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Note

Cupric sulphate as a charring agent in the TLC of lipids

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In order to overcome the problem of uniform charring of lipid fractions, Fewster *et al.*¹ sprayed developed plates with 3% cupric acetate solution in 8% aqueous orthophosphoric acid. Walker² proposed the simple technique of charring with ammonium sulphate, which was incorporated into the silica gel layer.

This paper describes the possibility of using cupric sulphate incorporated into the silica gel layer as a charring agent.

MATERIALS AND METHODS

Thin-layer plates were prepared as follows: 10 g of silica gel G (E. Merck, Darmstadt, G.F.R.) were mixed with 20 ml of water or 22 ml of 1, 5 or 10% aqueous cupric sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) solution. The slurries were spread on the glass plates (20 × 20 cm) to a thickness of 0.25 mm with a Desaga spreader. The coated plates were allowed to stand at room temperature for 15 min and then activated at 110° for 60 min. After cooling, the plates were developed in diethyl ether to the top of the plate, then dried at 110° for 30 min and stored in a desiccator until required.

Spots of 10 μl of a 1% standard solution of neutral lipids in chloroform were placed on the plates and then developed in light petroleum (b.p. 40–60°)–diethyl ether–acetic acid (80:20:1). The plates, impregnated with cupric sulphate, were directly heated at 180° for 1 h, while non-impregnated plates were dried at 110° for 30 min, sprayed with 50% v/v sulphuric acid and then heated at 180° for 1 h.

RESULTS AND DISCUSSION

The intensity of the brown spots of cuprous oxide on the plates impregnated with the 1% solution of cupric sulphate was much less than that of the spots on the plates impregnated with 5% or 10% cupric sulphate solution or the black spots of carbon on the plates sprayed with 50% sulphuric acid. The brown spots of cuprous oxide were stable for several months. The R_F values for neutral lipid fractions on non-impregnated plates and the plates impregnated with 1% or 5% cupric sulphate solution were similar (Fig. 1). Impregnation with 10% cupric sulphate solution increased the R_F values of triglycerides and cholesterol esters by too great an extent.

In conclusion, it can be said that the results of Fewster *et al.*¹ and our char-

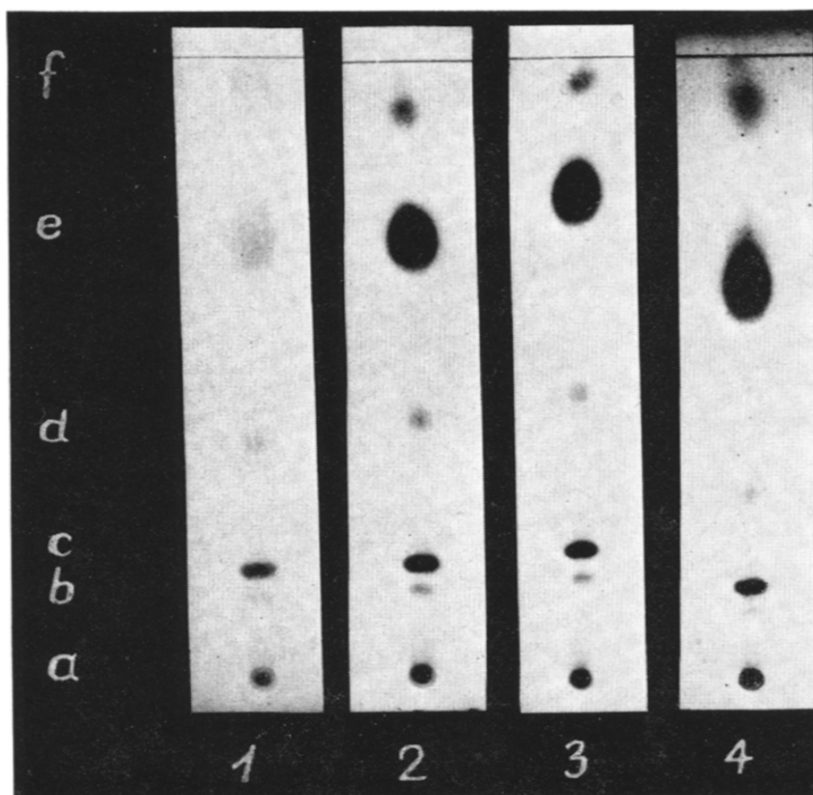


Fig. 1. Thin-layer chromatography of a standard mixture of neutral lipids. Strips: 10 g of silica gel G with 22 ml of: (1) 1% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution; (2) 5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution; (3) 10% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution; (4) 20 ml of water. Lipid fractions: a, monoglycerides + phospholipids; b, diglycerides; c, cholesterol; d, fatty acids; e, triglycerides; f, cholesterol esters.

ring technique involving cupric sulphate incorporated in the silica gel layer indicate that this is a simple method for the qualitative and quantitative TLC of lipid fractions.

REFERENCES

- 1 M. E. Fewster, B. J. Burns and J. F. Mead, *J. Chromatogr.*, 43 (1969) 120.
- 2 B. L. Walker, *J. Chromatogr.*, 56 (1971) 320.