Acknowledgement

We thank Mr. GLEN F. BAILEY for confirming the identity of some of the derivatives by ultraviolet spectrophotometric analysis.

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Received January 17th, 1964

J. Chromatog., 15 (1964) 111–115

Notes on semimicro preparative thin-layer chromatography

Details of preparative thin-layer chromatographic techniques used in this laboratory and mentioned in two recent papers[1,2] are described here since they are of general application. They have been used in the isolation of 2- to 5-mg quantities of material for subsequent identification by ultraviolet and infrared spectral studies and by chromatographic comparison.

Overdeveloping thin-layer plates

For the separation of contiguous spots and bands on thin-layer plates, a continuous, descending method was developed. It gave much better separation at higher loading than BRENNER AND NIEDERWIESER's method[3] and was easier in operation and gave better separation than repeated, ascending development. It resembles the continuous, descending methods by which STANLEY *et al.*[4] washed plates and which MISTRYUKOV recently described[5], as well as the descending method mentioned by BIRKOFER *et al.*[6]. It requires less special equipment than these published methods and makes use of the weight of the plate itself for providing contact with a soft, cloth wick. Although the apparatus is not as simple as that recently reported by BENNETT AND HEFTMANN[7], the method requires less handling of the delicate plates and is easier for routine use.

Plate coatings of activated alumina and unbonded fluorescent silica (silica gel HF_{254}, Brinkmann Instruments, Inc., New York) were employed at thicknesses of 0.25 and 0.50 mm. To minimize side effects during development and to facilitate handling, approximately 1 cm wide bands of surface layer were removed from each vertical edge of the plates. Application of sample and of chromogenic reagents was accomplished by means of a precision streaking device[2,8].

The developing apparatus, whose important details are shown in Fig. 1, consisted of the usual thin-layer plate rack of stainless steel modified so as to support a standard 8.5-in. paper chromatographic trough in its 9-in. metal cradle, bearing the appropriate antisiphon and anchor rods. Leading from each side of the solvent trough and extending over the antisiphon rods are wicks against which thin-layer plates are leaned,

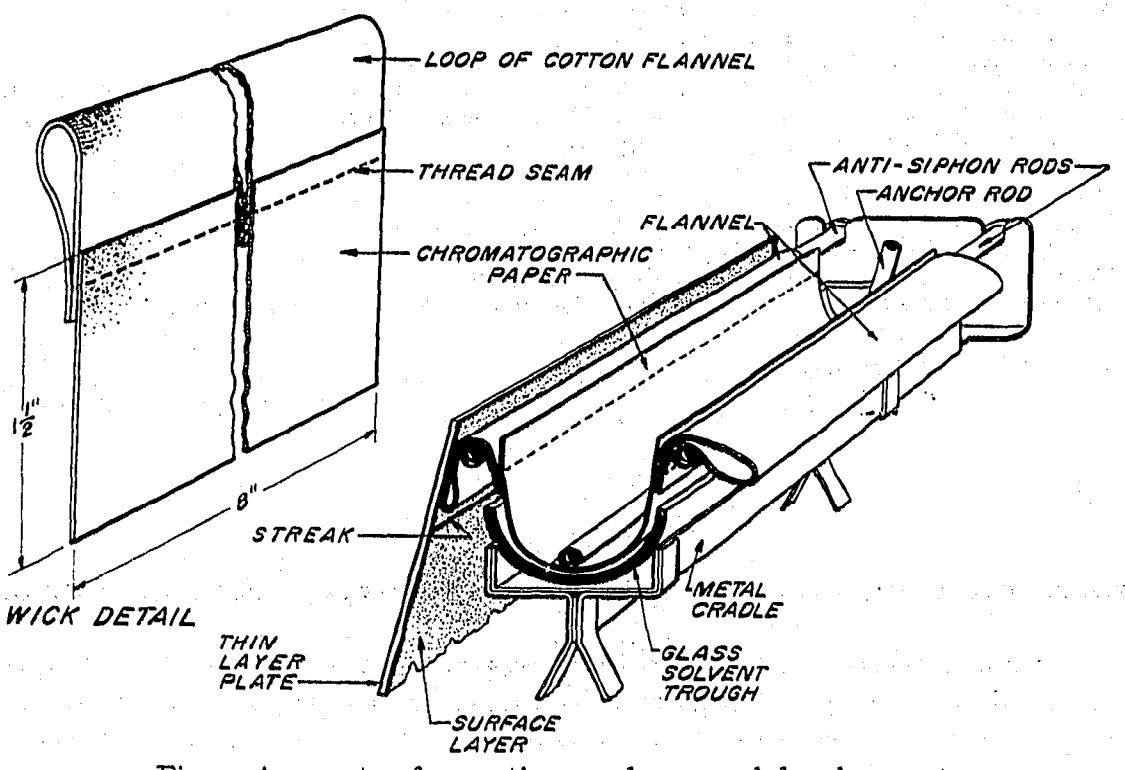

Fig. 1. Apparatus for continuous downward development.

layer side inward, at an approximate angle of 70°. The bottom edges of the plates rest on absorbent mats of heavy filter paper. The entire assembly is contained in a suitably sized chromatographic tank with a well-fitted cover. The wicks consist of a loop of fuzzy cloth (white cotton flannel) sewn to a strip of chromatographic paper (such as Whatman No. 1)—see separate wick detail in Fig. 1. This construction prevents marring of the thin-layer surface while affording good contact, the paper providing a slow solvent feed. The wicks are prewashed with solvent using spare plates.

In operation, two sample-streaked plates are lowered into the solvent saturated tank and pressed firmly against the wicks so that contact is made above the streak. Development begins at once. The time required for the solvent front to migrate to the bottom of the plates is noted and a total time of some multiple of this is tried.

The plates are then removed and dried and band separation observed in ultraviolet light (longwave and shortwave) or by cross streaking at the very edge of the surface layer with a chromogenic reagent. Should band separation be inadequate, the chromogenic-streaked zone is wiped from the plates and development continued until the desired separation is attained (7–18 h has been required with some systems). The

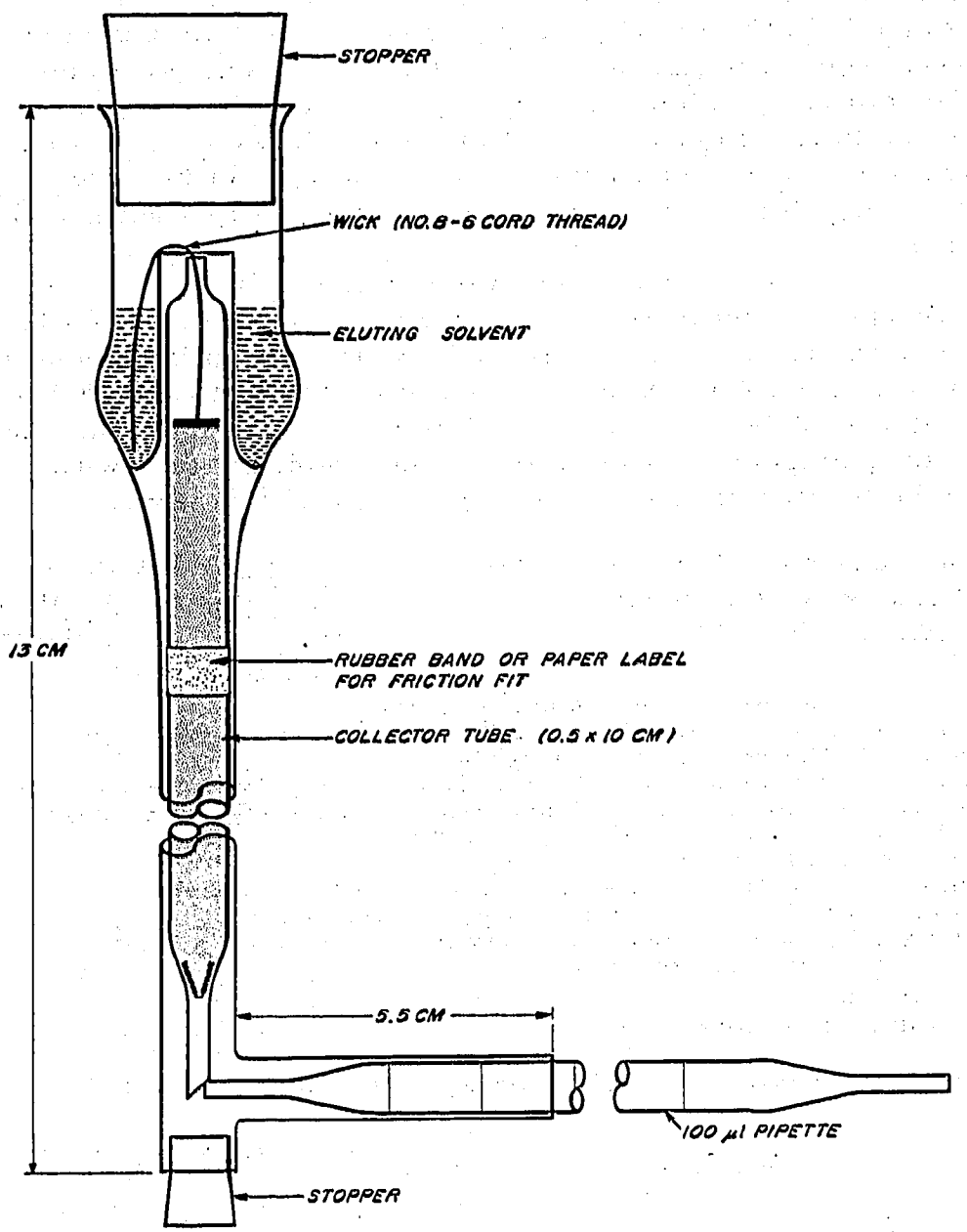

Fig. 2. Apparatus for elution with volatile solvents.

plates are then air dried to remove solvent, and chromogenic reagents if necessary are streaked at intervals in a direction perpendicular to the separated sample bands. The sample bands between the indicating streaks are removed from the plates as described below.

Removal and elution of bands

Bands developed on thin-layer plates in isolation work were removed from the plates and the adsorbed material eluted by the method developed for quantitative thin-layer chromatography[2], with suitable changes in the apparatus to accommodate larger amounts of surface layer and volatile organic solvents. The vacuum-cleaner-type collection tube was made longer and sometimes of greater diameter; a 10 × 0.5 cm (O.D.) tube would hold the absorbent from a 10 × 1.5 to 2 cm band of 0.5 mm thick absorbent layer. The eluting apparatus (see Fig. 2) was extended and fitted with a sidearm for the pipette, and both open ends of the apparatus were loosely stoppered. By removing the lower stopper, micro drops of eluate were taken to spot on thin-layer plates in order to test for completeness of elution. Wicks were washed in the organic solvent and stored dry. More than one thread was used if elution did not start in $^1/_2$–1 h.

Sesquiterpene alcohols on alumina were eluted with methylene chloride; the materials were similarly recovered from potassium bromide pellets which had been used in the determination of their infrared spectra, the pellet being ground to a powder prior to elution. Approximately 1 mg was obtained from each 10 × 0.5 cm tube, 200 μl of solvent being required to elute it from alumina, much less from potassium bromide (compare JANAK's elution from a capillary tube with a few drops[9]). A *p*-coumaroyl ester was similarly eluted from silica with acetone.

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Received January 17th, 1964

* Maintained in cooperation with the University of Wisconsin.

Column chromatography of tryptophan and some related indoles

A chromatographic separation of 5-hydroxytryptophan and tryptophan dissolved in water has been described by CONTRACTOR[1]. This note shows that the procedure can be extended to the quantitative separation of a greater range of indoles in water or in a complex biological system.