

THIN-LAYER CHROMATOGRAPHY USING THE DESCENDING TECHNIQUE WITH NON-BOUND ALUMINA PLATES

E. A. MISTRYUKOV

Institute of Organic Chemistry, U.S.S.R. Academy of Science, Moscow (U.S.S.R.)

(Received July 2nd, 1962)

Thin-layer chromatography on non-bound alumina has recently been proved as a very useful method for rapid analytical and quantitative separation of various mixtures¹⁻⁴. But as far as separation for preparative purposes is concerned, this method suffers one disadvantage, namely the necessity to apply too great an excess of alumina to make it practicable for chromatography of intermediate quantities of mixtures. The reason is that, with solvent systems of great elution power, alumina displays lower adsorption capacity (as is obvious from the higher R_F values) and with the less polar solvents the R_F values are too low to permit effective separation of zones in a single run. This disadvantage is overcome as shown below by applying a flow of solvent (as in column chromatography) which permits effective separation and more effective use of adsorbent. Moreover, when the thin layer (0.4-0.5 mm) is applied, this descending technique gives good analytical separation of compounds otherwise inseparable by the usual one-run method⁴. The idea is somewhat similar to that of BRENNER AND NIEDERWIESER⁵, except that the chromatograms are run in a closed tank and a solvent flow is secured not by evaporation but by gravity forces.

EXPERIMENTAL

The simple device for descending plate chromatography consists of a development chamber, usually a shallow round dish (a, Fig. 1) 34 cm in diameter and 10 cm in height with a ground cover, a solvent tank (b) 24 × 3.5 × 3 cm in size which is placed as high as the cover of the chamber permits (for the given size of dish, 4 cm from the bottom), and a frame (c). The frame (c) serves as a support for the glass plate (d) (24 × 24 cm) when alumina is being spread and samples placed, and for securing the solvent flow from the solvent tank into the chromatographic chamber. The frame (c) can be made of any sort of material of sufficient corrosive resistance; aluminium proved quite satisfactory for all solvent systems using alumina. Two modifications of a "pumping" system were tried, the first with a strip of filter paper inserted into the upper and lower slits (e), the second with the width of the slits (e) being carefully regulated to ensure the rise of the solvent by capillary forces up to the alumina layer and down by gravity into the chamber (0.1-0.2 mm). Both systems are equally satisfactory.

The chromatography is carried out as follows. The glass plate (d) is placed on the frame (c). The upper and lower planks (f and g) are fixed at a height which will give the desired thickness of alumina layer (the play between the edges of the glass plate and

the side-pieces (f) and (g) should not exceed 0.2–0.5 mm), and a uniform layer of alumina is spread with the help of the simple device (h) (or by a glass rod thickened at the ends) 10–15 mm from the upper edge of the plate. The margin so formed is filled with alumina (3–4 ml) on which the sample is first adsorbed (by evaporation of the mixture of compounds, alumina and appropriate solvent on the film evaporator).

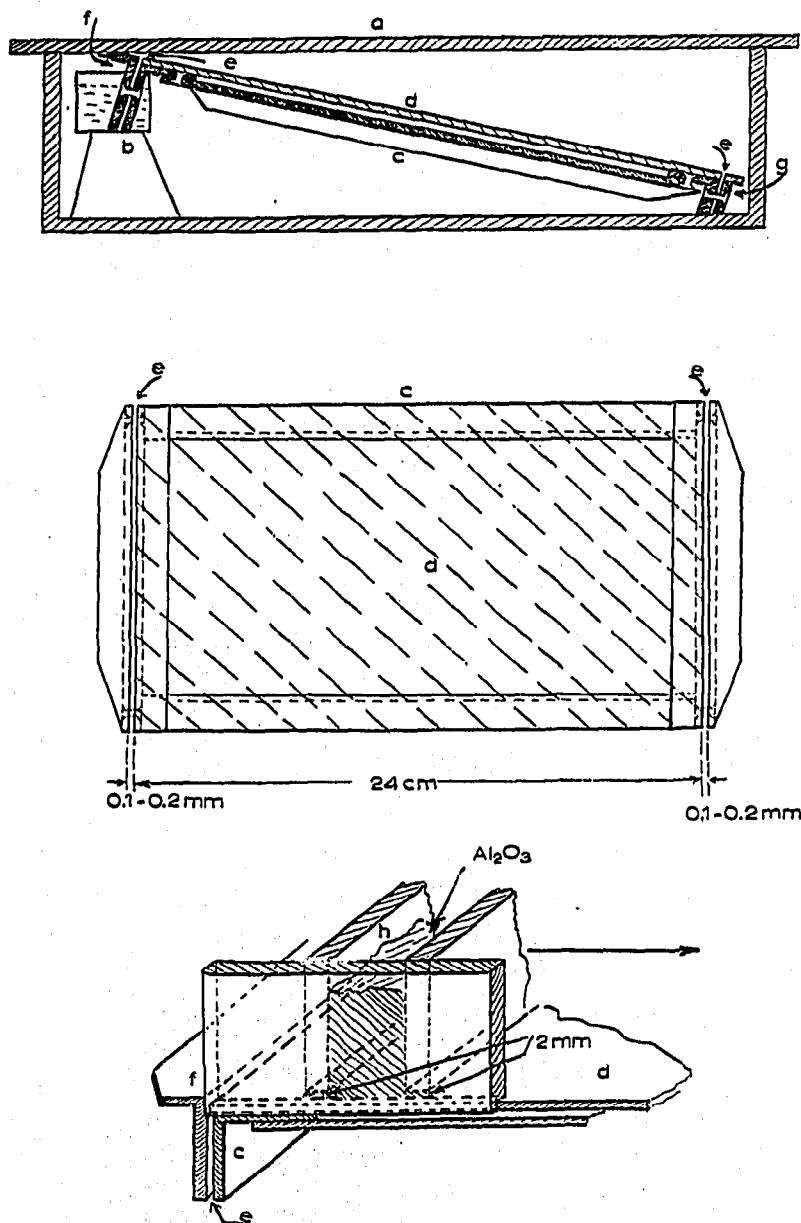


Fig. 1. Chromatographic chamber and its arrangement.

To secure compact zones on the chromatogram, the Al_2O_3 with adsorbed compound should be spread uniformly (spreading by spatula is usually satisfactory). The chromatoplate so prepared is placed with the frame in the chamber (a) with the side-piece (f) dipping into the solvent tank, and the lower end of the frame (g) resting on the bottom of the chamber.

The quantity of sample and the solvent system are determined by preliminary runs on the analytical plates⁴, and it is quite obvious that for compounds of close R_F values the charge should be lower and the solvent system less polar than for great R_F differences. For separation of compounds of close R_F values better results are obtained by the method of placing the sample adopted in column chromatography, *i.e.* a solution of the mixture in solvent of the lowest possible polarity is poured into the solvent tank (b) and when all solvent has been drained out, the tank is filled with the developing mixture. For good separation it is sometimes necessary to recirculate the solvent, or to wash out of the plate some components of the mixture. A combination of data from analytical chromatograms and flow-rate determinations for the given system should be carefully considered, especially for compounds invisible under U.V. light. For such compounds detection of the zone position is made either by spraying with an appropriate reagent^{2,3} or by iodine vapour. In the latter case, a slow flow of moist air saturated with iodine vapours is used and a narrow band so "painted" along the plate is then examined under U.V. light*.

RESULTS

A typical example of separation is given below. A mixture of two isomeric alcohols (R_F 0.25 and 0.48 on alumina, activity II, in chloroform-acetone, 3:1), prepared by NaBH_4 reduction of 0.71 g N-benzoyl-4-keto-*trans*-decahydroquinoline (R_F 0.85), and 4 ml of alumina was spread in a uniform layer at the upper end of the plate with a layer of alumina 2 mm thick (activity II, ~ 80 c.c.) Development of the chromatogram with chloroform (200 ml) was watched under U.V. light and stopped when the zone of one isomeric alcohol reached the end of the plate (traces of the starting material at this moment were washed out). After drying, the zones were collected on a sintered glass suction filter (closed by a cork with an inlet tube of 1-2 mm diameter), using a water pump. The adsorbed material was extracted with methanol and 0.59 g of one isomer (m.p. 104°) and 0.14 g of the other isomer (m.p. 140°) of N-benzoyl-4-hydroxy-decahydroquinoline were obtained.

SUMMARY

A convenient procedure for a descending method of analytical and preparative scale thin-layer chromatography on non-bound alumina is described.

REFERENCES

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* Good results were obtained by detecting the zones with filter paper "prints"—narrow filter paper strips (5-6 mm) which are gently pressed against the wet chromatograms and then treated with an appropriate reagent.