Paper chromatography in extra-long tanks*

Longer than the 60 cm usual size tanks for paper chromatography described by REINEKE[1] and FUKUSHIMA *et al.*[2] have been used by the authors for the quantitative determination of $C_{21}O_5$ and $C_{21}O_6$ corticosteroids from guinea pig urine[3]. With the chromatographic system used, however, great difficulties were experienced with the running of the mobile phase, which would sometimes not advance the desired distance. The observations suggested that successful runs in long tanks apparently occur only after proper saturation. This paper describes a solvent circulating device which allows a high degree of reproducibility of mobile phase running in extra-long tanks in a number of partition systems. In addition, other factors, determining the reproducibility and quality of the chromatographic zones, are described.

The circulation device described in this paper is similar to that described by BAKER, DOBSON AND MARTIN[4] for the saturation of 60 cm long tanks used for systems containing volatile solvents and heavily buffered papers.

Materials and methods

The tanks used were Pyrex cylindrical jars of 30.5 cm O.D. and 120 cm high (Corning Glass Works). Most of the experiments with the solvent saturating device were done with the ethylene chloride–methanol–water (200:17:33, v/v) system previously described[3]. As a rule, 1 in. strips of Whatman No. 2 paper were used. The separation of $C_{21}O_5$–$C_{21}O_6$ steroids such as cortisol, 2α-, 2β- and 6β-hydroxycortisol was studied.

Solvent saturating device

Solvent is pumped from the bottom of the tank to an upper distributor from which it runs down the inner wall (Fig. 1). The distributor was constructed of $^1/_4$ in. O.D. polyethylene tubing joined to a glass T with $^1/_{16}$ in. diameter holes drilled to direct solvent to the wall of the tank. A double layer curtain of fiber glass cloth was hung over the full circumference of the distributor and sewed into a closed bag to fit the inside of the tank. The chromatography papers, hung in trays supported on a platform (Will Scientific Co., Cat. No. 8437) inside the bag were thus protected from solvent spray. Solvent was introduced through the distributor by a bronze gear pump (Oberdorfer Foundries, Inc., Syracuse, N.Y., No. IP 326), fitted with carbon bearings. The pump was driven by an air motor (Gast Mfg. Corp., Benton Harbor, Mich., Model 2AMFCC-1). The pump and air motor assembly were placed on top of the tank cover. Connections to the pump were made with Imperial Eastman brass Poly-Flo fittings. The distributor and stainless steel intake tubing were fitted through the cover with brass bulkhead unions (Imperial Eastman No. 282-PT) and Teflon gaskets.

Chromatographic procedure

A chromatographic tank is prepared by introducing into the bottom 3 l of

* This investigation was supported (in part) by U.S.P.H.S. Research Grant CA-05466 and Career Development Award 1-K3-CA-3949 (to S.B.) from the National Cancer Institute and AM-02672 from the National Institutes of Arthritis and Metabolic Diseases, and Research Grant P-293 from the American Cancer Society.

mobile phase and 0.5 l of stationary phase which have been previously equilibrated at the running temperature of the tank. The mobile phase (whether upper or lower) is circulated by proper adjustment of the height of the intake tube.

Adequate tank saturation is achieved by circulating solvent for at least 1 h. After the paper chromatograms are placed in the tank, solvent is again circulated for 2 min and the papers left to equilibrate. Equilibration of papers for 1, 2, and 4 h resulted in equally good runs with sharp, straight zones and no appreciable edging.

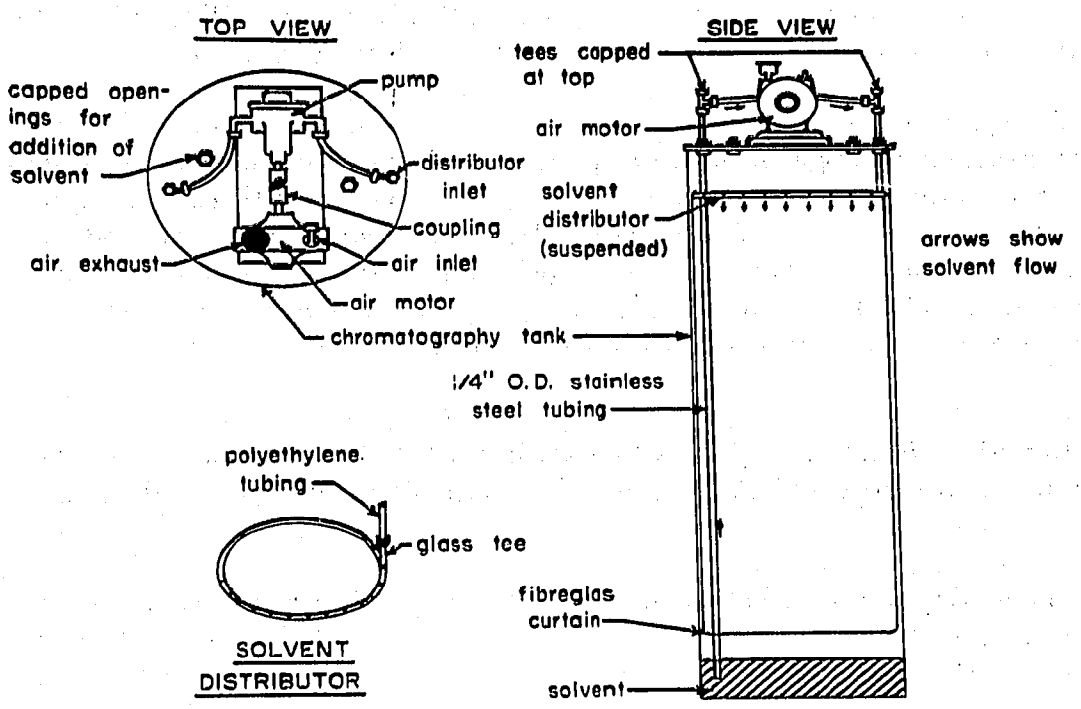

Fig. 1. Diagram showing construction of chromatography tank and solvent equilibration device.

When papers were immersed in water and immediately blotted prior to placing in tanks, good runs were also obtained without any paper equilibration. Similar results were recently described by BUSH AND CROWSHAW[5]. After adding the solvent to the trays (through holes in the covers of the tanks), solvent is again circulated for 2 min. It is not necessary then to circulate solvent again during a run of 17 h. With longer runs intermittent solvent circulation for 1 min every 10 h assures continuous advancing of solvent front. However, continuous circulation causes broad distorted zones. Tanks which were not used for chromatography for 48 to 72 h even when kept at a temperature of $\pm 1°$ are not suitable for chromatography without solvent recirculation for one hour.

The actual operating temperature of the tank was not critical (25° or 31°) but constancy in respect to time and space (within $\pm$ 1°) was important. A temperature gradient between the top and the bottom of the tank greater than 1° prevents in certain systems the proper advancing of the solvent front. Excessive temperature gradients may form if the tanks are kept in bottom heated cabinets and where there is a large difference (greater than 3°) between the temperature inside the cabinet and the room.

Reproducibility

In the procedure previously employed for quantitative paper chromatography of $C_{21}O_5$–$C_{21}O_6$ corticosteroids[3] using long tanks, papers were routinely dipped in water[6,7] and blotted prior to placing in tanks. This procedure seemed to lead in the solvent-uncirculated tanks to sharper resolution of the zones. Using the circulating device, it was found that wetting the paper was not necessary to achieve good runs.

At 25° ± 1°, 1 h equilibration and 16 h running time, cortisol travelled 69.3 ± 2.7 and 2α-hydroxycortisol 31.1 ± 0.8 cm (mean ± S.D.) (7 determinations) from the starting line. With wetted paper, there was a higher variability with the corresponding compounds: 73.9 ± 5.7 and 30.3 ± 3.8 cm (7 determinations).

Other chromatographic systems

Difficulties of running in long tanks not using the circulation device were also experienced in other chromatographic systems. The use of the equilibration device with three other systems was tried. These were: benzene–methanol–water (BUSH, B-5)[8], chloroform–formamide (ZAFFARONI)[9], and isooctane–*tert.*-butanol–water (EBERLEIN-BONGIOVANNI, E_2B)[10]. With the equilibration device good runs were achieved in all these systems including the difficultly running E_2B system.

Acknowledgements

The able technical assistance of JOHN NOBLE is gratefully acknowledged. The authors express their thanks to Dr. IAN E. BUSH for helpful discussions and suggestions.

Worcester Foundation for Experimental Biology, Shrewsbury, Mass. (U.S.A.)

HOWARD L. KIMBALL
NEAL L. MCNIVEN
SHLOMO BURSTEIN

1 L. M. REINEKE, *Anal. Chem.*, 28 (1956) 1853.
2 D. K. FUKUSHIMA, H. L. BRADLOW, L. HELLMAN, B. ZUMOFF AND T. F. GALLAGHER, *J. Biol. Chem.*, 235 (1960) 2246.
3 S. BURSTEIN AND H. L. KIMBALL, *Anal. Biochem.*, 4 (1962) 132.
4 P. B. BAKER, F. DOBSON AND A. J. P. MARTIN, *Analyst*, 75 (1950) 651.
5 I. E. BUSH AND K. CROWSHAW, *J. Chromatog.*, 19 (1965) 114.
6 E. SCHENKER, A. HUNGER AND T. REICHSTEIN, *Helv. Chim. Acta*, 37 (1954) 680.
7 M. M. PECHET, *Science*, 121 (1955) 39.
8 I. E. BUSH, *J. Biochem.*, 50 (1952) 370.
9 A. ZAFFARONI, R. B. BURTON AND E. H. KEUTMAN, *Science*, 111 (1950) 6.
10 W. EBERLEIN AND A. M. BONGIOVANNI, *Arch. Biochem. Biophys.*, 59 (1955) 90.

Received August 30th, 1965