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Vapor-Programmed Thin-Layer Chromatography, Development and Applications

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I. INTRODUCTION

During the past ten years thin-layer chromatography (TLC) has become a very useful and versatile tool in analytical chemistry. Despite some confusion at the beginning, the performance of the technique has developed into a uniform procedure that is discussed in the many books on the subject. It is remarkable, however, that the theoretical aspects of the TLC processes are still not fully understood.

We have been using the general procedures of TLC for several years in pharmaceutical science for the analyses of drugs with reasonable success. However, when investigating the TLC behavior of hypnotics and sedatives we observed two striking phenomena: a more efficient separation with the aid of the so-called unsaturated chamber and, furthermore, complete disappearance of a given separation in the sandwich chamber; the normal chamber provided complete resolution.

These two phenomena, for which the existing theories could not provide a suitable explanation, led to the investigations described in this chapter. After examining the influence of solvent vapor in TLC, a much better insight could be gained into the different processes affecting the migration of the spots, thus allowing a simple explanation of the two phenomena mentioned. Moreover, the better understanding of the TLC processes resulted in the development of a chamber type by which a new thin-layer chromatographic technique became possible, this technique we call vapor-programmed TLC. The development of the new apparatus is described in detail and examples of its capabilities are presented here. The results indicate that vapor-programmed TLC is a valuable contribution to the existing possibilities of TLC.

II. EXPERIMENTAL DATA

The chromatographic experiments described in this chapter were carried out according to the standard procedures and apparatus described below, unless stated otherwise in the text.

Adsorbents

(*a*) Silica gel GF 254 (E. Merck), 30 g/60 ml demineralized water are carefully mixed and stirred for 1 min in a mortar to give a homogenous suspension for the preparation of 5 plates.

(*b*) Aluminum oxide GF 254 (E. Merck), 30 g/60 ml demineralized water are carefully mixed and stirred for 10 min in a mortar to give a homogenous, slightly viscous suspension for the preparation of 5 plates.

The preparation of the suspensions can also be done with distilled water.

Spreading apparatus: Desaga.

Plate size: 20 × 20 cm.

Layer thickness: 0.25 mm when spread.

Plate drying: After preparation 15 min in air, then 30 min at 110°C in an oven with a fan. Cooling and storage is done in a desiccator over blue silica gel.

Demineralized water: Conductivity $< 0.4 \times 10^{-4}\ \Omega^{-1}\ m^{-1}$.

Solvents: "Pro Analysi" grade (E. Merck). N-chambers contained 100 ml of solvent, S-chambers contained 20 ml of solvent. Solvent mixtures were prepared immediately before development and were used once. The compositions of these mixtures are given by volume.

Substances: The following 0.2% w/v solutions were used in the chromatographic experiments: hypnotics and sedatives in chloroform, color dyes in benzene, sulfonamides in acetone, and local anaesthetics in 96% ethanol (as HCl salts). The identities of the drugs were established by melting points and, if possible, by examining their identity reactions as described in the various pharmacopoeiae. The purity of the substances was examined chromatographically. All components proved to be more than 99.0% pure, except cyclobarbital which showed a slight decomposition. The azo-dyes were available as a solution in benzene (Desaga).

Sample load: 0.005 ml with the aid of 0.01 ml micropipets (Desaga), corresponding with 10 μg solute. Mixtures of substances are composed in a way that 0.005 ml contains 10 μg of each component.

Starting points: 1.5–2.5 cm from the bottom edge of the plate.

Length of run: 10 or 15 cm over the starting points.

Temperature: 21° ± 1°C.

Relative humidity: 27–51%. In this range reproducibility of the chromatograms was observed. The experiments with azo-dyes were done at a relative humidity of 26–30%.

Development chambers: Normal tank chambers (N-chambers), 21 × 21 × 9 cm (Desaga type) and sandwich chambers (S-chambers), 20 × 20 × 0.1 cm (Camag type). In the sandwich chamber a plain glass plate was used to cover the adsorbent plate and no special arrangements were made for saturation procedures.

Detection: Ultraviolet light of 254 nm by means of a Universal lamp, Sylvania Germicidal G 8 T, with UV-G5 filter (Camag).

Photography: In UV light of 254 nm with two lamps (as under Detection) on either side of the plate, exposure 15 sec, aperture 5.6, distance 70 cm. Camera: Asahi Pentax SV, Super Takumar 1:1.8/55 lens with 49 mm ghostless filter (Asahi). Film: Agfacolor CT 18 diapositive (Agfa).

Saturated chambers: N-chambers lined with filter paper, with 100ml of solvent. After a saturation time of 30–45 min the plate is introduced.

Unsaturated chambers: N-chambers without filter paper, with 100 ml of solvent. The plate is placed into the chamber immediately after introduction of the solvent.

Glass troughs: 19 × 1.5 × 1.5 cm.

Balances: Mettler K7 and Mettler BCH.

Special conditions: The spotting period, that is, the time between removal of the plate from the desiccator and the introduction into the chamber, is at least 15 min.

Plates for N-chambers are stripped 0.5 cm wide at the side edges; plates for S-chambers are stripped 0.5 cm at the side edges and the top edge to ensure suitable fitting of the spacer-frame; plates for the vapor-programming chamber are stripped 0.5 cm at the side edges and the bottom edge.

III. THE ROLE OF SOLVENT VAPOR IN TLC*

A. Introduction

In TLC, development of the plate is usually performed after saturation of the chamber with solvent vapor. This procedure is recommended in every book on TLC in order to obtain more reproducible R_f values (30) and to avoid the appearance of edge effects as described by Demole (9) and Stahl (34). Although this procedure has proved to give suitable results in many instances, some authors recommend the use of unsaturated chambers, particularly in the separation of multicomponent mixtures of closely related substances (26,1,46,29). In our experiments with hypnotics and sedatives we also obtained improved separation by using an unsaturated chamber (39). This observation was recently confirmed by Hermans and Kamp (16).

A suitable explanation of these observations was not readily available. Von Arx and Neher (1) presumed that in saturated chambers the solvent

* In this section the influence of adsorbed water vapor on the adsorbent is left out of consideration for the sake of clearness. If experiments are done under standardized relative humidity and according to our working procedure, this water vapor influence will be constant.

ascends too fast, thus preventing sufficient selectivity in the adsorption processes by the adsorbent. Zinkel and Rowe (46) ascribed the improved separations to the prolonged time of run due to solvent evaporation from the plate, in combination with the occurrence of gradient development. These presumptions were not tested experimentally and the absence of a theoretical explanation led us, therefore, to a more detailed investigation into the various TLC processes. In our opinion, the use of single-component solvents, which are generally preferred in the more theoretical studies, would be incorrect because an explanation of the phenomena herewith is not valid for multicomponent solvents. Therefore, we started to work with a binary solvent system because the processes with these systems are easily applicable to both single- and multicomponent systems. A further reason to start with a binary solvent is that in practical TLC analysis multicomponent solvents are more frequently used than single-component solvents.

B. Examination of Some TLC Processes

In TLC the solvent is ascending in a "dry" adsorbent. It is well known that during this process multicomponent solvents can "demix" on the plate (as in frontal analysis for instance). The front of the ascending liquid contains 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, with the components having an increasing affinity for the adsorbent in the order $A < B < C$. The demixing causes a stepwise gradient elution which was applied by Niederwieser and Brenner (28) under the name "polyzonal TLC."

In normal TLC, using volatile multicomponent solvents, the situation is, however, much more complex because apart from the adsorption of the solvent, liquid-vapor equilibria play a role as well as the adsorption of solvent components from 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 will be formed, followed by the binary mixture.

2. In the dry part of the plate adsorption of solvent vapor will take place and, ether being more strongly adsorbed than chloroform, the adsorbate will mainly consist of ether.

3. In the wet part of the plate, which is already covered by the ascending solvent, absorption of vapor as well as evaporation of solvent will take place.

As a consequence of the adsorption in the dry part and the absorption in the wet part of the plate, the front zone will not be pure chloroform. Furthermore, it will be obvious that the extent to which processes *2* and *3* will affect the development depends on many factors, such as vapor pressure and relative affinity of the solvent components for the adsorbent, the geometry of the chamber, the temperature and, especially, whether or not the chamber has been saturated before development.

C. Experiments with Vapors

Because our main interest lies in the differences between unsaturated chambers and saturated chambers, the experiments are aimed at elucidating the influence of these parameters. In order to obtain data on the amount of adsorbed vapor and on the velocity of this process, the increase in plate weight was measured when a dry plate was brought into a saturated or unsaturated vapor atmosphere of one of the solvent components. This was done in a plastic box in which a Mettler K7 top-balance was placed. In the case of unsaturation, the plate and 4 glass troughs filled with 20 ml of a solvent component each were brought into the box at the same instant. In the case of saturation the 4 filled troughs were placed in the box first, followed by the plate after 30 min. In both cases vapor adsorption was measured over a 30-min period.

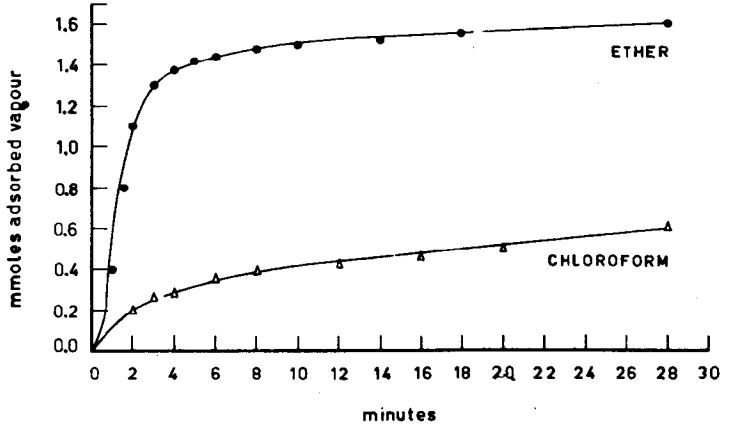

Fig. 1. Vapor adsorption of ether and chloroform by silica gel plates from an unsaturated atmosphere. Plate size 20 × 20 cm, layer thickness 0.25 mm. Weight measurement with a Mettler K 7 top balance as described in the text. Figures 1–12 are reproduced from R. A. de Zeeuw, "The role of solvent vapour in thin layer chromatography," "*J. Chromatog.*, **32**, 43 (1968), with permission of the publisher.

Evaporation of adsorbed vapor was measured by placing a dry plate into a saturated vapor atmosphere for 5 min (e.g., an N-chamber with a trough filled with a solvent component) and by observing the decrease in weight on a Mettler BCH. This was done by hanging the plate mounted in a wire frame onto the balance. The adsorbed vapor could freely evaporate in the balance cabinet. Due to the large volume of this cabinet the evaporated components produced only a small partial pressure which, in turn, had little influence on the evaporation process.

All weight measurements were repeated 5 times, each with different plates. In all cases identical adsorption and evaporation curves were obtained with the corresponding values showing less than 7% variation. Corrections for changes in upward pressure could be neglected.

The adsorption of chloroform and ether vapor from unsaturated and saturated atmospheres are given in Figures 1 and 2. The amounts of adsorbed vapor have been expressed in millimoles rather than in milligrams because of the differences in molecular weight. It can be seen, especially in the beginning, that the amount of adsorbed vapor from saturated atmospheres is higher. It should be noted that this period of about 20 min is usually an important part of the time needed for development. The evaporation curves of ether and chloroform vapor are shown in Figure 3. From these three figures it is obvious that, for this adsorbent (silica gel), the adsorption of ether is much stronger than that of chloroform. It will be clear that the shape of the curves in Figures 1, 2, and 3 are dependent on the geometry of the space in which they were measured, particularly in the

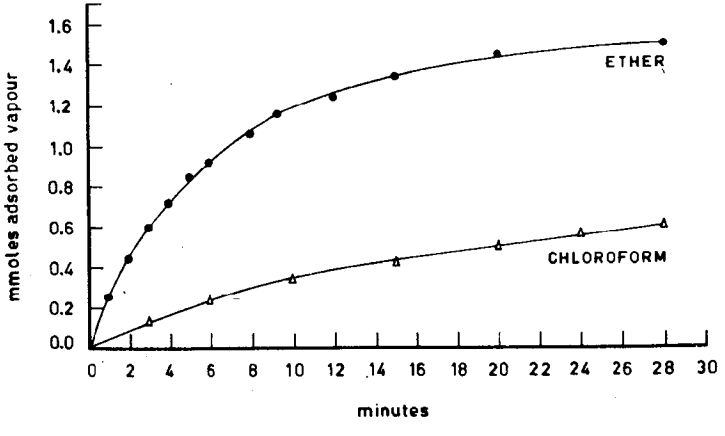

Fig. 2. Vapor adsorption of ether and chloroform by silica gel plates from a saturated atmosphere (details as in Fig. 1).

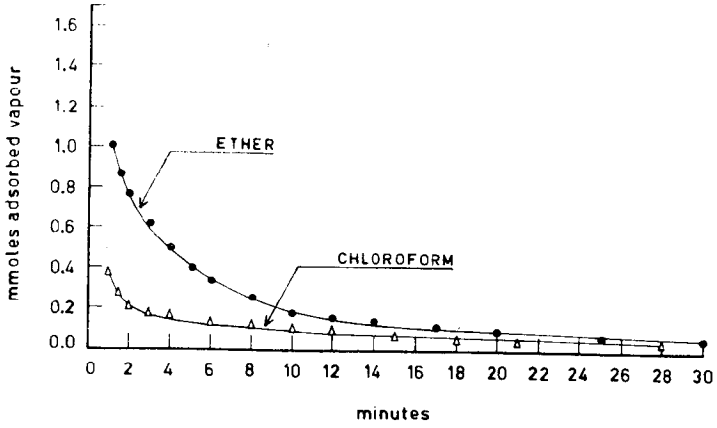

Fig. 3. Evaporation of adsorbed ether- and chloroform vapor from silica gel plates after a 5 min vapor uptake in a saturated atmosphere. Weight measurement with a Mettler BCH balance as described in the text.

beginning. Thus, the shapes in a normal chromatography tank may be different from those mentioned above, but the maximum adsorption values will remain the same.

The vapor pressures of ether and chloroform at 20°C are 435 and 154 mm, respectively (36). The amounts of ether and chloroform vapor being adsorbed by the plate at these vapor pressures and in a plastic box having a volume of about 10 l are approximately 58 and 67 cm^3 respectively. It is clear, then, that only very small quantities of the available vapors are adsorbed. For 20 × 20 cm plates, for example, the volume of vapor adsorbed is less than that contained in the 1 cm layer lying just above the entire plate surface.

The next chromatographic experiments were all done with a series of hypnotics numbered as follows: 1 = heptobarbital, 2 = phenobarbital, 3 = allobarbital, 4 = hexobarbital, 5 = methylphenobarbital, 6 = bromisoval. Also, a mixture of the components 3 + 4 + 5 + 6 was used.

Figure 4 illustrates the separation in a saturated chamber with chloroform-ether (75 + 25). The separation of the mixture 3–6 is incomplete and the spreading of the spots is limited to the lower part of the plate. In an unsaturated chamber the chromatogram of Figure 5 is obtained using the same solvent composition. Comparing Figures 4 and 5 it is clear that the selectivity of the separation shown in Figure 5 is much better. The mixture 3–6 is completely separated and the spread of the spots is enlarged. Furthermore, it can be seen that the location of the spots in Figure 5 is higher, due

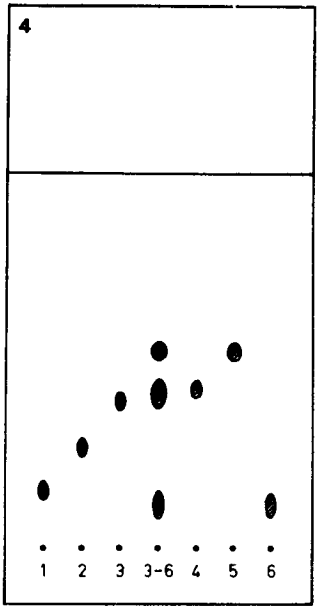

Fig. 4. Separation of hypnotics with chloroform-ether (75 + 25) in a saturated chamber. 1 = heptobarbital; 2 = phenobarbital; 3 = allobarbital; 4 = hexobarbital; 5 = methylphenobarbital; 6 = bromisoval; 3–6 = mixture of 3 + 4 + 5 + 6.

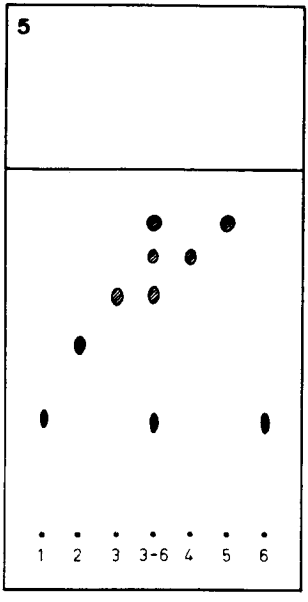

Fig. 5. Separation of hypnotics with chloroform-ether (75 + 25) in an unsaturated chamber; numbering as in Figure 4.

to solvent evaporation from the plate during the run. Hence, more solvent is needed to complete the 10 cm run, resulting in a longer duration of the development and a higher location of the spots.

Figure 6 shows the separation with chloroform and in Figure 7 ether is used as solvent, both in saturated chambers. The more polar character of ether results in higher R_f values but a closer examination of Figures 6 and 7 shows two differences in the separation sequence. In Figure 7, with ether as solvent, allobarbital (spot 3) moves faster than hexobarbital (spot 4) whereas bromisoval (spot 6) moves slower than heptobarbital, phenobarbital, and allobarbital (spots 1, 2, and 3).

With regard to Figures 4 and 5 we may conclude that in unsaturated chambers the influence of ether is smaller, since hexobarbital moves faster than allobarbital and bromisoval has the same migration rate as heptobarbital. This can be explained as follows. Ether is more polar than chloroform and the adsorbent has a greater affinity for ether vapor. However, in

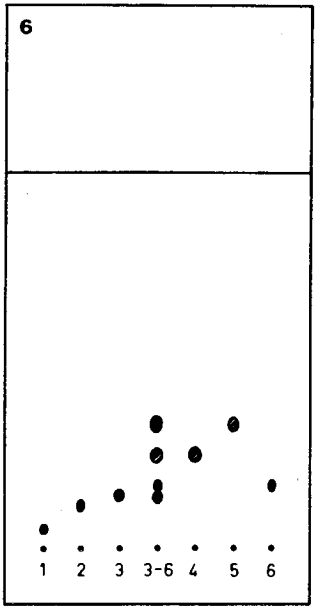

Fig. 6. Separation of hypnotics with chloroform in a saturated chamber; numbering as in Figure 4.

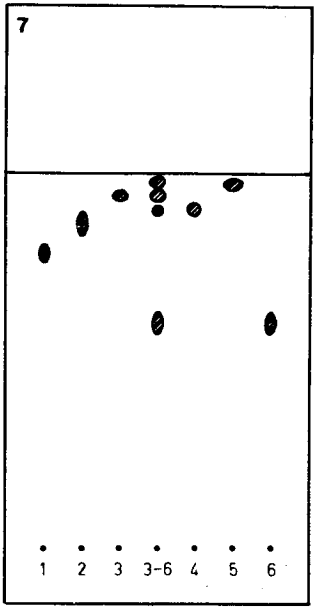

Fig. 7. Separation of hypnotics with ether in a saturated chamber; numbering as in Figure 4.

unsaturated chambers the amount of ether vapor available is less than in saturated chambers, especially in the beginning of the run and this results in a smaller ether vapor adsorption. This is in full agreement with the adsorption data of Figures 1 and 2.

From these experiments it becomes obvious that the amount of adsorbed ether vapor greatly determines the separation since the amount of ether in the solvent has not been changed. It should be noted that at the same time there is also an adsorption of the chloroform vapor but, as a consequence of the greater affinity of ether, this process will be of minor importance. Moreover, the effects of chloroform can be considered to be our base line from which the ether influence is examined. Therefore, it does not make a great difference if we replace the chloroform in the solvent by a less polar compound—benzene, for example. We should only take into account that benzene is less strongly adsorbed than chloroform. Accordingly the amount of adsorbed ether vapor will be higher. This is clearly shown in Figures 8 and 9. With benzene-ether (75 + 25) in unsaturated chambers the mixture 3–6 does not separate due to the greater influence of the ether. Further-

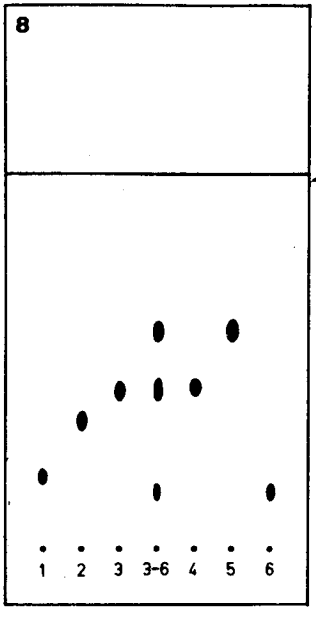

Fig. 8. Separation of hypnotics with benzene-ether (75 + 25) in an unsaturated chamber; numbering as in Figure 4.

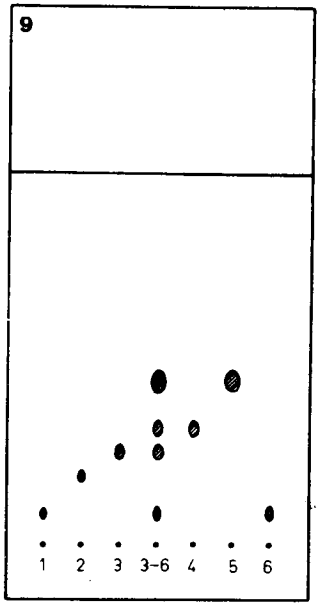

Fig. 9. Separation of hypnotics with benzene-ether (85 + 15) in an unsaturated chamber; numbering as in Figure 4.

more, bromisoval has a lower R_f value than heptobarbital. The mixture is fully separated, however, when the solvent ratio is changed to benzene-ether (85 + 15), with heptobarbital and bromisoval having the same migration rate. On the other hand, when using benzene-ether (85 + 15) in saturated chambers a chromatogram is obtained that is almost identical to the one in Figure 8 with the only difference being lower R_f values.

The role of the ether vapor can also be demonstrated using the following experiments. The separation of Figure 10 was obtained by developing the plate in an unsaturated chamber with chloroform only, but during development a trough containing 10 ml ether was present at the bottom of the chamber. The chloroform, the trough with ether, and the plate were placed in the chamber quickly one after the other. As no ether is present in the solvent, any influence of the ether can only be due to its vapor. This influence is evident if Figure 10 is compared with Figure 6, in which we also used chloroform as the solvent, but without a trough of ether. It can also be observed that the separation in Figure 10 is almost the same as in Figure 5, where we used chloroform-ether (75 + 25) as solvent.

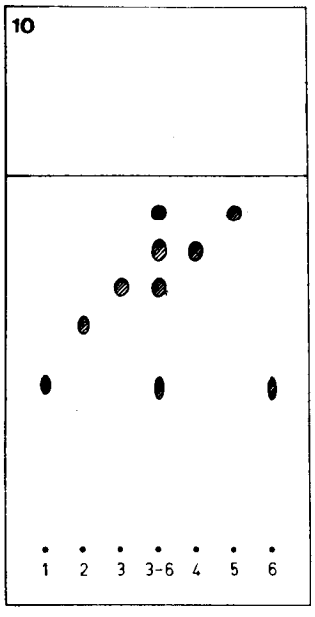

Fig. 10. Separation of hypnotics with chloroform in the presence of a trough with ether; unsaturated chamber; numbering as in Figure 4.

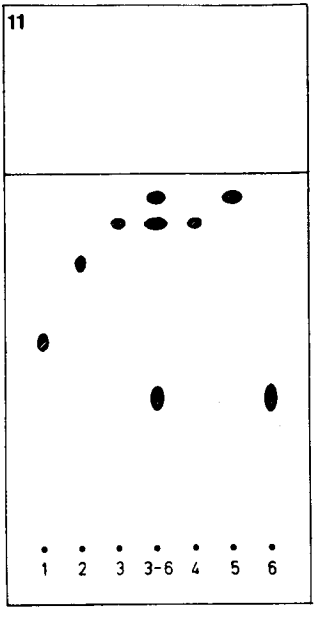

Fig. 11. Separation of hypnotics; presaturation of the plate with vapor by a trough with ether for 5 min, followed by development with chloroform; numbering as in Figure 4.

In the next experiment the amount of ether vapor available 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 solvent chloroform was added by means of a small tube inserted through the cover of the chamber. The separation is shown in Figure 11. The influence of ether is greater here because allobarbital and hexobarbital are not separated and bromisoval travels slower than heptobarbital. Presaturation with ether vapor for 30 min, followed by development in an S-chamber with chloroform resulted in the chromatogram of Figure 12. The effect of ether here apparently becomes so large that the separation is comparable with that in Figure 7 when we used ether only as solvent. For this experiment an S-chamber was preferred because it gives:

(*a*) decreased evaporation of adsorbed ether from the plate;
(*b*) decreased removal of adsorbed ether by chloroform vapor;
(*c*) decreased ether absorption by the solvent.

It becomes obvious from this experiment that quite small amounts of ether are responsible for great changes in the separation. After 30-min presaturation the plate has adsorbed about 1.5 mM of ether vapor, which is about 112 mg. Measurements with the solvent chloroform-ether (75 + 25) showed that 3.10 g was needed 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 on the separation, which can be seen in Figure 5, is far less than the influence of the adsorbed 112 mg ether vapor which causes the separation of Figure 12. These data once again confirm our presumption that the amount of adsorbed ether vapor determines the nature of the separation to a large extent, whereas the amount of ether present in the initial solvent is of minor importance.

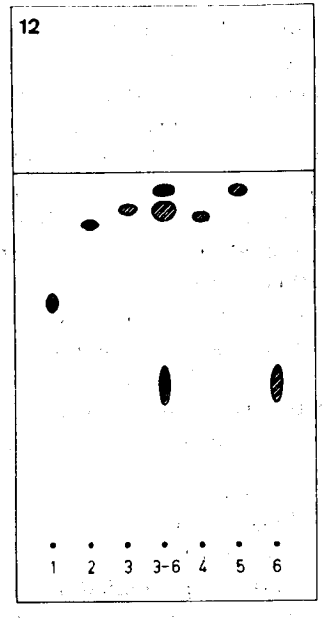

Fig. 12. Separation of hypnotics; presaturation of the plate with vapor by a trough with ether for 30 min, followed by development with chloroform in an S-chamber; numbering as in Figure 4.

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

D. Conclusions

The above investigations have shown that solvent vapor plays a very important role in TLC and, furthermore, it is clear that the different processes involved are generally rather complex. Therefore, it may be useful to summarize the different processes that take place during development in a scheme particularly for those processes which can be influenced by the investigator. We are aware of the shortcomings of this scheme but it might be valuable for further investigations. It should also be noted that transitions are given in the scheme and not chemical reactions.

The chromatographically important processes start at time 0 with the introduction of the solvent in the chamber (the beginning of vapor genera-

tion). The next step is the introduction of the plate which immediately begins to adsorb the generated vapor. The degree of adsorption will depend on the affinity of the vapor components for the adsorbent and on the rate of chamber saturation. The development of the plate then starts at moment I with the ascending of the solvent through the dry adsorbent. This will cause a partial adsorption of the solvent by the adsorbent (*cf.* frontal analysis). The adsorbed part of the solvent, together with the underlying adsorbent will now act as the stationary phase S. Although there are other interpretations existing for the stationary phase in TLC we believe that there are sufficient reasons to justify this term in our concept. The remaining nonadsorbed part of the solvent forms the mobile phase M, whereby Klinkenberg and Bayle (24) and Smit and Van den Hoek (32) have shown the existence of an equilibrium between both phases, so $S \leftrightarrows M$. We also make use of the following abbreviations:

Solvent = L
Vapor = V
Adsorbent = A
Vapor-impregnated adsorbent = iA
Mobile and stationary phase influenced by vapor = M^+ and S^+

The first transition at moment 0 is:

$$0^1 \qquad L \rightarrow V_0$$

When the plate is introduced adsorption will occur:

$$0^2 \qquad V_0 + A \rightarrow iA_0$$

The development of the plate then starts at moment I:

$$I^1 \qquad L + iA \rightarrow S_I + M_I$$

At the same time vapor equilibria play a role:

$$I^2 \qquad M_I,\ V_0, \text{ and } L \rightarrow V_I$$

$$I^3 \qquad V_I + iA_0 \rightarrow iA_I$$

$$I^4 \qquad V_I + M_I + S_I \rightarrow M_I^+ + S_I^+$$

The last four transitions describe the equilibria that tend to form between the solvent, the vapor phase, the adsorbent, and the mobile and stationary phase. The next transition represents the further ascending of the solvent:

$$I^5 \qquad M_I^+ + iA_I \rightarrow S_{II} + M_{II}$$

At the same time, however, new supply of solvent is necessary and, moreover, new equilibria will tend to form between the various phases (II^1–II^4). Transition II^5 again represents the further ascending of the solvent, followed by transition III^1, and so on:

II^1 $\quad L + S_I \rightarrow S_I^1 + M_I^1$

II^2 $\quad M_{II}, M_I^1, V_I$, and $L \rightarrow V_{II}$

II^3 $\quad V_{II} + iA_I \rightarrow iA_{II}$

II^4 $\quad V_{II} + M_{II} + S_{II} \rightarrow M_{II}^+ + S_{II}^+$
$\quad V_{II} + M_I^1 + S_I^1 \rightarrow M_I^{1+} + S_I^{1+}$

II^5 $\quad M_{II} + iA_{II} \rightarrow S_{III} + M_{III}$
$\quad S_{II}^+ + M_I^{1+} \rightarrow S_{II}^1 + M_{II}^1$

III^1 $\quad L + S_I^{1+} \rightarrow S_I^2 + M_I^2 \ldots$ etc.

As it may be difficult to obtain a sufficiently clear insight in the various transitions, the illustrations of Figure 13 can be helpful. When using multicomponent solvents there will be no equilibrium during development between V, M, and S in both unsaturated and saturated chambers. When using a single-component solvent in the unsaturated chamber, there is again no equilibrium between V, M, and S in the beginning of the run, although the qualitative composition of the phases will be the same. Only the use of a single-component solvent in a fully saturated chamber will provide equilibrium during development between V, M, and S.

The transitions in the scheme and in the drawings once again underline the important role of solvent vapor. The adsorbed vapor can be considered the preliminary stationary phase because the adsorbate will mainly consist of the more polar solvent component(s). When the ascending solvent covers the vapor-impregnated areas this preliminary phase will be completed to a normal stationary phase. We further presume that solute separation will take place by interactions of the solute molecules with the stationary phase, with the mobile phase being the transport medium. So, the character of the stationary phase is very important for the separation and the experiments described above have clearly shown that this character can be highly influenced by vapor adsorption. For example, small amounts of adsorbed ether vapor on a plate which is developed with chloroform as solvent are causing such marked effects that we might think that ether was used as development solvent instead of chloroform.

In general, it can be concluded that the influence of the more polar solvent components upon the separation is mainly due to its vapor. Hence, it is possible to replace a great many multicomponent solvents by a system

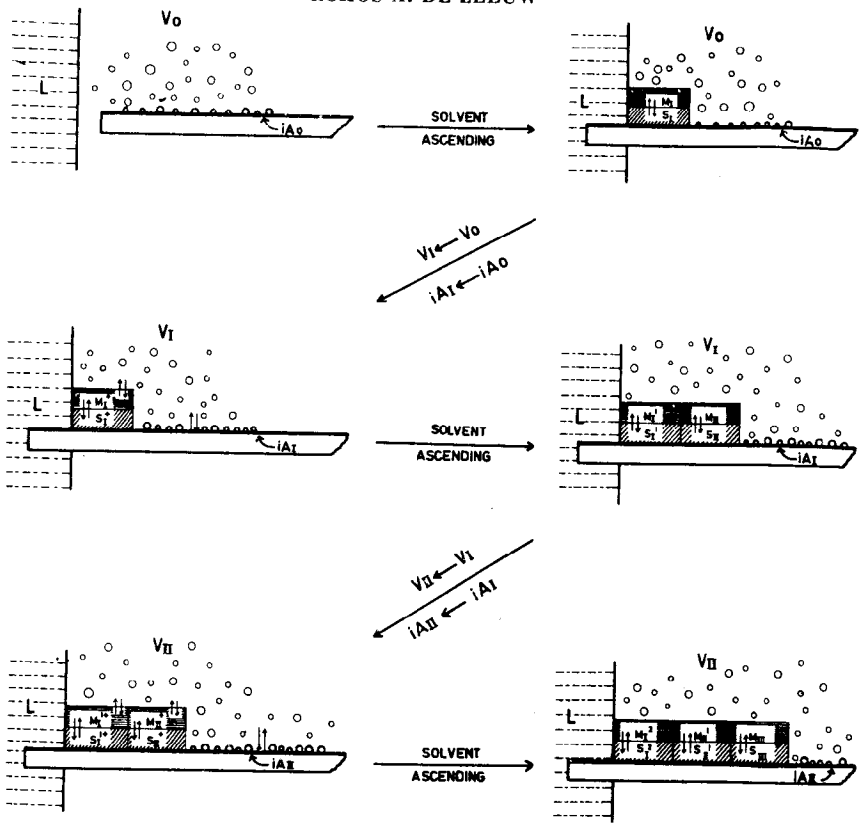

Fig. 13. Schematic illustration of the main transitions taking place during development of a plate in TLC. L = solvent; V = vapor; iA = vapor-impregnated adsorbent; S = stationary phase; M = mobile phase.

of a single-component solvent and one or more troughs with the more polar liquid components. The use of troughs also enables the investigation of the vapor effects of liquid components which cannot always be used in solvent systems. Components such as water and ammonia, which frequently cause solvent demixings, can then be investigated up to high vapor concentrations.

It was also shown that the best separations are not a priori obtained with saturated chambers, but that optimal conditions have to be established experimentally, especially with regard to the influence of solvent vapor. The use of unsaturated chambers or presaturation of the plate with one or more vapor components or the use of troughs containing liquids can be valuable.