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Note

Sensitive fluorogenic visualization reagent for the detection of lipids on thin-layer chromatograms*

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Users of thin-layer chromatography (TLC) must have at their command a variety of visualization techniques. Two broad classes of visualization reagents may be distinguished: those yielding color with the compound(s) of interest and those producing fluorescence under ultraviolet excitation[1]. Many such reagents are available for the detection of lipids of biological interest after TLC separation. Chromogenic reagents include phosphomolybdic acid, phosphotungstic acid, sulfuric acid, bromothymol blue, iodine, malachite green, and copper(II) sulfate. Fluorogenic reagents are fewer in number and include 2′,7′-dichlorofluorescein, Rhodamine B, and Rhodamine G. Fluorogenic agents for lipids have some advantages over chromogenic agents such as improved sensitivity, milder conditions for the visualization process, and often a more rapid treatment procedure.

8-Anilino-1-naphthalenesulfonate (ANS), as the ammonium salt, finds use as a fluorescent probe for proteins[2], detergent micelles[3] and phospholipid aggregates[4]. It has also been used as a fluorescent spray reagent to detect phospholipids on TLC plates[5] and to quantify phospholipids after TLC separation[6]. We report here a brief study of the detection limits for both neutral lipids and phospholipids of biological interest after TLC separation on silica gel flexible sheets using ANS and some common visualization reagents.

EXPERIMENTAL

Silica gel IB2 flexible TLC sheets, Baker-flex® 20 × 20 cm (J. T. Baker, Phillipsburg, N.J., U.S.A.) were used without activation. The sheets were developed in an unsaturated tank, 8½ × 4 × 9 in.

Lipids were obtained from various commercial sources. Visualization reagents and solvents were from J. T. Baker. The phosphomolybdic acid reagent was used as a 10% solution in water, 2′,7′-dichlorofluorescein as a 0.03% solution in methanol and Rhodamine B as a 0.05% solution in ethanol. A 0.1% solution of ANS ammonium salt was prepared in water. If stored in a brown bottle in a refrigerator, this reagent is stable for several weeks.

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Stock solutions of the lipids were made up in benzene. A TLC sheet was spotted 2 cm from the bottom with 1 μl of the lipid solutions and air dried. The sheet was developed to a solvent height of 10 cm from the origin in an unsaturated tank with a developing solvent suitable for the separation of the common physiological neutral lipids (hexane–diethyl ether–acetic acid, 80:20:1) or the phospholipids (chloroform–methanol–acetic acid–water, 600:150:2:18). The sheet was then removed from the tank and dried in an oven at 110° until solvent removal was complete. The sheet was then sprayed with the selected visualization reagent. With phosphomolybdic acid, the sheet was heated at 110° for 10 min after spraying. With the fluorogenic visualizing agent, the sheets were examined either while wet or immediately after evaporation of the spray solvent. The sheets were inspected in an ultraviolet visualization chamber (Chromato-Vue, Model CC-20; Ultraviolet Products, San Gabriel, Calif., U.S.A.). Spots sprayed with ANS showed up better under "long-wave" ultraviolet excitation. The detection limits were established by serial dilution of the lipid solution and TLC development until the spot could no longer be visualized with the selected reagent.

RESULTS AND DISCUSSION

The R_F values and detection limits found for four neutral lipids are listed in Table I. It will be seen that for at least three of the compounds the detection limits with ANS are better than with 2',7'-dichlorofluorescein or Rhodamine B. The results with the phospholipids sphingomyelin and lecithin are summarized in Table II. ANS

TABLE I

R_F VALUES OF NEUTRAL LIPIDS AND DETECTION LIMITS OF VISUALIZATION REAGENTS

Solvent system: hexane–diethyl ether–acetic acid (80:20:1).

Lipid	R_F	*Detection limit (μg)*		
		Dichlorofluorescein	*Rhodamine B*	*ANS**
Cholesterol	0.18	0.1	0.025	0.05
Oleic acid	0.32	0.4	0.5	0.1
Triolein	0.67	0.1	0.2	0.05
Cholesteryl acetate	0.93	0.3	0.5	0.1

* Sheet examined while still wet with spray reagent.

TABLE II

R_F VALUES OF PHOSPHOLIPIDS AND DETECTION LIMITS OF VISUALIZATION REAGENTS

Solvent system: chloroform–methanol–acetic acid–water (600:150:2:18).

Lipid	R_F	*Detection limit (μg)*	
		Phosphomolybdic acid	*ANS**
Sphingomyelin	0.12	0.1	0.05
Lecithin	0.37	0.5	0.2

* Sheet examined while still wet with spray reagent

was found to be more sensitive than phosphomolybdic acid, which is probably the most frequently used visualization reagent for this class of compounds.

The superiority of ANS over 2',7'-dichlorofluorescein and Rhodamine B can probably be explained by the improved background of the sheet after spraying. The spots with these last two reagents show a fluorescent color similar to the color of the dark background (pink and gold, respectively). In comparison, ANS gives yellow-green fluorescent spots on a dark, uncolored background that is only faintly fluorescent. This high contrast, it should be emphasized, is only achieved if all of the developing solvent has been removed from the sheet and the sheet is examined while still wet with the ANS spray reagent. Some contrast is lost on subsequent drying after visualization.

ANS, consequently, is recommended as a sensitive, fluorogenic spray reagent for the visualization of various classes of lipids on TLC sheets.

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