Protoporphyrin IX dimethyl ester was obtained commercially (Sigma Chemical Company, St. Louis, Mo., U.S.A.). Magnesium was quantitatively inserted into protoporphoryin IX dimethyl ester by the method of BAUM *et al.*[5]. Zinc was quantitatively incorporated into protoporphyrin IX dimethyl ester by dissolving the porphyrin in methanol and adding solid zinc nitrate. After waiting approximately 5 min, the methanol solution was added to an equal volume of diethyl ether, and then the methanol and residual zinc nitrate were washed out with distilled water.

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A two-dimensional pipette for sample application in preparative thin-layer chromatography

The undisputed necessity of thin, straight, and homogeneous sample application bands in preparative thin-layer chromatography prompted us to construct a two-dimensional pipette based on the principle employed in the Desaga broad band pipette[1]. Two identical stainless steel plates were machined and hand-lapped with abrasive so that surface S was as plane as possible and knife-edge K was razor-sharp, nick-free, linear, and unwarped (Fig. 1). (Poor results from a glass prototype, owing

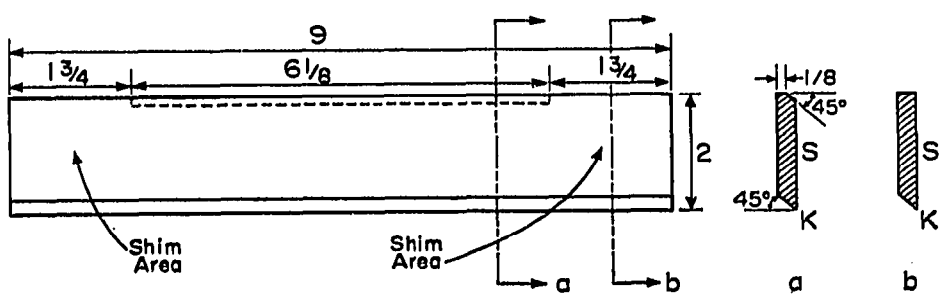

Fig. 1. Two-dimensional pipette. Dimensions in inches.

to the difficulty of producing flat glass surfaces and the impossibility of obtaining perfect knife-edges, demanded these precautions.)

Flat stainless steel or tantalum shims of measured thickness (25–600 μ) cut to fit each 1$\frac{3}{4}$ in. end shim surface create a thin, virtually two-dimensional 1$\frac{5}{8}$ × 6$\frac{1}{8}$ in. gap. With the two knife-edges flush and parallel, the two plates and shims are clamped tightly together at each end with a No. 35 pinch ball-and-socket screw-compressed clamp using one or two small rectangular pieces of thin wooden lath between the outside of the plates and the clamp, in order to apply homogeneous pressure to the shim areas. A crossbar is clamped to a two-masted ringstand. Two burette holders clamped to the crossbar support the assembled two-dimensional pipette—reasonably erect, with knife-edges down and horizontal—by means of the protruding grips of the ball-and-socket clamps.

Depending upon the specific gravity of the solution to be applied to the preparative thin layer, 0.25–1.0 ml aliquots may be added into the channel formed at the top edge of the assembly. A 20 × 20 cm preparative thin-layer plate reproducibly positioned on the top platform of a laboratory jack is elevated for a brief (about $\frac{1}{2}$ sec.) contact with the knife-edges, whereupon a narrow 6$\frac{1}{8}$ in. line of solution is printed. (It is convenient to adjust the apparatus so that shoving the jack base against the ringstand base automatically positions the line to be printed the desired distance, perhaps $\frac{5}{8}$ in., from an edge of the thin layer.) Owing to the length of the line, S-chambers can be employed and edge effects rarely occur.

Initial care should be taken to insure that the adsorbent surface and the knife-edges are parallel at the instant of contact. Quite a narrow line (2 mm) can be printed, even after repeated applications interspersed with drying periods, providing the pipette is not over-charged. Gaps 50 μ or less consistently clogged with adsorbent; gaps 254 μ or more wide never clogged and performed about equally well. However, with certain solvents (*e.g.*, chloroform), the wider gaps tended to print a wider and uneven line. Our experience has been that a gap width of 254 μ is acceptable and has never proven unsatisfactory under a wide variety of conditions. In our hands the only other pipette suitable for preparative work[2], although admirable in its simplicity, proved non-reproducible, unsuitable for convenient quantitative transfer (owing to the necessity of dipping the glass application edges into the solution), and somewhat subtle to operate.

Virtually no practice is required to operate the two-dimensional pipette. With it an operator can quickly and quantitatively apply milliliter amounts of solutions for large-scale preparative thin-layer chromatography. Far less exotic and expensive than several brushing, streaking, spraying, or multiple-capillary applicators in use[1], it incorporates for the most part commonly available laboratory apparatus.

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