

Metal Ions Separation Via Paper Chromatography: Enhanced Methods Using Eluting Solutions

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Abstract Chromatography is an essential physical technique that allows the constituent components of a mixture to be identified, separated, and purified in preparation for qualitative examination. Paper chromatography (PC) is a sort of planar chromatography, which refers to a stationary phase that is a solid, flat surface. In this illustration stationary phase is a particular kind of paper (Whatman quantitative filter paper grade 41). The fundamental idea behind paper chromatography is the differential passage of a mixture's constituent parts through filter paper or chromatography paper. A quick method for separating mixtures of metal ions, amino acids, carbohydrates, colors, and pharmaceuticals is paper chromatography (PC). For this qualitative analysis, only a very small sample is needed. Metal cation separation has seen increased by the use of the PC approach. Here, the four experiments show how PC may be used to separate metal ions [(Pb²⁺ & Cu²⁺), (Ni²⁺ & Co²⁺), (Cu²⁺ & Fe³⁺), (Cu²⁺ & Co²⁺)] based on their colored spots and the retardation factors or retention factors (R_f) values by using different eluting solutions.

Keywords: qualitative analysis, green developer, chromatographic jar, spotting capillaries, colored spots, retention factor, under graduate experiment

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1. Introduction

The separation of chemical species is an important stage in many chemical operations and processes. Paper chromatography (PC) can be used to swiftly and efficiently separate materials such as pigments, metal ions, and other substances. Paper chromatography is used to separate the metal cations in the aqueous mixture. Nad et al. described the chromatographic separation and identification of Fe³⁺, Ni²⁺, Co²⁺, Cu²⁺, Pb²⁺, and Hg²⁺ [1]. During this study, rubeanic acid, Dimethylglyoxime (DMG) and sodium sulphide were used as eluting solutions. S.C. Das [2] described the separation and identification of inorganic cations (Co²⁺, Ni²⁺, and Cu²⁺) by using ethanolic solution of rubeanic acid as spraying reagent.

Till now, no such works have been done by taking water as the universal mobile phase (developer) for the separation of group II metal ions (Pb²⁺ & Cu²⁺) with 10% KI solution as eluting agent; group IV metal ions (Ni²⁺ & Co²⁺) with 5% NH₄OH solution as spraying agent; group II and group III metal ions (Cu²⁺ & Fe³⁺) with 100 ml, 1N aqueous solution of potassium ferrocyanide K₄[Fe(CN)₆] as spraying agent; and group II and group IV metal ions (Cu²⁺ & Co²⁺) with 10% NH₄OH solution as eluting agent. Thus it was thought of interest to undertake four experiments of paper chromatography (PC) for the

separation of metal ions [(Pb²⁺ & Cu²⁺), (Ni²⁺ & Co²⁺), (Cu²⁺ & Fe³⁺), (Cu²⁺ & Co²⁺)] for UG students.

2. PC Experiment-1: Separation of Group II Metal ions (Pb²⁺ & Cu²⁺) by 10% KI Solution

2.1. Materials and Method

i) Experimental

Requirements

A. Apparatus & chemical required

(i) Chromatographic jar ii) 10% KI Solution iii) Measuring cylinder iv) Lead nitrate Pb(NO₃)₂ v) Copper sulfate CuSO₄.5H₂O vi) Spotting capillaries vii) Small test tubes viii) 10ml and 250ml beaker ix) Whatman quantitative filter paper grade 41 x) Distilled water

(B) Solution required

(i) Metal salts solution: Prepared saturated solution of lead nitrate and copper sulfate by dissolving them in distilled water (1mg/mL) in the 10ml beaker.

(ii) Detector: Prepared 10% KI solution in distilled water in the 250ml beaker.

(C) Developer

200ml distilled water in the 250ml beaker.

ii) Procedure

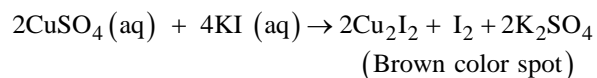
The chromatographic jar had a suspended Whatman grade 41 filter paper strip. Place a dot on the side of the line that you drew on this stripe, around 0.5 cm from the bottom. Development will start at this end, which will be the strip's bottom. Using a small capillary to pass through two (02) places on the filter paper, the saturated solutions of Pb^{2+} and Cu^{2+} were applied separately. For every remedy, a brand-new capillary was used. The filter paper with the 02 spots was then allowed to dry outside. After that, the dried and stained filter paper strip was once more suspended in the distilled water-filled chromatographic jar, with the top end secured to the steel rod and the bottom end touching the developer (water). The strip is shown to be vertical. Always place the point above the level of the developer. As the developer (solvent front) approaches the upper end of the filter paper (Figure 1b), the developer (water) is let to climb along the filter paper (Figure 1a). After taking the filter paper out of the chromatography jar, the solvent front was indicated with a pen. The developer was then removed from the paper by drying it. 10% KI solution was taken in a sprayer and applied to the dry filter paper as a separating solvent or spraying reagent. With the KI reaction, one yellow and one brown colored spot appeared right away (Figure 1c), signifying the identification of Pb^{2+} and Cu^{2+} ions, respectively. Pencils were used to mark the colored zones.

2.2. Results and Analysis

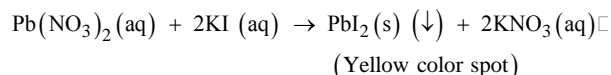
2.2.1. Reactions Involved During Formation of Colour Spots by Interaction with Solute Zone

In a redox reaction, 10% potassium iodide (KI) added to a copper sulfate solution causes the copper sulfate to combine with the KI solution, the iodide ions reduce Cu(II)

to Cu(I) , and the iodide (I^-) ion oxidizes to iodine (I_2), forming a brown color spot on the Whatman 41 filter paper. The solute zone (Cu^{2+} ion) traveled a distance where the Cu^{2+} reaction with KI happened, and since I_2 escaped quickly, this was immediately noted by marking the brown color spot with a pen.



However, potassium iodide (KI) and lead nitrate [$\text{Pb}(\text{NO}_3)_2$] interacted to form an ion exchange that resulted in the production of potassium nitrate (KNO_3) and a yellow-colored spot of lead iodide (PbI_2).



2.2.2. Data Analysis

Observed the colored spots corresponding to two different cations. Two cations (Pb^{2+} and Cu^{2+}) were identified and separated by comparing color spots and retention factor values. The first spot appeared as brown due to the liberation of I_2 after the reaction of KI with $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ indicated the distance traveled by one solute zone as Cu^{2+} (ds_1) and the second spot Pb^{2+} appeared as yellow indicating the distance traveled by another solute zone (ds_2). Then, measured the distance of the color zones of each solute from the point of application. Also measured the distance between the solvent front (water) and the starting line, labeled this distance as distance traveled by the solvent (dm). Then calculated retardation factors or retention factors (R_f) values of each cation (Table 1) by the following equation:

$$\text{Retention Factor } (R_f) = \frac{\text{Distance travelled by the centre of solute zone in cm } (\text{ds})}{\text{Distance travelled by the solvent front in cm } (\text{dm})}$$

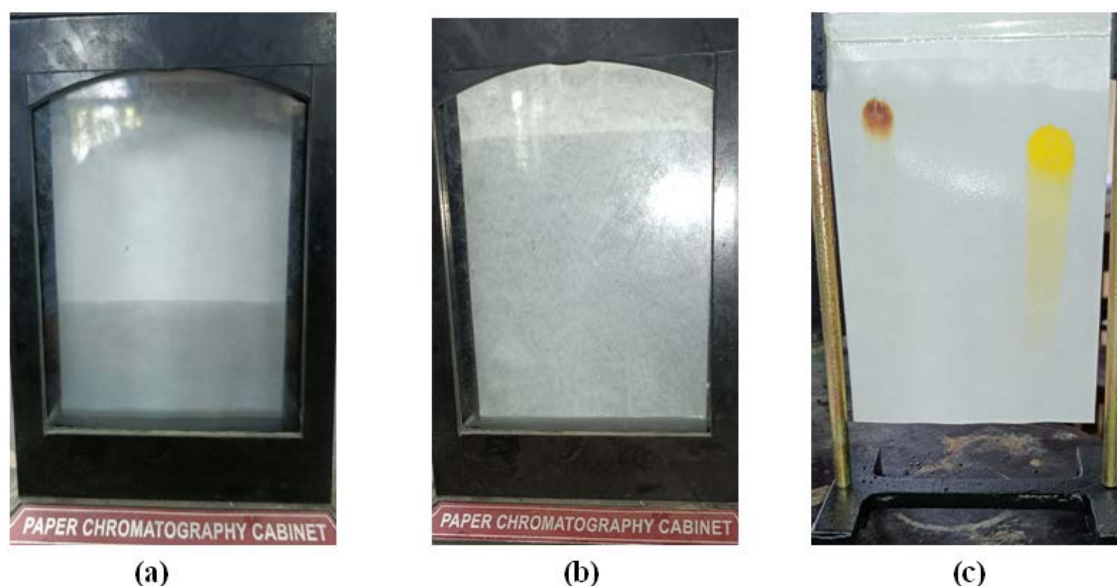


Figure 1. Separation of metal ions (Pb^{2+} and Cu^{2+}) by paper chromatography

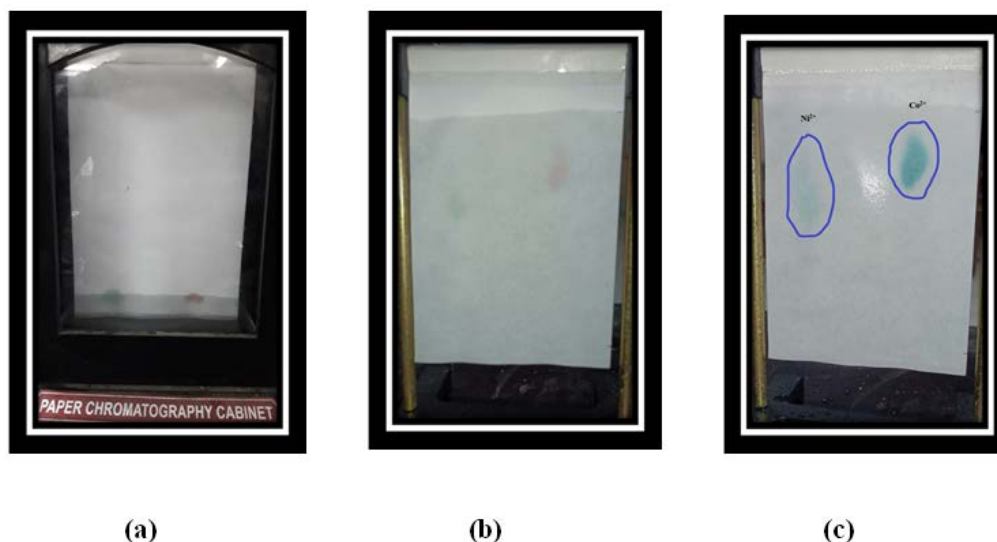


Figure 2. Separation of metal ions (Ni^{2+} and Co^{2+}) by paper chromatography by using 5% NH_4OH solution

3. PC Experiment-2: Separation of Group IV Metal ions (Ni^{2+} & Co^{2+}) by 5% NH_4OH solution

3.1. Materials and Method

i) Experimental

Requirements

A. Apparatus & chemical required

(i) Chromatographic jar ii) 5% NH_4OH Solution iii) Measuring cylinder iv) Nickel nitrate hexahydrate $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ v) Cobalt nitrate $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ vi) Spotting capillaries vii) Small test tubes viii) 10ml, 100ml, 250ml beaker ix) Whatman quantitative filter paper grade 41 x) Distilled water

(B) Solution required

(i) Metal salts solution: Prepared saturated solution of both metal nitrates by dissolving them in distilled water (1mg/mL) in the 10ml beaker.

(ii) Detector: Prepared 5% NH_4OH solution in distilled water in the 100ml beaker.

(C) Developer

200ml distilled water in the 250ml beaker.

ii) Procedure

Whatman grade 41 filter paper strip was suspended in the chromatographic jar. On this stripe drawn a line at about 1cm from the bottom and put dot in the side of the line. This end will be the bottom of the strip and development will take place from this end. The saturated solution of Ni^{2+} and Co^{2+} applied separately on the filter paper with help of a fine capillary through two (02) spots. A fresh capillary was used for each solution. Then, filter paper containing 02 spots was dried in open air. The spotted and dried filter paper strip was then suspended again in the chromatographic jar containing distilled water with the upper end pinned to the steel rod and lower end touching the developer (water). It is observed that the strip is vertical. The spot should always be above the developer

level. The developer (water) is allowed to rise along the filter paper (Figure 2a) and wait till developer (solvent front) reaches near the upper end of the filter paper along with rise of two metal ions (Figure 2b). The filter paper was then removed from the chromatography jar and marked the solvent front with the help of pen. Then the paper was dried to evaporate the developer. As a separating solvent or spraying reagent 5% NH_4OH solution was taken in a sprayer and scattered it on the dried filter paper. Green gel and bluish green colored spots developed immediately (Figure 2c) with the reaction of NH_4OH corresponding to the identification of Ni^{2+} and Co^{2+} ions respectively. The coloured zones were marked with pen.

Attempt was made in the PC experiment initially by using 1% NH_4OH solution but not achieved satisfactory result for Ni^{2+} (Figure 3).

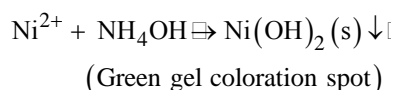
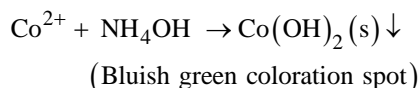


Figure 3. Separation of metal ions (Ni^{2+} and Co^{2+}) by paper chromatography by using 1% NH_4OH solution

3.2. Results and Analysis

3.2.1. Reactions Involved During Formation of Colour Spots by Interaction with Solute Zone

Nickel and cobalt (II) nitrate solutions are mixed with 5% NH_4OH solution to generate metal hydroxide, which results of colored spots in the Whatman grade 41 filter paper that are green gel and bluish green, respectively [3,4].



3.2.2. Data Analysis

Two cations (Ni^{2+} and Co^{2+}) were identified and separated by comparing their color spots and retention factor values. First spot appeared as green gel due to formation of nickel (II) hydroxide $\text{Ni}(\text{OH})_2$ indicated the distance travelled by one solute zone as Ni^{2+} (ds_3) and second spot Co^{2+} appeared as bluish green due to formation of cobalt (II) hydroxide $\text{Co}(\text{OH})_2$ indicated the distance travelled by another solute zone (ds_4). Then calculated retardation factors or retention factors (R_f) values (Table 1).

4. PC Experiment-3: Separation of Group II and Group III Metal ions (Cu^{2+} & Fe^{3+}) by 1(N) $\text{K}_4[\text{Fe}(\text{CN})_6]$ solution

4.1. Materials and Method

i) Experimental

Requirements

A. Apparatus & chemical required

(i) Chromatographic jar (ii) Spraying bottle (iii) 100 ml, 1N aqueous solution of potassium ferrocyanide $\text{K}_4[\text{Fe}(\text{CN})_6]$ (iv) Ferric chloride, FeCl_3 (v) Copper sulfate pentahydrate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (vi) Spotting capillaries (vii) Small test tubes (viii) 100ml Measuring cylinder (ix) 10ml, 250ml beaker (x) Whatman quantitative filter paper grade 41 (xi) Distilled water

(B) Solution required

(i) Metal salts solution: Prepared saturated solution of ferric chloride and copper sulfate by dissolving them in distilled water (1mg/mL) in the 10ml beaker.

(ii) Eluting solution: Prepared 100 ml, 1N aqueous solution of potassium ferrocyanide $\text{K}_4[\text{Fe}(\text{CN})_6]$ in the 250ml beaker.

(C) Developer

200ml distilled water in the 250ml beaker.

ii) Procedure

In the chromatographic jar, a strip of Whatman grade 41 filter paper was suspended. Place a dot to the side of the line you drew on this stripe, about 1 centimeter from the bottom. Development will start at this end, which is the bottom of the strip. Using a tiny capillary that passed through two (02) locations, the saturated solutions of Fe^{3+} and Cu^{2+} were applied individually to the filter paper. Every solution was applied to a brand-new capillary. The filter paper with the 02 spots was then allowed to dry outside. The filter paper strip that had been spotted and dried was then suspended once more in the chromatographic jar filled with distilled water, with the lower end coming into contact with the developer (water) and the top end being pinned to the steel rod. The strip is shown to be vertical. Always place the point above the level of the developer. Allow the developer (water) to rise along the filter paper (Figure 4a) and wait for the two metal ions to rise together with the developer (solvent front) to approach the upper end of the filter paper (Figure 4b).

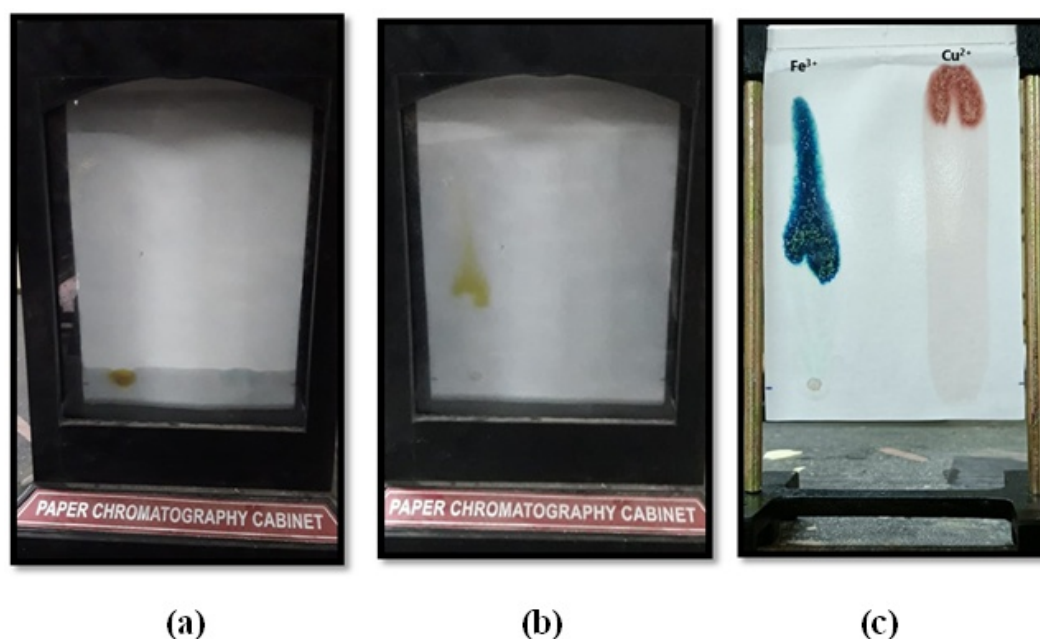


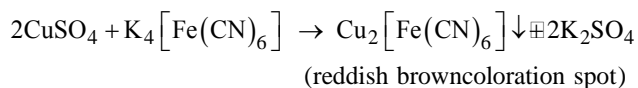
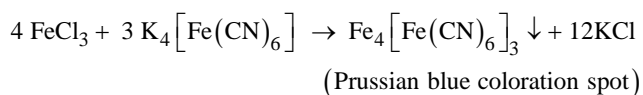
Figure 4. Separation of metal ions (Fe^{3+} and Cu^{2+}) by paper chromatography by using 1N aqueous $\text{K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$ solution

After taking the filter paper out of the chromatography jar, the solvent front was indicated with a pen. The developer was then removed from the paper by drying it. Potassium ferrocyanide 1N aqueous solution was used as an eluting or spraying reagent; it was applied to the dry filter paper using a sprayer. Following the interaction with the aqueous solution of $K_4[Fe(CN)_6]$, prussian blue and reddish-brown spots appeared right away (Figure 4c), signifying the identification of Fe^{3+} and Cu^{2+} ions, respectively. Pen was used to indicate the colored zones.

4.2. Results and Analysis

4.2.1. Reactions Involved During Formation of Color Spots by Interaction with Solute Zone

Ferric chloride solution was mixed with 1N aqueous solution of $K_4[Fe(CN)_6]$ formed Prussian blue or Berlin blue coloration spot, iron(III)hexacyanidoferrate(II), $Fe_4[Fe(CN)_6]_3$ in the Whatman grade 41 filter paper strip.



On the other hand, copper sulfate reacted with 1N aqueous solution of $K_4[Fe(CN)_6]$ formed reddish brown coloration spot, cupric ferrocyanide, $Cu_2[Fe(CN)_6]$ in the Whatman grade 41 filter paper strip.

4.2.2. Data Analysis

Observed the colored spots corresponding to two different cations (Cu^{2+} and Fe^{3+}). Two cations (Cu^{2+} and Fe^{3+}) were identified and separated by comparing their color spots and

retention factor values. First spot appeared as prussian blue due to formation of iron(III)hexacyanidoferrate(II), $Fe_4[Fe(CN)_6]_3$ indicated the distance travelled by one solute zone as Fe^{3+} (ds_5) and second spot for Cu^{2+} appeared as reddish brown due to formation of cupric ferrocyanide, $Cu_2[Fe(CN)_6]$ indicated the distance travelled by another solute zone Cu^{2+} (ds_6). Then calculated retardation factors or retention factors (R_f) values (Table 1).

5. PC Experiment-4: Separation of Group II and Group IV Metal ions (Cu^{2+} & Co^{2+}) by 10% NH_4OH solution

5.1. Materials and Method

i) Experimental

Requirements

A. Apparatus & chemical required

(i) Chromatographic jar (ii) 10% NH_4OH Solution (iii) Measuring cylinder (iv) Copper sulphate $CuSO_4 \cdot 5H_2O$ (v) Cobalt nitrate $Co(NO_3)_2 \cdot 6H_2O$ (vi) Spotting capillaries (vii) Small test tubes (viii) 10ml, 100ml, 250ml beaker (ix) Whatman quantitative filter paper grade 41 (x) Distilled water (xi) FT-IR spectrophotometer

(B) Solution required

(i) Metal salts solution: Prepared saturated solution of copper (II) sulfate and cobalt (II) nitrate by dissolving them in distilled water (1mg/mL) in the 10ml beaker.

(ii) Detector: Prepared 10% NH_4OH solution in distilled water in the 100ml beaker.

(C) Developer

Taken 200ml distilled water in the 250ml beaker.

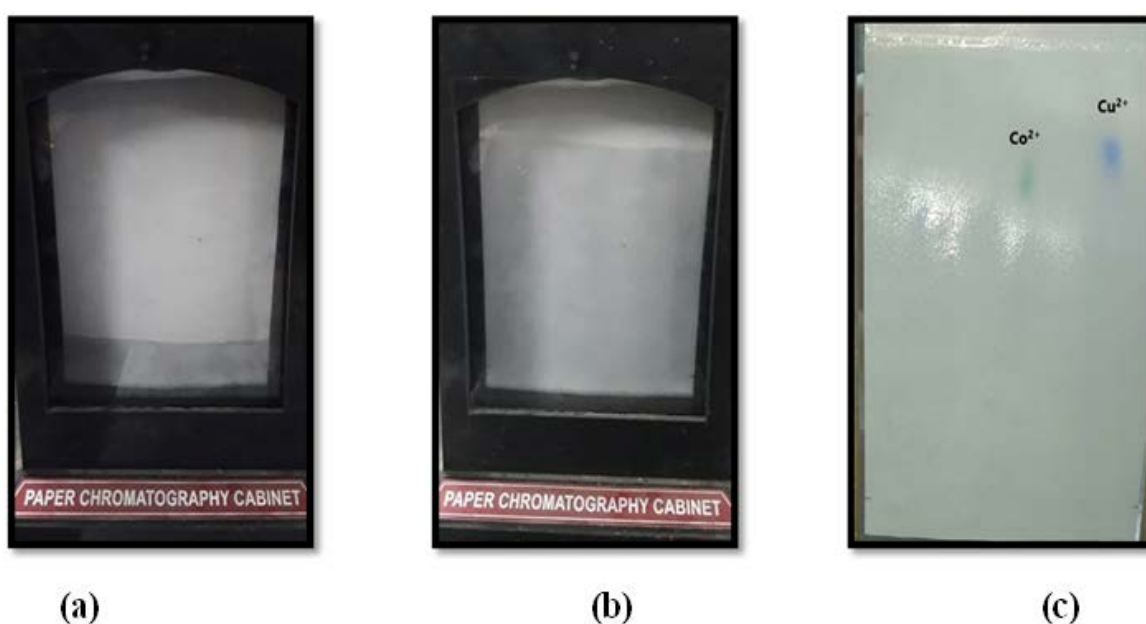


Figure 5. Separation of metal ions (Cu^{2+} and Co^{2+}) by paper chromatography by using 10% NH_4OH solution

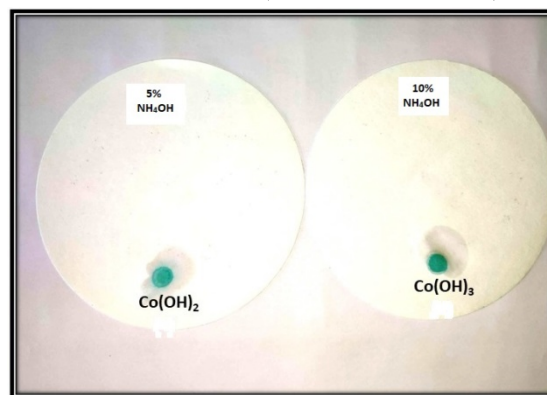
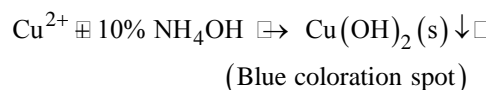
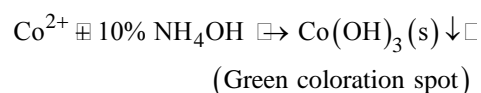
ii) Procedure

In the chromatographic jar, a strip of Whatman grade 41 filter paper was suspended. Place a dot to the side of the line you drew on this stripe, about 1 centimeter from the bottom. Development will start at this end, which is the bottom of the strip. Cu^{2+} and Co^{2+} saturated solutions were administered individually to the filter paper through two (02) locations using a tiny capillary. For every remedy, a brand-new capillary was used. The filter paper with the 02 spots was then allowed to dry outside. The filter paper strip that had been spotted and dried was then suspended once more in the chromatographic jar filled with distilled water, with the lower end coming into contact with the developer (water) and the top end being pinned to the steel rod. The strip is shown to be vertical. Always place the point above the level of the developer. Developer (water) is allowed to rise along the filter paper (Figure 5a); two metal ions rise along the filter paper as the developer (solvent front) approaches the upper end of the paper (Figure 5b). After taking the filter paper out of the chromatography jar, the solvent front was indicated with a pen. The developer was then removed from the paper by drying it. 10% NH_4OH solution was used in a sprayer as a separating solvent or spraying reagent, and the dried filter paper was covered with it. As soon as 10% NH_4OH was added, green and blue colored spots appeared (Figure 5c), signifying the presence of Co^{2+} and Cu^{2+} ions, respectively.

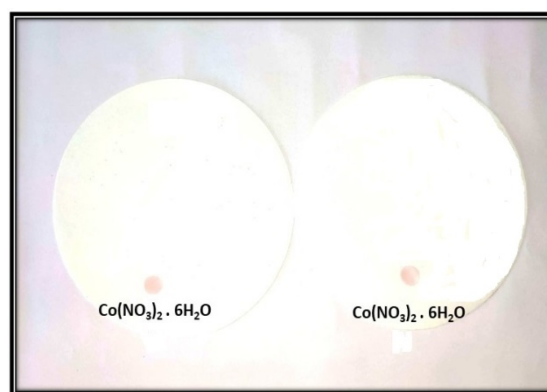
5.2. Results and Analysis

5.2.1. Reactions Involved During Formation of Colour Spots by Interaction with Solute Zone

When copper salt reacts with 10% NH_4OH solution gives copper (II) hydroxide, $\text{Cu}(\text{OH})_2$, which results of blue colored spot in the Whatman grade 41 filter paper. When $\text{Co}(\text{II})$ nitrate reacts with 10% NH_4OH solution gives $\text{Co}(\text{III})$ hydroxide, $\text{Co}(\text{OH})_3$, results green colored spots in the Whatman grade 41 filter paper instead of bluish green $\text{Co}(\text{II})$ hydroxide $\text{Co}(\text{OH})_2$, which obtained by the reaction of $\text{Co}(\text{II})$ nitrate with 5% NH_4OH solution. The cobalt(II) ion is more stable because it cannot quickly oxidize to the far less stable cobalt(III) state. All cobalt(II) salts are therefore stable, however cobalt(II) is readily converted to cobalt(III) in basic solutions (50 ml 10% NH_4OH) [5]. With increasing concentration of NH_4OH solution (5% to 10%), basic nature of the reaction medium increases and with increasing concentration of basic medium, air oxidation of cobalt(II) to cobalt(III) takes place relatively easily [5,6]. Thus, $\text{Co}(\text{II})$ hydroxide (bluish green) oxidizes to $\text{Co}(\text{III})$ hydroxide results green colored spot. That's why after eluting with 10% ammonium hydroxide solution bluish green coloured of $\text{Co}(\text{OH})_2$ immediately converted into green coloured $\text{Co}(\text{OH})_3$. It was further confirmed by the spot analysis of cobalt(II) nitrate solution with 5% and 10% NH_4OH solution individually in the Whatman grade 41 filter paper (Figure 6.). It was observed that during spot test of cobalt (II) nitrate solution with 5% NH_4OH , bluish green coloured developed (Figure 6b Left) and with 10% NH_4OH the green colour developed immediately (Figure 6b Right).



(b)



(a)

Figure 6. Spot analysis of cobalt(II) nitrate in the Whatman grade 41 filter paper (a) before addition of NH_4OH (b) after addition of 5% and 10% NH_4OH solution

5.2.2. FT-IR Analysis of Green colored Spot

FT-IR analysis of green spot carried out within the range $4000\text{--}400\text{ cm}^{-1}$ by Bruker Alpha II-E spectrophotometer. The stretching and bending vibrations of the OH (hydroxyl) group in $\text{Co}(\text{OH})_3$ typically occur in the infrared (IR) region of the electromagnetic spectrum. The strong broad band for -OH stretching vibrations typically occurs at 3384 cm^{-1} , while strong bending vibrations occurs around 1653 cm^{-1} [7], suggesting the presence of -OH group in cobalt (III) hydroxide. IR frequency of a Co-O bond generally occurs within the range $400\text{ to }600\text{ cm}^{-1}$. But in green spot, i.r. band for Co-O bond occurs at 620 cm^{-1} . A positive shift in the IR frequency of a Co-O bond, typically happens when the cobalt atom is in a higher oxidation state, such as $\text{Co}(\text{III})$ or $\text{Co}(\text{IV})$. In these higher oxidation states, the Co-O bond becomes stronger, leading to a higher vibrational frequency [8]. So the observe i.r. frequency of Co-O bond suggests the presence of (+3) oxidation state of cobalt in the cobalt hydroxide, $\text{Co}(\text{OH})_3$ for which green color spot developed.

Table 1. Separation of metal ions by paper chromatography

Experiment No.	Solution used (Cation Present)	Eluting Solution used	Color of the spot	Distance travelled by solute (ds) (in cm)	Distance travelled by solvent (dm) (in cm)	R _f value = ds/dm
Exp. 01	CuSO ₄ .5H ₂ O (Cu ²⁺ ion)	aqueous solution of 10% KI	Brown	14 (ds ₁)	15.5	0.903
	Pb(NO ₃) ₂ (Pb ²⁺ ion)		Yellow	13 (ds ₂)	15.5	0.838
Exp. 02	Ni(NO ₃) ₂ .6H ₂ O (Ni ²⁺ ion)	5% NH ₄ OH solution	Green gel	12.2 (ds ₃)	15.2	0.8026
	Co(NO ₃) ₂ .6H ₂ O (Co ²⁺ ion)		Bluish green	12.7 (ds ₄)	15.2	0.8355
Exp. 03	FeCl ₃ (Fe ³⁺ ion)	1N aqueous solution of K ₄ [Fe(CN) ₆]	Prussian blue	13.2 (ds ₅)	15.8	0.835
	CuSO ₄ .5H ₂ O (Cu ²⁺ ion)		Reddish brown	14.3 (ds ₆)	15.8	0.905
Exp. 04	Co(NO ₃) ₂ .6H ₂ O (Co ²⁺ ion)	10% NH ₄ OH solution	Green	12.2 (ds ₇)	14.5	0.8413
	CuSO ₄ .5H ₂ O (Cu ²⁺ ion)		Blue	13.4 (ds ₈)	14.5	0.9241

5.2.3. Data Analysis

Two cations (Cu²⁺ and Co²⁺) were identified and separated by comparing their color spots and retention factor values. Observed the coloured spots corresponding to two different cations (Cu²⁺ and Co²⁺). First spot appeared as green due to formation of cobalt (III) hydroxide Co(OH)₃ indicated the distance travelled by one solute zone, Co²⁺ (ds₇), where in basic medium it is readily oxidized into Co³⁺ and second spot Cu²⁺ appeared as blue due to formation of copper (II) hydroxide Cu(OH)₂ indicated the distance travelled by the another solute zone, Cu²⁺ (ds₈). Then calculated retardation factors or retention factors (R_f) values (Table 1).

6. Conclusion

Using water as mobile phase (developer), separation of metal ions [(Pb²⁺ & Cu²⁺), (Ni²⁺ & Co²⁺), (Cu²⁺ & Fe³⁺), (Cu²⁺ & Co²⁺)] have been done by taking different eluting agents like aqueous solution of 10% KI, 5% NH₄OH solution, 1N aqueous solution of K₄[Fe(CN)₆] and 10% NH₄OH solution respectively based on their colored spots and the retardation factors or retention factors (R_f) values. Thus, by using these improved techniques, make paper chromatography easy to separate two cations from the same or different groups of the analytical table.

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