AN APPROACH TO KINETIC STUDIES
USING RADIOCHROMATOGRAPHY ON THIN LAYERS

PART I.

C. J. MOYE

C.S.R. Research Laboratories, Roseville, Sydney (Australia)

(Received May 9th, 1963)

INTRODUCTION

A common technique used in biosynthetic studies, is that of counting radioactive samples at "infinite thickness". When obtained by counting at infinite thickness, the activities of samples derived from a common parent compound, can be related to one another and the parent compound with the minimum of correction[1].

It occurred to us that the same technique applied to thin-layer chromatography should offer considerable advantages in semi-quantitative kinetic studies of complex reactions. One would require that:

(i) the adsorbent layers be uniformly thin, but at infinite thickness to the radiation being emitted,

(ii) the compounds be uniformly distributed in depth through the thin layer before and after chromatography, and

(iii) all the reaction products be well separated by the chromatographic procedure, so that they do not interfere with one another when they are counted. The separation necessary would depend on the resolution of the scanner.

In order to test the above hypothesis, we required a scanning unit sufficiently sensitive to detect ^{14}C and ^{35}S radiation* emitted from a thin-layer chromatogram. The unit described was suitable for this purpose.

It soon became apparent in preliminary studies, that the technique could be improved by using more uniform thin layers. Better results were also obtained when the thickness of the adsorbing layer was adjusted to give a weight of adsorbent of 22–25 mg/cm². This was probably due to difficulties in obtaining uniform distribution of sample when spotting on thicker layers. We therefore designed the second unit described herein, which is a modification of a spreader described in the literature[2] and commercially available**. Both units are simply constructed and inexpensive. Results of kinetic studies using this new technique, will be described elsewhere.

---

* The technique is uniquely suited to studies with these isotopes, (and others emitting β particles of similar energy), since *ca.* 22 mg/cm² represents infinite thickness of an absorber for these isotopes, and this thickness is suitable for thin-layer chromatography.

** From Camag, Chemie Erzeugnisse und Adsorptions Technik, A.G., Hombergerstr. 14, Muttenz B.L., Switzerland.
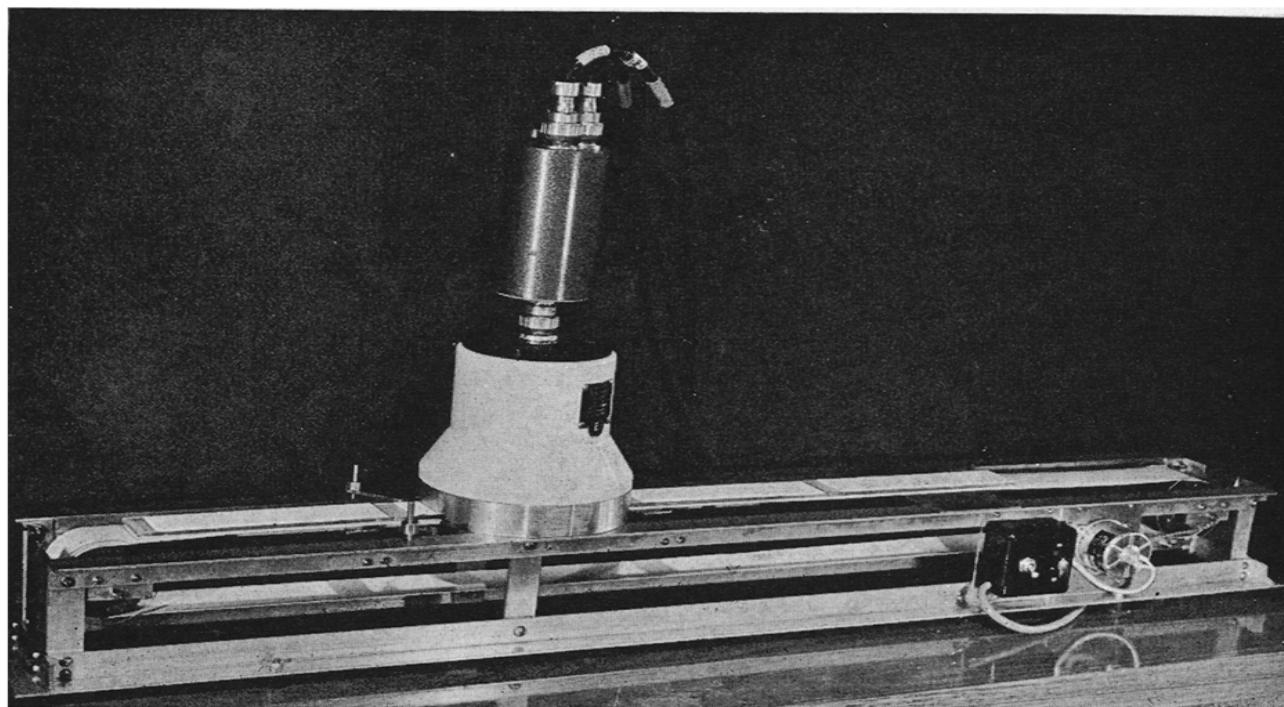
Fig. 1. Scanner unit in operation. Thin-layer chromatoplates are centered and pass from left to right under a thin window G.M. tube mounted in the EKCO castle head.

DESCRIPTION OF APPARATUS

Scanning unit

The scanning unit was built to utilise the head of an EKCO G.M. counting unit. Other G.M. castle units could be similarly modified. It consists of a continuous belt driven by a synchronous motor, and an adaptor to mount the EKCO head unit on the framework enclosing the belt and drive mechanism, and in close proximity to the belt. The unit in operation, is pictured in Fig. 1, and the adaptor, dimensioned for our EKCO head, in Fig. 2.

The scanner support assembly is shown in Fig. 3, and consists of three main parts. There is the belt running on adjustable rollers, a synchronous motor which can be readily interchanged when a change of scanning speed is required, and a smooth flat platform whose height can be adjusted, and above which is placed two adjustable guides for centering the plates on the belt. Dimensioning of this unit would depend on the requirements of the users. Our unit will handle over three hours counting without requiring attention, if run-on is provided at one end and a run-off at the other end of the belt. A continuous automatic device for handling the plates after counting would allow overnight operation. The unit is not as efficient as the windowless unit recently described[3], but is suitable for studies of isotopes other than tritium, and does not require any special ancillary equipment.

Thin-layer applicator

The modification to our existing applicator was suggested by the aforementioned experiences and by an observation that it should be possible to trim dry chromatoplates by repassing them under the applicator blade. Accurate setting of this blade would give a layer of excellent uniformity, free of variations that can arise during
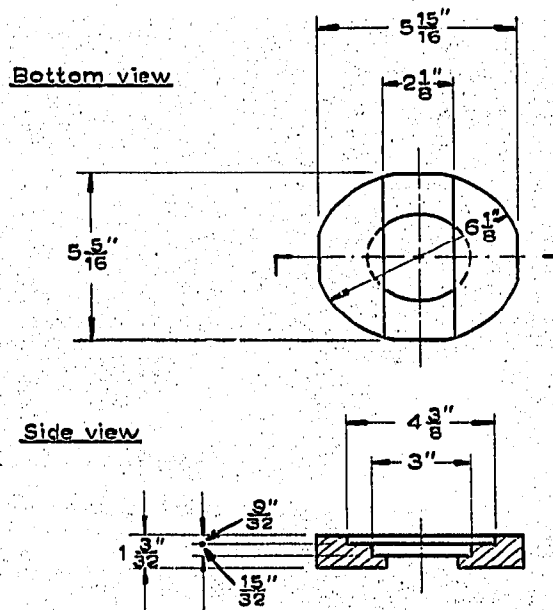
Fig. 2. Adaptor. Bottom view and side view showing dimensions in inches.

preparation and drying. Two simple micrometer depth gauges were fitted to the applicator blade of our unit to serve this purpose. They were located outside the adsorber well to prevent rusting, and the method of mounting is clearly shown in Fig. 4.

Accurate setting of both the micrometers was very simply achieved. Glass chromatographic plates were placed under the applicator blade, which was lowered onto them with the micrometers wound back. The micrometers were next wound down
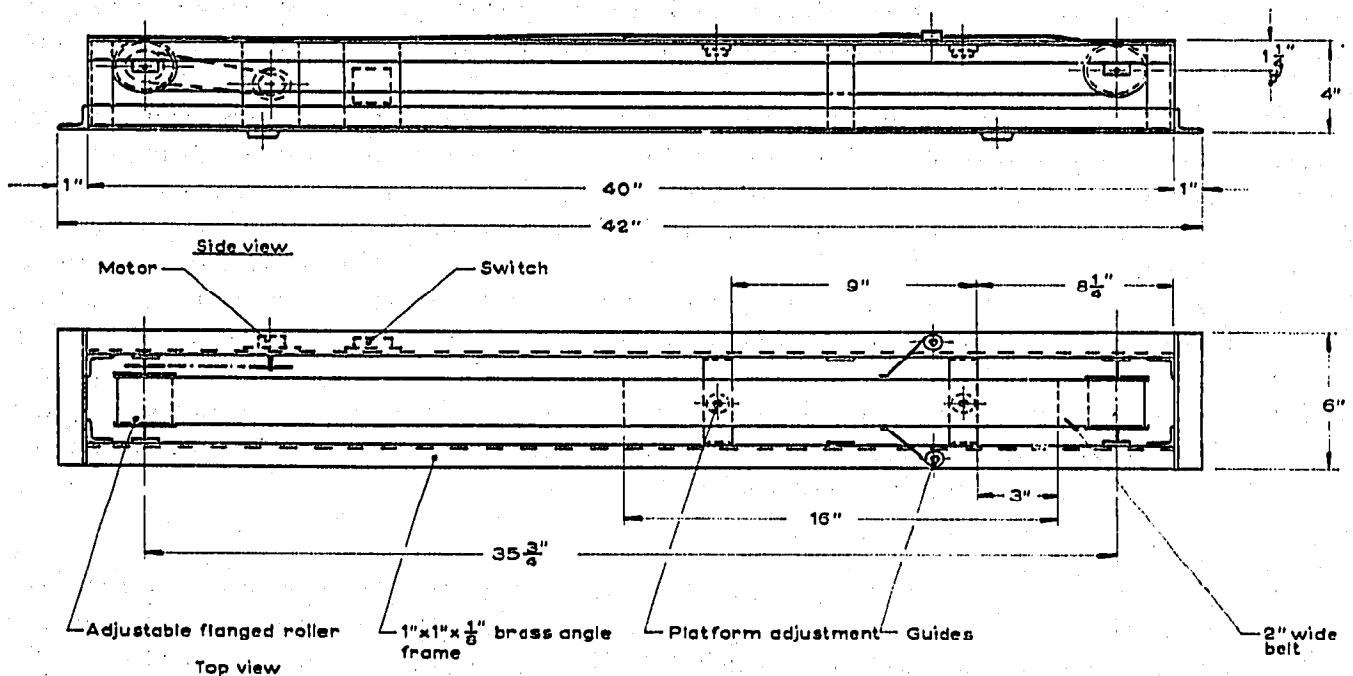

Fig. 3. Scanner assembly. Top view and side view showing dimensions in inches.
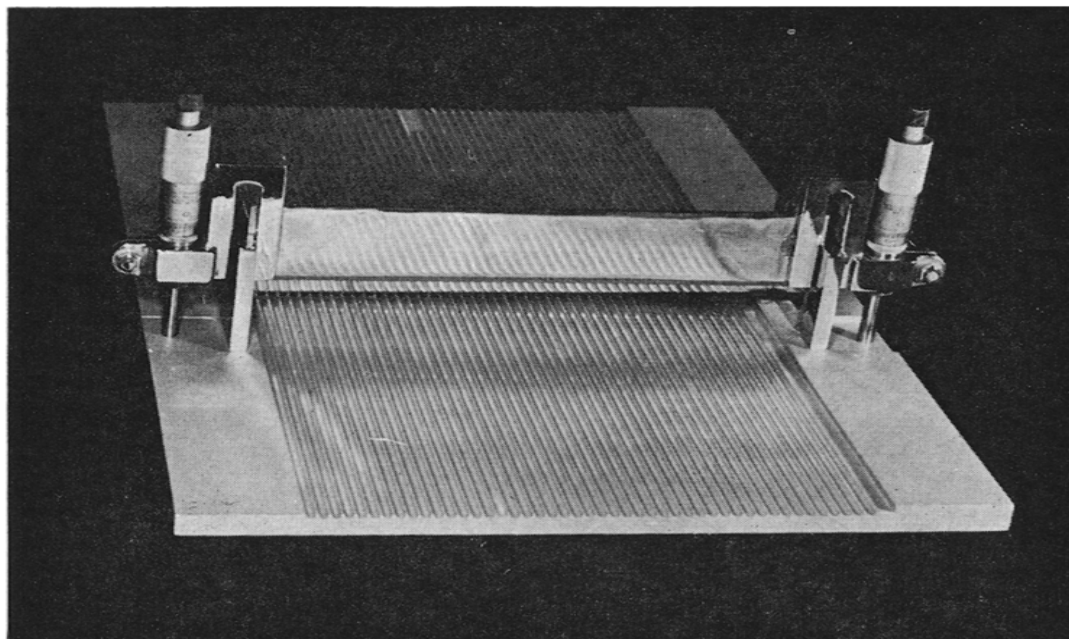
Fig. 4. Improved thin-layer applicator. Showing the means of mounting the two micrometers outside the adsorbent trough.

by their ratchet stop knobs, while pressure was applied to the blade, until they were set on the base plate. Both micrometers were now adjusted to the desired height by screwing the barrel in the normal way.

EXPERIMENTAL

We have recorded in Table I, the results of an investigation into the uniformity of the thin layers obtained by the above procedure. Three plates (cut from polished plate glass) were prepared in the one operation. Two of the plates, plates 2 and 3 (Table I) were repassed under the blade after drying, and all plates were divided into 1 cm² areas by marking with a razor blade. The individual square centimeters were taken from the plates and weighed. The results show clearly that the uniformity of the thin layers on the chromatoplates was very much improved. The results in Table I were obtained using aluminium oxide G adsorbent (40 g aluminium oxide, 60 ml water, stirred for 90 sec) and a similar improvement was obtained with Silica Gel G (30 g silica gel, 60 ml water, stirred for 90 sec). The plates were thoroughly dried at 100° in an oven in the approved way and then equilibrated overnight in the laboratory atmosphere before the weighings were commenced. The variation shown in measurements on the untreated plate is probably greater than one would normally obtain, as the knife setting was 1 mm; but regardless of the uniformity of the untreated plate it is evident that an excellent plate can be prepared from it by the "dry trimming" technique.

The error in the result, 25.3 ± 1.5 mg/cm², is thought to be largely due to the difficulty experienced in accurately marking the plates in square centimeters and then removing the adsorbent from these squares. The uniformity of the plates was checked by taking six micrometer readings along the length and in the middle of the plates.

TABLE I

RESULTS OF THE MEASUREMENT OF ADSORBER UNIFORMITY

	Plate 1 Weight/cm² of surface area (mg/cm²)			Plate 2 Weight/cm² of surface area (mg/cm²)			Plate 3 Weight/cm² of surface area (mg/cm²)		
	1	2	3	1	2	3	1	2	3
1	75.8	73.8	72.3	60.0	61.0	61.0	25.5	23.7	23.9
2	78.0	73.6	69.6	64.0	63.0	60.1	25.5	24.6	25.0
3	86.2	82.4	79.1	64.1	66.2	62.7	25.7	25.6	25.4
4	109.6	104.8	100.4	62.7	60.0	61.2	25.3	24.4	25.0
5	112.4	112.5	107.8	58.8	58.7	56.9	25.3	24.5	25.0
6	109.4	105.5	106.8	63.3	58.6	60.2	26.8	25.4	26.7
7	103.2	103.2	107.2	55.1	55.9	55.7	25.6	24.8	25.6
8	88.2	88.4	89.4	56.0	53.9	53.2	26.5	25.2	25.8
9	81.6	80.1	83.2	55.5	56.7	56.6	24.6	24.0	25.0
10	75.1	74.7	78.7	52.0	50.7	52.6	26.0	25.2	26.3
11	67.2	67.2	70.6	55.3	56.2	56.8	25.6	24.4	25.5
12	61.5	62.3	62.3	56.9	54.8	58.9	26.2	24.5	25.8
Column total	1048.2	1028.5	1027.4	703.7	695.7	695.9	308.6	296.3	305.0
Number	12	12	12	12	12	12	12	12	12
Column average	87.3	85.7	85.6	58.6	58.0	58.0	25.7	24.7	25.4
Plate average	86.2 ± 26.3 mg/cm²			58.2 ± 8.0 mg/cm²			25.3 ± 1.5 mg/cm²		
Plate particulars	Plate uniformity = 3.827 ± 0.009 mm (six measurements along centre) Size = 18 cm × 4 cm Knife set = 1 mm (Untouched after plating)			Plate uniformity = 3.827 ± 0.012 mm (six measurements along centre) Size = 18 cm × 4 cm Knife set = 0.75 mm (One pass under blade)			Plate uniformity = 3.826 ± 0.004 mm (six measurements along centre) Size = 18 cm × 4 cm Knife set = 0.35 mm (Reduced from 1 mm in steps —five passes under blade)		

This would not detect all the slight surface irregularities, which would further contribute to the error.

SUMMARY

The construction of two instruments, a simple scanning unit for thin-layer chromatograms and a modified spreader for obtaining very uniform thin layers, is described.

REFERENCES

1 C. J. MOYE, *Ph.D. Thesis*, Manchester University, 1958, pp. 10–12.
2 E. G. WOLLISH, M. SCHMALL AND M. HAWRYLYSHYN, *Anal. Chem.*, 33 (1961) 1138.
3 P. E. SCHULZE AND M. WENZEL, *Angew. Chem. Intern. Ed. Engl.*, 1 (1962) 580.