# Chromatography with curved flow way

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A new method suited for continuous separation of neutral substances on chromatographic paper has been developed. This method is convenient for the purpose of preparation. The form of paper gets the substances to proceed on a curved way.

Был разработан метод разделения нейтральных веществ континуальным способом на хроматографической бумаге, который подходит для препаративных целей. Форма бумаги обуславливает движение веществ по искривленной траектории.

Present chromatography does not enable us to separate a greater amount of neutral substances in a continuous or semicontinuous manner. Only substances of ionic character may be separated by electrophoresis [1—6] in a continuous manner and the existing continuous chromatography with rotating carrier [1, 4, 5, 7, 8] makes possible to separate only small amounts of substances. The shortcoming of my original preparative method also consisted in the fact that the fraction collector had to move simultaneously with the moving fractions on the paper [9].

## Experimental

### *Separation of a mixture containing two coloured water-soluble substances*

3 g of the dye Saturn yellow L4G and 3 g of the dye Saturn blue L3R (VCHZ), Pardubice-Rybitví) were weighed and dissolved in 500 cm³ of the mobile phase $S_1$ (isoamyl alcohol, *ca.* 26% aqueous solution of ammonia, pyridine (1 : 1 : 1.3)) [10]. On chromatographic paper Whatman 2 in this system, the $R_f$ values of the Saturn yellow L4G and the Saturn blue L3R were 0.54 and 0.24, respectively. Three pieces of chromatographic paper

Whatman 2 of equal size cut according to Fig. 1 (*A*) were put on each other and situated on the glass plate *B* and *P* (Fig. 1). About 200 cm³ of the mobile phase $S_1$ were poured on the paper which got thoroughly wet. By rolling movement of a glass tube, the paper was pressed

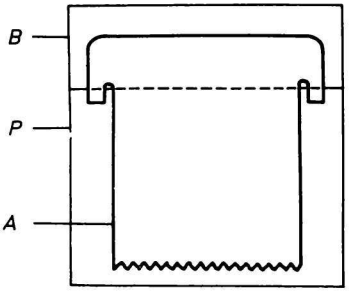


*Fig. 1.* Shape of the chromatographic paper *A* put on the glass plates *B*, *P* for soaking.

on the glass. By rolling the tube on the paper, the air was pressed out and the wet paper got clung to the glass base. Then the glass plate *B* as well as paper was carefully taken up so that the rest of paper got detached from the glass plate *P*. The glass plate *B* was hold at the bottom in one hand, a part of paper was situated on the plate and other part of paper hung from the plate. The drops of solvent which were on the glass beside the paper were drained with other hand by means of filter paper. The borders of the paper which did not get attached to the glass were also pressed with a piece of paper. Beforehand, the support *N* was put on the bars $O_1$ and $O_2$. The cells *E* forming communicating vessels were put on this support (Fig. 2). Then the glass plate *B* with the papers *A* was laid into the chamber on the bars $C_1$ and $C_2$. The plate *B* with the papers *A* was put into the chamber so that the start of the papers $D_1$ and $D_2$ reached into the vessels *E*. The hanging part of paper was placed over the vessel *K*, where the eluate dropped from the paper. The pure mobile phase $S_1$ saturating the chamber was poured in its bottom *G*, where the fraction collector *K* with the outlets *L*, *M* was placed. The fraction collector could be slid forward or backward owing to which a narrower or wider part of eluate was to be taken from the central part of the paper.

Fig. 3 shows the schematic form of chromatogram, where *I* designates the most rapidly proceeding zone which is continuously caught from the aperture *L* and *II* denotes the slower component of mixture which gets out of the aperture *M* and is given back by the device *F* into the vessels *E* for repeated separation. The chamber is covered with the plate *H* and the device *F* is put inside through the hole of this plate to reach into connecting tube of the vessels *E*. Thus the apparatus is ready for working. Then 100 cm³ of the mobile phase $S_1$ is poured through the device *F* into the vessels *E* and when the moisture in paper has been balanced (on the next day at best), a mixture of the dyes dissolved in 500 cm³ of $S_1$ is added through the device *F* into the vessels *E*. The paper sucks the solution of dye mixture from the vessels *E* and the mixture is separated on paper. The more movable yellow fraction proceeded in front. As the mixture was sucked symmetrically from both sides of paper, the more movable fraction was concentrated in the centre of paper and trickled down into the underlaid collector *K*. The eluate (*I*) was outlet through the exit *L* (Fig. 3). The less movable portion (*II*) of the mixture flowed in the second section of the collector *K* and

subsequently it was discharged through the hole $M$ (Fig. 3). As this fraction contained a mixture which had not been separated yet, it was given back into vessels $E$ through the device $F$. Simultaneously, the remainder of the stock solution of dye mixture was added into the device $F$. When the dye mixture subjected to separation was consumed, the pure mobile

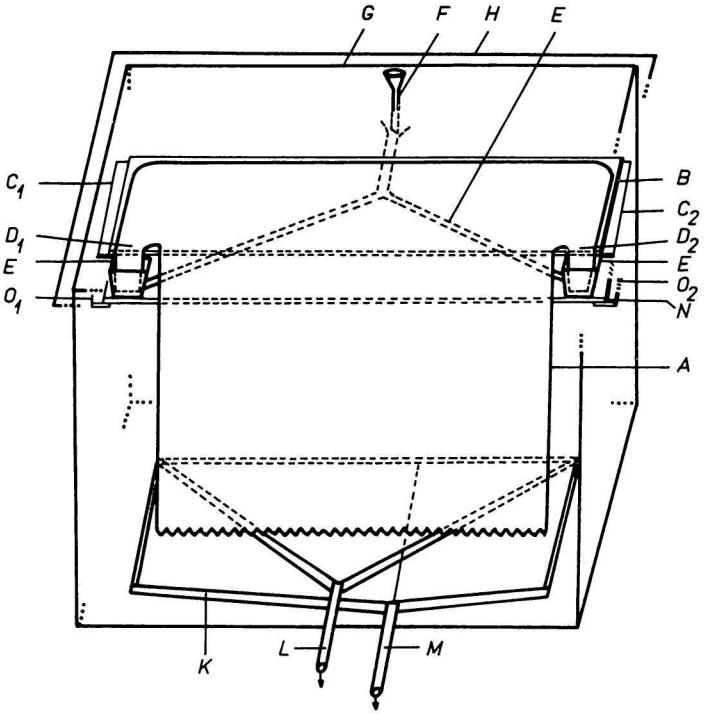


*Fig. 2.* Apparatus for chromatography with curved flow way.

$A$ — Chromatographic paper; $B$ — glass support; $C_1$, $C_2$ — metal plate bars, sealed to plate chamber, on which the glass support $B$ is situated; $D_1$, $D_2$ — start of chromatogram; $E$ — communicating vessels for separated mixture; $F$ — device for pouring solution containing the mixture or pure solvent; $G$ — metal plate chromatographic chamber. A part of the front wall is replaced by glass, at the bottom of the front wall there are two holes in which the outlets of fractions reciprocate from the front backwards and the other way round. The top borders of the metal plate bear a round groove with rubber packing to which the glass covering plate $H$ clings; $K$ — fraction collector situated under the paper in the chamber. It has two compartments: the more movable fraction which proceeds through the tube $L$ flows from the centre of the paper into the triangular compartment. The less movable fraction which proceeds through the tube $M$ flows from the sides of the paper into the second compartment. This fraction is given back through the device $F$ into the communicating vessels $E$ for further separation. The fraction collector may reciprocate from the front backwards and the other way round owing to which a greater or smaller portion of eluate from the paper may be caught; $L$ — exit of the more movable fraction; $M$ — exit of the less movable fraction; $N$ — support of the communicating vessels $E$; $O_1$, $O_2$ — metal plate bars for putting the support $N$.

phase $S_1$ was poured into the vessels $E$. After the eluation of the yellow component, the subsequent most movable component appeared in the centre (Saturn blue). Since only two dyes were taken and the yellow component had been separated, the blue component filled the whole paper. Then the procedure could be finished and the blue dye was extracted in a faster way.

### *Separation of a mixture containing two in water insoluble coloured substances*

Two dispersion dyes, *i.e.* 50 mg of Foron brillant orange E-RL-200% and 50 mg of Foron rot S-FL, were weighed. The dyes were dissolved in 5 cm³ of the mobile phase $S_{8a}$ (mixture of pyridine and water (2:1) saturated with $\alpha$-bromonaphthalene). Two pieces of the chromatographic paper Whatman 2 of the shape represented in Fig. 1 were drawn in a trough filled with a 20% solution of $\alpha$-bromonaphthalene in petroleum ether. The paper was allowed to dry for 15 min in air. As the paper in this state did not sit tight on glass but became detached, it was soaked with 100 cm³ of the mobile phase $S_{8a}$, pressed with a glass tube and put into the chamber. First of all, 50 cm³ of the solvent $S_{8a}$ were poured in the vessels $E$ and on soaking, the solution of dyes was added. Then the pure mobile phase was added and the subsequent procedure was like that described in the preceding experiment. The red component was the first to proceed.

### *Separation of a mixture containing more than two components*

The mixture is separated into intermediate fractions containing two components and these fractions are separated on a new paper in the same manner as described in the first case. The working procedure may be modified as follows. The paper is soaked with water, pressed with

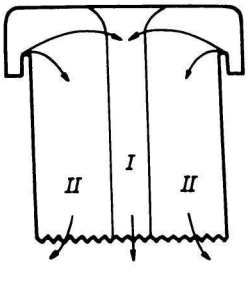


*Fig. 3.* Form of chromatogram.
*I* — The more movable fraction. This fraction coming from both starts is concentrated in the middle of the paper; *II* — the less movable fraction which cannot get in the centre before the more movable fraction has been eluated from the paper.

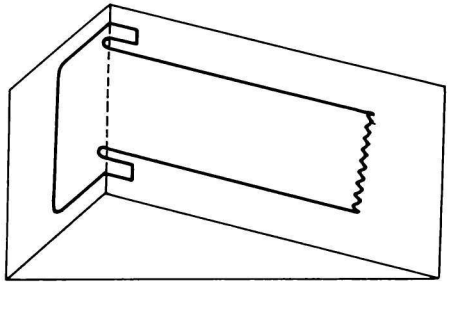

*Fig. 4.* Support for putting and wetting a greater number of chromatographic papers.

a tube, put into the chamber and the pure mobile phase is poured into the vessels $E$. The paper is allowed (usually overnight) to get wet with the mobile phase. Afterwards, the solution containing the investigated substances is added and it is proceeded as described before.

### *Separation of a mixture containing colourless substances*

The procedure is similar to that used in preceding cases, the only difference being that the collecting trough is so put under the paper that it corresponds to 20—25% (or less) of the paper. For instance, if the paper has 21 tips, the more movable fraction is taken from the five central tips and the united eluate from the 16 tips (*i.e.* 8 tips on the right side and 8 tips on the left side of the centre) is given back for repeated separation. The more movable fraction is collected and analyzed in small amounts as usual in the separation of colourless substances by column chromatography.

## Discussion

The developed method completes the kinds of continuous separation of substances in greater amounts. In contrast to electrophoresis and magnetophoresis, it enables us to separate even neutral substances. In comparison with the *Solms* continuous method [7] involving rotating paper, the efficiency is enhanced because 10 and even more papers may be put upon each other.

The paper capacity may be estimated as follows. 1 $dm^2$ of the Whatman 2 paper weighs 0.92 g. The separation capacity of 100 g of the paper for the use of 2% solution is about 3.5 g of a substance. 100 g of the paper supplies about 10—40 $cm^3$ of the eluate in one hour.

The flow in the paper is influenced mainly by these factors: The closer to the paper is the level of liquid in the vessel $E$, the greater is the rate of flow in the paper. Therefore it is the best, to keep the maximum level by proportioning excess solution (*e.g.* by micropump) so that the excess drips back into stock solution. It is more useful to put the vessels under the glass on which the paper is laid because this paper sucks as much solution as it is able to separate. If the vessels are over the paper, too much solution can trickle on the paper which is not able to separate it and the mixture gets diffused on the paper.

The paper put horizontally on the glass brings about the highest rate of flow whereas the paper put obliquely or vertically with respect to the glass reduces the rate of flow. The longer is the upright part of paper, the greater is the rate of flow. The wider is the start of the paper, the richer is the flow.

The volume of eluate depends on the number of the papers which are put upon each other on the glass. The experiments have shown that it is possible to work even with a layer of 20 papers. If a greater number of papers is used, after wetting

they do not remain upon each other at the bending where they hang from the glass. This inconvenience may be eliminated if a rectangular support holding two glass plates on which the papers are put is used for soaking the papers (Fig. 4). In this case, the transport of the glass plates and papers does not result in a change of position of the papers and no deformation appears at the bending.

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