Apparatus for applying samples to thin-layer chromatograms

T. W. Scott and J. W. U. Beeston

C.S.I.R.O., Division of Animal Physiology, Ian Clunies Ross Animal Research Laboratory, Prospect, New South Wales, Australia

SUMMARY A simple device is described for the rapid application of samples to thin-layer plates. It permits quantitative application of relatively large lipid samples in an extremely short time. Neutral lipids of sheep thyroid were separated.

KEY WORDS apparatus · rapid application · large samples · thin-layer chromatography

The quantitative application of relatively large samples of lipid to thin-layer chromatograms is tedious and time-consuming. To expedite this procedure we constructed a device which allows the application of 0.5 ml of a chloroform solution of lipid in 2–3 min.

The apparatus (Fig. 1) consists of a polished stainless steel slide (*a*) and back guide (*b*), which support the thin-layer plate. The sample is introduced into a stainless steel reservoir (*c*) having a removable hypodermic needle (26 gauge is a convenient size) fitted to the bottom. The reservoir may be moved to any point along the channel *d*, which is also adjustable for height and angular position. As the sample drops from the needle on to the plate it is rapidly dried by a stream of warmed nitrogen (37°C) emerging from the orifice *e*. The nitrogen is heated in a coiled stainless steel tube ($^1/_8$ inch diameter), which is wrapped with glass tape and further insulated with an aluminum cover (*f*). One end of the tube terminates in the orifice *e* and the other at a $^1/_8$ inch needle valve (*g*); this in turn is connected to the nitrogen source. A 100 watt transformer (*h*) supplies 3 v across the coiled tube (the resistance being about 0.3 ohm), which itself is the heating element. A voltage controller (*j*) on the input side of the transformer controls the temperature of the nitrogen. During the application the thin-layer plate may be moved backwards and forwards to insure even deposition of the sample.

After incubation of ovine thyroid slices with uniformly labeled glycerol-^{14}C the lipids were extracted with chloroform–methanol 2:1 (v/v) and partitioned into neutral lipids and phospholipid fractions by silicic acid column chromatography. The neutral lipids were eluted with

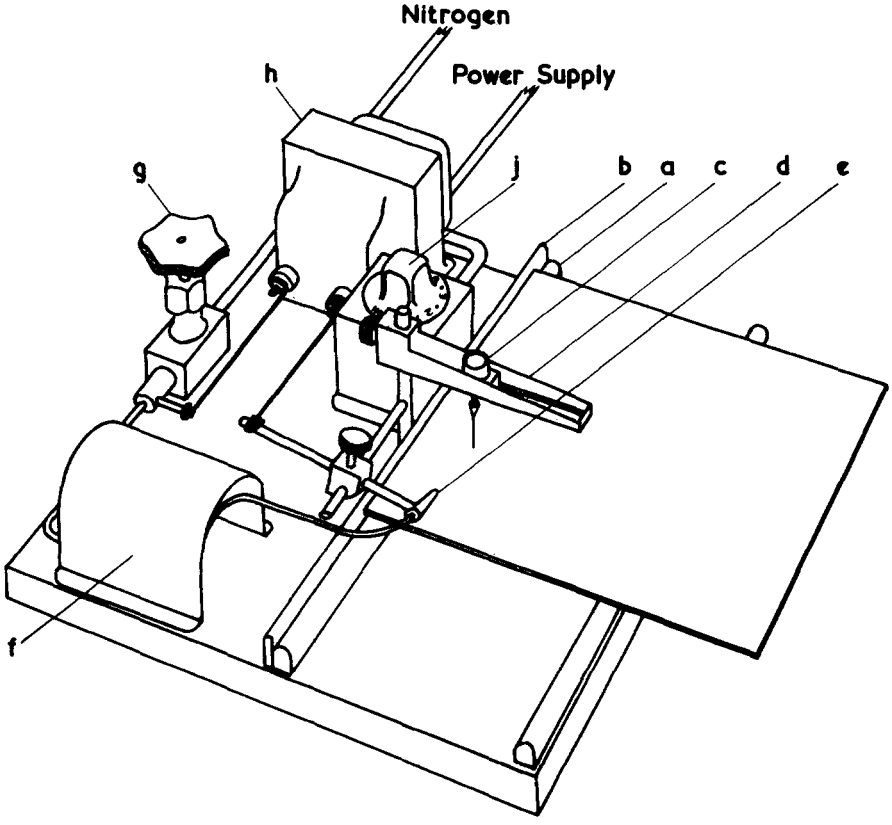

Fig. 1. Apparatus for applying samples to thin-layer chromatographic plates: *a*, stainless steel slide; *b*, back guide; *c*, reservoir; *d*, channel; *e*, orifice; *f*, aluminum cover; *g*, needle valve; *h*, transformer; *j*, voltage controller.

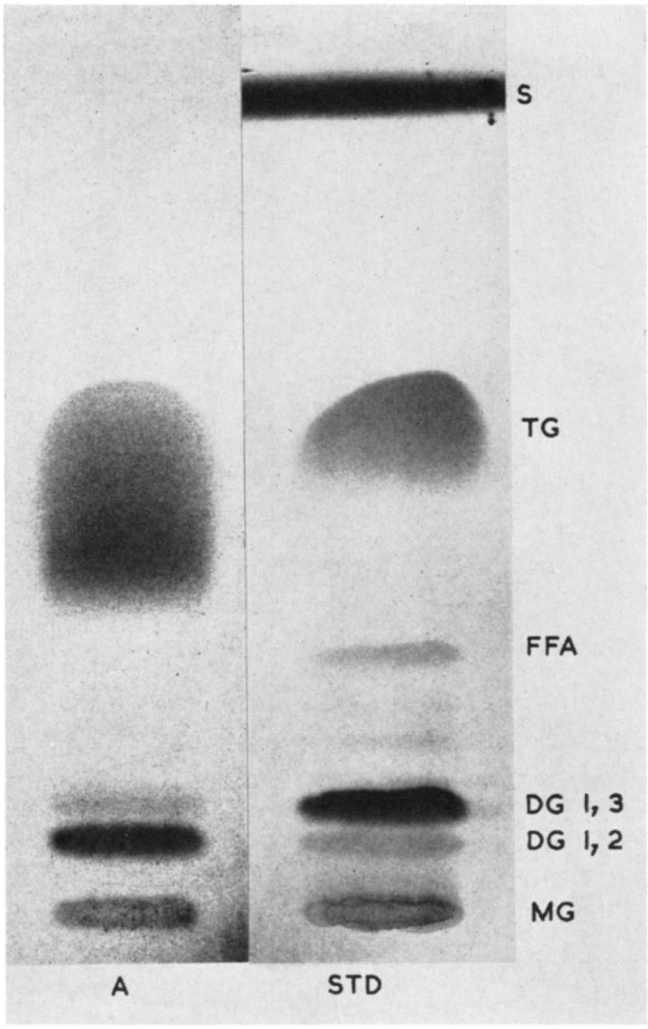

FIG. 2. Photograph of an autoradiograph of a thin-layer chromatogram of ovine thyroidal neutral lipids (*A*) labeled with glycerol-^{14}C. *STD*, standard lipid mixture chromatographed and exposed to iodine vapor; *MG*, monoglyceride; *DG*, diglyceride; *FFA*, free fatty acid; *TG*, triglyceride; *S*, solvent front.

chloroform and the phospholipids with methanol. The neutral lipid extract was evaporated to dryness in vacuo and redissolved in chloroform. An aliquot (0.5–1.0 ml) was pipetted into the reservoir *c* and applied to the thin-layer plate (Silica Gel G) as described above; the system was rinsed with chloroform to ensure quantitative transfer of the sample. A standard mixture of neutral lipids was applied next to the sample; these were detected by exposure to iodine vapor. The plate was developed with petroleum ether–diethyl ether–acetic acid 90:10:1. Fig. 2 is a photograph of an autoradiograph of the plate together with the plate with standards. It demonstrates that monoglyceride, 1,2- and 1,3-diglycerides, and triglyceride were effectively separated. The apparatus is easy to construct and by its use samples are rapidly and accurately applied.

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