A technique for quantitative recovery of lipids from chromatoplates*

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[Manuscript received May 13, 1963; accepted June 4, 1963.]

» Thin-layer chromatography (TLC) as described by Stahl et al. (1) provides a rapid and convenient means for separating and identifying microgram quantities of a wide variety of lipids (2). However, on a preparative scale, separation of milligram quantities necessitates removal of the adsorbent and subsequent elution for recovery of lipid. Although preparative TLC can be exceedingly useful, conventional methods for removing the adsorbent are tedious. Furthermore, silicic acid is easily spilled during transfer and tends to blow away when organic solvents are added for elution.

One method for circumventing these difficulties has been described by Ritter and Meyer (3), who recovered appropriate bands of adsorbent from the plate by direct suction into the thimble of a Soxhlet extractor.

In the course of studies on triglyceride synthesis in rat adipose tissue, a simple method was developed for the quantitative recovery of approximately 30 mg of lipid from chromatoplates. A central feature of this method is the use of a simple device to assure quantitative recovery of silicic acid particles. The adsorbent is cleanly separated from the glass by a few firm strokes from a single-edged razor blade and collected into a mound, using a black background to assist in visualizing traces of uncollected material. The particles of adsorbent are then sucked into a glass aspirator[1] (Figs. 1, 2),

* Supported in part by PHS Research Grant HE-06222-02 from the National Institutes of Health, U.S. Public Health Service.

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[1] Obtainable from Kopp Laboratory Supplies, Inc., 70-13 35th Road, Jackson Heights 72, New York.

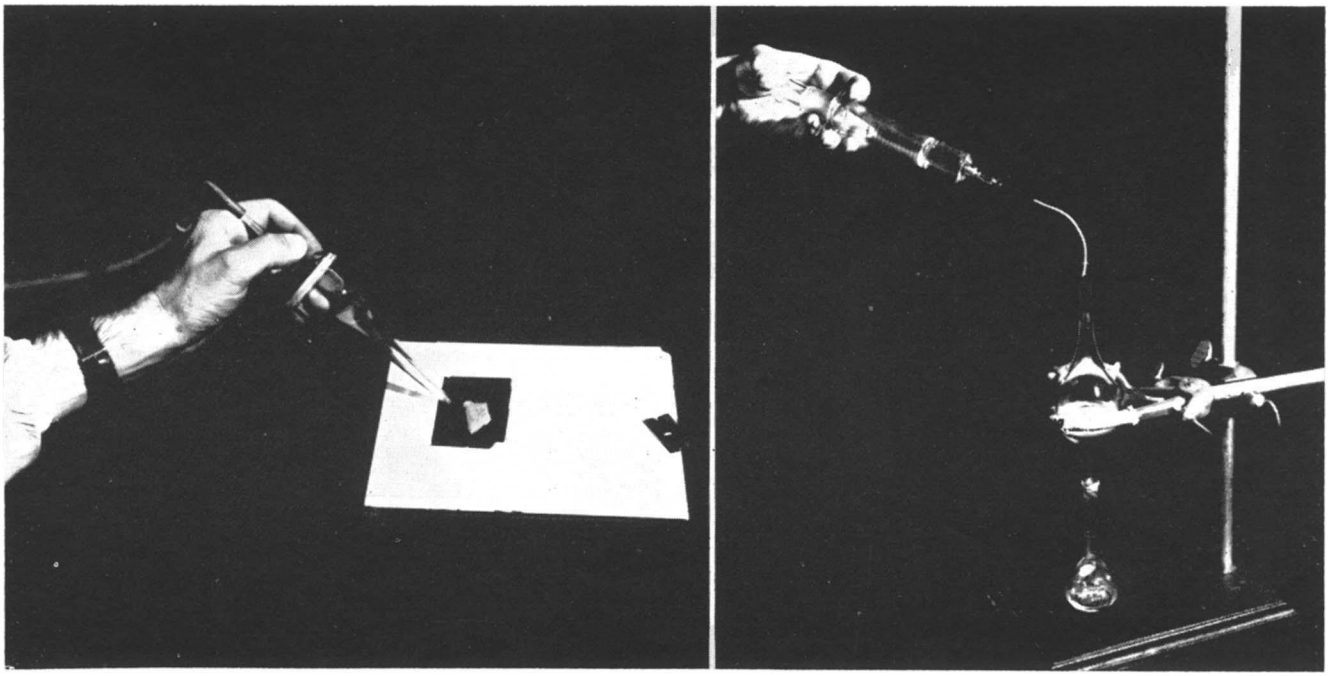

FIG. 1. Use of glass aspirator for quantitative removal of adsorbent from chromatoplates and elution of lipid from adsorbent.

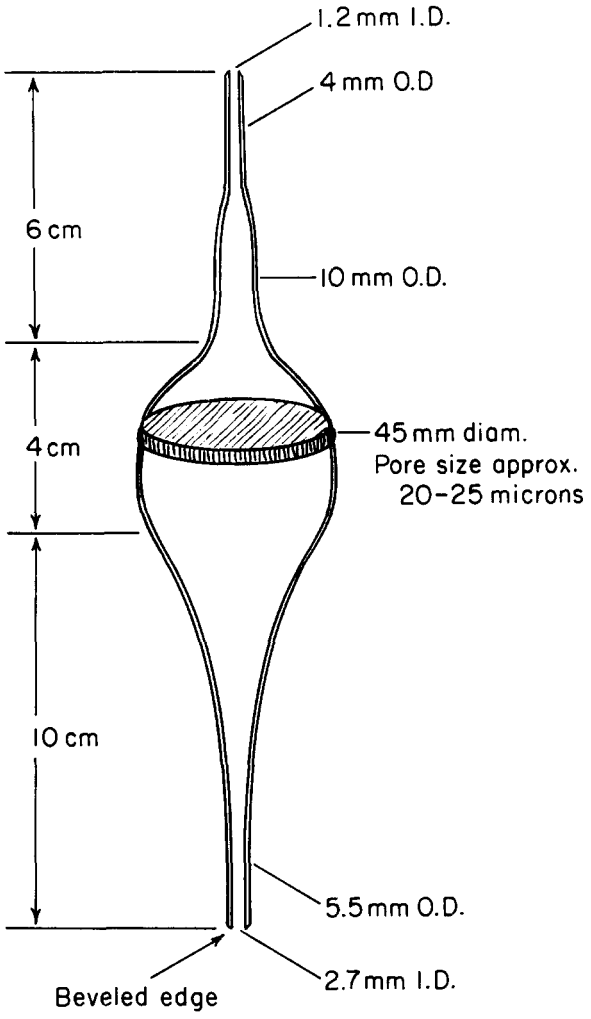

FIG. 2. Dimensions of glass aspirator.

consisting of heavy glass tubing drawn out at both ends with a sintered glass filter (pore size 20–25 μ) in the expanded center portion. With the shorter end attached to a conventional water suction apparatus, a large mound of silicic acid can be drawn into the device within a few seconds. Thereafter, the glass aspirator is disconnected from the vacuum, held vertically, and tapped to displace any silicic acid adhering to its mouth. Solvents are slowly introduced from a syringe via teflon tubing (Fig. 1) to prevent particles blowing back as air is displaced. Elution is then carried out in a few seconds with the aid of moderate pressure from a cylinder of compressed nitrogen. The pore size of the sintered glass disk is critical; minor deviations will result in poor suction or inadequate retention of silicic acid.

By the use of this device, it has been possible to assure quantitative recovery of many polar and non-polar lipids from thin layers made with several different adsorbents. Details of the application of this method to the separation of adipose tissue lipids are described below.

From 5 to 20 μl of adipose tissue lipid was applied to the silicic acid layer in a linear series of small spots by means of a microliter syringe.[2] A similar quantity was put aside for carboxyl ester and radioactivity determinations. Separation was achieved with petroleum ether (b.p. 60–70°)–diethyl ether–acetic acid 67.8:32.0:0.2 (v/v). The origin was then covered with a plastic ruler and the remainder of the plate sprayed with 0.001% aqueous Rhodamine 6G.[3] The positions of the lipid fractions were identified under an ultraviolet lamp. The silicic acid layer from 0.5 cm below the origin to 1.0 cm beyond the solvent front was divided into four areas containing (1) triglycerides plus sterol esters, (2) free fatty acids, (3) 1,2- and 1,3-diglyceride plus free sterols, and (4) phospholipids plus monoglycerides at the origin. Each of these areas was recovered as described above and eluted separately. Phospholipids and monoglycerides were eluted with a total of 25 ml of methanol in three separate washings while the other fractions were eluted in a similar fashion with ethyl ether. Methanol, but not diethyl ether, eluted the Rhodamine as well as lipid; hence, the origin was protected from the spray. Aliquots of the filtrate were taken for carboxyl ester determination (4) and C^{14} determination. Radioactivity was determined by the addition of toluene (with 4 g 2,5-diphenyloxazole and 50 mg 1,4-di-2-(5-phenyloxazolyl)benzene/liter) to the dried aliquot and counting in a Tri-Carb scintillation counter (Model 314, AX, Packard Instrument Co., LaGrange, Ill.).

For a total of 40 separate chromatographic separations, the recovery of carboxyl ester averaged 98.7% with a standard deviation of 6.68. Corresponding figures for recovery of C^{14} counts were 99.5% ± 6.97.

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[2] Hamilton Company, Whittier, California.
[3] National Aniline Division, Allied Chemical Corporation.