

A Comparative Analysis of Ink Composition under Acidic, Basic, And Neutral Hydrolysis Conditions Using Thin-layer Chromatography (TLC)

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ABSTRACT

Ink analysis plays a vital role in forensic science, particularly in the field of question document examination for purposes such as authentication of documents, investigation of forgeries, and other criminal cases involving written evidence. This study focused on the chemical breakdown of ink composition under acidic, basic, and neutral hydrolyzed conditions using thin-layer chromatography (TLC) as an instrument. This study explored three chemical hydrolyzed conditions: acidic (Nitric acid -HNO₃), basic (Sodium Hydroxide – NaOH), and neutral (distilled water). And observed how these conditions affect the chemical composition of ink by identifying the changes in resulting RF (retention factor) values. For this analysis, the ink was extracted from the Reynolds 045 fine CarbuRe blue ballpoint pen, and RF values for each component were measured from the silica gel-coated TLC plates and compared the RF values of standard ink (non-hydrolyzed) and hydrolyzed ink samples. The analysis revealed significant changes in the ink composition, with shifts in RF values when standard (non-hydrolyzed) ink sample was compared with those hydrolyzed ink samples.

Keywords: Chemical breakdown of ink, Acidic hydrolysis of ink, Basic hydrolysis of ink, Neutral hydrolysis of ink, Thin-layer chromatography (TLC), Retention Factor (RF)

INTRODUCTION

Forensic science plays a vital role in investigating crimes and criminals and gathering evidence. A document analysis is considered a crucial aspect in the field where the document is in question. One of the significant aspects of the document examination was ink analysis, which helps forensic experts to determine whether a document has been altered by any physical or chemical methods, fabricated, or tampered with, especially in cases such as forgery, documents that require authentication, and other criminal cases involving written or printed evidence. Ink is a liquid or paste that contains dyes and pigments, it was widely used for writing or drawing by using a pen or brush for making a text, design, image or colored surfaces. Ink is usually composed of solvent, pigment, dye, resins, solubilizer, surfactant, fluorescence and other materials [1]. Various analytical techniques are used for ink analysis in the case of document examination. This study used thin-layer chromatography (TLC) as an instrument for ink analysis because of its capability to separate and identify the mixture of dyes and pigments found on

the ink and for its efficiency and cost-effectiveness. TLC, which allows for the separation of ink components based on their solubility and their interaction with the stationary phases [2]. In this study, Reynolds 045 fine carbure blue ballpoint pen ink was used for analysis. Usually, ballpoint pen ink is composed of oil and dyes. This will contribute to the ink thickness, and the oil components in the ink are responsible for the quick drying of ink and the reason for the resistance of the ink in water [3]. This study focuses on the chemical breakdown of ink composition. Ink degradation was the main reason for the chemical breakdown of ink. Ink degradation can occur due to various environmental and chemical factors, which will alter or affect the ink composition and its stability over time. The most common cause of ink degradation is chemical hydrolysis, which means that when we add water, it reacts with organic molecules to break the molecules and form two or more smaller organic molecules. By using this chemical hydrolysis method, the ink in the document can be chemically altered, erased, forged, or tampered with [4]. This study analyzed the chemical breakdown

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of ink composition under various chemical hydrolysis conditions such as (acidic, basic, and neutral) using thin-layer chromatography as an instrument.

Three chemical hydrolysis conditions were analyzed in this study:

1. Ink hydrolyzed by acid:

Nitric acid (HNO_3) was utilized for this procedure, which has ($\text{pH} < 7$). Since it breaks bonds and supplies protons (H^+) ions, particularly in the ink's dyes and pigments.

2. Ink hydrolyzed by base:

sodium hydroxide (NaOH) was utilized in this procedure, which has ($\text{pH} > 7$). The bonds in the ink composition may be broken by the release of (H^-) ions from NaOH .

3. Neutral hydrolysis of ink:

This was done to determine the ink's water solubility and stability using distilled water, which has (pH of 7). For TLC analysis, the ink was chemically extracted from a Reynolds 045 fine carbure blue ballpoint pen, which is commercially available. The ink sample is subjected to three chemical hydrolysis conditions (acidic, basic, and neutral). The extracted ink was applied on the per-coated silica gel TLC plate (stationary phase) by using capillary action [5], and then the plate was developed using a mixture of the solvent system "(i.e., ethyl acetate: ethanol: distilled water - 70:35:30 ml)" [6][7], which was termed the mobile phase. TLC allows for the separation of components based on their affinity towards the stationary phase when it comes in contact with the mobile phase. Components with higher affinity for the stationary phase move slower, and components with lower affinity for the stationary phase move faster [5]. The retention factor (RF) value is an important parameter in a TLC analysis, and it is a direct measurement method to measure the amount of materials and mixtures in each spot. The RF value was calculated by measuring the ratio of the distance traveled by the solute to the distance traveled by the solvent front. [8].

RF = $\frac{\text{Distance of center of spot from the baseline}}{\text{Distance of solvent front from the baseline}}$ [8]

In this study, the RF value for each component that is separated from the ink samples is measured, and the RF value of the standard (non-hydrolyzed) ink sample was compared with those hydrolyzed ink samples, which were hydrolyzed under acidic, basic, and neutral conditions. Analyzing these changes in RF value will help the experts to authenticate the document containing ink by identifying chemical alteration or tampering in the ink. The result will help forensic document examiners to distinguish between genuine and altered documents by authenticating them. This study provides valuable insights into how different chemical factors affect or alter the composition of the ink; from this, the examiner could easily identify chemically altered ink. This study will help to authenticate the document under question while in legal proceedings [9].

Significance:

In this analysis, the document examiner can determine whether any specific chemical agents are subjected to the ink compositions. In forensic analysis, this study helps to determine whether the ink in the document has been chemically altered, erased, forged or tampered with. "Application of TLC in ink analysis is a simple, rapid, cost-effective and efficient technique" [10]. Apart from the forensic investigation, this study helps to understand the chemical breakdown of ink composition under various chemical hydrolysis conditions such as (acidic, basic and neutral).

METHODOLOGY

Solvent preparation (mobile phase)

The mobile phase is the component that helps the analyte to interact with the stationary phase [11]. For this research, a solvent system composed of "ethyl acetate, ethanol, and distilled water in a ratio of 70:35:30 was used" [6][7] due to its efficiency in separating and identifying the compounds present in the ink.

Ink sample preparation

Ink sample 1

The blue ink was obtained from a commercially available Reynolds 045 fine carbure ballpoint pen, and the ink was extracted in "ethanol as an extraction solvent" [11] [12] This study focused on the chemical

breakdown of the ink composition. For this, three chemical hydrolyzed conditions were used;

Ink sample 2 - The ink was hydrolyzed under an acidic solution:

For this, 1 ml of concentrated nitric acid (HNO₃) was diluted in 9 ml of distilled water to create a total of 10 ml of reagent. A mixture of 5 ml of extracted ink and 5 ml of the prepared acidic reagent was taken in the plate.

Ink sample 3 - The ink was hydrolyzed under a basic solution:

For this, 1 ml of 10n NaOH (sodium hydroxide) was diluted in 9 ml of distilled water to create a total of 10 ml of reagent. A mixture of 5 ml of extracted ink and 5 ml of the prepared basic reagent was taken in the plate.

Ink sample 4 - The ink was hydrolyzed under neutral conditions:

For this, the mixture of 5 ml of distilled water and 5 ml of extracted ink sample was taken in the plate.

TLC Procedure

Place the solvent system (“i.e., ethyl acetate: ethanol: distilled water) in the proportion of 70:35:30” [6][7],

in the TLC chamber. Prepared ink samples are spotted on the pre-coated silica gel TLC plate about 2 cm above the bottom of the plate with the help of capillary tubes. Four distinct ink samples are spotted separately to each TLC plate, sized (5 × 10 cm). The plate was placed in the developing chamber that contained the solvent system (mobile phase) at room temperature. It took 30-45 minutes for the solvent system to reach the top of the plate. Once the solvent traveled within a cm of the top of the plate or 3/4 in the TLC plate, the plate was removed from the chamber. The solvent front and the chromatographic spots were observed under daylight (direct detection method) and marked using a pencil and recorded as a photograph under daylight. Finally, the qualitative analysis of thin-layer chromatography was measured by calculating the respective retention factor of each spot obtained in the plate [6].

RESULT AND DISCUSSION

Figures 2 to 5 show the results of the TLC separation of the ink composition of the prepared standard (non-hydrolyzed) and hydrolyzed ink samples using the developing solvent system of “(i.e., ethyl acetate: ethanol: distilled water) in the ratio of (70:35:30)” [6][7].

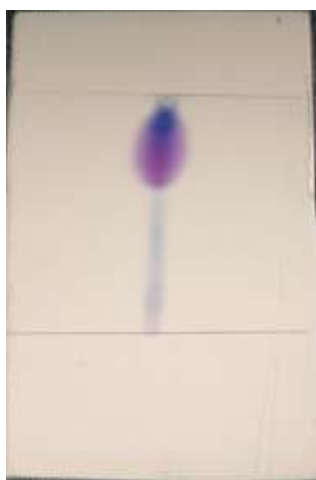


Figure 1. The thin-layer chromatogram for standard ink (ink sample 1) obtained from a Reynolds 045 fine-carbure blue ballpoint pen (Photographed under daylight).



Figure 2. The thin-layer chromatogram obtained for ink sample 2 (acidic hydrolyzed condition), which was hydrolyzed in nitric acid (HNO₃) (photographed under daylight).



Figure 3. The thin-layer chromatogram obtained for ink sample 3, which was hydrolyzed in sodium hydroxide (NaOH) (photographed under daylight).



Figure 4. The thin-layer chromatogram obtained for ink sample 4, which was hydrolyzed using distilled water (photographed under daylight).

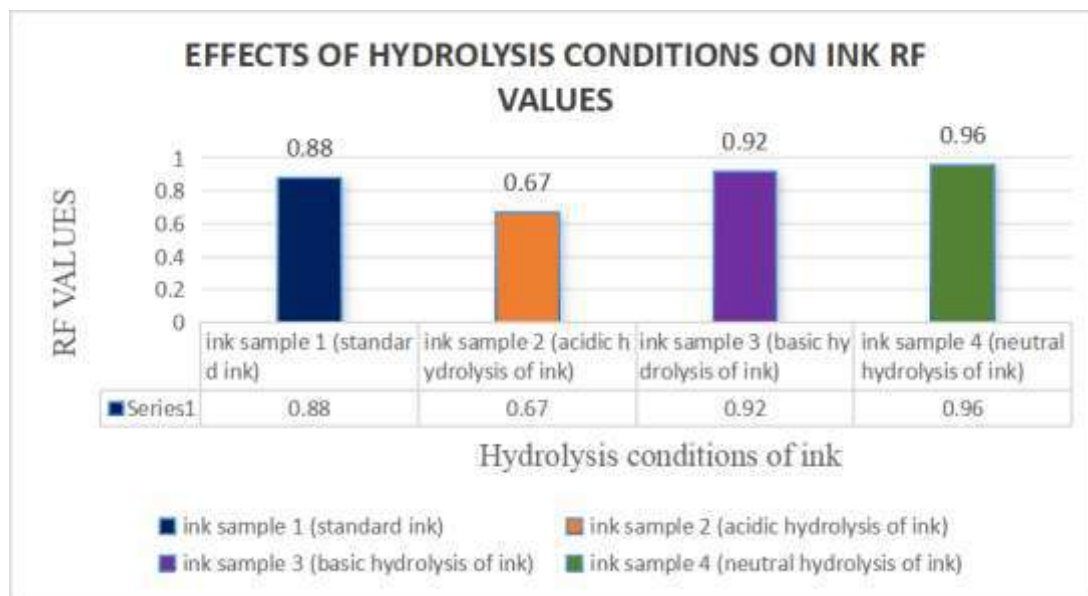
Table 1. Results for ink samples 1 to 4 by qualitative analysis through thin-layer chromatography (TLC).

Name of the pen sample	Ink samples	Thin-layer chromatography		
		Solvent front (cm)	Spot traveled (cm)	Retention factor (RF) values

Reynolds 045 fine carbure blue ballpoint pen	Ink sample 1	5.1	4.5	0.88
	Ink sample 2	5.2	3.5	0.67
	Ink sample 3	5.2	4.8	0.92
	Ink sample 4	5.2	5	0.96

The Retention factor (RF) value for ink samples 1 to 4 were measured as shown in Table 1. The variations in the RF values were observed for distinct ink samples. This indicates that there are changes in ink

composition when the ink is subjected to acidic, basic, and neutral chemical hydrolysis conditions.



Graph 1. represents comparative analysis of effects of ink composition under standard, acidic, basic, and neutral hydrolysis conditions using RF values.

This study aimed to compare the retention factor (RF) value of standard (non-hydrolyzed) ink with those of ink subjected to acidic, basic, and neutral chemical hydrolysis conditions, which was clearly distinguished in Graph 1.

- The standard ink (non-hydrolyzed) which was labeled as ink sample 1, was extracted from Reynolds 045 fine-carbure blue ballpoint pen and run under TLC for 30 minutes; it gave RF values of 0.88 as shown in the Graph 1, which was consistent with the previously published results from [13]. Indicates the reliability and reproducibility of ink composition in chromatography. This serves as a reference for comparison with hydrolyzed ink samples.

Comparing the standard (non-hydrolyzed) ink sample 1, with ink sample 2, subjected to nitric acid (acidic chemical condition):

For this condition, the extracted ink was subjected to nitric acid (HNO_3) and run under TLC for 30 minutes;

it gave an RF value of 0.67. The RF value was decreased when it was compared with the standard (non-hydrolyzed) ink sample1, RF value as shown in the Graph 1. This indicates that components in the ink experienced certain chemical alterations, which will lead to the degradation of the ink composition in an acidic environment.

Comparing the standard(non-hydrolyzed) ink sample1, with ink sample 3, subjected to sodium hydroxide (basic chemical condition):

For this condition, the extracted ink was subjected to sodium hydroxide (NaOH) and run under TLC for 30 minutes; it gave an RF value of 0.92. The RF value was slightly increased when it was compared with the standard (non-hydrolyzed) ink sample 1, RF value as shown in the Graph 1. It indicates that the dye components in the ink composition may be unchanged in an alkaline environment. However, It may have led to the breakdown of certain ink compositions and

allowed them to travel slightly further on the TLC plate.

Comparing standard (non-hydrolyzed) ink sample 1, with ink sample 4, subjected to distilled water (neutral chemical condition) :

For this condition, the extracted ink was subjected to distilled water and run under TLC for 30 minutes, which gives an RF value of 0.96. It indicates that water alone does not make any chemical changes in ink composition, but the result shows that there was a slight solubilization of components in the ink. It will lead to a slight increase in the RF value of the TLC plate while comparing it with ink sample 1, as shown in the Graph 1.

DISCUSSION:

The results of RF values for ink samples 1 to 4 were measured, and the standard (non-hydrolyzed) ink sample, which was labeled as ink sample 1, was compared with ink samples 2 to 4, which were subjected to acidic, basic, and neutral environmental conditions. The results of RF values are shown in table 1. The comparison results of RF values are demonstrated in Graph 1. This study provides the results for the standard ink and for ink that was subjected to acidic, basic, and neutral chemical conditions. For ink sample 1 (standard ink), it gives a resulting RF value of 0.88, which serves as a comparative point for other ink samples. In ink sample 2, the extracted ink was subjected to nitric acid, HNO_3 (acidic conditions), which will give the resulting RF value of 0.67, indicating that acid will break down the ink composition and reduce its mobility on the TLC plate while comparing it with ink sample 1. Ink sample 3 was subjected to sodium hydroxide, NaOH (basic condition), which will give a resulting RF value of 0.92. It shows a slight increase in RF values when it was compared with ink sample 1 and indicates that basic chemical environmental conditions make the ink composition more soluble in the mobile phase and allow it to travel further. And it may affect the ink stability. Unlike acidic conditions, this will cause only minor changes in ink composition by showing slight shifts in RF values and does not degrade the ink components. In ink sample 4, the extracted ink was subjected to distilled water (neutral condition); it shows the resulting RF value of 0.96, which indicates that there is a slight increase in RF

value while comparing it with ink sample 1. It shows that water does not chemically alter the ink composition, but it causes slight changes in the ink stability. This will lead to a slight increase in their mobility, and this shift in RF indicates that some ink components may be more soluble in water, which will reduce the retention on the stationary phase and increase their mobility on the TLC plate. This will provide crucial information in a forensic investigation by showing the results on the effects of chemical exposure on ink composition and its stability. These findings will help to authenticate the document, which contains ink evidence. By these findings, we can identify whether the document was exposed to any chemical environment with the help of thin-layer chromatography (TLC). TLC acts as an effective technique to analyze the effects of different chemical hydrolyzed conditions in the ink composition [10].

CONCLUSION:

In conclusion, this study analyzed the effects of chemical conditions such as acidic, basic, and neutral hydrolysis on ink composition and its stability. For this analysis, the ink from a Reynolds 045 fine-carbure blue ballpoint pen was extracted using ethanol and labeled as ink sample 1, kept as a standard reference, and the extracted ink was subjected to acidic, basic, and neutral chemical hydrolyzed conditions to analyze the effects of chemical exposure on ink composition. For this analysis, the extracted ink was subjected to nitric acid (HNO_3) for acidic conditions; it was labeled as ink sample 2. For the basic chemical condition, the extracted ink was subjected to sodium hydroxide (NaOH) and labeled as ink sample 2, and for neutral chemical conditions, the extracted ink was subjected to distilled water and labeled as ink sample 4. The ink samples 1 to 4 were separately spotted on the TLC plate and run under thin-layer chromatography with the solvent system of "ethyl acetate, ethanol, and distilled water in the ratio of (70:35:30)" [6][7] for 30 minutes at room temperature. Finally, the retention factor (RF) values of ink samples 1 to 4 were measured and shown in Table 1. For ink sample 1, the resulting RF value was 0.88, and for ink sample 2 it was 0.67; for ink sample 3, the RF value was 0.92, and for ink sample 4, the RF value was 0.96. In this study, ink sample 1 (standard ink) was compared with those of hydrolyzed ink samples (i.e., ink samples 2 to 4), which was shown

in graph 1. The results concluded that acidic conditions caused significant degradation in the ink composition, which will lead to a reduction in RF values when comparing it with ink sample 1. Ink sample 2 (basic hydrolysis conditions) shows slight alterations in ink composition, which gives results of a slight shift in RF values when it is compared with ink sample 1. Ink sample 4 (neutral hydrolysis conditions) gives the results of water never making major changes or alterations in the ink composition, but it may affect the ink stability by solubilizing the ink composition under water. This will lead to a slight shift in RF value while comparing it with ink sample 1. These findings highlight the stability of ink composition under different environmental conditions, especially in chemical environments. This study concludes that whether the ink was exposed to certain chemicals, it will create alterations in ink composition, and it may affect the ink stability over time. This can be identified by thin-layer chromatography (TLC). TLC provided effective results and was considered a cost-effective technique for the analysis of ink. This study provides valuable information in forensic examinations, especially in cases such as forgery, document authentication, and other cases involving ink evidence [4]. This study provides a primary understanding of the effects of chemical hydrolysis on ink composition. However, there is a wide range of scope for future research based on this study. This can include the usage of various types of ink brands and types in the analysis because different ink formulations may exhibit different behavior under chemical exposure; this will provide an extensive database for forensic analysis, and advanced analytical techniques such as “HPTLC” [14], “Raman spectroscopy and FTIR” [15] could also be effectively used for ink analysis to understand these chemical effects in ink composition. Since ink composition can also be influenced by the type of paper used, future research could examine how different paper materials have an impact on the degradation of ink composition under chemical exposure

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