CHAPTER -III
THIN LAYER CHROMATOGRAPHIC SEPARATION, IDENTIFICATION AND DETERMINATION OF CERTAIN ANIONS
3.1 INTRODUCTION

Of the various chromatographic techniques, thin-layer chromatography (TLC) has recently gained popularity not only because of advancements in technique and instrumentation and improvement in efficiency but also because of its relatively low cost and speed of analysis compared with other highly selective and efficient chromatographic techniques such as high performance liquid chromatography and gas chromatography. The differential migration of species in TLC is due to varying degrees of affinity of the components in the stationary and mobile phase mixtures. The exact separation mechanisms involved depend on the nature of the two phases and the solutes. Chromatographic retention and selectivity depend upon interactions such as hydrogen bonding and electron pair donor and acceptor, ion-ion, ion-dipole, and van der Waals interactions.

As extensive literature survey (1-10) on TLC was undertaken; it is surprising to note that very little work has been reported on the analysis of anionic mixtures as compared with that of cationic mixtures. As a continuation of our previous work on the TLC of anions (11-13), this chapter describes a simple method for identification, separation, and determination of \( \Gamma^- \), SCN\(^-\), NO\(_2^-\), Br\(^-\), BrO\(_3^-\), IO\(_4^-\), IO\(_3^-\), CrO\(_4^{2-}\), and PO\(_4^{3-}\) under various experimental conditions.

3.2 EXPERIMENTAL

Apparatus: A TLC applicator (Toshniwal, India) was used for preparation of 20×3.5 cm glass plates. The chromatography was performed in 24×6 cm glass jars.

Chemicals: Silica gel G (particle size between 10 and 40 \( \mu \)m; Catalog No.
27335) and methanol were obtained from Glaxo Laboratories (India). kieselguhr (particle size between 10 and 40 \mu m; Catalog No. 015037), kaolin (Catalog No. 033059) and cellulose microcrystalline (particle size less than 30 \mu m; Catalog No. 027984) were obtained from CDH Laboratories (India). All other reagents were of Analytical Reagent grade.

**Test Solutions:** The test solutions (1% w/v) were sodium salts of nitrate, nitrite; fluoride and potassium salts of iodide, iodate, periodate, bromide, bromate, permanganate, chromate, dichromate, ferricyanide, ferrocyanide, chloride, phosphate, and sulfite; thiocyanate and molybdate were taken as their ammonium salts. Double distilled water with a specific conductivity (K = 2 \times 10^{-6} \text{ ohm}^{-1} \text{ cm}^{-1} \text{ at } 25^\circ \text{C}) was used for the preparation of salt solutions.

**Detection Reagents:** For the detection of various anions, the following reagents were used:

(a) Saturated AgNO₃ solution in the methanol for I⁻, Br⁻, Cl⁻, F⁻, SO₃⁻, CrO₂⁻, CrO₄²⁻ and PO₄³⁻.

(b) Diphenylamine (0.2-0.5%) in 4M H₂SO₄ for NO₂⁻, NO₃⁻, IO₄⁻, IO₃⁻, BrO₃⁻, MnO₄⁻ and WO₄²⁻.

(c) Ferric chloride (10%) in 2.0M HCl for SCN⁻, Fe(CN)₆³⁻ and Fe(CN)₆⁴⁻.

(d) Alcoholic pyrogallol (0.5%) for MoO₄²⁻ and Mo₇O₂₄⁶⁻.

**Mobile Phases:** The following solvents were investigated: M₁, 0.1M HCl-acetone (1:9); M₂, 1.0M formic acid; M₃, 1.0M sodium formate; M₄, double distilled water.

**Stationary Phases:** The following sorbent layers were used: S, silica
gel impregnated with aqueous 1% CuSO₄ solution; S₂, silica gel G; S₃, alumina; S₄, cellulose microcrystalline; S₅, kaolin; S₆ kieselguhr G; S₇, alumina + cellulose (2:1); S₈, kieselguhr + cellulose (1:2,2:1).

Procedure:
(a) Preparation of TLC plates: Silica gel, alumina, and kieselguhr TLC plates were prepared by mixing the adsorbent with double distilled water in a 1:3 ratio by weight. The resultant slurry was mechanically shaken for 10 min after which it was applied to well-cleaned glass plates with the help of TLC applicator to give a layer of approximately 0.25 mm thickness. The plates were air dried at room temperature and then heated at 100± 5°C for 1 h to activate them. After activation, the plates were stored in a desiccator. Cellulose or kaolin coated plates were prepared in a similar fashion by using a slurry made by mixing cellulose or kaolin with double distilled water in a 1:4 ratio by weight. No additional binder was added to the slurry. For impregnated silica gel layers, a slurry was made by mixing silica gel with an aqueous solution of 1% CuSO₄ in a 1:3 ratio. Thin layers were then prepared as described previously.

Qualitative analysis: A sample volume (1.0-10 μL) containing a sufficient amount of analyte (0.1-10 μg) was applied using a micropipette about 2.0 cm above the lower edge of the chromatoplates. The spots were dried, and the plates were developed in glass jars containing the mobile phase using a one-dimensional ascending technique. Before developing the plates, the glass jars that contained the mobile phase were covered with a lid for about 20 min so that the glass jars would get pre-saturated with the mobile phase vapors.
The mobile phase (solvent) was allowed to migrate up to 10 cm from the starting line in all cases. After development, the plates were dried at room temperature and the anion spots were visualized using the appropriate spray reagent (Figure 3.2).

Quantitation: For semi-quantitative determination of $I^-$, $Br^-$ and $NO_2^-$, 0.01 ml of various standard solutions of $KI$, $KBr$ and $NaNO_2$ (2.5-40%) were spotted on silica gel layers impregnated with a 1% $CuSO_4$ solution. The chromatoplates were developed with $M_1$. After detection, the spot was copied onto tracing paper from the chromatoplate and then the spot-area was calculated graphically of triplicate tests.

The limit of detection of various anions was determined by spotting different amounts of anionic solutions on the chromatoplates. The plates were developed and the spots were detected as described above. The method was repeated with successive lowering of the amount of anion until spots could no longer be detected. The minimum amount of anion that was able to be detected was taken as the limit of detection.

A volumetric procedure was applied for the quantitative determination of iodate after its TLC separation from periodate. A standard volumetric method (14) was set up using 0.01M sodium thiosulfate solution as an intermediate solution. For the determination of $IO_3^-$ in the presence of $IO_4^-$ various samples containing a mixture of $KIO_3$ and $KIO_4$ in variable amounts were prepared. Using a lambda pipette, 0.01 ml of the resultant mixture was loaded on the chromatoplates. The plates were developed with double distilled water (15). A pilot plate was employed simultaneously in order to locate the exact position of the spot on the working plate. The area
occupied by IO₃⁻ was scraped and IO₃⁻ was extracted with 1.0M HCl. The adsorbent was separated from the solution by filtration and washed with 1.0 M HCl to ensure complete extraction of IO₃⁻. The filtrate was added to a blank that was prepared by mixing 2 ml KIO₃ (1%). The contents were titrated with a 0.01 M Na₂S₂O₃ solution, the blank was also simultaneously titrated with 0.01M sodium thiosulfate and the difference between the volume of the Na₂S₂O₃ solution consumed in both cases was taken for the determination of IO₃⁻ in the sample.

3.3 RESULTS AND DISCUSSION

Tables 3.1 and 3.2 show that the chromatographic systems can be successfully applied for the separation of several anions from their binary and ternary mixtures. The results in Table 3.1 indicate that well resolved ternary separations of IO₄⁻ and IO₃⁻ can be realized from a synthetic mixture containing I⁻ or SCN⁻ and NO₂⁻, Br⁻, BrO₃⁻, CrO₄²⁻ or PO₄³⁻. The proposed method is well-suited for microgram detection of anions on cellulose microcrystalline layers. It is clear from Table 3.2 that distilled water can also be used as a non-toxic eluant for some binary separations of anions. In addition to qualitative separations, quantitative and semi-quantitative determination of certain anions with preliminary separation on thin layers can also be made. Table 3.3 presents the results of quantitative determination of iodate in the presence of periodate. It is evident from Table 3.3 that the proposed method is accurate (percent error, ± 2.2) and reproducible (relative standard deviation, 18 ppt).

We attempted to semiquantitatively determine IO₄⁻, IO₃⁻, NO₂⁻, Br⁻, and I⁻ by measuring the size of the spots. We outlined the spots on a piece of
Table 3.1 R<sub>f</sub> Ranges (sample size, n=3) Involved in Ternary Separation on Cellulose Microcrystalline Thin Layers Using 0.1 M HCl - Acetone (1:9) as Eluant*

<table>
<thead>
<tr>
<th>Anions Separated</th>
<th>Lower detection limit (µg)</th>
<th>R&lt;sub&gt;f&lt;/sub&gt;</th>
<th>Accompanying anions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I&lt;sup&gt;-&lt;/sup&gt;</td>
<td>7.64</td>
<td>0.66-0.75</td>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, IO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, NO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, Br&lt;sup&gt;-&lt;/sup&gt;, BrO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;3-&lt;/sup&gt;, CrO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
</tr>
<tr>
<td>SCN&lt;sup&gt;-&lt;/sup&gt;</td>
<td>3.81</td>
<td>0.73-0.78</td>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, IO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, NO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, BrO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;3-&lt;/sup&gt;, CrO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
</tr>
<tr>
<td>NO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>3.33</td>
<td>0.35-0.41</td>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, IO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, SCN&lt;sup&gt;-&lt;/sup&gt;, Br&lt;sup&gt;-&lt;/sup&gt;, I&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>Br&lt;sup&gt;-&lt;/sup&gt;</td>
<td>6.71</td>
<td>0.37-0.38</td>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, IO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, I&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>BrO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>3.83</td>
<td>0.27-0.32</td>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, IO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, SCN&lt;sup&gt;-&lt;/sup&gt;, I&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>PO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;3-&lt;/sup&gt;</td>
<td>11.58</td>
<td>0.32-0.38</td>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, IO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, SCN&lt;sup&gt;-&lt;/sup&gt;, I&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>CrO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>4.06</td>
<td>0.35-0.42</td>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, SCN&lt;sup&gt;-&lt;/sup&gt;, Br&lt;sup&gt;-&lt;/sup&gt;, I&lt;sup&gt;-&lt;/sup&gt;</td>
</tr>
<tr>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>4.15</td>
<td>0.04-0.06</td>
<td>I&lt;sup&gt;-&lt;/sup&gt;, SCN&lt;sup&gt;-&lt;/sup&gt;, NO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, Br&lt;sup&gt;-&lt;/sup&gt;, BrO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, PO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;3-&lt;/sup&gt;, CrO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
</tr>
<tr>
<td>IO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>4.08</td>
<td>0.04-0.07</td>
<td>I&lt;sup&gt;-&lt;/sup&gt;, SCN&lt;sup&gt;-&lt;/sup&gt;, NO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, Br&lt;sup&gt;-&lt;/sup&gt;, BrO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;, PO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;3-&lt;/sup&gt;</td>
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</table>

*Standard R<sub>f</sub> values of individual ions: I<sup>-</sup>, 0.73; SCN<sup>-</sup>, 0.83; NO<sub>2</sub><sup>-</sup>, 0.41; Br<sup>-</sup>, 0.45; BrO<sub>3</sub><sup>-</sup>, 0.40; IO<sub>4</sub><sup>-</sup>, 0.07; IO<sub>3</sub><sup>-</sup>, 0.08; CrO<sub>4</sub><sup>2-</sup>, 0.74; PO<sub>4</sub><sup>3-</sup>, 0.43.
Table 3.2: Separations Achieved Experimentally on Different Sorbent Layers Using Distilled Water as Eluant

<table>
<thead>
<tr>
<th>Sorbent system</th>
<th>Separation($R_L$-$R_T$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica gel 'G'</td>
<td>$IO_4^-(0.00)-IO_3^-,BrO_3^-,NO_2^-,I^-,WO_4^{2-}$, Fe( CN)$_6^{3-}$, Fe( CN)$_6^{4-}(1.0-0.85)$</td>
</tr>
<tr>
<td>Alumina</td>
<td>$NO_3^-(1.0-0.82)-WO_4^{2-},MnO_4^-,PO_4^{3-}(0.00)$, $NO_3^-(1.0-0.82)-CrO_4^{2-}$ or $Cr_2O_7^{2-}(0.18-0.00)$, $NO_3^-(1.0-0.86)-Fe( CN)_6^{3-}(0.1-0.00)$, $SCN^-(1.0-0.86)-CrO_4^{2-}$ or $Cr_2O_7^{2-}(0.19-0.00)$</td>
</tr>
<tr>
<td>Cellulose</td>
<td>$MnO_4^-(0.00)-IO_4^-,IO_3^-,CrO_4^{2-}$ or $Cr_2O_7^{2-}(1.0-0.90)$</td>
</tr>
<tr>
<td>Alumina + Cellulose (2:1)</td>
<td>$MnO_4^-(0.00-0.00)-IO_3^-(0.45-0.30)-NO_2^-(1.0-0.89)$</td>
</tr>
</tbody>
</table>

*R$_L$ is the $R_F$ of the leading front and $R_T$ is the $R_F$ of the trailing front of the spot.

Table 3.3: Determination of Iodate with Preliminary TLC Separation from Periodate on Silica Gel G Layers with Distilled Water as the Eluant

<table>
<thead>
<tr>
<th>Amount of $IO_4^-$ taken (mg)</th>
<th>Amount of $IO_3^-$ loaded (mg)</th>
<th>Amount of $IO_3^-$ recovered* (mg)</th>
<th>Error (%)</th>
<th>Relative standard deviation (ppt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.415</td>
<td>0.405</td>
<td>0.412</td>
<td>-1.7</td>
<td>18.1634</td>
</tr>
<tr>
<td>0.365</td>
<td>0.450</td>
<td>0.441</td>
<td>+2.0</td>
<td>15.3794</td>
</tr>
<tr>
<td>0.307</td>
<td>0.500</td>
<td>0.489</td>
<td>+2.2</td>
<td>13.0410</td>
</tr>
<tr>
<td>0.232</td>
<td>0.590</td>
<td>0.601</td>
<td>-1.9</td>
<td>12.6718</td>
</tr>
</tbody>
</table>

*Each value is the average of five determination (n = 5)
paper and determined the weight of the paper strip covering the spot-area. A linear realationship was obtained when the weight of the paper covering the spot-area was plotted against the area of the spot (Figure 3.2). The linearity was maintained up to 160 mg of NO$_2^-$, 80 mg of I$, and 100 mg of Br$^-$. The deviation from linearity started if the loading amount exceeded the upper limit. The reproducibility of data plotted in figure 3.2 was checked by two independent analysts. The values obtained by both the analysts differ by ±15% from the average value as plotted in Figure 3.2. Thus, the method can safely be applied for semi-quantitative determination of Br$^-$, I$, and NO$_2^-$.

However, the semi-quantitative method was unsuitable for IO$_3^-$ and IO$_4^-$ because of the lower solubility of KIO$_3$ or KIO$_4$ in water.

A plot of loading amount versus spot-area for NO$_2^-$, Br$^-$, and I$^-$ follows the equation $y = mx + c$, where $c$ has positive values. However, a plot of $c/m$ values versus atomic weight / molecular weight of anion / anionic salt shows a linear relationship (Figure 3.3). Thus $c/m$ is the molecular weight of the anion salt or the multiple of molecular weight of the anionic salt and a constant. This relationship may be useful to correlate the spot-area with the atomic weight of anions or molecular weight of anionic salts.

3.4 CONCLUSION

The chromatographic system consisting of cellouse thin layers and 0.1M HCl-acetone (1:9) is the most suitable solvent system for the identification, separation, and quantitation of several anions. The determination of IO$_3^-$ with preliminary separation from IO$_4^-$ is important, as iodate is reduced to iodide in alkaline medium.
Figure 3.1: Diagram of some ternary separations: 1, IO$_3^-$ - BR$^-$ - I$^-$; 2, IO$_4^-$ - BRO$_3^-$ - I$^-$; 3, IO$_4^+$ - NO$_2^-$ - SCN$^-$; 4, IO$_4^-$ - BrO$_3^-$ - SCN$^-$. Conditions: stationary phase, cellulose; mobile phase, HCl-acetone (1:9).
Figure 3.2: Plot of spot area versus weight of paper covering the spot area. Conditions: stationary phase, silica gel impregnated with 1% CuSO₄; eluant, HCl-acetone (1:9).
Figure 3.3: Plot of $c/	ext{m}$ values versus atomic weight/molecular weight of anion/anionic salt. Conditions: stationary phase, silica gel impregnated with 1% CuSO$_4$; eluant, HCl-acetone (1:9).
REFERENCES


