A practical support for ascending and descending paper chromatography

A wide variety of equipment for ascending and descending paper chromatography has been described in the literature[1–9]. As well, chromatography racks and assemblies for holding solvent trays in chromatography chambers are commercially available. To various degrees all of these suffer disadvantages in cost, in design complexity, in convenience, in being difficult to adjust or limiting in use or in requiring special construction techniques, such as spot-welding, silver soldering and glass blowing. Even though the latter technique is a popular one, not many inexperienced workers can utilize such procedures to produce a rack described by one group of workers[5]. Considering the importance of leveling the solvent trough, such home-made glass racks are not at all simple to work with. In addition, considering cost, commercial solvent racks made of glass break easily; hence, they are expensive.

While the apparatus described by SINGER AND KENNER[6] accommodates a large number of papers, a disadvantage is noted in that no provision is allowed for leveling. This is a serious handicap since it has been found in this laboratory that the indented handles, forming part of the jar used by these workers, are not level with respect to one another. Furthermore, there is considerable variation among different jars with respect to placement of these handles.

Another prominent disadvantage is noted in the use of metal stands made partially or totally of steel. When these stands are allowed to stand in contact with solvent contained in the bottom of chromatography chambers, they invite rust and corrosion of the metal with a consequent contamination of the chamber. This requires frequent cleaning of both chamber and stand. Often the thumbscrews used for leveling these stands[9] become "frozen" due to rust and require the use of pliers for loosening them.

Stainless steel racks have, thus, become popular since this material is inert to many common chromatography solvents. This is a desirable feature of this rack type. Many of these units are designed to stand on the bottom of the chromatography jar; and the platforms holding the solvent troughs are leveled by four leveling screws, which are a part of the rack. Since practically all commercially-available jars are not completely flat bottomed, however, the use of these racks causes much difficulty in stability and leveling. In view of the extreme importance of leveling in the development of multi-limbed or mixed chromatograms, this disadvantage is a significant one.

Considering the difficulties described above, a single unit-support, adaptable for use with all commercially available cylindrical chromatography jars, has been designed and is presented in this report.

The support is constructed of 20* gauge stainless steel (Type 302) and utilizes the expanding-collar principle used in a tray support previously described[8]. It differs from the latter support in being much simpler to construct in that no specialized

* Twenty gauge stainless steel is approximately 0.036 in. in thickness. A thinner gauge stainless steel, 22 or 24 may be used, but a heavier gauge is not recommended since this is more difficult to cut with conventional tools.

techniques, such as spot-welding and silver soldering, are required. The support is simply cut* from a pattern, such as that described in Fig. 1. Right angle bends are then

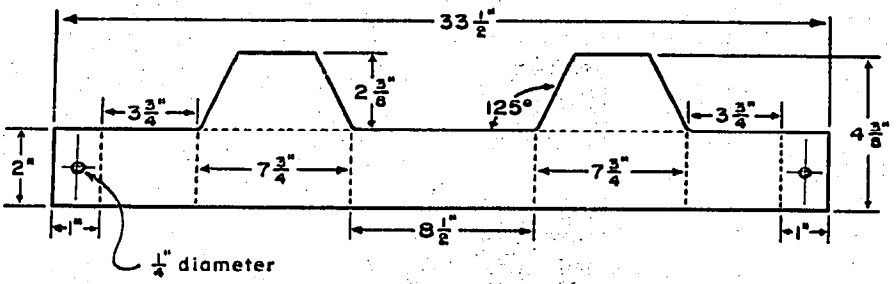

Fig. 1. Cut out pattern for support.

made in a vise, as shown by the dotted lines in the figure, to form the two platforms and ends, as illustrated in Fig. 2. Following this, the collar is shaped by hand to form the cylindrical collar (Fig. 3). A stainless steel stove bolt**, is inserted through the holes contained in the two bend end sections. A wing nut and hexagon-shaped nuts are placed, and the assembly is complete (Fig. 3).

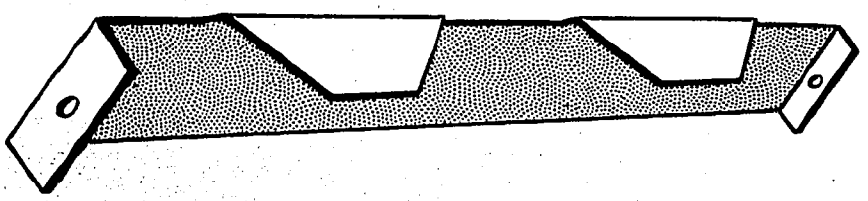

Fig. 2. Support after cut out showing right angle bends in platforms and ends.

The dimensions presented in Fig. 1 will suffice for any jar with an 11 inch inside diameter, regardless of height. A variation of these dimensions allows for an adaption of the support so that it may be used in jars of any diameter. In calculating the overall length of stainless steel to use, one may use the equation, $C = \pi d + 1$, where C = circumference to be measured and d = inside diameter of the jar to be used. This formula compensates for the space containing the wing nut and the two (bent) end sections, which accept the stove bolt.

In use, the collar is closed to its smallest dimension by backing up the wing nut. The unit is then lowered into the jar and placed at any desired distance from the top of the chamber. At this level the expanding collar is tightened by use of the wing nut to a degree sufficient to just contain the unit. It is then leveled, using a small spirit level, following which the wing nut is securely tightened by hand. One or two solvent troughs are then placed upon the platforms for descending chromatography (Fig. 4). Alternatively, filter paper strips may be suspensed downward from the protruding

* The pattern may be cut using a hand hack saw or a power saw using a fine blade with approximately 32 teeth to the inch. The lighter weight may be cut with a pair of heavy-duty hand shears. Rough edges should be filed smooth to prevent accidental cuts.

** For a chamber of 11 in. inside diameter, a 2 1/2 × 1/4 in. stainless steel stove bolt can be used. For chambers of different diameters, the size of the bolt may be adjusted. Any size bolt which will allow closure and expansion of the collar in the desired chamber may be used.

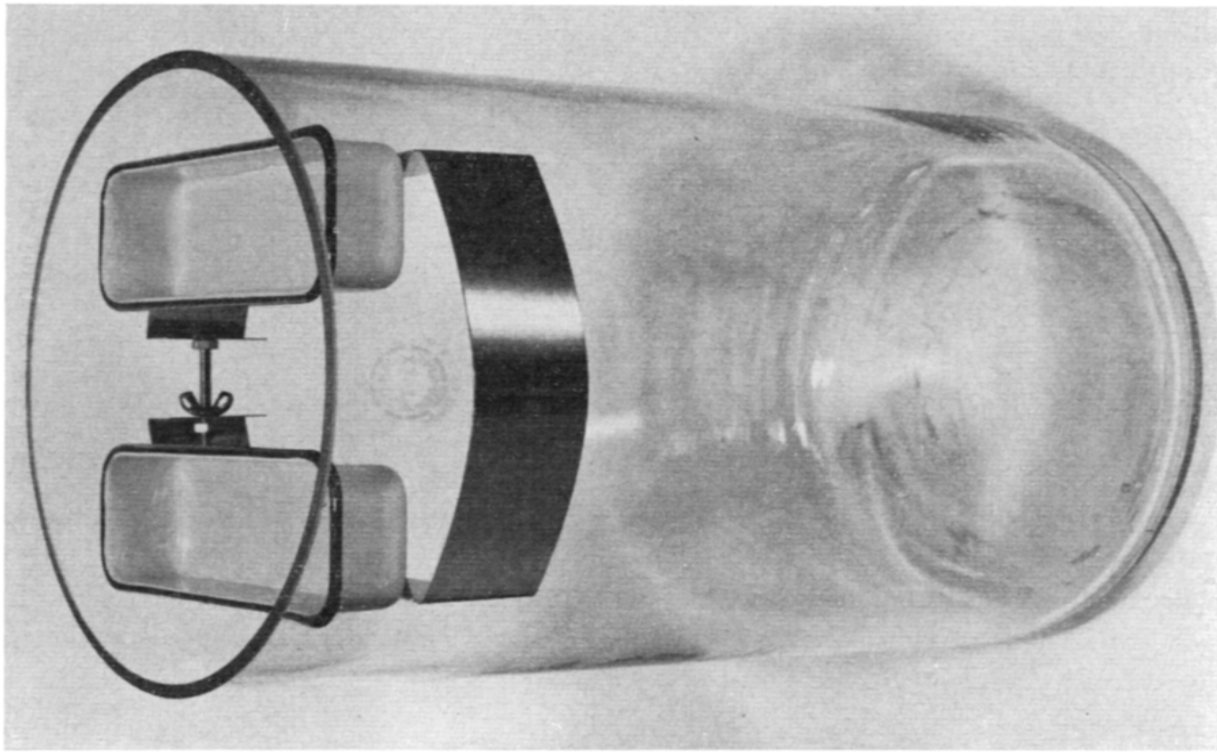

Fig. 4. Support with expanding collar set against cylinder surface.

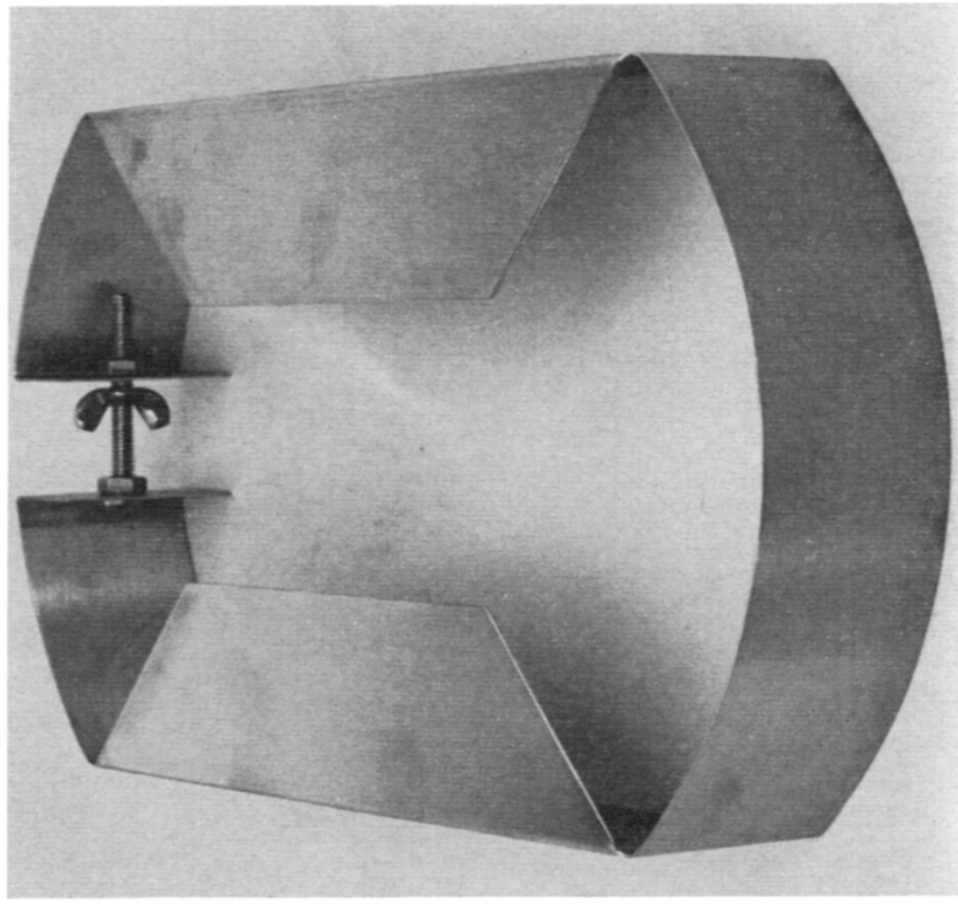

Fig. 3. Support after forming to cylindrical shape.

edges of the platforms into the solvent at the bottom of the chamber and developed by ascending chromatography*. The chamber, thus prepared, and after equilibration with appropriate solvents, is ready for chromatography. The use of this support, as described, gives a clear and unobstructed area beneath the tray support.

Acknowledgement

This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York. Illustrative material by Leon Schwartz and Gerald Cooper, Department of Illustration and Photography, University of Rochester Atomic Energy Project.

Departments of Radiation Biology and Biochemistry, University of Rochester, School of Medicine and Dentistry, Rochester, N.Y. (U.S.A.)

FRANK M. GANIS

1 R. CONSDEN, A. H. GORDON AND A. J. P. MARTIN, *Biochem. J.*, 38 (1944) 224.
2 W. H. LONGNECKER, *Anal. Chem.*, 21 (1949) 1402.
3 F. C. STEWARD, W. STEPKA AND J. F. THOMPSON, *Science*, 107 (1948) 451.
4 J. L. BUCHAN AND R. I. SAVAGE, *Analyst*, 77 (1952) 401.
5 A. JEANES, C. S. WISE AND R. J. DIMLER, *Anal. Chem.*, 23 (1951) 415.
6 A. J. SINGER AND L. KENNER, *Anal. Chem.*, 23 (1951) 387.
7 R. M. MA AND T. D. FONTAINE, *Science*, 110 (1949) 232.
8 W. L. PORTER, *Anal. Chem.*, 26 (1954) 439.
9 R. B. BURTON, A. ZAFFARONI AND E. H. KEUTMANN, *J. Biol. Chem.*, 188 (1951) 763.

Received September 1st, 1961

* Strips of glass plate or beakers may be used for anchoring the paper strips.

Chromatography of unstable materials of biological origin

Many materials of biological origin, especially when purified, undergo changes on contact with gaseous oxygen or on prolonged standing in aqueous solution. Thus they cannot be subjected to many of the procedures normally applied to materials during their chromatographic or electrophoretic separation unless special precautions are taken.

Column chromatography and continuous electrophoresis frequently offer the only practical means of preparative purification of these substances, yet the introduction of dissolved gaseous oxygen with the eluting agent during the separation process and the action of atmospheric oxygen on the collected purified fractions frequently causes a substantial loss in the biological activity sought. This loss is often compounded by changes in the functionality of the dissolved biological material with time. In many instances these changes occur even in the absence of gaseous oxygen and in a refrigerated environment. Thus it is desirable to carry out the separation of such sensitive