THE ROLE OF SOLVENT VAPOUR IN THIN-LAYER CHROMATOGRAPHY*

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INTRODUCTION

In thin-layer chromatography (TLC) development of the plate is usually performed after saturation of the chamber with solvent vapour. Although this procedure has appeared to be valuable in many instances, some authors recommend the use of unsaturated chambers, especially in the separation of multicomponent mixtures of closely related compounds. VON ARX AND NEHER[1] reported better separation of amino acids if development of the plate was started immediately after introduction of the solvent. Their results were recently verified by JONES AND HEATHCOTE[2], while DE ZEEUW[3] showed improved separation of hypnotics by using an unsaturated chamber.

VON ARX AND NEHER[1] supposed that in saturated chambers the solvent ascends too fast, whereas DE ZEEUW suggested that the improved separation might be due to changes in the composition in the solvent during development. It is the purpose of this paper to show that this suggestion holds true.

In TLC the solvent is ascending in a "dry" adsorbent. It is wellknown that during this process multicomponent mixtures can demix on the plate (as for instance in frontal analysis). The front of the ascending liquid is a single component (A) which is followed by a zone of a binary mixture (A + B) then by a zone of a ternary mixture (A + B + C) and so on. The non-homogeneity causes a stepwise "gradient elution". The method was developed by NIEDERWIESER AND BRENNER[4] and they gave it the name of "polyzonal" TLC. It should be noted that the components of the solvent mixture have an increasing affinity to the (silica gel) adsorbent in the order, A, B, C and so on.

In TLC, using volatile multicomponent solvents the situation is, however, much more complex, because apart from the adsorption of the solvent, liquid vapour equilibria play a role as well as the adsorption of solvent components in the gas phase.

If, for example, we take a binary mixture of chloroform and ether as the solvent we may conceive the following processes:

(1) By capillary action of the porous adsorbent solvent ascends and by a process comparable to frontal analysis a certain zone of pure chloroform is followed by the binary mixture.

(2) In the dry part of the plate adsorption of solvent vapour will take place and,

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ether being more strongly adsorbed than chloroform, the adsorbate will mainly consist of ether.

(3) In the wet part of the plate absorption of vapour as well as evaporation of solvent takes place.

As a consequence of the absorption due to the solvent and adsorption of vapour the leading edge will not be pure chloroform.

It will be obvious that the extent to which the processes mentioned under (2) and (3) will affect the development depends upon many factors, such as vapour pressure and relative affinity of the solvent components to the adsorbent, the geometry of the chambers and especially whether the chamber has been saturated or not.

It should be observed that in the wet part of the plate there is no difference between ether adsorbed from the vapour phase and ether adsorbed from the solvent.

EXPERIMENTAL

Materials and equipment

Substances: 0.2 % w/v solutions in chloroform of: (1) heptobarbital; (2) phenobarbital; (3) allobarbital; (4) hexobarbital; (5) methylphenobarbital; (6) bromisoval; (3–6) mixture of substances 3 + 4 + 5 + 6, 0.2 % w/v each. Names and qualities of the substances according to the Netherlands Pharmacopœia, Ed. VI (1958).

Load: 0.005 ml (corresponding with 10 μg of each substance).

Adsorbent: Silica Gel GF 254 (Merck), 30 g/60 ml distilled water to prepare five plates.

Plate size: 20 × 20 cm.

Layer thickness: 0.25 mm when spread.

Activation: Air dried for 15 min, heated for 30 min at 110° in an oven with fan, cooled and stored in a desiccator over blue silica gel.

Solvents: Chloroform, ether, benzene, "Pro Analyse" (Merck).

Length of run: 10 cm, spots applied 1.5 cm from the bottom of the plate.

Temperature: 20–22°.

Relative humidity: 27–48 %. In this range no changes in the chromatograms were observed as a consequence of different humidities.

Apparatus: Spreading device: Desaga; N-chambers, 21 × 21 × 9 cm: Desaga; S-chamber, 20 × 20 × 0.1 cm: Camag; glass troughs, 19 × 1.5 × 1.5 cm.

Detection: U.V. light of 254 nm: Camag.

Balances: Mettler K 7 and Mettler BCH.

Saturation conditions

Saturation of the chamber atmosphere with solvent vapour was achieved by lining the walls of the N-chamber with filter paper. After a saturation time of 30 min development was started.

Unsaturated chambers were obtained by placing the plates into the chambers immediately after the solvent was added. No filter paper was used in these cases. It should be noted that when the solvent edge has reached the 10 cm line, more solvent was used in the case of unsaturated chambers due to evaporation of the solvent; accordingly solute spots will have run higher than in the case of saturated chambers.

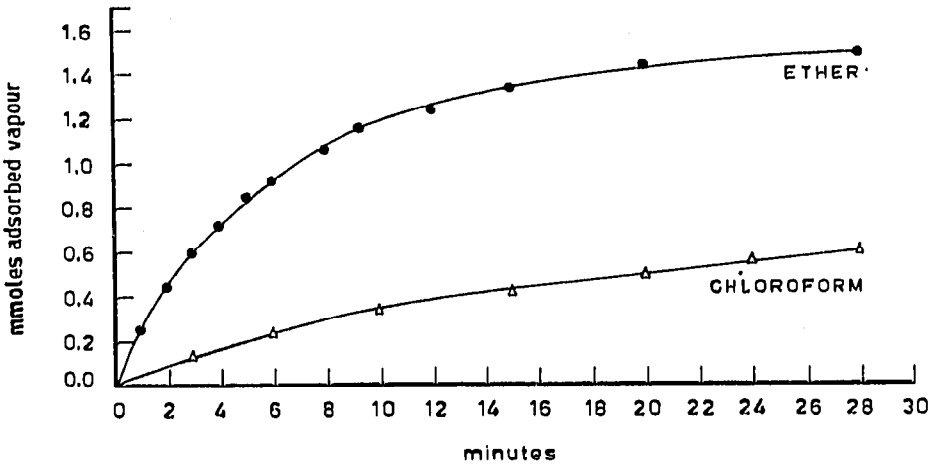

Fig. 1. Vapour adsorption of ether (●–●) and chloroform (△–△) by silica gel plates from an unsaturated atmosphere. Plate size 20 × 20 cm, layer thickness 0.25 mm. Weight measurement with a Mettler K 7 top balance as described in the text.

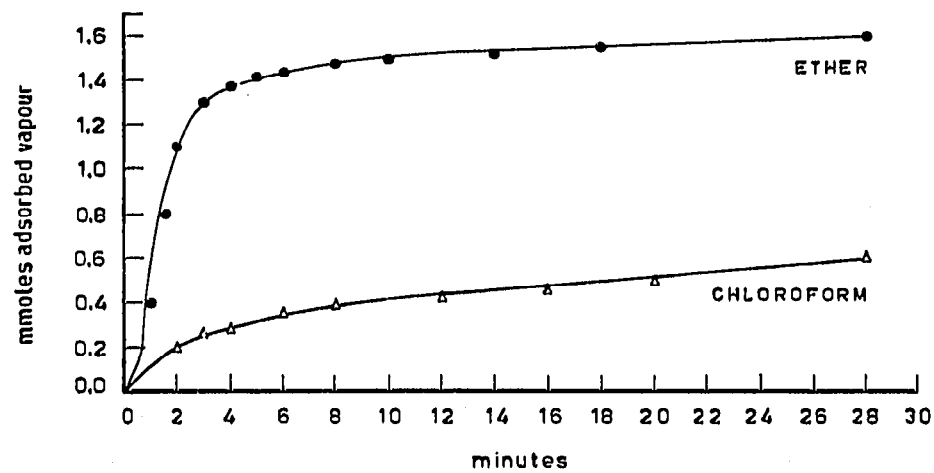

Fig. 2. Vapour adsorption of ether (●–●) and chloroform (△–△) by silica gel plates from a saturated atmosphere. Details as in Fig. 1.

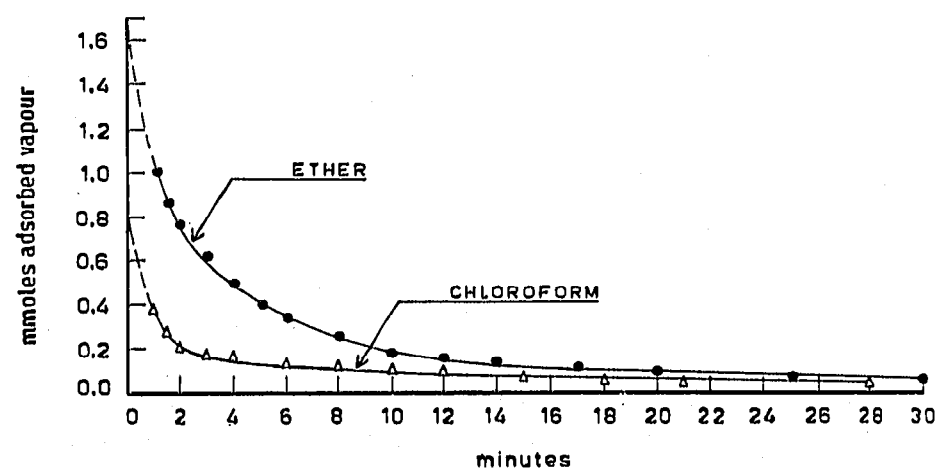

Fig. 3. Evaporation of adsorbed ether vapour (●–●) and chloroform vapour (△–△) from silica gel plates after a 5 min vapour uptake in a saturated atmosphere. Weight measurement with a Mettler BCH balance as described in the text.

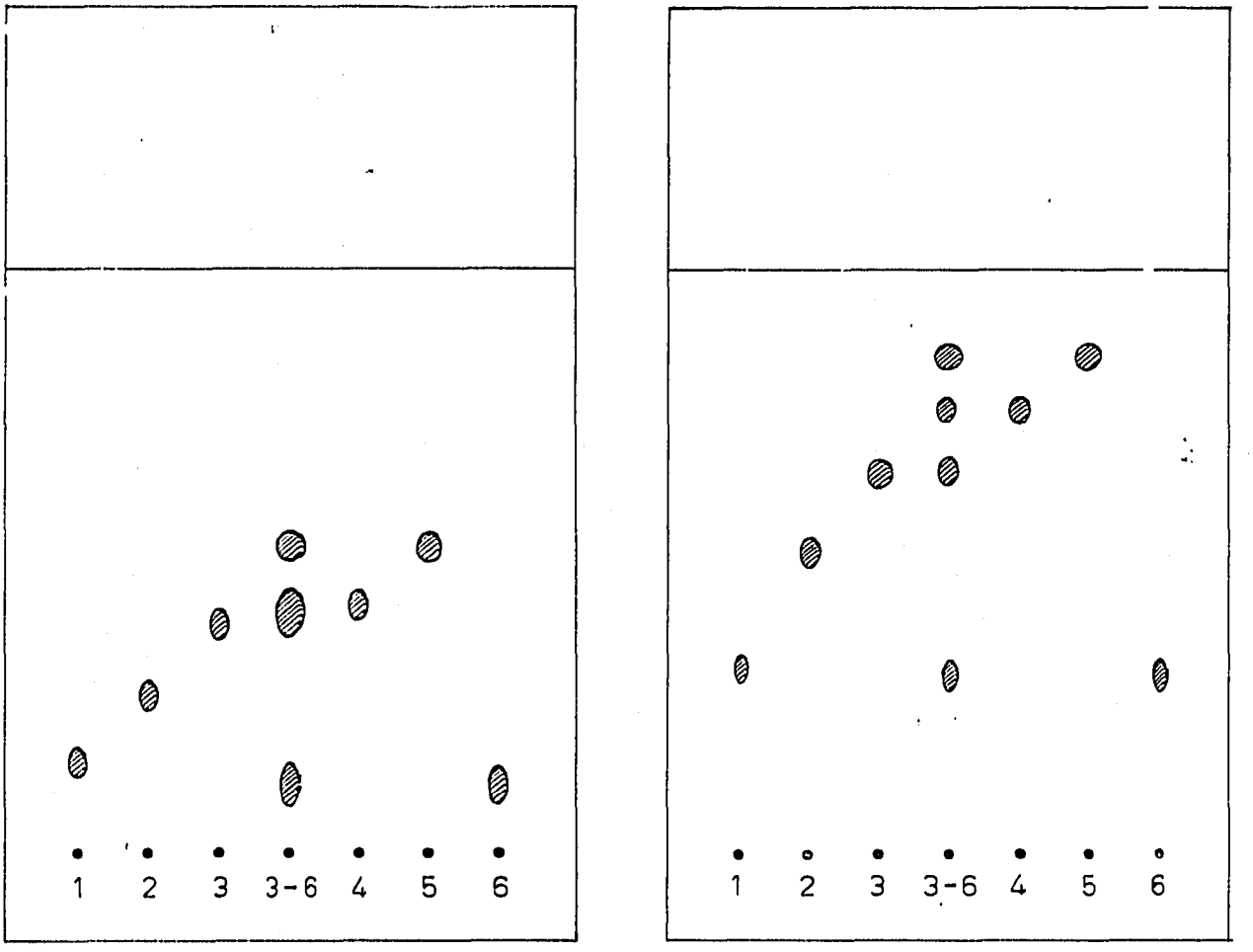

Fig. 4. Separation of hypnotics with chloroform–ether (75:25, v/v). Saturated chamber. 1 = heptobarbital; 2 = phenobarbital; 3 = allobarbital; 4 = hexobarbital; 5 = methylphenobarbital; 6 = bromisoval; 3–6 = allobarbital + hexobarbital + methylphenobarbital + bromisoval.

Fig. 5. Separation of the hypnotics with chloroform–ether (75:25, v/v). Unsaturated chamber. For numbering, see Fig. 4.

Weight measurement of adsorbed vapour

In order to obtain data about the amount of adsorbed vapour, the increases of plate weight were measured when a dry plate was brought into a saturated or unsaturated vapour atmosphere of one of the solvent components. For this purpose a plastic box was used in which a Mettler K 7 top-balance was placed. In the case of unsaturation, the plate and 4 troughs (19 × 1.5 × 1.5 cm) filled with a solvent component were brought into the box at the same instant; in the case of saturation the 4 troughs were placed into the box first, followed by the plate after 30 min.

Evaporation of adsorbed vapour was measured by placing a dry plate into a saturated vapour atmosphere for 5 min (*e.g.* an N-chamber with a trough filled with a solvent component) and by observing the decrease of weight on a Mettler BCH balance.

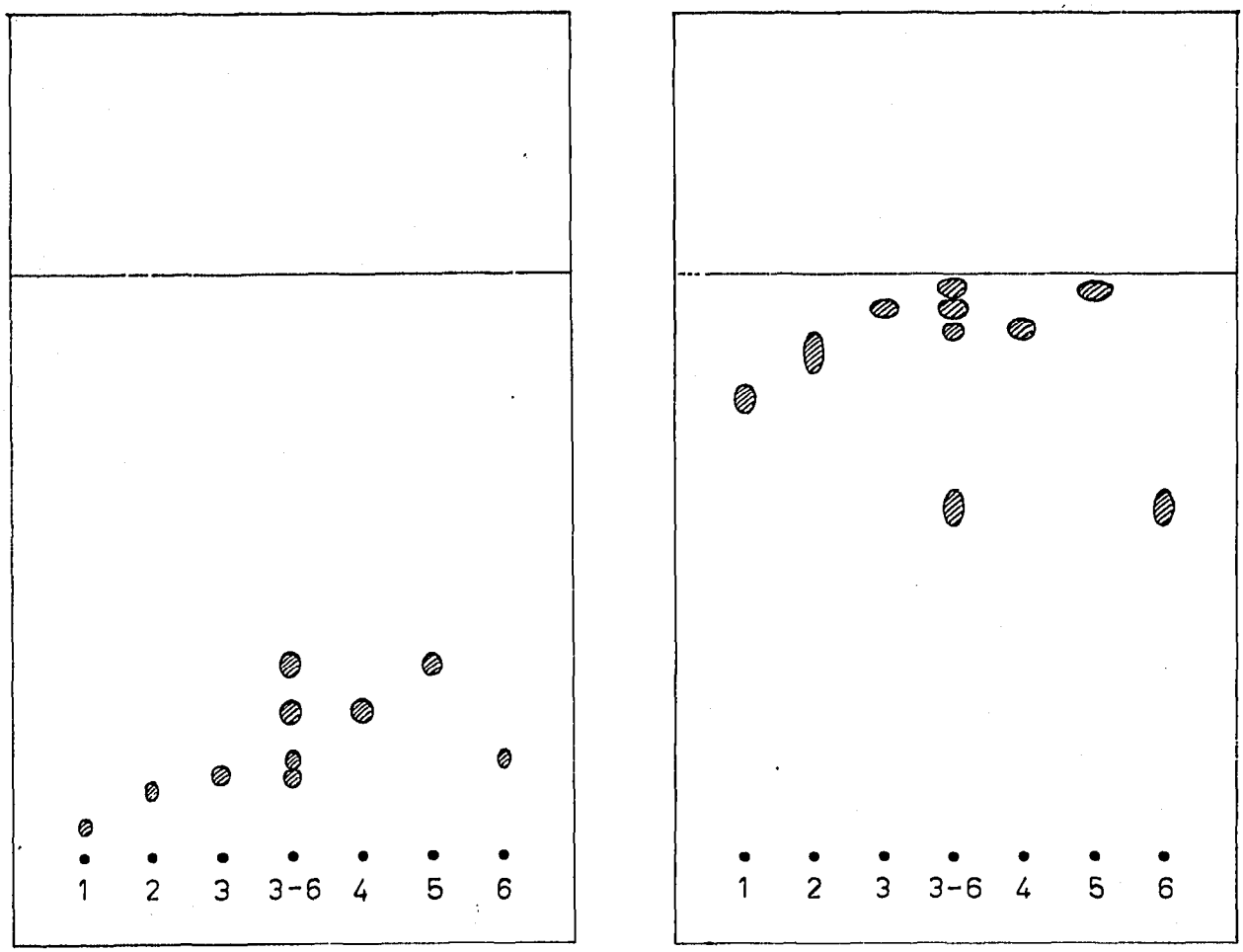

Fig. 6. Separation of hypnotics with chloroform. Saturated chamber. For numbering, see Fig. 4.

Fig. 7. Separation of hypnotics with ether. Saturated chamber. For numbering see Fig. 4.

RESULTS AND DISCUSSION

The adsorption of chloroform- and ether-vapour from unsaturated and saturated atmospheres is illustrated in Figs. 1 and 2. The amounts of adsorbed vapour have been expressed in millimoles, rather than in milligrams because of the differences in molecular weight. It can be seen that, especially in the beginning, the amount of adsorbed vapour from saturated atmospheres is higher. The evaporation curves of chloroform and ether vapour are shown in Fig. 3. From these three figures it is obvious that the adsorption of ether is much stronger than that of chloroform.

The chromatographic experiments were all done with the substances and the mixture mentioned under "Materials and Equipment".

Fig. 4 illustrates the separation in saturated chambers with chloroform–ether (75:25, v/v) as solvent. The separation of mixture 3–6 is incomplete. In unsaturated chambers the chromatogram as in Fig. 5 is obtained. Although the location of the spots is higher than in Fig. 4 due to the evaporation of solvent from the plate during the run, it is clear that the selectivity of the separation in Fig. 5 is much better, especially for the mixture 3–6.

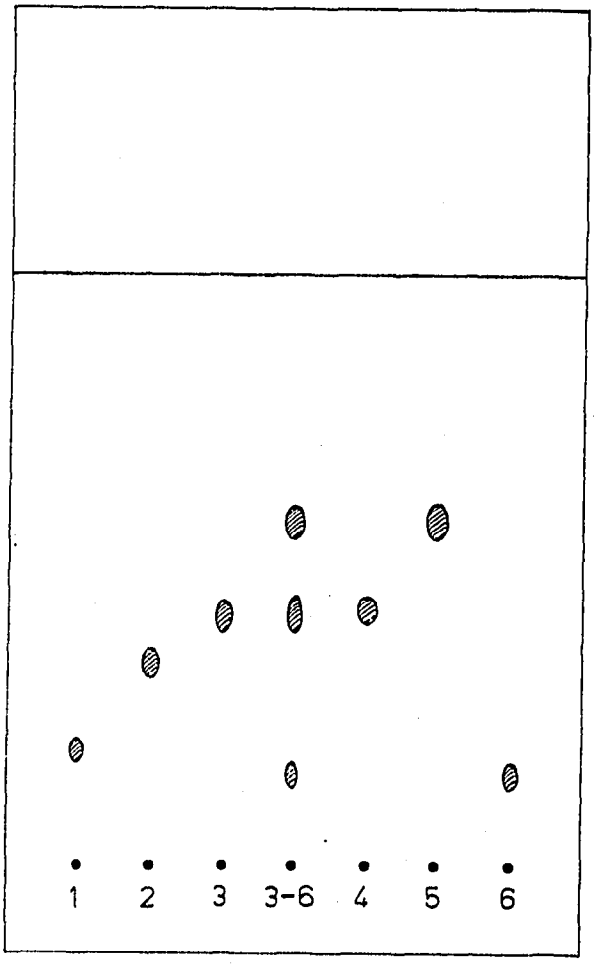

Fig. 8. Separation of hypnotics with benzene–ether (75:25, v/v). Unsaturated chamber. For numbering, see Fig. 4.

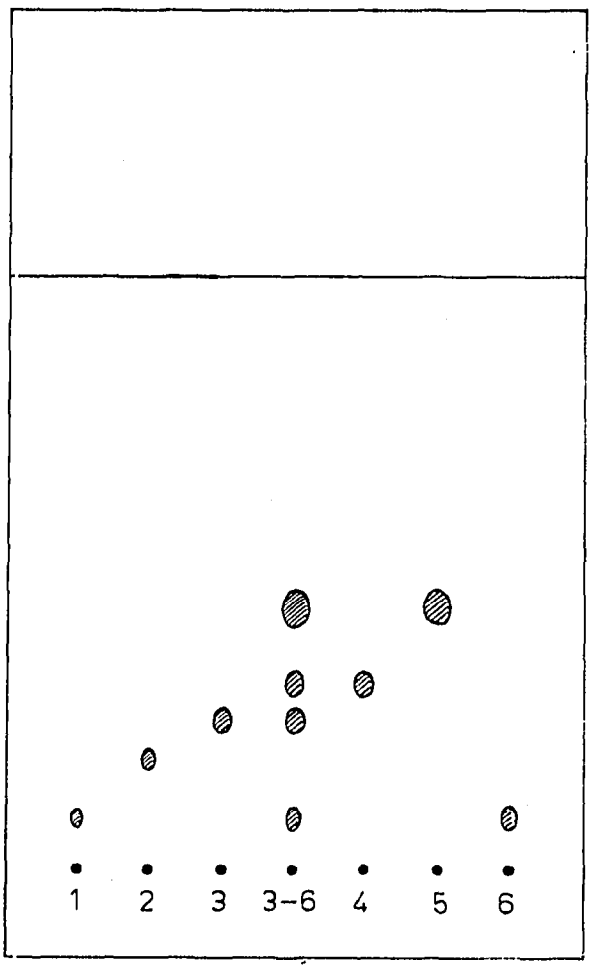

Fig. 9. Separation of hypnotics with benzene–ether (85:15, v/v). Unsaturated chamber. For numbering, see Fig. 4.

Fig. 6 shows the separation with chloroform and in Fig. 7 ether is used as solvent, both in saturated chambers. Comparing Figs. 6 and 7, two differences can be observed. In Fig. 7, allobarbital (spot 3) runs faster than hexobarbital (spot 4) and bromisoval (spot 6) runs slower than allobarbital, phenobarbital and heptobarbital (spots 3, 2 and 1).

With regard to Fig. 4 and Fig. 5 we may conclude that in unsaturated chambers the influence of ether is smaller since hexobarbital runs faster than allobarbital and bromisoval has the same migration rate as heptobarbital. Ether is more polar than chloroform and the adsorbent has a greater affinity to ether vapour. However, in unsaturated chambers the amount of ether vapour available will be less than in saturated chambers, especially at the beginning of the run and this will result in a decrease of ether vapour adsorption. This is in full agreement with the adsorption data of Figs. 1 and 2.

From these experiments it becomes obvious that the amount of adsorbed ether vapour greatly determines the separation since the amount of ether in the solvent has not been changed. At the same time, there is also an adsorption of the chloroform

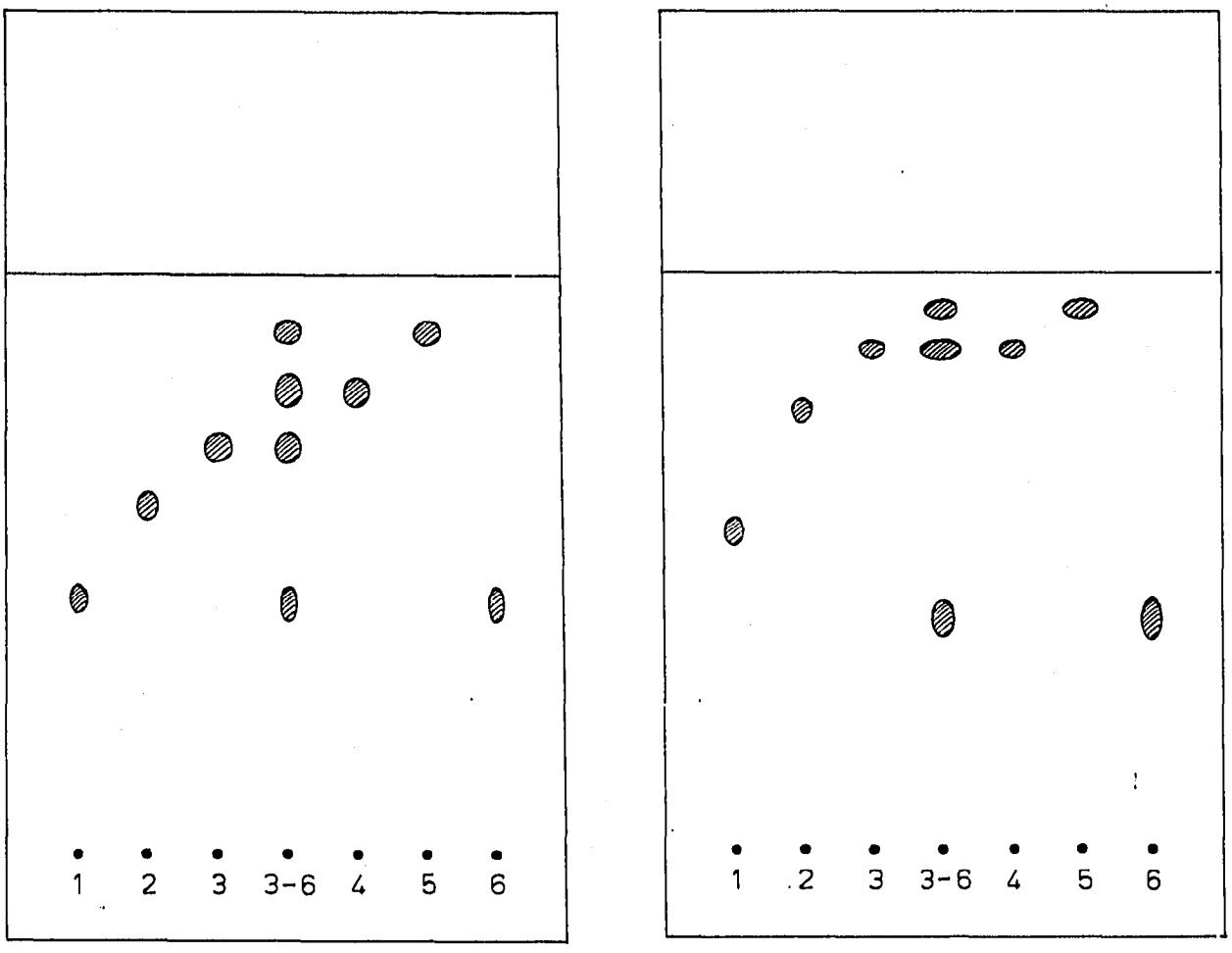

Fig. 10. Separation of hypnotics with chloroform in the presence of a trough containing ether. Unsaturated chamber. For numbering, see Fig. 4.

Fig. 11. Separation of hypnotics. Presaturation of the plate with vapour by a trough containing ether for 5 min, followed by development with chloroform. For numbering, see Fig. 4.

vapour but this is of minor importance as we are considering chloroform to be our base-line from which the influence of the ether is examined. Therefore it does not make a great difference when we replace the chloroform in the solvent by a less polar compound, *e.g.* benzene. We only have to take into account that benzene will be less strongly adsorbed than chloroform and accordingly the amount of adsorbend ether vapour will be higher. This is clearly shown in Figs. 8 and 9. With benzene–ether (75:25, v/v) in unsaturated chambers the mixture 3–6 is not separated due to the greater influence of the ether. The mixture is fully separated however, when we change the solvent ratio of benzene–ether to 85:15, v/v.

The role of the ether vapour in the separation could also be demonstrated in the following experiments. The separation of Fig. 10 was obtained by developing the plate in an unsaturated chamber with chloroform only, but a trough containing 10 ml ether was present at the bottom of the chamber during development. The chloroform, the trough with the ether and the plate were placed in the chamber immediately one after the other. As no ether was present in the solvent, any influence of the ether on the separation can only be due to its vapour. This influence is evident if Fig. 10 is com-

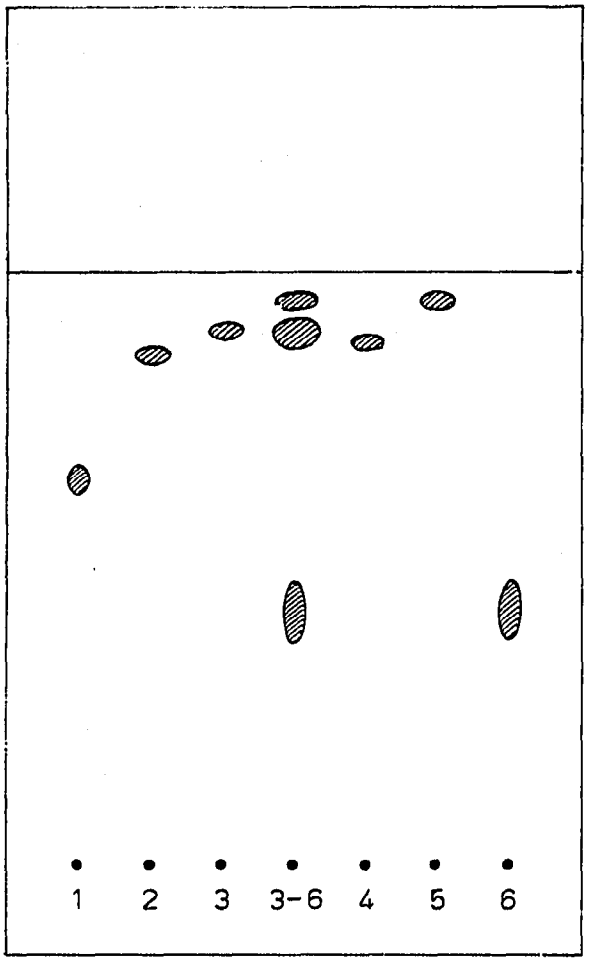

Fig. 12. Separation of hypnotics. Presaturation of the plate with vapour by a trough containing ether for 30 min, followed by development with chloroform in an S-chamber. For numbering, see Fig. 4.

pared with Fig. 6 (solvent chloroform, no trough). It can also be observed that the separation in Fig. 10 is almost the same as in Fig. 5, where we used chloroform–ether (75:25, v/v) as solvent.

The amount of ether vapour in the chamber was increased by putting the plate and the trough with ether together in the chamber for presaturation. After a 5 min presaturation time, the chloroform was added by means of a small tube inserted in the cover of the chamber. The separation is shown in Fig. 11. The effect of the ether is greater here since allobarbital and hexobarbital are not separated and bromisoval runs slower than heptobarbital. Presaturation with ether vapour for 30 min, followed by development in an S-chamber with chloroform resulted in the separation as shown in Fig. 12. The influence of the ether here becomes apparently so large that the separation is comparable with that in Fig. 7, when we used ether only as solvent. For this experiment the S-chamber was preferred for three reasons:

(1) Decreased evaporation of adsorbed ether from the plate;
(2) Decreased removal of adsorbed ether by chloroform vapour;
(3) Decreased ether absorption by the solvent.

It thus becomes obvious from this experiment that quite small amounts of adsorbed ether are responsible for great changes in the separation. After 30 min

presaturation the plate had adsorbed about 1.5 mmoles of ether vapour, which is about 112 mg. When we use for example chloroform–ether (75:25, v/v) as solvent, we needed 3.10 g to wet 11.5 cm of the plate, in which about 415 mg of ether can be found. However, the influence of these 415 mg ether, which can be seen in Fig. 5, is far less than the influence of the adsorbed 112 mg ether vapour which causes the separation shown in Fig. 12. These data underline our presumption that the amount of adsorbed ether determines the separation to a very high degree and that the amount of ether present in the initial solvent is of minor importance.

It should be remembered that the influence of the vapour is not restricted to vapour adsorption on the dry plate. In addition, there is also absorption of vapour by the solvent on the wet part of the plate. In our opinion however, the influence of the latter process will be rather small in comparison to the influence of vapour adsorption.

CONCLUSIONS

The investigations have shown that the role of solvent vapour in TLC is very important. With multicomponent solvents the best separations are not *a priori* associated with saturated chambers. Optimal conditions have to be established experimentally, especially with regard to the influence of solvent vapour. The use of unsaturated chambers or presaturation of the plate with one or more vapour components or the use of troughs containing solvent can be very valuable. The trough technique can also be used to examine the vapour influence of other liquids not present in the solvent. Thus, components which frequently cause immiscibility can be used, *e.g.* water or ammonia.

Since there is little or no possibility at all for vapour impregnation in column chromatography, the assumed similarity between CC and TLC becomes doubtful. This may be the reason that several separations in TLC have proved to be impossible on a column. Also dry columns, *e.g.* those described by DAHN AND FUCHS[5], are not comparable with thin layers since the diffusion of vapour is very limited.

It is clear that after these experimental observations it is necessary to obtain a better understanding of the fundamentals playing a role in these complex processes in TLC. For this purpose, and to improve TLC separations by making a fruitful use of the influence of vapours, further investigations are being carried out.

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SUMMARY

The role of solvent vapour in thin-layer chromatography (TLC) was investigated. Unsaturated chambers proved to give better separations than saturated chambers, due to the differences in the amounts of vapour present during development.

It was also shown that vapour not originating from the solvent could highly affect the separations. These non-solvent vapours were introduced by making use of troughs. With this trough technique new possibilities for testing the influence of many different liquids become available.

The results indicate that the influence of vapours can be a valuable factor in improving TLC separations.

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