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A VERSATILE TWIN-TROUGH DEVELOPING TANK FOR THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A novel twin-trough developing tank is described. The bottom of the tank is separated into two troughs so that the amount of solvent can be reduced to approximately 20 ml, which is sufficient to saturate the tank and develop the chromatogram. After development, practically no solvent remains in the trough. A revision or change of existing thin-layer chromatographic methods is usually not necessary; the results remain unchanged.

Compared with the conventional tank, employing 100 ml of solvent, the new tank has the following advantages: a saving of 50 to 80% on solvents; the avoidance of solvent waste (*i.e.*, a contribution by analysts towards environmental protection); "automatic" adjustment of the solvent development distance owing to the amount of solvent chosen; and an improvement in the separation and its reproducibility by means of layer pre-loading or by adjustment of the relative humidity in the tank.

INTRODUCTION

A possible scarcity, and the increasing price, of solvents as well as the costs involved in destroying solvent waste in an environmently acceptable manner were the reasons for the search for a new developing tank that would permit the use of smaller amounts of solvent. The results of this search are described below.

In conventional thin-layer chromatography (TLC), the chromatogram is usually developed in a rectangular tank containing about 100 ml of solvent mixture. The amount of solvent that is needed for saturation of the tank and development of the chromatogram is only about 20 ml, depending on the size and type of the tank and the volatility of the solvent. The thickness of the layer (± 20%) and the temperature (19–26°) usually exert only a small influence in this regard.

The remaining solvent, approximately 80 ml, which must be removed after completion of the development, is needed only to achieve a sufficiently high solvent level in the tank, thus allowing the immersion of the plate in the solvent during the whole development period (see Table I). It is a fact that other tanks that enable one to use a smaller amount of solvent exist, but these are usually not employed for routine work.

TABLE I

SOME EXAMPLES OF SOLVENT CONSUMPTION AND SOLVENT WASTE

V = Solvent consumption for the saturation of the tank without paper (1 h); VS = Solvent consumption for the saturation of the tank with a paper lining (1 h); VS + P = Effective consumption for chromatography (layer, Kieselgel G (0.25 mm); tank saturation, 1 h with paper; length of run, 10 cm; room temperature, 22–23°).

Solvent	*Conventional TLC tank*			*Amount wasted* (ml)*
	V (ml)	*VS (ml)*	*VS + P (ml)*	
Ammonia (25%)	4	33	42	58
Acetone	4	17	24	76
Diethyl ether	16	30	38	62
Chloroform	4	17	24	76
Dichloromethane	—	—	26	74
Ethyl acetate	2	15	21	79
Methanol	2	18	25	75
Toluene	1	14	19	81
Chloroform–methanol–water (80:20:2)	3	19	27	73
Chloroform–acetic acid (80:20)	3	15	23	77
Cyclohexane–chloroform–acetic acid (50:40:10)	3	16	23	77
Ethyl acetate–acetone–ammonia (25%) (70:30:6)	4	19	25	75

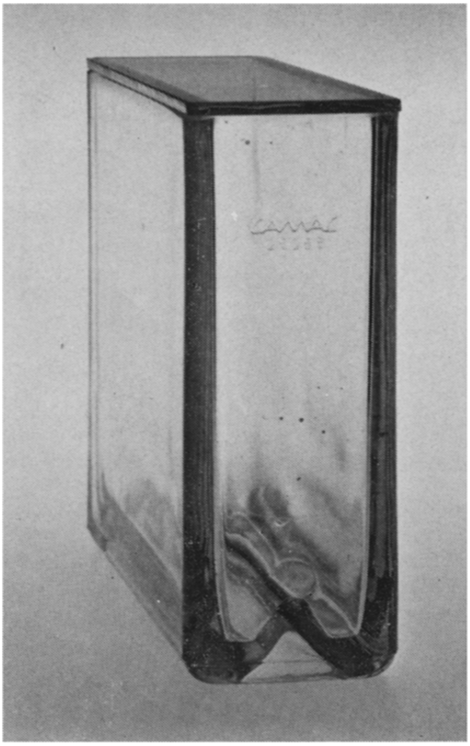
* When 100 ml of solvent are added to the tank.

CHARACTERISTICS OF THE NEW TANK AND EXAMPLES OF ITS APPLICATION

The size of the new twin-trough developing tank (TTT) (Camag, Muttenz, Switzerland) (Fig. 1) is 220 × 230 × 75 mm. The bottom of the tank is separated into two troughs of equal shape by means of a 4 cm high undulation running the whole length of the tank (hence twin trough). Such a shape reduces the amount of solvent required and yet provides a solvent level sufficiently high to develop the chromatogram. In this way, the amount of solvent, almost all of which is utilized and is sufficient to saturate the tank and develop the chromatogram, can be reduced to approximately 20 ml (in the absence of saturation even less can be used).

In a non-paper lined tank in which a 0.25 mm thick Kieselgel G layer is used, 5 ml of methanol produce a solvent development distance of approximately 11 cm and 5 ml of chloroform–ethanol (1:1) approximately 8 cm. It is now possible to state the amount of solvent required to yield optimum tank saturation and a desired solvent development distance. In more than 90% of our experiments, the results (*i.e.*, separation, R_F value, shape of the spots) were not changed when 20–25 ml of solvent was used in a TTT. A revision or a change of existing TLC techniques is usually not necessary, which is important, especially in routine applications. In Figs. 2 and 3, chromatograms obtained by using 100 and 25 ml of solvent, respectively, are illustrated.

In approximately 10% of our procedures, the reduction of solvent volume to 20 ml caused a deterioration in the degree of separation. In these instances the solvent contained a small volume of ammonia, which is very volatile. In such cases there are two ways of achieving a separation as good as that obtained with larger volumes. The first is to increase the amount of the volatile component as illustrated in the



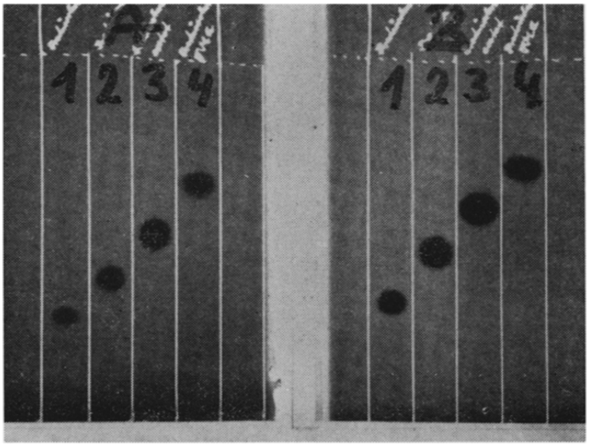


Fig. 1. Twin-trough developing tank for TLC.

Fig. 2. Chromatographic separation of four different steroids. In A, the solvent volume was 26 ml and in B, 101 ml. Zone: 1, prednisolone; 2, prednisone; 3, prednisolone acetate; and 4, prednisolone pivalate. The samples, 50 μg of each, were applied to silica gel G plates with the aid of a EVA-Chrom applicator (W + W Electronic, Basel, Switzerland), separated in the solvent system[1] dichloromethane–diethyl ether–methanol–water (77:15:8:1.2) and the spots made visible by spraying with alkaline tetrazolium blue in methanol.

following example: for normal, a solvent volume of 106 ml and the mixture ethyl acetate–acetone–ammonia (25%) in the proportions 70:30:6 are used; for reduced 26.5 ml, in the proportions 17.5:7.5:1.5; and for corrected, 28 ml, in the proportions 17.5:7.5:3. Alternatively, it is possible to add the reduced amount of solvent (of the original composition) to the first trough and to add to the second trough a small volume of the volatile component (in the above example, this would be 2 ml of 25% ammonia solution).

In one of our separations, the material of interest is separated by a two-step technique (first in the solvent system benzene–acetone (18:2) and then in ethyl acetate. A development distance of 10 cm is usual in each instance. The separation proved to be better when both solvents were placed simultaneously in their respective troughs of the TTT and the layer was developed consecutively.

The parameters[3] of relative humidity and of saturation conditions in a tank influence the obtained chromatographic results very readily and lead to their variations.

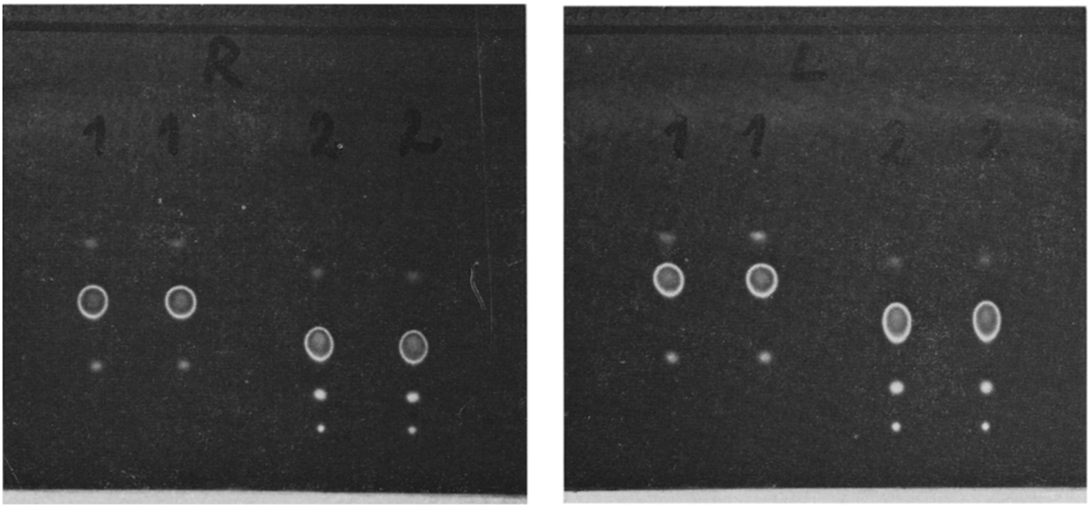


Fig. 3. Chromatographic separation of desipramine hydrochloride (substance 1, Pertofran/AS) and imipramin hydrochloride (substance 2, Tofranil/AS). In L, the solvent volume was 20 ml and in R, 100 ml. The samples, 50 μg of each, were applied (see Fig. 2 for details) to chromatograms and separated in acetic acid (glacial)–ethyl acetate–water–HCl, (11:7:1:1)[2]. After separation, the zones were made visible by inspection under ultraviolet light (366 nm), after spraying with potassium dichromate–sulphuric acid (0.5% $K_2Cr_2O_7$ in 20% H_2SO_4) reagent.

The TTT system described above enables a good saturation to be achieved, even when one solvent is incompatible with the other or with the substance to be chromatographed. In this situation a solvent different from that used for developing the plate can be placed in the second trough, which results in good saturation and layer pre-loading.

In certain other instances, the reproducibility of the separation can be improved by adjusting the relative humidity in the tank. Using the twin trough tank, an appropriate sulphuric acid–water mixture can simply be added to the second trough and then after adequate humidity equilibrium has occurred, the solvent can be added to the first trough.

Since its development and introduction in January, 1975, the TTT system has been so successful in our laboratory that it has virtually replaced all other types of tanks.

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