PLANAR CHROMATOGRAPHIC SEPARATIONS OF ORGANIC AND INORGANIC COMPOUNDS

THESIS

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Dedicated To My Adorable Parents

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Certificate

This is to certify that the work embodied in this thesis entitled, "Planar Chromatographic Separations of Organic and Inorganic Compounds" is the original contribution of Mr. Yasir Hamid Sirwal, carried out under my guidance & supervision, and is suitable for the award of degree of Doctor of Philosophy in Applied Chemistry of Aligarh Muslim University, Aligarh.

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LIST OF PUBLICATIONS

1. Thin-Layer Micellar Chromatography of Coinage Metal Cations, A. Mohammad and **Yasir Hâmid Sirwal,** ***J. Planar Chromatogr. – Mod. TLC*** **(Hungary), 15, 107-115 (2001).**

2. Identification of Amino Acids on Alumina TLC Plates Developed with Aqueous Anionic Surfactant Solutions Containing Inorganic and Organic Additives: Selective Separation of L-Proline from other Amino Acids, A. Mohammad, **Yasir Hamid Sirwal** and V. Agrawal, ***Acta Universitatis Cibiniensis, Seria F Chemia*** **(Romania) Accepted.**

3. A New Anionic Micellar Mobile Phase System for Normal-Phase Thin-Layer Chromatography of Amino Compounds: Simultaneous Separation of Aminophenol Isomers, A. Mohammad and **Yasir Hamid Sirwal,** ***J. Analytical Chemistry*** **(Russia) Revised.**

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GENERAL INTRODUCTION

1.1 INTRODUCTION

Analytical chemistry has extensive applications in the analysis of organic and inorganic compounds, pharmaceuticals, bio-chemicals, body fluids, polluted water, food and many other areas. With the global awareness in health hazards and environmental pollution, analytical chemistry has played key role to unveil its causes. Modern sophisticated computerized instrumental techniques make possible to elucidate the microstructure of molecular species and thereby the reaction mechanics taking place onto the species, studies of rare and artificial radioactive elements and to obtain substances in the highest state of purity. This branch usually begins by placing chemical analysis in the broader prospective of chemical science, describing different methods of analysis e.g. **qualitative** (deals with finding what constituent or constituents are in analytical sample) and **quantitative** (deals with the determination of how much of given substance is in the sample) on macro to micro level and can also be applied to the routine analysis. Chemical analysis is an important part of many of most exciting scientific projects being carried out throughout the world, because on this basis one can understand the properties of materials of our interest. According to the type of process used to perform the analysis, methods used for chemical analysis can be categorized as given in **Figure 1.1**.

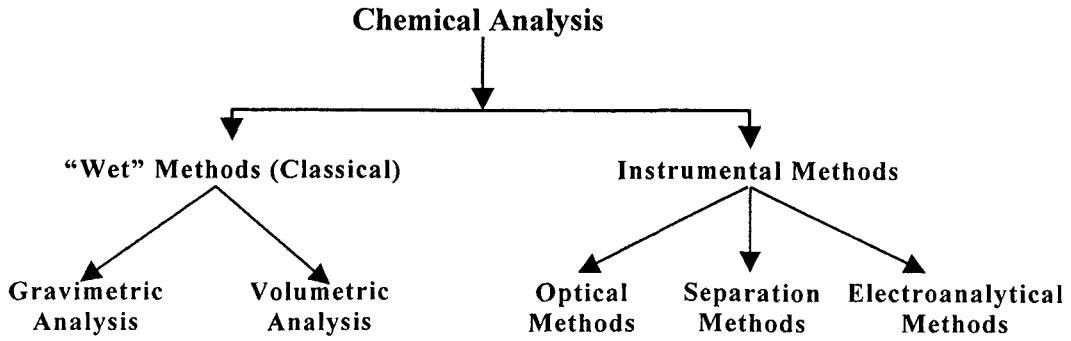

Figure 1.1: The Major Categories of Chemical Analysis

Instrumental as well as non-instrumental (or classical) methods are being employed for the analysis of air, water and soil pollutants. Since instrumental analysis is becoming increasingly automatic and research oriented, the need for analytical chemistry is also rising as newer problems are erupting up in view of the challenging demands of the contemporary world. As a matter of fact, analytical chemistry involving the development of new methods and modifications to classical methods is most suited to meet the requirements of mankind. Despite the advantages offered by the instrumental methods in various fields, their wide spread adoption has not rendered the classical methods obsolete for the simple reasons that firstly, they cannot be applied if the analyte is present in large concentration and secondly, it is absolutely imperative to use classical methods (volumetric or gravimetric) of analysis for standardization of newer methods. In fact classical methods deserve to be strengthened because they are simple, inexpensive and versatile.

1.2 SEPARATION TECHNIQUES

The identification and separation of various species can be achieved by an array of systematic procedures. Among the most versatile analytical separation techniques, ring-oven technique, ion-exchange, dialysis, electrophoresis and chromatography have wider applicability. These techniques are briefly described below:

(i) ***Ring-Oven Technique***: The ring-oven technique has been developed as pseudo-chromatographic method for testing a single drop of solution for several components on filter paper. The sample to be tested is placed at the centre of the ring formed by the solute, the carrier solvent evaporates on approaching the heated ring-zone after transportation through the pores of the filter paper. Separations are based on the varying behaviors of solutes with a

solvent resulting in differential migration fronts from the starting point. The method is applicable to microanalytical separation and has future prospects.

(ii) ***Dialysis***: Dialysis is another differential migration technique, which involves the selective migration of components through the membrane by diffusion process. In dialysis the separation is based upon the exploitation of difference in relative rates of diffusion of two species through the membrane. This process is very useful for separating ionic substances from organic impurities of larger particle size.

Dialysis has become a practical means for the separation of salts from colloidal suspensions. The combination of cation and anion-exchange membranes in electrolytic cells make it possible to desalt saline waters and separate many ionic substances from the mixture. When an electric field is applied across the membrane, the process is called electrodialysis.

(iii) ***Ion-Exchange***: Ion-exchange is a process in which reversible stoichiometric interchange of ions of the same sign take place between an electrolyte solution (solvent system or moving phase) or molten salt and a solid phase (adsorbent or stationary phase). The selectivity of an exchanger depends upon the nature of the ion-exchanger and the composition of the liquid phase, which is in contact with it. It is therefore, possible to increase the separation potential of the ion-exchange process by proper selection of exchanger and eluent. The exchanger phase may be inorganic or organic in nature.

It is difficult to say when ion-exchanger was actually discovered. *Helfferich* has given an interesting historical account (1) tracing the discovery of ion-exchange as far back as the times

of *Moses*. The earliest references found in *Holy Bible* establish *Moses's* priority of preparing drinking water from brackish water by ion-exchange method (2).

Ion-exchange process is well suited for the separation of inorganic ions (cations and anions) because the separation is based on the exchange of ions in stationary phase, as given below:

Cation Exchange:

$$\underline{2NaX} + CaCl2\ (aq.) = \underline{CaX2} + 2NaCl\ (aq.)$$

Anion Exchange:

$$\underline{2XCl} + Na2SO4(aq.) = \underline{X2SO4} + 2NaCl\ (aq.)$$

Where X represents a structural unit of the ion-exchanger, solid phases are underlined: aq. indicates that the electrolyte is in the aqueous solution.

(iv) ***Electrophoresis***: Electrophoresis, which involves the migration of particles through a solution under the influence of an electric field, was first reported (3) by *König* in 1937. Later on, *König* and *Vön Klobusitzky* (4) used paper electrophoresis for separation of a yellow pigment from snake venom. Electrophoresis is helpful in separating and identifying microamounts of high molecular weight substances such as proteins, which are often difficult to separate by chromatography alone. Varying with the properties of the medium, the separations may result primarily from electrophoretic effect or from a combination of electrophoresis and adsorption, ion-exchange or other distribution equilibria. Electrochromatrography, zone electrophoresis, electro-migration and ionophoresis are other methods based upon electrophoresis.

Clinical chemists and biochemists have exploited electro-chromatographic methods for fractionating biological materials e.g. separation of proteins and other large molecules present in serum, wine, spinal fluid, gastric juices and other body fluids. Inorganic ions are conveniently separated by this technique.

1.3 CHROMATOGRAPHY

In spite of the popular belief and general acceptance of the contribution of *Tswett* as being the real discoverer of chromatography (literally "colour writing" from the Greek), the starting of chromatography predated to the work of *F.F. Runge* who investigated the separation of coloured substances (i.e. dyes) on paper (5). The work carried out by *Goppelsroeder* (6) and *Schonbein* (7) on chromatographic separation of substances on filter paper has been included in a report (8) published by *Fischer* and *Schmidner* in 1892. However, the concept of separation on columns may be attributed to *Reed's* work, which was followed by *Day* who separated petroleum fractions with the help of columns (9,10). The paper published in 1906 by *M. Tswett*, a lecturer of Botany at the University of Warsaw provided the first description in nearly modern terms of chromatographic separation (11). He described the resolution of different components of pigments as colored bands like spectrum of light rays on a calcium carbonate column and termed it as "*Chromatogram*".

The actual importance of *Tswett's* work remained dormant until about 1931, when separations of plant carotene pigments were reported by prominent organic chemist *Kuhn* (12,13). His research attracted much attention and adsorption column chromatography became invaluable tool in the field of natural product chemistry.

In 1941, *Martin* and *Synge* (14,15) laid another milestone in development of chromatography by reporting their discovery of liquid-liquid partition chromatography. One liquid was used as adsorbent and another liquid was allowed to percolate through the former, thus making the technique as a chromatographic process. This work initialized the development of other forms of chromatography. The chronological development of separation techniques after *Tswett's* discovery of chromatography are presented in **Table 1.1**.

Table 1.1: Chronological Development of Chromatographic Separation Techniques

Authors	Year of Origin	Separation Technique
M.S. Tswett	1906	Adsorption Chromatography
P. Konig	1937	Electrophoresis
N.A. Izmailov and *M.S. Schraiber*	1938	Thin-Layer Chromatography (Adsorption)
A.J.P. Martin and *R.L.M. Synge*	1941	Liquid-Liquid Partition Chromatography
A.J.P. Martin, R. Consden and *A. H. Gordon*	1944	Paper Chromatography
L.C. Craig	1944	Counter Current Chromatography.
S. Claesson	1946	Gas-Solid Chromatography
S.W.Mayer and *E.R. Tompkins*	1947	Ion-Exchange Chromatography
J.G. Kirchner, J.M. Miller and *G.J. Keller*	1951	Thin-Layer Chromatography (Partition)
A.T. James and *A.J. P. Martin*	1952	Gas-Liquid Chromatography
J.Porath and *P.Flodining*	1959	Gel-Filtration Chromatography or Size-Exclusion Chromatography
J.J. Kirkland, Cs Horvath and *J.P.K. Huber*	Late1960	High-Performance Liquid Chromatography (HPLC)
E. Klesper, A.H. Carwin and *D.A. Turner*	1962	Super Critical Fluid Chromatography (SFC)
J.C. Moore	1964	Gel-Permeation Chromatography
H.Small, T.S. Stevens and *W.C. Bauman*	1975	Ion-Chromatography
A. Zlatkis and *R.E. Kaiser*	1976	High-Performance Thin-Layer Chromatography
E. Tyihak, E. Mincsovics and *H. Kalasz*	1979	Over-Pressurized Layer Charomatography (OPLC)

Chromatography is a phenomenon in which two or more compounds in a mixture are physically separated by distributing between two phases: *(i) a stationary phase which can be a solid or a*

liquid supported on solid and (ii) a mobile phase (either a gas or a liquid) which flows continuously through the stationary phase. Differences in the affinity of individual components lead to their separation. Chromatography is a collective term, which is applicable to all methods that appear diverse in some regards but share certain common features. The basis of several related separating methods is the differential migration from a narrow initial zone of mixture with suitable combination of driving force and resistive action of which either one or both must be selective in order to achieve effective separations. The chromatrographic systems can be classified according to

(1) State of aggregation of the phases,

(2) Physical arrangement of the phases and

(3) Mechanism underlying the distribution equilibrium.

Chromatographic systems generating from solid, liquid and gaseous phases are (a) liquid-liquid (b) liquid-solid (c) gas-liquid and (d) gas-solid. If the mobile phase is a gas, the technique is known as "*Gas Chromatography*" and if it is a liquid then the technique is called "*Liquid Chromatography*". The stationary phase may be in the form of flat bed consisting of adsorbent spread uniformly on a sheet of glass or aluminium (thin-layer chromatography) or a sheet of cellulose (paper chromatography) or packed into a glass or metal column (column chromatography). According to the mode of separation of mechanism, chromatography can be adsorption, partition, ion- exchange, size exclusion, electrochromatography etc. A simple classification of chromatographic methods is summarized in **Table 1.2**.

Table 1.2: Classification of Chromatographic Methods

S.No.	Type of Chromatography	Examples
1.	Adsorption Chromatography	Column Chromatography, Thin-Layer Chromatography, Gas-Solid Chromatography
2.	Partition Chromatography	Paper Chromatography, Reversed-Phase Thin-Layer Chromatography, Classical Liquid-Liquid Chromatography
3.	Modified Partition (or Bonded Phase Chromatography)	High-Performance Liquid Chromatography (HPLC) and High-Performance (HP) TLC
4.	Ion- Exchange Chromatography	Cation and Anion Exchange Chromatography
5.	Exclusion Chromatography	Ion-Exclusion and Gel Permeation Chromatography, Molecule Sieve Chromatography
6.	Electrochromatography	Capillary and Zone Electrophoresis

The common liquid chromatography techniques are being described below:

Adsorption: In adsorption chromatography, the retention behavior of the solute is consequence of the interaction with the surface of solid adsorbent. The adsorbent surface has a rigid structure making this type of chromatography useful for separation and identification of geometrically and structurally similar compounds. For example, fatty acids have been easily separated by adsorption column chromatography.

Partition: Liquid-Liquid partition chromatography was first reported by *Martin* and *Synge* (14,15). The distribution of solutes takes place between two immiscible liquids. In normal-phase chromatography, more

polar liquid (e.g. water rich) is the stationary phase whereas reverse (i.e. less polar stationary phase and more polar mobile phase) is used in the case of reversed-phase partition chromatography. It is a powerful tool for the separation of members of a homologous series.

Bonded Phase: Applications of liquid chromatography are now made on chemically modified (bonded phase) silica layers or columns. The surface modification is done by chemical reaction between silanol groups or compounds having cyano, amino and other groups in the alkyl chain. The column or layer materials bearing bonded alkyl chains are used for reversed-phase chromatography to achieve more selective separation of substances of very close polarities.

Ion-Exchange: The stationary phase in-ion exchange chromatography is microporous polymer to which anionic and cationic exchange groups are attached. The retention or separation of solutes is based upon differences in the exchange potential between various ions of the ion-exchanger packed in the column.

Size Exclusion: In size exclusion chromatography the solid support is a porous polymer with a controlled pore size. The components are separated according to their size or molecular geometry in solution. The macro compounds are excluded by the stationary phase and emerge out first whereas the smaller constituents are retained in pores of the adsorbent. The size exclusion may be performed in aqueous systems (gel filtration), or in non-aqueous systems (gel permeation).

Electrochromatography: In this case, separation is based upon the difference in mobility of different ions under the application of an external DC potential. Ions with higher mobility (e.g. Na^{+}) move faster compared to those with low mobility under the influence of direct electric current facilitating good separations. Capillary zone electrophoresis allows separation of metals at nanogram concentration

levels. Most of the separation methods of this category can not be carried out using columns, plates or capillary tubes and hence these can be better classified as "Electrophoresis methods".

Since the work presented in this thesis is mainly based on the use of thin layer chromatography as an analytical tool, it is necessary to mention the salient features of this technique. The following paragraphs are devoted to cover all-important aspects of the development and current state-of-art procedure of thin-layer chromatography as used for the analysis of organic and inorganic substances.

1.4 THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography (TLC), a subdivision of liquid chromatography is carried out on a flat surface and hence it is sometimes referred to as *planar chromatographic separation technique*. In TLC, the mobile phase (a liquid) migrates through the stationary phase (thin layer of porous sorbent on a flat inert surface) by capillary action. This technique is simple, versatile and inexpensive means of separating and identifying the components of complex mixtures of inorganic, organic and biochemical substances.

The beginning of TLC can be ascribed to the report of Dutch biologist, *Beyerink* (16), who separated hydrochloric and sulfuric acids in the form of fine rings on thin layer of gelatin using a visualizing agent. Following the same method, *Wijsman* (17) identified the presence of two enzymes in malt *diastase* using a fluorescent method for detecting separated enzymes on thin layer. He used the bacteria obtained from sea water as fluorescent agent. However, the invention of TLC is usually credited to two Russian Scientists, *N.A. Izmailov* and *M.S. Schraiber*, who used binder free horizontal thin layers (2mm thick) of alumina spread on glass plate to the analysis of pharmaceutical preparations which led to the publication of their classical paper (18) on

"*A Spot Chromatographic Method of Analysis and its Application in Pharmacy*" in 1938. Since their method consists of depositing a drop of sample solution being investigated and the development by the application of several drops of solvent on flat surface of adsorbent before observing the separated zones, it was called "*Drop Chromatography or Spot Chromatography*". They also pointed out the usefulness of this method for preliminary testing of sorbent properties before their utilization in the form of column. Though *Izmailov* is best known for his fundamental work on TLC, his main field of interest was electrochemistry for which he received the *Mendeleiv* Prize of the Academy of Science of USSR in 1961.

In 1939, *Brown* developed a useful technique called "*Circular Paper Chromatorgraphy*" which involves the placing of filter paper between two glass plates and the application of sample and the developing solvent through a small hole of the upper plate. To obtain stronger adsorbent, he proposed the use of a thin layer of alumina between two sheets of paper. In 1940, *Lapp* and *Erali* used a loose layer of alumina spread on a glass slide that was supported on an inclined aluminium sheet. This sheet was cooled at its upper end and heated at the lower end. The sample was placed at the top of the adsorbent layer and gradually developed by solvent descending movement. The use of heat at the lower end of the layer increased the evaporation rate of the solvent so that increased development could take place (19). It is interesting that, in 1949, two American Chemists, *Meinhard* and *Hall* (20) gave the concept of "*Surface Chromatography*" and described their work on the use of microscope slides coated with a mixture of alumina (an adsorbent) and celite (a binder) to separate Fe^{2+} and Zn^{2+}. Their work was probably the first application of TLC for the separation of inorganic ions.

A great advancement in the development of TLC was made after the work of *Kirchner* and his associates (21-23) who introduced the use of larger plates. However, it was not until 1958, when *Stahl* of University of Mainz, Germany (24) described equipment and efficient sorbents for the preparation of thin-layer plates, that the practical effectiveness of the technique for separation was realized. The first book "*Thin Layer Chromatography*" on the subject by *Stahl* appeared in 1965 (25). Since those days, TLC has passed through several stages of development until at present, owing to the efforts of many researchers, it has become a powerful, sensitive and highly effective instrumental analytical method, which in many respects is not inferior to HPLC.

Some of the advantages of TLC over HPLC, worth mentioning here include less solvent consumption, low operational cost, easier sample preparation, more rapid throughput, greater detection possibilities and the use of disposable plates. TLC also permits the simultaneous analysis of many samples in the same time period required for one HPLC analysis. The samples and the standards in TLC are analyzed under identical experimental conditions rather than serially as in the case of HPLC. Due to advancements in instrumentation and practice in the late 1970s and early 1980s, high-performance (HP) TLC, overpressured (OP) TLC, centrifugal layer chromatography (CLC), and reversed-phase (RP) TLC were originated.

HPTLC emerged around mid 1970's, has expanded the horizon of TLC application to almost all the important fields of study. The commercially available HPTLC plates being made of more uniformly distributed finer particles of sorbent provide faster separations, reduced zone diffusion, lower detection limits, less solvent consumption and better separation efficiency. Typically, eighteen to thirty-six samples can be run on a single HPTLC plate with development time of 3-20 min. for a migration distance of 2-7cm. Further, the enhanced sensitivity and

separation efficiency may be achieved using HPTLC plates containing a concentration zone or those suitable for use in reverse-phase TLC. Some of the differences between TLC and HPTLC are compared in **Table 1.3**. The influence of environmental conditions on the reproducibility of R_F values has been the major problem of TLC.

The status of TLC amongst the other chromatographic techniques varies from country to country. In India, TLC in its classical variant is

Table 1.3: Comparison of TLC and HPTLC

Parameter	TLC	HPTLC
Plate size	20 x 20 cm	10 x 20 or 10 x 10 cm
Average particle size	20 μm	5 μm
Adsorbent layer thickness	100 – 250 μm	200 μm
Plate height	30 μm	12 μm
Sample volume	1 – 5 μL	0.1 – 0.2 μL
Solvent migration distance	10 – 15 cm	3 – 6 cm
Separation time	30 – 200 min	3 – 6 min
Samples per plate	10	18 or 36
Diameter of separated spots	6 – 15 mm	2 – 6 mm
Detection limits	–	–
(a) Absorption	1 – 5 ng	0.1 – 0.5 ng
(b) Fluorescence	0.05 – 0.1 ng	0.005 – 0.01 ng

HPTLC provides faster separation, reduced zone diffusion, better separation efficiency and higher sensitivity.

the most widely used chromatographic method because of the following reasons. (a) The availability of limited number of liquid chromatographs in research laboratories, (b) simplicity of the technique, (c) possibility of simultaneous analysis of a large number of samples, (d) low cost and (e) the ease of operation by a researcher with little experience. Numerous publications on TLC/HPTLC applications attest to the versatility and applicability of this technique in all branches of science. It has opened new fields of exploration and become an invaluable aid to separation scientists.

TLC can be used for (a) qualitative analysis (to identify the presence or absence of a particular substance in a mixture, (b) quantitative analysis (to determine precisely and accurately, the amount of a particular substance in a sample mixture) and (c) preparative analysis (to purify and isolate a particular substance for subsequent use). All three cases require the common procedures of sample application, chromatographic separation and sample component visualization. However, analytical TLC differs from preparative TLC as the sample solution/or amount is applied on thinner layers in the former case, whereas thicker TLC plates are used for preparative TLC.

1.5 TLC PROCEDURE

The TLC process is an off-line process in which all the procedural steps, depicted in **Figure 1.2** are carried out independently. The basic TLC procedure involves the spotting of sample mixture (5-10 μL for classical TLC and 1-2 μL for HPTLC) at about 2 cm above the lower edge of the TLC plate, drying the spot (usually at room temperature), development of plate with suitable mobile phase to a distance of 8-10 cm (classical TLC) and 2-6 cm (HPTLC) inside a cylindrical or rectangular closed chamber by ascending technique, withdrawing plate from the developing chamber, drying the layer at room temperature to

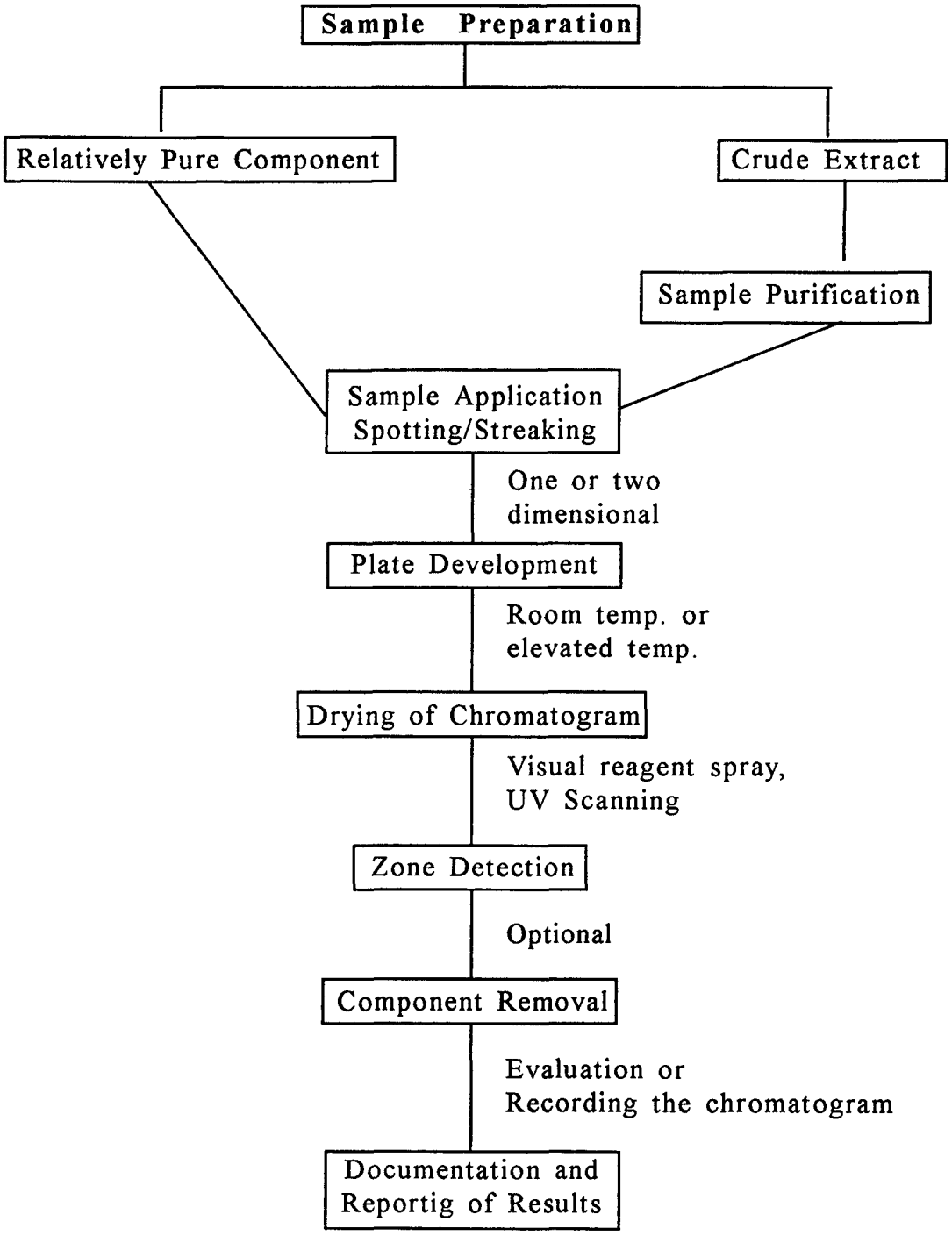

Figure1.2:Scheme of Typical Thin-layer Chromatographic Process

remove the mobile phase, detection of spots on TLC plate using suitable detection reagent, measurement of R_F values of the resolved spots and the quantitative estimation of the analyte after extraction from the layer with suitable extractant. The differential migration of components results due to varying degrees of affinity of the components in a mixture for stationary and mobile phases.

Sample Preparation: Standard methods for sample preparation, identification and separation of analyte present in a variety of samples such as plants, food, biological, geological and environmental samples have been reported. In general, metal cation solutions are prepared by dissolving their corresponding salts in 0.1 M HCl (or HNO_3) to a final metal concentration of 0.1-0.2 M. Anion solutions are prepared in distilled water, dilute acid or alkali solutions. Amines and phenols are used as freshly prepared solutions in ethanol or acetone.

TLC Plate Preparation or Coating Procedures: The contemporary trend is of using commercially available pre-coated plates. The manual preparation of layers involves the coating of slurry of the adsorbent (silica gel, alumina and soil) on glass, aluminium or plastic sheet (20×20 or 20×10 cm) with the help of TLC applicator. The thickness of dried layer for analytical purposes is kept to 0.2-0.3 mm. A binder (starch, gypsum, dextrin or polyvinyl alcohol) is usually added to the layer material to provide better adhesion, mechanical stability and durability.

1.6 SAMPLE APPLICATION

Sample application is one of the most important steps in the technology of TLC. Improperly applied samples result in poor chromatograms. Sample can be applied as spot or streak using micropipette, microsyringe, melting point capillaries etc. A number of automatic spotters of varying design are available for sample application. The nanoapplicator (Nanomat) is an example of micrometer

controlled syringes which has a dynamic volume range of 50-230 nL. Another applicator (Linomat) allows sample application in narrow bands. The application of sample as streak or band provides more efficient separations. The sample should be completely dried before placing the plate in the developing chamber. Dilute solutions can be applied to the layer either with sorbent drying between successive applications or after bringing the sample solution to proper concentration.

1.7 DEVELOPMENT MODES

The process of migration of mobile phase through the sorbent layer to effect separation of the sample substance is called development. Ascending development has been the most commonly used mode of development in TLC. Other development modes such as multiple, stepwise, circular two-dimensional and reversed-phase partition development have also been used to limited extent. The distance for the migration mobile phase has been kept to 10-12 cm for conventional TLC. While performing the development one should take care of the angle of the development and saturation of chamber apart from other factors.

1.8 CHROMATOGRAPHIC SYSTEMS

The stationary and mobile phases together comprise the chromatographic system. The proper selection of stationary and mobile phase conditions decides the degree to which effective separations of components in a mixture can be achieved.

Stationary Phase (Layer Sorbent): Silica gel, an amorphous and porous sorbent has been the most preferred layer material followed by alumina and cellulose. Thin layers of silica gel G (gypsum binder) and silica gel S (starch binder) with or without, "fluorescent indicator" have been used more frequently. Silica gel is slightly acidic in nature. At the

surface of silica gel the free valences of the oxygen are connected either with hydrogen (Si-OH, silanol groups) or with another silicon atom (Si-O-Si, siloxane groups) **(Figure 1.3)**. The silanol groups represent adsorption active surface centres that are able to interact with solute molecules. On the other hand, alumina (aluminium oxide) is basic and is more reactive than silica gel. Adsorption is the separation mechanism in both silica gel and alumina. Cellulose, an organic material is used as a sorbent in TLC when it is convenient to perform a given paper chromatographic separation by TLC with decreased development time and increase in the sensitivity of detection. The various types of layer materials used may be broadly classified as:

(a) ***Untreated Sorbents or Unmodified Layers*:** Silica gel (G, H or LS), alumina, cellulose, kieselguhr, polyamide, sephadex (a cross-linked polymeric dextran gels) and polyacrylonitrile etc.

(b) ***Impregnated Sorbents*:** In order to achieve improved selectivity the above mentioned and other available sorbents have been used as layer materials after impregnation with a variety of organic (high molecular weight amines, organophosphorous compounds, organic chelating agents etc.) and inorganic (aqueous salt solutions) impregnants.

(c) ***Bonded Sorbents*:** In recent years a trend of using both hydrophilic and hydrophobic modified sorbent phases is receiving wide acceptance. These phases are superior than those mentioned above under (a) and (b). The hydrophobic modified sorbents contain organo-functional groups like methyl (RP-2), octyl (RP-8), dodecyl (RP-12), octadecyl (RP-18) and phenyl residues. The hydrophilic modified sorbents possess amino-, cyno-, and diol residues as a functional group. The polar functional groups are bonded to the silica matrix via short-chain non-polar spacer.

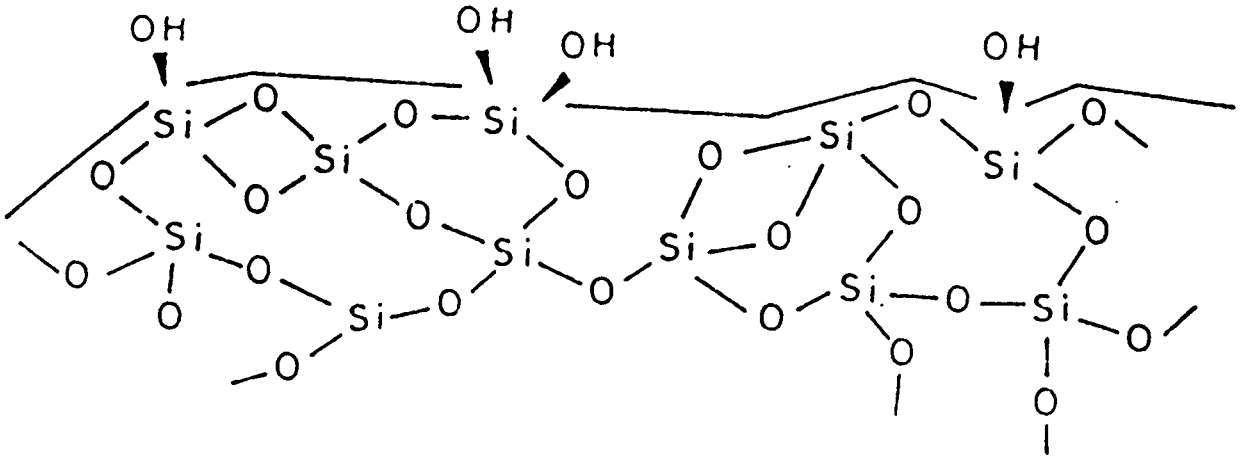

Figure 1.3: Structure of Silica Gel

(d) ***Mixed Sorbents***: Mixed layers (impregnated and non-impregnated) have been used by several workers for achieving enhanced resolution of components. Mixed layers are usually of medium activity as compared to the separated phases. The addition of kieselguhr in silica generally reduces the activity of silica, resulting in a new sorbent layer with altered activity that is capable of providing peculiar separations, not possible on separated phases. The mixed layers of upto three or four sorbents have been occasionally prepared and used for specific TLC applications. However, binary (or biphasic) sorbent layers have been more commonly used for routine analysis of organic as well as inorganic mixtures.

(e) ***Ion-Exchangers***: A variety of inorganic and organic ion-exchangers have been used in TLC. These include the use of stannic silicate, zirconiumpho-sphoantimonate, zincferrocyanide, stannic sulfosalicylate, zirconium (IV) antimonate, hydrous antimony (V) oxide and polyethyleneimine (PEI) and diethylamino ethyl (DEAE) celluloses.

(f) ***Miscellaneous Sorbents***: Certain layer materials, not covered above include silufol; silufol with a layer of silica gel; soil; soil and fly ash mixture; soil treated with neutral; alkaline and saline solutions; polychrom [porous copolymer of mixed 1,4- and 1,5-di(methacryloyl-oxymethyl) naphthalene and styrene]; silica gel H slurried in 4% ammonium nitrate solution containing 1% sodium carboxymethyl cellulose; chitin, diatomite and chicken egg powder.

Mobile Phase (Solvent System): In TLC the separation of ions is usually governed by the physical interactions of the adsorbent and the coordinative properties of the mobile phase. The mixture of organic solvents containing some aqueous acid, base or a buffer are, in general, well suited for the separation of ionic species whereas anhydrous organic solvents and water containing mobile phases have been found more useful for separating nonionic species. Mobile phase should be as

simple as possible and prepared from the purest grade of solvent. The use of mixtures composed of more than four components of mobile phase should be avoided because of problems associated with reproducible preparations. In contrast to mobile phases of higher volatility, which are capable to evaporate quickly from the sorbent layer, better reproducibility is achieved with mobile phases of lower volatility. The mobile phases used as developers in TLC may be categorized into following groups.

(a) ***Inorganic Solvents***: Solutions of mineral acids, bases, salts and mixture of acids, bases and /or their salts.

(b) ***Organic Solvents***: Acids, bases, hydrocarbons, alcohols, amines, ketones, aldehydes, organo phosphates and their mixture in different proportions.

(c) ***Mixed Solvents***: Above mentioned organic solvents mixed with water, mineral acids, inorganic bases or dimethyl sulphoxide and buffered salt solutions.

(d) Surfactant mediated - aqueous and hybrid solutions of cationic, anionic and non-ionic surfactants.

SURFACTANT – MEDIATED SYSTEMS

These systems contain surfactant as one of the components of the mobile phase. Surfactants in the aqueous mobile phase can be used in the following ways:

a) As monomer surfactants where the concentration of surfactant in aqueous mobile phase is restricted to well below the critical micelle concentration (CMC) of the surfactant. These mobile phases are most suited to separate ionic species by ion-pair chromatography (IPC). In this technique, a small concentration of ion-pairing reagent, which has an opposite charge to the ionic

solutes (i.e. cationic surfactant for anionic solutes and anionic surfactant for cationic solutes), is added to the aqueous mobile phase and its concentration is kept low to avoid the formation of micelles.

b) As surfactant micelles where the surfactant concentration is kept well above its CMC value. In such cases, the mobile phase is composed of surfactant molecules in the form of monomers and aggregates (or micelles). These mobile phases are very useful for simultaneous separation of ionic and non-ionic compounds by micellar liquid chromatography (MLC).

c) As microemulsion where surfactant in the presence of water, an oil (hydrocarbon) and co-surfactant (i.e. medium chain length amine or alcohol) is used as transparent solution.

Surfactants are long chain amphiphilic organic or organometallic molecules containing a highly polar (hydrophilic or lipophobic) or "ionic head group" attached to a non-polar (hydrophobic or lipophilic) hydrocarbon tail of varying chain length. The "head group" is either cationic (e.g. ammonium or pyridinium ion), anionic (e.g. hydroxy compounds) or zwitterionic (e.g. amine oxide, carboxylate or sulphonate betain) and the hydrocarbon tail which may contain at least 8 carbon atoms. Depending upon the nature of hydrophilic group, surfactant can be classified as anionic [$R\text{-}X^{-}M^{+}$]; cationic [$R\text{-}N^{+}(CH_3)_3X^{-}$]; zwitterionic [$R\text{-}(CH_3)_2\ N^{+}CH_2X^{-}$] and nonionic [$R(OCH_2\ CH_2)]_mOH$, where R is a long aliphatic hydrocarbon chain, M^{+} is a metal ion, X^{-} is a halogen, COO^{-} or SO_4^{2-} and m is an integer. A list of some common surfactants is provided in **Table 1.4**.

Table 1.4: Some Typical Surfactants, Formulae and their CMCs

Surfactant	Formulae	CMC (M)
Aqueous (normal)		
Anionic		
Sodium dodecyl sulfate (SDS)	$CH_3(CH_2)_{11}OSO_3^-Na^+$	8.1×10^{-3}
Potassium perfluoroheptanoate	$C_7F_{15}COO^-K^+$	3.0×10^{-2}
Sodium polyoxyethylene (12)-dodecyl ether	$CH_3(CH_2)_{11}(OCH_2CH_{12})_{12}\ OSO^-_3\ Na^+$ (SDS12EO)	2.0×10^{-4}
Cationic		
Cetylpyridinium chloride	$C_{16}H_{33}N^+C_5H_5Cl^-$	1.2×10^{-4}
Cetyltrimethyl ammonium-bromide (CTAB)	$CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$	9.0×10^{-4}
Nonionic		
Polyoxyethylene (6) dodecanol	$CH_3(CH_2)_{11}(OCH_2CH_2)_6OH$	9.0×10^{-5}
Polyoxyethylene (23)- dodecanol (Brij-35)	$CH_3(CH_2)_{11}(OCH_2CH_2)_{23}OH$	1.0×10^{-4}
Zwitterionic		
N-Dodecyl-N, N-dimethyla-mmonium-3-propane-l-sulfonic acid (SB-12)	$CH_3(CH_2)_{11}N^+(CH_3)_2(CH_2)_3SO_3^-$	3.0×10^{-3}
N,N-Dimethyl-N (carboxy-methyl) octylammonium salt,	$C_8H_{17}N^+(CH_3)_2CH_2COO^-$ (Octylbetaine)	25×10^{-2}
Nonaqueous (reversed)		
Bis(2-ethylhexyl) sodium sulfosuccinate (AOT)	$NaO_3SCH(CH_2COOC_8H_{17})COOC_8H_{17}$	6.0×10^{-4}

MICELLES

Surfactant (or amphiphilic) molecules comprising of hydrophobic and hydrophilic moieties tend to exhibit a considerable degree of self-organization when dissolved in aqueous solutions. Above a certain concentration level, termed as critical micelle concentration (CMC), the surfactant molecules in solutions (water or organic solvents) aggregate to form micelles. The process of micelle formation is called "micellization". Micelles do not exist at all concentrations and temperatures. There is a very small concentration range below which aggregation to micelles is absent and above which association leads to micelle formation. This narrow concentration range during which

micelle formation occurs is called the CMC. At low concentration i.e. below CMC and at temperature above the critical micelle concentration (e.g. Kraft temperature), the surfactant is dispersed in the aqueous media at the molecular level as a monomer. The average number of monomers per micelle is called the aggregation number (N). At 25° C and 1 atmospheric pressure, the CMC is typically less than 20 mM, with each micelle consisting of 40-140 monomers. A conventional model of micelles is that proposed by *Hartly* **(Figure 1.4)** which is very useful for visualization of a micelle. The various structures formed in aqueous solution on increasing the concentration of surfactant are illustrated in **Figure 1.5.**

There are mainly two types of micelles:

a) ***Normal Micelles***: The molecular organization of surfactant molecules in aqueous solutions results in the formation of normal micelles. Above CMC, the surfactant molecules are self aggregated in such a manner that the hydrophobic moieties (i.e. hydrocarbon tails) are oriented inward forming a non-polar core and hydrophilic (polar) head groups are outward keeping themselves in contact with the bulk aqueous phase. Normal aqueous micelles are generally formed from singly-chain surfactants and chain branching inhibits micellization.

Micelles are considered to be dynamic in nature, with continuous exchange of surfactant molecules, in and out of the aggregates occurring in the milliseconds to microsecond range. Thus, individual surfactant molecules (called monomers) are thought to be distributed throughout the aqueous phase surrounding the micelles.

b) ***Reverse Micelles***: In contrast to the normal micelles which are formed in polar (i.e. aqueous media) solvents, reverse micelles are formed in non-polar solvents like hexane or chloroform and a

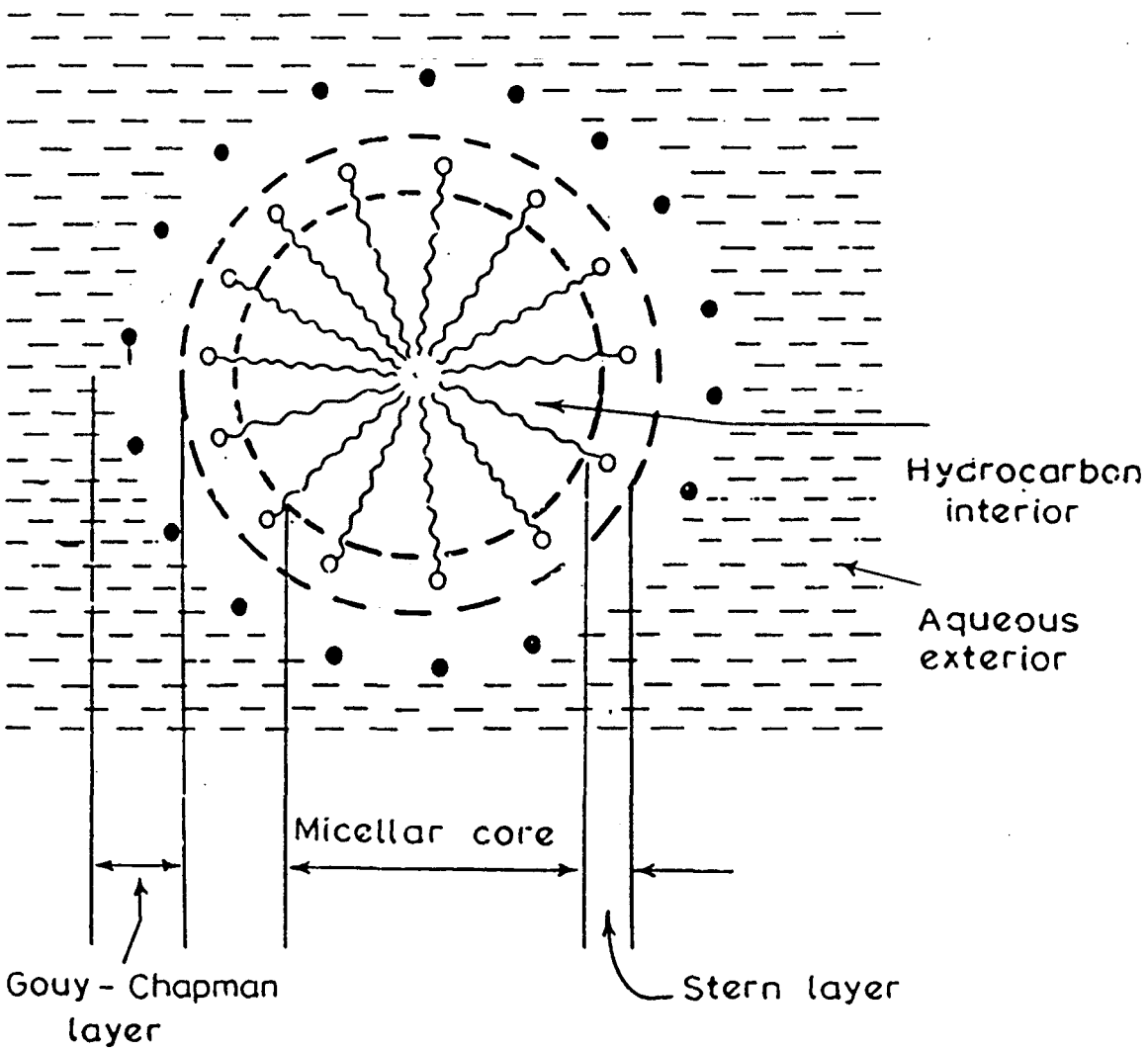

Figure 1.4: Hartley's Model of a Spherical Micelle

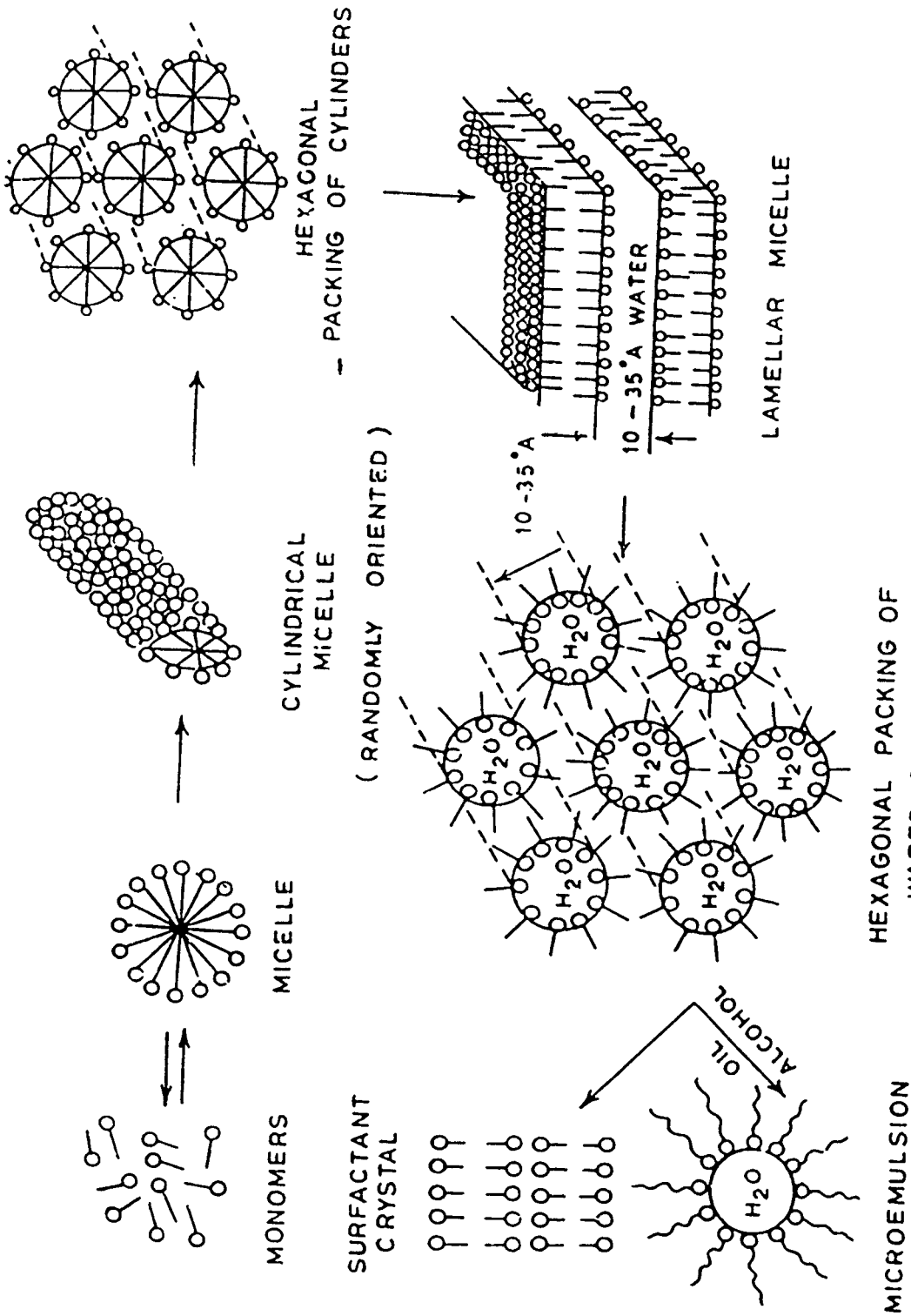

Figure 1.5: A Schematic Illustration for the Formation of Various Structures in Surfactant Solutions upon Increasing the Concentration of Surfactant

trace of water where the polar head groups of the surfactant are directed towards the interior of the aggregate and the hydrocarbon chains are in contact with the non-polar solvent. Compared to normal micelles, reverse micelles are more complex and less understood. Reverse micelles offer the same potential advantages for analysis as do normal micelles i.e. the ability to solubilize polar species that would be excluded from normal micelles. An interesting aspect of reverse micelles is their capability to solubilize water in the interior of micelle structure.

From macroscopic perspective, micellar solutions are homogeneous and cannot be filtered. However, the unique characteristics of micellar aggregates stem from their microscopically non-homogeneous nature i.e., they provide a microenvironment, which is distinctly different from the bulk solvent. The most important property of micelles is their ability to solubilize substances that are otherwise insoluble (or sparingly soluble) in water.

1.9 VISUALIZATION

Physical, chemical, enzymatic or biological detection methods are commonly used in TLC. A book by *Jork et al.* (26) is an excellent source of general information about physical and chemical methods of detection. Physical method of detection involves the use of spectroscopy or autoradiography, X-ray fluorescence micro analysis with a scanning collimated primary X-ray beam, UV radiation etc. Among the physical methods, visualization under UV-light is most common. The chemical detection methods involve the spraying of plates with a suitable reagent, which forms coloured compounds with the separated species. Alternatively, the reagent can also be taken in the mobile phase or in the adsorbent. In some cases, the detection is completed by inspecting the TLC plate after spraying with a suitable detection reagent under UV-

light. Both selective and non-selective reagents may be used depending upon the requirement. However, reagents giving sufficiently sensitive colour reactions with several species are generally preferred. The biological detection methods (bio-autography) are useful for specific detection of compounds with a certain physiological activity. An example is the detection of antibiotics on TLC plates using triphenyl-tetrazolium chloride and a microorganism that is sensitive to the antibiotic to be detected. Similarly, to detect antifungal compounds by TLC, inhibition of fungal growth was assessed by the detection of dehydrogenase activity with thiazolyl blue. In addition to these techniques, enzyme inhibition, immunostaining and flame ionization detection methods have also been used.

1.10 QUALITATIVE ANALYSIS

(a) ***Identification*****:** In TLC, the identification of separated compounds is primarily based on their mobility in a suitable solvent, which is described by the R_F value of each compound. Where

$$R_F = \frac{\text{Distance of solute motion from the origin}}{\text{Distance of solvent motion from the origin}}$$

The factors which influence the magnitude of R_F are nature of sorbent and mobile phases, layer thickness, activation temperature, sample volume, chamber saturation, relative humidity and mode of development technique. Another term R_M, which is the logarithmic function of the R_F value (i.e. $R_M = \log 1/R_F - 1$) is more useful as it bears a linear relationship to some TLC parameters or structural element of the analyte. However, in case of continuous and multiple development, where the solvent front is not measured, the term

R_X [R_X = $\frac{\text{Distance moved by solute}}{\text{Distance moved by standard}}$] is used.

R_F value ranges from 0.0 for a zone not leaving the point of application to 0.999 (≈1.0) for zone migration with solvent front. Unlike R_F, R_X value can be greater than 1.0.

(b) ***Separation*:** When two or more analytes have differential migration with the same chromatographic system, they are mixed thoroughly, the mixture is spotted on the TLC plate and chromatographed. The separated components of mixture are detected and their R_F values are recorded. Some of the basic requirements for a good separation are (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 complexation should occur between/among separable species and (d) chromatography of individuals and the mixture should be performed under identical experimental conditions.

1.11 QUANTITATIVE ANALYSIS

The three main approaches related to quantitation TLC include visual estimation and spot-size measurement, zone elution and *in-situ densitometry*.

a) ***Visual Estimation and Spot-Size Measurements*:** This is the simplest method of semiquantitative analysis. TLC plates with a definite sample aliquot along side standards containing known weights of analyte are simultaneously developed. After detection, the weight of analyte in the sample is estimated by visual comparison of the size and intensity of the standards and sample zones. The visual comparison works well if the applied amounts of sample are kept close to the detection limit and the sample is accurately bracketed with standards.

The accuracy and reproducibility of this method falls in the range of 10-30%.

To standardize the quantification methods in TLC, *Mohammad* and *Fatima* (27-28) *Mohammad* and *Tiwari* (29), *Nanda* and *Devi* (30) and *Mlodzikowski* (31) have established a linear relationship between the size of the spot and the amount of the analyte.

(b) ***Zone Elution*:** The zone elution method includes (a) drying the layer, (b) locating the separated analyte zones, (c) scraping off the portion of sorbent layer containing the analyte from the chromatogram and (d) measurement against standards by an independent microanalytical method such as spectrophotometry, gas chromatography, voltammetry or titrimetry. Scraping and elution processes are usually performed manually. Spectrophotometry has been the most widely used technique for quantification of eluted species.

(c) ***In-situ Densitometry*:** *In-situ densitometry*, a preferred technique for quantitative TLC involves the measurement of ultraviolet or visible absorbance, emitted fluorescence upon excitation with UV -light or fluorescence quenching directly on the layer. Absorption of UV-light is measured either on regular layers or on layers incorporated with phosphor. Video densitometers are now available for quantitative densitometric analysis. Modern optical densitometric scanners are linked to computer and are capable of automated peak location. A double beam densitometer equipped with a TLC scanner, an integrator and a microcomputer has been used for simultaneous determination of light rare earths in monazite sand and the CAMAG turner fluorometric scanner was used for the estimation of cadmium ion (32).

1.12 ADVANTAGES OF TLC

TLC is the most versatile and flexible chromatographic method. It is rapid because pre-coated layers are available for use as received,

without preparation. It has highest sample throughout, because up to 30 individual samples and standards can be applied to a single plate and separated at the same time. The automated sample applications and developers allow high accuracy and precision in quantification. There is a wide choice of layers, developers and detection methods. The wide choice of detection reagents leads to unsurpassed specificity. Less pure samples can be successfully analyzed, as the layers are normally not reused. Being an "off line" method, different steps of the procedure are carried out independently.

1.13 COMBINATION OF TLC WITH OTHER ANALYTICAL TECHNIQUES

The careful combination of TLC with other analytical techniques is more useful to collect information regarding the analysis of a complex sample. Spectrophotometry, high-performance liquid chromatography and gas chromatography, in conjugation with TLC are the three most widely used techniques. However, mass/GC, infrared and thermal analytical techniques in combination with TLC has also been used. One of the newest techniques used in combination with TLC is photoaccuoustic spectrometry, which is capable to locate compounds *in –situ* on the plate. *Issaq* and *Barr* (33) combined TLC with flameless atomic absorption spectrometry (FAAS) to identify an inorganic compound in an impure organometallic complex and to determine the recovery and purity of organometallic samples.

The examples cited above reveal, how the separation methods of TLC complement the analytical methods necessary for the absolute identification of a substance. TLC provides an excellent purification method for separating a substance of interest from other contaminants in the sample. Analytical techniques can then be applied to identify the separated substances.

1.14 LITERATURE

The research work performed on TLC analysis of organic and inorganic substances has been well documented in the form of several reviews, monographs, books and articles (34-44). *CRC Handbook of Chromatography series* started in 1972 under the joint editorship of *G. Zweig* and *J. Sherma* and continued since 1991 by the latter and the *Handbook of Thin Layer Chromatography* published in 1992 and 1996 under the editorship of *B. Fried* and *J. Sherma* have covered nicely the literature of TLC. Further, the latest work carried out on TLC is continuously being reviewed binnially in the Fundamental Reviews of Analytical Chemistry by *J. Sherma*. The last review of this series has appeared recently (45). The work published on TLC of metal ions, amines and phenols during the last decade has been presented briefly in **Tables 1.5** and **1.6**.

Table 1.5: Literature on TLC Studies of Nitrogen Containing Compounds Performed During 1991-2001

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Several amino acids	—	—	A new reagent, acetylacetone – formaldehyde is proposed for sensitive detection of separated amino acids on TLC plate under U.V. light.	46
Fifteen dansylated amino acids derivatives	Aqueous solutions of formic, acetic, propionic and perchloric acids	—	Salting-out and salting-in effects in the reversed-phase TLC of dansylated amino acid derivatives.	47
Phenyalanine, tryptophan and tyrosine	Sodium sulfate	Cellulose	Simulataneous determination of amino acids by densitometry.	48
Several amino acids	—	—	Use of D- camphor-10-sulphonic acid-ninhydrin as spray reagent for the identification of amino acids. Limit of detection of amino acids were 0.4-2 μg and 0.2-1 μg under cold and hot conditions respectively.	49
Amino acids	Eleven binary and ternary mobile phases	Polyamide PA-6 and polyamide P-6D	Separation of amino acids derivatized by 4-dimethylamino-L-nitrobenzene-4-sulfonyl chloride.	50
Fifteen amino acids	Eighteen mixed solvent systems	Silica gel	Thiohydration derivatives were resolved and visualized as orange spots on silica layers	51
Amino acids enantiomers	—	—	Combination of two- dimensional TLC and HPLC for enantiomeric analysis of amino acids of mammalian tissue.	52

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
L- and D- amino acids	Mixtures of MeCN – H_2O and MeOH or PrOH in different proportions	HPTLC precoated chiral plate with concentrating zone after impregnation with Cu salt and optically active amino acid	Determination of optical purity of L-amino acids by TLC. Amino acids with D-configuration have lower R_F compared to amino acids with L- configuration.	53
Tryptophan enantiomers	Mixed aqueous-organic solutions containing bovine serum albumin	Several type of reversed-phase layers	Reversed-phase TLC separation of enantiomers of tryptophans.	54
Enantiomers of tryptophans and phenyl tryptophans	Aqueous solvents	Microcrystalline and native cellulose	Separation of D- and L-tryptophans and D- and L-phenyltryptophans by adsorption chromatography	55
Several amino acids	—	—	A new spray reagent (3,5-dinitrobenzoylchloride) was used for sensitive detection of amino acids (sensitivity, 4-5μg). The detection was performed under U.V. light.	56
PTH-amino acids	—	Polyamide sheets	Two dimensional TLC, clear separation of all PTH amino acids in a relatively short time.	57
Several amino acids	Aqueous, non-aqueous and mixed solvent systems	Antimony (V) phosphate-silica gel 'G'	Quantitative separation of amino acids from two drugs (astymin- forte and santevini (plus).	58
L-lysine, L-homoserine and L-threonine	—	Sorbfil plates	Quantitative analysis of amino acids in biological fluids.	59
L- and D- isomers of amino acids	Binary and ternary mobile phases	Chitin and transition metals impregnated chitin.	Separation of optical isomers of amino acids.	60

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Twelve dansyl DL-amino acids	Aqueous organic solutions	$RP_{18}W/ UV_{254}$, RP_{18} W/F_{254} and sil $C\text{-}18_{50} UV_{254}$	Reversed-phase plannar chromatographic separation of dansyl DL-amino acids.	61
Substituted Tryptophan enantiomers	Mixed aqueous and organic solvents	Microcrystalline celulose	Adsorption chromatography	62
Hydroxamates of arginine, threonine and histidine	Pure and mixed organic solvents containing acetonitrile	—	Reversed-phase HPTLC separation	63
Forteen dansylated amino acids	Aqueous solution of Li, Na, K Rb and Cs chlorides	—	Reversed-Phase TLC and salting-out effect on the mobility of amino acids.	64
Racemic amino acids	n-Butanol-acetonitrile-water (6+2+3).Chloroform-methanol-propionic acid (15+6+4) and actonitrile–methanol-water (92+2+1)	Silica gel impregnated with a complex of copper and L-proline	Resolution of racemic amino acids	65
Eighteen PTH- amino acids	Pyridine – benzene (2.5+20), methanol – CCl_4 (1+20) and acetone – dichloromethane (0.3+8)	—	New mobile phase systems for TLC resolution.	66
Several amino acids	—	—	p-Dichlorodicyanobenzoquinone was proposed as a new chromogenic reagent for sensitive detection of amino acids (detection limit, 0.1–1μg) on TLC plates.	67
Tryptophan and its fluoro and methyl derivatives	Aqueous solution of α-cyclodextrin	Cellulose	Chiral separations	68
Several amino acids	—	Silica gel impregnated with transition metal ions and their anions	Improved resolution of amino acids was realized as a result of complexation between amino acids and transition metals.	69

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Tryptophan derivatives	Acetonitrile-MeOH-H_2O (4+1+1)	Chiral plates	Ascending one-dimensional TLC procedure for separation of 1,1-ethylidene-bis (L-tryptophan), L-tryptophan), L-tryptophan and D-tryptophan.	70
Several amino acids	—	Cellulose	Identification of free amino acids in extracts of medicinal plants.	71
Amino acids	MeOH - water (1+1, 1+3 or 1+5)	RP-18 Silica plates	Spectrophotometric determination of certain amino acids with preliminary TLC separation.	72
Amino acids	—	—	Kinetic fluorescence detection of glycine and glutamine after TLC separation.	73
Amino acids	—	Cellulose	Two-dimensional TLC for ascertaining the presence of free amino acids in extracts.	74
Thirty amino acids, twenty nucleotides and related compounds	—	—	Two-dimensional TLC for simultaneous separation.	75
Several amino acids	Acetate buffer (0.3 M, pH-6.0) – acetonitrile – n-butanol (12+5+10)	Silica gel impregnated with Cu ions	Utilization of effectiveness of Cu ions in TLC separation of amino acids and the comparison of TLC results with those obtained by RP-HPLC.	76
Racemic aromatic amino acids and aromatic amino alcohols	Highly concentrated solutions of α-or β-cyclodextrin	Cellulose	Qualitative analysis	77

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Amino acids	—	—	Quantitative TLC of industrial amino acids employing video densitometric analytical technique.	78
Amino acids derivatives	Different concentrations of ethyl acetate in heptane and chloroform	Silica gel	Separation of derivatives of amino acids on HPTLC silica layers following multiple gradient development technique.	79
Amino acids	n-Butanol – glacial acetic acid – water (3+1+1)	Silica gel 'G'	A rapid and reproducible TLC method for detection of amino acids (detection limit ≈ 0.02 μg).	80
Valine, leucine, butyrine and phenylalanine	Urea and dicarboxylic acid containing mobile phase	Silica modified with β-cyclodextrin	Separation of DL-enantiomers of amino acids.	81
Histidine, arginine, tryptophan and methionine	n-Butanol – acetic acid – water (4+1+1) and C_2H_2OH – water (70+3)	Silica gel	Application of TLC in combination of derivative spectroscopy for the determination of amino acids in baby foods.	82
Seven DL-mixtures of amino acids	Three-component mobile phases	Chitin, chitosan, Cu impregnated chitin	Qualitative analysis	83
Amino acid enantiomers	H_2O - THF- chloroaniline-MeOH (6+5.8+8.2+80)	Silica gel treated with L-arginine and copper acetate	Resolution of amino acid enantiomers by ligand exchange TLC.	84
Enantiomers and dansyl derivatives of amino acids	Mixtures of 0.5 M aqueous NaCl and MeCN	Silica gel impregnated with (1R, 3R, 5R – azobicyclo [3,3,0] octan-3-carboxylic acid	Resolution of enantiomers of amino acids and their dansyl derivatives.	85
Amino acid enantiomers	—	Silica gel treated with L-arginine and Cu acetate	Qualitative analysis	86

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Several amino acids	—	—	Separation, extraction and refinement of components of natural products using HPTLC method.	87
Amino acids	n-Butanol – MeOH – CH_3COOH (8+1+3) and n-butanol – CCl_4 –CH_3COOH (8+3+1)	Silica gel impregnated with various ammonium salts	Separation of amino acids via ion-pair formation of cationic amino acids with the anions of impregnant.	88
Aromatic amino acids	Acetonitrile – MeOH – H_2O (4+1+1 or 2)	Chiral plates	Qualitative analysis	89
Ten PTH-amino acids	Acetonitrile and acetate buffer of pH – 4.0 (for reversed-phase) and chloroform – acetonitrile and chloroform – THF (for normal-phase) TLC	Untreated silica gel and C_{18} RP silica plate	Separation by normal–phase and reversed phase TLC. Reversed-phase HPLC was also tried.	90
D- and L-isomers of amino acids	—	Chiral plates	TLC separation of amino acid enantiomers. Distinction between L- and D-isomers on the basis of proposed topological indexes.	91
Dansyl amino acid enantiomers	Acetonitrile- 1% triethyl ammonium acetate- acetic acid	β-cyclodextrins bonded stationary phase	Normal and reversed- phase TLC used for complete resolution of 8 pairs of dansyl amino acid enantiomers.	92
Methionine and selenomethionine	—	—	Qualitative analysis	93
15 α-Amino acids	—	Silica gel	Detection, separation and analysis of α-amino acids using 4-diethylamino-diazobenzene-4-isothioicyanate as complexing agent.	94

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
DL-Arginine, DL-histidine, DL-lysine, DL-valine and DL-leucine	Acetonitrile-MeOH-H_2O	Silica gel	Enantiomers resolution of basic DL-amino acids using a pharmaceutical industry waste as chiral impregnating reagent.	95
L-Tyrosine and L-dopa	—	—	TLC separation and quantitation of L-dopa and L-tyrosine in mixtures.	96
Dansyl amino acids	Acetonitrile- 0.5M NaCl 10+4 and 14+3 (v/v)	Silica gel impregnated with vancomycin (macryocyclic antibiotic)	Racemic resolution by normal-phase TLC.	97
L-Lycine, L-threonine, L-homoserine and cobalamines	Mixed-aqueous organic solvents contain NH_3	Sorbfil TLC plates	Quantitative analysis of amino acids	98
Amino acids	Water-in-oil microemulsion consisting of SDS, BuOH, n-hexane and water	Silica gel	TLC analysis of amino acids using microemulsion systems as mobile phase.	99
L-Tryptophan	Propan-2-ol — 25% aqueous HN_3	Sorbfil TLC plates	Quantitative analysis	100
Aliphatic and aromatic amino acids	1% CTAB solution prepared in water + butanol (95 + 5)	Plain alumina	Separation of L-proline from other aliphatic and aromatic amino acids by micellar thin-layer chromatography.	101
Amino acids	Oil-in-water microemulsion	Plain alumina and Li^+, Na^+, NH^{4+} impregnated alumina	Qualitative separation of aliphatic and aromatic amino acids.	102

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Several amines and their derivatives	Several aqueous and non-aqueous solvent systems	Silica gel, alumina, cellulose and copper sulphate impregnated silica gel	Indole has been successfully separated from carbazole and diphenylamine.	103
Alkyl and aryl amines	Methanol-water (1+1) with added salt and various buffers	—	Determination of lipophilicity of amines with reversed-phase TLC.	104
Several amines	Mixed organic solvents containing acetone	1% sodium carboxy-methyl cellulose plus silica gel 'G'	Separation ad determination of nature of color of spots, lowest detectable amount and R_F values of amines.	105
Ten aromatic and aliphatic amines	Seven aqueous systems	Polyacrylonitrile	TLC of amines shows that an increase in the hydrophobic part of amines results in their increased retention on polyacrylonitrile layer.	106
Twenty aromatic amines	Various organic solvents at different concentration levels	Silica gel impregnated with ammonium cerium (IV) nitrate	Effect of impregnant concentration, type of mobile phase and concentration of organic solvent in the mobile phase on the mobility of amines was investigated.	107
Several aromatic amines	Ethyl acetate, 1-hexane, ethyl alcohol and/or CCL_4	—	Use of 5-chloro-4, 6-dinitrobenzofurazon as a new reagent for highly sensitive detection of aromatic amines on TLC plates.	108
Several aromatic amines	—	Silica gel	New analytical technique (TLC-spectrophotometry under the influence of temperature gradient) for the simultaneous determination of aromatic amines.	109

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Fourteen amines	—	—	The comparative study of mapping of derivatised structurally diverse amines by two- dimensional TLC.	110
Fourteen chiral amino alcohols and amines	—	RP-18 WF_{254} HPTLC plates	Separation of chiral amino alcohols and amines after derivatization with Marfey's reagent.	111
Diphenylamine and its nitrate derivatives	—	—	Application of TLC for isolation and identification of diphenylamine and its nitrate derivatives from smokeless gunpowder samples.	112
Thirteen aromatic amines	Benzene – chloroform (4+6); 2% SDS; 3% Tween-20; 2% CTAB and chloroform – acetone – CCl_4 mixtures	Plain silica gel 'G' and silica gel impregnated with 1% CTAB	TLC of aromatic amines using surfactants in stationary as well as in mobile phase. Best separations were with SDS.	113
Twenty–seven aromatic amines	Cyclohexane – benzene (1+4)	Silica gel 'G', silica gel impregnated with aqueous solutions of various sodium salts	Qualitative analysis and examination of effects of the nature of substituent groups and the number of C-atoms in the molecule of amines on the retardation factor of various amines.	114
Nitrosoamines and amines	Cyclohexane – dioxane; heptane; EtOAc; iso-octane, cyclohexane, 2-propanol	Diol- and CN- bonded sorbent plates	Application of computer-aided optimization technique to sample clean up of nitrosoamines and amines by solid-phase extraction.	115
Eight biogenic amines	Benzene– triethylamine (5+1); benzene–triethylamine–acetone (10+2+1)	—	Determination and separation of biogenic amines in fish samples by two – dimensional TLC.	116

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Aniline derivatives	Various mobile phases	Silica gel	Prediction of chromatographic parameters for some anilines by molecular connectivity. Multivariable regression analysis of the connectivity functions gives a correct prediction of the experimental elution sequence of anilines.	117
Several aromatic amines	0.1% Aqueous copper acetate	Silica gel, alumina, cellulose, cellulose – silica gel (4+1)	Identification and separation of aromatic amines.	118
Twenty- six primary aromatic amines	Aqueous acidic and salt solutions	Iron (III) tungstophosphate	TLC of primary aromatic amines and their qualitative separations.	119
Several amines	—	—	Detection of trace quantities (≈ng) of amines on TLC plates with the use of enzymic reactions.	120
Isomers of nitroaniline	—	—	Determination of m-, p- and o-nitroanilines using spectrophotometric and thermal gradient chromatographic methods.	121
Sixteen primary aromatic amines	Sodium nitrate and hydrochloric acid solutions	Zirconium molybdophosphate mixed with silica gel 'G'	Qualitative and quantitative analysis of primary aromatic amines.	122
Several amines	Ethylacetate – methanol – 6% acetic acid (5+3+2)	Silica gel F_{254}	Determination of chlorphenamine meleate in Xiaojieling granules by scanning the spot at 264nm appeared on TLC plate. The recovery and RSD were 98.2% and 3.5% respectively.	123

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Biogenic amines	—	Silica gel	Simple, valid and rapid TLC method for separation and *in situ* densitometric determination as dansyl derivetives in food samples.	124
Isomers of aromatic amines	Water-in-oil microemulsion consisting of SDS or CTAB, water, heptane and 1-pentanol or butanol	Alumina, cellulose, silica gel 'G' and silica gel 'H'	Qualitative separation of m-isomers of aromatic amines from corresponding o- and p-isomers that moved faster giving higher R_F values.	125
Several aromatic amines	(A) Isopropanol – n-hexane and (B) Methanol – water	Silica gel, RP- 8 and RP- 18 silica	Adsorption (with eluent A) and partition (with eluent B) TLC separation of aromatic amines which are the reduction products in silica gel and reversed-phase layers.	126
Biogenic amines	Dichloromethane–trimethyl-amine (10+1)	Silica gel ^{60} F_{254}	Quantitative analysis of biogenic amines in fish meals.	127
Amine compounds	—	—	4-Chloro-5,7-dinitrobenzofurazan and 7-chloro-4,6-dinitrobenzofuraxan are reported as spray reagents for detection of amine compounds.	128
Primary and secondary aliphatic, fatty aromatic and heterocyclic amines	Isonitrosoacetyl acetone	—	A qualitative test for amines derived from acetone.	129
Biogenic amines, alkaloids and their derivatives	Binary mobile phases compose of acetate buffer and organic (MeOH, acetonitrile, THF) solvents	Silica gel, bonded amine, diol plates and RP-18	Adsorption and partition chromatography	130

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
p-Aminophenol	—	—	Determination of p-aminophenol in various medicinal forms by spectro-photometry and TLC.	131
Secondary and tertiary amines	1,4-dioxane + 25% aq. solution of ammonia and 1,4-dioxane + 25% ammonia aqueous solution	Silufol - 254 - UV	Identification of twenty amines. Dependence of R_F on [ammonia]/ [C_6H_6] ratio and on amine's polarity.	132
Biogenic amines	MeOH	—	Extraction and purification of histamine and tyramine from homogenized fish meat.	133
Aromatic amines	Silica gel 'G', alumina, kieselguhr and microcryst-alline cellulose	Carbon tetrachloride, cyclohexane and dieth-ylether	Separation of p-dimethylaminobenzal-dehyde from p-dimethylcinnamaldehyde has been achieved on silica layers after their derivetization with diphenylamine on TLC plate.	134
Aromatic amines	Aqueous solutions of TX-100 plus SDS	Silica gel 'G'	Examination of migration behavior of aromatic amines using non-ionic surfactant containing mobile phase systems.	135
Aromatic amines	CTAB- alcohol- water	Silicagel 'G'	Separation of indole from p-dimethyl-aminobenzaldehyde and diphenylamine.	136

Table 1.6: Literature on TLC Studies of Inorganic Ions and Metal Complexes Performed During 1991–2001

Analyte	Mobile Phase	Stationary Phase	Remarks/ Comments	Ref.
Tris (β-diketonato) complexes of Co^{3+}, Cr^{3+} and Ru^{3+}	Single and multi component organic and aqueous-organic solvent systems	Silica gel, Silica gel $^{60}F_{254}$ HPTLC plates	Qualitative analysis	137
Forty-nine inorganic ions	Aq. H_2SO_4 (0.01-1.0 M) and H_2SO_4+ ammonium sulfate systems	p-Aminobenzyl cellulose	Qualitative separation	138
Inorganic ions	Aq. HCl and HCl + ammonium chloride systems	Diethyl- (2-hydroxypropyl)-aminoethyl QE-cellulose	Selective separation of Re (VII) from many inorganic ions.	139
Zr and Hf	HNO_3+ HCl or H_2SO_4 containing different concentrations of hydrogen peroxide	Silica gel	Complete separation of Zr from mixtures containing Zr:Hf ratios ranging from 20:1 to 1:40.	140
Several metal ions	Eleven neutral and acidic solvent systems	Cellulose and synthesized carbamide formaldehyde polymer (aminoplast)	Separation of metal ions of different valency states.	141
Fe, Ni, Zn, Cu, Pb and Mn	Aq. solutions of sodium thioglycolate (0.01- 0.2 M)	Silica gel 'G'	Separated metal ions were determined by atomic absorption spectroscopy.	142
Forty-nine inorganic ions	Aq. HCl and HCl-ammonium chloride mixtures	p-Aminobenzyl cellulose	Qualitative TLC	143

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Ce^{2+}, Ce^{4+}, Nd, Eu, Gd, Tb, Yb, Y, Ti, V, Zr and Th	Citric acid (0.01-1.0 M)	Silica gel coated with different concentrations of primine JM-T	The R_F values of the lanthanide ions increase with increasing concentration of citric acid in the mobile phase.	144
Alkali metals	Aq. ammonium nitrate	Zinc ferrocyanide	Qualitative separations	145
Rare-earth elements	Solutions of acids, bases and salts	Diatomite	Qualitative separations	146
Cu, Co, Cd, Hg, Ni and Ag	1.0 M Inorganic salt solutions in aqueous methanol	Chitin/ chitosan	Qualitative separations and determination of chromatographic parameters as a function of the concentrations of MeOH, NH_3, and inorganic salts in the mobile phase.	147
Ni, Cu, Zn, Pd, Cd, Cr, Fe, Ru, Rh, La, Au, Tl, Zr, Pt, Nb, Ta, Mn, Ag, Hg, Co, Mo and W	Mixed aqueous-organic solvents containing DMSO	Silica gel 'G', silica gel impregnated with DMSO	A correlation between R_F values on impregnated layers developed with DMSO-THF (1+10) and the atomic numbers of the metal ions exists.	148
Transition metal ions	Several aq. mobile phases	Chitin/chitosan	Qualitative TLC	149
Twenty-one inorganic cations	Acetylacetone + acetone + conc. HCl (5+5+1)	Cellulose	Separation and identification of cations using six detection reagents.	150

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
IO^{3-}, IO^{4-}, BrO^{3-}, I^-, MoO_4^{2-} and $Fe(CN)_6^{4-}$	Distilled water	Silica gel	Effect of heavy metals on the chromatographic separation of periodate from other oxyanions and cyanoferrates.	151
Eighteen anions	Mixed acidic-organic solvents containing formic acid	Alumina, alumina plus silica gel	Investigations of the effect of transition metals on Cl^-, Br^-, I^- and NO^{2-}, NO^{3-} separations.	152
Seventeen anions	Aq. organic acids	Anhydrous antimony (V) oxide	Qualitative separations	153
Halides, oxyanions, hexacynoferrate, thiocyanate and phosphate	Acetone mixed with DMSO, formic or mineral acid	Silica gel impregnated with aq. salt solutions of Cu, Zn, Ni or Co	Microgram detection and separation of anions.	154
Fe, Cu and Mn	Ethanol + isobutanol + conc. HCl + water	Cellulose	Qualitative separations	155
Pb, Cd and Zn	—	Microcrystalline cellulose	Determination of heavy metals by TLC-square-wave anodic stripping voltammetry.	156
Mn, Co, Ni, Cu, Zn, Fe, Cr, Ti and V	Aq. succinic acid	Silica gel coated with high molecular weight amines	Reversed-phase TLC for qualitative identification of 3d metal ions.	157
Eleven metal ions	—	Silica gel impregnated with a mixture of alizarin red S and aliquat 336	Study of retention behavior of metal ions from aqueous solutions (pH 1-7) on impregnated silica layers.	158

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Metal ions	Sixteen different solvent systems	Mixtures of silica and inorganic ion-exchanger gels	Qualitative separations	159
Mg, Al, Ca, V, Cu, Zn, Ge, Y, Zr, Mo, Ag, Cd, In, La, Ce, Eu, Tb, Tl, Pb and Bi	n- Butanol + benzene + 1 M HNO_3 + 1 M HCl (75 + 69 + 4 + 2, v/v) or acetone + 3 M HCl (99 + 1, v/v)	Porous glass sheet	Detection limits and R_F values of fluorescent cations separated on porous glass sheet.	160
Forty-nine inorganic ions	Aq. sulfuric acid and sulfuric acid-ammonium sulphate	Diethyl-(2-hydroxypropyl) aminoethyl cellulose	Separation of Se(III), rare-earth (III), Y(III), Th(IV) and V(VI) from other ions.	161
Fe, Co, Zn, Cd, Cu and Ni	—	Silica gel modified with analog of dibenzo-18 - crown- 6	Application for the analysis of alloys and natural water samples.	162
Metal ions	Distilled water	Soil with different characteristics	Study of the influence of soil properties and constituents on the mobility of cadmium by soil TLC.	163
Rare- earth elements	0.1 M $H_2C_2O_4$, 2 M NH_4Cl, 5.0 M HCl, 0.5 M ammonium citrate	Fixion 50x 8	Preconcentration of rare-earths by circular TLC for subsequent ICP-AES determination in geological samples.	164
Toxic metals	DMSO-HNO_3 and DMSO - HCl systems	Plain and silica gel loaded with various concentrations of EDTA or TBP	Normal-phase, reversed-phase and chelation TLC of metal ions. Quantitative separation of Pb from synthetic alloys.	165

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Twenty-six transition and alkali metal ions	Four mobile phases	Cellulose	Qualitative separations	166
Heavy metals	—	Stannic sulfosalicylate	Quantitative separations of Fe (III), Cu (II) and Pb (II) from other metals.	167
Some anions	—	—	Use of acid phosphates for detection and determination of certain anions.	168
Some anions	—	Silica gel 'G', alumina, cellusose microcrystalline, alumina + silica gel 'G'	Separation of anions in the presence of hardness causing salts. Identification of NO_2^- in artificial sea water.	169
Cl^-, Br^-,I^-, ClO_3^-, ClO_4^-, $H_2PO_4^-$, NO_2^-, NO_3^-, SCN^- and SO_4^{2-}	Acetone + chloroform (3+1)	Trimethyl hydroxy-propylamine cellulose	Separation of inorganic anions as diantipyrilmethane using radial or ascending technique.	170
Forty-nine inorganic ions	Aq. sulfuric acid and sulfuric acid + ammonium sulphate media	Arsenosilicates of Sn (IV), Cr (III) and Sb (V)	R_F values increase with increasing acid or sulphate concentration in the mobile phase.	171
3d Series transition metal ions	Different concentrations of HNO3	Silufol	Examination of mobility pattern of metal ions as a function of concentration of HNO_3	172
Uranium	Mixtures of DMF and HNO_3 or HCl	Silica gel impregnated with high molecular weight amines	Selective separation of uranium from synthetic mixture of several metal ions.	173

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Cations with some anions	Aq. MeOH containing tributylphosphate and formic acid	Silica gel impregnated with mono-2-ethyl hexyl acid phosphate	Qualitative separation of cations and anions.	174
Inorganic ions	Sulfuric acid and organic solvents	Silica gel impregnated with mono-2-ethyl hexyl acid phosphate	Separation of polyvalent ions and tervalent rare-earths which tend to form anionic sulphate complexes.	175
Inorganic metal cations	Aqueous solutions of formic acid and sodium formate	Surface-modified silica layers	Study on migration behaviour of metal cations under the influence of pH of mobile phase and the concentration of impregnants.	176
Copper	MeOH + acetic acid	Silica gel 'G'	TLC of Cu after extraction from biological tissues by dry oxidation.	177
Hg, Cu and Cd	Benzene+acetone+DMF (5:4:1)	Silica gel 60	UV spectroscopic determination of metal ions after elution with H_2O.	178
Au, Ru, Rh, Pd, Dy and Pt	HCl + acetylacetone	Silica gel	Evaluation of resolution for several pairs of ions and the estimation of their detection limits.	179
Inorganic ions	Isobutyl methyl ketone (IBMK) + formic acid (FA)	Silica gel	Examination of relationship between concentration of IBMK/ FA and the R_F values of ions.	180

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Inorganic ions	HCOONa (1.0 M) + KI (1.0 M) in 1:9 ratio	Microcrystalline cellulose	Study of inorganic ions with organic reagent on the basis of thin layers.	181
Rare-earths	NH_4Cl solution	Silica gel pretreated with amines	R_F values of each metal decreased with increasing pKa value of amine used for pretreatment.	182
Actinides	—	Silica gel impregnated with polyethylene glycol	Separation on the basis of diff-erent sorption behaviour of acti-nides in their ter- and penta-valency states.	183
Heavy metals	HCOOH (1.0 M), HCOONa (1.0 M) and their mixtures	Silica gel and alumina impregnated with LiCl	Qualitative separation and detection (limit of detection, 0.22-3.4 μg).	184
Cd, Zn, Cu and Pb	HCOONa (1.0 M) + KI (1.0 M) in 1:9 ratio	Silica gel	Quantitative separation of Cu (II) by AAS after TLC separation from other metals.	185
Transition metals	Pyridine + benzene + acetic-acid + H_2O (6:5:8:4, 5:5:4:1) and BuOH + benzene + formic acid (5:10:9)	Silica gel impregnated with EDTA, dimethyl-glyoxime or 1, 10-phenan-throline	Separation of eight component mixtures and quantitative esti-mation by AAS.	186
I^-, IO_3^-, IO_4^-, Br^-, BrO_3^-, NO_2^-, SCN^-, CrO_4^{2-}, PO_4^{3-}, MnO_4^- and WO_4^{2-}	Distilled water, aq. HCOOH or HCOONa and acetone plus HCl	Silica gel impregnated with $CuSO_4$, alumina, cellulose containing alumina or kieselguhr	Ascending technique, semiquanti-tative determination of I^-, Br^-, and NO_2^- by spot-area measurement.	187

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
I^-, IO_3^-, IO_4^-, Br^-, BrO_3^-, NO_2^-, MnO_4^- and CrO_4^{2-}	Water-in-oil microemulsion	Silica gel, cellulose, alumina 'G', kieselguhr and their mixtures	Semiquantitative determination of IO_4^- by peak height measurement.	188
Thirty cations	Fifteen solvent systems including NH_4OH(0.5 M)	Ce (III) Silicate	Selective separation of Pt	189
Cd, Cu and Pb	—	—	Detection limits for Cd and Pb were 1 and 4 μg respectively.	190
Heavy metals	—	—	Identification of metals in human bones, placenta, milk and air by adsorption and IE-TLC.	191
Ni, Co and Cu	—	—	Determination of metals in rock samples by TLC/photodensitometry.	192
Co, Fe and Cu	Aqueous or organic solvents of different pH values	Silica gel impregnated with sodium salt of condroitin sulphate	TLC of metal ions, use of plasma polymerization technique for coating impregnant on the layer material and TLC of metal ions.	193
Al, Ca, Co, Cr, Cu, Fe Mg, Mn, Mo, Ni, Pb, Sn, Ta, Ti, V, Y and Zr	—	TBP coated polymeric supports	ICP-AES determination of Zr in Zr-U Alloys after separation by TLC.	194
Metal cations	—	—	Determination of Fe in process media by employing 8-hydroxy quinoline as complexing agent.	195

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Nine-anions and eleven cations	NH_4OH (1.0 M) + acetone (1:9, 3:7, 1:1, 7:3, 9:1)	Cellulose microcrystalline, kieselguhr and cellulose plus kieselguhr	TLC separation and colorimetric determination of SCN^- in water and wastewater.	196
Twenty-eight metal ions	Aqueous and mixed solvent systems	Lanthanum silicate ion-exchanger	Separation of several metal ions from their multicomponent mixtures.	197
Sixty-four ions	HNO_3 and $HNO_3 + H_2O_2$	Silica gel	Selective separation of Zr (IV), Hf (IV) and many other ions	198
Metal cations	—	—	Use of 8-hydroxy quinoline as complexing reagent and lumogallion as detector for Al.	199
Toxic metals	—	—	Qualitative and selective separation of toxic heavy metals.	200
Several bi-, tri-, tetra- and penta- valency ions	—	Microcrystalline cellulose and silica gel	Selective separation and identification of metals of different valency states.	201
Mo, V and W	—	Alumina	Selective separation of Mo from vanadium and tungsten.	202
Pb and Cd	—	—	Separation of lead and cadmium from humic acid.	203
Metal chlorosulphates	Acid containing mobile phases and aqueous ammonium sulphate solution (1.0 M)	Chicken egg shell powder and egg shell mixed with cellulose or silica gel 'H'	Examination of mobility and selective separation of metal chlorosulphates.	204

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Metal ions	*DMSO- HNO_3 systems*	*Stannic selenite silicate*	*Quantitative separation of Mo^{6+} after separation from Cr^{3+}, Cu^{2+}, Fe^{3+} and Co^{2+}.*	205
Thirty-three metal ions	Methylisobutyl ketone and formic acid	Silica gel	The study of retention sequence of metal ions on silica layers.	206
Metal complexes	—	—	The study and application of TLC of Co and Ni complexes was detected on thin layers.	207
Fe and Cr	—	Molecular sieves NaX	The separation of Fe^{3+} and Cr^{3+} as well as some anions of elements.	208
d-Block metals	Buffered EDTA solutions	Stannic phosphate silicate layers	Binary and ternary separations	209
Metal cations	—	Microcrystalline cellulose	Separation and identification of ten cations.	210
Cr and Ni	Acetone + hydrochloric acid + water (43 + 4 + 3, v/v)	Microcrystalline cellulose	TLC separation and determination of Cr and Ni in high steel.	211
Heavy metals	Tetra ammonium bromide (1%) plus phosphate buffer (pH 8.0, 2.0 M)	Silica gel impregnated with EDTA (2%)	Separation and quantification of individual metal from a five-component mixture of heavy metal ions.	212

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
U and Th	Isopropyl dithiophosphoric acid	—	Separation and determination of U and Th (conc. range 2.5 - 3.0 μg) in the presence of other metal ions.	213
Twenty inorganic cations	(a) 1-Butanol saturated with a 1:1 mixture of 3 M HNO_3 and 1 M HCl (b) MeOH + 36% HCl (10 + 3, v/v)	Microcrystalline cellulose	Detection of inorganic cations by two-dimensional TLC.	214
Metals and minerals	—	—	Detection of trace amounts of metals and minerals.	215
Heavy metal cations	Aqueous micellar solutions	Microcrystalline cellulose	Selective separation of heavy metal cations by micellar TLC.	216
Hg, UO_2^{2+}, Fe, Pb, Cd and Zn	Aq. formic acid-sodium chloride systems	Silica gel impregnated with KSCN	Identification of Hg^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+} in synthetic sludge by micellar TLC.	217
Co and Cr complexes	—	Polyacrylonitrile	Some qualitative separations were achieved.	218
Co and Ni	1.0 M Aqueous potassium thiocyanate	Mixed stannic arsenate gel and silica gel 'G' in (10:1, w/w) layer impregnated with 0.2 M tributyl-phosphate	Mutual separation of Co^{2+} and Ni^{2+}.	219
Thirteen metal ions	Methanol	Silica gel mixed with Sn(IV) arsenosilicate and impregnated with tributylamine	Detection and separation of heavy metals.	220

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Several metal ions including Ag	Ammonia, acetic acid and sodium or ammonium salt solutions	Microcrystalline cellulose, alumina 'G' and their binary mixtures	Selective separation of Ag^{+} from binary, ternary and quarternary mixtures of metal ions.	221
Toxic metals	Lower alcohols and benzene	Cellulose MN, silica gel modified with mercapto-propyl trimethoxysilane and silica gel 'R' impreg-nated with piperazine	Separation of Cd^{2+}, Pb^{2+}, Bi^{3+}, Hg^{2+}, Co^{2+} and Cu^{2+}.	222
1,3-Diketonates of heavy metals	Micellar mobile phases containing sodium dodecyl sulfate	Silufol and plasma-chrom plate	The study of 1-3 diketonates and other metal ions.	223
Co^{3+} diamine complexes	Aqueous solutions of ammonium sulfate at various concentration levels	Silica gel, cellulose and polyacrylonitrile	Investigation of effect of chelate complexes on their mobility by salting- out TLC.	224
Cu^{+}, Cu^{2+} and Co	—	Silica gel impregnated with metal salts	The study of colorful salts. Cu (I), Cu (II), Co (III) were mainly chosen.	225
Forty-three inorganic ions	—	Layered double hydroxides	Qualitative TLC ; a new parameter SR_F was introduced to quantify the separating power of the sorbent.	226

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Several metal ions	—	Cellulose 'R'	Qualitative separation of a mixture of cations and their identification with 0.01% 9-formylacridine solution in dichloromethane.	227
Metal ions	—	—	Separation of metal ions from their binary mixtures using TLC plates impregnated by mixed oxides.	228
UO_2^{2+}, Cu, Ni and Cd	Mixtures of acetone, water and acetic acid or HCl.	Silica gel 'G'	Separation of Cd^{2+} and UO_2^{2+} from Cu^{2+}, Co^{2+} and Ni^{2+} from their binary, ternary and quarternary mixtures. Radiofrequency values for different cations were also calculated.	229
d- and f-Block metal ions	BuOH – 8 M HNO_3 and aq. HNO_3	Plain as well as TBA impregnated silica gel 'G'	Quantitative separation of Zr^{4+} and W^{6+} from binary mixtures and from synthetic alloy component systems.	230
Cu, Ni, Co^{2+}, Co^{3+}, Fe ions and their 1,3- diketonates	2.5 x10^{-2} M aq. SDS	Silufol and RP-Plazmachrom	TLC separation of metal ions and their 1, 3- diketonates.	231
Toxic metals	—	Mixed bed of titania and silica.	Analytically important separations of toxic metals on mixed bed of oxides of Ti and Si.	232
Metal-peptidoglycan monomer complexes	—	Cellulose	Quantitative TLC, identification and separation of some metals and their peptidoglycan monomer complexes.	233

Analyte	Mobile Phase	Stationary Phase	Remarks/Comments	Ref.
Mg and Al	0.5 - 2.0 M HCl and HNO_3	Amberlite IRP-69 and microcrystalline cellulose mixed in different ratio	Ion-exchange TLC separation of Mg and Al. Quantitative estimation of Mg in Al-alloys.	234
Thirty metal ions	Aqueous and mixed mobile phases	Titanium (IV) silicate ion-exchanger	Rapid separation of Al^{3+}, V^{5+}, Hg^{2+} Cd^{2+} and other ions from numerous metal ions.	235
Co, Ni and Cu	0.1 M KSCN	Stannic arsenate ion-exchanger plus silica gel (10:1, w/w) impregnated with 0.2 M TBP	Reversed-phase TLC for separation and identification of co-existing Co^{2+}, Ni^{2+} and Cu^{2+}	236
Metal ions	CTAB - ethanol (1:2, w/v) with added water in 1: 99 ratio	Silica gel 'G'	Identification and separation of Zn^{2+}, Cd^{2+} and Hg^{2+} using surfactant-containing mobile phase systems.	237
I^-, IO_3^- and IO_4^-	Mixtures of NH_4OH and CH_3COCH_3 in 1:9, 3:7, 1:1, 7:3 and 9:1 ratios	Aluminium oxide 'G'	Separation and determination of iodide and its oxyanions	238
Fourteen heavy metal ions	Various aqueous and alcoholic organic solvents	Plain, mixed and TBA impregnated silica gel 'G' layers	Sorption behavior of metal ions in normal-phase and reversed-phase TLC using layers prepared from silica-zirconium tungstophosphate gels	239
Inorganic ions	Tri-n-butylphosphate-water-formic acid system	Stannic arsenate or tin (IV) molybdosilicate plus silica gel, alumina or cellulose (1:9)	Qualitative separation of IO_3^- from NO_2^- and BrO_3^-; quantitative estimation of IO_3^- on mixed stannic arsenate-alumina layer (1:9).	240

Chlorosulfates of Mn, Fe, Co, Ni, Cu and Zn	Doubled-distilled water	Silica gel-cellulose (2:1, w/w)	Separation of nickel chlorosulfate from manganese, iron, copper or zinc chlorosulfate.	241
Au, Ag, Cu, Ni, Cd, Cr, Hg and Zn	Aq. CTAB (1.2 mM) and aq. $(NH_4)_2 SO_4$ (2.5 M)	Silica gel 'G' and alumina 'G'	Thin-layer chromatography coupled with spectrophotometry and titrimetry for quantitative separation of Au^{3+} and Ag^{+} from accompanying metal ions.	242

The metal ions were in their usual valency states.

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Chapter-2

A NEW ANIONIC MICELLAR MOBILE PHASE SYSTEM FOR NORMAL-PHASE THIN-LAYER CHROMATOGRAPHY OF AMINO COMPOUNDS: SIMULTANEOUS SEPARATION OF AMINOPHENOL ISOMERS

2.1 INTRODUCTION

Since the first report (1,2) by *Armstrong* and co-workers about the use of aqueous micellar solutions as mobile phase in thin-layer chromatography (TLC), micellar mobile phases (MMP) have been the focus of numerous separation studies (3-5) because of their distinct advantages such as unusual selectivity, sensitivity, cost effectiveness, low toxicity and safe handling. MMP have been shown to increase fluorescent quantum efficiencies, thereby lowering limit of detection (6).

The MMP consisting of surfactant molecules in the form of monomers and aggregates (or micelles) are capable to provide a complex combination of hydrophobic, electrostatic, hydrogen bonding, and steric interactions to the solute, which results in, enhanced separation selectivities of structurally similar substances. The unusual separation possibilities in micellar liquid chromatography are arised as a result of combined effects of relative partitioning of solute between (a) micelles and water (b) stationary phase and water and (c) stationary phase and micelles, which is distinctly different from conventional liquid chromatography where relative distribution of solute between a stationary phase and a liquid mobile phase is responsible for the separation. The chromatographic performance of MMP can be further improved by addition of appropriate additives (7-10) such as organic solvents, inorganic metallic salts, cyclodextrins and ion-pairing agents.

The separation and identification of amino compounds is important because of their increasing industrial, pharmaceutical, toxicological and pesticidal applications. As they are often used in the form of raw or intermediate materials in chemical synthesis, amino compounds are easily transported into the environment via wastewater. On the other hand, aminophenols are used as antioxidants in organic polymers. It is, therefore, not surprising that several chromatographic procedures (11-18) have been developed for the analysis of nitrogen-

containing compounds. The electron donor properties of aromatic amines have been cleverly utilized in thin-layer chromatographic separation (TLC) of amines in the form of charge-transfer complexes with poly-nitro compounds (19,20) using less polar organic solvent systems as mobile phases. The weak physical bonding between amines (n- and π-electron donor) and aromatic nitro compounds (π-electron acceptor) is susceptible to rupture by polar solvents and adsorptive forces, which is one of the probable reasons of limited studies on TLC separation of amines via charge-transfer complexation with aromatic nitro compounds. Alternative attempts to separate amines on silica gel layers impregnated with metallic salts did not produce satisfactory results because of formation of occasional tailed spots, restricted choice of mobile phase systems because of co-migration of impregnated salt during development of TLC plates and the tendency to absorb moisture by adsorbent layer. On the other hand, TLC methods involving the use of mixed organic mobile phase systems containing hexane, benzene, dioxane etc. are not useful for routine analysis because of toxic effects of solvents (13, 21, 22).

The close inspection of literature available on TLC indicates that good separations of isomers of toluidine, anisidine, chloroaniline, bromoaniline and xylidine on silica layer impregnated with cadmium sulphate and/or acetate (23,24), o- and p-isomers of nitroaniline on silica – cellulose (1:1) mixed layer (13), and closely related aromatic amines such as carbazole, indole, diphenylamine, p-dimethylamino-benzaldehyde and p-dimethylaminocinnamaldehyde (25,27) have been achieved. To the best of our knowledge, not a single paper is reported documenting well-resolved spots of all three isomers of aminophenols.

Mitchell and *Waring* (28) have reported a modified chromogenic reagent obtained by substitution of $ZnCl_2$ for $FeCl_3$ in ferric ferricyanide spray reagent for the detection of aminophenols and related compounds

(detection limit 2-5 μg) on TLC plates coated with silica gel. This method is useful for preliminary visual identification of isomeric compounds, which are difficult to resolve. The distinguished colored spots revealed by o-, m-, and p-aminophenols with modified reagent were dark brown, mauve and blue respectively. *Dhillon* and co-workers (29) have also detected aminophenols with poor resolution as brown spots on silica TLC plates containing nitrite.

The present communication reports a new mobile phase that is composed of aqueous solutions of an anionic surfactant, sodium dodecyl sulfate (SDS) and calcium chloride (0.1 M). It is capable to promote excellent resolution of aminophenol isomers on silica HPTLC plates and has superiority over the reported TLC methods, as the solvent system used is odorless, non-volatile, inexpensive and non-toxic. The proposed method is rapid, reproducible and sensitive.

2.2 EXPERIMENTAL

Chemicals and Reagents: Silica gel 60 F_{254} 'HPTLC' plates (Merck, Darmstadt, Germany); sodium dodecyl sulfate (SDS) (BDH, India); N-cetyl-N,N,N-trimethyl ammonium bromide (CTAB), Triton X-100, and aminophenols (Loba Chemie, India); methanol, propanol and butanol (Qualigens, India) and acetonitrile, calcium chloride, cobalt chloride and amines (CDH, India) were used. All other reagents were Analytical Reagent grade.

Amines Studied: Diphenylamine (DPA), o-chloroaniline (o-CAL), m-chloroaniline (m-CAL), p-chloroaniline (p-CAL),o-nitroaniline (o-NAL), m-nitroaniline (m-NAL), p-nitroaniline (p-NAL), aniline (AL), indole (ID), p-dimethylaminobenezaldehyde (p-DAB), and DL-tryptophan (DL-TTP), o-toluidine, (o-TDL) and m-toluidine (m- TLD).

Aminophenols Studied: o-aminophenol (o-APH), m-aminophenol (m-APH) and p-aminophenol (p-APH).

Test Solutions: The test solutions (1% w/v) of all amines and aminophenols were prepared in methanol. The aqueous solutions (1% w/v) of metal ions (Fe^{3+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Be^{3+} and Hg^{2+}) were prepared from their chlorides, nitrates or sulfate salts and phenols (1% w/v) were used for interference studies.

Detection Reagents: All amines were detected by exposing the HPTLC plates to iodine vapors. DL-tryptophan was detected with 0.5% (w/v) ninhydrin solution prepared in acetone.

Stationary Phase: Silica gel 'HPTLC' plates

Mobile Phases: The following solvent systems were used as mobile phase

Symbol	Composition
M_1	H_2O
M_2	0.01 and 0.001M aqueous SDS
M_3	0.01 and 0.001M methanolic SDS
M_4	0.01 and 0.001M methanolic CTAB
M_5	0.01 and 0.001M methanolic Triton X-100
M_6	0.01 M SDS in MeOH + H_2O (9:1, 1:9, 7:3, 3:7 and 5:5)
M_7	0.01 M CTAB in MeOH + H_20 (9:1, 1:9, 7:3, 3:7 and 5:5)
M_8	0.01 M Triton X-100 in MeOH+ H_20 (9:1, 1:9, 7:3, 3:7 and 5:5)
M_9	0.01 M SDS in propanol + H_2O (7:3 and 3:7)
M_{10}	0.01 M SDS in butanol + H_20 (7:3)
M_{11}	0.01 M SDS in acetonitrile + H_20 (7:3 and 3:7)
M_{12}	Methanol + H_2O (7:3 and 3:7)
M_{13}	0.01 M SDS in MeOH + 0.1 M aqueous $CoCl_2$ (7:3 and 3:7)
M_{14}	0.01 M SDS in MeOH + 0.1 M aqueous $CaCl_2$ (7:3 and 3:7)
M_{15}	0.01 M SDS in MeOH + 0.1 M aqueous NaCl (7:3 and 3:7)
M_{16}	0.01 M SDS in MeOH + 0.1 M aqueous LiCl (7:3 and 3:7)

Procedure: Test solutions (approx. 5µl) were applied on high-performance thin-layer plates with the help of micropipets. The plates

were developed in the chosen solvent system by the ascending technique. The solvent ascent was fixed to 5 cm in all cases for the determination of R_F value of individual amino compound. After development, the plates were withdrawn from glass jars and dried at room temperature. HPTLC plates were then exposed to iodine vapors for about 10 min. The amines and aminophenols were detected as yellowish brown spots. The R_L (R_F of leading front) and R_T (R_F of trailing front) values for each spot were determined and the R_F value was calculated

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

Separation: For the separation, equal amounts of aminophenols to be separated were mixed and 5μL of the resultant mixture was loaded on the HPTLC plates. The plates were developed to 5cm height, the spots were detected and the R_F values of the separated amines and aminophenols were determined.

Limit of Detection of Aminophenols: The limit of detection of aminophenols was determined by spotting different amounts of aminophenols on the HPTLC plates, developing the plates and detecting the spots using iodine and zinc ferrocyanide separately as detection reagents. Zinc ferrocyanide was prepared following the method reported by *H.B. Weiser et al.* (30). This reagent was also used to determine the limit of detection of aminophenols on laboratory prepared TLC plates coated with silica gel 'G' (E. Merck India). The method was repeated with successive lowering the amount of aminophenols until no spots were detected. The minimum amount of aminophenol detectable on the HPTLC plates was taken as the limit of detection.

Interference: For examining the effect of impurities such as heavy metal cations, phenols and amines, one drop (5μl) of each o-, m-, p-aminophenols and impurity solution was spotted successively at the same spot on the line of application of HPTLC plates. The spot was completely dried after each spotting. After drying, TLC plate was

developed with M_{14} and the R_F values of the resolved spots were determined.

2.3 RESULTS AND DISCUSSION

Results of the present study have been summarized in **Tables 2.1 – 2.5** and **Figures 2.1 – 2.4**.

The mobility of amino compounds obtained with water (zero surfactant) and in anionic, cationic and non-anionic surfactants mediated mobile phases (M-M_5) has been shown in **Table 2.1**. In water, most of the amines show little mobility whereas in 0.01 M aqueous SDS, amines such as o- or m-APH, o-TLD, DL-TTP, m-NAL and o-NAL move faster giving R_F values in the range 0.40 - 0.77. All other amines remained at the point of application (i.e. R_F = 0.0 - 0.08). Thus, 0.01 M aqueous SDS can discriminate only between two amines under the given set of experimental conditions to facilitate binary separations. This separation potentiality of SDS is hampered when methanol is used as solvent instead of water. With 0.01M methanolic SDS, all amines are solubilized and migrate to the solvent front giving high mobility (R_F > 0.80). DL-TTP is the exception, which produces a badly tailed spot. Results with 0.01 M methanolic CTAB, a cationic surfactant was almost similar to those obtained with 0.01M methanolic SDS. All amines, except p-NAL, DL-TTP and p-APH show high mobility. p-NAL and DL-TTP form diffused spots whereas p-APH gives mid R_F (R_F = 0.56) and hence it can be separated from other amines. The mobility pattern of amines does not differ much on using a non-ionic surfactant (0.01M Triton X-100) instead of 0.01 M SDS or CTAB.

These observations indicate that the mobility of certain amines **(Table 2.1)** alters marginally with the change in head group charge of surfactant in methanolic solution. For example, R_F values of p-CAL, o-TLD and m-TLD are decreased from 0.95, 0.96 and 0.95 to 0.86, 0.82 and 0.84 respectively on substitution of 0.01 M alcoholic CTAB

Table 2.1: R_F Values of Amino Compounds Obtained on Silica HPTLC Plates Developed with Water and Surfactant Containing Mobile Phases

Amino Compound	Water	0.01M Aqueous SDS	0.01M SDS in MeOH	0.01M CTAB in MeOH	0.01M Triton X-100 in MeOH
DPA	0.10	0.07	0.92	0.95	0.75
o-CAL	N.D	N.D	N.D	N.D	N.D
m-CAL	0.10	0.00	0.90	0.90	0.90
p-CAL	0.08	0.08	0.86	0.95	0.92
o-NAL	0.47	0.52	0.93	0.95	0.81
m-NAL	0.29	0.41	0.95	0.72	0.95
p-NAL	0.29T	0.25	0.95	0.41T	0.90
AL	0.00	0.00	0.90	0.91	0.81
o-TLD	0.00	0.59	0.82	0.96	0.77
m-TLD	0.00	0.00	0.84	0.95	0.92
ID	0.28T	0.50	0.69	0.93	0.77
p-DAB	0.18T	0.15	0.95	0.95	0.77
DL-TTP	0.78	0.77	0.35T	0.25T	0.4T
o-APH	0.15	0.67	0.95	0.95	0.90
m-APH	0.55T	0.74	0.95	0.95	0.90
p-APH	0.00	0.00	0.95	0.94	0.90

T = Tailed spot ($R_L - R_T > 0.3$)

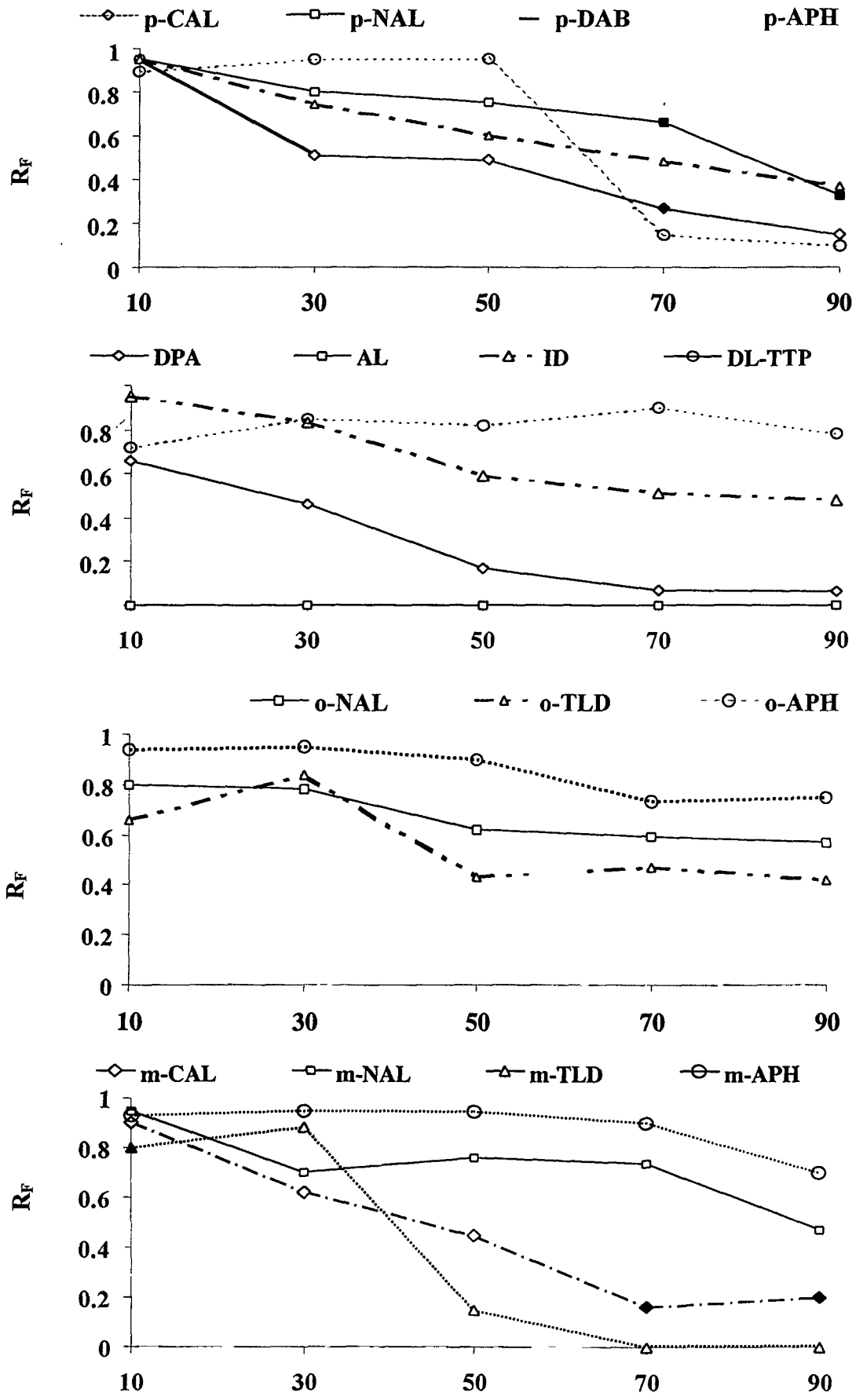
(a cationic surfactant with positive head group charge) by 0.01 M alcoholic SDS (an anionic surfactant with negative head group charge). However, a reversed trend i.e. increase in R_F value from 0.72 (in methanolic CTAB) to 0.95 (in methanolic SDS) was observed in the case of m-NAL. DPA, o-NAL, AL, o-TLD and p-DAB have the lower R_F values (R_F values fall in the range 0.77 to 0.81) in 0.01 M alcoholic Triton X-100 (a non-ionic surfactant) compared to 0.01 M alcoholic SDS or CTAB. Furthermore, when the concentration of SDS, CTAB or Triton X-100 is decreased from 0.01 M (which is above their CMC values) to 0.001 M (below their CMC values) in the mobile phase, no significant change in R_F value (or migration) of amines was noticed. The results (not shown in **Table 2.1**) obtained with 0.001 M methanolic surfactants (SDS, CTAB or Triton X-100) showed a slight decrease in R_F value for IND, p-DAB and DL-TTP and slight increase in R_F value for m-NAL, IND and p-DAB compared to their values in 0.01M methanolic SDS and Triton X-100 respectively. In the case of methanolic CTAB and aqueous SDS, the mobility of amines was found the same regardless the concentration of surfactant (0.01 or 0.001 M). The development time for 10cm ascent was in the order:

0.01 M aqueous SDS > 0.01 M methanolic Triton X-100 >

0.01 M methanolic SDS or CTAB.

It is evident from **Table 2.1** that alcoholic surfactants are non-effective to provide desired separations of amines. Therefore, various solvent systems (M_6-M_7) were formulated by adding different volumes of water into 0.01 M methanolic SDS, CTAB or Triton X-100 in order to achieve differential migration (i.e. different R_F values) for the separation of amines. The results obtained with these mobile phases are listed in **Figures 2.1 – 2.3**.

From the results presented in **Figures 2.1 – 2.3**, the following conclusions may be drawn:



Volume of Added H_2O (%) in M_6 (0.01 M SDS in Methanol)

Figure 2.1: Plot of R_F Vs Volume of Added H_2O in M_5 (0.01M SDS in Methanol)

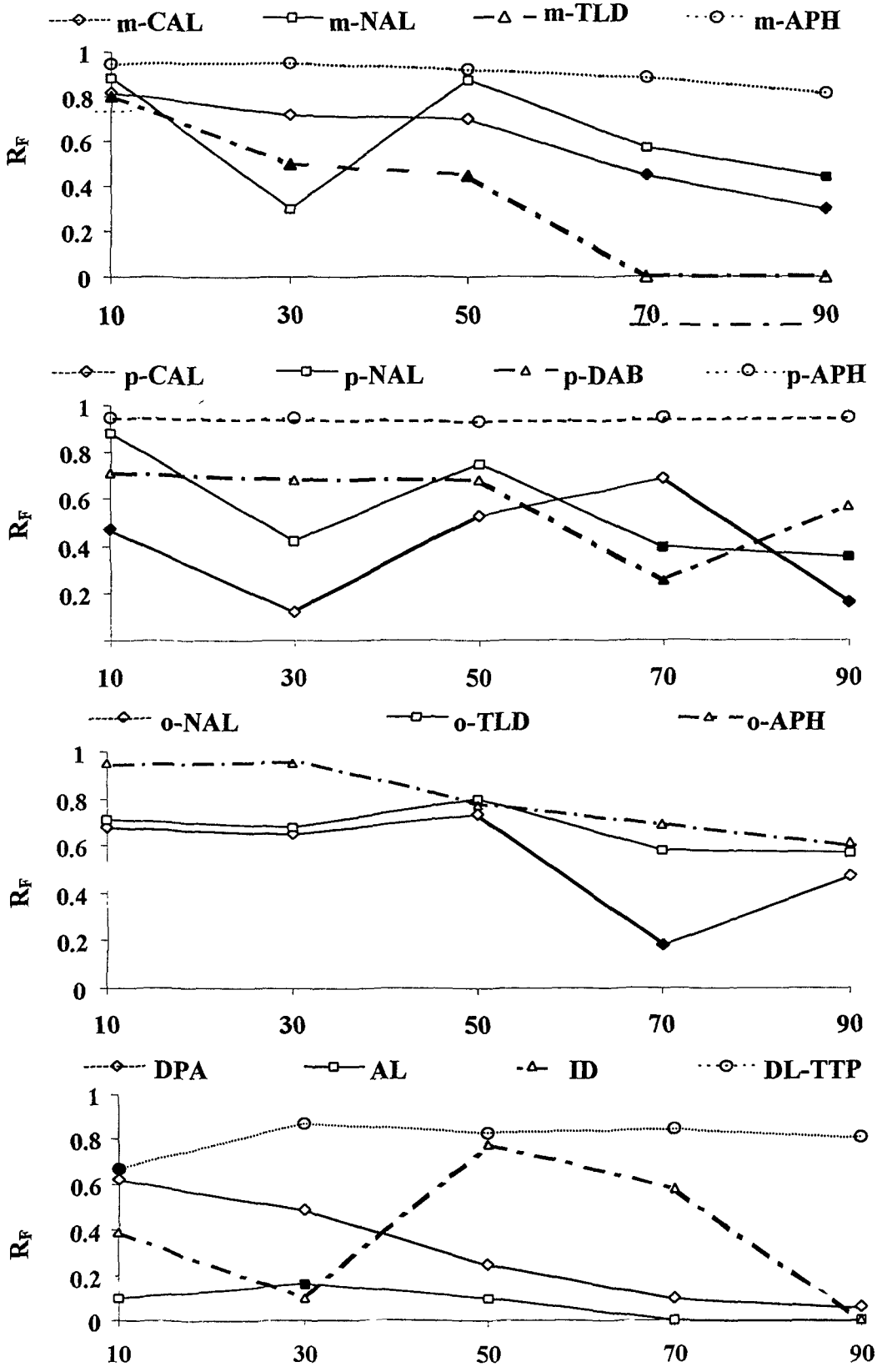


Figure 2.2: Plot of R_F Vs Volume of Added H_2O in M_7 (0.01 M CTAB in Methanol)

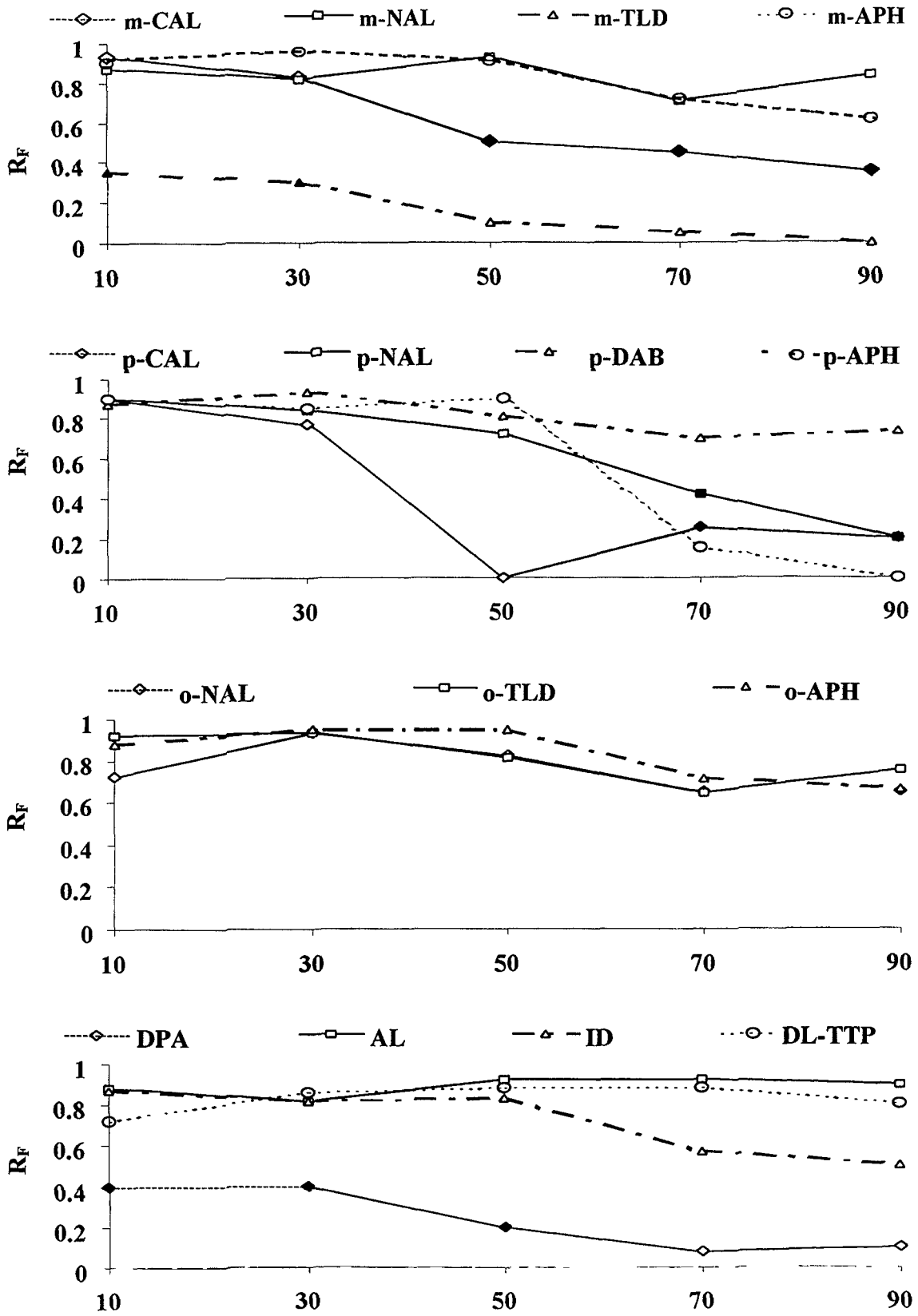


Figure 2.3: Plot of R_F Vs Volume of Added H_2O (%) in M_8 (0.01M Triton X-100 in Methanol)

(i) The mobility of amines generally decreases with the increase in water content in the mobile phase regardless the nature of surfactant. The mobility (R_F value) of amines such as p-CAL, p-NAL, p-DAB, p-APH, DPA, ID, o-NAL, o-TLD, o-APH, m-CAL, m-NAL, m-TLD, and m-APH is lower in mobile phase systems, M_6-M_8 containing 90% added water compared to their mobility in M_6-M_8 containing 10% added water.

(ii) Highly compact and well-formed circular spots for all amines (except DPA and m-TLD) appeared when TLC plates were developed with mobile phases consisting of 10 or 30% H_2O and 90 or 70% methanolic surfactant (SDS, CTAB, or Triton X-100).

(iii) Certain amines (m-CAL, p-NAL, p-CAL, p-DAB, DPA, m-TLD) produce tailed spots with mobile phases (M_6-M_8, 5:5 and 3:7) consisting of 50-70% water content. DPA and m-TLD also produce tailed spots with M_8 (9:1 and 7:3).

(iv) In most of the mobile phases the separation of amines from their two-component mixtures is always possible.

(v) The development time increases with the increase in water content in the mobile phase. A three fold increase in development time was noticed on increasing the water content from 10% (development time = 15 min to 90% (development time = 45 min).

To examine the effect of nature of alcohols on the mobility of amines, methanol was substituted by propanol-1, butanol-1 or acetonitrile in M_6 [SDS in MeOH + H_2O, (3:7 or 7:3)] and the mobility

of amines was investigated using resulted mobile phases (M_9-M_{11}). All the amines move with the solvent front ($R_F > 0.9$) with these mobile phases. Thus, these solvent systems can only be used to elute the amines from the matrices. However, m-CAL ($R_F = 0.07$), can only be selectively separated from all other amines ($R_F > 0.90$) in M_{11} (3:7). DPA produced tailed spot in M_{11}. o-CAL could not be detected on TLC plates developed with M_9-M_{11} eluents. Thus, methanol (strongly hydrogen bonded) alcohol has better separation efficiency in micellar systems compared to moderately hydrogen-bonded alcohols like propanol or butanol.

Figure 2.4 shows how the mobility of amines is modified in the presence of SDS. In this Figure, ΔR_F [R_F obtained with 0.01 M SDS in MeOH+H_2O (3:7 or 7:3) minus R_F measured in MeOH+H_2O (3:7 or 7:3)] was plotted. The positive and negative values of ΔR_F reveal the net effect of SDS on the mobility of amines. The most effected amines are AL and o-TLD. The positive value of ΔR_F in the case of o-TLD (ΔR_F = +0.84) indicates that SDS activates the solulibilization of o-TLD in alcoholic water whereas the solubility of AL is decreased in the presence of SDS in alcohol – water mixture as evident from negative ΔR_F value (ΔR_F = –0.83). An interesting aspect of this study is that the negative magnitude of ΔR_F value of amines corresponds to their basicity. AL being stronger base ($K_b = 4.2 \times 10^{-10}$) compared to m-CAL ($K_b = 0.30 \times 10^{-10}$), p-CAL ($K_b = 0.10 \times 10^{-10}$), m-NAL ($K_b = 0.029 \times 10^{-10}$), p-NAL ($K_b = 0.001 \times 10^{-10}$), o-NAL ($K_b = 0.00006 \times 10^{-10}$) and DPA ($K_b = 0.0006 \times 10^{-10}$) has higher negative ΔR_F (ΔR_F = –0.83) compared to above mentioned amines. Aminophenols being acidic show

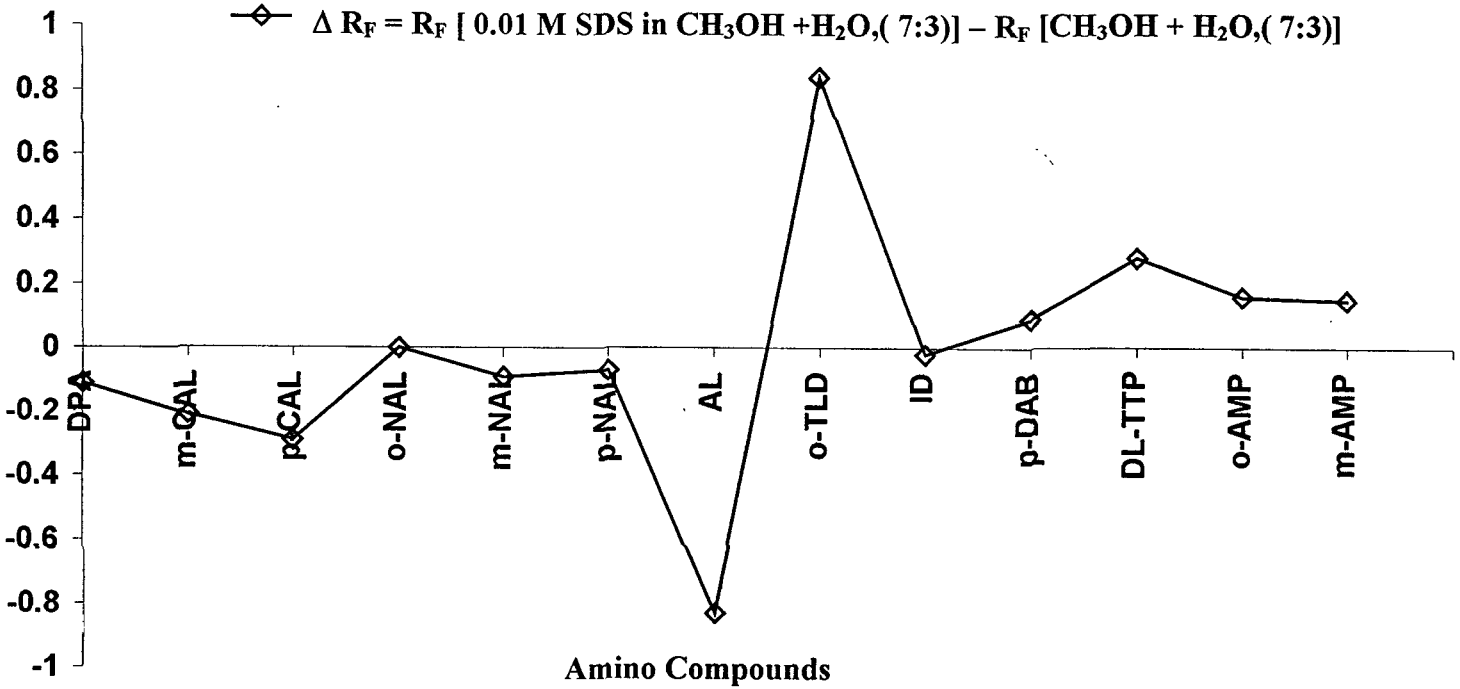


Figure 2.4: Plot of Δ R_F Vs Amino Compounds

positive ΔR_F values. ID being a weaker base show zero ΔR_F. The positive ΔR_F values for o-TLD and p-DAB may be attributed to the presence of methyl groups in their molecules. TTP containing both $-NH_2$ and –COOH groups show positive ΔR_F value.

The addition of electrolytes (i.e. inorganic salts) in micellar systems has been proved advantageous for achieving enhanced selectivity. The added ionic species modify the microenvironment of micellar systems. To examine the effect of inorganic additives on the mobility of amines, 0.1 M aqueous solution of NaCl, LiCl, $CaCl_2$ or $CoCl_2$ was added into 0.01 M methanolic SDS solution in the ratio of 7:3 and 3:7. The results presented in **Table 2.2** show that the mobility of all amines is decreased on increasing the concentration of salt from 30% - 70% in the mobile phase. This lowering in mobility with the increase in salt concentration may be attributed to the salting-out effect. Amongst several analytically important possible separations with these solvent systems, the separation of o-, m- and p-aminophenols with 0.01 M SDS $_{(CH3OH)}$ + 0.1 M $CaCl_{2\ (aqueous)}$ (3:7) is of great interest. The R_F values, given in parenthesis follow the order: p-APH (0.15) < o- APH (0.62) < m-APH (0.94). It shows that $CaCl_2$ is the most effective additive compared to NaCl, LiCl or $CoCl_2$ to discriminate among amines, facilitating their simultaneous separations from multicomponent mixtures. In the case of $CoCl_2$, separation of coexisting o-, m-, and p-aminophenols is also possible as the order of R_F values, given in parenthesis was p-APH (0.10) < o-APH (0.55) < m-APH (0.98). The comigration of cobalt salt at 0.1 M concentration level leaves the pink coloration up to the middle of TLC plate during development of the

Table 2.2: Effect of Added Inorganic Electrolytes in 0.01 M Methanolic SDS on the Mobility (i.e. R_F Values) of Amino Compounds*

Amino Compound	0.01 M SDS (CH_3OH) + 0.1 M NaCl		0.01 M SDS (CH_3OH) + 0.1 M LiCl		0.01 M SDS (CH_3OH) + 0.1 M $CaCl_2$		0.01 M SDS (CH_3OH) + 0.1 M $CoCl_2$	
	7:3	3:7	7:3	3:7	7:3	3:7	7:3	3:7
DPA	0.84	0.05	0.62	0.15	0.38T	0.19T	0.77	0.15
p-CAL	0.89	0.00	0.90	0.25T	0.56	0.48	0.80	0.33
o-NAL	0.91	0.55	0.92	0.78	0.73	0.70	0.89	0.75
m-NAL	0.80	0.25T	0.95	0.78	0.83	0.61	0.78	0.64
p-NAL	0.88	0.30T	0.93	0.50T	0.89	0.73	0.92	0.64
AL	0.85	0.36	0.39	0.00	0.50T	0.64	0.51	0.37
o-TLD	0.76	0.00	0.00	0.00	0.50T	0.73	0.00	0.00
m-TLD	0.72	0.00	0.90	0.50T	0.40T	0.10	0.79	0.00
ID	0.76	0.55	0.95	0.76T	0.72	0.73	0.74	0.05
p-DAB	0.80	0.00	0.95	0.50T	0.77	0.55	0.87	0.53
DL-TTP	0.81	0.76	0.95	0.40T	0.85	0.83	0.89	0.57
o-APH	0.74T	0.50T	0.95	0.73	0.84	0.62	0.87	0.55
m-APH	0.87	0.5T	0.95	0.92	0.84	0.94	0.95	0.98
p-APH	0.15	0.00	0.85	0.15	0.00	0.15	0.85	0.10

T = Tailed spot ($R_L - R_T > 0.3$)

** With all above-mentioned mobile phase systems, m-CAL remained at the point of application ($R_F = 0.0$) and o-CAL could not be detected.*

plate which clouds the sharpness of the detection of the o- and p-aminophenols. Therefore, we preferred $CaCl_2$ containing SDS mobile phase for the present study.

When 0.01 M SDS (CH_3OH) and 0.1 M $CaCl_2$ solutions were mixed in 1:7 ratio instead of 3:7 (i.e. when the ratio of 0.01 M SDS to 0.1 M $CaCl_2$ is decreased), and the system was used as mobile phase, a decrease in R_F value of o-isomer (R_F = 0.55) was observed. This lowering in R_F value improves its separation from m- aminophenol (R_F = 0.94). When 0.1 M $CaCl_2$ in the mobile phase M_{14} (3:7) was substituted with 0.1 M Ca $(NO_3)_2$, it was observed that mobility of o-aminophenol increases to that extend where the separation of o- (R_F = 0.80) from m-isomer (R_F = 0.83) becomes difficult and the separation of p- isomer (R_F = 0.05) is only possible either from o- or m-aminophenol. Furthermore, the decrease in R_F value of indole in Ca $(NO_3)_2$ containing mobile phase, did not affect its separation from DL-tryptophan, the mobility of which remains the same as was in 0.1 M $CaCl_2$.

From the results summarized in **Table 2.3**, it is clear that the mobility of o-APH is influenced by the presence of Fe^{3+}, Co^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, indole, DL-tryptophan, p-DAB, phloroglucinol and gallic acid. Such impurities are capable to transform the compact spot of o-APH into an elongated form. Consequently, a poor separation of o-APH from m-APH results in the presence of these impurities. The separation of o-APH (R_F = 0.35) from m-APH (R_F = 0.69) or p-APH (R_F = 0.05) is also influenced in the presence of m-cresol as a result of lowering in R_F values (or mobility) of both o- and m- aminophenols.

The results presented in **Table 2.4** show that o-, m- and p-aminophenols could be successfully separated in the presence of five-fold concentration of

Table 2.3: Separation of o-, m- and p-Aminophenols from their Mixtures in the Presence of Metal Cations, Amines and Phenols on HPTLC Plates Developed with 0.01 M SDS (MeOH) + 0.1 M Calcium Chloride (3:7)

Added Impurities	Separation (R_F)		
	o-APH	m-APH	p-PH
Fe^{3+}	0.45T	0.95	0.05
Cu^{2+}	0.57	0.94	0.05
Co^{2+}	0.49T	0.94	0.05
Ni^{2+}	0.53	0.88	0.05
Zn^{2+}	0.44T	0.82	0.05
Cd^{2+}	0.44T	0.82	0.05
Hg^{2+}	0.52T	0.90	0.05
Pb^{2+}	0.53	0.92	0.05
Bi^{3+}	0.56	0.95	0.05
Indole	0.40T	0.90	0.05
DL-TTP	0.49T	0.87	0.05
Diphenylamine	0.45	0.92	0.10
Aniline	0.45	0.82	0.05
p-DAB	0.42T	0.82	0.10
Pyrocatechol	0.56	0.84	0.10
Phloroglucinol	0.44T	0.90	0.05
Resorcinol	0.42	0.84	0.05
Gallic acid	0.45T	0.88	0.05
o-Cresol	0.45	0.88	0.05
m-Cresol	0.35	0.69	0.05
p-Cresol	0.50	0.78	0.05

In the absence of impurities R_F values (given in parenthesis) of aminophenols from their mixtures were o- APH (0.63), m- APH (0.88) and p- APH (0.05).

Table 2.4: Separation of o-, m- and p-Aminophenols at Their Different Concentration Levels in the Mixture

Mixture	Concentration Ratio	Separation (R_F)
o-APH + m-APH	1:5	o-APH (0.63) — m-APH (0.95)
	5:1	o-APH (0.62) — m-APH (0.85)
o-APH + p-APH	1:5	o-APH (0.63) — p-APH (0.25T)
	5:1	o-APH (0.5) — p-APH (0.05)
m-APH + p-APH	1:5	m-APH (0.89) — p-APH (0.25T)
	5:1	o-APH (0.89) — p-APH (0.05)
o-APH + m-APH + p- APH	1:1:5	o-APH (0.62) — m-APH (0.89) — p-APH (0.25T)
	1:5:1	o-APH (0.66) — m-APH (0.92) — p-APH (0.05)
	5:1:1	o-APH (0.64) — m-APH (0.92) — p-APH (0.00)

T = Tailed spot ($R_L - R_T > 0.3$)

each isomer in their binary or ternary mixtures. The formation of tailed (or elongated) spots in the case of p-APH at its higher concentration level (e.g. 5 times than other isomers) is indicative of poor separation whereas no such problem is arised in case of o- and m- APH. It is therefore concluded that for better separation of aminophenol isomers, the concentration of p-APH must be kept low in their mixtures.

The separation of coexisting o-, m-, and p-aminophenols is very important and the proposed method is the only procedure to provide excellently resolved spots for all the three isomers of aminophenol on TLC plate **(Figure 2.5)**. It is to be noted that the R_F values of aminophenol isomers in their mixture differ marginally from those obtained by spotting them individually. The TLC methods reported earlier **(Table 2.5)** was capable only to separate two isomers and the simultaneous separation of three isomers was either impossible or there was the possibility of very poor separation.

The limits of detection of aminophenols given in parenthesis for o-APH (0.416 μg), m-APH (1.25 μg) and p-APH (0.83 μg) indicates that the proposed system is most suitable for sensitive detection of aminophenols. None of the isomers could be detected on HPTLC plate if Zn-ferrocyanide is used as detector instead of I_2 vapors. However, this reagent produce colored spots with o-, m- and p-APH on TLC plates coated with commercially available silica gel G. The limit of detection and the colored pattern were almost identical to those reported by *Mitchell et al.* (28).

Table 2.5: List of TLC Systems for the Separation of Aminophenol Isomers

Stationary Phase	Mobile Phase	R_F Values of o-, m- and p-Aminophenols			Remarks	Reference
		o-	m-	p-		
S_1	M_1	1	2	1	No separation	24
	M_2	27	27	12		
S_2	M_3	58	50	18		
	M_1	2	1	0		
	M_2	11	11	4		
	M_3	29	24	3		
S_1	M_4	13	13	13	Binary but poor separation	29
S_3		22	15	17		
S_4		20	15	18		
S_5		15	9	13		
S_6	M_5	0	0	0	Binary separation or poor ternary separation	31
	M_6	91	71	65		
	M_7	87	87	87		
S_7	M_8	63	88	05	Efficient ternary separation	Present study, ternary separation of o-, m- and p-APHS

Stationary Phases: *S_1 = Non- impregnated silica gel 'G' layer; S_2 = Silica gel 'G' layer impregnated with $CdSO_4$; $S_3 - S_5$ = Silica gel 'G' layer impregnated with 5%, 10%, 15% sodium nitrite respectively; S_6 = Aluminum RP - 18 F_{254} plates; S_7 = Silica gel 60, F_{254} plates*

Mobile Phases: *M_1 = Benzene + acetic acid (9:1); M_2 = Benzene + acetic acid + methanol (8:1:1); M_3 = Acetic acid + ethyl acetate + isopropyl ether (2:25:25); M_4 = Benzene + ethyl acetate (20:1); M_5 = Toluene; M_6 = Toluene + MeOH (8:2); M_7 = Acetonitrile + water (70 + 30, v/v); M_8 = 0.01 M SDS (MeOH) + 0.1 M aqueous $CaCl_2$ (3:7)*

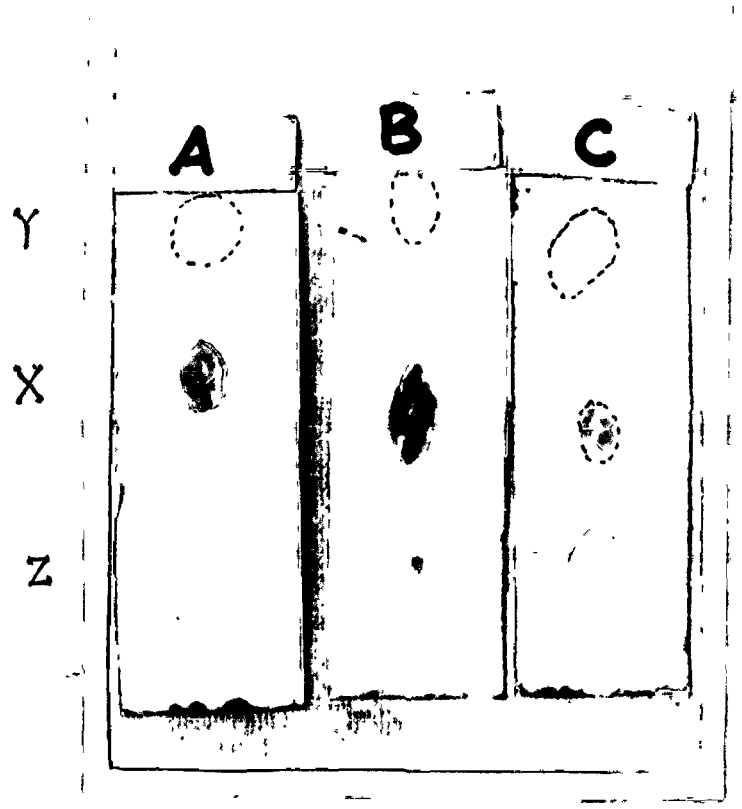


Figure 2.5: Resolved Spots of o- (X), m- (Y), and p-Aminophenol (Z) on Silica Gel ^{60} F_{254} 'HPTLC' Plates Developed with

A. 0.01 M SDS in Methanol plus 0.1 M $CaCl_2$ (3:7)

B. 0.01 M SDS in Methanol plus 0.1 M $CaCl_2$ (3:7) – 1% Aniline in Methanol (1:1)

C. 0.01 M SDS in Methanol plus 0.1 M $CaCl_2$ (3:7) – 1% Pyrocatechol in Methanol (1:1).

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Chapter-3

IDENTIFICATION OF AMINO ACIDS ON ALUMINA TLC PLATES DEVELOPED WITH AQUEOUS ANIONIC SURFACTANT SOLUTIONS CONTAINING INORGANIC AND ORGANIC ADDITIVES: SELECTIVE SEPARATION OF L-PROLINE FROM OTHER AMINO ACIDS

3.1 INTRODUCTION

Thin-layer chromatography (TLC) being simple and cost effective has been used by several workers (1-4) as an analytical tool for rapid analysis of amino acids and heavy metal cations. Most of the workers have used silica gel (5-7), alumina (8-10), cellulose and cellulose derivatives (11-12), chitin and chitosan (13-14), polyamide (15), as layer materials in combination with aqueous, mixed – organic and mixed aqueous – organic solvents as mobile phase. Salting-out reversed-phase TLC has been successfully applied for rapid analysis of dansylated amino acids (16). Interesting separations of racemic aromatic amino acids have been reported on cellulose layers using concentrated aqueous solutions of α- or β-cyclodextrins (17-18). *Ravi Bhushan et al* have achieved improved separations of closely related amino acids on silica gel surface-modified with metallic salt solutions (19-20).

Micellar liquid chromatography (MLC) involving the use of surfactant ions above their critical micelle concentration (CMC) as mobile phase has been the focus of numerous studies (21-25) for controlling the retention of a variety of solutes since it was first proposed by Armstrong and co-workers in 1977 (26). Taking into consideration the advantageous features such as inexpensiveness, non-toxicity and non-inflammability of micellar mobile phases, we have utilized a novel microemulsion system (27) consisting of sodium dodecyl sulfate (SDS) as one of the components to achieve certain important separations of amino acids on silica gel layer. Traditionally, the separation efficiency of micellar systems has been enhanced by adding small quantities of organic additives e.g. 1-propanol or 1-pentanol (28,29). In this regard, the present investigation deviates from earlier studies and we have achieved the improved separation by adding copper salt solution into anionic SDS micellar solution. As a result, a

copper salt solution into anionic SDS micellar solution. As a result, a selective separation of proline has been achieved on alumina layer using 0.01M aqueous SDS plus 0.1M copper sulfate solutions in 9:1 ratio. To the best of our knowledge, no work has been reported on the use of SDS micellar solution containing metallic salts as mobile phase in the analysis of amino acids by alumina TLC.

3.2 EXPERIMENTAL

***Apparatus*:** A thin-layer chromatographic applicator (Toshniwal, India), 20 x 3cm glass plates and 24 x 6cm glass jars were used for the development of chromatographic plates. A glass sprayer was used to spray reagent on the plate to detect the spot.

***Chemicals and Reagents*:** Amino acids, aluminium oxide 'G', nickel chloride, lithium chloride, sodium nitrate and copper sulfate (CDH, India); sodium molybdate (Qualigens, India), cobalt chloride and thiourea (E. Merck, India); sodium chloride and urea (G.S.C, India) and sodium dodecyl sulfate (BDH, India), were used. All reagents were of Analar Reagent grade.

***Amino Acids Studied*:** L-valine (A1), DL-valine (A2), L-isoleucine (A3), DL-isoleucine (A4), L-arginine (A5), L-arginine- monohydrochloride (A6), L-tryptophan (A7), DL-tryptophan (A8), L-methionine (A9), DL-methionine (A10), L-cystine (A11), L-cystein hydrochloride (A12), L-serine (A13), DL-serine (A14), DL-nor- leucine (A15), L-leucine (A16), DL-alanine (A17), DL-phenylalanine (A18), 3-(3,4-dihydroxy-phenyl) DL-alanine (A19), L-proline (A20), DL-threonine (A21) and DL-aspartic acid (A22).

Test Solution: Test solutions (1%) of amino acids were prepared in double-distilled water.

Detector: A 0.3% ninhydrin solution in acetone was used to detect all the amino acids.

Stationary Phase: Aluminium oxide 'G'

Mobile Phases: The following solvent systems were used as mobile phase

Symbol	Composition
M_1	0.001 M, 0.005 M, 0.01 M and 0.05 M aq. SDS
M_2	0.01 M aq. SDS + 0.1 M aq. $CuSO_4$ (9:1, 5:5 and 1:9)
M_3	0.01 M aq. SDS + 0.1 M aq. $CoCl_2$ (9:1 and 1:9)
M_4	0.01 M aq. SDS + 0.1 M aq. $NiCl_2$ (9:1 and 1:9)
M_5	0.01 M aq. SDS + 0.1 M aq. LiCl (9:1 and 1:9)
M_6	0.01 M aq. SDS + 0.1 M aq. NaCl (9:1 and 1:9)
M_7	0.01 M aq. SDS + 0.1 M aq. $NaNO_3$ (9:1 and 1:9)
M_8	0.01 M aq. SDS + 0.1 M aq. $NaMoO_4$ (9:1 and 1:9)
M_9	0.01 M aq. SDS + 0.1 M aq. urea (9:1 and 1:9)
M_{10}	0.01 M aq. SDS + 0.1 M aq. thiourea (9:1 and 1:9)
M_{11}	0.001 M aq. SDS + 0.001 M aq. $CuSO_4$ (9:1 and 1:9)
M_{12}	0.001 M aq. SDS + 0.001 M aq. $CoCl_2$ (9:1 and 1:9)
M_{13}	0.001 M aq. SDS + 0.001 M aq. $NiCl_2$ (9:1 and 1:9)
M_{14}	0.001 M aq. SDS + 0.001 M aq. LiCl (9:1 and 1:9)
M_{15}	0.001 M aq. SDS + 0.001 M aq. NaCl (9:1 and 1:9)
M_{16}	0.001 M aq. SDS +0.001 M aq. $NaNO_3$ (9:1 and 1:9)
M_{17}	0.001 M aq. SDS + 0.001 M aq. $NaMoO_4$ (9:1 and 1:9)
M_{18}	0.001 M aq. SDS + 0.001 M aq. urea (9:1 and 1:9)
M_{19}	0.001 M aq. SDS + 0.001 M aq. thiourea (9:1 and 1:9)

Preparation of TLC Plates: The plates were prepared by mixing alumina with water in 1:3 volume ratios with constant shaking until homogeneous slurry was obtained. The resultant slurry was applied on the glass plates with the help of a Toshniwal applicator to give a 0.25 mm-thick layer. The plates were dried in air at room temperature and then activated by heating for 1 h at 100 ± 5° C in an electrically controlled oven. The activated plates were stored in a close chamber at room temperature until used.

Procedure: Test solutions (approx. 10 µl) were applied by means of micropipets approximately about 2.0cm above the lower edge of the plates. The plates were developed in the chosen solvent system by the ascending technique. The solvent ascent was fixed to 10 cm in all cases. After development was complete, the plate was withdrawn from glass jars and dried at room temperature followed by spraying with freshly prepared ninhydrin solution. All amino acids except L-proline appeared as violet spots on heating TLC plates for 15–20 minutes at 100 ± 5°C. L-proline gives yellow spot. The R_L (R_F of leading front) and R_T (R_F of trailing front) values for each spot were determined and the R_F value was calculated as:

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

Separation: For the separation, equal amounts of L-proline and other amino acids were mixed and 20 µl of the resultant mixture was loaded on the TLC plates. The plates were developed, the spots were detected and the R_F values of the separated amino acids were determined.

Limit of Detection: Procedure for the limit of detection of amino acids as described in chapter 2 for amino compounds. ***Semiquantitative Determination by Spot-Area Measurement***: For semiquantitative determination by spot-area measurement method,

0.01 mL from a series of standard solutions (0.1-1.5%) of DL-alanine, DL-phenylalanine, 3-(3,4-dihydroxyphenyl) DL-alanine and L-proline were spotted on alumina layers. The plates were developed with 0.01M aqueous SDS plus 0.1M aqueous $CuSO_4$ in volume ratio of 9:1. After detection, the spots were copied onto tracing paper from the chromatoplates and then the area of each spot was calculated.

3.3 RESULTS AND DISCUSSION

Alumina was selected as layer material for TLC separation of aliphatic and aromatic amino acids because of its following properties:

- It has rigid structure, which undergoes little swelling or shrinking in water or solutions containing electrolyte.
- The hydroxyl groups (OH^-) and oxide (O^{2-}) ions constituting active sites on the surface are responsible for selective chromatographic separations.
- The pH of alumina slurry is about 7.0, around which most of the aliphatic amino acids exist as zwitter ions.

The results of the present study are summarized in **Tables 3.1 - 3.5** and **Figures 3.1 - 3.3.** From the data listed in **Table 3.1**, following conclusions are drawn.

(a) In pure water (zero surfactant concentration), most of the amino acids produce tailed spots i.e. $R_L - R_T > 0.3$.

(b) At SDS concentration (0.001M), which is below critical micelle concentration (CMC) value of the surfactant (CMC of SDS, 0.008 M), the mobility of the amino acids is slightly improved but certain amino acids still yield tailed spots.

(c) At SDS concentration (0.01M) in the mobile phase i.e. near CMC of the surfactant, the mobility of amino acids is

Table 3.1: Mobility of Amino Acids on Alumina Layers Developed with Pure Water and Different Concentrations of SDS in Water

Amino Acid	Water	SDS Concentration (M)		
		0.001	0.01	0.1
L-valine	0.60T	0.57 T	0.61 T	0.50 T
DL-valine	0.52T	0.54	0.55 T	0.35 T
L-isoleucine	0.55 T	0.68 T	0.75	0.58
DL-isoleucine	*0.57 T*	*0.66 T*	*0.72*	*0.65*
L-arginine	0.10	0.10	0.11	0.15 T
L-arginine monohydrochloride	0.12	0.10	0.11	0.20
L-tryptophan	0.40 T	0.30	0.44 T	0.55 T
DL-tryptophan	0.33 T	0.29	0.41	0.54
L-methionine	0.37 T	0.40	0.49	0.48 T
DL-methionine	0.40 T	0.38	0.47	0.47 T
L- cystine	0.04	0.04	0.13	0.13
L-cystein hydrochloride	0.03	0.02	0.12	0.13
L-serine	0.20 T	0.10	0.10	0.13
DL-serine	0.19	0.16	0.10	0.12
DL-nor-leucine	0.64 T	0.65 T	0.67	0.65 T
L-leucine	0.60 T	0.55 T	0.67	0.64
DL-alanine	0.25	0.55 T	0.57	0.50
DL-phenylalanine	0.29	0.45 T	0.50	0.52
3-(3,4dihydroxyphényl)-DL-alanine	0.60	0.58 T	0.63	0.65
L-proline	0.63 T	0.70	0.77	0.77
DL-threonine	0.05	0.10	0.10	0.11
DL-aspartic acid	0.05	0.10	0.10	0.11

T = Tailed spot ($R_L - R_T > 0.3$)

further enhanced slightly and the spots become compact (i.e. $R_L - R_T < 0.3$).

(d) At surfactant concentration much above the CMC value of SDS (i.e. 0.1M), the number of amino acids producing tailed spots is increased as compared with those producing tailed spots at 0.01 M SDS concentration.

Taking into consideration the compactness and the clearer detection of spots, the mobile phase containing 0.01M SDS in double-distilled water was selected. The microenvironment of surfactant-mediated system is greatly influenced by the presence of added electrolyte (30). Therefore, mobile phases comprising of 0.01M SDS and aqueous copper sulfate (0.1M) solution in different volume ratio (9:1, 1:9 and 1:1) were tested with the aim of obtaining better separations of amino acids. The R_F values of amino acids obtained on alumina layer developed with these mobile phases are summarized in **Table 3.2**.

It is clear from **Table 3.2** that a slight decrease in R_F value occurs on the increase of $CuSO_4$ concentration. Other amino acids experience only minor influence of presence of Cu^{2+} ions on their mobility. However, the presence of Cu^{2+} ions in the mobile phase improves the selective separation of L-proline from other amino acids. The increase in R_F of L-proline from 0.77 **(Table 3.1)** in 0.01 M SDS to 0.88 **(Table 3.2)** in 0.01M SDS + 0.1M $CuSO_4$ (9:1) provides an opportunity to achieve improved separation of L-proline from closely associated amino acids.

In order to examine the influence of different organic and inorganic compounds on the mobility of amino acids, $CuSO_4$ in M_2 (0.01M SDS + 0.1M $CuSO_4$, 9:1 and 1:9) was replaced by 0.1M $CoCl_2$, $NiCl_2$, LiCl, NaCl, $NaNO_3$, Na_2MoO_4, urea and thiourea and the resultant mobile phases were used to determine the mobility of amino acids on alumina layer. The results obtained with M_3-M_{10} (9:1) are

Table 3.2: Mobility (R_F Value) of Amino Acids on Alumina Layer Developed with Mixed Mobile Phases Consisting of 0.01 M Aqueous SDS plus 0.1 M Aqueous $CuSO_4$ in Different Ratio

Amino Acid	0.01 M Aqueous SDS + 0.1 M Aqueous $CuSO_4$		
	9:1	5:5	1:9
L-valine	0.68	0.65T	0.62
DL-valine	0.66	0.66 T	0.63
L-isoleucine	0.71	0.63 T	0.61 T
DL-isoleucine	0.72	0.66 T	0.66 T
L-arginine	0.30	0.27	0.12
L-arginine monohydrochloride	0.30	0.27	0.11
L-tryptophan	0.56	0.55 T	0.30 T
DL-tryptophan	0.52	0.52 T	0.38 T
L-methionine	0.50	0.44T	0.34
DL-methionine	0.44	0.32	0.34
L- cystine	0.10	0.06	0.03
L-cystein hydrochloride	0.09	0.04	0.11
L-serine	0.18	0.17 T	0.14
DL-serine	0.18	0.16 T	0.14
DL-nor-leucine	0.62	0.66 T	0.66
L-leucine	0.66	0.65 T	0.64
DL-alanine	0.56	0.54 T	0.54
DL-phenylalanine	0.60	0.47	0.53
3-(3,4dihydroxyphenyl)- DL-alanine	0.63	0.55 T	0.53
L-proline	0.88	0.69	0.70
DL-threonine	0.14	0.05	0.05
DL-aspartic acid	0.07	0.05	0.05

T = Tailed spot ($R_L - R_T > 0.3$)

presented in **Table 3.3**, from where it is evident that the mobility of amino acids is modified by the presence of ionic as well as molecular species of the additives. For example, the mobilities of L- and DL-isoleucines (R_F = 0.43 and 0.46 respectively), L-methionine (R_F = 0.70) in LiCl containing mobile phase are different from those achieved in $CoCl_2$, $NiCl_2$ and NaCl containing mobile phases. Similarly, the mobilities of L- and DL-serines ($R_F \approx 0.55$) in $NiCl_2$ differ from that obtained with $CoCl_2$ and LiCl containing mobile phases. These observations indicate that the nature of cations present in micellar SDS system play an important role in controlling the mobility of certain amino acids. This variation in mobility of amino acids is probably caused due to the fact that these cations interact differently with the anionic micellar system to provide the microenvironment of different nature. Furthermore, the nature of anions in the mobile phase was also found to influence the mobility of amino acids as evident from the results obtained with NaCl and $NaNO_3$ containing mobile phases. The mobilities of L-serine (R_F = 0.47) and DL-serine (R_F = 0.43) are higher in NaCl system compared to their mobilities (L-serine, R_F = 0.13 and DL-serine, R_F = 0.12) in $NaNO_3$ containing mobile phases. However, the mobilities of most amino acids achieved in $NaMoO_4$ were found almost identical to the mobilities obtained in $NaNO_3$ mobile phase in spite of the fact that MoO_4^{2-} is stronger complexing agent compared to NO_3^-. The mobilities of amino acids obtained in urea and thiourea containing mobile phases are almost comparable which indicate that the higher electronegativity of nitrogen (urea) compared to sulfur (thiourea) does not exert significant effect on the mobility of amino acids.

From above discussion, it is apparent that the mobility of most of amino acids does not differ much in SDS micelllar systems containing different inorganic and organic additives. However, in certain cases such

Table 3.3: Effect of Different Inorganic and Organic Additives in 0.01 M Aqueous SDS on the Mobility of Amino Acids

Amino Acid	Mobile Phase (0.01M SDS + 0.1M Additives, 9:1)							
	$CoCl_2$	$NiCl_2$	LiCl	NaCl	$NaNO_3$	Na_2MoO_4	Urea	Thiourea
L-valine	0.70	0.66	0.76	0.62	0.70T	0.65T	0.55	0.56
DL-valine	0.69	0.63	0.77	0.65	0.75T	0.66	0.60	0.58
L-isoleucine	0.75	0.61	0.43	0.61	0.75T	0.64	0.60	0.75
DL-isoleucine	0.74	0.63	0.46	0.65	0.72T	070	0.62	0.75
L-arginine	0.36	0.25	0.30	0.25	0.20T	0.14	0.21	0.28
L-arginine monohydrochloride	0.37	0.25	0.30	0.26	0.20T	0.14	0.21	0.26
L-tryptophan	0.60	0.51	0.62	0.51	0.47	0.53	0.49	0.43
DL-tryptophan	0.65	0.50	0.57	0.49	0.42	0.50	0.45	0.45
L-methionine	0.47	0.47	0.70	0.44	0.45T	0.38T	0.36	0.43
DL-methionine	0.47	0.46	0.65	0.41	0.35T	0.38T	0.39	0.39
L- cystine	0.05	0.13	0.05	0.12	0.08	0.10	0.07	0.05
L-cystein hydrochloride	0.05	0.12	0.05	0.12	0.10	0.10	0.08	0.06
L-serine	0.14	0.54	0.15T	0.47	0.13	0.13	0.10	0.12
DL-serine	0.10	0.55	0.15T	0.43	0.12	0.12	0.11	0.12
DL-nor-leucine	0.50	0.65	0.50	0.63	0.64	0.66	0.55	0.60
L-leucine	0.50	0.64	0.59	0.59	0.62	0.64	0.48	0.57
DL-alanine	0.42	0.53	0.57	0.56	0.66	0.60	0.44	0.48
DL-phenylalanine	0.45	0.51	0.54	0.57	0.59	0.59	0.43	0.27T
3-(3,4dihydroxyphenyl)- DL-alanine	0.55	0.55	0.65	0.56	0.67	0.57	0.53	0.60
L-proline	0.80	0.84	0.88	0.76	0.82	0.78	0.78	0.86
DL-threonine	0.05	0.10	0.05	0.05	0.14	0.10	0.10	0.10
DL-aspartic acid	0.05	0.05	0.05	0.05	0.05	0.09	0.09	0.05

T = Tailed spot ($R_L - R_T > 0.3$)

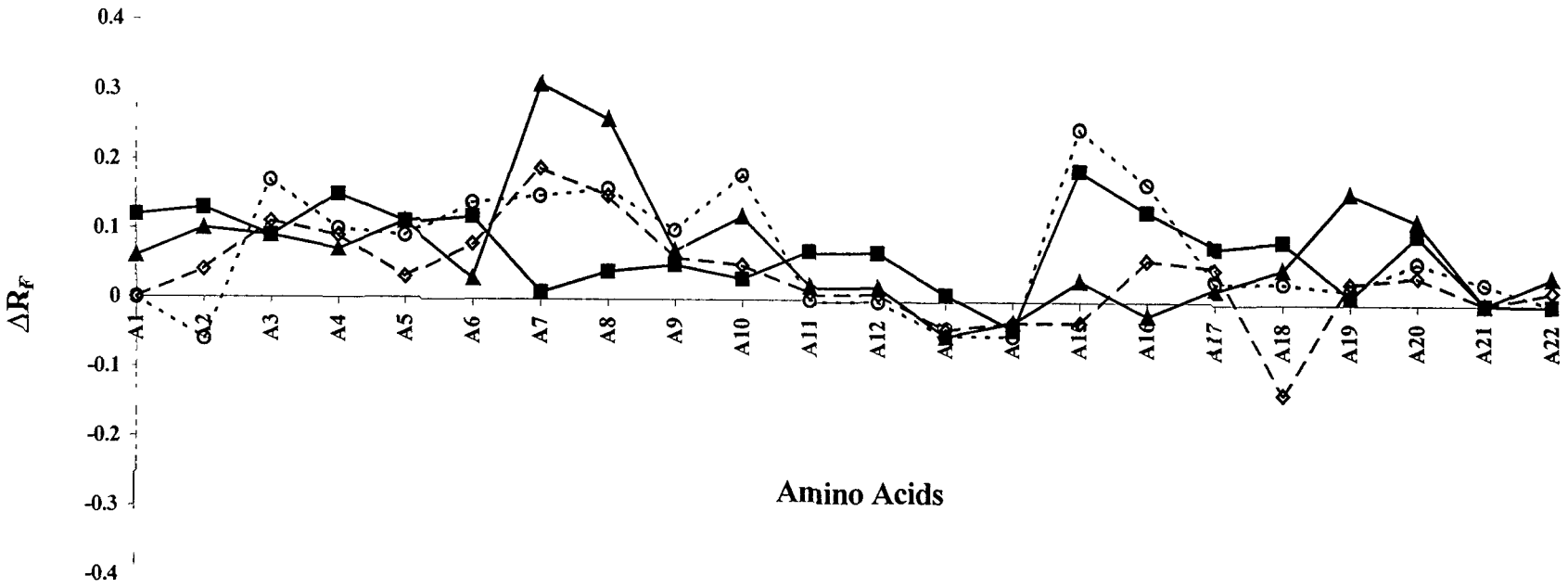
as isoleucine, methionine and serine, the modified mobility in the presence of additives facilitates new separations of amino acids.

To examine the effect of concentration of additives on the mobility of amino acids, the R_F values of amino acids were also determined in M_2–M_{10} (9:1) containing 90% SDS and 10% additives and the results so obtained were compared with those achieved in M_2–M_{10} (1:9) containing 10% SDS and 90% additives. For this purpose, difference in R_F values i.e. ΔR_F [ΔR_F = R_F of amino acids in 0.01 M SDS plus 0.1 M additives (9:1) minus R_F of amino acids in 0.01 M SDS plus 0.1 M additives (1:9)] were calculated. The ΔR_F values listed in **Table 3.4** indicate that the mobilities of most of amino acids is influenced on increasing the additives concentration from 10 - 90% as indicative by positive or negative values of ΔR_F for all amino acids. Tryptophan, methionine, L-proline and arginine in $CuSO_4$; leucine, nor-leucine and alanine in $CoCl_2$; arginine in $NiCl_2$; valine, isoleucine, arginine and methionine in LiCl; serine in NaCl; arginine in $NaNO_3$, DL-alanine in Na_2MoO_4; tryptophan and isoleucine in urea and isoleucine in thiourea are the most effected amino acids as indicative by higher magnitude of ΔR_F value. The increase or decrease in R_F value of amino acids on the increase of the concentration of additives in the mobile phase facilitates some new separations of amino acids.

The simultaneous lowering of concentration of SDS from 0.01M and that of additives from 0.1M to 0.001M in the mobile phase (M_{11}–M_{19}) results in the formation of tailed spots with simultaneous reduction in mobility of most of the amino acids. A representative plot of ΔR_F [ΔR_F = {R_F of amino acids in 0.01M SDS plus 0.1M additive (9:1)} – {R_F of amino acids in 0.001 M SDS + 0.001M additive (9:1)}] is shown in **Figure 3.1**. It is clear from **Figure 3.1** that the mobility (R_F) of most of the amino acids is decreased on lowering the concentration of

Table 3.4: List of ΔR_F [ΔR_F = R_F in 0.01 M SDS plus 0.1 M Salt Solution (9:1)] – [R_F in 0.01 M SDS + 0.1 M Salt Solution (1:9)] Values of Amino Acids
Stationary Phase: Alumina

Amino Acid	ΔR_F Value								
	$CuSO_4$	$CoCl_2$	$NiCl_2$	LiCl	NaCl	$NaNO_3$	Na_2MoO_4	Urea	Thiourea
L-valine	+0.06	+0.13	+0.06	+0.36	-0.03	+0.14	-0.04	+0.01	+0.06
DL-valine	+0.03	+0.09	+0.04	+0.37	-0.07	+0.20	-0.05	+0.07	+0.09
L-isoleucine	+0.10	+0.11	-0.02	-0.22	-0.06	+0.05	-0.09	+0.24	+0.23
DL-isoleucine	+0.06	+0.04	0.03	-0.22	-0.03	+0.02	+0.02	+0.24	+0.26
L-arginine	+0.18	-0.06	-0.37	+0.16	+0.02	-0.30	-0.01	+0.09	+0.04
L-arginine monohydrochloride	+0.19	-0.05	-0.08	+0.16	0.00	-0.30	-0.01	+0.08	+0.02
L-tryptophan	+0.26	+0.03	-0.03	+0.07	-0.04	-0.03	+0.03	+0.34	+0.10
DL-tryptophan	+0.14	+0.08	0.00	+0.12	-0.04	-0.06	0.00	+0.30	+0.17
L-methionine	+0.16	0.00	+0.10	+0.28	+0.04	+0.06	-0.04	-0.15	+0.08
DL-methionine	+0.10	-0.01	+0.08	+0.31	+0.06	-0.03	+0.01	-0.05	+0.10
L- cystine	+0.07	-0.03	+0.01	-0.02	-0.01	-0.03	-0.04	+0.02	0.00
L-cystein hydrochloride	-0.02	-0.03	+0.01	-0.04	-0.01	-0.02	-0.03	+0.03	-0.01
L-serine	+0.04	-0.11	+0.10	+0.01	+0.37	-0.08	-0.02	0.02	+0.02
DL-serine	+0.04	-0.11	+0.11	+0.01	+0.33	-0.12	-0.02	0.00	+0.02
DL-nor-leucine	-0.04	-0.17	-0.02	-0.14	0.00	-0.02	-0.05	-0.13	-0.08
L-leucine	+0.02	-0.17	+0.02	-0.03	-0.03	-0.03	-0.02	-0.14	-0.03
DL-alanine	+0.02	-0.23	-0.01	+0.07	+0.01	+0.02	+0.18	-0.13	-0.13
DL-phenylalanine	+0.07	-0.11	-0.02	+0.06	-0.01	+0.07	+0.04	-0.13	-0.14
3-(3,4dihydroxyphenyl)- DL-alanine	+0.1	-0.12	-0.02	+0.07	-0.10	-0.04	+0.05	-0.08	+0.04
L-proline	+0.18	+0.08	+0.06	+0.08	+0.04	+0.12	-0.04	+0.08	+0.06
DL-threonine	+0.09	-0.13	-0.02	-0.10	-0.08	+0.03	-0.03	0.00	-0.08
DL-aspartic acid	+0.02	-0.03	-0.02	0.00	+0.05	0.00	-0.01	-0.02	0.00



—■— ΔR_F =[R_F in 0.01 M SDS plus 0.1 M NaCl (9:1)] - [R_F in 0.001 M SDS plus 0.001 M NaCl(9:1)]

—▲— ΔR_F =[R_F in 0.01 M SDS plus 0.1 M Urea (9:1)] - [R_F in 0.001 M SDS plus 0.001 M Urea(9:1)]

- - ○ - - ΔR_F =[R_F in 0.01 M SDS plus 0.1 M Thiourea (9:1)] - [R_F in 0.001 M SDS plus 0.001 M Thiourea(9:1)]

– ◈ – ΔR_F =[R_F in 0.01 M SDS plus 0.1 M $CoCl_2$ (9:1)] - [R_F in 0.001 M SDS plus 0.001 M $CoCl_2$(9:1)]

Figure 3.1: Plot of Δ R_F Vs Amino Acids

both SDS and additives in the mobile phase as indicative by positive values of ΔR_F. The solvent systems (M_{11}-M_{19}) were found unsuitable from the separation point of view of amino acids.

The lowest possible detectable microgram amounts along with dilution limits of amino acids (given in parenthesis) on alumina layer were L-isoleucine (6.0, 1:1.6x10^2), DL-isoleucine (8.0, 1: 1.2 x10^2), DL-alanine (8.0, 1:1.2x10^2), DL-phenylalanine (5.0, 1:2x10^2) and 3-(3,4-dihydroxyphenyl) DL-alanine (4.0, 1:2.5 x10^2). The present method is superior for sensitive detection of DL-phenylalanine compared to the reported method (31).

An attempt has also been made for the semiquantitative determination of DL-alanine, DL-phenylalanine, 3-(3,4-dihydroxyphenyl) DL-alanine and L-proline by spot-area measurement method. For this purpose, 0.01 mL of standard solutions of these amino acids (10 -150 μg) was spotted on alumina layer. The plates were developed and the spots were detected on TLC plates. The spots obtained were copied directly on tracing paper from the chromatoplates and the spot area was measured. A relationship between the spot area and microgram quantities of amino acids follows the empirical equation ζ = km, where ζ is the spot area, m is the spotted amount and k is constant. The linearity is maintained up to 150 μg/spot of DL-alanine, DL-phenylalanine, 3-(3,4-dihydroxyphenyl) DL-alanine and L-proline. At higher concentration a negative deviation from linear law in all cases was observed. The precision and accuracy was ± 15%. A representative calibration curve for semiquantitative determination of L- proline is shown in **Figure 3.2**.

All the amino acids were clearly detected and reliable separations were achieved on alumina layer developed with several mobile phase systems consisting of SDS and organic as well as inorganic additives.

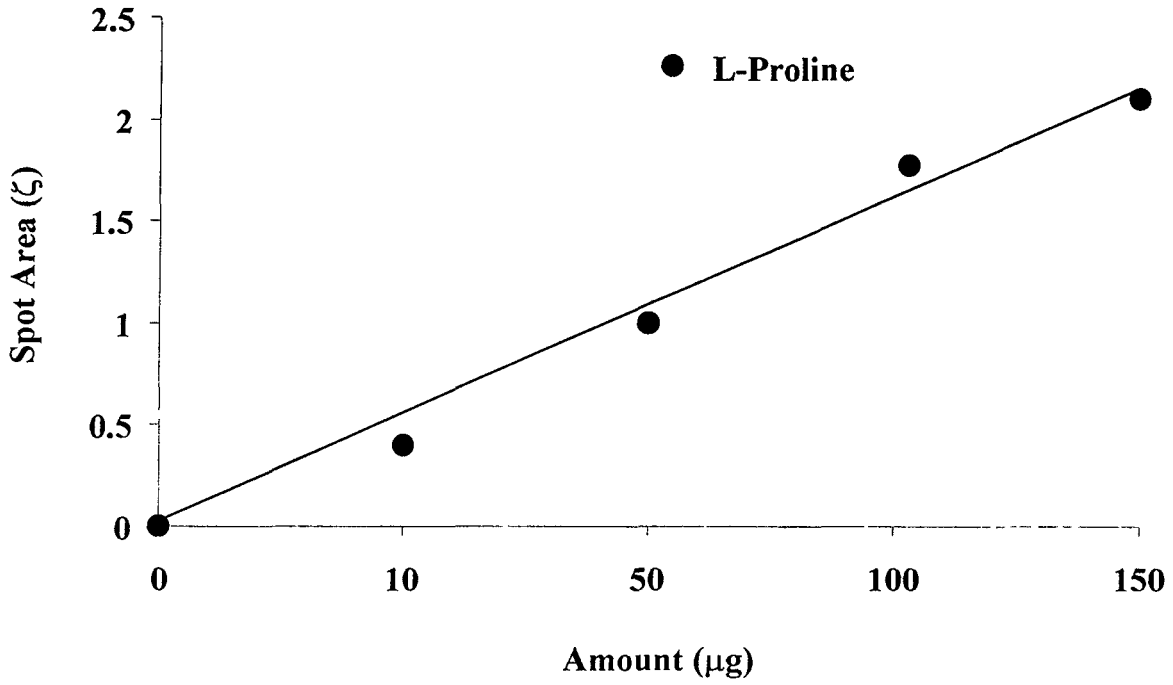


Figure 3.2: Calibration Curve for Semiquantitative Determination of L- Proline

Table 3.5: Experimentally Achieved Separations of Amino Acids on Plain Alumina Layers Developed with Different Mobile Phases

Mobile Phase	Separation (R_F)
0.01 M aq. SDS + 0.1 M aq. $CuSO_4$ (9:1)	L-proline (0.87) — DL-alanine/L-tryptophan/L-leucine or DL-nor-Leucine (0.54)/DL- tryptophan (0.50)/DL-phenylalanine/3-(3,4-dihydroxyphenyl) DL-alanine (0.61)/ L-methionine or DL-methionine (0.33)/DL-threonine (0.13)/DL- aspartic acid/ L-cystine or L-cystein hydrochloride (0.06)/L-serine or DL-serine (0.11)
0.01 M aq. SDS + 0.1 M aq. LiCl (9:1)	L-proline (0.87) — DL-alanine/DL-phenylalanine /L-leucine or DL-tryptophan (0.56)/L- tryptophan (0.60)/L-isoleucine or DL-isoleucine (0.45)/DL-nor-leucine (0.50)/L-arginine or L-arginine monohydrochloride (0.29)/L-cystine or L-cystein hydrochloride/ DL-threonine or DL-aspartic acid (0.06)
0.01 M aq. SDS + 0.1 M aq. urea (9:1)	L-proline (0.80) — L-valine (0.53)/DL-valine (0.58)/L-cystine or L-cystein hydrochloride (0.06)/ L-serine/DL-serine/DL-threonine or DL-aspartic acid (0.10)

The experimentally obtained separations are listed in **Table 3.5**. It is clear from this Table that the developed method is most suitable for selective separation of L-proline from other amino acids. The identification and separation of L-proline is important as it has physiological significance, maintaining the nitrogen equilibrium in man (32) and the growth of chick (33). The high concentration of proline in blood causes renal disease, convulsions and mental retardation.

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Chapter-4

THIN-LAYER MICELLAR CHROMATOGRAPHY OF COINAGE METAL CATIONS

4.1 INTRODUCTION

Mutual Separation of copper ($_{29}$Cu), silver ($_{47}$Ag) and gold ($_{79}$Au), the elements of IB group of the periodic table, is analytically important because of their similar chemical properties. These metals with general configuration (n–1) $d^{10}ns^1$ have a tendency to form complex salts in which the metal can be a complex cation or complex anion. Copper is associated with silver in copper glance $(Cu,Ag)_2S$ ore and hence separation is needed to isolate pure silver from the ore. The presence of silver ions has been found to reduce the rate of adsorption of Au^{3+} from thiourea solution by activated carbon (1), and in other ways also the presence of one metal in small quantities has deleterious effects on performance of other metals. Because of the industrial, commercial and medicinal importance of these metals, several analytical techniques have been developed for the separation and determination of Au^{3+}, Cu^{2+} and Ag^{+} from a variety of matrices. These include ion-exchange chromatography (2,3,4), potentiometry (5,6), capillary zone electrophoresis (7), solvent extraction (8,9), single-sweep oscillopolarography (10), ion-pair – reversed-phase high-performance liquid chromatography (11), size-exclusion chromatography (12), titrimetry (13,14), reversed-phase column chromatography (15,16), reflectance spectroscopy (17), flame or graphite furnace atomic absorption spectrometry (18,19), laser-excited atomic fluorescence spectroscopy (20), neutron activation analysis, ion - pair (21,22) and foam plastic (23) chromatography, in addition to hyphenated techniques, e.g. thin-layer chromatography – spectro-photometry (24), solvent extraction – atomic absorption spectroscopy/ flame atomic absorption spectroscopy (25,26), inductively coupled plasma atomic emission spectroscopy and inductively coupled plasma mass spectroscopy (27,28) and ion-exchange chromatography–photometry (29). Ion-chromatography of Au-cyanide complexes and

problems arising during analysis of gold by titrimetric and spectrophotometric methods has been reviewed (30, 31).

New materials, including chelate-forming plastics containing amino-thiourea (32), VS-II anion-exchange fiber (33), ion-exchange resin loaded with bismuthiol (34), silica gel-bound thia-crown ether (35), silica gel chemically modified with p- dimethylaminobenzylidene-rhodanine (36), chitosan treated with dithiocarbamates (37), and nanofilter membrane (38) have recently been developed and used for preconcentration and separation of gold, silver and copper.

Among the analytical techniques used thin-layer chromatography (TLC), which is being inexpensive and versatile, is still popular among analytical scientists, especially those working in India, China, Japan and European countries. As a result, TLC systems comprising ECTEOLA–cellulose and HCl + NaCl + H_2O for separation of gold, platinum and palladium from associated base metals (39), chitin and aqueous buffer solutions for separation of Cu^{2+} and Ag^{+} (40), alumina and aqueous solutions of both organic and inorganic acids and some sodium salts for rapid separation of Au^{3+} from Te^{4+} and Se^{4+} (41,42) and silica gel containing sodium or ammonium acetate and toluene for separation of Cu^{2+} complex from transition metal complexes (43) have been reported.

The analytical techniques listed above have been successfully used to separate Au^{3+} from either Ag^{+} or Cu^{2+} but the work on the mutual separation of these metal ions from their three-component mixtures is lacking. As far as we are aware no reference is available on the separation of mixtures of Au^{3+}, Cu^{2+} and Ag^{+} by TLC with surfactant-containing mobile phases (or micellar mobile phases). During our previous study (44) on the micellar thin-layer chromatography of heavy metal cations we realized that micellar mobile phases have unique separation capabilities and provide unusual selectivity, enhanced

detection sensitivity and faster analysis. The efficiency of micellar systems for the separation of cations (45) and anions (46) has been reported and reviewed by *Okada* (47). Surprisingly, very little work seems to have been performed on the use of micellar mobile phases in the TLC of inorganic species (48, 49) and none of these studies has examined the separation of metal cations. It was therefore decided to identify novel micellar mobile phases enabling highly selective separations of metal cations. As a result, simultaneous separation of Au^{3+}, Cu^{2+} and Ag^{+} from their mixtures has been achieved on silica layers by use of a buffered anionic micellar mobile phase with added amino acids. The proposed TLC method is selective and rapid, with development times averaging 5 min.

4.2 EXPERIMENTAL

***Chemicals and Reagents*:** Silica gel 'G' (Merck, India); sodium dodecyl sulfate (BDH, India); L-arginine, L-histidine and DL-phenylalanine, phenols, amines and anions (CDH, India); and L-tryptophan (Loba – chemie, India), were used. All reagents were of Analar Reagent grade.

***Metal Cations Studied*:** Fe^{3+}, Cu^{2+}, Ni^{2+}, Co^{2+}, UO_2^{2+}, VO^{2+}, Cd^{2+}, Zn^{2+}, Ag^{+}, Pb^{2+}, Tl^{+}, Bi^{3+}, Hg^{2+}, Al^{3+}, Ti^{4+} and Au^{3+}.

***Test Solutions*:** 1.0% aqueous solutions of following salts were used as test solution:

- Nitrates of Cd^{2+}, Zn^{2+}, Pb^{2+}, Tl^{+}, Bi^{3+}, Al^{3+} and Ag^{+}.
- Chlorides of Ni^{2+}, Co^{2+}, Fe^{3+}, Hg^{2+}, Ti^{4+} and Au^{3+}.
- Sulfates of Cu^{2+}, VO^{2+} and UO_2^{2+}.

All the solutions were prepared in demineralized water with a specific conductivity ($K = 2 \times 10^{-6}$ ohm^{-1} at 25°C). To limit the extent of hydrolysis small quantities of corresponding acid were added to

solutions of the nitrates of lead, silver and bismuth and the chloride of mercury.

Solutions (1%) of anions were also prepared by dissolving the sodium salts of NO_2^-, NO_3^-, MoO_4^{4-} and PO_4^{4-}; the potassium salts of I^-, IO_3^-, IO_4^-, $S_2O_8^{2-}$, $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$, and the ammonium salt of $C_2O_4^{2-}$ in demineralized water. Aqueous solutions (1%) of the potassium and ammonium salts of SCN^- were also prepared. Solutions (1%) of a variety of amines and phenols were prepared in methanol.

Buffer Solutions:

S. No.	Composition	Volume Ratio	pH
1	0.04 M Boric acid — 0.04 M phosphoric acid	50:50	2.3
2	0.02M Boric acid — 0.04M phosphoric acid — 0.24 M NaOH	50:50:8	3.4
3	0.04M Boric acid — 0.04M phosphoric acid — 0.24 M NaOH	50:50:10	5.7
4	0.04M Boric acid — 0.04M phosphoric acid — 0.24M NaOH	50:50:14	7.0
5	0.04M Boric acid — 0.04M phosphoric acid — 0.24M NaOH	50:50:60	11.9

Detection Reagents: The reagents used for detection of the cations were:

- $8x10^{-3}$% (w/v) Dithizone in carbon tetrachloride for Cd^{2+}, Zn^{2+}, Ag^+, Pb^{2+}, Tl^+, Bi^{3+} and Hg^{2+}.
- Aqueous potassium ferrocyanide (1%) for Fe^{3+}, Cu^{2+}, UO_2^{2+}, VO^{2+} and Ti^{4+}.

- Dimethylglyoxime (0.2%) for Ni^{2+} and Co^{2+}.
- Aluminon (1%) for Al^{3+} and
- Yellow ammonium sulphide for Au^{3+}.

Stationary Phase: Silica gel 'G'

***Mobile Phases*:** The following Solvent systems were used as mobile phases

Symbol	Composition
M_1	0.001, 0.005, 0.01 or 0.05 M aqueous SDS
M_2	0.001, 0.005, 0.01 or 0.05 M buffered SDS (pH 2.3)
M_3	0.001, 0.005, 0.01 or 0.05 M buffered SDS (pH 3.4, 5.7, 7.0 or 11.9)
M_4	0.01 M SDS (pH 2.3) + 0.01 M L-arginine (1:9, 3:7, 5:5, 7:3 or 9:1)
M_5	0.01 M SDS (pH 2.3) + 0.01 M DL-phenylalanine (1:9, 3:7, 5:5, 7:3 or 9:1)
M_6	0.01 M SDS (pH 2.3) + 0.01 M L-tryptophan (1:9, 3:7, 5:5, 7:3 or 9:1)
M_7	0.01 M SDS (pH 2.3) + 0.01 M L-histidine (1:9, 3:7, 5:5, 7:3 or 9:1)
M_8	0.001, 0.005 or 0.05 M SDS (pH 2.3) + 0.01 M L-arginine (9: 1)
M_9	0.01 M SDS (pH 2.3) + 0.001 M L-arginine, DL-phenylalanine, L-tryptophan or L-histidine (1:9, 9:1)

***Preparation of TLC Plates*:** Silica gel TLC plates were prepared by the procedure as described in chapter 3.

Preparation of Test Materials: The materials used in separation tests were:

- Gold-plated printed circuit board (GPCB; containing Au, Ni and Cu) from Toyama Electric, Bangalore, India.
- Silver mirror scrap (SMS; containing Ag and Cu) and Silver mirror spent solution (SMSS; containing Ag and Cu), both from Ship Mirror Industries, Bangalore, India.
- High-copper dental amalgam (HCDA; containing Ag, Hg, Cu, Zn and Sn) from the Dental College, A.M.U., Aligarh, India.
- Synthetic sterling silver scrap (SS; containing Ag and Cu).

Peeling: Peeling of silver mirror scrap (specimen surface area 19 cm^2) was performed with concentrated formic acid (90%, w/w) in a glass beaker. The acid was heated at 110°C and the scrap material was added into it. On completion of peeling (within 1 min) the solution was separated and the peeled material was used as the 'source material' for silver.

Leaching: Leaching of silver mirror scrap (specimen surface area 19 cm^2) was performed with 50% nitric acid in a glass beaker. On completion of leaching (within 1 min) the solution was separated from the leached residue and used for silver separation. A similar leaching procedure was used for other silver-containing material (0.153g sterling silver scrap and 0.25g high-copper dental amalgam).

Leaching of gold-plated printed-circuit board (specimen surface area 72.42cm^2 containing 16 large pins and 14 small pins) was performed with aqua-regia in a glass beaker. On completion of leaching the solution was separated from the residue and used for gold separation.

Procedure: For the determination of R_F value of metal ions as described in chapter 3 for amino acids.

Separation: The test solutions (0.01mL) of mixtures of copper, silver and gold were spotted on the TLC plate coated with silica gel 'G' and the chromatography was performed using 0.01M SDS (pH 2.3) + 0.01M L-tryptophan (1:9), or 0.01 M SDS (pH 2.3) + 0.01 M L-histidine (1:9) as mobile phase. The resolved spots for these metal cations were observed on TLC plates after spraying with chromogenic reagents. The R_F values of Au^{3+}, Cu^{2+} and Ag^{+} in their mixture were found to vary marginally from their individual R_F values.

Interference: To investigate interference of inorganic anions, amines and phenols on the R_F values of Au^{3+}, Cu^{2+} and Ag^{+}, an aliquot (0.01mL) of impurity solution was spotted with the mixture (0.01mL) of Au^{3+}, Cu^{2+} and Ag^{+} and chromatography was performed as described above. The spots were detected and the R_F values of separated metal ions were determined.

Limit of Detection: The limits of detection for identification of the cations as described in chapter 2 for amino compounds.

Applications

(i) Chromatography of Unspiked Materials: Chromatography of leachate from unspiked dental amalgam and from a printed circuit board was performed with 0.01 M SDS (pH 2.3) + 0.01 M L-histidine (1:9), as mobile phase. Spots of Ag^{+}, Hg^{2+}, Zn^{2+}, Cu^{2+} and Au^{3+} were detected and the R_F value of each cation was determined.

(ii) Chromatography of Spiked Materials: Spiked samples of PCB, dental amalgam, and silver mirror scrap leachate, silver mirror spent solutions, and sterling silver was prepared as follows:

(a) PCB or dental amalgam solution (1mL) was mixed with silver test solution (1.0%, 1mL) and the chromatography was performed on

0.01mL (1%) of the mixture.

(b) Gold solution (1mL) was added to SMC, SMSS or SS solution (1.0mL of each) and 0.01mL of the mixture was used for chromatographic separation of Au^{3+}, Cu^{2+} and Ag^{+}. The spots were identified by their respective R_F values.

4.3 RESULTS AND DISCUSSION

The results of this study have been summarized in **Figures 4.1 – 4.3** and **Tables 4.1 – 4.6**. The unique features of this study are:

(1) Selection of micellar mobile phases containing an anionic surfactant, sodium dodecyl sulfate (SDS), which is negatively charged and tends to attract positively charged species, including metal cations.

(2) Use of polar (arginine and histidine) and non-polar (phenylalanine and tryptophan) amino acids as additives.

(3) Separation of Au^{3+}, Cu^{2+} and Ag^{+} from their mixtures and investigation of the effects of phenols, cations and anions on the separation of mixtures of Au^{3+}, Cu^{2+} and Ag^{+} ions.

(4) Application of the method to the analysis of several real and synthetic samples to determine the presence of gold, silver and/or copper.

Effect of Concentration and pH of the SDS Solution

Results obtained by use of different concentrations of unbuffered aqueous SDS (M_1) and buffered SDS (M_2 and M_3) solutions reveal the following trends:

(i) Metal ions such as Al^{3+}, Ti^{4+}, VO^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Bi^{3+} and UO_2^{2+} show either no mobility ($R_F = 0.0$) or very little mobility ($R_F \approx 0.05$) at all concentration levels as well as over entire pH range of SDS solutions. A slightly higher mobility

(R_F = 0.30) in the case of Zn^{2+} was observed when 0.05 M SDS solution of pH 2.3 was used as mobile phase.

(ii) Ni^{2+}, Co^{2+} and Tl^{+} produce badly tailed spots ($R_L - R_T > 0.3$) almost with all the mobile phases used with the exception of 0.01 M SDS (pH 2.3). Compared to buffered SDS solutions (pH 3.4, 5.7, 7.0 and 11.9), higher mobility for these cations was realized with SDS solution (pH 2.3).

(iii) Au^{3+} as well-formed spot, always migrates with solvent front ($R_F > 0.9$) regardless the concentration or pH of SDS solution and hence it can be selectively separated from binary mixtures of other metal ions.

(iv) The mobility of certain metals (Cu^{2+}, Zn^{2+}, Ag^{+}, Bi^{3+} and Al^{3+}) was increased marginally on substitution of 0.001 or 0.005 M buffered SDS (pH 2.3) with 0.01 or 0.05 M SDS (pH 2.3).

(v) The development time for 10cm ascent was typically short i.e. 10-12 min for all mobile phase systems used.

The R_F data of metal ions obtained with buffered SDS (pH 2.3) at different concentrations (M_2) levels are compared and presented in **Figure 4.1**. It is clear from this figure that the mobility of metal ions is slightly influenced by the concentration of SDS in the mobile phase.

Effect of Added Amino Acids

As micellar SDS solutions (buffered as well as non-buffered) were found to resolve only limited number of two component mixtures of metal cations, it was decided to improve the separation efficiency of SDS mobile phase system. Several workers have reported (50-54) the improvement in chromatographic efficiency of micellar systems as a result of alteration in micellar properties in the presence of organic and inorganic additives. The additives tested so far include alcohols, diols, dipolar aprotic solvents (DMSO, dioxane), alkylnitriles, alkanes, urea,

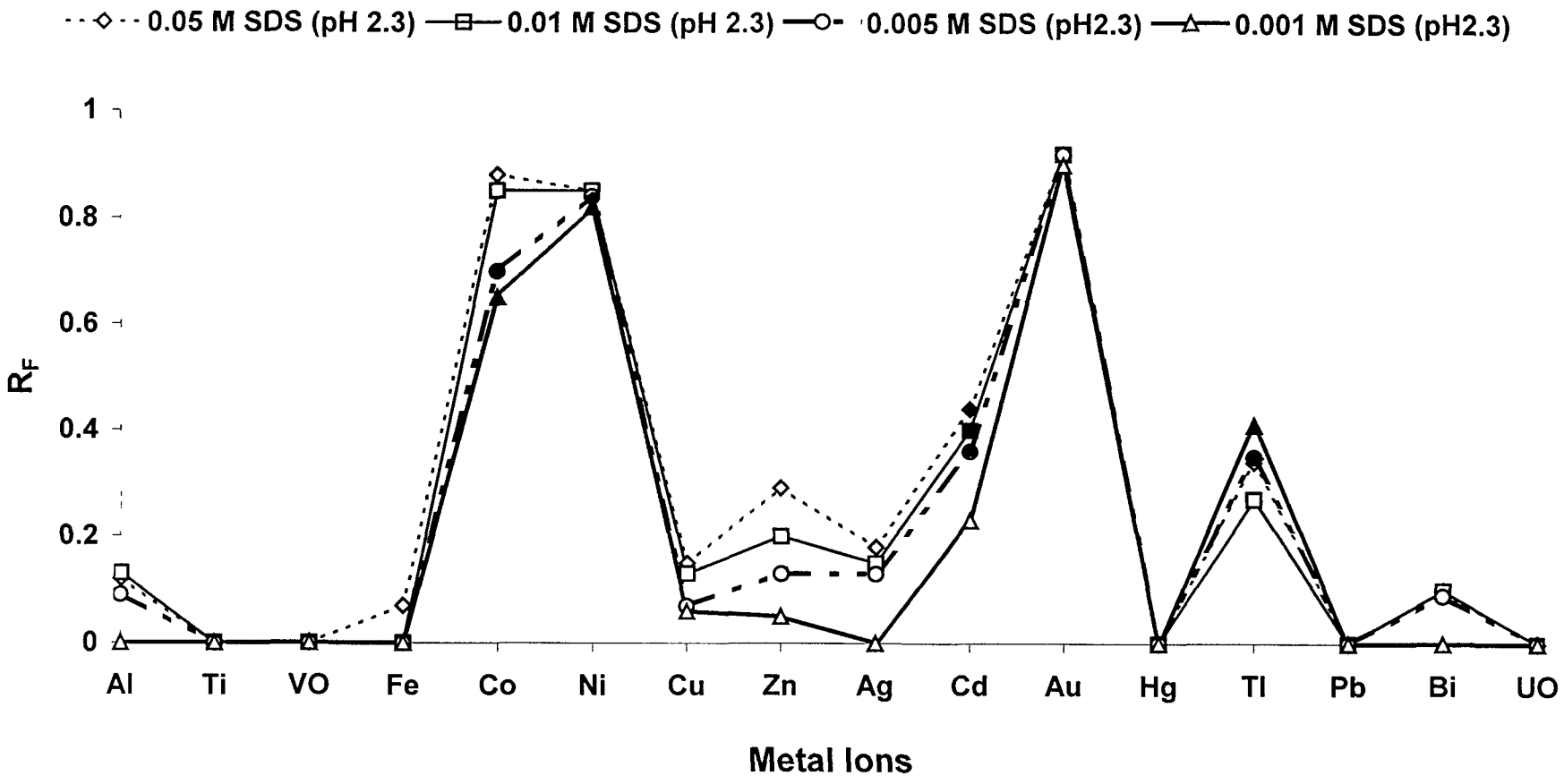

Figure 4.1: Effect of SDS Concentration, at Fixed pH, on the Mobility of Metal Cations
Filled Symbols Represent Tailed Spots

NaCl, and acetone etc. It is surprising that not a single reference is available on the use of amino acids as additives in micellar TLC of inorganics. Amino acids being amphiphilic substances are supposed to provide unique selectivities in the separation of metal cations. Amino acids were chosen as additives for the following reasons:

(i) Amino acids contain both amino ($-NH_2$) and carboxylic acid (R-COOH) functional groups providing nitrogen and oxygen as electron donor centers to coordinate with metals (55).

(ii) Amino acids form complexes with Cu^{2+} (56) and the presence of ionic surfactant in the medium influences the binding of Cu^{2+} with certain amino acids (57). Cu^{2+} and Ag^{+} have greater tendency to form coordination compounds with nitrogen than that with oxygen (58).

(iii) In solution, amino acids exist in the following protonic equilibrium:

$$R\text{-}COOH \Leftrightarrow R - COO^{-} + H^{+}$$

$$R - N^{+}H_3 \Leftrightarrow R - NH_2 + H^{+}$$

Thus, the pH depended protonated (R-COOH and $R\text{-}N^{+}H_3$) and proton acceptor ($R\text{-}COO^{-}$ and $R\text{-}NH_2$) groups may influence the migration trend by modifying the properties of micellar mobile phase.

(iv) The aromatic R-groups of phenylalanine and tryptophan are hydrophobic, a property that has important consequences for the ordering of water molecules in the mobile phase.

In the present study, the mobile phase systems consisting of variable concentrations of amino acids (L-arginine, DL-phenylalanine, L-histidine or L-tryptophan) and buffered SDS (pH 2.3) were formulated by adding required volumes of 0.01 M amino acids into SDS solution (0.001 – 0.05M) in volume ratio of 1:1, 3:7, 7:3, 9:1 and 1:9 keeping total volume constant in each case. The chromatography performed with these mobile phase systems (M_4-M_9) reveals the following trends:

(a) The solvent systems [M_4-M_7 (3:7, 5:5, 7:3)] containing amino acids (arginine, phenylalanine, tryptophan or histidine) at concentration levels of 30 – 70%, produce tailed spots ($R_L - R_T > 0.3$) for all the metal ions except Al^{3+}, Pb^{2+} and Fe^{3+} which stayed at the point of application and Au^{3+} that moved with the solvent front. Thus, the presence of amino acids in SDS containing mobile phases causes tailing in the spots of metal cations probably due to competitive interactions among cations, charged amino acid and anionic SDS. Since the experiments were performed at pH 2.3, the amino acids in this study are supposed to bear a net partial positive charge.

(b) In case of mobile phases composed of either 10% SDS and 90% amino acids [M_4-M_7, (1:9)] or 10% amino acids and 90% SDS [M_4-M_7, (1:9)], number of cations producing tailed spots was decreased as compared to mobile phases discussed above in (a). The results are compared in **Table 4.1**. Compared to mobile phases containing 90% SDS (0.01M, pH 2.3) plus 10% amino acids (0.01M), better separation possibilities were with those containing 10% SDS (2.3 pH) plus 90% amino acids.

(c) Against our hope, arginine (aliphatic amino acid with side chain containing basic group) which has been utilized satisfactorily for resolution of amino acid enantiomers via ligand exchange TLC (59) was found unsuitable for separating metal cations. Similarly, phenylalanine (a non-cyclic aromatic amino acid) was found ineffective to produce satisfactory results. However, heterocyclic aromatic amino acids such as tryptophan and histidine were found capable to provide better-resolved spots of metal cations. On the basis of results presented in **Table 4.1**, the chromatographic performance (or separation efficiency) of amino acids was in the following decreasing order:

Table 4.1: R_F Values of Metal Cations on Silica Gel 'G' Layers Developed with Micellar Mobile Phases Containing Buffered SDS (pH 2.3) and Amino Acids

Metal Ion	0.01M SDS (pH 2.3) + 0.01M L-Arginine			0.01M SDS (pH 2.3) + 0.01M DL-Phenylalanine			0.01M SDS (pH 2.3) + 0.01M L-Tryptophan			0.01M SDS (pH 2.3) + 0.01M L-Histidine		
	9:1	1: 9	5:5	9:1	1: 9	5:5	9:1	1: 9	5:5	9:1	1: 9	5:5
Al^{3+}	0.05	0.05	0.10	0.14	0.05	0.00	0.00	0.00	0.00	0.10	0.00	0.11
Ti^{4+}	0.15	0.30T	0.25T	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20T
VO^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fe^{3+}	0.08	0.07	0.10	0.00	0.25T	0.06	0.06	0.03	0.06	0.00	0.00	0.06
Co^{2+}	0.75	0.70	0.75	0.17T	0.58	0.22T	0.68	0.69	0.50T	0.57	0.55	0.78T
Ni^{2+}	0.65T	0.50T	0.50T	0.17T	0.65	0.40T	0.80T	0.69	0.50T	0.57	0.52	0.80T
Cu^{2+}	0.47T	0.13	0.25	0.05	0.36T	0.15	0.55	0.68	0.29	0.18	0.57	0.25T
Zn^{2+}	0.25T	0.12	0.20T	0.10	0.17	0.09	0.60	0.00	0.25T	0.17	0.05	0.22T
Ag^{+}	0.30T	0.23T	0.20T	0.00	0.05	0.15	0.35T	0.15	0.26T	0.16T	0.05	0.13
Cd^{2+}	0.35T	0.28T	0.30T	0.10	0.18	0.20T	0.17T	0.45T	0.45T	0.19	0.18	0.30T
Au^{3+}	0.90	0.89	0.90	0.41T	0.90	0.70T	0.90	0.90	0.90	0.75	0.95	0.92
Hg^{2+}	0.43T	0.30T	0.22T	0.05	0.17T	0.27T	0.55T	0.45T	0.34T	0.25T	0.56	0.45
Tl^{+}	0.35T	0.25T	0.30T	0.35	0.2T	0.17T	0.20T	0.20T	0.15	0.43	0.25	0.30
Pb^{2+}	0.15	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bi^{3+}	0.25T	0.25T	0.25T	0.00	0.00	0.09	0.00	0.20T	0.19T	0.00	0.00	0.00
VO_2^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

T = Tailed spot ($R_L - R_T > 0.3$)

L-histidine ≥ L-tryptophan > DL-phenylalanine > L-arginine Thus, the micellar solvent systems containing heterocyclic amino acids like L-tryptophan or L-histidine proved superior to solvents containing aromatic or aliphatic amino acids like phenylalanine or arginine for separation of metal cations.

(d) The solvent system consisting of 0.01 M buffered SDS (pH 2.3) and 0.01 M histidine (M_7) or tryptophan (M_6) in 1:9 ratios was found most suitable for separating co-existing Au^{3+}, Cu^{2+} and Ag^{+} ions **(Table 4.2)**.

(e) When chromatography was performed using mobile phases (M_8) obtained by mixing 0.001, 0.005 or 0.05 M SDS (pH 2.3) with 0.01 M amino acid (L-arginine) in 9:1 ratio, less tailed spots for Ni^{2+}, Co^{2+}; slightly higher mobility for Cu^{2+} and Tl^{+} and increased compactness for Zn^{2+} and Cd^{2+} was noticed with the increase in SDS concentration of mobile phase. However, the mobility of Fe^{3+}, Pb^{2+}, Bi^{3+}, Hg^{2+}, Al^{3+}, Ti^{4+}, UO_2^{2+} or VO^{2+} (R_F = 0.0) and Au^{3+} (R_F = 0.9) remained unchanged over the entire SDS concentration range.

(f) When 0.01 M amino acid in the mobile phases M_4-M_7 was substituted with 0.001 M, little increase in mobility of Cu^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Zn^{2+}, and Tl^{+} was noticed with mobile phases containing L-arginine or DL-phenylalanine. However, an opposite trend (i.e. decrease in the mobility) for these metal ions was realized with L-tryptophan containing mobile phase. The mobility of all other metal ions was found to remain unchanged. In the case of L-histidine, mobility of all metal ions remains almost the same irrespective of the concentration of histidine (0.01 or 0.001 M).

In order to provide a clearer picture about the variation of R_F values of the metal ions as a function of concentration of amino acids in the mobile phase, a representative plot of R_F Vs volume fraction of

Table 4.2: Separations Achieved Experimentally on Silica Gel 'G' Layers Developed with Different Mobile Phases

Mobile Phase	Separation (R_F x 100 values) [a)]
0.01 M SDS in 2.3 pH buffer	Ni^{2+} or Co^{2+} (82) – Al^{3+} (10) or Ag^{+} (16)
	Ni^{2+} (82) – Fe^{3+}, Hg^{2+}, VO^{2+}, Ti^{4+}, UO_2^{2+} or Pb^{2+} (00)
	Co^{2+} (82) – Tl^{+} (30) or Cd^{2+} (32)
	Au^{3+}(90) – Ag^{+} (16)
	Au^{3+}(90) – Tl^{+}(30) or Cd^{2+} (32)
	Tl^{+} (30) – Al^{3+}(10)
0.01 M SDS (2.3 pH) + 0.01 M DL- phenylalanine (1:9)	Au^{3+} (92) – Zn^{2+} (17) or Ag^{+} (05)
	Co^{2+} (80) – Zn^{2+} (17)
	Ni^{2+} (80) – Ag^{+} (05)
	Co^{2+} (80) – Bi^{3+}, VO^{2+}, UO_2^{2+} or Pb^{2+} (00)
0.01 M SDS (2.3 pH) + 0.01 M L- tryptophan (1:9)	Au^{3+} (95) – Cu^{2+} (65) – Ag^{+} (05)
	Au^{3+} (95) – Co^{2+} (68) – Fe^{3+} (00)
	Au^{3+} (95) – Ni^{2+} (68) – Zn^{2+} or Pb^{2+} (00)
0.01 M SDS (2.3 pH) + 0.01 M L- histidine (1:9)	Au^{3+} (95) – Cu^{2+}(53) – Ag^{+} (05)
	Au^{3+} (95) – Cu^{2+} (53) – Zn^{2+} (05)
	Au^{3+} (95) – Cu^{2+} (53) – Bi^{3+}, Fe^{3+}, VO^{2+}, UO_2^{2+}, Ti^{4+} or Al^{3+} (00)

[a)] ***The R_F values of metal ions in their mixtures are slightly different from their individual R_F values because of mutual interactions.***

0.01 M L-histidine was constructed **(Figure 4.2)**. The curves shown in **(Figure 4.2)** pass through maxima and minima exhibiting the variation of R_F values (or mobility) of metal cations without any regular pattern. This situation is probably arised due to the formation of occasional tailed spots in certain cases as a result of multiple interactions.

In **Figure 4.3**, the ΔR_F [R_F in M_6 (1:9) minus R_F in M_7 (1:9)] was plotted against metal cations to show the net effect of change in microenvironment of micellar mobile phase on the mobility of metal ions. It is clear from this Figure that metal ions either migrate faster (positive ΔR_F value) in tryptophan containing micellar system or show the same mobility (ΔR_F values $\pm$ 0.05) compared to their mobility in histidine containing micellar system. Thus, an enhanced mobility of cations is possible with micellar systems containing amino acid with large non-polar side chain (e.g. tryptophan) compared to the micellar systems having amino acid with polar side chain (e.g. histidine).

Separation of metal ions obtained on silica layer with micellar systems in the presence and absence of amino acids has been listed in **Table 4.2**. To widen the applicability of the proposed method, the separation of Au^{3+}, Cu^{2+} and Ag^{+} was examined in the presence of organic (phenols, urea, thiourea) and inorganic (cationic and anionic) impurities.

The results presented in **Tables 4.3** and **4.4** indicate that all the impurities are ineffective in bringing about the change in the mobility of Au^{3+} and Ag^{+}. Conversely, the mobility of Cu^{2+} is influenced by the impurities. The variation in R_F value of Cu^{2+} was from 0.30 (o-nitro-phenol impurity) to 0.60 (thiourea impurity). However, the simultaneous separation of Au^{3+}, Cu^{2+} and Ag^{+} from their mixtures was always possible. The poorest separation was in the presence of $K_2S_2O_8$ and ammonium oxalate because of the formation of tailed spots of Cu^{2+}. From the data provided in **Table 4.3**, it seems that the position of

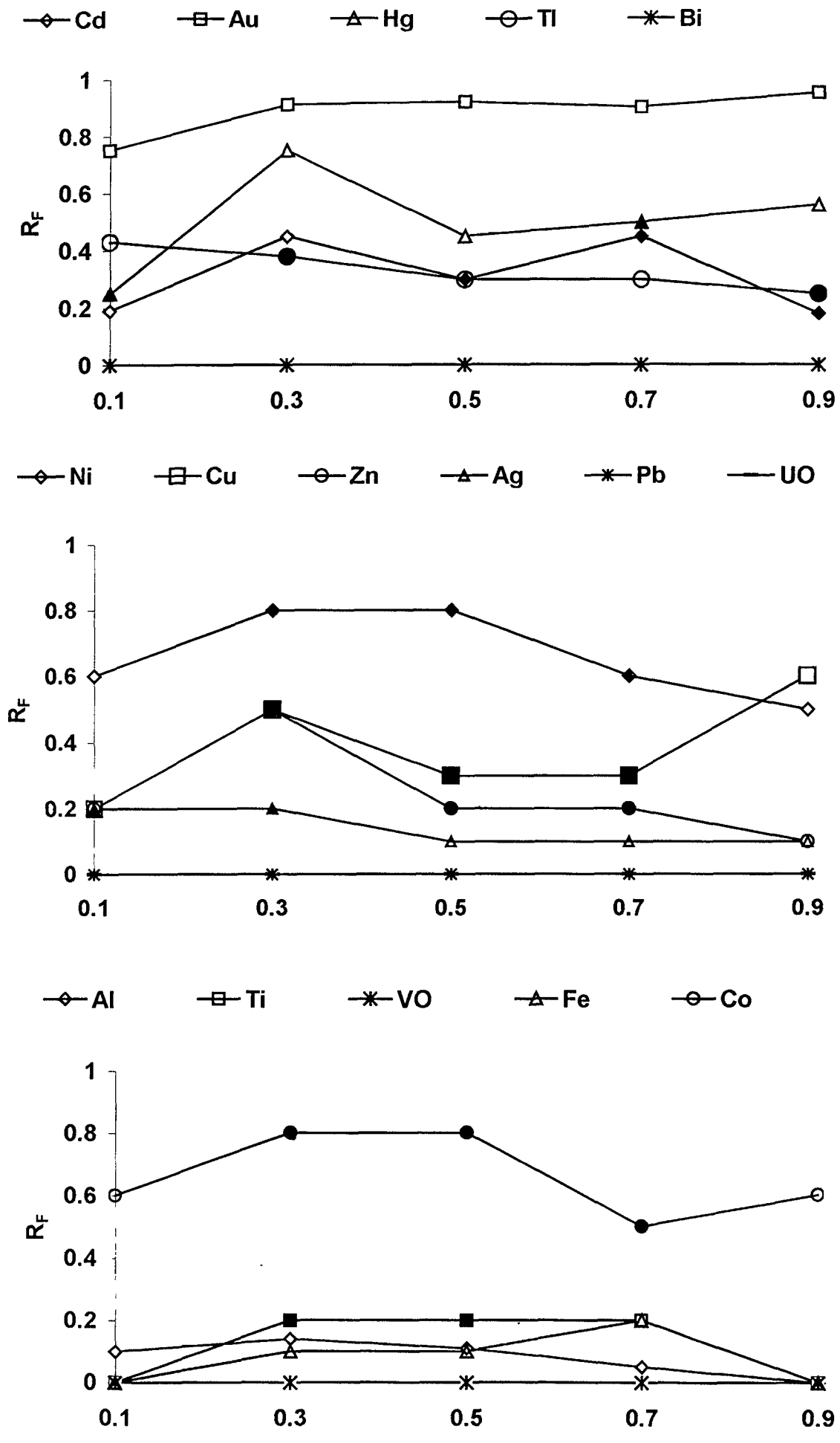

Figure 4.2: Dependence of R_F on Volume Fraction of 0.01 M L-Histidine Filled Symbols Represent Tailed Spots

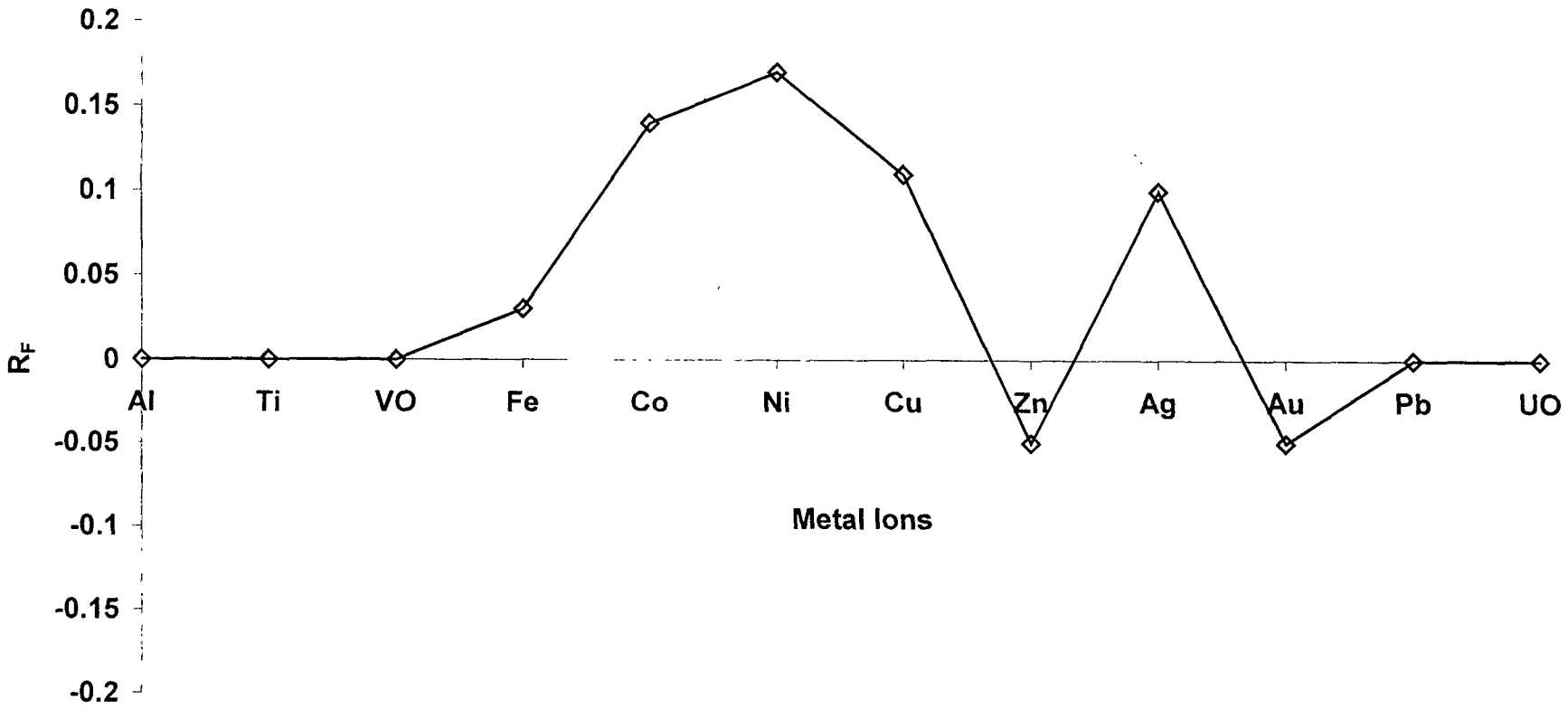

Figure 4.3: Differences, ΔR_F, between R_F Values Obtained for the Metal Cations by Use of Mobile Phases M_6 (1:9), and M_7 (1:9) [$\Delta R_F = R_F \{M_6 (1:9)\} - R_F \{M_7 (1:9)\}$]

Table 4.3: Effect of Organic and Inorganic Impurities on the Separation of Au^{3+}, Cu^{2+} and Ag^{+} on Silica Gel 'G' Layers Developed with 0.01 M SDS (2.3 pH) + 0.01 M L-Histidine (1: 9)

1% Aqueous Solution of Different Impurity	Separation (R_F)		
	Au^{3+}	Cu^{2+}	Ag^{+}
Urea	0.94	0.56	0.05
Thiourea	0.95	0.60	0.05
$Na NO_3$	0.92	0.44	0.00
$Na NO_2$	0.93	0.52	0.00
$Na MoO_4$	0.94	0.44	0.00
NH_4SCN	0.94	0.55	0.05
NaH_2PO_4	0.93	0.58	0.00
KIO_3	0.92	0.50	0.05
KIO_4	0.95	0.44	0.05
KI	0.95	0.55	0.05
K_4 [Fe (CN) $_6$]	0.93	0.52	0.00
K_3 [Fe (CN) $_6$]	0.95	0.52	0.05
KSCN	0.94	0.55	0.05
$K_2S_2O_8$	0.92	0.52T	0.00
Ammonium oxalate	0.95	0.52T	0.00

T = Tailed spot ($R_L - R_T > 0.3$)

Table 4.4: Separation of Au^{3+}, Cu^{2+} and Ag^{+} Ions from their Mixtures, in the Presence of Phenolic Compounds as Impurities on Silica Gel 'G' Layer Developed with 0.01 M SDS (2.3 pH) + 0.01 M L-Histidine (1:9)

Phenolic Compounds as Impurity	Separation (R_F)		
	Au^{3+}	Cu^{2+}	Ag^{+}
Phenol	0.93	0.55	0.05
Phloroglucinol	0.95	0.56	0.06
Pyrogallol	0.94	0.51	0.00
m-Nitrophenol	0.96	0.50	0.00
o-Nitrophenol	0.96	0.30	0.00
p-Nitrophenol	0.95	0.53	0.05
Vaniline	0.95	0.57	0.05
Pyrocatechol	0.95	0.49	0.00
m-Hydroxyacetophenone	0.95	0.52	0.05
Gallic acid	0.95	0.56	0.05
Orcinol	0.93	0.35	0.00
Picric acid	0.96	0.59	0.05
Hydroquinone	0.95	0.55	0.05
Resorcinol	0.93	0.53	0.00
o-Cresol	0.96	0.55	0.05
m-Cresol	0.95	0.53	0.00
p-Cresol	0.92	0.45	0.05

substituent groups in the benzene ring control the mobility of Cu^{2+}. For example, the order of increase in R_F value of Cu^{2+} (given in parenthesis) in the presence of o-, m -, and p-isomers of nitrophenols and cresols was:

o-nitrophenol (0.3) < m-nitrophenol (0.50) < p-nitrophenol (0.53) and

p-cresol (0.45) < m-cresol (0.53) < o-cresol (0.55) .

This reverse order in the mobility of Cu^{2+} can be attributed to the opposite effects of NO_2 (an electron withdrawing group) and CH_3 (an electron releasing group) attached to benzene ring. The ΔR_F values (difference in R_F values of resolved spots from binary mixtures of metal cations) obtained for Au^{3+} – Ag^+, Au^{3+} – Cu^{2+} and Cu^{2+} – Ag^+ pairs on silica gel layer were 0.95, 0.30 and 0.65 respectively with 0.01 M SDS and 0.01 M L-tryptophan (1:9) eluent and 0.95, 0.42 and 0.53 respectively with 0.01 M SDS + 0.01 M L-histidine (1:9) eluent. From these data, it can be safely concluded that SDS – tryptophan system is better for resolving Cu^{2+} – Ag^+ (Δ R_F = 0.65) whereas SDS – histidine system is more useful for resolving Au^{3+} – Cu^{2+} mixture (Δ R_F = 0.42). The detection limit (μg), given in parenthesis for Cu^{2+} (6.6), Ag^+ (1.6), Fe^{3+} (0.76), Zn^{2+} (0.022), Cd^{2+} (0.038), and Hg^{2+} (0.029) indicates that the proposed method is highly sensitive to detect heavy metals at trace levels. Zn^{2+} and Hg^{2+} down to 0.02 and 0.03 (μg) levels respectively can be easily detected on TLC plates.

The data presented in **Table 4.5** clearly demonstrate that the individual solvent systems are not of much practical utility for separation purposes. However, buffered SDS (pH 2.3) in combination of amino acids (histidine/tryptophan), as discussed above has enormous analytical potentialities to facilitate analytically important separations.

The proposed method was successfully applied for identification and separation of Au^{3+}, Cu^{2+}, Ag^+, Ni^{2+} and Hg^{2+} in a variety of matrices. The results are summarized in **Table 4.6**.

Table 4.5: Mobility Trends of Metal Cations on Silica Gel 'G' Layers Developed with Water, Unmodified Micellar Mobile Phases and Amino Acid-Containing Micellar Mobile Phases

Metal Ion	Water	0.01M SDS	0.01M SDS in pH 2.3	0.01M L-Arginine	0.01M DL-Phenylalanine	0.01M L-Tryptophan	0.01M L-Histidine
Al^{3+}	0.00	0.00	0.13	0.00	0.00	0.00	0.00
Ti^{4+}	0.40T	0.00	0.00	0.10	0.00	0.00	0.20 T
VO^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fe^{3+}	0.14	0.00	0.00	0.22	0.22T	0.08	0.12
Co^{2+}	0.30T	0.80T	0.85	0.40T	0.40T	0.19T	0.81T
Ni^{2+}	0.50T	0.80T	0.85	0.45T	0.50T	0.20T	0.83T
Cu^{2+}	0.15	0.00	0.13	0.22	0.22T	0.14	0.12
Zn^{2+}	0.10	0.00	0.20	0.13	0.05	0.05	0.15
Ag^{+}	0.25T	0.15	0.15	0.30T	0.20T	0.30T	0.18T
Cd^{2+}	0.20T	0.14	0.40T	0.15	0.25T	0.17T	0.35T
Au^{3+}	0.90	0.88	0.92	0.85	0.75T	0.77T	0.85
Hg^{2+}	0.50T	0.05	0.00	0.50T	0.35T	0.50T	0.50T
Tl^{+}	0.20T	0.05	0.27	0.18T	0.18T	0.15T	0.25T
Pb^{2+}	0.00	0.00	0.00	0.00	0.00	0.06	0.00
Bi^{3+}	0.40T	0.00	0.10	0.20T	0.15	0.00	0.25T
UO_2^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00

T = Tailed spot ($R_L - R_T > 0.3$)

Table 4.6: Simultaneous Separation of Au^{3+}, Cu^{2+} and Ag^{+} from Real and Spiked Samples on Silica gel 'G' Layers with 0.01 M SDS (pH 2.3) + 0.01 M L-Histidine (1:9), as Mobile Phase

Sample	Separation (R_F)	Sample	Separation of Ions Added to Spiked Sample (R_F)		
			Au^{3+}	Cu^{2+}	Ag^{+}
High-copper dental amalgam (HCDA)	Ag^{+} (0.04) – Cu^{2+}(0.52) Zn^{2+} (0.14) – Cu^{2+}(0.52) Ag^{+}(0.0) – Hg^{2+}(0.57) Zn^{2+}(0.09) – Hg^{2+}(0.55)	High-copper dental amalgam (HCDA)	0.91	0.52	0.05
Printed circuit board (PCB)	Cu^{2+} (0.52) – Au^{3+} (0.91)	Printed circuit board (PCB)	0.92	0.53	0.05
Silver mirror scrap (SMS)	Ag^{+} (0.05) – Cu^{2+} (0.52)	Silver mirror scrap (SMS)	0.92	0.52	0.05
Silver mirror spent solution (SMSS)	Ag^{+} (0.05) – Cu^{2+}(0.53)	Silver mirror spent solution (SMSS)	0.92	0.53	0.05
Sterling silver (SS)	Ag^{+} (0.05) – Cu^{2+}(0.53)	Sterling silver (SS)	0.91	0.52	0.05

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Chapter-5

NOVEL SOLVENT SYSTEM FOR THE SEPARATION OF Cr^{6+} FROM Cr^{3+} AND ASSOCIATED HEAVY METAL CATIONS BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

5.1 INTRODUCTION

Heavy metals have received considerable attention of analysis in recent past because of their physical and environmental importance (1-2). According to our present state of knowledge, metals such as Pb, Cd, Hg, Ni, Cu, Zn, As and Cr^{6+} are toxic and harmful to human health. These metals are capable to form stable complexes with bio-ligands containing oxygen, nitrogen, or sulphur atoms (3) and control several redox processes in living organisms. The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in aquatic life. Industrial waste constitutes the major source of various kinds of metal pollution in aqueous systems. The major sources of chromium to the aquatic environment are electroplating and metal finishing industrial effluents, sewage and wastewater treatment plant discharge, and chromates from cooling water. Chromium exists in several oxidation states (e.g. di-, tri-, penta-, and hexa-) but only Cr^{3+} and Cr^{6+} are biologically important. Chromium in the aquatic environment tends to speciate into Cr^{3+} and Cr^{6+}, with the trivalent ion oxidizing into the hexavalent form or precipitating out of solution.

The various analytical techniques available for the detection, determination and separation of chromium include normal-phase and reversed-phase thin-layer chromatography (4-6), ion-chromatography (7-8), extraction chromatography (9), ion-exchange chromatography (10-11), reversed-phase high-performance liquid chromatography (12-13), micellar electrokinetic chromatography (14), precipitation flotation (15), solid-phase extraction (16), titrimetry (17-18), capillary electrophoresis (19-20), spectrophotometry (21-22)], atomic absorption spectroscopy (23-24), atomic emission spectroscopy (25), neutron activation analysis (26-27), flame atomic absorption spectroscopy (28-29), graphite furnace atomic absorption spectroscopy (30-31) and

hyphenated techniques such as ion-exchange chromatography – flame atomic absorption spectroscopy (32), ion-chromatography – thermal lens spectrometry (33-34), gas chromatography – neutron activation analysis (35), inductively coupled plasma mass spectroscopy (36-37), inductively coupled plasma mass spectroscopy – atomic emission spectroscopy (38-39), ion-exchange chromatography – flame atomic absorption spectroscopy (40), solid-phase extraction – flame atomic emission spectroscopy (41), liquid chromatography – inductively coupled plasma mass spectroscopy (42), high-performance liquid chromatography – inductively coupled plasma mass spectroscopy (43) and ion-chromatography – inductively coupled plasma mass spectroscopy (44).

Of the various separation procedures, thin-layer chromatography (TLC) is probably the most versatile as it can be used for the selective separation of metal cations on micro as well as on a macro scale. The use of high- performance TLC plates has further enhanced the efficiency of this technique. The exhaustive survey of literature of last thirty years (45) shows that tremendous progress has been made in developing rapid and selective TLC methods for the separation of toxic heavy metals (Cu, Ni, Co, Pb, Cd, Zn, Hg, Cr, Fe, and Al) from interfering elements using a variety of acidic developers containing mineral or carboxylic acid as one of the components. The systematic examination of published data on the use of acidic mobile phase systems in the analysis of metal cations demonstrated the following preferential trend about the use of acids:

$HCl > HNO_3 > H_2SO_4 > H_3PO_4 > CH_3COOH > HCOOH >$ other carboxylic acids

The most frequent use of HCl is understandable as it forms chloro-complexes with almost all the heavy metal cations. Perchloric acid has been rarely used. On the other hand, use of formic acid (FA) as eluent in TLC of metal cations has received little attention (46-50) in spite of the facts that (a) it does not permit oxidation of cations during analysis, (b) FA containing developers are less affected by silica gel

properties (51), (c) it provides excellent resolution of aflatoxins (52) and metal cations (46-50) and (d) it is sufficiently acidic [K_a (H_2O) at $25^0C = 1.77 \times 10^{-4}$] to prevent hydrolysis of salts.

All the studies with FA containing eluents were performed using conventional, laboratory made TLC plates. It was therefore decided to utilize the analytical potential of FA as eluent and precoated HPTLC silica plates as stationary phase in the analysis of heavy metal cations. As a result several analytically important separations of heavy metals were realized. The separation of different valency states of chromium is industrially important as Cr^{3+} is converted to Cr^{6+} in alkaline peroxide media.

5.2 EXPERIMENTAL

Chemicals and Reagents: Silica gel 60 F_{254} 'HPTLC' plates (Merck, Darmstadt, Germany); dimethylamine (s.d.fine chemicals Ltd, India); dimethylaniline, inorganic salts, amines and phenols (CDH, India); o-aminophenol (Loba Chemie, India); formic acid (Merck, India), methanol and acetone (Qualigens, India) were used. All the reagents used were of Analytical Reagent grade.

Test Solutions: Standard aqueous solutions (1.0%) of the chloride, nitrate or sulfate salts of Ni^{2+}, Co^{2+}, Hg^{2+}, Cr^{3+}, Cd^{2+}, Pb^{2+}, Tl^{+}, Bi^{3+}, Al^{3+}, Ag^{+}, VO^{2+} and Cr^{6+} were used as test solutions.

Detection Reagents: Conventional chromogenic reagents as mentioned in chapter 4 were used for the detection purposes of metal ions. Cr^{6+} was detected with saturated alcoholic solution of $AgNO_3$ and Cr^{3+} with 1% methanolic solution of alizarin red 'S'.

Stationary Phase: Silica gel 60 F_{254}'HPTLC' plates

Mobile Phases: The following solvent systems were used as mobile phase

Symbol	Composition
M_1	Methanol (MeOH)
M_2	Dimethylamine (DMA)
M_3	Formic acid (FA)
M_4	Acetone
M_5	Dimethylaniline (DMAL)
M_6	ortho-Aminophenol (o- APH)
M_7	Methanol + dimethylamine (8:2)
M_8	Methanol + formic acid (8:2)
M_9	Water + dimethylamine (8:2)
M_{10}	Water + formic acid (8:2)
M_{11}	Dimethylamine + methanol + formic acid (2:8:2)
M_{12}	Dimethylamine + methanol + formic acid (4:8:2)
M_{13}	Dimethylamine + methanol + formic acid (8:8:2)
M_{14}	Dimethylamine + methanol + formic acid (10:8:2)
M_{15}	Dimethylamine + acetone + formic acid (2:8:2)
M_{16}	Dimethylamine + acetone + formic acid (4:8:2)
M_{17}	Dimethylamine + acetone + formic acid (8:8:2)
M_{18}	Dimethylamine + acetone + formic acid (10:8:2)
M_{19}	Dimethylamine + water + formic acid (2:8:2)
M_{20}	Dimethylamine + water + formic acid (4:8:2)
M_{21}	Dimethylamine + water + formic acid (8:8:2)
M_{22}	Dimethylamine + water + formic acid (10:8:2)
M_{23}	Dimethylaniline + methanol + formic acid (2:8:2)
M_{24}	Dimethylaniline + methanol + formic acid (4:8:2)
M_{25}	Dimethylaniline + methanol + formic acid (8:8:2)
M_{26}	Dimethylaniline + methanol + formic acid (10:8:2)
M_{27}	o-Aminophenol + methanol + formic acid (2:8:2)
M_{28}	o-Aminophenol + methanol + formic acid (4:8:2)
M_{29}	o-Aminophenol + methanol + formic acid (8:8:2)
M_{30}	o-Aminophenol + methanol + formic acid (10:8:2)

Procedure: The chromatography of heavy metal cations was performed following the procedure as described for amino compounds in chapter 2.

Separation: The test solution (0.01 mL) containing metal ions to be separated were spotted on HPTLC plates and the chromatography was performed using various mobile phases. The resolved spots for these metal cations were observed on HPTLC plates after spraying chromogenic reagents and the R_F values of the separated metal ions were determined.

Interference: For investigating the interference of inorganic ions, phenols and surfactants on the R_F values (mobility) of Cr^{6+} and Cr^{3+}, an aliquot (0.01mL) of impurity solution was spotted with each metal ion as mixture on HPTLC plate and chromatography was performed as described above. The spots were detected and the R_F values of separated metal ions were determined.

Effect of pH: Effect of pH on the mutual separation of Cr^{6+}, Ni^{2+} and Co^{2+} was investigated by adding required amount of acid to the mixture of the three metals and subjected to HPTLC process as has been described earlier.

Limit of Detection: The detection limits of Ni^{2+}, Co^{2+}, Cu^{2+} and Pb^{2+} were determined by the procedure as described in chapter 2 for amino compounds.

Semiquantitative Determination by Spot–Area Measurement: For semiquantitative determination by sport- area measurement method, 0.01 mL volumes from a series of standard solutions (0.5 – 2.0%) of Ni^{2+} and Cr^{6+} were spotted on HPTLC plates. The plates were developed with M_7 [methanol + dimethylamine (8:2)] and M_{13} [dimethylamine + methanol + formic acid (8:8:2)]. After detection the spots were copied onto tracing paper from the chromatoplates and then the area of each spot was calculated.

***Semiquantitative Determination by Visual Comparison*:** Aliquot (0.01 mL) of standard solutions of different concentrations (0.5 – 2.0%) of potassium dichromate were spotted on HPTLC plates alongwith the spotting of 0.01mL of industrial wastewater sample. After completing the chromatographic process, the color intensity and the R_F value of analyzed industrial wastewater sample was matched with the colored spots of standard reference solutions of potassium dichromate. The amount of chromium present in the industrial sample was determined according to color intensity of its spot on HPTLC plate after visual comparison with the color intensities of standard solutions.

***Chromatography of Spiked Wastewater Samples*:** The spiked samples of industrial wastewater were prepared as follows:

(a) Wastewater sample (sample 1) containing Cr^{6+} was spiked with aqueous solution of Cr^{3+} (1%) in 1:1, v/v ratio. About 0.01 mL of the resultant spiked sample was subjected to HPTLC on silica layers using M_7 as mobile phase and R_F values of the resolved spots of Cr^{6+} and Cr^{3+} were determined.

(b) Industrial wastewater samples (samples 2 and 3) containing Ni^{2+} were spiked with aqueous solutions of Cr^{6+} (1%) and Cr^{3+} (1%) in 1:1:1, v/v ratio and 0.01 mL of the resultant sample was subjected to chromatography using M_{13} as mobile phase. The R_F values of resolved spots of Ni^{2+}, Cr^{6+} and Cr^{3+} were determined.

5.3 RESULTS AND DISCUSSION

The results of the present study have been summarized in **Tables 5.1 – 5.7** and **Figures 5.1 – 5.3**. The mobility of metal cations obtained with pure single-component organic mobile phases (M_1-M_6), two-component mixed organic (M_7-M_8) and aqueous – organic (M_9-M_{10}) solvent systems has been summarized in **Table 5.1**. It is clear from **Table 5.1** that only formic acid containing mobile phases (M_3, M_8

Table 5.1: Mobility (R_F Value) of Heavy Metal Ions on HPTLC Silica Gel Layer using Single-Component (M_1-M_6), Two-Component Organic (M_7, M_8) and Two-Component Aqueous – Organic (M_9, M_{10}) Mobile Phase Systems

Metal Ion	Mobile Phase									
	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9	M_{10}
VO^{2+}	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00
Cr^{6+}	0.34	0.86*	0.72	0.00, 0.97[a]	0.01	0.00	0.86	0.63	0.85	0.78
Ag^{+}	0.00	0.00	0.15	0.00	0.03	0.02	0.00	0.15	0.00	0.09
Hg^{2+}	*0.45T*	*0.00*	*0.85*	*0.00*	*0.10*	*0.02*	*0.30*	*0.82*	*0.04*	*0.94*
Pb^{2+}	0.00	0.00	0.68	0.00	0.00	0.00	0.00	0.49	0.10	0.93
Cd^{2+}	0.08	0.00	0.78	0.00	0.05	0.05	0.02	0.72	0.00	0.94
Tl^{+}	0.10	0.00	0.82	0.00	0.00	0.00	0.05	0.76	0.00	0.78
Ni^{2+}	0.05	0.00	0.87	0.00	0.00	0.04	0.02	0.81	0.00	0.84
Co^{2+}	0.04	0.06	0.75	0.00	0.00	0.03	0.04	0.68	0.00	0.88
Bi^{3+}	0.09	0.00	0.16	0.09	0.02	0.40T	0.06	0.16	0.04	0.23
Cr^{3+}	0.05	0.05	0.70	0.05	0.05	0.05	0.05	0.60	0.05	0.75

[a] ***Double spot, T = Tailed spot (R_L-R_T >0.3), *Detection clarity is poor***

and M_{10}) is capable to induce the migration of metal cations. Cr^{6+} is the exception, which shows higher mobility with certain mobile phases (M_1-M_4, M_7 and M_9) in the absence of formic acid. The use of pure acetone as mobile phase (M_4) resulted in the formation of double spots of Cr^{6+}. The peculiar behavior of formic acid as tailing reducer and mobility activator opens new separation opportunities of metal cations. Keeping this in mind, we added a third component methanol, acetone or water in the mixture of amines and formic acid. The resultant three-component mobile phase systems (M_{11}-M_{26}) were investigated as developer in TLC of metal cations. Amines were selected because of our past experience (52) as they provide highly compact and well-resolved spots of metal cations on silica layer.

The results obtained with mixed aqueous – organic solvent systems (M_{11}-M_{22}) containing different concentrations of DMA and fixed concentrations of FA and organic modifier (M_{11}-M_{18}) or water (M_{19}-M_{22}) are tabulated in **Table 5.2**. On the basis of these results, the metal cations can be grouped into following three categories:

(a) Metal cations such as VO^{2+}, Ag^{+} and Bi^{3+} are strongly retained by the stationary phase and remain near the point of application irrespective of the concentration of dimethylamine and the nature of the organic modifier (methanol or acetone).

(b) Metal cations such as Cr^{3+} and Cr^{6+} showed an increase in the mobility with the increase of dimethylamine concentration in the mobile phase irrespective of the fact either the mobile phase contains methanol (M_{11}-M_{14}), acetone (M_{15}-M_{18}) or water (M_{19}-M_{22}) in combination with formic acid and dimethylamine.

Table 5.2: Migration Behavior of Heavy Metal Cations on Precoated HPTLC Silica Gel Plates using Dimethylamine, Formic Acid and/or Acetone, Methanol, Water in Various Volume Ratios as Mobile Phase Systems

	Mobile Phase											
Metal Ion	**M_{11}**	**M_{12}**	**M_{13}**	**M_{14}**	**M_{15}**	**M_{16}**	**M_{17}**	**M_{18}**	**M_{19}**	**M_{20}**	**M_{21}**	**M_{22}**
VO^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.05	0.02	0.00	0.00
Cr^{6+}	0.62	0.65	0.91	0.92	0.55	0.63	0.68	0.90	0.53	0.53	0.59	0.89
Ag^{+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.11	0.00
Hg^{2+}	0.82	0.72	0.62	0.52	0.88	0.86	0.79	0.76	0.95	0.59	0.17	0.10
Pb^{2+}	0.63	0.52	0.28	0.15	0.84	0.81	0.77	0.00	0.86	0.57	0.02	0.00
Cd^{2+}	0.89	0.66	0.42	0.29	0.84	0.80	0.78	0.76	0.93	0.84	0.26	0.09
Tl^{+}	0.86	0.78	0.13	0.10	0.78	0.61	0.09	0.04	0.78	0.64	0.35	0.02
Ni^{2+}	0.87	0.68	0.44	0.25	0.77	0.68	0.56	0.42	0.93	0.84	0.23	0.00
Co^{2+}	0.81	0.66	0.05	0.00	0.73	0.67	0.42	0.00	0.89	0.81	0.35	0.00
Bi^{3+}	0.15	0.08	0.00	0.00	0.14	0.13	0.05	0.00	0.15	0.11	0.06	0.03
Cr^{3+}	0.60	0.68	0.90	0.90	0.53	0.75	0.70	0.90	0.50	0.55	0.60	0.86

(c) Metal cations such as Hg^{2+}, Pb^{2+}, Cd^{2+}, Tl^{+}, Ni^{2+} Co^{2+}, and Bi^{3+} showed decrease in R_F with the increase of dimethylamine concentration in the mobile phase systems (M_{11}-M_{22}).

Thus, these mobile phase systems facilitate the selective separation of several metal cations by virtue of their variable mobility trends. For example, Cr^{3+} and Cr^{6+} can be selectively separated from all other metal cations using M_{13}, M_{14}, M_{18}, M_{21} and M_{22} because of their higher mobility compared to other metal cations in these mobile phases. Similarly, M_{13} and M_{18} can be utilized for achieving an analytically important Ni^{2+}– Co^{2+}– Cr^{6+} separation from their mixtures. This separation could not be achieved with pure organic (M_1-M_6) and two-component mixed organic (M_7, M_8) as well as mixed aqueous – organic (M_9, M_{10}) mobile phase systems.

In order to examine the effect of nature of amino compounds on the mobility of metal cations, dimethylamine was replaced by dimethylaniline (M_{23}-M_{26}) and ortho-aminophenol (M_{27}-M_{30}) in the solvent systems (M_{11}-M_{14}) maintaining the volume ratio of methanol and formic acid the same. The resultant mobile phase systems (M_{23}-M_{30}) were used to determine the R_F value of metal cations. The results obtained are encapsulated in **Table 5.3**. It is clear from **Table 5.3** that the nature of amino compound has pronounced effect on the R_F value (or mobility) of metal cations. VO^{2+}, Ag^{+} and Bi^{3+} show little mobility with all the mobile phase systems (M_{23}-M_{30}).

The following changes in R_F values of the cations were noticed due to the substitution of dimethylamine with dimethylaniline:

(a) R_F value of Cr^{6+} drops from 0.91 (M_{13}) and 0.92 (M_{14}) to 0.07 (M_{25}) and 0.05 (M_{26}).

(b) R_F value of Pb^{2+} decreases from 0.52 (M_{12}) to 0.27 (M_{24}).

Table 5.3: R_F Values of Metal Cations on HPTLC Silica Plates with Mixed Three-Component Mobile Phase Systems Consisting of Methanol, Formic Acid and Dimethylaniline or o-Aminophenol in Different Volume Ratio

Metal Ion	Mobile Phase							
	M_{23}	M_{24}	M_{25}	M_{26}	M_{27}	M_{28}	M_{29}	M_{30}
VO^{2+}	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Cr^{6+}	0.60	0.56	0.07	0.05	0.41	0.81	0.83	0.86
Ag^{+}	0.15	0.15	0.12	0.05	0.15	0.12	0.04	0.02
Hg^{2+}	0.72	0.66	0.56	0.42	0.80	0.64	0.52	0.40
Pb^{2+}	0.61	0.27	0.15	0.06	0.25	0.25	0.30	0.32
Cd^{2+}	0.48	0.39	0.28	0.15	0.55	0.65	0.75	0.85
Tl^{+}	0.85	0.80	0.62	0.41	0.70	0.60	0.48	0.32
Ni^{2+}	0.72	0.64	0.52	0.16	0.64	0.87	0.90	0.92
Co^{2+}	0.70	0.52	0.42	0.30	0.60	0.64	0.70	0.76
Bi^{3+}	0.16	0.10	0.09	0.07	0.15	0.17	0.17	0.18
Cr^{3+}	0.60	0.55	0.10	0.05	0.40	0.60	0.80	0.82

(c) R_F value of Cd^{2+} decreases from 0.89 (M_{11}), 0.66 (M_{12}), 0.42 (M_{13}) and 0.29 (M_{14}) to 0.48 (M_{23}), 0.39 (M_{24}), 0.28 (M_{25}) and 0.15 (M_{26}).

(d) R_F value of Tl^{+} increases form 0.13 (M_{13}) and 0.10 (M_{14}) to 0.62 (M_{25}) and 0.41 (M_{26}).

(e) R_F value of Cr^{3+} decreases from 0.90 (M_{13}, M_{14}) to 0.10 (M_{25}) and 0.05 (M_{26}).

Similarly, on the substitution of dimethylaniline in M_{11}-M_{14} by o-aminophenol caused a decrease in R_F value of Pb^{2+} from 0.63 (M_{11}) and 0.52 (M_{12}) to 0.25 (M_{27}, M_{28}), whereas the R_F of Cd^{2+} increased from 0.29 (M_{14}) to 0.85 (M_{30}). The R_F values of Ni^{2+} and Co^{2+} from 0.00 (M_{14}) to 0.92 and 0.76 (M_{30}) respectively. A decrease in R_F value of Cr^{3+} from 0.60 (M_{11}) to 0.40 (M_{27}) was also observed.

It is clear form above observations that amine – methanol – formic acid systems have enormous analytical potentiality for achieving selective separations of heavy metal cations form their multi-component mixtures because the nature of the added amine has profound influence on the mobility of cations. Some separations of metal cations achieved experimentally using different mobile phase systems have been encapsulated in **Table 5.4**.

Tables 5.5 and **5.6** summarize the effect of various inorganic ions, surfactants and phenolic impurities on the separation of co-existing Cr^{6+} and Cr^{3+}. It is evident from **Table 5.5** that inorganic ions bring about a marginal change in the mobility of Cr^{6+} without influencing the mobility of Cr^{3+}. Thus, the separation is possible in all cases. Amongst heavy metal ions, Hg^{2+} and Al^{3+} influence the mobility of Cr^{6+} resulting in the formation of tailed spot. A significant lowering in R_F value of Cr^{6+} was noticed in the presence of MoO_4^{2-} where R_F value is decreased from 0.85 to 0.71. A tailed spot of Cr^{6+} is also

Table 5.4: Experimentally Achieved Separations of Heavy Metal Cations on Silica Gel HPTLC Plates Developed with Different Mobile Phase Systems

Mobile Phase	Separation (R_F)
FA	Hg^{2+} or Ni^{2+} (0.86)/Cd^{2+} or Co^{2+}(0.75)/Cr^{6+} or Cr^{3+} (0.71)/Pb^{2+} (0.67) – V^{2+} (0.00)/Bi^{3+}(0.15)
MeOH + DMA (8:2)	Cr^{6+}(0.85) – Cr^{3+} (0.05)
	Cr^{6+} (0.85) – $V0^{2+}$, Ag^{+} or Pb^{2+} (0.00)/Cd^{2+} or Ni^{2+} (0.02)/Co^{2+}, Tl^{+} or Bi^{3+} (0.05)
MeOH + FA(8:2)	Hg^{2+} or Ni^{2+} (0.81)/Cd^{2+} (0.71)/Tl (0.75)/Cr^{6+} or Cr^{3+} (0.76) – Bi^{3+} or Ag^{+} (0.15)/VO^{2+} (0.00)
Water + FA (8:2)	Hg^{2+}, Pb^{2+}or Cd^{2+}(0.93)/Ni^{2+}or Co^{2+}(0.86)/Cr^{6+}or Cr^{3+}(0.76)– Bi^{3+}(0.21)/Ag^{+}(0.10)/VO^{2+}(0.00)
DMA + MeOH + FA (8:8:2)	Cr^{6+} (0.90) – Ni^{2+}(0.45) – Co^{2+}(0.00)
	Cr^{6+} or Cr^{3+} (0.90) – Hg^{2+} (0.60)/Cd^{2+} – $V0^{2+}$, Ag^{+} or Bi^{3+} (0.00)
DMA + acetone + FA (2:8:2)	Hg^{2+} (0.88)/Pb^{2+} or Cd^{2+} (0.85)/Tl^{+} or Ni^{2+} (0.78) – Cr^{6+} or Cr^{3+} (0.54) – Bi^{3+} (0.15)/VO^{2+} or Ag^{+} (0.0)
DMA + acetone + FA (10:8:2)	Cr^{6+} (0.90) – Ni^{2+}(0.40) – Co^{2+}(0.00)
	Cr^{6+} or Cr^{3+} (0.90)/Hg^{2+} or Tl^{+} (0.75) – VO^{2+}, Ag^{+}, Pb^{2+} or Bi^{3+} (0.00)
DMA + water + FA (10:8:2)	Cr^{6+} or Cr^{3+} (0.87) – Hg^{2+} or Cd^{2+} (0.10)/VO^{2+}, Ag^{+}, Pb^{2-}, Ni^{2+}, or Co^{2+} (0.00)
o-APH + MeOH + FA (10:8:2)	Ni^{2+}(0.92)/Cr^{6+} or Cd^{2+} (0.85) – Hg^{2+} (0.39)/Pb^{2+} or Tl^{+} (0.33) – Bi^{3+} (0.16)/VO^{2+} (0.05) or Ag^{+} (0.02)

Table 5.5: Effect of Inorganic Impurities on the Separation of Co-existing Cr^{6+} and Cr^{3+} from Their Mixture on Silica HPTLC Plates Developed with M_7 [Methanol + Dimethylamine (8:2, v/v)]

Impurities	Separation (R_F)	
	Cr^{6+}	Cr^{3+}
Ni^{2+}	0.83	0.05
Co^{2+}	0.83	0.05
Cd^{2+}	0.78	0.05
Zn^{2+}	0.79	0.05
Ag^{+}	0.84	0.05
Pb^{2+}	0.84	0.05
Tl^{+}	0.88	0.05
Bi^{3+}	0.78	0.05
Hg^{2+}	0.80T	0.05
Al^{3+}	0.80T	0.05
$NaNO_2$	0.75	0.05
$NaNO_3$	0.79	0.05
NaH_2PO_4	0.79	0.05
KI	0.81	0.05
KIO_3	0.90	0.05
KBr	0.67T	0.05
$K_3[Fe(CN)_6]$	0.81	0.05
$K_4[Fe(CN)_6]$	0.86	0.05
NH_4SCN	0.81	0.05
$(NH_4)_2MoO_4$	0.71	0.050
Without impurit	0.85	0.05

T = Tailed spot ($R_L - R_T > 0.30$)

Table 5.6: Effect of Surfactants and Phenolic Impurities on the Separation of Cr^{6+} and Cr^{3+} from Their Mixture on Silica HPTLC Plates Developed with M_7 [Methanol + Dimethylamine (8:2, v/v)]

Impurities	Separation (R_F)	
	Cr^{6+}	Cr^{3+}
Sodium dodecyl sulphate (SDS)	0.67T	0.05
N-Cetyl-N,N,N-trimethyl ammonium bromide (CTAB)	0.64T	0.05
Polyoxyethylene dodecyl ether (Brij- 35)	0.75T	0.05
Polyoxyethylene (20) cetyl ether (Brij- 58)	0.67T	0.05
Polyoxyethylene (20) stearyl ether (Brij- 78)	0.70T	0.05
Polyoxyethylene (20) oleyl ether (Brij- 98)	0.67T	0.05
Polyoxyethylene (20) sorbitan monolaurate (Cween- 20)	0.75	0.05
Polyoxyethylene (4) sorbitan monopalmitate (Cween- 40)	0.67T	0.05
Sorbitane monostearate (Span- 60)	0.69T	0.05
Orcinol	0.80	0.05
Resorcinol	0.76	0.05
Pyrocatechol	0.80	0.05
Phloroglucinol	0.83	0.05
Pyrogallol	0.81	0.05
o-Aminophenol	0.80	0.05
m-Aminophenol	0.86	0.05
p-Aminophenol	0.50T	0.05
Without impurity	0.85	0.05

T = Tailed spot ($R_L - R_T > 0.30$)

formed in the presence of Br^-. The results presented in **Table 5.6** clearly demonstrate that the effect of surfactants is similar to that of inorganic ions on the mobility of chromium ions. Mobility of Cr^{3+} remains unaffected whereas the mobility of Cr^{6+} is marginally changed. The compactness of Cr^{6+} spot is converted into elongated shape in the presence of surfactant irrespective of the nature of the surfactant (anionic, cationic or non-ionic). However, the separation of Cr^{6+} from Cr^{3+} is always possible. Phenolic impurities were also found to influence the mobility of Cr^{6+} slightly whereas the mobility of Cr^{3+} remained unaffected. The separation of Cr^{6+} from Cr^{3+} is possible in the presence of all phenols except p-aminophenol, which cause significant tailing in Cr^{6+} and hampers its separation from Cr^{3+}.

Effect of pH on the R_F values of Ni^{2+}, Co^{2+} and Cr^{6+} and on the mutual separation of Ni^{2+}- Co^{2+}- Cr^{6+} has been shown in **Figure 5.1**. It is clear from the figure that the best separation of Ni^{2+}, Co^{2+} and Cr^{6+} from their mixture can be obtained in the pH range 2.5 – 3.5. At pH 1.5, the mobility of Ni^{2+} is reduced such that it can not be separated from Cr^{6+} and Co^{2+}. At pH 6.0, the R_F value of Ni^{2+} is increased and hence its separation from Cr^{6+} and Cr^{3+} is poor. At pH 8.0, mobility of Ni^{2+} is increased much higher than that of Cr^{6+} giving poor separation from the mixture of Cr^{6+}, Ni^{2+} and Co^{2+}.

The lowest detectable microgram amounts along with dilution limits (given in parenthesis) of metal ions achieved on HPTLC plates developed with M_7 solvent system were for Ni^{2+}(0.028, 1: 3.5 x 10^5), Co^{2+}(0.11, 1: 9 x 10^4), Cu^{2+}(2.09, 1:4.784 x 10^3) and Pb^{2+}(0.08, 1:1.25 x 10^5). It is clear from these data that the proposed method is highly sensitive for the detection of the cations.

In addition to the qualitative analysis, a quantitative evaluation of the metal ions is often required to ascertain the level of the toxic metals in environment samples. A relatively less accurate but the

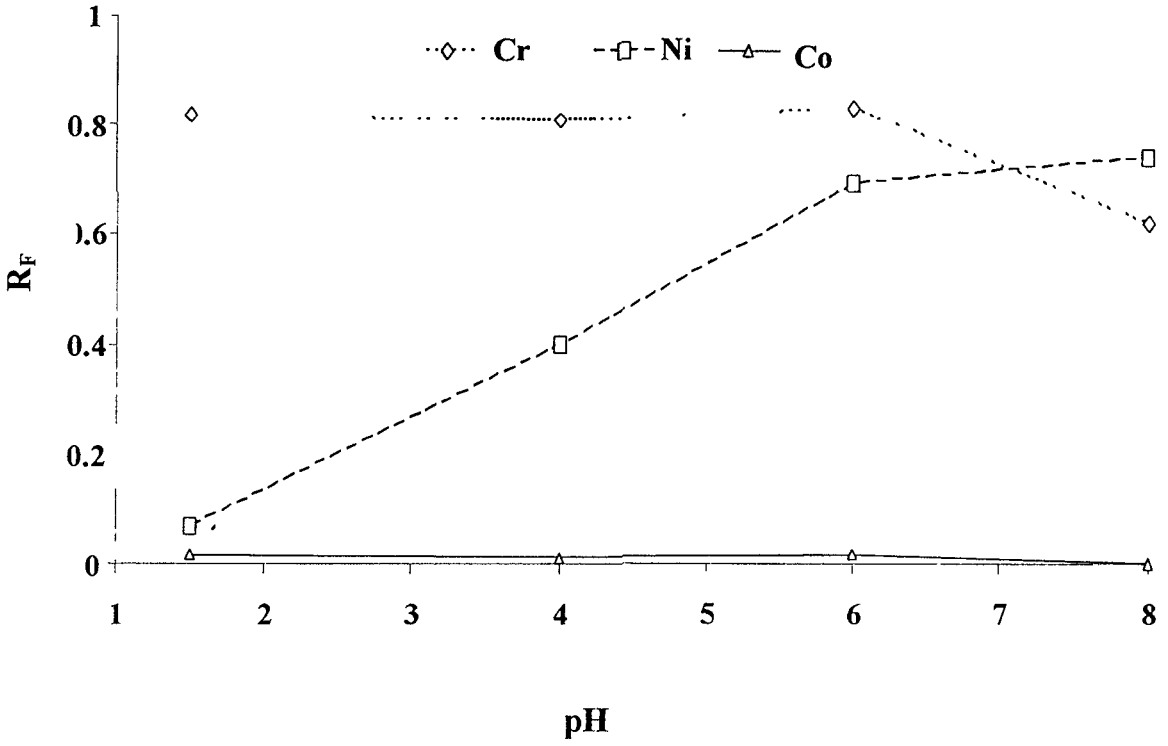

Figure 5.1: Effect of pH on the Separation of Cr^{6+} – Ni^{2+} – Co^{2+} Developed in M_{13} (Dimethylamine – Methanol – Formic Acid, 8:8:2) Mobile Phase on HPTLC Silica Plate

simplest method for quantitation is based on the measurement of the size of the spot by drawing the outline of the spot on a piece of tracing paper. Therefore, an attempt was made to achieve semiquantitative determination of metal ions by measuring the spot area. A linear relation obtained when the amount of the sample spotted was plotted against the area of the spot **(Figures 5.2 and 5.3)** follows the empirical equation $\xi = km$, where ξ is the area of the spot, m is the amount of the solute and k is a constant. The linearity is maintained up to 200 μg/ spot of Ni^{2+} and Cr^{6+}. At higher concentration, a negative deviation from linear law in both the cases was observed. The standard curve constructed for semiquantitative determination of Cr^{6+} **(Figure 5.2)** was used to find out the amount of chromium present in industrial wastewater sample. The accuracy and precision were below $\pm$ 15%. The results of semiquantitative determination by visual comparison method were applied for estimating the Cr^{6+} present in industrial wastewater. The analyzed industrial wastewater samples (chrome wastewater) were found to contain chromium content in the range 5-75 μg/ L.

The proposed method was successfully applied for identification and separation of heavy metal ions in spiked industrial wastewater samples. The results presented in **Table 5.7** clearly demonstrate the applicability of the method for identification of Cr^{6+}, Cr^{3+}, Ni^{2+} and Co^{2+} as well as the mutual separation of coexisting Cr^{6+} from Cr^{3+} and of Cr^{6+} from Ni^{2+} and Co^{2+} in a variety of industrial wastewater samples.

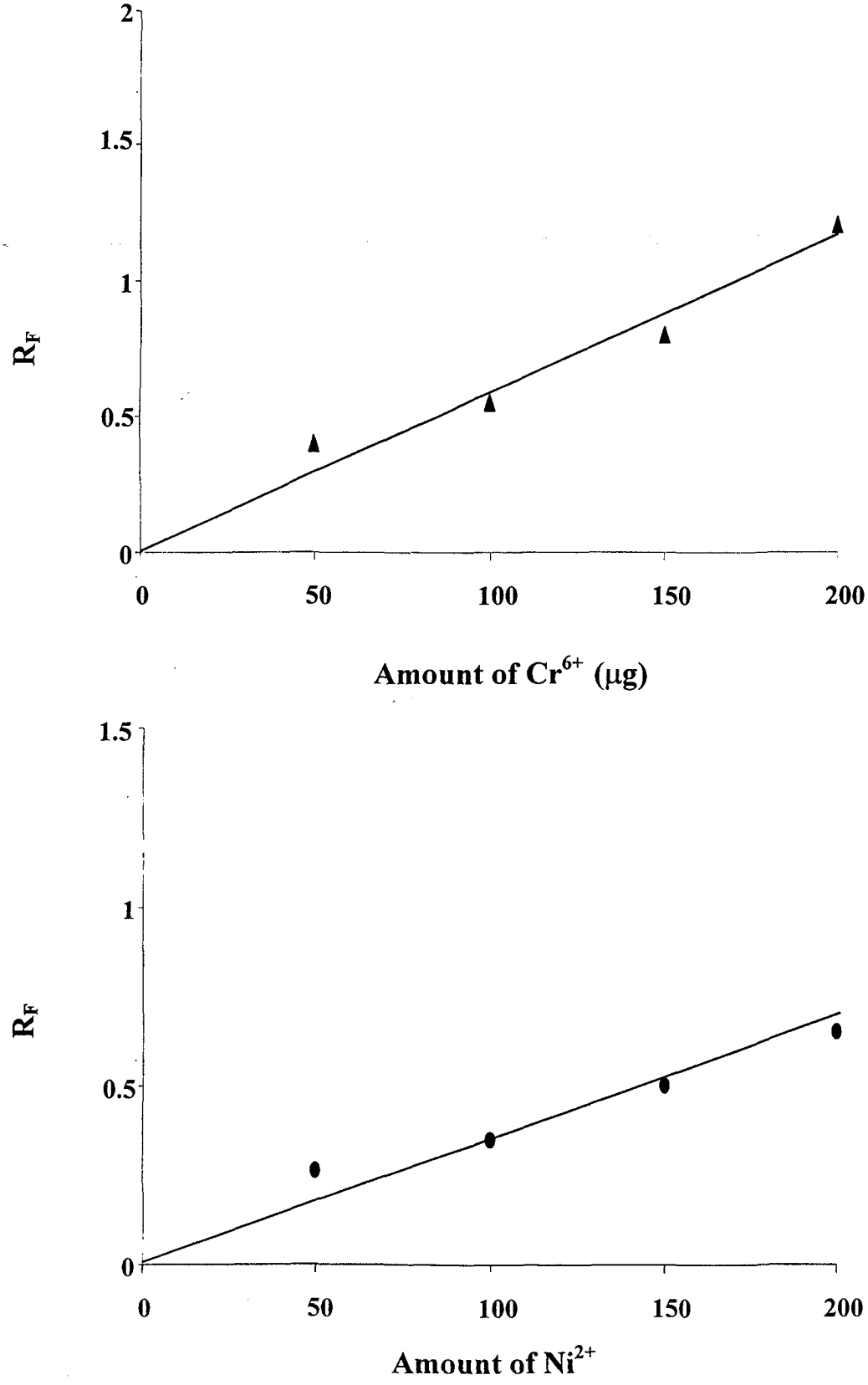

Figure 5.2: Calibration Curves for Semiquantitative Determination of Cr^{6+} and Ni^{2+} Developed in M_7 (Methanol – Dimethylamine, 8:2) Mobile Phase System on HPTLC Silica Plate

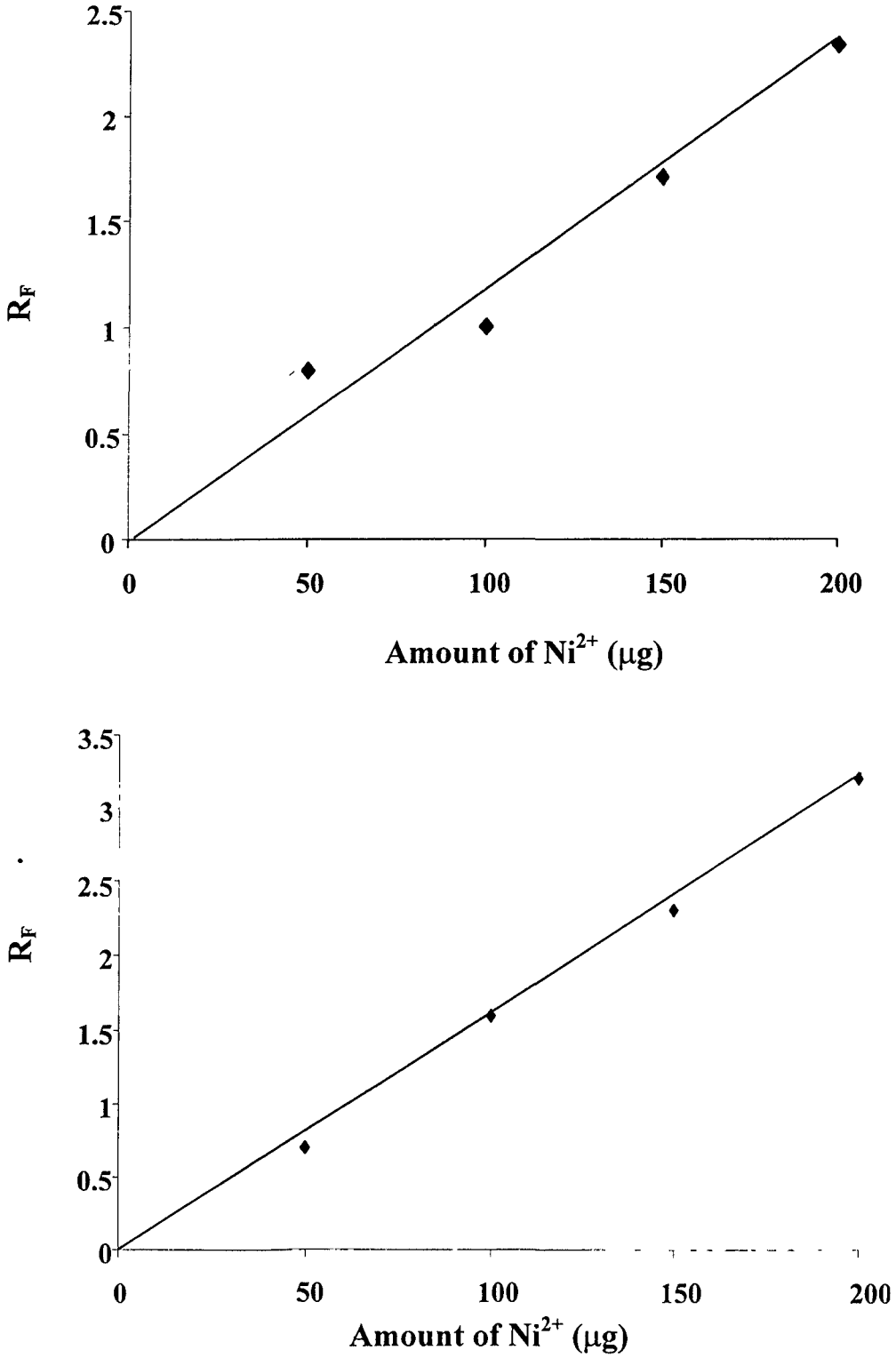

Figure 5.3: Calibration Curves for Semiquantitative Determination of Ni^{2+} and Cr^{6+} Developed in M_{12} (Dimethylamine – Methanol – Fomic Acid, 8:8:2) Mobile Phase System on HPTLC Silica Plate

Table 5.7: Application of Proposed Chromatographic System (HPTLC Silica Gel – M_7 and M_{13}) in Identification and Separation of Certain Heavy Metals from Spiked Industrial Wastewater Samples

Mobile Phase	Industrial Wast	Separation (R_F)
M_7	Sample 1	Cr^{6+} (0.86) – Ni^{2+} (0.45) – Co^{2+} (0.00)
M_{13}	Sample 2	Cr^{6+} (0.87) – Ni^{2+} (0.41) – Co^{2+} (0.03)
	Sample 3	Cr^{6+} (0.87) – Ni^{2+} (0.43) – Co^{2+} (0.00)

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Chapter-6

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY OF HEAVY METAL CATIONS WITH FORMIC ACID CONTAINING MOBILE PHASES: EFFECT OF ADDED ORGANIC SOLVENTS AND SURFACTANTS ON THE MOBILITY OF CATIONS

6.1 INTRODUCTION

Because of unique properties, formic acid (FA) has been used as a promising medium for chromatographic separation of metal cations by ion-exchange column chromatography (1-3). The favorable chromatographic features of FA as an eluent in inorganic chromatography include (a) capability to form formate complexes with certain metal cations (4), (b) sufficiently acidic nature [K_a (H_2O) at 25° C = 1.77 x 10^{-4}] to check the hydrolysis of metal salts, (c) reducing properties to prevent oxidation of cations during analysis and (d) a clearer detection of metal cations on silica TLC plates after development (5). Our investigations on laboratory made plain and surface modified silica layers have shown that FA offers unusual possibilities in thin-layer chromatographic analysis of cations (5-7).

After careful survey of recent literature on planar chromatographic analysis of metal cations (8-11), we reached to the conclusion that the analytical potential of FA containing eluents has not been fully utilized. It was therefore, decided to use aqueous formic acid solutions with added surfactants and organic solvents as mobile phase in TLC analysis of heavy metal cations on precoated silica HPTLC plates. As a result, many important separations of metal cations from their multicomponent mixtures have been realized within 3 - 5 min on micro-HPTLC plates. The proposed method has been applied for identification of heavy metals in synthetically prepared hydroxide sludge and spiked industrial wastewaters.

6.2 EXPERIMENTAL

Chemicals and Reagents: Silica gel 60 F_{254} 'HPTLC' plates (Merck, Darmstadt, Germany); sodium dodecyl sulfate (BDH, India); N-cetyl-N, N,N-trimethyl ammonium bromide (CTAB) and Triton X-100 (Loba Chemie, India); methanol, ethanol and acetone (Qualigens, India);

1,4-dioxane and formic acid (Merck, India), dimethyl sulfoxide, acetonitrile and inorganic salts (CDH, India) were used. All the reagents used were of Analytical Reagent grade.

Test Solutions: Standard aqueous solutions (1.0%) of the chloride, nitrate or sulfate salts of Ni^{2+}, Co^{2+}, Fe^{3+}, Hg^{2+}, Ti^{4+}, Cd^{2+}, Pb^{2+}, Tl^{+}, Bi^{3+}, Al^{3+}, Ag^{+}, Cu^{2+}, VO^{2+} and UO_2^{2+} were used as test solutions.

Detection Reagents: Conventional chromogenic reagents as mentioned in chapter 4 were used for the detection purposes of metal ions. Cr^{6+} was detected with saturated alcoholic solution of $AgNO_3$.

Stationary Phase: Silica gel 'HPTLC' Plates

Mobile Phase: The following solvent systems were used as mobile phase

Symbol	Composition
M_1	1.0 M HCOONa
M_2	1.0 M HCOOH
M_3	1.0 M HCOOH + 1.0 M HCOONa (3:7)
M_4	1.0 M HCOOH + 1.0 M HCOONa (1:1)
M_5	1.0 M HCOOH + 1.0 M HCOONa (7:3)
M_6	0.01 M HCOOH
M_7	0.01 M HCOONa
M_8	1.0 M HCOOH + CH_3OH (7:3)
M_9	1.0 M HCOOH + C_2H_5OH (7:3)
M_{10}	1.0 M HCOOH + acetone (7:3)
M_{11}	1.0 M HCOOH + acetonitrile (7:3)
M_{12}	1.0 M HCOOH + DMSO (7:3)
M_{13}	1.0 M HCOOH + 1,4-dioxane (7:3)

M_{14}	1.0 M HCOOH + 0.1 M aq. SDS (7:3, 3:7)
M_{15}	1.0 M HCOOH + 0.1 M aq. CTAB (7:3, 3:7)
M_{16}	1.0 M HCOOH + 0.1 M aq. Triton X-100 (7:3, 3:7)
M_{17}	1.0 M HCOOH + 0.0001 M aq. SDS (7:3)
M_{18}	1.0 M HCOOH + 0.0001 M aq. CTAB (7:3)
M_{19}	1.0 M HCOOH + 0.0001 M aq. Triton X-100 (7:3, 3:7)

***Preparation of Spiked Industrial Wastewater and River Water*:** A 50 mL volume of industrial wastewater (pH 2.98) collected from lock industries, Aligarh, India or river water (pH 7.48) obtained from Ganga river at Naraura, India was spiked with 100 μg each of Ni^{2+}, Cd^{2+} and Ag^{+} salts. About 30 mL of 0.5% thioacetamide solution was added into the spiked sample. The resultant precipitate of Ni, Cd and Ag sulfides was washed with distilled water, centrifuged and dissolved in minimum possible volume of concentrated HCl. The acid was completely removed by evaporation and the residue was dissolved in 5 mL of distilled water. An aliquot (5μL) of each sample was applied on TLC plate and chromatography was performed as done for the standard samples.

***Preparation of Heavy Metal Hydroxide Sludge*:** Synthetic heavy metal sludge of Ni, Cd and Ag was prepared by adding sufficient volume of 1% NaOH solution into a mixture containing 1% solution of these metal salts in equal volumes. The metal hydroxide precipitate so obtained was filtered, dried and dissolved in a minimum volume of concentrated hydrochloric acid. The acid was completely evaporated, the residue was dissolved in 5 mL of distilled water and TLC was performed having 5 μL sample.

Procedure: For the determination of R_F value of metal ions as described in chapter 2 for amino compounds.

Separation: For the separation of heavy metal cations, the procedure followed is as described in chapter 5.

Limit of Detection: The limits of detection for identification of cations as described in chapter 2 for amino compounds.

Semiquantitative Determination by Spot–Area Measurement: For semiquantitative determination by spot- area measurement method, 0.01 mL volumes from a series of standard solutions (0.5 – 2.0%) of Ni^{2+}, Fe^{2+}, Tl^{+} and Pb^{2+} were spotted on HPTLC plates. The plates were developed with M_5 i.e. 1.0 M HCOOH + 1.0 M HCOONa (7:3). After detection the spots were copied onto tracing paper from the chromatoplates and then the area of each spot was calculated.

Semiquantitative Determination by Visual Comparison: Aliquot (0.01 mL) of standard solutions of different concentrations (0.5 – 2.0%) of nickel chloride were spotted on HPTLC plates alongwith the spotting of 0.01mL of industrial wastewater sample. After completing the chromatographic process, the color intensity and the R_F value of analyzed industrial wastewater samples were matched with the colored spots of standard reference solutions of nickel chloride. The amount of nickel present in the industrial samples was determined according to color intensity of its spot on HPTLC plate after visual comparison with the color intensities of standard solutions.

6.3 RESULTS AND DISCUSSION

The results have been presented in **Tables 6.1 – 6. 5** and **Figures 6.1 – 6.2**. The R_F values of metal cations obtained on silica HPTLC plates developed with 1.0 M HCOOH (pH ≈ 2.3), 0.01 M HCOONa (pH > 7.0) and 1.0M HCOOH plus 1.0 M HCOONa (3:7, 1:1, 7:3) are summarized in **Table 6.1**. With 1.0 M HCOONa (M_1), most of the cations remain near the point of application whereas Tl^+ shows appreciable mobility and thus can be selectively separated from other metal ions on HPTLC silica plate developed with 1.0 M HCOONa. Conversely, Cu^{2+} and Cd^{2+} have much higher mobility in 1.0 M HCOOH (M_2) compared to 1.0 M HCOONa and can be separated from Fe^{3+}, UO_2^{2+}, VO^{2+}, Bi^{3+} and Ti^{4+}. The combinations of these two solvent systems in different proportions further increase the separation potentiality of formic acid by modifying the retention behavior of cations and several metal cations can be selectively separated with M_3-M_5 mobile phases. The lowering of HCOOH or HCOONa concentration from 1.0M to 0.01M (M_6, M_7) results in the decrease in R_F value of cations. The results presented in **Figure 6.1** as ΔR_F (R_F in 1.0 M HCOOH or HCOONa – R_F in 0.01 M HCOOH or HCOONa Vs metal cations) plots clearly indicate this effect. At high acid concentration (1.0 M HCOOH), the large number of H^+ ions compete with the cations for the exchange sites of silica gel leading to high mobility (i.e. high R_F values) for metal cations.

Effect of Added Organic Solvents

In order to understand the role of organic solvents on the mobility of metal ions, the R_F values of metal cations were determined with mobile phases M_8-M_{13} obtained by mixing methanol, ethanol, acetone, DMSO, dioxane and acetonitrile with 1.0 M aqueous FA respectively in 7:3 ratios by volume. The ΔR_F values (obtained by subtracting R_F

Table 6.1: Mobility (R_F Values) of Heavy Metal Cations on Silica HPTLC Plates Developed with Formate Ion Containing Mobile Phases

Metal Ion	Mobile Phase				
	M_1	M_2	M_3	M_4	M_5
Fe^{3+}	0.00	0.10	0.00	0.00	0.00
Cu^{2+}	0.00	0.77	0.72	0.30T	0.51
Ni^{2+}	0.28	0.87	0.72	0.68	0.84
Co^{2+}	0.30	0.87	0.51	0.70	0.84
UO_2^{2+}	0.00	0.00	0.00	0.00	0.08
VO^{2+}	0.00	0.15	0.00	0.00	0.06
Cd^{2+}	0.00	0.58	0.36	0.52	0.48
Ag^{+}	0.00	0.18T	0.00	0.00	0.00
Pb^{2+}	0.00	0.40T	0.03	0.12	0.30T
Tl^{+}	0.68	0.62	0.67	0.55	0.35
Bi^{3+}	0.05	0.05	0.00	0.00	0.07
Hg^{2+}	0.10	0.46T	0.00	0.00	0.20T
Al^{3+}	0.00	0.35T	0.00	0.00	0.28T
Ti^{4+}	0.05	0.00	0.05	0.05	0.05
Cr^{6+}	0.66T	0.69	0.62	0.90	0.68

T = Tailed spot ($R_L - R_T > 0.30$)

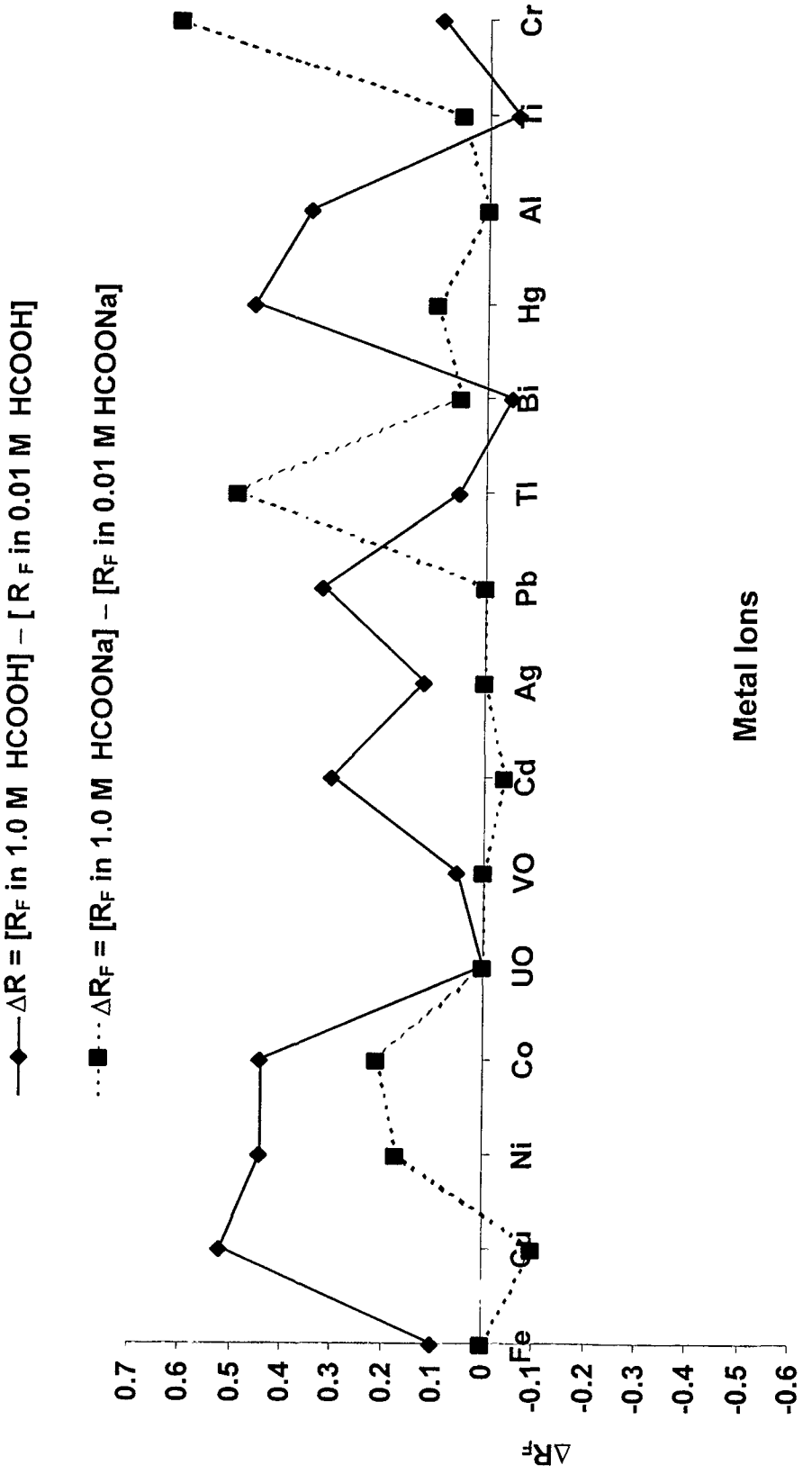

Figure 6.1: Plot of ΔR_F Vs Metal Ions

values realized in 1.0 M FA from the R_F values achieved in 1.0 M FA plus organic additives) are presented in **Table 6.2**. The positive and negative values of ΔR_F clearly demonstrate the effect of added organic solvents on the mobility trend of metal cations. Several workers (10-17) have mostly used the above mentioned organic solvents as one of the components of mixed–aqueous or organic mobile phase systems for TLC analysis of inorganic ions. Amongst these, DMSO is aprotic dipolar; MeOH and EtOH are polar proton donor; dioxane is proton acceptor; acetone being non-polar favors the formation of non- dissociated metal complexes and acetonitrile bearing CN group is an useful complexing agent. Thus, the inherent diverse properties of the added organic solvents were found to favorably influence the mobility pattern of metal cations leading to the opening of new possibilities for the separation of metal cations. The mobility of heavy metal cations decreases upon the addition of alcohol or ketone (e.g. MeOH, EtOH and acetone) in the mobile phase. This trend is indicative by negative value of ΔR_F **(Table 6.2)** for most of the metal cations. Conversely, the addition of 1,4-dioxan, DMSO and acetonitrile in the mobile phase enhances the mobility of cations as indicative by positive ΔR_F value.

Effect of Added Surfactants

The use of surfactant – mediated systems, as mobile phase in liquid chromatography has been the choice of analytical chemists because of their advantages of enhanced selectivity, low cost and reduced toxicity. Since the first report by *Armstrong* and *Henry* (18), interest in surfactant–mediated mobile phases under the name of ion-pair chromatography (IPC) or micellar liquid chromatography (MLC) has grown rapidly (19-24). Keeping in mind the unusual selectivity of surfactant eluents, we have used anionic (SDS), cationic (CTAB) and anionic (Triton X-100) surfactants in combination with 1.0 M FA as

Table 6.2: Effect of Added Organic Solvents in 1.0 M HCOOH on the Mobility of Metal Ions

	$\Delta R_F = R_F$ in 1.0 M HCOOH plus Organic Solvents (7:3) – R_F in 1 M HCOOH					
Metal Ion	**Methanol**	**Ethanol**	**Acetone**	**Acetonitrile**	**DMSO**	**Dioxane**
Fe^{3+}	+0.10	-0.10	-0.10	-0.05	-0.03	-0.05
Cu^{2+}	-0.67	-0.64	-0.50	-0.14	-0.02	-0.07
Ni^{2+}	-0.63	-0.26	-0.22	+0.06	-0.11	-0.11
Co^{2+}	-0.63	-0.16	-0.20	+0.08	+0.10	+0.11
UO_2^{2+}	+0.05	+0.30	+0.25	+0.21	+0.40	+0.48
VO^{2+}	-0.10	+0.06	-0.05	+0.35	+0.04	+0.29
Cd^{2+}	+0.06	-0.05	-0.48	+0.08	+0.21	+0.12
Ag^{+}	-0.13	-0.18	-0.18	-0.18	-0.18	-0.18
Pb^{2+}	-0.12	-0.29	-0.25	+0.20	+0.13	+0.18
Tl^{+}	+0.09	-0.18	+0.09	+0.07	+0.02	+0.10
Bi^{3+}	-0.05	-0.05	-0.05	-0.05	+0.02	+0.02
Hg^{2+}	-0.08	-0.46	-0.46	+0.30	+0.03	+0.36
Al^{3+}	+0.17	+0.07	+0.02	0.00	+0.20	+0.20
Ti^{4+}	0.00	0.00	0.00	0.00	0.00	0.00
Cr^{6+}	+0.21	-0.22	+0.21	-0.40	-0.26	+0.11

Pb^{2+}, UO_2^{2+}, Cu^{2+} and VO^{2+} in 1.0 M HCOOH plus methanol, ethanol, acetone or dioxane (7:3); Ag^{+}, Pb^{2+}, Hg^{2+} are tailed in 1.0 M HCOOH and Al^{3+} is tailed in all mobile phases (M_8 - M_{13}) produced tailed spots.

eluent. The mobility pattern of metal cations obtained on HPTLC plates developed with surfactant containing FA mobile phase is provided in **Table 6.3**. The R_F values data listed in **Table 6.3** show that the mobility of the cations is modified by the presence of surfactants in the mobile phase.

From the results presented in **Table 6.3,** following trends are noticeable:

(a) Fe^{3+}, Ag^{+}, Bi^{3+} and Ti^{4+} remain near the point of application *irrespective of the nature of surfactant (cationic, anionic or non-ionic)* and the concentration of surfactant.

(b) Al^{3+} produces tailed spot at all concentration of the surfactants.

(c) Cr^{6+}, Pb^{2+}, UO_2^{2+} and Cd^{2+} show occasional tailing.

(d) Most of the metal cations show higher mobility in mobile phases comprising of volume ratio 7:3 of formic acid and surfactant solution compared to the mobile phase consisting of formic acid and surfactant in 3:7 volume ratio.

(e) Lowering of surfactant concentration from 0.1 M (M_{14}-M_{16}) to 0.0001 M (M_{17}-M_{19}) in the mobile phase leads to minor change in mobility of metal ions.

The formic acid containing mobile phases were found capable to resolve several metal cations from their multicomponent mixtures. As a result some important separations of metal cations realized experimentally have been summarized in **Table 6.4**.

The lowest detectable microgram amounts along with dilution limits (given in parenthesis) of metal ions achieved on HPTLC plates developed with M_5 solvent system were for Cu^{2+} (2.07μg, 1: 4.830 x 10^3), Ni^{2+} (0.026μg, 1: 3.8 x 10^5), Co^{2+} (0.09μg, 1: 1.1 x 10^5) and Pb^{2+} (0.1μg, 1: 10 x 10^4). It is clear from these data that the proposed method is highly sensitive for the detection of the cations.

Table 6.3: Effect of Added Surfactants in 1.0 M Aqueous Formic Acid [a] on the Mobility of Metal Cations

Stationary Phase: Silica Gel 'HPTLC' Plates

Metal Ion	M_{14}		M_{15}		M_{16}		M_{17}	M_{18}	M_{19}
	7:3	3:7	7:3	3:7	7:3	3:7	7:3	7:3	7:3
Fe^{3+}	0.10	0.10	0.05	0.05	0.05	0.05	0.05	0.06	0.05
Cu^{2+}	0.76	0.45	0.74	0.47	0.64	0.44	0.58	0.61	0.52
Ni^{2+}	0.76	0.59	0.88	0.62	0.78	0.63	0.64	0.66	0.61
Co^{2+}	0.78	0.60	0.88	0.64	0.74	0.61	0.70	0.66	0.72
UO_2^{2+}	0.35	0.09	0.28T[b]	0.10	0.35	0.20	0.41	0.45	0.22
VO^{2+}	0.66	0.41	0.70	0.33	0.67	0.42	0.67	0.78	0.60
Cd^{2+}	0.72	0.62	0.74	0.66	0.57	0.45	0.68T	0.68	0.56
Ag^{+}	0.10	0.10	0.10	0.10	0.10	0.10	0.06	0.05	0.14
Pb^{2+}	0.35T	0.18T	0.66	0.36T	0.48	0.22	0.47	0.47T	0.39
Tl^{+}	0.64	0.55	0.68	0.57	0.64	0.59	0.61	0.60	0.68
Bi^{3+}	0.08	0.09	0.88	0.05	0.06	0.06	0.09	0.12	0.09
Hg^{2+}	0.75	0.59	0.90	0.90	0.69	0.57	0.30	0.05	0.66
Al^{3+}	0.53T	0.23T	0.62T	0.44T	0.44T	0.40T	0.34T	0.44T	0.55T
Ti^{4+}	0.12	0.15	0.10	0.10	0.05	0.05	0.05	0.08	0.08
Cr^{6+}	0.50T	0.50T	0.53	0.86	0.72	0.80T	0.70T	0.72T	0.75T

[a] *R_F values of metal ions in 1.0 M HCOOH, given in parenthesis were Fe^{3+} (0.10), Cu^{2+} (0.77), Ni^{2+} (0.87), Co^{2+} (0.87), UO_2^{2+} (0.0), VO^{2+} (0.15), Cd^{2+} (0.58), Ag^{+} (0.18T), Pb^{2+} (0.40T), Tl^{+} (0.62), Bi^{3+} (0.05), Hg^{2+} (0.46T), Al^{3+} (0.35T), Ti^{4+} (0.0), and Cr^{6+} (0.69).*

[b] *T = Tailed Spot ($R_L - R_T > 0.30$)*

Table 6.4: Experimentally Achieved Separations of Metal Ions on High-Performance Thin-Layer Chromatographic Plates Developed with Different Mobile Phases

Mobile Phase	Separation (R_F)
1.0 M HCOOH	Ni^{2+} or Co^{2+} (0.86)/Cu^{2+} (0.75)/ Cr^{6+}(0.67) — VO^{2+}(0.13), Fe^{3+}(0.08) /Bi^{3+}(0.05), UO_2^{2+}, Ti^{4+}(0.00)
1.0 M HCOOH+ 1.0 M HCOONa (7:3)	Ni^{2+}, Co^{2+} (0.84)/Cr^{6+} (0.67) — Tl^{+} (0.35) — UO_2^{2+}, VO^{2+} or Bi^{3+} (0.05), Fe^{3+} or Ti^{4+} (0.00)
	Ni^{2+} (0.84) — Cd^{2+} (0.46) — Ag^{+} (0.00)
1.0 M HCOOH + CH_3OH (7:3)	Cr^{6+} (0.88) — Cd^{2+} (0.60), Hg^{2+} (0.35) — Cu^{2+}(0.10)/UO^{2+} or VO^{2+} (0.06), Fe^{3+}, Bi^{3+} or Ti^{4+} (0.00)
1.0 M HCOOH + C_2H_5OH (7:3)	Cr^{6+} (0.88) or Co^{2+}(0.70) — Cd^{2+} (0.51) or Tl^{+} (0.42) — Cu^{2+} or Pb^{2+} (0.10), Fe^{3+}, Ag^{+} Bi^{3+}, Hg^{2+} or Ti^{4+} (0.00)
1.0 M HCOOH + acetone (7:3)	Cr^{6+} (0.88)/Ni^{2+} or Co^{2+} (0.66), Tl^{+} (0.70) — UO_2^{2+} (0.23)/Pb^{2+} (0.14), VO^{2+} or Cd^{2+} (0.09) and Fe^{3+}, Ag^{+} Ti^{4+}, Bi^{3+} or Hg^{2+} (0.00)
1.0 M HCOOH + DMSO (7:3)	Cd^{2+}, Ni^{2+}, Co^{2+} or Cu^{2+} (0.76) — Pb^{2+} or Hg^{2+} (0.50), UO_2^{2+} (0.38) — Fe^{3+} or Bi^{3+} (0.05)/Ag^{+} or Ti^{4+} (0.00)
1.0 M HCOOH+ 1,4–dioxane (7:3)	Hg^{2+} (0.80)/Ni^{2+} or Co^{2+} (0.75)/Cu^{2+} , Cd^{2+} or Tl^{+} (0.71) — UO_2^{2+} (0.45) — Fe^{3+} or Cu^{2+} (0.05)
1.0 M HCOOH + 0.1 M SDS (7:3)	Hg^{2+}, Ni^{2+}, Co^{2+} or Cu^{2+} (0.76) — UO_2^{2+} (0.33) — Fe^{3+}, Ag^{+} or Ti^{4+} (0.12)
1.0 M HCOOH+ 0.1M CTAB (7:3)	Hg^{2+}, Ni^{2+} or Co^{2+} (0.89) — Cr^{6+} (0.50) — Fe^{3+} , Bi^{3+}, Ag^{+} or Ti^{4+} (0.05)
1.0 M HCOOH+ 0.1M Triton X–100 (7:3)	Ni^{2+}, Co^{2+} or Cr^{6+} (0.74)/Hg^{2+} (0.70)/Tl^{+} or Cu^{2+} (0.63)/UO_2^{2+} (0.35) — Fe^{3+} , Ti^{4+} or Bi^{3+} (0.05)/Ag^{+} (0.10)

In addition to the qualitative analysis, a quantitative evaluation of the metal ions is often required to ascertain the level of the toxic metals in environment samples. A relatively less accurate but the simplest method for quantification is based on the measurement of the size of the spot by drawing the outline of the spot on a piece of tracing paper. Therefore, an attempt was made to achieve semiquantitative determination of metal ions by measuring the spot area. A linear relationship obtained when the amount of the sample spotted was plotted against the area of the spot follows the empirical equation $\xi = km$, where ξ is the area of the spot, m is the amount of the solute and k is a constant. Representative plots for Ni^{2+} and Pb^{2+} are shown in **Figure 6.2**. The linearity is maintained up to 200 μg/ spot of Ni^{2+}, Pb^{2+}, Fe^{3+}, and Tl^{+}. At higher concentration, a negative deviation from linear law in all cases was observed. The standard curve **(Figure 6.2)** was used to find out the amount of nickel present in industrial wastewater samples. The accuracy and precision of the method was about ± 15%. The results of semiquantitative determination by visual comparison method were applied for estimating the nickel present in industrial wastewater. The analyzed industrial wastewater samples (chrome, bright and wastewaters) were found to contain nickel content in the range 7-100 μg/ L.

Amongst TLC systems examined, the system comprising of silica HPTLC plates developed with M_5 i.e. 1.0 M HCOOH plus 1 M HCOONa (7:3) mobile phase was identified as the most useful for the analysis of heavy metal cations. This system was applied for identification and separation of certain metal cations from industrial wastewater and metal hydroxide sludge samples. The results summarized in **Table 6.5** clearly demonstrate that the proposed method can be successfully applied for identification and the separation of Ni^{2+}, Cd^{2+} and Ag^{+} from their mixtures.

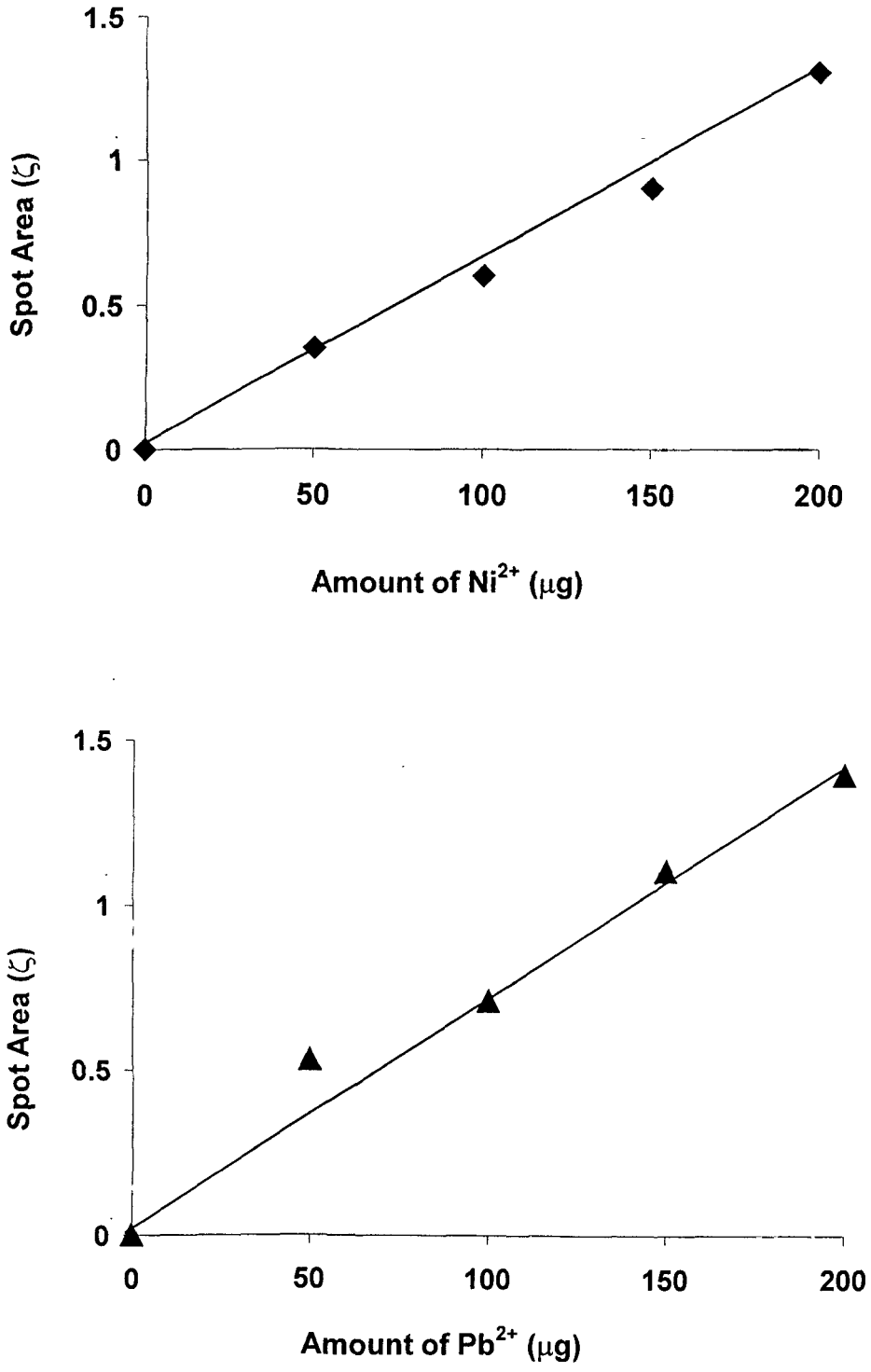

Figure 6.2: Calibration Curves for Semiquantitative Determination of Ni^{2+} and Pb^{2+}

Table 6.5: Identification and Separation of Mixtures of Ni^{2+}, Cd^{2+} and Ag^{+} from Spiked Water and Heavy Metal Sludge Samples

Spiked/ Synthetic Sample	Separation (R_F)		
	Ni^{2+}	Cd^{2+}	Ag^{+}
River water	0.84	0.45	0.00
Industrial wastewater	0.82	0.43	0.00
Sludge	0.83	0.46	0.00

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Micellar Thin-Layer Chromatography of Coinage Metal Cations

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Summary

Chromatography of some metal cations has been performed on silica gel layers with micellar mobile phases containing sodium dodecyl sulfate (SDS), an anionic surfactant. The effects on the mobility of metal ions of SDS concentration, mobile phase pH, and the presence of amino acids (l-arginine, dl-phenylalanine, l-tryptophan, and l-histidine) in the mobile phase were examined. Although amino acids caused diffusion (or tailing) of the metal ion spots, use of l-histidine or l-tryptophan facilitates the analytically important separation of a mixture of Au^{3+}, Cu^{2+}, and Ag^{+} ions. The TLC system comprising silica gel G as stationary phase and 0.01 m SDS (pH 2.3)–0.01 M l-tryptophan or l-histidine, 1 + 9, as mobile phase was identified as the most suitable for the separation of mixtures of Au^{3+}, Cu^{2+}, and Ag^{+}. The interference of impurities such as amines, phenols, and inorganic anions on the mobility and separation of a mixture of Au^{3+}, Cu^{2+}, and Ag^{+} ions was also examined. The lower limit of detection of some metal ions, viz. Fe^{3+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+} was determined. The proposed method has been used for identification and separation of Au^{3+}, Cu^{2+}, and Ag^{+} in a variety of spiked samples.

1 Introduction

Mutual separation of copper ($_{29}$Cu), silver ($_{47}$Ag), and gold ($_{79}$Au), the elements of group IB of the periodic table, is analytically important because of their similar chemical properties. These metals, with the general configuration $(n-1)d^{10}ns^{1}$ have a tendency to form complex salts in which the metal can be a complex cation or a complex anion. Copper is associated with silver in copper glance $(Cu,Ag)_2S$ ore and hence separation is needed to isolate pure silver from the ore. The presence of silver ions has been found to reduce the rate of adsorption of Au^{3+} from thiourea solution by activated carbon [1] and in other ways also the presence of one metal in small quantities has deleterious effects on performance of other metals. Because of the industrial, commercial, and medicinal importance of these metals, several analytical techniques have been developed for the separation and determination of Au^{3+}, Cu^{2+}, and Ag^{+} from a variety of matrixes. These include ion-exchange chromatography [2, 3], potentiometry [4], capillary zone electrophoresis [5], solvent extraction [6], single-sweep oscillopolarography [7], ion pair-reversed-phase HPLC [8], size-exclusion chromatography [9], titrimetry [10], reversed-phase column chromatography [11], reflectance spectroscopy [12], flame or graphite furnace atomic absorption spectrometry [13], laser-excited atomic fluorescence spectroscopy [14], neutron activation analysis, ion-pair [15, 16] and foam plastic [17] chromatography, in addition to hyphenated techniques, e.g. TLC–spectrophotometry [18], solvent extraction–AAS/FAAS [19], ICP–AES and ICP–MS [20, 21], and ion-exchange chromatography–photometry [22]. Ion chromatography of Au–cyanide complexes and problems arising during analysis of gold by titrimetric and spectrophotometric methods have been reviewed [23, 24].

New materials, including chelate-forming plastics containing amino-thiourea [25], VS-II anion-exchange fiber [26], ion-exchange resin loaded with bismuthiol [27], silica gel-bound thia-crown ethers [28], silica gel chemically modified with *p*-dimethylaminobenzylidenerhodanine [29], chitosan treated with dithiocarbamates [30], and nanofilter membrane [31] have recently been developed and used for preconcentration and separation of gold, silver, and copper.

Among the analytical techniques used thin-layer chromatography (TLC), which is inexpensive and versatile, is still popular among analytical scientists, especially those working in India, China, Japan, and European countries. As a result, TLC systems

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comprising ECTEOLA–cellulose and HCl + NaCl + H_2O for separation of gold, platinum, and palladium from associated base metals [32], chitin and aqueous buffer solutions for separation of Cu^{2+} and Ag^+ [33], alumina and aqueous solutions of both organic and inorganic acids and some sodium salts for rapid separation of Au^{3+} from Te^{4+} and Se^{4+} [34], and silica gel containing sodium or ammonium acetate and toluene for separation of Cu^{2+} complex from transition metal complexes [35] have been reported.

The analytical techniques listed above have been successfully used to separate Au^{3+} from either Ag^+ or Cu^{2+} but the work on the mutual separation of these metal ions from their three-component mixtures is lacking. As far as we are aware no reference is available on the separation of mixtures of Au^{3+}, Cu^{2+}, and Ag^+ by TLC with surfactant-containing mobile phases (or micellar mobile phases). During our previous study [36] on the micellar thin-layer chromatography of heavy metal cations we realized that micellar mobile phases have unique separation capabilities and provide unusual selectivity, enhanced detection sensitivity, and faster analysis. The efficiency of micellar systems for the separation of cations [37] and anions [38] has been reported and reviewed by *Okada* [39]. Surprisingly, very little work seems to have been performed on the use of micellar mobile phases in the TLC of inorganic species [40, 41] and none of these studies has examined the separation of metal cations. It was therefore decided to identify novel micellar mobile phases enabling highly selective separations of metal cations. As a result, simultaneous separation of Au^{3+}, Cu^{2+}, and Ag^+ from their mixtures has been achieved on silica layers by use of a buffered anionic micellar mobile phase with added amino acids. The proposed TLC method is selective and rapid, with development times averaging 5 min.

2 Experimental

2.1 Chemical Reagents and Samples

Silica gel G was from Merck (India), sodium dodecyl sulfate from BDH (India), L-argir ine, L-histidine, DL-phenylalanine, phenols, amines, and anions from CDH (India), and L-tryptophan from Loba-chemie (India). All reagents were of analytical-reagent grade.

The metal cations studied were Fe^{3+}, Cu^{2+}, Ni^{2+}, Co^{2+}, UO_2^{2+}, VO^{2+}, Cd^{2+}, Zn^{2+}, Ag^+, Pb^{2+}, Tl^+, Bi^{3+}, Hg^{2+}, Al^{3+}, Ti^{4+}, and Au^{3+}. Aqueous test solutions (1.0%) were prepared from the nitrates of Cd^{2+}, Zn^{2+}, Pb^{2+}, Tl^+, Bi^{3+}, Al^{3+}, and Ag^+, the chlorides of Ni^{2+}, Co^{2+}, Fe^{3+}, Hg^{2+}, Ti^{4+}, and Au^{3+}, and the sulfates of Cu^{2+}, VO^{2+}, and UO_2^{2+}. All the solutions were prepared in demineralized water with a specific conductivity, K, of 2×10^{-6} ohm^{-1} at 25°C. To limit the extent of hydrolysis small quantities of the corresponding acid were added to solutions of the nitrates of lead, silver, and bismuth and the chloride of mercury.

Solutions (1%) of anions were also prepared by dissolving the sodium salts of NO_2^-, NO_3^-, MoO_4^{4-}, and PO_4^{4-}; the potassium salts of I^-, IO_3^-, IO_4^-, $S_2O_8^{2-}$, $Fe(CN)_6^{3-}$, and $Fe(CN)_6^{4-}$, and the ammonium salt of $C_2O_4^{2-}$ in demineralized water. Aqueous solutions (1%) of the potassium and ammonium salts of SCN^- were also prepared. Solutions (1%) of a variety of amines and phenols were prepared in methanol.

2.1.1 Detection Reagents

The reagents used for detection of the cations were:

– 8×10^{-3} % (*w/v*) dithizone in carbon tetrachloride for detection of Cd^{2+}, Zn^{2+}, Ag^+, Pb^{2+}, Tl^+, Bi^{3+}, and Hg^{2+};

– aqueous potassium ferrocyanide for detection of Fe^{3+}, Cu^{2+}, UO_2^{2+}, VO^{2+}, and Ti^{4+};

– dimethylglyoxime for detection of Ni^{2+} and Co^{2+};

– aluminon for detection of Al^{3+}; and

– yellow ammonium sulfide for detection of Au^{3+}.

2.1.2 Preparation of Test Materials

The materials used in separation tests were:

– gold-plated printed-circuit board (GPCB; containing Au, Ni, and Cu) from Toyama Electric, Bangalore, India;

– silver mirror scrap (SMS; containing Ag and Cu) and silver mirror spent solution (SMSS; containing Ag and Cu), both from Ship Mirror Industries, Bangalore, India;

– high-copper dental amalgam (HCDA; containing Ag, Hg, Cu, Zn, and Sn) from the Dental College, A.M.U., Aligarh, India; and

– synthetic sterling silver scrap (SS; containing Ag and Cu).

Peeling

Peeling of silver mirror scrap (specimen surface area 19 cm^2) was performed with concentrated formic acid (90% *w/w*) in a glass beaker. The acid was heated at 110°C and the scrap material was added into it. On completion of peeling (within 1 min) the solution was separated and the peeled material was used as the 'source material' for silver.

Leaching

Leaching of silver mirror scrap (specimen surface area 19 cm^2) was performed with 50% nitric acid in a glass beaker. On completion of leaching (within 1 min) the solution was separated from the leached residue and used for silver separation. A similar leaching procedure was used for other silver-containing material (0.153 g sterling silver scrap and 0.25 g high-copper dental amalgam).

Leaching of gold-plated printed-circuit board (specimen surface area 72.42 cm^2 containing 16 large pins and 14 small pins) was performed with aqua-regia in a glass beaker. On completion of leaching the solution was separated from the residue and used for gold separation.

2.2 Chromatography

Chromatography was performed on silica gel G with buffered micellar mobile phases. The mobile phases and buffers used are listed in **Tables 1** and **2**, respectively.

Table 1

The mobile phases used.

Symbol	Composition
M_1	0.001, 0.005, 0.01, or 0.05 M aqueous SDS
M_2	0.001, 0.005, 0.01, or 0.05 M buffered SDS (pH 2.3)
M_3	0.001, 0.005, 0.01, or 0.05 M buffered SDS (pH 3.4, 5.7, 7.0, or 11.9)
M_4	0.01 m SDS (pH 2.3)–0.01 M L-arginine (1 + 9, 3 + 7, 5 + 5, 7 + 3, or 9 + 1)
M_5	0.01 m SDS (pH 2.3)–0.01 M DL-phenylalanine (1 + 9, 3 + 7, 5 + 5, 7 + 3, or 9 + 1)
M_6	0.01 m SDS (pH 2.3)–0.01 M L-tryptophan (1 + 9, 3 + 7, 5 + 5, 7 + 3, or 9 + 1)
M_7	0.01 m SDS (pH 2.3)–0.01 M L-histidine (1 + 9, 3 + 7, 5 + 5, 7 + 3, or 9 + 1)
M_8	0.001, 0.005, or 0.05 M SDS (pH 2.3)–0.01 m L-arginine (9 + 1)
M_9	0.01 m SDS (pH 2.3) + 0.001 M L-arginine, DL-phenylalanine, L-tryptophan, or L-histidine (1 + 9, 9 + 1)

Table 2

The buffer solutions used.

No.	Components	Volume ratio	pH
1	0.04 M Boric acid–0.04 M phosphoric acid	50:50	2.3
2	0.02 M Boric acid–0.04 M phosphoric acid–0.24 M NaOH	50:50:8	3.4
3	0.04 M Boric acid–0.04 M phosphoric acid–0.24 M NaOH	50:50:10	5.7
4	0.04 M Boric acid–0.04 M phosphoric acid–0.24 M NaOH	50:50:14	7.0
5	0.04 M Boric acid–0.04 M phosphoric acid–0.24 M NaOH	50:50:60	11.9

2.2.1 Preparation of TLC Plates

Silica gel plates were prepared by mixing the adsorbent with doubly-distilled water in the ratio 1:3 (*w/w*). The resulting slurry was shaken mechanically for 10 min then applied to well-cleaned 20 cm × 3.5 cm glass plates by means of a Toshniwal (India) TLC applicator to give layers approximately 0.25 mm thick. The plates were dried in air at room temperature and then activated by heating at 100 ± 5°C for 1 h. After activation the plates were stored in a desiccator.

2.2.2 Chromatography of the Individual Cations

Test solutions (approx. 10 µL) were applied by means of micropipets approximately 2.0 cm above the lower edge of the plates. The spots were dried and the plates were developed, by the one-dimensional ascending technique, in 24 cm × 6 cm glass jars previously saturated with mobile phase vapor by equilibration for approximately 20 min. The development distance was always 10 cm from the origin. After development the plates were dried again and the cations were visualized as colored spots by application of appropriate detection reagents, by means of a glass sprayer. The cations were identified on the basis of their R_F values, calculated from the R_L (R_F of leading front) and R_T (R_F of trailing front) of each spot.

2.2.3 Chromatography of Mixtures of Copper, Silver, and Gold

Test solutions (10 µL) of mixtures of copper, silver, and gold were spotted on the plates and chromatography was performed with 0.01 M SDS (pH 2.3)–0.01 M L-tryptophan, 1 + 9, or 0.01 M SDS (pH 2.3)–0.01 M L-histidine, 1 + 9, as mobile phase. The resolved spots of these metal cations were observed on the plates after spraying with chromogenic reagents. The R_F values of Au^{3+}, Cu^{2+}, and Ag^+ in their mixture were found to vary marginally from their individual R_F values.

2.2.4 Interference

To investigate interference by inorganic anions, amines, and phenols on the R_F values of Au^{3+}, Cu^{2+}, and Ag^+, an aliquot (approx. 10 µL) of impurity solution was spotted with the mixture (approx. 10 µL) of Au^{3+}, Cu^{2+}, and Ag^+ and chromatography was performed as described above. The spots were detected and the R_F values of separated metal ions were determined.

2.2.5 Limit of Detection

The limits of detection for identification of the cations were determined by spotting different amounts of solutions of the cations on the plates. The plates were then developed and the spots detected as described above. The method was repeated with successive reduction of the amounts of cations until the spots could no longer be detected. The minimum amount of cation that could be detected was taken as the limit of detection.

2.3 Applications

2.3.1 Chromatography of Unspiked Materials

Chromatography of leachate from unspiked dental amalgam and from a printed circuit board was performed with 0.01 M SDS (pH 2.3)–0.01 M L-histidine, 1 + 9, as mobile phase. Spots of Ag^+, Hg^{2+}, Zn^{2+}, Cu^{2+}, and Au^{3+} were detected and the R_F value of each cation was determined.

2.3.2 Chromatography of Spiked Materials

Spiked samples of PCB, dental amalgam, and silver mirror scrap leachate, silver mirror spent solutions, and sterling silver were prepared as follows:

(i) PCB or dental amalgam solution (1 mL) was mixed with silver test solution (1%, 1 mL) and chromatography was performed on 10 µL of the mixture.

(ii) Gold solution (1 mL) was added to SMC, SMSS, or SS solution (1.0 mL of each) and the mixture (10 μL) was used for chromatographic separation of Au^{3+}, Cu^{2+}, and Ag^{+}. The spots were identified from their respective R_F values.

3 Results and Discussion

The results of this study have been summarized in Figures 1–3 and Tables 3–8. The unique features of this study are:

(i) Selection of micellar mobile phases containing an anionic surfactant, sodium dodecyl sulfate (SDS), which is negatively charged and tends to attract positively charged species, including metal cations.

(ii) Use of polar (arginine and histidine) and non-polar (phenylalanine and tryptophan) amino acids as additives.

(iii) Separation of Au^{3+}, Cu^{2+}, and Ag^{+} from their mixtures and investigation of the effects of phenols, cations, and anions on the separation of mixtures of Au^{3+}, Cu^{2+}, and Ag^{+} ions.

(iv) Application of the method to the analysis of several real and synthetic samples to determine the presence of gold, silver, and/or copper.

3.1 Effect of the Concentration and pH of the SDS Solution

Results obtained by use of different concentrations of unbuffered aqueous SDS (M_1) and buffered SDS (M_2 and M_3) solutions reveal the following trends:

(i) The metal ions Al^{3+}, Ti^{4+}, VO^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Bi^{3+}, and UO_2^{2+} are either immobile ($R_F = 0.0$) or only slightly mobile ($R_F \approx 0.05$) at all concentrations and over entire the pH range of SDS solutions. The mobility of Zn^{2+} was slightly higher (R_F = 0.30) when 0.05 m SDS solution of pH 2.3 was used as mobile phase.

(ii) Badly tailing spots ($R_L - R_T > 0.30$) were obtained for Ni^{2+}, Co^{2+}, and Tl^{+} with all the mobile phases used except 0.01 M SDS (pH 2.3). The mobility of these cations was higher when pH 2.3 SDS solution was used as mobile phase than when the SDS solution was buffered at pH 3.4, 5.7, 7.0, or 11.9.

(iii) Au^{3+} always migrated, as a well-formed spot, with the mobile phase front ($R_F > 0.90$) irrespective of the concentration or pH of the SDS solution. It can, therefore, be selectively separated from binary mixtures of other metal ions.

(iv) The mobility of Cu^{2+}, Zn^{2+}, Ag^{+}, Bi^{3+}, and Al^{3+} was marginally increased by substitution of 0.001 or 0.005 M buffered SDS (pH 2.3) with 0.01 or 0.05 m SDS (pH 2.3).

(v) The development time for 10 cm ascent was typically short – 10 to 12 min for all the mobile phases used.

Metal ion R_F data obtained with buffered SDS (pH 2.3) containing different concentrations of SDS (mobile phase M_2) are compared in **Figure 1**. It is clearly apparent from this figure that the mobility of the metal ions is barely affected by the concentration of SDS in the mobile phase.

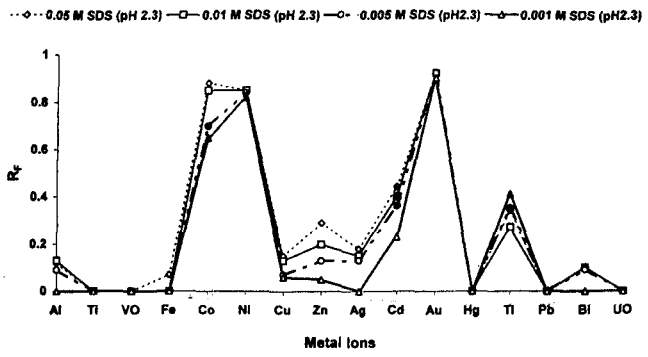

Figure 1

Effect of SDS concentration, at fixed pH, on the mobility of the metal cations. Filled symbols indicate tailing spots.

3.2 Effect of Added Amino Acids

Because buffered and non-buffered micellar SDS solutions resolved only a limited number of two-component mixtures of the metal cations, it was decided to improve the separation efficiency of the SDS mobile phase system. Several workers have reported [42–46] improvement of the chromatographic efficiency of micellar systems as a result of alteration of the micellar properties of the mobile phase by use of organic and inorganic additives such as alcohols, diols, dipolar aprotic solvents (DMSO, dioxane), alkylnitriles, alkanes, urea, NaCl, and acetone. It is surprising that no references are available on the use of amino acids as additives in the micellar TLC of inorganic species – because amino acids are amphiphilic substances their use might provide unique selectivity in the separation of metal cations.

In this study mobile phases containing different concentrations of amino acids (L-arginine, DL-phenylalanine, L-histidine, or L-tryptophan) in buffered SDS (pH 2.3) were prepared by adding 0.01 M amino acid solutions to SDS solution (0.001–0.05 M) in the volume ratios 1:1, 3:7, 7:3, 9:1, and 1:9, always keeping the total volume constant. Chromatography performed with these mobile phases (M_4–M_9) revealed the following trends:

(i) Mobile phases M_4–M_7 (3:7, 5:5, and 7:3) containing the amino acids arginine, phenylalanine, tryptophan, or histidine at concentrations of 30–70% resulted in tailing spots ($R_L - R_T > 0.3$) for all the metal ions except Al^{3+}, Pb^{2+}, and Fe^{3+}, which remained at the point of application, and Au^{3+}, which moved with the mobile phase front. The presence of amino acids in SDS-containing mobile phases probably causes tailing of the spots of metal cations because of competitive interactions among cations, charged amino acid, and anionic SDS. Because the experiments were performed at pH 2.3, the amino acids in this study bear a net partial positive charge.

(ii) With mobile phases containing either 10% SDS and 90% amino acids (M_4–M_7, 1 + 9) or 90% SDS and 10% amino acids (M_4–M_7, 9 + 1) for several of the cations spot tailing was reduced compared with use of the mobile phases discussed above in (i). The results are compared in **Table 3**. Better separation was obtained by use of mobile phases containing 10% SDS

Table 3

R_F values of metal cations on silica gel G layers developed with micellar mobile phases containing buffered SDS (pH 2.3) and amino acids.

Metal ion	0.01 M SDS (pH 2.3)–0.01 M L-arginine			0.01 M SDS (pH 2.3)–0.01 M DL-phenylalanine			0.01 M SDS (pH 2.3)–0.01 M L-tryptophan			0.01 M SDS (pH 2.3)–0.01 M L-histidine		
	9 + 1	1 + 9	5 + 5	9 + 1	1 + 9	5 + 5	9 + 1	1 + 9	5 + 5	9 + 1	1 + 9	5 + 5
Al^{3+}	0.05	0.05	0.10	0.14	0.05	0.00	0.00	0.00	0.00	0.10	0.00	0.11
Ti^{4+}	0.15	0.30T	0.25T	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20T
VO^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fe^{3+}	0.08	0.07	0.10	0.00	0.25T	0.06	0.06	0.03	0.06	0.00	0.00	0.06
Co^{2+}	0.75	0.70	0.75	0.17T	0.58	0.22T	0.68	0.69	0.50T	0.57	0.55	0.78T
Ni^{2+}	0.65T	0.50T	0.50T	0.17T	0.65	0.40T	0.80T	0.69	0.50T	0.57	0.52	0.80T
Cu^{2+}	0.47T	0.13	0.25	0.05	0.36T	0.15	0.55	0.68	0.29	0.18	0.57	0.25T
Zn^{2+}	0.25T	0.12	0.20T	0.10	0.17	0.09	0.60	0.00	0.25T	0.17	0.05	0.22T
Ag^{+}	0.30T	0.23T	0.20T	0.00	0.05	0.15	0.35T	0.15	0.26T	0.16T	0.05	0.13
Cd^{2+}	0.35T	0.28T	0.30T	0.10	0.18	0.20T	0.17T	0.45T	0.45T	0.19	0.18	0.30T
Au^{3+}	0.90	0.89	0.90	0.41T	0.90	0.70T	0.90	0.90	0.90	0.75	0.95	0.92
Hg^{2+}	0.43T	0.30T	0.22T	0.05	0.17T	0.27T	0.55T	0.45T	0.34T	0.25T	0.56	0.45
Tl^{+}	0.35T	0.25T	0.30T	0.35	0.2T	0.17T	0.20T	0.20T	0.15	0.43	0.25	0.30
Pb^{2+}	0.15	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bi^{3+}	0.25T	0.25T	0.25T	0.00	0.00	0.09	0.00	0.20T	0.19T	0.00	0.00	0.00
VO_2^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

T = tailing spot ($R_L - R_T > 0.3$)

Table 4

Separations achieved experimentally on silica gel G layers developed with different mobile phases.

Mobile phase	Separation ($R_F \times 100$)[a]
0.01 M SDS in 2.3 pH buffer	Ni^{2+} or Co^{2+} (82)–Al^{3+} (10) or Ag^{+} (16)
	Ni^{2+} (82)–Fe^{3+}, Hg^{2+}, VO^{2+}, Ti^{4+}, UO_2^{2+}, or Pb^{2+} (0)
	Co^{2+} (82)–Tl^{+} (30) or Cd^{2+} (32)
	Au^{3+} (90)–Ag^{+} (16)
	Au^{3+} (90)–Tl^{+} (30) or Cd^{2+} (32)
	Tl^{+} (30)–Al^{3+} (10)
0.01 M SDS (2.3 pH)–0.01 M DL-phenylalanine, 1 + 9	Au^{3+} (92)–Zn^{2+} (17) or Ag^{+} (5)
	Co^{2+} (80)–Zn^{2+} (17)
	Ni^{2+} (80)–Ag^{+} (05)
	Co^{2+} (80)–Bi^{3+}, VO^{2+}, UO_2^{2+}, or Pb^{2+} (0)
0.01 M SDS (2.3 pH)–0.01 M L-tryptophan, 1 + 9	Au^{3+} (95)–Cu^{2+} (65)–Ag^{+} (5)
	Au^{3+} (95)–Co^{2+} (68)–Fe^{3+} (0)
	Au^{3+} (95)–Ni^{2+} (68)–Zn^{2+} or Pb^{2+} (0)
0.01 M SDS (2.3 pH)–0.01 M L-histidine, 1 + 9	Au^{3+} (95)–Cu^{2+} (53)–Ag^{+} (05)
	Au^{3+} (95)–Cu^{2+} (53)–Zn^{2+} (05)
	Au^{3+} (95)–Cu^{2+} (53)–Bi^{3+}, Fe^{3+}, VO^{2+}, UO_2^{2+}, Ti^{4+} or Al^{3+} (0)

[a]The R_F values of metal ions in their mixtures are slightly different from their individual R_F values because of mutual interactions.

(0.01 M, pH 2.3) plus 90% amino acids than with those containing 90% SDS (0.01 M, pH 2.3) plus 10% amino acids (0.01 M).

(iii) Despite our hopes, arginine (an aliphatic amino acid with a side-chain containing a basic group), which has been used satisfactorily for resolution of amino acid enantiomers by ligand-exchange TLC [47], was found unsuitable for separating metal cations. Similarly, use of phenylalanine (a non-cyclic aromatic amino acid) led to unsatisfactory results. Heterocyclic aromatic amino acids such as tryptophan and histidine were, however, found to provide better-resolved spots of the metal cations. On the basis of the results presented in Table 3 the suitability of amino acids for separation of two- or three-component mixtures of metal cations was in the order: L-histidine = L-tryptophan > DL-phenylalanine > L-arginine Thus micellar mobile phases containing heterocyclic amino acids such as L-tryptophan or L-histidine proved superior to mobile phases containing aromatic or aliphatic amino acids such as phenylalanine or arginine for the separation of metal cations.

(iv) The mobile phase 0.01 M buffered SDS (pH 2.3)–0.01 M histidine (M_7) or tryptophan (M_6), 1 + 9, was found most suitable for separating mixtures of Au^{3+}, Cu^{2+}, and Ag^{+} ions (**Table 4**).

(v) When chromatography was performed with mobile phases (M_8), obtained by mixing 0.001 M, 0.005 M, or 0.05 M SDS (pH 2.3) with 0.01 M amino acid (L-arginine) in the ratio 9:1, less tailed spots for Ni^{2+}, Co^{2+}, slightly higher mobility for Cu^{2+} and Tl^{+}, and increased compactness for Zn^{2+} and Cd^{2+} were observed as the concentration of SDS in the mobile phase was increased. The mobility of Fe^{3+}, Pb^{2+}, Bi^{3+}, Hg^{2+}, Al^{3+}, Ti^{4+}, UO_2^{2+}, and VO^{2+} (R_F = 0.0) and Au^{3+} (R_F = 0.90), however, remained unchanged over the entire SDS concentration range.

(vi) When 0.01 M amino acid in the mobile phases M_4–M_7 was substituted with 0.001 M amino acid little increase in the mobility of Cu^{2+}, Fe^{3+}, Ni^{2+}, Co^{2+}, Zn^{2+}, and Tl^{+} was noticed with mobile phases containing L-arginine or DL-phenylalanine, whereas the opposite trend (i.e. reduced mobility) was observed for these metal ions when the mobile phase contained L-tryptophan. The mobility of the other metal ions was unchanged. When L-histidine was used as mobile phase additive the mobility of all the metal ions was almost constant, irrespective of histidine concentration (0.01 or 0.001 M).

To provide more information about the variation of the R_F values of the metal ions as a function of the concentration of amino acids in the mobile phase, a representative plot of R_F against the volume fraction of 0.01 M L-histidine was constructed (**Figure 2**). The curves shown in this figure pass through maxima and minima which reveal that the R_F values (or mobility) of the metal cations vary without any regular pattern. This situation probably arose because of the occasional formation of tailing spots as a result of multiple interactions.

Differences, ΔR_F, between R_F values obtained for the metal cations by use of mobile phases M_6, 1 + 9, and M_7, 1 + 9, are plotted in **Figure 3**, to show the net effect of changing the microenvironment of the micellar mobile phase on the mobility

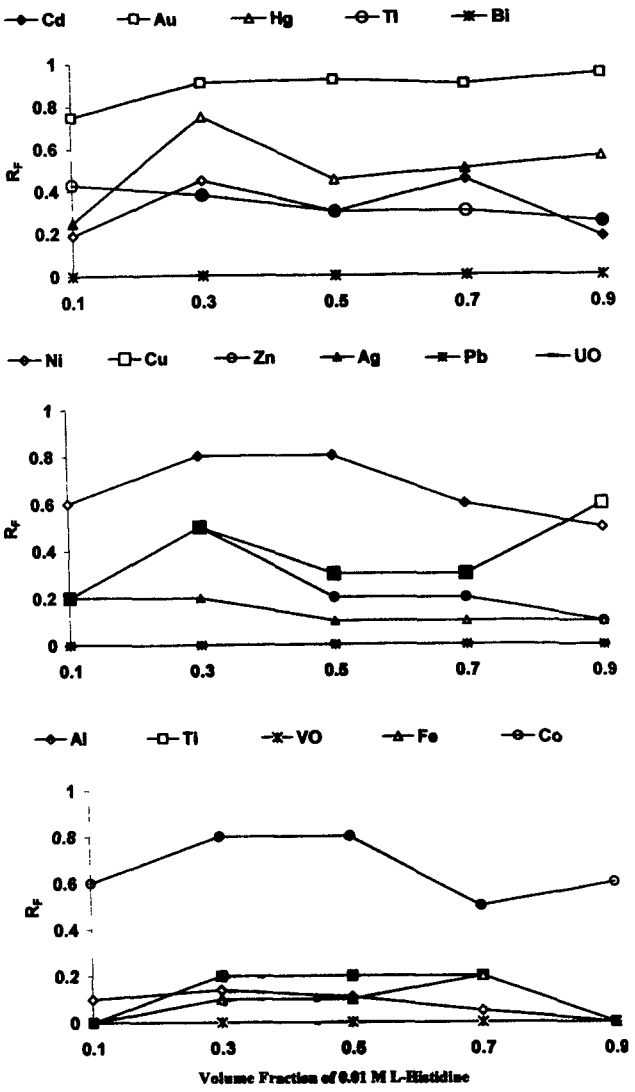

Figure 2

Dependence of R_F on the volume fraction of 0.01 M L-histidine. Filled symbols indicate tailing spots.

of metal ions. It is clear from this figure that metal ions either migrate faster (positive ΔR_F value) or have the same mobility (ΔR_F values ±0.05) when the micellar mobile phase contains tryptophan (5- or 6-membered heterocyclic amino acid) rather than histidine (5 membered heterocyclic amino acid). Thus the mobility of the cations is enhanced by use of micellar mobile phases containing an amino acid with a large non-polar side chain (e.g. tryptophan) rather than an amino acid with a polar side chain (e.g. histidine).

3.3 Separations

Results from separation of the metal ions on silica layers with micellar systems in the presence and absence of amino acids are listed in Table 4. To widen the applicability of the method the separation of Au^{3+}, Cu^{2+}, and Ag^{+} was examined in the presence

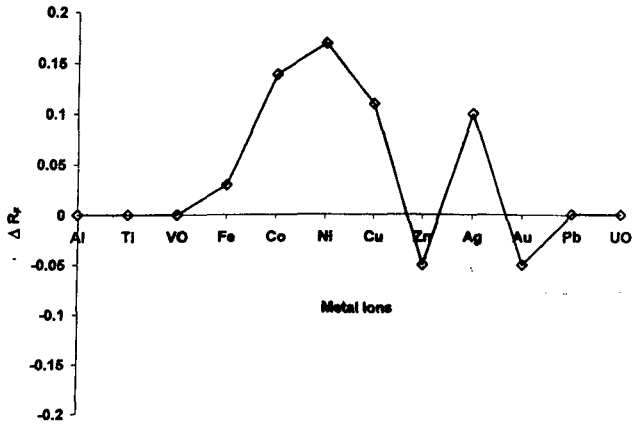

Figure 3

Differences, ΔR_F, between R_F values obtained for the metal cations by use of mobile phases M_6, 1 + 9, and M_7, 1 + 9 ($\Delta R_F = R_{FM6,1+9} - R_{FM7,1+9}$).

of organic (phenols, urea, thiourea) and inorganic (cationic and anionic) impurities.

The results presented in **Tables 5** and **6** indicate that the impurities have no effect on the mobilities of Au^{3+} and Ag^+ but that the mobility of Cu^{2+} is affected by the impurities – the R_F of Cu^{2+} varied from 0.30 (*o*-nitrophenol impurity) to 0.60 (thiourea impurity). Despite this the separation of Au^{3+}, Cu^{2+}, and Ag^+ from their mixtures was always possible. The poorest separation was in the presence of $K_2S_2O_8$ and ammonium oxalate, because of the formation of tailing of Cu^{2+}.

Table 5

Effect of organic and inorganic impurities (1% aqueous solutions) on the separation of Au^{3+}, Cu^{2+}, and Ag^+ on silica gel G layers developed with 0.01 M SDS (2.3 pH)–0.01 M L-histidine, 1 + 9.

Impurity	Separation (R_F)		
	Au^{3+}	Cu^{2+}	Ag^+
Urea	0.94	0.56	0.05
Thiourea	0.95	0.60	0.05
$NaNO_3$	0.92	0.44	0.00
$NaNO_2$	0.93	0.52	0.00
$NaMoO_4$	0.94	0.44	0.00
NH_4SCN	0.94	0.55	0.05
NaH_2PO_4	0.93	0.58	0.00
KIO_3	0.92	0.50	0.05
KIO_4	0.95	0.44	0.05
KI	0.95	0.55	0.05
$K_4Fe(CN)_6$	0.93	0.52	0.00
$K_3Fe(CN)_6$	0.95	0.52	0.05
KSCN	0.94	0.55	0.05
$K_2S_2O_8$	0.92	0.52T	0.00
Ammonium oxalate	0.95	0.52T	0.00

T = tailing spot ($R_L - R_T > 0.3$)

Table 6

Separation of Au^{3+}, Cu^{2+}, and Ag^+ ions from their mixtures, in the presence of phenolic compounds as impurities, on silica gel G layers developed with 0.01 M SDS (2.3 pH)–0.01 M L-histidine, 1 + 9.

Impurity	Separation (R_F)		
	Au^{3+}	Cu^{2+}	Ag^+
Phenol	0.93	0.55	0.05
Phloroglucinol	0.95	0.56	0.06
Pyrogallol	0.94	0.51	0.00
m-Nitrophenol	0.96	0.50	0.00
o-Nitrophenol	0.96	0.30	0.00
p-Nitrophenol	0.95	0.53	0.05
Vanillin	0.95	0.57	0.05
Pyrocatechol	0.95	0.49	0.00
m-Hydroxyacetophenone	0.95	0.52	0.05
Gallic acid	0.95	0.56	0.05
Orcinol	0.93	0.35	0.00
Picric acid	0.96	0.59	0.05
Hydroquinone	0.95	0.55	0.05
Resorcinol	0.93	0.53	0.00
o-Cresol	0.96	0.55	0.05
m-Cresol	0.95	0.53	0.00
p-Cresol	0.92	0.45	0.05

From the data listed in Table 6 it seems that the mobility of Cu^{2+} is affected by the position of substituent groups on the benzene ring. For example, the order of the increase in the R_F of Cu^{2+} (given in parentheses) in the presence of the *o*, *m*, and *p* isomers of nitrophenols and cresols was:

o-nitrophenol (0.30) < *m*-nitrophenol (0.50) < *p*-nitrophenol (0.53)

and

p-cresol (0.45) < *m*-cresol (0.53) < *o*-cresol (0.55).

This reversal of the order of the mobility of Cu^{2+} can be attributed to the opposite effects of NO_2 (an electron-withdrawing group) and CH_3 (an electron-releasing group) attached to benzene ring. The ΔR_F values (differences between the R_F values of resolved spots from binary mixtures of metal cations) obtained for Au^{3+}–Ag^+, Au^{3+}–Cu^{2+}, and Cu^{2+}–Ag^+ pairs on silica gel layers were 0.95, 0.30, and 0.65, respectively, when 0.01 M SDS–0.01 M L-tryptophan, 1 + 9, was used as mobile phase and 0.95, 0.42, and 0.53, respectively, when 0.01 M SDS–0.01 M L-histidine, 1 + 9, was used as mobile phase. From these data it can be safely concluded that the SDS–tryptophan system is better for resolving Cu^{2+} from Ag^+(ΔR_F = 0.65) whereas the

Table 7

Mobility trends of metal cations on silica gel G layers developed with water, unmodified micellar mobile phases, and amino acid-containing micellar mobile phases.

Metal ion	Water	0.01 M SDS	0.01 M SDS at pH 2.3	0.01 M L-arginine	0.01 M DL-phenylalanine	0.01 M L-tryptophan	0.01 M L-histidine
Al^{3+}	0.00	0.00	0.13	0.00	0.00	0.00	0.00
Ti^{4+}	0.40T	0.00	0.00	0.10	0.00	0.00	0.20 T
VO^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fe^{3+}	0.14	0.00	0.00	0.22	0.22T	0.08	0.12
Co^{2+}	0.30T	0.80T	0.85	0.40T	0.40T	0.19T	0.81T
Ni^{2+}	0.50T	0.80T	0.85	0.45T	0.50T	0.20T	0.83T
Cu^{2+}	0.15	0.00	0.13	0.22	0.22T	0.14	0.12
Zn^{2+}	0.10	0.00	0.20	0.13	0.05	0.05	0.15
Ag^{+}	0.25T	0.15	0.15	0.30T	0.20T	0.30T	0.18T
Cd^{2+}	0.20T	0.14	0.40T	0.15	0.25T	0.17T	0.35T
Au^{3+}	0.90	0.88	0.92	0.85	0.75T	0.77T	0.85
Hg^{2+}	0.50T	0.05	0.00	0.50T	0.35T	0.50T	0.50T
Tl^{+}	0.20T	0.05	0.27	0.18T	0.18T	0.15T	0.25T
Pb^{2+}	0.00	0.00	0.00	0.00	0.00	0.06	0.00
Bi^{3+}	0.40T	0.00	0.10	0.20T	0.15	0.00	0.25T
UO_2^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.00

T = tailing spot ($R_L - R_T > 0.3$)

Table 8

Simultaneous separation of Au^{3+}, Cu^{2+}, and Ag^{+} from real and spiked samples on silica gel G layers with 0.01 M SDS (pH 2.3)–0.01 M L-histidine, 1 + 9, as mobile phase.

Sample	Separation (R_F)	Separation of ions added to spiked sample (R_F)		
		Au^{3+}	Cu^{2+}	Ag^{+}
High-copper dental amalgam	Ag^{+} (0.04)–Cu^{2+} (0.52)	0.91	0.52	0.05
	Zn^{2+} (0.14)–Cu^{2+} (0.52)			
	Ag^{+} (0.0)–Hg^{2+} (0.57)			
	Zn^{2+} (0.09)–Hg^{2+} (0.55)			
Printed circuit board	Cu^{2+} (0.52)– Au^{3+} (0.91)	0.92	0.53	0.50
Silver mirror scrap	Ag^{+} (0.05)–Cu^{2+} (0.52)	0.92	0.52	0.05
Silver mirror spent solution	Ag^{+} (0.05)–Cu (0.53)	0.92	0.53	0.50
Sterling silver	Ag^{+} (0.05)–Cu (0.53)	0.91	0.52	0.05

SDS–histidine system is more useful for resolving Au^{3+} from Cu^{2+} ($\Delta R_F = 0.42$). The detection limits [μg, in parentheses] for Cu^{2+} (6.6), Ag^{+} (1.6), Fe^{3+} (0.76), Zn^{2+} (0.022), Cd^{2+} (0.038), and Hg^{2+} (0.029) enable highly sensitive detection of the heavy metals at trace levels – Zn^{2+} and Hg^{2+} down to 0.02 and 0.03 μg, respectively, can easily be detected on TLC plates.

The data presented in **Table 7** clearly demonstrate that although the unmodified mobile phases are of little practical utility for separation purposes, buffered SDS (pH 2.3) combined with amino acids (histidine or tryptophan) have enormous analytical potential to facilitate analytically important separations of two-component mixtures of cations.

3.4 Applications

The proposed method has been successfully used for identification and separation of Au^{3+}, Cu^{2+}, Ag^{+}, Ni^{2+}, and Hg^{2+} from a variety of matrixes. The results are summarized in **Table 8**.

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Densitometric Thin-Layer Chromatography of Polycyclic Aromatic Sulfur Compounds

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Key Words:

TLC
Densitometry
Aromatic sulfur compounds
Determination
Separation

Summary

Thin-layer chromatography has been used to separate a mixture of sulfur polynuclear aromatic hydrocarbons (S-PAH), heterocyclic compounds with mutagenic and carcinogenic properties. S-PAH were oxidized to give the sulfone derivatives and then S-PAH standards, their oxidized forms, and PAH were applied to silica gel and RP-18 plates and developed in a horizontal chamber with different mobile phases. After chromatography the plates were observed in UV light at $\lambda = 254$ nm and scanned densitometrically at the same wavelength. R_F values were determined for the compounds.

1 Introduction

Aromatic sulfur compounds (e.g. thiaarenes, sulfur polynuclear aromatic hydrocarbons) are heterocyclic compounds with antiestrogenic, mutagenic, and carcinogenic properties [1–5]. Thiaarenes are ubiquitous in the environment, because of their thermal and photochemical stability. Sulfur polynuclear aromatic hydrocarbons (S-PAH) occur with PAH and enter the atmosphere and then soil, water, and sludges mainly as a result of fuel combustion [4, 6]. Because their concentrations are smaller than those of PAH, and because many thiaarene isomers are possible, their determination is very complicated [2] and is sometimes omitted in complex analysis of environmental samples.

Determination of the heterocyclic sulfur compounds present in mixtures obtained from natural and anthropogenic sources involves two steps:

– extraction of concentrates of sulfur compounds from those mixtures; and

– separation of the extracted concentrates and qualitative and/or quantitative determination of individual compounds.

The first step has been described elsewhere [7]. A variety of chromatographic techniques has been used for the second step [1–4, 6–8].

The aim of this work was selection of optimum conditions for the densitometric thin-layer chromatographic analysis of selected biologically active sulfur compounds that might be present in environmental samples.

2 Experimental

2.1 Standards and Materials

The composition of the standard mixture of S-PAH used is shown in **Table 1**. A standard mixture of PAH (Supelco No 4-9156) containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)-fluoranthene, benzo(*a*)pyrene, benzo(*a*)anthracene, benzo(*ghi*)-perylene, and indeno(123-*cd*)pyrene was also used. 3-Chloroperbenzoic acid was from Fluka.

Chromatography was performed on 20 cm × 20 cm aluminum-backed silica gel (Merck, Darmstadt, Germany) and RP-18 (Macherey–Nagel, Düren, Germany) TLC plates. Methanol, dichloromethane, chloroform, acetonitrile, acetone (POCh, Gliwice, Poland), pentane, *n*-hexane (Fluka), and redistilled deionized water were used as mobile phases.

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Sorry, but it was a mistake: your article will be published in our journal from 2002 (not 2003), vol.4, no. 1.

Sincerely yours,
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"Acceptance Letter for Chapter V"

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Dear Dr. Mohammad,

This is only to let you know briefly, that your most recent paper submitted for publication in "Acta Chromatographica" ("Novel solvent system for the separation of Cr6+ from Cr3+ and associated heavy metal cations by high-performance thin-layer chromatography") was reviewed and recommer for publication in AC (No.13, 2003, to appear some time in mid-2003).

Formal acceptance letter will be airmailed to your postal address very soon.

Best regards, sincerely yours,

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