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LAYER PRE-LOADING IN PARTITION THIN-LAYER CHROMATOGRAPHY OF AMINO ACIDS

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SUMMARY

It was shown by a number of examples that layer pre-conditioning techniques, hitherto used in adsorption chromatography, are also useful for influencing partition chromatographic separations.

INTRODUCTION

Geiss and coworkers[1–3] have demonstrated practically and explained theoretically that interactions between the stationary phase and the ambient atmosphere are important parameters in thin-layer chromatography (TLC). Obviously, the same holds true in all instances of flat bed chromatography, *i.e.*, when the chromatographic medium is exposed in the surrounding gas atmosphere.

De Zeeuw[4] used solvent pre-loading effects in order to influence empirically the chromatographic behaviour of migrating substances. For the systematic investigation of such pre-loading effects, it is necessary that the conditions can be varied in the direction transverse to that of chromatography. A number of examples in which the influence of pre-conditioning is demonstrated by transverse gradients have been published[1–3]. Such gradients were: relative humidity, *i.e.*, pre-loading with water, pre-loading with solvents of higher and lower polarity than that of the mobile phase, and pre-conditioning with volatile reagents, including acids and bases. Experiments of this kind are suitable for optimizing the chromatographic conditions for a particular separation problem.

So far, all the known examples of the systematic investigation of pre-loading effects have concerned adsorption chromatography. Stewart[5] investigated theoretically the influence of the gas atmosphere on the stationary phase in liquid–liquid chromatography. Lofts *et al.*[6] studied the role of the individual components of complex partition chromatographic solvents in the separation of amino acids. They postulated "movers, homogenizers, sharpeners, pH controllers", etc. We have tried to extend the influence of similar compounds via the gas phase in liquid–liquid TLC.

EXPERIMENTAL

Separations of amino acids and Dansyl-amino acids* were carried out on silica gel pre-coated plates (Camag DF-B), which were developed in a Vario KS chamber** after equilibration for 30 min with the liquids contained in the 10-subdivision tray. Before development was started, a separation slide (Sandwich slide) was inserted between the layer and the conditioning tray in order to prevent uncontrolled interaction between the migrating solvent and the equilibration liquids. Amino acids were stained with ninhydrin, and Dansyl-amino acids were observed under longwave ultraviolet light (366 nm).

Chromatograms with uniform pre-loading, obtained for the identification of individual substances, were developed in a Sandwich chamber with a counter plate soaked with the pre-conditioning liquid.

RESULTS

A mixture of the amino acids glycine, alanine, valine, leucine, methionine and

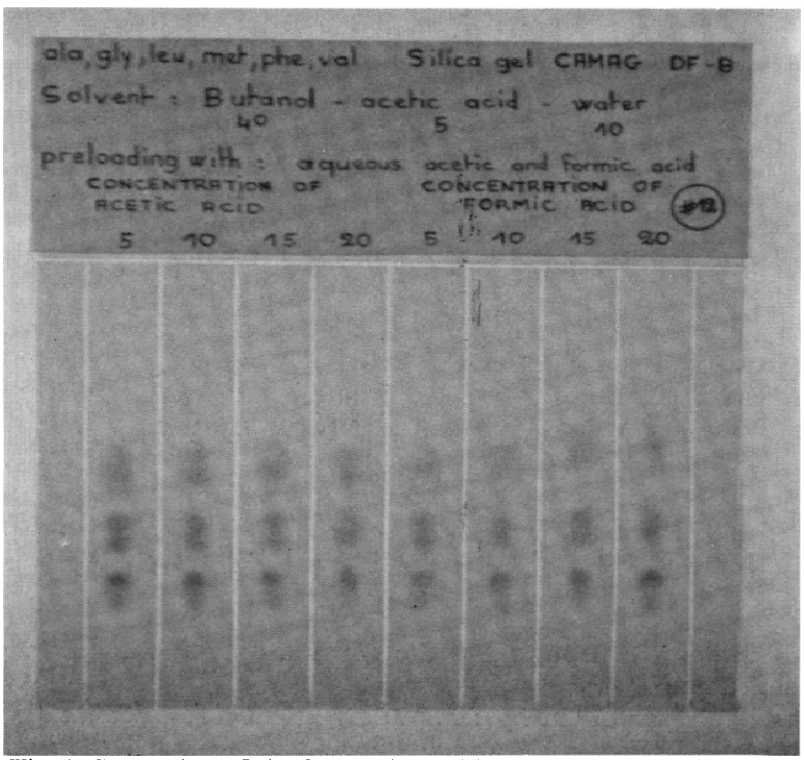

Fig. 1. Separation of the free amino acids glycine, alanine, valine, leucine, methionine and phenylalanine on silica gel with butanol-acetic acid–water (40:5:10) after pre-loading with different concentrations of acetic and formic acids.

* Dansyl = 5-dimethylaminonaphthalene-1-sulphonyl chloride.
** Licensed by Euratom, manufactured by Camag.

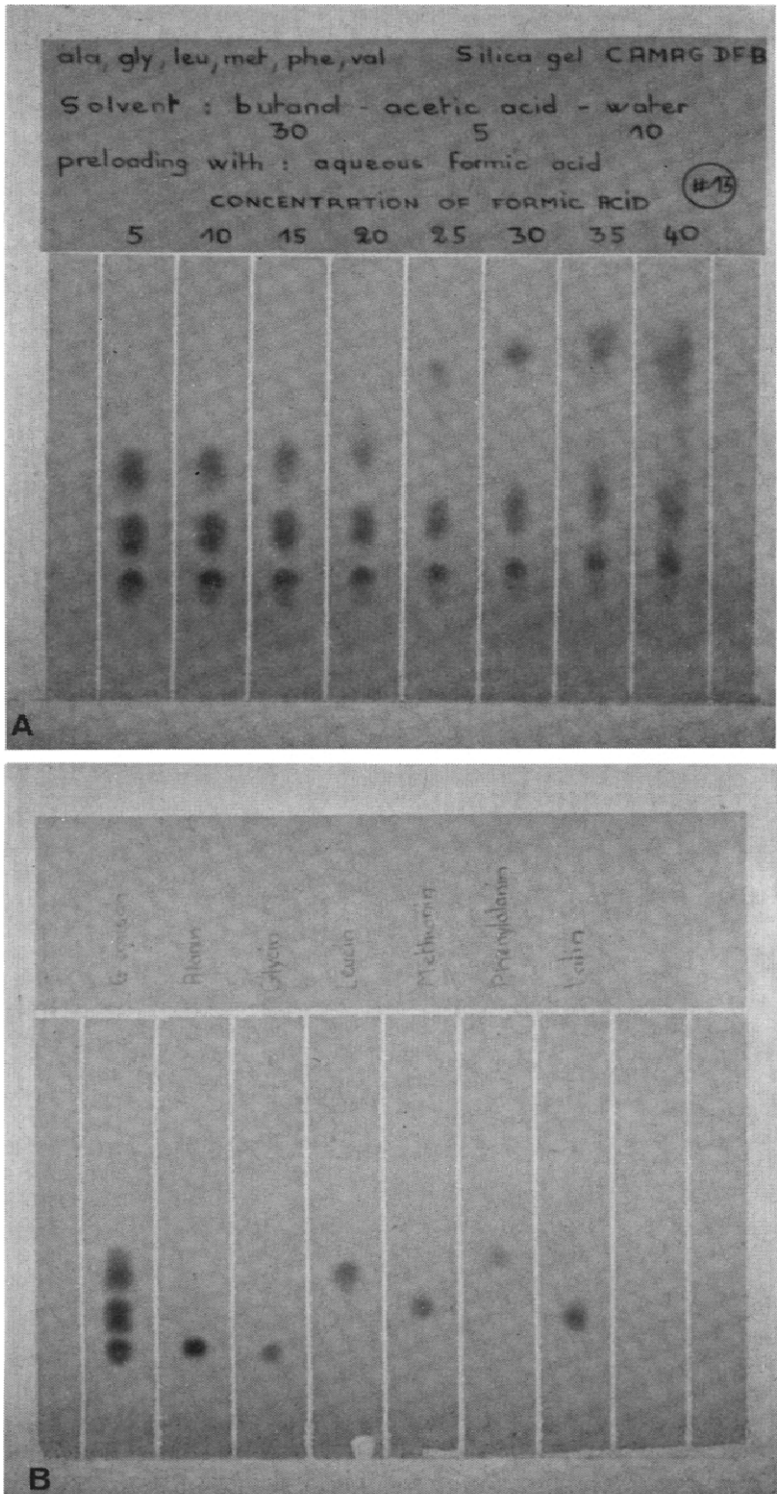

Fig. 2. (A) Separation of the free amino acids glycine, alanine, valine, methionine, leucine and phenylalanine on silica gel with butanol–acetic acid–water (30:5:10) after pre-loading with a formic acid gradient of 5–40%. (B) Chromatography of individual amino acids with solvent as in (A) after pre-loading with 10% of formic acid.

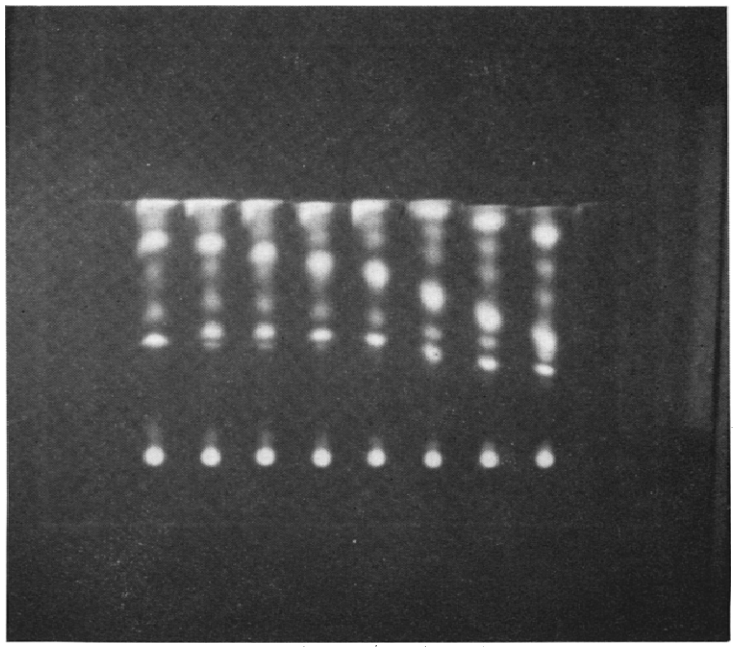

Fig. 3. Separation of the Dansyl derivatives of glycine, alanine, valine, methionine, leucine and phenylalanine with ethyl acetate–chloroform–ethanol–acetic acid (60:45:4:4) after pre-loading with a formic acid gradient of 5–40%.

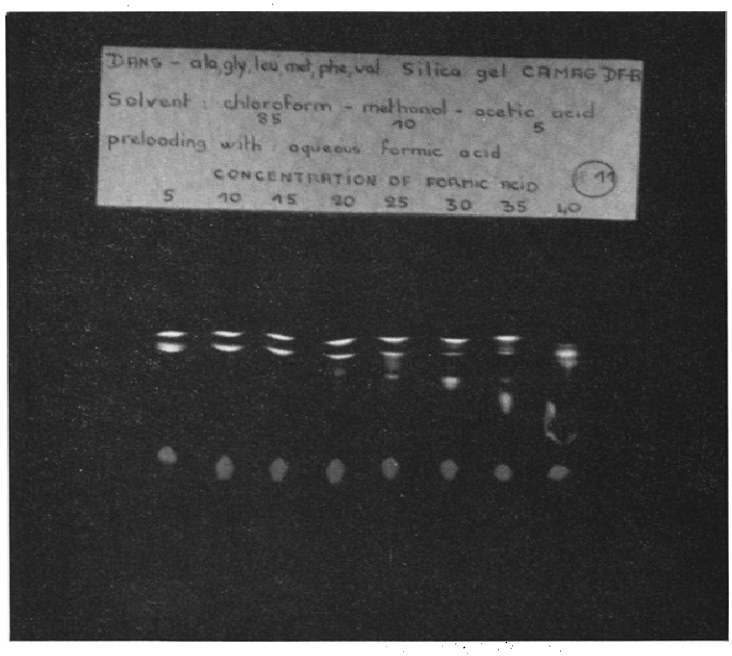

Fig. 4. Separation of the Dansyl derivatives of glycine, alanine, phenylalanine, leucine and valine with chloroform–methanol–acetic acid (85:10:5) after pre-loading with an aq. formic acid gradient of 5–40% with 10% of methanol in all mixtures.

phenylalanine was developed with butanol–acetic acid–water (40:5:10) after pre-equilibration with different concentrations of acetic and formic acids (Fig. 1). Variation of the acetic acid concentration in the pre-loading liquid had little influence. Only the two fastest moving amino acids, phenylalanine and leucine, become less sharply separated when the acid content of the stationary phase is increased. With formic acid pre-loading, this pair of amino acids is not separated at any concentration.

A slight variation in the developing solvent to butanol–acetic acid–water (30:5:10) and a formic acid pre-loading gradient from 5–40% gave the separation shown in Fig. 2. The sequence of amino acids in the order of decreasing R_F values is phenylalanine, leucine, methionine, valine, alanine and glycine. Fig. 2B shows the identification chromatogram pre-conditioned with 10% formic acid.

Dansyl derivatives of amino acids have the advantage that they can be observed under longwave UV light while the chromatogram develops. The separation of the Dansyl derivatives of glycine, alanine, valine, methionine, leucine and phenylalanine with ethyl acetate–chloroform–ethanol–acetic acid (60:45:4:4) after pre-loading with a formic acid gradient of 5–40% was very poor at all concentrations. Heavy trailing obscured the resolution of the compounds. The addition of 10% of methanol to the pre-loading liquids greatly improved the result (Fig. 3).

Fig. 4 shows the chromatogram of a mixture of the Dansyl derivatives of valine, leucine, phenylalanine, alanine, glycine (order of decreasing R_F values) developed with chloroform–methanol–acetic acid (85:10:5) after pre-loading with an aq. formic acid gradient of 5–40% containing 10% of methanol in all mixtures. In the absence of methanol, trailing was again observed.

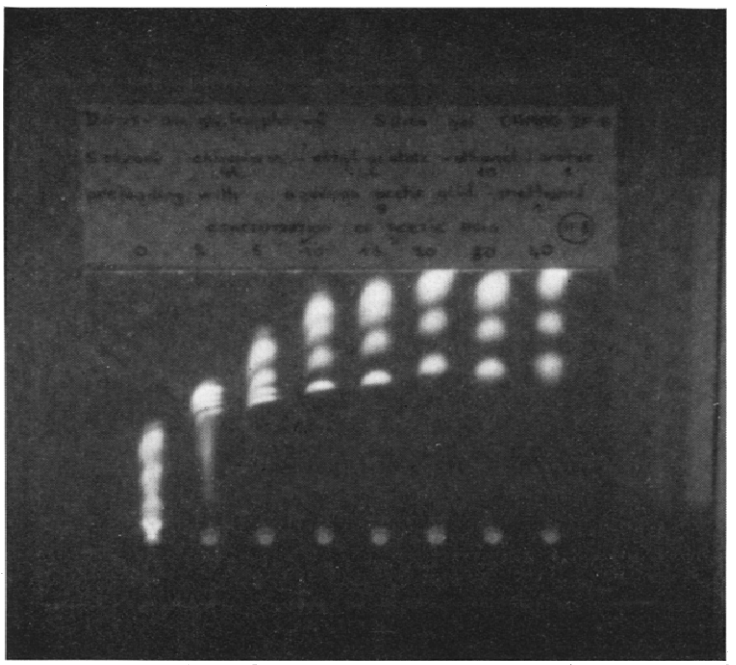

Fig. 5. Separation of the Dansyl derivatives of glycine, alanine, phenylalanine, leucine and valine with chloroform–ethyl acetate–ethanol–water (45:45:10:1) after pre-loading with an acetic acid gradient of 0–40% containing 10% of methanol.

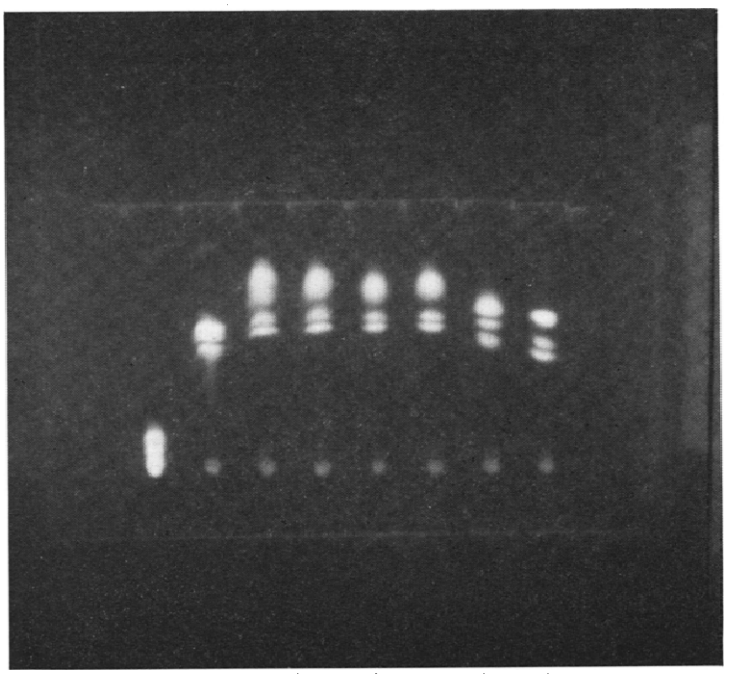

Fig. 6. The same separation as in Fig. 5 but with pre-loading with a formic acid gradient of 0–40% containing 10% of methanol.

Figs. 5 and 6 show the same mixtures of Dansyl-derivatives as in Fig. 4 chromatographed with chloroform–ethyl acetate–ethanol–water (45:45:10:1), in Fig. 5 after pre-conditioning with an acetic acid gradient of 0–40%, and in Fig. 6 with a formic acid gradient of 0–40%, containing 10% of methanol in all instances. A comparison between these two chromatograms shows that the influence of the acid pre-loading is not simply an effect of pH, because the more volatile and stronger formic acid is less effective than acetic acid and even shows the opposite effect at higher concentrations.

These few examples show that layer pre-conditioning in partition TLC appears to be promising for improving and controlling separations of complex mixtures such as amino acids, and are worthy of further investigation.

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