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An arrangement for simultaneous elution of one thin-layer chromatography plate with several solvents

In the "Vario-KS-Chamber"* described by GEISS AND SCHLITT[1] the chromatographic layer can be conditioned in a controlled manner, before and during development with certain chromatographically effective vapours. We describe here an accessory of this chamber, useful for the concurrent elution of a 20 × 20 cm TLC plate with up to 5 different single solvents.

The solvent tank of the Vario-KS-Chamber is exchanged for another which is divided into 5 smaller compartments. The solvent transport is obtained by 5 correspondingly smaller paper wicks for the solvent. Because of the different velocities of the different solvents through the layer, some adsorbent must be scraped from the plate, so that the layer, on the plate, appears as 5 rectangular bands 16.5 × 2.5 cm. This avoids the movement of faster solvents towards the bands of the slower solvents. Furthermore, in order to avoid mixing of vapour phases between neighbouring compartments, a sandwich slide, made of 6 polypropylene barriers of 1 mm thickness and glued together (see Fig. 1), is inserted between the bands before the elution.

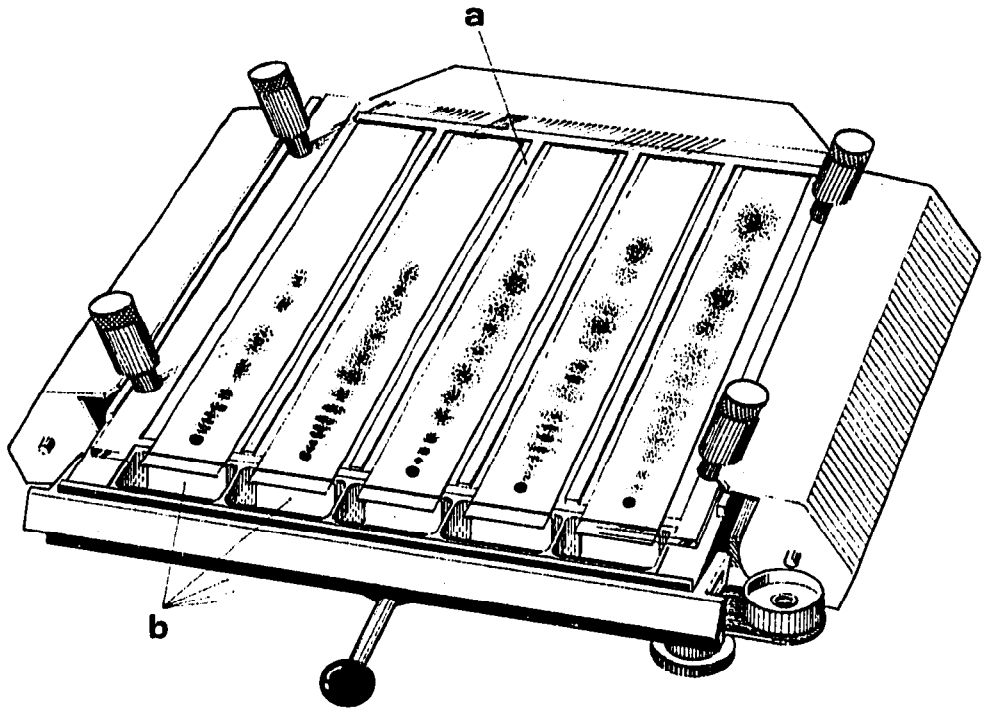

Fig. 1. Vario-KS-Chamber modified for elution with an orthogonal solvent gradient. (a) barriers; (b) solvent tanks.

Such an arrangement represents a form of gradient elution and is an "orthogonal" solvent gradient following the definition of NIEDERWIESER[2]; continuous gradient elution was first described by the same author[3].

* Manufacturer: Camag, Muttenz (Switzerland).

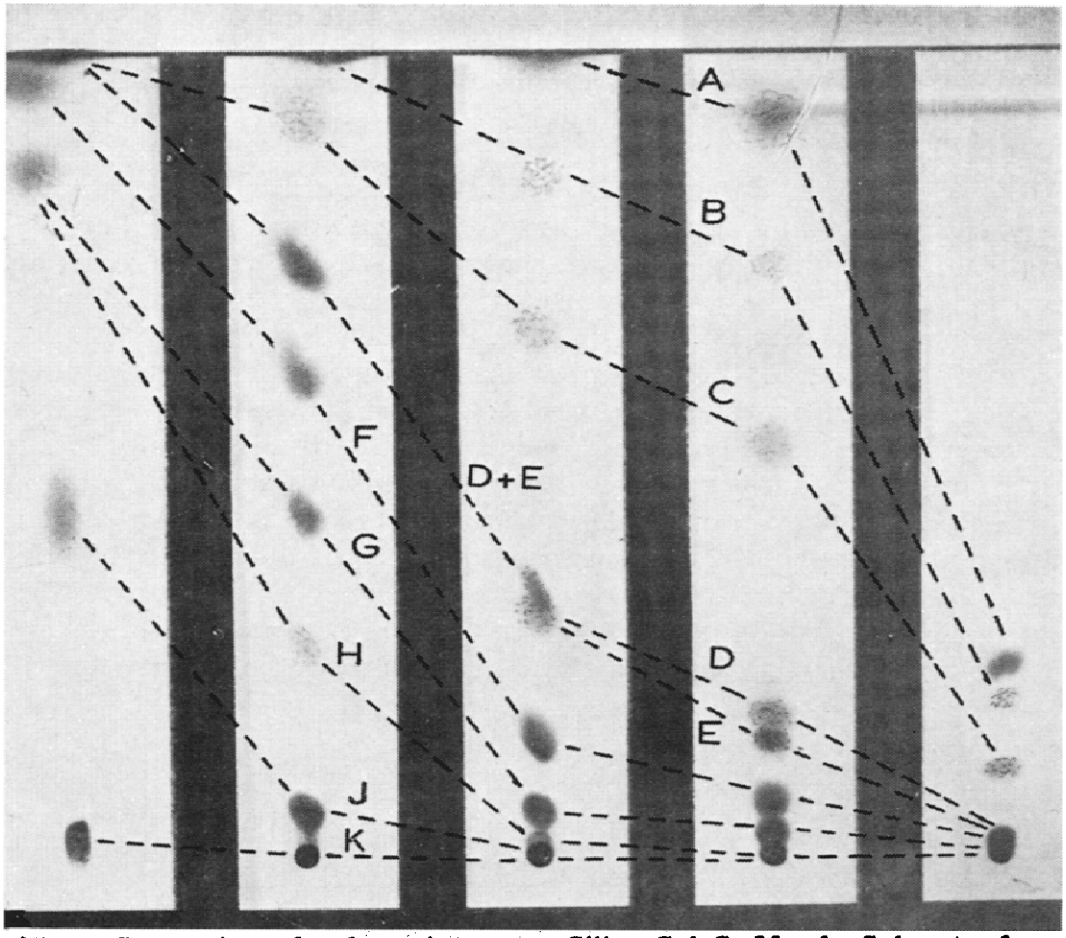

Fig. 2. Separation of a dye mixture on Silica Gel G, Merck. Solvents, from left to right: ethyl acetate; 1,2-dichloroethane; benzene; toluene and carbon tetrachloride; relative humidity 43%. Substances: Fat Red (A), *p*-methoxyazobenzene (B), Butter Yellow (C), Acetorange (D), Indophenol (E), F 11 Ciba (F), F 8 Ciba (G), F 22 Ciba (H), F 5 Ciba (J), F 34 Ciba (K).

This orthogonal solvent gradient is useful in new problems of separation in TLC when solvent systems have to be tested for their optimal effects. Five different solvents can be tested simultaneously on the same plate, one beside the other, under the same conditions. Naturally this gradient can be easily combined with all other gradients which can be used in the Vario-KS-Chamber.

In Fig. 2, a chromatogram of a complex dye mixture is shown; this could be obtained in 80 min. This is an example of the programmed resolution chromatography of a complex sample mixture whose components vary widely in polarity. It demonstrates, in addition, that for the separation of different groups of substances different solvents must be chosen. The pair Indophenol/Acetorange (D/E), for example, is only separated with toluene, under these conditions.

A further modification of the solvent tank makes it possible to work with a continuous solvent gradient. The barriers between the various liquid chambers are lowered in such a way that the various paper solvent wicks are able to exchange solvents between themselves in the gaseous phase before the beginning of the development. As very different mixtures are formed among the pure solvents, the R_F values are not changed in a stepwise but in a continuous way. NIEDERWIESER[3] obtained

such a gradient by combining the BN-Chamber with a complex arrangement of mixing reservoirs, which also allowed the control of the composition of the solvent along the gradient.

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1 F. GEISS AND H. SCHLITT, *Chromatographia*, 1 (1968) 392.
2 A. NIEDERWIESER, *Chromatographia*, 2 (1969) 23.
3 A. NIEDERWIESER, *Chromatographia*, 2 (1969) 362.

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Thin-layer chromatography of cyclic adenosine 3′, 5′-monophosphate on tetraborate-impregnated silica gel layers

A number of procedures for the separation of cyclic adenosine 3′,5′-monophosphate (c-AMP) from other adenine derivatives on thin-layer chromatograms have been published[1–3]. These procedures generally employ cellulose thin layers and a multi-component solvent system, *e.g.* *n*-butanol–acetone–acetic acid–ammonium hydroxide–water.

The tendency of borate ions to form complexes with the 2′,3′-*cis*-diol grouping on simple sugars[4] was utilised to develop a simple and efficient procedure for the separation of c-AMP from other adenine derivatives.

Methods

Plates were prepared using a slurry made up of Silica Gel GF_{254} (Fluka) (30 g) and 5% (w/v) aqueous sodium tetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$, 60 ml). Film thickness was 250 μ and the plates were heated at 110° for 30 min. Substrates (10 μl, 0.05% solution in 50% aqueous ethanol) were applied using a microsyringe. The developing solvent was 50% aqueous ethanol, and the development time was approximately 4 h for a 20 × 20 cm plate. After development the plates were dried at room temperature and viewed under UV light.

Results

The use of tetraborate-impregnated layers gave a very satisfactory separation of c-AMP from other adenine derivatives, as shown in Table I. c-AMP and theo-