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PROGRAMMED MULTIPLE DEVELOPMENT

LATERAL SPOT RECONCENTRATION

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SUMMARY

Normal programmed multiple development (PMD) causes longitudinal spot reconcentration, but does not counter lateral diffusion. If the periodic evaporation of solvent characteristic of PMD is caused to occur preferentially along the center line of the developing chromatogram, solvent from each side flows with a component toward that first-drying center line. This flow produces lateral spot reconcentration. Normal PMD complemented by lateral spot reconcentration is called centered PMD. An easily reproducible and usable mask produces centered PMD, which is demonstrated on ordinary precoated plates. Grooves in the thin-layer bed can also produce centered PMD. A centered-PMD spot produced on an ordinary pre-coated plate has a minimized, round area 1–3 mm in diameter. With a given plate, this area reflects the whole of a given molecular population. Such a spot does not spread with longer developments, but merely separates further from its neighbors.

INTRODUCTION

Programmed multiple development (PMD)[1–9] is a form of thin layer chromatography (TLC)[10,11]. In PMD, the TLC plate remains at all times in contact with the solvent. Therefore throughout each PMD the solvent moves by capillary action toward the solvent front. The location of the solvent front is governed by the rate of solvent evaporation.

The rate of solvent evaporation is varied automatically, in accordance with the given PMD program chosen by the operator. In consequence, the solvent front moves (characteristically for PMD) farther up the plate with each solvent advance, but returns to the point of spot deposition with each solvent removal.

Each time the solvent front moves up or down across a spot, the spot becomes reconcentrated. During solvent advance, molecules behind the front move toward molecules not yet reached. During solvent removal, molecules behind the front move toward molecules that have been deposited by the receding solvent front. Thus, twice per PMD cycle, each spot is reconcentrated longitudinally.

Heretofore, no method has been reported for countering the lateral diffusion

of spot molecules, except channeled plates. Channels mechanically limit the lateral diffusion of the spot molecules. However, channels do not cause lateral spot reconcentration, in which the spot molecules actually move laterally toward the centers of their respective spots.

This paper reports a convenient PMD method that, while preserving normal PMD longitudinal spot reconcentration, at the same time achieves lateral spot reconcentration on conventional, precoated, unchanneled TLC plates.

EXPERIMENTAL

Conventional TLC equipment

Pre-coated silica gel G TLC plates, calibrated micropipettes, and dye mixtures were purchased from Camag, New Berlin, Wisc., U.S.A. For use, the plates were cut to the sizes desired.

PMD equipment

The Model 2000 Programmer, the Model 222 Developer, PTFE spacers, and facing plates, all from the Regis Chemical Company, Morton Grove, Ill., U.S.A., were used.

Masks

The masks were hand-made, cut by a razor from brown kraft paper and household aluminum foil.

Each mask was made as wide as the plate with which it was to be used, but 20 mm less in length. Thus, in use, a mask does not dip into the solvent.

Each mask contained three or more slots, mutually aligned, and vertical in use. Each slot was 3 mm wide and extended to about 5 mm from the top and the bottom of the mask. The slot centers were spaced regularly, 15 mm or more apart (see Fig. 1).

Test solutions

The dye mixture to be used was prepared by mixing approximately equal volumes of Camag dye mixtures I, II, and IIN. From this full-strength composite, a one-tenth-strength solution was prepared by dilution with reagent-grade benzene.

Spotting

Each spot was made by applying 1 μl of the chosen solution to a point 25 mm from the edge of the plate.

If a mask was to be used, it was placed under the plate during spotting. The spots were then placed over the mask slots, which could be easily seen through the plate.

Mask use

In use, a mask is held between the TLC plate and the radiator, its reflective side toward the radiator.

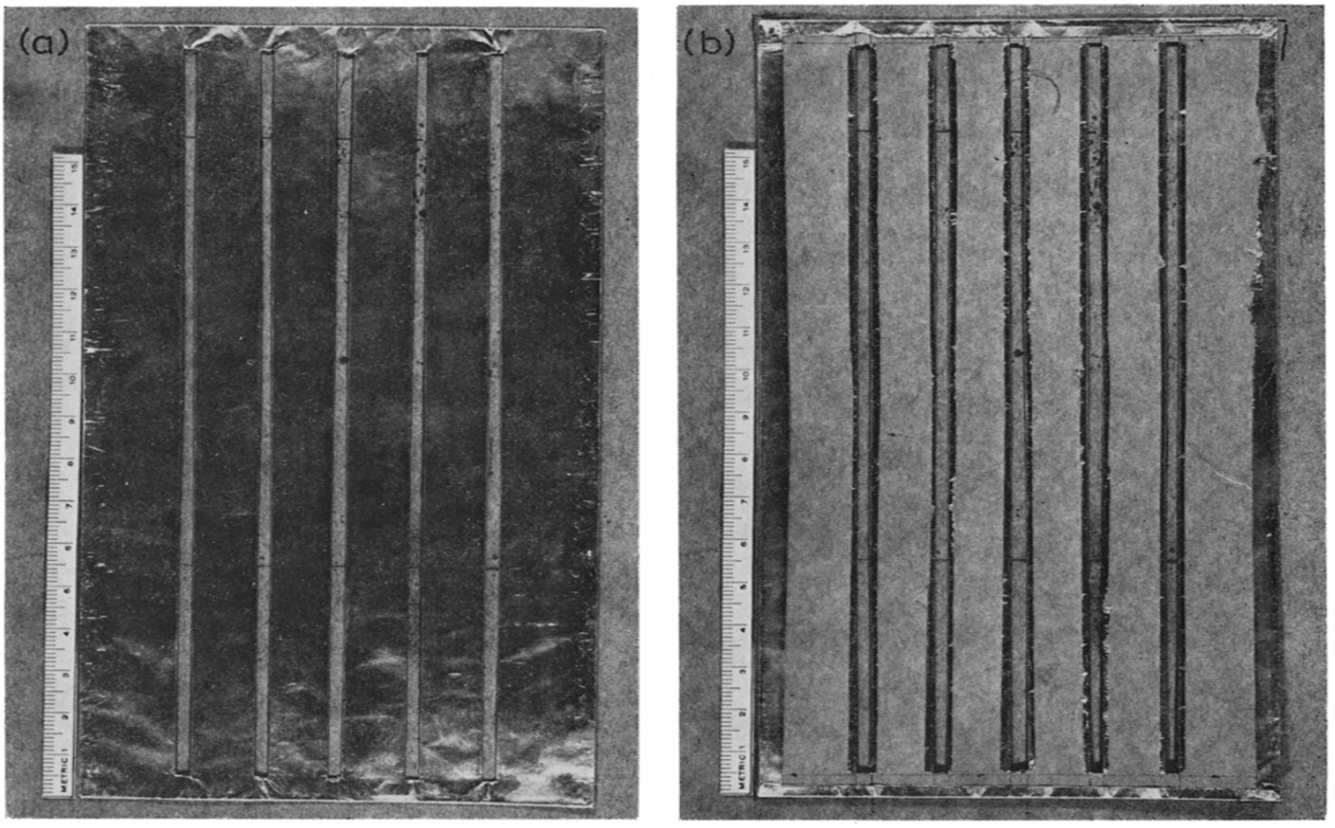

Fig. 1. Usable mask, made from kraft paper covered by aluminum foil. (a) Reflective aluminum surface faces radiators in use; (b) back of mask faces TLC plate in use.

TLC plate preparation and use

The TLC plates were prepared and developed in the fashion normal for PMD. A 1-mm wide strip of bed is removed 10 mm from each vertical edge of the plate. In use, the TLC plate is separated from a matching glass facing plate by a PTFE spacer; spring clamps hold the assembly, including a mask if one is used (Fig. 2).

Also, a vapor-trapping 15-mm wide aluminum foil strip around the outside of the assembly is advisable with either conventional or PMD developments if the plate measures 200 mm in the vertical direction. Otherwise, the solvent loss as vapor through the sides of the chamber can approach or equal the solvent flow upward when the solvent front is far up the plate; the solvent front will in consequence either advance abnormally slowly or stop completely. (The outside strip stops short of dipping into the solvent.)

Developments

The development conditions for the plates are given in Table I.

A rough guide for correlation of the developed spots is given in Table II.

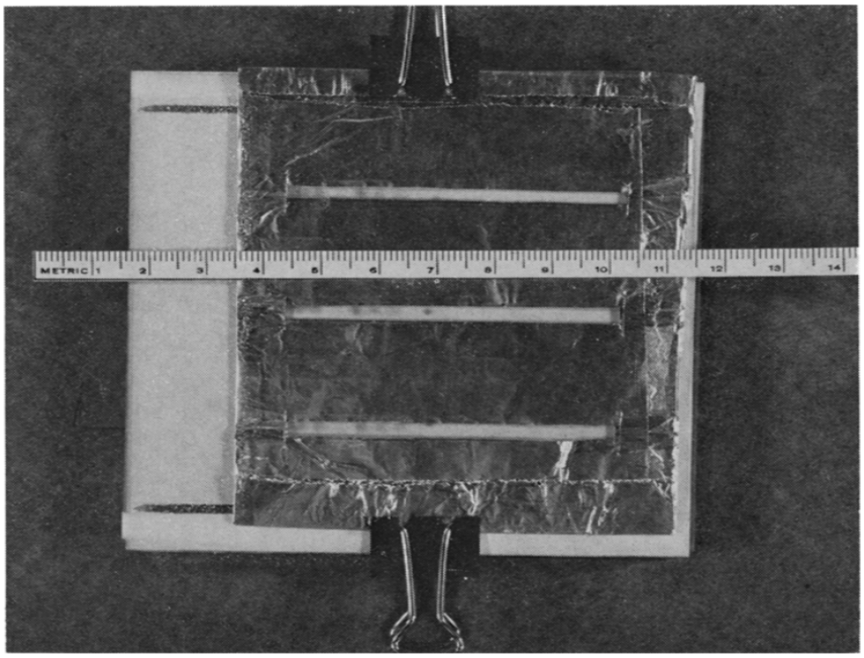

Fig. 2. The mask in use. The mask is then merely included as part of the plate assembly. The reflective foil is placed away from the plate, toward the radiator. The spots are placed under the slots in the mask.

TABLE I

DEVELOPMENT CONDITIONS FOR THE PLATES

Figure	*Method*	*Cycles*	*Unit times (sec)*		*Overall time (h)*
			Solvent advance	*Solvent removal*	
3	Conv.*				1.3
4	PMD**	9***	100	100§	1.5
5	PMD**	32***	100	20§§	17.6

* Developed by benzene; solvent front marked.

** Developed by chloroform–carbon tetrachloride (1:2).

*** Cycles in Mode 1. In Mode 1, the solvent advance time T in a given cycle is $T = n\,t$, for a given cycle of number n and unit advance time t. Thus for the unit advance time of 100 sec, the successive advance times are 100, 200, 300, $\cdots$ (100 n) sec.

§ Fixed time. Fixed solvent removal times are invariant.

§§ Scheduled time. Scheduled solvent removal times follow the solvent advance Mode law***.

RESULTS AND DISCUSSION

We shall call that PMD that includes lateral as well as longitudinal spot reconcentration, centered PMD.

The results of applying centered PMD are illustrated in Figs. 4 and 5. It can

TABLE II

CORRELATION OF THE DEVELOPED SPOTS

*Spot**	*Position (mm)*		
	Fig. 3	*Fig. 4*	*Fig. 5*
4, 5	97	45	98 (spot 4), 91 (spot 5)
6	80	42	85
7, 8	70	38	77 (spot 7), 73 (spot 8)
9	60	35	62
10	50	32	51
11	37	28	43

* For further correlation and origin of spot numbers, see Figs. 6 and 7 in ref. 12.

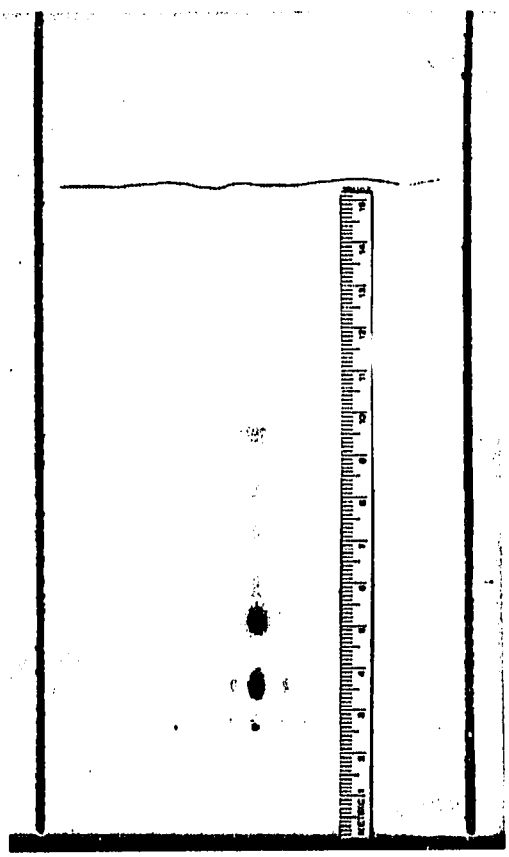

Fig. 3. Three conventionally developed, 80-min chromatograms. The center chromatogram was made from the full-strength dye solution; the outer two chromatograms were made from the same dye mixture at one-tenth strength.

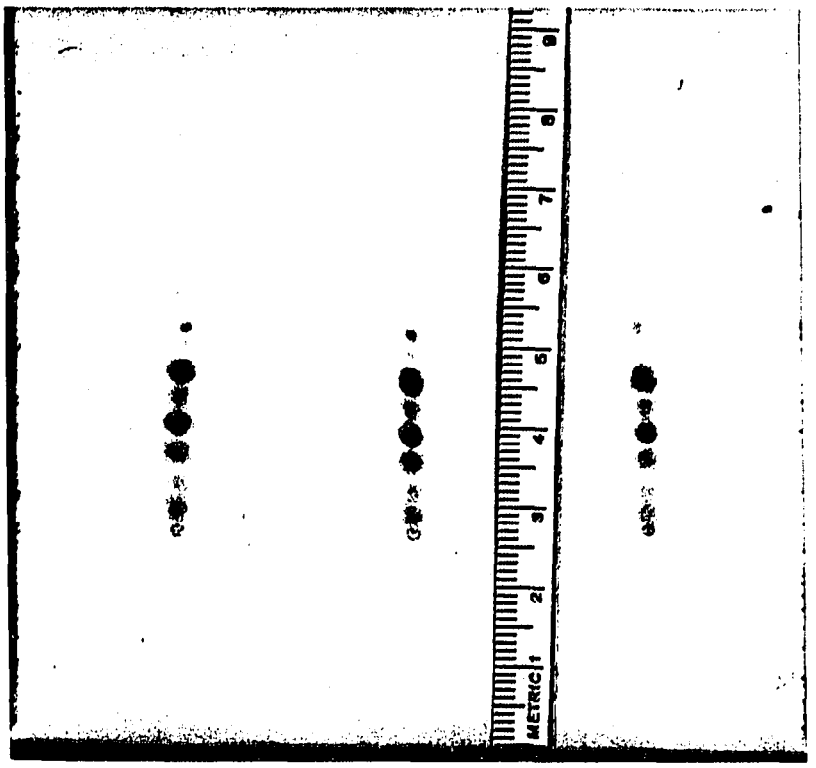

Fig. 4. Centered-PMD chromatograms, 90 min, each corresponding to each of the two outer chromatograms of Fig. 3. The spot diameter roughly indicates the relative concentration of a dye component, as can be seen. The minimized spot diameter, shown by spots at approximately 50 and 52 mm, is 1 to 2 mm. Chromatograms are all made from one-tenth-strength dye solution.

be seen that with centered PMD, spot area greater than a certain minimum reflects only one variable: spot loading.

Mechanism

The mechanism that leads to lateral spot reconcentration is easily explained. The thin-layer bed on each side of the developing chromatogram is shielded from direct infrared radiation. Therefore, when the radiator comes on to effect solvent removal, the solvent under each slot is directly heated by the radiation. This solvent begins to evaporate considerably faster than the cooler, shaded solvent to each side.

The solvent front under each slot soon develops a characteristic dip. This dip shows the desired, preferential evaporation and ultimate depletion of solvent from the line of the chromatogram.

The dips in the solvent front deepen as the radiant heating continues. Solvent, however, always flows normally with respect to the local solvent front. The direction of solvent flow into either side of one of these characteristic dips therefore has a lateral, chromatogram-centered component. Moving with such solvent flow, spot molecules move toward their respective centers. All the spots along the chromatogram are laterally reconcentrated as the cusped solvent front recedes to the origin.

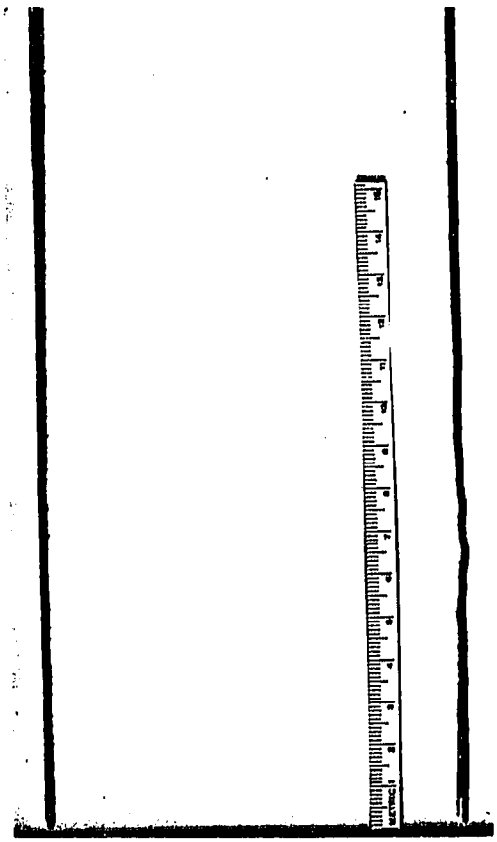

Fig. 5. Centered-PMD chromatograms, 17 h, corresponding to the chromatograms of Fig. 4 and also to the outer two chromatograms of Fig. 3. The oblong, Fig. 4 spot at 45–47 mm has become the Fig. 5 spots at 93 and 98 mm. Spot diameters are not functions of R_F or development time, but primarily of molecular population. Chromatograms are all made from one-tenth-strength dye solution

After solvent removal, the radiator is usually turned off for solvent advance. It need not be. The chromatogram shown in Fig. 4, for instance, was made with the radiator kept on at lowest power (1.25% of maximum) during solvent advance. However, the advancing solvent front was not cusped. The effect of such radiant heating during solvent advance is essentially the same with centered as with normal PMD: the chromatogram is compressed longitudinally. Thus, lateral spot reconcentration seems to be primarily a result of interaction of the receding solvent front with the spot.

Solvent removal has been shown to be more effective than solvent advance in longitudinal spot reconcentration involving heating[9]. Also, the slower the solvent removal, the more effective the resultant longitudinal spot reconcentration[1,9]. Therefore both longitudinal and lateral spot reconcentration are more effective if solvent removal is carried out with scheduled rather than fixed time.

Techniques

The slots in the mask apparently need not be narrower than those used here. Slot widths from 1 mm to 10 mm were tried, though not tested rigorously. The 3-mm

slots are reasonably easy to make, reproducible from slot to slot and level to level, and wider than the spots they produce. The lateral width of the spots from 3-mm wide slots depends primarily on loading, as shown particularly in Fig. 5.

Spots that are reconcentrated both longitudinally and laterally are usually round. With normal PMD, the top-to-bottom spot width approaches a minimum of perhaps 25 particle diameters with zero loading. With plates such as these, that is about 0.8 mm. Thus the round spots generated here similarly show diameters that approach an 0.8 mm minimum.

The spacing of the slots was not investigated. A spacing closer than the 15 mm minimum used here may be quite adequate. However, enough slots should be used to cover the width of the plate, so as to have a reasonably uniform (though cusped) receding solvent front.

For those developments in which the solvent does not advance more than 30 or 40 mm past the spot origin, the narrow rectangular slots of the masks used in this work are adequate. For longer solvent advances, slots shaped narrow at the top and wide at the bottom would be better. With these, solvent front recession would start desirably slowly but eventually, and necessarily for PMD, continue to the origin.

Spot area and molecular population

The centered-PMD chromatograms of Figs. 4 (90 min) and 5 (17 h) were made with one-tenth-strength dye solution, as were the outer two conventional chromatograms (80 min) in Fig. 3. Diffusion, uncountered either longitudinally or laterally in the two outer conventional chromatograms, quickly renders them essentially unusable. Even with normal PMD, uncountered lateral diffusion would seriously diminish the sensitivity of higher-R_F spots if the development were continued for a number of hours, say, over 10.

Under centered PMD, molecules can no longer leave their respective spots. Therefore developing spots, once isolated, thereafter remain constant in area. The molecular populations of fully separated spots change only by adventitious, well-known, but always unwelcome mechanisms such as photodecomposition. One must accept this possibility and be ready to take counter-measures such as, with photodecomposition, protecting the developing plate from possibly harmful ultraviolet or even visible light.

Centered PMD makes possible the use of conventional unchanneled plates for separations that require very high numbers of multiple developments, without any loss of sensitivity through diffusion. Fig. 5 suggests the potential of this approach, which has not previously been available. Therefore a further demonstrative study of such an extended separation has been prepared[12].

Centered PMD further improves the sensitivity enhancement of normal PMD[3]. This improvement will be more closely defined in a forthcoming study.

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