

INTERNSHIP REPORT

A project on

Fabrication of channels for Paper Chromatography

carried out at

Indian Institute of Science



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Introduction:

Chromatography is an analytical technique commonly used for separating a mixture of chemical substances into its individual components. There are many types of chromatography techniques e.g., liquid chromatography, gas chromatography, paper chromatography and so on. The emphasis is on paper chromatography in this project.

Paper chromatography is a chromatography technique used to separate mixture of chemical substances into its individual components. Paper chromatography consists of two phases, a mobile phase and a stationary phase. The stationary phase which is also known as substrate is the paper which is used for chromatography. The paper used for chromatography is composed of pure cellulose, while many other papers contain structure-reinforcing-additives that are potentially detrimental in analytical assays. The mobile phase is solvent.

In this project, the analysis of active molecules such as R6G (Rhodamine 6G), methylene blue, malachite green has been carried out. The solvent used is ethanol. The procedure of paper chromatography followed throughout the project is as follows:

- i. A thin strip of the chromatography paper about 1cm wide and 3cm long was cut.
- ii. A drop of solution (3 μ l) was pipetted on the paper.
- iii. The paper was then dipped in the solvent (well above the drop).
- iv. Enough time was provided so as to achieve maximum rise of the active molecules.

The paper was cut with a triangular shape at the bottom (about 1 cm in height) so as to fasten the wicking of the molecules. The sample of the paper used for chromatography is as shown in the figure.



Figure 1: Sample of the chromatography paper used

Objectives:

The objectives of the project were as follows:

1. To fabricate channels to facilitate the movement of active molecules.
2. To optimize the width of the channel to achieve least amount of time required for the molecules to reach the maximum height.
3. To optimize the height of rise of the molecules.
4. To minimize the dispersion of molecules away from the channel.
5. To optimize the method of fabricating the channels by considering the environment, cost, availability of resources, ease of fabrication and other aspects.
6. To optimize the time of exposure, temperature of exposure of the substrate.

Literature Survey:

A number of ways have been adapted to fabricate channels. Some of the ways which were considered for the fabrication of channels are as follows:

1. Wax printing:

Wax printing is one of the most widely used method used for fabrication of hydrophobic layers on paper. In this method, wax is printed on the paper using a wax printer. The process uses wax printing to form hydrophobic barriers (wax) on hydrophilic substrates (filter paper). The barriers segment the paper and form channels. The wax printing technique requires two steps:

- i. Print wax on the surface of the paper
This step requires a wax printer in which molten wax is deposited on the paper and automatically cooled to prevent spreading.
- ii. Spread the wax in an oven or on a hot plate
This method requires an oven or a hot plate which can help the wax to penetrate into the depth of the paper

This is a simple yet efficient way of making the surface of the paper hydrophobic. This process requires less than 5 minutes. Channels as thin as 300 μm can be achieved by this method.

This method was however not adapted in the project due to the high cost of wax printer and also due to the non-availability of wax printers.

2. Printing of toluene on the paper

In this approach, the paper substrate is first converted into an entirely hydrophobic material by soaking it into a polystyrene solution (1.0 wt% in toluene) and drying it at room temperature for 2h. The hydrophobic paper is then exposed to the inkjet printing of picoliter volumes of toluene, thereby leading to a channel width of about 550 μm . However, the toluene used as the ink was considered a disadvantage to the user and

environment, because of its classification as a flammable and volatile organic compound (VOC). Hence, this method was not adapted.

3. Use of wax crayons

In this method, wax crayons are colored on the paper in a suitable manner so as to make a channel. The colored region of the paper is then heated to allow penetration of the wax into the paper. This method can lead to a channel width of about 1mm depending upon the person who is coloring the paper. A number of ways can be used to heat the wax, such as hair dryer (blower), hot plate, oven.

This method was adapted in the project due to its simplicity and low cost. A number of optimizations can be done in this method which will be discussed in the following sections.

4. Inkjet printing of Tween 20 solution:

When paper was dipped in pure tween 20 solution and dried, it was found that water did not spread on the paper. Using this as basis, inkjet printing of dilute solutions of tween 20 was carried out which will be discussed in the following sections.

Fabrication of hydrophobic surface using wax crayons:

As discussed earlier, in this method, a number of parameters could be changed to fabricate hydrophobic surface on paper. The parameters that were checked for optimization are as follows:

1. Quantity of wax deposited on the paper.
2. Method of heating the wax.
3. Temperature of heating.
4. Time of exposure to high temperatures.
5. Width of the channel.

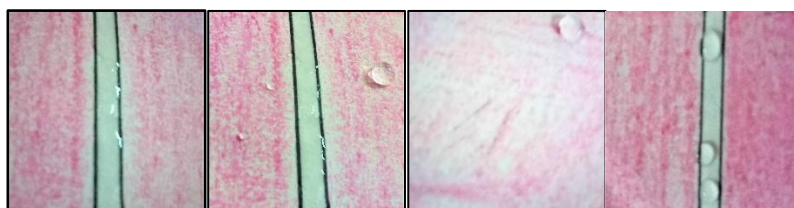


Figure 2: From left to right: i) An ideal case of a hydrophilic channel ii) Hydrophilic channel with hydrophobic surrounding iii) Hydrophobic back iv) A non-ideal case of hydrophobic channel

As shown in the figures above, it was desired to obtain a hydrophilic channel with a hydrophobic surrounding. The front and the back surface of the paper was desired to be hydrophobic except the channel. Hence, all the optimization was carried out based on these objectives.

The dependency of the parameters stated above for creating a hydrophobic surface is as follows.

1) Quantity of wax deposited on the paper:

The quantity of the wax deposited on the paper plays a crucial role in the decision of other parameters. The equipment used for heating wax to decide the optimum quantity of wax to be deposited on the paper was a hair dryer. Hair dryer was used as it is easy to vary the time of exposure keeping the temperature constant. It is also an easy and a cost-effective method to heat wax. The following are the observations based on the number of layers of wax crayon on the paper:

Number of layers	Time required to make channel hydrophobic (min)
1	23
2	16
3	6
4	5

The number of layers were same on both the sides of the paper. The paper was completely colored using wax crayons (except the channel), this corresponds to one layer. The second layer was similarly colored after the first and so on. From the table above, it can be seen that there is not much difference in the time for channel to become hydrophobic between 3 and 4 layers. Hence, 3 layers was chosen as the optimum quantity of wax to be deposited on the paper. The number of layers in all the following sections will be three.

2) Method of heating the wax:

The various methods of heating the wax in this project are:

- i) Hair dryer: The hair dryer was used as an equipment to blow hot air. The temperature of the air blown by the hair dryer was about 55 °C. Due to the low temperature that can be attained by a hair dryer, the time of exposure was observed to be large. As seen earlier, it took 6 minutes for the channel to become hydrophobic for a three-layered paper. The hair dryer was only used to in the earlier stages of the project and not throughout.
- ii) Hot plate: A hot plate facilitates direct and uniform heating of the paper as compared to a hair dryer. It was necessary to be able to provide higher temperatures for the melting of wax to reduce the time of exposure. The channel was found to be hydrophobic in a minute by heating the paper at 70 °C. The time of exposure reduced drastically when moved from hair dryer to hot plate. However, hot plate was not used throughout the project as the paper sticks to the plate. The plate was covered with Aluminium foil to overcome this problem. The paper however stuck on the Aluminium foil. This was attributed to the direct contact to the heating surface. Hence, hot plate could not be used throughout.
- iii) Oven: An oven was used which served as an alternative to hot plate. Hot oven could overcome the difficulties and disadvantages discussed earlier and also

reduced the time of exposure as the external factors which hindered the heating of wax were now absent. The oven took 30 seconds for the channel to become hydrophobic for three layers at 70 °C. Hence, the oven was used as a heating equipment throughout the project.

3) Temperature of heating

Temperature of heating plays a vital role in the heating of wax. The temperature of heating affects the time of exposure. When the temperature of heating is high, the time of exposure is low. This was also found from the experiments conducted. The experiment was conducted for a paper colored with three layers of wax. The observations are as follows:

Temperature of heating	Time of exposure
40 °C	330 s
60 °C	120 s
70 °C	30 s

It can be concluded from the observations that as the temperature of heating increases the time of exposure reduces. 70 °C was used as the optimum temperature for heating wax as the time of exposure was less for this temperature.

4) Time of exposure to high temperature:

Time of exposure to high temperature is the deciding factor for penetration of wax into the depth of the paper. The experiments were conducted so as to minimize the time of exposure. Less time of exposure is favored so that the wax can be penetrated into the paper faster. As seen in the sections above. An oven was chosen as a heating equipment for heating of wax to minimize the time of exposure. Higher temperatures are chosen so as to reduce the time of exposure.

The time of exposure to high temperatures is inversely proportional to the temperature of heating i.e., as the temperature of heating increases, the time of exposure reduces.

5) Width of channel:

Width of channel is similar to quantity of wax. A paper 1 cm wide and 3 cm long was used for this set of experiments. It was found that with 4 minutes of heating in the oven at 70 °C, the lateral penetration was 0.5 mm on both sides of the channel. Whereas, as the channel width was reduced, the lateral penetration increased as the quantity of wax available for spreading was increased. It was found that for a channel width of 3 mm, the lateral penetration of the wax was 0.5 mm on either side of the channel. When the channel width was 2 mm, the channel would become hydrophobic due to uneven lateral penetration. A channel width of 1 mm blocked the channel. Hence, an optimum channel width of 3 mm was used throughout the project.

Inkjet printing of Tween 20 solution

Tween 20 is a polysorbate-type nonionic surfactant formed by the ethoxylation of sorbitan before the addition of lauric acid. Its stability and relative nontoxicity allows it to be used as a detergent and emulsifier in a number of domestic, scientific, and pharmacological applications. As the name implies, the ethoxylation process leaves the molecule with 20 repeat units of polyethylene glycol; in practice these are distributed across 4 different chains leading to a commercial product containing a range of chemical species.

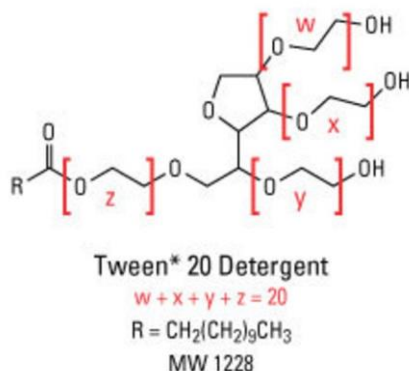


Figure 3: Structure of Tween 20

It was found that drop casting of Tween 20 on paper and heating it for 20 minutes at 70 °C made the paper hydrophobic. This led to the thought of printing Tween 20 solution on paper and forming a well-defined channel as it was found that there was no lateral spreading in the case of Tween 20.

Preparation of Tween 20 solution:

Three different solutions of 10 %, 20%, 30% and 40% wt./vol compositions were prepared for testing the inkjet procedure for fabrication of channels.

The procedure followed for preparation of these solutions are as follows:

- i) Tween 20 is a liquid with 1228 g/gmol and density of 1095 kg/m³.
- ii) The solution prepared was of 10 ml volume. Hence, 1, 2, 3 and 4 grams of Tween 20 was measured for 10%, 20%, 30% and 40% wt./vol solutions respectively.
- iii) The solution was thoroughly mixed.
- iv) The solution was heated for 15 minutes at 60 °C.
- v) The solution was let aside to attain room temperature and the solution thus formed was used as an ink for inkjet printing.

Procedure for Inkjet printing of Tween 20 solution:

The following steps were followed for printing of the solutions:

- i) A black ink cartridge was washed with tap water and then washed with DI water.

- ii) The cartridge was then sonicated for a period of 20 mins.
- iii) A number of test prints were carried out using DI water as ink to empty any traces of ink present in the cartridge.
- iv) 200 μ l of Tween 20 solution used as ink was filled in the cartridge and a number of test prints were carried out so as to ensure the solution is uniformly spread in the cartridge.
- v) Six 2cm wide and 3 cm long rectangular shapes were printed on a paper.
- vi) The paper once printed was to dry under a fan for 10 minutes.
- vii) The cartridge was removed from the printer, wiped with a Kimwipe paper dipped in iso-propanol after every print.
- viii) 50 μ l of solution was refilled after every print.
- ix) The procedure was repeated for 10 times to get ten layers of Tween 20 on the paper.

The thus dried paper was then heated in an oven for 20 minutes at 70 °C to evaporate the solvent.

However, the paper did not show any hydrophobicity. Several reasons were expected to hinder the formation of a hydrophobic layer. They are:

- a) Lack of penetration of the solution into the depth of the paper: Printing is a surface phenomenon. Thus, the Tween 20 solution could not have penetrated to the depth of the paper.
- b) Improper evaporation of the solvent: Since the paper was heated at 70 °C, the solvent, i.e., water in this case, might not have completely evaporated which could be one of the reasons the hydrophobic layer was not formed.

Hence, the inkjet printing of Tween 20 solutions could not be used as a method to create hydrophobic layer. However, pure Tween 20 could not be printed due to its high viscosity. Drop casting pure Tween 20 was not a uniform technique that could be continued due to uncertain amounts of the chemical to be dropped on the paper and it also was a costlier process. Hence, considering these disadvantages, inkjet printing of Tween 20 was not continued for creating a hydrophobic layer on paper in this project.

Paper Chromatography:

Since it is already known that these channels are being formulated as a substrate for paper chromatography, it is important to know the advantages of creating a hydrophobic surface around the channel.

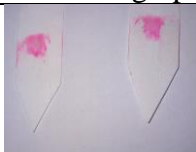



In paper chromatography, the sample mixture is applied to a piece of filter paper, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by capillary action. Components of the mixture are carried along with the solvent up the paper to varying degrees, depending on the compound's preference to be adsorbed onto the paper versus being carried along with the solvent.

It was desired to achieve maximum rise of the sample i.e., the height of rise of active molecules. The active molecules in this case was pesticide swabbed from food products like vegetables, dal, rice etc., Swabbing of food products meant there would be very less amount of sample available for analysis. When these swabbed sample was analyzed using paper chromatography, there were chances that these molecules would get penetrated into the depth of the paper. This was undesirable and also unnecessary as the molecules were analyzed by Surface Enhanced Raman Spectroscopy (SERS) which required the molecules to be concentrated on the surface of the paper. Hence, by making the surface around the channel hydrophobic it could be ensured that the molecules would not spread across the paper and could be concentrated in a confined area of interest. This way, the rise of the molecules could also be increased and the sample could also be concentrated in a small area.







Along with reducing the area of spread of the molecules, it was necessary to increase the rise of the sample.

By paper chromatography of several dyes like Rhodamine 6G, methylene yellow and malachite green using ethanol as solvent, it was found that the color of the wax crayons was soluble in ethanol. Hence, the project was continued using candle wax as a substitute for wax. It was also found that heating of the paper after covering both the front and backside of the paper was disadvantageous since over heating of wax at the back of the paper resulted in a hydrophobic channel. Hence, it was desired to cover the paper with wax at the front of the paper, heat it and then cover and heat the wax at the back of the paper. This ensured longer heating time of wax in the front of the paper and lower heating time in the back.

A number of heating time combinations for the front and back side of the paper was carried out which yielded the following results:

Front side heating time	Backside heating time	Chromatography result
30 seconds	15 seconds	
30 seconds	30 seconds	
1 minute	15 seconds	
1 minute	30 seconds	

Fabrication of channels for Paper Chromatography

2 minutes	15 seconds		
2 minutes	30 seconds		
3 minutes	15 seconds		
3 minutes	30 seconds		
4 minutes	15 seconds		
4 minutes	30 seconds		

From the above table, it can be observed that heating the back side of the paper for 15 seconds yielded better results than heating it for 30 seconds. This is because of greater penetration of wax into the depth of the paper that it reaches the front side and makes the channel hydrophobic. Hence, all the combinations with heating time of 30 seconds on the back side of the paper were eliminated.

All the channels in this experiment were of 3 mm width. Heating time greater than 1 minute resulted in lateral penetration of the wax and blocked the channel due to which the sample was left as a drop on the paper. The chromatography of these samples leads to uneven rise. Hence, all the combinations with heating time greater than 1 minute were eliminated.

The two combinations that could be continued further were samples which were heated for 30 seconds and 1 minute on the front side and for 15 seconds on the back side. Out of these two samples, the sample which was heated for 30 seconds had a rise of 1.8 cm and the sample which was heated for 1 minute had a rise of 1.3 cm.

All the samples were dipped in the solvent for 30 minutes in a constant volume of solvent (1 ml).

From the results of this experiment, the optimum combination of time of heating wax was concluded to be 30 seconds on the front side and 15 seconds at the back side.

After optimization of time combinations, it was required to estimate the height of rise of the molecules. For this, R6G was used as the dye and ethanol was used as a solvent. The shape of the chromatography paper used is as shown in the figure.



Figure 4: Shape of the chromatography paper used

The above figure shows the shape of the chromatography paper used in the experiment. the outline of the chromatography paper is inkjet printed silver nanowire, the procedure of which will be discussed in the following section. It was desirable for the molecules to reach the tip of the paper to help in the analysis of molecules using Surface Enhanced Raman Spectroscopy (SERS). However, the molecules did not reach the tip of the paper since the paper was 3 cm long. Hence, a number of samples were tested to estimate the height of the rise of molecules. Six samples were tested out of which the height of rise of the molecules were 2, 1.8, 1.8, 1.6, 1.8, 1.8 cm. 1.8 cm being the most repeated result was used as the optimum height of rise of the molecules.

The shape of the paper was kept so as to reduce the surface area per unit volume towards the starting and the end of the chromatography. This way, the wicking is faster and the molecules approach the tip faster.

Since the height of the rise was estimated, the next step was to formulate SERS active substrate.

Paper based SERS active substrate:

SERS is a label-free, point-of-use spectroscopic technique capable of exhibiting high selectivity and sensitivity even at single molecule detection levels. The surface enhancement comprises an electromagnetic effect and a charge-transfer effect.

Fabrication of SERS substrates includes a number of steps which are as follows:

Preparation of D-76 developer:

- i) For preparation of 100 ml D-76 developer solution, 276 mg of metol, 667 mg of hydroquinone, 267 mg of Borax salt and 13.3 g of anhydrous sodium sulphite were measured.
- ii) These chemicals were transferred into a conical flask and it was diluted with 100 ml of DI water.
- iii) It was stirred thoroughly to form a clear solution.
- iv) This solution was sonicated for 20 minutes.

Preparation of KX solution:

2 M solution of KX was prepared. KX contains 95% by weight KBr and 5% by weight of KI. KI is added to increase the photosensitivity of the solution. The molecular weight of KX was found out by mixture rule and was calculated to be 121.35204 g/gmol.

- i) The weight of the chemical required for 100 ml of 2 M solution was calculated to be 24.27 using the molecular weight calculated by mixture rule.
- ii) Out of 24.27 g, 23.0565 g of KBr (95% of 24.27g) and 1.2135 g of KI (5% of 24.27g) were measured and transferred into a conical flask.
- iii) The solution was then diluted using DI water to make 100 ml solution.
- iv) The solution was then mixed thoroughly to make it a clear solution.
- v) The solution was then sonicated for 20 minutes.
- vi) The sonicated solution was passed through a 0.22 μm pore size filter.
- vii) It was then sonicated again for 20 minutes.

Preparation of AgNO_3 solution:

- i) 20 ml solution of 1M AgNO_3 was prepared using 3.3966 g of AgNO_3 (molecular weight = 169.87 g/gmol)
- ii) The solution was mixed thoroughly.
- iii) The container was covered with an aluminium foil and sonicated for 20 min.
- iv) The solution was passed through a 0.22 μm pore size filter.
- v) The solution was sonicated for 20 min.

2:1 molarity of KX: AgNO_3 was used so as to ensure complete reduction of silver. The KX and AgNO_3 thus prepared were used as ink for inkjet printing of the substrates. The paper used for printing was chromatography paper. The shapes printed are as follows:



Figure 5: Shapes printed

Inkjet printing of SERS active substrate:

- i) Two black 803 cartridges were used for printing.
- ii) The cartridges were opened by making a hole. The sponge containing the black ink was removed.
- iii) The cartridges were washed thoroughly first with tap water and then with DI water.
- iv) The cartridges were sonicated for a period of 20 minutes.
- v) They were then filled with DI water and test prints were carried out until all the black ink was washed out.
- vi) The two cartridges were labelled KX and AgNO₃.
- vii) 200 µl of the solution was filled in the respective cartridges.
- viii) Test prints were carried out to ensure uniform filling of the solution in the pores.
- ix) For the printing of the substrate, two layers of KX was printed first so that it could penetrate into the pores and silver would be on the surface.
- x) After two layers of KX, AgNO₃ was printed.
- xi) Alternative layers of KX and AgNO₃ were printed.
- xii) After printing every layer, the paper was dried under a fan for 8-10 minutes.
- xiii) The cartridge was wiped with a KimWipe tissue dipped in Iso-propyl alcohol after every print.
- xiv) Separate KimWipe tissues were used for the two cartridges.
- xv) The cartridge was filled with 50 µl of the solution when the solution in the cartridge was empty.
- xvi) After printing, the cartridges were washed thoroughly, sonicated, filled with DI water and kept aside.

After printing, the paper was exposed to halogen light for 20 minutes which was kept 15 cm away from the source. The halogen exposure generates latent silver clusters within silver halide film. The paper was dipped in the developer solution for a period of 20 minutes, this was done so that the silver clusters formed after halogen exposure can be developed into nanowires. The paper was then rinsed in DI water and then let to dry.

After drying, the printed area was checked for conductivity. The resistance on the printed area was found to be 11.5 Ω.

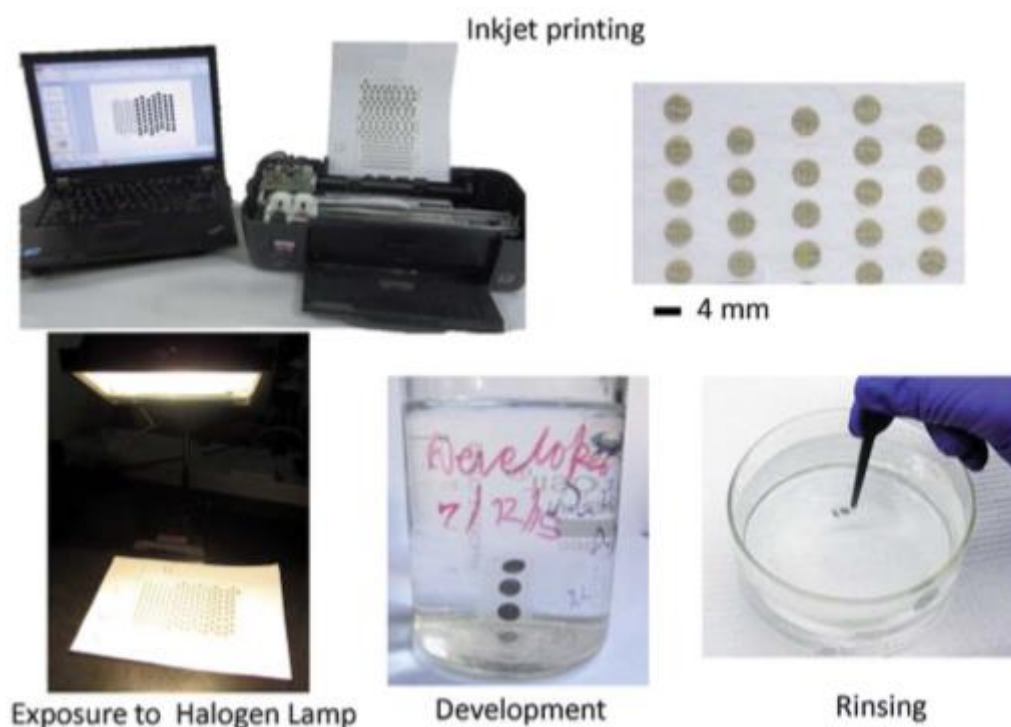


Figure 6: Representative photographs illustrating the sequence used for fabrication of SERS active substrate

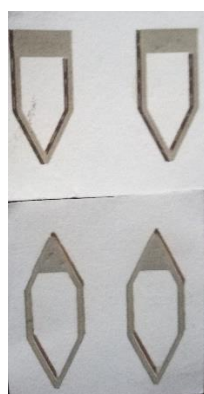


Figure 7: Sample of SERS active substrate formulated

Chromatography on SERS active substrate:

Chromatography was carried out on the SERS active substrate to make sure the molecules attach on the SERS active region (silver printed area). R6G was the molecules that were to be analyzed which acted as the SERS probe. The silver printed paper was the SERS substrate. Once the molecules reached the tip of the paper and adsorbed on the silver area, the paper was let to dry. The dried paper was to be analyzed, the steps of which will be discussed below.

SERS analysis:

After formulation of SERS active substrate, chromatography was carried out on the paper and made sure that the molecules reached the tip of the paper. The paper was then dried.

Aluminium foil was stuck on a glass slide on which the sample to be analysed was stuck as follows:



Figure 8: SERS sample analyzed

The sample was then analyzed under a Surface Enhanced Raman Spectrometer. The spectrometer uses a laser light of 532 nm. The integration was set to 1 second with a laser power of 2 which constitutes to 0.66 μW . The spectrometer was under rast mode which covers a larger area than a normal laser dot. Analysis of the waxed area without the molecules, with molecules and on the edge were conducted. The background effects were eliminated to get peaks constituting to change in bond shape due to absorption of energy from laser source and excitation of molecules.

The results obtained from the spectrometer is as follows:

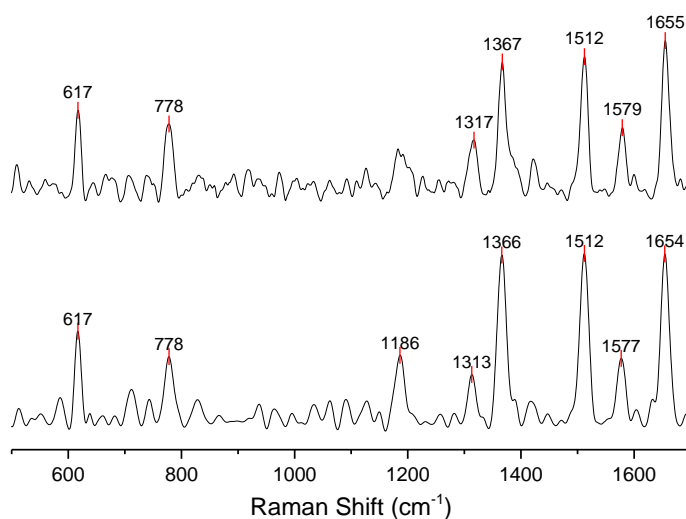


Figure 7: Raman shift of R6G using a flat tip

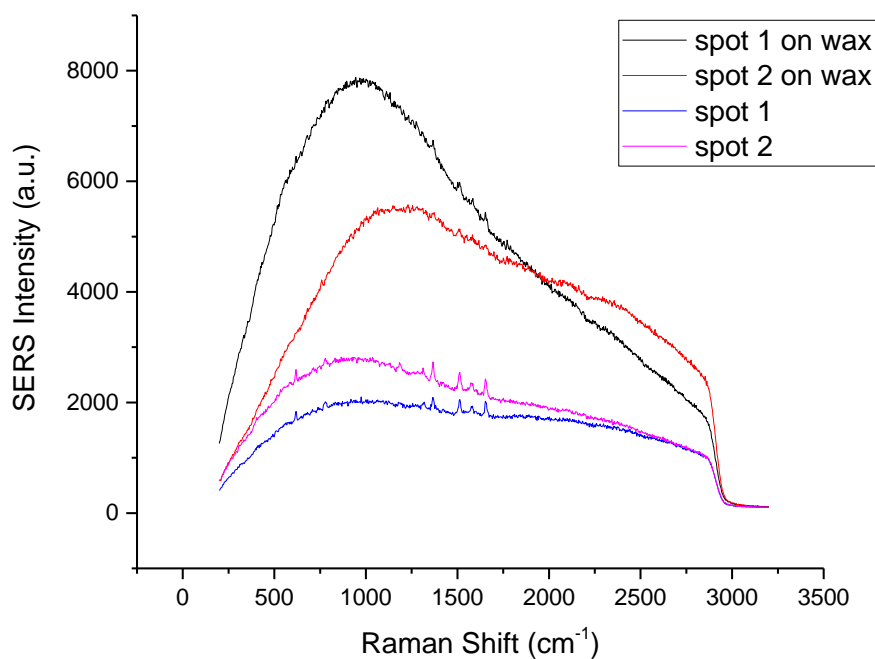


Figure 8: Graph of SERS intensity v/s Raman Shift using a flat tip

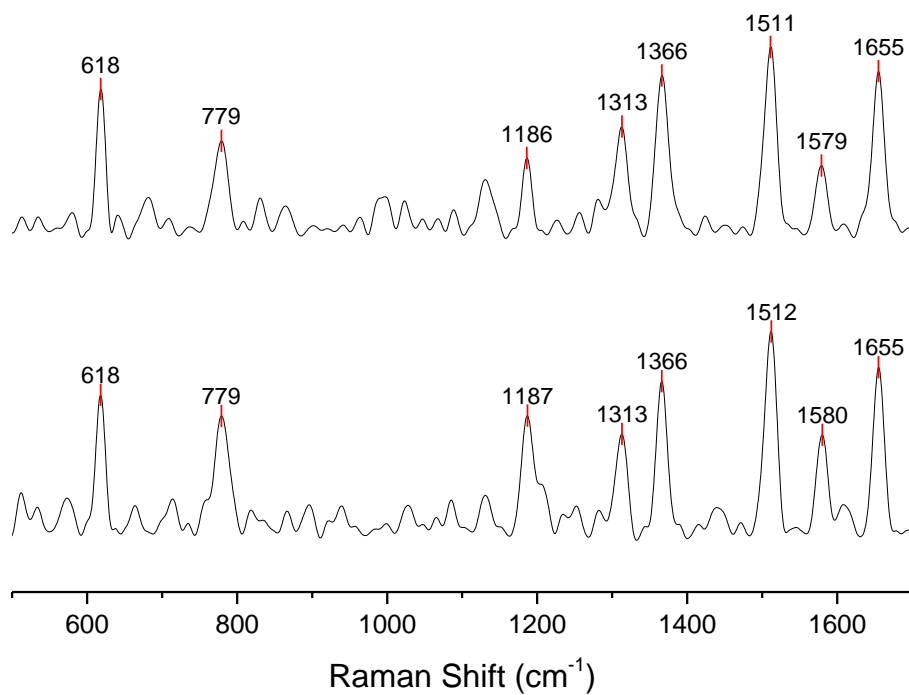


Figure 9: Raman Shift of R6G using pointed tip

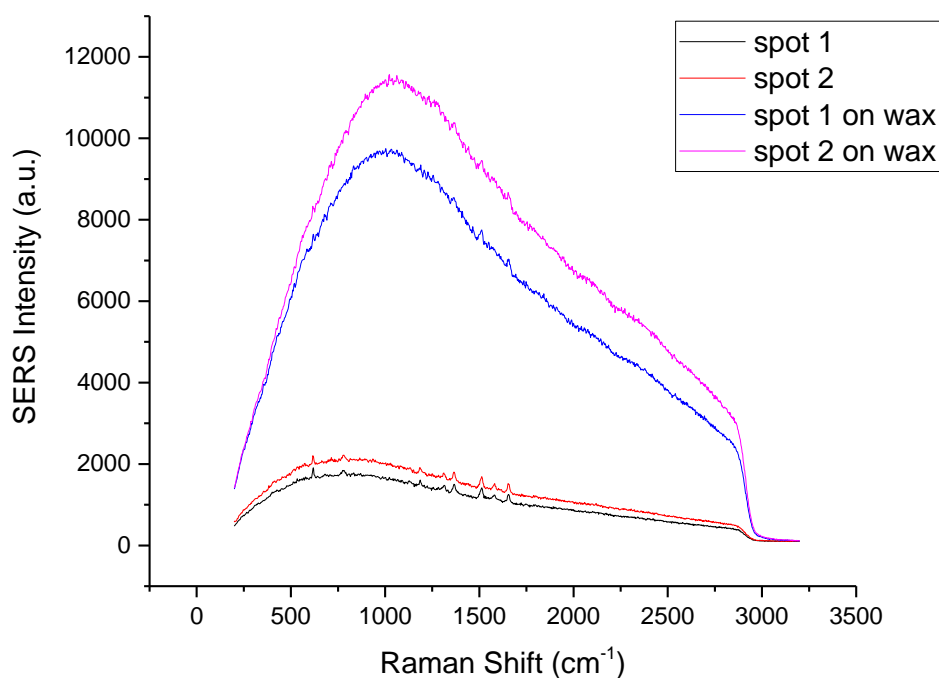


Figure 10: Graph of SERS intensity v/s Raman Shift using a pointed tip

Results and Conclusion

It was found that waxing the paper caused higher rise in paper chromatography. Separation of molecules can also be achieved by paper chromatography using suitable solvents. Oven was concluded to be the best way of heating the wax. Different combinations of time of exposure of the waxed paper were analyzed. SERS active substrates were printed on the paper. The height of rise of R6G molecules were optimized by different methods. The height of rise of R6G molecules on plain chromatography paper was found to be 0.6 cm using ethanol as solvent and after optimization using all the parameters discussed was found to be 1.8 cm. Optimum channel width of 3 mm was considered throughout.

It can be concluded that paper chromatography is one of the easiest and cost-effective method for separating molecules. The paper and the solvent can be optimized to rise the active molecule to the SERS active site to enable SERS analysis which can be used to detect pesticides in food products.