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NOTE

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Abstract

An apparatus is described which allows a sample spotted on a thin-layer plate to be concentrated into a narrow line parallel to the solvent front advance. Reduction of the size of the starting spot in this way improves resolution in the resulting chromatogram. The apparatus has been tested out using dye mixtures on high-performance and classical thin-layer plates, and the results compared with separations on a preabsorbent-type thin-layer plate. The apparatus is found to be effective in multiple development chromatography.

INTRODUCTION

Resolution in thin-layer chromatography (TLC) can be improved by reducing the size of the sample spot and increasing the separation between the developed spots. The former approach is the basis of high-performance thin-layer chromatography (HPTLC) (*1*), while the latter is embodied in the technique known as multiple development (*2*), which is effective in improving resolution in the lower R_F range.

The least complicated way of producing a small spot is to load a small volume. For quantitative work this volume must be reproducible. Small platinum-tipped pipettes with fixed volumes of 100 and 200 nl are available for HPTLC (Camag, Switzerland) (*1*). The main drawback to loading small volumes is the requirement that the solution be as concentrated as possible in order to obtain sufficient sensitivity. In cases like pesticide residue analysis, however, this is seldom practicable.

The technique of multiple development chromatography has been improved since the unidimensional multiple chromatography (UMC) approach was investigated (*2*). Instead of removing and drying the plate each time the solvent front reaches a predetermined position, as in UMC, the solvent front is forced down by a stream of air, or radiant heat, after which it is allowed to run free again (*3*). By forcing the solvent front down, a double concentration of the developing spots results since the solvent front washes through the spots twice in each cycle. The developed spots become concentrated in a narrow line parallel to the solvent front.

The entire process of multiple development is flexible as regards distance and portion developed each time, as well as the number of developments, and is thus amenable to electronic programming. Such a process is embodied in the Programmed Multiple Development apparatus (Regis Chemical Co.).

These two approaches to improving resolution in thin-layer chromatography are invariably considered separately in published works. It is an object of this contribution to describe an apparatus that can combine reducing the starting spot size with subsequent multiple development. The use of the apparatus in obtaining improved resolution in conventional HPTLC while loading larger volumes than normally allowed is also described.

EXPERIMENTAL

Description of Apparatus

The apparatus is illustrated in Figs. 1 and 2. A T-piece made out of metal tubing contains a number of evenly spaced angled holes in the head. The T-piece, closed off at the ends of the head, is attached to a stepping motor and passes through an oversize hole in the lid covering the developing chamber. A slit in the lid aligned parallel to and directly above the thin-layer plate allows the removal of air from the chamber. Attached to the tail of the T-piece is a rubber hose which connects to a small air-pump. An in-line heater (between pump and T-piece) is available for use in the case of high boiling point solvents.

By means of an electronic control unit the T-piece can be made to progress down or up at a widely varying rate. The control unit and stepping motor employed are derived from the Camag U-chamber system (Camag, Switzerland).

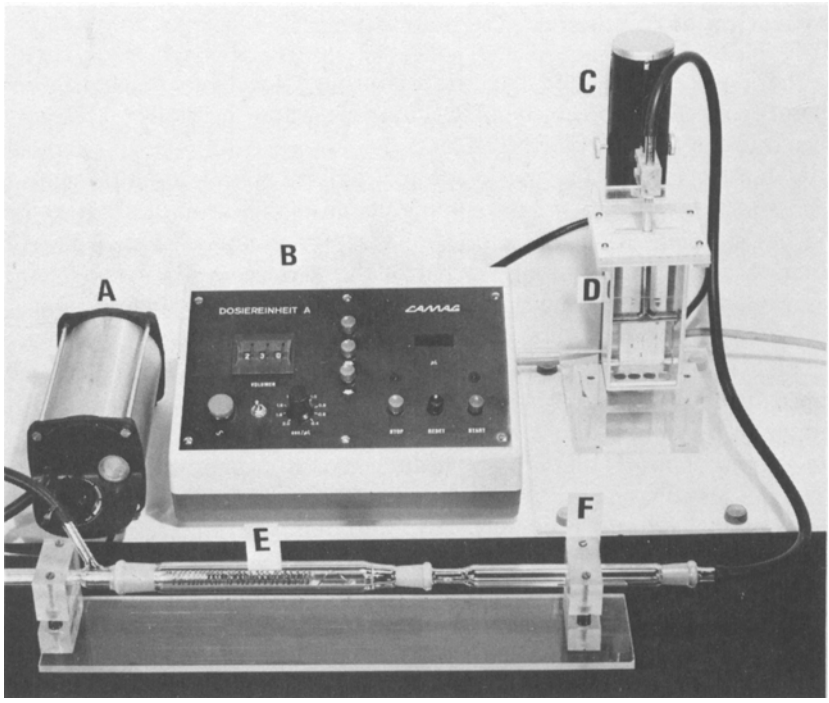

FIG. 1. Apparatus used for concentrating samples spotted on TLC plates and carrying out multiple development. A: air pump; B: stepping motor control unit; C: stepping motor mast; D: development chamber showing positioning of air inlet T-piece; E: in-line heating element with connection hose to development chamber which is normally insulated with asbestos material.

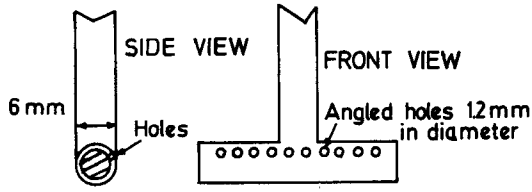

FIG. 2. T-piece geometry.

Operation of Apparatus

The T-piece is inserted backwards, through the oversize hole in the chamber lid, fixed to the stepping motor arm, and the rubber hose connected to the tail.

A thin-layer plate, previously spotted, is fixed rigidly in an aluminum cage and lowered into the developing chamber. The T-piece head, with the holes facing the plate, is positioned approximately 2 mm from the latter. A cover, sliding along the tail of the T-piece, is lowered over the slit in the lid, thus making the chamber airtight.

In order to reduce the size of the initial sample spot with the apparatus, the plate is placed in a solvent and the T-piece is run down and positioned approximately 0.5 cm above the spot. A polar solvent, carrying the sample in the solvent front, is drawn up the plate by capillary action to a position opposite the T-piece head where it evaporates due to the stream of air coming from the holes in the T-piece head. When the sample has been concentrated optimally into a thin line, the T-piece head is run down (forcing the solvent front with it) for 0.5 cm, and the plate removed rapidly from the chamber and dried under a hair drier.

In the multiple development mode the development of the plate in normal solvent is allowed to proceed to a set distance, the sliding cover removed, anchored, and a stream of air from the T-piece used to force the solvent front down again. The speed of the stepping motor is adjusted to force the front down as fast as practicable or required.

RESULTS ON DYE MIXTURES

Two lipophilic dye mixtures and various types of thin-layer plates were used to test the effectiveness of the apparatus.

One microliter of each dye was spotted on Merck silica gel HPTLC, classical TLC, and preadsorbent TLC plates (E. Merck, Darmstadt), and developed in toluene. The dye concentration was 0.45% (w/v) in toluene.

A Vitatron TLD Densitometer (Vitatron N. V., Holland) in the transmission mode was employed to obtain chromatogram curves from the developed plates.

The results are shown in Tables 1 and 2 and Figs. 3 and 4.

In all cases where the original spot has been concentrated into a line using *n*-butanol, the resolution of the developed chromatogram is improved compared to conventional spotting (i.e., simple draining of a

TABLE 1

Comparison of Resolution under Different Conditions between Adjacent Peaks in Two Dye Mixtures: Conventional Development[a]

Plate type	Spotting method	Resolution between peaks								
		Dye A						Dye B		
		1/2	2/3	3/4	4/5	5/6	6/7	1/2	2/3	3/4
HPTLC	1	2.16	1.52	1.90	1.20	0.63	2.46	1.47	2.05	1,93
		2.16	1.58	2.00	1.25	0.57	2.40	1.65	2.10	2.00
	2	3.25	1.87	2.17	1.18	0.78	2.62	1.72	2.50	2.14
		3.57	1.86	2.24	1.44	0.77	2.72	1.65	2.40	2.05
TLC	1	2.80	1.57	1.55	1.14	—	2.11	1.42	1.88	1.80
	2	3.28	1.79	1.98	1.24	—	2.13	1.51	2.23	2.09
Preadsorbent TLC	1	3.06	1.66	1.63	—	—	2.03	1.09	2.44	2.24
		3.06	1.94	1.67	1.04	0.78	2.16	1.32	2.40	2.14

[a] HPTLC = high-performance thin-layer plate. TLC = classical thin-layer plate. 1 = sample developed from spot. 2 = sample concentrated into a line, before development, using *n*-butanol. — = peaks not sufficiently resolved for measurement.

pipette). This occurs in both conventional (single) development and multiple development. The improvement in resolution is highest for the components with low R_F values (up to 58% better) and lowest for high R_F values.

With the apparatus used in the multiple development mode, the improvement in resolution of multiple development compared to conventional development, using the same spotting technique, is greatest for the lowest R_F spot by a factor of up to 3.5. This relative improvement decreases with increasing R_F until, in the region $R_F = 0.4$ to 0.5, the methods give comparable results. With higher R_F values the reverse situation occurs but at a slower rate.

The type of multiple development employed depends on the R_F values of the sample components and the result required. The maximum improvement in resolution for the lowest R_F spot is obtained using Mode b in Table 2. The disadvantage of multiple development is that it destroys resolution in the R_F range 0.5 to 1.0, and the more cycles one uses the worse the situation becomes.

The results obtained using the preadsorbent plate show that it marks a step forward in classical TLC, since it results in improved resolution. The resolution using the plate virtually matches that on the HPTLC

TABLE 2

Comparison of Resolution under Different Conditions between Adjacent Peaks in Two Dye Mixtures: Multiple Development[a,b]

Plate type	Spotting method	Resolution between peaks								
		Dye A						Dye B		
		1/2	2/3	3/4	4/5	5/6	6/7	1/2	2/3	3/4
HPTLC	1[a]	5.76	1.65	2.00	0.98	0.59	1.74	2.98	2.30	1.84
		6.26	1.68	1.75	1.33	1.04	1.51	3.54	2.69	1.65
	1[b]	6.61	2.03	2.23	0.93	0.34	1.12	3.51	3.45	1.90
		7.22	1.94	1.92	1.00	0.40	1.27	3.89	2.96	1.50
	2[a]	8.07	2.33	2.14	1.13	0.65	1.98	3.60	2.91	1.82
		7.29	2.60	2.15	1.11	—	1.61	3.12	3.02	1.75
TLC	1[a]	6.91	2.13	1.61	—	—	1.04	2.86	2.64	1.66
	2[a]	7.80	2.41	2.17	1.19	0.55	1.65	3.04	3.16	1.92
Preadsorbent TLC	1[a]	9.36	2.34	1.70	—	—	1.50	2.92	2.86	1.84
		7.82	2.39	2.00	1.37	0.75	1.92	3.62	3.37	2.15

[a] See footnotes to Table 1.

[b] a = multiple development as follows: developed to progressively higher distances between cycles: 2, 3, 4, 5 cm (i.e., 4 cycles). b = multiple development as follows: developed for 5 cm solvent front forced down for 5 cm; repeated 3 times. The figure of four for the number of developments is the optimum above which resolution improves only slowly (*4*, *5*). 15 °C of heat via the in-line heater was used in conjunction with air to force the solvent front down.

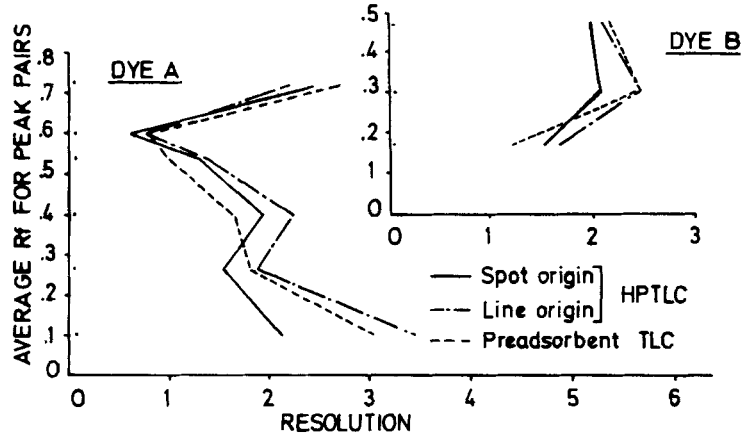

FIG. 3. Comparison of resolution in HPTLC and preadsorbent TLC: conventional development.

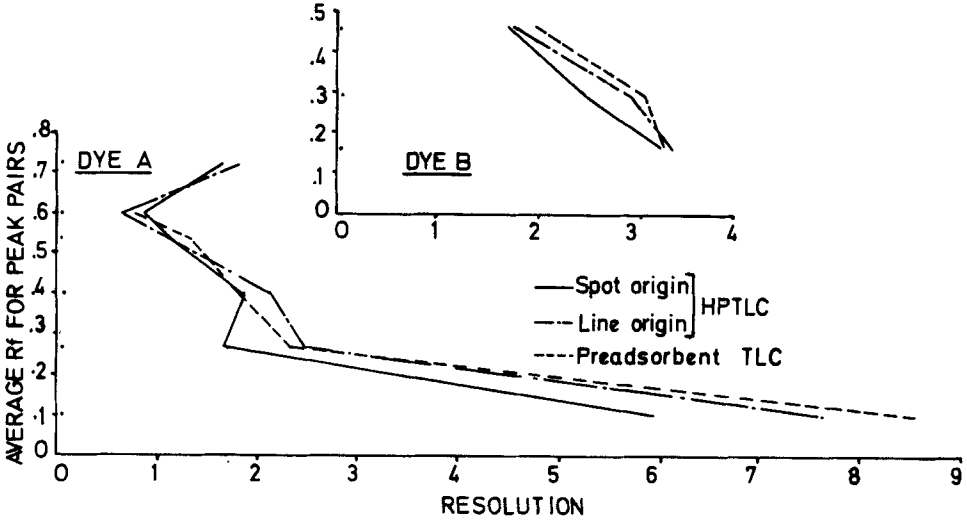

FIG. 4. Comparison of resolution in HPTLC and preadsorbent TLC: multiple development.

plate using the spot concentration technique, and is clearly better than in the case of conventional spotting on HPTLC plates.

CONCLUSIONS

The spot concentration technique described has been shown to improve resolution significantly in both conventional and multiple development compared to conventional spotting. Improvement occurred on both classical TLC and HPTLC plates. The approach will be advantageous where solvent demixing is a problem and multiple development cannot be used. The technique should be especially useful in situations dealing with dilute solutions when larger than normal volumes are required to be loaded.

The spot concentration approach also offers advantages in multiple development since the number of cycles required for a given resolution is now reduced. Resolution will, in fact, be improved by comparison in the higher R_F range since, with fewer cycles, the higher components are not forced into a smaller area.

The new preadsorbent TLC plate in effect embodies the spot concentration technique and is less complicated. The present preadsorbent

plate is, however, coated with classical-type adsorbent, and the development of the same principle using HPTLC adsorbent will offer definitive advantages in terms of resolution and the larger amount of material that can be loaded.

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