notes on methodology

Simple devices for the application of samples as narrow streaks for thin-layer chromatography

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SUMMARY The construction and use of devices, based on the design of Achaval and Ellefson, for the application of samples as 1–12 cm streaks at the origin of thin-layer chromatograms is described. These devices are simple to make, and rapid and quantitative in their operation.

KEY WORDS rapid · quantitative · sample applicator · thin-layer chromatography

A number of devices for the application of lipid samples as thin streaks at the origin of thin layers have been described in recent years. In general these devices fall into three groups: (*a*) expensive, commercial streakers, such as those supplied by Camag and Applied Sciences Laboratories Inc.; (*b*) relatively simple designs which, presumably, can be readily built in the laboratory (1–6); and (*c*) designs of intermediate complexity that require more skill in construction to ensure satisfactory performance (7–9).

Several reported devices (1–3, 7) have been tried in this laboratory but, since we wanted to apply three or four different leaf lipid samples quantitatively as 3–4 cm streaks on a 20 cm^2 layer, all of these were too slow in operation for our purpose. Other disadvantages were also apparent. Uneven application of the sample (1, 2) and disturbance of the layer at the origin (3, 7) were encountered particularly when a moderate volume (50 μl) had to be applied to the layer in several passes with the streaker. Other reported methods for applying samples (10–12) were not considered applicable for our purposes.

A recently reported design for a trough-type applicator that applies an 18 cm streak (13) promised both quantitative application and unprecedented speed. This design was slightly modified to simplify construction of an applicator for a 4 cm streak. The immediate success of this prompted the construction of several other applicators capable of applying streaks of 1–12 cm (Fig. 1) which are now in routine use in this laboratory. The 1 cm streaker has proved particularly useful in monitoring column effluents. It provides a rapid and quantitative alternative

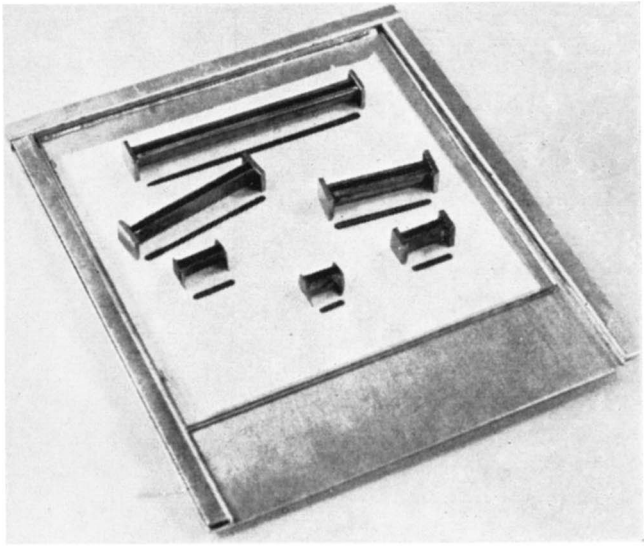

Fig. 1. Some of the sample streakers in routine use in this laboratory arranged on a (20 cm)^2 layer (250 μ) of Silica Gel H. The thin streaks produced by the devices are displayed in front of each one.

to the application of several small, closely spaced spots over the same distance, a technique normally used to improve the resolution of a chromatogram over that obtained when the same amount of sample is applied as a single spot (14, 15). Fig. 2 shows the type of resolution achieved by the use of the 2- and 4-cm applicators.

The ability of the streakers to apply a lipid sample quantitatively was demonstrated as follows. 20 and 50 μl of a chloroform solution of leaf lipids, containing 0.273 μmole of phosphorus per 50 μl, were transferred to Silica Gel H (E. Merck A.G., Darmstadt, Germany) layers (previously developed in chloroform–methanol–acetic acid–water 85:15:10:3) by means of the 2- and 4-cm streakers, respectively. A similar volume of pure solvent was used to rinse out the micropipettes and applicators. Lipid-loaded zones were scraped (without chromatography) into digestion tubes and the phosphorus was determined in the presence of adsorbent by a modification of the method of Parker and Peterson (16). In 10 determinations with the 4 cm streaker the mean recovery of lipid phosphorus was 0.263 ± 0.007 (SD) μmoles, or 96.5%; with the 2 cm streaker, 0.107 ± 0.005 (SD) μmoles, or 97.2%. The 12 cm device had already been shown to give quantitative transfer when used for the analysis α-tocopherol in leaves (17).

The main object of this report, however, is to emphasize the ease with which this type of device can be made. No particular precision equipment is required and a single streaker can be made in 1–2 hr. 0.125 inch thick stainless steel used in the end plates gave greater surface area in contact with the layer, thus minimizing damage to the layer. Silica Gel G was rarely, and Silica Gel H never

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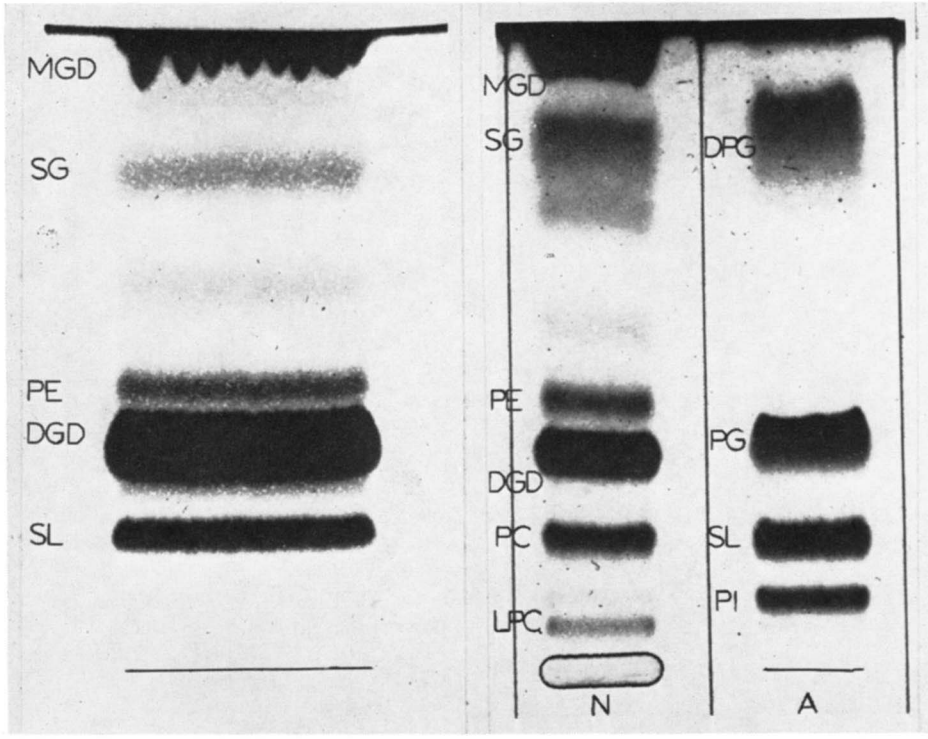

FIG. 2. Examples of chromatograms obtained using sample applicators of the type designed by Achaval and Ellefson (13). Left, glycolipid fraction equivalent to 200 mg (fresh weight) of white clover leaves from a Florisil column (10) streaked across 4 cm. Right, neutral, including dipolar (N) and acidic (A) lipid fractions from a DEAE-cellulose column. Neutral lipids equivalent to 50 mg of white clover leaves and acidic lipids equivalent to 200 mg of white clover leaves, streaked across 2 cm.

MGD, monogalactosyl diglyceride; *SG*, sterol glycoside; *PE*, phosphatidyl ethanolamine; *DGD*, digalactosyl diglyceride; *SL*, sulfolipid; *PI*, phosphatidyl inositol; *PG*, phosphatidyl glycerol; *DPG*, diphosphatidyl glycerol; *LPC*, lysophosphatidyl choline.

affected by careful use of the devices. The side plates were made of 0.125 inch or 0.04 inch stainless steel. There appeared to be no great advantage, however, in using the heavier material: a 7 cm streaker with sides of 0.04 inch steel was quite rigid.

Some details of the construction procedure are presented to show its simplicity. The sides were made about 0.25 inch longer than the required streak, and their lower edges filed to an angle of about 15° before the end bevels were filed in (14). A wedge or former was made by bending sheet brass so that when the side plates of the streaker were placed against the former and clamped with a bulldog clip they lay at the suggested 15° angle. With the weir gap kept as close and even as possible, the whole assembly was then held in position on one of the end plates with a suitable clamp, and the sides were carefully fixed to the end with two drops of molten "hard" solder (Easy Flow). The other end was then fixed in position, care being taken that no solder ran into the trough or into the gap. The former was removed and the bases of the end plates and the gap were then filed flat throughout. This treatment tended to open the gap, but judicious squeezing and tapping closed it up again. An old feeler gauge (0.004 inch) was then pushed down through the gap to push out the furred edges, which were removed by rubbing the unit over a piece of fine emery paper laid flat. The outside, 15° angle was then carefully filed to give even, gap-defining edges 0.008–0.01 inch in width. The end bevels were touched up and checked to ensure that they were of sufficient width to prevent the sample from running onto the end plates and that the weir gap was of the desired length. The streaker was then ready for testing. Streaks were usually applied at a 90° angle to the direction of spreading of the thin-layer.

50 μl of each of three different lipid samples in chloroform, along with 50 μl of washings, could be applied from a single 4 cm streaker in 60 sec.

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