

## A solvent equilibrator inside the descending chromatography tank

### Automatic application of mobile phase

For paper chromatography in volatile systems composed of two solvent phases such as are used for the separation of steroids<sup>1</sup>, the prepared paper sheets generally require some hours' equilibration in the tank. To start the run, a volume of upper phase solvent pre-equilibrated with the lower phase is drawn from a nearby separator and introduced into the trough from which the paper hangs inside the tank. During the transfer the solvent frequently turns cloudy, indicating a disturbance of equilibrium. Furthermore, automatic starting becomes desirable when it is necessary to introduce the solvent at inconvenient times during the night so as to finish the run within the following day.

Few of the automatic starting systems described in the literature are adaptable for two-phase solvent chromatography. To ensure a completely closed system for such volatile solvents, ABELSON AND FOX<sup>2</sup> linked the solvent reservoir directly with the tank through a tap, which was automatically operated by means of a battery-powered electrical circuit comprising a solenoid and anti-flash unit triggered by a timer. It would be more desirable to equilibrate the running solvent actually inside the tank and without the complication of an electrical system. For this purpose, the apparatus shown in Fig. 1 was devised in this laboratory. It consists of a siphon inside a reservoir tube shaped to fit into the solvent trough of a chromatography tank, the measurements given being appropriate to a tube for the 12-inch Shandon model. Charging the equilibrator tube with solvent through the side-arm is greatly facilitated by inclining the tube at about 45° to the vertical. The volumes of the two phases are so chosen that only the upper (mobile) phase feeds the internal siphon when the tube is placed in the dry trough to clamp the paper in position. The tank is sealed by a glass lid with a centre hole to accommodate the side-arm which carries a sleeve to make the tank sufficiently air-tight. During equilibration the side-arm is kept closed; but momentary application of pressure to it, such as by a rubber bulb, starts the run by causing upper phase solvent to siphon into the trough. At the end of the run, suction is applied at the side-arm to drain the solvent in the trough back through the siphon. The tube is then removed and the paper lifted out.

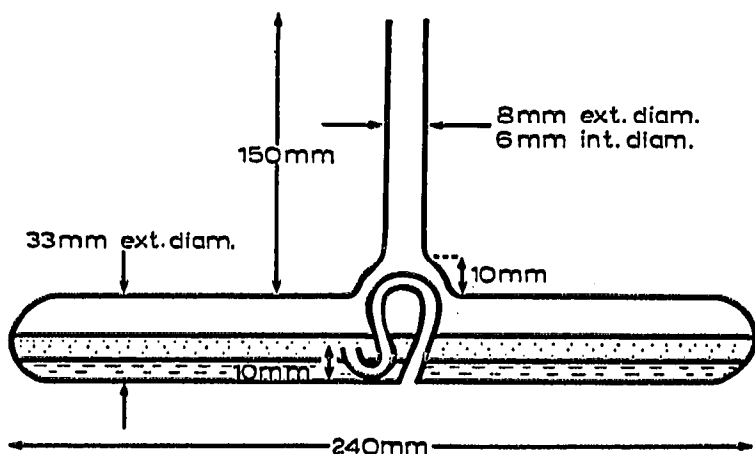


Fig. 1. Solvent equilibrator.

An adaptation of the simple alarm-clock device of KRABISH AND SJÖVALL<sup>3</sup> permits automatic addition of the running solvent (Fig. 2). When the alarm rings, the winding screw turns to lower the sinker into the reservoir and siphons the water it contains into the funnel and *via* its overflow tube into the separator. The air displaced transfers this volume of running solvent from the equilibrator (shown here in cross-section) into the trough and thereby starts the run. When the run is completed, some of the water in the separator is drawn off by opening the tap and the resultant reduction in pressure drains the solvent in the trough back into the equilibrator tube through the siphon.

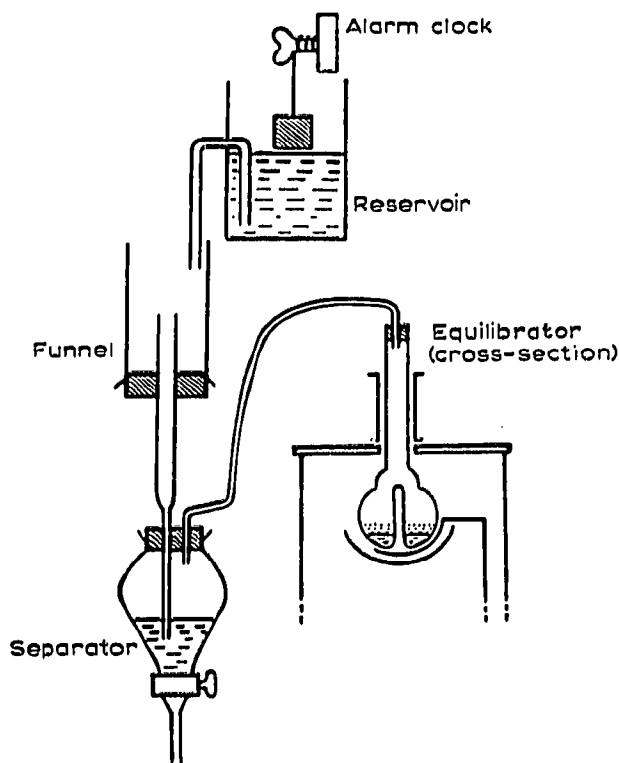


Fig. 2. Arrangement for automatic starting of chromatography.

The equilibrator is robust and made entirely of glass. It can be constructed to fit most standard chromatography apparatus. There are no mechanically moving parts inside the tank. The central position of the siphon in the tube minimises the chance of lower phase solvent entering its orifice while easing the tube into the trough. By its upwards extension into the side-arm the siphon is increased in height to avoid inadvertent starting during equilibration. A feature of the equilibrator is that it allows drainage of the solvent from the trough after the run; this facilitates withdrawal of the paper chromatogram. Without material alteration the equilibrator may be used equally well for monophasic systems.

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### **Paper chromatography of higher esters of fatty acids with saccharose**

Analyses of the samples taken in the course of the reaction of methyl esters of fatty acids with saccharose in dimethylformamide or dimethylsulfoxide<sup>1-14</sup>, and those of the finished product for determining the mono- and diester content, are based on the specific rotation of the butanol solution<sup>3</sup>. This method is not reliable, assuming the presence of only two components, the mono- and diester. In fact, the reaction is more intricate, as higher esters of saccharose or esters of fatty acids together with split products of saccharose, especially glucose, form comparatively easily, as shown by earlier work studying the kinetics of the reesterification of saccharose dipalmitate with saccharose<sup>15</sup>.

These classic methods may serve as orienting tests, but they do not comply with the requirements for accurate determinations of the composition of the product or of more detailed studies of the reaction. Therefore our attention was called to analytical studies of higher and lower esters of saccharose.

The determination of mono- and diesters of fatty acids with saccharose by paper chromatography has been described in an earlier paper<sup>16</sup>. A mixture of the substances or the reaction solution was separated on Whatman No. 1 paper by eluting with the system benzene-*n*-propanol (10:3, v/v). The descending technique was used and for a good separation an atmosphere of 90 % relative moisture in the chamber was very important.

On drying, the paper was washed in a petroleum ether solution of paraffin, m.p. 50° (concentration 10 g/l) and on evaporating the solvent in a drier, the spots were detected by dipping the paper into a water bath at 27° for 2 min. The mono- and diesters emulsified the paraffin and the spots became transparent. The sensitivity of the method was between 0.55 and 1 µg of mono- and diester.

The higher esters of saccharose showed a much lower emulsifying ability. The monoesters of glucose and fructose behaved similarly. Although those esters could be

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