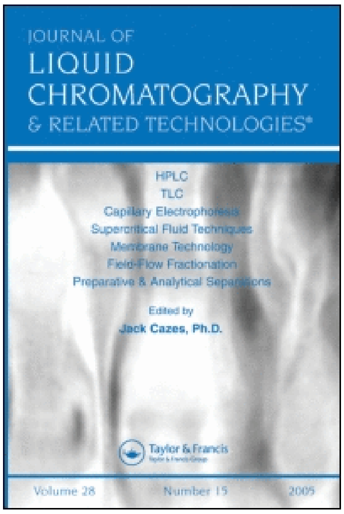

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A SIMPLE DEVICE FOR GRADIENT ELUTION IN EQUILIBRIUM SANDWICH CHAMBERS FOR CONTINUOUS THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

The separation efficiency of various thin-layer chromatographic techniques was investigated using a horizontal sandwich tank for continuous elution with a glass distributer. Complex extract of camomile flowers (Azulane) was used as the test mixture and the number of separated spots as the measure of efficiency. Best results were obtained when gradient elution with chloroform-ethyl acetate mixtures was applied. A simple miniaturized device without mechanical mixer was employed, the spontaneous mixing being enhanced by gravitational flow of the components and their association on the molecular level.

INTRODUCTION

Modifications of thin-layer chromatography have been frequently used to enhance the efficiency of separation and to shorten the duration of analysis. Thus, multiple development, programmed multiple

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development (PMD) and short bed - continuous development (SBCD)(1-7) were reported to give much better results than conventional TLC. Complex samples which contain compounds of wide differences in polarity cannot be separated by isocratic development and require the use of solvent - programmed elution. Numerous devices for the generation of gradients in TLC have been proposed (4,5); most of them require the application of mechanical stirrers. A simple device for sandwich TLC has been reported by Sioufii et al.(9); stepwise gradients can also be generated without a mixing device by simple change of solvent container, when equilibrium sandwich chambers with a glass distributer are used (10,11).

Since continuous gradients provide better separation of complex samples and their application is relatively scarce owing to rather complex devices required for the generation of reproducible gradients, attempts have been made to simplify the equipment; especially to eliminate the mixer and to miniaturize the eluent containers. To enhance spontaneous mixing, two restrictions were assumed for the component solvents A and B (B - solvent of higher eluent strength):

1 The densities of solvents A and B are different. If $d_B < d_A$, solvent B (stronger) is introduced

at the bottom of container A and tends to migrate upwards.

2. Solvents A and B interact, e.g., have opposite electron donor-acceptor properties.

The delivery of the eluent to the thin layer is relatively slow, therefore, it was assumed that convection (especially for mixtures having positive heat of mixing) and interaction of molecules would be sufficient to cause spontaneous mixing of the two solvents.

EXPERIMENTAL

Modification of horizontal sandwich tank of the Brenner-Niederwieser chamber was used (Fig 1), the eluent being delivered by an inverted U-tubing (S, 0.8 mm ID) and spread across the plate by a glass distributer D glued to the lower surface of the smaller cover plate C (10,12,13). Two glass containers A and B were used, connected with a short PTFE tubing. The connection could be severed with a spring clip L.

A patent registration has been claimed for the device and its modifications as well as the principles of its applications.

The procedure of gradient elution was as follows. The two containers were filled with solvents A and B (with the connection severed by the clip L) to levels corresponding to hydrostatic equilibrium (i.e., $h_A d_A =$

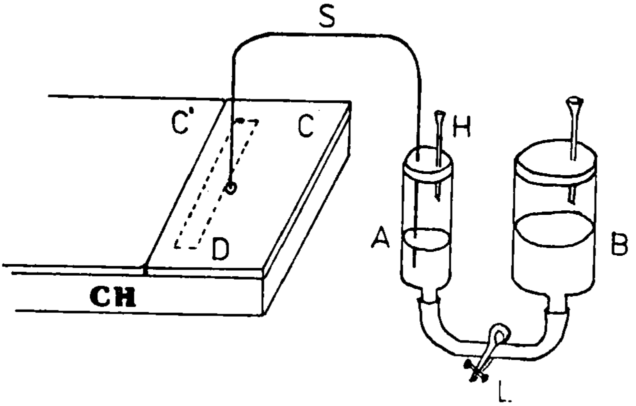

FIGURE 1 Double container for generation of gradients with spontaneous mixing of component solvents A weak and B strong . C,C'- cover plates; CH-tray; D-distributer; H-hypodermic needle; L-spring clip; S-siphon connection

$h_B d_B$). By a slight overpressure (with a hypodermic syringe, through the needle H) the siphon connection between the container A and the slit under the distributer D is produced The cover plate C is moved forward to contact the distributer with the edge of the layer, Additional pressure is applied when necessary (if the consumption of eluent from the distributer is too rapid in the first stage). The flow of solvent becomes soon regular and then the connection between the containers is opened and solvent B begins to flow into the container A, In preliminary experiments dilute solutions of dyes in solvent B were used which enabled to confirm that under conditions stated above (e.g., A = chloroform and B = ethyl acetate) spontaneous mixing takes place when the delivery of mixed eluent is slow as in the usual development process.

As the complex test mixture, oontaining numerous components of differentiated polarity, Azulane was used. Azulane (produced by Herbapol, Warsaw, Poland) is the extract of the flowers of camomile (Matricaria chamomilla). 10 ml of the extract was evaporated and the residue dissolved in 2 ml of ethyl acetate, discarding the insoluble fraction. The criterion of efficiency of the technique was the number of separated components visible under UV light or after exposition to iodine vapours

Glass plates, 200 x 50 x 1.3 mm, covered with Silica Si 60 (E.Merck, Darmstadt, FRG) or precoated aluminium sheets were used. Equilibrium sandwich chambers for 200 x 5 mm plates, produced by Polish Reagents Lublin (14), were employed.

RESULTS AND DISCUSSION

In Fig.2 the chromatograms obtained by isocratic elution with heptane-ethyl acetate mixtures are shown. The components visible under UV lamp are numbered 1-8 and could be distinguished owing to characteristic fluorescence; other components could be detected by exposure to iodine vapours.

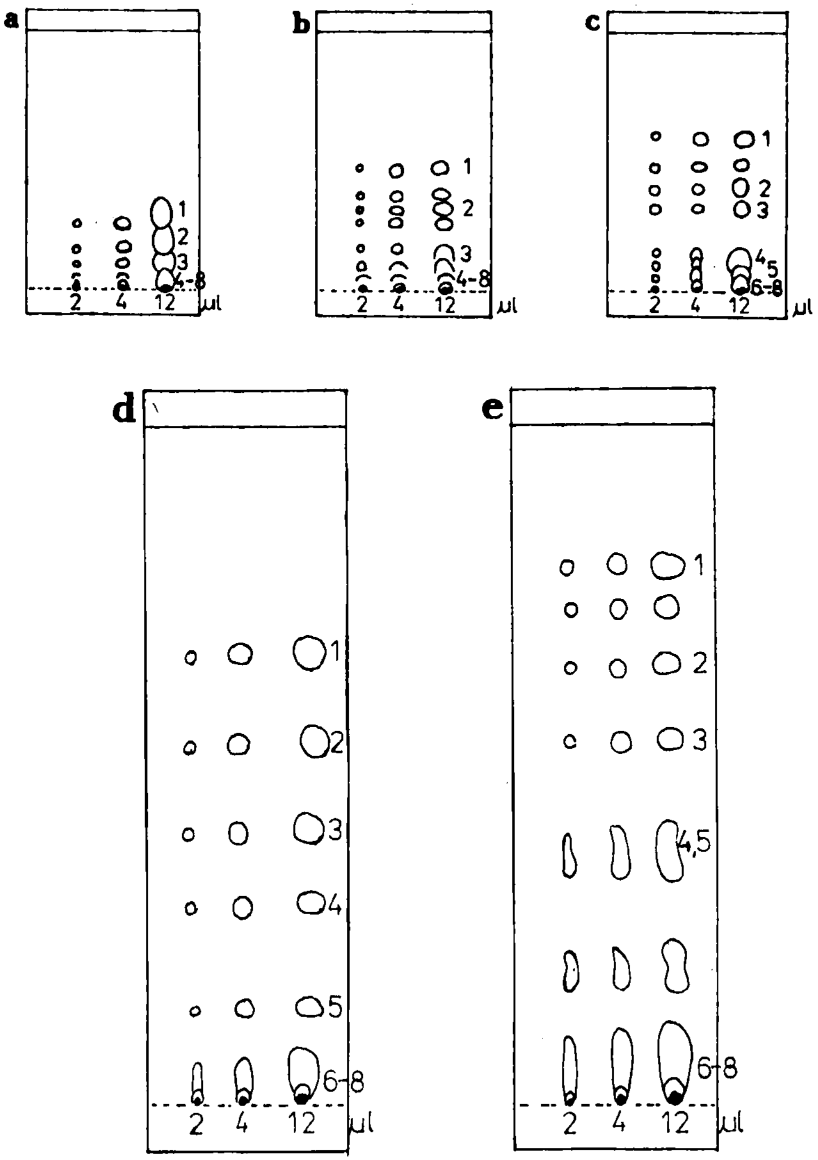

FIGURE 2. Separation of Azulene extract on thin layers of silica. Concentration of ethyl acetate in the eluent: a-10%; b-20%: c,d-30%; e-50%. Diluent - n-heptane. Development distance: 7 cm (a-c) or 16 cm(d,e).

The binary heptane-ethyl acetate separated well the components 1-3 while the R_F values of compounds 4-8 were too low even at 50% content of ethyl acetate. The elongation of the elution distance to 19 cm only partly improved the separation (Fig.2).

Therefore, in the next series of experiments heptane was substituted by chloroform. The increase of the solvent strength results in much better separation of the more polar components at the cost of inferior separation of the less polar components 1 and 2 (Fig 3)

Since it was expected that some components remain on the start line with compound No 8, the continuous development of the chromatograms was applied in the

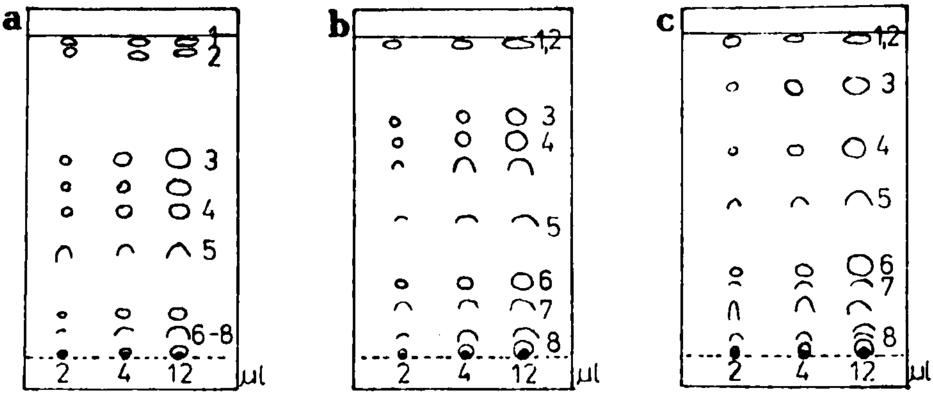

FIGURE 3 Effect of concentration of ethyl acetate on the separation of Azulene extract: a-5%; b-10%; c-15%. Diluent-chloroform. Distance of development: 7 cm. Spots No 1-8 correspond to components of characteristic fluorescence in UV light, other spots are visible after exposition to iodine vapours

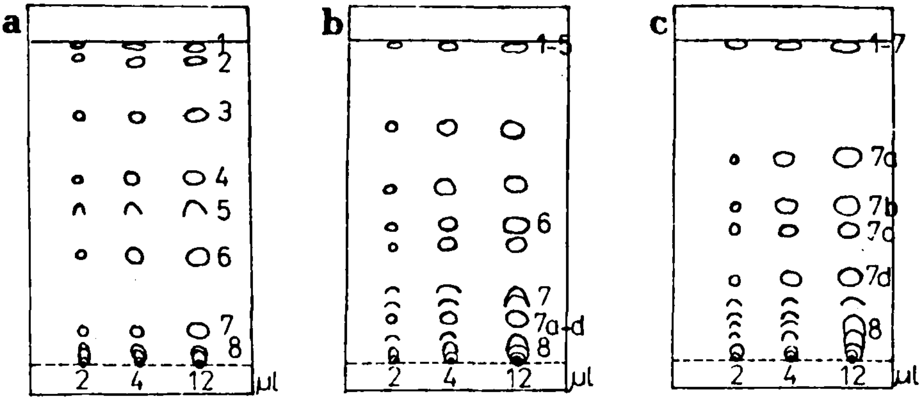

FIGURE 4 Short-bed - continuous development of Azulane extract: a-ethyl acetate - heptane, 30:70; b-ethyl acetate - chloroform, 5:95; c-ethyl acetate - chloroform, 10:90. Distance of development - 4 x 7 cm (determined from the migration of marker spots).

next series of experiments. As demonstrated by Perry(3), prolonged elution on a shorter distance with eluents of low strength results in improvement of separation and sensitivity of detection (3,6,7). Elution with four interstitial (void) volumes was applied and the results are shown in Fig 4. For heptane-ethyl acetate (70:30) the chromatogram (Fig.4a) was similar to that obtained for single development with 15% ethyl acetate in chloroform (Fig 3c). SB-CD with four void volumes of 5% or 10% ethyl acetate in chloroform resulted in flattening of the less polar compounds on the end line and partial or complete separation of a number of polar compounds (Fig.4 b,c) which remained on the start line in the former experiments.

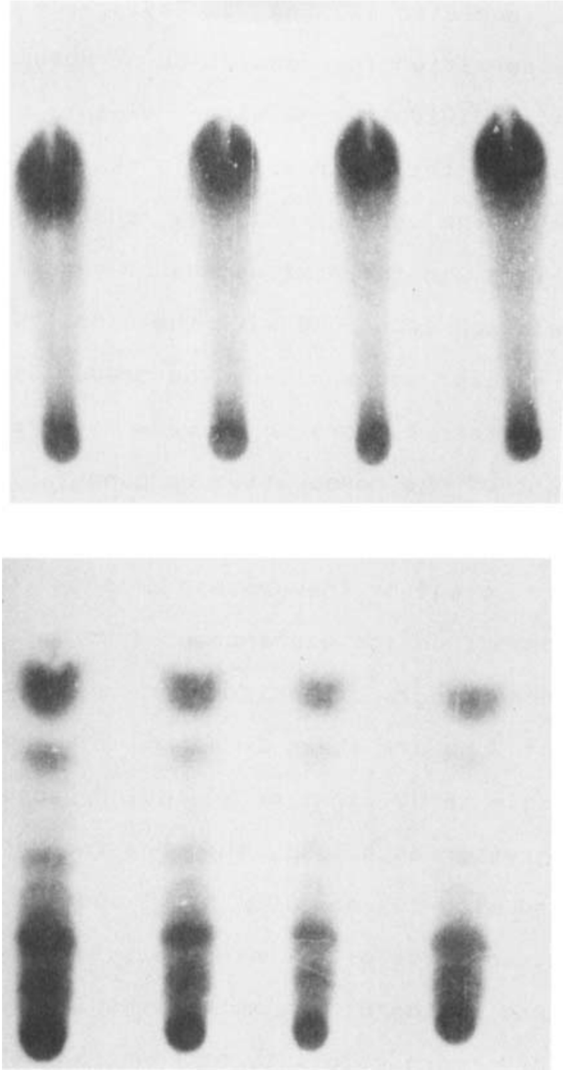

FIGURE 5. Initial stage of development on 3 cm distance: a-isocratic elution, ethyl acetate - chloroform 3:7, b-gradient elution, A-5% ethyl acetate, B-30% ethyl acetate in chloroform.

Thus, isocratic elution, including the SB-CD technique, permitted the resolution of about 8-10 components. Individual runs with eluents of different strengths are required to separate the components in the various polarity ranges.

Therefore, in the next series of experiments the gradient elution technique with the simple device described earlier was applied. The gradual increase of the eluent strength seems to be very favourable for the displacement of the consecutive components from the starting zone and the separation is much better already in the first stage of the process as shown in Fig. 5b for development on the distance of 3 cm.

The chromatograms obtained for development on the distance of 7 cm are shown in Fig.6. The number of spots visible in UV light or in iodine vapours is markedly greater especially when the two containers were filled with 10% and 100% ethyl acetate. It was found that in spite of the simplicity of the device, the standardization of the method resulted in satisfactory reproducibility of the gradient profile and R_F values. The first results reported here are thus very promising.

The following observations have been made during the experiments.

Owing to marked differences in the adsorption affinities of solvents A and B used in gradient

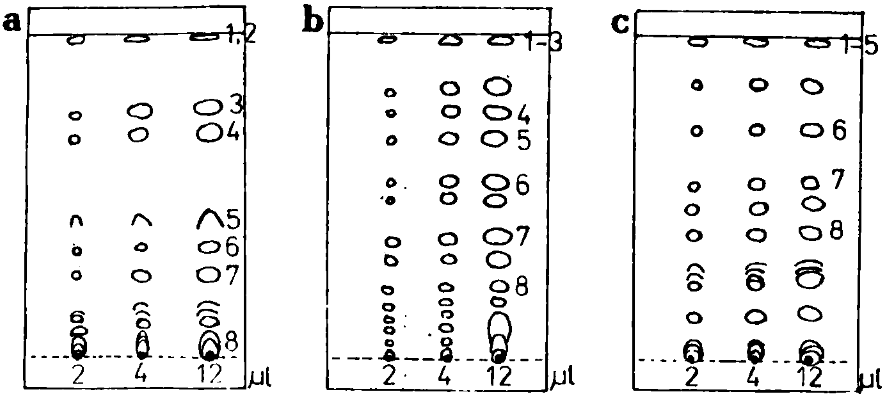

FIGURE 6. Gradient elution of Azulane extract, ethyl acetate - chloroform mixtures: a-5% (A) and 30% (B); b-10% (A) and 100% (B); c-50% (A) and 100% (B).

elution, the generated gradient profile tends to become much steeper in the layer due to solvent demixing effects (preferential adsorption of solvent B). Formation of a sharp demixing front results in unfavourable accumulation of flattened spots at the front. The R_F value of the solvent demixing front is low especially at the beginning of the gradient program when the concentration of solvent B is low (15), therefore, to eliminate this phenomenon it is much better to start not from zero concentration of B but from, e g., 10% B (in container A) and to spot the sample on the wet plate 2-3 cm behind the solvent front, using pure solvent B in the larger container. For this purpose the smaller cover plate C is shifted back by ca 2 mm to severe the contact of the layer with the distributer and the samples are spotted through the gap between the cover plates C and C′.

An alternative simplified procedure can also be used. The slit under the distributer is filled through the orifice using a micropipette (100 μl for 5 x 50 mm distributer or 200 μl for 5 x 100 mm distributer) with 10% solution of solvent "B" in solvent "A and the single distributer volume of eluent is introduced into the layer. The smaller cover plate is shifted back and the samples spotted onto the layer through the gap between the cover plates C and C′. The siphon connection between the container A and the distributer is produced, the plate C′ shifted forward to introduce the eluent into the layer and the elution process is thereby started; the connection between containers A and B is then opened.

When the range of elution strengths of the mixtures is too high (too high R_F values of most components of the sample), less polar solvents A and B should be used (e.g., A = trichloroethylene, B = diisopropyl ether).

The generated gradient profile depends on the ratio of diameters of the two containers, volumes of the two solvents in the containers and the volume of eluent absorbed by the layer (i.e., its dimensions). To secure the reproducibility of the eluent composition program, these parameters must by standarized. Steeper profiles are obtained when the diameter (volume) of container A is smaller relative to B. Less steep gradients can be obtained using mixtures of A and B (e.g., 1:1) in the second vessel instead of pure solvent B.

The simplified gradient TLC technique requires further verification for various multicomponent samples and eluent - sorbent systems as well as investigation of the generated gradient profiles and parameters which determine their shape. The investigations will be reported in the future communications.

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