SEPARATION AND IDENTIFICATION OF TRANSITION METAL IONS BY PAPER CHROMATOGRAPHY: IMPROVED QUALITATIVE INORGANIC ANALYSIS

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Abstract

Paper chromatography (PC), a set of low-cost, straightforward experiments has been designed to teach the fundamentals of chromatography to undergraduate students studying introductory inorganic chemistry. These experiments detect and separate metal ions belonging to different groups in the analytical table. Through these PC experiments, undergraduate students will learn about the separation and identification of metal ions from various metal salts, the comparison of color spots and retention factor values during the separation of metal ions, the impact of alkalinity during the oxidation of metal ions, among other ideas. Paper chromatography (PC) and planar chromatography both use immobile phases that are solid, plane surfaces. A specific type of paper is used in these experiments as the stationary phase (Whatman quantitative grade 41 filter paper). A very small amount of sample is used for this qualitative study. Using various eluting agents, PC may be utilized to separate, identify transition metal ions (Fe^{3+} & Cr^{3+}) in the analytical group III, (Pb^{2+} & Cu^{2+}) in group II, (Co^{2+} & Ni^{2+}) in group IV, (Co^{2+} & Cu^{2+}) in group (IV) and group (II) and (Mo^{6+} & W^{6+}) in the group VI based on their colorful spots and retention factors (R_f) values. This qualitative study facilitated undergraduate students to realize the impact of PC to identify and separate pair of metal ions through different color spots and their retardation factor (R_f) values. *[African Journal of Chemical Education—AJCE 14(3), July 2024]*

INTRODUCTION

Paper chromatography experiments are frequently utilized in beginning laboratory courses both in organic and inorganic chemistry because they help students learn fundamental chromatographic principles as well as concepts related to polarity. Common experimental applications in organic chemistry comprise the investigation and detection of most important organic acids like citric acid, malic acid, tartaric acid and lactic acid in wine and fruit juices by paper chromatography (PC) [1], partition of food dyes, indicator dyes [2-4], the visualization of ninhydrin in amino acids [5,6] and tomato extracts [7]. In inorganic chemistry, metal ions can be quickly and effectively identified and separated by using PC. Qureshi et. al. experiment is based on rapid quantitative partition of Fe(II) and Fe(III) by paper chromatography. They used a mixture of 4M HCl, n-butanol, acetic acid and acetone (1:1:1:1) as developing solvent and detected by either NH_3 gas or 1,10-phenanthrolene [8]. Berg et. al. separated Co^{3+}, Cu^{2+} and Ni^{2+} ions as acetylacetonates by PC using a mixture of cyclohexane (84%), dioxane (10%) and methanol (6%) as developing solvent [9]. Bhatnagar et. al. described a new mechanism of PC of the partition of Ag^{+1}, Cu^{2+}, Ni^{2+}, Co^{2+}, Hg^{2+}, Pb^{2+} & Fe^{3+} with impregnated papers with aqueous glycine (2%, W/V), ammonium thiocyanate (4%, W/V) solution using polar and non-polar solvents like alcohols, ketones and chloroform [10]. Another method of separation of Pb^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, Ni^{2+}, Co^{2+} & UO_3^{2+} cations by stannic phosphate impregnated paper was reported by Qureshi et. al. [11]. Stevens separated Mo(VI) and Mo(V) ions by PC as oxinates and examined by UV spectroscopy [12]. The metal cations in the aqueous mixture which could contain Fe^{3+}, Ni^{2+}, and Cu^{2+} are separated using paper chromatography [13]. During this study, ammonia vapour and Dimethylglyoxime (DMG) used to develop the chromatogram, acetone and 8M HCl was used as eluting solution. Five metal cations Ag^{+}, Fe^{3+}, Co^{2+}, Cu^{2+}, and Hg^{2+} have been examined by paper chromatography [14]. The experiment involved treating an aqueous solution of KI and $K_4[Fe(CN)_6]$ as the eluting solution, and treating an aqueous HCl with ethyl and butyl alcohol as the mobile phase. The NCERT book describes how to separate the components of a combination of inorganic compounds including two cations, Pb^{2+} and Cd^{2+}, using chromatographic method [15]. The identification and isolation of inorganic cations (Co^{2+}, Ni^{2+}, and Cu^{2+}) were explained by S.C. Das [16]. The chromatographic separation and identification of Co^{2+}, Ni^{2+}, and Cu^{2+} ions were described by Nad et al. [17]. S.C. Das and Nad et al. both covered the use of an etanolic solution of rubeanic acid as a spraying reagent in a PC-based method of separating inorganic cations (Co^{2+}, Ni^{2+}, and Cu^{2+}). Earlier our research group identified and separated metal ions (Pb^{2+} & Cu^{2+}),(Co^{2+} & Ni^{2+}), (Cu^{2+} & Fe^{3+}), and (Co^{2+} & Cu^{2+}) by using different using solvent like 10% aqueous KI solution, 5% NH_4OH solution, 1N aqueous solution of potassium ferrocyanide and 10% NH_4OH solution as eluting agents, respectively. Four PC experiments namely (Pb^{2+} & Cu^{2+}), (Co^{2+} & Ni^{2+}), (Cu^{2+} & Fe^{3+}), and (Co^{2+} & Cu^{2+}) have been done by using higher concentration of different eluting solvents [18], later on concentration of eluting agents were minimized along with insertion of two new PC experiments and are described in this article.

Hence, in this work, green solvent like water is used as the universal mobile phase (developer) along with potassium ferrocyanide $K_4[Fe(CN)_6]$ as eluting agent during separation of metal ions like (Fe^{3+} and Cr^{3+}) , metal ions (Pb^{2+} & Cu^{2+}) are separated using 1% aqueous KI solution as the eluting solvent, (Co^{2+} & Ni^{2+}) group IV metal ions are separated using 4% NH_4OH solution as the spraying solvent, (Co^{2+} & Cu^{2+}) group (IV) and group (II) cations are separated using 6% NH_4OH solution as the eluting agent, and aqueous solution of $CuCl_2 . 2H_2O$ is used as eluting agent during separation of (Mo^{6+} and W^{6+}).

METHODOLOGY

i) Experimental

A. Required chemicals and apparatus

(i) Jar for chromatography, (ii) Measuring cylinder, (iii) Capillary, (iv) Tiny test tube, (v) Beakers (10mL, 100mL, and 500mL) , (vi) Grade 41 Whatman quantitative filter paper, (vii)

Chromium (III) oxide, (viii) Ferric chloride, (ix) Lead nitrate, (x) Copper sulfate, (xi) Nickel nitrate, (xii) Cobalt nitrate, (xiii) Sodium molybdate, (xiv) Sodium tungstate, (xv) 1(N) $K_4[Fe(CN)_6]$ solution, (xvi) 1% KI solution, (xviii) 4% NH_4OH solution, (xviii) 6% NH_4OH solution, (xix) 1(N) $CuCl_2 . 2H_2O$ solution.

(B) Required solution

(i) Solution of metal salts/oxide: To make a saturated solution, metal salts/oxide were dissolved in 1 mg/mL of distilled water in a 10 mL beaker.

Metal salts/oxide used:

(a) $FeCl_3$ & Cr_2O_3 (**PC experiment 1**)

(b) $Pb(NO_3)_2$ & $CuSO_4.5H_2O$ (**PC experiment 2**)

(c) $Ni(NO_3)_2.6H_2O$ & $Co(NO_3)_2.6H_2O$ (**PC experiment 3**)

(d) $Co(NO_3)_2.6H_2O$ & $CuSO_4.5H_2O$ (**PC experiment 4**)

(e) $Na_2MoO_4. 2H_2O$ & $Na_2WO_4. 2H_2O$ (**PC experiment 5**)

(ii) Eluting agents used:

(a) 100ml 1(N) $K_4[Fe(CN)_6]$ solution was prepared in a 250 mL beaker with distilled water (**PC experiment 1**); (b) 1% KI solution was prepared in a 100 mL beaker with distilled water (**PC experiment 2**); (c) 4% NH_4OH solution was prepared in a 100 mL beaker with distilled water (**PC**

experiment 3); (d) 6% NH_4OH Solution was prepared in a 100 mL beaker with distilled water (**PC experiment 4**); (e) 100ml 1(N) copper (II) chloride solution was prepared in a 250 mL beaker with distilled water (**PC experiment 5**).

(C) Green developer: Distilled water (500 mL).

ii) Experimental procedure

A Whatman 41 grade filter paper strip was perched inside the chromatographic jar. At about 0.5 centimeters from the bottom of the line created on this stripe, place a dot. This end, the bottom of the strip, will be the site of development. The saturated solutions of metal salts/oxide were administered independently by capillaries on 02 locations in the top of the chromatographic paper. Each time used fresh capillary. After that, the chromatographic paper containing two metal spots left exterior for dried out. Then, the dehydrated and spotted chromatographic paper strip perched in the jar of chromatography crammed with green solvent (distilled water) once more, bottom end contacted the green solvent and the upper end fastened to the steel bar. Water (green developer) allowable to ascend through the chromatographic paper strip (Figure 1a, 2a) until it approached the uppermost portion of the paper strip (Figure 1b, 2b). The solvent front was identified by using a pen after removing the chromatographic paper from the jar of chromatography. After that, chromatographic paper strip dehydrated to remove the developer. Then by sprayer, eluting agents as specified above were used over dry filter paper. In the PC experiment 1, one prussian blue and light brown spot immediately emerged with the 1(N) $K_4[Fe(CN)_6]$ reaction (Figure 1c), indicating the identification of Fe^{3+} and Cr^{3+} ions, respectively. In the PC experiment 2, one brown colored and one yellow colored spot immediately emerged with the 1% KI reaction (Figure 2c), indicating the identification of Pb^{2+} and Cu^{2+} ions, respectively. In the PC experiment 3, green gel coloration and bluish green coloration spot immediately emerged through the reaction of 4% NH_4OH (Figure 3c), indicating the identification of Ni^{2+} and Co^{2+} ions, respectively. In the PC experiment 3, initially, 1-2% NH_4OH solution was used but the results for Ni^{2+} was not adequate. With 3% NH_4OH, color spots obtained for both the metal ions but intensity of Ni^{2+} color spot was not very prominent (Figure 4). In the PC experiment 4, green and blue spot instantaneously appeared with the 6% NH_4OH reaction (Figure 5c), indicating the detection of Co^{2+} and Cu^{2+} ions, respectively. In the PC experiment 5, green coloration and light sky blue coloration spot immediately emerged through the reaction of 1(N) copper (II) chloride $CuCl_2$ solution (Figure 8c), indicating the identification of Mo^{6+} and W^{6+} ions, respectively. All colorful zones were marked with pencil.

RESULTS AND DISCUSSION

PC Experiment-1

In the chromatographic filter paper strip, berlin blue or prussian blue colored spot appeared [19] due to the formation of $Fe_4[Fe(CN)_6]_3$, iron(III) hexacyanidoferrate(II), when metal salt, $FeCl_3$ was combined with eluting solvent 1(N) $K_4[Fe(CN)_6]$.

$$4\,FeCl_3 + 3\,K_4[Fe(CN)_6] \rightarrow Fe_4[Fe(CN)_6]_3 \downarrow + 12KCl$$

(**Prussian blue spot**)

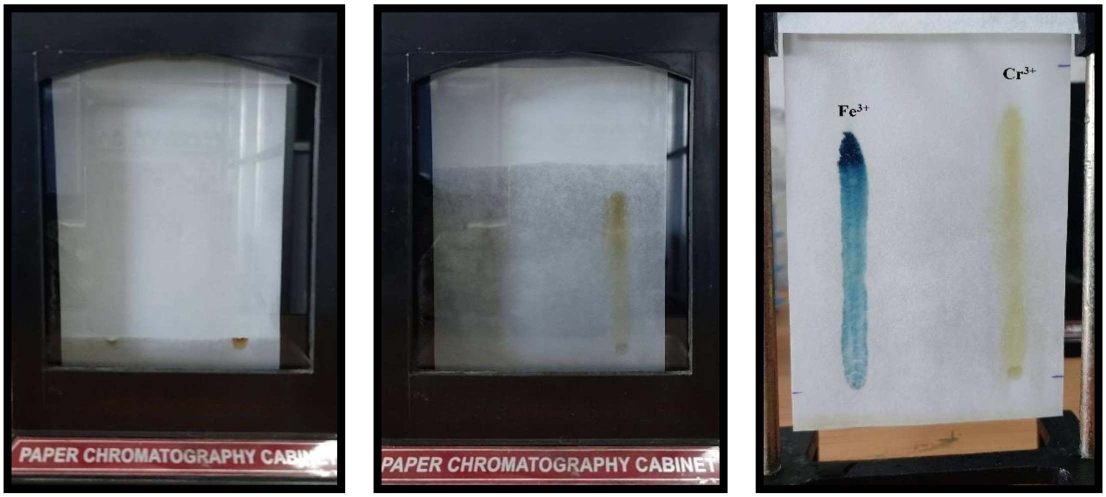
$$2Cr^{3+} + K_4[Fe(CN)_6] \rightarrow \{Cr[Fe(CN)_5OH]\}^{-1} \downarrow + 2\,CN^-$$

(**Brown spot**)

Conversely, in the filter paper strip, Cr^{3+} ion combined with a 1(N) aqueous solution of $K_4[Fe(CN)_6]$ to generate $\{Cr[Fe(CN)_5OH]\}^{-1}$, a light brown coloring spot [20].

By contrasting the retention factor values and color spots of the two cations (Fe^{3+} and Cr^{3+}), they were distinguished from one another. Because $Fe_4[Fe(CN)_6]_3$, formed, the first spot looked as prussian blue. The distance traveled by the solute zone, Fe^{3+} (ds_1), was indicated by the reaction of aqueous solution of 1 (N) $K_4[Fe(CN)_6]$ with $FeCl_3$. The second spot for Cr^{3+}, emerged as light brown owing to the development of $\{Cr[Fe(CN)_5OH]\}^{-1}$.The reaction of aqueous solution of 1 (N) $K_4[Fe(CN)_6]$ with Cr^{3+} in Cr_2O_3 showed the space travelled by another solute zone, Cr^{3+} (ds_2). We next calculated retention factors (R_f) or retardation factors (**Table-1**). By comparing color spots and retardation factors, two cations (Fe^{3+} and Cr^{3+}) were recognized and distinguished from one another.

$$\text{Retardation factor } (R_f) = \frac{\text{The solute zone center's travel distance in cm (ds)}}{\text{The solvent front's transit distance in cm (dm)}}$$



(a) **(b)** **(c)**

Figure 1. Separation of transition metal ions (Fe^{3+} and Cr^{3+}) by PC

PC Experiment-2

When 1% KI reacted with metal salt, $CuSO_4.5H_2O$ solution, a brown spot formed on the chromatographic paper as a result of the redox reaction. In this case $CuSO_4$ interacts with KI, Cu(II) converted into Cu(I) with I^- ions, and simultaneously I^- ions oxidized into I_2. Since I_2 fled swiftly, the Cu^{2+} ion (solute zone) moved a space where Cu^{2+} reacts with potassium iodide. This was promptly observed by writing a pen mark on the brown color spot.

$$\mathbf{2CuSO_4\ (aq) + 4KI\ (aq) \rightarrow 2Cu_2I_2 + I_2 + 2K_2SO_4}$$

(Brown spot)

But lead nitrate [$Pb(NO_3)_2$] and potassium iodide (KI) combined to create an ion exchange that produced potassium nitrate (KNO_3) and a yellow-colored lead iodide spot (PbI_2).

$$\mathbf{Pb(NO_3)_2\,(aq) + 2KI\ (aq) \rightarrow PbI_2(s)\ (\downarrow) + 2KNO_3(aq)}$$

(Yellow spot)

By comparing color spots and retardation factors, two cations were recognized and distinguished from one another.

$$\text{Retardation factor } (R_f) = \frac{\text{The solute zone center's travel distance in cm (ds)}}{\text{The solvent front's transit distance in cm (dm)}}$$

Then, determine the metal cations by looking at the colorful patches that matched two distinct cations. As a result of I_2 being released following KI's reaction with $CuSO_4$, the first spot looked brown. One solute zone's travel distance represented by Cu^{2+} (ds_1), and another solute zone's travel distance, represented by Pb^{2+} (ds_2). In every experiment, the solvent's travel distance (dm) was calculated by measuring the separation between the starting line and the solvent front (water). The values of retardation factors (R_f) were then calculated (**Table-1**).

Figure 2. Cu^{2+} and Pb^{2+} separation using paper chromatography by 1% KI solution

PC Experiment-3

Green gel and bluish green spots on the filter paper are the result of mixing nickel and cobalt nitrate solutions with 4% NH_4OH solution to create metal (II) hydroxide [21].

$$\mathbf{Ni^{2+} + NH_4OH \rightarrow Ni(OH)_2(s)\downarrow}$$

(Green gel spot)

$$\mathbf{Co^{2+} + NH_4OH \rightarrow Co(OH)_2\,(s)\downarrow}$$

(Bluish green spot)

By contrasting their color spots and retention factor values, two cations Co(II) and Ni(II) were recognized and distinguished from one another. When 4% NH_4OH solution reacted with $Ni(NO_3)_2.6H_2O$, nickel (II) hydroxide $Ni(OH)_2$ was formed, and the first spot emerged as green gel. After $Co(NO_3)_2.6H_2O$ reacted with a 4% NH_4OH solution, $Co(OH)_2$ was produced, which is what gave the appearance of second spot (Co^{2+}) as bluish green. One solute zone's travel distance represented by Ni^{2+} (ds_3), and another solute zone's travel distance, represented by Co^{2+} (ds_4). The values of retardation factors (R_f) were then calculated (**Table-1**).

(a) **(b)** **(c)**

Figure 3. Ni^{2+} and Co^{2+} separation using paper chromatography by 4% NH_4OH solution

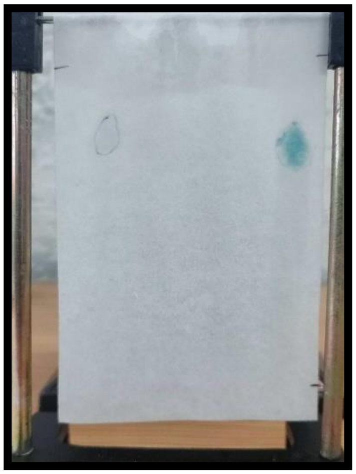


Figure 4. Ni^{2+} and Co^{2+} separation using paper chromatography by 3% NH_4OH solution

PC Experiment-4

Copper (II) hydroxide $Cu(OH)_2$ is produced when metal salt and 6% NH_4OH solution react, producing a blue stain on the Whatman filter paper [19]. Rather than the blue green $Co(OH)_2$ that occurs from the reaction of Co(II) nitrate with 4% NH_4OH solution (PC experiment 2), green colored spots are produced when Co(II) nitrate combines with 6% NH_4OH solution to form Co(III) hydroxide, or $Co(OH)_3$. Generally, cobalt(II) salts are stable; however, in basic solutions (100 ml 6% NH_4OH), cobalt(II) is easily transformed to cobalt(III). The basic character of the reaction medium increases with increasing NH_4OH solution concentrations (4% to 6%), and air oxidation of Co(II) to Co(III) occurs reasonably quickly with increasing basic medium concentrations [22,23].

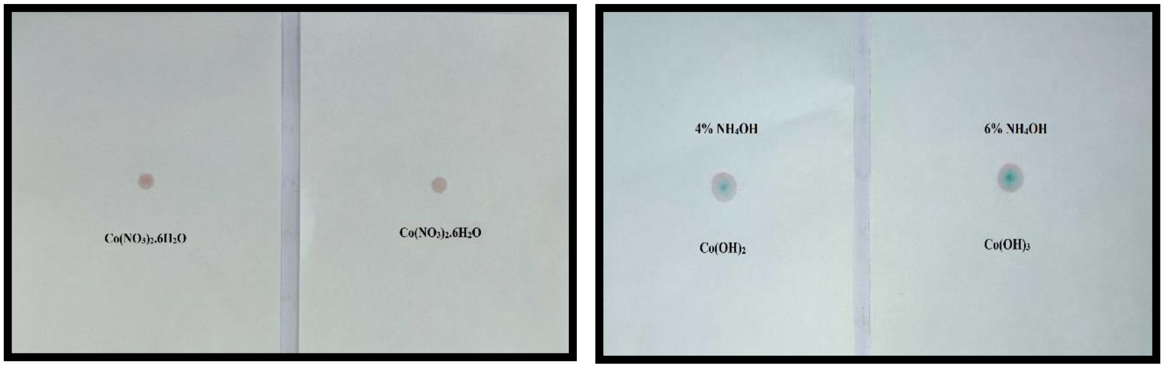
Consequently, a green colored spot arises from the oxidation of bluish green colored cobalt(II)hydroxide to cobalt(III)hydroxide. It was further verified using the Whatman chromatography paper's spot test of $Co(NO_3)_2.6H_2O$ solution with separate solutions of 4% and 6% NH_4OH (**Figure 6**). When cobalt (II) nitrate solution (**Figure 6a**) was spot tested with 4% NH_4OH, it was found that a blue green color appeared (**Figure 6b** Left), and when 6% NH_4OH was added, a green color developed right away (**Figure 6b** Right).

$$Co^{2+} + 6\%\ NH_4OH \rightarrow \underset{\textbf{(Green spot)}}{Co(OH)_3\,(s)} \downarrow$$

$$Cu^{2+} + 6\%\ NH_4OH \rightarrow \underset{\textbf{(Blue spot)}}{Cu(OH)_2(s)} \downarrow$$

(a) **(b)** **(c)**

Figure 5: Co^{2+} and Cu^{2+} separation using paper chromatography by 6% NH_4OH solution



(a) **(b)**

Figure 6: $Co(NO_3)_2.6H_2O$ spot detection within the Whatman chromatography strip (a) prior to and (b) with the addition of 4% and 6% NH_4OH solution

The green spot underwent FT-IR analysis using a Bruker Alpha II model spectrometer, operating in the 4000–400cm^{-1} range. The hydroxyl assembly in the Co(III) hydroxide typically experiences stretching and bending vibrations in the IR region. About 1638 cm^{-1}, a bending vibration is seen, and within 3490-3499 cm^{-1} is often where the tough broad band for the -OH stretching vibration occurs [24]. These vibrations indicate that the cobalt(III)hydroxide containing –OH group. For a Co-O bond, the average range of an IR frequency is 400–600cm^{-1}. However, in the green area, the Co-O bond's IR band is located at 647 cm^{-1} (Figure 7). In the higher oxidation state of cobalt atom, such (+3) or (+4), there is usually a (+ve) shift in the i.r. frequency of the cobalt-oxygen bond (Co-O). Metal-oxygen bond gets stronger in higher oxidation states, which raises the vibrational frequency [25]. The presence of cobalt in the (+3) state in cobalt(III)hydroxide, is therefore suggested by the observed i.r. frequency of the cobalt-oxygen linkage, resulted green color spot.

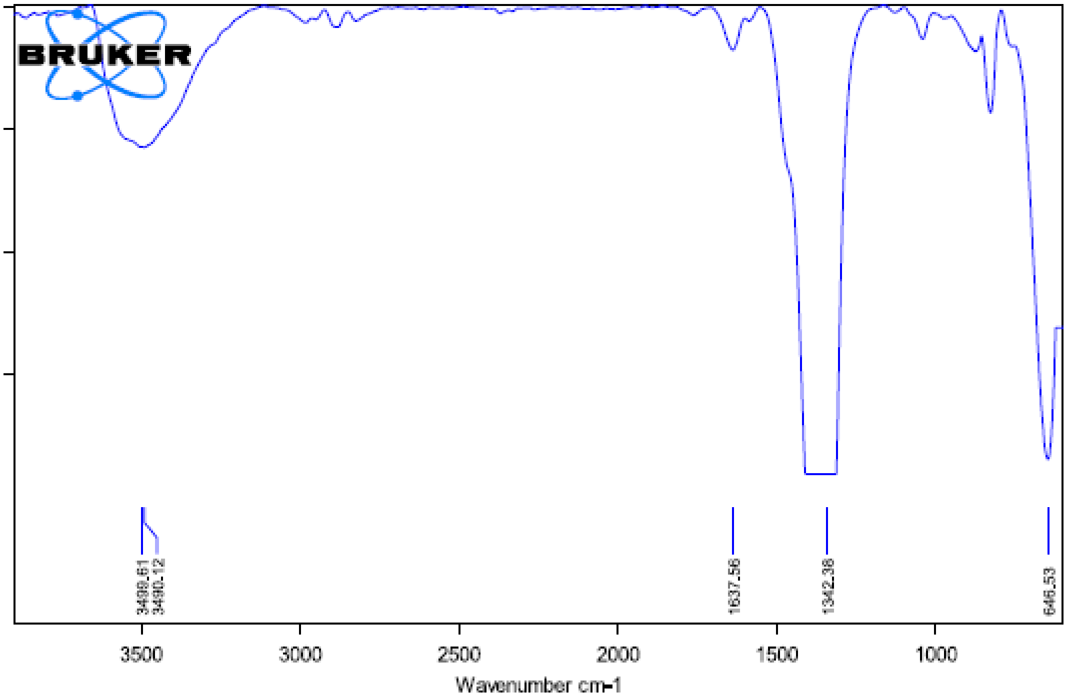


Figure **7.** IR spectrum of green ppt of $Co(OH)_3$

The color spots and retention factor values of two cations (Co^{2+} and Cu^{2+}) were compared in order to identify and distinguish them. The first spot turned green as a result of the reaction between the $Co(NO_3)_2.6H_2O$ and 6% NH_4OH solution, which produced the cobalt (III) hydroxide $Co(OH)_3$.

Cobalt(II), one solute zone, which is easily oxidized into cobalt(III) in basic medium, traveled the distance indicated by (ds_7). The second spot (Cu^{2+}) became blue because $CuSO_4.5H_2O$ and 6% NH_4OH solution reacted to generate copper (II) hydroxide $Cu(OH)_2$. Cu^{2+}, the other solute zone, traveled the distance indicated by (ds_8). We next calculated retardation factors (R_f) (**Table-1**).

PC Experiment-5

Green and light sky blue coloration spots on the filter paper are the result of mixing sodium molybdate, $Na_2MoO_4.\ 2H_2O$ and sodium tungstate, $Na_2WO_4.\ 2H_2O$ with copper (II) chloride $CuCl_2$ solution to create $CuMoO_4$ and $CuWO_4$ respectively [26-29].

$$CuCl_2 + Na_2MoO_4 \rightarrow CuMoO_4 \downarrow + 2\ NaCl$$

(Green spot)

$$CuCl_2 + Na_2WO_4 \rightarrow CuWO_4 \downarrow + 2\ NaCl$$

(Sky Blue spot)

By contrasting their color spots and retention factor values, two cations Mo(VI) and W(VI) were recognized and distinguished from one another. When aqueous solution of copper (II) chloride reacted with sodium molybdate, $Na_2MoO_4.\ 2H_2O$, cupric molybdate $CuMoO_4$ was formed, and the first spot emerged as green (Mo^{6+}). After sodium tungstate, $Na_2WO_4.\ 2H_2O$ reacted with aqueous solution of copper (II) chloride, $CuWO_4$was produced, which is what gave the appearance of second spot (W^{6+}) as light sky blue. One solute zone's travel distance represented by Mo^{6+} (ds_3), and another solute zone's travel distance, represented by W^{6+} (ds_4). The values of retardation factors (R_f) were then calculated (**Table-1**).

(a) **(b)** **(c)**

Figure 8. Separation of transition metal ions (Mo^{6+} and W^{6+}) by PC

Table 1: Metal ions separation and identification using paper chromatography

Experiment Number	**Metal salt/oxide (Cation Present)**	**Eluting agent**	**Color spots**	**Distance travelled by solutes (ds in cm)**	**Distance travelled by mobile phase (dm in cm)**	**R_f value = ds/dm**
1	$FeCl_3$ (Fe^{3+})	1(N) solution of $K_4[Fe(CN)_6].3H_2O$ (aq)	Prussian blue	10.7 (ds_1)	13.5	0.79
	Cr_2O_3 (Cr^{3+})		Light brown	11.8 (ds_2)	13.5	0.87
2	$CuSO_4.5H_2O$ (Cu^{2+})	1% KI solution (aq)	Brown	12.8 (ds_1)	14	0.91
	$Pb(NO_3)_2$ (Pb^{2+})		Yellow	11.6 (ds_2)	14	0.83
3	$Ni(NO_3)_2.6H_2O$ (Ni^{2+})	4% NH_4OH solution (aq)	Green gel	12.4 (ds_3)	15.0	0.82
	$Co(NO_3)_2.6H_2O$ (Co^{2+})		Bluish green	12.8 (ds_4)	15.0	0.85
4	$Co(NO_3)_2.6H_2O$ (Co^{2+})	6% NH_4OH solution (aq)	Green	12.1 (ds_7)	14.2	0.85
	$CuSO_4.5H_2O$ (Cu^{2+})		Blue	13.2 (ds_8)	14.2	0.93
5	$Na_2MoO_4. 2H_2O$ (Mo^{6+})	1N solution of $CuCl_2.2H_2O$ (aq)	Green	12.5 (ds_3)	13.1	0.95
	$Na_2WO_4. 2H_2O$ (W^{6+})		Light sky blue	11.7 (ds_4)	13.1	0.89

EVALUATION OF LEARNING OUTCOMES

These five (05) experiments were carried out with two different groups of second year undergraduate students at the inorganic chemistry laboratory at the Bir Bikram Memorial College (20 students in total). The identification and separation of metal ions using paper chromatography usually requires a combination of critical thinking, practical skills, and theoretical understanding as learning goals. The following are the learning target for these experiments: 1. Help students comprehend the fundamentals of paper chromatography. 2. Describe the elements (basic medium strength) that influence the metal ion separation process using paper chromatography. 3. Use chromatogram interpretation to find and examine metal ions. 4. Connect color patches to metal ion properties. 5. To compare the separation of various metal ions qualitatively, use R_f values. 6. Identify and resolve typical problems that could come up throughout the chromatographic procedure. 7. Assess the accuracy and dependability of the experimental findings. 8. Clearly and succinctly present your findings. 9. Use caution when handling chemicals and scientific equipment. These learning objectives provide a thorough grasp of the identification and separation of metal ions using paper chromatography by addressing a variety of skills and knowledge domains. Depending on the particular aims and objectives of the educational institution or course, adjustments can be necessary. When comparing the experimental R_f values to the student average R_f values shown in Table 2, it was determined that the largest difference between the student average R_f values for two trials and our experimental R_f values was 0.05. Students answered several chromatography theory-related post-lab questions properly (90% accurate) and correctly identified metal ions (85% correct). After completing paper chromatography experiments, students believed that "the experiment worked well so that they got good results," according to their survey replies. They gave these experiments 9 out of 10, saying it was "interesting to perform," and they would "recommend others to do the lab”.

Table 2. Comparison of the student average R_f values with our experimental R_f values

Experiment Number (Cations present)	**Experimental R_f value**	**Student’s R_f averages**	**Difference**
1 (Fe^{3+} & Cr^{3+})	Fe^{3+} - 0.79 Cr^{3+} - 0.87	Fe^{3+} - 0.81 Cr^{3+} - 0.92	Fe^{3+} - (± 0.02) Cr^{3+} - (± 0.05)
2 (Cu^{2+} & Pb^{2+})	Cu^{2+} - 0.91 Pb^{2+} - 0.83	Cu^{2+} - 0.92 Pb^{2+} - 0.87	Cu^{2+} - (± 0.01) Pb^{2+} - (± 0.04)
3 (Ni^{2+} & Co^{2+})	Ni^{2+} - 0.82 Co^{2+}- 0.85	Ni^{2+} - 0.80 Co^{2+} - 0.83	Ni^{2+} - (± 0.02) Co^{2+} - (± 0.02)
4 (Co^{2+} & Cu^{2+})	Co^{2+} - 0.85 Cu^{2+} - 0.93	Co^{2+} - 0.84 Cu^{2+} - 0.88	Co^{2+} - (± 0.01) Cu^{2+} - (± 0.05)
5 (Mo^{6+} & W^{6+})	Mo^{6+} - 0.95 W^{6+}- 0.89	Mo^{6+} - 0.91 W^{6+} - 0.86	Mo^{6+} - (± 0.04) W^{6+} - (± 0.03)

HAZARDOUS STATEMENT

Ensuring the safe handling of corrosive and hazard chemicals, students should advise to wear appropriate eye protection like chemical splash goggles and disposable latex gloves for the safety of skin along with a face shield. Keeping in mind about the hazardness of NH_4OH, we minimize the concentration of the eluting agent NH_4OH, diluting with water, which is less toxic and less volatile and for the sake of additional safety precaution minimizing concentration of NH_4OH may also be done under a fume hood.

INSTRUCTOR NOTES

For stockroom preparation

(i) Solution of metal salts/oxide: To make saturated solution of metal salts/oxide, they are dissolved in 1 mg/mL of distilled water in a 10 mL beaker.

ii) Eluting agents preparation:

Preparation of 1N 100mL $K_4[Fe(CN)_6].3H_2O$ solution (PC experiment 1): Weight out 10.556g $K_4[Fe(CN)_6].3H_2O$ and dissolve in distilled water and then make up the volume 100 mL.

Preparation of 1% KI 100 mL solution (PC experiment 2): Weight out 1g KI and dissolve in 100 mL distilled water.

Preparation of 4% NH_4OH 100 mL solution (PC experiment 3): Take 16 mL of 25% NH_3 solution and mix with it 84 mL distilled water.

Preparation of 6% NH_4OH 100 mL solution (PC experiment 4): Take 24 mL of 25% NH_3 solution and mix with it 76 mL distilled water.

Preparation of 1N 100mL copper (II) chloride, $CuCl_2.2H_2O$ solution (PC experiment 5): Weight out 8.524g $CuCl_2.2H_2O$ and dissolve in distilled water and then make up the volume 100 mL.

Answer key to five experiments is given in Table 3.

Table 3. Answer key to solutes (PC Experiment 1 to PC Experiment 5)

Experiment Number (Metal ions present)	**Color spots**	**Experimental R_f value (R_f value = ds/dm)**
1 (Fe^{3+}& Cr^{3+})	Fe^{3+} - Prussian blue	0.79 (± 0.02)
	Cr^{3+}- Light brown	0.87 (± 0.05)
2 (Cu^{2+} & Pb^{2+})	Cu^{2+}- Brown	0.91 (± 0.01)
	Pb^{2+}-Yellow	0.83 (± 0.04)
3 (Ni^{2+} & Co^{2+})	Ni^{2+}- Green gel	0.82 (± 0.02)
	Co^{2+}- Bluish green	0.85 (± 0.02)
4 (Co^{2+} & Cu^{2+})	Co^{2+}- Green	0.85 (± 0.01)
	Cu^{2+} - Blue	0.93 (± 0.05)
5 (Mo^{6+} & W^{6+})	Mo^{6+} - Green	0.95 (± 0.04)
	W^{6+}- Light sky blue	0.89 (± 0.03)

CONCLUSIONS

In these experiments, paper chromatography allows for the separation of distinct metal ions according to how far apart they migrate on the chromatography paper. It is possible to ascertain the identification of the metal ions from their retardation factor (Rf) values. Visualizing metal ions [(Fe^{3+}, Cr^{3+}), (Pb^{2+} & Cu^{2+}), (Ni^{2+} & Co^{2+}), (Co^{2+} & Cu^{2+}), and (Mo^{6+}, W^{6+})] on a chromatogram, they frequently display distinctive colors. These distinct identifiers along with different retardation factor (R_f) values facilitate the identification procedure and increase the analysis accuracy. Through these experiments, separation and identification of transition metal ions [(Fe^{3+}, Cr^{3+}), (Pb^{2+} & Cu^{2+}), (Ni^{2+} & Co^{2+}), (Co^{2+} & Cu^{2+}), and (Mo^{6+}, W^{6+})] from different analytical groups have been carried out using water as the universal mobile phase (green developer) with various eluting agents through different color spots and R_f values by paper chromatography. Consequently by utilizing these improved techniques, make paper chromatography easier for UG students to separate and identify metal ions in the qualitative analysis.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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REFERENCES

[1] Samarasekara, D., Hill, C., Mlsna, D. Analysis and identification of major organic acids in wine and fruit juices by paper chromatography. *J. Chem. Educ.*, **2018**, 95(9), 1621-1625. https://doi.org/10.1021/acs.jchemed.8b00129.

[2] Birdwhistell, K. R., Spence, T. G. A New Glow on the Chromatography of M&M candies. *J. Chem. Educ.*, **2002**, 79(7), 847. https://doi.org/10.1021/ed079p847.

[3] Markow, P. G. The ideal solvent for paper chromatography of food dyes. *J. Chem. Educ.*, **1988**, 65(10), 899. https://doi.org/10.1021/ed065p899.

[4] Sharma, V., McKone, H. T., Markow, P. G. A global perspective on the history, use, and identification of synthetic food dyes. *J. Chem. Educ.*, **2011**, 88(1), 24-28. https://doi.org/10.1021/ed100545v.

[5] Stoffyn, P. J., Jeanloz, R. W. Identification of amino sugars by paper chromatography. *Arch. Biochem. Biophys.*, **1954**, 52(2), 373-379. https://doi.org/10.1016/0003-9861(54)90137-X.

[6] Fowden, L. The quantitative recovery and colorimetric estimation of amino-acids separated by paper chromatography. *Biochem. J.*, **1951**, 48(3), 327. https://doi.org/10.1042/bj0480327.

[7] Wu, M. A., Burrell, R. C. Flavonoid pigments of the tomato (Lycopersicum esculentum Mill.). *Arch. Biochem. Biophys.*, **1958**, 74(1), 114-118. https://doi.org/10.1016/0003-9861(58)90205-4.

[8] Qureshi, M., Akhtar, I., Varshney, K. G. Fast Quantitative Separation of Iron (II) and Iron (III) by Paper Chromatography. *Anal. Chem.*, **1966**, 38(10), 1415-1417. https://doi.org/10.1021/ac60242a036.

[9] Berg, E. W., Strassner, J. E. Paper Chromatography of Cobalt (III), Copper (II), and Nickel (II) Acetylacetonates. *Anal. Chem.*, **1955**, 27(1), 127-129. https://doi.org/10.1021/ac60097a041.

[10] Bhatnagar, R. P., Bhatnagar, N. P. Mechanism of Paper Chromatography of Inorganic Ions on Impregnated Papers: Separation of Ag (I), Cu (II), Ni (II), Co (II), Hg (II), Pb (II) & Fe (III),” *Indian J. Chem.*, **1977**, 15(A), 1089-1091. https://nopr.niscpr.res.in/bitstream/123456789/53344/1/IJCA%2015A%2812%29%201089-1091.pdf.

[11] Qureshi, S. Z. Chromatography of some metal ions on paper impregnated with stannic phosphate. *J. Chromatogr. A*, **1966**, 22, 198-200. https://doi.org/10.1016/S0021-9673(01)97090-6.

[12] Stevens, H. M. The separation of molybdenum valencies by paper chromatography part II1. *Anal. Chim. Acta*, **1956**, 14, 126-130. https://doi.org/10.1016/0003-2670(56)80138-4.

[13] Altig, J. CHEM 121 L. Section 1, Partner: Mr. Magoo, Paper chromatography of a Metal cation *Mixture*, PDF file, Available at (https://www.nmt.edu/academics/chemistry/people/jaltig/docs/ModelChromReport.pdf), 2009 (accessed Dec 08, **2023**).

[14] Paper Chromatography-Separation and identification of Five Metal Cations (Experiment), PDF file. Available at (chem.libretexts.org), (online Library), Available at https://batch.libretexts.org/print/url=https://chem.libretexts.org/Ancillary_Materials/Laboratory_Experiments/Wet_Lab_Experiments/General_Chemistry_Labs/Online_Chemistry_Lab_Manual/Chem_11_Experiments/03%3A_Paper_Chromatography-_Separation_and_Identification_of_Five_Metal_Cations_(Experiment).pdf , (University of California, Davis, US), 2021 (accessed Dec 08, **2023**).

[15] Science laboratory manuals. PDF File, NCERT Unit 5, Chromatography, 34-39, Available athttps://ncert.nic.in/pdf/publication/sciencelaboratorymanuals/classXII/chemistry/lelm105.pdf (accessed Dec 08, **2023**).

[16] Das S.C., "*Advanced Practical Chemistry*," 6th Edition, **2017**, 417-419, ISBN:81-901944-0-2.

[17] Nad, A. K. "*An Advanced Course in Practical Chemistry*," **2003**, 84-86, ISBN: 978 81 7381 302 3.

[18] Das A., Sharma D., Debnath P., Paul B. Metal Ions Separation Via Paper Chromatography: Enhanced Methods Using Eluting Solutions, *World J. chem. Educ.*, **2023**, 11(4), 134-140. https://pubs.sciepub.com/wjce/11/4/2/index.html.

[19] Kawatake, K., Shigemoto, N. Preparation of potassium iron (III) hexacyanoferrate (II) supported on activated carbon and Cs uptake performance of the adsorbent. *J. Nucl. Sci. Technol.*, **2012**, 49(11), 1048-1056. https://doi.org/10.1080/00223131.2012.730894.

[20] Bembi, R., Malik, W. U. Reaction of Cr (III) with Potassium Hexacyanoferrate (II). *Z. Phys. Chem.*, **1975**, 256(1), 865-869. https://doi.org/10.1515/zpch-1975-256108.

[21] The University of Colorado Boulder, Equilibrium – Complex ions – Metal + ammonia complexes, (Library webpage), Available at (https://www.colorado.edu/lab/lecture-demo-manual/e740-equilibrium-complex-ions-metal-ammonia-complexes), p.E740 (accessed Dec 08, **2023**).

[22] Kavitha, K., "Effect of ammonia on the redox reaction of cobalt(II) with potassium ferricyanide in alkaline medium" *Int.. J. Adv. Res. Innov. Technol.*, **2018**, 4, 766-770, https://www.ijariit.com/manuscripts/v4i4/V4I4-1451.pdf.

[23] Clark, J., Chemistry of cobalt. Available at (chem.libretexts.org)., https://chem.libretexts.org/Bookshelves/Inorganic_Chemistry/Supplemental_Modules_and_Websites_(Inorganic_Chemistry)/Descriptive_Chemistry/Elements_Organized_by_Block/3_d-Block_Elements/Group_09%3A_Transition_Metals/Chemistry_of_Cobalt(University of California, Davis, US), (accessed Dec 08, **2023**).

[24] *The nature of vibrational spectroscopy*, Michigan State University, (webpage), Available athttps://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/spectrpy/infrared/irspec1.htm(accessed Dec 08, **2023**).

[25] Andris, E., Navrátil, R., Jašík, J., Srnec, M., Rodríguez, M., Costas, M., Roithová, J. M− O Bonding Beyond the Oxo Wall: Spectroscopy and Reactivity of Cobalt (III)-Oxyl and Cobalt (III)-Oxo Complexes. *Angew. Chem. Int. Ed.*, **2019**, 58(28), 9619-9624, https://doi.org/10.1002/ange.201904546.

[26] Sciencemadness Discussion Board, Synthesis of an artificial analogue of a molybdenum mineral, **2007**. https://www.sciencemadness.org/talk/viewthread.php?tid=8643.

[27] Tan, W., Luan, J. Investigation into the synthesis conditions of CuMoO 4 by an in situ method and its photocatalytic properties under visible light irradiation. *RSC Adv.*, **2020**, 10(16), 9745-9759. https://doi.org/10.1039/D0RA00496K.

[28] Unadkat, S., Solanki, G. K. Growth and Structural Characterization of CuWO4 Crystals by Flux Growth Method. *Res. Appl. Emerg. Technol.*, **2020**, 2(2), 7-12. http://hbrppublication.com/OJS/index.php/RAET/article/view/1349.

[29] Kannan S., Mohanraj K., Siva Kumar G. Effect of Annealing on the Preparation of $CuWO_4$ Particles, Juniper Online Journal Material Science, **2017**, 2(1): 555577. https://doi.org/10.19080/JOJMS.2017.02.555577.