STUDIES ON THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF INORGANIC IONS

SUMMARY

THESIS
SUBMITTED FOR THE DEGREE OF
Doctor of Philosophy
IN
APPLIED CHEMISTRY

BY
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LIST OF PUBLICATIONS


SUMMARY

The study was aimed to develop thin layer chromatographic systems for the identification, separation and determination (semi-quantitative/quantitative) of inorganic anions (halides, halates, CN-containing and oxyanions of metals) and cations of p- and d-block metals. Ions were selected on the basis of their industrial, environmental, geological, biological and agricultural importance. To achieve the target, the work presented in this thesis was performed. Several thin layer chromatographic systems were developed by using; (i) plain commonly available adsorbents i.e. silica gel, cellulose microcrystalline, aluminium oxide, kieselguhr, kaolin; (ii) binary mixtures of these adsorbents in various ratios and (iii) impregnated layers with complexing salts. The layer thickness of 0.25 mm has been kept constantly. TLC plates were prepared manually by using a TLC applicator (Toshniwal, India). Mobile phase plays a crucial role to make the chromatographic systems more selective. Low cost solvents (double distilled water, acetone, mineral and carboxylic acids, ammonia and its salts; and their binary mixtures) were used as mobile phases. Mode of chromatogram development strongly affect the development time. Ascending, the most widely used mode has been adopted for the entire thin layer chromatographic studies. Some important separations were achieved by employing newly developed chromatographic systems followed by the quantitative studies. The results have been summarized in seven chapters, discussed briefly as:
CHAPTER - I GENERAL INTRODUCTION

This chapter provides a wider information about the general aspects of chemistry, analytical chemistry, chromatography and thin - layer chromatography (TLC). Chromatography was classified on the basis of phase distribution process. Some chromatographic techniques have been enlisted alongwith their origin and salient features (Table 1.1). Analyte species have also been classified (Figure 1.1).

![Figure 1.1: Classification of Analyte Species](image)

Yearwise developments in the history of TLC have been summarized (Table 1.2). Semi - empirical models of chromatography correlating the $R_p$ (retardation factor) with various physical parameters have been described briefly under the heading , 'Theory and Principle of TLC'. All the basic steps of a TLC analysis ranging from sample preparation to quantitative determination, have been discussed in this chapter.

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CHAPTER - II : THIN - LAYER CHROMATOGRAPHY OF INORGANIC IONS SINCE 1980

This chapter presents a literature survey for last seventeen years (1980-1996) on the thin layer chromatographic analysis of inorganic ions. There are two hundred and fifty-four references cited in this chapter. Relative studies have been made to compare the work done (number of publications) on the TLC of inorganic anions and cations (Figure 2.1). Figure 2.2 shows that most of publications dealing with the TLC of inorganic ions, have been appeared in Journal of planar chromatography.

![Figure 2.1 : Relative Study of Work Done on the TLC of Inorganic Anions and Cations](image1)

![Figure 2.2 : Top Ten Journals Regarding the Number of Publications on TLC of Inorganic Ions (1980-1996)](image2)
CHAPTER III: TLC SEPARATION, IDENTIFICATION AND DETERMINATION OF CERTAIN ANIONS

This chapter describes the retention studies of nineteen inorganic anions on plain, impregnated and mixed sorbent phases of silica gel, cellulose, microcrystalline kaolin, alumina and kieselguhr G have been examined in aqueous solvent systems. Analytical potential of water, a non-toxic eluant has been explored for its applicability as a simple, inexpensive and easily available developer in liquid layer chromatography. Better separation possibilities of anions on cellulose with HCl-acetone (1:9) eluant and on cellulose + alumina (1:2) mixed bed with water, were realized. Some ternary separations are shown in figure 3.1.

Figure 3.1: Diagram of some ternary separations: 1. IO₃⁻ - Br⁻ - SCN⁻; 2. Br⁻ - I⁻ - NO₂⁻; 3. IO₃⁻ - NO₂⁻ - SCN⁻; 4. IO₃⁻ - BrO₃⁻ - SCN⁻. Conditions: stationary phase, cellulose; mobile phase, HCl-acetone (1:9).

The limits of identification for I⁻, Br⁻, NO₂⁻, SCN⁻, BrO₃⁻, IO₃⁻, IO₄⁻, CrO₄²⁻ and PO₄³⁻ have also been determined semi quantitative determination I⁻, Br⁻ and NO₂⁻ have been attempted by spot-area measurement method.
CHAPTER IV: W/O MICROEMULSION AS MOBILE PHASE IN THIN LAYER CHROMATOGRAPHIC RETENTION STUDIES OF ANIONS

A water-in-oil microemulsion, consisting of SDS/1-pentanol/water/n-heptane, has been used as mobile phase for the separation of inorganic anions from their binary mixtures. The weight ratio, SDS/1-pentanol was kept constant at 1/2.46 for all compositions. The retentions efficiency of anions on layers of silica gel G, alumina, cellulose microcrystalline, kieselguhr G and mixtures of kieselguhr and cellulose in 4:1 and 3:2 ratios, has been examined with the W/O microemulsion system. Thin layers of kieselguhr were most useful for differential migration of anions. Quantitative separation of $\text{IO}_4^-$ from accompanying ions, limits of determination and dilution of few anions have been reported. The effects of amines, phenols and heavy metals on the separation efficacy of $\text{IO}_4^-$ also have been investigated. Semi-quantitation of $\text{IO}_4^-$ by peak-height measurement has been performed (Figure 4.1).

Figure 4.1: Standard calibration curve for semiquantitative determination of $\text{IO}_4^-$ by peak-height measurement method.
CHAPTER - V: TLC SEPARATION, COLORIMETRIC DETERMINATION AND RECOVERY OF THIOCYANATE FROM PHOTOCYANIC WASTE, RIVER AND SEA WATERS

This chapter summarizes the results of thin layer chromatographic retention studies of some inorganic anions (SCN⁻, NO₂⁻, MnO₄⁻, CrO₄²⁻, Cr₂O₇²⁻, MoO₄²⁻, Mo₇O₂₄⁶⁻, Fe(CN)₆³⁻ and Fe(CN)₆⁴⁻) and metal ions (Fe³⁺, Zn²⁺, Cu²⁺, Cd²⁺, Co²⁺, Hg²⁺, UO₂²⁺, VO²⁺, Ag⁺, Mn²⁺ and Bi³⁺). Inorganic ions were chromatographed on plain and mixed adsorbents containing cellulose and/or kieselguhr using mixtures of 0.1 M NH₄OH and CH₃COCH₃ in various ratios as mobile phases. The effect of proportional composition of mobile phase on the hRₚ values of inorganic anions was examined. Cations remained at the point of application. Some anion - cation separations (Ag⁺ - SCN⁻, Fe³⁺ - SCN⁻, Co²⁺ - SCN⁻) were achieved. Effect of additives and pH was examined on the TLC detection of SCN⁻.

The proposed TLC system was coupled to the spectrophotometry of SCN⁻ using Fe³⁺ - SCN⁻ color reaction in acidic medium. The optical density was measured at 460 nm and the Beer’s law was followed upto 11.18 ppm concentration of SCN⁻.

To test the applicability, proposed TLC - colorimetric method was applied to the fortified samples of photogenic waste, river water and seawater. During entire experimental work, relative standard deviation varies from 2.124 to 10.442 pph.
CHAPTER VI: A NEW CHROMATO-IODOMETRIC METHOD FOR THE SEPARATION AND DETERMINATION OF IODIDE AND ITS OXYANIONS

This chapter encapsulates the results showing the retention behavior of \( I^- \), \( IO_3^- \) and \( IO_4^- \) ions on plain sorbent phases (silica gel G, cellulose microcrystalline, alumina G and kieselguhr G) using mixtures of 0.1 M \( \text{NH}_4\text{OH} \) and \( \text{CH}_3\text{COCH}_3 \) as mobile phases. The chromatographic system consisting of alumina and 0.1 M \( \text{NH}_4\text{OH-CH}_3\text{COCH}_3 \) (9:1) was found to be best as it provides more compact and well resolved spots of anions. Effect of time was examined on the detection of \( IO_4^- \) in a ternary mixture with \( IO_3^- \) and \( I^- \) (Figure 6.1).

A new iodometric method was developed for the quantification of \( I^- \), \( IO_3^- \) and \( IO_4^- \). The titrant was 2.0% aqueous acetone for all analytes. The proposed TLC-iodometric method was applied to recover iodide, iodate and periodate from fortified water samples. The LOQ for \( I^- \), \( IO_3^- \) and \( IO_4^- \) were 76.4, 16.3 and 16.6 \( \mu g \) respectively.
CHAPTER VII: COLORIMETRIC DETERMINATION OF Ag(I) IN ORES WITH PRELIMINARY TLC SEPARATION FROM ASSOCIATED METAL IONS

This chapter encapsulates the results of TLC separation of metal ions on layers of cellulose microcrystalline, alumina G and their binary mixtures. Aqueous solutions of NH₃, AcOH, AcONa, AcONH₄ and NaNO₃ were used as mobile phase. Ag⁺ was found separated from other metal ions on mixed layer using 1.0 M NH₄OH as mobile phase. Some important binary, ternary and quaternary separations were achieved. Effect of acid-base equilibrium was examined on the retention behavior of Ag⁺. Figure 7.2 shows the formation of double spot of Ag⁺.

![Figure 7.2: Formation of double spot of Ag⁺. M₁-M₅ are mixed mobile phases containing AcOH and NH₄OH in 10:0, 7:3, 1:1, 3:7 and 0:10 respectively.](image)
After TLC separation, Ag⁺ was quantitatively determined by using dithzone solution (in acetone) as chromogenic reagent. The optical density was measured at 480 nm to sketch the standard calibration curve (Figure 7.3). The TLC-colorimetric method was applied to the horn silver (AgCl) and argentite (Ag₂S) for the recovery of Ag⁺.

![Figure 7.3: Standard calibration curve for the colorimetry of Ag(I).](image)
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DEDICATED

to (Late) Professor M. Qureshi,
the Founder of Analytical Chemistry
at Aligarh Muslim University, Aligarh (India)
CERTIFICATE

Certified that the work incorporated in this thesis is the original contribution of **Mr. Jay Pal Singh Chahar** and is suitable for submission to the award of Ph. D degree.

(ALI MOHAMMAD)
Supervisor

Res: Iqra Colony, New Sir Syed Nagar, Aligarh-202002 (INDIA)
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(JAY PAL S. CHAHAR)
LIST OF PUBLICATIONS


CHAPTER-1
GENERAL INTRODUCTION
Chemistry, the branch of science is concerned with the studies of properties, composition and structure of matter. The term 'Chemistry' was originated from the word 'Chemia' which means black color. The soil of Egypt was called as chemia since it was black in color. Chemistry deals with a wide field of knowledge, so for the ease of studies it became imperative to classify it into various branches e.g. inorganic, organic, physical, industrial, agricultural and analytical chemistry.

1.1 ANALYTICAL CHEMISTRY

Analytical chemistry has emerged among the branches of science to be the most interdisciplinary. It is concerned with the theory and practice of methods used to determine the composition of matter. Analytical chemistry is also concerned with the techniques employed in chemical analysis. The analysis may either be qualitative (study of physical / chemical nature of analyte species) or quantitative (determination of how much of a particular substance is present in a sample). Analyte species may be classified as shown in Figure 1.1.

In recent years, technological developments has create analytical problems which demand increasingly sophisticated instrumentation and wider knowledge for their solution. As a result of advancements made, numerous instrumental and non-instrumental techniques (e.g. gravimetry, titrimetry, solvent extraction, spectroscopy, polarography, potentiometry, electrophoresis and chromatography) are available today for analysts' use.

1.2 CHROMATOGRAPHY

The term 'Chromatography' is derived from two Greek words 'Chroma' and 'Graphy' meaning color and writing respectively. Hence, the chromato-
Figure 1.1: Classification of Analyte Species
graphy is a branch of analytical chemistry where the studies are based on color. Although, meaning of the term is largely understood by chemists, a good definition of chromatography is difficult to formulate. It is a collective term to methods which appear diverse in some regards but share certain common features. A definition should emphasize that components of sample are distributed between two phases but this alone is inadequate because we do not wish the term to embrace all the separation processes. Chromatography defined by Cassidy (1) and Keulemans (2) received wide acceptance in the past. However, the IUPAC has recently defined chromatography as: A method used primarily for the separation of components of a sample, in which the components are distributed between two phases, one of which is stationary, while the other moves. The stationary phase may be a solid, a solid supported liquid or a gel. The mobile phase may be a liquid or a gas. The separation occurs because each analyte distributes differently between both the phases.

1.3 HISTORY OF CHROMATOGRAPHY

At about the end of 19th century, Davy observed the compositional changes in crude petroleum when it came in contact with rocks. A Russian botanist M. Tswett was attracted towards the fact that the crude petroleum is derived from the plants which were buried millions of years ago under the earth's crust while a rock is not much different from CaCO$_3$ from the chemical point of view. Being inspired from Davy's observation, M. Tswett packed a narrow glass tube with CaCO$_3$ and passed petroleum ether extract of chlorophyll and other plant pigments through that column. M. Tswett
<table>
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<th>Originator</th>
<th>Technique</th>
<th>Salient features</th>
</tr>
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<tbody>
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<td>M.S.Tswett (1906)</td>
<td>Adsorption Chromatography</td>
<td>Solid S.P.$^1$ (column); flow of liquid mobile phase under CA$^2$; adsorption phenomena.</td>
</tr>
<tr>
<td>N.A.Izmailov and M.S.Schraiber (1938)</td>
<td>Thin-Layer Chromatography (Adsorption)</td>
<td>Solid S.P. (planar); liquid mobile phase flows under CA; adsorption.</td>
</tr>
<tr>
<td>L.C.Craig (1944)</td>
<td>Countercurrent Chromatography</td>
<td>Liquid-liquid partition Chromatography.</td>
</tr>
<tr>
<td>Barrer (1945)</td>
<td>Gel-Permeation Chromatography</td>
<td>Solid S.P.(column); Solvent moves under CA; size-exclusion phenomena.</td>
</tr>
<tr>
<td>S.Claesson (1946)</td>
<td>Gas-Solid Chromatography</td>
<td>Solid (column) S.P.; gas mobile phase moves under FF$^3$.</td>
</tr>
<tr>
<td>Mayer and Thompkins</td>
<td>Ion-Exchange Chromatography</td>
<td>Solid (ion-exchange resin) S.P. in the shape of column; liq-</td>
</tr>
<tr>
<td>Originator</td>
<td>Technique</td>
<td>Salient Features</td>
</tr>
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<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>J.G.Kirchner; G.J.Keller</td>
<td>Thin-Layer Chromatography (partition)</td>
<td>Solid Supported liquid (planar) S.P.; liquid mobile phase flows under CA; partition phenomena</td>
</tr>
<tr>
<td>J.M.Miller and G.J.Keller</td>
<td></td>
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<td>(1951)</td>
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<td>(1952)</td>
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<tr>
<td>H. Small, T.S. Stevens and W.C.Bauman</td>
<td>Ion Chromatography</td>
<td>Bonded liquid (column) S. P.; liquid mobile phase moves under FF; suitable for ions.</td>
</tr>
<tr>
<td>(1975)</td>
<td></td>
<td></td>
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<tr>
<td>A.Zlatkis and R.E.Kaises</td>
<td>High-performance Thin-layer Chromatography</td>
<td>Bonded liquid (planar) S. P.; liquid mobile phase moves under CA; modified partition phenomena; instrumental technique with computerized documentation.</td>
</tr>
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<td>(1976)</td>
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<tr>
<td>E.Tyihak, E.Mincsovics and H.Kalasz</td>
<td>Over-pressurized layer Chromatography</td>
<td>Solid (planar) S. P.; liquid mobile phase moves under FF. OPLC is TLC with a constant optimized mobile phase flow speed.</td>
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<tr>
<td>(1979)</td>
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</tbody>
</table>
found plant pigments separated and he published two research papers (3,4). Tswett's work was realized in 1931 by a prominent organic chemist R. Kuhn and his co-workers when they used chromatographic system similar to that used by Tswett and separated natural products (5,6). This research attracted more attention and the adsorption phenomena was started to be used in the field of natural product chemistry. When chromatography became capable of solving critical analytical problems, analysts were attracted towards it and made improvements in this field with regards to the separation efficiency, selectivity and sensitivity of chromatographic system, lesser retention time etc. As a result, various chromatographic techniques have been developed and of them, some are presented in Table 1.1.

1.4 CLASSIFICATION OF CHROMATOGRAPHY

All the known types of chromatography can be put into four groups: (a) liquid-solid, (b) gas-solid, (c) liquid-liquid and (d) gas-liquid. Considering various classification factors e.g. nature and physical state of stationary and mobile phases, mode of mobile phase movement and shape of stationary phase, chromatographic techniques may also be classified. Besides these factors, chromatography may also be classified on the basis of phase distribution process, as described below;

(a) Adsorption Chromatography: Adsorption is the tendency of molecules, ions or atoms in solution to interact with the surface of a solid. The chromatography involving adsorption of analyte species as retention phenomenon on a stationary phase of a surface active granular solid is known as adsorption chromatography. The surface of an adsorbent has rigid structure and free valencies to make the chromatographic system more useful
to separate geometrical and structural isomers. Silica has been the most common adsorbent since long. At present, a variety of silicas and other customary materials such as CaCO₃, MgCO₃, kieselguhr, alumina, starch, cellulose, and organic polymers are available to use as stationary phase.

The use of more polar stationary phases with less polar mobile phases was considered to be the normal phase adsorption chromatography. A different approach utilizing non-polar adsorbents and more polar mobile phases has become dominant in recent years and is termed as reversed phase chromatography.

*(b) Partition Chromatography:* The chromatography in which the distribution of solute takes place between two nearly immiscible liquids (stationary and mobile phases) is known as partition chromatography. The stationary liquid is supported by a highly porous solid of large surface area. In 1941, Martin and Synge (7,8) employed silica gel as a solid support to immobilize water as stationary phase in a chromatographic column. Chemically bonded stationary phases are viewed by many chromatographers as liquid films on solid supports, or at least analogs there of; reversed phase operations with such column packings are then treated as liquid-liquid partition. The bonded phases eliminate problems related to stripping of stationary liquid from the column by the mobile phase. In principle, this is circumvented by pre-saturating the mobile phase with the stationary liquid, but difficulty ensues if it is desired to alter the composition of mobile phase during the experiment (i.e. gradient elution).
(c) **Ion-Exchange Chromatography**: In this type of liquid chromatography, ion-exchange resins are used as stationary phase. A resin is a solid, insoluble co-polymer, capable of exchanging ions of similar charge present in a solution. The resins are prepared by introducing ionizable groups into an organic polymer matrix, of which the most common is the cross-linked polystyrene. A table of ion-exchange resins and their properties has been presented by Dorfner (9). The major types of ion-exchange layers in use includes, polyester sheets coated with a mixture of silica gel and anion /cation exchange resin with an inert binder (Polygram ionex-5), polyethyleneimine (PEI) cellulose, diethylaminoethyl (DEAE) cellulose, mixed layer of DEAE cellulose and unmodified cellulose etc.

(d) **Size-Exclusion Chromatography**: In size-exclusion chromatography, the stationary phase consists of small particles having pores. If certain molecules are sufficiently small to move into the pores, they will be held up the stationary phase and those particles which are too large to enter the pores will pass the stationary phase readily on percolating the mobile phase. Size-exclusion chromatography is also known as gel-filtration chromatography or gel-permeation chromatography. This is of greater use in liquid chromatography for the separation of molecules having vapour pressure too small to be separated by the gas chromatography.

The process of size-exclusion is concerned with the distribution of solute between the aqueous phase within the gel and external water. Two important types of column materials useable in aqueous media are cross-linked dextrans (Sephadex G) and polyacrylamides (Bio-gel F).
1.5 THIN-LAYER CHROMATOGRAPHY

TLC is a type of liquid chromatography in which the stationary phase is in the form of layer supported by glass plate, aluminium foil or plastic sheet. As originally developed in 1938 and still widely practiced today, classical capillary action TLC is an inexpensive, rapid, simple and highly effective analytical technique requiring little instrumentation. A suitable development chamber containing mobile phase and a TLC plate is all that required to carry out qualitative as well as quantitative analysis. TLC is highly selective and flexible because of the availability of a great variety of layer materials and wider choice of mobile phases. Following are some purposes for which the thin-layer chromatography is of help.

(i) Identification of substance.
(ii) Separation of two or more components of a mixture.
(iii) Determination of amount of a particular species present in a sample.
(iv) Preconcentration and preparation of a sample.
(v) Study of relative polarity of any solid-liquid or liquid-liquid phase.

1.6 HISTORY OF TLC

In 1966, Pelick et al. (10) presented a tabulation of significant developments in TLC and provided translation of classical studies made by Izmailov and Schraiber and by Stahl. In continuation, the history of TLC was reviewed by Stahl (11), Heftmann (12), Kirchner (13-15), Jork and Wimmer (16), Wintermeyer (17) and Sherma (18). From the historical point of view, countable achievements made in the history of TLC, are enlisted in Table 1.2.
### Table 1.2: Important Years in the History of TLC.

<table>
<thead>
<tr>
<th>Year</th>
<th>Chromatographer(s) and their work</th>
<th>Ref.(s)</th>
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<tbody>
<tr>
<td>1938</td>
<td>Izmailov and Schraiber used unbound alumina layers and applied the drops of solvent to the plate to separate certain medicinal compounds. The procedure was called “drop chromatography”.</td>
<td>19</td>
</tr>
<tr>
<td>1949</td>
<td>Meinhard and Hall, using drop chromatography, separated Fe^{2+} and Zn^{2+} on microscope slides coated with alumina (adsorbent) + starch (binder).</td>
<td>20</td>
</tr>
<tr>
<td>1951</td>
<td>Kirchner et al. used glass plates to support the layer, developed the plates by ascending technique, and coined the term chomatostrip for his layers.</td>
<td>21</td>
</tr>
<tr>
<td>1958</td>
<td>E. Stahl introduced the term chromatography and standardized the materials, procedures and nomenclature involved in TLC.</td>
<td>22,23</td>
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<tr>
<td>1965</td>
<td>Przybylowicz et al. discussed the importance of precoated TLC plates.</td>
<td>24</td>
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<tr>
<td>1976</td>
<td>Zlatkis and Kaiser modernized TLC in the form of a highly instrumental technique and named as high performance thin-layer chromatography (HPTLC).</td>
<td>25</td>
</tr>
<tr>
<td>1979</td>
<td>Tyihak et al. applied extra force for the movement of solvent and introduced over-pressurized layer chromatography (OPLC).</td>
<td>26</td>
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</tbody>
</table>
1.7 THEORY AND PRINCIPLE OF TLC

In the liquid chromatography, the solvent phase moves through interparticular space available among the particles of stationary phase. When passes through the point (where the analyte has been spotted), the mobile phase tries to keep the solute with it. The migration of solute over stationary phase depends upon the relative interactions of stationary/mobile phase on the solute. In a TLC system, the $R_p$ coefficient is a basic quantity used to express the migration of analyte species on the chromatoplate. It can be calculated as,

$$R_F = \frac{\text{Distance travelled by the analyte}}{\text{Distance travelled by the solvent front}}$$

Both the distances are measured from the point of application of analyte.

In other words,

$$R_F = \frac{R_L + R_T}{2}$$

Where, $R_L$ and $R_T$ values are $R_F$ of leading and trailing fronts of the spot respectively. The $R_F$ values vary from 0.00 (analyte remain at the spotting line) to 0.99 (analyte migrates with the solvent front).

Practically, all solutes can be adsorbed on a microporous solid surface or be partitioned between two immiscible liquids, but, there are some thermodynamic quantities, generally used in physical chemistry, are coupled with the empirical parameters of retention as in the form of 'semi-empirical models of chromatography'.

Snyder (27,28) and Soczewinski (29) established a semi-empirical model in late 1960's to get relationship between the retention coefficient
(R_F) and the thermodynamic adsorption coefficient as,

\[ R_F = \frac{1}{K_{th} \cdot (Va \cdot Wa) / Vm} \]

Where, Vm is total volume of mobile phase; Va is the volume of adsorbed mobile phase per unit mass of adsorbent and Wa is the considered mass of adsorbent. The thermodynamic adsorption coefficient (K_{th}) was defined by Scott and Kucera (30,31) with the help of following equation,

\[ K_{th} = \frac{Fp' f_1(T) \cdot Cp' + Fd' f_2(T) \cdot Cd'}{Fp f_3(T) \cdot Cp + Fd f_4(T) \cdot Cd} \]

Where, Fp' and Fd' are polar and dispersive forces respectively between the solute molecules and the stationary phase; Fp and Fd are polar and dispersive forces respectively between solute molecules and the mobile phase; Cp', Cp and Cd are the concentrations of polar moieties and dispersive moieties in the stationary and mobile phases respectively. Cd is proportional to the density of Dispersive medium which can be expressed as concentration in terms of the mass per unit volume. Cd = A.d, where, A is a constant.

Martin and Synge (8,32) established the Martin-Synge model of partition chromatography to ascribe thermodynamic meaning to the retention parameter (R_F). According to this model, R_F value can be viewed as,

\[ R_F = \frac{1}{1 + K (Vm / Vs)} \]

Where, Vm and Vs are the volumes of mobile and stationary phases respectively.

In order to develop a quantitative measure the solvent's relative ability
to intermolecularly interact with the solutes as proton acceptors, proton donors and strong dipoles, Snyder (33,34) established a new model coupling the solvent's polarity index ($P'$) with solubility constants ($Kg''$) of the selected test solutes; ethanol (a model proton donor), dioxan (a model proton acceptor) and nitromethane (a model strong dipole). The main relationship of this approach is,

$$P' = \log (Kg'')_e + \log (Kg'')_d + \log (Kg'')_a$$

where, $(Kg'')_e$, $(Kg'')_d$ and $(Kg'')_a$ are measures of the excess retention of given solute (i.e. ethanol, dioxane and nitromethane respectively) relative to an n-alkane of equivalent molar volume.

1.8 BASIC STEPS OF TLC ANALYSIS

The thin layer chromatographic analysis includes the identification, separation, and quantitative determination of a substance involving the following basic steps:

(I) SAMPLE PREPARATION

Sample preparation procedures for TLC are similar to those for other chromatographic techniques. The sample solutions to be analysed must be sufficiently pure and concentrated so that the analyte can be easily detected and separated as a discrete, compact spot/zone. If the substance is present at low level in a complex sample, solvent extraction, purification and concentration procedures usually precede TLC. Preparative TLC may also be used for the same purpose. An informative chapter on sample preparation for TLC was written by J. Sherma (18). A. Mohammad listed some solvent systems used to prepare the samples of inorganics and organometallics (35).
For the preparation of metal ion solutions, their nitrates, chlorides or sulfates of analytical reagent grade are dissolved in double distilled water, 0.1M HNO$_3$ or 0.1M HCl to a final metal ion concentration of 0.05-0.2M. Anion standards are solutions prepared from sodium, potassium or ammonium salts of the corresponding acids using distilled water, dilute acids or alkalies. The metal complexes are generally taken as freshly prepared solutions in ethanol, acetone, chloroform or distilled water. The complexes were occasionally dissolved in the corresponding solvent system being used as mobile phase.

(II) TLC PLATE PREPARATION

The available procedures for the manual preparation of thin layers from sorbents (manufactured specially for TLC) include spreading, dipping, pouring or spraying the slurry on a glass or plastic plate. Of these procedures, the spreading one is the most common and several types of TLC applicators are available for this purpose. Manually prepared layers should have uniform, bright and translucent appearance when viewed in incident and transmitted light and the layer should also be sufficiently bound to the supporting sheet. Standard TLC plates are 20×20 or 20×10 cm$^2$ size and the thickness of dried layer for analytical purposes is kept to 0.1-0.3 mm. The most common layer thickness in use at present is 0.25 mm.

There has been a tremendous improvement in the technology of layer materials since 1956 when silica gel with standardized and reproducible properties became available for hand made thin layers primarily due to Stahl's constant efforts. A large number of layer materials have been used as the stationary phase in inorganic TLC but silica gel, as usual has been the
much favoured layer material. The layer materials used so far in inorganic TLC / HPTLC may be categorized as under:

(a) **Non Surface Modified Layers**: These are layers prepared from silica gel G / H, alumina, cellulose, polyacrylonitrile (PAN), kieselguhr etc.

(b) **Chemically Modified and Bonded Layers**: This group of layers include lipophilic C\textsubscript{18} bonded silica gel, aminopropyl silica gel, octadecyl silica gel, ECTEOLA cellulose, carboxymethyl cellulose, aminobenzyl cellulose, diethyl-(2-hydroxypropyl) aminoethyl cellulose, fixion 50x8 etc.

(c) **Inorganic Ion-Exchange Layers**: Stannic silicate, zirconium phosphoantimonate, zinc ferrocyanide, Stannic sulfosalicylate and binder free zirconium antimonate in H\textsuperscript{+} form have been used as cation exchange layer materials whereas hydrous antimony (V) oxide has been the most commonly used anion exchanger.

(d) **Impregnated Layers**: For the preparation of impregnated layers generally, inorganic salts, acids, chlorobenzene, amines, dimethylsulfoxide (DMSO), tributylamine (TBA), tributylphosphate (TBP), EDTA, mixture of alizarin red S and aliquat 336 are used as impregnantes in the inorganic TLC.

(e) **Mixed Layers**: The binary layers that have been used in inorganic TLC include silica gel + microcrystalline cellulose, silica gel + ion-exchange gels, microcrystalline cellulose + modified silica gel H, silica gel + alumina/antimonic acid and kieselguhr + cellulose.

(f) **Miscellaneous Layers**: These materials include silufol, silufol UV 254, silufol with a layer of silica gel, soil, soil + flyash, polychrome A,
diatomite, chitin, chitosan, kieselguhr, kaolin, and polyamide have also been used as layer materials.

(III) SAMPLE APPLICATION

Sample application is a critical step for obtaining good resolution and quantification in TLC. Manual spotting methods, which involve the use of a variety of sample applicators are most frequently employed in quantitative TLC.

The simplest technique to apply samples spotwise is to use a fixed volume pipette of 100 nL and 200 nL that are available in the form of platinum-iridium capillaries melted in a glass holder. Larger volumes can be applied with fixed volume glass capillaries available for volumes of 500 nL and greater. As an alternative to capillary pipettes, a micrometer controlled syringe can be used for the same purpose. One aspect of the syringe type dosage device is its selectable volume. The nanoapplicator (Nanomat) is an example of micrometer controlled syringe which has a dynamic volume range of 50-230 nL.

An advantageous alternative to sample application as spots is the application of sample in the form of narrow band or streak. This provides the highest resolution attainable with a particular TLC system (36). The Linomate, an applicator, allows sample application in narrow bands by a spray-on technique. By employing this technique, the gain in precision of quantitative TLC analysis can be expected to be in the range of 30%. With the aid of this device, sample volumes ranging from 2-100 µL can be applied onto HPTLC plate. A valuable aid for sample application in large volumes of very dilute samples is the concentrating zone (Fig. 2.2) which
consists of a chromatographically inactive adsorbent (Kieselguhr). The substances to be separated are concentrated to a small band at the interface between concentrating zone and the chromatographically active adsorbent (Silica). Another method of sample concentration is a short pre-elution (few mm) with a solvent, in which all substances have higher $R_F$ values.

![Diagram of TLC application](image)

**Figure 1.2**: Bandwise ($A_1$) and spotwise ($A_2$) application of sample (mixture of substances $S_1$ and $S_2$).

The automatic TLC sampler III' is the latest version of this kind. It applies sample automatically from a rack of sample vials. The samples are desponded from a steel capillary, spotwise by contact transfer or bandwise by spray-on technique. The capillary moves at a lateral speed of 25 cm/s. The sample dispensing speed is selectable between 10 and 1000 nL/s. The application module is controlled by an IBM-compatible PC, normally the same computer that controls densitometric evaluation of chromatogram.

After sample application, the solvent of the sample is allowed to evaporate completely before the start of development of TLC plate. It can be
achieved either allowing the spot to air dry at room temperature or by blowing the cold or hot air.

(IV) DEVELOPMENT OF CHROMATOGRAM

The movement of mobile phase through sorbent bed is known as its development. In TLC, various factors i.e mobile phase composition, temperature, humidity and chamber saturation strongly affect the retention of analyte. Numerous modes are available for the development of chromatogram, some are discussed below:

(a) Ascending: This is the most frequently used technique for linear development. Following sample application and drying of the zone, TLC plate is placed in a pre-saturated jar in such a manner that the mobile solvent phase must be below the point of application of the sample. The mobile phase is then allowed to ascend by capillary action, usually to a distance of 10-15 Cm above the origin on 20×20 Cm TLC plate and 3-7 Cm on smaller HPTLC plates.

Covered glass tank in which the TLC plate is placed for development is known as chamber. Various chambers i.e N-chamber, S-chamber, Twin trough chamber (an N-chamber modified with an inverted V-shaped ridge at the bottom dividing the tank into two sections) are available. A computer controlled automatic developing chamber has been designed to provide programmable, reproducible linear ascending development without operator's attention.

(b) Descending: Downward movement of mobile phase occurs in this type of development. For descending development, the mobile phase must be fed to the top of a vertical inclined plate through a wick arrangement.
This technique has no significant advantages over ascending one and is rarely used for the thin-layer gel filtration chromatography (TLG) for proteins, peptides and nucleic acids. By employing this mode, the development time can be reduced slightly as the capillary forces and gravitational forces function in the same direction.

(c) Horizontal: In this type of development, a continuous supply of solvent must be transferred from a capillary arrangement. Development can be carried out from one as well as from both sides. The developing solvent held in narrow troughs, is carried to the layer through capillary slits formed between the trough walls and glass slides. The chamber is covered with glass plate during pre-equilibration and development can be operated in N-type or S-type configurations. The horizontal developing chamber permits simultaneous development from opposite edges to the middle of sixty sample spots on 20×20 cm HPTLC plate or thirty samples on a 10×10 cm plate.

(d) Two-Dimensional: In fact, two-dimensional development is a technique but not a mode of chromatogram development for which, the ascending mode is adopted for the full length of the plate to achieve maximum resolution. The plate is air dried, rotated at 90° angle and redeveloped usually with a second solvent having a diverse, complementary selectivity. Resolution (spot capacity) in 2-D TLC is greatly improved as compared to 1-D TLC because sample components are resolved over the entire area of the plate. Poole et al. (37) have reviewed methods for combining different retention mechanisms in 2-D TLC.

(e) Radial: For radial or circular development, the mobile phase is slowly applied to the center of ring of spots in the middle of a horizontal TLC plate.
Sample components move outward in the form of concentric arcs under the influence of a negative solvent gradient that causes the rear of each zone to move relatively faster than the front. The relation between linear and circular migration is given \((38,39)\) as:

\[
R_F^{\text{linear}} = R_F^{\text{circular}}^2
\]

Circular chromatography can be carried out with forced flow as well as under capillary action flow of mobile phase. No special plate preparation is required for off-line radial OPLC or RPC, but for on-line radial OPLC, a sector must be isolated by scraping the layer and then impregnated with solvent\((40)\).

In addition to above mentioned modes of chromatogram development, multiple\((41)\), continuous\((42-45)\), gradient\((46,47)\) and anti-circular\((48)\) development techniques are also available.

**MOBILE PHASE:**

In liquid chromatography including TLC, the mobile phase exerts a decisive influence on the separation. Various optimization schemes (Windows diagram, Overlapping resolution maps, the Simplex method and PRISMA model) are proposed for normal phase and reversed phase TLC. A large number of mobile phases have been reported, of them some are enlisted below.

**(a) Organic Solvents:** The single component mobile phase comprising of acetone, acetonitrile, benzene, carbon tetrachloride, chloroform, dioxane, ethanol, ethylacetate, methanol, o-xylene, petroleum ether, toluene, n-octanol, n-nonane, cyclohexanes and binary/ternary mixtures of alcohols, amines, ketones, phenols, haloalkanes have been used.
(b) *Inorganic Solvents*: Being non-toxic and non-volatile, solvent systems of this group have been widely used in TLC of inorganics and organometallics. This group includes the solution of mineral acids, alkalis and inorganic salts prepared in double distilled water or water-methanol mixture.

(c) *Mixed Solvents*: Mixtures of two or more different solvents, most of which have either a base (NaOH, NH₄OH, R-NH₃) or an acid (mineral or carboxylic) as a component, are used to develop the TLC plate.

(d) *Complexing Solvents*: Solutions of surfactants (SDS, CTAB, or Triton X-100), buffered EDTA and DMSO have been used as mobile phase in the TLC of inorganics.

(V) DETECTION AND IDENTIFICATION

After development, TLC plate is removed from developing chamber, dried at room temperature and then activated to make it free from mobile phase. The resolved analyte(s) on plate are detected by their original color, natural fluorescence, quenching of fluorescence or as colored zones produced as a result of chemical reaction of analyte with the detection reagent.

The following detection procedures are available for the visualization of spots on TLC plate.

(a) *Self Detection*: The analytes which are colored or produce colored complexes with stationary/mobile phase, may be viewed in daylight without any treatment. This procedure has limited applicability and is not suitable for the visualization of trace amounts of an analyte on TLC plate.

(b) *Chromogenic Detection*: When a complex (colored) forming reagent is applied onto the TLC plate either by spraying or dipping the plate, The
analyte is visualized on the basis of colored spot and this detection procedure is termed as chromogenic detection. Various types of chromogenic reagents are available for the visualization of inorganics, organics and organometallics (49,50).

(c) UV Detection: Analytes with native fluorescence are viewed as bright zones on a dark background under UV light. For this purpose, shortwave (254 nm) and longwave (366 nm) UV lamps are available for use in a dark room. Viewing cabinets incorporating these lamps are also available for inspecting chromatograms under UV light in an undarkened room (51).

Compounds that absorb UV radiation on an "F-layer" containing a phosphor or fluorescent indicator (often zinc silicate), when irradiated with 254 nm UV light, absorbing compounds quench the uniform layer fluorescence and detected as dark violet spots on bright background.

(d) Flame Ionization Detection: A novel detection method for TLC involves the use of a FID in conjunction with sorbent coated rods (52,53). The coated rods are developed in a saturated N-chamber, dried and advanced automatically at a constant speed through the hydrogen flame of the FID. The separated analytes on the rod are ionized by the flame and the ions generate an electric current, proportional to the amount of substance entering the flame. Detection procedures for a number of compounds, are well documented by J. Sherma (54).

(e) Enzymatic Detection: Enzymatic reactions can be monitored on a TLC plate and the end product can be detected. Many pesticides inactivate or inhibit enzymes associated with animal nervous tissue. The presence of minute amounts of such cidal substances may interfere with the indoxyl
acetate substrate reaction, producing colorless spots on background (55,56).

(f) Biological Detection: Biological test procedures are useful for the specific detection of compounds with a certain physiological activity. As a recent example, an agar overlay assay using *Candida abicans* as the indicator organism was devised for the detection and activity-guided fractionation of antifungal compounds by TLC. Inhibition of fungal growth was assessed with thimole blue. The technique was compatible with diol and $C_{18}$ plates (57).

(VI) SEPARATION

When two or more analytes have differential migration with the same chromatographic system, they are mixed thoroughly. The mixture is loaded onto the TLC plate and chromatographed. The separated components of mixture are detected and their $R_f$ values are recorded. Following are some basic requirements for a good separation,

(a) Each spot should be compact ($R_L - R_T < 0.3$).

(b) The difference in $R_f$ values of two adjacent spots should be at least 0.1.

(c) No complex formation should be taken place between/among separable species.

(d) Chromatography of individuals and the mixture should be performed in identical manner.

(VII) QUANTITATION

When the amount of an analyte is determined and the method gives an accuracy and reproducibility in the range of 10-30%, the procedure is considered as semi-quantitative analysis. For more accurate results, method termed as quantitative analysis is performed which gives relatives standard
deviation between 3 and 10%. Following procedures are available for semi-quantitative (a,b) and quantitative (c,d) analyses.

(a) Visual Comparison: The simplest type of in-situ quantitative TLC is based on visual comparison of colored, fluorescent or quenched spots. This method is quick and requires no equipment. Comparison is easier on TLC plates coated with adsorbents of wider range of particle's size. Following two characteristics of analyte spot are useful for semi-quantitative determination.

When total forces (polar and dispersive) of stationary phase acting on analyte are highly greater than that of mobile phase, the substance is strongly adsorbed and the intensity of spot increases instead of its size, with increasing amount of substance loaded on TLC plate. Under such conditions, the amount of the unknown sample is determined by matching the intensity of its spot with those of standards.

When forces of stationary and mobile phases acting on analyte are nearly equal, the size of spot increases with increasing loading amount of analyte and the spot's size of unknown amount is compared visually with that of standards.

(b) Spot-Area Measurement: The methods based on the measurement of spot-area can reduce the error of quantification to the range 5-15%. The spot-area can be measured by anyone of the following methods;

In graphical method, the size and shape of the spot is copied from TLC plate onto the graph paper and the number of squares inside the spot's periphery are counted. This method has highest probability of error be cause an square is counted only when its half or more portion is inside the spot-
area otherwise the square is left, as shown in figure 1.3.

![Graphical Measurement](image)

Figure 1.3: Spot-area Measurement by Graphical Method (Area = 43 mm²).

In area-weight method, size of spot is copied onto the filter sheet followed by cutting and weighing of the filter paper corresponding to the spot-area. The area-weight (weight of filter paper covering the spot-area) is plotted against the loading amount of analyte species to sketch the calibration curve.

Algebraic method avoids the plotting of calibration curves and is based on the three analyses (a sample, diluted sample and a standard) with the same chromatographic system. The calculation of unknown amount is made on the basis of following equation.

\[
\log W = \log W_s + \left[ \frac{\sqrt{A} - \sqrt{A_s}}{\sqrt{A_d} - \sqrt{A}} \right] \log D
\]

Where \( W \) and \( W_s \) are weights of solute in sample (before dilution) and standard respectively; \( D \) is the dilution limit; \( A, A_d \) and \( A_s \) are spot-areas of solute in sample (before dilution), diluted sample and in standard, respectively.

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The RSDs for analyses using this method have been reported between 3 and 10%. Linear relationships have been found between spot-areas and log weight (58) as well as between square root of spot-area and log weight (59). The exact relationship yielding a linear plot has been found to depend on the layer thickness, nature of the solute and the detection reagent (60). As an attempt to standardize the quantitative TLC, Mohammad and Fatima (61,62), Nanda and Devi (63) and Mlodzikowski (64) have established a linear relationship between the spot-size and the amount of substance.

(c) In-Situ Densitometry: It is the most preferred technique for quantitative analysis where the determination of the sample is performed by in-situ measurement of absorbance or fluorescence of a separated zone using an optical beam in the form of a rectangular slit. A schematic diagram of a single beam scanner arranged for measurement of absorption in the reflectance mode is shown in Figure 1.4.

The purpose of scanner is to convert the spot on a layer into a chromatogram consisting of a series of peaks similar in appearance to GC or HPLC. Halogen or tungsten lamps are used to provide light of 400-800 nm range (visible absorption), deuterium lamp for scanning directly or as quenched zones on ‘F’ layers in the 190-400 nm range (UV region) while for fluorescence scanning, a high intensity xenon or mercury lamp is used as the source. Filters or monochromators (prism or grating) are employed for wavelength selection and a photomultiplier (photodiode) for signal measurement. The plate, mounted on a movable stage is scanned with a fixed beam of monochromatic light. Reflected or transmitted light is measured with
Figure 1.4: Schematic Diagram of Optical Path for Densitometric Absorption Scanning. W, Tungsten lamp; D, Deuterium lamp; M, Mercury lamp; MC, Monochromator; S, Mirror; P, Plate; PM, Photomultiplier detector; R, Recorder; and I is Integrator.
either single beam, double beam or single beam - dual wavelength optical systems. The modern scanner with a computer controlled motordriven monochromator, allows automatic recording of *in-situ* absorption with fluorescence excitation spectra. The method based on fluorescence has been preferred over absorption for quantitative densitometric analysis because of the higher sensitivity, better selectivity, wider linear range of calibration curve (peak height Vs concentration).

Transmission or reflectance scanning can also be used for photometric evaluation of substances. TLC quantification by *in-situ* densitometry (65,66) and sources of error in densitometric evaluation (67) have been reviewed. The relative standard deviation of *in-situ* densitometry can be maintained below 2%. It is a reliable analytical technique for quantification (68).

(d) **Zone - Elution and Photometry**: In 1958, Kirchner et al. (69) first reported the application of TLC to the quantification in combination with spectrophotometric measurements. As a result of achievements made in this field, reflectance photometry, emission spectroscopy, AS etc. have been combined with TLC for the quantitative analysis.

The TLC-spectrophotometry involves drying the layer, locating the resolved zones, scraping off the separated zones of sample and standards and elution of the analyte from the layer material with a suitable solvent. The eluted substances are concentrated and analyzed by any Independent microanalytical method. Spectrophotometry has been the most widely used technique for the quantification of eluted inorganic species (70-75).
REFERENCES


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CHAPTER -II

THIN - LAYER CHROMATOGRAPHY OF INORGANIC IONS SINCE 1980
2.1 INTRODUCTION

Thin-layer chromatography (TLC) has always been recognized as a practical and effective technique for purifying materials before their analysis with sophisticated instruments. It was one of the chromatographic techniques applied for the routine analysis in preparative chemical laboratories.

In recent years, TLC has grown much in status and has experienced a dramatic surge due to its simplicity, versatility, and low cost. It is now being used as an efficient and rapid detection technique. The combination of speed, sensitivity and simplicity with resolution which exceeds that of low pressure column Chromatography and paper chromatography ensures continued interest in TLC. With optimization of techniques and materials, TLC can be applied for the quantitation of various compounds present in environmental and geological matrices.

The work on TLC of inorganics published up to the end of 1972 has admirably reviewed by Brinkman et al. (1) and that appeared during 1972-1980 has been presented by Kuroda and Volynet (2). The latest work on TLC of inorganics and organometallics covering the period 1978-1995 has been described in chapters (3,4) of the Handbook of Thin-layer chromatography edited by J. Sherma and B. Fried.

The majority of work on inorganics belongs to cations as compared to anions (Figure 2.1). Transition and inner transition metals have been the popular choice because of their technological importance whereas inorganic anion TLC was performed on halides, helates, cyanides, S-, N-, P-containing and multiple anions.
In a recent report (5) J. Sherma has excellently discussed the techniques and instrumentation of HPTLC. Regarding publications on its applications, the interested readers are advised to consult the reviews (6-11) and books (12-20). The information regarding general features, comparative performance of stationary and mobile phases, detection methods, development modes and data processing of inorganic TLC can be found in various review articles (21-35). Besides inorganic TLC, some important applications of TLC along with its theory, principle and instrumentation have been well presented by E. Heftmann (36).

The purpose of this chapter is to present a summary of work done on TLC of inorganic ions during 1980-1996. Total number of papers published in a journal during this period, is plotted against the journal (Figure 2.2). Top ten journals are;

1. *J. Planar Chromatogr.* (J_1)
2. *J. Liq. Chromatogr.* (J_2)
3. *Chromatographia* (J_3)
5. *J. Chromatogr.* (J_5)
7. *Fenxi Huaxue.* (J_7)
9. *J. Chromatogr. Sci.* (J_9)

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Figure 2.1: Relative Study of Work Done on the TLC of Inorganic Anions and Cations

Figure 2.2: Top Ten Journals Regarding the Number of Publications on TLC of Inorganic Ions (1980-1996)
<table>
<thead>
<tr>
<th>Ion / metal</th>
<th>S.P.</th>
<th>M.P.</th>
<th>Comment</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba, Sr, La, Ce, Pr, Nd, Pm, Sm, Eu, Y, Te, Mo, Zr, U Cu, Zn, Ni</td>
<td>S₁</td>
<td>M₁</td>
<td>Two-dimensional development; qualitative analysis of lanthanides on 2mm silanized silica gel layers</td>
<td>37</td>
</tr>
<tr>
<td>Forty-nine ions including W, Ha, Tl, Mo, Re, Sn, Pb, Cr, Ni, Cd, Zr, Co, Te, Sc, Sb, Cu, Ag, As, Be, Fe, Mg, Th, Y, Ge Bi, Th, Zr, Ce, Cr, Al, Ti, W, UO₂, Sn, Fe, Sb, V</td>
<td>S₃</td>
<td>M₃</td>
<td>Development time 35-45 min; layer thickness 0.25 mm; run 17 cm; qualitative separations</td>
<td>39</td>
</tr>
<tr>
<td>Eighteen metal ions Cations</td>
<td>S₅</td>
<td>--</td>
<td>Identification and separation of inorganic cation.</td>
<td>41</td>
</tr>
<tr>
<td>Forty-eight metal ions Rare-earth metals</td>
<td>S₆</td>
<td>M₅</td>
<td>Qualitative separations.</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>S₇</td>
<td>M₆</td>
<td>Qualitative separations.</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>--</td>
<td>M₇</td>
<td>Separation of rare-earth metals in ores (monazite) rocks, pure Ce(NO₃)₃ and irradiated nuclear fuels, separation of rare-earths from uranium</td>
<td>44</td>
</tr>
<tr>
<td>V, Th, As, Pb, Zr, Se</td>
<td>S₈</td>
<td>--</td>
<td>A correlation between R_f values of metal ions on impregnated layers and the stability constant is discussed</td>
<td>45</td>
</tr>
<tr>
<td>Twenty-two cations</td>
<td>S₄</td>
<td>--</td>
<td>Qualitative analysis</td>
<td>46</td>
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</tbody>
</table>
Twenty-three metal ions
Inorganic metal ions
Group I, IIA, IIB, and IIIB cations
Several metal ions
Some anions

Fe (CN)$_6^{3-}$, Fe(CN)$_4^{4-}$, MnO$_4^-$, MoO$_4^{2-}$, S$_2$O$_8^{2-}$, S$_2$O$_7^{2-}$, SeO$_3^{2-}$, SeO$_4^{2-}$, WO$_4^{2-}$, CrO$_4^{2-}$, Cr$_2$O$_7^{2-}$, VO$_3^-$, BrO$_3^-$, IO$_3^-$, ClO$_4^-$, ClO$_3^-$, ClO$_4^-$, NO$_2^-$, NO$_3^-$, Cl$^-$, Br$^-$, NO$_3^-$, SCN$^-$, I$^-$

Qualitative Separations
TLC of 22 anions and 25 cations; detection of CN$^-$ as Hg(CN)$_2$.

Determination of condensed phosphates in commercial liquid polyphosphate fertilizers
Separation by anion-exchange TLC
Separation on silica gel was poor; addition of ion exchange resin markedly improved the resolution.
<table>
<thead>
<tr>
<th>Ion / metal</th>
<th>S.P. M.P.</th>
<th>Comment</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au, Ir(III), Ir(IV), Pt, Pd, Ru, Rh</td>
<td>$S_{17}$ $M_{18}$</td>
<td>Qualitative separations</td>
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<td>Rare-earth metals</td>
<td>$S_{1}$ $M_{19}$</td>
<td>Separation and detection of rare-earth metals in a variety of mixtures</td>
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</tr>
<tr>
<td>Forty metal ions</td>
<td>$S_{19}$ $M_{20}$</td>
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<td>Transition metal ions</td>
<td>$S_{7}$ $M_{21}$</td>
<td>Separation of Fe(III), Ce(IV), Cu(II), Co(II), Cr(III) and Ni(II)</td>
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<td>Rare-earth metals</td>
<td>$S_{1}$ $M_{22}$</td>
<td>Qualitative separations</td>
<td>62</td>
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<td>Mo, Y, La</td>
<td>$S_{2}$ $M_{23}$</td>
<td>Chromatographic behavior of La, Y and Mo was investigated.</td>
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<td>Ni, Cu, Hg, Pb, Bi, As, Ce, Tl</td>
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<td>Cr(III), Cr(VI)</td>
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<td>Optimum condition for quantitative separation of Cr(III) and Cr(VI) from their binary mixtures.</td>
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</tr>
<tr>
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<td>$S_{26}$ $M_{26}$</td>
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<td>Ion / metal</td>
<td>S.P.</td>
<td>M.P.</td>
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<td>Ion / metal</td>
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<tr>
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<td>Ion / metal</td>
<td>S.P.</td>
<td>M.P.</td>
<td>Comment</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Ion / metal</td>
<td>S.P.</td>
<td>M.P.</td>
<td>Comment</td>
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<tr>
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<td>Ion / metal</td>
<td>S.P.</td>
<td>M.P.</td>
<td>Comment</td>
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<tr>
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</tr>
<tr>
<td>Ag, Al, As, Au, Ba, Be, Bi, Ca, Ce, Ce⁴⁺, Co, Cr, Cu, Dy, Fe, Ga, Ge, Hg⁺, Hg, Ln, La, Mg, Mn, Mo⁶⁺</td>
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<td>M₇₅</td>
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</tr>
<tr>
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<td>M₇₆</td>
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</tr>
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</tr>
<tr>
<td>Ag, Al, Cu, Th, Mo, Zn, Cd, Pb, Se, Zr, Ni, W, UO₂²⁺, VO²⁺, Fe, Ti⁺, Co</td>
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<td>Semiquantitative determination of Pb, Ag, and Th by spot area measurement method and spectrophotometric determination of UO₂²⁺ after separation from other metal ions.</td>
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<tr>
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<td>S₄₉</td>
<td>M₈₀</td>
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<tr>
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<td>--</td>
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<td>Qualitative separations.</td>
</tr>
<tr>
<td>Ion / metal</td>
<td>S.P.</td>
<td>M.P.</td>
<td>Comment</td>
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<tr>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
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<tr>
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<td>M₈₉</td>
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<tr>
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<tr>
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</tr>
<tr>
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<td>M₉₂</td>
<td>Separations of Zn(II) from Cd(II) in presence of certain anions in the sample solutions of different pH.</td>
</tr>
<tr>
<td>Ion / metal</td>
<td>S.P.</td>
<td>M.P.</td>
<td>Comment</td>
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<tr>
<td>Fe²⁺ and Fe³⁺</td>
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<td></td>
<td>Ascending technique; run 10 cm; development time 30 min; 0.25 mm layer; sample pH 1.3; separation of Fe²⁺ from Fe³⁺ in presence of Cl⁻, Br⁻, SO₄²⁻, SO₃⁻, PO₄³⁻, and CH₃COO⁻ ions.</td>
</tr>
<tr>
<td>Polyphosphates</td>
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<td></td>
<td>Densitometric determination of polyphosphates in meat products.</td>
</tr>
<tr>
<td>Fe, Cd, Zn, Cu, Bi, Tl⁺, Ag, Hg, Pb, Se, Mn, UO₂²⁺, Ni, Al, Zr, Ti, Th</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Al, Fe²⁺, Fe³⁺ Co, Ni, Cu, Zn, Ag, Cd, Hg, Tl, Pb, Bi Ni, Co, Zn, Cd, Cu, UO₂²⁺, V⁴⁺, Fe²⁺, Fe³⁺, Al, Th, Ti, Mo⁶⁺, Sc, W, Hg, V⁵⁺, Tl⁺, Pb, Bi, Ag UO₂²⁺, Ti, Al, W, Ni, Fe, Co, Mn, Cu, Bi, Zn, Cd, Ta, Se, Pb, Ce, Ag, Th, Zr, Hg, Tl Th, Zr, UO₂²⁺</td>
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<td>M₉₆</td>
<td>Ascending technique; run 10 cm; quantitative separation of Ni from Fe, Zn, Cd, and Pb.</td>
</tr>
<tr>
<td></td>
<td>S₇</td>
<td>M₉₇</td>
<td>Ascending technique; run 10 cm; 0.25 mm layer; loading volume 5 mL; examination of the effect of sample pH, presence of anions in the sample and the presence of alumina in the stationary phase on the separation of V⁵⁺ from W⁶⁺ and Mo⁶⁺.</td>
</tr>
<tr>
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<td></td>
<td>Ascending technique; run 10-12 cm; development time 1-2 h; qualitative separations.</td>
</tr>
<tr>
<td></td>
<td>S₆₀</td>
<td>M₉₈</td>
<td>Ascending technique; run 10 cm; 0.25 mm layer; studies on the effect of anions on the separations Th⁴⁺-Zr⁴⁺-</td>
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<td>M.P.</td>
<td>Comment</td>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Rare-earth elements</td>
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<td>All rare-earths except Pm</td>
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<td>$S_{24}$</td>
<td>$M_{162}$</td>
<td>Qualitative separations ; effect of CaCl$_2$, MgCl$_2$ and NaHCO$_3$ on the separation of anions ; identification of NO$_2^-$ in artificial sea water .</td>
</tr>
<tr>
<td>Cl$^-$, Br$^-$,I$^-$,ClO$_3^-$, ClO$_4^-$, H$_2$PO$_4^-$, NO$_2^-$, NO$_3^-$, SCN$^-$, SO$_4^{2-}$</td>
<td>$S_{107}$</td>
<td>$M_{178}$</td>
<td>Separation of inorganic anions as DMA (diantipyrimethane ) complexes and salts of protonated DMA using radial or ascending technique ; determination of anion content by</td>
</tr>
<tr>
<td>Ion / metal</td>
<td>S.P.</td>
<td>M.P.</td>
<td>Comment</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>------</td>
<td>------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Forty-nine inorganic ions</td>
<td>$S^{102}$</td>
<td>$M^{172}$</td>
<td>planimetry using calibration plots of zone area against amount of anion. $R_F$ value increases with increasing acid or sulfate in the mobile phase.</td>
</tr>
<tr>
<td>3d series transition metal ions</td>
<td>$S^{103}$</td>
<td>$M^{173}$</td>
<td>Examination of the effect of the concentration of $H_2$MEHP and $HNO_3$ on $R_F$ values.</td>
</tr>
<tr>
<td>Uranium</td>
<td>$S^{104}$</td>
<td>$M^{175}$</td>
<td>Ascending technique; selective separation of uranium from synthetic mixture of several metal ions.</td>
</tr>
<tr>
<td>Cations with some anions</td>
<td>$S^{79}$</td>
<td>$M^{176}$</td>
<td>TLC of cations and anions, qualitative separations.</td>
</tr>
<tr>
<td>Inorganic ions</td>
<td>$S^{105}$</td>
<td>$M^{177}$</td>
<td>Separation of polyvalent ions and trivalent rare earths which tend to form anionic sulfate complexes.</td>
</tr>
<tr>
<td>Inorganic metal ions</td>
<td>$S^{108}$</td>
<td>$M^{179}$</td>
<td>The effect of impregnants and of mobile phases of different pH on the mobility of inorganic ions.</td>
</tr>
<tr>
<td>Copper</td>
<td>$S^{7}$</td>
<td>$M^{180}$</td>
<td>Layer thickness 0.25 mm; plate activation at $110^\circ C$ from biological tissue by dryoxidation and ashing, then chromatography on silica.</td>
</tr>
<tr>
<td>Hg(II),Cu(II), Cd(II)</td>
<td>$S^{47}$</td>
<td>$M^{181}$</td>
<td>Temperature $25^\circ C$, UV spectroscopy determination of metal ions after elution with $H_2$O.</td>
</tr>
<tr>
<td>Au(III), Ru(III), Rh(III), Pd(II), Os(IV), Pt(IV)</td>
<td>$S^{5}$</td>
<td>$M^{182}$</td>
<td>$R_S$ value of each pair of ions is 1.0 except that of Ru(III) and Pd(II), limit of detection is 4.0 $\mu g$.</td>
</tr>
<tr>
<td>Inorganic ions</td>
<td>$S^{55}$</td>
<td>$M^{183}$</td>
<td>Relationship between volumetric % concentration (Cp) of IBMK/FA and the $R_F$ values of ions is obtained.</td>
</tr>
<tr>
<td>Inorganic ions</td>
<td>$S^{9}$</td>
<td>--</td>
<td>Reactions of inorganic ions with organic reagents are studied on thin layers.</td>
</tr>
<tr>
<td>Rare-earths</td>
<td>$S^{110}$</td>
<td>$M^{184}$</td>
<td>$R_F$ value of each metal decreased with increasing pKa value of amine used for pretreatment.</td>
</tr>
<tr>
<td>Ion / metal</td>
<td>S.P.</td>
<td>M.P.</td>
<td>Comment</td>
</tr>
<tr>
<td>--------------------</td>
<td>------</td>
<td>------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Actinides</td>
<td>$S_{111}$</td>
<td>$M_{185}$</td>
<td>Actinides are separated on the basis of different sorption behavior in (III) and (V) oxidation states.</td>
</tr>
<tr>
<td>Th$^{4+}$, Fe$^{3+}$, Al$^{3+}$, Bi$^{3+}$, UO$_2^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Ni$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Pb$^{2+}$, Ag$^{+}$, Tl$^{+}$</td>
<td>$S_{112}$</td>
<td>$M_{185}$</td>
<td>Ascending technique ; run 10 cm ; layer thickness 0.25 mm ; limits of detection falls in the range of 0.22 - 3.4 $\mu$g.</td>
</tr>
<tr>
<td>Cd$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, Pb$^{2+}$</td>
<td>$S_{5}$</td>
<td>$M_{186}$</td>
<td>Ascending technique ; run 10 cm. ; quantitation of Cu(II) by AAS after separation from other metal ions.</td>
</tr>
<tr>
<td>Transition metal ions</td>
<td>$S_{113}$</td>
<td>$M_{187}$</td>
<td>Separation of eight component mixture ; quantitative estimation by AAS.</td>
</tr>
<tr>
<td>I$^{-}$, IO$$_3^-$, IO$$_4^-$, Br$^-$, BrO$$_3^-$, NO$_2^-$, SCN$^-$, CrO$_4^{2-}$, PO$_4^{3-}$, MnO$_4^-$, WO$_4^{2-}$</td>
<td>$S_{114}$</td>
<td>$M_{188}$</td>
<td>Ascending technique ; semiquantitation of I$^{-}$, Br$^{-}$ and NO$_2^-$ by spot area measurement.</td>
</tr>
<tr>
<td>I$^{-}$, IO$_3^-$, IO$_4^-$, Br$^-$, BrO$_3^-$, NO$_2^-$, MnO$_4^-$, CrO$_4^{2-}$</td>
<td>$S_{5}$, $M_{189}$, $S_{115}$</td>
<td>$S_{9}$, $S_{21}$</td>
<td>Ascending technique ; run 10 cm. ; semiquantitation of IO$_4^-$ by peak height measurement.</td>
</tr>
<tr>
<td>Thirty cations</td>
<td>$S_{116}$</td>
<td>$M_{190}$</td>
<td>Development time 19min. ; separation of Pt from 27 other cations.</td>
</tr>
<tr>
<td>Cd(II), Cu(II), Pb (II)</td>
<td>--</td>
<td>--</td>
<td>Detection limits for Cd (II) and Pb is 1 and 4 $\mu$g respectively ; on plate square wave stripping voltametry.</td>
</tr>
<tr>
<td>Heavy metal</td>
<td>--</td>
<td>--</td>
<td>Separation and identification of metals in human bones ; placenta ; milk and air by adsorption and IE-TLC.</td>
</tr>
<tr>
<td>Ni, Co, Cu</td>
<td>--</td>
<td>--</td>
<td>Determination in rock samples by TLC / photodensitometry ; relative error ranging from 2 % to 38%.</td>
</tr>
<tr>
<td>Co(II), Fe(II), Cu(II)</td>
<td>$S_{117}$</td>
<td>$M_{191}$</td>
<td>Some new TLC plates are suggested to immobilize various ion - attractive materials.</td>
</tr>
<tr>
<td>Ion / metal</td>
<td>S.P.</td>
<td>M.P.</td>
<td>Comment</td>
</tr>
<tr>
<td>------------</td>
<td>------</td>
<td>------</td>
<td>---------</td>
</tr>
<tr>
<td>Al, Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Sn, Ta, Ti, V, Y, Zr</td>
<td>$S_{118}$</td>
<td>--</td>
<td>ICP-AES determination in Zr-U alloys after separation by TLC.</td>
</tr>
<tr>
<td>Metal cations</td>
<td>--</td>
<td>--</td>
<td>Determination of Fe in process media by employing 8-hydroxyquinolone as complexing agent; Lumogallion was used in Al detection.</td>
</tr>
<tr>
<td>SCN$^-$, MnO$_4^-$, NO$_2^-$, CrO$_4^{2-}$, CrO$_7^{2-}$, MoO$_4^{2-}$, Mo$<em>7$O$</em>{24}^{6-}$, Fe(CN)$_6^{4-}$, Fe(CN)$_6^{3-}$, Fe$^{3+}$, Zn$^{2+}$, Cu$^{2+}$, Ca$^{2+}$, Co$^{2+}$, Hg$^{2+}$, UO$_2^{2+}$, VO$^{2+}$, Ag$^+$, Mn$^{2+}$, Bi$^{3+}$</td>
<td>$S_{119}$ M$_{192}$</td>
<td>TLC separation and colorimetric determination of SCN$^-$ as applied to water and wastewater.</td>
<td>254</td>
</tr>
</tbody>
</table>

S.P. : Stationary Phase

M.P. : Mobile Phase
Table 2.2 : List of stationary phases used

<table>
<thead>
<tr>
<th>( S_i )</th>
<th>Phase Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Silanized silica gel (Merck, Darmstadt) and polygram plate</td>
</tr>
<tr>
<td>2</td>
<td>Precoated plates; Fixion 50×8 (H⁺ or Na⁺) saturated with mobile phase vapours</td>
</tr>
<tr>
<td>3</td>
<td>Cellulose phosphate (Whatman P41, U.K.) and cellulose phosphate + microcrystalline cellulose (3:1)</td>
</tr>
<tr>
<td>4</td>
<td>Silica gel H</td>
</tr>
<tr>
<td>5</td>
<td>Silica gel</td>
</tr>
<tr>
<td>6</td>
<td>Cellulose</td>
</tr>
<tr>
<td>7</td>
<td>Silica gel G</td>
</tr>
<tr>
<td>8</td>
<td>Silica gel impregnated with diethylenetriamine</td>
</tr>
<tr>
<td>9</td>
<td>Cellulose microcrystalline</td>
</tr>
<tr>
<td>10</td>
<td>Zirconium tungstate</td>
</tr>
<tr>
<td>11</td>
<td>Cellulose MN 300 G Containing 10% CaSO₄</td>
</tr>
<tr>
<td>12</td>
<td>Lanthanum antimonate (binder-free)</td>
</tr>
<tr>
<td>13</td>
<td>Hydrated stannic oxide</td>
</tr>
<tr>
<td>14</td>
<td>Silufol 254</td>
</tr>
<tr>
<td>15</td>
<td>Silica gel with starch binder and fluorescent indicator</td>
</tr>
<tr>
<td>16</td>
<td>DEAE Cellulose</td>
</tr>
<tr>
<td>17</td>
<td>Ecteola-cellulose</td>
</tr>
<tr>
<td>18</td>
<td>Silica gel and vionit CS-32 ion-exchange resin</td>
</tr>
<tr>
<td>19</td>
<td>Dowex 1 or 50 mixed with cellulose (Avicel SF)</td>
</tr>
<tr>
<td>20</td>
<td>Dowex 50×8 cation-exchange resin</td>
</tr>
<tr>
<td>21</td>
<td>Alumina G</td>
</tr>
</tbody>
</table>
S22 Sulfoethyl cellulose (Serva, Heidelberg, FRG)
S23 Ti (IV) antimonate (H+ form)
S24 Alumina
S25 Glass powder
S26 Corn starch
S27 Cellulose MN 300
S28 Mixture of silica gel, starch and NH4NO3
S29 Silica gel with hydrous MnO2
S30 Stannic arsenate
S31 Silica gel impregnated with 0.5% sulfaguanidine
S32 Silica gel impregnated with 8-hydroxyquinoline and dibenzoylmethane
S33 Sulfoethyl cellulose (strongly acidic) cation exchanger
S34 Silica gel R and 15% vionit CS ion exchanger
S35 Silica gel impregnated with Aq. sodium molybdate
S36 Silica gel impregnated with organophosphorus compounds
S37 Cellulose phosphate in H+ form (Whatman P 41, U.K.)
S38 Silica gel impregnated with 0.5% aqueous 2, 2-dipyridyl and iminodiacetic acid
S39 Hydrous zirconium oxide
S40 Silica gel impregnated with 30% s- or t-butylamine
S41 Silica gel impregnated with crystalline antimonic (V) acid-
p-sulfochlorophosphonazo
S42 Tin (IV) arsenosilicate and arsenophosphate
S_{43}  Semicrystalline Sn phosphate ion - exchanger + silica gel G
S_{44}  Silica gel impregnated with fluorescein
S_{45}  Zirconium (IV) molybdate
S_{46}  Polyamide
S_{47}  Silica gel 60
S_{48}  Silica gel (Wakogel B-0)
S_{49}  PEI cellulose
S_{50}  Silica gel G mixed with chitosan
S_{51}  Cellulose with azopyrocatechol group
S_{52}  Silica gel impregnated with 0.5 M NH\textsubscript{4}Cl and a saturated Aq. solution of barium nitrate
S_{53}  Silica gel - ammonium nitrate - CM cellulose (5.0 : 0.64 : 0.16 w/w)
S_{54}  Silica gel H- ammonium nitrate - CM cellulose (5.0 : 0.64 : 0.16 w/w)
S_{55}  Tin pyrophosphate and silica gel containing sodium CM cellulose as binder
S_{56}  Silica gel- sodium CM cellulose - ammonium nitrate
S_{57}  Silica gel - ammonium nitrate - sodium CM cellulose - water, (33 : 4 : 1 : 100 w/w)
S_{58}  Silica gel impregnated with hydroxybenzoic acid
S_{59}  Silica gel impregnated with 0.1 M Aq. sodium nitrite, sodium molybdate and potassium dihydrogen orthophosphate
S_{60}  Silica gel loaded with various concentrations of TBA
S_{61}  Silica gel - starch - ammonium rhodanate (2.8 : 0.15 : 0.5 w/w)
S_{62}  C_{18} - bonded silica reversed phase layer
S\textsubscript{63} C\textsubscript{18} - bonded silica

S\textsubscript{64} Binder free Zr (IV) antimonate in the H\textsuperscript{+} form, silica gel G and mixture of Zr (IV) antimonated and silica gel G (1:1)

S\textsubscript{65} Silica gel H - microcrystalline cellulose - ammonium nitrate

S\textsubscript{66} Microcrystalline cellulose modified with silica gel G

S\textsubscript{67} Antimonic acid

S\textsubscript{68} Cellulose (MN 300) impregnated with PEI

S\textsubscript{69} Silica gel impregnated with 0.1 - 1.0 M Aq. solutions of NaCl, NH\textsubscript{4}Cl, KBr or KI

S\textsubscript{70} Silica gel treated with acid

S\textsubscript{71} Stannic silicate

S\textsubscript{72} Silica gel H and sodium CM cellulose

S\textsubscript{73} Silufol UV\textsuperscript{254} impregnated with 5 % paraffin oil in hexane

S\textsubscript{74} Polychrom (poros polymer, particle size 10- 40 \( \mu \)) in combination with polyvinyl alcohol

S\textsubscript{75} Chitin

S\textsubscript{76} Chitosan

S\textsubscript{77} Silufol sheets (aluminium- backed silica gel)

S\textsubscript{78} Zirconium phosphoantimonate

S\textsubscript{79} Silica gel impregnated with different concentrations of mono - 2 - ethylhexyl acid phosphate (H\textsubscript{2}MEHP)

S\textsubscript{80} Fixion 50 \( \times \) 8

S\textsubscript{81} Silica gel coated with high molecular weight amines (Primine JM-T, Amberlite LA - 1, Alumina 36, Aliquat 336)
S\textsubscript{82} Silica gel impregnated with salicylic, syringic or o- phthalic acids
S\textsubscript{83} Silica gel impregnated with copper sulfate solution
S\textsubscript{84} p - Amino benzyl cellulose
S\textsubscript{85} Diethyl - (2 - hydroxypropyl) aminoethyl Q E - cellulose (strong basic anion exchanger)
S\textsubscript{86} Synthesized carbamide - formaldehyde polymer (amino plast)
S\textsubscript{87} Silica gel coated with different concentration of Primine JM - T
S\textsubscript{88} Zinc ferrocyanide
S\textsubscript{89} Diatomite
S\textsubscript{90} Silica gel impregnated with DMSO
S\textsubscript{91} Alumina - silica gel G (1 : 1, 1 : 2, 2 : 1)
S\textsubscript{92} Anhydrous antimony (V) oxide
S\textsubscript{93} Silica gel impregnated with 0.1 % Aq. solution of copper sulfate, zinc sulfate, nickel chloride, cobalt chloride, Co (NH\textsubscript{3})\textsubscript{6}Cl
S\textsubscript{94} CM cellulose
S\textsubscript{95} Silica gel impregnated with a mixture of alizarin red S and aliquat 336
S\textsubscript{96} Silica gel modified with analog of dibenzo- 18 - crown - 6
S\textsubscript{97} Mixtures of silica and inorganic ion-exchange gels
S\textsubscript{98} Poros glass sheets
S\textsubscript{99} Diethyl - (2 - hydroxypropyl) aminoethyl cellulose
S\textsubscript{100} Twentytwo soils with different characteristics
S\textsubscript{101} Silica gel impregnated with TBP
S\textsubscript{102} Arsenosilicates of Sn (IV), Cr (III) and Sb (V)
Silufol
Silica gel impregnated with high molecular weight amines
Silica gel impregnated with mono-2-ethylhexyl acid phosphate (H⁺ - MEHP)
Stannic sulfosalicylate
Trimethyl hydroxypropylamine cellulose (QA)
Surface-modified sorbent layers
Silica gel GF245
Silica gel pretreated with amines {(iminodi-2 ethanol; tris (hydroxymethyl)aminomethane; nitrilotri-2-ethanol and 1, 3-diazole}
Silica gel G impregnated with polyethylene glycol
Silica gel and alumina impregnated with 0.1-1.07 LiCl
Silica gel plain and impregnated with EDTA (2%), DMG(1%) or 1,10-phenanthroline (1%)
Silica gel plain and impregnated with 1% Aq. CuSO₄/Alumina, Kieselguhr, Kaolin, Alumina + cellulose (2:1) Kieselguhr + cellulose (1:2,2:1)
Kieselguhr / Kieselguhr + Cellulose (4:1,3:2)
Ce(III) silicate
Silica gel impregnated with sodium salt of condroitin sulfate
TBP coated polymeric supports
Cellulose microcrystalline / cellulose + Kieselguhr (4:1, 3:2, 1:1); Kieselguhr
Table 2.3: List of Mobile Phases Used

<table>
<thead>
<tr>
<th>$M_i$</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_1$</td>
<td>Et$_2$O-bis (2-ethylhexyl) phosphate-HNO$_3$ (100+3+2); diiso-Pr$_2$O-THF-HNO$_3$ (25+20+1); Et$_2$O-bis (2-ethylhexyl) phosphate-HNO$_3$ (50+2+1)</td>
</tr>
<tr>
<td>$M_2$</td>
<td>1.0 - 2.0 M Sodium nitrate</td>
</tr>
<tr>
<td>$M_3$</td>
<td>Aq. acetic acid (0.1-3.0 M) and mixtures of acetic acid and ammonium acetate solutions</td>
</tr>
<tr>
<td>$M_4$</td>
<td>N-butyric acid - conc. HCl (100+1.5)</td>
</tr>
<tr>
<td>$M_5$</td>
<td>Polar and nonpolar solvents</td>
</tr>
<tr>
<td>$M_6$</td>
<td>Me$_2$SO-HCl (1-6 M) (1+9, 3+7, 5+5, 7+3 and 9+1, v/v)</td>
</tr>
<tr>
<td>$M_7$</td>
<td>Et$_2$O-bis (2-ethylhexyl) phosphate-HNO$_3$ (100+1+3.5), Et$_2$O-THF-bis (2-ethylhexyl) phosphate-KNO$_3$ (10+15+1+3.5)</td>
</tr>
<tr>
<td>$M_8$</td>
<td>Ten Aq. and mixed solvent systems</td>
</tr>
<tr>
<td>$M_9$</td>
<td>Mixture of short-chain alcohols and/or acetone with strong or weak acids</td>
</tr>
<tr>
<td>$M_{10}$</td>
<td>Seventeen solvent mixtures</td>
</tr>
<tr>
<td>$M_{11}$</td>
<td>Dioxane</td>
</tr>
<tr>
<td>$M_{12}$</td>
<td>Buffered Aq. ammonia (pH 10)</td>
</tr>
<tr>
<td>$M_{13}$</td>
<td>Concentrated Aq. ammonia-n-propanol (1+2), pyridine-water-n-butanol (3+3+5)</td>
</tr>
<tr>
<td>$M_{14}$</td>
<td>MeOH-benzene-ethylacetate-EMK-Aq. ammonia (6+6+6+2+1)</td>
</tr>
<tr>
<td>$M_{15}$</td>
<td>Acetone-benzene (1+1)</td>
</tr>
<tr>
<td>$M_{16}$</td>
<td>5M HCl with different concentrations of hydrogen peroxide</td>
</tr>
</tbody>
</table>
M_{17} Mixtures of Me_2CO , HCl and water in various ratios

M_{18} Different concentrations of HCl and Aq. chloride solutions of Li, Mg, Na, Ca, Sr and Ac

M_{19} Et_2CO - bis (2 - ethylhexyl )phosphate - HNO_3 - Et_2 CO - THF , THF - bis (2 - ethylhexyl )phosphate - HNO_3 and iso - Pr_2 CO - THF - HNO_3

M_{20} HOAc - HCl

M_{21} Me_2CO - EtOAc - C_6H_6 , (7+1+3)

M_{22} Iso - Pr_2 O - THF - conc. HNO_3 - H_2 O (100+70+4.21+3.45)

M_{23} Aq. HCl

M_{24} Polar solvent systems

M_{25} Water , Aq. solutions of NH_3 , KCl , NaF , KNO_3 , K_2SO_4 , KClO_4 , Na_2CO_3 and K_3PO_4 at different molar concentrations

M_{26} Distilled water , Aq. sodium chloride (0.1-1% )

M_{27} Butanol - acetone - water (9+9+2)

M_{28} Acetone - water (10+1), methanol - water - butanol (3+1+1), butanol saturated with 2N HNO_3

M_{29} 1.0 M acetic acid and mixtures of 0.1 M acetic acid and ammonium acetate ( pH 4.7, 5.7, 6.5 and 6.7 )

M_{30} Butanol - propanol - water (1+3+1)

M_{31} Many single - component and mixed solvent systems containing polyhydric alcohols , methylamine , Aq. ammonia , DMF , water , Ketones , pyridine and esters

M_{32} Acetone - butanol - 10% Aq. ammonia - water ( 13+4+2+1)

M_{33} HNO_3 (0.001-1.0 M) , DMF - 0.1M HNO_3 ( 1+0, 4+1,1+4, 4+6, 6+4)
M₃₄ 0.1 M ammonium oxalate, DMSO - 1M HCl (1+9)

M₃₅ Binary solvent systems

M₃₆ 3 or 5 M HCl containing NaClO₄

M₃₇ Different solvent mixtures

M₃₈ Aq. and Aq. - organic binary mixtures in various ratios

M₃₉ TBP - EMK, EtOAc and HNO₃

M₄₀ Dil. HNO₃

M₄₁ Aq. HCl

M₄₂ Isopropanol - ethylacetate - DMF - acetic acid - water (10+6+1+1+2),
iso - propanol - ethylacetate - DMF - acetic acid - water
(60+30+3+5+10)

M₄₃ Propanol - Aq. ammonia (2+1)

M₄₄ THF - acetic acid - water (10+1+1)

M₄₅ Acidic solvent systems

M₄₆ Butanol - ethyl acetate - acetic acid (4+1+1) and ethyl acetate - formic acid - water - pyridine (3+1+1+0.5)

M₄₇ 0.05 M H₂SO₄ - methanol or acetone (1+0,4+1,3+2 or 2+3 v/v)

M₄₈ Oxalic acid - Oxalate systems

M₄₉ Mixtures containing ether, THF, bis (2-ethyl hexyl) phosphate, and HNO₃ in different ratios

M₅₀ Me₂CO - HCl - water (45+2+3) and BuOH - HCl - water (45+4+6)

M₅₁ Monotetradecyl phosphate (P 538), ethylacetate and HNO₃

M₅₂ 6M HCl - acetylacetone - 9M H₂SO₄ (60+2+1)

M₅₃ Demineralized water, mixtures of sodium formate and formic acid solutions in different ratios

69
M₅₄  Acetone - n - butanol - conc. Aq.ammonia - water (3+4+2+1)
and n- butanol - ethanol - conc. Aq. ammonia - water (2+2+1+1)
M₅₅  20% acetic acid - dioxane - 88% isopropanol - 9% trichloroacetic acid - methanol (1+2+3+5+10)
M₅₆  MeOH - 6N HNO₃ - acetylacetone (4+3+1)
M₅₇  0.5M H₂SO₄ - acetone or methanol (1+0, 4+1, 3+2 and 2+3 )
M₅₈  Isoamyl alcohol - water - AcOH (2+1+1) ,methanol - benzene - AcOH ( 4+2+3)
M₅₉  Aq. solutions of inorganic acids (HClO₄, HCl, HBr, HNO₃, H₂SO₄ and H₃PO₄), their mixtures and some of their salts
M₆₀  Aq. HCl and HNO₃ of different pH and their mixtures with organic solvents in different ratios
M₆₁  1.0M HCOOH , 1.0M HCOONa and their binary mixture
(1+1,2+8, 4+6, 6+4 , 8+2)
M₆₂  Aliphatic alcohol - HCl systems
M₆₃  Isopropyl ether - THF - tributyl phosphate - HNO₃ (10+6+1+1)
M₆₄  Iso - PrOH - Et₂O - EtOAc - HNO₃ (20+20+10+1 ) ,iso - PrOH-THF-HNO₃ (30+10+1)
M₆₅  Potassium pyrophosphate solution
M₆₆  Buffered EDTA solution
M₆₇  Pyridine - water -n- butanol - Aq. ammonia ( 4+8+2+1)
M₆₈  Toluene - acetic acid (5+2)
M₆₉  Butanol - formic acid (1+1)
M₇₀  Chloroform or Toluene
M₇₁  Mixture of organic solvents
M_{72} 20.0 M formic acid in butanol

M_{73} Ammonium nitrate solutions of different concentrations (0.5 - 6.0M) with constant pH (4.9+0.1)

M_{74} Ethanol - HCl (4+1, 9+1); ethanol - HNO_3 (9+1); isopropanol - HCl (9+1) and ternary mixtures of organic, Aq. and mineral acids in different ratios

M_{75} 0.01 - 1.0M HCl

M_{76} n- butanol - HCl (2+1,3+1, 4+1, 9+1, 17+3 )

M_{77} Aq. solutions of HCl (0.1 - 0.5 M) buffer solutions of different pH, Aq. - organic mixtures of different composition

M_{78} Aq. solutions HCOOH, HCOONa, CH_3COOH, CH_3COONa, CH_3COOH- CH_3COONa (1+1) and HCOOH - HCOONa (1+1)

M_{79} Aq. formic acid solutions containing CH_3COOH, DMSO or EMK; IBMK saturated with formic acid

M_{80} Aq. H_2SO_4 (0.01 - 0.1 M) and H_2SO_4 - (NH_4)_2 SO_4 (0.01 - 1.0 M) solutions

M_{81} Phosphate - Et_2O - HNO_3 (40+40+0.8 ; 20+42+2.5)

M_{82} P_2O_4 - dioxane - EtOAc - HNO_3 (1+1+2 ; 0+2.4+4.0)

M_{83} TBP - THF - Et_2O - HNO_3 (1+9+9+1.5)

M_{84} Hydrocarbons and their mixtures in different ratios

M_{85} 0.05 - 2.0 M formic acid in butanol

M_{86} Mixtures of 0.05 M HCl and 0.01 - 1.0 M NH_4SCN in different ratios

M_{87} Acids( nitric, tartaric, citric, perchloric, formic), bases {NH_4OH, (CH_3)_3N}, neutral compounds (NH_4Cl, NH_4NO_3, AcONH_4) or mixture of these with organic solvents (EtOH, MeOH, n- PrOH, Me_2CO)
Monotetradecylphosphoric acid - Et₂O - conc. HNO₃ (20+42+2.5 ; 40+40+0.8)

Et₂O - THF - bis (2-ethylhexyl) phosphate - HNO₃

P₅₀₇ - IBMK - iso - (Pr₂O - HNO₃ (1+12+6+2.4 v/v)

n-propyl alcohol - ethyl acetate - conc. HNO₃ (10+30+1), n-propyl alcohol - dichloromethane - conc. HNO₃ (10+30+1, 30+10+1),
n-propyl alcohol - cyclohexane anhydrous alcohol - conc. HNO₃ (20+15+15+1), n-propyl alcohol - petroleum ether - THF - conc. HNO₃ (25+15+15+1)

Mixed organic solvents containing s-butylamine

10M HCOOH in butanol - ethylemethyl ketone (1+1,2+1, 1+3,3+1)

Isopropanol - 13.5 % trichloroacetic acid - 25% Aq. ammonia (140+40 +0.6)

Aq. HCOONa (10⁻³, 10⁻²,0.1, 1.0 and 5.0M), 0.1M HCOONa- 0.1M HCOOH (1+9 ,3+7,1+1,7+3 and 9+1), 1.0M HCOONa - 1.0M NaCl (1+1,1+2,2+1,1+9 and 9+1) mixture of 0.1 or 1.0M HCOONa and 0.1 or 1.0M NaCl, KBr, KI or KBrO₃ in different ratios

Mixtures of Aq. 1.0M formic acid and alkaline salt solutions

Ternary mixtures of different ratios of TBA, formic acid, and acetate

Aq. formic acid (10⁻³ - 2.0M), Aq. sodium formate (10⁻³-5M) and mixtures of 1.0M formic acid and 1.0M sodium formate (1+1,4+6,6+4,2+8 and 8+2)

Binary mixtures containing DMA and distilled water, methanol; or butanol; ternary mixtures containing DMA, acetone or ethyl acetate and formic acid
M₁₀₀ Trialkylmethyl ammonium chloride -n- octyl alcohol - petroleum ether - conc. HNO₃ (60+7+25+1)

M₁₀₁ Tri -n- octylamine or tri- iso- octylamine -4- methyl -2- pentanone - isopropyl ether - isopropyl alcohol - NHO₃ (1+8+8+6+0.75,v/v)

M₁₀₂ Tri methyl ammonium chloride -n- octyl alcohol - petroleum ether-HCl (2+10+30+1, 2+6+30+1.2 and 2+7+30+1 ,v/v)

M₁₀₃ P₂₀₄ - P₅₀₇ - tributyl phosphate - THF - HNO₃ - diisopropyl ether (111+52+5+521+86+1000 and 68+43+27+460+103 +1000 ,v/v)

M₁₀₄ I. Bis (2 - ethylhexyl ) phosphate - diisopropyl ether - diethyl ether - nitric acid (1+10+6+1.1)

II. Mono ( 2- ethylhexyl ) phosphate - isopropyl ether - ethyl ether - nitric acid (1+8+8+1.1)

M₁₀₅ Methanol - lactate media

M₁₀₆ Mixed Aq. - hydroxyisobutyric acid solutions containing methanol

M₁₀₇ Methanol - CH₂Cl₂ (1+99)

M₁₀₈ HNO₃ (10²⁴ - 1.0M ) DMSO - 0.1M HNO₃ (1+0, 4+1, 3+2, 1+4), dioxane - 0.1M HNO₃ (1+0, 4+1,3+2,2+3,1+4)

M₁₀₉ Aq. alkali metal nitrate solutions

M₁₁₀ Bis- ( 2- ethylhexyl) phosphate [P₂₀₄ + 2 - ethylhexyl ( 2- ethyl) phosphoric acid ( P₅₀₇ ) - THF - HNO₃ - isopropyl ether ,3+2+90+19+280, 17+2+110+18+20 , v/v ]

M₁₁₁ Mixed solvent systems consisting of mono (2- ethylhexyl phosphate, 4- methyl pentanone , nitric acid , isopropyl ether and /or THF in different ratios

M₁₁₂ n- Butanol - HCl - acetone (100+1+100)

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M_{113} Aq. organic acids

M_{114} Aq. sodium salicylate

M_{115} (I) P_{507} - mono (2-ethylhexyl) phosphonate - diethyl ether - conc. HNO_3 (1.5+43.5+2) and

(II) P_{507} - diethyl ether - conc. HNO_3 (3+42+1)

M_{116} Formic acid - butanol systems

M_{117} Aq. solutions (0.01 -0.2M) of acetic acid and chlorosubstituted (mono-, di- and trichloro) acetic acids

M_{118} Aq. solutions of sodium acetate

M_{119} Mixed HCl - organic solvent systems (0.1M HCl - ethanol or acetone (1+0, 7+3, 1+1, 3+7)

M_{120} Mixed H_2SO_4 - organic (methanol or acetone) solvent systems

M_{121} 0.1M HCl - dioxane (7+7, 5+5, 3+7 v/v)

M_{122} Isopropanol - THF - 50% HNO_3 (60+20+3)

M_{123} Demineralized water, 0.1M formic acid; 1.0M KI, KBr or NaCl; 0.1M formic acid - 0.1M KI or KBr (1+9), 1.0M formic acid - 1.0M HBr (1+9 and 9+1), 1.0M formic acid - 1.0M NaCl, NH_4Cl, KBr or KI (1+9,3+7,1+1,7+3 and 9+1)

M_{124} Ethyl acetate - acetone, formic acid - water (4+1, 8+7)

M_{125} Thirty six solvent systems

M_{126} 0.2M acetic acid - 0.2M sodium acetate systems

M_{127} Organic solvent mixtures consisting of di-(2-ethylhexyl) phosphate, mono-(2-ethylhexyl) phosphonate, tributyl phosphate, THF, nitric acid and isopropylether in different ratios

M_{128} 10% ammonium acetate with Aq. ammonia

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M\textsubscript{129} n- BuOH - IBMK - p\textsuperscript{20} - HCl (140+100+15+18) and
\hspace{1cm} (140+100+15+1)

M\textsubscript{130} Mixtures of EtOH and Aq. NaCl or HCl

M\textsubscript{131} Aq. methanolic solutions (1+1.3+1, v/v) of ammonia or AcOH with
ammonium nitrate, or sometimes ammonium acetate

M\textsubscript{132} Aq. solutions of sodium malonate and sodium malate

M\textsubscript{133} Distilled water and buffer solutions (pH 5.2, 7.2 or 9.2)

M\textsubscript{134} 0.05M glycolic acid at pH 2.5 (adjusted with HCl)

M\textsubscript{135} Aq. solutions of HCl, HBr, H\textsubscript{2}SO\textsubscript{4}, HNO\textsubscript{3}, H\textsubscript{3}PO\textsubscript{4} and various
organic acids

M\textsubscript{136} MeOH - EtOH - NH\textsubscript{4}Cl - Aq. ammonia

M\textsubscript{137} Buffered EDTA solutions

M\textsubscript{138} HNO\textsubscript{3} (0.05 - 3.0M)

M\textsubscript{139} Mixed H\textsubscript{2}SO\textsubscript{4} and dioxane systems

M\textsubscript{140} Oxalic acid and NH\textsubscript{4}Cl solutions

M\textsubscript{141} Aq. solutions of organic and inorganic acids

M\textsubscript{142} Aq. solutions of alkali-metal chlorides

M\textsubscript{143} Mixtures of 2.5M HCl, 2.5M NaCl and 0.6% hydrogen peroxide in
different ratios

M\textsubscript{144} 0.03M citric acid

M\textsubscript{145} Distilled and tap water

M\textsubscript{146} Sixty-nine organic solvent systems (single component, two compo-
nent, and three component systems comprising amine, alcohols,
ketones or phenols and formic acid)

M\textsubscript{147} Water - ethanol - 2-methyl propanol - 2-propanol - Aq. ammo
nna - trichloro acetic acid (150+175+75+107+2+25)

$M_{148}$ Acetone mixed with 0.1M Aq. HCl, NaCl, HBr, formic acid

or Aq. ammonia (1+9 or 9+1)

$M_{149}$ H$_2$SO$_4$ (0.01 - 1.0M ) and H$_2$SO$_4$ - ammonium sulfate (0.01 - 1.0M)

systems

$M_{150}$ Aq. HCl and HCl - ammonium chloride (0.01 - 1.0M ) systems

$M_{151}$ HNO$_3$ - HCl or H$_2$SO$_4$ containing different concentrations of hydrogen peroxide

$M_{152}$ Eleven neutral and acidic solvent systems

$M_{153}$ Aq. solutions of sodium thioglycolate (0.01 - 0.2M )

$M_{154}$ Aq. HCl and Aq. HCl - ammonium chloride mixtures

$M_{155}$ 0.01 - 1.0M citric acid

$M_{156}$ Aq. ammonium nitrate

$M_{157}$ Solutions of acids, bases and salts

$M_{158}$ 1.0M inorganic salt solutions in Aq. methanol

$M_{159}$ DMSO - 1.0M HNO$_3$ (1+1) ; DMSO - THF (1+10) ; n - butanol-acetone - HNO$_3$ (6+6+1) ; diisopropyl ether - DMSO (10+1) and

DMSO- THF - diisopropyl ether systems

$M_{160}$ Several Aq. mobile phases

$M_{161}$ Acetylacetone - acetone - conc. HCl (5+5+1)

$M_{162}$ Distilled water

$M_{163}$ Mixed acidic - organic solvent systems containing formic acid

$M_{164}$ Acetone - DMSO or formic acid, acetone - DMSO - formic acid and

acetone - mineral acid mixtures

$M_{165}$ Ethanol - isobutanol - conc. HCl - water (12+6+1+1)

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M_{166} 0.001 - 0.1 M Aq. solutions of succinic acid

M_{167} Sixteen different solvent systems

M_{168} n-Butanol - benzene - 1M HNO_3 - 1M HCl (75+69+4+2 v/v) or acetone - 3M HCl (99+1 v/v)

M_{169} Aq. sulfuric acid and sulfuric acid - ammonium sulfate media

M_{170} 0.1M H_2C_2O_4, 2M NH_4Cl , 5.0M HCl, 0.5 M ammonium citrate

M_{171} DMSO - HNO_3 and DMSO - HCl systems

M_{172} Sulfuric acid, sulfuric acid - ammonium sulfate mixture

M_{173} HNO_3 at different concentrations

M_{174} Four mobile phases

M_{175} Mixtures of DMF and HNO_3 or HCl

M_{176} Aq. MeOH containing tributylphosphate and formic acid

M_{177} Sulfuric acid and organic solvents

M_{178} Acetone - chloroform (3+1)

M_{179} Formic acid and sodium formate

M_{180} MeOH - AcOH (100+0.3 v/v)

M_{181} Benzene - acetone - DMF (100:80:20)

M_{182} Mixture HCl - acetylacetone

M_{183} IBMK- formic acid

M_{184} NH_4Cl solution

M_{185} Formic acid (1.0M); HCOONa (1.0 M) and their mixture

M_{186} HCOONa (1.0 M) - KI (1.0 M) in the ratio 1:9

M_{187} Pyridine - benzene - HOAc - H_2O (6:5:8:4, 5:5:4:1); BuOH - benzene - formic acid (5:10:9)

M_{188} Formic acid (1.0 M); HCOONa (1.0 M); Double distilled water;
HCl (1.0 M) - acetone (1:9)

M_{189} Water - oil microemulsion

M_{190} 15 solvent systems including NH_4OH (0.5 M)

M_{191} Aq. or organic solvents of different pH

M_{192} NH_4OH (1.0 M) - acetone (1:9, 3:7, 1:1, 7:3, 9:1)
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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$_5^-$, 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 μm; Catalog No. 97
27335) and methanol were obtained from Glaxo Laboratories (India). Kieselguhr (particle size between 10 and 40 μm; Catalog No. 015037), kaolin (Catalog No. 033059) and cellulose microcrystalline (particle size less than 30 μm; 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= 2×10⁻⁶ ohm⁻¹ cm⁻¹ at 25°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⁻, Cr₂O₇²⁻, 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, 1.0 M 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 IO$_3^-$ was scraped and IO$_3^-$ 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 IO$_3^-$ . The filtrate was added to a blank that was prepared by mixing 2 ml KIO$_3$ (1%) . The contents were titrated with a 0.01 M Na$_2$S$_2$O$_3$ solution , the blank was also simultaneously titrated with 0.01M sodium thiosulfate and the difference between the volume of the Na$_2$S$_2$O$_3$ solution consumed in both cases was taken for the determination of IO$_3^-$ 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 IO$_4^-$ and IO$_3^-$ can be realized from a synthetic mixture containing I$^-$ or SCN$^-$ and NO$_2^-$ , Br$^-$ , BrO$_3^-$ , CrO$_4^{2-}$ or PO$_4^{3-}$ . 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 IO$_4^-$ , IO$_3^-$ , NO$_2^-$ , 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>
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<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;,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>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;,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>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>
</tr>
</tbody>
</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$_2$O$_7^{2-}$ (0.18-0.00)</td>
</tr>
<tr>
<td></td>
<td>NO$_3^-$ (1.0-0.86) - Fe(CN)$_6^{3^-}$ (0.1-0.00) NO$_3^-$ (1.0-0.86) - CrO$_4^{2-}$ or Cr$_2$O$_7^{2-}$ (0.19-0.00)</td>
</tr>
<tr>
<td>Cellulose</td>
<td>MnO$_4^-$ (0.00) - IO$_4^-$, IO$_3^-$, CrO$_4^{2-}$ or Cr$_2$O$_7^{2-}$ (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>

*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 NO₂⁻, 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 NO₂⁻. However, the semi-quantitative method was unsuitable for IO₃⁻ and IO₄⁻ because of the lower solubility of KIO₃ or KIO₄ in water.

A plot of loading amount versus spot-area for NO₂⁻, 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 IO₃⁻ with preliminary separation from IO₄⁻ is important, as iodate is reduced to iodide in alkaline medium.
Figure 3.1: Diagram of some ternary separations: 1, IO₃⁻ - Br⁻ - I⁻; 2, IO₄⁻ - BrO₃⁻ - I⁻; 3, IO₄⁻ - NO₂⁻ - SCN⁻; 4, IO₄⁻ - BrO₃⁻ - 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/m values versus atomic weight/molecular weight of anion/anionic salt. Conditions: stationary phase, silica gel impregnated with 1% CuSO₄; eluant, HCl-acetone (1:9).
REFERENCES


CHAPTER -IV
W/O MICROEMULSION AS MOBILE PHASE IN THIN LAYER CHROMATOGRAPHIC RETENTION STUDIES OF ANIONS
4.1 INTRODUCTION

Microemulsions are isotropic, clear or translucent thermodynamically stable dispersions of oil, water, surfactant and often a small amphiphilic molecule, called a cosurfactant (1). The droplet diameters in microemulsions range from 100 to 1000 Å. The microemulsion systems are usually transparent because of their much smaller droplet size compared to macroemulsions. One can distinguish three different types of microemulsions: Oil microdroplets enclosed in a surfactant-cosurfactant film are dispersed in an aqueous continuous phase (oil-in-water, O/W type); water droplets are dispersed in the oil-continuous phase (water-in-oil, W/O type); and if oil and water droplets overlap in each other, the system is called bicontinuous (2). Water-in-oil (W/O) microemulsions are complex fluids that have wide-ranging applications; for example, as novel lubricants, reaction media for new synthetic chemistry and mobile phases in separation science (3,4). Since the first report by Armstrong and Henry (5), micellar chromatography has been a subject of interest to many analytical chemists (6-8). It has recently been shown that W/O microemulsions could be used as normal liquid chromatographic mobile phases (9). Interesting selectivities, along with poor efficiencies, were obtained. It must be noted that the W/O microemulsions used did not contain any alcohol cosurfactants. It was reported earlier that the addition of 1-pentanol to the mobile phase greatly improves efficiencies by reducing the adsorbed amount of emulsifier (surfactant). In this work, a W/O microemulsion with 1-pentanol as cosurfactant was used as eluant because use of such phases in thin-layer chromatography (TLC) is an unexplored area of research. In these systems,
water microdroplets, enclosed in surfactant/cosurfactant film, are dispersed in an oil-continuous phase (10). One characteristic feature of microemulsions is their wide domain of existence in the pseudoternary phase diagram (9,11).

In continuation of our earlier studies on TLC of anions (12-14), this report describes simple methodology for identification and separation of anions under optimal experimental conditions with a W/O microemulsion as novel eluant. The use of such phases may offer unique solutions for the change in retention data due to the influence of water on the stationary phase (15) because water is present in the core of W/O microemulsion droplets.

4.2 EXPERIMENTAL

Apparatus: As reported in 3.2.

Chemicals: Specially pure sodium dodecyl sulfate (SDS) and n-heptane (99%) were obtained from BDH (Poole, United Kingdom) and n-pentanol was Riedel product (99%). Demineralized water, redistilled from alkaline potassium permanganate was used. Alumina, microcrystalline cellulose, and kieselguhr were obtained from CDH Laboratories (India), while silica gel G was obtained from Glaxo (India). All other chemicals (Analar grade) were used as supplied.

Test Solutions: The test solutions (1%) were either Na or K salts of all anions studied, except SCN⁻, which was used as ammonium thiocyanate. Solutions of the nitrates of lead, silver and bismuth, and the chloride of mercury were prepared in demineralized water, which contained small quantities of the corresponding acid to limit the extent of hydrolysis. The solutions (1%) of various amines and phenols used were prepared in methanol.
Detection Reagents: The spray reagents used for the detection of various anions were similar to those presented in 3.2.

Mobile Phase: The W/O microemulsion, used as mobile phase, was prepared by titrating a coarse emulsion of n-heptane (160 ml), water (8 ml), and SDS (8 gm) with n-pentanol (24 ml). Heptane was chosen for the oil phase because of low toxicity of the odd-carbon number alkanes. The microemulsion was produced at 30°C.

Stationary Phases: The following sorbent layers were used as stationary Phases; Silica gel G (S₁), Alumina (S₂), Microcrystalline cellulose (S₃), Kieselguhr G (S₄), Kieselguhr + cellulose (4:1,3:2) (S₅).

Procedure

Preparation of TLC plates: The TLC plates were prepared by adopting the method as given in 3.2.

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

The identification limits of various anions were determined by spotting different amounts of anionic solutions on the chromatoplates. The plates
were developed and the spots were detected as described in 3.2. The method was repeated with successive lowering of the amount of anion until spots could no longer be detected. The minimum amount of anion just detectable was taken as the limit of detection.

4.3 RESULTS AND DISCUSSION

We have tried identification and separation of anions on various adsorbents (S₁-S₅). Kieselguhr thin layers were highly effective for differential migration of anions. Table 4.1 summarizes some binary separations of IO₄⁻ and MnO₄⁻ from accompanying anions by using the W/O microemulsion as mobile phase and kieselguhr as the stationary phase. Separations achieved are well resolved and compact in this chromatographic system. It is an important aspect of this study because our past experience (14) has shown that kieselguhr, being only slightly active, fails to provide any fruitful data when used as stationary phase for normal TLC of anions with organic or mixed aqueous-organic solvent systems. This indicates that the W/O microemulsion plays a crucial role in enhancing the differential migration of anions on kieselguhr. This is possibly due to the presence of a water core in W/O microemulsion droplets which provide a restricted volume (16) and are responsible for specific selectivities. The hRᶠ values of separated anions are given in Table 4.1. The Rᶠ value of each individual anion changes slightly when it is chromatographed in mixture with other anions.

Table 4.2 presents the detection and dilution limits of anions. The proposed method is highly sensitive for most ions, except Br⁻, I⁻ and IO₄⁻. The most interesting behavior is exhibited by MnO₄⁻, which can be detected easily, even if it is present at 1 µg. Similarly, CrO₄²⁻ and BrO₃⁻ can be
detected easily. This may well be attributed to the fact that, in W/O microemulsion, these solutes are localized in the hydrophilic core, which is responsible for some sort of preconcentration of anions (17). Further, the anionic charge on the microemulsion droplet is responsible for fast transfer of anionic species out of the microemulsion droplet and easily available to interact with the detection reagents.

Table 4.3 presents some quantitative separations of anions. KBr, up to 5 mg, and NaNO$_2$, up to 1.25 mg, can be easily separated from KIO$_4$ (100 μg). Separation of KIO$_4$ from milligram quantities of KI does not take place, KIO$_4$ remains undetected while KI produces a tailed spot. Thus increasing the KI amount produces a deleterious effect and causes poor or no detection of KIO$_4$. This may possibly be due to reduction of KIO$_4$ to iodine in solutions of moderate acidity. Because of this fact, we carried out the separation of IO$_4^-$ from NO$_2^-$, Br$^-$, and I$^-$.

Finally, Table 4.4 summarizes the effect of various additives on the separation efficacy of IO$_4^-$ from Br$^-$ and NO$_2^-$, Amines by and large do not influence the separations, but there are drastic changes in the $R_f$ values of ions, compared with their standard $R_f$ values. The separation mixture precipitates by the addition of amines. In case of β-naphthylamine, Br$^-$ remains undetected because the entire spot ($R_f$ range of Br$^-$) is superimposed by the solvent uptake of β-naphthylamine (deep purple spot). The situation for NO$_2^-$ is similar, but the spot of NO$_2^-$ is clear. Spots of Br$^-$, when chromatographed with diphenylamine and 2-nitroaniline, appeared as streaks after 1/2 h. Phenols neither hamper separation nor produce precipitation. However, addition of pyrogallol produces slight turbidity, and IO$_4^-$ remains
undetected when separated from NO$_2^-$ or Br$^-$. In this case, the $R_f$ range of IO$_4^-$ gets superimposed by pyrogallol. Heavy metals also do not hinder the separation except silver, which causes dense precipitation as a result of which Br$^-$ remains undetected. Addition of lead to the separation mixture of IO$_4^-$ and Br$^-$ leads to poor detection of Br$^-$. Mercury shows no effect on the separation of IO$_4^-$ from Br$^-$ or NO$_2^-$.

An attempt has been made for the semi-quantitative determination of IO$_4^-$ by employing a peak-height measurement procedure. For this purpose, 0.1 ml standard aqueous solution of KIO$_4$ (0.02 - 0.1, %) were spotted on kieselguhr thin layers. The chromatograms were developed and detected. The spots obtained were copied directly on tracing paper from the chromatoplates. A linear relationship was obtained when the square of peak-heights of the spots, was plotted against microgram quantities of KIO$_4$ (Figure 4.1).
Table 4.1: Binary Separations Achieved Experimentally on Kieselguhr Layers Developed with W/O Microemulsion

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>Separations (hR&lt;sub&gt;f&lt;/sub&gt; = R&lt;sub&gt;f&lt;/sub&gt; × 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kieselguhr G</td>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (5.75) – NO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (89.5)</td>
</tr>
<tr>
<td></td>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (6.75) – BrO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (89.25)</td>
</tr>
<tr>
<td></td>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (7.25) – I&lt;sup&gt;-&lt;/sup&gt; (88.25)</td>
</tr>
<tr>
<td></td>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (7.5) – Br&lt;sup&gt;-&lt;/sup&gt; (94.0)</td>
</tr>
<tr>
<td></td>
<td>MnO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (5.0) – BrO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (91.0)</td>
</tr>
<tr>
<td></td>
<td>MnO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (5.0) – NO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (90.0)</td>
</tr>
<tr>
<td></td>
<td>MnO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt; (2.5) – Br&lt;sup&gt;-&lt;/sup&gt; (93.25)</td>
</tr>
</tbody>
</table>

Table 4.2: Detection and Dilution Limits of Anions as Their Salts on Kieselguhr Layers Developed with W/O Microemulsion

<table>
<thead>
<tr>
<th>Anion</th>
<th>Lower detection limit (µg)</th>
<th>Dilution limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>20</td>
<td>1:1000</td>
</tr>
<tr>
<td>IO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>10</td>
<td>1:2000</td>
</tr>
<tr>
<td>BrO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>2</td>
<td>1:10,000</td>
</tr>
<tr>
<td>I&lt;sup&gt;-&lt;/sup&gt;</td>
<td>20</td>
<td>1:1000</td>
</tr>
<tr>
<td>Br&lt;sup&gt;-&lt;/sup&gt;</td>
<td>20</td>
<td>1:1000</td>
</tr>
<tr>
<td>MnO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>1</td>
<td>1:20,000</td>
</tr>
<tr>
<td>CrO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>2</td>
<td>1:10,000</td>
</tr>
</tbody>
</table>

* Dilution limit = 1: (Volume of test solution × 10<sup>6</sup> [Limit of detection (µg)] *)

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Table 4.3: Quantitative Separation of $\text{IO}_4^-$ (20–100µg) from Milligram Amounts of $\text{NO}_2^-$, $\text{Br}^-$, and $\text{I}^-$ with Chromatographic System as in Table 4.1

<table>
<thead>
<tr>
<th>Accompanying anion salt</th>
<th>Amount of salt (mg)</th>
<th>Separations ($R_L - R_F$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₂</td>
<td>1.25</td>
<td>$\text{IO}_4^-$ (0.15–0.00) – $\text{NO}_2^-$ (1.0–0.46)</td>
</tr>
<tr>
<td>KBr</td>
<td>1.25</td>
<td>$\text{IO}_4^-$ (0.11–0.00) – $\text{Br}^-$ (1.0–0.76)</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>$\text{IO}_4^-$ (0.10–0.00) – $\text{Br}^-$ (1.0–0.68)</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>$\text{IO}_4^-$ (0.10–0.00) – $\text{Br}^-$ (1.0–0.48)</td>
</tr>
<tr>
<td>KI</td>
<td>1.0</td>
<td>$\text{IO}_4^-$ (ND) – $\text{I}^-$ (1.0–0.70)</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>$\text{IO}_4^-$ (ND) – $\text{I}^-$ (1.0–0.65)</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>$\text{IO}_4^-$ (ND) – $\text{I}^-$ (1.0–0.20)</td>
</tr>
</tbody>
</table>

*Spots of $\text{Br}^-$ appear after 20 min; ND = Not detected*
Table 4.4: Effect of Additives on Some Binary Separations of $\text{IO}_4^-$ from $\text{NO}_2^-$ and $\text{Br}^-$

<table>
<thead>
<tr>
<th>Additives</th>
<th>Separations ($R_f$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Amines</strong></td>
<td></td>
</tr>
<tr>
<td>(a) $\beta$-Naphthylamine</td>
<td>$\text{IO}_4^-$ (0.05) – $\text{NO}_2^-$ (0.85) ; $\text{IO}_4^-$ (0.06) – $\text{Br}^-$ (ND)</td>
</tr>
<tr>
<td>(b) Diphenylamine</td>
<td>$\text{IO}_4^-$ (0.21) – $\text{NO}_2^-$ (0.88) ; $\text{IO}_4^-$ (0.21) – $\text{Br}^-$ (0.74)</td>
</tr>
<tr>
<td>(c) 2-Nitroaniline</td>
<td>$\text{IO}_4^-$ (0.20) – $\text{NO}_2^-$ (0.65) ; $\text{IO}_4^-$ (0.17) – $\text{Br}^-$ (0.78)</td>
</tr>
<tr>
<td><strong>2. Phenols</strong></td>
<td></td>
</tr>
<tr>
<td>(a) Phenol</td>
<td>$\text{IO}_4^-$ (0.03) – $\text{NO}_2^-$ (0.82) ; $\text{IO}_4^-$ (0.10) – $\text{Br}^-$ (0.85)</td>
</tr>
<tr>
<td>(b) Resorcinol</td>
<td>$\text{IO}_4^-$ (0.07) – $\text{NO}_2^-$ (0.90) ; $\text{IO}_4^-$ (0.07) – $\text{Br}^-$ (0.81)</td>
</tr>
<tr>
<td>(c) Pyrogallol</td>
<td>$\text{IO}_4^-$ (ND) – $\text{NO}_2^-$ (0.80) ; $\text{IO}_4^-$ (ND) – $\text{Br}^-$ (0.81)</td>
</tr>
<tr>
<td><strong>3. Heavy metals</strong></td>
<td></td>
</tr>
<tr>
<td>(a) $\text{Hg}^{2+}$</td>
<td>$\text{IO}_4^-$ (0.05) – $\text{NO}_2^-$ (0.92) ; $\text{IO}_4^-$ (0.08) – $\text{Br}^-$ (0.83)</td>
</tr>
<tr>
<td>(b) $\text{Pb}^{2+}$</td>
<td>$\text{IO}_4^-$ (0.03) – $\text{NO}_2^-$ (0.90) ; $\text{IO}_4^-$ (0.01) – $\text{Br}^-$ (ND)</td>
</tr>
<tr>
<td>(c) $\text{Ag}^+$</td>
<td>$\text{IO}_4^-$ (0.05) – $\text{NO}_2^-$ (0.94) ; $\text{IO}_4^-$ (0.05) – $\text{Br}^-$ (ND)</td>
</tr>
<tr>
<td>(d) $\text{Bi}^{3+}$</td>
<td>$\text{IO}_4^-$ (0.04) – $\text{NO}_2^-$ (0.94) ; $\text{IO}_4^-$ (0.06) – $\text{Br}^-$ (ND)</td>
</tr>
</tbody>
</table>

Standard $R_f$ values of individual ions are: $\text{IO}_4^-$ (0.00), $\text{NO}_2^-$ (0.92), and $\text{Br}^-$ (0.90).
Figure 4.1: Standard calibration curve for semiquantitative determination of I$_7$ by peak-height measurement method.
REFERENCES

CHAPTER -V
THIN LAYER CHROMATOGRAPHIC SEPARATION, COLORIMETRIC DETERMINATION AND RECOVERY OF THIOCYANATE FROM PHOTOCYANIC WASTE, RIVER AND SEA WATERS
5.1 INTRODUCTION

Identification, separation and quantification of SCN⁻ is important due to its application in photography, catalysis, agri-chemicals, rust-inhibition and dyeing-printing of textiles (1). At high acidities, thiocyanate produces cyanides in the presence of oxidants and thus causes lethal damage to aquatic life when SCN⁻ containing waste is discharged into rivers. Numerous methods (2-8) have been developed for analysing SCN⁻ and CN⁻ but most of them were not applied to polluted water and effluents. A spectrophotometric method based on the color reaction of Fe³⁺ with SCN⁻ has been used for the quantification of SCN⁻ but it suffers from cationic, anionic and phenolic interferences. An extensive literature survey on the quantitative determination of SCN⁻ (1981-1992) with respect to the techniques used, shows that out of twenty research papers published, only one dealt with spectrophotometry.

TLC has been a widely used technique for the separation of SCN⁻ (9-12). The literature survey (13) shows that out of forty research papers published so far on TLC of SCN⁻, none examined the separation of SCN⁻ from complexing cations (Ag⁺, Fe³⁺, Co²⁺, Cu²⁺, etc.). This chapter reports a reliable TLC-colorimetric method for the determination of SCN⁻ in the presence of cationic and anionic impurities. The proposed method is applicable to the determination of SCN⁻ in photogenic waste, river water and seawater.

5.2 EXPERIMENTAL

Apparatus: TLC applicator, glass plates and jars were as reported in 3.2. Spectrophotometer (Elico, India, Model Ultra Spec. CL-54) and pH meter
(Elico, India, Model LI-10T) were used.

**Chemicals**: Ammonium thiocyanate (Merck, India); ferric chloride and ammonia solution (Qualigens); acetone, cellulose microcrystalline and Kieselguhr G (CDH, India) were used. All other chemicals were of Analytical Reagent grade.

**Test Solutions**: Aqueous solutions (1.0% w/v) of following were used:

(a) Salts of anions were as reported in 3.2
(b) Nitrates of Ag⁺, Bi⁺, UO₂²⁺ and Zn²⁺
(c) Chlorides of Fe³⁺ and Hg²⁺
(d) Sulfates of Cu²⁺, VO²⁺, Co²⁺ and Mn²⁺

**Detection Reagents**: To locate the spots of analyte ions, the reagents used were:

(a) For anions detection reagents were as mentioned in 3.2.
(b) 1.0% K₄Fe(CN)₆ in water for Fe³⁺, Cu²⁺, VO²⁺ and UO₂²⁺.
(c) 1.0% Dimethylglyoxime in ethanol for Co²⁺.
(d) 1.0% Dithizone in benzene for Zn²⁺, and Cd²⁺.
(e) Saturated H₂S in water for Ag⁺, Hg²⁺ and Bi³⁺.

**Mobile Phases**: Mixtures of 0.1 M NH₄OH and CH₃COCH₃ in 1:9 (M₁), 3:7 (M₂), 1:1 (M₃), 7:3 (M₄) and 9:1 (M₅) ratios were used as solvent systems.

**Stationary Phases**: Plain cellulose microcrystalline (S₁), Kieselguhr G (S₂) and their mixture in 4:1 (S₃), 3:2 (S₄) and 1:1 (S₅) ratios were used as adsorbent materials.

**Procedure**: The entire methodology was carried out as follows:

*Preparation of TLC plates*: Plates were prepared as mentioned in 3.2.
Preparation of fortified samples: Four samples of SCN⁻ solutions (5.09-12.92 mM) were prepared in seawater (collected from Anjuna Beach, Goa, India). Similarly, spiked river water (Ganga, Rajghat) and photogenic waste (Aligarh) were prepared to examine the recovery of SCN⁻.

Chromatography of ions: To study the retention behavior of some inorganic ions, 0.01 ml of the test solution was spotted onto the TLC plate, air dried and then developed with M₁⁻ to M₅ upto 10 cm. The plate was air dried, analyte ions were detected as colored spots and then identified on the basis of their Rf values. For mutual separations, an aliquot (0.02 ml) of binary or 0.03 ml of ternary mixture of analytes was loaded onto the plate and the chromatography was performed. In order to examine the effect of sample pH on the separation of SCN⁻, various mixtures of test solutions were brought to the required pH value (3.0-12.25) by adding either glacial acetic acid or NaOH solution. The effect of amines (2-nitroaniline, β-naphthylamine and diphenylamine) and phenols (phenol, resorcinol and pyrogallol) were examined on the detection and separation of SCN⁻ by mixing 1% alcoholic solution of additive with binary/ternary mixture containing SCN⁻.

Colorimetry of thiocyanate: For quantitative determination of SCN⁻ by TLC-colorimetry, 0.1 ml of standard ammonium thiocyanate (2.55-25.48 mM SCN⁻) was spotted onto the plate (S₂) along with foreign ions and developed with M₁. A pilot plate was simultaneously run. SCN⁻ containing portion of adsorbent from the working plate was scraped off and SCN⁻ was extracted with 10 ml of distilled water followed by washing of the adsorbent with 2 ml of water. Thus the total volume of solution was kept to 12 ml in each case. To the filtrate, 0.5 ml of chromogenic reagent solution
(8.0% ferric chloride in 2 M HCl) was added. Absorbance was measured at 460 nm and the absorbance was plotted against the concentration (ppm) of SCN\(^{-}\) solution to sketch the standard calibration curve which was used to determine the concentration of SCN\(^{-}\) in a water sample with preliminary separation from accompanying inorganic ions.

Recovery from fortified samples: For the recovery of SCN\(^{-}\) from spiked waste (photogenic) and water (river and sea) samples, 0.1 ml of the solution was spotted onto TLC plate (chromatographic system; S\(_2\), M\(_1\)) and the chromato-colorimetry was performed in the similar manner as that for standards. The loaded and recovered amounts of SCN\(^{-}\) were compared and the relative standard deviation (\(\%\)), relative error and percent recovery were calculated.

5.3 RESULTS AND DISCUSSION

Qualitative Studies: Inorganic ions were chromatographed on plain and mixed adsorbents containing cellulose and /or Kieselguhr (S\(_1\) - S\(_3\)) using mixtures of 0.1 M NH\(_4\)OH and CH\(_3\)COCH\(_3\) in various ratios as mobile phases (M\(_1\)-M\(_3\)) . The results of this study are summarized in Tables 5.1 and 5.2.

The effect of proportional composition of mobile phase on the hR\(_f\) values of inorganic anions is examined and the results are reported in Table 5.1 where only plain adsobents (S\(_1\) and S\(_3\)) were used. Cations were also chromatographed with these chromatographic systems, cations remain at the point of application. However, some metal ions yield occasional tailed spots. To achieve difference in migration of anions, S\(_1\) and S\(_3\) were mixed together to get a set of adsorbents (S\(_2\)-S\(_4\)) on which the anions were chromatographed using M\(_1\) as mobile phase. The hR\(_f\) values of anions are
enlisted in Table 5.2. Out of three mixed adsorbents, cellulose-Kieselguhr (4:1, S2) is selected because it provides sufficient difference in hRf values of SCN⁻ and others. It also provides compact spots because increasing Kieselguhr proportion results in the tailing of spots. Hence, the combination of S2 and M₁ is the best chromatographic system which is utilized for the selective separation of SCN⁻ from all ions studied. These ions strongly interfere in the colorimetric method of SCN⁻ quantification due to the formation of colored complexes either with SCN⁻ or Fe³⁺ (14). For Ag⁺-SCN⁻ separation, the white precipitate of AgSCN formed on mixing of both ions was dissolved in NH₃ solution and the clear solution was spotted on the TLC plate. Fe³⁺-SCN⁻ separation is of great importance, especially when SCN⁻ is to be determined colorimetrically by using FeCl₃ as chromogenic reagent.

For this separation, 1.0 % aqueous NaF solution was added to red colored complex to decolorize it. FeF₃, a more stable complex was formed and SCN⁻ became free and was detected on TLC plate. In the Co²⁺-SCN⁻ separation, Co²⁺ could not be detected. In general, for metal ion-SCN⁻ separation, the amount of SCN⁻ was taken x times more than that of metal ion (x is the number of unit charges present on the metal ion). Effects of sample pH, phenols and amines on the detection of SCN⁻ have been examined and SCN⁻ was clearly detected in all cases. The Rf value of SCN⁻ remained unchanged over the pH range (3.0-12.25) of sample solution. No change in the mobility of SCN⁻ was noticed on being chromatographed in the presence of amines and phenols.
Quantitative Studies: The proposed TLC system (S₂, M₁) was coupled to the spectrophotometry of SCN⁻ using Fe³⁺-SCN⁻ color reaction in an acidic (2 M HCl) medium. The complex absorbs incident radiation of 460 nm and follows the Beer's law up to 11.18 ppm. The optical density (A) plotted against the concentration (ppm) of SCN⁻ is an average of A₁ (SCN⁻ after separation from NO₂⁻), A₂ (from CrO₄²⁻) and A₃ [from Fe(CN)₆³⁻]. The R.S.D. varies from 2.124-0.442 %.

To test the applicability, proposed TLC-colorimetric method was applied to the fortified samples of photogenic waste, river water and seawater. The results are summarized in Table 5.3. This table shows that the relative recoveries of SCN⁻ are almost the same.
Table 5.1: Effect of Mobile Phase Composition on the Mobility of Inorganic Ions

<table>
<thead>
<tr>
<th>Anion</th>
<th>Stationary phase</th>
<th>hR&lt;sub&gt;f&lt;/sub&gt; values achieved with M₁</th>
<th>M₂</th>
<th>M₃</th>
<th>M₄</th>
<th>M₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN⁻</td>
<td>S₁</td>
<td>49</td>
<td>91</td>
<td>91</td>
<td>93</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>S₅</td>
<td>85</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>92</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>S₁</td>
<td>21</td>
<td>76</td>
<td>82</td>
<td>91</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>S₅</td>
<td>88</td>
<td>89</td>
<td>90</td>
<td>90</td>
<td>93</td>
</tr>
<tr>
<td>MnO₄⁻</td>
<td>S₁</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>S₅</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fe(CN)&lt;sub&gt;6&lt;/sub&gt;³⁻</td>
<td>S₁</td>
<td>6</td>
<td>54</td>
<td>89</td>
<td>92</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>S₅</td>
<td>83</td>
<td>90</td>
<td>90</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>Fe(CN)&lt;sub&gt;6&lt;/sub&gt;⁴⁻</td>
<td>S₁</td>
<td>0</td>
<td>6</td>
<td>78</td>
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<td>96</td>
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<td></td>
<td>S₅</td>
<td>8</td>
<td>17</td>
<td>80</td>
<td>91</td>
<td>93</td>
</tr>
<tr>
<td>CrO₄⁻²⁻</td>
<td>S₁</td>
<td>10</td>
<td>23</td>
<td>70</td>
<td>90</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>S₅</td>
<td>41</td>
<td>91</td>
<td>91</td>
<td>93</td>
<td>94</td>
</tr>
<tr>
<td>Cr&lt;sub&gt;2&lt;/sub&gt;O₇⁻²⁻</td>
<td>S₁</td>
<td>8</td>
<td>20</td>
<td>78</td>
<td>88</td>
<td>92</td>
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<tr>
<td></td>
<td>S₅</td>
<td>43</td>
<td>89</td>
<td>90</td>
<td>90</td>
<td>94</td>
</tr>
<tr>
<td>MoO₄⁻²⁻</td>
<td>S₁</td>
<td>9</td>
<td>24</td>
<td>83</td>
<td>90</td>
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<td></td>
<td>S₅</td>
<td>36</td>
<td>88</td>
<td>90</td>
<td>91</td>
<td>96</td>
</tr>
<tr>
<td>Mo₇O₄⁻²⁴⁻</td>
<td>S₁</td>
<td>12</td>
<td>30</td>
<td>80</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>S₅</td>
<td>38</td>
<td>90</td>
<td>91</td>
<td>95</td>
<td>95</td>
</tr>
</tbody>
</table>

*Metal ions remain at the point of application in all of the above mentioned chromatographic systems.*
Table 5.2: Effect of Stationary Phase Composition (Cellulose / Kieselguhr Proportion) on the Mobility of Inorganic Ions* when Developed with M<sub>1</sub>  

<table>
<thead>
<tr>
<th>Anion</th>
<th>h&lt;sub&gt;R&lt;/sub&gt;&lt;sub&gt;F&lt;/sub&gt; values achieved on</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S&lt;sub&gt;1&lt;/sub&gt;</td>
<td>S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>S&lt;sub&gt;3&lt;/sub&gt;</td>
<td>S&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>SCN&lt;sup&gt;-&lt;/sup&gt;</td>
<td>49</td>
<td>79</td>
<td>80</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>NO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>21</td>
<td>21</td>
<td>32</td>
<td>86</td>
<td>88</td>
</tr>
<tr>
<td>MnO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fe(CN)&lt;sub&gt;6&lt;/sub&gt;&lt;sup&gt;3-&lt;/sup&gt;</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>12</td>
<td>83</td>
</tr>
<tr>
<td>Fe(CN)&lt;sub&gt;6&lt;/sub&gt;&lt;sup&gt;4-&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CrO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>10</td>
<td>20</td>
<td>24</td>
<td>28</td>
<td>41**</td>
</tr>
<tr>
<td>Cr&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>8</td>
<td>19</td>
<td>19</td>
<td>25</td>
<td>43</td>
</tr>
<tr>
<td>MoO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>9</td>
<td>11</td>
<td>17</td>
<td>21</td>
<td>36**</td>
</tr>
<tr>
<td>Mo&lt;sub&gt;7&lt;/sub&gt;O&lt;sub&gt;24&lt;/sub&gt;&lt;sup&gt;6-&lt;/sup&gt;</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td>23</td>
<td>38</td>
</tr>
</tbody>
</table>

*All metal ions studied remain at the point of application (h<sub>R</sub><sub>F</sub> =0.0 ).

**Tailed spots (h<sub>R</sub><sub>L</sub>- h<sub>R</sub><sub>T</sub> > 30 ).
<table>
<thead>
<tr>
<th>Sample (pH)</th>
<th>Amount of SCN⁻ loaded (μg)</th>
<th>Amount of SCN⁻ recovered (μg)</th>
<th>Relative recovery (%)</th>
<th>Relative standard deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photogenic waste (9.4)</td>
<td>29.60</td>
<td>30.19</td>
<td>102.00</td>
<td>9.38</td>
</tr>
<tr>
<td></td>
<td>44.40</td>
<td>45.18</td>
<td>102.44</td>
<td>5.60</td>
</tr>
<tr>
<td></td>
<td>59.20</td>
<td>60.64</td>
<td>102.44</td>
<td>5.42</td>
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<td></td>
<td>74.00</td>
<td>76.46</td>
<td>103.32</td>
<td>10.70</td>
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<tr>
<td>River water (8.2)</td>
<td>29.60</td>
<td>28.12</td>
<td>94.99</td>
<td>9.91</td>
</tr>
<tr>
<td></td>
<td>44.40</td>
<td>42.62</td>
<td>96.00</td>
<td>7.84</td>
</tr>
<tr>
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<td>74.00</td>
<td>73.03</td>
<td>98.69</td>
<td>5.63</td>
</tr>
<tr>
<td>Sea water (8.0)</td>
<td>29.60</td>
<td>27.38</td>
<td>92.50</td>
<td>9.91</td>
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<td></td>
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<td>41.64</td>
<td>93.78</td>
<td>7.57</td>
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<td>59.20</td>
<td>56.23</td>
<td>94.99</td>
<td>6.59</td>
</tr>
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<td></td>
<td>74.00</td>
<td>71.53</td>
<td>96.66</td>
<td>5.45</td>
</tr>
</tbody>
</table>
REFERENCES


CHAPTER -VI

A NEW CHROMATO-IODOMETRIC METHOD FOR
THE SEPARATION AND DETERMINATION OF
IODIDE AND ITS OXYANIONS
6.1 Introduction

The determination of anions has undergone substantial changes in recent years. Significant advances have been made in the area of chromatographic separation and determination of inorganic species (1-4). However, there is a need for rapid clean-up, identification and quantitation of inorganic anions present in environmental samples. Thin-layer chromatography (TLC) being inexpensive and simple, can be utilized as a reasonably rapid analytical tool to complement modern instrumental techniques.

The literature survey of last thirty-five years on TLC of inorganics reveals that out of 148 papers published on inorganic anions (5), 62 papers report the TLC of one or two anion(s) of iodine. There were only 19 papers which described the TLC of all three iodine anions (iodide, iodate and periodate). As regards to ternary separation of these anions under optimized experimental conditions, few workers have used silica gel (6-8), alumina (9,10) and mixed alumina (11) layers. All the papers published on TLC of iodide and its oxyanions describe only their qualitative studies, not their quantitative determination. Furthermore, none of these workers have examined the effect of transition metal ions on the separation of iodine anions. The work reported here is aimed at solving the problem of determining iodide, iodate and periodate after their rapid clean-up on alumina ‘G’ layer using NH₄OH - CH₃COCH₃ mixture as mobile phase.

A new iodometric method is developed for simultaneous determination of I⁻, IO₃⁻ and IO₄⁻. This method requires lesser reagents because analytes are used as iodine producing agents for one another and the CH₃COCH₃ is utilized as titrant as well as a component of mobile phase. The proposed
method is applicable to the spiked double distilled water for the recovery of analytes.

6.2 EXPERIMENTAL

Apparatus: TLC Applicator, plates and jars used were as given in 3.2.

Chemicals: The chemicals used were KIO₃, KIO₄ and CH₃COCH₃ (CDH, India); HNO₃ and NH₃ solution (Qualigens). All other chemicals used were of analytical reagent grade.

Test Solutions: The test solutions were 1.0% (except IO₄⁻ which was taken as 0.5%) aqueous solutions of potassium salts of iodide, iodate and periodate.

Detection Reagents: Following chromogenic reagents were used to locate the anionic spots on TLC plate:
(a) 0.3% (C₆H₅)₂NH in 4 M H₂SO₄ for IO₃⁻ and IO₄⁻
(b) Saturated AgNO₃ in CH₃OH for I⁻

Mobile Phases: Mixtures of 0.1M NH₄OH and CH₃COCH₃ in 1:9 (M₁), 3:7 (M₂), 1:1 (M₃), 7:3 (M₄) and 9:1 (M₅) ratios were used.

Stationary Phases: Plain adsorbents i.e. aluminium oxide ‘G’ (S₁), cellulose microcrystalline (S₂), kieselguhr ‘G’ (S₃) and silica gel ‘G’ (S₄) were used. All adsorbents were supplied by CDH (India).

Procedure: The complete methodology involves following steps:

Preparation of TLC plates: TLC plates were prepared by adopting the method as given in 3.2.

Identification of analytes: 0.01 ml of test solution was spotted onto TLC plate, spot was air dried and then developed by ascending mode fixing ascent upto 10 cm. After development, plate was dried again and then the anions were visualized as colored spots by spraying with appropriate
detection reagent. The analyte species were identified on the basis of their 
R_f values, calculated from R_L (R_f of leading front) and R_T (R_f of trailing 
front) for each spot.

Separation: The test solutions of iodide, iodate and periodate were mixed 
in proper ratio, the resultant mixture was spotted onto TLC plate coated 
with alumina and the chromatography was performed using M_5 as mobile 
phase. To examine the pH and time effects on the resolution of all three 
components from their mixtures, stock solutions of mixture were prepared 
by mixing KI, KIO_3 and KIO_4 solutions in 5:1:1 ratio and their pH was 
adjusted to a definite level (pH range 4-10) by adding AcOH or NaOH 
solutions. An aliquot (0.1 ml) of each mixture was spotted on TLC plate at 
different time intervals. The plates were developed and the resolved spots 
were detected. For investigating the effect of transition metal ions on the 
separation of I^-, IO_3^- and IO_4^-, 1.0% aqueous solution of metal ion was 
mixed with ternary mixture of analytes to get a solution consisting of metal 
ion, iodide, iodate and Periodate in 2:5:1:1, ratio by volume. Of this solu­
tion, 0.1 ml was spotted onto TLC plate. In the case of precipitation, filtrate 
(0.1 ml) was spotted. After separation, analyte species were detected.

Quantitative determination: 0.1 ml of standard solution of KI (0.1 - 
1.0%) , KIO_3 (0.02- 0.20%) or KIO_4 (0.02 -0.20%) was spotted sepa­
rately onto the alumina coated plate (working plate) and the plate was 
developed with M_5. A pilot plate was simultaneously run to locate the exact 
position of analyte spot. After development, TLC plate was completely 
dried at 60°C to make it free from traces of the developer and the area of 
adsorbent corresponding to analyte spot was scraped off and taken into a
conical flask followed by the addition of 10 ml of the following solution;

(a) 0.05% (w/v) KIO₃ in 0.1M HNO₃ for I⁻.

(b) 0.05% (w/v) KI in 0.1M HNO₃ for IO₃⁻ and IO₄⁻.

Brown color was produced which was titrated directly (without filtration) with 2.0 M aqueous CH₃COCH₃ (v/v) until complete decolorization occurs. The volume of titrant so consumed was plotted against the amount of analyte substance.

*Recovery from fortified water samples*: The solutions of KI (0.2-0.6%) KIO₃ (0.04-0.12%) and KIO₄ (0.04-0.12%) were prepared in double distilled water from stock solutions of KI (10%), KIO₃ (1%) and KIO₄ (0.5%). Three test samples were prepared by mixing equal volumes of KI, KIO₃, and KIO₄ solutions of the following concentrations.

(a) KI (0.2%), KIO₃ (0.04%) and KIO₄ (0.04%)

(b) KI (0.4%), KIO₃ (0.08%) and KIO₄ (0.08%)

(c) KI (0.6%), KIO₃ (0.12%) and KIO₄ (0.12%)

0.1 ml of one of above mixtures was spotted onto the TLC plate, chromatography was performed and the analytes were titrimetrically determined after mutual separation. The chromatography and titrimetry were performed in similar manner to that for standards. Percent recovery and relative standard deviation were calculated.

6.3 RESULTS AND DISCUSSION

*Qualitative Studies*: In order to find out the most suitable stationary phase for the separation of iodide, iodate and periodate, commonly used adsorbents i.e. silica gel, alumina, cellulose and kieselguhr were tried. Out of these, the satisfactory separation was achieved only on alumina layer when deve-
loped with mobil phases (M<sub>2</sub> to M<sub>5</sub>), giving best separation with M<sub>5</sub> (Table 6.1). Considering the resolution of detected spots, the chromatographic system comprising of S<sub>1</sub> and M<sub>5</sub> as stationary and mobile phases was selected for detailed study.

Alumina exhibiting both cation and anion exchange characteristics has many desirable properties such as rigid structure, little swelling/shrinking in water or solutions containing electrolyte and organic modifiers, reasonable resistance to strong oxidizing and reducing agents and good thermal stability. Its low resistance towards strong acids and bases make it useful for applications under mild acidic or basic conditions. It is assumed that in our case, the retention of analyte anion on alumina follows anion exchange as shown below:

\[
\text{Al}^+ \text{O}^- \text{H}^+ + X^- \rightleftharpoons \text{Al}^+ X^- + \text{OH}^-
\]

The separation of iodide, iodate and periodate is of great importance as these anions are interconvertible. Hence, this separation is selected for detailed qualitative as well as quantitative studies. It was always possible to detect iodide and iodate at all pH values (pH 4 - 10) in a three component mixture whereas periodate could not be detected when a sample (mixture of iodide, iodate and periodate) of pH 4.0 was chromatographed after 30 minutes of mixing time. Thus, all three components (iodide, iodate and periodate) can be resolved, detected and quantified on TLC plates from a single sample upto 30, 60 and 75 min at pH values of 3, 6 - 7 and 10 respectively Fig. 6.1. It seems that the reduction of IO<sub>4</sub><sup>-</sup> into IO<sub>3</sub><sup>-</sup> is slowed down in alkaline medium. Effect of some transition metal ions (VO<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>3+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>) which are encountered in a variety of cases, examined
on the separation, $IO_4^-$ - $IO_3^-$ - I$^-$. The mixture consisting of KI, KIO$_3$ and KIO$_4$ in 5:1:1 ratio (pH = 7) was chromatographed with and without metal ions using S$_1$/M$_5$ chromatographic system. These metal ions remained at the point of application ($R_F$ = 0) when chromatographed individually with the same system. All three anions were resolved on TLC plate in the absence of metal ions. The addition of metal ions to the mixture leads to the formation of little precipitation at the bottom of glass tube in each case. Chromatography of supernatant shows the presence of metal ions along with well resolved spots of iodide and iodate. However, no clear detection of periodate was observed. The precipitate was dissolved in minimum amount of an acid (Fe$^{3+}$, Co$^{2+}$) or NH$_4$OH (Cu$^{2+}$, Zn$^{2+}$ and Ni$^{2+}$) and the TLC of this solution shows the presence of metal ions in the form of well formed spots.

Though, periodate was clearly detected on TLC plate after chromatography of dissolved precipitate produced in the case of Fe$^{3+}$, Cu$^{2+}$, Zn$^{2+}$, Ni$^{2+}$ and Co$^{2+}$ but it could not be detected in the case of VO$^{2+}$ and Mn$^{2+}$. It seems that VO$^{2+}$ and Mn$^{2+}$ being reducing agents, convert periodate into iodate. This assumption finds support from the observation that the mixture of periodate and metal ion when chromatographed and detected, a spot corresponding to the $R_F$ value of iodate was observed on TLC plate and periodate was found absent. It clearly indicates the transformation of periodate into iodate. VO$^{2+}$ and Mn$^{2+}$ were clearly detected at their original position ($R_F$ = 0).

**Quantitative Studies**: I$^-$ (0.1-1.0 mg as KI), $IO_3^-$ (20-200 µg as KIO$_3$) and $IO_4^-$ (20-200 µg as KIO$_4$) have been quantitatively determined.

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titrimetrically using 2.0% aqueous CH\textsubscript{3}COCH\textsubscript{3} as titrant at room temperature (20 ± 2°C). The analyte solutions produce yellow/brown color of iodine on addition of appropriate reagent. The addition of acetone to the solution results in the decolorization due to the iodination of acetone. The equivalence point is reached when brown/yellow color is changed to colorless. Volumes of titrant so consumed are plotted against the amounts of analyte salts. It is expected that experimentally more volume of acetone be consumed for each mole of analyte as required theoretically on the basis of reaction mechanism. In the presence of an acid or base an equilibrium between keto and enol forms of acetone exists as a result of direct shift of hydrogen from carbon to oxygen and vice-versa, as:

\[
\begin{align*}
\text{CH}_3\text{COCH}_3 & \overset{\text{OH}}{\rightleftharpoons} \text{CH}_3\text{C}==\text{CH}_2 \\
\text{Keto Form} & \quad \text{Enol Form}
\end{align*}
\]

The keto form is more stable and the percentage of enol content of acetone is 1.5 x 10\textsuperscript{-4} (12). The extent of enolization is affected by solvent, concentration and the temperature. Water is capable to reduce the enol concentration by hydrogen bonding with carbonyl group. In the present case, enolic form is solely responsible for iodination and therefore the consumption of acetone taken as 2.0 % aqueous solution is much larger than the theoretical value. The standard calibration curves for KI, KIO\textsubscript{3}, and KIO\textsubscript{4} were constructed (Figure 6.2) which were used for the estimation and recovery of I\textsuperscript{-}, IO\textsubscript{3}\textsuperscript{-} and IO\textsubscript{4}\textsuperscript{-} after their separation from mixtures. To determine the lower limit of quantification (LOQ), different amounts (µg)
of an anion were spotted on TLC plate. chromatographed, area of adsorbent corresponding to the anionic spot was scraped off and then the analyte anions was determined by titrimetry. The lowest amount, below which the chromatoo-iodometric method is not applicable, is taken as LOQ. The LOQ values for iodide, iodate and periodate are 76.4, 16.3 and 16.6 µg respectively.

Recovery from Spiked Water: The results presented in Table 6.2 show that the recovery of IO₃⁻ is always higher than 100% and that of IO₄⁻ is much lesser than 100% after 30 min which reduced to zero after 70 min of mixing time of the components. This phenomenon may be explained on the basis of conversion of IO₄⁻ into IO₃⁻. The recovery of I⁻ was always fairly good. During quantitative determination and recovery of analytes, the relative standard deviation varies from 1.54 to 13.29 pph.
Table 6.1: hR<sub>f</sub> Values of I<sup>-</sup>, IO<sub>3</sub><sup>-</sup> and IO<sub>4</sub><sup>-</sup> with Different Chromatographic Systems.

<table>
<thead>
<tr>
<th>Anion Studied</th>
<th>Stationary phase</th>
<th>hR&lt;sub&gt;f&lt;/sub&gt; Values achieved with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M&lt;sub&gt;1&lt;/sub&gt; M&lt;sub&gt;2&lt;/sub&gt; M&lt;sub&gt;3&lt;/sub&gt; M&lt;sub&gt;4&lt;/sub&gt; M&lt;sub&gt;5&lt;/sub&gt;</td>
</tr>
<tr>
<td>I&lt;sup&gt;-&lt;/sup&gt;</td>
<td>S&lt;sub&gt;1&lt;/sub&gt;</td>
<td>65 70 86 90 97</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>56 91 92 92 94</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;3&lt;/sub&gt;</td>
<td>95 96 97 97 98</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;4&lt;/sub&gt;</td>
<td>90 92 97 98 98</td>
</tr>
<tr>
<td>IO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>S&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0 0 8 24 30</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0 55 72 85 94</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;3&lt;/sub&gt;</td>
<td>89 90 90 92 94</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0 75 92 90 95</td>
</tr>
<tr>
<td>IO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>S&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0 60 74 85 95</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;3&lt;/sub&gt;</td>
<td>* * * * *</td>
</tr>
<tr>
<td></td>
<td>S&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0 0 0 0 0</td>
</tr>
</tbody>
</table>

* Badly tailed (hR<sub>L</sub> – hR<sub>T</sub> = 100 ) spot
Table 6.2: Recovery of Anions from Ternary Mixtures Prepared in Double Distilled Water

<table>
<thead>
<tr>
<th>Anion</th>
<th>Amount Loaded (µg)</th>
<th>Recovery after 30 Min of Mixing of Components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount Recovered (µg)</td>
<td>Relative Recovery (%)</td>
</tr>
<tr>
<td>I⁻</td>
<td>152.89</td>
<td>142.35</td>
</tr>
<tr>
<td></td>
<td>305.79</td>
<td>289.39</td>
</tr>
<tr>
<td></td>
<td>458.68</td>
<td>417.44</td>
</tr>
<tr>
<td>IO₃⁻</td>
<td>32.69</td>
<td>45.01</td>
</tr>
<tr>
<td></td>
<td>65.35</td>
<td>79.22</td>
</tr>
<tr>
<td></td>
<td>98.08</td>
<td>110.79</td>
</tr>
<tr>
<td>IO₄⁻</td>
<td>33.24</td>
<td>12.95</td>
</tr>
<tr>
<td></td>
<td>66.40</td>
<td>25.59</td>
</tr>
<tr>
<td></td>
<td>99.60</td>
<td>38.64</td>
</tr>
</tbody>
</table>

* Not detected
Figure 6.1: TLC Detection of $I^-$, $IO_3^-$ and $IO_4^-$ under optimized pH and Mixing Time. pH: 4.0 (Plate No. 1,2), 7.0(3,4) and 10.0(5,6); Mixing Time: 30 Min(1), 35 Min(2), 60 Min(3), 65 Min(4), 75 Min(5) and 80 Min(6).
Figure 6.2. Standard Calibration Curves for the TLC-Iodometry of Iodide (A) and its Oxyanions (B).
REFERENCES


CHAPTER VII
COLORIMETRIC DETERMINATION OF SILVER IN ORES WITH PRELIMINARY TLC SEPARATION FROM ASSOCIATED METAL IONS
7.1 INTRODUCTION

In recent years, thin-layer chromatography (TLC) has grown much in status and has enjoyed popularity due to its simplicity, versatility and low cost. TLC with optimization of techniques and layer materials, can be applied for the identification, separation and quantitation of various organic and inorganic species present in pharmaceutical, environmental, geological and biological samples. As a result, it has found interesting applications in the analysis of river, sea and industrial wastewater for heavy metal contents (1-7); characterization of hazardous wastes (8); determination of heavy metals in water, industrial sewage and aquatic plants (9-11); concentration and detection of heavy metal compounds in fresh water (12); identification of heavy metals in the human placenta (13); separation of heavy metals in tubewell water samples (14); recovery of SCN⁻ in photogenic waste (15) and pesticide residue analysis (16).

The majority of TLC has been performed on silica gel layers with some use of chemically modified and bonded layers, inorganic ion exchangers, cellulose, silufol, polyamides, chitosan, alumina, cellulose derivatives and mixed sorbent layers. In general, mixtures of organic solvents containing some aqueous acids, bases or a buffer have been found most suitable for the separation of ionic species.

As evident from the literature survey of last twenty-two years (17-19) on TLC of inorganics, few workers have used mixed layers. The binary layers that have been used include silica gel-microcrystalline cellulose (MCC) - NH₄NO₃, silica gel G-MCC, modified silica gel H-MCC and silica gel-ion exchange gels or Cr (IV) antimonate for cations; Silica gel -alumina or antimonnic acid and kieselguhr - cellulose for anions.
There appears to be no report of using alumina-cellulose mixed layers (plain and impregnated) for the separation of metal ions.

It is heartening that out of more than two hundred references cited on TLC of inorganics and organometallics in a chapter of the Handbook of Thin-Layer Chromatography, only one report refers to the use of alumina mixed with cellulose as layer materials in the analysis of anions (18).

In continuation of our work on the TLC of inorganics ions on mixed layers (15, 20-22), this paper reports a simple and reliable method for the identification, separation and determination of silver.

7.2 EXPERIMENTAL

Apparatus: TLC applicator, plates and jars used were as presented in 3.2 Spectrophotometer (Elico, India, Model SL 171) was used for quantitative studies.

Chemicals: Silver nitrate, ammonia solution (Qualigens); acetone (CDH, India), ammonia (BDH) were used. All other Chemicals used were of Analytical Reagent (AR) grade.

Test Solutions: 1.0% aqueous solutions of following salts were used as test solutions.
(a) Nitrates of Pb\(^{2+}\), Hg\(^{2+}\), Ag\(^{+}\), Tl\(^{+}\), Cd\(^{2+}\), Fe\(^{3+}\) and Bi\(^{3+}\)
(b) Chlorides of Ni\(^{2+}\), Co\(^{2+}\) and Zn\(^{2+}\)
(c) Sulfate of Cu\(^{2+}\)

Detection Reagent: 8 x 10\(^{-3}\) % (W/V) Dithizone in acetone was used as spray reagent for the visualization of all metal ions studied.

Mobile Phases: Double distilled water; aqueous solutions (1.0M) of NaNO\(_3\), AcONa, AcONH\(_4\); and 0.01 - 1.0 M aqueous ammonia were used as eluants.
Stationary Phases: Following plain and mixed layer materials were used as stationary phases.

(a) Alumina G (S₁)

(b) Mixtures of alumina and microcrystalline cellulose in 4:1 (S₂), 3:2 (S₃), 1:1 (S₄), 1:2 (S₅) and 1:4 (S₆) ratios.

(c) Microcrystalline cellulose (S₇).

Procedure:

Preparation of TLC Plates: TLC plates were prepared by adopting the method described in 3.2.

Chromatography: A fixed volume (5 μL) of test solution was spotted on TLC plate at the point of application (3.0 cm above the lower edge), the plate was air dried and then developed with 20 ml of chosen mobile phase in a glass jar. After development of plate by ascending technique upto 10 cm from the point of application, it was air dried and then the analyte substance was visualized as colored spot by spraying the detection reagent on the plate. Each analyte was identified on the basis of its Rₚ (retardation factor) value.

When two or more metal ions have differential migration (different Rₚ values) their test solutions were mixed together thoroughly and 10μL, 15μL, and 20μL of binary, ternary or quaternary mixtures was spotted on TLC plate and the chromatography was performed. Separated metal ions on TLC plate were appeared at their original positions. For investigating the effect of inorganic anions (S²⁻, CrO₄²⁻, WO₄²⁻, MoO₄²⁻, I⁻, Fe(CN)₆⁴⁻) on the Ag⁺-Cu²⁺ separations, 1% aqueous solution of anion concerned was mixed with binary mixture of Ag⁺ and Cu²⁺ in 1:2 ratio. In case of complexation, the colored precipitate was dissolved either in ammonia solution or nitric acid.
The clear solution was spotted onto the plate and then chromatographed.

**Quantitative studies**: Five standard solutions containing concentration of AgNO₃ in the range 200 - 1000 ppm were used. 0.1 ml of each solution was spotted onto the TLC plate (stationary phase; S₄), air dried and then developed with 1.0 M NH₄OH. After air drying the plate, the spot of silver was visualized by spraying the plate with dithizone solution in acetone. The spot was scraped off the plate and then extracted with 8 ml of acetone followed by washing with 2 ml of acetone. The volume of filtrate was made to 10 ml with acetone and the optical density of the filtrate was measured at 480nm (λ max.) to sketch the calibration curve. Each value of optical density taken for the construction of calibration curve was the average of five observations.

**Recovery of Silver from Ores**: For the synthesis of horn silver(AgCl), Sufficient amount of KCl was added to each aliquot (10 ml) of 0.04, 0.06 or 0.08% AgNO₃ solution. To get the complete precipitation of AgCl. The supernatant was discarded and the white curdy precipitate (Settled down at the bottom of glass tube) was air dried, heated at 50°C for 30 min and then dissolved in 13.36M ammonia (specific gravity, 0.91). The solution was made upto 10ml with 1.0M NH₄OH. 0.1 ml of each solution was spotted onto TLC plate, chromatographed (with S₄, 1.0M NH₄OH) and quantitatively determined by using the method similar to that for standards. The relative recovery (pph) and the relative standard deviation (pph) were calculated.

Agentite (Ag₂S) was synthesized by passing H₂S Separately in each solution of 0.04, 0.06 or 0.08% AgNO₃. After discarding the supernatent, the resultant black precipitate was dried, digested with concentrated HNO₃ and then diluted upto 10ml with water. Similar to horn silver, three solutions
(Concentration of Ag₂S; 0.04, 0.06 and 0.08%) were prepared, which were chromatographed and colorimetrically determined. Relative recovery and relative standard deviation were calculated for each concentration of sample solution.

7.3 RESULTS AND DISCUSSION:

Qualitative Studies: All experiments were performed at room temperature (20°C) and carried out at least in triplicate. The development time for 10 cm ascent was 12-15 min and the results showed good reproducibility. The TLC plates, prepared from mixed adsorbents were found more stable as compared to those coated with alumina. The results of this study are summarized in tables (7.1 - 7.3) and figures (7.1 - 7.3).

Our aim behind this study was to develop a selective TLC-spectrophotometric method for the identification and quantification of silver ion in the presence of transition metal ions i.e. Fe³⁺, Cu²⁺, Ni²⁺, Co²⁺, Zn²⁺, Cd²⁺, Hg²⁺, Pb²⁺, Tl⁺ or Bi³⁺. The literature survey of twenty-two years (17-19, 23-25) on TLC of inorganic substances showed that Ag⁺ is remained at the point of application in most of the cases and its separation from Fe³⁺, Cu²⁺, Hg²⁺, Pb²⁺, Tl⁺ and Bi³⁺ is generally difficult. There are a few chromatographic systems (26-30) which allow the maximum migration of Ag⁺. The Chromatographic system developed by us is a novel system as it allows the rapid and reproducible separation of Ag⁺ from all metal ions studied. Thus, Ag⁺ can be removed from any matrix containing heavy metals e.g. Silver alloys and geological samples. The selected mobile and stationary phases are simple and inexpensive.

Alumina G (S₁) and microcrystalline cellulose (S₇) were used as stationary phases in conjunction with 1.0M aqueous solutions of NaNO₃,
AcONa, AcONH₄ or NH₄OH as mobile phase to study the retention behaviour of eleven metal ions. It was observed that metal ions migrate up and showed maximum R_p values on S₇ layer when developed with NaNO₃, AcONa or AcONH₄. Bi³⁺ is the exception which is strongly retained by stationary phase. The different R_p values of metal ions on S₇ with 1.0M NH₄OH providing higher separation possibilities. As regards to S₁, all metal ions remain at the point of application except Tl⁺ (hR_p = 20) when developed with NaNO₃ and AcONa. However, Ag⁺ showed higher mobility (hR_p = 75) on this stationary phase when developed with 1.0M NH₄OH whereas other metal ions remained near the point of application.

In search of better adsorbent, S₁ and S₇ were mixed together in various ratios to get a set of mixed beds (Table 7.1). Of these stationary phases (S₂ - S₆), Cu²⁺, Ni²⁺ and Ag⁺ give tailed spots on S₅ and S₆ and no sufficient difference in R_p values of metal ions was achieved on S₂ and S₃. Thus S₂, S₃, S₅ and S₆ can not be used for a selective and reproducible separation of metal ions. Out of these six mixed adsorbents, S₄ was found to be the best in terms of detection clarity and selective separation possibilities of metal ions. To find out the optimum concentration of NH₄OH as mobile phase, aqueous solutions containing 0.01 - 1.0 M ammonia were tested. 1.0M NH₄OH was found the most suitable mobile phase for providing differential migration of metal ions (Figure 7.1). By using S₄ (Stationary Phase) alongwith 1.0M NH₄OH (mobile phase), we achieved experimentally several binary, ternary and quaternary separations (Table 7.2). Effect of acid-base equilibrium (AcOH - NH₄OH) on the mobility of Ag⁺ was examined. A peculiar behaviour of Ag⁺ migration was observed (Figure 7.2). With M₁ and M₂ (AcOH > 50 %) Ag⁺ gives a single spot with hR_p value
around 45 but at \( M_3 (\text{NH}_4\text{OH- AcOH}, 1:1) \) two spots of silver were appeared instead of single spot due to the existence of Ag\(^+\) \((hR_f = 45)\) and diammine argentate ion \((hR_f = 70)\). Double spots formation was also observed with 1.0M\text{AcONH}_4\). With mobile phases having more then 50%. \text{NH}_4\text{OH}( M_4 and M_5), a single spot \((hR_f = 85)\) is appeared. It may be attributed to the existence of Ag(\text{NH}_3)_2\(^+\) species. It appears that below 50% concentration of \text{NH}_4\text{OH}, silver migrates as Ag\(^+\); at 50%, it migrates both as Ag\(^+\) and Ag(\text{NH}_3)_2\(^+\) species and above 50% the migrating species is the Ag(\text{NH}_3)_2\(^+\). This observation is important as it highlights the applicability of TLC in studies of chemical speciation.

**Quantitative Studies:**

Silver (upto 100\( \mu \)g) was spectrophotometrically determined using dithizone as chromogenic reagent. Optical densities were measured at 480 nm and plotted against \( \mu \)g amounts of AgNO\(_3\) to sketch the standard calibration curve (Figure 7.3). This curve was used to study the recovery of Ag(I) from its ores (horn silver and argentite). The results are presented in Table 7.3. It was observed that the % recovery is below 100 from horn silver (AgCl) while above 100 from argentite (Ag\(_2\)S). The later is due to the presence of sulfide ion, as this ion produces colour with dithizone. During entire study. The relative standard deviation remained below 9.09 pph.
Table 7.1: $h R_f$ Values of Metal Ions on Plain and Mixed Sorbent Layers Using 1.0M NH$_4$OH as Eluant.

<table>
<thead>
<tr>
<th>Metal Ion</th>
<th>hR$_f$ Values on S$_1$</th>
<th>S$_2$</th>
<th>S$_3$</th>
<th>S$_4$</th>
<th>S$_5$</th>
<th>S$_6$</th>
<th>S$_7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag$^+$</td>
<td>75</td>
<td>78</td>
<td>82</td>
<td>90</td>
<td>92</td>
<td>92</td>
<td>95</td>
</tr>
<tr>
<td>Tl$^+$</td>
<td>7</td>
<td>12</td>
<td>15</td>
<td>17</td>
<td>30</td>
<td>45</td>
<td>32</td>
</tr>
<tr>
<td>Hg$^{2+}$</td>
<td>37</td>
<td>35</td>
<td>44</td>
<td>60</td>
<td>72</td>
<td>90</td>
<td>BT</td>
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<td>Pb$^{2+}$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>12</td>
<td>15</td>
<td>17</td>
<td>20T</td>
<td>BT</td>
<td>BT</td>
<td>BT</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>10</td>
<td>12</td>
<td>27</td>
<td>45</td>
<td>50</td>
<td>50T</td>
<td>80T</td>
</tr>
<tr>
<td>Co$^{2+}$</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>10</td>
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<td>Zn$^{2+}$</td>
<td>2</td>
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<td>17</td>
<td>17</td>
<td>36</td>
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</tr>
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<td>Cd$^{2+}$</td>
<td>8</td>
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<td>25</td>
<td>27</td>
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<td>Fe$^{3+}$</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Bi$^{3+}$</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

T; Tailed spot ($h R_L - h R_T > 30$)

BT; Badly Tailed spot ($h R_L - h R_T > 50$)

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Table 7.2: Experimentally Achieved separations of Metal Ions on $S_4$
Using 1.0M $NH_4OH$ as Eluant. Development Time = 15 min

<table>
<thead>
<tr>
<th>Type of Mixture</th>
<th>hR_L - hR_T of Separated Ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binary</td>
<td>$Ag^+ (97 - 70)$, $Fe^{2+}$, $Co^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Zn^{2+}(34 - 0)$</td>
</tr>
<tr>
<td></td>
<td>$Ni^{2+}(50 - 20)$, $Cd^{2+}(45 - 15)$ or $Hg^{2+}(62-35)$</td>
</tr>
<tr>
<td></td>
<td>$Tl^+(24 - 8)$, $Pb^{2+}$, $Bi^{3+}(0 - 0)$ or $Hg^{2+}(63 - 40)$</td>
</tr>
<tr>
<td></td>
<td>$Hg^{2+}(76-54)$, $Pb^{2+}(0 - 0)$</td>
</tr>
<tr>
<td></td>
<td>$Ni^{2+}(52 - 24)$, $Co^{2+}(0 - 0)$</td>
</tr>
<tr>
<td>Ternary</td>
<td>$Ag^+(95 - 75)$, $Hg^{2+}(63 - 35)$, $Pb^{2+}(0 - 0)$</td>
</tr>
<tr>
<td></td>
<td>$Ag^+(90 - 65)$, $Ni^{2+}(48 - 25)$, $Co^{2+}(0 - 0)$</td>
</tr>
<tr>
<td></td>
<td>$Hg^{2+}(73 - 45)$, $Tl^+(25 - 12)$, $Pb^{2+}(0 - 0)$</td>
</tr>
<tr>
<td></td>
<td>$Hg^{2+}(72 - 53)$, $Tl^+(27 - 12)$, $Bi^{3+}(0 - 0)$</td>
</tr>
<tr>
<td>Quaternary</td>
<td>$Ag^+(95 - 85)$, $Hg^{2+}(76 - 50)$, $Tl^+(27 - 12)$, $Pb^{2+}(0 - 0)$</td>
</tr>
</tbody>
</table>

Table 7.3: Recovery of Ag (I) from its Ores.

<table>
<thead>
<tr>
<th>Ore</th>
<th>Amount Loaded ($\mu g$)</th>
<th>Amount Recovered ($\mu g$)</th>
<th>Relative Recovery (%)</th>
<th>Relative S.D* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horn Silver</td>
<td>25.40</td>
<td>22.28</td>
<td>87.72</td>
<td>9.09</td>
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<td>38.10</td>
<td>35.10</td>
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<td>50.80</td>
<td>46.75</td>
<td>92.02</td>
<td>4.83</td>
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<td>Argentite</td>
<td>25.40</td>
<td>32.28</td>
<td>127.09</td>
<td>6.58</td>
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<td>38.10</td>
<td>44.18</td>
<td>115.96</td>
<td>3.83</td>
</tr>
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<td></td>
<td>50.80</td>
<td>52.12</td>
<td>102.60</td>
<td>5.52</td>
</tr>
</tbody>
</table>

*S.D. = Standard Deviation
Figure 7.1: Mobility of metal ions on alumina G, under the influence of NH$_4$OH concentration of mobile phase. All other metal ions remain at the point of application.
Figure 7.2: Formation of double spot of Ag⁺. M₁-M₅ are mixed mobile phases containing AcOH and HH₄O⁺ in 10:0, 7:3, 1:1, 3:7 and 0:10 respectively.
Figure 7.3: Standard calibration curve for the colorimetry of Ag(I).
Thin-Layer Chromatographic Separation, Identification, and Determination of Certain Anions

Ali Mohammad*, Sharad Tiwari, and Jay Pal Singh Chahar
Analytical Laboratory, Department of Applied Chemistry, Z.H. College of Engineering and Technology, Aligarh Muslim University, Aligarh-202002, India

Abstract
The retention behavior of 19 anions on plain, impregnated, and mixed adsorbent layers of silica gel G, alumina, cellulose microcrystalline, kaolin, and kieselguhr G are examined in aqueous solvent systems. The analytical potential of water, a nontoxic eluant, is explored for its applicability as a simple, inexpensive, and easily available developer in liquid layer chromatography. Better separations of anions are achieved on cellulose with HCl-acetone (1:9) as the eluant and on a cellulose and alumina (1:2) mixed bed with water. The semiquantitative determination of I\(^-\), SCN\(^-\), NO\(_2\)\(^-\), Br\(^-\), BrO\(_3\)\(^-\), IO\(_4\)\(^-\), IO\(_3\)\(^-\), CrO\(_4\)\(^{2-}\), and P\(_2\)O\(_5\)\(^{3-}\) are also determined.

Introduction
Of the various chromatographic techniques, thin layer chromatography (TLC) has recently gained popularity not only because of advancements in technique and instrumentation and improvements 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 depends upon interactions such as hydrogen bonding and electron pair donor and acceptor, ion–ion, ion–dipole, and van der Waals interactions.

An 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 shown in Figure 1. As a continuation of our previous work on the TLC of anions (11–13), this paper describes a simple method for identification, separation, and determination of I\(^-\), SCN\(^-\), NO\(_2\)\(^-\), Br\(^-\), BrO\(_3\)\(^-\), IO\(_4\)\(^-\), IO\(_3\)\(^-\), CrO\(_4\)\(^{2-}\), and P\(_2\)O\(_5\)\(^{3-}\) under various experimental conditions.

Experimental
Reagents
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. 015036), 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, and fluoride and potassium salts of iodide, iodate, periodate, bromide, bromate, permanganate, chromate, dichromate, ferricyanide, ferrocyanide, chloride, phosphate, and sulfite, except thiocyanate and molybdate, which were taken as their ammonium salts. Double distilled water with a specific conductivity \(K = 2 \times 10^{-6}\) ohm\(^{-1}\) cm\(^{-1}\) at 25°C was used for the preparation of salt solutions.

Preparation of thin layer 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 20-\(\times\)3.5-cm\(^2\) glass plates with the help of a TLC apparatus (Toshniwal, India) 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 previously described.

Detection reagents

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

- Saturated AgNO₃ solution in methanol for I⁻, Br⁻, Cl⁻, F⁻, SO₄²⁻, CrO₄²⁻, Cr₂O₇²⁻, and PO₄³⁻.
- Diphenylamine (0.2-0.5%) in 4M H₂SO₄ for NO₂⁻, NO₃⁻, IO₃⁻, IO⁻, BrO₃⁻, MnO₄⁻, and WO₄²⁻.
- Ferric chloride (10%) in 2.0M HCl for SCN⁻, Fe(CN)₆³⁻, and Fe(CN)₆⁴⁻.

Mobile phases

The following solvents were investigated:

- S₁, 0.1M HCl-acetone (1:9);
- S₂, 1.0M formic acid;
- S₃, 1.0M sodium formate;
- S₄, double distilled water.

Stationary phases

The following sorbent layers were used:

- A₁, silica gel impregnated with aqueous 1% CuSO₄ solution;
- A₂, silica gel G;
- A₃, alumina;
- A₄, cellulose microcrystalline;
- A₅, kaolin;
- A₆, kieselguhr G;
- A₇, alumina + cellulose (2:1);
- A₈, kieselguhr + cellulose (1:2, 2:1).

Procedure

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 (24 x 6 cm²) 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 presaturated 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 2).

<table>
<thead>
<tr>
<th>Anions separated</th>
<th>Lower detection limit (µg)</th>
<th>Rf</th>
<th>Accompanying anions</th>
</tr>
</thead>
<tbody>
<tr>
<td>I⁻</td>
<td>7.64</td>
<td>0.66 - 0.75</td>
<td>IO₃⁻, IO₄⁻, NO₂⁻, Br⁻, BrO₃⁻, PO₄³⁻, CrO₄²⁻</td>
</tr>
<tr>
<td>SCN⁻</td>
<td>3.81</td>
<td>0.73 - 0.78</td>
<td>IO₃⁻, IO₄⁻, NO₂⁻, Br⁻, BrO₃⁻, PO₄³⁻, CrO₄²⁻</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>3.33</td>
<td>0.35 - 0.41</td>
<td>IO₃⁻, IO₄⁻, SCN⁻, Br⁻, I⁻</td>
</tr>
<tr>
<td>Br⁻</td>
<td>6.71</td>
<td>0.37 - 0.38</td>
<td>IO₃⁻, IO₄⁻, I⁻, J⁻</td>
</tr>
<tr>
<td>BrO₃⁻</td>
<td>3.83</td>
<td>0.27 - 0.32</td>
<td>IO₃⁻, IO₄⁻, SCN⁻, Br⁻, I⁻</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>11.58</td>
<td>0.32 - 0.38</td>
<td>IO₃⁻, IO₄⁻, SCN⁻, Br⁻, I⁻</td>
</tr>
<tr>
<td>CO₃²⁻</td>
<td>4.06</td>
<td>0.35 - 0.42</td>
<td>IO₃⁻, SCN⁻, Br⁻, I⁻</td>
</tr>
<tr>
<td>IO₃⁻</td>
<td>4.15</td>
<td>0.04 - 0.06</td>
<td>I⁻, SCN⁻, NO₂⁻, Br⁻, BrO₃⁻, PO₄³⁻, CrO₄²⁻</td>
</tr>
</tbody>
</table>

| * Standard Rf values of individual ions: I⁻, 0.73; SCN⁻, 0.83; NO₂⁻, 0.41; Br⁻, 0.43; BrO₃⁻, 0.46; IO₃⁻, 0.07; IO₄⁻, 0.08; CrO₄²⁻, 0.42; PO₄³⁻, 0.43.

Figure 1. Graphical representation of the yearwise publication of papers on thin-layer chromatography of cations and anions. Data obtained from the bibliography section of the Journal of Chromatography.

Figure 2. Diagram of some ternary separations: 1, IO₃⁻ - BR⁻ - I⁻; 2, IO₄⁻ - BrO₃⁻ - I⁻; 3, IO₃⁻ - NO₂⁻ - SCN⁻; 4, IO₄⁻ - BrO₃⁻ - SCN⁻. Conditions: stationary phase, cellulose; mobile phase, HCl-acetone (1:9).
For semiquantitative determination of I−, Br−, and NO2−, 0.01 mL of various standard solutions of KI, KBr, and NaN3O2 solution. The chromatoplates were developed with S1. After detection, the spot was copied onto tracing paper from the chromatoplates, and then the spot area was calculated graphically. Spot areas reported in this paper represent the averages 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 IO3− 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 IO3− in the presence of IO4−, various samples containing a mixture of KIO3 and KI04 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 1.0M HCl to ensure complete extraction of IO3−. The filtrate was added to a blank that was prepared by mixing 2 mL KI (1%), 2 mL concentrated HCl, and 0.2 mL KIO3 (1%). The contents were titrated with a 0.01M 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.

**Results and Discussion**

Tables I and II 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 I indicate that well-resolved ternary separations of IO4− and IO3− can be realized from a synthetic mixture containing I− or SCN− and NO3−, 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 II that distilled water can also be used as a nontoxic eluant for some binary separations of anions. In addition to qualitative separations, quantitative and semiquantitative determination of certain anions with preliminary separation on thin layers can also be made. Table III presents the results of quantitative determination of iodate in the presence of periodate. It is evident from Table III that the proposed method is accurate (percent error, ± 2.2) and reproducible (relative standard deviation, 18 ppt).

We attempted to semiquantitatively determine IO4−, IO3−, NO3−, Br−, and I− by measuring the size of the spots. We outlined the spots on a piece of paper and determined the weight of the paper strip covering the spot area. A linear relationship was obtained when the weight of the paper covering the spot area was plotted against the area of the spot (Figure 3). The linearity was maintained up to 160 mg of NO3−, 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 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. Thus, the method can safely be applied for semiquantitative determination of Br−, I−, and NO3−. However, the semiquantitative method was unsuitable for IO4− and IO3− because of the lower solubility of KIO3 or KIO4 in water.

A plot of loading amount versus spot area for NO3−, 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 4). Thus c/m is the molecular weight of the anion salt or the molecular weight of the anionic salt times a constant.

### Table II. Separations Achieved Experimentally on Different Sorbent Layers Using Distilled Water as Eluant

<table>
<thead>
<tr>
<th>Sorbent system</th>
<th>Separations (Rf-Rt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica gel 'G'</td>
<td>I03− (0.00) − I04−; BrO3−; NO3−; I−; WO42−; Fe(CN)64− or Fe(CN)63− (1.0−0.85)</td>
</tr>
<tr>
<td>Alumina</td>
<td>NO3− (1.0−0.82) − WO42−; MnO4−; PO42− (0.00)</td>
</tr>
<tr>
<td></td>
<td>NO3− (1.0−0.82) − CrO42− or CrO32− (0.18−0.00)</td>
</tr>
<tr>
<td></td>
<td>NO3− (1.0−0.86) − Fe(CN)63− (0.1−0.00)</td>
</tr>
<tr>
<td></td>
<td>SCN− (1.0−0.86) − CrO42− or CrO32− (0.19−0.00)</td>
</tr>
<tr>
<td>Cellulose</td>
<td>MnO4− (0.00) − I03−; I04−, CrO42− or CrO32− (1.0−0.90)</td>
</tr>
<tr>
<td>Alumina +</td>
<td>MnO4− (0.00−0.00) − I03− (0.45−0.30) − NO3− (0.1−0.89)</td>
</tr>
<tr>
<td>Cellulose (2:1)</td>
<td>MnO4− (0.00−0.00) − I03− (0.41−0.24) − BrO3− (1.0−0.86)</td>
</tr>
</tbody>
</table>

* Rf is the Rf of the leading front and Rt is the Rf of the trailing front of the spot.

### Table III. Determination of Iodate with Preliminary TLC Separation on Silica Gel G Layers with Distilled Water as the Eluant

<table>
<thead>
<tr>
<th>Amount of IO4− taken (mg)</th>
<th>Amount of IO3− loaded (mg)</th>
<th>Amount of IO3− 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>
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<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 determinations (n = 5).
This relationship may be useful to correlate the spot area with the atomic weight of anions or molecular weight of anionic salts.

Conclusion

The chromatographic system consisting of cellulose thin layers and 0.1M HCl-acetone (1:9) as the eluant is the most suitable 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.

Acknowledgment

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References


Water-in-Oil Microemulsion as Mobile Phase in Thin-Layer Chromatographic Retention Studies of Anions

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ABSTRACT: A water-in-oil (W/O) microemulsion, consisting of sodium dodecyl sulphate (SDS)/1-pentanol/water/heptane, has been used as mobile phase for the separation of inorganic anions from their binary mixtures, for example, $\text{IO}_4^->\text{NO}_2^-$, $\text{IO}_3^>\text{BrO}_3^-$, $\text{IO}_4^>\text{Br}^-$, $\text{MnO}_4^>\text{BrO}_3^-$, $\text{MnO}_4^>\text{NO}_2^-$, and $\text{MnO}_4^>\text{Br}^-$. The weight ratio, SDS/n-pentanol was kept constant at 1/2.46 for all compositions. The retention efficiency of anions on layers of silica gel G, alumina, microcrystalline cellulose, kieselguhr G, and mixtures of kieselguhr and cellulose in 4:1 and 3:2 ratios has been examined with the W/O microemulsion system as a mobile phase. Thin layers of kieselguhr were most useful for differential migration of anions. Quantitative separation of $\text{IO}_4^-$ from accompanying ions, limits of identification, and dilution of few anions are reported. The effects of amines, phenols, and heavy metals on the separation efficacy of $\text{IO}_4^-$ also have been investigated.

KEY WORDS: Detection, identification, inorganic anions, kieselguhr adsorbent, microemulsion, peak height, periodate determination, SDS, separation, TLC.

Microemulsions are isotropic, clear, or translucent thermodynamically stable dispersions of oil, water, surfactant, and often a small amphiphilic molecule, called a cosurfactant (1). The droplet diameters in microemulsions range from 100 to 1000 Å. The microemulsion systems are usually transparent because of their much smaller droplet size compared to macroemulsions. One can distinguish three different types of microemulsions: Oil microdroplets enclosed in a surfactant-cosurfactant film are dispersed in an aqueous continuous phase (oil-in-water, O/W type); water droplets are dispersed in the oil-continuous phase (water-in-oil, W/O type); and if oil and water droplets overlap in each other, the system is called bicontinuous (2). W/O microemulsions are complex fluids that have wide-ranging applications, for example, as novel lubricants, reaction media for new synthetic chemistry, and mobile phases in separation science (3,4). Since the first report by Armstrong and Henry (5), micellar chromatography has been a subject of interest to many analytical chemists (6–8). It recently has been shown that W/O microemulsions could be used as normal liquid chromatographic mobile phases (9). Interesting selectivities, along with poor efficiencies, were obtained. It must be noted that the W/O microemulsions used did not contain any alcohol cosurfactants. It was reported earlier that the addition of 1-pentanol to the mobile phase greatly improves efficiencies by reducing the adsorbed amount of emulsifier (surfactant). In this work, a W/O microemulsion with 1-pentanol as cosurfactant was used as eluent because use of such phases in thin-layer chromatography (TLC) is an unexplored area of research. In these systems, water microdroplets, enclosed in surfactant/cosurfactant film, are dispersed in an oil-continuous phase (10). One characteristic feature of microemulsions is their wide domain of existence in the pseudoternary phase diagram (9,11).

In continuation of our earlier studies on TLC of anions (12–14), this report describes simple methodology for identification and separation of anions under optimal experimental conditions with a W/O microemulsion as novel eluent. The use of such phases may offer unique solutions for the change in retention data, due to the influence of water on the stationary phase (15) because water is present in the core of W/O microemulsion droplets.

EXPERIMENTAL PROCEDURES

Chemicals. Specially pure sodium dodecyl sulphate (SDS) and n-heptane (99%) were obtained from BDH (Poole, United Kingdom), and n-pentanol was a Riedel product (99%). Demineralized water, redistilled from alkaline potassium permanganate, was used. Alumina, microcrystalline cellulose, and kieselguhr were obtained from CDH Laboratories (India), while silica gel G was obtained from Glaxo (India). All other chemicals (Analar grade) were used as supplied.

Test solutions. The test solutions (1%) were either Na or K salts of all anions studied, except SCN$, which was used as ammonium thiocyanate. Solutions of the nitrates of lead, silver, and bismuth, and the chloride of mercury were prepared in demineralized water, which contained small quantities of the corresponding acid to limit the extent of hydrolysis. The
solutions (1%) of various amines and phenols used were prepared in methanol.

**Chromatography.** A TLC apparatus (Toshniwal, India) was used for the preparation of 20 x 3.5 cm² glass plates. The chromatography was performed in 24 x 6 cm² glass jars.

**Detectors.** The following spray reagents were used for the detection of various anions. (i) Saturated AgNO₃ solution in methanol for I⁻, Br⁻, Cl⁻, F⁻, CrO₃²⁻, Cr₂O₇²⁻, SO₄²⁻, and PO₄³⁻; (ii) diphenylamine (0.2–0.5%) in 4 M H₂SO₄ for NO₃⁻; NO₂⁻, IO₃⁻, IO₂⁻, BrO₃⁻, MnO₄⁻, and WO₄²⁻; (iii) ferric chloride (10⁻² M) in 2 M HCl for SCN⁻, Fe(CN)₆³⁻, and Fe(CN)₅⁴⁻; (iv) alcoholic pyrogallol (0.5%) solution for MoO₄²⁻ and Mo₇O₂₄²⁻.

**Chromatographic systems.** The following sorbent layers were used as stationary phases: S₁ = silica gel G; S₂ = alumina; S₃ = microcrystalline cellulose; S₄ = kieselguhr G; S₅ = kieselguhr + cellulose (4:1), (3:2).

**Mobile phase.** The W/O microemulsion, used as mobile phase, was prepared by titrating a coarse emulsion of n-heptane (160 mL), water (8 mL), and SDS (8 g) with n-pentanol (24 mL). Heptane was chosen for the oil phase because of low toxicity of the odd-carbon number alkanes. The microemulsion was produced at 30°C.

**Preparation of TLC plates.** The TLC plates were prepared by mixing adsorbents with demineralized water in a 1:3 ratio by weight (1:4 ratio for S₅). The resultant slurry was mechanically shaken for 10 min, after which it was applied on well-cleaned glass plates to give a uniform layer of 0.25 mm thickness. The plates were air dried at room temperature and then heated at 100 ± 5°C for 1 h. After activation, the plates were stored in an air-tight chamber. No binder was added during the preparation of plates.

**Procedure.** A sample volume (1.0–10 μL) with a sufficient amount of analyte (0.1–10 μg) was applied with the aid of micropipette about 2.0 cm above the lower edge of the TLC plate. The spots were dried, and the plates were developed by the one-dimensional ascending technique, in glass jars (24 x 6 cm²) that contained the mobile phase. Before the development of plates, the glass jars, containing mobile phase, were covered with a lid for about 20 min, so that the glass jars may get presaturated with the mobile phase vapors. The solvent was allowed to migrate up to 10 cm from the starting line in all experiments. After development, the plates were dried at room temperature, and the spots were visualized with the appropriate spraying reagent.

The identification limits of various anions were 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 just detectable was taken as the limit of detection.

**RESULTS AND DISCUSSION**

We have tried identification and separation of anions on various adsorbents (S₁–S₅). Kieselguhr thin layers were highly effective for differential migration of anions. Table 1 summarizes some binary separations of IO₃⁻ and MnO₄²⁻ from accompanying anions by using the W/O microemulsion as mobile phase and kieselguhr as the stationary phase. Separations achieved are well resolved and compact in this chromatographic system. It is an important aspect of this study because our past experience (14) has shown that kieselguhr, being only slightly active, fails to provide any fruitful data when used as stationary phase for normal TLC of anions with organic or mixed aqueous-organic solvent systems. This indicates that the W/O microemulsion plays a crucial role in enhancing the differential migration of anions on kieselguhr. This is possibly due to the presence of a water core in W/O microemulsion droplets which provides a restricted volume for each individual anion changes slightly when it is chromatographed in mixture with other anions.

Table 2 presents the detection and dilution limits of anions. The proposed method is highly sensitive for most ions, except Br⁻, I⁻, and IO₃⁻. The most interesting behavior is exhibited by MnO₄²⁻, which can be detected easily, even if it is present at 1 μg. Similarly, CrO₃²⁻ and BrO₃⁻ can be detected easily. This may well be attributed to the fact that, in W/O microemulsion, these solutes are localized in the hydrophilic core, which is responsible for some sort of preconcentration.

### Table 1

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>Separations (hRf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kieselguhr G</td>
<td>IO₃⁻ (5.75)–NO₂⁻ (89.3)</td>
</tr>
<tr>
<td></td>
<td>IO₃⁻ (6.75)–BrO₃⁻ (89.25)</td>
</tr>
<tr>
<td></td>
<td>IO₃⁻ (7.25)–I⁻ (88.25)</td>
</tr>
<tr>
<td></td>
<td>IO₃⁻ (7.5)–Br⁻ (94.0)</td>
</tr>
<tr>
<td></td>
<td>MnO₄²⁻ (5.0–BrO₃⁻ (91.0)</td>
</tr>
<tr>
<td></td>
<td>MnO₄²⁻ (5.0–NO₂⁻ (90.0)</td>
</tr>
<tr>
<td></td>
<td>MnO₄²⁻ (2.5–Br⁻ (93.25)</td>
</tr>
</tbody>
</table>

*Note: hRf = Rf x 100, Rf = Rp of leading front of spot, Rb = Rp of trailing front of spot.

<table>
<thead>
<tr>
<th>Table 2 Detection and Dilution Limits of Anions as Their Salts on Kieselguhr Layers Developed with Water-in-Oil Microemulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anion</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>IO₃⁻</td>
</tr>
<tr>
<td>IO₃⁻</td>
</tr>
<tr>
<td>BrO₃⁻</td>
</tr>
<tr>
<td>I⁻</td>
</tr>
<tr>
<td>Br⁻</td>
</tr>
<tr>
<td>MnO₄²⁻</td>
</tr>
<tr>
<td>CrO₃²⁻</td>
</tr>
</tbody>
</table>

* Dilution limit = 1/(volume of test solution x 10⁶) limit of detection (μg).
WATER-IN-OIL MICROEMULSION AS MOBILE PHASE

TABLE 3
Quantitative Separation of IO4\(^{-}\) (20-100 \(\mu\)g) from Milligram Amounts of NO2\(^{-}\), Br\(^{-}\), and I\(^{-}\) with Chromatographic System as in Table 1

<table>
<thead>
<tr>
<th>Amount of accompanying ion salt (mg)</th>
<th>Separations (Rf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO2</td>
<td>IO4(^{-}) (0.01-0.00)-NO2(^{-}) (1.0-0.46)</td>
</tr>
<tr>
<td>KBr(^{+})</td>
<td>IO4(^{-}) (0.11-0.00)-Br(^{-}) (1.0-0.76)</td>
</tr>
<tr>
<td>KI</td>
<td>IO4(^{-}) (0.10-0.00)-Br(^{-}) (1.0-0.68)</td>
</tr>
<tr>
<td>2.5</td>
<td>IO4(^{-}) (0.10-0.00)-Br(^{-}) (1.0-0.48)</td>
</tr>
<tr>
<td>5.0</td>
<td>IO4(^{-}) (0.90-0.80)-Br(^{-}) (1.0-0.65)</td>
</tr>
<tr>
<td>4.0</td>
<td>IO4(^{-}) (0.80-0.65)-Br(^{-}) (1.0-0.20)</td>
</tr>
</tbody>
</table>

Spots of Br\(^{-}\) appear after 20 min. \(^{\#}\)ND = not detected.

of anions (17). Further, the anionic charge on the microemulsion droplet is responsible for fast transfer of anionic species out of the microemulsion droplet and easily available to interact with the detection reagents.

Table 3 presents some quantitative separations of anions. KBr, up to 5 mg, and NaNO2, up to 1.25 mg, can be easily separated from KIO4 (100 \(\mu\)g). Separation of KIO4 from milligram quantities of KI does not take place, KIO4 remains undetected while KI produces a tailed spot. Thus, increasing the KI amount produces a deleterious effect and causes poor or no detection of KIO4. This possibly may be due to reduction of KIO4 to iodine in solutions of moderate acidity. Because of this fact, we carried out the separation of IO4\(^{-}\) from NO2\(^{-}\), Br\(^{-}\), and I\(^{-}\).

Finally, Table 4 summarizes the effect of various additives on the separation efficacy of IO4\(^{-}\) from Br\(^{-}\) and NO2\(^{-}\). Amines by and large do not influence the separations, but there are drastic changes in the Rf values of ions, compared with their standard Rf values. The separation mixture precipitates by the addition of amines. In case of β-napthylamine, Br\(^{-}\) remains undetected because the entire spot (Rf range of Br\(^{-}\)) is superimposed by the solvent uptake of β-napthylamine (deep purple color spot). The situation for NO2\(^{-}\) is similar, but the spot of NO2\(^{-}\) is clear. Spots of Br\(^{-}\), when chromatographed with diphenylamine and 2-nitroaniline, appeared as streaks after one-half hour. Phenols neither hamper separations nor produce precipitation. However, addition of pyrogallol produces slight turbidity, and IO4\(^{-}\) remains undetected when separated from NO2\(^{-}\) or Br\(^{-}\). In this case, the Rf range of IO4\(^{-}\) gets superimposed by pyrogallol. Heavy metals also do not hinder the separations except silver, which causes dense precipitation as a result of which Br\(^{-}\) remains undetected. Addition of lead to the separation mixture of IO4\(^{-}\) and Br\(^{-}\) leads to poor detection of Br\(^{-}\). Mercury shows no effect on the separation of IO4\(^{-}\) from Br\(^{-}\) or NO2\(^{-}\).

An attempt has been made for the semiquantitative determination of IO4\(^{-}\) by employing a peak height measurement procedure. For this purpose, 0.1 mL standard aqueous solu-

TABLE 4
Effect of Additives on Some Binary Separations of IO4\(^{-}\) from NO2\(^{-}\) and Br\(^{-}\)

<table>
<thead>
<tr>
<th>Additives</th>
<th>Separations Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Amines</td>
<td>IO4(^{-}) (0.05)-NO2(^{-}) (0.85)</td>
</tr>
<tr>
<td>(a) β-Napthylamine</td>
<td>IO4(^{-}) (0.06)-Br(^{-}) (0.85)</td>
</tr>
<tr>
<td>(b) Diphenylamine</td>
<td>IO4(^{-}) (0.21)-NO2(^{-}) (0.88)</td>
</tr>
<tr>
<td>(c) 2-Nitroaniline</td>
<td>IO4(^{-}) (0.20)-NO2(^{-}) (0.65)</td>
</tr>
<tr>
<td>2. Phenols</td>
<td>IO4(^{-}) (0.17)-Br(^{-}) (0.78)</td>
</tr>
<tr>
<td>(a) Phenol</td>
<td>IO4(^{-}) (0.03)-NO2(^{-}) (0.82)</td>
</tr>
<tr>
<td>(b) Resorcinol</td>
<td>IO4(^{-}) (0.10)-Br(^{-}) (0.85)</td>
</tr>
<tr>
<td>(c) Pyrogallol</td>
<td>IO4(^{-}) (0.07)-NO2(^{-}) (0.90)</td>
</tr>
<tr>
<td>3. Heavy metals</td>
<td>IO4(^{-}) (0.07)-Br(^{-}) (0.81)</td>
</tr>
<tr>
<td>(a) Hg(^{2+})</td>
<td>IO4(^{-}) (0.05)-NO2(^{-}) (0.92)</td>
</tr>
<tr>
<td>(b) Pb(^{2+})</td>
<td>IO4(^{-}) (0.08)-Br(^{-}) (0.83)</td>
</tr>
<tr>
<td>(c) Ag(^{+})</td>
<td>IO4(^{-}) (0.03)-NO2(^{-}) (0.90)</td>
</tr>
<tr>
<td>(d) Bi(^{3+})</td>
<td>IO4(^{-}) (0.01)-Br(^{-}) (ND)</td>
</tr>
</tbody>
</table>

Standard Rf values of individual ions are: IO4\(^{-}\) (0.00), NO2\(^{-}\) (0.92), and Br\(^{-}\) (0.90).

FIG. 1. Standard calibration curve for semiquantitative determination of IO4\(^{-}\) by peak height measurement method.
tions of KIO₄ (0.02–0.1%) were spotted on kieselguhr thin layers. The chromatograms were developed and detected. The spots obtained were copied directly onto tracing paper from the chromatoplates. A linear relationship was obtained when the square of peak heights of the spots was plotted against microgram quantities of KIO₄ (Fig. 1).

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REFERENCES


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Thin-layer chromatographic separation, colorimetric determination and recovery of thiocyanate from photogenic waste, river and sea waters

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Thin-layer chromatographic separation, colorimetric determination and recovery of thiocyanate from photogenic waste, river and sea waters

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Abstract

TLC was used to study the retention behaviour of some inorganic anions \( \text{SCN}^- \), \( \text{NO}_3^- \), \( \text{MnO}_4^- \), \( \text{CrO}_4^{2-} \), \( \text{MoO}_4^{2-} \), \( \text{Fe(CN)}_6^{3-} \) and \( \text{Fe(CN)}_6^{4-} \) and metal ions \( \text{Fe}^{3+} \), \( \text{Zn}^{2+} \), \( \text{Cu}^{2+} \), \( \text{Cd}^{2+} \), \( \text{Co}^{2+} \), \( \text{Hg}^{2+} \), \( \text{UO}_2^{2+} \), \( \text{VO}^{2+} \), \( \text{Ag}^+ \), \( \text{Mn}^{2+} \) and \( \text{Bi}^{3+} \) on cellulose, Kieselguhr and their mixtures. Mixed solvent systems containing 0.1 M \( \text{NH}_3\text{OH} \) and \( \text{CH}_3\text{COCH}_3 \) were used as eluents. SCN\(^-\) separated from other ions was determined by spectrophotometry at 460 nm using acidic \( \text{FeCl}_3 \) solution as chromogenic reagent. Beer's law was followed up to 11.84 ppm of SCN\(^-\). The proposed TLC-colorimetric method was applied to fortified samples of photogenic waste, river and seawaters.

Keywords: Water analysis; Environmental analysis; Thiocyanate; Inorganic anions; Inorganic cations

1. Introduction

Identification, separation and quantification of SCN\(^-\) is important due to its application in photography, catalysis, agri-chemicals, rust-inhibition and dyeing–printing of textiles [1]. At high acidities, thiocyanate produces cyanides in the presence of oxidants and thus causes lethal damage to aquatic life when SCN containing waste is discharged into rivers. Numerous methods [2–8] have been developed for analysing SCN\(^-\) and CN\(^-\) but most of them were not applied to polluted water and effluents. A spectrophotometric method based on the colour reaction of Fe\(^{3+}\) with SCN\(^-\) has been used for the quantification of SCN\(^-\) but it suffers from cationic, anionic and phenolic interferences. An extensive literature survey on the quantitative determination of SCN\(^-\) (1981–1992) with respect to the techniques used, shows that out of twenty research papers published, only one dealt with spectrophotometry.

Thin-layer chromatography (TLC) has been a widely used technique for the separation of SCN\(^-\) [9–12]. The literature survey [13] shows that out of forty research papers published so far on TLC of SCN\(^-\), none examined the separation of SCN\(^-\) from complexing cations (Ag\(^+\), Fe\(^{3+}\), Co\(^{2+}\), Cu\(^{2+}\) etc.). This paper reports a reliable TLC-colorimetric method for the determination of SCN\(^-\) in the presence of cationic and anionic impurities. The proposed meth-
od is applicable to the determination of SCN⁻ in photogenic waste, river water and seawater.

2. Experimental

2.1. Apparatus

TLC applicator (Toshniwal, India); 20×3.5 cm glass plates, 24×6 cm jars, spectrophotometer (Elico, India, Ultra Spec. Model CL-54) and pH meter (Elico, India, Model LI-10T) were used.

2.2. Test solutions

Aqueous solutions (1.0%, w/v) of following were used.
1. Potassium salts of Fe(CN)₆³⁻, Fe(CN)₄²⁻, MnO₄⁻, CrO₇²⁻ and Cr₂O₇²⁻.
2. Sodium salts of NO₃⁻ and MoO₄²⁻.
3. Ammonium salts of SCN⁻.
4. Nitrates of Ag⁺, Bi⁺⁺, UO₂²⁺ and Zn²⁺.
5. Chlorides of Fe⁺⁺ and Hg⁰⁺.
6. Sulphates of Cu²⁺, VO²⁺, Co⁺⁺ and Mn²⁺.

2.3. Chemicals

Ammonium thiocyanate (Merck, India), ferric chloride and ammonia solution (Qualigens), acetone, cellulose microcrystalline and Kieselguhr G (CDH, India) were used. All other chemicals were of analytical reagent grade.

2.4. Detection reagents

To locate the spots of analytic ions, the reagents used were:
1. 0.3% Diphenylamine in 4 M H₂SO₄ for NO₃⁻, MnO₄⁻, CrO₇²⁻ and Cr₂O₇²⁻.
2. 8.0% FeCl₃ in 2 M HCl for SCN⁻, Fe(CN)₆³⁻ and Fe(CN)₄²⁻.
3. 0.5% Pyrogallol in methanol for MoO₄²⁻ and Mo₆O₄⁶⁻.
4. 1.0% K₄Fe(CN)₆ in water for Fe⁺⁺, Cu⁺⁺, VO²⁺ and UO₂²⁺.
5. 1.0% Dimethylglyoxime in ethanol for Co⁺⁺.
6. 1.0% Dithizone in benzene for Zn²⁺ and Cd²⁺.
7. Saturated H₂S in water for Ag⁺, Hg⁰⁺ and Bi³⁺.

2.5. Stationary phases

Plain cellulose microcrystalline (S₁), Kieselguhr G (S₄) and their mixtures in 4:1 (S₂), 3:2 (S₃) and 1:1 (S₅) ratios were used as adsorbent materials.

2.6. Mobile phases

Mixtures of 0.1 M NH₄OH and CH₃COCH₃ in 1:9 (M₃), 3:7 (M₄), 5:5 (M₅), 7:3 (M₆) and 9:1 (M₇) ratios were used as solvent systems.

2.7. Preparation of chromatoplates

Cellulose, Kieselguhr G or their mixture was slurried with double distilled water in 1:3 and the resultant slurry (without additional binder) was applied onto glass plates with the aid of an applicator to give a layer of 0.25-mm thickness. Plates were dried at room temperature, activated at 100±2°C and then stored in a desiccating chamber until use.

2.8. Preparation of fortified samples

Four samples of SCN⁻ solutions (5.09–12.92 mM) were prepared in seawater (collected from Anjuna Beach, Goa). Similarly, spiked river water (Ganga, Rajghat) and photogenic waste (Aligarh) were prepared to examine the recovery of SCN⁻.

2.9. Procedure

The entire methodology was carried out as follows.

2.9.1. Chromatography of inorganic ions

To study the retention behaviour of some inorganic ions, 0.01 ml of the test solution was spotted onto the TLC plate, air dried and then developed with M₁–M₇ up to 10 cm. The plate was air dried, analytic ions were detected as colored spots and then identified on the basis of their Rₚ values. For mutual separations, an aliquot (0.02 ml) of binary or 0.03 ml of ternary mixture of analytes was loaded onto the plate and the chromatography was performed. In order to examine the effect of sample pH on the separation of SCN⁻, various mixtures of test solu-
tions were brought to the required pH values (3.0-12.25) by adding either glacial acetic acid or NaOH solution. The effects of amines (2-nitroaniline, β-naphthylamine and diphenylamine) and phenols (phenol, resorcinol and pyrogallol) were examined on the detection and separation of SCN⁻ by mixing 1% alcoholic solution of additive with binary/ternary mixture containing SCN⁻.

2.9.2. Colorimetry of thiocyanate

For quantitative determination of SCN⁻ by TLC-colorimetry, 0.1 ml of standard ammonium thiocyanate (2.55-25.48 mM SCN⁻) was spotted onto the plate (S₁) along with foreign ions and developed with M₁. A pilot plate was simultaneously run. SCN⁻ containing portion of adsorbent from the working plate was scraped off and SCN⁻ was extracted with 10 ml of distilled water followed by washing of the adsorbent with 2 ml of water. Thus, the total volume of solution was kept to 12 ml in each case. To the filtrate, 0.5 ml of chromogenic reagent solution (8.0% ferric chloride in 2 M HCl) was added, absorbance was measured at 460 nm and the absorbance was plotted against the concentration (ppm) of SCN⁻ solution to sketch the standard calibration curve which was used to determine the concentration of SCN⁻ in a water sample with preliminary separation from accompanying inorganic ions.

2.9.3. Recovery from fortified samples

For the recovery of SCN⁻ from spiked waste (photogenic) and water (river and sea) samples, 0.1 ml of the solution was spotted onto TLC plate (chromatographic system; S₂, M₁) and the chromatocolorimetry was performed in the similar manner as that for standards. The loaded and recovered amounts of SCN⁻ were compared and the relative standard deviation (%), relative error and percentage recovery were calculated.

3. Results and discussion

3.1. Qualitative studies

Inorganic ions were chromatographed on plain and mixed adsorbents containing cellulose and/or Kieselguhr (S₁-S₄) using mixtures of 0.1 M NH₄OH and CH₃COCH₃ in various ratios as mobile phases (M₁-M₄). The results of this study are summarized in Tables 1 and 2. The effect of proportional composition of mobile phase on the hRᶠ values of inorganic anions is examined and the results are reported in Table 1 where only plain adsorbents (S₁ and S₄) were used. Cations were also chromatographed with these chromatographic systems, cations remain at the point of application. However, some metal ions yield occasional tailed spots. To achieve difference in migration of anions, S₁ and S₄ were mixed together to get a set of adsorbents (S₂-S₄) on which the anions were chromatographed using M₁ as mobile phase. The hRᶠ values of anions are enlisted in Table 2. Out of three mixed adsorbents, cellulose-Kieselguhr (4:1, S₄) is selected because it provides sufficient difference in hRᶠ values of SCN⁻ and others. It also provides compact spots because increasing Kieselguhr proportion results in the tailing of spots. Hence, the combination of S₄ and M₁ is the best chromatographic system which is utilized for the selective separation of SCN⁻ from all ions studied. These ions strongly interfere in the colorimetric

<table>
<thead>
<tr>
<th>Anion</th>
<th>Stationary phase</th>
<th>hRᶠ values achieved with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M₁</td>
<td>M₂</td>
</tr>
<tr>
<td>SCN⁻</td>
<td>49</td>
<td>91</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>85</td>
<td>90</td>
</tr>
<tr>
<td>MnO₄⁻</td>
<td>88</td>
<td>89</td>
</tr>
<tr>
<td>Fe(CN)₆³⁻</td>
<td>6</td>
<td>54</td>
</tr>
<tr>
<td>Fe(CN)₅⁶⁺</td>
<td>83</td>
<td>90</td>
</tr>
<tr>
<td>CrO₇³⁻</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Cr₂O₇⁶⁻</td>
<td>41</td>
<td>91</td>
</tr>
<tr>
<td>MoO₄³⁻</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Mo₆O₁₉⁶⁻</td>
<td>43</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>90</td>
</tr>
</tbody>
</table>

* Metal ions remain at the point of application in all of the above mentioned chromatographic systems.
Effect of stationary phase composition (cellulose/Kieselguhr proportion) on the mobility of inorganic ions when developed with M1.

<table>
<thead>
<tr>
<th>Ion studied</th>
<th>( aR_s ) values achieved on</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN(^-)</td>
<td>49 79 80 85 85</td>
</tr>
<tr>
<td>NO(_3)^-</td>
<td>21 21 32 86 88</td>
</tr>
<tr>
<td>MnO(_4)^-</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>Fe(CN)(_6)^3^-</td>
<td>6 6 7 12 83</td>
</tr>
<tr>
<td>Fe(CN)(_6)^4^-</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>CrO(_4)^2^-</td>
<td>10 20 24 28 41*</td>
</tr>
<tr>
<td>MoO(_4)^2^-</td>
<td>8 19 19 25 43</td>
</tr>
<tr>
<td>MoO(_4)^3^-</td>
<td>9 11 17 21 36*</td>
</tr>
<tr>
<td>MoO(_4)^4^-</td>
<td>12 15 15 23 38</td>
</tr>
</tbody>
</table>

* All metal ions studied remain at the point of application (\( aR_s = 0.0 \)).

** Tailed spots (\( aR_s - aR_f > 30 \)).

The method of SCN\(^-\) quantification due to the formation of coloured complexes either with SCN\(^-\) or Fe\(^{3+}\) [14]. For Ag\(^+\)—SCN\(^-\) separation, the white precipitate of AgSCN formed on mixing of both ions was dissolved in NH\(_3\) solution and the clear solution was spotted on the TLC plate. Fe\(^{3+}\)—SCN\(^-\) separation is of great importance, especially when SCN\(^-\) is to be determined colorimetrically by using FeCl\(_3\) as a chromogenic reagent.

For this separation, 1.0% aqueous NaF solution was added to red coloured complex to decolourize it. FeF\(_3\), a more stable complex was formed and SCN\(^-\) became free and was detected on TLC plate. In the Co\(^{2+}\)—SCN\(^-\) separation, Co\(^{2+}\) could not be detected. In general, for metal ion—SCN\(^-\) separation, the amount of SCN\(^-\) was taken \( x \) times more than that of metal ion (\( x \) is the number of unit charges present on the metal ion). Effects of sample pH, phenols and amines on the detection of SCN\(^-\) have been examined and SCN\(^-\) was clearly detected in all cases. The \( R_f \) value of SCN\(^-\) remained unchanged over the pH range (3.0—12.25) of sample solution. No change in the mobility of SCN\(^-\) was noticed on being chromatographed in the presence of amines and phenols.

### Table 3

<table>
<thead>
<tr>
<th>Sample (pH)</th>
<th>Amount of SCN(^-) loaded (( \mu g ))</th>
<th>Amount of SCN(^-) recovered (( \mu g ))</th>
<th>Relative recovery (%)</th>
<th>Relative standard deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Photogenic waste</strong></td>
<td>29.60</td>
<td>30.19</td>
<td>102.00</td>
<td>9.38</td>
</tr>
<tr>
<td>(9.4)</td>
<td>44.40</td>
<td>45.48</td>
<td>102.44</td>
<td>5.60</td>
</tr>
<tr>
<td></td>
<td>59.20</td>
<td>60.64</td>
<td>102.44</td>
<td>5.42</td>
</tr>
<tr>
<td></td>
<td>74.00</td>
<td>76.46</td>
<td>103.32</td>
<td>10.70</td>
</tr>
<tr>
<td><strong>River water</strong></td>
<td>29.60</td>
<td>28.12</td>
<td>94.99</td>
<td>9.91</td>
</tr>
<tr>
<td>(8.2)</td>
<td>44.40</td>
<td>42.62</td>
<td>96.00</td>
<td>7.83</td>
</tr>
<tr>
<td></td>
<td>59.20</td>
<td>57.22</td>
<td>96.66</td>
<td>7.09</td>
</tr>
<tr>
<td></td>
<td>74.00</td>
<td>73.03</td>
<td>98.69</td>
<td>5.63</td>
</tr>
<tr>
<td><strong>Sea water</strong></td>
<td>29.60</td>
<td>27.38</td>
<td>92.50</td>
<td>9.91</td>
</tr>
<tr>
<td>(8.0)</td>
<td>44.40</td>
<td>41.64</td>
<td>93.78</td>
<td>7.57</td>
</tr>
<tr>
<td></td>
<td>59.20</td>
<td>56.23</td>
<td>94.99</td>
<td>6.59</td>
</tr>
<tr>
<td></td>
<td>74.00</td>
<td>71.53</td>
<td>96.66</td>
<td>5.45</td>
</tr>
</tbody>
</table>
samples of photogenic waste, river water and sea water. The results are summarized in Table 3. This table shows that the relative recoveries of SCN⁻ are almost the same.

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References