A PRESSURELESS APPARATUS WITH CENTRAL-SPOT DEVELOPMENT FOR CENTRIFUGAL CHROMATOGRAPHY

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Several devices have been constructed for chromatography accelerated by centrifugal force[1–6]. In these devices the system of delivering the developing solvent is either central, without continuous influx of the mobile phase[6], or excentric with continuous influx of the mobile phase[1–5]. Both these methods have certain advantages and disadvantages; in the case of the method without continuous influx of the mobile phase the development is irregular and the supply of the mobile phase does not permit chromatography over long periods with overflow. Only MCDONALD's apparatus, which has the distributor in an excentric position is not subject to this disadvantage. One imperfection of this apparatus must, however, be pointed out, *viz.*, it cannot ensure conventional development of circular chromatograms and requires in addition such a great quantity of accurately adjustable overpressure that this cannot be supplied hydrostatically.

The above-mentioned reasons inspired us to construct a new type of apparatus in which the imperfections of the previous ones are eliminated. In this prototype the distributor of the mobile phase[7] is pressureless or, properly speaking, it is sufficient to supply merely hydrostatic pressure for accurate regulation of the mobile phase influx. This apparatus can be said to be adaptable to different chromatographic methods. It allows the performance of adsorption and partition chromatography, and also of special methods, such as the overflow technique or gradient elution.

Description of the apparatus

The apparatus for centrifugally accelerated chromatography consists of the following parts: the distributor of the mobile phase (Fig. 1), which consists of a capillary tube, the tip of which is ground out to form a conical aperture. This is in fact a bearing for a stainless steel ball, which is in direct contact with the paper. The bottom half of the ball bearing consists of a disc made of hardened PVC, at the centre of which there is a hollow with the same radius of curvature as the ball. The distributor is supported at two points and may be displaced vertically when it is necessary to change the paper. The influx of the mobile phase can be regulated directly by a brass needle valve "Regula" (in the case of non-corrosive phases) or indirectly, by means of

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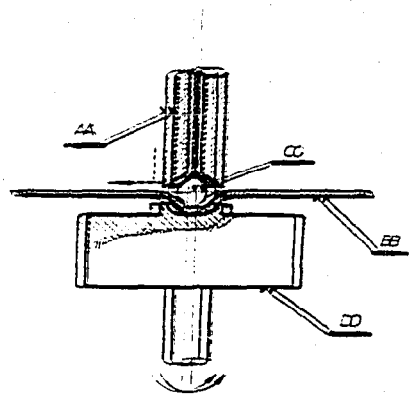


Fig. 1. Distribution of the mobile phase. A = capillary supply tube; B = disc of chromatographic paper; C = ball of the distributor; D = bottom part of the bearing. Dotted arrows: direction of flow of the mobile phase. Solid arrow: direction of rotation.

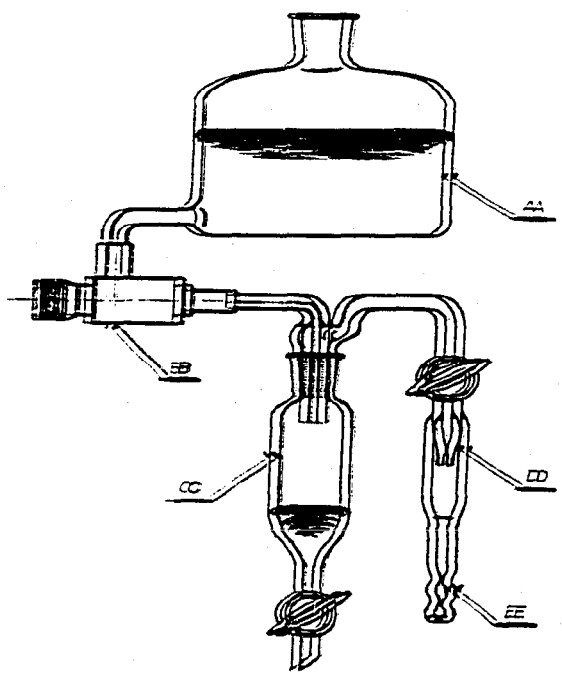


Fig. 2. Scheme of indirect regulation of the movement of the mobile phase by means of mercury. A = mercury reservoir; B = needle valve; C = reservoir of mobile phase with capillary supply of mercury; D = drop counter; E = mobile phase inlet of the chromatograph.

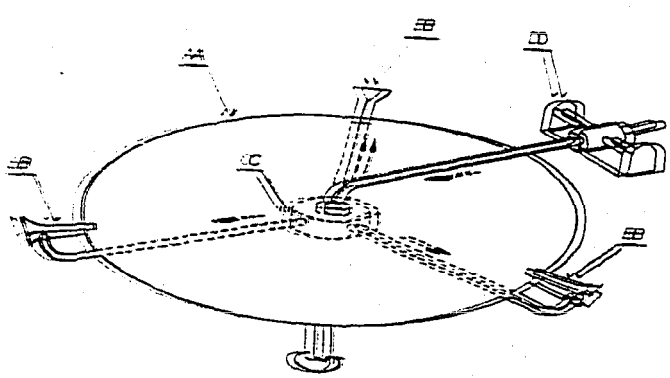


Fig. 3. Method of fastening the chromatographic paper in the apparatus. A = chromatographic paper; B = crocodile clips fixing the position of the paper; C = capillary distributor of the mobile phase with the ball; D = mounting of the distributor.

mercury (Fig. 2). If a glass needle valve is available, this can be used to regulate any mobile phase directly. The flow-rate is indicated by a common type of drop counter. All connections must be made by means of polyethylene or, preferably, polytetrafluoroethylene (teflon) tubes.

The hydrodynamic conditions of chromatography in the centrifugal field are perfectly reproducible for a given mobile phase, speed of rotation and paper. The flow-rate calibration of the apparatus can be made once and for all.

The mobile phase flows in at a steady rate, passing over the ball and the chromatographic paper. The paper is fixed at the periphery by means of crocodile clips, which are placed at angles of 120° along the periphery, as can be seen in Fig. 3. The chromatographic paper, which has a diameter of 20 cm, is revolved at a speed of 750 r.p.m. This size of paper proved to be sufficient for our purpose. The position of the paper is determined only by the pressure of the capillary (mobile phase inlet) and by the clips mentioned above. An aluminium tank can be used as the chromatographic chamber. The lid of this tank is made of plexiglass and is coated with silicone varnish to make it resistant to organic solvents.

The chromatographic chamber can be saturated very simply by placing basins with the solvents at the bottom. The saturation process is very rapid, because the arms bearing the crocodile clips function simultaneously as a ventilator.

Testing of the apparatus

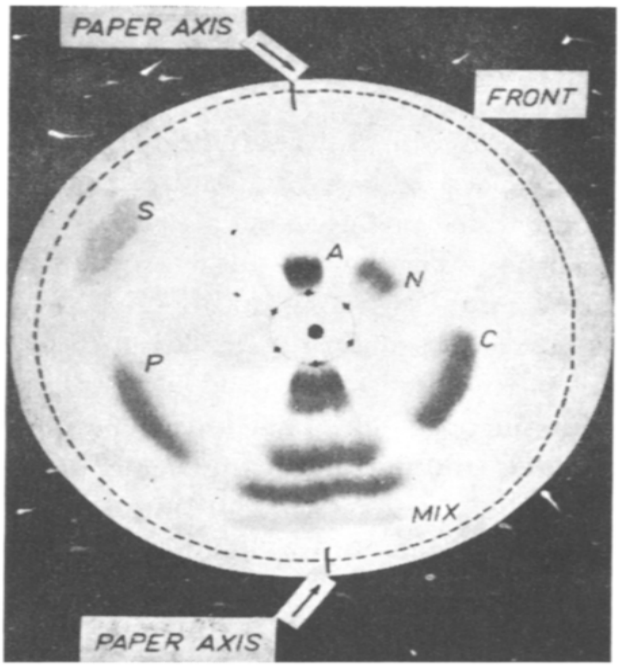
The properties of the apparatus were tested with colour standards (Schleicher & Schüll), using various types of chromatographic papers. The following types of paper proved to be the best for separating these colours: Whatman No. 1 and 3, S & S 2045 b Gl and Ederol 225 and 226. Some difficulty was encountered when using papers of lower capacity, such as, *e.g.*, Whatman No. 1; this consisted in inaccurate regulation of the mobile phase influx. By ensuring that the needle valve functions precisely it is possible to eliminate this difficulty.

The quality of the separation can be improved by using heavy papers. It is a general rule that chromatograms on heavy paper are developed longer and that the quality of the separation is better. A comparison of the developing times is given in Table I.

TABLE I

DEVELOPING TIME FOR VARIOUS KINDS OF CHROMATOGRAPHIC PAPER

Type of paper	*Developing time (min)*	*Quality of separation*
Ederol 225	12	adequate
Ederol 226	16	adequate
Whatman No. 3	22	good
Whatman No. 1	35	good
S & S 2045 b Gl	45	excellent



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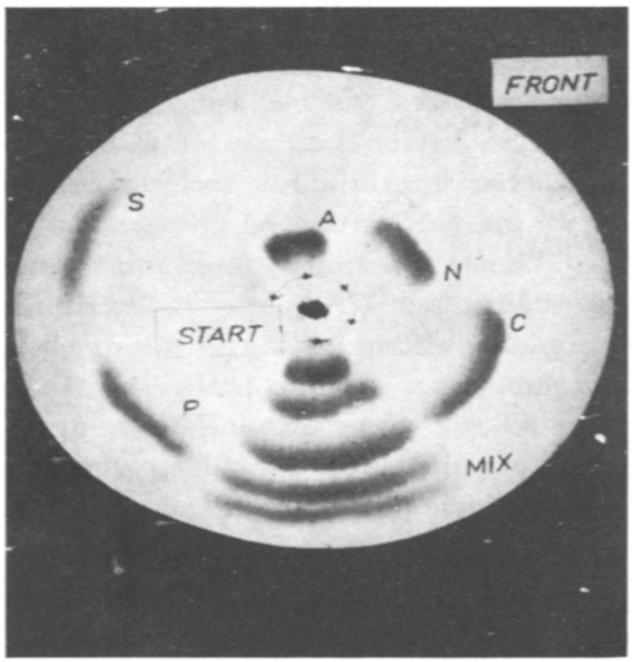

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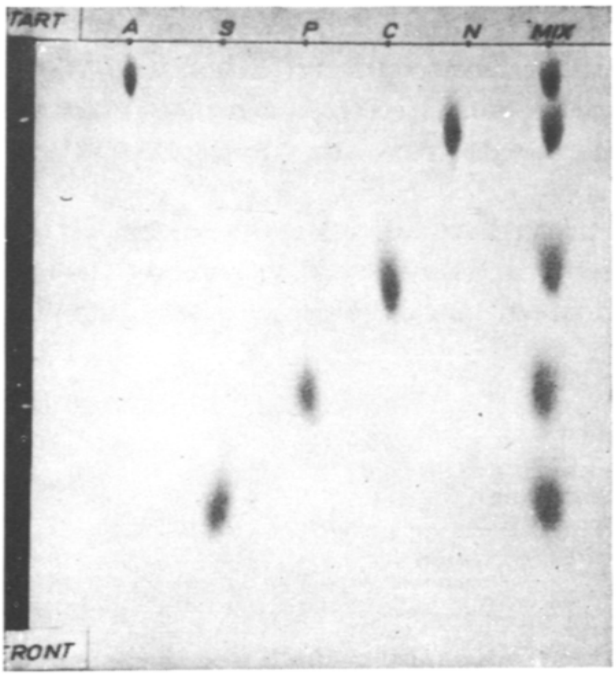

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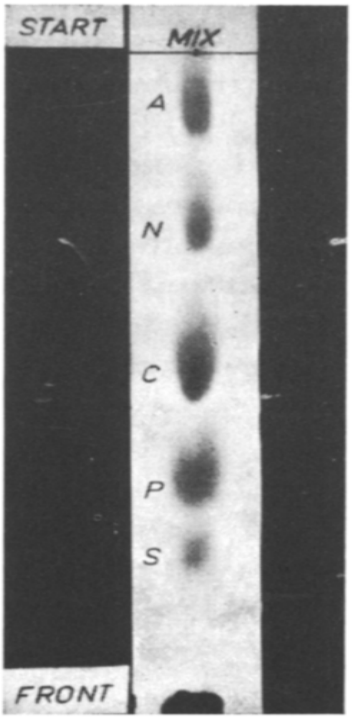

D

Fig. 4. A comparison of chromatograms obtained with different developing techniques. A: in a centrifugal field; B: by the radial technique; C: by the descending technique; D: by the ascending technique.

Operating procedure

Samples of the colours were applied in quantities of 0.1 ml to the start which was 5.5 cm from the centre of the chromatographic disc. Six samples were applied at the same time (five standards and a mixture of these standards).

Development was realized with 2% sodium citrate in 25% ammonia, the chamber being saturated with aqueous ammonia. A comparison of the results obtained with different chromatographic techniques is given in Fig. 4.

The R_F values differ slightly from those obtained with the conventional descending technique, as can be seen in Table III.

TABLE III

COMPARISON OF R_F VALUES OBTAINED WITH DIFFERENT DEVELOPING TECHNIQUES

Dye	Developing technique			
	Centrifugal	Radial	Ascending	Descending
M	0.15	0.16	0.09	0.06
N	0.28	0.31	0.27	0.23
C	0.50	0.57	0.49	0.36
P	0.71	0.72	0.65	0.56
S	0.83	0.83	0.77	0.73

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SUMMARY

A pressureless apparatus with centrally developed centrifugally accelerated chromatography was constructed and tested. With this apparatus chromatographic separations are accelerated without any additional equipment (pressure supply source) being necessary. Application of all the conventional chromatographic methods is possible. This apparatus is now being patented.

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