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Sensitive non-destructive visualization of isoprenoid quinones on reversed-phase thin-layer chromatograms

In adsorptive thin-layer chromatography (TLC) of isoprenoid quinones, the preparation of Silica Gel G layers uniformly impregnated with the fluorescent dye Rhodamine 6 G (ref. 1) offers a rapid and sensitive non-destructive visualization of these compounds on the developed plate[2,3]. No analogous technique for reversed-phase TLC (RPTLC) has, hitherto, been reported. This is because the solvent mixtures commonly used in RPTLC tend to carry the dye up the plate, which results in a high concentration of dye near the solvent front, and insufficient dye elsewhere. Instead the plates have been sprayed with either Rhodamine 6 G (ref. 2) or sodium fluorescein[3] after development.

In this communication it is reported that impregnated plates can be used for RPTLC if some of the dye is included in the developing solvent. This technique was not found to be suitable when either Silica Gel G or cellulose was used as a support for the stationary phase, because of partial adsorption of dye from solution, resulting in a high concentration of dye near the origin, and near the solvent front, if the plate had previously been impregnated, and very little dye in between. It worked perfectly well, however, with Kieselguhr G, provided that the developing solvent was sufficiently polar: acetone–water mixtures containing up to 97.5% of acetone gave quite adequate visualization under the 360-nm lamp (visualization was also possible at 250 nm, though not so effective), when the dye employed was Rhodamine 6 G. When sodium fluorescein was employed, satisfactory background fluorescence was obtained with acetone concentrations up to 95%.

Experimental and results

Thin-layer plates, 20 × 20 × 0.025 cm were prepared by using a ratio of Kieselguhr G (Merck, ref. 8129): aqueous dye solution (0.003% (w/v) Rhodamine 6 G or 0.05% (w/v) sodium fluorescein) of between 0.50:1 and 0.55:1. They were left horizontal at room temperature for 15 min, and then dried at 100° for over 30 min. Medicinal paraffin (25 g) was mixed with 250 ml of light petroleum (b.p. 40–60°, AnalaR, dried over sodium) and passed through a column containing 25 g of aluminium oxide (Woelm acid, Brockman Grade O). More petroleum was passed through the same column into the filtrate, until the total volume was 500 ml.

The plates were carefully dipped into this 5% (w/v) solution of paraffin in a shallow dish, and gently agitated, until no visible pattern remained on the layer. They were then lifted out, and the petroleum allowed to evaporate.

Chromatograms were developed with aqueous acetone which had been saturated with medicinal paraffin, and contained the same dye as that with which the plates had been impregnated (0.003% (w/v) Rhodamine 6 G or 0.04% (w/v) sodium fluorescein; the use of higher concentrations leads to a marked loss of sensitivity). The sensitivity of detection was evaluated with side-chain-labelled [1′,2′-^{14}C]ubiquinone-9 (Q-9), specific activity 10 μCi/mg (ref. 4). A solution of [1′,2′-^{14}C]Q-9 in cyclohexane (*ca.* 1 μg/ml) was used to give a series of spots of differing concentration, and the chromatogram was developed in 90% (Q-9, R_F 0.3) or 95% (Q-9, R_F 0.6) aqueous

acetone (containing dye). The acetone was removed in a stream of compressed air, and visualization attempted at 360 nm. The spots were then scraped off, eluted, and counted, using a liquid scintillation counter. In one experiment, the chromatogram was developed in the absence of dye, the plate sprayed with a 0.005% (w/v) solution of Rhodamine 6 G in acetone, and visualized at 360 nm, after which the chromatogram was developed again in 90% aqueous acetone containing Rhodamine 6 G, and visualized as before. (Visualization was at its most sensitive when carried out before all the water had evaporated, when the background fluorescence was red. When the background fluorescence became pink, some of the spots became less easy to see.) From these, and other experiments, it was concluded that when 0.003% (w/v) Rhodamine 6 G was included in the solvent, any spot containing more than 10 ng of Q-9 was easily visible, and spots containing 5 ng were visible under favourable conditions. Less than half this sensitivity was obtained for Q-9 on 0.003% (w/v)–0.01% (w/v) Rhodamine 6 G-sprayed Kieselguhr G RPTLC plates (spraying with 0.2% Rhodamine 6 G completely masked all spots examined), and less than one-fifteenth on similarly sprayed Silica Gel G RPTLC plates. Spraying was, in addition, less rapid, and less convenient. When sodium fluorescein (uranin) was included in the developing solvent, the corresponding limit of detection was estimated to be at 40 ng of Q-9.

In another experiment, Q-9 (1 μg) was applied in a band across the plate. It was easily visible at 360 nm, after development of the plate in aqueous acetone, containing Rhodamine 6 G or sodium fluorescein.

It will be evident, then, that incorporation of fluorescent dye into the developing solvent offers greater sensitivity, rapidity, and convenience in detection, than spraying the plate with dye after development. The best results are obtained when the plate is visualised before all the water (but after all the acetone) has evaporated from the plate.

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1 J. AVIGAN, D. S. GOODMAN AND G. STEINBERG, *J. Lipid Res.*, 4 (1963) 100.
2 D. R. THRELFALL AND G. R. WHISTANCE, *Methods Enzymol.*, 18, Part C (1971) 369.
3 P. J. DUNPHY AND A. F. BRODIE, *Methods Enzymol.*, 18, Part C (1971) 407.
4 O. ISLER, R. RÜEGG AND A. LANGEMANN, *Chem. Weekbl.*, 56 (1960) 613.

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