanoparticles. After preparing 0.1 mM of 3 ml Au solution and combining it with 2 ml of 13 mM sodium citrate, the mixture is heated sustainably for two minutes at 80 0C with a power of up to 150 W. The colour changes from light yellow to burgundy or pink. After 15 minutes of cooling, use for the reaction.

2.4 Synthesis of pSC4 – Au Nps with Salivary Nicotine

Different concentrations of dissolved nicotine in water were tested, ranging from 5 ppm to 250 ppm. 2 ml of each solution was used for the reaction, and equal amounts of 2 ml of Au NPs and 2 ml of Para-Sulphonato Calixarene were added. The reaction was carried out using various dilutions. After letting the reaction mixture remain for a while, the mixture's hue changed from red to blue.

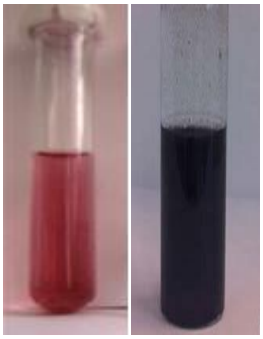


Figure 1 (a)- Au Nps and 1 (b) Au Nps with Salivary Nicotine

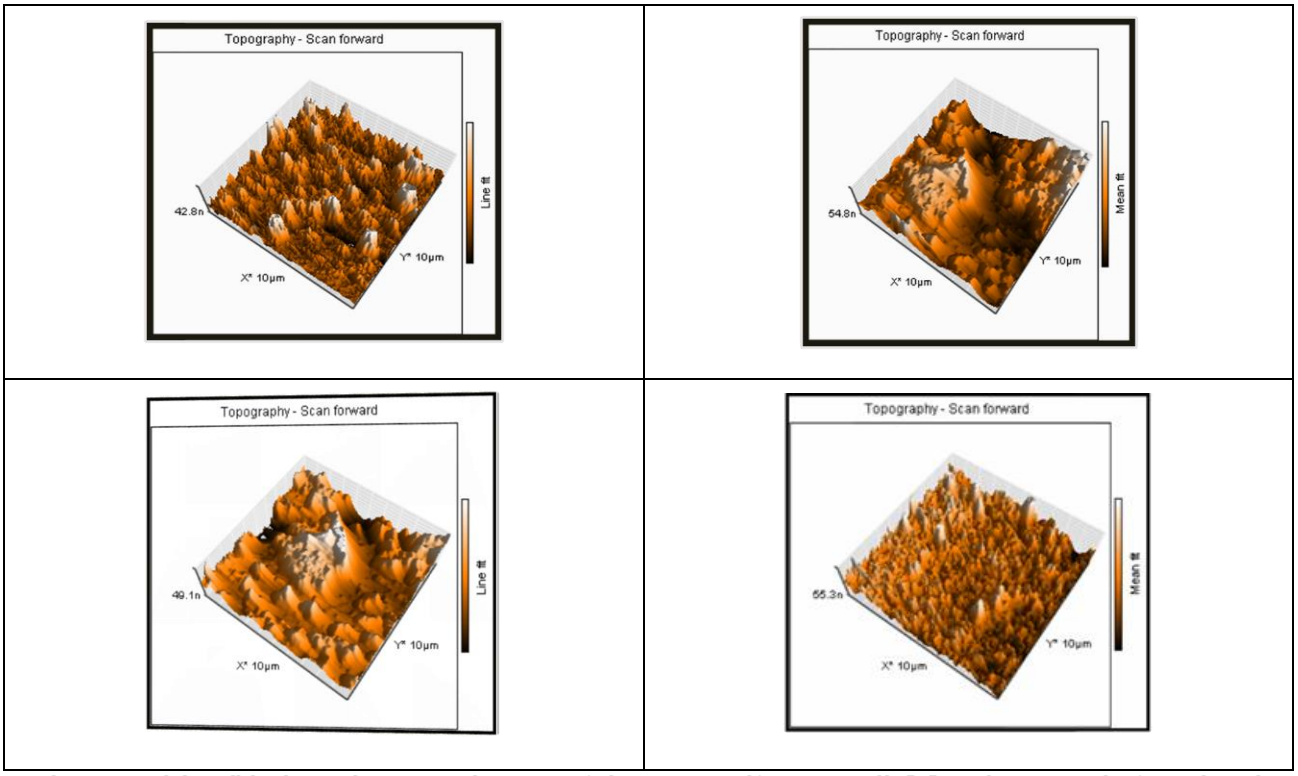
2.5 Analytical Methods

UV Spectrometry was performed at Chemistry Department of Gujarat University. Fourier Transform Infrared Radiation (FTIR) was performed on the instrument Jasco 5000 AFM was performed at Physics Department of Gujarat University. And DLS was performed at Research building of Chemistry Department of Gujarat University.

3. RESULTS AND DISCUSSION

3.1 Spectral Characterizations

The pSC4-Au Nps were produced in water by reacting 1 ml of 0.1 mM Au Nps with 1 ml of 0.1 mM Para Sulfonato calix[4]arene solution at room temperature for 5 and 10 minutes, respectively. The results were characterised using AFM, DLS, FTIR, and UV-Vis Spectroscopy.



Figures 2 (a) & (b) show the AFM pictures of the Para Sulfonato Calix[4] and Au Nps before the nicotine sample was added. In this instance, there is no aggregation and the particle size is reduced. On the other hand, once the nicotine sample was added, the AFM images of Au Nps and Para Sulfonato Calix[4]arene are displayed in figures 2(c) and (d). It displays larger particles as a result of aggregation.

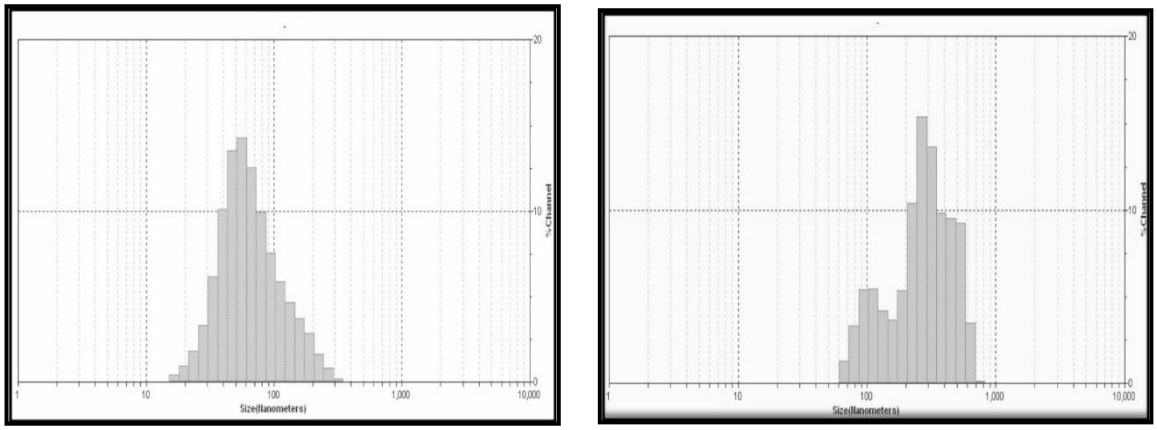


Figure 3 (a) shows the DLS graph of Au + pSC4 before the addition of clothianidin, where the particle size appears to be 65 nm. And the figure 3 (b) shows the DLS graph of Au + pSC4 after the addition of the Nicotine sample, where the particle size appears to be 265 nm..

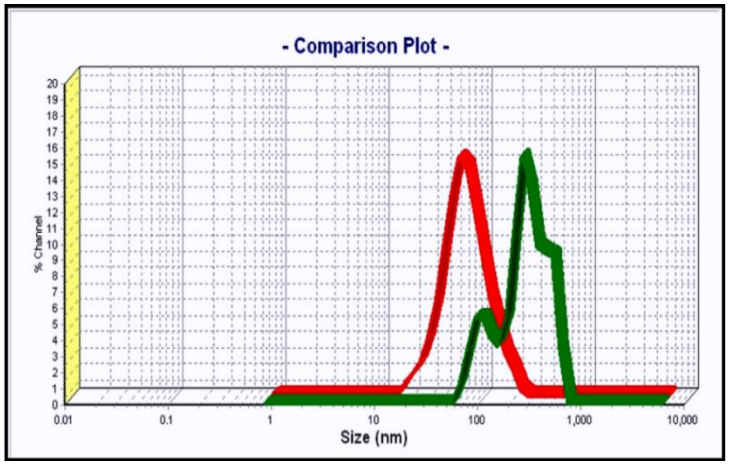
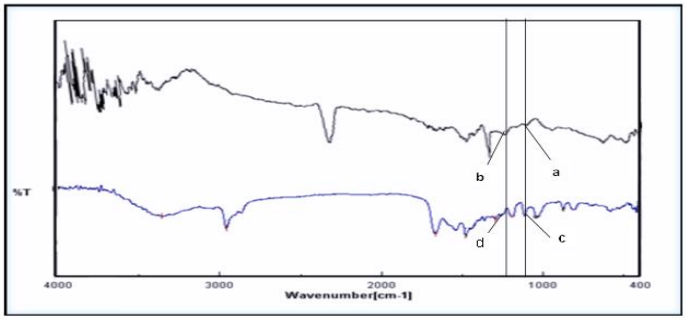


Figure – 3 (c) Red plot shows the size of Au + Calixarene, i.e. 65nm and the green plot shows the size of Au + Calixarene + Clothianidin, i.e. 265 nm, showing that the aggregation occurs in the solution after addition of Clothianidin and so the size of the molecules increases.



Para – Sulfonato Calix[4]arene — Au + Para Sulfonato Calix[4]arene

a – 1178 cm^{-1}; b – 1036 cm^{-1}; c – 1187 cm^{-1}; d – 1049 cm^{-1}

Figure – 4 (a) displays the Au-para Sulfonato calixarene and para Sulfonato calixarene FTIR spectra. When pSC4 – Au Nps is generated, the SO_3^- groups of pSC-4 coordinate with the gold atoms on the surface of Au NPs, as can be shown by comparing the peaks of SO_3^- at 1187 and 1049 cm^{-1} seen in pure pSC4 to 1178 and 1036 cm^{-1} in the graph of Au Nps + pSC4.

3.2 Colorimetric Detection of Nicotine Samples

To explore the molecular bonding abilities of pSC4 – Au Nps, a variety of plant extracts were added to the pSC4 – Au Np solution. Various plant extracts were analyzed for the colour change of the pSC4 – Au Nps. But it was observed that no major changes in the absorbance were observed in pSC4 – Au nps on the addition of various samples as shown in figure 4; except that of the salivary nicotine sample which showed a prominent boosting up in the absorbance of pSC4 – Au Nps i.e. from 529 nm to 568 nm; showing the change in colour form Red to blue as shown in figure. The selectivity towards salivary nicotine sample shown by pSC4 – Au Nps is accredited by the aggregation pSC4 – Au Nps induced by salivary nicotine sample; which is supported by AFM images and DLS graphs which shows the increase in the size of aggregated particles from 65 nm to 265 nm. The scheme of aggregation is shown in the figure 6.

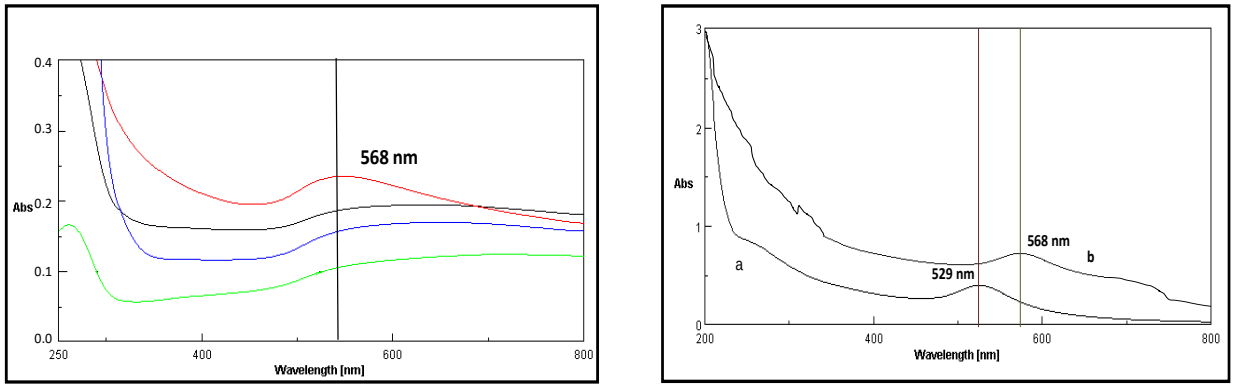


Figure – 5 (a) UV Vis Spectra of pSC4 – Au Nps with salivary nicotine sample at different concentrations 5 ppm, 10 ppm, 50 ppm & 250 ppm in order from top Figure – 5(b) UV Vis Spectra showing the shift of wavelength from 529 nm (a) to 568 nm (b) on the addition of salivary nicotine sample

Salivary nicotine samples and para Sulfonato Calix[4]are subject to an aggregation process that might be caused by electrostatic contact or host-guest interaction. Salivary nicotine samples are bound into the para sulfonato calix[4]arene cavity during host-guest contact, where they undergo aggregation. Because para-sulfonato calix[4]arene has an electron-rich cyclic cavity of SO_3^-, it may draw clothianidin's positively charged NH+, causing an electrostatic contact between the two.

Further, we checked our reaction with different PH conditions, the reaction remains stable in basic medium for weeks to months.

From this results, it can be found that whenever salivary nicotine sample is present in water in the detection limit of 0.05 ppm concentration, the positive result can be detected by Calixarene based gold nanoparticles.

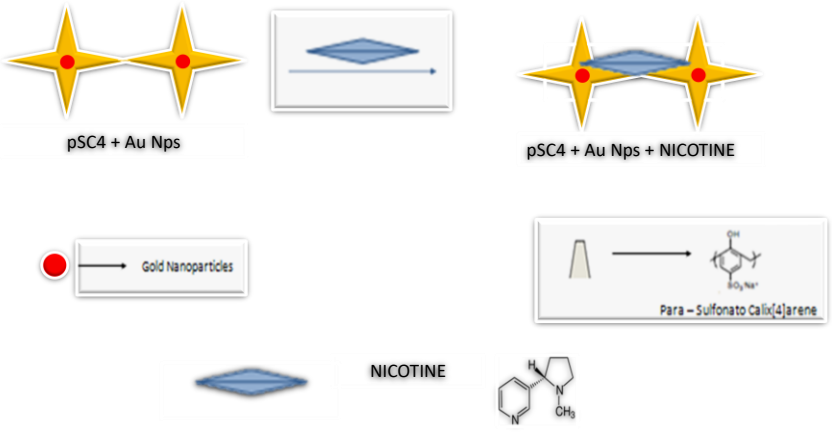


Figure 6- Schematic Representation of Salivary Nicotine Samples induced aggregation of pSC4- Au Nps.

4. CONCLUSION

The findings suggested that AFM, DLS, FTIR, and UV Vis Spectroscopy were used to effectively generate and characterize water soluble pSC4-Au Nps. The detection of salivary nicotine samples extracted from water and soil may be effectively accomplished using the novel innovation of Para Sulfonato Calix[4]arene - Au Nps, making colorimetric assay analysis simpler, quicker, and faster up to a concentration limit of 0.05 ppm. with a great deal of promise for the in-situ, real-time detection of nicotine in saliva samples.

5. ACKNOWLEDGMENT

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