Submitted to Analytical Biochemistry

LBL-7963
Preprint
C.2

ZONE COLLECTOR AND TRANSFER DEVICE FOR THIN-LAYER CHROMATOGRAPHY

Steven G. Platt

RECEIVED
LAWRENCE
BERKELEY LABORATORY

SEP 7 1978

LIBRARY AND
DOCUMENTS SECTION

July 1978

Prepared for the U. S. Department of Energy
under Contract W-7405-ENG-48

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 6782

LBL-7963
C.2

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

Short Communication: Analytical Biochemistry

ZONE COLLECTOR AND TRANSFER DEVICE FOR THIN-LAYER CHROMATOGRAPHY

Steven G. Platt

*Western Regional Research Center,

Science and Education Administration,

United States Department of Agriculture,

Berkeley, California 94710

and

Laboratory of Chemical Biodynamics,

Lawrence Berkeley Laboratory,

University of California,

Berkeley, California 94720

*Address for correspondence

FOOTNOTE

[1] Reference to a company and/or product names by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

ZONE COLLECTOR AND TRANSFER DEVICE FOR THIN-LAYER CHROMATOGRAPHY

Recently I have begun to use two-dimensional thin-layer chromatography (TLC) in place of paper chromatography for the kinetic analysis of photosynthetic incorporation of tracer carbon into plant metabolites. Because our general experimental approach (1-3) generates a large number of TLC fractions of widely different radioactivity, an economical, convenient and rapid technique for collecting and transfering TLC zones to liquid-scintillation vials was required. Many zone collectors have been described (4-11), but were judged inadequate because of one or more of the following disadvantages: potential contamination by adsorbent from previous use adhering to the device, clogging of fritted glass filters, need for cleaning and drying prior to reuse, or need for a separate device for each sample. This report describes a device which is free of these disadvantages and is suitable for rapid collection and transfer of a series of zones.

MATERIALS AND METHODS[1]

TLC Zone Collector

The collector (Fig. 1) is constructed from a sealing tube with a sealed-in 10-mm diameter coarse fritted disk (Corning Glassworks, Corning, New York). One side of the filter tube is cut and ground to the plane of the fritted disk and the other end of the tube is attached to a one-way glass push-pull valve (12). The side arm of the collector is connected to a vacuum line with rubber tubing. The vacuum valve is open when the plunger is in the "pulled" position and closed in the "push" position.

Procedure for Sample Collection and Transfer

The collector valve is opened, and a 14-mm diameter disk of Whatman GF/A glass-fiber filter paper is centered on the fritted disk. The filter covers the frit and extends past the edge of the device. (A number of 14-mm disks can be cut simultaneously from a stack of larger filter circles with a cork borer.) GF/A filters offer little resistance to air flow although they have an effective retention of particles 1.6 μm in diameter. A TLC zone, located, e.g., by radioautography, is scraped loose from the plate with a scalpel blade, then the collector is held over the loosened adsorbent. The air flow through the glass-fiber filter is strong enough to cause the adsorbent to adhere to the filter. As soon as a fraction has been collected, the end of the collector is inserted into the mouth (16 mm diameter) of a liquid-scintillation vial. The valve plunger is now pushed in, shutting off the vacuum and causing the adsorbent and filter to fall into the vial. The collector is withdrawn and is now ready to be used for the next sample; it requires no cleaning. The scalpel is wiped with tissue paper prior to reuse.

Determinations of Recovery and Cross-Contamination

Glass TLC plates precoated with silica gel and cellulose (Table 1) were used to determine recovery and cross contamination with the collector. Aliquots (4-15 μℓ) of radioactive sucrose in water (8159 DPM/μℓ, prepared from $^{14}CO_2$ by photosynthesis (3)) were spotted on the plates. When they were dry, the adsorbent areas containing the labeled sucrose were collected and transferred to scintillation vials by the procedure described above. Periodically, collection of adsorbent containing tracer was followed immediately by collection of a blank zone on the chromatographic plate to determine cross-contamination. After collection of the

TABLE 1

Recovery of ^{14}C-Sucrose and Cross-Contamination

TLC plates	DPM applied	Recovery[a] (percent)	Blank[b] DPM
Cellulose, MN 300, 250 μm layer[c]	32636	99.6 ± 1.4	
	122385	99.9 ± 1.6	39, 43
Cellulose, Avicel, 250 μm layer[c]	32636	101.9 ± 1.3	
	122385	99.7 ± 2.4	39, 60
Silica gel, Adsorbasil 5, 250 μm layer[d]	32636	97.5 ± 1.4	
	122385	97.9 ± 1.0	44, 41

[a] Mean ± standard deviation, 3-6 determinations.
[b] From each plate, blank zones were collected immediately following two radioactive (122385 DPM) zones.
[c] Analtech, Newark, Delaware.
[d] Applied Science Laboratories, State College, Pennsylvania.

entire series of TLC zones, the samples were eluted by adding 5 ml of water to each vial and shaking them simultaneously in a water-bath at 30°C for 1.5 hr. This was followed by addition of 15 cc of Aquasol 2 (New England Nuclear, Boston, Mass.), thorough shaking, and finally, scintillation counting. The counting efficiency, determined by adding a ^{14}C-toluene standard, was not significantly changed by the presence of the filter and adsorbent in the vials.

RESULTS AND DISCUSSION

The collector is effective for both cellulose and silica gel TLC plates, and is probably suitable for use with other adsorbents as well. The glass-fiber filter is transferred along with the collected adsorbent, and a fresh filter is used for each zone. Recovery is essentially quantitative and cross-contamination is negligible (Table 1), because the adsorbent does not come in contact with the collector.

The design shown in Fig. 1 has been found to be most convenient for rapid, repetitive use. The glass push-pull valve makes it easy to hold the device and shut off the vacuum with one hand. It allows rapid and complete control without visual alignment, as required by rotary glass stopcocks. A less convenient, but operationally adequate, modified device can be constructed without the aid of a glass blower. It consists of a 10-mm OD glass tube, with a cheesecloth support for the GF/A filter taped over one opening. The second opening is attached to a vacuum line by a rubber hose. The vacuum is controlled by use of a stopcock.

The liquid scintillation procedure described in the MATERIALS AND METHODS was carried out to test the performance of the collector. The device is, of course, also applicable to other procedures requiring the transfer of TLC zones. Once collected, the TLC fraction could, for

example, be transferred to a Soxhlet thimble for continuous extraction, or to a test tube for elution and then filtration. The eluted material can then be examined by the usual range of analytical techniques.

ACKNOWLEDGMENTS

The author gratefully acknowledges the excellent technical assistance of L. Rand and thanks E. Heftmann for reviewing this manuscript. The collection device shown was constructed by C. W. DeGroot.

This work was supported by the U. S. Department of Energy.

REFERENCES

1. Platt, S. G., Plaut, Z. and Bassham, J. A. (1976) Plant Physiol. 57, 69-73.
2. Platt, S. G. and Bassham, J. A. (1977) J. Chromatog. 133, 396-401.
3. Platt, S. G., Plaut, Z. and Bassham, J. A. (1977) Plant Physiol. 60, 739-742.
4. Janak, J. (1962) Nature 195, 696-7.
5. Ritter, F. J. and Meyer, G. M. (1962) Nature 193, 941-2.
6. Goldrick, B. and Hirsch, J. (1963) J. Lipid Res. 4, 482-3.
7. Spikner, J. E. and Towne, J. C. (1963) Chemist-Analyst 52, 50.
8. Nagel, J. N. and Dittmer, J. C. (1969) J. Chromatog. 42, 121-3.
9. Levitt, M. J. (1971) Chromatographia 4, 75-6.
10. Sudilovsky, O. and Hinderaker, P. H. (1972) Anal. Biochem. 45, 525-9.
11. Preiss, B. (1977) Anal. Chem. 49, 671-672.
12. Platt, S. G. Erwin, W., and DeGroot, C. W. (1978) J. Chem. Ed., In Press.

Steven G. Platt*

Western Regional Research Center,
Science and Education Administration,
United States Department of Agriculture,
Berkeley, California 94710

and

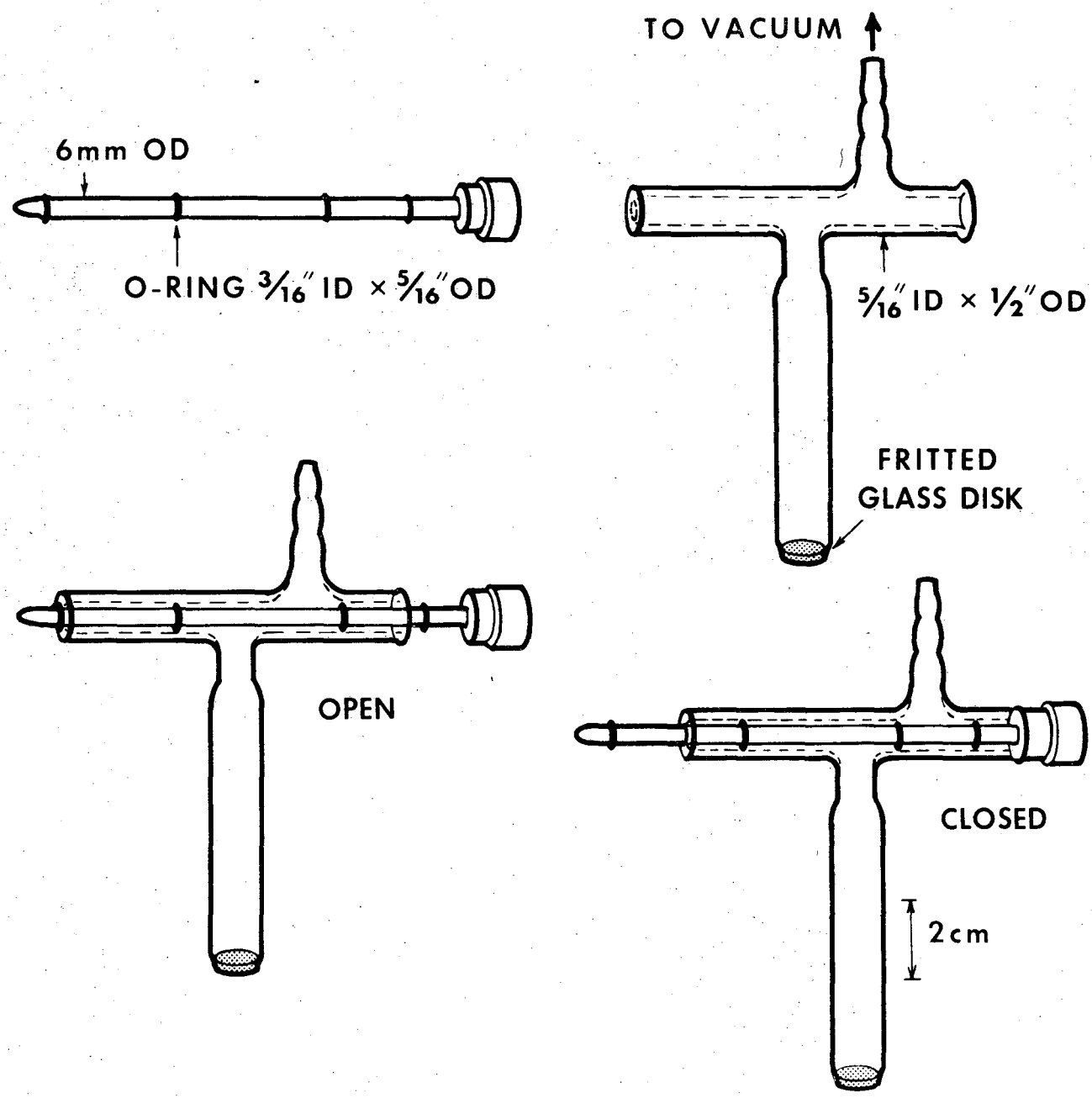
Laboratory of Chemical Biodynamics,
Lawrence Berkeley Laboratory,
University of California,
Berkeley, California 94720

---

* Reprint requests should be sent to the first address given.

FIGURE LEGEND

Figure 1. Zone collector and transfer device for TLC. The valve is shown disassembled, opened and closed. The O-rings fit in grooves in the glass plunger and provide seals between it and the inner wall of the glass tube.



XBL 788-9979

Fig. 1

This report was done with support from the Department of Energy. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the Department of Energy.

Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

TECHNICAL INFORMATION DEPARTMENT
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720