CHROM. 6272

## An automatic quantitative spotter for thin-layer and paper chromatography

Purines and pyrimidines are not very soluble in water and in order to apply a suitable quantity onto a chromatogram a large volume needs to be spotted. Heat cannot be used to accelerate the process, because of the instability of some of the compounds and, when several spots are needed the procedure becomes tedious and takes a great deal of time. To surmount this difficulty an instrument for the automatic spotting of aqueous solutions was developed as part of a research program on the radiation and photochemistry of nucleic acids.

When applying samples to thin-layer chromatograms it is important that the spots are small and that none of the absorptive layer is removed or damaged. Compounds with low solubilities require a large number of repeated applications to a single spot and any instrument must be able to operate for several hours unattended. Various semi-automatic devices have been reported (some of which are available commercially) for the loading of samples onto paper and thin-layer plates either as a streak[1-16] or as spots[17-27]. These devices employed melting point or capillary tubing[1,2, 17-25] or micro-syringes[3-9, 26,27] to apply the solution. These narrow-bore tubes all tend to become blocked after a period by air bubbles or by picking up adsorbent from the plate with consequent damage to the layer. After trials with hollow needles, all of which suffered from the clogging problem, a standard applicator which holds a drop of liquid by surface tension was devised. A 4-mm diameter Pyrex glass rod was heated and simultaneously rotated allowing a concentric taper to be drawn out. The resultant solid needle was cut at a diameter of 0.6 mm and the end ground flat in a lathe using SL-58 H500-OP carborundum

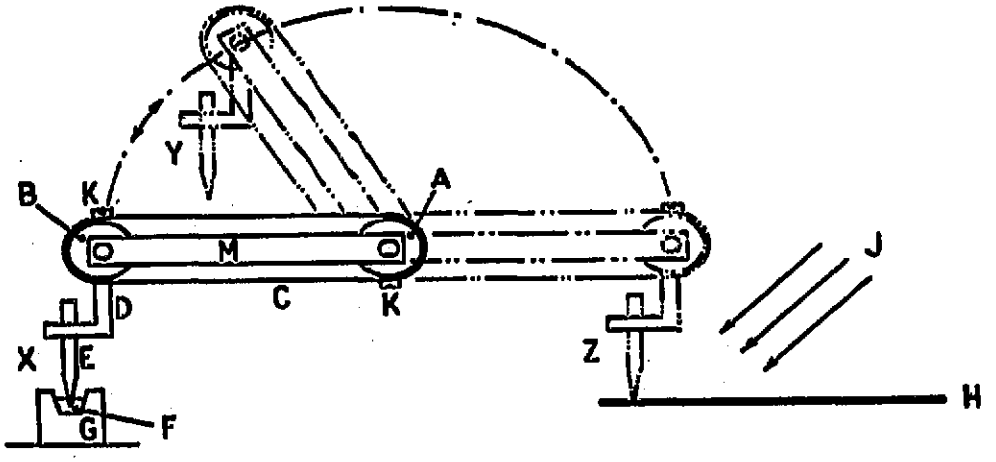


Fig. 1. Schematic diagram showing successive positions of the solid needles. Position X, collecting drop from sample reservoir; position Y, in transit; position Z, applying drop to layer or paper. A = roller fixed to base; B = roller fixed to needle support bar; C = stainless-steel band, keeps needle support, D, in vertical position through 180° movement; D = needle support; E = solid glass needle; F = sample solution; G = PTFE reservoir cup; H = thin-layer or paper sheet; J = supply of cool air; K = stainless-steel band anchor screws; M = motor-driven bar.

paste to give a completely flat surface. These solid needles can be readily cleaned and with reasonable care proved to have a long service life.

A row of these solid needles is attached to a motor-driven bar (M) that repeatedly transfers the needles from PTFE reservoir cups (Fig. 1, G) containing the sample solution (F) to the thin-layer plate (H) and back again. The vertical position of the needle is maintained throughout the transfer by two rollers (A and B) coupled by a thin stainless-steel band (C). The latter prevents wobble and a too rapid and heavy descent of the needle onto the plate. A variable speed motor is used to adjust the rate of the transfers. The residence time of the needles in the reservoir and on the plate is set by the gearing shown schematically in Fig. 2. The maximum speed of one transfer cycle is 14.7 sec, when the needle rests above the layer for 2 sec and in the reservoir for 8.3 sec.

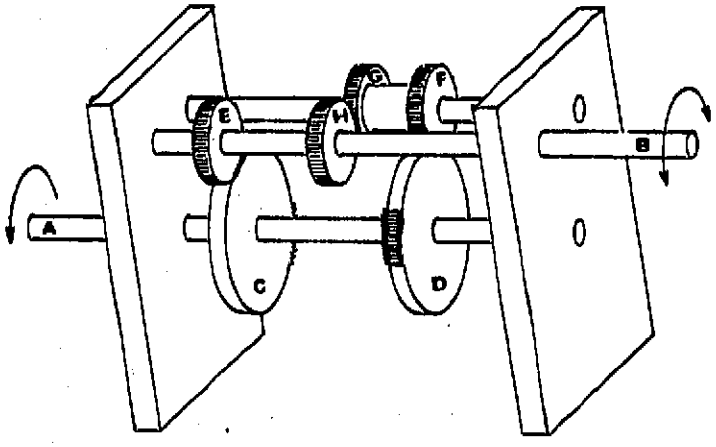


Fig. 2. 'Lost motion' gear box. A = Continuously rotating drive shaft coupled to the motor. B = Output shaft reciprocating through 180°. The needle bar is coupled directly to this shaft. C and D = Toothed wheels having a large fraction of the teeth ground away. Sufficient teeth are left to rotate shaft B through only 180° when meshing with wheel E or gear train F, G, H. The elapsed time between the forward and reverse strokes is set by the relative position of the toothed sections of C and D.

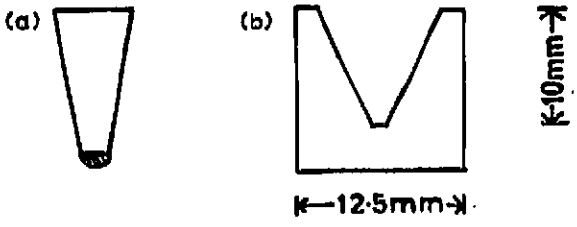


Fig. 3. (a) Diagram of the needle tip showing the appearance of the liquid drop. (b) Cross section of the PTFE reservoir cups.

The volume of sample applied to the layer in a single transfer is related to the needle tip diameter, and the spot size obtained varies accordingly, *e.g.* 0.62- and 1.25-mm needle diameters gave 1-mm and 3-mm diameter spots, respectively. 20 $\mu$l of aqueous solution can be spotted in 75 min as a 1-mm diameter spot. Aqueous solutions are held on the needle tip by surface tension as a drop (Fig. 3a). The approximate volume was calculated from measurements of the needle diameter, $2r$, and the depth of the drop, $h$, inserted into the formula for the volume of a spherical segment.

$$V = \frac{\pi h}{6} \times (3r^2 + h^2)$$

From this, drop volumes transferred by a needle of 0.7-mm diameter were found to be: water, 0.04 $\mu$l; benzene 0.02 $\mu$l; and ethanol, 0.01 $\mu$l. The organic

solvents were so volatile that most of the drop evaporated before it could be transferred. The machine is suited only to the spotting of materials in aqueous and relatively non-volatile solvents. For volatile solvents conventional techniques are usually adequate.

Sample solutions were measured into PTFE reservoir cups. In addition to being inert and readily cleaned, the non-wettability of PTFE facilitates complete sample transfer. The reservoir cups have a cone-shaped recess of volume *ca.* 0.35 ml; this particular shape proved to be the best of several designs tried. The tip of the cone was flattened off to a diameter of 1.25 mm (Fig. 3b). The reservoirs are supported within recesses in individual aluminium platforms. There is a height adjustment and a centering adjustment for each pot. Side-ways movement of the needle holder gives the third adjustment, enabling precise alignment of the needle in the centre of the reservoir. This proved to be vital for quantitative transfer of sample solution to the layer.

The thin-layer plate is supported on a horizontal aluminium platform and held in position by a lever-actuated bar, with foam padding on the underside to prevent damage to the layer. This bar is divided into two independent sections, enabling simultaneous spotting of two plates of different thickness. Having positioned the plate, the needles are clamped in position, resting lightly on the surface of the thin-layer plate; the plate support platform is then dropped 0.08 mm so that the needle does not actually touch the layer. The needle-supporting rod is then lifted over to the reservoirs and adjustment made so that the needle tips are located centrally in the cups.

Complete evaporation of the spotted liquids between transfers is assisted by a fan mounted on a bar above the thin-layer plate. A vertical aluminium shield prevents forced evaporation in the reservoir pots. The complete instrument is shown in Fig. 4.

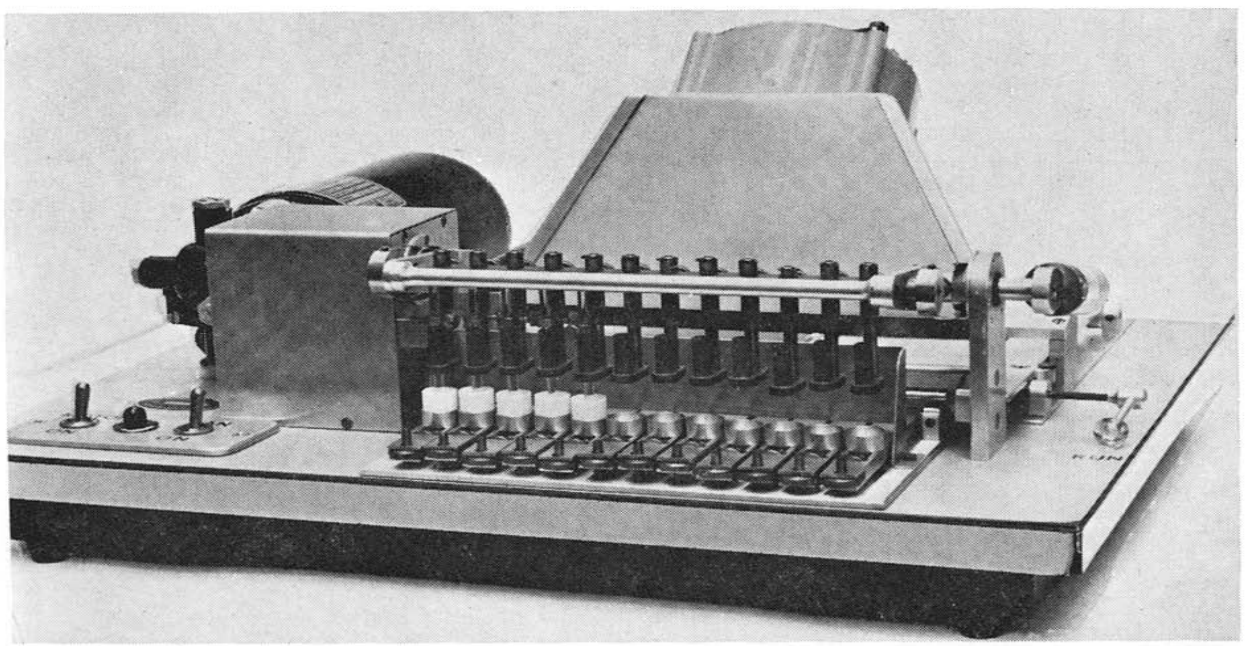

Fig. 4. Photograph of the complete machine set-up with five needles only.

Tests on the quantitative transfer of sample solutions were carried out using an aqueous tritiated thymine solution (specific activity 1.75 $\mu$Ci$\mu$/mole). 20 $\mu$l of $10^{-3}$ *M* thymine was pipetted into the reservoir and spotted onto cellulose sheet Kodak 6065. Further quantitative tests were carried out in which 5, 10, 15, 25, 30, 40, and 200-$\mu$l volumes were transferred. In each case 10 $\mu$l of water were placed in the pots after spotting the solution. This water was also spotted onto the plate to transfer evaporated residues. After transfer the pots and needles were washed with 0.5 ml of water; 10 ml of scintillator (composed of 30 parts of Tergitol TP9 and 70 parts of 15 g Intertechnique Butyl PBD per 2.5 l of toluene) were added and the solutions counted on an Intertechnique liquid scintillation counter.

The average of fifty-three tests showed that 0.8 % and 2.1 % of the starting solution were retained in the pot and on the needle, respectively, giving an average transfer of 97.1 % of the thymine to the thin-layer plate.

The machine is readily modified to spot paper by slotting the paper through a piece of glass rod, bent through three U turns, which can be mounted onto the aluminium platform (Fig. 5).

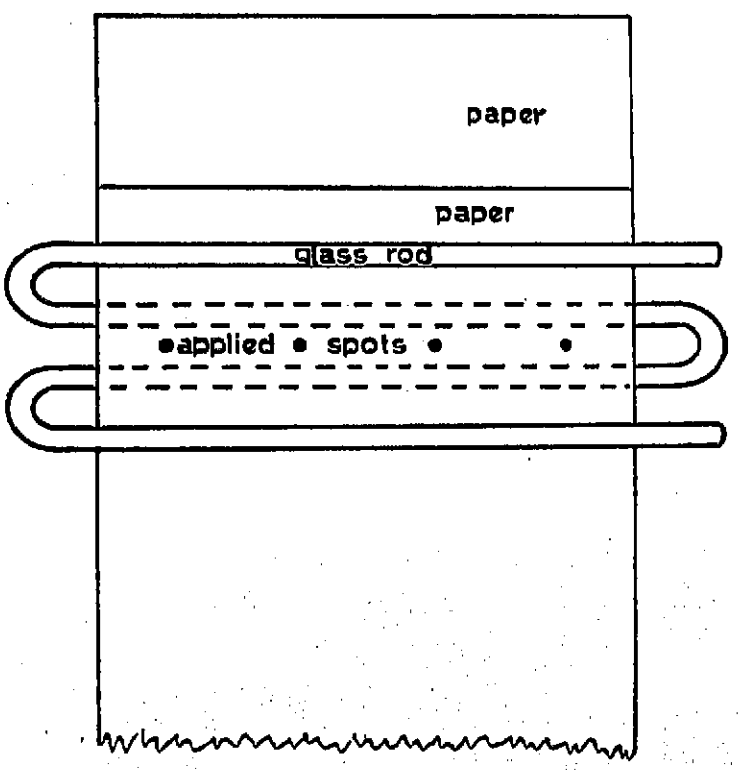


Fig. 5. Paper holder used when spotting paper chromatograms.

Acknowledgements are due to the M. R. C. and C. R. C. for financial support. One of us (Z. TRAMER-ZAREBSKA) would like to acknowledge an I.A.E.A. visiting

fellowship held at the Institute of Cancer Research. The skilled technical assistance of Mr. K. JONES in the construction of the instrument was very much appreciated.

*Division of Physics,*
*Institute of Cancer Research,*
*Clifton Avenue, Belmont,*
*Sutton, Surrey (Great Britain)*

J. W. BOAG
P. S. BOND
E. M. FIELDEN
H. HODT
Z. TRAMER-ZARĘBSKA*

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Received July 24th, 1972

* Permanent address: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.