

Notes

Thin-layer chromatography under controlled conditions

Within the past few years thin-layer chromatography (TLC) has developed into an important chromatographic technique. Although TLC has many advantages, the fact that it is, in principle, chromatography with "open" columns gives rise to a number of problems which cannot be solved without special precautions and suitable equipment.

One of these problems is caused by atmospheric humidity which can influence separation on chromatoplates. Though this phenomenon is now fairly well known, its effect can be more disadvantageous than hitherto recognized. In our work on the separation of 2,4-DNPs* of aliphatic carbonyl compounds into classes^{1,2} it was found that although an oven-dried plate was used even the humidity of the air in a chromatoplate jar was sufficient to prevent separation. Separation could only be obtained when a chromatoplate jar was used in which it was possible to work in complete absence of atmospheric humidity. Results are given in Figs. 1A and 1B.

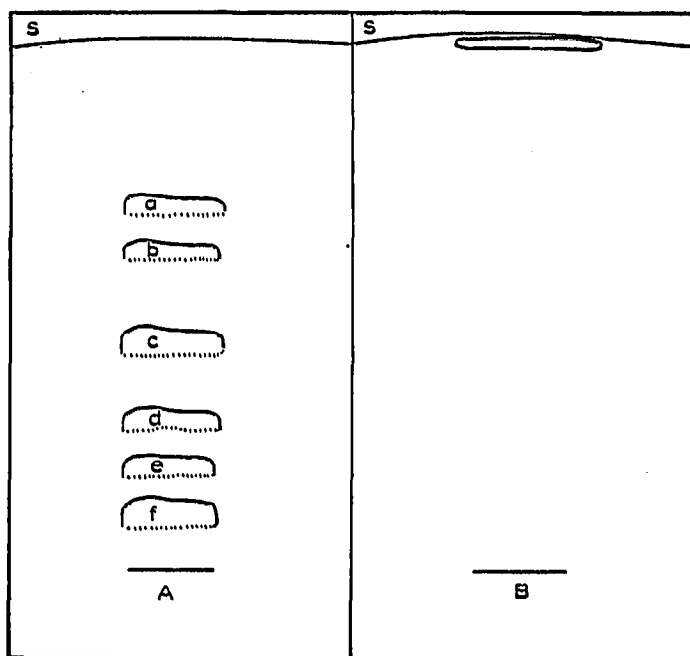


Fig. 1. Separation of 2,4-DNPs into classes by thin-layer adsorption chromatography on basic zinc carbonate. Mobile phase petroleum ether bp. 60–70°–benzene–pyridine (7:1:2) containing 0.2% abs. ethanol. A. Chromatography in absence of water vapour: a = hexanone-2; b = pentanone-3; c = heptanal; d = hept-2-enone-4; e = oct-2-enal; g = deca-2,4-dienal; s = solvent front; plate 8 × 18 cm. B. Chromatography in presence of water vapour. The same mixture of DNPs as in (A) is not resolved and moves close to the solvent front.

* 2,4-Dinitrophenylhydrazones.

Another important source of problems is the presence of oxygen during the chromatography of lipids. In TLC the risk of autoxidation of lipids is even greater than in column chromatography. To demonstrate this effect, a mixture of fatty acid methyl esters was run on two chromatoplates, the first in an atmosphere of nitrogen and the second in air. The methyl esters were extracted from the two adsorbent layers and the fatty acid composition was determined by gas chromatography. Results are given in Table I. It can be seen that there is a significant decrease in the amount of higher unsaturated fatty acids as a result of autoxidation.

In order to overcome the above problems we designed the chromatoplate chamber shown in Fig. 2. It consists of a glass tank ($20 \times 20 \times 5$ cm inner dimensions) closed by a glass plate with a hole to which a female joint was cemented. In one side of the jar an opening was drilled for the introduction of the mobile phase from a funnel fastened to the side of the chamber. To obtain an air-tight seal with the covering plate, some silicone grease was applied to the polished rim of the tank.

TABLE I

INFLUENCE OF OXYGEN (DURING TLC) ON THE COMPOSITION OF A MIXTURE OF FATTY ACID METHYL ESTERS

The figures refer to peak areas of fatty acid methyl esters separated by TLC relative to methyl stearate (= 100).

	Composition of the mixture of fatty acid methyl esters		
	before TLC	after TLC in N_2	after TLC in air
Methyl stearate	100	100	100
Methyl oleate	31	31	31
Methyl linoleate	92	91	87
Methyl linolenate	115	115	108
Methyl arachidonate	82	83	72

When carrying out a separation, the spotted plate was placed in the chamber (with a piece of filter paper covering the walls of the jar if the chamber had to be saturated with the solvent vapour). The lid was closed and, using the two openings in the chamber, dried nitrogen (or air) was passed through the chamber. After displacement of moisture (and oxygen) the mobile phase was introduced while the chamber was tilted backwards in order to wet the piece of filter paper. When the atmosphere in the jar was saturated with vapour, the jar was placed in a vertical position so as to bring the plate in contact with the mobile phase which began to ascend. The development of too high a pressure in the chamber was prevented by opening the tap at the side of the funnel.

Using the equipment described, it was possible to prevent the undesirable effects of humidity and of oxygen. R_F values were very reproducible as can be seen in Fig. 3, *e.g.*, where the results of separation of 2,4-DNPs according to chain length² are given.

It was also possible to carry out separations on chromatoplates of lower activity by leading air (or nitrogen) of fixed humidity through the chromatoplate chamber.

In our experiments plates of different size were coated with a thin layer of ad-

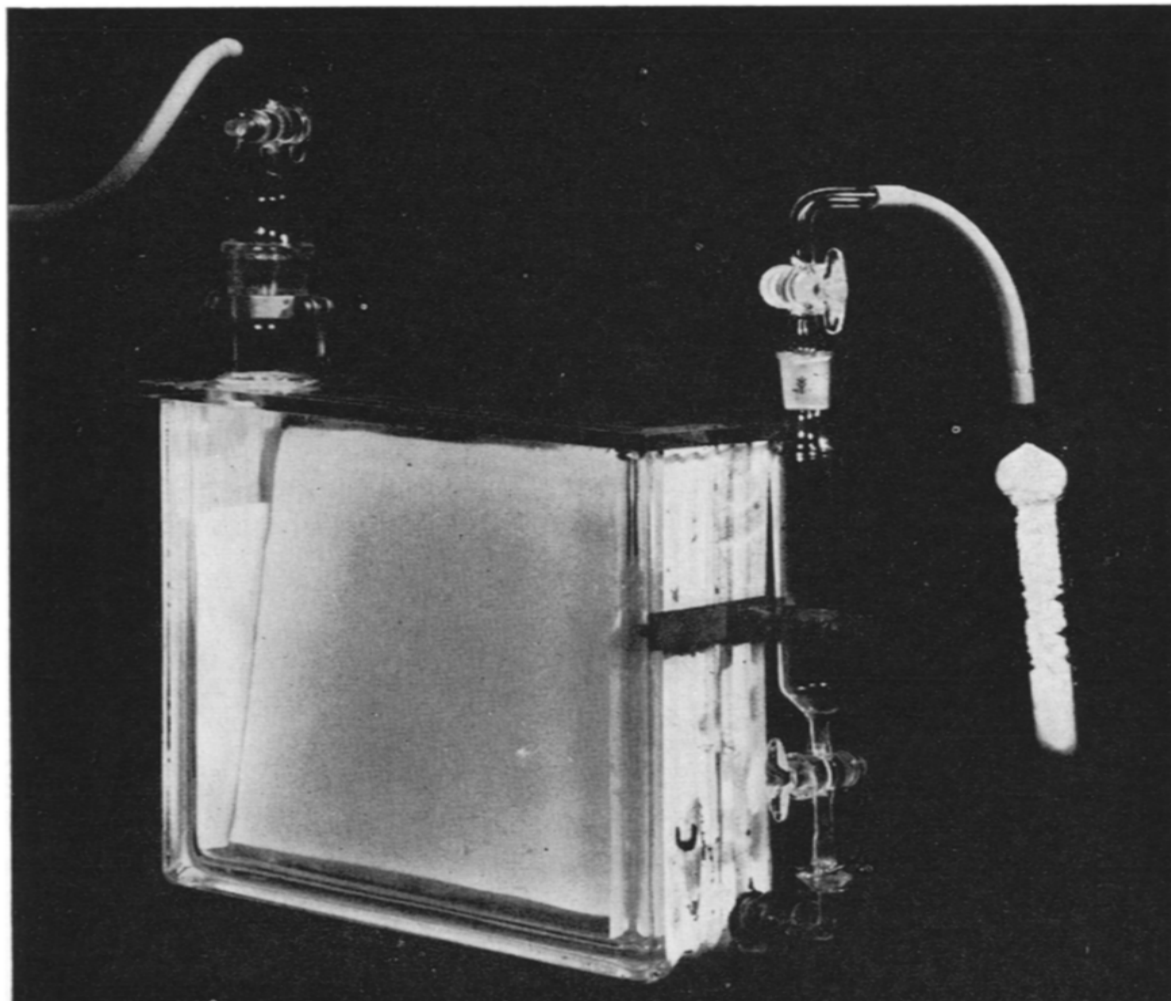


Fig. 2. Chromatoplate chamber designed for TLC under controlled conditions.

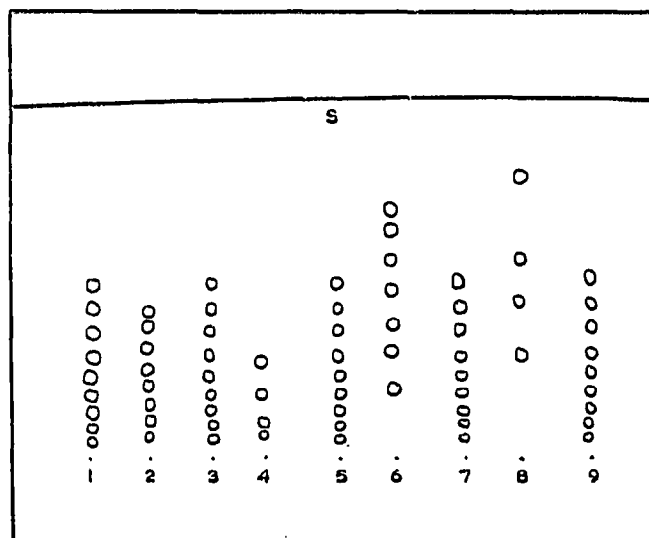


Fig. 3. Separation of 2,4-DNPs according to chain length by thin-layer partition chromatography on Carbowax-400 impregnated plates of basic zinc carbonate. Eluent = petroleum ether, bp. 100–120°. 1 = alkanals C_2 to C_{10} incl.; 2 = alk-2-enals C_4 to C_{11} incl.; 3 = same as in 1; 4 = alka-2,4-dienals C_6 , C_7 , C_9 , C_{11} ; 5 = same as in 1; 6 = alkanones-2 C_3 to C_9 incl.; 7 = same as 1; 8 = alk-2-enones-4 C_5 , C_6 , C_7 , C_{10} ; 9 = same as 1; s = solvent front; plate 15 × 15 cm.

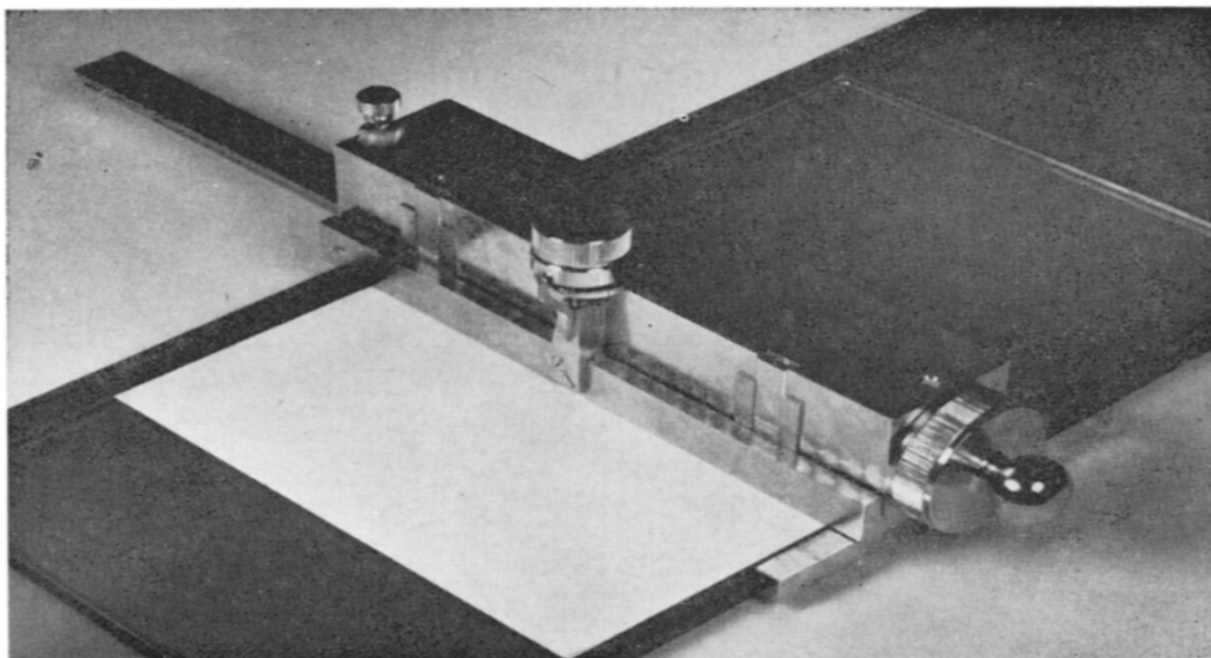


Fig. 4. Applicator for thin-layer chromatography.

sorbent using an applicator of our own design. With this applicator, shown in Fig. 4, a layer of the desired thickness (max. 2 mm) can be applied to plates of different widths. The slide on the left side of the applicator is extended according to the width of the plate, while the slide at the front of the instrument is moved vertically to adjust the adsorbent layer to the desired thickness.

I wish to thank Mr. C. DE GRAAF and Mr. A. M. BAKKENES for carrying out the construction of the designed chromatoplate chamber and the applicator respectively.

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Received September 20th, 1963