CHROM. 4962

Letter to the Editor

Dear Sir,

I should like to comment on a paper by R. A. DE ZEEUW *et al.* (*J. Chromatog.*, 47 (1970) 382) entitled *Improved separation in thin-layer chromatography using a single-component solvent in an unsaturated chamber.*

Therein several statements are made:

(1) "For the system under investigation, using a single-component solvent, unsaturated chambers generally yielded better separation [expressed as $(R_F)_{\max} - (R_F)_{\min}$] than did saturated chambers."

(2) "Although a possible explanation for the occurrence of improved separations when using multi-component solvents in unsaturated chambers has been given[1], an explanation is not yet available for single-component solvents."

(3) "In unsaturated chambers, solvent evaporation during the run will take place to a higher extent than in saturated chambers, which require an extra solvent supply from the solvent reservoir."

(4) "As a consequence of this solvent supplementation more solvent will be transported across the lower parts of the plate than across the higher parts, which, in turn, would be expected to result in a higher migration of the lower spots. Hence this "pushing up" would have an adverse effect on the separation and on the speed of the spots. However, the findings described here indicate that other factors must also be involved, ..."

(5) "In general it appeared that unsaturated chambers yielded lower *a* values (constant of the Galanos–Kopoulas equation) than did saturated chambers."

ad (1)

For judging "separation" of two compounds one should better refer to their resolution, $R_s = \Delta R_F/[2(\sigma_1 + \sigma_2)]$, taking into account also the band width, than only to ΔR_F.

To judge the improvement of separation in unsaturated chambers with respect to saturated ones, rather *distant spots with relatively elevated R_F values* must not be considered: Going from saturated to unsaturated their ΔR_F values may remain constant or decrease, as shown by examples III and IV in Fig. 1. When considering only the upper two spots in DE ZEEUW's table (with high R_F values), the ΔR_F values are less in favour of the unsaturated N-chamber (Table I). Also from a practical aspect *only the separation of neighbouring spots is of interest* (the separation of wide polarity range mixture, requiring gradients, is disregarded here).

The ΔR_F values of *closely adjacent pairs of spots* (*e.g.* II in Fig. 1) in almost all cases must increase when passing from a saturated to an unsaturated N-chamber, or to continuous TLC (see *ad. 4*).

Since much improved separation can be duplicated as well by continuous TLC (this is not exactly true for multi-component solvents), one should prefer the latter technique, which is easier to control.

TABLE I

ΔR_F *saturated chamber*	ΔR_F *unsaturated chamber*	ΔR_F *saturated chamber*	ΔR_F *unsaturated chamber*
0.12	0.17	0.17	0.15
0.14	0.16	0.16	0.18
0.11	0.13	0.12	0.24
0.11	0.10	0.13	0.15
0.11	0.02	0.16	0.13
0.13	0.24	0.18	0.14
0.10	0.15	0.09	0.26
0.08	0.21	0.09	0.12
0.15	0.16	0.11	0.08
		0.16	

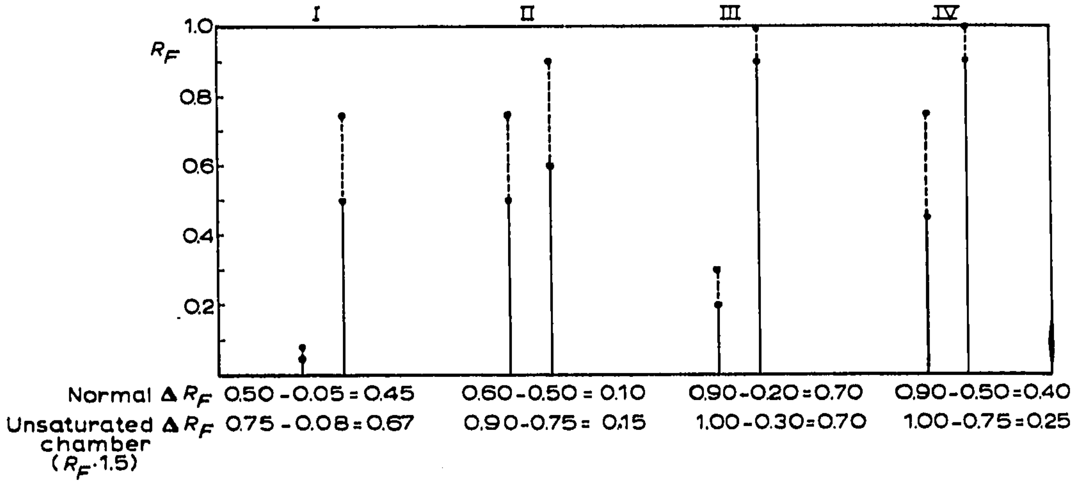

Fig. 1. ΔR_F of two spots in saturated N-chamber (solid line) and unsaturated N-chamber (or continuous TLC) (solid line + dotted line), assuming that the integral solvent flow in the latter one is 50 % higher. Schematical. For high R_F values ΔR_F may decrease in unsaturated chamber.

ad (2)

Both for single-component and multi-component solvents, improved separations in unsaturated N-chambers are essentially based upon the same effect: increased total amount of solvent transported through the layer, as shown in the literature[2,3]. The mechanism postulated by DE ZEEUW for multi-component solvents[1] (gradient of decreasing activity in the sense of migration) has become untenable[2,4-6].

ad (3)

Solvent evaporation during the run only takes place in large-volume unsaturated chambers, so-called (unsaturated) N-chambers, but not in (small-volume) S-chambers that are (ideally) unsaturated as well. Thus, "unsaturated chamber" in this case must be specified further. Nevertheless R_F values (and hence ΔR_F) in S-chambers are (for single-component solvents) slightly higher than in saturated ones, all other conditions being constant. This is not due to solvent evaporation from the S-chamber (there is almost no evaporation because of the tiny volume) but to the absence of vapour pre-adsorption by the dry layer.

ad (*4*)

Even in unsaturated N-chambers, the saturation of the lower and medium zones of the trough starting from the bottom is almost accomplished within 15 min[2]. Thus the solvent will evaporate only from the zones of the layer close to the front from a height at which the chamber is still rather unsaturated. Hence the entire solvent supplementation for loss by evaporation is transported up to the front integrally through all zones of the layer (except the parts close to the front). Therefore the same effect is achieved as in continuous ("flow-through") TLC (Fig. 2): Increased solvent transport (to the front) increases *proportionally* the migrating distances of all spots.

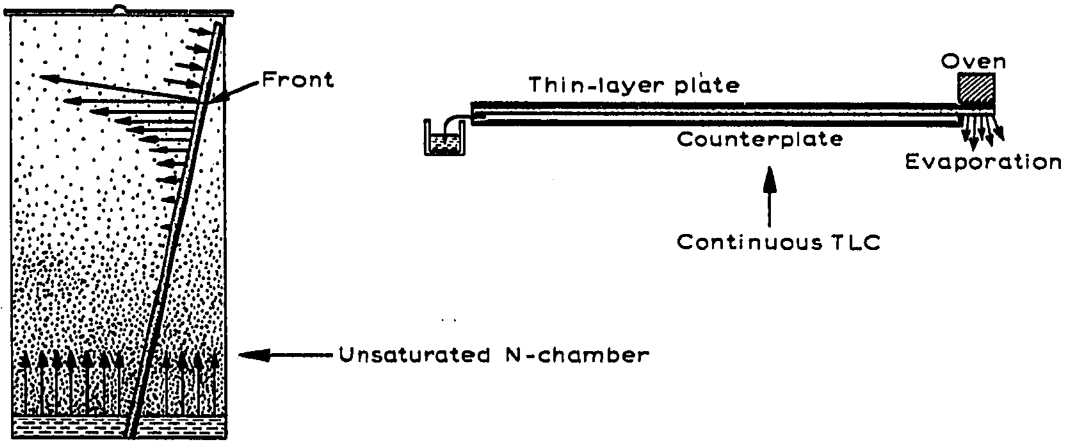

Fig. 2. Similarities of "unsaturated N-chamber" and continuous TLC. In each system integral solvent flow is increased by solvent evaporation from the upper parts of the layer.

Thus one makes a better use of the theoretical plates along the layer (= increase of the number of effective plates)! Result: proportional increase of all R_F values *and* improvement of resolution. Only a *simultaneous* evaporation of solvent *from all wetted parts* of the layer would have lifted superproportionally ("pushed up") the R_F value of the lower spot, as postulated by De Zeeuw *et al.*, and reduced the distances of the extreme R_F values.

ad (*5*)

This is only due to the casual choice of a reference data set for a *saturated* chamber. If a set from an unsaturated N-chamber had been designed as a standard, the opposite would be true; then the *a* values from saturated chambers would be the lower ones. The constant *a* essentially corrects for differences of integral solvent flow between the two systems.

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