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## ADAPTING A STANDARD THIN-LAYER CHROMATOGRAPHY APPARATUS FOR LABORATORY USE<sup>1</sup>

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### ABSTRACT

The Eastman Chromagram apparatus was employed in thin-layer chromatographic studies involving United States species of *Polymnia* (Compositae). Certain technical modifications of this apparatus were made in an attempt to more efficiently saturate the chamber with vapors of the eluents. Results indicated that a critical level of reproducibility could be achieved only with modified apparatus, which included the insertion of saturated "□"-shaped filter paper, with a glass rod across the base of the "□", all held securely between the two glass plates by additional clips.

Thin-layer chromatography (TLC) has been widely used as a research tool for at least ten years. However, certain recent changes in details of apparatus design have made possible greater reproducibility of results.

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One apparatus which is relatively new to the field of TLC and which purports to achieve greater reproducibility of results is the "B-N" chamber produced by Brinkman Instruments, Westbury, New York. By completely saturating the chamber, partial evaporation of solvents from the developing layer prevents adverse migration characteristics caused by the vapor phase.

This paper is concerned with a somewhat less sophisticated apparatus, the Eastman Chromagram system manufactured by Distillation Products Industries, Rochester, New York. The basic apparatus consists of a wire stand which supports a plastic-covered glass trough and two 23-cm<sup>2</sup> glass plates, whose ground margins are held in close contact by two metal clips.

The Eastman apparatus is frequently used for preliminary chromatography and is not intended to eliminate the need for analytical apparatus such as gas chromatographs. However, in an attempt to perform chemical taxonomic studies of the genus *Polymnia* (Compositae) using TLC, a high degree of reproducibility was achieved with the Eastman Chromagram (TM) system, but only with the modifications described below.

Initially a total of six sets of plates with ground-glass margins, including two sets specially ground by the manufacturer were tried, but all produced chromatograms which this writer judged of greater than nominal distortion in spot distribution (Eastman Chromagram sheets coated with alumina were used exclusively). The basic problem appeared to be large-chamber characteristics, such as too much space which did not become saturated with vapors of the developing solvent. One of the advantages of the TLC technique is supposed to be rapid saturation of a small chamber. Thus, with the Eastman system, the unsatisfactory results achieved by this author might have been due to certain characteristics of design rather than to faulty manufacture. I regularly observed *convex* solvent fronts with *concave* distribution of spots, particularly toward the more non-polar end of the chromatogram, which indicated an "edge" effect. Solvent was apparently evaporating from the solvent front into the unsaturated chamber space, from which some of these vapors likely diffused between the ground edges of the plates to the outside. Thus, complete saturation of the chamber, in all probability, was never achieved. Capillary action alone would not have been sufficient to maintain a wet solvent seal along the total ground-glass perimeter of the plates. This may have been brought about by ground-glass margins which were not sufficiently parallel. Intermittent soaking of all exposed edges with solvent from a pipette reduced the degree of variance in  $R_f$  values, as evidenced by the more horizontal solvent front, which itself can serve as an indicator of chamber saturation.

The successful modification of the original apparatus involved addition of the following items: three clips like those supplied with the original apparatus, a buret, a 3-mm diameter glass rod 12 cm long, and four thicknesses of Whatman #1 filter paper cut in the shape of a "□," whose outside dimensions were 20 cm wide by 23 cm long. The two 23-cm-long side arms were 5 cm wide and the connecting arm was 3 cm wide. Because the glass rod was positioned parallel to and along the surface of the solvent in the trough, a straight meniscus was formed, which helped insure a horizontal solvent front at the outset. (All spots were located above the glass rod.) The filter paper, which was soaked in the solvent system, helped maintain a saturated atmosphere within the chamber.

Spotting was carried out in the usual manner and dry air helped maintain minimal spot diameters. Eastman Chromagram sheets cut to 10 cm wide and 17.5 cm long were of sufficient size to be closely surrounded by, but not to be in contact with, the filter paper. A 3-mm-wide strip of adsorbent was scraped from the top and two side edges so as to prevent solvent which had condensed on the plates from moving onto the developing layer by capillary action. If perchance some condensate did get onto the chromatogram, a second adsorbent-free barrier

was set up approximately 1 cm interior to the two lateral edges and the top edge. These two barriers proved adequate. Activation of adsorbent layers was carried out after their preparation in the above manner. Treatment was 105°C for 30 minutes, after which sheets were removed to a dry atmosphere until used.

Assembly and pretreatment of the chromatogram followed. The assembled apparatus with modifications is shown in Figure 1. The four thicknesses of filter paper were soaked in the developing solvent, then placed around the adsorbent-coated sheet in such manner that the free ends of the filter paper protruded from between the bottom edges of the two glass plates. The 12-cm-long glass rod

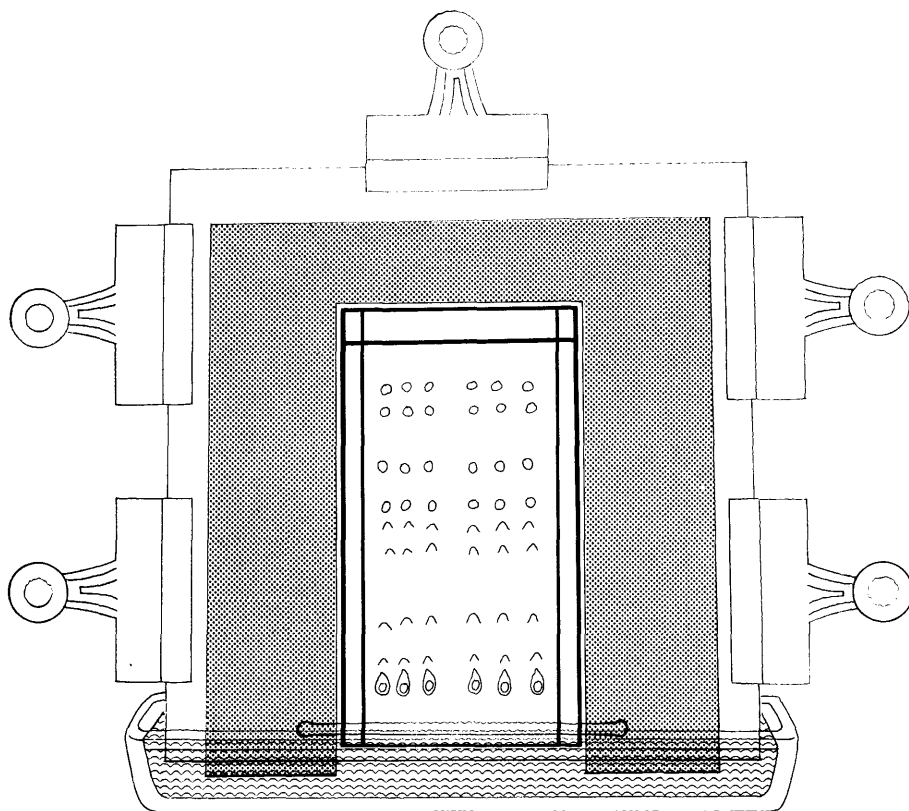


FIGURE 1. Diagram of a modified TLC apparatus showing "sandwich" of Chromagram sheet and  $\Gamma$ -shaped filter paper (stippled) between two glass plates, which are held together by five clips.

was placed horizontally on the adsorbent-coated surface 1 cm from the bottom edge of the sheet. This rod was of sufficient thickness to flatten out the developing sheet, which had become curved during activation. The clips, two on each side and one at the top, provided closer contact along the ground-glass margins. The "sandwich" was then propped up on the front edge of the uncovered trough, which contained 150 ml of developing solvent, with the free ends of the filter paper dipping down into the trough. Thus a wick effect, due to the continuously wetted free ends of the filter paper, helped insure chamber saturation prior to development. Additional insurance of chamber saturation came from the use of a buret to hold the eluent, which was allowed to discharge at the rate of 1 drop/3-8 sec. onto the

top edge of the "sandwich" where the two plates joined. This created a completely saturated seal throughout the ground-glass perimeter during development. Any evaporation would now take place only outside and not inside, hence edge effects were greatly reduced.

Prior to development, which was in an ascending manner, the chamber atmosphere was allowed 30 minutes in which to become saturated. A uniform initial exposure of the bottom edge of the chromatogram to the developing solvent was achieved by allowing the bottom edges of the plates to be lowered into the solvent in a horizontal manner. (Vertical positioning of the "sandwich" during this maneuver is not required.) Constant contact of the bottom leading edge of the front plate with the inner front side of the trough aided in the horizontal descent of the "sandwich" into the solvent-filled trough. The 150 ml of solvent was just sufficient to contact the horizontal glass rod; thus a straight meniscus was insured throughout development. Without the rod to flatten out the sheet, which became curved during activation, a curved meniscus usually resulted, causing a convexity in the solvent front and consequently distortion in  $R_f$ 's of the spots.

This modified Eastman Chromagram system would appear to be adaptable to most laboratory procedures where TLC is to be carried out using pre-coated sheets. This apparatus seems quite suitable for one-dimensional development or for step-wise elution. The changes in the basic apparatus suggested herein render it less flexible to two-dimensional procedures, but the need for such development may be obviated through step-wise methods.

Reproducibility, with low variations in  $R_f$  values, can be achieved with relative ease. In preliminary work carried out at Cranbrook, a step-wise development was employed, using azulene as a standard reference dye. The steps were: step 1) benzene: ethyl acetate 6:4 for 9 cm, and step 2) benzene: ethanol 97:3 for 14 cm. In 50 chromatograms, the azulene moved upward an average of 12.3 cm from the starting point. The least movement was 11.9 cm and the greatest was 12.4 cm. All runs were at 21-23°C.

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