REPRODUCIBILITY OF R_F VALUES IN UNSATURATED CHAMBERS AND RELATED DEVELOPMENT TECHNIQUES

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SUMMARY

The use of unsaturated chambers in thin-layer chromatography is considered with respect to the reproducibility of the separation. It was shown that the reproducibility in unsaturated chambers is as good as that in saturated chambers, when other factors influencing reproducibility are kept constant. Since there are reasons to believe that, particularly with multicomponent solvents, better separations can be expected from unsaturated chambers, this technique is to be preferred to the use of saturated chambers.

In thin-layer chromatography (TLC) development of the plate is usually performed after saturation of the chamber atmosphere with solvent vapour. In many instances the tanks are lined with filter paper to decrease the saturation time. This technique should give more reproducible R_F values[1], and should also avoid the appearance of edge effects[2].

However, there are some authors who recommend the use of unsaturated chambers to obtain better separation and clearer spots[3–7]. It was felt that more attention should be paid to this technique because of our general interest in improving separations. Therefore, in this paper the advantages of unsaturated chambers and the reproducibility of the separations obtained in this way are considered.

EXPERIMENTAL

Saturated chambers

Tanks lined with filter paper. After a saturation period of 45 min the plate was introduced and development started.

Unsaturated chambers

Development is started immediately after introduction of the solvent. No filter paper is used in these cases.

Materials and equipment

Barbiturates. 0.2% w/v solutions in chloroform. Names and qualities according to The Netherlands Pharmacopoeia, Ed. VI (1958). Load: 0.005 ml.

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Azo-dyes. Test mixture according to STAHL. Load: 0.003 ml.

Adsorbent. Silica Gel GF_{254} (Merck, Darmstadt), 30 g/60 ml distilled water for 5 plates, plate size 20 × 20 cm, layer thickness 0.25 mm when spread.

Activation. Air dried for 15 min, then heated for 30 min at 110° in an oven fitted with a fan, cooled and stored in a desiccator.

Solvents. Pro Analysi (Merck, Darmstadt).

Apparatus. Desaga (Heidelberg).

Temperature. 22°. Relative humidity: 45–51%.

Documentation. Direct photography after activation with ammonia vapour, under two U.V. lamps (Camag, Muttenz), at 254 nm. Camera: Asahi Pentax, type SV, with a Super Takumar 1:1.8/55 lens and a 49 mm U.V. ghostless filter. Distance between camera and plate 70 cm, aperture 5.6, exposure 15 sec. Film: Agfa Color CT-18 diapositive.

RESULTS AND DISCUSSION

In previous papers[8,9] the role of solvent vapour in TLC, particularly with multicomponent solvents, was discussed and it was found that the separation was greatly affected by the nature and the amount of adsorbed vapour. It will be clear that the amount of vapour available for adsorption from unsaturated chambers is very small at the beginning of development and increases during the run. Although it is far beyond the purpose of this Symposium to discuss the full theoretical details, which is done elsewhere[9], we have reason to believe that the process of vapour adsorption from unsaturated chambers will give rise to a concentration gradient of adsorbed vapour, showing increasing polarity when going upwards over the plate. This gradient will provide better separations, as it will increase the migration of the faster running spots, so that they are separated to a greater extent. So, theoretically,

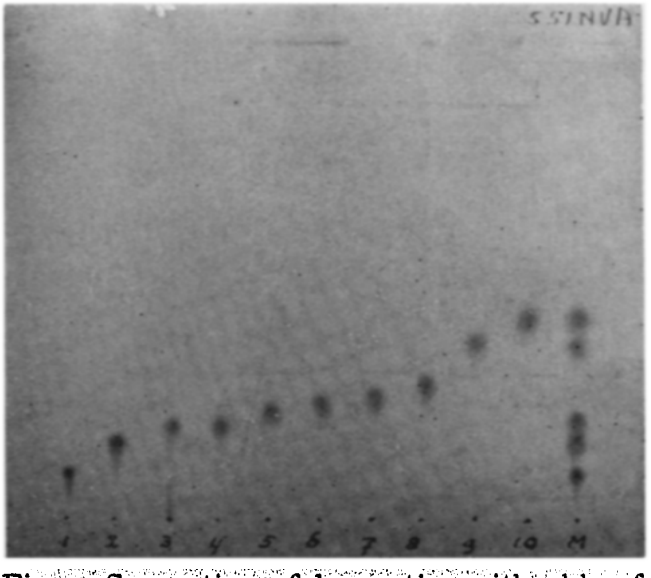

Fig. 1. Separation of hypnotics with chloroform–acetone (90:10) in saturated chambers. 1 = Heptobarbital; 2 = phenobarbital; 3 = cyclobarbital; 4 = allobarbital; 5 = butobarbital; 6 = pentobarbital; 7 = itobarbital; 8 = secobarbital; 9 = hexobarbital; 10 = methylphenobarbital; M = Mixture of 1 + 2 + 4 + 9 + 10.

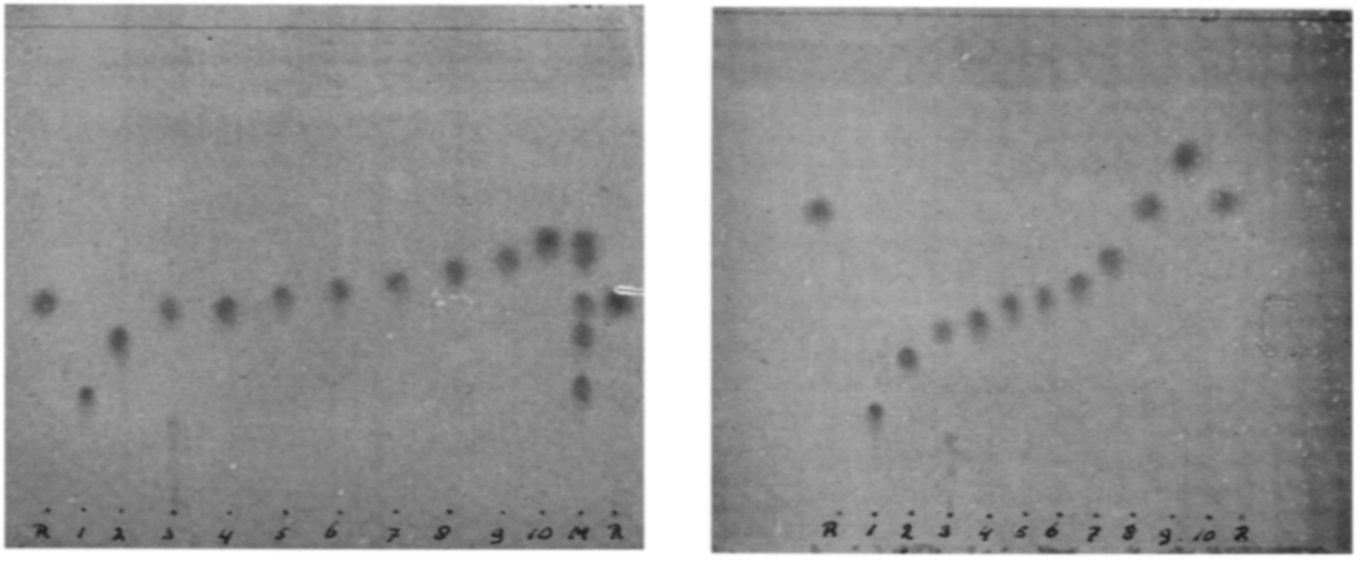

Fig. 2. Separation of hypnotics with chloroform–acetone (80:20) in saturated chambers. M = Mixture of 1 + 2 + 4 + 9 + 10. R = Reference substance 4-nitroaniline. For further explanation, see the legend to Fig. 1.

Fig. 3. Improved separation of hypnotics with chloroform–acetone (90:10) due to development in unsaturated chambers. Photography under U.V. light of 254 nm after activation with ammonia vapour. For explanation of figures etc., see the legends to Figs. 1 and 2.

improved separations are to be expected from unsaturated chambers when multi-component solvents are used. A good example of this improvement can be seen in Figs. 1–3. Fig. 1 shows the separation of a selection of barbiturates in saturated chambers with the well known solvent chloroform–acetone (90:10). A higher acetone concentration in the solvent does not improve the separation as can be seen in Fig. 2. In Fig. 3 the separation from unsaturated chambers is shown with chloroform–acetone (90:10) and it is obvious that this is much better. The time of development in unsaturated chambers is of course somewhat longer, but this is fully compensated by the fact that no time is needed for the saturation of the chamber. So, the overall time for unsaturated chambers is shorter than for saturated chambers.

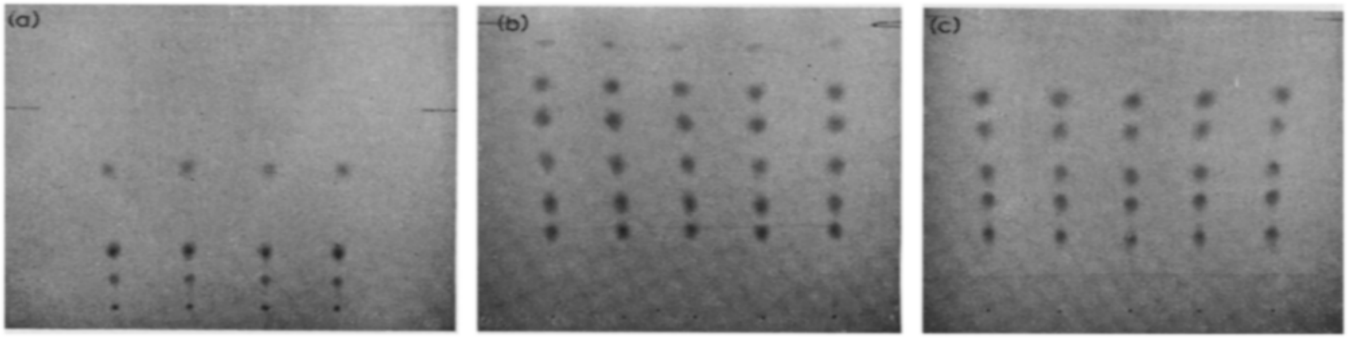

Fig. 4. Absence of edge effects after development in unsaturated chambers. (a) Test mixture according to STAHL, developed with benzene. (b) Mixture of hypnotics (heptobarbital, phenobarbital, allobarbital, hexobarbital, methylphenobarbital, bromisoval) developed with chloroform–isopropanol–25% ammonia (45:45:10). (c) Mixture of hypnotics (see under b) developed with chloroform–ether (75:25). Photography under U.V. light of 254 nm, b and c after activation with ammonia vapour.

The shape of the spots in unsaturated chambers is at least equally as good as those in saturated chambers and in many cases, *e.g.* sulfonamides, alkaloids, barbiturates and local anaesthetics, they are even more compact and distinct.

The appearance of edge effects, as reported by STAHL[10] and DEMOLE[11] could not be confirmed in our investigations as can be seen from Fig. 4. Sometimes a very slight difference could be observed in R_F values of the same solute, but this was not found to present any difficulty in identification procedures. It is possible that the earlier observations of edge effects were caused by other factors.

The reproducibility of results in unsaturated chambers is now considered. We know that reproducibility is dependent upon many factors, such as the quality of the adsorbent and solvent, temperature, relative humidity, geometry of the chamber and especially whether or not the chamber has been saturated with solvent vapour prior to development. Assuming that all the first mentioned factors can be kept constant, the remaining factor is the saturation of the chamber. We have found[9] that saturation of the chamber not only decreases evaporation of the solvent from the plate during the run, but a much more important effect is that, when the plate is placed in an atmosphere of vapour, it will adsorb a certain amount of that vapour, depending upon the amount of vapour available, that is to say the degree of saturation. So, when the chamber is completely saturated, the nature and the amount of adsorbed vapour will always be the same and the separations, being greatly dependent on this adsorbed vapour, will be reproducible.

In unsaturated chambers the amount of adsorbed vapour will of course be lower than in the saturated chambers, but if we are able to provide a fixed type of unsaturation in our chambers it is obvious that as a consequence these lower amounts of adsorbed vapour will also be fixed and we can then also expect reproducible results. This type of fixed unsaturation is obtained when development of the plate is started immediately after introduction of the solvent. In this case, no vapour is present at the beginning of the run, and keeping other factors constant, there will always be reproducible vapour conditions during development. The R_F values obtained by this

TABLE I

R_F VALUES × 100 FROM UNSATURATED CHAMBERS

Solvent	*Substance*	$R_F \times 100$				
Chloroform–ether (75:25, v/v)	Heptobarbital	25	24	26	25	25
	Phenobarbital	37	38	36	39	37
	Allobarbital	47	50	46	49	48
	Hexobarbital	63	65	61	64	63
	Methylphenobarbital	73	76	72	75	75
Isopropanol–chloroform–25% ammonia (45:45:10, v/v)	Heptobarbital	29	30	31	30	31
	Phenobarbital	37	39	39	38	38
	Allobarbital	53	53	53	53	51
	Hexobarbital	69	68	67	67	67
	Methylphenobarbital	80	80	78	79	77
Benzene	Indophenol	13	14	15	14	14
	Sudan Red G	29	28	31	29	29
	Butter Yellow	70	70	73	70	70

technique show the same reproducibility as in saturated chambers as can be seen in Table I.

So, in our opinion unsaturated chambers are to be preferred to saturated chambers, when other conditions are standardized. With multicomponent solvents the separation will be improved in many cases and although with single component solvents no improvement can be expected, the saving of time because no saturation period is needed, can be valuable.

It should be observed that the same holds true for the reproducibility in similar unsaturated techniques in which troughs with liquid at the bottom of the chamber are used. This trough technique has been described in previous papers[8,9].

REFERENCES

1 K. RANDERATH, *Dünnschicht-Chromatographie*, 2nd Ed., Verlag Chemie, Weinheim, 1965, p. 70.
2 E. STAHL (Editor), *Dünnschicht-Chromatographie*, 2nd Ed., Springer Verlag, Berlin, Heidelberg, New York, 1967, p. 67.
3 H. K. MANGOLD, *J. Am. Oil Chemists' Soc.*, 38 (1961) 708.
4 E. VON ARX AND R. NEHER, *J. Chromatog.*, 12 (1963) 329.
5 K. JONES AND J. G. HEATHCOTE, *J. Chromatog.*, 24 (1966) 106.
6 R. A. DE ZEEUW AND M. T. FEITSMA, *Pharm. Weekblad*, 101 (1966) 957.
7 G. PATAKI, *J. Chromatog.*, 29 (1967) 126.
8 R. A. DE ZEEUW, *Pharm. Weekblad*, 102 (1967) 113.
9 R. A. DE ZEEUW, *J. Chromatog.*, 32 (1968) 43.
10 E. STAHL, *Arch. Pharm.*, 292 (1959) 411.
11 E. DEMOLE, *J. Chromatog.*, 1 (1958) 24.