

Advanced Methods for the Separation and Identification of p and d block elements by Paper Chromatography

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Abstract:

The objective of this study was to utilize paper chromatography (PC) as a cost-effective and accessible method to impart fundamental principles of chromatography to undergraduate students in introductory inorganic chemistry courses. The experiments focused on detecting and separating metal ions from various groups in the analytical table. Specifically, the study aimed to educate students on the separation and identification of metal ions, comparing color spots and retention factor values, and understanding the influence of alkalinity during metal ion oxidation. Paper chromatography and planar chromatography share the use of solid, flat stationary phases. In this study, Whatman quantitative grade 41 filter paper was employed as the stationary phase. Minimal sample quantities were utilized for qualitative analysis. Through the use of different eluting agents, PC was employed to separate and identify transition metal ions such as (Fe^{3+} & Cr^{3+}) in group III, (Pb^{2+} & Cu^{2+}) in group II, Co^{2+} & Ni^{2+} in group IV, (Co^{2+} & Cu^{2+}) in group IV and II, (Cu^{2+} & Fe^{3+}) in group II and III, and (Mo^{6+} & W^{6+}) in group VI, based on their distinct color spots and retention factor (R_f) values. This qualitative investigation enabled undergraduate students to appreciate the efficacy of PC in identifying and separating pairs of metal ions through observation of different color spots and their corresponding R_f values.

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Introduction:

Paper chromatography (PC) experiments are frequently employed in introductory laboratory courses for both organic and inorganic chemistry, serving as valuable tools for teaching fundamental chromatographic principles and polarity concepts. In organic chemistry, PC finds applications in various experiments, including the detection of organic acids like citric acid, malic acid, tartaric acid, and lactic acid in wine and fruit juices (Samarasekara et al., 2018), partitioning of food and indicator dyes (Birdwhistell et al., 2002; Markow, 1988; Sharma et al., 2011), visualization of ninhydrin in amino acids and tomato extracts (Stoffyn et al., 1954; Fowden, 1951; Wu et al., 1958). In the realm of inorganic chemistry, PC serves as an effective method for the rapid identification and separation of metal ions. Qureshi et al. demonstrated a method for the quantitative partition of Fe(II) and Fe(III) using PC with a mixture of 4M HCl, n-butanol, acetic acid, and acetone (1:1:1:1) as the developing solvent (Qureshi et al., 1966). Berg et al. separated Co^{3+} , Cu^{2+} and Ni^{2+} ions as acetylacetonates using PC with a developing solvent comprising cyclohexane (84%), dioxane (10%), and methanol (6%) (Berg et al., 1955). Bhatnagar et al. (1977) described a novel mechanism for PC involving the partition of various metal ions viz. Ag^+ , Cu^{2+} , Ni^{2+} , Co^{2+} , Hg^{2+} , Pb^{2+} & Fe^{3+} using impregnated papers with aqueous glycine (2%, W/V) and ammonium thiocyanate (4%, W/V) solutions, employing polar and non-polar solvents such as alcohols, ketones, and chloroform (Bhatnagar et al., 1977). Furthermore, PC has been utilized for the separation of Pb^{2+} , Cu^{2+} , Fe^{3+} , Fe^{2+} , Ni^{2+} , Co^{2+} & UO_3^{2+} cations using stannic phosphate impregnated paper (Qureshi et al., 1966), as well as for the separation of Mo(VI) and Mo(V) ions by Stevens as oxinates examined via UV spectroscopy (Stevens, 1956). Recent studies explored the separation and examination of various metal cations Ni^{2+} , and Cu^{2+} , Ag^+ , Fe^{3+} , Co^{2+} , Cu^{2+} , and Hg^{2+} using PC, employing different eluting and developing solutions (Altig, 2023; Paper Chromatography pdf, 2023). NCERT Unit 5 provided insights into separating inorganic compounds, including Pb^{2+} and Cd^{2+} cations, via chromatographic methods (NCERT Unit 5 pdf, 2023). Additionally, Das (2017) and Nad et al. (2003) detailed the chromatographic separation and identification of Co^{2+} , Ni^{2+} , and Cu^{2+} ions, utilizing an ethanolic solution of rubeanic acid as a spraying reagent (Das, 2017; Nad, 2003). The p and d block elements play a vital structural and functional role, influencing function of enzymes and structures of biomolecules (Bhattacharjee et al. 2022; Adhikari et al. 2020; Singh et al. 2018; Nath et al. 2024). Thus, the easy methods of separation and identification of p and d block elements plays a critical role in chemistry.

In our own research (Das et al., 2023), we identified and separated metal ions such as (Pb^{2+} & Cu^{2+}), (Co^{2+} & Ni^{2+}), (Cu^{2+} & Fe^{3+}), and (Co^{2+} & Cu^{2+}) using different eluting agents including 10% aqueous KI solution, 5% NH_4OH solution, 1N aqueous solution of potassium ferrocyanide, and 10% NH_4OH solution, respectively. These experiments were further refined and supplemented with two new PC experiments in this study (Das et al., 2023).

Hence, in this work, green solvent like water is used as the universal mobile phase (developer) along with potassium ferrocyanide $\text{K}_4[\text{Fe}(\text{CN})_6]$ 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, group II and group III metal ions (Cu^{2+} & Fe^{3+}) with 1N aqueous solution of potassium ferrocyanide $\text{K}_4[\text{Fe}(\text{CN})_6]$ as spraying agent, and aqueous solution of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ is used as eluting agent during separation of (Mo^{6+} and W^{6+}) metal ions.

Paper Chromatography Experiment-1: Separation of Group III Metal ions (Fe^{3+} and Cr^{3+}) by 1(N) $\text{K}_4[\text{Fe}(\text{CN})_6]$ Solution

Experimental

Required chemicals and apparatus

(i) Chromatography Jar, (ii) Measuring cylinder, (iii) Capillary, (iv) Tiny test tube, (v) Beakers (10 mL, 100 mL, and 500 mL), (vi) Grade 41 Whatman quantitative filter paper, (vii) Chromium (III) oxide, (viii) Ferric chloride, (ix) 1(N) $\text{K}_4[\text{Fe}(\text{CN})_6]$ solution.

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: FeCl_3 & Cr_2O_3
(ii) Eluting agents used: 100 mL 1(N) $\text{K}_4[\text{Fe}(\text{CN})_6]$ solution was prepared in a 250 mL beaker with distilled water

Green developer

500 mL distilled water is used as green developer.

Experimental procedure

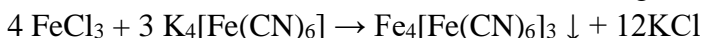
A strip of Whatman 41 grade filter paper was carefully positioned inside the chromatographic jar, with a dot placed approximately 0.5 centimetres from the bottom as the starting point for development. Using fresh capillaries for each application, saturated solutions of metal salts/oxides were individually deposited onto two locations at the top of the chromatographic paper. Subsequently, the paper was left exposed to air to allow the spotted areas to dry. Once dry, the chromatographic paper strip was placed back into the chromatography jar filled with a green solvent (distilled water), with the bottom end submerged in the solvent and the upper end secured to a steel bar. The solvent (green developer) was allowed to ascend through the paper strip until it reached the uppermost portion. The movement of the solvent front was then marked using a pen upon removal of the chromatographic paper from the jar. Afterwards, the chromatographic paper strip was dried to remove any remaining developer. Eluting agents, as specified previously, were then applied over the dry filter paper using a sprayer. In the first paper chromatography experiment, the reaction with 1(N)

$K_4[Fe(CN)_6]$ produced one Prussian blue spot and one light brown spot, indicating the presence of Fe^{3+} and Cr^{3+} ions, respectively. Each distinct zone of color was carefully outlined with a pencil for reference.

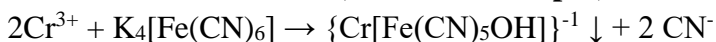
Results and Analysis

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

In the chromatographic filter paper strip, berlin blue or Prussian blue colored spot appeared (Kawatate et al., 2012) 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) $K_4[Fe(CN)_6]$.



(Prussian blue spot)



(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 (Bembi et al., 1975).

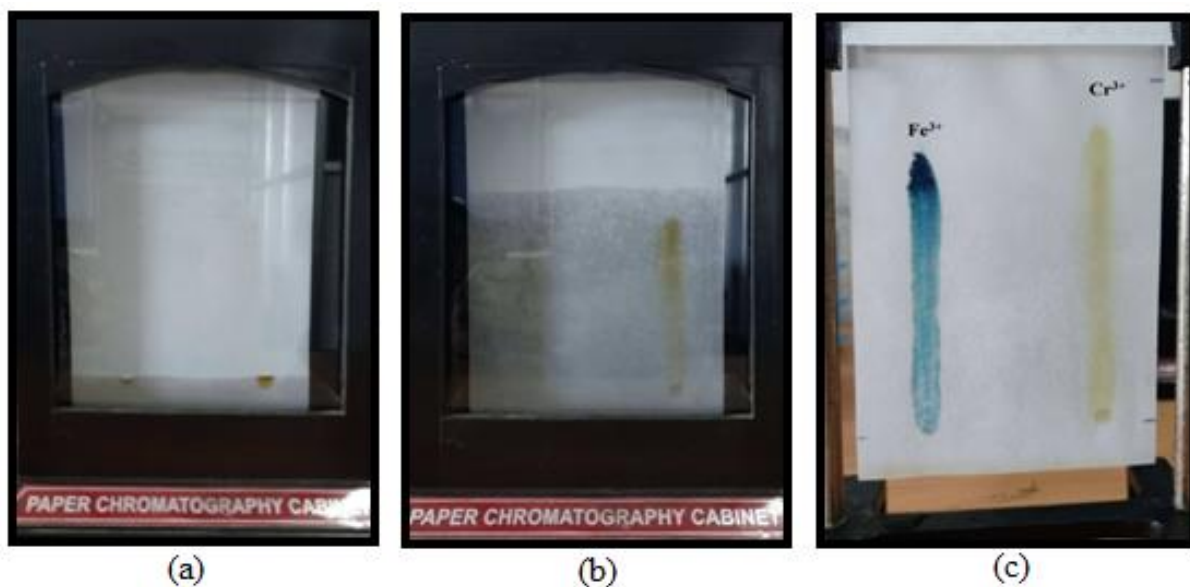


Figure 1. Separation of transition metal ions (Fe^{3+} and Cr^{3+}) by PC

Data Analysis

By comparing the retention factor values and observing the color spots of the two cations, (Fe^{3+} and Cr^{3+}), clear distinctions between them were made. The first spot, appearing as prussian blue, was attributed to the formation of $Fe_4[Fe(CN)_6]_3$. The distance travelled by the solute zone of Fe^{3+} (ds_1) was determined by the reaction between $FeCl_3$ and an aqueous solution of 1 (N) $K_4[Fe(CN)_6]$. The second spot, representing Cr^{3+} , emerged as light brown due to the formation of $\{Cr[Fe(CN)_5OH]\}^{-1}$, formed from the reaction of Cr^{3+} in Cr_2O_3 with an aqueous solution of 1 (N) $K_4[Fe(CN)_6]$, indicating the distance travelled by another solute zone

(ds₂). Retention factors (R_f), or retardation factors, were then calculated (see Table-1). By comparing the color spots and R_f values, the two cations (Fe^{3+} and Cr^{3+}) were successfully identified and distinguished from one another.

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

Paper Chromatography Experiment-2: Separation of Group II Metal ions (Pb^{2+} and Cu^{2+}) by 1% KI Solution

Experimental

Required chemicals and apparatus

(i) Jar for chromatography, (ii) Measuring cylinder, (iii) Capillary, (iv) Tiny test tube, (v) Beakers (10 mL, 100 mL, and 500 mL), (vi) Grade 41 Whatman quantitative filter paper, (vii) Lead nitrate, (viii) Copper sulfate, (ix) 1% KI solution

Required solution

- (i) Solution of metal salts: To make a saturated solution, metal salts were dissolved in 1 mg/mL of distilled water in a 10 mL beaker. Metal salts: $Pb(NO_3)_2$ & $CuSO_4 \cdot 5H_2O$
 (ii) Eluting agents used: 1% KI solution was prepared in a 100 mL beaker with distilled water

Green developer

500 mL distilled water is used as green developer.

Experimental procedure

A strip of Whatman 41 grade filter paper was positioned inside the chromatographic jar, with a dot placed approximately 0.5 centimetres from the bottom as the starting point for development. Saturated solutions of metal salts/oxides were carefully applied at two distinct locations near the top of the paper strip, each time using a fresh capillary. Once the spots were applied, the chromatographic paper was left outside to dry. Subsequently, the dried paper strip was suspended in the chromatography jar filled with green solvent (distilled water), ensuring the bottom end made contact with the solvent while the upper end was attached to a steel bar (Figure 2a). The green solvent was allowed to ascend through the paper strip until it reached the top, indicating the solvent front, which was marked with a pen upon removal from the jar (Figure 2b). Afterwards, the chromatographic paper was dried to remove excess solvent. Eluting agents, as specified, were sprayed onto the dry filter paper. In PC experiment 2, distinct brown and yellow spots emerged immediately upon reaction with 1% KI solution (Figure 2c), indicating the presence of Pb^{2+} and Cu^{2+} ions, respectively. These colorful zones were carefully marked with a pencil for identification.

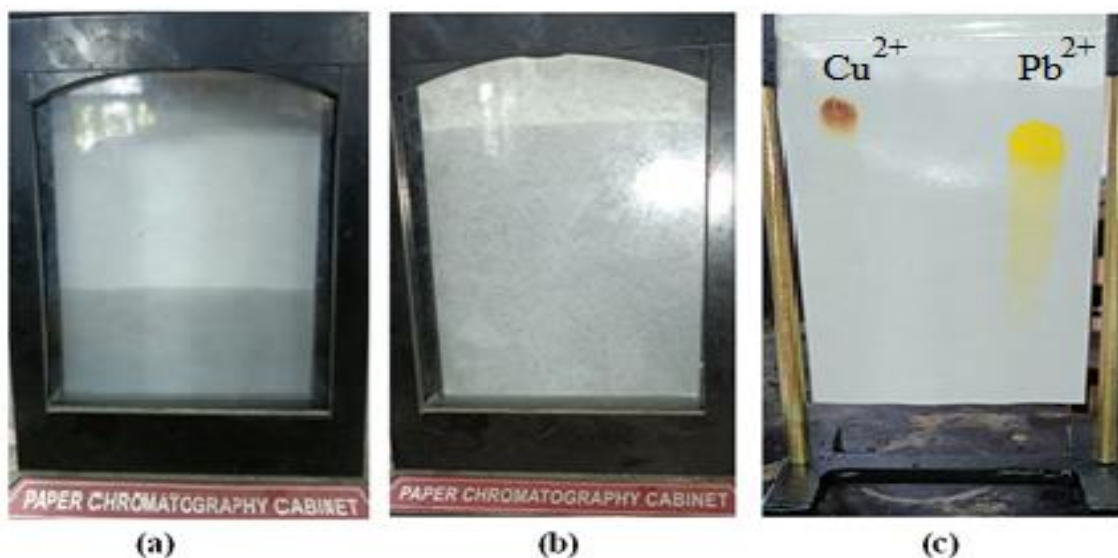


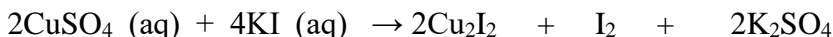
Figure 2. Cu^{2+} and Pb^{2+} separation using paper chromatography by 1% KI solution

Results and Analysis

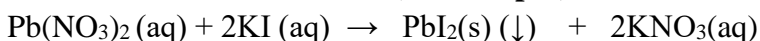
Reactions Involved During Formation of Color Spots by Interaction with Solute Zone

When 1% KI reacted with the metal salt $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution, a distinctive brown spot appeared on the chromatographic paper due to a redox reaction. In this chemical interaction, CuSO_4 interacted with KI, leading to the conversion of Cu(II) ions into Cu(I) ions through the action of I^- ions. Simultaneously, the I^- ions themselves were oxidized into I_2 . Since I_2 is volatile, it quickly vaporized, leaving behind a zone where Cu^{2+} ions (the solute) reacted with potassium iodide. This phenomenon was promptly noticed, and a pen mark was made on the brown-colored spot for easy identification.

But lead nitrate [$\text{Pb}(\text{NO}_3)_2$] 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).



(Brown spot)



(Yellow spot)

Data Analysis

By comparing the color spots and their respective retardation factors, two distinct cations were identified and differentiated from each other. The metal cations were determined by examining the colorful patches that matched these two distinct cations. Following the release of I_2 due to the reaction of KI with CuSO_4 , the first spot exhibited a brown hue. The travel distance of one solute zone, represented by Cu^{2+} (d_{s1}), and another solute zone, represented by Pb^{2+} (d_{s2}), were measured. Additionally, in each experiment, the solvent's travel distance (d_m) was calculated by measuring the separation between the starting line and the solvent front

(water). Subsequently, the values of retardation factors (R_f) were computed, as detailed in Table-1.

Paper Chromatography Experiment-3: Separation of Group IV Metal ions (Ni^{2+} and Co^{2+}) by 4% NH_4OH Solution

Experimental

Required chemicals and apparatus

(i) Jar for chromatography, (ii) Measuring cylinder, (iii) Capillary, (iv) Tiny test tube, (v) Beakers (10 mL, 100 mL, and 500 mL), (vi) Grade 41 Whatman quantitative filter paper, (vii) Nickel nitrate, (viii) Cobalt nitrate, (ix) 4% NH_4OH solution.

Required solution

- (i) Solution of metal salts: To make a saturated solution, metal salts were dissolved in 1 mg/mL of distilled water in a 10 mL beaker. Metal salts: $Ni(NO_3)_2 \cdot 6H_2O$ & $Co(NO_3)_2 \cdot 6H_2O$.
- (ii) Eluting agents used: 4% NH_4OH solution was prepared in a 100 mL beaker with distilled water.

Green developer

500 mL distilled water is used as green developer.

Experimental procedure

A Whatman 41 grade filter paper strip was positioned inside the chromatographic jar, with a dot placed approximately 0.5 centimetres from the bottom as the starting point for development. Saturated solutions of metal salts/oxides were separately administered using fresh capillaries at two locations near the top of the chromatographic paper. Afterwards, the chromatographic paper containing the metal spots was left to dry outside. Once dried, the spotted paper strip was suspended again in the chromatography jar filled with green solvent (distilled water), with the bottom end submerged in the solvent and the upper end secured to a steel bar. The green solvent, acting as the developer, was allowed to ascend through the paper strip until it reached the topmost portion, indicating the solvent front. Upon removal from the chromatography jar, the solvent front was marked using a pen for reference. The chromatographic paper strip was then dehydrated to remove excess solvent. Following this, eluting agents, as specified, were sprayed over the dry filter paper. In PC experiment 3, the reaction with 4% NH_4OH resulted in the immediate emergence of green gel coloration and bluish-green coloration spots, indicating the presence of Ni^{2+} and Co^{2+} ions, respectively (Figure 3c). Initially, 1-2% NH_4OH solution was used, but the results for Ni^{2+} were not satisfactory. However, with 3% NH_4OH , color spots were obtained for both metal ions, although the intensity of the Ni^{2+} color spot was not very prominent (Figure 4). All colorful zones were carefully marked with a pencil for identification purposes.

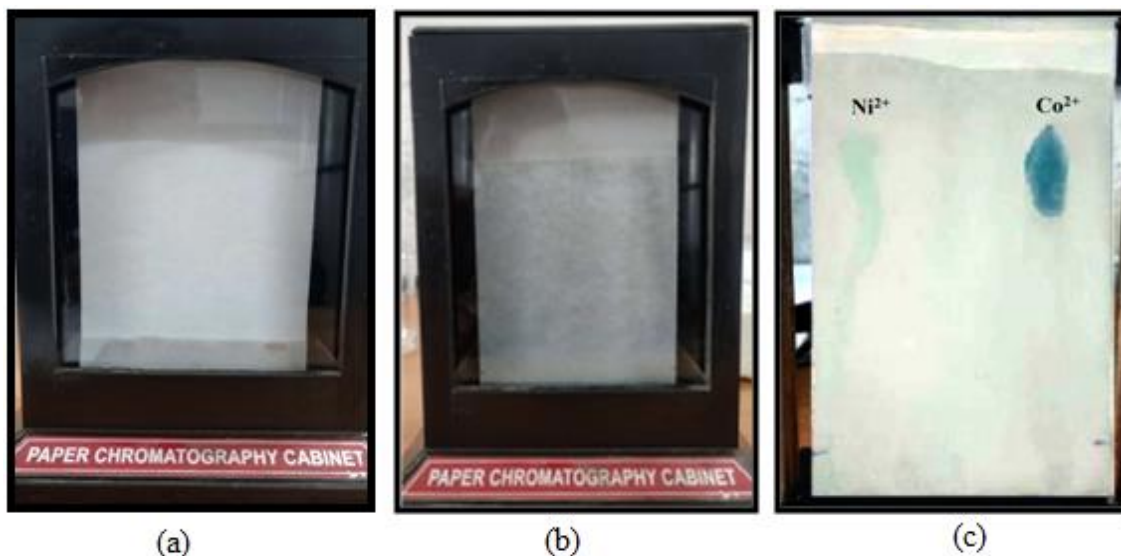


Figure 3. Ni²⁺ and Co²⁺ separation using paper chromatography by 4% NH₄OH solution

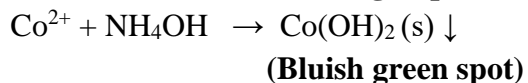
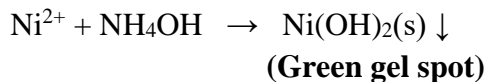


Figure 4. Ni²⁺ and Co²⁺ separation using paper chromatography by 3% NH₄OH solution

Results and Analysis

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

Green gel and bluish green spots on the filter paper are the result of mixing nickel and cobalt nitrate solutions with 4% NH₄OH solution to create metal (II) hydroxide (The University of Colorado pdf, 2023).



Data Analysis

By comparing their color spots and retention factor values, two cations, Co(II) and Ni(II), were distinguished from one another. When 4% NH_4OH solution reacted with $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, nickel (II) hydroxide $\text{Ni}(\text{OH})_2$ formed, resulting in the first spot appearing as green gel. On the other hand, when $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ reacted with a 4% NH_4OH solution, $\text{Co}(\text{OH})_2$ was produced, giving rise to the appearance of the second spot (Co^{2+}) as bluish green. The travel distance of one solute zone, represented by Ni^{2+} (d_3), and the travel distance of another solute zone, represented by Co^{2+} (d_4), were measured. Subsequently, the values of retention factors (R_f) were calculated, as detailed in Table-1.

Paper Chromatography Experiment-4: Separation of Group IV and Group II Metal ions (Co^{2+} and Cu^{2+}) by 6% NH_4OH Solution.

Experimental

Required chemicals and apparatus

(i) Jar for chromatography, (ii) Measuring cylinder, (iii) Capillary, (iv) Tiny test tube, (v) Beakers (10 mL, 100 mL, and 500 mL), (vi) Grade 41 Whatman quantitative filter paper, (vii) Copper sulfate, (viii) Cobalt nitrate, (ix) 6% NH_4OH solution.

Required solution

- (i) Solution of metal salts: To make a saturated solution, metal salts were dissolved in 1 mg/mL of distilled water in a 10 mL beaker. Metal salts: $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ & $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.
 (ii) Eluting agents used: 6% NH_4OH Solution was prepared in a 100 mL beaker with distilled water.

Green developer

500 mL distilled water is used as green developer.

Experimental procedure

A strip of Whatman 41 grade filter paper was carefully placed inside the chromatographic jar, with a dot positioned approximately 0.5 centimetres from the bottom, serving as the starting point for development. Saturated solutions of metal salts/oxides were applied independently at two locations near the top of the chromatographic paper using fresh capillaries for each application. Subsequently, the chromatographic paper containing the two metal spots was left to dry externally. Once dried, the spotted paper strip was repositioned in the chromatography jar, submerged in green solvent (distilled water), with the bottom end in contact with the solvent and the upper end secured to a steel bar. The green solvent, acting as the developer, ascended through the paper strip until it reached the topmost portion, indicating the solvent front. After removal from the chromatography jar, the solvent front was marked using a pen for reference. The chromatographic paper strip was then dehydrated to remove excess developer. Following

this, eluting agents, as specified, were sprayed over the dry filter paper. In PC experiment 4, upon reaction with 6% NH_4OH , a green and blue spot instantaneously appeared (Figure 5c), indicating the detection of Co^{2+} and Cu^{2+} ions, respectively. All colorful zones were marked with pencil for identification purposes.

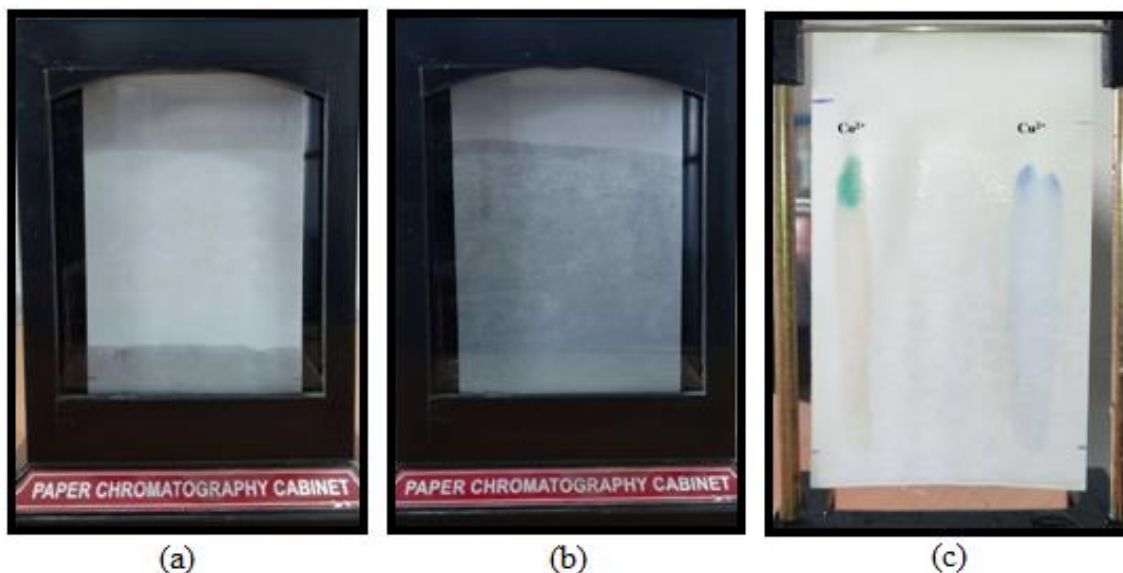
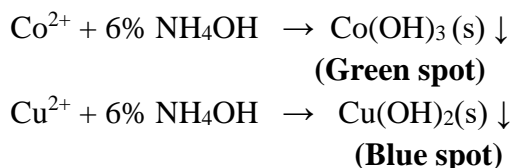


Figure 5. Co^{2+} and Cu^{2+} separation using paper chromatography by 6% NH_4OH solution

Results and Analysis

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

When a metal salt reacts with a 6% NH_4OH solution, it produces copper (II) hydroxide $\text{Cu}(\text{OH})_2$, resulting in a distinct blue stain on the Whatman filter paper (Kawatake et al., 2012). This stands in contrast to the blue-green $\text{Co}(\text{OH})_2$ formation from the reaction of $\text{Co}(\text{II})$ nitrate with a 4% NH_4OH solution (PC experiment 3). Instead, when $\text{Co}(\text{II})$ nitrate combines with a 6% NH_4OH solution, it forms green-colored spots due to the production of $\text{Co}(\text{III})$ hydroxide or $\text{Co}(\text{OH})_3$. Typically, cobalt(II) salts exhibit stability; however, in basic solutions such as a 6% NH_4OH solution, cobalt(II) readily transforms into cobalt(III). The basic nature of the reaction medium increases with higher NH_4OH solution concentrations (from 4% to 6%), leading to relatively rapid air oxidation of $\text{Co}(\text{II})$ to $\text{Co}(\text{III})$ (Kavitha et al., 2018; Clark, 2023; Adhikari et al. 2023). Consequently, the oxidation of bluish-green cobalt(II) hydroxide to cobalt(III) hydroxide results in the appearance of a green-colored spot. This phenomenon was further confirmed by spot testing $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solution with separate solutions of 4% and 6% NH_4OH using Whatman chromatography paper (Figure 6). When cobalt (II) nitrate solution was spot tested with 4% NH_4OH , a blue-green color was observed (Figure 6b Left), while the addition of 6% NH_4OH resulted in immediate development of a green color (Figure 6b Right).



The green spot underwent FT-IR analysis using a Bruker Alpha II model spectrometer, which operated in the range of 4000–400 cm^{-1} . In cobalt(III) hydroxide, the hydroxyl group typically exhibits stretching and bending vibrations in the infrared region. A bending vibration is observed around 1638 cm^{-1} , while the broad band for the -OH stretching vibration is typically found within the range of 3490–3499 cm^{-1} (The Nature Vibrational Spectroscopy Webpage, 2023). These vibrational modes confirm the presence of hydroxyl groups in cobalt(III) hydroxide. The infrared spectrum also revealed the presence of the Co-O bond, which typically exhibits frequencies in the range of 400–600 cm^{-1} . However, in the green spot, the IR band corresponding to the Co-O bond was observed at 647 cm^{-1} (Figure 7). This higher frequency suggests a stronger Co-O bond, characteristic of higher oxidation states of cobalt atoms (+3 or +4) (Andris et al., 2019). In higher oxidation states, there is a positive shift in the IR frequency of the cobalt-oxygen bond due to the increased strength of the metal-oxygen bond. Therefore, the observed IR frequency of the cobalt-oxygen linkage at 647 cm^{-1} supports the presence of cobalt in the +3 oxidation state in cobalt(III) hydroxide, consistent with the green color of the spot.

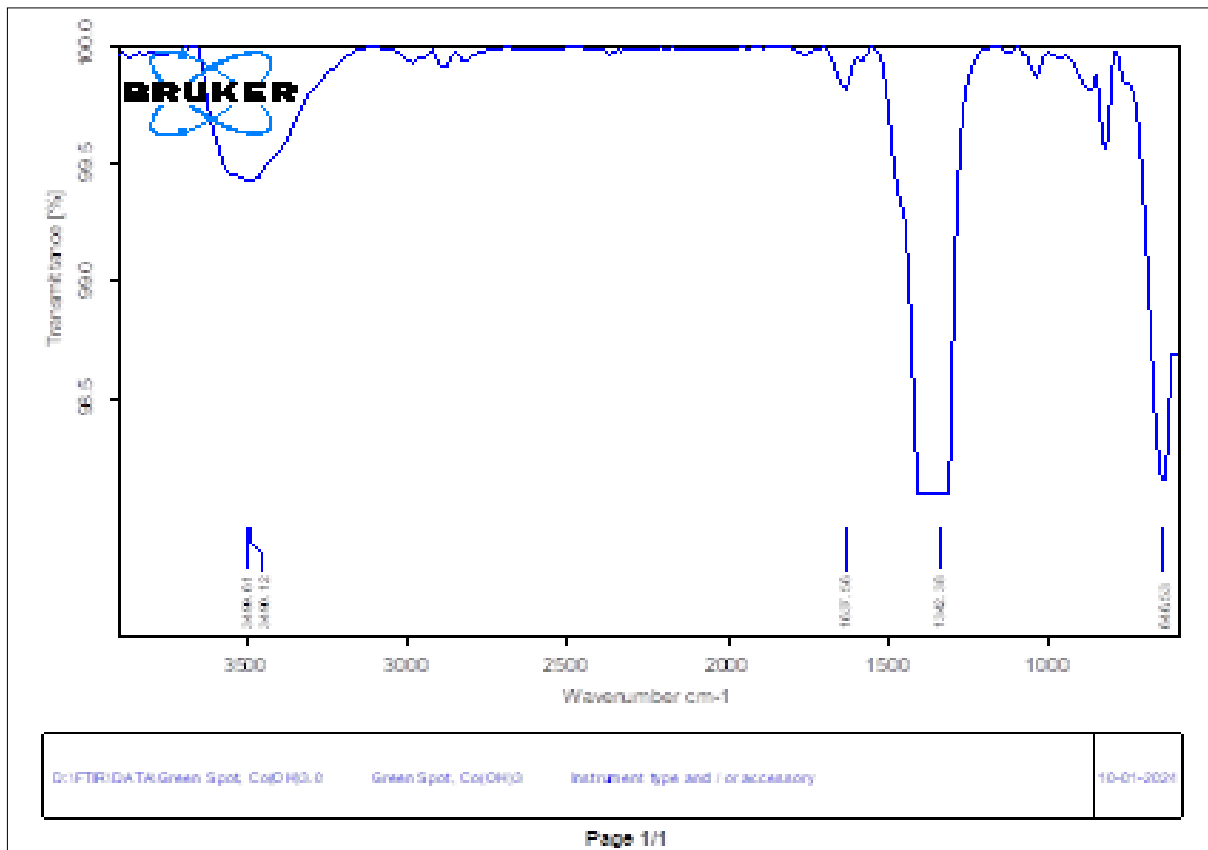


Figure 6. $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ spot detection within the Whatman chromatography strip (a) prior to and (b) with the addition of 4% and 6% NH_4OH solution

Data Analysis

The color spots and retention factor values of two cations, Co^{2+} and Cu^{2+} , were compared to identify and distinguish them. The first spot turned green due to the reaction between $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and a 6% NH_4OH solution, resulting in the formation of cobalt(III) hydroxide $\text{Co}(\text{OH})_3$. Cobalt(II), being easily oxidized to cobalt(III) in a basic medium, travelled a distance indicated by (ds_7). On the other hand, the second spot (Cu^{2+}) appeared blue as a result of the

reaction between $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and a 6% NH_4OH solution, leading to the production of copper(II) hydroxide $\text{Cu}(\text{OH})_2$. Cu^{2+} , representing the other solute zone, travelled a distance indicated by (d_{s8}). Subsequently, we calculated the retardation factors (R_f), as summarized in Table-1. This comparison of color spots and R_f values facilitated the clear identification and differentiation of the Co^{2+} and Cu^{2+} ions.



Paper Chromatography Experiment-5: Separation of Group II and Group III Metal ions (Cu^{2+} and Fe^{3+}) by 1(N) $\text{K}_4[\text{Fe}(\text{CN})_6]$ Solution

Experimental

Required chemicals and apparatus

(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) 100 mL Measuring cylinder, (ix) 10 mL, 250 mL beaker, (x) Whatman quantitative filter paper grade 41, (xi) Distilled water

Required solution

(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 $K_4[Fe(CN)_6]$ in the 250 mL beaker.

Green developer

500 mL distilled water is used as green developer.

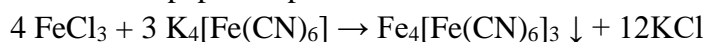
Experimental procedure

A strip of Whatman grade 41 filter paper was suspended in the chromatographic jar, with a dot placed about 1 centimeter from the bottom, serving as the starting point for development. Using separate capillaries, saturated solutions of Fe^{3+} and Cu^{2+} were individually applied to the filter paper at two distinct locations, ensuring each solution was administered with a fresh capillary. After spotting, the filter paper with the two spots was left to dry outside. Once dried, the spotted filter paper strip was re-suspended in the chromatography jar filled with distilled water. The lower end made contact with the developer (water), while the upper end was pinned to a steel rod. The strip was positioned vertically, ensuring the point was always above the level of the developer, allowing the water to rise along the filter paper. As the water ascended, carrying the metal ions, the solvent front approached the upper end of the filter paper. After removing the filter paper from the chromatography jar, the solvent front was marked using a pen. The paper was then dried to remove the developer. Subsequently, potassium ferrocyanide 1N aqueous solution was used as an eluting or spraying reagent. It was applied to the dry filter paper using a sprayer. Upon interaction with the aqueous solution of $K_4[Fe(CN)_6]$, prussian blue and reddish-brown spots appeared immediately (Figure 8c), indicating the identification of Fe^{3+} and Cu^{2+} ions, respectively. Colored zones were indicated using a pen for clear identification.

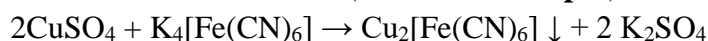
Results and Analysis

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

When ferric chloride solution was mixed with 1N aqueous solution of $K_4[Fe(CN)_6]$, it resulted in the formation of a Prussian blue or Berlin blue coloration spot, corresponding to iron(III)hexacyanidoferrate(II), $Fe_4[Fe(CN)_6]_3$, on the Whatman grade 41 filter paper strip. Conversely, when copper sulfate reacted with 1N aqueous solution of $K_4[Fe(CN)_6]$, it led to the formation of a reddish-brown coloration spot, representing cupric ferrocyanide, $Cu_2[Fe(CN)_6]$, on the filter paper strip.



(Prussian blue spot)



(Reddish brown spot)

Data Analysis

The colored spots corresponding to two different cations, (Cu^{2+} and Fe^{3+}), were observed and analyzed. By comparing their color spots and retention factor values, both cations were identified

and separated successfully. The first spot appeared as prussian blue, indicating the formation of iron(III)hexacyanidoferrate(II), $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$. This spot represented the distance travelled by one solute zone, identified as Fe^{3+} (d_{s9}). Conversely, the second spot for Cu^{2+} appeared as reddish brown, indicating the formation of cupric ferrocyanide, $\text{Cu}_2[\text{Fe}(\text{CN})_6]$. This spot represented the distance travelled by another solute zone, Cu^{2+} (d_{s10}). Subsequently, the retardation factors or retention factors (R_f) values were calculated and summarized in Table 1. This comparison facilitated the clear identification and separation of Cu^{2+} and Fe^{3+} ions.

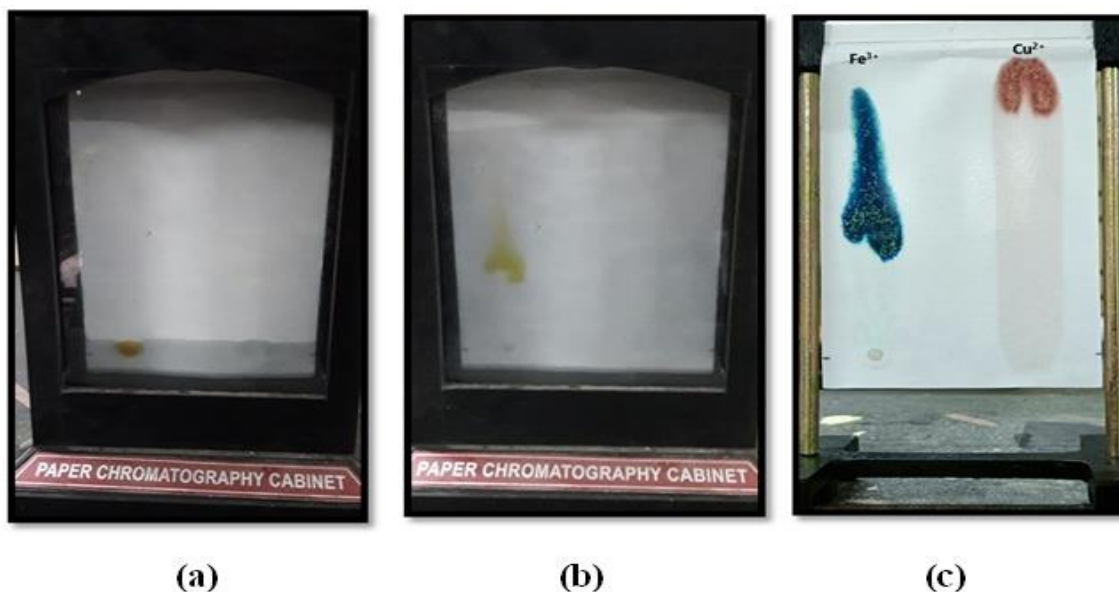


Figure 8. Separation of metal ions (Fe^{3+} and Cu^{2+}) by paper chromatography by using 1N aqueous $\text{K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$ solution

Paper Chromatography Experiment-6: Separation of Group VI Metal ions (Mo^{6+} and W^{6+}) by 1(N) $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ Solution

Experimental

Required chemicals and apparatus

(i) Jar for chromatography, (ii) Measuring cylinder, (iii) Capillary, (iv) Tiny test tube, (v) Beakers (10 mL, 100 mL, and 500 mL), (vi) Grade 41 Whatman quantitative filter paper, (vii) Sodium molybdate, (viii) Sodium tungstate, (ix) 1(N) $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ solution.

Required solution

(i) Solution of metal salts: To make a saturated solution, metal salts were dissolved in 1 mg/mL of distilled water in a 10 mL beaker. Metal salts: $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ & $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$
(ii) Eluting agents used: 100 mL 1(N) copper (II) chloride solution was prepared in a 250 mL beaker with distilled water.

Green developer

500 mL distilled water is used as green developer.

Experimental procedure

A Whatman 41 grade filter paper strip was positioned inside the chromatographic jar, with a dot placed approximately 0.5 centimetres from the bottom, serving as the starting point for development. Saturated solutions of metal salts/oxides were individually administered using fresh capillaries at two locations near the top of the chromatographic paper. Subsequently, the chromatographic paper containing the two metal spots was left to dry outside. Once dried, the spotted paper strip was re-suspended in the chromatography jar filled with green solvent (distilled water). The bottom end made contact with the solvent, while the upper end was fastened to a steel bar. As the green solvent ascended through the paper strip, carrying the metal ions, it reached the topmost portion of the paper strip. Upon removal from the chromatography jar, the solvent front was marked using a pen. The paper strip was then dehydrated to remove excess solvent. Following this, eluting agents as specified were sprayed over the dry filter paper. In PC experiment 5, upon reaction with 1N copper (II) chloride CuCl_2 solution, a green coloration and a light sky-blue coloration spot emerged immediately (Figure 9c), indicating the identification of Mo^{6+} and W^{6+} ions, respectively. All colorful zones were marked with pencil for identification purposes.

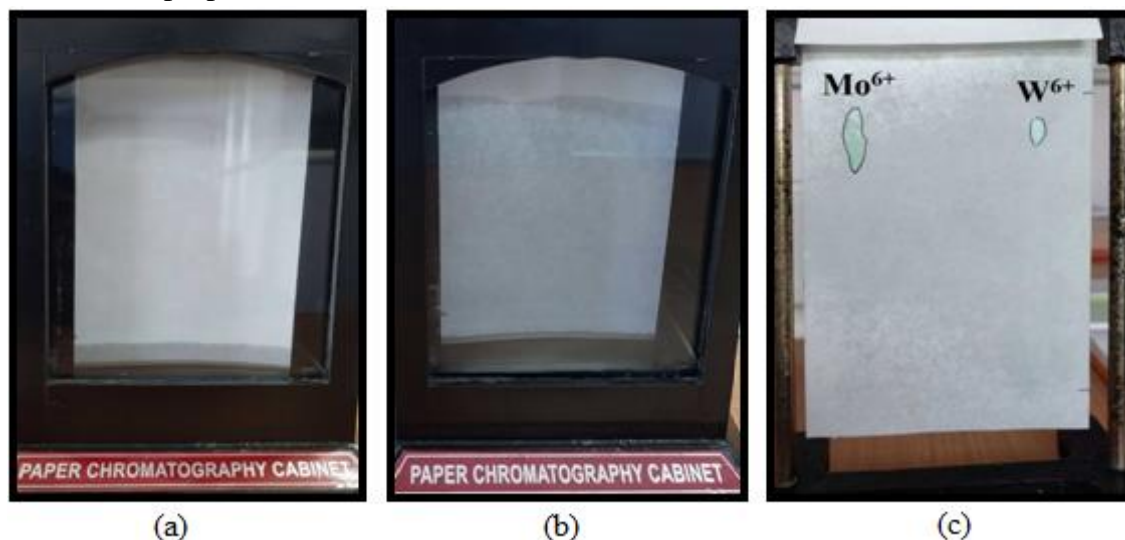
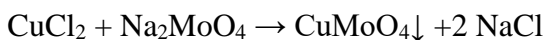


Figure 9. Separation of transition metal ions (Mo^{6+} and W^{6+}) by PC

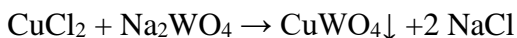
Results and Analysis

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

Green and light sky-blue coloration spots on the filter paper are the result of mixing sodium molybdate, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ and sodium tungstate, $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ with copper (II) chloride CuCl_2 solution to create CuMoO_4 and CuWO_4 respectively (Science madness Discussion Board, 2007; Tan et al., 2020; Unadkat et al., 2020; Kannan et al., 2017).



(Green spot)



(Sky Blue spot)

Data Analysis

By contrasting their color spots and retention factor values, two cations, Mo(VI) and W(VI), were recognized and distinguished from each other. When the aqueous solution of copper (II) chloride reacted with sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$), cupric molybdate (CuMoO_4) was formed, resulting in the emergence of the first spot as green (Mo^{6+}). On the other hand, when sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) reacted with the aqueous solution of copper (II) chloride, CuWO_4 was produced, giving rise to the appearance of the second spot (W^{6+}) as light sky blue. The travel distance of one solute zone represented by Mo^{6+} (ds_{11}), and the travel distance of another solute zone represented by W^{6+} (ds_{12}) were measured. Subsequently, the values of retardation factors (R_f) were calculated and summarized in Table-1. This comparison facilitated the clear identification and differentiation of Mo(VI) and W(VI) ions.

Safety Hazards

To ensure the safe handling of corrosive and hazardous chemicals, it is imperative to advise students to wear appropriate eye protection, such as chemical splash goggles, along with disposable latex gloves to protect the skin. Additionally, a face shield should be worn to provide extra protection. Given the hazardous nature of NH_4OH , precautions should be taken to minimize risks. One such measure is to dilute the concentration of the eluting agent NH_4OH with water. Water is less toxic and less volatile, reducing the potential for harm. Furthermore, to enhance safety precautions, the dilution of NH_4OH should ideally be carried out under a fume hood to minimize exposure to harmful vapors. This approach ensures the safety of students and minimizes the risks associated with handling corrosive chemicals.

Evaluation of Learning Outcomes

Two groups of second-year undergraduate students, totalling 20 individuals, conducted a series of six experiments in the inorganic chemistry laboratory at Bir Bikram Memorial College. These experiments focused on the identification and separation of metal ions using paper chromatography, aiming to achieve specific learning objectives: i. Help students comprehend the fundamentals of paper chromatography, ii. Describe the factors influencing the

separation of metal ions using paper chromatography, iii. Utilize chromatogram interpretation to locate and analyze metal ions, iv. Establish connections between color patches and metal ion properties, v. Compare the qualitative separation of various metal ions using R_f values, vi. Identify and address common issues encountered during chromatographic procedures, vii. Evaluate the accuracy and reliability of experimental findings, viii. Present findings clearly and succinctly, ix. Emphasize caution when handling chemicals and scientific equipment. The sele-

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 ₂ O (aq)	Prussian blue	10.7 (ds ₁)	13.5	0.79
	Cr ₂ O ₃ (Cr ³⁺)		Light brown	11.8 (ds ₂)		0.87
2	CuSO ₄ .5H ₂ O (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)		0.83
3	Ni(NO ₃) ₂ .6H ₂ O (Ni ²⁺)	4% NH ₄ OH solution (aq)	Green gel	12.4 (ds ₃)	15.0	0.82
	Co(NO ₃) ₂ .6H ₂ O (Co ²⁺)		Bluish green	12.8 (ds ₄)		0.85
4	Co(NO ₃) ₂ .6H ₂ O (Co ²⁺)	6% NH ₄ OH solution (aq)	Green	12.1 (ds ₇)	14.2	0.85
	CuSO ₄ .5H ₂ O (Cu ²⁺)		Blue	13.2 (ds ₈)		0.93
5	FeCl ₃ (Fe ³⁺ ion)	1(N) solution of K ₄ [Fe(CN) ₆].3H ₂ O (aq)	Prussian blue	13.2 (ds ₉)	15.8	0.835
	CuSO ₄ .5H ₂ O (Cu ²⁺ ion)		Reddish brown	14.3 (ds ₁₀)		0.905

6	Na ₂ MoO ₄ . 2H ₂ O (Mo ⁶⁺)	1N solution of CuCl ₂ .2H ₂ O (aq)	Green	12.5 (ds ₁₁)	13.1	0.95
	Na ₂ WO ₄ . 2H ₂ O(W ⁶⁺)		Light sky blue	11.7 (ds ₁₂)	13.1	0.89

-arning objectives cater to a comprehensive understanding of metal ion identification and separation through paper chromatography, encompassing critical thinking, practical skills, and theoretical understanding. When comparing the experimental R_f values with the student average R_f values shown in Table 2, the largest difference observed was 0.06. Post-lab questions related to chromatography theory were answered correctly by students with a 90% accuracy rate, and metal ions were identified correctly 85% of the time. According to survey responses, students expressed satisfaction with the experiments, rating them 9 out of 10 and finding them interesting to perform. They also indicated a willingness to recommend the lab to others, suggesting a positive learning experience overall.

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⁺ - 0.87	Co ²⁺ - (- 0.01) Cu ²⁺ - (- 0.06)
5 (Cu ²⁺ & Fe ³⁺)	Cu ²⁺ - 0.83 Fe ³⁺ - 0.90	Cu ²⁺ - 0.85 Fe ³⁺ - 0.89	Cu ²⁺ - (+ 0.02) Fe ³⁺ - (- 0.01)
6 (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)

Conclusions

The separation and identification of metal ions [(Fe³⁺, Cr³⁺), (Pb²⁺ & Cu²⁺), (Ni²⁺ & Co²⁺), (Co²⁺ & Cu²⁺), (Cu²⁺ & Fe³⁺), and (Mo⁶⁺, W⁶⁺)] were conducted using water as the mobile phase (green developer) in paper chromatography. Various eluting agents such as aqueous solution of 1N K₄[Fe(CN)₆], aqueous solution of 1% KI, 4% NH₄OH, 6% NH₄OH, aqueous solution of 1N K₄[Fe(CN)₆], and 1N CuCl₂ solution were employed. Through these improved qualitative analysis techniques, undergraduate students can effectively utilize paper chromatography (PC) to separate and identify different transition metal ions like [(Fe³⁺, Cr³⁺), (Pb²⁺ & Cu²⁺), (Ni²⁺ & Co²⁺), (Co²⁺ & Cu²⁺), (Cu²⁺ & Fe³⁺), and (Mo⁶⁺, W⁶⁺)] in the analytical table. This approach facilitates hands-on learning and enhances understanding of transition metal ion separation and identification processes.

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