CHAPTER I: INTRODUCTION

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

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

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

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 type of ink is referred to as secret ink it can be made from common foodstuffs, for instance the British used rice starch to manufacture Invisible ink during the Indian mutiny in 1857.[8]

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.

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 aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium 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 Significances 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 in the 1940s. However, paper chromatography was gradually replaced by thin layer chromatography (TLC), which has one of the most routinely used chromatography techniques (Ettreand 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.

TLC is a quick, sensitive, and inexpensive technique that only requiresa few micrograms ofsample for one successful analysis. TLC is commonly used todetermine the number ofcomponents in a mixture, verify the identity and purity ofa compound, monitor theprogress of a reaction, determine the solvent compositionfor preparative separations; andanalyse the fractions obtained from columnchromatography.

Like all forms of chromatography, TLC involves a dynamic and rapid equilibrium ofmolecules between the two phases (mobile phase and stationary phase). However, TLCdiffers 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 ofthe mobile phase and itsvapor, an equilibrium will be established gradually (also calledchamber saturation). The partof the stationary layer that is already wetted with mobilephase also contributes to the formation of the equilibrium. During development, molecules are continuously moving back and forth between thefree and adsorbed states. A balance of intermolecular forces determines theposition of equilibrium and thus the ability of the solvent to move the solute up the plate.

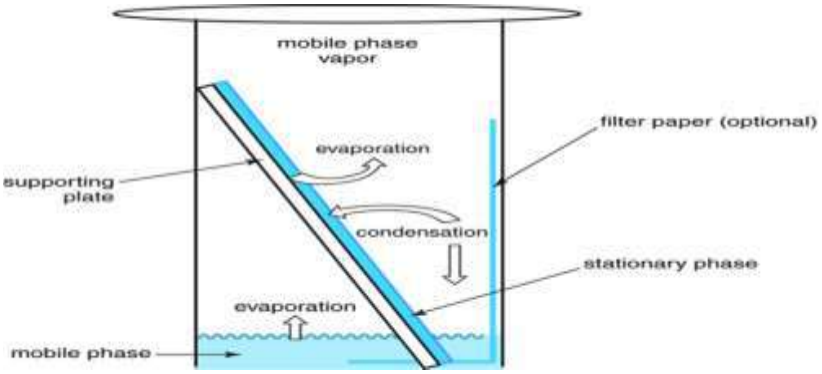


Figure 1: Schematic representation of ascending development chamber for conventional TLC

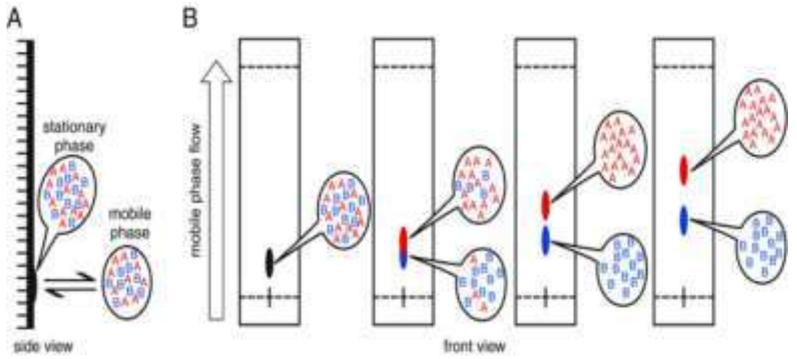


Figure 2:(**A**) Mixture of A and B adsorbed on the stationary phase and free in mobile phaseand (**B**) schematic representations of the principle of separation.

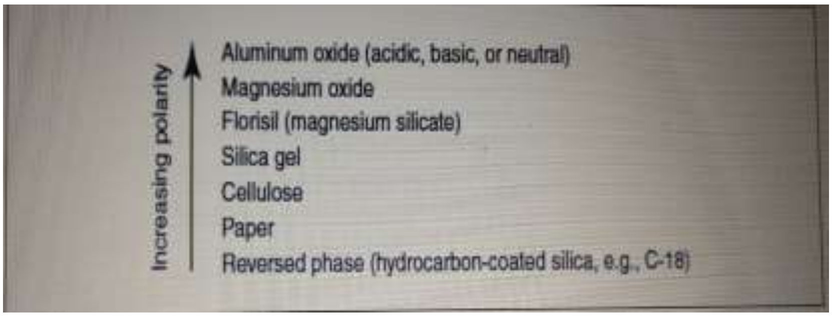
This balance depends on (1) the polarity of the TLC coating material, (2) the polarity of the development solvent, and (3) the polarity of the sample molecule(s). For example, with a sample consisting of two compounds A and B, if the molecules A spend more time in the mobile phase, they will be carried 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 mostcommonly used adsorbent in TLC, although for some very sensitive substances lessactive adsorbents such as aluminium oxide is preferred to prevent sample decomposition.Moreover, in the early days, the use of cellulose, polyamide, and Florisil (magnesiumsilicate) as adsorbent agents was also described.

Based on these considerations it is recommended that:

I. for lipophilic substances: silica, aluminium oxide, acetylated cellulose, polyamideshould be used.

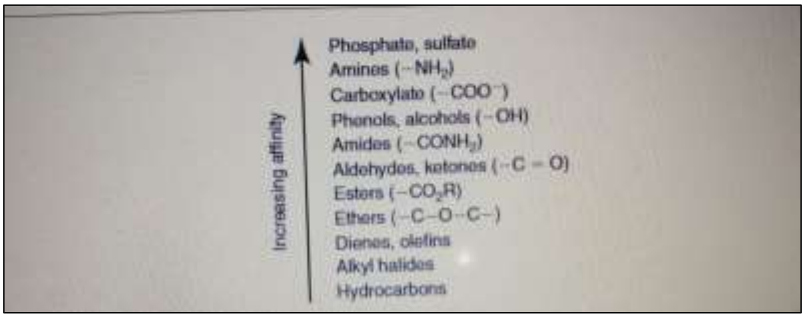
II. for hydrophilic substances: cellulose, cellulose ion exchangers, polyamide, andreversed-phase silica should be used.[10]



Increasing polarity

Aluminum oxide (acidic, basic, or neutral)
Magnesium oxide
Florisil (magnesium silicate)
Silica gel
Cellulose
Paper
Reversed phase (hydrocarbon-coated silica, e.g., C-18)

Figure 3: TLC stationary phase polarities.



Increasing affinity

Phosphate, sulfate
Amines ($-NH_2$)
Carboxylate ($-COO^-$)
Phenols, alcohols ($-OH$)
Amides ($-CONH_2$)
Aldehydes, ketones ($-C=O$)
Esters ($-CO_2R$)
Ethers ($-C-O-C-$)
Dienes, olefins
Alkyl halides
Hydrocarbons

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 casesdoes the solvent consist of only one component, and mixtures of up to five componentsare commonlyused. No matter how many components are present, the prepared solventsystem 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 thesample. The desired mobile phase will be able to provide the greatest solubility whilebalancing the sample affinity for the solvent and the stationary phase to achieve separation.

Resolution is improved by optimizing the affinity between sample, solvent, and stationaryPhase. Most TLC solvent systems contain a polar solvent and a chromatographically lesspolar solvent.[10]

CHAPTER II: 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 ageingtechnique) 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 coloured inks, paints, fibres and other materials of forensicinterest). 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 animproved high-performance liquid chromatography system for the analysis of basic dyes in forensic casework. 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 coloured inks of inkjet printer cartridges by thin layer chromatography and Highperformance liquid chromatography. Examination of the writing inks on questioned documents using a non-destructive spectroscopic method by observing colourproperties of inks under different light sources and selected filters was reported by Osbom [I]. Chemical spot tests were also reported for detecting metal components such as iron, copper and vanadium in fluid inks [I]. 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 [2-81. Among them,thin layer chromatography (TLC), requiring no special apparatushas widely been used.

However, this method is not very sensitive and requires a large sample size. The use of more sensitiveinstrumental analytical methods including high pressure liquid chromatography (HPLC) [9-151, capillary electrophoresis (CE)[16, 171 and mass spectrometry [18, 191 has been reported.

Cedric Neumann et.al. (2007) 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 analyzed by HPLC.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 analysing ink samples by high-performance thin layer chromatography (HPTLC) in reproducible way, when ink samples are analysed 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.

Maarten R. van Bommel et al. (2007) studied onHigh-performance liquid chromatography and non-destructive three-dimensional fluorescence analysis of early syntheticdyes.Chromatographicand 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 behaviour of synthetic dyes

DjavanshirDjozan et.al. (2008) studied on Forensic discrimination of blue ballpoint pen inks based on thinlayer 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.

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.

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CHAPTER III: AIM & OBJECTIVES

AIM

To analyze the blue ink at various temperatures using TLC.

OBJECTIVES

- To determine solubility of various solvents in the Ink.
- To determine the RF values of various Ink and compare it.
- To find out the variation in RF value according to various temperature.

CHAPTER IV: MATERIALS & METHODOLOGY

MATERIALS:

Apparatus

1. TLC plate
2. TLC Chamber
3. Blue 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]

Instruments

1. Hot Plate
2. U.V Chamber

Figure 5: Hot Plate

Figure 6: Mini U.V Cabinet

METHODOLOGY:

- Ink sample of same brand is collected. Ink sample is dissolved in Methanol solvent.
- Solvent system is prepared by dissolving 70ml of ethyl acetate, 35ml of absolute ethanol and 30ml of distilled water and pour into the TLC plate.
- One to two drops of ink samples are placed on TLC plate.
- In these way five samples from one brand of ink were run in the solvent system.
- The RF values of ink is calculated.

Figure 7: Blue Ink Sample collected in Test Tube by dissolving 10ml of Methanol

Figure 8: Heating of the Sample using Hot Plate

Figure 9: TLC Chamber containing samples

CHAPTER V: OBSERVATIONS

Observation Table & Images

At Normal Temperature

RFValue 15min	Average	RF Value 30min	Average	RF Value 60min	Average
0.85	0.82	0.87	0.87	0.83	0.86
0.85		0.83		0.77	
0.81		0.88		0.83	
0.83		0.88		0.94	
0.76		0.87		0.83	

Figure 10: **Under White Light in U.V Chamber at Normal Room Temperature**

At 10°CTemperature

RF Value 15min	Average	RF Value 30min	Average	RF Value 60min	Average
0.87	0.82	0.94	0.90	0.92	0.91
0.77		0.89		0.93	
0.85		0.91		0.83	
0.81		0.83		0.90	
0.78		0.91		0.96	

Figure 11: Under White Light in U.V Chamber at 10°cTemperature

At 30°CTemperature

RF Value 15min	Average	RF Value 30min	Average	RF Value 60min	Average
0.78	0.84	0.82	0.85	0.79	0.86
0.84		0.90		0.90	
0.77		0.86		0.88	
0.90		0.90		0.89	
0.91		0.80		0.84	

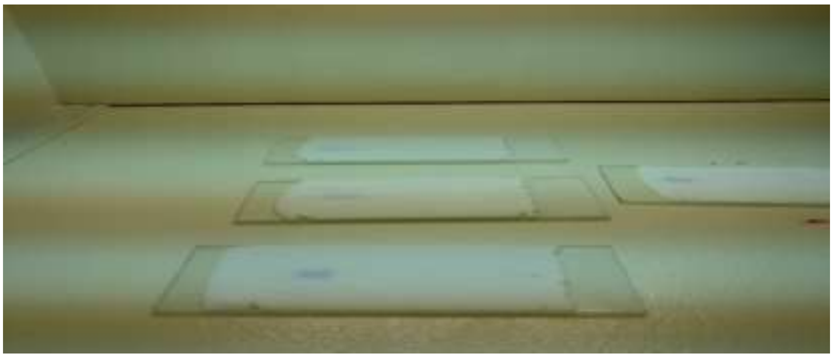

Figure 12: **Under White light in U.V Chamber at 30°c Temperature**

At 50°C Temperature

RF Value 15min	Average	RF Value 30min	Average	RF Value 60min	Average
0.90	0.88	0.93	0.86	0.90	0.88
0.84		0.86		0.93	
0.90		0.80		0.91	
0.90		0.85		0.77	
0.86		0.84		0.90	

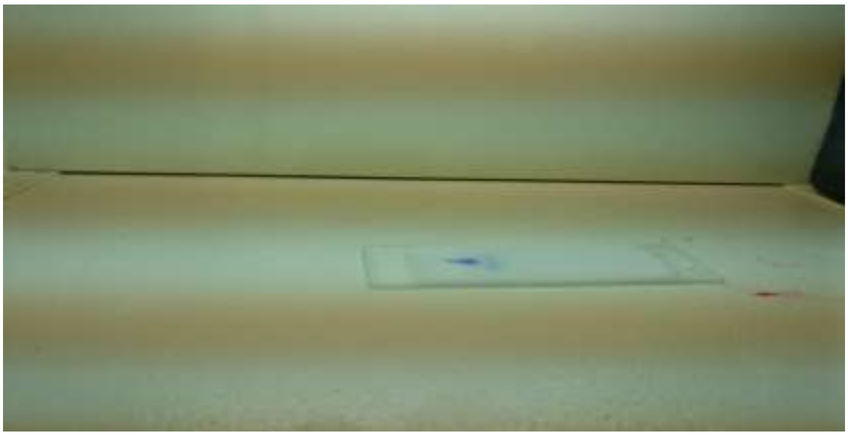

Figure 13: **Under White Light in U.V Chamber at 50°c Temperature**

At 80°C Temperature

RF Value 15min	Average	RF Value 30min	Average	RF Value 60min	Average
0.80	0.86	0.86	0.88	0.90	0.85
0.88		0.87		0.88	
0.88		0.85		0.82	
0.86		0.90		0.72	
0.88		0.90		0.93	

Figure 14: **Under White Light in Mini U.V Chamber at 80°c Temperature**

At 100°CTemperature

RF Value 15min	Average	RF Value 30min	Average	RF Value 60min	Average
0.85	0.83	0.79	0.82	0.86	0.83
0.84		0.83		0.81	
0.79		0.89		0.81	
0.79		0.85		0.84	
0.88		0.77		0.84	

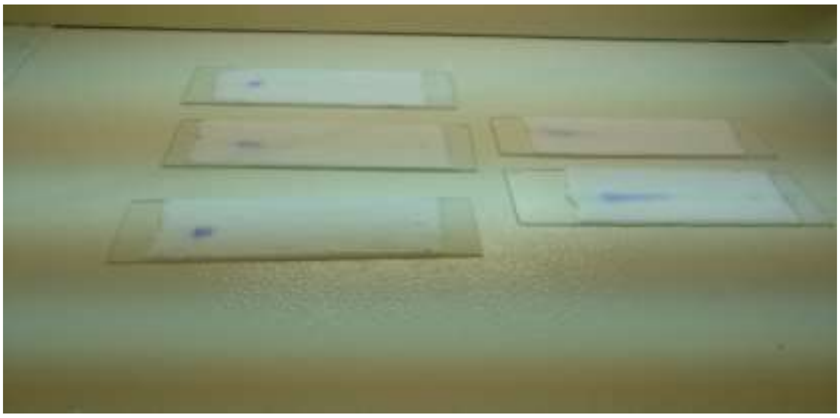

Figure 15: **Under White Light in U.V Chamber at 100°c Temperature**

CHAPTER VI: RESULT & CONCLUSION

Result

Average Rf Values of Blue Ink at Normal temperature is 0.82 in 15 minutes, 0.87 in 30 minutes and 0.86 in 60 minutes.

Average Rf Values of Blue Ink at 10°c is 0.82 in 15 minutes, 0.90 in 30 minutes and 0.91 in 60 minutes.

Average Rf Values of Blue Ink at 20°c is 0.84 in 15 minutes, 0.85 in 30 minutes and 0.86 in 60 minutes.

Average Rf Values of Blue Ink at 50°c is 0.88 In 15 minutes, 0.86 In 30 minutes and 0.88 In 60 minutes.

Average Rf Values of Blue Ink at 80°c is 0.86 in 15 minutes, 0.88 in 30 minutes and 0.85 in 60 minutes.

Average Rf Values of Blue Ink at 100°c is 0.83 in 15 minutes, 0.82 in 30 minutes and 0.83 in 60 minutes.

CONCLUSION

In present study the variations of Rf Values of same brand pen at various temperature is seen to be varied with respect to time.

The study needs to analyse under UV Spectroscopy to find out the variation of Rf Values at different Temperatures.

CHAPTER VII: REFERENCES

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