

CHAPTER I

THEORY AND LITERATURE SURVEY

## 1.1 INTRODUCTION

The practice of chromatography is much older than a knowledge of its theory. The technique of chromatography was invented by M.Tswett,<sup>1</sup> a botanist, in 1906 in Warsaw. This ingenious adsorptive method provided scientists with an effective and efficient procedure to separate chemical compounds in a state of high purity.

The name chromatography (Greek word, Chroma means colour and graphy means writing) means colour writing and evidently Tswett did not comprehend its use beyond coloured materials. Although he was unsuccessful in applying the technique to colourless substances, later chromatographers have successfully extended its potentialities for the separation of colourless substances. The main problem was the detection of the colourless zones on the adsorbent materials. Now many ingenious techniques are coupled to locate these zones. As for example, the development of fluorescence by UV irradiation or alternately by spraying with suitable reagents and developing coloured complexes. So the word chromatography has come to be a misnomer 'kinetic partition' would be a better term, but the word chromatography is so popular that it is unlikely to be superceded. A gamut of physico-chemical phenomena such as adsorption, coagulation, precipitation, ion-exchange, counter-current extraction and liquid partition between the stationary and the mobile phases are operative in chromatography.

Chromatography is an analytical technique used for the resolution of different solutes by their differential migration

rates in a porous medium. The migration is due to the flow of solvents.

## 1.2 CLASSIFICATION

Chromatography can be classified on the basis of the physico-chemical forces operative in the separation techniques. They are broadly adsorption, electric charge, steric factor and partition.

### (i) Adsorption chromatography :

Adsorption chromatography is based on the differences in the adsorption coefficients. When separation happens continuously between a liquid and a solid phase, it is called hypersorption.<sup>2</sup> Separations can also be between a gaseous and solid phase or at the interface between gaseous and liquid phase.

### (ii) Electrophoretic and ion exchange chromatography :

The electric charge on the substances can be correlated to their dissociation constants. Based on this, separation can be effected in two ways by ion-exchange and by electrophoresis.

### (iii) Gel-permeation chromatography :

In gel-filtration, gel permeation and allied ion- or molecular sieving, the size (steric factor) of the ions or molecules diffusing into the sieve structure become important.

(iv) Partition chromatography :

If one of the two phases - stationary - is supported on a suitable carrier and the other phase is allowed to flow through slowly-mobile phase; the mixture can be separated by partition chromatography. Partition can be between liquid and gaseous phases constitutes the gas-liquid chromatography. Partition can also be between two liquid phases usually one of the liquids (the more polar) is anchored on a suitable solid carrier such as silica gel or a cellulose base, while the other less polar phase is allowed to flow through slowly.

If silica gel is replaced by a filter paper, it makes paper chromatography. Thus paper chromatography is a special case of partition chromatography in which the adsorbent column is a paper strip.

### 1.3 PAPER CHROMATOGRAPHY

Paper chromatography is today considered not only as one of the best among the modern analytical tools, but rightly as the ultimate in the separation techniques. It is particularly useful in the separation of complex mixtures, labile substances and closely related compounds such as isomers, homologues, isotopes and multiple valences.

The credit for the present fullfledged status of paper chromatography goes to A.J.P.Martin<sup>3</sup> and his coworkers<sup>4</sup> R.Consden, A.H.Gordon and R.L.M.Synge. Consden, Gordon and Martin<sup>5</sup> showed that filter paper sheets and strips can also be

used as support of a stationary phase in partition chromatography. This technique, called as paper chromatography is probably the most versatile method for analytical work on a micro scale. The other names proposed are papyrography<sup>6</sup> and partography.<sup>7</sup>

Originally paper chromatography was used to separate mixture of organic substances such as dyes and amino acids only. But now method has been perfected to separate cations and anions of inorganic substances as well.

The technique of paper chromatography consists essentially of three stages :

- (i) spotting the mixture on a cellulose paper
- (ii) elution with a solution or solvent, and
- (iii) identification of the bands through their natural colours or physically or chemically developed colours.

The whole mechanism of separation takes place on the paper. Therefore, the filter paper plays an important role in the success of paper chromatography. Cellulose makes the filter paper. It is manufactured exclusively from the purest form of shorter cotton fibres termed as linters. Whatman filter papers commonly used for chromatographic purpose, have a content of 99 % of  $\alpha$ -cellulose. The mineral content varies from 0.07 % to 0.01 %. Inorganic trace matter composed largely of complex silicates<sup>8-9</sup> may range from 0.04 - 0.07 % of the total chromatographic papers also contain lipophilic substances - fats, waxes and resins - upto 0.025 %. These are generally washed out by the mobile solvent to the solvent front during chromatography.

The choice of paper is dependent on the type of the problem under investigation. The prime factors that govern the choice are whether the paper is to be used for quantitative or qualitative analysis, for analytical or preparative chromatography or whether the substance used are hydrophilic or lipophilic, neutral or charged species. Ordinary filter paper will serve most purposes.

To speed-up chromatographic analysis, coarser and faster papers can be used when substances to be separated have sufficiently wide-apart  $R_F$  values. Whatman No.31 Et is about four times faster than Whatman No.1. Slow papers are rarely employed, but they, of course, facilitate a better resolution of substances with close  $R_F$  values. Heavy papers such as Whatman No.3 MM are suited for preparative purposes. The slow papers available are Whatman 20 Schleicher and Schull 2045 a, Macherey Nagel 261 and Edrol 208. With Schleicher and Schull 2071, a single sheet has been used for the separation of gram quantities of substances.

Characteristics of Whatman chromatography papers are summerized in following table.

Papers	Rate of flow		
	Fast	Medium	Slow
Thin	No. 4	No. 7	No. 2
	No. 54	No. 1	No. 20
	No. 540		
Thick	No. 31	No. 3	
	No. 17	No. 3 MM	

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The flow rate on the paper is dependent both on the nature of the paper as well as on the solvent. To a larger extent, the flow rate of the solvent is governed by its viscosity. Whatman No. 1 and 4 have the same characteristics, but the flow rate of the latter is twice as fast as that of the former.

For the efficient separation of polar substances, the exchange capacity of the paper is increased by increasing the carboxyl content (1.4 %) by partial oxidation.

It is possible to increase the capillarity of the paper by partial hydrolysis which is achieved by soaking the filter paper for 24 hours in 7 % hydrochloric acid and washing successively with water and ethanol.

It is possible to increase the rigidity of the paper by acetylation, esterification and other chemical methods.

Although several minor physico-chemical forces such as ion-exchange and adsorption are operative in all chromatographic work, the most plausible mechanism is a partition between two immiscible phases, one stationary and the other mobile.<sup>10-11</sup> The stationary phase is made up of the solvent held by the cellulose of the paper, while the mobile phase is the irrigating eluent or moving liquid or developing solvent. The two phases are in contact over a very large interface on the filter paper, facilitating rapid attainment of equilibrium in distribution between the two phases. A partition mechanism requires two immiscible liquids. But the solvent mixtures used in the paper

chromatography are very often miscible with water and sometimes water itself serves as the eluent.<sup>12-14</sup>

Generally water or hydroxylated polar solvents are used as stationary solvents and more or less non-polar solvents as mobile phases.

#### 1.4 MIGRATION PARAMETERS

By employing the concept of a theoretical plate, Martin et al.<sup>15</sup> developed an equation to correlate the rate of movement of a zone with a partition coefficient viz.

$$R_F = \frac{A_L}{A_L + A_S}$$

where,

$\alpha$  = partition coefficient

=  $\frac{\text{concentration in water phase}}{\text{concentration in lipid phase}}$

$A_S$  = The cross-sectional area occupied by the stationary phase

$A_L$  = The cross-sectional area occupied by the mobile phase

$R_F$  =  $\frac{\text{Distance travelled by the solute zone}}{\text{Distance travelled by the solvent zone}}$

$R_F$  value is a function of the partition coefficient. The  $R_F$  values have been shown to agree well with those calculated from the partition coefficients measured by static methods. Also the degree of separation of two substances of known



partition coefficients can be calculated for a given length of column or paper strip. The  $R_F$  value measures the velocity of movement of the zone relative to that of the developer front.

$R_F$  value is influenced by so many physical conditions. It is a constant for a given substance, provided the conditions of chromatographic system are kept constant with respect to temperature, type of paper flow rate, geometry of the paper, duration and direction of development, nature and the shape and size of the wick used (i.e. radial chromatography), the amount of liquid in the reservoir, humidity, etc.

The  $R_F$  defines the movement of the substance relative to the solvent front in a given chromatographic system.

The  $R_F$  value of a substance depends upon a number of factors which are :

- (1) the solvent employed;
- (2) the medium used for separation i.e. the quality of the paper in case of paper chromatography;
- (3) the nature of the mixture;
- (4) the temperature and
- (5) the size of the vessel in which the operation is carried out.

Keeping the above factors constant, it is possible to compare the  $R_F$  values of different substances. The  $R_F$  value is never greater than unity.  $R_F$  values apparently larger than unity may be encountered under exceptional conditions when a solvent produces two distinct fronts.<sup>16</sup> The  $R_F$  value

is calculated on the basis of the front near to the origin (the aqueous wet solvent front). The solute ions in this case can travel beyond aqueous front to the volatile organic dry solvent front. Those components of the mixture to be separated which are most readily soluble in the organic mobile phase will have  $R_F$  values near, or equal to unity. Those components which have a lower solubility in the organic phase will have  $R_F$  values near to zero. The  $R_F$  value is a characteristic of a particular species in any given type of separation and is sometimes used for the qualitative identification of the unknown species.

Besides the  $R_F$  values, the chromatograms are indicated by other different terms such as  $R_X$ ,  $R_M$  and  $R_C$ . These parameters are also qualitative and quantitative parameters, characteristic of a substance.

$R_X$  values : Whenever greater reliability is required, reference standards are used in parallel runs or as internal standards. In some cases, the solvent front runs off the end of filter paper, the movement of a substance in such cases is expressed as  $R_X$  but not  $R_F$ .

$$R_X = \frac{\text{Distance travelled by the substance from the original line}}{\text{Distance travelled by the standard substance X from the original line}}$$

$R_M$  values<sup>17, 18</sup> : The  $R_F$  values of chemically related compounds are very close. The influence of the individual functional groups was presumed to be added to a rough approximation.

According to Bate-Smith and Westall,<sup>17</sup>  $R_M$  is defined as follows :

$$R_M = \log \left[ \frac{1}{R_F} - 1 \right]$$

The term  $R_M$  is additive and is composed of the partial  $R_M$  values of the individual functional groups or other grouping of atoms in the molecules.<sup>18,19</sup>

$R_C$  values : In many cases, deviation from the rule of additivity has been disclosed. To obviate this difficulty, Decker suggested calibration of the gradient (one of the causes responsible for the deviation) with substances from standard homologous series.<sup>20,21</sup>

### 1.5 ELUTION TECHNIQUES

There are variety of elution techniques in paper chromatography.

#### (1) Descending chromatography :

When the development of the paper is done by allowing the solvent to travel down the paper, it is known as descending technique. The advantage of this technique is that the development can be continued indefinitely eventhough the solvent runs off at the other end of the paper.

#### (2) Ascending chromatography<sup>22,23</sup> :

When the development of the paper is done by allowing the solvent to travel up the paper, it is known as ascending technique.

(3) Ascending-descending chromatography<sup>24</sup> :

It is the hybrid of the above two techniques. Herein the upper part of the ascending chromatogram can be folded over a glass rod allowing the ascending development to change over into the descending after crossing the glass rod.

(4) Horizontal-linear development<sup>25</sup> :

This type of development requires only a limited space. The chromatogram is placed horizontally on glass rods or stretched synthetic fibres (nylon) or aluminium plates. The advantage of this method lies in its reproducible  $R_f$  values<sup>26</sup> and the only possible disadvantage is the diffusion due to surface phenomenon at the interface between the moist paper and the plates.

(5) Spiral development :

Herein the solvent system is introduced in a direction perpendicular to the axis of the spiral. The advantage is it eliminates irregularities and uneven saturation caused by high tanks. One disadvantage of this technique is that the solvent front is not visible during development.

(6) Radial development :

This is also known as circular paper chromatography. This makes use of radial development.<sup>27,28</sup> The gap in the circular paper will produce geometrical irregularities in the chromatogram.

(7) Conical chromatography<sup>29-31</sup> :

In this technique, the paper is formed into a cone and the substances to be resolved are applied to its base. The substances get concentrated towards the apex, even as in the filtration process, although the set-up of the filter paper is reversed.

(8) Wedge-strip and related techniques<sup>32-34</sup> :

This technique is a combination of radial and linear (ascending) developments. A large number of wedge strips can be run simultaneously on a single chamber.

(9) Multiple chromatography :

This includes procedures in which irrigation is repeated either in the same direction as in the first run-multiple development - or in a direction perpendicular to the flow of the first system - two dimensional chromatography. This technique <sup>is good for</sup> (better) the separation of complex mixtures. Fractional chromatography is a special aspect of multiple chromatography.

(10) Two-dimensional chromatography<sup>5</sup> :

The paper can be a square or rectangle. The sample is applied to one of the corners. The second development performed at right to the direction of the first run. This type of chromatography can be carried out with identical solvent system in both directions or by two solvent systems.



## 1.6 APPLICATIONS

### 1.6.1 Conditions to be satisfied

- (1) The composition of the flowing solvent should be kept constant throughout the development.
- (2) The developing solvent should flow at a slow rate which depends on temperature, paper used, etc.
- (3) The solute should be so chosen that the components to be separated have a small but definite solubility in it, because besides the absorption, the movement of a substance in a paper is a function of the solubility in the developing agent (solvent).
- (4) The relative movement of the solutes are conveniently given by their  $R_f$  values.

### 1.6.2 Versatility of paper chromatography

Paper chromatography has a wide and versatile field of applications, because it is mildest of all the separation techniques. Biochemical, organic, inorganic, natural and synthetic substances can easily be separated by this analytical technique.

Advantages of this technique are various -

- (1) The equipment is very simple which can be easily manipulated and fabricated.
- (2) It has a high efficiency of separation hardly surpassed by other techniques.

(3) Separation can be effected both on the micro and semimicro scale verging on to the macro scale.

(4) Closely related homologues, isotopes, isomers, multivalencies and very labile and reactive substances, polar and non-polar substances can be separated with stability.

(5) Consistant results are obtained.

(6) The case with which a large number of analysis can be performed.

1.6.3 Limitations

The limitations of paper chromatography are -

(1) Very large quantities can not be handled conveniently.

(2) The upward flow is slower than downward flow in descending chromatography.

(3) Inconsistant results are obtained after the solvent has travelled 28 cms.

1.7 IMPREGNATED FILTER PAPERS

For the efficient separation of polar substances such as cations, amines, amino acids, etc. the exchange capacity of the paper is enhanced by buffered and/or treated papers as Whatman-phosphate, Whatman-citrate, carboxy-methyl cellulose, amino-ethyl cellulose, diethylaminoethyl cellulose papers, etc.

For effecting the separation of hydrophobic substances, kieselguhr filter paper<sup>35</sup> is suitable. Silicon impregnated

papers as Whatman No. 1,4,8 and 20 act as carriers of the lipophilic phase in reverse-phase chromatography.<sup>36-39</sup> Papers treated with active adsorbents such as silicic acid or aluminium hydroxide are also used. Sometimes cellulose is used as a substrate for various other adsorbents. Alumina and silicic acid have been precipitated in the pores of the filter paper to produce a thin sheet of adsorbent which combine the flexibility of paper with the adsorbent characteristics of the precipitates. Filter paper impregnated with starch, oxine, DMG, alkali chlorides, etc. find more use in spot- test techniques. When used in paper chromatography, they work on the principle of precipitation chromatography.

The technique is improved for obtaining the reproducibility of  $R_F$  values in impregnated paper chromatography.<sup>40</sup>

Papers impregnated with ion exchangers give cleaner and faster cation separations than untreated papers. The separations now depend not only on partition, but also on high selectivities shown by the ion exchangers for various metal ions.<sup>41</sup> This outstanding property can be utilized in chromatography by impregnating papers with these materials. For this purpose, the inorganic material has definite advantages over organic resins.

- (1) Its synthesis and impregnation on paper is easier.
- (2) It does not shrink significantly in contact with aqueous solvents or exchanging ions.



- (3) The amount of the ion-exchanger on the paper can be varied and papers with different ion-exchange capacities can be easily prepared.
- (4) It is more selective and many difficult separations can be made easily.

When used in conjunction with mixed solvent systems, the inorganic ion exchange papers become even more effective for the separation of cations.

Previous studies suffer from two limitations :

- (1) Only pure inorganic solvents have been exclusively studied. Mixed solvent systems which have shown very attractive possibilities on ion exchange columns have not been adequately tested on ion exchange papers.
- (2) Numerous metal ions have not been chromatographed in a large number of solvent systems, and hence the useful number of separations developed is not large.

Few separations of one cation from numerous metal ions using inorganic ion exchange papers have been reported in the literature, although Sherma and coworkers<sup>42-45</sup> have reported a number of specific separations on papers impregnated with organic ion exchangers.

Chromatography has therefore been performed on papers impregnated with zirconium phosphate<sup>46-51</sup> selenite,<sup>52</sup> ammonium phosphomolybdate<sup>48,53</sup> and tungstate.<sup>54-59</sup> Some interesting

possibilities for the separation of alkaloids and amino-acids on zirconium phosphate papers have also been reported.<sup>60</sup>

From the earlier reports<sup>61-64</sup> it was observed that these papers are highly selective and they give rapid separations with simple aqueous systems.<sup>65</sup> Quantitative separations were also achieved by Qureshi and coworkers.<sup>66</sup>

Several elements Fe, Cs, Ce, Cr, As, V, Mo and Hg are separated and detected in different valency state on paper impregnated with zirconium phosphate.<sup>51</sup> The adsorption of metal ions -  $U^{6+}$ ,  $Th^{4+}$ ,  $Cu^{2+}$ , and  $La^{3+}$  from organic solvents such as methanol, ethanol, isopropyl alcohol and acetone mixed with acids such as HCl,  $HNO_3$  or  $H_2SO_4$  was studied on cellulose paper impregnated with zirconium phosphate, sulphonic acid resins and quaternary ammonium compounds.<sup>67</sup>

Papers impregnated with inorganic ion exchangers tin(IV), tungstate and selenite have been shown to be useful for ion exchange chromatography of several metal ion mixtures.<sup>63</sup> The ion exchange separation of selenium<sup>68</sup> and the quantitative separation of selenium from important cations on tin(IV) tungstate papers has been investigated.<sup>66</sup> The electrochromatography of metal ions in different background electrolytes on stannic tungstate papers was investigated by Qureshi and coworkers.<sup>69</sup>

Mixed solvents have been effectively used to obtain analytically difficult separations of cations by chromatography on stannic phosphate<sup>65</sup> and stannic tungstate papers.<sup>70,71</sup>

Papers impregnated with stannic phosphate<sup>61</sup> have also been used recently for some preliminary chromatographic work. Electrochromatographic separations of metal ions on tin(IV) phosphate papers in twelve background electrolytes are described.<sup>72</sup> The  $R_F$  values of several metal ions are obtained on Whatman No. 1 chromatographic paper with different amounts of tin(IV) phosphate by ascending solvent development.<sup>73,74</sup>

Electrochromatographic separation of binary and ternary mixtures and paper chromatographic separation of several metal ions in stannic(IV) antimonate papers were investigated.<sup>75-77</sup> The specific separation and  $R_F$  values of inorganic metal ions were compared on papers impregnated with tin(IV)-molybdate, phosphate and tungstate.<sup>78</sup>

The usefulness of stannic arsenate impregnated paper for chromatography of metals was demonstrated by application of several metal ions by using Butanol-50 % nitric acid developing solvent.<sup>79</sup>

The chromatographic behaviour of metal ions on titanium arsenate, stannic arsenate and Whatman No. 1 papers in  $10^{-5}$  - 3.0 M nitric acid has been studied along with the effect of pH.<sup>80</sup> They found that the selectivity sequence for cations on titanium arsenate papers is not the same as that on titanium arsenate columns and they defined a new quantity  $R_i$ .  $R_i = R_F$  on untreated paper -  $R_F$  on treated paper which they proposed as a measure of the ion-exchange effect. Few studies of such systems have been reported.<sup>51,72,81-83</sup> Qureshi et al.<sup>84</sup> chromatographed

several metal ions on paper impregnated with titanium arsenate in  $10^{-5}$  - 4 M nitric acid. The effect of pH and of the Ti/As ratio on the  $R_F$  values has also been studied.

Sn(IV) was separated from Sn(II) by ascending chromatography on paper impregnated with titanium tungstate.<sup>85</sup>

Rapid separation and  $R_F$  values of several metal ions were studied on papers impregnated with titanium molybdate,<sup>86</sup> zirconium antimonate,<sup>87</sup> ammonium molybdophosphate,<sup>88</sup> ammonium tungstophosphate,<sup>89</sup> ammonium thiocyanate,<sup>90</sup> zirconium tungstoarsenate,<sup>91</sup> collidinium tungstoarsenate<sup>92</sup> and thorium phosphate.<sup>93</sup>

Filter paper impregnated with ion exchange resins have also been studied by dipping it in an aqueous suspension of a colloidal form of the resin. Some rare earths,  $Co^{+2}$ ,  $Cu^{+2}$  and  $Fe^{+2}$  are separated by partition chromatography on paper impregnated with Dowex-50 cation exchange resin with the use of acetone-hydrochloric acid-water, but plane filter gives a similar results.<sup>94</sup> Paper impregnated with Dowex-2 anion exchange resin demonstrates the sequence changes of  $Co^{+2}$ ,  $Cu^{+2}$  and  $Fe^{+2}$  with variation in the concentration of the aqueous HCl used as solvent.

$R_F$  values of numerous metal ions are given for HCl + 1.0 N  $HClO_4$  mixture on SB-2 anion exchange resin paper and for  $HNO_3$  + 1.0 N  $HClO_4$  on SB-2 and SA-2 cation exchange paper.<sup>95</sup>

Singh and Tandon<sup>96</sup> prepared hydrous zirconium oxide paper by treating Whatman No. 1 chromatographic paper strip with zirconium oxychloride and aqueous ammonium hydroxide and achieved rapid separation of several binary and ternary metal ions on this impregnated paper.

Papers impregnated with hydrated titanium oxide<sup>97</sup> was used to study the chromatographic behaviour of various inorganic cations and anions. For the separation of metals, Papers impregnated with tetraheptylammonium nitrate and nitrate salts of Hyamine-1622 and Hyamine 10-X were used.<sup>98</sup>

The separation of metal ions by selective reduction is studied on Whatman No.1 paper loaded with amalgams.<sup>99</sup> A filter paper impregnated with copper iodide can be used in qualitative analysis such as spot tests or in electrophoresis, paper chromatography and thin layer chromatography.<sup>100</sup>

The migration of ions  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Fe}^{3+}$  on filter papers impregnated with aqueous glycine and ammonium thiocyanate solutions was studied by Bhatnagar and Bhatnagar.<sup>101</sup>

The separation of  $\text{Al}^{+3}$ ,  $\text{Ga}^{+3}$ ,  $\text{In}^{+3}$ ,  $\text{Tl}^{+3}$ ,  $\text{Fe}^{+2}$ ,  $\text{Co}^{+2}$  and  $\text{Ni}^{+2}$  on paper impregnated with bis(2-ethylhexyl)hydrogen phosphate was studied using 10 M to 0.1 mM HCl as developer.<sup>102</sup> Pande and Joshi<sup>103</sup> were used the papers impregnated with 1-phenyl-3-thiobenzoyl thiocarbamide at pH 5.0 for the separation of  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$ .

Chromatographic analysis of  $\text{Ag}^+$ ,  $\text{Hg}^+$ ,  $\text{Tl}^+$ ,  $\text{Cu}^+$  and  $\text{Pb}^{2+}$  was achieved on paper impregnated with 0.1 M trioctylamine thiocyanate in benzene.<sup>104</sup> Factors affecting the chromatographic separation of  $\text{Pb}^{2+}$ ,  $\text{Ag}^+$  and  $\text{Hg}^+$  on filter paper strips impregnated with KI and  $\text{K}_2\text{CrO}_4$  were studied by Chatterji and Bhagwan.<sup>105</sup> They have shown that  $R_F$  and S values can be controlled by altering the concentration of KI and  $\text{K}_2\text{CrO}_4$  and of the colloid in the impregnating solution. S value-spreading factor is defined as the ratio of final band width to the initial band width.

Whatman No.1 paper impregnated with 5 % methyl trioctyl ammonium chloride in benzene solution or with trioctylamine solution was used in reversed phase chromatography of uranium, zirconium, hafnium and transition metals of the iron group.<sup>106</sup>

A paper peak chromatographic method for determining Cd and Te in cadmium telluride employs paper impregnated with 0.165 % Bismuthol II.<sup>107</sup> Fluorine compound is determined by atmospheric air pollution by a contact method using filter paper impregnated with  $\text{Ca}(\text{OH})_2$ .<sup>108</sup>

Microgram amounts of iodine, bromine, chlorine and thiocyanate were determined by chromatography on paper impregnated with slightly soluble silver compounds.<sup>109</sup> Microgram amounts of copper was determined by peak paper chromatography on paper impregnated with 0.1 % solution of phthalate of Schiff base of aniline with p-dimethyl amino benzaldehyde.<sup>110</sup> The chromatogram was developed with methyl

alcohol and then immersed in aqueous 5 % potassium ferricyanide solution.

Microgram quantities of magnesium<sup>111</sup> was determined by paper impregnated with 0.05 % quinalizarin in alcohol, while aluminium<sup>112</sup> was determined by paper impregnated with a mixture of NaBPh<sub>4</sub> and aluminon. The chromatogram was developed with 4:1 PrOH-H<sub>2</sub>O.

Varshney and coworkers<sup>113</sup> studied the electrophoresis of metal ions on paper impregnated with tin(IV) hexacyano ferrate(II). R<sub>F</sub> values have been obtained for several cations developed by the ascending solvent technique on Whatman No.1 paper impregnated with liquid cation exchanger such as dinonyl naphthalene sulphonic acid.<sup>114</sup>

Micro amounts of arsenate and phosphate ions are determined by peak paper chromatography.<sup>115</sup> In one method, phosphate ion was determined on paper treated with 0.5 % lead nitrate and 1 % potassium iodide and for arsenate, 0.2 % lead nitrate was used. In second method, the paper was treated with 0.5 % or 0.2 % lead nitrate respectively and the chromatograms are developed with 2 % KI for PO<sub>4</sub><sup>3-</sup> and 3 % KI for AsO<sub>4</sub><sup>3-</sup>.

Chen Bor Kuan and Horvath Csaba<sup>116</sup> were chromatographed a series of phenyl acetic acid and cinnamic acid derivatives on Whatman No. 4 paper impregnated with ethanolic 40 % formamide containing 5 % of formic acid and developed with benzene cyclohexane (1:1).

Relationships between solute retention and eluent composition were determined for various organic compounds in reversed phase chromatographic systems on Whatman No. 4 paper strips impregnated with 20 % solution of oleyl alcohol in benzene.<sup>117</sup>