An improved method for charring lipids on thin-layer chromatograms

Lipids separated on thin-layer plates may be visualised by destructive "charring" with one of the dehydrating acids (sulphuric, phosphoric or chromic). This is normally sprayed on to the thin-layer plate; the plate is then heated to 120–200° to char lipids present.

The major advantage of this method is that the position of the lipid is determined unambiguously; disadvantages are that further analysis is impossible; the surface of the thin layer is pitted by the small but heavy droplets of acid and spraying may be uneven. The latter two facts caused some concern during our attempts to quantitate lipids by a method[1] involving thin-layer separation on microscope slides, charring, and determination of resultant optical density.

We therefore attempted to generate the acid on the thin layer by interaction of a volatile halide or oxide with water vapour. Any of the following compounds can be hydrolysed to the acid named:

(1) $PCl_5 + H_2O \longrightarrow POCl_3 + 2\ HCl$
Phosphorus pentachloride (solid, sublimes 162°) → Phosphorus oxychloride

$POCl_3 + 3\ H_2O \longrightarrow H_3PO_4 + 3\ HCl$
Phosphorus oxychloride (b.p. 105°C) → Phosphoric acid

(2) $CrO_2Cl_2 + 2\ H_2O \longrightarrow H_2CrO_4 + HCl$
Chromyl chloride (liquid, b.p. 117°) → Chromic acid

(3) $SO_2Cl_2 + 2\ H_2O \longrightarrow H_2SO_4 + 2\ HCl$
Sulphuryl chloride (liquid, b.p. 69°) → Sulphuric acid

(4) $SO_3 + H_2O \longrightarrow H_2SO_4$
Sulphur trioxide (α-form liquid, b.p. 44.5°; β-form solid, sublimes 50°; α-form is slowly converted to β-form on standing). → Sulphuric acid

As we perform sensitive phosphorus analyses in our laboratory, the use of volatile phosphorus compounds was contra-indicated. Chromyl chloride was satisfactory from the aspect of application but on hydrolysing and charring the whole thin layer was blackened.

Sulphuryl chloride and sulphur trioxide worked equally well, and in view of the difficulty in both obtaining and handling sulphur trioxide or fuming sulphuric acid, we decided on the former compound for simplicity.

Thin-layer chromatograms on microscope slides (3 in. × 1 in.) were placed in a Coplin jar, in the bottom of which 1 ml of sulphuryl chloride was placed. Slides did not touch the liquid. After 2-min exposure to the vapour, slides were rapidly removed and held over a steaming water bath for 30 sec. They were then placed on a hot plate,

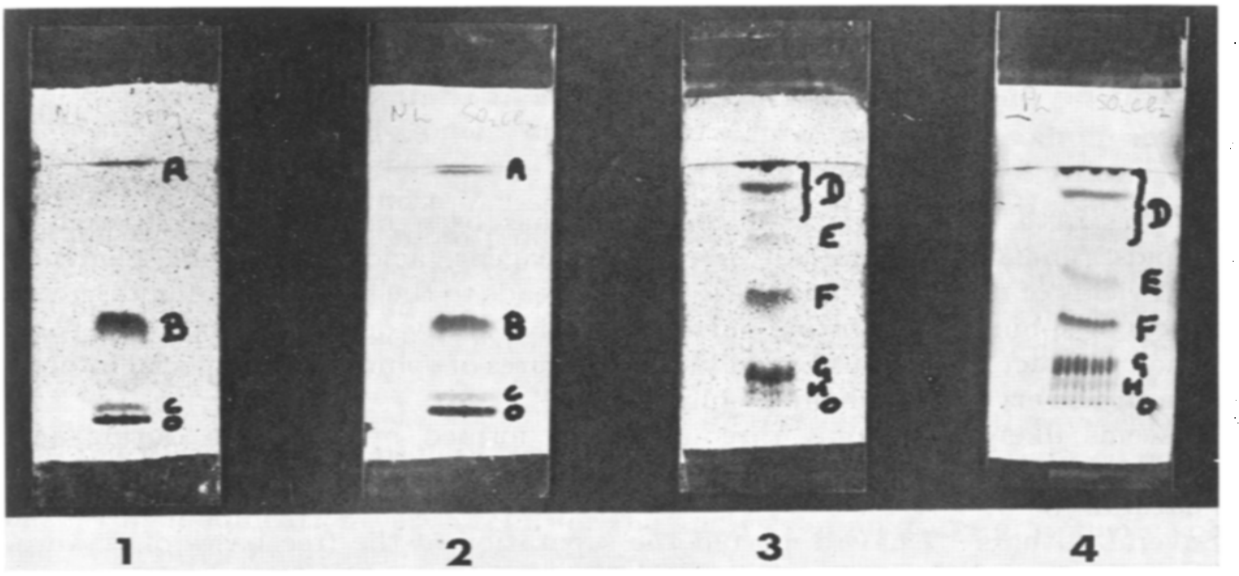

Fig. 1. Neutral lipids (1 and 2) and phospholipids (3 and 4) from chicken liver. Slides 1 and 3 were sprayed with concentrated sulphuric acid. Slides 2 and 4 were exposed to sulphuryl chloride vapour. Identity of lipids — Slides 1 and 2: A = cholesterol esters; B = triglycerides; C = cholesterol; O = origin (containing phospholipids). Slides 3 and 4: D = neutral lipids; E = phosphatidic acid (?); F = phosphatidyl ethanolamine; G = phosphatidyl choline; H = sphingomyelin; O = origin.

thermostatically controlled at 200°. Lipids were charred in a few seconds (Fig. 1).

This method therefore overcomes the disadvantage of uneven spraying and poor surface.

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1 C. M. VAN GHENT AND C. J. F. BÖTTCHER, unpublished results.

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