FABRICATION OF SILVER NANOWIRES BY INKJET PRINTING AND USING THEM FOR SERS DETECTION OF MOLECULES

Report submitted under
SUMMER INTERNSHIP PROGRAMME – JULY, 2019
IN THE FACULTY OF CHEMICAL ENGINEERING

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Acknowledgement

I would like to thank Dr. S. Venugopal for his continuous guidance and support throughout this project. I am grateful to him for making my first research experience valuable and making me broaden my horizon on what it takes to enter the field of research.

I would like to thank the other faculty members of the chemical department for taking time out of their work to give us insightful talks and lecture on the various fields they are working in and helping us get a better understanding of what we would like to pursue.

I would like to thank my mentor Nikita for her constant support and guidance throughout the project and in helping me getting a better understand of the topic I was working on. A special thanks to lab seniors Sushant, Khantesh, Madhavan, Abhishek, Dhisesh and Pritam for their constant encouragement and helping in any way possible.

I am thankful to my fellow summer interns Koushik, Varshan, Tharun, Vasu, Aishwarya, Harishwar, Rakshit and Hariharan for their support and for providing a pleasant and comfortable atmosphere around the lab.

I am grateful to all the faculty and staff of the IISc for providing such a privilege to work in this prestigious institution and for their support during entire Internship period. Also, a special to CEA committee for making us part of CEA nite and accepting us as part of the Department.

Introduction:

Pesticides are used in a wide range of settings with one of the most important areas being in agriculture. They allow more food to be produced on a given area of land which as a result increases yields and improves farm revenues. Without them crop losses due to pests and diseases are between 30 and 50% depending on the crop grown. By protecting crops, pesticides contribute to the production of a plentiful supply of high-quality and affordable food and contribute to food security.

However, as pesticides are used to kill or control harmful organisms, unwanted pests, weeds, etc. they have the capacity to harm people, other non-target organisms (wildlife) and the environment. With an ever-growing population, there is a need for clean air, water, food and health care.

Food adulteration is the addition or removal of any substances to or from food, so that the natural composition and quality is affected. Adulterated food is impure, unsafe and not wholesome. Food can be adulterated intentionally and accidentally. Unintentional adulteration is a result of ignorance or the lack of facilities to maintain food quality. This may be caused by spill over effect from pesticides and fertilisers. Inappropriate food handling and packaging methods can also result in adulteration.

Intentional food adulteration is usually done for financial gain. The most common form of intentional adulteration is colour adulteration. Some examples of intentional adulteration are addition of water to liquid milk, extraneous matter to ground spices, or the removal or substitution of milk solids from the natural product. Natural adulteration occurs due to the presence of certain chemicals, organic compounds or radicals naturally occurring in foods which are injurious to health and are not added to the foods intentionally or

unintentionally. Some of the examples are toxic varieties of pulses, mushrooms, green and other vegetables, fish and seafoods. About 5,000 species of marine fish are known to be poisonous and many of these are among edible varieties.

FOOD ARTICLE	ADULTERANT	HARMFUL EFFECTS
Bengal gram dhal & thoor dhal	Kesai dhal	Lathyrism cancer
Tea	Used tea leaves processed and coloured	Liver disorder
Coffee powder	Tamarind seed, date seed powder	Diarrhoea
	Chicory powder	Stomach disorder, giddiness and joint pain
Milk	Unhygienic water & starch	Stomach disorder
Khoa	Starch & less fat content	Less - nutritive value
Wheat and other food grains (Bajra)	Ergot (a fungus containing poisonous substance)	Poisonous
Sugar	Chalk powder	Stomach – disorder
Black powder	Papaya seeds and light berries	Stomach, liver problems
Mustard powder	Argemone seeds	Epidemic dropsy & glaucoma
Edible oils	Argemone oil	Loss of eyesight, heart diseases, tumours
	Mineral oil	Damage to liver, carcinogenic effects
	Karanja oil	Heart problems, liver damage
	Castor oil	Stomach problem
Asafoetida	Foreign resins galbanum, colophony resin	Dysentery
Turmeric powder	Yellow aniline dyes	Carcinogenic
	Non-permitted colourants like metanil yellow	Highly carcinogenic
	Tapioca starch	Stomach disorder
Chilli powder	Brick powder, saw dust	Stomach problems
	Artificial colours	Cancer
Sweets, Juices, Jam	Non-permitted coaltar dye, (metanil yellow)	Metanil yellow is toxic and carcinogenic
Jaggery	Washing soda, chalkpowder	Vomiting, diarrhoea
Pulses (green peas and dhal)	coaltar dye	Stomach pain, ulcer
Suapari	colour and saccharin	Cancer
Honey	Molasses sugar (sugar plus water)	Stomach disorder
Carbonator water beverages	Aluminium leaves	Stomach disorder
Cloves	Cloves from which volatile oil has been extracted	Cheating, waste of money

Table 1: Adulteration in Food And Its Harmful Effects

Food Safety and Standards Authority of India (FSSAI) is the government body which sets standards for articles of food and regulates their manufacturing, storage, distribution, sale and import to ensure availability of safe and wholesome food for human consumption in India. FSSAI ensures the quality of food by testing them in their laboratories

The current gold standards for trace detection utilize techniques such as High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) for chemicals, and bioassays such ELISA and Western blot for biological agents. While sensitive, these tests are often time consuming, expensive and require skilled technicians. Hence, there is a definite need for low-cost technologies that can enable point of use adulterant detection

SERS has emerged as a practical trace detection technology enabled by a portable detector and a cleverly-engineered substrate.SERS is faster and easier to perform compared to the most commonly used techniques such as those which use mass spectrometry and gas chromatography which maintains measurement sensitivity and reliability while solving issues of cost and complexity.

Surface Enhanced Raman Spectroscopy (SERS) is a capable technique for rapid, on-field detection of Raman spectra of molecules. The vibrational signature encoded in the Raman spectra can be used as a unique identifier of molecular structure. A plasmonic nanostructured substrate is required for amplification, by several orders of magnitude, of the weak Raman signal (almost exclusively emitted from the adsorbed molecular layer closest to the surface), and such 'surface-enhancement' renders SERS capable of detecting the presence of a single molecule. Silver-based nanostructures have optimal plasmonic properties giving rise to higher SERS enhancement factors as compared to other materials.

Benefits of SERS

While the current widespread trace detection technologies offer similar detection performance, SERS has a number of appealing features:

• Inexpensive, portable equipment

• Simultaneous detection of multiple sample constituents

• Minimal false positives

• Portable, detection at the point of sample collection

• Mitigates fluorescence issues found in real-world samples

Amongst the various methods of fabricating SERS substrate, the most cost-effective method would be the use of additive drop on demand inkjet printing technology.

Inkjet printing is a material-conserving deposition technique used for liquid phase materials. These materials, or inks, consist of a solute dissolved or otherwise dispersed in a solvent. Inkjet-printing jets the single ink droplet from the nozzle to the desired position, therefore, no waste is created, resulting in an economical fabrication solution and the printing is uniform throughout the substrate.

As mentioned earlier, there are many techniques to detect adulterants. In this present work we have developed a simple and inexpensive but exceptionally sensitive portable chemical and biological sensing platform through the innovative use of Kim wipe combined with Surface Enhanced Raman spectroscopy (SERS). A chromatographic methodology was used to separate some dyes and the separated dyes can be detected using SERS.

MATERIALS USED:

- CHEMICALS (For fabricating SERS substrate)

- Silver Nitrate
- Ascorbic Acid

- Potassium Bromide
- Potassium Iodide
- Isopropyl Alcohol
- Ethanol
- Sodium Sulphite anhydrous
- Metol(Monomethyl p-aminophenol hemisulphate)
- Quinol
- Sodium Carbonate
- De-Ionised Water

➢ DYES

- Rhodamine 6G (R6G)
- Methyl Orange
- Methylene Blue

➢ SOLVENTS FOR CHROMATOGRAPHY

- Chloroform
- Hexane
- Toulene
- IPA
- Methanol
- Ethanol
- DI water
- Di-chloromethane
- Isopropyl alcohol
- Toluene

➢ APPARATUS

- Deskjet printer (HP 1010 series)
- Black Ink Cartridges (HP 802)
- Halogen Lamp (Crompton Greaves J240V 500 W R7S, 9500 Lumens)
- Laminar Hood (Esco)
- Sonicator (Bransonic® Ultrasonic Cleaner)
- Syringe Filter (Millex ® GV Filter unit)

➢ SOFTWARES

- Microsoft Office PowerPoint 2016
- OriginPro

➢ SOLUTION PREPARATION

All the chemicals used were used as received from SDFCL, Fisher Scientific and Merck Chemicals and are of higher purity. All the solutions were prepared using DI water. The molar ratio of $AgNO_3$ to KCL was always 2:1 to ensure complete conversion of AgCl on Kimwipe.2M $AgNO_3$ and 4M KCl solution was prepared to print over kimwipe to get AgCl layer.

KX solution was made using 5% (by weight) KI and 95% (by weight) KBr.

The developer solution was prepared by dissolving 18g Sodium Sulphate, 32g Sodium Carbonate, 4.8g Quinol, 0.8g Potassium bromide and 1.2g Metol in 200ml deionized water.

Experimental Methods:

Fabrication of SERS substrate:

A HP Deskjet 1010 series printer was used for printing the precursor salt solutions on kimwipe tissue stuck on a A4 copier paper. Two separate HP 802 black ink cartridges were used for printing KX and AgNO3 solutions. The cartridges were thoroughly cleaned removing the sponge holding the black ink and rinsing the reservoir in flowing tap water, finally with DI water. If required, sonication was done, and the DI water was filled in reservoir and some test patterns were printed on paper until there was no evidence of black ink.

The loading of the cartridge was found out by weighing the cartridge before and immediately after printing with DI water. This was repeated for five printings and the average of difference in their weights was noted. Knowing the density of water, the volume to be used per print could be found out. The loading of 2M of $AgNO_3$ was found to be 0.1976 ±0.009 mg/cm^2. The DI water loading was found to be 1.831 mg/cm^2.

Water Loading (µl/Cmsq)	Weight of AgNo3(mg)	mg/25cmsq	Wt of Silver/25cmsq	Wt of Silver/cmsq
1.8112	0.308	7.7	4.89	0.196
1.984	0.337	8.425	5.35	0.214
1.7784	0.302	7.55	4.794	0.192
1.7848	0.303	7.575	4.81	0.192
1.8015	0.306	7.65	4.858	0.194

Table 2: Silver Loading Calculation

In this work, plausible print-expose-develop mechanism was followed. 40 1cm diameter (each) circle patterns were drawn on Microsoft Office Word 2013 and printed alternatively with 2M $AgNO_3$ and 4M KCL (for nanoparticles)/4M KX (for nanowires) for six times. (In order to get a silver Loading of 1mg).

The paper should be dried properly after each print, otherwise printing on wet paper may block the print head and lead to cartridge failure. Another precaution that was taken to prevent cartridge failure was to wipe the printer head with tissue soaked in IPA. This prevents the formation of Ag precipitates on the printer head and prevent clogging of the cartridge.

After all the printings, the printed area was then exposed under a halogen lamp for approximately 10 minutes. Keeping the sample for prolonged time under the lamp showed no appreciable effect. The sample was then sequentially immersed for 10 min in a standard photographic developer solution (D-72), which is a reservoir of electrons and hence reduces Ag+ ions to Ag. These ions on reduction change its lattice structure by breaking the clusters and forming Ag nanoparticles on paper.

Fig.1. Alternate Layers of $AgNO_3$ and KCL being printed on Kimwipe Tissue

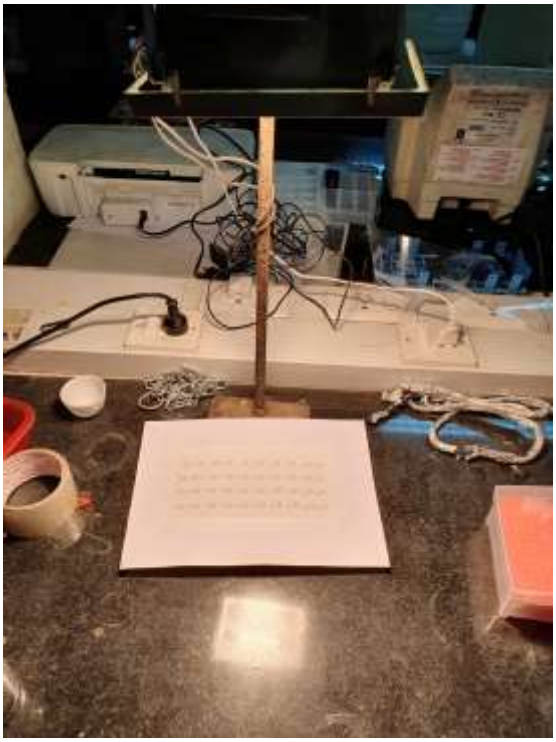


Fig.2. Printed Kimwipe Exposed Under Halogen Lamp

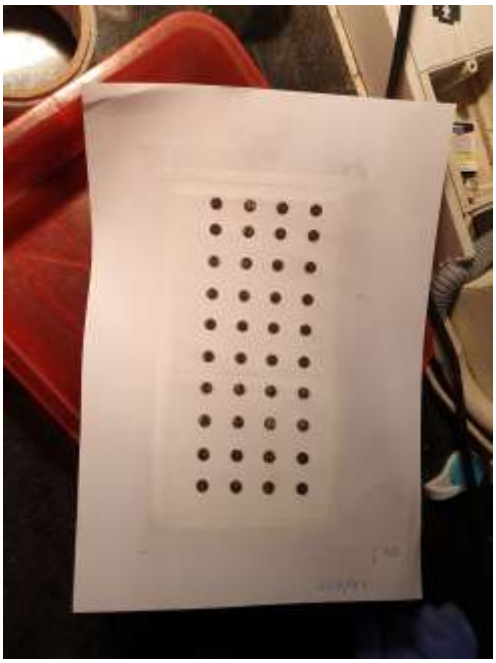


Fig.3. After Development of Nanowires

SEM Images:

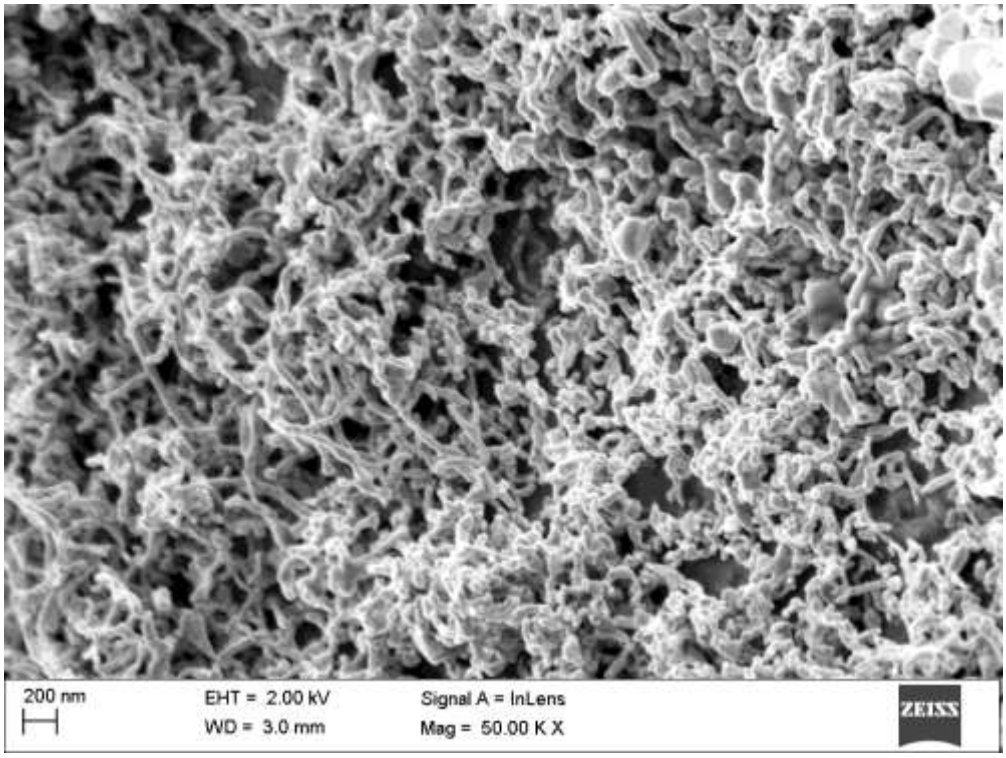


Fig.4. Nanowires Formation

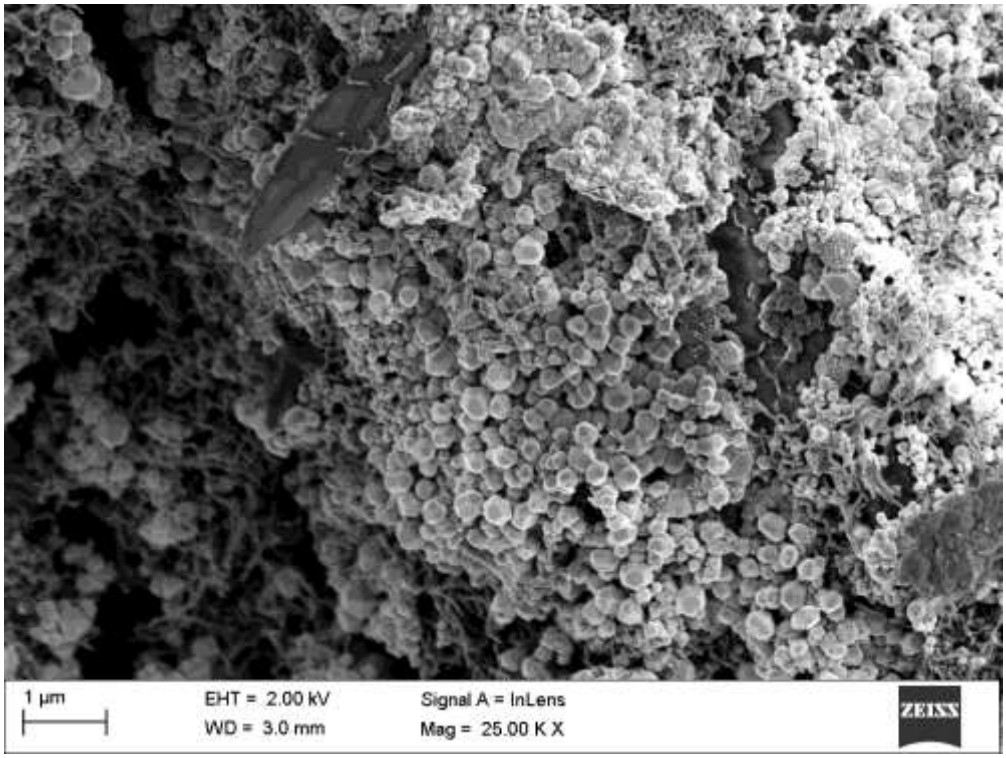


Fig.5. Nanoparticle Formation

Chromatography:

Chromatography was the process used to separate mixtures of substances into its singular components. In paper chromatography, the stationary phase was a very uniform absorbent paper. The mobile phase wa a suitable liquid solvent or mixture of solvents. Chromatography paper was used as the stationary phase to separate the dyes. The dye mixture consisted of Methylene Blue, Rhodamine 6G, Metanil Yellow.

Fig.6.Dye Mixtures

Fig.7. Chromatography Setup

To perform chromatographic separation of a sample, a pipette was used to place a small drop of the sample onto a strip of chromatography paper. This attached to a pen/pencil/tweezer was kept hanging over a beaker with its bottom submerged into the solvent. The mobile phase solvents differ depending on the sample and target components being separated. The level of the mobile phase was adjusted so that the meniscus was 1 cm below that of the position of the applied droplet. A constant run time of 30 minutes was maintained. After 30 mins the strip was removed and air dried. In certain cases, 2D chromatography was required to separate closely related species. This was done by rotating the strip by 90 degrees (after drying) and then performing another separation step using a second mobile phase.

Various Solvents were used to determine the most appropriate solvent require for separation of the dye mixtures.

Fig.8. Chromatograms after separation of dye mixture in various solvents

OBSERVATIONS:

- No separation takes place when we use non-polar solvents like Toluene or Hexane. This shows that the mixture was highly polar in nature and hence had a strong interaction with the cellulose in chromatography paper.

CONCLUSION:

For proper separation of the 3-dye mixture consisting of R6G, MY and MB chromatography must be done using methanol and ethanol in 2 different ratios:

- First chromatography was used 75% Ethanol+25% Methanol solution as solvent to separate the MB from the mixture.

Fig.9. MB being separated from mixture

- Second chromatography process was used a 60% Methanol + 40% Ethanol solution to separate R6G and MY.

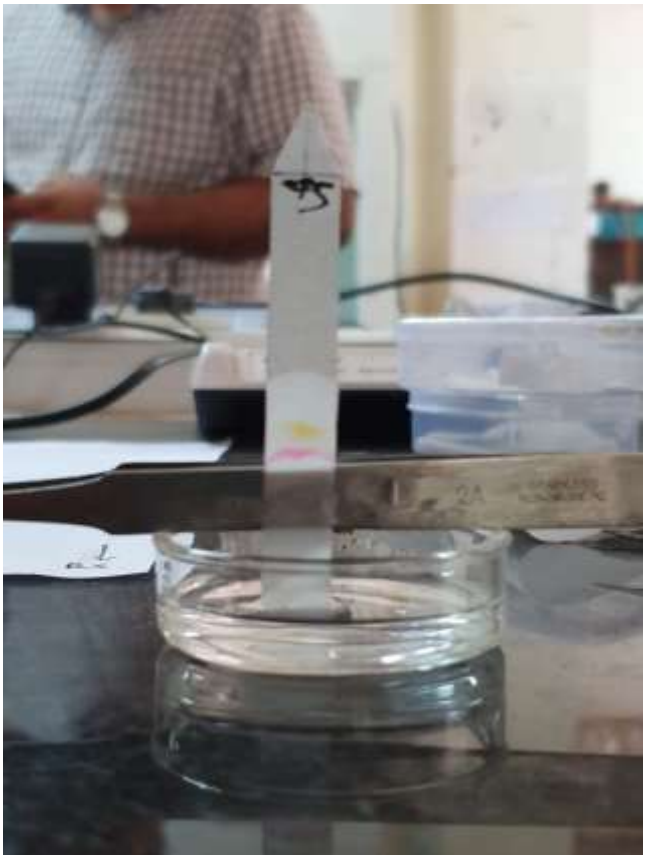


Fig.10. R6G and MY being separated

Transference of Dye to Silver Substrate:

Before doing the SERS measurement of the separated dyes, they first needed to be transferred on to the silver nanostructure to enhance the sensitivity of the Raman spectrum.

The Separated dyes on chromatography paper dried and the cut. The strip was then attached to the kimwipe tissue and then dipped in a suitable solvent. While the solvent did concentrate on the tip of the chromatography strip, transference of the dye was not taking place consistently (as seen in the image below). This was due to presence of tape that was hold the two substrates in place.

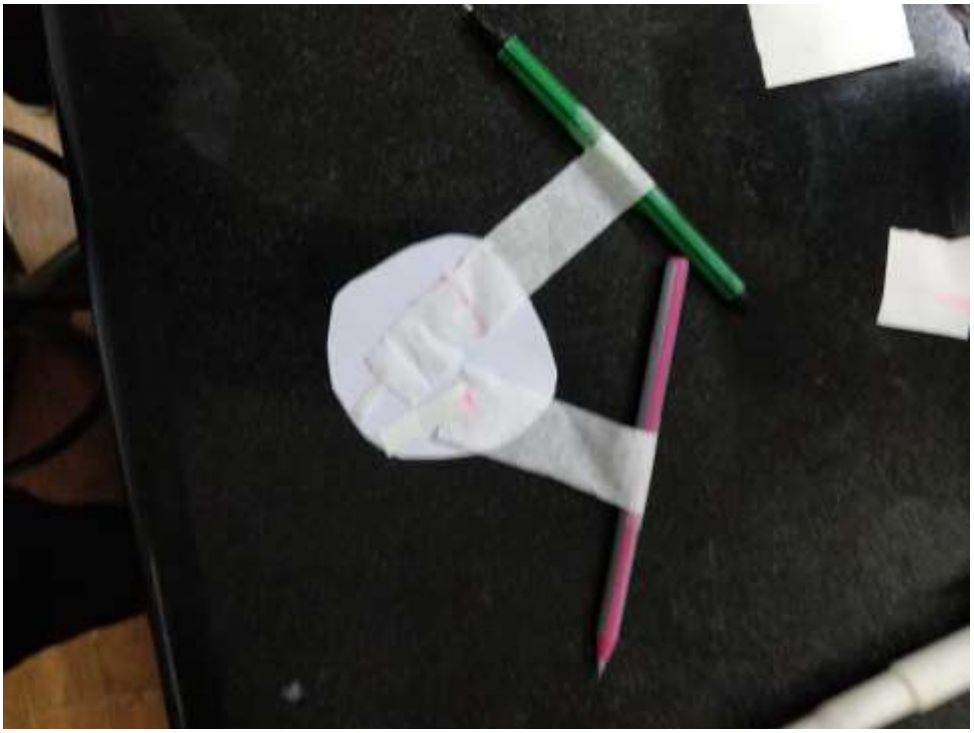


Fig.11. Transference of Dye on to kimwipe tissue

So, the next method was trying to transfer the dyes while manually holding the substrates in place. In this set-up, the cut off strip of chromatography sheet containing the separated dye in cut in a triangular shape on top to concentrate the dye to a single point for transference.

Using Acetonitrile as the solvent, the two substrates were kept in contact with each other for 10 mins. This showed promising results and was the method that was decided upon to be used for transference.

Fig.12. R6G Transferred on to the Silver Substrate

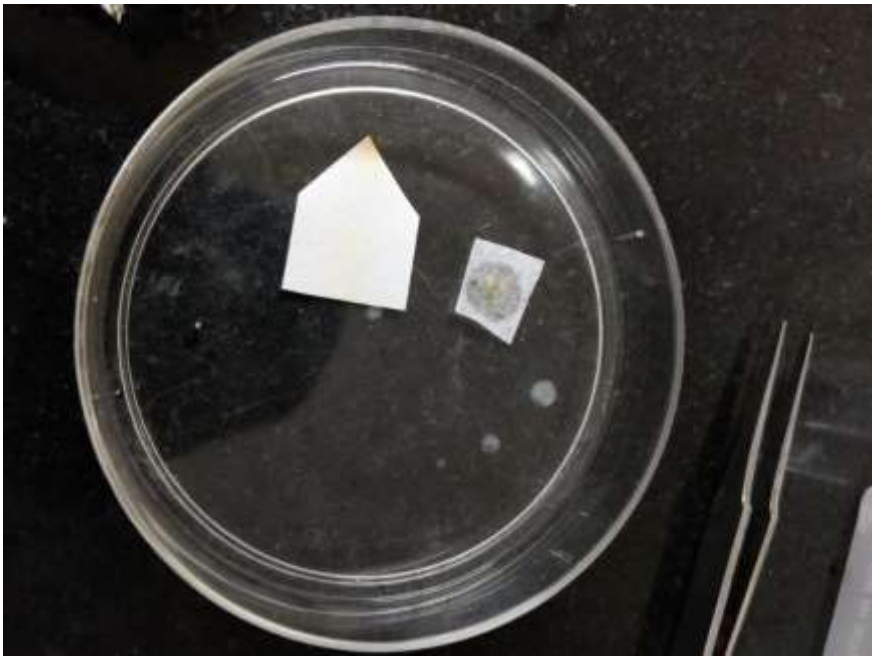


Fig.13. MY Transferred on to the Silver Substrate

SERS Measurement:

The Separated dyes transferred on to the Silver substrate was placed and stuck on a glass slide. Drop-casted MY, MB and R6G on to silver substrate and stuck on glass slide for SERS measurement.

SERS measurement was performed using a Sierra Raman Spectrometer (Snowy Range Instruments). It had a laser of 532 nm with adjustable laser power and a spectral range of 200-3000 cm^{-1}. The optimal distance was found to be at 11.8 mm. Raster was ON and a laser power of 16.5 mW and Integration time of 5 seconds. To determine the signal intensity, the height of the most prominent peak from the Raman bands was calculated.

Fig.14. Portable Raman Spectrometer

Analysis of SERS Spectrum:

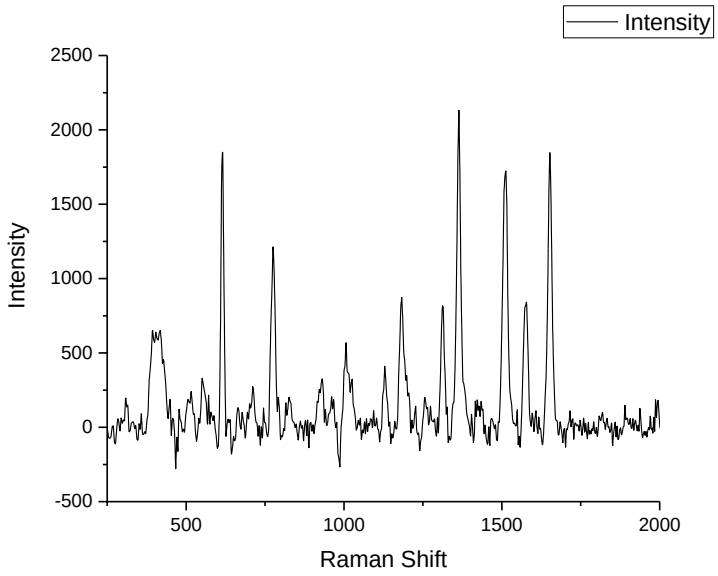
The averaged spectra of the data from three trials were plotted using ORIGIN PRO software. Background contributions from the substrates were accounted for by manually subtracting the SERS signals obtained from blank samples and compared. As the obtained spectra was not intense enough to compare with the standard spectrum (due to various factors like fluorescence), ORIGIN PRO was used to cancel the background noise and to create a baseline curve.

Using the origin software, spectra of dyes from various samples are compared using the pure dye spectrum (which have been drop-casted on to the silver substrate and measured) as a reference one. With the standard spectra of pure dyes, our obtained signals can be compared for the peaks at specific wavelengths and hence they can be detected.

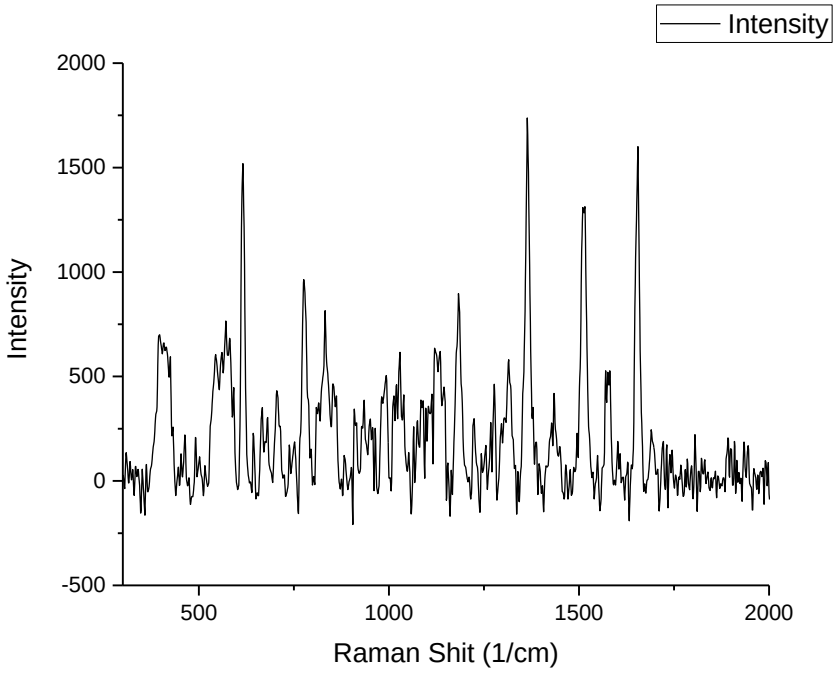
Fig.15. Raman spectra of all the dyes separated from the mixture and comparing them with the reference spectra of pure dyes

a) **R6G** (Characteristic Peak is present at 616cm^{-1})

Drop-casted R6G (integrated Area = 20020.29061)

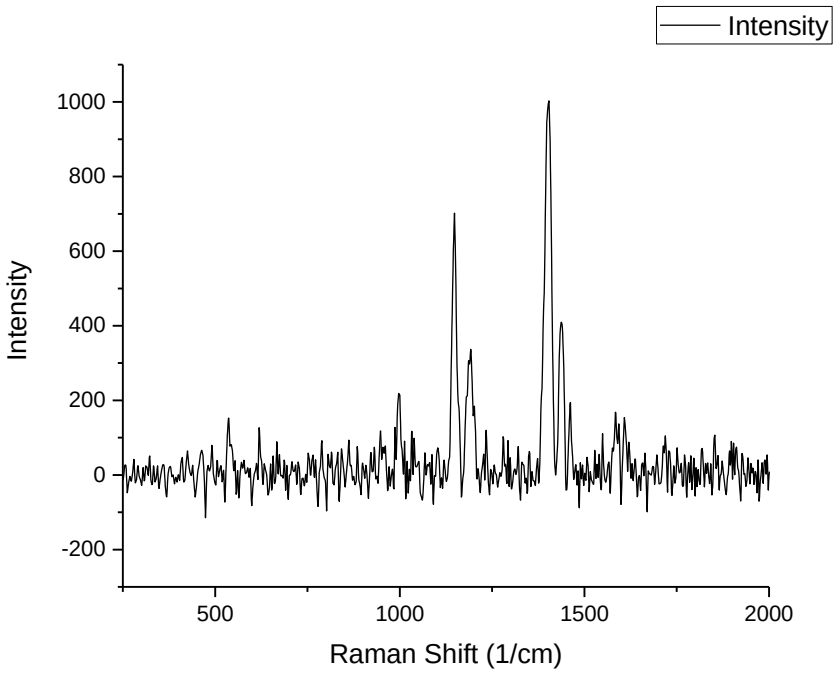


Transferred R6G (Integrated Area = 17218.82862)

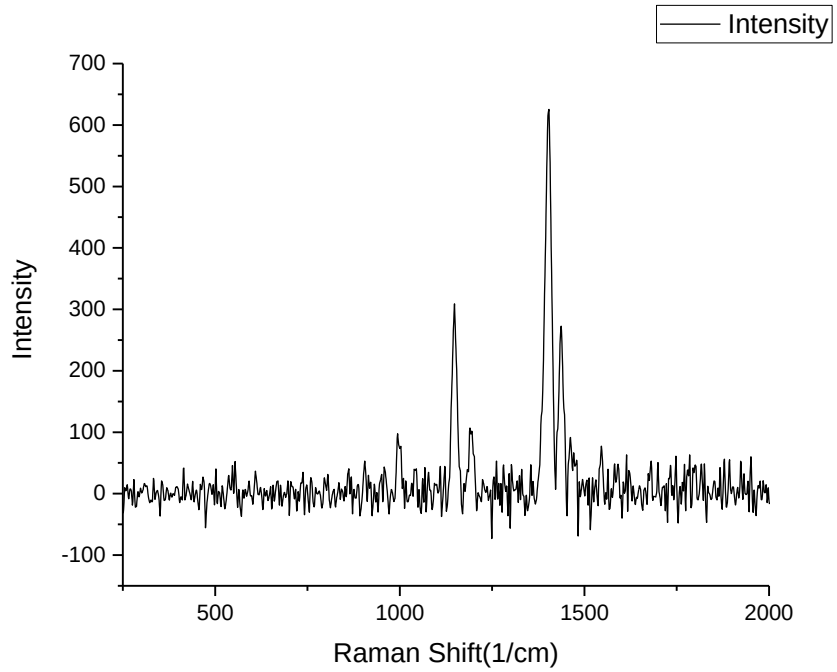


b) MY (Characteristic peaks in the SERS Spectra: 1193 cm^{-1} & 1437 cm^{-1} (ν(N=N)), 1147 cm^{-1} (ν(C-Nazo) stretching) ,1406 cm^{-1} (S=O stretching))

Drop-casted MY (Integrated area = 10697.73801)

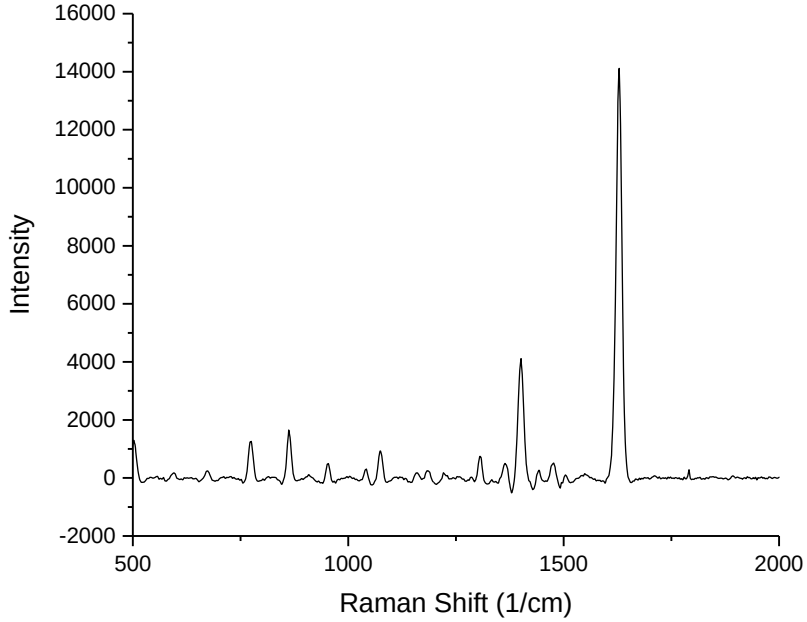


Transferred MY (Integrated area = 8225.4329)

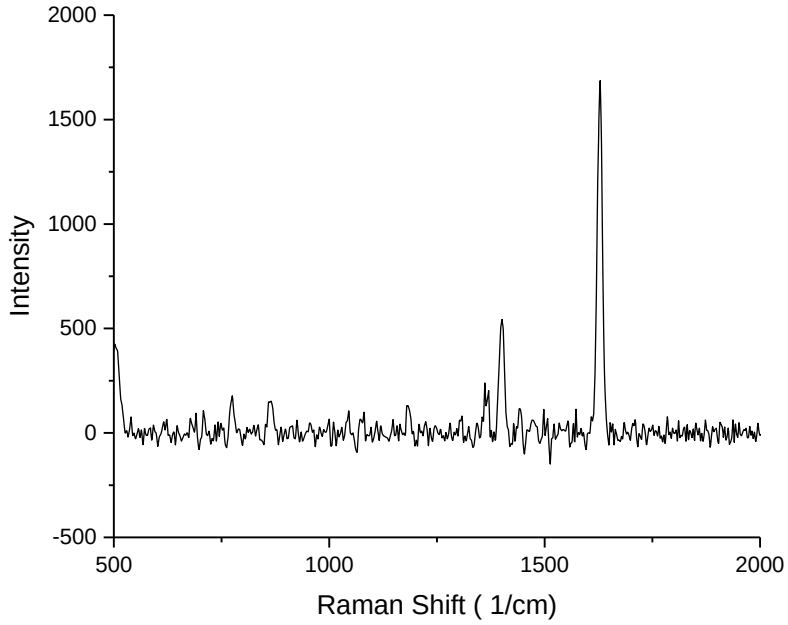


c) MB (Characteristic Peak is present at at around 1621, 1385 cm^{-1})

Drop-casted MB (Integrated area = 33374.7813)



Transferred MB (Integrated area = 29552.30355)



As we can we are able to identify the transferred dye using their characteristic peaks on the spectrum. We can also see that there was a decrease in intensity between the drop-casted dye spectrum and then transferred dye spectrum indicating that all of the dye was not transferred onto the substrate and some of it has spread over the chromatography paper.

Summary and conclusion:

Use of adulterants to make fruits and vegetable is a major cause of concern due to its effects on human body. The current methods of detecting adulterants is time consuming and complex. As a result, taking immediate preventative measure to prevent the sales of adulterated food into the market is difficult.

The use of inkjet printed SERS substrate on Kimwipe tissue paper and portable Raman spectrometer to detect dyes shows promising results and is economical. With little changes (like the solvent used) we can use this same process to identify and analyse traces of pesticides present on fruits and vegetables.

Pesticides are usually mixed with a surfactant and a Pesticides are mixed with surfactants and Preservatives before applying them on crops and fruits. So, if the active ingredient is not separated from the additives the spectra, we get could be inconclusive. Hence separation process is very important when we need to detect pesticides.

Future Studies:

While the separation and detection process yield a positive result, there are certain difficulties encountered for which we need to come up with a method of overcoming them.

One of the major issues that we encountered is the separation of the dye mixture of lower concentration. Pesticides and adulterants found in food items are usually present at very low concentrations.

Certain adulterants and pesticides do not emit any colour and are essentially invisible to the naked eye once dropped on to the chromatography paper for separation. Methods to detect such pesticides needs to be found and should be used to optimize the separation process. Using iodine (iodine reacts with most organic compounds to produce colour) or UV light to detect invisible pesticides are the current procedures that are being studied upon.

References:

1. Wei W. Yu and Ian M. White , **Inkjet Printed Surface Enhanced Raman Spectroscopy Array on Cellulose Paper** , Anal. Chem. 2010, 82, 9626-9630

2. Li Zhang , Bin Wang , Guang Zhu , Xia Zhou , **Synthesis of silver nanowires as a SERS substrate for the detection of pesticide thiram**, L. Zhang et al. / Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 133 (2014) 411–416

3. D. Fernand, D. Berg´e-Lefranc, C. Pardanaud, T.N.T. Phan, A. Merlen,and V. Hornebecq , **Detection of Rhodamine 6G at low concentrations using Raman Spectroscopy : A comparison between Ag and Au-based nanoporous substrates,** Eur. Phys. J. Special Topics 224, 2001–2010

4. Pushkaraj Joshi and Venugopal Santhanam **, Paper-based SERS active substrates on demand ,** RSC Adv., 2016, 6, 68545–68552

5. Irina Geiman,B.S.; Marco Leona, Ph.D.; and John R. Lombardi,Ph.D. **Application of Raman Spectroscopy and Surface-Enhanced Raman Scattering to the Analysis of Synthetic Dyes Found in Ballpoint Pen Inks**

6. Eric P. Hoppmann, Wei W. Yu and Ian M. White,**Highly sensitive and flexible inkjet printed SERS sensors on paper,** 17 July 2013

7. Dmitry Kurouski, and Richard P. Van Duyne,**In situ Detection and Identification of Hair Dyes Using Surface-Enhanced Raman Spectroscopy (SERS)**, February 6, 2015

8. Kneipp, K. et al. **Single molecule detection using surface-enhanced Raman scattering (SERS),** Physical Review Letters 78, 1667–1670 (1997)

9. Tong Zhang, Yuan-Jun Song, Xiao-Yang Zhang and Jing-Yuan Wu, **Synthesis of Silver Nanostructures by Multistep Methods**, Sensors 2014, 14, 5860-5889

10. Bo Li, Shengrong Ye, Ian E. Stewart,Samuel Alvarez, and Benjamin J. Wiley,

11. **Synthesis and Purification of Silver Nanowires To Make Conducting Films with a Transmittance of 99%,** American Chemical Society,2015

12. Jordan F. Betz,ab Wei W. Yu,ab Yi Cheng,bc Ian M. Whiteab and Gary W. Rubloff, **Simple SERS substrates: powerful, portable,and full of potential**, Phys. Chem. Chem. Phys., 2014, 16, 2224-2239

13. L. Polavarapu and L. M. Liz-Marzán, "**Towards low-cost flexible substrates for nanoplasmonic sensing**," *Phys. Chem. Chem. Phys.*, vol. 15, no. 15, p. 5288, Mar. 2013.

14. W. Sers, "**P - SERS Technology Technical Paper P-SERS— Trace detection overcoming the cost and usability limitations of traditional SERS technology**," pp. 1–8, 2015.

15. S. K. Parmar and V. Santhanam, "**In situ formation of silver nanowire networks on paper**," *Current Science*, vol. 107. Current Science Association, pp. 262–269.

16. P. Joshi and V. Santhanam, "**Paper-based SERS active substrates on demand,**" *RSC Adv.*, vol. 6, no. 72, pp. 68545–68552, Jul. 2016.

17. S. Dhakal, K. Chao, W. Schmidt, J. Qin, M. Kim, D. Chan, Evaluation of Turmeric Powder Adulterated with Metanil Yellow Using FT-Raman and FT-IR Spectroscopy., Foods (Basel, Switzerland). 5 (2016). doi:10.3390/foods5020036

18. B. V Sudheer, M.K. Lakshmidevi, D. V Krishna Rao, Adulteration of the pulses in coastal region of Andhra Pradesh, J Evol. Med Dent Sci J. Evol. Med. Dent. Sci. 4 (2015) 6187–6192. doi:10.14260/jemds/2015/902