The absolute R_F values on a mixed cellulose–silica gel layer differ from those on a plain silica gel or cellulose layer for a given solvent. This is because the active adsorbent has been diluted with an essentially inert support. Similar R_F values can be obtained by varying the solvent ratios. For example, ethyl acetate–toluene (1:4) on a mixed layer gives similar R_F values to ethyl acetate–toluene (2:1) on silica gel. Likewise, acetonitrile–water (1:9) gives similar R_F values to acetonitrile–water (1:3) on cellulose.

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Adaptation of Swinny filter holders for the collection and elution of samples from thin-layer plates

The collection of fractions from thin-layer chromatograms with a vacuum into Soxhlet thimbles in special holders[1] or onto the sintered glass plates of filter tubes[2] has been described. We present here an alternative procedure that utilizes the Swinny adapters which were designed for attachment to hypodermic syringes and are primarily used with membrane filters. The use of Swinny filter holders for collection of samples fractionated by gas–liquid chromatography has been reported[3].

Method and materials

Filter paper (Whatman No. 42) or glass fiber filter (Reeve Angel No. 934AH) discs were mounted on the side of the support grid of the holder indicated in Fig. 1(A). The male leur fitting of the holder was attached to a vacuum line with a No. 13 hypodermic needle inserted into a length of tubing (Fig. 1(B)). The sample was aspirated up with the female leur fitting of the holder being used as a nozzle. After the fraction was collected, the assembly was inverted with the vacuum still attached, and the barrel of a syringe was connected to the female leur fitting of the holder. The vacuum was then detached and the adsorbed compound eluted directly by pouring a suitable solvent into the syringe (Fig. 1(C)). If an adequate flow rate was not obtained, the plunger of the syringe was inserted and pressure applied.

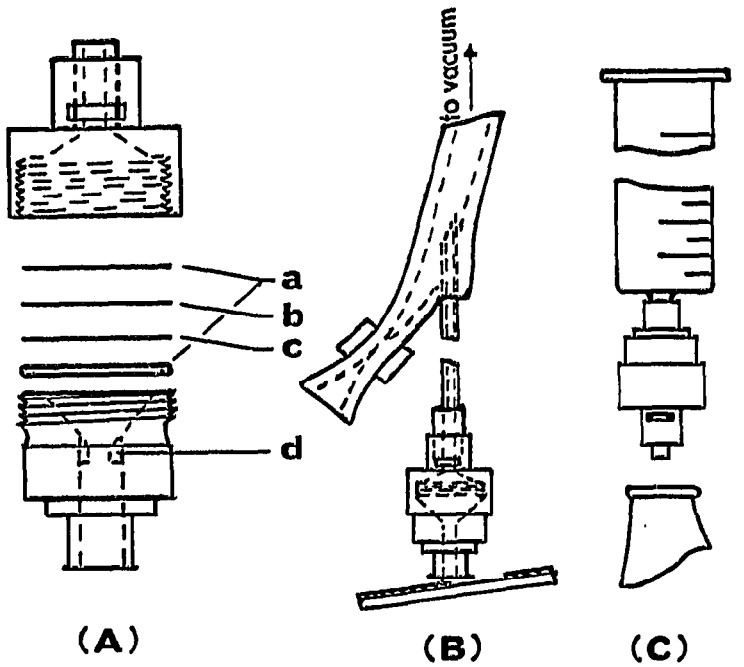

Fig. 1. (A) Disassembled Swinny filter holder showing order of assembly of components as follows: a, Teflon gaskets; b, grid support; c, filter paper or glass fiber filter disc. The constriction at "d" must be drilled out. (B) Assembled holder as used for collecting sample. (C) Holder inverted and connected to syringe for elution of sample.

TABLE I

RECOVERY OF LIPIDS SPOTTED ON SILICA GEL THIN-LAYER PLATES

Lipid spotted and amount in μmoles	*Eluting solvents*[a]		*Recovery (%)*[b]		
	I	*II*	*Solvent I*	*Solvent II*	*Total*
Phosphatidylethanolamine					
0.08	1:19	1:19	91.1	3.7	94.8
0.79	2:1	1:19	91.1	2.5	93.6
0.79	1:19	1:19	96.2	2.5	98.7
Diphosphatidylglycerol					
0.08	1:19	1:19	53.5	8.2	61.7
0.84	1:19	1:19	70.2	8.3	78.5
0.84	2:1	1:19	92.8	4.7	97.5
Phosphatidylglycerol					
0.08	1:19	1:19	88.0	4.6	92.6
0.84	2:1	1:19	92.8	4.7	97.5
[^{14}C]Tripalmitin					
2.1 (2,270 c.p.m.)	2:1	1:19	94.7	2.3	97.0
[^{14}C]Palmitic acid					
3.5 (461 c.p.m.)	2:1	1:19	94.0	3.1	97.1
[^{14}C]Cholesterol					
1.0 (2,574 c.p.m.)	2:1		97.1		97.1

[a] Five milliliters of each solvent were used for each elution except cholesterol was eluted with 10 ml. The solvents consisted of mixtures of chloroform and methanol in the portions shown (v/v).

[b] Determined by analysis of phosphorus[4] with the phospholipids and by recovery of radioactivity with the ^{14}C-labeled neutral lipids.

Some holders have a constriction immediately inside the female leur fitting and it was found essential that it be drilled out to the same diameter as the bore of the leur fitting (Fig. 1(A)). This was readily accomplished with a 4 mm (5/32 in.) drill bit and power drill. Filter paper or glass fiber filter discs 13 mm in diameter for the small size holders were conveniently cut with a No. 9 cork borer. The large size holders required 25 mm diameter discs. The neutral lipids for which recoveries are given were all commercially available compounds. The three phospholipids were isolated from *Escherichia coli* by a fractionation procedure to be described in detail elsewhere. Their identification and purity were established by analysis of hydrolysis products[4].

Results and discussion

The small holders efficiently collected up to 7 cm^2 of 0.5 mm thick adsorbent from a plate. Large holders were used for up to 38 cm^2 without a barrel extension or over 400 cm^2 of adsorbent with a single barrel extension which is available from the manufacturer (Millipore Corp., Bedford, Mass.). There appeared to be no limit to the amount of adsorbent collected when additional extensions were used in series. The elution solvents and volumes and recoveries for a variety of compounds collected on 4 cm^2 of silica gel (0.5 mm) with the small size holder are given in Table I. It should be emphasized that these recoveries are for compounds applied to the plate and then aspirated. Obviously, recoveries from developed plates will also reflect the efficiency of the fractionation as well. These experiments were designed to demonstrate the feasibility of the collection and elution system and its application to specific fractionations should be in every case independently checked.

The initial cost of the small holders is about the same as sintered glass filter tubes that have been used for the same purpose[2]. The Swinny holders offer several advantages including their rugged metal construction, larger capacity, and the fact that they can be disassembled for cleaning.

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