AUTOTRANSFER CHROMATOGRAPHY: FACILE TWO-DIMENSIONAL CHROMATOGRAPHY IN WHICH THE STATIONARY PHASE IS CHANGED

I. AUTOTRANSFER FROM THIN-LAYER TO PAPER

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INTRODUCTION

In the course of work on unidentified metabolites excreted by mental patients[1,2] the need for simple coupling of different chromatographic procedures was felt. Particularly pressing was the need for some method to permit automatic spotting of all TLC fractions onto paper chromatograms. We had found that labile metabolites in low concentrations were not efficiently handled by the methods of scraping off the spots, eluting, concentrating, and re-spotting, as there could be losses at several stages (*e.g.* incomplete removal of the spot, incomplete elution, losses on glassware, volatilization during reconcentration of the eluate). For a series of unknown compounds, these sources of error were not easily checked. In addition, such methods for transferring each fraction were time-consuming, tedious, and used much glassware. An attempt was therefore made to simplify and "automate" the transfer of materials from one chromatographic system to another.

The simplicity of the techniques developed to fill this need, and the exceptional clarity and interpretability of the resultant composite chromatograms prompt us to use the specific term "autotransfer chromatography" for the general procedure. Autotransfer chromatography (ATC) is the direct and essentially automatic coupling, in two or more dimensions, of chromatographic systems which differ in their stationary phases.

Several workers have reported methods involving some form of autotransfer chromatography as just defined. For example, NIGAM *et al.*[3] and JANAK[4] have both reported autotransfer from GLC to TLC, while KAUFMAN AND MAKUS[5], and KNIGHT[6] have both reported two-dimensional methods closely related to autotransfer. Of these latter methods, one alters the stationary phase by treatment with a phase-reversing agent after development in the first direction is completed[5], and the other modifies the stationary phase (ion exchange paper) between dimensions by adding an ionization suppressing agent. A brief review of the chromatography literature, however, revealed very little work specifically concerned with the complete and automatic coupling of TLC with paper chromatography.

APPARATUS AND PROCEDURE

The apparatus developed for ATC, depicted in Figs. 1 and 2, consists of a

vapour-tight glass tank containing specially-designed glass structures to support the TLC plate and chromatography paper, a weighted, precisely dimensioned polypropylene cylinder to press the transfer edges of paper and plate together, and a trough for the transfer solvent.

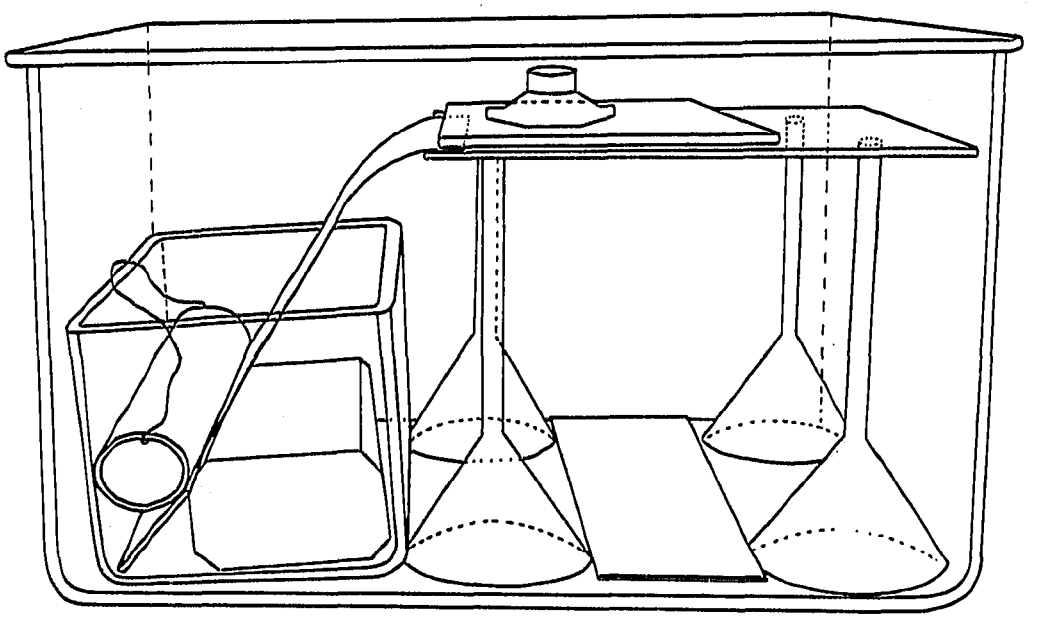

Fig. 1. Autotransfer chamber. Transfer–solvent ascent is from the lower left side, diagonally upward and toward the right, where the paper is held between a heavy glass plate (paperweight) and a glass shelf supported on funnels. Paper and TLC plate are pressed together between the cylinder and an octagonal bottle inside the solvent vessel.

To effect a transfer, the TLC plate (already spotted, run in a suitable solvent and dried) is placed against the octagonal support, with one side immersed in a few ml of solvent selected to "elute" these components from the thin layer. A strip of chromatography paper equal in width to the length of the TLC plate is pre-cut to the desired length, and about 1 mm of its transfer edge is curved upwards, to avoid damaging the thin layer in the next operation. With the distal end of the paper anchored between the glass paperweight and high glass shelf, the transfer end is gently applied to the upper surface of the TLC plate, and secured in place by lowering the polypropylene cylinder, using a loop of fine cord. The chamber is closed as quickly as possible, and the progress of transfer noted; if the solvent chosen is satisfactory for both transfer and subsequent paper chromatography of the substances under study, then the entire procedure can be carried out in the autotransfer chamber; if it is desirable to chromatograph with a solvent different from that necessary to transfer all the components from the thin layer, or if many ATC's are to be run with few autotransfer chambers, then the paper is removed, marked to show the solvent front, and air-dried after the transfer solvent has travelled only a few cm. The resulting paper is submitted to ascending or descending chromatography, the transfer solvent front corresponding with the line of application in conventional chromatograms. When the final chromatogram is read, the original TLC plate should also be read in the same way.

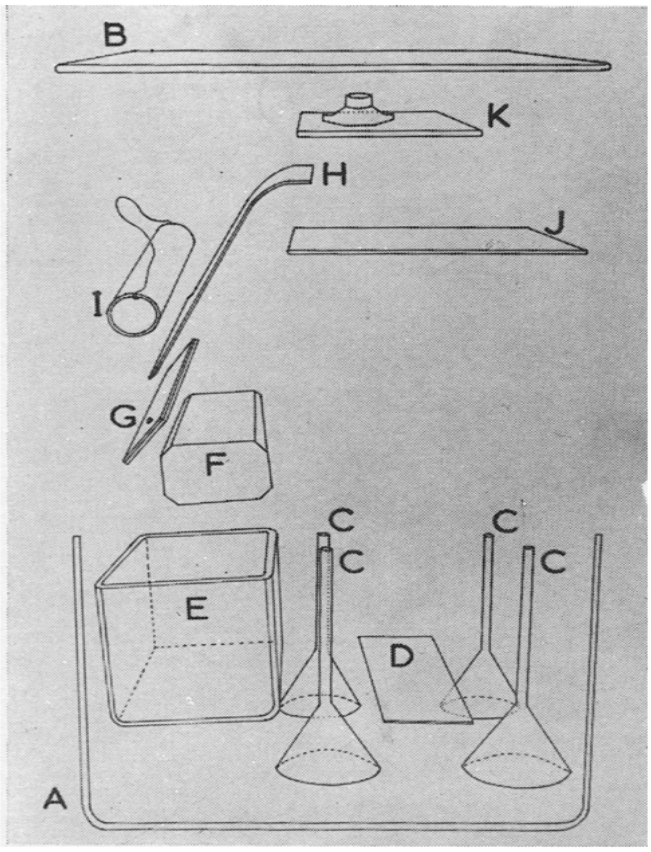

Fig. 2. Details of autotransfer chamber (exploded view, in perspective). A = glass tank (inside dimensions: 26.5 cm wide × 37 cm long × 25 cm deep), *e.g.* Shandon Universal Chromatank, Smith 10-in. Model; B = glass lid; C = four funnels (max. diameter 81 mm, total height 20 cm); D = glass stabilizing plate (7 cm wide × 25.5 cm long × 3 mm thick); E = transfer solvent trough (inside dimensions: 12.0 cm wide × 25 cm long × 12 cm deep), *e.g.* Shandon overflow tank for Apparatus No. 2520; F = octagonal bottle (outside dimensions: 77 mm wide × 20 cm long × 55 mm thick; bevel surfaces 15 mm wide); G = standard narrow TLC plate (5 cm wide × 20 cm long × 4 mm thick); H = chromatography paper (20 cm wide × 22 cm min. length); I = polypropylene cylinder (37 mm O.D. × 24.5 cm; filled with glass bars); J = glass shelf (22 cm wide × 25 cm long × 3 mm thick); K = glass paperweight (14 cm wide × 23 cm long × 6 mm thick; with knob).

RESULTS

Our general experience with this technique has shown that:

(1) Modified and impregnated papers may be used in the second dimension.

(2) Selected portions of AT chromatograms may be further subjected to autotransfer, back to layers or on to modified papers. These analogous ATC procedures will be described in further communications.

(3) The transfer occurs more quickly if the solvent is also placed on the floor of the entire chamber.

(4) The "transferred" paper may be subjected to electrophoresis instead of paper chromatography.

(5) It must not be simply assumed that transfer is complete, or that the position of each component is at the transfer–solvent front. If the R_F in the paper dimension must be precisely known, the position of the spot immediately following transfer must

be determined; alternatively, pertinent reference substances can be introduced onto the TLC plate after drying it but before the transfer step. However, the detailed behavior of substances is exceptionally easy to monitor in this procedure.

(6) While it is generally desirable to effect a complete transfer of all components from the plate to the transfer–solvent front on the paper, it is also advantageous in some work to selectively transfer from the plate, using an appropriate solvent.

(7) When desirable the "transferred" paper sheet may be cut into strips to be run in different solvents particularly useful for the resolution of groups of substances "classified" by TLC.

DISCUSSION

Autotransfer chromatography, thin-layer to paper (ATC, TL → P), has the following advantages:

(1) The rather different separating capacities of TLC and paper chromatography are both applied to the same sample, *e.g.* (a) lipids may be separated according to functional group by TLC, and then further separated within each group using the paper chromatography dimension, and (b) primarily adsorption techniques may be coupled with primarily partition techniques.

(2) The transfer of material from TLC plate to paper chromatogram is virtually automatic, requires no scraping, elution, solvent stripping, or re-spotting.

(3) The transfer involves no test tubes or other glassware, and all parts of the system may be viewed under ultraviolet light and sprayed with chromogenic agents to reveal exactly what has taken place. Such phenomena as failure to elute the spots from the plate, or decomposition are readily apparent.

(4) For the reason given under (2) and (3), results are more easily interpreted, there being no problems in determining the effectiveness of "elution", and no possibility of losses on external glassware, or losses through volatilization during flash evaporation of eluting solvents.

(5) Strongly coloured thin-layer materials (*e.g.* charcoal) can be used since the compounds may be revealed by conventional U.V. and chromogenic techniques in the subsequent paper dimension.

(6) Small amounts of compounds difficult to reveal on TLC plates with selective sprays may be separated by TLC, then transferred to paper and more easily detected.

(7) The same basic procedure and equipment may be used to carry out analogous chromatographic procedures, but changing fixed phases as follows: paper to TLC; one sort of paper (*e.g.* siliconized) to another type of paper (*e.g.* alumina-impregnated); or TLC plate to another TLC plate coated with a different adsorbent. These methods will be treated more fully in subsequent publications.

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SUMMARY

Apparatus and procedures for one subtype of automatic transfer chromatography are described. In this technique, materials fractionated on a thin-layer chromatography plate are automatically transferred at right angles onto a paper chromatogram. The method minimizes operator time, eliminates nearly all glassware, allows complete monitoring of the entire system by simple ultra-violet or chromogenic spray techniques, eliminates certain losses, and provides patterns of excellent resolution and enhanced interpretability in terms of chemical structure.

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