

CONTINUOUS PREPARATIVE THIN-LAYER CHROMATOGRAPHY

R. VISSER

Department of Chemical Technology, Technological University Twente, Enschede (The Netherlands)

(Received November 1st, 1966)

Chromatography has its main application in solving analytical problems, but since it can produce essentially pure compounds, preparative methods are of interest for synthetic work, for those identification techniques which need mg amounts and for isolation of minor constituents in mixtures. "Scaling-up" of the separation can be effected by enlargement of dimensions, by reiteration of a given cycle, if possible by automatic methods, and by continuous or semi-continuous procedures. Semi-continuous methods are regarded as consisting of a series of physically separated non-continuous systems following each other at constant time intervals.

For gas-liquid chromatography the first two methods, *i.e.* with large columns¹⁻⁴ and with a repeated cycle⁵⁻⁷ are well-known. Recently, some continuous methods have been described⁸⁻¹⁰, based on the counter-current principle, but their application seems to be limited. A semi-continuous method ("simultaneous multi-column analysis") has also been reported¹¹. In paper chromatography both large-scale¹²⁻¹⁶ and continuous^{17,18} procedures are applied. For column chromatography large-scale techniques^{19,20} and a semi-continuous method have been described²¹. Elegant methods for large-scale²² and continuous^{23,24} electrophoresis are well-known. Finally, large-scale preparative thin-layer chromatography has recently been developed²⁵.

Repetitive and continuous techniques are mainly based on the elution method, large-scale techniques both on the elution method (paper- and column chromatography) and on the fundamental procedure (paper-, column- and preparative-layer chromatography and block-electrophoresis).

The principal condition for a continuous method is movement of the complete system to be separated perpendicular to the direction in which the separation takes place. Thus, in continuous electrophoresis the separation is performed along a horizontal axis and the system as a whole is descending. In continuous cylindrical paper- and column chromatography the separation is realized parallel with a vertical axis, whilst the system is rotating around this axis, each point moving along a horizontal circle. In continuous circular paper chromatography the separation takes place along the radii, whilst each point of the system is moving along a circle.

Thin-layer chromatography has been widely used for analytical purposes, but no continuous preparative methods have so far been described. Both principles, cylindrical and circular, are at present being studied but in this paper only the analogue of a cylindrical system will be discussed.

DESCRIPTION OF THE APPARATUS

A diagram of the apparatus is shown in Fig. 1. Separations are performed on a belt of impermeable material (A) resistant to a temperature of 120° and to chemicals normally used in thin-layer chromatography. For this purpose PTFE on a glass-fibre base (thickness 0.35 mm) was chosen. As there were no endless belts commercially available, the ends of a rectangular sheet (width 0.25 m) were connected with cotton yarn and the seam covered with a strip of FEP (polyfluorethylenepropylene), heated under slight pressure to 275° . The belt passes around two horizontal cylinders (B and C) with a diameter of 74 mm, positioned in one horizontal plane, distance 0.60 m. The upper part is led over a flat plane, which is the upper surface of a series of compartments (D, E and F) between the upper and lower parts of the belt.

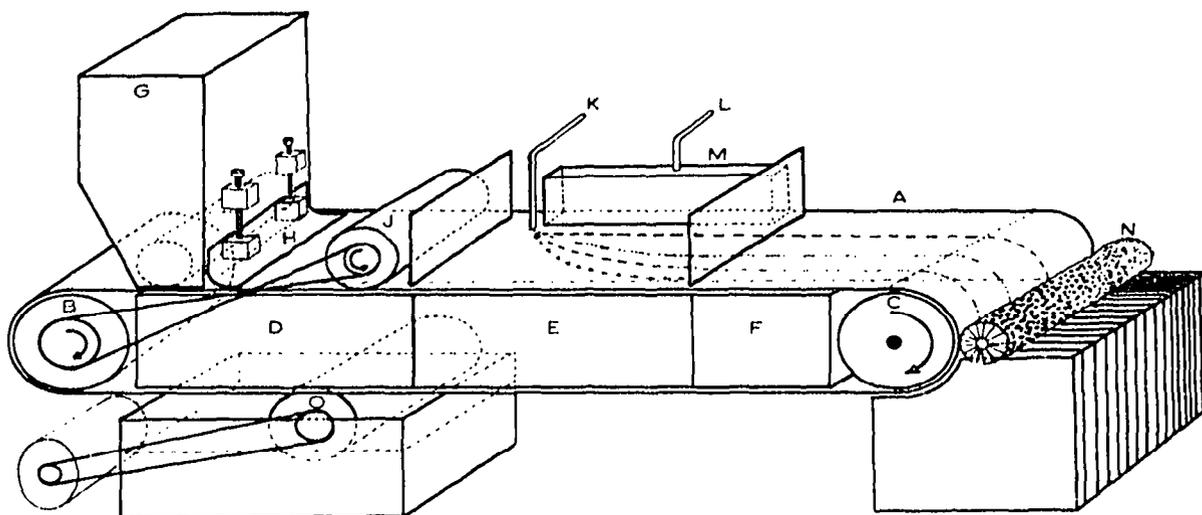


Fig. 1. Schematic view of the apparatus. The characters are explained in the text.

At the starting point on the upper surface of the belt a tank (G) is placed, containing dry silica gel, aluminium oxide or another adsorbent. Through an adjustable slit (H) a continuous layer of e.g. 1-mm thickness is spread on the moving belt. This layer is compressed by a cylinder (J) and, if desired, activated by heating by means of electric heaters mounted in the first compartment (D) (length about 0.20 m); moreover, the rest of the compartment can be heated by a circulating liquid.

At the beginning of the second part a continuous strip of the mixture to be separated is applied to the surface through a stainless steel capillary tubing (K), the distance to one edge being about 40 mm. In this part (length 0.23 m) the separation is also performed. To this end a continuous constant stream of eluant is fed through a tube (L) into a container (M) about 0.16 m long, placed along the edge at the same side as the point where the mixture to be separated is applied. This container is filled with cotton or glass wool and has a slit through which a strip of thick filter paper passes. This strip touches the border-line of the adsorbent layer to make contact for the solvent. Separation takes place perpendicular to the moving direction of the belt, the solvent going to the opposite edge. In order to perform the separation at a constant

temperature, the compartment under this part of the belt (E) can be connected with a circulating thermostat bath.

In order to obtain a reproducible separation dosage pumps were used for the application of both the mixture to be separated (Braun type Unita IIb) and the eluant (I.K.B, type 4900, all-teflon model).

In the third part the solvent is evaporated by heating and venting. The layer is removed by a rotating brush (N) with spindle in the same horizontal plane as the shaft of cylinder (C), and divided over a series of containers.

Finally the belt is cleaned by a rotating cylinder of foam plastic, partially immersed in a tank filled with running water (O).

The belt is driven by a synchronous motor equipped with a gearbox and a clutch. The normal speed is 0.125 m/h.

Most of the construction was done with stainless steel. The upper side is covered with glass plates. Since the distance between these plates and the upper surface of the belt is about 60 mm, a closed box of teflon and glass is positioned in the developing compartment. This reduces the volume to be saturated with the eluant vapour, leaving about 5 mm between the upper surface of the layer and the box.

RESULTS

As an example, the results of the separation of a mixture of three azo dyes are given. Some data are listed in Table I. The separation was performed by application of 0.678 ml of a solution containing 0.5% w/v of each of the three dyes in benzene during 1.5 h. Benzene was used as an eluant, and the flow rate was 51 ml/h. Kieselgel H (Merck) was used as an adsorbent; the thickness of the layer was 0.6 mm after compression. Longer runs were subject to some improvements in the apparatus.

The determinations were carried out by means of UV-spectrophotometry, after extraction of the fractions with ethanol in a soxhlet apparatus. The ethanol extract of one receiver between the front line and the first dye was used as a blank.

The separation was slightly better than separation performed with the same concentration on an analytical plate (ascending development). However, only receivers

TABLE I

AMOUNTS OF THREE AZO DYES FOUND IN A SERIES OF RECEIVERS AFTER SEPARATION

Number of receiver	<i>p</i> -Amino-azobenzene (mg)	<i>p</i> -Phenyl-azoaniline-2-naphthol (mg)	<i>p</i> -Dimethyl-amino-azobenzene (mg)
7	0.016	—	—
8	0.302	—	—
9	2.275	—	—
10	0.525	1.400	—
11	—	1.125	—
12	—	0.761	0.655
13	—	—	2.000
14	—	—	0.198
15	—	—	0.010
Total	3.118	3.286	2.863

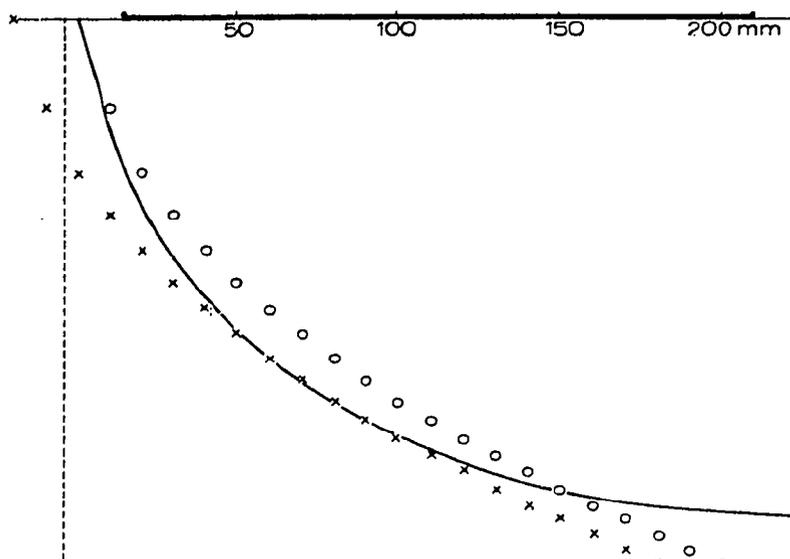


Fig. 2. Diagram of solvent front. The thick part of the y-axis indicates the length of the solvent contact strip.

with a width of 10 mm were available; as a result the border lines between two receivers did not coincide with the borders between two zones and overlap was found.

The shape of the front was studied (Fig. 2). If the y-axis is the edge of the layer (at the side of the eluant tank), the x-axis perpendicular to it and the origin of the coordinate system in the intersection of the front-line with the edge of the adsorbent layer, fair agreement could be found between the experimental curve (drawn line) and a parabola of the formula:

$$x^2 = a + c \cdot y$$

with $a = -1000 \text{ mm}^2$ and $c = 210 \text{ mm}$ (circles). Since the speed along the y-axis is 125 mm/h this curve is identical with

$$x^2 = a + b \cdot t$$

(t in h) with $b = 26\,000 \text{ mm}^2/\text{h}$. The values of a and b are equal to those found in the literature²⁰.

Improved agreement was reached by shifting the theoretical curve 20 mm to

TABLE II

R_F VALUES FOUND FOR A PREPARATIVE AND A STANDARD ANALYTICAL SEPARATION

Compound	R_F found	
	This experi- ment	Analytical plate
<i>p</i> -Aminoazobenzene	0.19	0.14
<i>p</i> -Phenylazoaniline-2-naphthol	0.29	0.23
<i>p</i> -Dimethylaminoazobenzene	0.41	0.41

the left (crosses). The discrepancies at both ends might be explained by slight evaporation close to the heated activation and drying compartments.

The results indicate that the elution pattern is essentially the same as with horizontal development of a standard analytical plate. This conclusion is supported by the R_F values obtained for this method and the standard ascending technique (Table II). Slight discrepancies may be explained by differences in the silica gel used (Kieselgel G for the standard plate) and in activation.

DISCUSSION

Continuous and semi-continuous preparative separations of non-volatile materials can be carried out with paper chromatography, thin-layer chromatography and column chromatography.

Comparison of continuous cylindrical and circular paper chromatography with continuous thin-layer chromatography leads to the conclusion that thin-layer chromatography has the following advantages. First, the stationary phase is continuously removed, so that no contamination with materials remaining from a former cycle can occur. Secondly, there is no need for long development times, *i.e.* it is not necessary to wait until the slowest compound has left the adsorbent; although this procedure leads fundamentally to a less efficient separation than elution analysis, the separation might come out better since shorter times and more homogeneous adsorbing materials lead to less diffusion (both longitudinal and "eddy" diffusion). Finally, the homogeneous adsorbent gives a more reproducible separation.

Column chromatography has, compared with thin-layer and paper chromatography, a great advantage in that the optimum plate number can be achieved by variation of the pressure upon the eluant²⁷. However, semi-continuous column chromatography needs a series of identical columns. If this is not realized, the overall efficiency might be worse than that for each individual column. Furthermore, the total time of a cycle is again determined by the time the slowest compound needs to leave the column.

Finally, continuous thin-layer chromatography compared with preparative-layer chromatography demands a smaller amount of labour; ideally, it works without control. A major problem is that the speed of elution is determined by the rate of the viscous flow of the eluant liquid across the adsorbent. This means that the speed of the belt, the eluant flow and the thickness of the layer are closely related. As a result the supply of the eluant is critical and an additional regulating device should be designed in order to obtain the optimum flow.

Future developments

In principle it is possible to use the apparatus described above for continuous preparative thin-layer electrophoresis (or preferably electrochromatography). The simplest way to realize this is to mount two electrodes parallel to the edges of the adsorbent layer, to supply the buffer solution in a zone in the development compartment parallel with and directly behind the separation wall to the activation compartment, and the supply of the mixture to be separated at a point immediately behind the supply of the buffer solution.

It should be noted that the apparatus, though developed for preparative work,

could also be used for routine analytical separation of a series of samples, as a part of an "Auto-analyzer" type apparatus.

The author is indebted to prof. E. A. M. F. DAHMEN for his interest in this investigation, and to personnel of the Central Technical Service of this university for design and construction of the apparatus.

SUMMARY

A short survey of preparative chromatographic methods is given. An apparatus for general use in continuous preparative thin-layer chromatography is described, and its performance compared with some related preparative techniques.

REFERENCES

- 1 D. E. M. EVANS, W. E. MASSINGHAM, M. STACEY AND J. C. TATLOW, *Nature*, 182 (1958) 591.
- 2 J. J. KIRKLAND, in V. T. COATES, H. J. NOEBELS AND I. S. FAGERSON (eds.), *Gas Chromatography*, Academic Press, New York, 1957, p. 203.
- 3 F. H. HUYTEN, W. VAN BEERSUM AND G. W. A. RIJNDERS, in R. P. W. SCOTT (ed.), *Gas Chromatography 1960*, Butterworths, London, 1961, p. 224.
- 4 E. BAYER, in R. P. W. SCOTT (ed.), *Gas Chromatography 1960*, Butterworths, London, 1961, p. 236.
- 5 E. HEILBRONNER, E. KOVÁTS AND W. SIMON, *Helv. Chim. Acta*, 40 (1957) 2410.
- 6 H. BOER, *J. Sci. Instr.*, 40 (1963) 121.
- 7 H. BOER, *J. Sci. Instr.*, 41 (1964) 365.
- 8 D. GLASSER, *Proc. 6th Intern. Symposium on Gas Chromatography, Rome, 1966*, to be published (preprint no. 10).
- 9 P. E. BARKER AND D. H. HUNTINGTON, *ibid.* (preprint no. 11).
- 10 P. E. BARKER AND D. H. HUNTINGTON, *J. Gas Chromatog.*, 4 (1966) 59.
- 11 D. DINELLI, S. POLEZZO AND M. TARAMASSO, *J. Chromatog.*, 7 (1962) 477.
- 12 H. K. MITCHELL AND F. A. HASKINS, *Science*, 110 (1949) 278.
- 13 W. L. PORTER, *Anal. Chem.*, 23 (1951) 412.
- 14 E. VON ARX AND R. NEHER, *Helv. Chim. Acta*, 39 (1956) 1664.
- 15 L. HAGDAHL AND C. E. DANIELSON, *Nature*, 174 (1954) 1062.
- 16 L. HAGDAHL AND K. D. LERNER, *Sci. Tools*, 5 (1958) 23.
- 17 J. SOLMS, *Helv. Chim. Acta*, 38 (1955) 1127.
- 18 M. PAVLIČEK, J. ROSMUS AND Z. DEYL, *J. Chromatog.*, 10 (1963) 497.
- 19 R. J. HALL, *J. Chromatog.*, 5 (1961) 93.
- 20 R. TERANISHI AND T. R. MON, *J. Chromatog.*, 12 (1963) 410.
- 21 H. SVENSSON, C. AGRELL, S. DEHLÉN AND L. HAGDAHL, *Sci. Tools*, 2 (1955) 17.
- 22 H. BLOEMENDAL, *Zone Electrophoresis in Blocks and Columns*, Elsevier, Amsterdam, 1963.
- 23 H. SVENSSON AND I. BRATTSTEN, *Arkiv Kemi*, 1 (1949) 401.
- 24 W. GRASSMANN AND K. HANNIG, in HOUBEN-WEYL, *Methoden der organischen Chemie*, Georg Thieme Verlag, Stuttgart, 1958, Vol. 1/1, p. 685.
- 25 H. HALPAAP, *Chem. Ing. Tech.*, 35 (1963) 488.
- 26 M. BRENNER, A. NIEDERWIESER, G. PATAKI AND R. WEBER, in E. STAHL (ed.), *Dünnschichtchromatographie*, Springer-Verlag, Berlin, 1962, p. 109.
- 27 J. F. K. HUBER AND J. A. R. HULSMAN, *Anal. Chim. Acta*, 38 (1967) 305.