

THE APPARENT INFLUENCE OF LAYER THICKNESS ON R_F VALUES OF THIN-LAYER CHROMATOGRAMS

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SUMMARY

Using wedged layers in combination with various developing techniques it could be demonstrated that the apparent influence of the layer thickness on the R_F -values in adsorption chromatography is to be attributed to solvent vapour phase saturation.

Widely varying opinions have been reported¹⁻⁹ on the influence of the layer thickness on the R_F values in thin-layer chromatography. Some authors mention this more or less in general terms, others report distinct relationships, and some have found that the layer thickness does not affect the R_F values.

Extremely thin layers are not included in the following considerations, such layers being of the order of only a few multiples of the grain diameter of the adsorbent, *i.e.* below 100 μ .

Some test chromatograms were run in order to demonstrate the relationship between layer thickness and R_F value. Attention is drawn to the fact that the results reported here are primarily pertinent to the adsorption-chromatographic system aluminium oxide with benzene as solvent, and some dyes as model compounds (Alumina DS-O CAMAG; dyes: Sudan Yellow G, Indophenol, Sudan III, Sudan II).

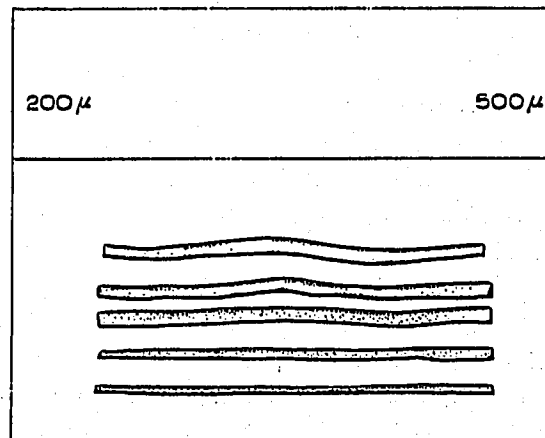
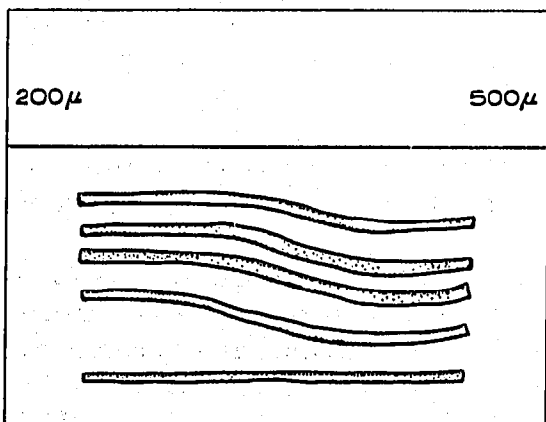


Fig. 1. Developing tank without saturation.

Fig. 2. Developing tank presaturated.

Plates 20×20 cm with a thickness gradient were prepared using a Camag TLC applicator. The floating gate controlling the layer thickness is adjusted by two set screws approximately 10 mm from either edge. In this case the one set screw was adjusted to 500μ , the other one to 200μ . Otherwise the plates were coated and dried as usual. The sample solution was applied with a "Chromatocharger" in the form of a straight line in the direction of the thickness gradient ($40 \mu\text{l}$ per 160 mm). Then the plates were chromatographed with benzene using different techniques:

Fig. 1 depicts a chromatogram developed in a classical jar without vapour phase saturation. Naturally the R_F values at the thinner side of the layer are considerably increased.

The chromatogram shown in Fig. 2 was developed in the same type of developing chamber, which was pre-saturated with the solvent vapour by lining the tank with benzene soaked filter paper.

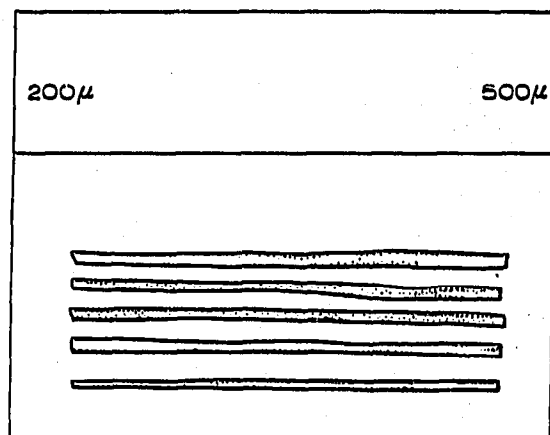
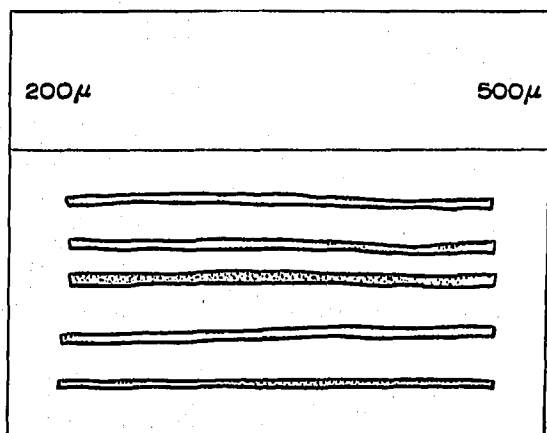


Fig. 3. Sandwich-chamber unsaturated.

Fig. 4. Sandwich-chamber presaturated.

Fig. 3 shows a chromatogram obtained by development in a non-saturated sandwich chamber with an internal width between the plates of 1.7 mm.

Development of the chromatogram in Fig. 4 was carried out in a "saturated sandwich". In this case a layered plate was used instead of the plain glass cover plate, whereby the layer of the counterplate was soaked with the solvent prior to assembling the sandwich.

Two conclusions can be drawn from these experiments:

(1) The apparent influence of layer thickness on the R_F values of thin-layer chromatograms can be attributed to the degree of vapour phase saturation, at least in the case of the system investigated here.

(2) The comparison of the "saturated jar" and the "saturated sandwich" illustrates that certain development techniques commonly considered to feature saturated conditions are far from such equilibration. This is so even with chromatographic conditions which were referred to as "oversaturated" in the literature.

These examples also show that such "wedged layers" are a simple but rather useful tool for investigating chromatographic systems with respect to vapour phase saturation.

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DISCUSSION

BOLLINGER: Has the layer thickness also been checked after drying? If not, can you exclude that the thickness gradient has not been smoothed or levelled out after the application in a horizontal position?

JÄNCHEN: The layer thickness of one plate was checked by positioning another glass plate on it and assessing their distance, *i.e.* their slope.

GRÜNE: In what way does the cardboard seal influence the chromatographic separation in the case of the S chamber, I mean with particular reference to its influence on saturation, temperature, etc.

GEISS: You have said, Dr. JÄNCHEN, that the absorbing cardboard sealing frame need not be harmful but, in many cases, can be of advantage, *e.g.* if the layer is to be presaturated with the solvent vapour. I believe, however, that it is always a disadvantage—except in the case of presaturation by wetted counter-layer—because the frame by its capillary behaviour inevitably induces lateral adsorption of solvent vapours by the layer which results in undesirable marginal effects. In the case of solvent mixtures this is particularly annoying since the component of greater eluting power which normally migrates at the bottom is lifted by suction at the edges and deactivates the marginal portions of the layer by its vapour. With regard to the origin line, the line which connects the spots of identical substances becomes thus concave in the case of one-component elution solvents and convex in the case of mixtures.

BRENNER: What is the ratio of the phases of the solvent and the absorbent in the counter-layer?

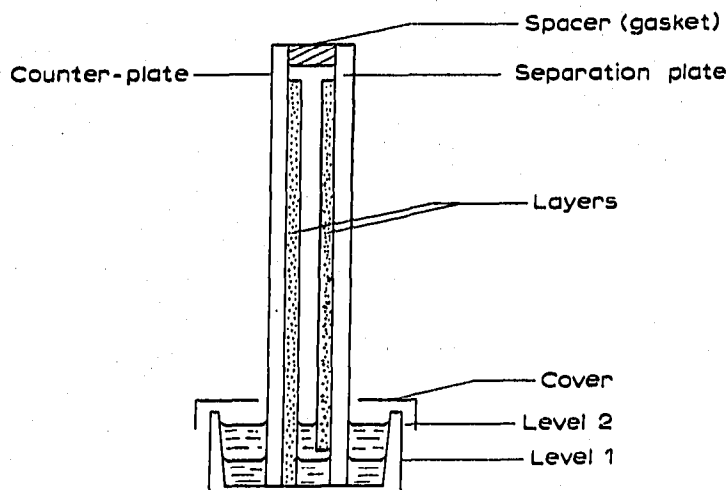
JÄNCHEN: The capacity of the saturation counter-layer for the solvent was very high in comparison with the adsorption capacity of the chromatographic layer.

BRENNER: Has anybody measured the ratio between the carrier material and solvent on a thickness-gradient plate?

DE GRAAF: I have measured this ratio along the plate in S and BN chambers, but not in plates with a thickness gradient.

GEISS: A high degree of saturation can be achieved if the solvent (see Fig. 5, level 1) is first allowed to ascend in the counter-layer (30–60 min) the lower edge of which has not been wiped off; the fluid level in the tank is then raised (level 2) until development begins in the actual separation plate.

However, I do not see any advantage in a uniform presaturation of the layer, thus only the running distances are proportionally shortened. There may be excep-



tions with solvent mixtures. The case is different with gradient-wise pretreatment with vapours of solvent mixtures involving deactivation which exploit the gradient as such; e.g. our KS chamber is suitable for this.

BRENNER: Does the equilibrium become established between the layer and counter-layer across the vapour phase?

GEISS: After some 10 h of equilibration the saturation of the layers with solvent vapours is almost finished. At this equilibrium for aluminium oxide G 25% and for Kieselgel G 60% of the free layer is filled. The remaining 75% or 40%, respectively, are filled by the ascending eluent, this making the layer dripping wet (klatschnass).

BRENNER: Thus before chromatographic development the equilibrium does not basically exist.

GEISS: There is an equilibrium, but not the one belonging to the dripping wet chromatography plate.

HUBER (Ludwigshafen): Go to a bathroom and let the water run into the tub. While it is running get saturated with water vapour. Then jump into the tub. The difference is considerable.

BRENNER: This is not true. (See, however, the subsequent paper.)

FREY: In addition to layer thickness, layer density must be taken in consideration as well. Depending on the layering technique, thicker layers can be more or less densely packed than thinner ones. It would thus be possible to understand both decreases and increases of R_F values accompanying the increase of layer thickness.