A REVIEW ON THIN LAYER CHROMATOGRAPHY: FORENSIC ANALYTICAL TECHNIQUE

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ABSTRACT

This article is to explain basic applications of thin layer chromatography in analytical methods. Thin layer chromatography is a rapid technique as it requires no sophisticated instrumentation for separation and identification of components. It is less time consuming and has wide application in identifying impurities in a compound. It requires only small treatments, give the progress of a reaction and identify compounds present in a given substance. TLC is a preferred method of analysis in many other sciences.

INTRODUCTION

Thin Layer Chromatography (TLC) is an analytical technique in forensic science. It is a chromatography technique used to separate mixtures. Chromatography was discovered by M. Tswett in 1906.[1] Thin layer chromatography (TLC) is a type of liquid chromatography in which the stationary phase is in the form of a layer on a glass, an aluminum, or a plastic support. The term "planar chromatography" is often used for both TLC and paper chromatography (PC) because each avail a planar stationary phase rather than a packed column. PC, which utilizes plain, modified, or saturated paper (cellulose) as the stationary phase, involves many of the same basic techniques as TLC, but it has not evolved into an efficient, sensitive, quantitative, instrument-based analytical method and has many disadvantages as compare to TLC [2].

TLC is highly selective and flexible because of the great variety of layers of stationary phase. It has proven to be as sensitive as HPLC in many analysis as the solvent usage per sample is very low [2].

PRINCIPLE OF THIN LAYER CHROMATOGRAPHY

Thin layer chromatography uses a thin glass plate coated with either aluminum oxide or silica gel as the solid phase. The mobile phase is a solvent chosen according to the properties of the components in the mixture to be analyzed. The principle of TLC is the distribution of a compound between a solid stationary phase (the thin layer) applied to a glass or plastic plate and a liquid mobile phase (eluting solvent) that is moving over the solid phase. A small amount of a compound or mixture is applied on the TLC plate just above the bottom of TLC plate. The plate is then developed in the developing chamber, containing solvent just below the level at which the sample was applied so that the spotted point do not dip in the solvent. The solvent start running through the particles on the plate through the capillary action. As the solvent moves over the mixture, each compound will either remain with the solid phase or dissolve in the

solvent and move in upward direction to the plate. Whether the compound moves up the plate or stays behind depend on the physical properties of that individual compound and thus depend on its molecular structure, especially functional groups. The solubility rule "Like Dissolves Like" is followed. The more similar the physical properties of the compound to the mobile phase, the longer it will stay in the mobile phase. The most soluble compounds will be carried out by the mobile phase farthest up the TLC plate. The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind. [3]

INSTRUMENTATION

Most chromatography methods work because of a difference in polarity. The stationary phase keeps the components of a mixture of similar polarity combined with it causing them to move more slowly than the mobile phase .Because of differences in solubility of compounds in the mobile phase, and because of the strength of attraction to the stationary phase ,some components move faster than others .This result in separation of the compounds. As the solvent rises, the compounds in the samples move to varying heights on the plate. The each component of compound appear as spots which are visualized either by UV light or an iodine chamber. [4]

The relative intensity of the spots is not an accurate indication of the amount of compound present .The distance the spot travels on the plate is expressed as "ratio to front" or Rf value given in the equation below.

Rf = Distance of center of spot from starting point / Distance of solvent front from starting point [1].

The Rf value can be affected by many experimental conditions. The only way to get identity of an unknown compound through TLC is identical to a known compound is to spot a solution of the known or standard compound on the same TLC plate.

APPLICATIONS AS AN ANALYTICAL TECHNIQUE IN FORENSICS

Identification of Drugs, Poisons and explosives: Thin-layer chromatography (TLC) is one of the most widely used techniques for the separation and identification of drugs, whether they come from proprietary preparations ,illicitly prepared material or biological samples. It is a supreme technique because of its simplicity, low cost and the selectivity of detection reagents. However, an analyst challenged with a new or unknown compound has the problem of choosing a suitable system or systems from the hundreds that have been proposed, sometimes without any knowledge of their effectiveness. During the last few years, much effort has been put into choosing the best TLC systems for general screening and identification purposes in order to create a number of standardised systems. The main advantages gained by such standardisation are that (a) analyses are performed more efficiently since only the more effective system are used, (b) chromatographic data built up by one laboratory are easily transferable to another laboratory and (c) any disagreements between the findings of two or more laboratories analysing the same sample should be minimised [5].

TLC of drugs and explosives is used as a preliminary examination test, to provide an indication of the nature of the sample, rather than being used for positive identification of a sample. It is useful because many of the colour change presumptive tests used will only provide an indication of a certain active group in the molecule. For example with explosives the Greiss reagent (a colour change presumptive test) will normally indicate that a nitrate or nitrite group is present whereas with TLC it is possible to differentiate

between different nitrate and nitrite containing compounds as they can have different R_f values and react to produce different coloured bands with the visualisation reagent.

As for example, it is possible to differentiate between trinitrotoluene (TNT), dinitrotoluene (DNT) and nitrotoluene (NT). All three of these explosive compounds contain nitrogen groups but in different numbers; three, two and one respectively. This variation is enough to produce visible differences, however, there can be some overlap of R_f values and colour is an independent measurement. TLC is therefore taken as a presumptive test to inform further analysis rather than producing a definitive result on its own.

To perform TLC with drugs and explosives the sample must first be dissolved in a solvent – unless it is already in a liquid state – and then it can be spotted onto a TLC plate and run in the same way as the TLC plates for dyes and inks. When using TLC to analyze drugs and explosives, directly compare an unknown sample with known standards on a single plate to give a presumptive identity of the unknown sample [6].

Identification Of Dyes And Inks: Document forgery is a common problem, especially using pen or ink in writing to forge or edit document and signatures .Most questionable documents consist of forged bank checks, bills, handwritten correspondence, contracts and others, which require analysis of ballpoint pen inks. Ink analysis is an important forensic procedure because it can reveal useful information for an investigation. Modern inks contain mixtures of various substances that are meant to improve ink characteristics (Rouxet al.,1999; Valia, 2017; Vogt, 1997). The most important component of coloring material comes in the form of dyes, pigments, and various combinations. Dyes are soluble in the vehicle that is a mixture of solvents, oils and resins. This carrier is an important component of the ink, which affects its flowing and drying characteristics [7].

Forensic document examination, especially the analysis of inks, can be divided into two approaches including non-destructive document and destructive document. Non-destructive analytical methods will choose specific characteristics of ink to serve as parameters, such as its colors, luminescence and radiation absorption. Questionable documents may be differentiated by properties of transmission, reflection and fluorescence spectra obtained for inks deposited on the paper surface. However, the methods of physic-chemical analysis can determine the type and composition of ink, leading to ink identification (Feraru and Meghea,2014). Destructive document analysis starts by removing a small section from the ink line with extraction solvent to open up more avenues of analysis. In particular, the chromatographic separation of colored pigments from component dyes can be useful. Even though a blue ballpoint pen can only write in one color, the ink is actually made from a mixture of different colored pigments. This method has proven highly productive for the comparison and matching of ink with the database of chromatograms (Ismailet al., 2014; Lewie, 1996; Zlotnick and Smoth, 1999; Samanidou et al., 2004) [7].

Liquid Dye Samples And Ink Within Pens Can Be Applied Directly To TLC Plates. When The Dye Is On Fibres, For Example From A Piece Of Clothing, The Dye Must Be Removed From The Fibre Into A Solvent Which Can Then Be Applied To The TLC Plate. This Is Usually Carried Out By Sealing The Fibre In A Tube With A Small Amount Of Solvent Then Heating The Tube For At Least An Hour. The Solvent Can Then Be Spotted Onto The TLC Plate.

When The Ink You Want To Test Is On A Piece Of Paper A Similar Method Is Followed Except That There Is No Need To Heat The Sample And Solvent. Simply Cut A Small Section Of The Paper With The Ink On, Place It In A Tube With Solvent And Leave It Until The Ink Has Dissolved In The Solvent

(There Should Be A Colour Change Visible As The Ink Moves Into The Solvent). Then Once Again The Solvent Is Simply Spotted Onto A TLC Plate.

Once A TLC Plate Has Been Run There Should Be Clear Bands Visible On The Plate Indicating The Separation Of Different Components Of The Ink Or Dye. The R_f Values And The Colour Of The Bands Can Be Recorded And In The Case Of Inks Compared To A Database Held By The United States Secret Service. This Can Help Identify The Ink Used And From This It May Be Possible To Identify A Specific Pen That Contains That Particular Ink. As Ink Manufacturers Add Different Compounds To New Inks These Specific Features Can Be Used To Date The Bands Seen On A TLC Plate. So Remarkably, A Quick Bit Of TLC Can Identify An Unknown Pen Ink (CSI Actually Got Something Right For Once).

If The Aim Was To Determine Whether Writing Was Made By A Specific Pen, Both Samples Should Be Run On The Same Plate And Then They Can Be Compared Directly. However, Caution Must Be Taken When Making Conclusions About The Results. While If The Two Samples Do Not Produce Matching Bands That Means That The Note Was Not Written Using That Pen, If The Bands Do Match This Means That The Note *Could* Have Been Written Using That Pen. Due To The Number Of Pens Produced That Will Contain The Same Ink It Is Not Possible To Say Conclusively From This Single Test That One Specific Pen Was Used To Write A Note. However, If This Result Is Combined With Others Suggesting The Same Thing, It Will Lend Weight To The Argument That It Is Likely That The Pen Was Used To Write The Note.

When Looking At Dyes Using TLC There Is Not A Similar Large Database Of Expected Band Patterns And Therefore It Is Generally Used For The Comparison Of Two Samples, Rather Than Attempting To Identify A Specific Dye From An Unknown. However As With The Comparison Of Pen Inks, The Same Issue Arises In That There May Be Multiple Sources Of Fibres Dyed With The Same Ink. This Can Be A Point Of Contention In Court, And So It Would Be Very Unusual To Have A Case Based Solely On This Sort Of Evidence And Those That Are Can Often Be Subject To Many Appeals. Usually Dye Analysis Would Be Used To Lend Weight To A Conclusion Suggested By Other Evidence [6].

Identification of Pesticides: TLC and HPTLC complement gas chromatography (GC) and high-performance column liquid chromatography (HPLC) for pesticide separation, detection, identification, and quantification because of their following unique advantages over column chromatography: single use of the layer simplifies sample preparation procedures; simplicity of development by dipping the plate into a mobile phase in a chamber; high sample through-put with low operating cost because multiple samples can be run simultaneously with standards on a single plate using a very low volume of solvent; high resolution through multiple development or two-dimensional (2D) development on a plate with a single adsorbent or dual adsorbents; selective and sensitive post chromatographic detection and identification with a very wide variety of chromogenic, fluorogenic, and biological reagents and coupled spectrometric techniques; high resolution and accurate and precise quantification achieved on HPTLC plates, especially with automated sample application, development, and densitometric scanning methods; visual observation and direct recording of the entire chromatogram including all sample components, the origin, and the mobile phase front; and the ability to repeat detection and quantification steps under different conditions. Thin layer radio chromatography (TLRC) is used routinely for metabolism, degradation, and other studies of pesticides in plants, animals, and the environment, and these applications will be covered as well as studies of lipophilicity and pesticide migrations through soils [8].

Most pesticide determinations are performed on silica gel TLC or HPTLC commercially pre-coated plates. The layers almost always contain a gypsum binder named G layers [9] or an organic binder. Silica gel H layers [10] are especially hard and rugged and permit the use of high concentrations of water in mobile phases without loss of adherence. Layers often contain a fluorescent indicator or phosphor to facilitate detection of compounds that absorb 254 nm UV light as dark zones on a fluorescent background. These are termed F layers or F 254 layers by the manufacturers. Cellulose, alumina [11], and kieselguhr layers are also used occasionally for pesticide TLC. Chemically bonded silica gel phases are being used with increasing frequency for TLC analysis of pesticides, most notably cyano, amino, and diol normal phase (NP) layers and C18 (octadecyl) and C18W (water wettable octadecyl) reversed phase(RP) layers. A novel, non-chromatographic use of C2 and C18 bonded layer plates works as extractants for pesticides in a passive sampling environmental screening method: a 50 mm × 51 mm plate is placed between a plastic mount and a wire guard cage, the sampler is placed for a number of days in the water source (e.g., river or creek) to be sampled, the layer is then removed, and the pesticides eluted for analysis by HPLC [12]. Impregnated layers are also sometimes applied for pesticide analysis, e.g., silica gel impregnated with mineral oil for the RP separation of pyrethrins and piperonyl butoxide [13]. The separation of a pesticide mixture containing metoxuron, deiso-propylatrazine, cyanazin, and trifluralin usually obtained in 260 s on an ultra-thin (10 μm) silica gel layer with a monolithic structure having a defined meso- and macropore structure. A volume of 20 nL of sample (0.1% solution in acetonitrile) is applied with an ATS 4 sample applicator, the layer developes over a distance of 2 cm with petroleum ether–acetone (7 + 3), and the chromatogram evaluates with a diode array densito-meter. These layers have advantages of short migration distances, short development times, low solvent consumption, and high sensitivity [14].

Lanthanum silicate or lanthanum tungstate ion exchange layers are used for the separation and detection of diazinon and ethion residues recovered from pistachio nuts by MSPD(Matrix phase solid dispersion). Mobile phases can be methanol–10% ammonia (95 + 5) and methanol–dichloromethane–10% ammonia (65 + 31 + 4), respectively, and visualization was obtained with palladium chloride reagent. Diazinon and ethion RF values are 0.79 and 0.69, respectively, on lanthanum silicate and 0.98 and 0.90 on lanthanum tungstate [15].

Mobile phases for the Normal Phase TLC of pesticides are less polar than the layer and are composed of aqueous–organic solvent mixtures or fully organic mixtures, such as heptane plus a polar modifier [ethyl acetate, tetrahydrofuran (THF), dioxane, or diisopropyl ether] for silica gel. Acid or base may be added to the mobile phase in a small amount to reduce zone tailing [9]. Mobile phases for RP TLC are more polar than the layer, e.g., acetonitrile–water, methanol–water, or THF (Tetrahydrofuran) –water with C18 bonded silica gel. Mobile phases are chosen by trial and error guided by literature searches, or by use of by a systematic, computer-assisted optimization method such as window diagrams [16].

Quantitative TLC of pesticides is usually performed by measuring the visible absorption, UV absorption, or fluorescence of standard and sample analyte zones in situ on a high-performance layer using a slit-scanning densitometer in the reflection mode. As an example, an HPTLC–densitometry method was developed for the quality control and stability analysis of commercial emulsifiable concentrate (EC) formulations of the synthetic pyrethroids cypermethrin, α -cypermethrin, and λ-cyhalothrin. Reference standards and the EC formulation, applied with a Linomat IV band applicator, were chromatographed on

an aluminum backed silica gel 60 F254 layer in a vapor-presaturated twin-trough chamber with hexane– toluene (1 + 1) mobile phase. Quantification was carried out by single wavelength reflectance scanning at 220 nm using a TLC Scanner II (Fig. 3). Calibration plots were linear in the range 8–24 μg, and the linearity correlation coefficients ranged between 0.97 and 0.99. Recoveries from laboratory-prepared test samples of the EC formulations were in the range 95–99% [17]. Similar methods were described for analysis of formulations of the pyrethroids fenvalerate and deltamethrin [18]. Calibration plots for these pesticides were linear in the range 3–23 μg, and recoveries from laboratory prepared EC formulations were 96–100%. In both cases, HPTLC results were comparable to those obtained using a more complex, slower, and more costly GC–flame ionization detection method.

A more recent approach is to perform imaging documentation [19] and quantification using a video densitometer consisting of a video documentation system incorporating quantification software.

Hence, Pesticide Analysis Remains One Of The Leading Applications Of TLC And HPTLC For Qualitative And Quantitative Analysis Of Foods And Crops [9]; Environmental Samples Such As Soil, Drinking Water [20], And Sand [21];Forensic And Medical Samples [22]; Biological Samples; And Commercial Formulations. In Addition, TLC Is Very Widely Used In A Variety Of Pesticide Studies, Such As Determination Of Quantitative Structure–Activity Relations (QSAR) That Describe How The Molecular Structure, In Terms Of Descriptors (Lipophilic, Electronic, Steric), Affects The Biological Activity Of A Compound.

CONCLUSION

As old and simple as TLC is, it has been found its way still in modern science as it can be used as a first indicator of the components, before more advanced techniques can be used such as HPLC, GC etc. It provides fast, low cost qualitative analyses and screening in order to obtain information such as sample stability, purity, and uniformity and to follow the course of a reaction . Samples that are difficult to prepare can be analyzed readily, and detection is especially flexible in the absence of the mobile phase and with a variety of parameters.TLC has been applied virtually in all areas of analysis, including chemistry, biochemistry, biology, industrial, agricultural, environmental, food, pharmaceutical, clinical, natural products, toxicology, forensics, plant science, bacteriology, parasitology, and entomology.

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