Microextraction for Paper Chromatography

The dimensions of plant analysis have changed very much since the invention of paper chromatography [R. Consden, A. H. Gordon, A. J. P. Martin, *Biochem. J.* **38**, 224 (1944)]. This paper describes a microextraction method with which serial extractions can be made from the very small amounts of tissue actually needed for paper chromatography.

An extraction apparatus of the Soxhlet type cannot be made very small because the capillarity of the glass tubing prevents proper working. Figure 1 shows an apparatus that uses the refluxing principle of the Soxhlet; however, the construction of this apparatus is simpler. The condensing solvent causes a continuous flow from the condenser through the side arm that contains the tissue pieces and back to the boiling solvent in the flask. The capacity of the flask is about 5 milliliters. The capacity of the side arm can be adapted to the size of tissue by the choice of the position (Fig. 1). To close flask and side arm, ground glass stoppers are the best; if cork stoppers are used, they must be wrapped with aluminum foil to prevent extraction of material from the cork. To prevent delayed boiling (bumping), the apparatus has to be shaken a little. The flask should contain one glass boiling ball, especially with the use of solvents with a high heat of vaporization such as water.

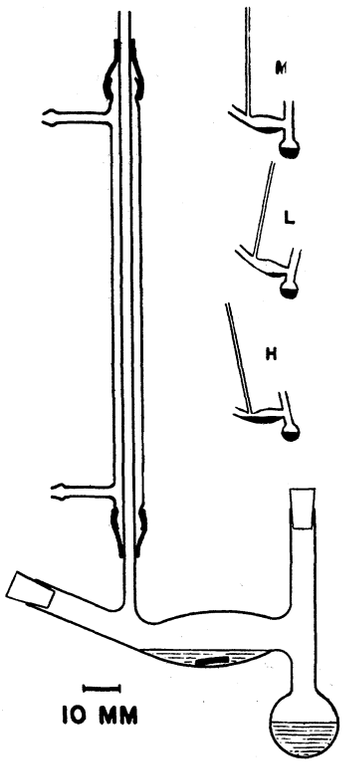

Fig. 1. Extraction apparatus. Positions for medium, low, and high capacity of side arm: *M*, *L*, and *H*, respectively.

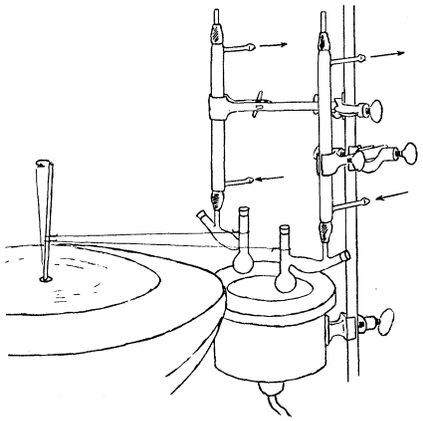

Fig. 2. Assembly for two simultaneous extractions.

Figure 2 shows the assembly for two simultaneous extractions. On the left side there is an old centrifuge running on slowest speed. A wooden stick is mounted slightly eccentric to the axis. The flasks are connected with the wooden stick by a thin wire. The slight back and forth movement of the flasks is actually a rotating one in an arc around the condenser jacket, which is fixed to the support. The flask, the side arm, and the inner tube of the condenser are one piece, the latter being connected with the condenser jacket by short pieces of rubber tubing. Five of these sets, consisting of two extractors with one heater on a support, can be placed around the centrifuge; this permits ten simultaneous extractions.

The whole assembly having been set up, the tissue is introduced into the side arm. Sufficient solvent should be pipetted into the side arm so that it runs over into the flask and fills it to the desired level (Fig. 1).

When the extraction is finished, the solution has to be concentrated either by turning the apparatus into position *H* (Fig. 1) or by taking the solvent out of the side arm with a pipette. The condensing solvent now does not reflux into the flask but stays in the side arm. When the solution in the flask has been concentrated (this moment is very critical), the apparatus is turned around the condenser (condenser jacket fixed) off the heater. Condensation of the solution still vaporized will clean the wall of the flask as soon as the apparatus is removed from the heat. The concentrated solution can now be transferred onto the paper chromatogram. The extractors can then be pulled out of the condenser jackets and replaced by new ones. During the next extraction, the first set can be cleaned. Therefore only one set of condenser jackets, but two sets of extractors, are required for continuous extraction.

We have been using this extraction method for several months in our laboratory to extract sugars with pyridine from 30-milligram (fresh weight) samples of plant tissue for paper chromatographic analysis. Extraction is complete after about 1 hour.

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Slide Projection from Lecture Table

For several years in our department, we have employed with satisfaction a device (Fig. 1) that enables a lecturer to project his own slides from the lecture table. Although we have some reservation concerning the originality of this arrangement, we have not seen it described by others. A schematic view of our original experimental installation has been published [*Southern Chemist* **13**, 73 (1953)]. Inasmuch as this device has interested many visitors to our department, we have decided to describe it for a larger audience.

We project the slide to a mirror suspended just below the ceiling of the room, the image being reflected to a screen behind the lecture table. If we employ the standard 3¼ by 4-inch slide in a Spencer delineascope with a lens of 14-inch focal length and with distances between lantern, mirror, and screen as shown, the image on the screen has outside dimensions of 56 by 71 inches. With a good quality plate glass mirror there is no appreciable distortion or light absorption.

The size of the exposed surface of our framed mirror is 24 by 29 inches. Local conditions determine the method of suspending the mirror from the ceiling. In our new building, we were able to obtain an excellent installation by having a gal-

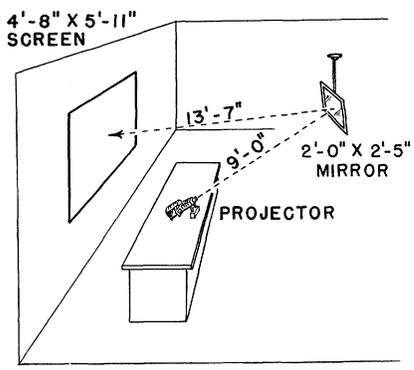

Fig. 1

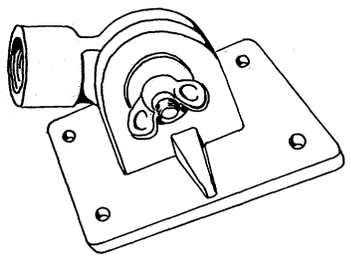

Fig. 2

vanized pipe nipple for 1½-inch pipe fixed in the concrete ceiling of the room when the slab was poured. A suitable length of pipe is attached to this nipple by a sleeve. The pipe screws into the heavy cast iron clamp illustrated in Fig. 2. This clamp (made to our specifications at a local foundry) is fastened by screws through the plate side to the back of the mirror frame. With this type of installation, the mirror is readily adjustable in both horizontal and vertical planes.

Inasmuch as our lantern cannot otherwise be tilted upward sufficiently, we mount it on a small sloped wooden platform. At the resulting angle, the light beam from lantern to mirror is not seen by the audience.

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27 June 1955

Anaerobic Cuvette

Studies of hemoproteins often require measurements of anaerobic reactions in a spectrophotometer. Recently Lazarow and Cooperstein (*1*) described a device that is especially suited for reactions involving catalytic hydrogenation. For some time, a somewhat different apparatus that allows anaerobic titrations, as well as additions from two sidearms, has been used in this laboratory (Johnson Foundation) and is described here because it may be useful for a variety of other studies as well (*2*).

The apparatus consists of two parts: the cuvette proper and a titration head (Fig. 1). The 1-centimeter cuvette fits into a Beckman cellholder. Two sidearms, B_1 and B_2, hold reagents to be mixed after the system has been gassed. There is a ground glass joint, *C*, between the cuvette and the titration head.

For anaerobic titrations, the sidearms and the burette *F* are left empty (*3*). The apparatus is evacuated and filled with an inert gas repeatedly, using stopcocks *D*. The burette *F* and the tip *G* are then filled through side tube *H* with the help of a two-way stopcock *E*. Titration is started and the drop hanging from the tip is stirred into the solution after each addition. This is accomplished by moving a magnetic flea between positions K_1 and K_2 with a magnet. In order to make an optical density measurement, the flea is stored in one of the sidearms (position K_3). Daylight is excluded from the apparatus with a black cloth.

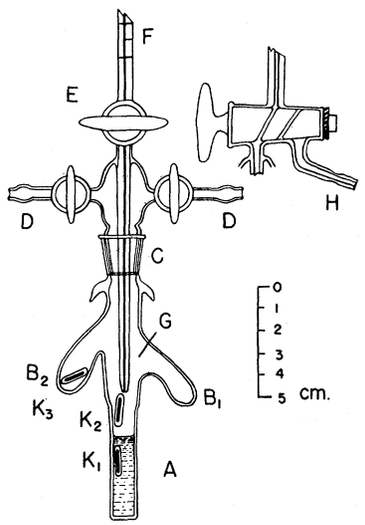

Fig. 1. Vacuum cuvette. Left, front view with upper end of the burette omitted. Right, side view of burette-filling mechanism. K_1, K_2, K_3, alternative positions of a magnetic flea. The other symbols are explained in the text.

If an addition of only one or two reagents is necessary, the titration head is removed and the gassing is performed by a two-way stopcock connected to a ground glass joint that fits into joint *C*. After anaerobic conditions have been obtained, the reagents in the sidearms are tipped in. This technique is essentially that of Ball *et al.* (*4*). Experimental results obtained using this system will be submitted elsewhere for publication.

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References and Notes

1. A. Lazarow and S. J. Cooperstein, *Science* 120, 674 (1954).
2. This research was supported by a grant from the Division of Grants and Fellowships, National Institutes of Health, U.S. Public Health Service.
3. In some cases, the removal of O_2 can be perfected by the presence of some $Na_2S_2O_4$ in one of the sidearms.
4. E. G. Ball, C. F. Strittmatter, O. Cooper, *J. Biol. Chem.* 193, 635 (1951).

2 May 1955

A great discovery is not a terminus, but an avenue leading to regions hitherto unknown. We climb to the top of the peak, and find that it reveals to us another higher than any we have yet seen and so it goes on. The additions to our knowledge of physics made in a generation do not get smaller or less fundamental or less revolutionary as one generation succeeds another. The sum of our knowledge is not like what mathematicians call a convergent series . . . where the study of a few terms may give the general properties of the whole. Physics corresponds rather to the other type of series called divergent, where the terms which are added one after another do not get smaller and smaller, and where the conclusions we draw from the few terms we know cannot be trusted to be those we should draw if further knowledge were at our disposal.—J. J. THOMSON.