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STEPWISE GRADIENT DEVELOPMENT IN THIN-LAYER CHROMATOGRAPHY

IV*. MINIATURIZED GENERATORS FOR CONTINUOUS AND STEPWISE GRADIENTS

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SUMMARY

A miniaturized generator of continuous gradients for equilibrium sandwich chambers with a glass distributor is described. The gradient profile is controlled by the initial concentration of the weaker eluent and its initial volume and by the composition and rate of delivery of the stronger eluent. Stepwise gradients are generated by delivery of eluents of increasing concentration of the stronger solvent from reservoirs in accordance with the gradient programme. Comparison of densitograms of thin-layer chromatograms from isocratic and gradient elution (continuous or stepwise) showed the considerable improvement in separation under gradient elution conditions.

INTRODUCTION

It was demonstrated in previous papers[1–3] that the equilibrium sandwich chamber with a glass distributor[4,5] is especially suitable for the miniaturization of gradient devices for thin-layer chromatography (TLC), owing to the pointwise delivery of the eluent to the distributor from a small container used as the mixing vessel[1,4]. For stepwise gradient elution, the eluent fractions can be introduced directly under the distributor[3]. Five eluent fractions of increasing eluent strength seem to be sufficient to avoid marked accumulation of bands on the fronts of increased concentrations of the stronger solvents in the mobile phase. Computer programs have been reported which present graphically the migration paths of individual solutes and calculate the final R_F values for given gradient progams[6,7]. The analysis of the migration paths permits the fine-tuning of stepwise gradient programs[7,8].

In the present paper, two simple and inexpensive devices are described: miniaturized generators of stepwise as well as of continuous gradients for the equilibrium sandwich chamber.

* For Part III, see ref. 6.

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EXPERIMENTAL

The horizontal equilibrium sandwich chamber with a glass distributor was obtained from Zaklad Odczynników Chemicznych, Polish Chemical Reagents, Lublin, Poland. The experimental technique is described in detail in Part II of this series[3].

Precoated 20 cm × 5 cm thin-layer plates (silica gel 60 F_{254}) or precoated high-performance (HP)TLC plates (10 cm × 10 cm) were obtained from E. Merck (Darmstadt, F.R.G.). The distance of development was 8–10 cm.

Continuous gradients were generated using the device shown in Fig. 1. The syringe pump (B) manufactured by Unipan, Warsaw, can be operated over a wide range of flow-rates depending on the diameter of the syringe; for a 10-ml syringe (15 mm I.D.) the delivery rates are in the range 0–20 ml/h. The 2-ml glass syringe (A) is filled with the stronger eluent and placed in a special holder. The bent long needle (M) is placed in the reservoir (R), which contains a small volume, *e.g.*, 2 ml of the weaker eluent. The capillary siphon (S) in the form of an inverted U-tube of stainless steel (1 mm I.D.) passes through an orifice in the cover plate and connects the reservoir with the slit under the distributor (D). A slight overpressure in the reservoir produced by a second syringe, temporarily connected to the short hypodermic needle (N) in the stopper, initiates the delivery. The chamber (Ch) is then shifted by several millimetres in the direction of the reservoir so that the distributor partly overlaps the edge of the layer. [In the starting possition the distributor is situated over the margin of the carrier plate scraped free of adsorbent[4].] The pump is then set into motion and the second syringe is removed from the short needle to allow equilibration of pressure inside the reservoir with the atmospheric pressure.

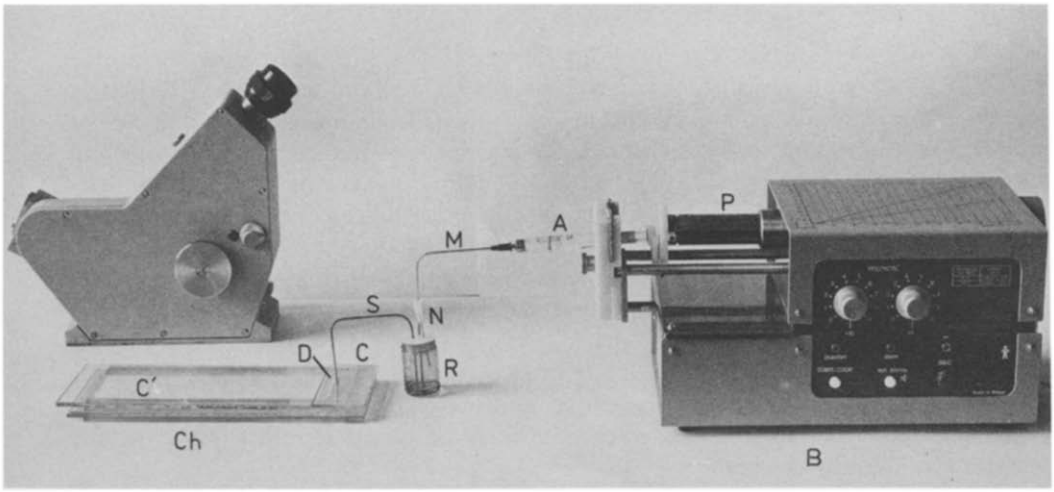

Fig. 1. Photograph of the syringe pump and the equilibrium sandwich chamber. A = glass syringe; B = infusion pump; R = reservoir of weaker solvent; S = capillary siphon; N = short injection needle; M = long injection needle; P = plunger; Ch = sandwich chamber; C = small cover plate with distributor welded to its lower surface; C′ = large cover plate; D = distributor.

To reduce or eliminate solvent-demixing effects[4] (formation of a solvent composition gradient along the direction of development —also in isocratic elution), it is best to wet the layer before elution with one distributor volume of the eluent from a micropipette through the orifice (D)[3]. (This corresponds to development over a distance of *ca.* 2 cm from the edge of the layer.) The samples can then be spotted through the slit between the two cover plates behind the front of the solvent. Then the siphon tip is introduced into the orifice (D), the siphon is connected and gradient elution is started.

Stepwise (five- or six-step) gradients were produced (*cf.*, also refs. 9–11) using a 2-m length of PTFE tubing (1 mm I.D.) wound in a spiral, on a carton or plastic disk, 20 cm in diameter, with eight radial cuts (Fig. 2). One end of the capillary was joined to a 2-ml hypodermic needle while the other end terminated in a bent stainless-steel capillary (1 mm I.D.).

Filling the PTFE tubing

The bent stainless-steel capillary is joined to the PTFE spiral as shown in Fig. 2a. Appropriate volumes of the eluent fractions are drawn consecutively into the PTFE tubing with the hypodermic syringe from a series of small test-tubes, according to the desired programme. Each fraction is separated from the preceding one by a small air-bubble. The fractions are introduced and stored in the capillary in the reverse order of delivery.

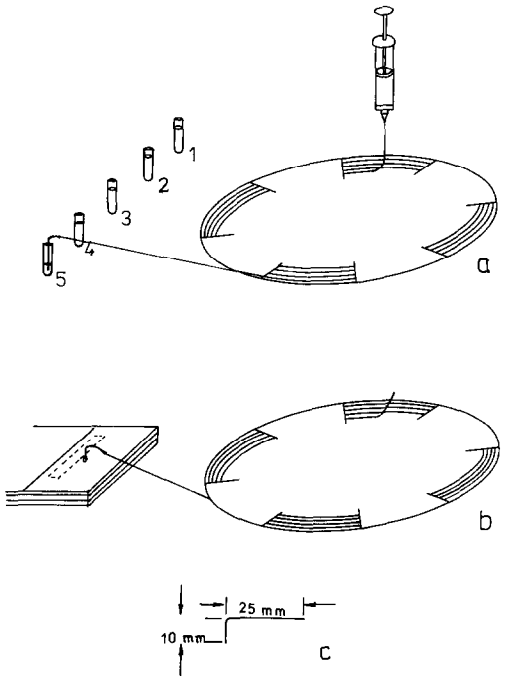

Fig. 2. Coiled PTFE capillary for storage of the eluent fractions. (a) Filling the capillary from a series of test-tubes; (b) the filled capillary is connected to the distributor of the sandwich chamber; (c) stainless-steel tip of the capillary.

Stepwise gradient elution

The bent stainless-steel capillary is inserted into the PTFE tubing with the longer arm (Fig. 2b) and its outlet (shorter arm) is inserted into the orifice of the chamber and thus brought into contact with the distributor. As before, the slit under the distributor is first filled with 0.1 ml of the weakest eluent by means of a micropipette. Then, one distributor volume of eluent is introduced into the layer and the samples are spotted between the cover plates on the pre-wetted layer. It is important that the coil be horizontal and on the same level as the adsorbent layer or slightly lower. The chamber and the flat coil are best placed next to each other on a table.

The flow of eluent from the PTFE tubing is started by slight overpressure, while the space under the distributor is filled. After starting the flow, the syringe at the end of the PTFE tubing is disconnected to allow the pressure in the tubing to equilibrate with the atmosphere.

The densitograms were obtained using a Shimadzu CS-930 densitometer.

As complex test samples, three drugs of plant origin were chosen: Seboren (*Fructus Pastinacae, Radix Bardanae, Radix Urticae, Rhizoma Calami*) from Herbapol Warsaw; Hemorigen (Erigeron Canadensis) from Herbapol Kłęka; Cholesol (*Cortex Frangulae, Herba Equiseti, Fructus Rosae, Anthudium Chamomilae, Fructus Corrandri, Fructus Juniperi, Herba Polygoni avic., Inflorescentia Helichrysi, Herba Hyperici, Intractum Teraxaci*) from Herbapol Wrocław. The separation of these mixtures was obviously incomplete, but the number of peaks discerned by the densitometer can be considered as a measure of the separation efficiency in the various elution modes.

A 250-mg amount of Seboren was evaporated to dryness. The oil residue was dissolved in ethyl acetate, filtered and evaporated again; 0.585 g of dry residue were dissolved in 3 ml of ethyl acetate and 8 μl of this solution were spotted. A similar procedure was used in the case of Hemorigen and Cholesol.

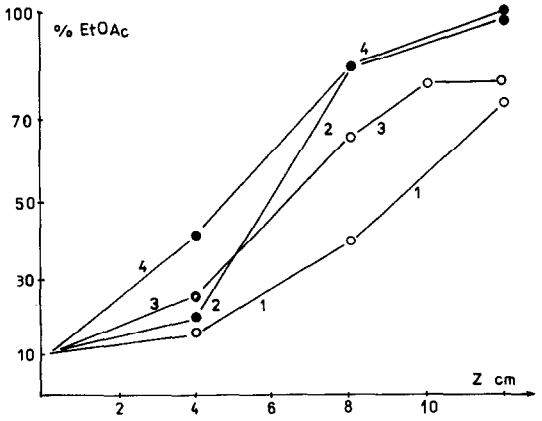

Fig. 3. Gradient profiles [percent ethyl acetate (EtOAc) plotted *vs.* migration distance of solvent front], determined refractometrically for various development conditions. Eluents: A = 10% ethyl acetate in chloroform; B = 100 ethyl acetate. Initial volume of eluent A: 1 and 2, 2 ml; 3 and 4, 1.5 ml. Flow-rate of eluent B: 1, 2 ml/h to a distance of 4 cm, then 4 ml/h; 2, 4 ml/h; 3, 4, 7 ml/h. 1 and 3, prepared layers; 2 and 4, precoated plates.

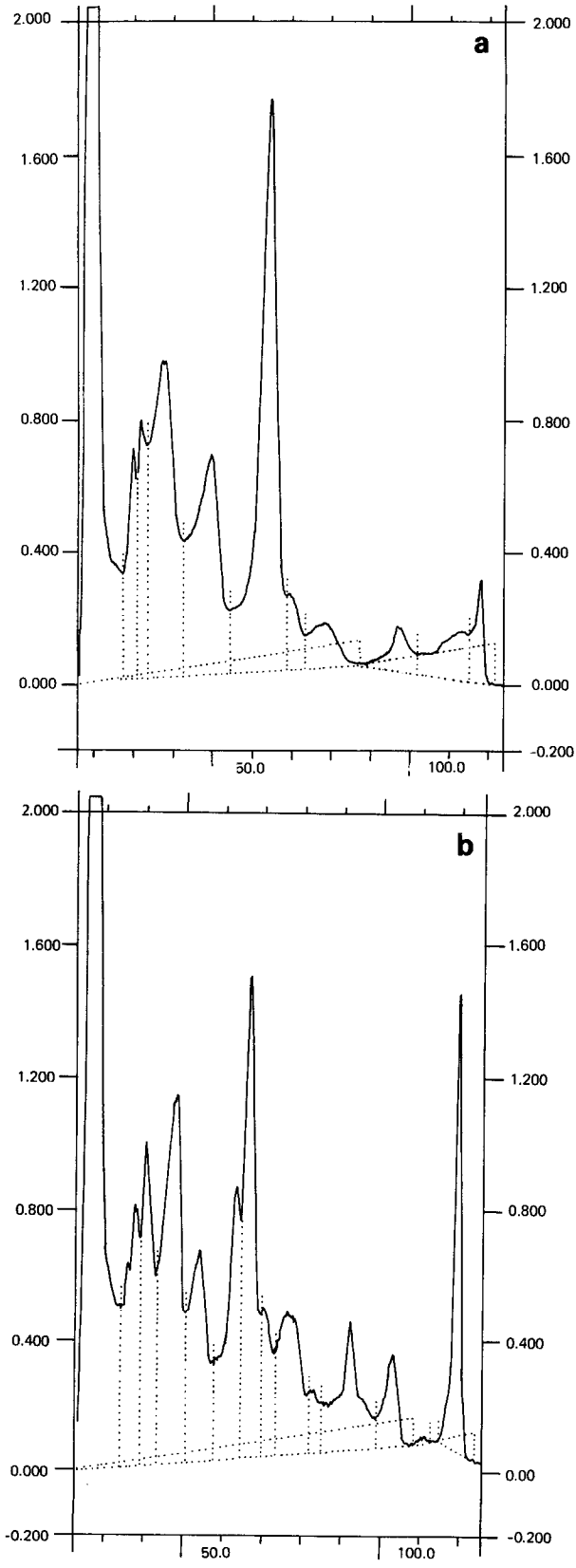

Fig. 4.

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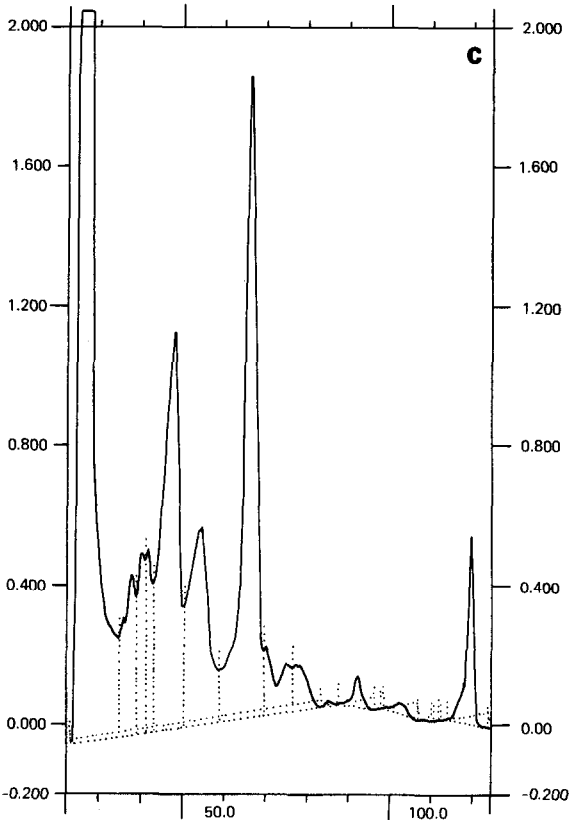

Fig. 4. Densitograms at 254 nm of Cholesol; (a) isocratic elution with 50% ethyl acetate in chloroform; (b) stepwise gradient with 0.1-ml fractions, 10 to 20 to 30 to 50 to 70% ethyl acetate in chloroform; (c) stepwise gradient 0.3 ml of 10% to 0.2 ml of 20% to 0.2 ml of 30% to 0.1 ml of 40% to 0.2 ml of 60% to 0.1 ml of 100% ethyl acetate in chloroform.

RESULTS AND DISCUSSION

In the case of continuous gradients, their profile could be varied by using various initial volumes of the weaker solvent in the reservoir (Fig. 1, R) and changing the composition and delivery rate of the stronger solvent from the syringe (Fig. 1, A). Several profiles for various delivery conditions are illustrated in Fig. 3.

Fig. 4a–c represent copies of densitometer printouts obtained for the Cholesol extract with two elution modes: isocratic and stepwise gradient. Comparison of the densitograms shows a marked increase in separation efficiency in the case of gradient elution. A still greater number of zones could be discerned visually under UV light (254 nm).

Chromatograms obtained by gradient elution of Hemorigen, Cholesol and Seboren extracts were photographed under UV light and are compared in Fig. 5.

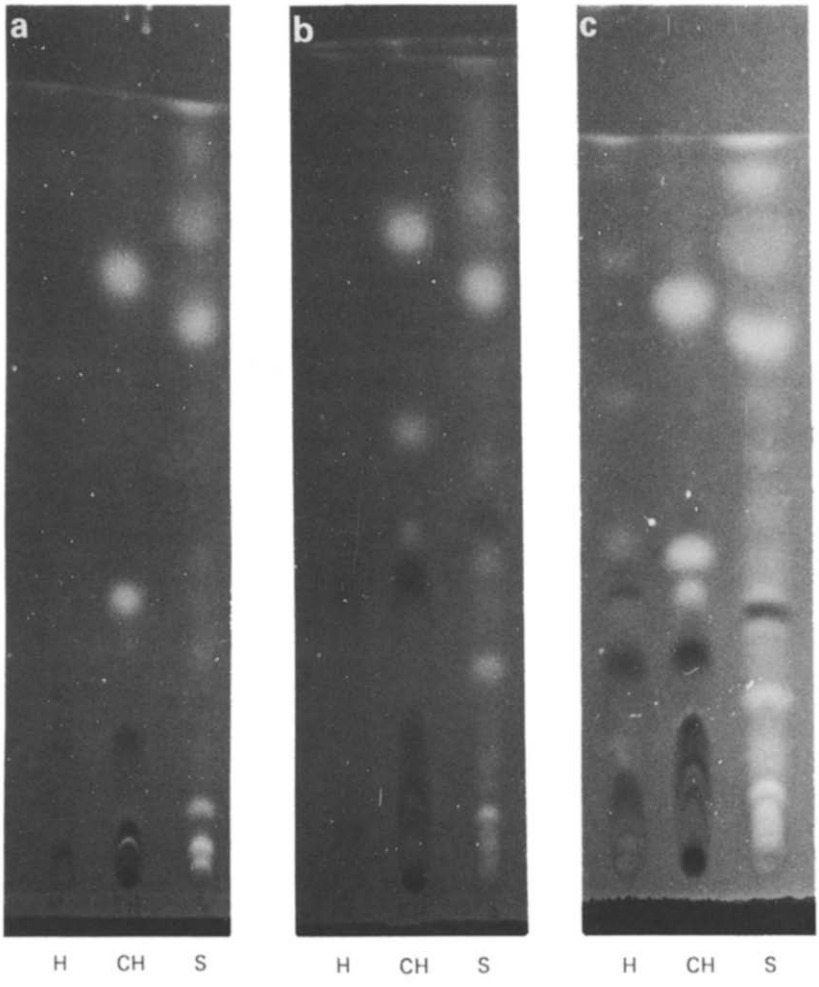

Fig. 5. Photographs under UV light (336 nm) of chromatograms of Hemorigen (H) , Cholestol (CH) and Soboren (S). (a) Isocratic elution, 20% ethyl acetate in chloroform; (b) isocratic elution, 50% ethylacetate in chloroform; (c) gradient elution, 10 to 70% ethyl acetate in chloroform.

CONCLUSIONS

Gradient elution, both continuous and stepwise, can easily be performed in sandwich chambers with a glass distributor.

Owing to the enhanced mutual displacement of the components of the sample and improved range of k' values the spots are compressed and the maxima in the densitograms more distinct so that an increased number of maxima is detected. Since the equipment is inexpensive (especially for stepwise gradients), the main advantage of TLC is retained. Solvent consumption is very limited to a few millilitres; such savings can be compared only to those reported by Siouffi *et al.*[12] who also employed an equilibrium sandwich chamber with a glass distributor. In other devices[13] a gradient

mixer is used and the excess overflowing eluent is discarded. It is worth pointing out that the increase in efficiency for micropreparative isolation purposes is even higher under overload conditions.

As demonstrated in an earlier paper[3], the reproducibility of R_F values in the gradient technique described is satisfactory ($\Delta R_F \leqslant 0.05$).

REFERENCES

1 E. Soczewiński and G. Matysik, *J. Liq. Chromatogr.*, 8 (1985) 1225.
2 E. Soczewiński, G. Matysik and K. Glowniak, in R. E. Kaiser (Editor), *Proc. 3rd Int. Symp. Instrumental High-Performance Thin-Layer Chromatography, Würzburg, 1985*, Institute for Chromatography, Bad Dürkheim, 1985, p. 413.
3 G. Matysik and E. Soczewiński, *J. Chromatogr.*, 369 (1986) 19.
4 E. Soczewiński, in R. E. Kaser (Editor), *Planar Chromatography*, Vol. 1, Hüthig, Heidelberg, 1986.
5 E. Soczewiński and T. Wawrzynowicz, *Chromatographia*, 11 (1978) 466.
6 E. Soczewiński and W. Markowski, *J. Chromatogr.*, 370 (1986) 63.
7 E. Soczewiński, G. Matysik and W. Markowski, *J. Liq. Chromatogr.*, 10 (1987) 1261.
8 E. Soczewiński, G. Matysik and W. Markowski, *Chem. Anal. (Warsaw)*, 33 (1988) in press.
9 A. Niederwieser and C. G. Honegger, *Adv. Chromatogr. (N.Y.)*, 2 (1966) 123.
10 C. Liteanu and S. Gocan, *Gradient Liquid Chromatography*, Ellis Horwood, Chichester, 1974, pp. 193–194.
11 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 218 (1981) 199.
12 A. M. Siouffi, T. Wawrzynowicz, F. Bressolle and G. Guiochon, in A. Zlatkis (Editor), *Advances in Chromatography 1979*, Chromatography Symposium, Houston, TX, 1979, p. 591.
13 L. C. Sander and L. R. Field, J. Chromatogr. Sci., 18 (1980) 133.