CENTRIFUGAL CHROMATOGRAPHY

V. APPARATUS FOR PREPARATIVE-SCALE PAPER CHROMATOGRAPHY IN THE CENTRIFUGAL FIELD*

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The three main problems in preparative-scale paper chromatography are (1) lengthy separation, (2) the small amounts of substance that can be separated and (3) the discontinuous character of the separation. Conventional apparatus for centrifugal chromatography which are suitable for rapid chromatographic separation (CARONNA[1], MCDONALD *et al.*[2], TATA AND HEMMINGS[3], PAVLÍČEK *et al.*[4,5], DAUPHIN *et al.*[6], HERNDON *et al.*[7], MATTHEWS AND CERVANTES[8]) are useful only for analytical purposes; the possibility of semimicro preparation is limited by the capacity of the chromatographic paper. Another disadvantage of these apparatus is the impossibility of carrying out a continuous chromatographic separation.

The technique of continuous paper chromatography and the device used have already been described by SOLMS[9]; this method is very efficient in obtaining preparative separation, but the time required for separation is extremely long.

For achieving quick and continuous paper chromatography the apparatus for centrifugal chromatography as described in one of the previous communications[5] was adapted here.

PRINCIPLE OF CONSTRUCTION AND USE OF THE APPARATUS

The sample is continuously deposited by means of a capillary tube onto the rotating disc of chromatographic paper, close to the mobile phase inlet. The capillary tube undergoes only a slow relative rotary motion (1 rev./20 min–6 h). Assuming that the capillary tube dispenses the sample at time t at a point x (see Fig. 1), then the chromatographic trace at this point is directly on the start. If the continuous chromatograph has been in motion for some time prior to time t, at this moment the mobile phase flow will have caused a partial (or complete) chromatographic separation at a point y, located counter to the relative rotary motion. The trace of each of the separated components is a spiral, the curvature of which depends on the R_F value or rather on the nature and form of the separating function. The next factor bearing on the

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course of the spiral is the rate of motion of the capillary tube with respect to the disc of chromatographic paper. If the period of one relative revolution of the capillary tube with respect to the paper disc just corresponds to the period within which the spot reaches the front of the paper, the spot (spiral) revolves once about an angle of

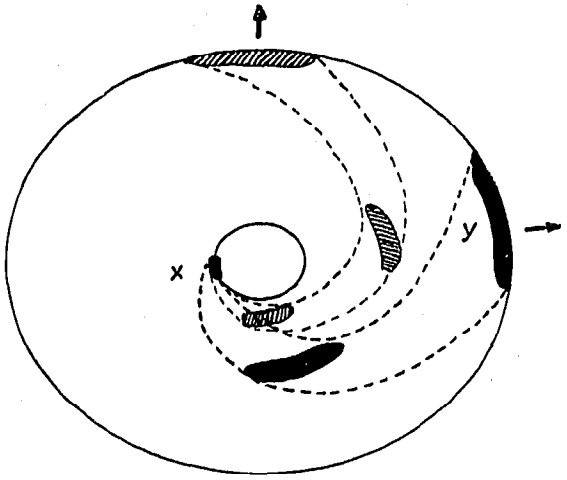


Fig. 1. Schematic representation of the continuous separation of two compounds with R_F values of 1.00 and 0.40.

360°; if this trace simultaneously corresponds to the substance with the highest R_F value the other substances (with lower R_F) revolve about an angle less than 360°. Practically speaking, it is not essential (because of unnecessary prolongation of the separation process and the possibility of the influence of diffusion or of the irregularity of the paper) that the substance with the highest R_F should revolve about 360°, but it may revolve about any central angle α, less than 360°, depending on the nature of the mixture to be separated. The scheme in Fig. 1 shows the separation of two substances of $R_F = 1.00$ and $R_F = 0.40$, the revolution time of the capillary tube being here just half the time necessary for the spot ($R_F = 1.00$) to reach the front of the paper under steady-state conditions.

The spiral of the substance to be separated follows a rotary course along the paper disc and the speed of the latter equals that of the capillary tube.

The fraction of the pure substance is collected from the front of the paper by various types of fraction collectors (see next section) which rotate at the same speed as the capillary tube. The capillary tube and a type of fraction collector form one construction unit. If the position of the capillary tube in this unit is denoted as radius vector r_0, then, according to a selected angular velocity of the capillary tube with respect to the paper disc, the fraction with the highest R_F in the fraction collector will be found lagging by the angle α behind the relative motion of the capillary tube, *i.e.* by the angle travelled by the capillary tube during the time required by the spot of the substance to cover the distance from the start to the front of the paper.

DESCRIPTION OF THE APPARATUS

The apparatus for the continuous paper chromatography is in principal the same as that described by PAVLÍČEK *et al.*[4,5]; the capillary type of apparatus has been used as distributor of the mobile phase[5].

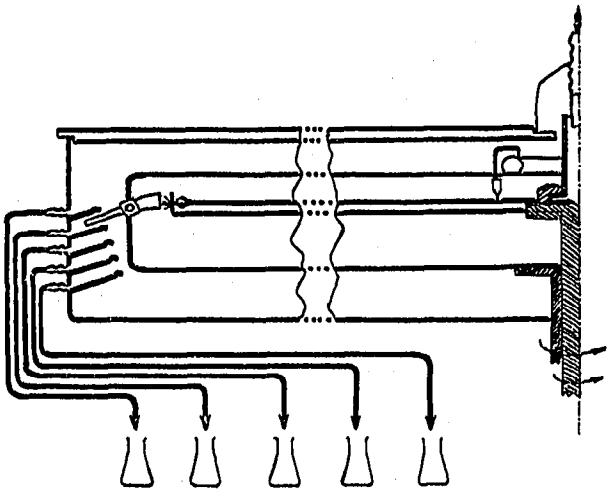


Fig. 2. Fraction collector; the separated fractions are collected along the wall of the chromatographic chamber.

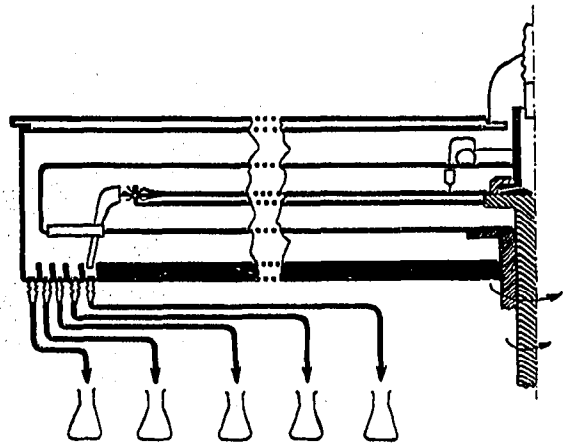


Fig. 3. Fraction collector; the separated fractions are collected on the bottom of the chromatographic chamber.

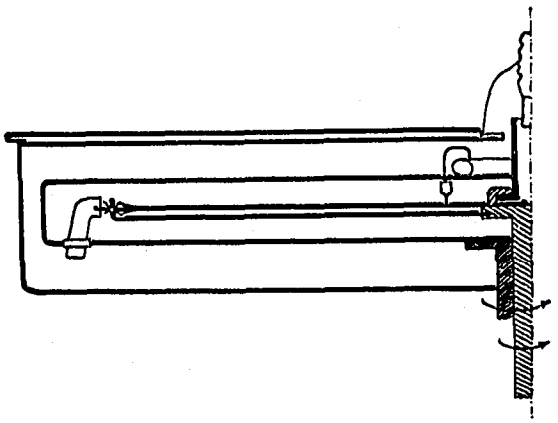
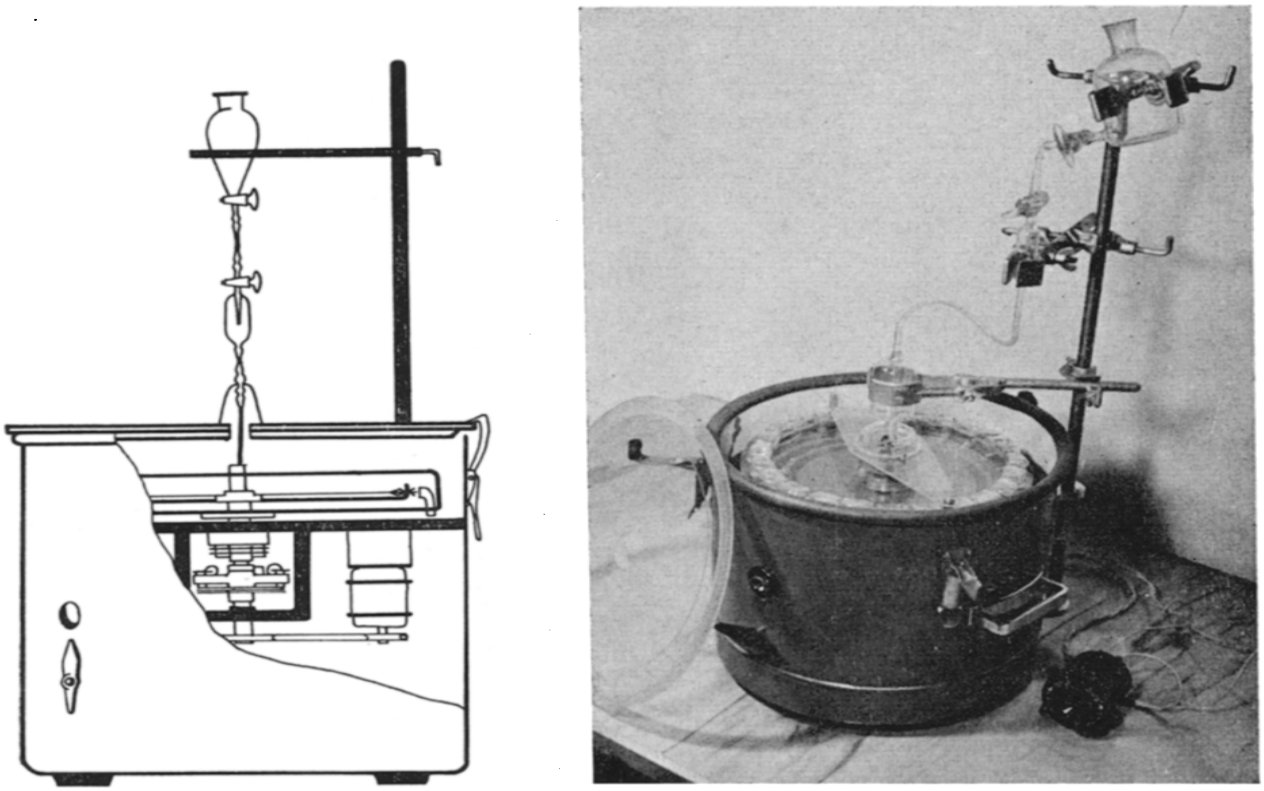


Fig. 4. Fraction collector; the separated fractions are collected in glass tubes along the disc of the chromatographic paper.

The apparatus is completed by one of three arrangements of fraction collectors; these are schematically shown in Figs. 2–4. The over-all scheme of the apparatus is given in Fig. 5. According to the arrangement of the fraction collector the separated fractions are collected either along the wall of the apparatus (Fig. 2), or on the bottom of the apparatus (Fig. 3). In the third arrangement the separated fractions remain in the glass tubes of the fraction collector. The last type is suitable only for separations where the volume of each fraction does not exceed approximately 1 ml.



a b

Fig. 5. (a) Overall scheme of the apparatus for continuous centrifugal chromatography. (b) Photograph. In this case the fraction collector shown in Fig. 4 is used.

The continuous centrifugal chromatography described reduces the time for separating 200–700 mg of substance to one-sixth in comparison with the descending technique.

SUMMARY

A simple technique and the necessary apparatus for quick preparative-scale paper chromatography is described. In order to reduce the time of separation the principle of centrifugal paper chromatography was used. The device permits a precise and controlled separation of the spotted sample into individual fractions within one-third to one-tenth of the time required for conventional chromatography.

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