CHAPTER I: INTRODUCTION

Ink is a liquid or paste that contains pigments or dyes and is used to color a surface to produce an image, text, or design. Ink is used for drawing or writing with a pen, brush, or quil. ink can be a complex medium, composed of solvents, pigments, dyes, resins, lubricants, solubilizers, surfactants, particulate matter, fluorescents, and other materials.

Logwood inks manufactured from extracts of the haematoxylon campecheartzim tree mixed with crystallized sodium carbonate and potassium chromate. The pieces of wood are purchased as small chips and they are steeped in boiled water to extract the dye. The ink dries easily into a purplish black who becomes a concentrated black on the surface and in the paper. It can be washed but when exposed to air it decomposes into small flakes.

Aniline Black Ink is containing aniline dye is used when permanence factor of ink is not important. It is used in stenciling and ticket writing.

Fountain Pen Ink is on capillary action. Fountain pen ink mostly water but contain several additives to give certain color, flow and characteristics and are prone to get washed by water

Ballpoint Ink Ballpoint pen was developed in Europe about 1939 and was initially distributed in Argentina about 1943. Ball point ink consists of synthetic dyes (sometimes carbon or graphite is also added for permanence) in various glycol solvents or benzyl alcohol. These ingredients consist of fatty acids, resins, surface active agents corrosion control ingredients and viscosity adjustors. The fatty acid (oleic is the most common) act as lubricants to the ball of the pen and they also help the starting characteristics of the ball point.

Invisible Ink is the type of ink which is referred to as secret ink it can be made from common food stuffs, for instance the British used rice starch to manufacture Invisible ink during the Indian mutiny in 1857.The most common of the secret ink is

one made from cobalt chloride when diluted before writing the ink dries to a very pale shade of pink, but once the ink   warmed ,it becomes a clear and distinct blue. When phenolphthalein is added to the ink it remains invisible unit it is exposed to ammonia vapours .[8]

Thin-layer chromatography is a chromatography technique used to separate non-volatile mixtures. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide, or cellulose. Chromatography works on the principle that different compounds will have different solubilities and adsorption to the two phases between which they are to be partitioned. Thin Layer Chromatography (TLC) is a solid-liquid technique in which the two phases are a solid (stationary phase) and a liquid (moving phase).[9]

The forensic significance of Ink are Detection of particular compound present in mixture, Establishing that two compounds from a different origin are the same, Determining the number compounds present in a mixture, Choosing the appropriate solvent for column chromatography to separate compounds, Monitoring reactions.

Ink analysis may be an important part of the investigation of questioned documents, including forged checks, wills, or altered records. Although all blue or black inks may look the same, there can be some important differences in their chemical composition.

The first widespread application of partition chromatography on a planar surface was paper chromatography, introduced n the 1940s. However, paper chromatography was gradually replaced by thin layer chromatography (TLC), which has one of the most routinely used chromatography techniques (Ettre and Kal'asz, 2001). TLC is also a liquid-solid adsorption technique where the mobile phase ascends the thin layer of stationary phase coated onto a backing support plate.

Like all forms of chromatography, TLC involves a dynamic and rapid equilibrium of molecules between the two phases (mobile phase and stationary phase). However, TLC differs from all other chromatographic techniques in the fact that a gas

phase is present, which can influence the results of separation significantly. Between the components of the mobile phase and its vapour, equilibrium will be established gradually (also called chamber saturation). The part of the stationary layer that is already wetted with mobile phase also contributes to the formation of the equilibrium.

During development, molecules are continuously moving back and forth between the free and adsorbed states. A balance of intermolecular forces determines the position of equilibrium and thus the ability of the solvent to move the solute up the plate.[10]
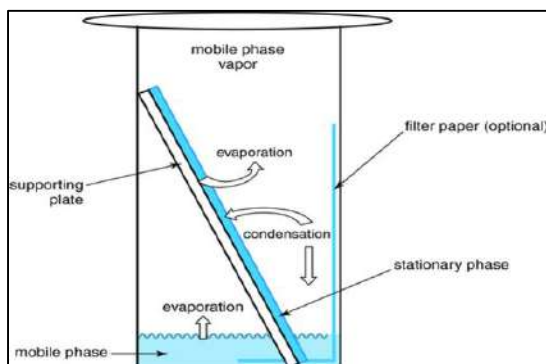

Figure1: Schematic representation of ascending development chamber for conventional TLC.
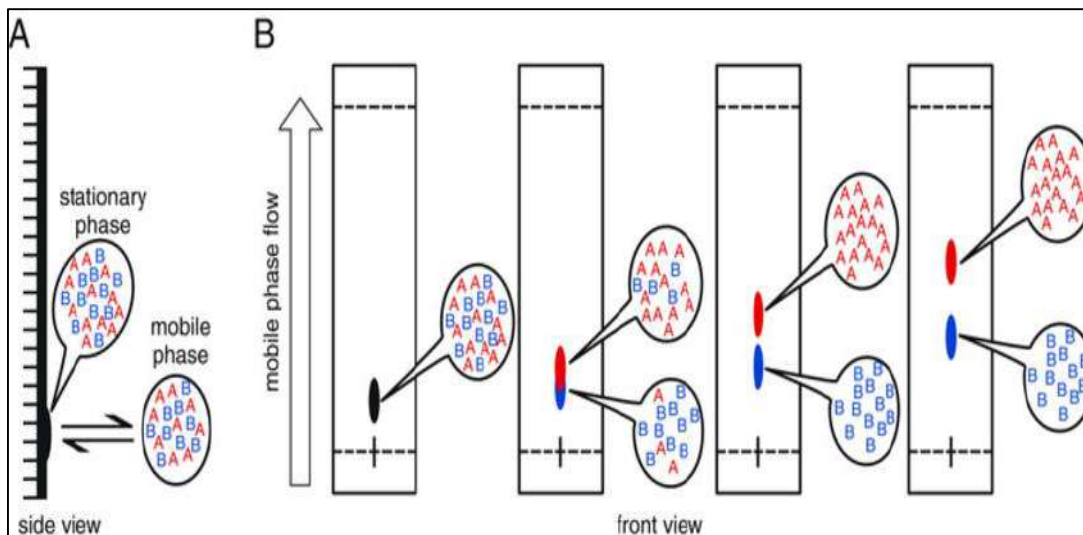

Figure 2: (A) Mixture of A and B adsorbed on the stationary phase and free in mobile phase and (B) schematic representations of the principle of separation.

This balance depends on (1) the polarity of the TLC coating material, (2) the polarity of the development solvent, and (3) the polarity of the sample molecule(s). For example, with a sample consisting of two compounds A and B as illustrated in Fig 6.3.2B, if the molecules A spend more time in the mobile phase, they will be carried through the stationary phase more rapidly and move further in a certain time. While molecules B are adsorbed to the stationary phase more than A, B molecules spend less time in the mobile phase and therefore move through the stationary phase more slowly, and do not move as far in the same amount of time. The consequence is that A is gradually separated from B as the mobile phase flows (ascends).

The standard silica coating (silica 60 with a mean pore diameter of 60 A°) is the most commonly used adsorbent in TLC, although for some very sensitive substances less active adsorbents such as aluminium oxide is preferred to prevent sample decomposition. Moreover, in the early days, the use of cellulose, polyamide, and Florisil (magnesium silicate) as adsorbent agents was also described. For selection of an adsorbent, one considers the properties of the compounds to be separated: first, the solubility of the sample compounds (hydrophilic or hydrophobic); then, whether the compounds can chemically react with the adsorbent or the eluent. Based on these considerations it is recommended that:

1. For lipophilic substances: silica, aluminium oxide, acetylated cellulose, polyamide should be used.

2. For hydrophilic substances: cellulose, cellulose ion exchangers, polyamide, and Reversed-phase silica should be used.
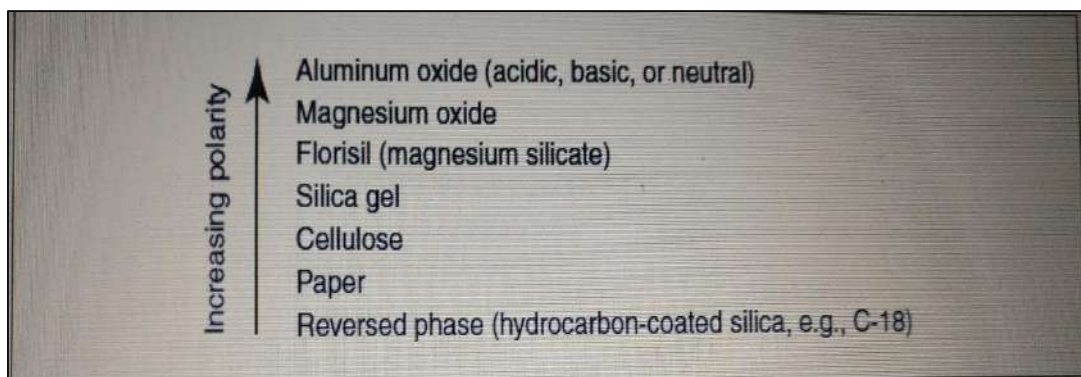
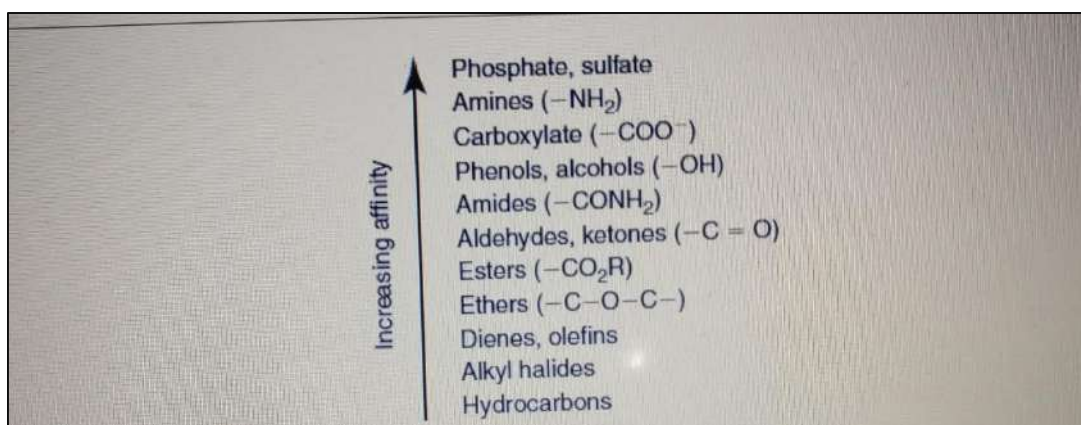**Figure 3: TLC stationary phase polarities.**

Figure 4: Affinity of common functional groups for silica gel (approximate).

Finding a suitable solvent system is usually the most difficult part of TLC experiments, and solvent system is the factor with the greatest influence on TLC. Only in a few cases does the solvent consist of only one component, and mixtures of up to five components are commonly used. No matter how many components are present, the prepared solvent system must be a homogenous system with no sign of cloudiness.

Three criteria are usually considered for choosing a solvent system: solubility, affinity, and resolution. The first step in solvent selection is to determine the solubility of the sample. The desired mobile phase will be able to provide the greatest solubility while balancing the sample affinity for the solvent and the stationary phase to achieve separation. Resolution is improved by optimizing the affinity between sample, solvent, and stationary Phase. Most TLC solvent systems contain a polar solvent and a chromatographically less polar solvent. [10]

In the present study, the red ink of various brands was analyzed by using TLC .In the past only one study was done related to this topic. In that study, ink of inkjet printer were analyzed by using TLC and HPLC.

CHAPTERII: LITERATURE REVIEW

Valery N. Aginsky (1993) studied on Determination of the age of ballpoint pen ink by gas and densitometric thin-layer chromatography. Two procedures for dating ballpoint inks are considered that use gas chromatography (a combination of the technique for determining the extent of extraction of ink volatile components and of the accelerated ageing technique) and densitometric thin-layer chromatography (separation of ink components and evaluation of the resulting chromatograms using a specially developed mass-independent technique that is also a very effective tool for the comparative TLC examination of similarly colored inks, paints, fibres and other materials of forensic interest). The procedures have been used in many real case situations and the results of the examinations were accepted as conclusive evidence by courts of law.

R.M.E. Griffin et.al. (1994) studied on an improved high-performance liquid chromatography system for the analyses of basic dyes in forensic case work Acrylic fibres are frequently encountered as physical evidence in casework in the Northern Ireland Forensic Science Laboratory. Separation and analysis of the basic dyes, used in the garment industry for the dyeing of acrylic fibres, was carried out by isocratic HPLC with a narrow bore column. The HPLC eluent used was a mixture of methanol and aqueous ammonium acetate solution (pH 9.76) in a ratio of 9:l. The sensitivity of the system is such that the dyes extracted from 0.2 cm of a black acrylic fibre could be detected using three injections on a single channel detector at wavelengths of 400, 500 and 600 nm (for yellow, red and blue dye components respectively).

NL Poon et.al. (2004) studied on Differentiation of colored inks of inkjet printer cartridges by thin layer chromatography and High performance liquid chromatography Examination of the writing inks on questioned documents using a non-destructive spectroscopic method by observing color properties of inks under different light sources

and selected filters was reported by Osbom. Chemical spot tests were also reported for detecting metal components such as iron, copper and vanadium in fluid inks. The development of more advanced chemical analyses of writing inks dates back well into the early 1950s. Since then, various analytical methods related to ink analysis have been reported in the literature. Among them, thin layer chromatography (TLC), requiring no special Apparatus has widely been used. However, this method is not very sensitive and requires a large sample size. The use of more sensitive instrumental analytical methods including high pressure liquid chromatography (HPLC), capillary electrophoresis (CE) and mass spectrometry has been reported.

Maarten R. van Bommel et al. (2007) studied on High-performance liquid chromatography and non-destructive three-dimensional fluorescence analysis of early synthetic dyes. Chromatographic and spectroscopic techniques are evaluated for the analysis of early synthetic dyes. The research focuses on the analysis of dyestuffs that were developed and used in the period 1850–1900, which has not been well investigated so far. The aim of this study was to develop and evaluate techniques that can be applied in the field of culture and art. A selection of 65 synthetic dyestuffs was chosen to investigate the usefulness of the analytical techniques applied. As a case study three embroideries designed by the well-known 19th century French painter Emile Bernard, were investigated to obtain more information about the use and behavior of synthetic dyes.

DjavanshirDjozanet.al. (2008) studied on Forensic discrimination of blue ballpoint pen inks based on thin layer chromatography and image analysis. This article aims to provide a new and fast method for differentiation of inks on a questioned document. The data acquisition was carried out by designing specific image analysis software for evaluating thin layer chromatograms (TLC-IA).The ink spot was extracted from the document using methanol and separated by TLC using plastic sheet silica gel 60 without fluorescent indicator, and a mixture of ethyl acetate, ethanol, and water (70:35:30, v/v/v) as mobile phase to discriminate between different pen inks, new software was designed on the basis of intensity profile of red, green, and blue (RGB)characteristic. In practice, after development of chromatogram, the chromatograms were scanned by ordinary

office scanner, intensity profiles of RGB characteristics on the development straight of each sample were produced and compared with the mentioned software. RGB profiles of ballpoint inks from various manufacturers showed that the patterns in most cases were distinctly different from each other. This new method allowed discriminating among different pen inks with a high reliability and the discriminating power of 92.8%. Blue ballpoint pen inks of 41 different samples available on the local market were successfully analyzed and discriminated.

Cedric Neumann et.al. (2009) studied on New perspectives in the use of ink evidence in forensic science Part II. Development and testing of mathematical algorithms for the automatic comparison of ink samples were analyzed by HPTLC. In the first part of this research, three stages were stated for a program to increase the information extracted from ink evidence and maximize its usefulness to the criminal and civil justice system. These stages are (a) develop a standard methodology for analyzing ink samples by high-performance thin layer chromatography (HPTLC) in reproducible way, when ink samples are analyzed at different time, locations and by different examiners; (b) compare automatically and objectively ink samples; and (c) define and evaluate theoretical framework for the use of ink evidence in forensic context. This report focuses on the second of the three stages. Using the calibration and acquisition process described in the previous report, mathematical algorithms are proposed to automatically and objectively compare ink samples. The performances of these algorithms are systematically studied for various chemical and forensic conditions using standard performance tests commonly used in biometrics studies. The results show that different algorithms are best suited for different tasks. Finally, this report demonstrates how modern analytical and computer technology can be used in the field of ink examination and how tools developed and successfully applied in other fields of forensic science can help maximizing its impact within the field of questioned documents.

Vishal Sharma et.al. (2017) studied on Fourier Transform Infrared Spectroscopy and High Performance Thin Layer Chromatography for Characterization and Multivariate discrimination of blue ballpoint pen ink for Forensic applications 57 blue ballpoint pen ink samples characterized & discriminated using ATR-FTIR.HPTLC is also used for discrimination. Multivariate analysis along with analytical method is explored.99.69 % discriminating power (DP) from non-destructive analysis of ATR–FTIR.DP from spectral comparison- 97.93% and destructive HPTLC- 93.80 %. Low cost, non–destructive, reliable and high resolution analysis

CHAPTER III : AIM and OBJECTIVE

AIM:

To analyze the red ink of various brands using TLC.

OBJECTIVE:

- To determine solubility of inks in various solvent systems.

- To find and compare the Rf of various inks

CHAPTER IV: MATERIALS & METHODOLOGY

Materials:

Apparatus:

1. TLC plate

2. TLC Chamber

3. Red ink

4. Beakers

5. Droppers

6. Capillary tubes

7. Measuring cylinder

Reagents

1. Methanol
2. Ethyl acetate / absolute ethanol / distilled water [70:35:30 ,v/v/v]

Instrument

- Combine: HEBER Model no: HME 63 Mini U.V Cabinet

Figure 5: Mini U.V Cabinet

Methodology

Ink sample of various brands was collected. Firstly, 3 ml of ink sample was dissolved in Methanol solvent. From each brand five samples were taken on five TLC plates.

For thin layer chromatography, slides were cleaned with acetone. Silica gel slurry was prepared by pouring the water in the ratio 2:1. Thin layer chromatography plates were prepared by mixing the adsorbent, such as silica gel and water. The slurry is spread on unreactive carrier sheet, like glass. The plate is dried and activated by heating in an oven at $110°$ C.

After Preparing the plate sample should be applied to silica plate. Using the small capillary a small spot of sample contacting solution should be applied to plate about 2cm from bottom of the plate. Then allow it to dry.

Then solvent system of 135ml was prepared that contains ethyl acetate: Absolute ethanol: Distilled water (70:35:30) and covered the solvent system with glass lid and was left for half an hour for saturation.

Then the plate was placed in the solvent chamber and lids was closed and allow it to run. The solvent moves up the plate by capillary action. The plate should be remove from the chamber after its running and then allow it to dry.

After the plate dried visualize the plate under UV Chamber. Then the RF value for various brands of ink samples was calculated.

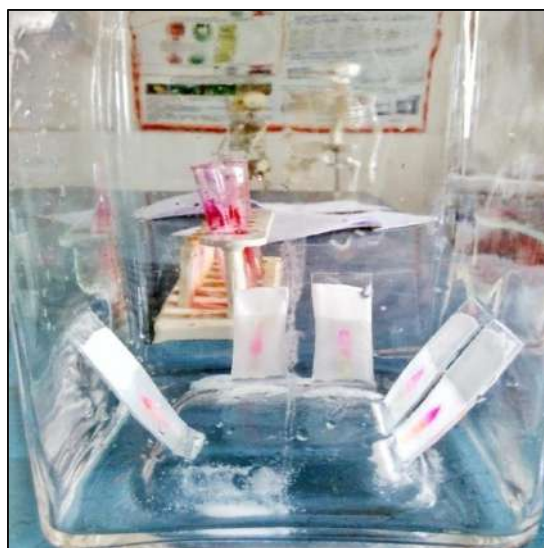**Figure 6**: **Red Ink Samples collected in test tube by dissolving 3ml of Methanol**

Figure 7: TLC Chamber

Figure 8: Samples kept in Mini U.V Cabinet

CHAPTER V: OBSERVATIONS

Table 1: Writek extra dark brand ink sample

SR.NO	SAMPLE	RF VALUE	AVERAGE
1	1	0.64	
2	2	0.66	
3	3	0.64	0.65
4	4	0.66	
5	5	0.66	

Figure 9: Under white light in Mini U.V Cabinet

Table 2: Linc Starline Trending brand ink sample

SR.NO	SAMPLE	RF VALUE	AVERAGE
1	1	0.8	0.81

2	2	0.77	
3	3	0.82	
4	4	0.84	
5	5	0.81	

Figure 10: Under white light in Mini U.V Cabinet

Table 3: Flair Q5 brand ink sample

SR.NO	SAMPLE	RF VALUE	AVERAGE
1	1	0.8	
2	2	0.74	
3	3	0.77	0.77
4	4	0.78	
5	5	0.74	

Figure 11: under white light in Mini U.V Cabinet

Table 4: Totem Meow brand ink sample

SR.NO	SAMPLE	RF VALUE	AVERAGE
1	1	0.65	
2	2	0.63	
3	3	0.75	0.67
4	4	0.67	
5	5	0.66	
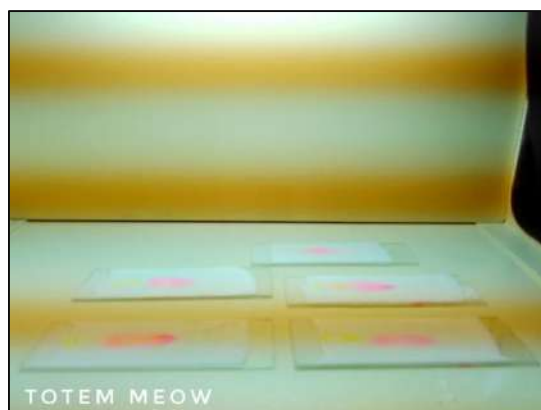
Figure 12: under white light in Mini U.V Cabinet

Table 5: Nataraj Superx brand ink sample

SR.NO	SAMPLE	RF VALUE	AVERAGE
1	1	0.74	
2	2	0.92	
3	3	0.77	0.82
4	4	0.84	
5	5	0.83	
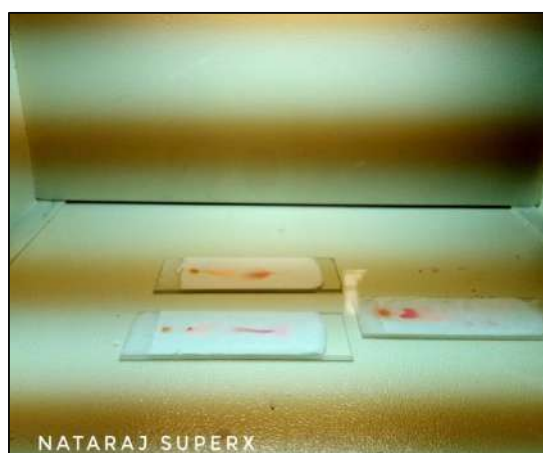
Figure 13: under white light in Mini U.V Cabinet

Table 6: Rorito Bkmax brand ink sample

SR.NO	SAMPLE	RF VALUE	AVERAGE
1	1	0.84	
2	2	0.70	
3	3	0.72	0.74
4	4	0.69	
5	5	0.74	
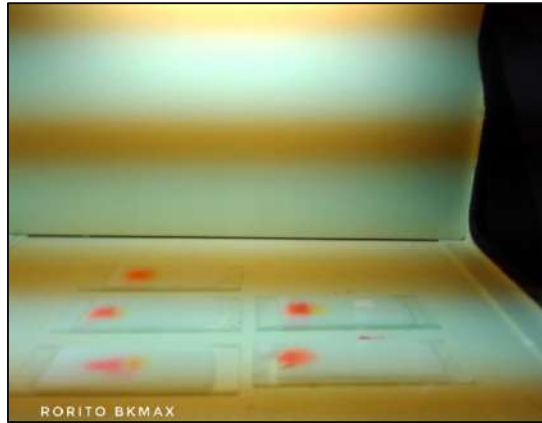
Figure 14: under white light in Mini U.V Cabinet

Table 7: Elkos Cuty brand ink sample

SR.NO	SAMPLE	RF VALUE	AVERAGE
1	1	0.86	
2	2	0.76	
3	3	0.76	0.80
4	4	0.73	
5	5	0.88	
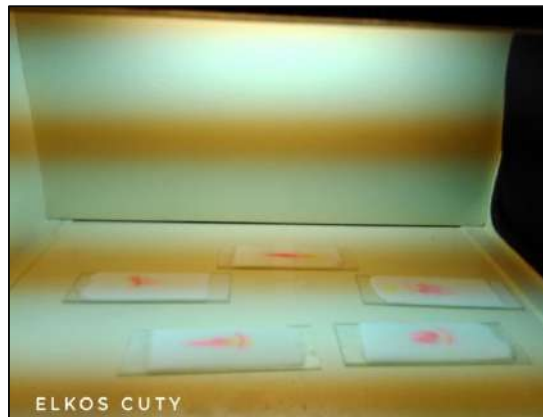
Figure 15: under white light in Mini U.V Cabinet

Table 8: Teamo – Bale brand ink sample

SR.NO	SAMPLE	RF VALUE	AVERAGE
1	1	0.81	
2	2	0.79	
3	3	0.82	0.79
4	4	0.78	
5	5	0.74	
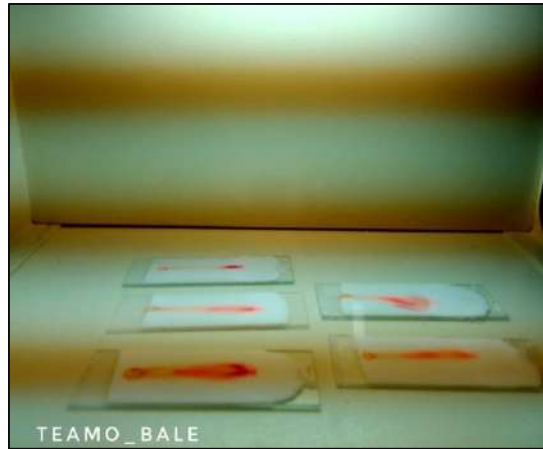
Figure 16: under white light in Mini U.V Cabinet

Table 9: Lexi 5n brand ink sample

SR NO	SAMPLE	RF VALUE	AVERAGE
1	1	0.82	
2	2	0.89	
3	3	0.81	0.87
4	4	0.88	

5	5	0.94	
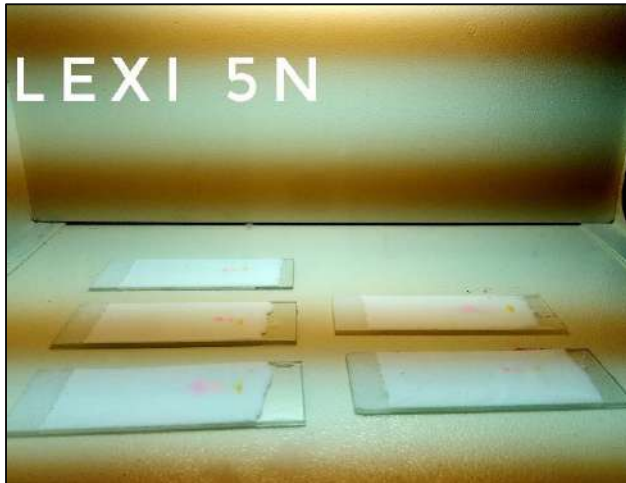
Figure 17: under white light in Mini U.V Cabinet

Table 10: Cello Winner brand ink sample

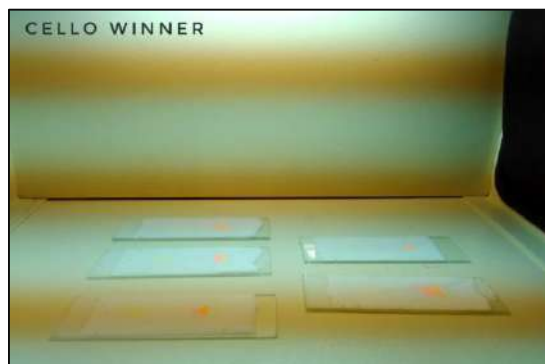| SR NO	SAMPLE	RF VALUE	AVERAGE
1	1	0.86	
2	2	0.92	
3	3	0.88	0.90
4	4	0.93	
5	5	0.93	

Figure 18: under white light in Mini U.V Cabinet

CHAPTER VI: RESULT & CONCLUSION

RESULT

Average Rf value for Writek Extra Dark brand red ink is calculated as 0.65. Average Rf value for Linc Starline Trending brand red ink is Calculated as 0.81. Average Rf value for FLAIR Q5 brand red ink is Calculated as 0.77.Average Rf value for Totem Meow brand red ink is Calculated as 0.67. Average Rf value for Nataraj Superx brand red ink is Calculated as 0.82. Average Rf value for Rorito Bkmax brand red ink is Calculated as 0.74. Average Rf value for Elkos Cuty brand red ink is Calculated as 0.80. Average Rf value for Teamo – Bale brand red ink is Calculated as 0.79. Average Rf value for LEXI 5n brand red ink is calculated as 0.87. Average Rf value for Cello Winner brand red ink is Calculated as 0.90

CONCLUSION

In present study the variation of Rf values for different brands of red ink are calculated and it is found that Rf value for red inks of various brands are different. The study needs to analyze various brands of red inks under U.V Spectroscopy to find out the absorbance of red ink or dye.

CHAPTER VII: REFERENCES

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