An Overview of Thin Layer Chromatography

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ABSTRACT

The present paper attempt to explain the basic ideas, significance and other parameters of Thin layer Chromatography (TLC) in different analytical methods. TLC can be performed with less complicated technique therefore it is less time consuming and economical, it has a wide application in pharmaceutical analysis. Thin layer chromatography can be used to note the progress of a reaction, determine the purity of a substance also it can be used to identify compounds present in a given mixture. Specific examples of these applications include: detection of pesticides or insecticides in food and water analyzing ceramides and fatty acids, analyzing the dye composition of fibers forensic , assaying the or identification of medicinal plants and their constituents or radiochemical purity of radiopharmaceutical. As 32 amino acids can be separated by TLC If it performed precisely. Also it can be used to identify the impurities in a compound. This method can be used as a preliminary analytical method prior to HPLC. TLC is very much preferred in most standard methods in environmental toxicology, industrial chemistry, food chemistry, water, inorganic and pesticide analysis, cosmetics, dye purity, herbal analysis and plant materials. In this review article the recent approach of TLC will be summarizes as an application of TLC based on Instrumental thin-layer chromatography, digitally enhanced TLC and Smartphone-based thin layer chromatography.

Key wards: Thin layer Chromatography, Heavy metal ions, separation and identification.

INTRODUCTION

The technique of thin layer chromatography was first introduced by Izamailov and Shraiber[1] in 1938 they called drop chromatography on horizontal thin layers. These workers used this technique for separating plant extract on 2mm thick and firm adhesive layer of alumina set on glass plate. Little notice was taken of the method until 10 years later, when two American Chemists describe the separation of terpenes, essential oil by this method [2]. Thin layer chromatography was presently known, began to attract attention through the work of Kirchner and his coworkers [3-5] starting in 1951, when Stahl[6] described equipment and efficient sorbents for the preparation of plates, that the effectiveness of this technique for separation was shown. This method is now one of the most frequently described separation techniques in quantitative as well as qualitative analysis. Thin layer chromatographic technique is considered to be superior technique than paper chromatography and column chromatography because of the certain specific reasons enumerated below.

1. In thin layer chromatography the separation is the sharpest as compared to other two method.
2. It requires less time (15-50 min) and less amount of substance(0.4mg).
3. Acid can be changes prayed on thin later chromatography plate for identification purpose which is not possible with other method.

4. Thin layer chromatographic plate can be heated to higher temperature without causing any damaged to it.
5. The individual spots are less diffused as compared to paper chromatography because of this fact the sensitivity of detection is increase several times.
6. In Thin layer chromatography we use silica, alumina coated glass palate, while paper chromatography is limited only for cellulose material and other media like silica, alumina coated plate cannot used.
7. It is possible to coat the plate with variety of specific reagents that would destroy paper chromatogram.
8. The capacity of thin layer absorbent is higher than that of paper.
9. The number of sample gas chromatography is single at one time while in thin layer chromatography twenty samples can be applied at one time.
10. Most of the metallic compounds, because of their ionic nature cannot be directly volatized at high temperature which occurs in commercially available chromatograph. Earlier studies centered around liquid phase techniques such as adsorption, partition or ion exchange modes in column and paper chromatography the later method were initially successful in separation and quantitative determination. However, they were subsequently been found tedious and time consuming. Hence the use of thin layer Chromatography manipulation because it gives faster and more accurate result permitting easier manipulation of plates and simple detection.

The success of thin layer chromatography as a highly efficient micro analytical separation method is based on a large number of advantageous properties[7-9].

This method is suitable for screening tests, High sample throughput in a short time, pilot procedure for HPLC. For a longer period of time the analytical information can be stored after the separation. The TLC method is ready-to-uses layer which acts as storage medium for data. Separated substances can be treated to succeeding analytical procedures (e.g. IRMS) at a later date and cost-efficient and rapid optimization of the separation is possible due to easy change of stationary and mobile phase in TLC method.

TLC system components consists of

1. **TLC plates,** preferably readymade with a stationary phase: These are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The coating of stationary phase on the plates is having uniform thickness and is in a fine particle size.
2. **TLC chamber.** The proper TLC chamber is needed for the development of TLC plate. The chamber used to controls a uniform environment inside it for proper development of spots on TLC plate. It also, keeps the process dust free and prevents the evaporation of solvents.
3. **Mobile phase.** This comprises of a solvent or solvent mixture.Particulate-free the mobile phase should be used and also it has the highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, a stationary phase.selection of a solvent system may include the cost, availability, quality, toxicity, volatility, and miscibility of the solvent or solvents chosen.One way of rating the interaction between a particular solvent and a sorbent is based on the eluting power of the solvent, which is defined by the solvent strength parameterε^0 and it is also known

as Eluotropic Series [10].For any given solvent, this parameter represents the adsorption energy per unit area of standard sorbent A larger the value of Eluotropic series indicates a greater interaction between the solvent and the sorbent. In a liquid-solid adsorption process, there is always a competition between solute and solvent molecules for a place on the sorbent surface. The solute molecules will be more readily displaced by a solvent of higher solvent strength. As a result, the R_f value of the solute will increase with an increase in the solvent strength parameter. A solvent that has a high solvent strength parameter on one sorbent, such as silica gel, may have a different solvent strength parameter on a different sorbent. Table 1.1 lists the relative strengths of different solvents on various adsorbents.

METHOD AND MATERIALS

Basic principal of Thin Layer Chromatography:

1. Preparation of plates

Slurry or paste of silica gel 'G' was prepared by mixing a double distilled water in the ratio of 1:2 with constant steering for about 10 minutes and then applied to the glass plate by using dipping method [21] and then dried over night at room temperature.

2. Running of TLC plates

Using the fine glass capillaries test solutions were spotted on the silica coated plates and then the plates were blow-dried with hot air. The mobile phase of varying concentration was adjusted to the desired pH using sodium hydroxide and hydrochloride acid solution. The plates were developed for about 15 min in the glass jar containing 15 ml aqueous solvent. Approximately very little amount of solvent i.e. 2 -3 ml was required to run the sample per plate.

3. Development of TLC plates

Plates were dried and different cations were detected by spraying various spot test reagent, which are as follows.

- Saturated alcoholic silver nitrate for Cr(IV)
- Saturated alcoholic alizarin red for Cr(IV),
- Dimethylglyoxime for Ni(II) and Co(II),
- Potassium ferrocyanide for Cu (II) and Fe(III) respectively.
- Dithiozone in carbon tetra chloride for Mo(VI), Zn(II), Cd(II), Hg(II), Bi(III), Ag(I) and Pb(II)(11).

At room temperature all the experiments were carried out at. In all such experiment, for each set of determinations the R_f values were measured. Various experiments were carried out to study the effect of concentration, effect of pH and effect of developing time of mobile phase (5 - 20 min) for the R_f values of the individual cations.

Table 1.1 Eluotropic Series

Solvent	ε^0	Solvent	ε^0
Fluoroalkanes	0.25	Methylene chloride	0.42
n-Pantane	0.0	Ethylene dichloride	0.44
Isooctane	0.01	Methyl ethyl ketone	0.51
Petroleum ethere	0.01	1-Nitropropane	0.53
n- Decane	0.04	Triethylamine	0.54
Cyclohexane	0.04	Acetone	0.56
Cyclopentane	0.05	Dioxane	0.56
1-pentene	0.08	Tetrahydrofuran	0.57
Carbon disulphide	0.15	Ethyl acetate	0.58
Carbon tetrachloride	0.18	Methyl acetate	0.60
Xylene	0.28	Diethylamine	0.63
1-Propylether	0.28	Nitromethane	0.64
1-Propyl chloride	0.29	Acetonitrile	0.65
Toluene	0.29	Pyridine	0.71
n-Propyl chloride	0.30	Dimethyl sulfoxide	0.75
Benzene	0.32	i-propanol, n-propanol	0.82
Ethyl bromide	0.35	Ethanol	0.88
Ethyl sulfiide	0.38	Methanol	0.95
chloroform	0.40	Ethylene glycol	1.1

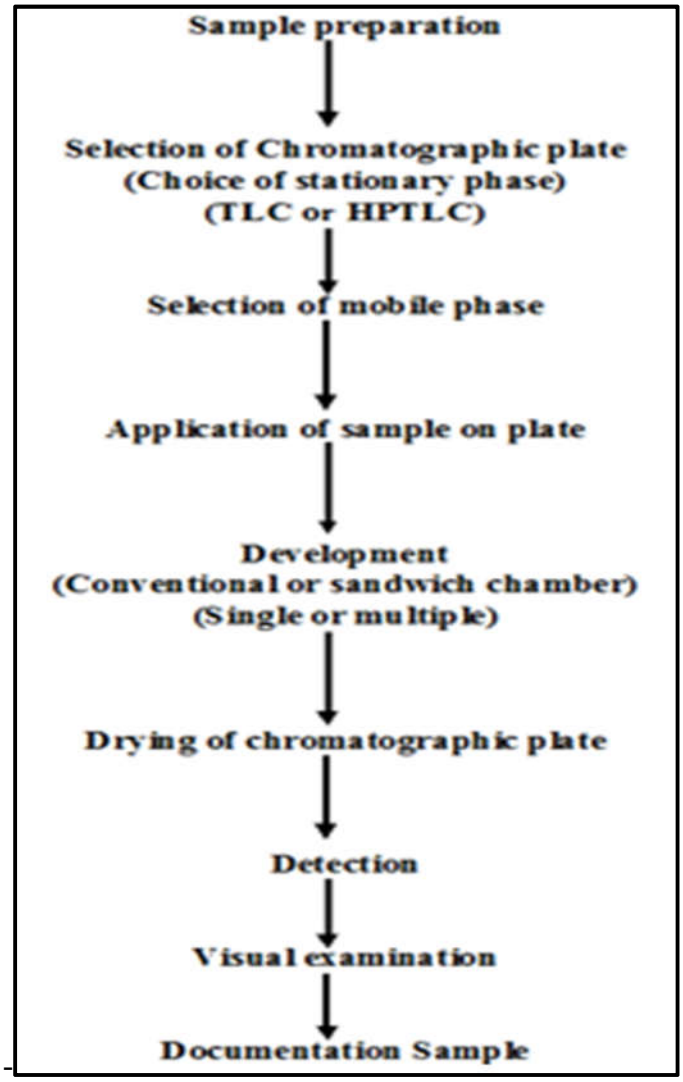

Fig. 1 : Flow Chart of Thin Layer Chromatography

Retardation factor

In addition to qualitative results, TLC can also provide a chromatographic measurement known as an $R_f$value. The $R_f$value is the "retardation factor" or the "ratio-to-front" value expressed as a decimal fraction.

The R_f value can be calculated as:

$$R_f = \frac{\text{Distance travelled by solute from the original line}}{\text{Distance travelled by solvent from the original line}}$$

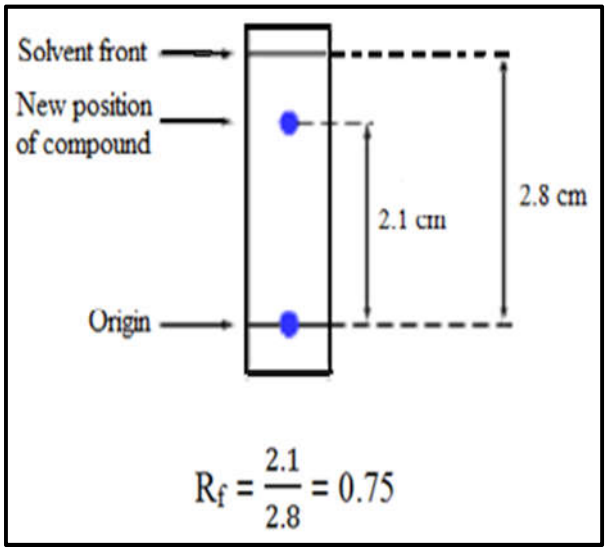

Figure 2. Paper Chromatography

From Fig. 2 if a compound travels 2.1 cm and the solvent front travel 2.8 cm, the R_f is 0.75. It means that if the compound has larger R_f value then it travels larger distance on the TLC plate. By comparing the two different compounds which run under identical chromatography conditions it was observed that the compound with the larger R_f value is less polar in nature because it is less strongly interacts with the polar adsorbent on the TLC plate than a polar compound run on the same TLC plate. The effect of pH of solvent, the effect of various concentration of mobile phase, effect of adsorbent and the effect of development of time on R_f value of various metal ions will be studied by using TLC method.

EXPERIMENTAL SECTION

Apparatus

Glass plates of size 4 x 20 cm which is coated with silica gel G and 20 x 25 cm glass jars for the development of glass plates, EI pH meter and glass sprayer for detecting reagents.

Chemicals and Reagents

(Merk India), silica gel- G (Merck, India), hydrochloric acid, sodium hydroxide and aqueous mobile phase.

Metal ion studied:

Cr(VI), Cr(III), Ni(II), Co(II), Cu(II), Fe(III), Zn(II), Cd(II), Hg(II), Bi(III), Ag(I), Pb(II), Mo(VI) and various other metal ions.

Stock Solutions:

Stock solutions of 1% of following salts were prepared in the 0.1 M hydrochloric acid.

1. Cr(VI) in the form of Potassium salt
2. Ni(II), Cr(III), Fe(III) and Zn(II) in form of Chloride.
3. Cu(II) in the form of Sulphate.
4. Mo(VI) in the form Trioxide.
5. Co(II) in the form Nitrate.

The suitable mobile phase was prepared in double distilled water.

Detection Reagents:
The following regents were used for the detection of various metal ions
1) 0.05 % Dithiozone in carbon tetrachloride.
2) Saturated alcoholic AgNO3.
3) Saturated alcoholic alizarin red.
4) 1% Alcoholic solution of DMG ie. Dimethylglyoxime.
5) 1% Aqueous potassium ferrocyanide.
Stationary phase: Silica gel –G.
Aqueous mobile phase

RESENT APPROACH OF THIN LAYER CHROMATOGRAPHY:

- The quantification method of the carbohydrate is proposed for samples of spot tests using the thin-layer chromatography (TLC) method which is based on the scanned image analysis (12).

- To identify and determine the amount of quercetin present in Berchemia lineate by adopting TLC method (13).

- Smartphone-based thin layer chromatography for the discrimination of falsified medicines i.e. toidentify and characterize the pharmaceutical products via TLC using a custom cradle that interfaces with a Smartphone (14).

- A smart thin layer chromatography system plates integrate thin film photo-sensors is used for the modeling of the photo-response of samples (15).

- Recently a mobile phone TLC analyser system is used for the quantitative analysis of pharmaceutical compounds (16).

- Recently if digital photography is combined with regular TLC, it can give highly improved qualitative analysis as well as accurate quantitative analysis. This novelty of digitally enhanced TLC (DE-TLC) is easily operated. A fluorescent TLC plate is illuminated by using UV light and picture of the plate is taken by using a digital camera. Then, on a computer, using either common photo-editing software or TLC Analyzer, the public domain software written for this work one can quickly produce multi-spectral scans, calibration curves and densitograms, output which is available previously only from more complex procedure or by using expensive equipment. Since this technique is less expensive than other quantitative chromatographic methods(17).

- According to Ahmed M. Galal et.al.anInstrumental thin-layer chromatography (ITLC) is based on standardized methodologies used for phytochemical analysis.

ITLC methods provide simplicity, faster separations, reproducibility, cost effectiveness, and versatility, in addition to this it is ecofriendly. Its very much advantages in the field of botanicals/dietary supplements also in the field of medicinal herbs include authentication and identification of herbs, discrimination of plant species, inspection of raw materials and finished products, detection of stability studies,evaluation and adulteration, of bioactivity(18).

CONCLUSION:

After establishing optimum conditions of pH of mobile phase, concentration of solvent and developing time quantitative separation of binary, ternary and quaternary mixtures of metal ions has been carried out in synthetic samples as well as in a environmental samples for example ground water sample, industrial effluent sample, effluent treated sludge sample, industrial soil sample and also in ore samples. This article will be helpful understanding principal and working of TLC in the field of research also this article give recent approach of TLC.

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