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OPEN TUBULAR THIN LAYER CHROMATOGRAPHY (OTTLC) IN THE ANALYSIS OF NATURAL PRODUCTS

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ABSTRACT

Open tubular thin layer chromatography (OTTLC) has been introduced in this study as a simple and inexpensive chromatogrphic technique. Methodology and application in analyses of natural products were also described. The new proposed form of TLC experiences some benefits over conventional TLC.

INTRODUCTION

Thin layer chromatography (TLC) is one of the most feasible and popular analytical tools especially in the area of natural products chemisty [1].

Meanwhile, many pharmacopeias, as the B.P., E.P. or U.S.P., have utilized TLC in both qualitative and quantitiative drug analyses or in quality control measures. Although TLC is an economic and versatile technique, there are still some improvements that could be brought in to provide additional potential to the method.

Partial or complete chamber saturation with vapors of the developing system is always necessary for reproducible resolutions. In general, once the space in the developing chamber is reduced to a minimum, the vapor atmosphere over the layer will be allowed to equilibrate very rapidly, and in the mean time, the contribution of the ambient conditions is decreased [2-5].

Sample application in TLC requires manual labor which if simplified or minimized, it will be of value when considering automating thin layer chromatography [6]. Sample resolution in conventional TLC is achieved only on a narrow strip of sorbent alongside the TLC plate. Consequently, unless TLC is used for multi-sample comparative analysis, the application of a small limited number of spots on a single TLC plate, usually ends up with an uneconomic use of the full resolving power of the total amount of the sorbent on the plate.

EXPERIMENTAL

Materials:

All solvents used were analytical grade. Test substances, atropine sulphate (Mafarian Smith LTD, Edinburgh); eserine salicylate (Sandoz Basil, Switzerland); cinnamon oil (Bush Boaka, Allen, London); methyl red and sudan III (Feinchmie, AG., Sehnitz); gentian violet and methylene blue (BDH Chemicals LTD, Poole, England) and picric acid (Aldrich, USA); extracts and fractions of natural products were prepared from *Ducrosia ismaelis* as well as its furocoumarin isolates, saxalin, oxypeucedanin hydrate and oxypeucedanin ethanolate [7]; silica gel 60 G254 (E. Merck, Darmstadt, Germany); normal glass and polyethylene tubings with variable sizes; 3, 4 or 5 mm internal diameters and 5, 10 or 15 cm in length; and five-dram vials were used as developing chambers.

Preparation of chromatotubes:

For OTTLC, layers were made by coating the inner surface of glass or polyethylene tubings with a slurry of silica gel (15 g) in 50 ml of water or in chloroform-methanol (1:1). Silica gel slurry was introduced into the tubes and then excess gel suspension was drained out. Afterwards, the tubes were rolled back and forth to allow even distribution of the sorbent layer. Chromatotubes were air dried then oven activated at 70°C for 1 hor before use.

Sample application:

Appropriate volume from the sample is delivered onto the sorbent layer by allowing the chromatotube to touch carefully the analyte solution for a moment (for about 1-2 mm distance) and then air dried. This proecess could be repeated as many times as needed for diluted samples and the outside of the tube is then wiped thoroughly.

A zone concentration step, for the applied sample, could be achieved by developing the chromatotube in a more polar organic solvent for few mm distance and then dried out.

Chromatotube development:

Small 5 dram-vials, the bottom of each lined with a small flat pad of cotton, were used as developing chambers.

Solvent systems were mixed thoroughly and enough volume (2-3 ml) of the solvent mixture were added into the developing vials to wet the cotton pad. OTTLC chromatotubes were developed in CH_2Cl_2-MeOH (9:1) or (8:2) for flavonoids and natural product extracts (fractions and isolates); ethyl acetate-acetone-diethylamine (6 : 4 : 0.2) for alkaloids; and $CHCl_3$-MeOH-HOAc (9 : 1 : 0.2) for dyes.

Visualization:

Normal visualization techniques used for conventional TLC were used. Colored materials were recorded under direct light or by using white light, while fluorescent materials or substances that do not absorb UV were detected under the 366 nm long wave or the 254 nm short wave UV, respectively.

Alternatively, iodine is also used for detection, either by placing the chromatotubes in iodine chamber, or by dipping or developing the TLC tubes in a 1% iodine solutions in CCl_4 followed by air-drying. Fractions of natural products containing flavonoids were examined by exposure to ammonia vapors, the OTTLC tubes were connected to a vacuum line and exposed to ammoina.

Alkaloids were analyzed by developing the chromatotubes in a solvent system containing 0.5% ninhydrin, followed by heating the chromatogram in an oven at 110°C [8].

RESULTS AND DISCUSSION

Changing the shape of TLC from the conventional planar form to a tubular system offers considerable advantages and adds great flexibility to the technique.

Although wall-coated open tubular micro-columns have been previously developed for GC to increase resolution, the application of an analogous sorbent structure in TLC achieves different and additional merits to the technique.

At first, since the sorbent layer is conserved on the inside of the tube (c.f. open planar chromatography), handling of the chromatogram is now becoming much easier and less liable to damage or deactivation especially by changes in the surrounding humidity. In addition, this design physically protects the layer from environmental contamination. Secondly, the space above the sorbent layer (the space within the tube) is also kept minimum which eliminates effect of changes in the ambient atmosphere and decreases the need for pre-equilibration with solvent vapor.

Moreover, regularly conventional TLC plates are developed in relatively large tanks, but in this technique they were replaced by simple small vials with the solvent consumption is substantially decreased (fig.1).

Small TLC plates are often daily used in most labs as a rapid routine analytical tool where in most cases such plates end with the application of a single or few spots.

Further and more important, open tubular TLC provides an efficient low cost operation thin-layer chromatography, since it permits full use of the minimum amount of sorbent of the chromatotube, in contrary to planar TLC where up to 50% of the plate area representing the marginal sides of the plate and/or interspot areas, are wasted.

Besides, the simple and trouble-free loading of the sample on TLC chromatotube without scratching or damaging the sorbent layer eliminates the need for application devices or high skills form the operator. This flexible technique conveniently allows the use of several, both non-destructive or destuctive, universal or selective methods to reveal the bands (c.f. spots in TLC) on the chromatogram (table 1).

OTTLC suffers from of the inconvenience of the unsuitability of the method for simultaneus use of reference materials on the same chromatotube. However, this disadvantage could be overcomed by running the reference materials on parallel tubes or by the use of an internal reference in the sample. The use of OTTLC in quantitative analyses or in preparative application is still under investigation.

Table (1) : Materials analyzed by OTTLC.

Tested Material	Method of Visualization
Alkaloids :	
- atropine	ninhydrin
- eserine	ninhydrin
- pilocrpine	ninhydrin
Colored compounds :	
- picric acid	visually, direct light
- sudan III	visually, direct light
- methylene blue	visually, direct light
- gentian violet	visually, direct light
- methyl red	visually, direct light
Flavonoids :	
- kaempferol	ammonia vapors
- quercetin	ammonia vapors
Furocoumarins :	
- saxalin	UV*; Iodine
- oxypeucedonin hydrate	UV*; Iodine
-oxypeucedonin ethanolate	UV*; Iodine
- crude fractions of *D. ismaelis*	UV*; Iodine
Cinnamon oil	Iodine

*** direct or by quenching**

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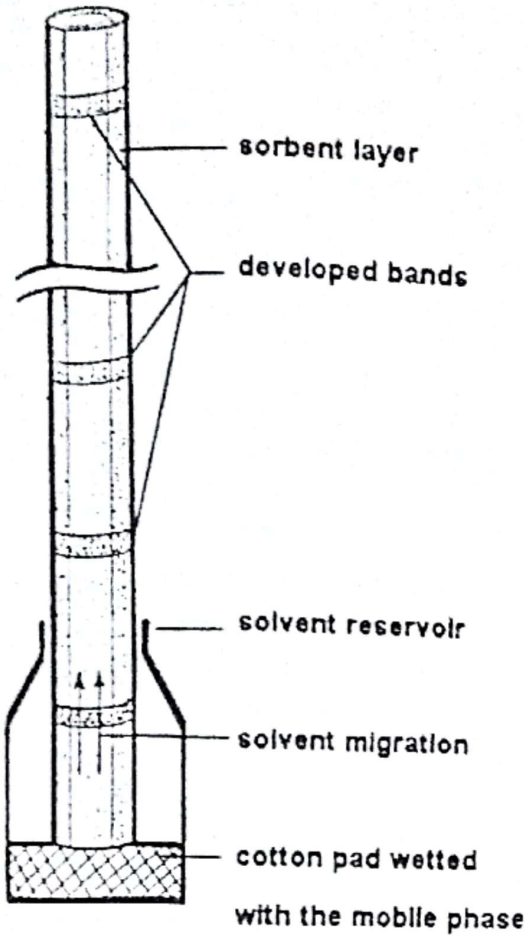

Fig. 1: An illustration of OTTLC

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إستخدام كروماتوجرافيا الطبقة الرقيقة الأنبوبية فى تحليل النواتج الطبيعية

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فى هذا البحث تم ابتكار طريقة تطبيقية جديدة لكروماتوجرافيا الطبقة الرقيقة. وتتلخص هذه الطريقة فى تبطين الجدار الداخلى لأنابيب مفتوحة الطرفين بطبقة رقيقة من مادة الأدمصاص. وقد تم استحداث هذه الطريقة لتسهيل بعض الخطوات والأستغناء عن خطوات أخرى مستخدمة فى كروماتوجرافيا الطبقة الرقيقة التقليدية. فقد اجرى تجربتها لتحليل مواد قياسية ومخاليط صناعية وخلاصات نباتية. ومن مزايا الطريقة الجديدة عدم الحاجة إلى عملية التنقيط واستخدام كميات قليلة من المذيبات العضوية والأقتصاد فى كميات مواد الأدمصاص وعدم الحاجة إلى وعاء مغلق مخصوص للتحليل أو تشبيع اناء التحليل بابخرة المذيبات قبل إجراء التجربة. وقد تم أيضاً استخدام الكواشف التقليدية المختلفة بصورة جديدة للكشف عن المواد المفصولة والتى تتبع مجاميع كيميائية مختلفة.