A Simple Device for Automatic Equilibration of Paper Chromatograms

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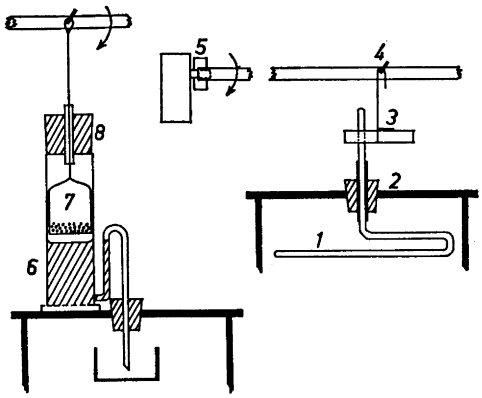
In paper chromatography, it is often necessary to equilibrate the papers with the vapours of the solvents in the chromatographic chamber before they are brought into contact with the mobile phase. Much time would be saved if this contact could be achieved automatically so that the chromatography could start at any time during the night. Delivery of mobile phase from reservoirs by the automatic turning of a stopcock has been described [1-3]. For the chromatography of bile acids [4] we have used a simple and inexpensive device which can be applied to ascending as well as descending chromatography and which can be adapted to suit most sizes of chromatographic vessels.

Since the completion of this work an automatic solvent addition funnel based on a siphon principle has been described [5].

Description of the apparatus. The figures refer to the diagram.

Ascending chromatography. The papers are suspended in the vessel from a bent glass rod (1) inserted into and emerging from a piece of glass tubing which is fixed in a cork (2) placed in a hole in the covering glass plate. The diameter of the glass tubing should be just large enough to permit free vertical movement of the glass rod. With the aid of a clothespin the glass rod is attached to the timer unit with a piece of bent brass wire (3) so that the lower ends of the chromatograms are about one cm above the mobile phase on the bottom of the chromatographic chamber. The timer unit consists of a brass tubing fitted with small spin (4) on which the glass rods with the papers are hung. One end of the brass tubing is connected with the winding screw for the alarm (5) of an alarm clock, the other end is loosely supported in a bearing. The connection between the brass tubing and the clock should be made so that it is easy to take the pieces apart. The clock is adjusted to a predetermined time and when the alarm rings the papers are lowered into the solvent. By a proper adjustment of the clothespin their fall is stopped when they have descended into the mobile phase.



Descending chromatography. The same timer unit is used as for the ascending technique. A siphon made of a measuring cylinder (6) is placed on the glass cover of the tank. The glass tubing is introduced through a cork in a hole in the covering plate, the tip being in position just above the chromatograms in the trough. The desired amount of mobile phase is introduced into the cylinder (maximum just below the bend of the siphon) and a cylindrical glass bulb containing leadshot (7) is cautiously introduced and suspended above the solvent surface in the cylinder. The bulb has a diameter about 3 mm less than the inner diameter of the cylinder. It is attached to the timer unit with a thin silver wire hung on one of the pins of the brass tubing. The measuring cylinder is stoppered with a cork and the silver wire passes through a capillary tube in the cork (8). When the alarm rings the glass bulb hanging above the solvent surface falls and the mobile phase is emptied into the trough. The larger the volume of the bulb, the smaller is the minimum volume that can be drained from the cylinder.

The device described has been in constant use in our laboratory for a year. As judged from the chromatograms, the entrance hole for the glass rod in ascending chromatography and the hole for the silver wire in descending chromatography do not cause changes in the vapour equilibration with our phase systems. The timer unit has the advantage that it can be used for several chromatographies at the same time,

irrespectively of whether the ascending or the descending technique is used. The use of an ordinary alarm clock makes the apparatus inexpensive but if the equilibration time must exceed eleven hours another type of timer is required.

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1. Martin, S. M. *Anal. Chem.* **30** (1958) 1890.
2. Abelson, D. M. and Fox, J. G. C. *J. Clin. Pathol.* **12** (1959) 375.
3. Decker, J. L. *J. Lab. Clin. Med.* **54** (1959) 476.
4. Sjövall, J. *Clin. Chim. Acta* **4** (1960) 652.
5. Van Duren, B. L. *Anal. Chem.* **32** (1960) 732.

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A Note on the Action of Firefly-Extracts on ATP

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Use of the enzyme system from *Photinus pyralis* for the determination of minor amounts of ATP has been suggested and further a method implying it has been described by Strehler and Totter [1]. In order to study the extent of the postulated linear dependence of light intensity on ATP concentration experiments were carried out with different amounts of firefly-extracts and of ATP.

The experiments were carried out in small test tubes closed with a rubber stopper. ATP solution, enzyme extract and either arsenate buffer or water was mixed by rapid shaking. Light intensity was then determined by a photometer. If we suppose that light intensity is a direct measure of the reaction velocity and accept that the phototube (type RCA 1-P-21) is linear in the spectral area in question [2], then the photometer readings directly express reaction velocity in arbitrary units. It is known that oxygen is required for the reaction but this is supposed to be present in a concentration that is sufficient for the reaction, for two reasons: First it

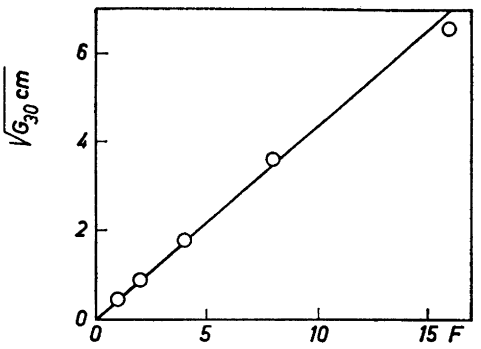

Fig. 1. Abscissa: Concentration of firefly-extract in arbitrary units. Ordinate: Square root of galvanometer deflection measured 30 sec. after start of experiment. Each experiment was made with a mixture of 500 μl water, 100 μl ATP solution (34 μg/100 μl) and 200 μl firefly-extract. t = 25.1°C.

is demonstrated by Hastings *et al.*[3] that only decreasing oxygen pressure to a very minute fraction of the normal has any influence on the reaction and secondly, we have demonstrated that bubbling of atmospheric air through the test tubes did not restore activity when the light intensity had fallen to approximately zero. Yet activity could be restored by the addition of either ATP or firefly extract de-

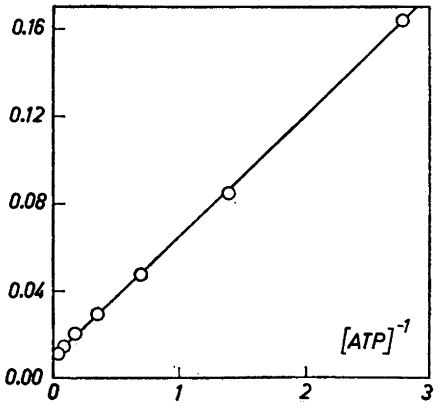

Fig. 2. Abscissa: Reciprocal of ATP concentration in ml · μg^{-1}. Ordinate: Reciprocal of galvanometer deflection. Experiments performed with 500 μl water, 250 μl enzyme solution and 250 μl ATP solution. t = 22.5°C.

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