CONTOUR THIN-LAYER CHROMATOGRAPHY

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Observation of the migrations of a variety of substances on thin-layer chromatograms has disclosed that they are altered in a manner to correspond with changes in the solvent front. Frequently, migrating spots move laterally reflecting shifts of the front, and parts of spots are shifted in sympathy with a localized increment of the front. This apparent rigid tandem between corresponding increments of spot and solvent front has led to consideration of its usefulness in expanding the applications and reliability of thin-layer chromatography.

There are a few instances of experiments reported in the literature in which investigators have endeavored to control spot and solvent movements on thin-layer panels. FISHBEIN AND FAWKES[1] reported the channel technique for quantitation of mareb and zineb, two agricultural fungicides using thin-layer scored panels. REINDELL AND HOPPE[2] described the "wedged-tip technique" of paper chromatography which leads to improved separation of substances which are forced to assume a band-like path and increases the time needed for the chromatogram because of the narrow band in contact with the solvent. RINK AND HERMANN, as reported by STAHL[3], utilized thin-layer panels in a variation of the wedge-tip technique, to advantage, in chromatographing sugars found in the analysis of urine.

It seemed possible that by directing the path of flow of the solvent, the substances carried by it could be induced to travel a prescribed course other than a straight line. This has been found to be the case as shown in the experiments to follow. In these experiments, possible means for directing spot migration were examined. The movements of the several spotting substances and solvent systems used were studied on panels and strips of silica gel thin-layer preparations.

Examinations were conducted of the migrations of some food color dyes on thin-layer panels of a variety of controlled shapes. The shapes studied were chosen to illustrate a variety of ways of directing solvent flow and examining the influence of the selected solvent path on movement of migrating substances by noting its progress, spread, and position.

MATERIALS AND METHODS

The thin-layer chromatograms were done with the Eastman Developing Apparatus and Eastman Chromagram Sheet type K301-R2 in 20 cm square size. The chamber is formed by two glass plates each with raised ground borders on the outer limits of the flat surface of one side. Two plates with raised borders aligned provide a thin small volume chamber slightly larger than 20 cm square on its flat dimension. The thin-layer panel after spotting is clamped into this chamber which is then supported at a slight angle in a solvent reservoir. Special figures including arcs and strips were cut out of the panels by scribing the outline of the desired shape on the silica gel coating with a compass point and cutting it with a scissors for curves or paper cutter for straight cuts. Cutting did not damage the coating. Strips and panels with non-rectangular shapes were cemented to the glass back wall of the developing chamber before a development run. Panels were activated in a 160° F oven for 15 min.

Substances chromatographed included methylrosaniline chloride (crystal violet) 5 % in 85 % ethanol and red and green dye mixtures of Schiller® containing Food Drug and Cosmetic Act (F.D.C.) colors[4] in a 15 % aqueous solution of propylene glycol v/v.

The red mixture contained F.D.C. yellow No. 5 (tartrazine) and red No. 2 (amaranth). These were combined to provide 2.5 % coloring material in the dilute propylene glycol solvent. This mixture provided two visible migrating spots on the chromatograms. The orange migrated to 70 % and the red to 50 % of the 10 cm solvent front distance for the solvent system used.

The green mixture contained F.D.C. yellow No. 5 and blue No. 1 (brilliant blue). These were also combined to form a 2.5 % solution in dilute propylene glycol. The green mixture provided two visible migrating spots, one fast blue one and a slower yellow spot.

Panels and strips were spotted using capillary tubes and dried spots were 2.82 mm in diameter. All spotting was done 1 in. above the bottom edge of the panel or strip; the solvent surface was approximately 1/2 in. above the bottom edge of the panel. All panels in the thin glass chambers were supported during solvent development at about 10° from vertical on the Eastman stand. In those cases where laterally curved strips or other nonlinear courses were to be followed, the study material was spotted on a vertically straight section 1 in. from the bottom with curvature starting at the point of spotting.

It was of interest to determine the degree of control of spots of color mixture applied to the thin layer panels in the experiments reported here. A panel was spotted ten times with the green color mixture. To accomplish this, 3 mm columns of the green solution in a 2 λ capillary pipet were expelled on the panel at 2-in. intervals in both dimensions. The 20 $\times$ 20 cm silica gel panel was then cut into 2-in. squares having a dried green spot at the center of each of ten squares. The diameters of these spots were measured directly using a machinist's etched rule, calibrated to 1/64th in., and read with a magnifying glass. All ten spots measured 7/64 in. Each 2-in. panel was placed in a slide projector with the image of the spot projected on the screen at 7 ft. The image was sharp and clear at this 19-fold magnification. The diameter of the projected image was measured on the screen converted to radius and the standard error determined. The mean radius and standard error for the 10 spots was 65.2 (S.E. = 1.10)/64 in. The area of the image was 3.27 sq. in. with a variation of 6.2 % from 3.19 to 3.40 the range of standard error.

$R_F \times 100$ values of curves were linear distances over spot path observed and projected. These were measured with a fine-link chain.

RESULTS

Strip width

Many studies appear in the literature[5–7] in which 10 or more spots are applied

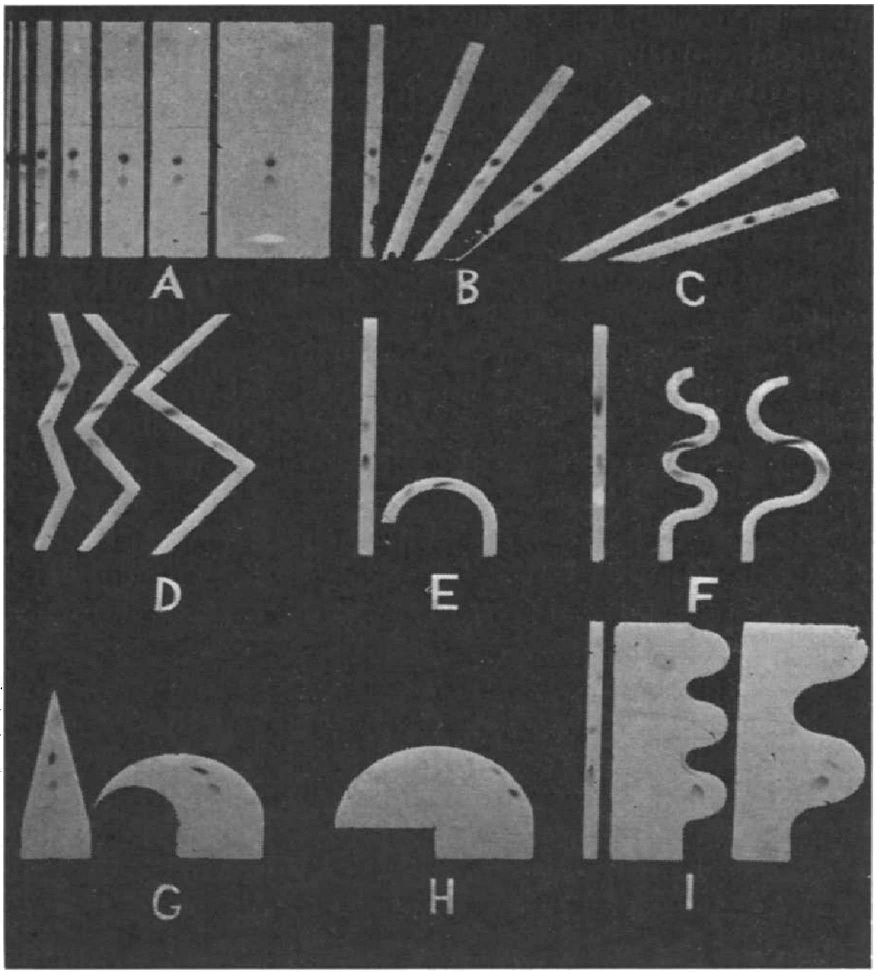

Fig. 1. Representative examples of angled and contoured panels. Each lettered group displays a panel or panels developed together as a single chromatogram. (A) Comparison of panel widths; (B and C) Angled strips; (D) Angled strips with reversal of direction; (E) 3 cm radius curved strip with vertical control; (F) Multiple curve strips with 1 or 2 cm radius with vertical control; (G) Tapered curve 6 cm outer curve radius with pyramid panel; (H) Hemidisc 7 cm radius; (I) Wide panel curved border panels 1 or 2 cm radius projections with a vertical control strip. Green color mixture was spotted except for groups (E), (H) and (I) which were spotted with red. Vertical strips are 20 cm high.

on a single 20 cm thin-layer panel, which suggests that migration on a strip 1 cm wide is practical. We conducted a series of experiments to compare the migration on panels of silica gel of varying widths. In each experiment test coloring was spotted 1 in. from the bottom edge in the center of several strips of different widths. In the first group, seven strips varying in width from 8 cm to 0.3 cm were developed together in a single chamber, illustrated in Fig. 1A. Green coloring was used which migrated in two spots as blue and yellow. The $R_F \times 100$ and measured front distances are shown in Table I, column I. The solvent system was *n*-propanol–ethyl acetate–water (60:10:30, v/v), hereinafter referred to as *trimix*.

In another group of seven strips, each was spotted with a solution of crystal violet instead of green color mix. These strips ranged in width sizes from 4 cm to 0.2 cm. The same solvent, trimix, was used and the results are recorded in Table I, Column II.

In a separate group crystal violet was spotted on strips also sized from 4 cm to

TABLE I

EFFECT OF STRIP WIDTH ON $R_F \times 100$

Solvent: (I) and (II) trimix; (III) *n*-propanol. Color mixture: (I) green; (II) and (III) crystal violet.

Strip width (cm)	*I*			*II*		*III*	
	$R_F \times 100$		*Front (cm)*	*$R_F \times 100$*	*Front (cm)*	*$R_F \times 100$*	*Front (cm)*
	Blue spot	*Yellow spot*					
8.0	68	50	8.2				
4.0	66	48	8.5	74	9.2	22	10.5
3.0	68	48	8.5			22	10.8
2.0	71	51	8.6	72	9.5	22	10.6
1.0	70	53	8.7	76	9.5	23	10.7
0.8				76	9.7		
0.5	70	52	8.3	85	9.4		
0.4				85	8.6	22	10.4
0.3	70	55	8.3				
0.2				89	9.4	24	9.7
Mean	69	51	8.4	79.6	9.3	22.5	10.5
Std. error	0.21	0.97	0.07	1.9	0.001	0.12	0.081

0.2 cm. Development was carried on in *n*-propanol as solvent and the results are recorded in Table I, Column III.

The combined results in Table I make it clear that it is feasible to use strips 1 cm wide to evaluate movements of the constituents of these coloring substances in these amounts because there is very little difference between $R_F \times 100$ values for migrating spots on 1 cm and wider panels. The slower migration of crystal violet on various strip widths utilizing *n*-propanol as solvent displayed the highest degree of reproducibility.

In strips 3 cm wide and larger the $R_F \times 100$ values were slightly lower in the cases of blue and yellow and crystal violet in trimix solvent.

The group of strips run in trimix showed that on narrow widths 1 cm and lower the migrating spots moved a little faster as indicated by slightly increasing $R_F \times 100$ values. This tendency was examined more closely by preparing sets of strips in pairs of 1 cm and 4 cm. These strips were developed with 3 or 4 pairs together in one development chamber. They were spotted with red coloring material which migrated as one orange and one slower spot in trimix solvent.

Table II shows this comparison for nine pairs of strips for the orange spots and for the red spots. The $R_F \times 100$ values for the spots on 1 cm and 4 cm strips are listed along with the computed T value. These figures show that the higher, more mobile orange spot resulted in $R_F \times 100$ values which average 7 % higher on 1 cm strips than on 4 cm strips. The slower red spot averaged 3 % higher on 1 cm strips. The $R_F \times 100$ values were statistically significantly higher at the 1 and 5 % level of probability for the orange spot.

Although these experiments show there is a slight influence on the movement of spots which attain $R_F \times 100$ values of more than 50 on 1 cm strips, this very practical strip width was utilized in a variety of experiments to be recorded here. The

TABLE II

COMPARISON OF 1 cm AND 4 cm STRIPS AND THEIR EFFECT ON $R_F \times 100$

No. of pair of strips		*Orange spot*		*Red spot*	
		Strip width 1 cm	*Strip width 4 cm*	*Strip width 1 cm*	*Strip width 4 cm*
1		65	71	44	51
2		68	73	47	50
3		69	70	48	47
4		68	77	45	41
5		67	70	46	45
6		61	68	36	41
7		67	68	41	43
8		65	64	38	35
9		64	71	37	41
	Mean	66	70	42.4	43.8
	Diff. means	4.0		1.4	
	Std. error of diff.	1.13		1.26	
	T calculated	3.45		1.11	
	Significance	positive at 5%		negative at 5%	
		positive at 1%		negative at 1%	

effect is predictable and small enough so that it does not alter the conclusions to be discussed.

In seeking an explanation for this effect it seems that there may be a pulling action associated with the attracting forces between the solvent and plastic backing material of the thin-layer panels and strips which is emphasized as strips are made narrow. On a few occasions migrating spots approached the edge on narrow strips and began a rather rapid ascension along the edge. When that happened it strung out the material and obliterated the $R_F \times 100$ determination. The few strips of this type were not included in the data presented in this paper.

Angled strips

Strips 1 cm in width were prepared for ascension thin-layer chromatography by fixing 3 or 4 strips in a chamber. One strip serving as control was arranged vertically —the others were angled to the horizontal at 75°, 60°, 45°, 30° and 15°. The vertical, 75°, 60° and 45° strips fitted easily in a single chamber, and the 30°, 15° and another vertical control fitted into a second chamber. Pairs of chambers were run at the same time, using a common solvent mix and time interval. The strips were fixed to the back glass chamber wall with plastic cement which was allowed to harden before solvent development was initiated. The flat glass chamber rested on a stand at 75° during development. Development time was 96 min to provide a front distance of 8 cm. In this series the green or red color mixes were used for spotting on the strips. A series of angled strips is illustrated in Fig. 1B and 1C.

It was found that the blue and yellow migrating spots from the green mixture and the orange and red spots from the red mixture moved along the strips in a manner very similar whether the strips were vertical or slanted. The spots typically remained centered on the strips and did not migrate to their borders. It was observed in these

TABLE IIIA

COMPARISON OF MIGRATION ON 1 cm STRIPS AT VARIED ANGLES

Strip angle (°)	$R_F \times 100$			
	Green color mixture		*Red color mixture*	
	Blue spot	*Yellow spot*	*Orange spot*	*Red spot*
Vertical	73 (3)*	49 (3)	76 (1)	52 (1)
75	74 (3)	51 (3)	74 (1)	49 (1)
60	73 (4)	52 (4)	79 (2)	55 (2)
45	74 (5)	54 (5)	79 (2)	56 (2)
30	69 (2)	45 (2)	78 (1)	51 (1)
15	72 (3)	47 (3)	72 (1)	47 (1)

* Values in parentheses indicate numbers of strips.

TABLE IIIB

COMPARISON OF MIGRATION ON 1 cm STRIPS WITH ANGLE REVERSED AT 5 cm INTERVALS

Strip angle (°)	$R_F \times 100$			
	Green color mixture		*Red color mixture*	
	Blue spot	*Yellow spot*	*Orange spot*	*Red spot*
Vertical	83 (2)*	59 (2)	94 (3)	72 (3)
75	77 (1)	54 (1)	84 (1)	67 (1)
60	72 (1)	51 (1)	71 (1)	60 (1)
45	84 (1)	57 (1)	88 (1)	64 (1)
30	81 (1)	54 (1)	90 (1)	70 (1)
15	82 (1)	56 (1)	89 (1)	72 (1)

* Values in parentheses indicate numbers of strips.

runs that the solvent progressed upward in a straight front perpendicular to the direction of the strip. This was the finding for all slanted strips, even those at 15° which presented the largest line of adsorbent material to the solvent surface.

The $R_F \times 100$ values of all of the migrating spots in Table IIIA are accumulated. These values show that the migration is constant for the various angles studied.

In the case of the blue spot, the fastest migrating of the two from green color, the mean $R_F \times 100$ values ranged from 69 to 74 which was remarkably consistent and suggests there was no appreciable difference over the whole range of angles tried. The lower 69 value, which was the mean for two strips set at 30°, was 6 % below the average of the means of the other groups and is not enough to alter the conclusion of lack of effect of angle on migration. The yellow, orange and red migrating spots exhibited similar small variation. These small variations did not display patterns of progressive differences in $R_F \times 100$ values with change in angle. The variations were random with respect to angle and probably are better explained on the basis of experimental error.

In another group of experiments variations of the slant strips were prepared to accomplish reversal of the spot movement. Strips were cut so that the spot would travel at angles to the solvent level similar to those described previously, but after about 5 cm of migration distance, the strip was cut so that the direction of travel was changed to form a path in the opposite direction still at the same angle from the solvent level. This change in direction produced a corner in the ascending strip, altering its course by an amount equivalent to twice the complement of the angle originally assigned for ascent. Thus, the 75° strip, after about 5 cm of travel, had a shift of 30° left so that it was now ascending an angle of 75° with the solvent surface on the other side. For the 60° strip the shift was 60°; for 45° the shift was 90°; for 30° the shift was 120°; and for 15° the shift was 150°. After 5 cm of travel in the shifted direction the strip was redirected with a shift back again by the same amount as previously done, so now the travel would be the same as at the start. This was done to show if there were any important influences on spot migration which could be attributed to imposed changes in direction of movement. Spots on reversal strips remained centered. Angled strips with reversal are illustrated in Fig. 1D.

Consultation of Table IIIB discloses that reversal of direction of movement of migrating spots did not alter the rate of spot migrations. As in the case previously described, where the migration was directed continuously at one angle, the spots displayed $R_F \times 100$ values close to the vertical strip controls run at the same time. In this group the $R_F \times 100$ values were consistently higher in all strips because the development time was increased above that used in previously recorded experiments to cause spots to pass two corners.

During the course of these reversal strip experiments it was found that a special effect was occurring in the 15° strips. At the corners there was an expanded area of adsorbent material due to the fact that, as you cut a 1 cm strip and change the angle of cut by 150°, there is an extensive increase of area because the cross section at the corner increases well over 1 cm. It was immediately apparent at the first trial that when the solvent front arrived at the first corner, there was a great deal of lateral spread involved in traversing it. This was a prolonged effect and persisted past the time the migrating spots rounded the corner so that as the spot started up the shifted limb of the strip it came under the influence of two fronts, one filling the corner angle and the other proceeding up the strip. This dual influence put a prolonged spread on the spot so that it elongated in a long thin band on the upper edge of the ascending limb of the strip. The lower spot was less elongated and tended to center on the shifted limb just below the first, faster spot. This effect was completely eliminated by removing excess adsorbent on the outside corner of the shift so that the adsorbent path was maintained at 1 cm width. The recorded $R_F \times 100$ values in Table III at 15° were taken from this corrected type strip. This effect was much less important in the 30° strips and was progressively less important for all strips at higher angles as the adsorbent excess diminished with each increase in angle. The other strips other than 15° were not corrected for this corner effect and this could account for apparent slight depression of $R_F \times 100$ values recorded. The movement of spots on other strips were normal to all appearances.

Curved strips

Silica gel panels were cut into curved strips in a manner which provided a 1 cm curved path of adsorbent. The strips prepared in this way included 180° arc strips

TABLE IV

THE EFFECT OF CURVED STRIPS ON $R_F \times 100$

Curve radius (cm)	*Green color mixture*			*Red color mixture*			*Red color mixture*		
	$R_F \times 100$		*Front (cm)*	*$R_F \times 100$*		*Front (cm)*	*$R_F \times 100$*		*Front (cm)*
	Blue spot	*Yellow spot*		*Orange spot*	*Red spot*		*Orange spot*	*Red spot*	
1 cm	74	46	5.0	58	38	5.2	67	37	5.2
control	74	46	4.6	68	47	3.4	66	39	5.6
2 cm	66	44	5.5	65	38	5.5	62	33	5.8
control	74	46	4.6	72	39	5.4	66	39	5.6
3 cm	73	54	10.9	76	53	11.0			
control				77	51	10.9			
4 cm	73	51	10.3	75	50	8.3	76	50	14.5
control	76	54	8.9	75	50	8.9	78	50	13.1
5 cm	78	58	13.9	79	64	13.6	77	56	15.6
control	90	68	12.2	—	—			61	12.6
5 cm							81	61	14.8
control							82	66	12.6
6 cm	75	54	15.0	87	66	11.9			
control	88	68	12.4	85	59	14.0			
7 cm	84	63	12.1	85	65	10.7	80	62	24.5
control	—	—		86	65	10.9	92	52	17.4

with inner radii of 1 through 7 cm at 1 cm intervals, making seven consecutive sizes with progressively longer paths consistent with increased radius. These curved strips were made with a 1-inch straight approach area with continuous adsorbent to lead solvent up from the supply receptacle. The color mixtures were spotted at the juncture of this 1-inch strip and the curve. Vertical strips 1 cm wide were run in the same chamber for comparison of migration of spots. Fig. 1E illustrates a 3 cm inner radius curve with control. Where possible, up to 3 cm radius, two curves and their control vertical strips were developed in a single chamber; 4 cm and larger were developed in separate chambers. Table IV shows the recorded linear $R_F \times 100$ values obtained in a series of experiments.

The several migrating spots followed the course of various curved strips 1 cm wide in a manner similar to that in which they followed the course of vertical and slanted strips. Typically, the solvent front proceeded over the course of the strip nearly perpendicular to the tangent at any point. Also, the spots remained centered or slightly above center and proceeded on an arc centered on the strip. There was a tendency for solvent front to advance on the inner, shorter curve which produced a slight shift of the migrating spots above center.

The data recorded in Table IV show a scatter of values for the $R_F \times 100$ which changes most consistently with increasing front distances. In all three series of curves of 1 through 7 cm for green and red color mixtures there is good agreement, in a predominant number of cases, between the $R_F \times 100$ values for both migrating spots and their respective controls and front distances achieved for both the curve and control vertical strips. The front had a tendency to go faster on the curved strip to produce a slightly higher front measurement and lower $R_F \times 100$ value. This happened in a preponderant number of cases. Eleven out of 16 instances the blue and orange spots had higher $R_F \times 100$ values on the control vertical strip than on the curved strip, and all of these eleven showed lower front distances for the control strip. This reflects the slight tendency of solvent front to gain as a result of faster progress on the shorter inner curve to bypass, to some extent, the migrating spot. It was also observed that there was a slight tendency for spots to migrate on an arc slightly above the center line of the curved strip. This was analyzed more fully in a following section on tapered curves.

Multiple curves

A separate series of panels was prepared to extend the findings of the simple curves. These 1 cm strips started like the simple curves but the arc was reversed at the high point with this resulting arc again reversed at its high point to provide an ascending series of S shapes. The multiple curves are illustrated in Fig. 1F with two examples having 1 cm and 2 cm radii respectively.

Solvent fronts and migrating spots followed the path of the multiple curves in a manner very similar to that for simple curves. Examination of linear front distances and migrating spot $R_F \times 100$ values discloses that the reversal of direction had a compensating influence, so the tendency of front increase and migration lag did not show up in measurement of final positions.

In several experiments it was shown that spots migrated at similar rates whether they were directed through curves with reversal of migration or on straight vertical strips. Differences between $R_F \times 100$ values for the multiple curve or straight panel show there is no constant or consistent pattern of difference between curved or straight migrations. This was the case for blue and yellow migrating spots from green color mix, for orange and red spots from red color mix, and for crystal violet, as shown in Table V.

Pyramids

A number of pyramid-shaped panels were run using green or red color mixes for spotting materials. These were studied to compare their influence on migration with that of curved tapered panels.

The pyramids were prepared so that base and vertical dimensions corresponded with base and centered linear curved path of the tapered curve, and these matched figures were developed in a chamber together. See Fig. 1G. The data show that $R_F \times 100$ values of migrated spots on tapered curves were lower than corresponding spots on pyramids and that these values for pyramids were below those for rectangular panels as recorded in Tables I and II preceding. A closer examination of the influence of the pyramid shape on spot migration was sought by separately accumulating the pyramid values (see Table VI).

In Table VI are listed $R_F \times 100$ values for migrating spots arranged to correspond with dimensions of pyramid panel and the amount of taper. The taper was computed by dividing one half the pyramid base by the altitude (vertical distance from original dye spot to apex) which measures the unit lateral displacement per unit

TABLE V

THE EFFECT OF MULTIPLE CURVES ON $R_F \times 100$

Solvent: Trimix in each case. Color mixture: (I) green; (II) red; (III) crystal violet.

Curve radius (cm)	*I*			*II*			*III*	
	$R_F \times 100$		*Front (cm)*	*$R_F \times 100$*		*Front (cm)*	*$R_F \times 100$*	*Front (cm)*
	Blue spot	*Yellow spot*		*Orange spot*	*Red spot*			
0.25	96	79	11.1	77	55	13.0	80	12.4
control	90	72	10.4	74	56	11.3	81	10.8
0.5	94	70	11.3	81	58	14.9	85	13.9
control	90	72	10.4	64	37	13.4	64	10.3
0.5	71	38	5.8					
control	69	48	4.8					
1.0	81	57	13.4	66	52	14.9	79	15.9
control				74	46	13.5	39	16.5
1.0	79	52	11.6					
2.0	85	62	13.8					
control	84	55	15.4					

TABLE VI

PYRAMIDS OF VARIED BASE SIZE AND ALTITUDES AND THEIR EFFECT ON $R_F \times 100$

Pyramid			*Green color mixture*			*Red color mixture*		
Base (cm)	*Altitude (cm)*	*Taper*	*$R_F \times 100$*		*Front (cm)*	*$R_F \times 100$*		*Front (cm)*
			Blue spot	*Yellow spot*		*Orange spot*	*Red spot*	
3	20	0.000	70	47	9.3			
3	20	0.000	70	50	12.8			
5	20	0.125				72	51	9.7
4	14	0.143	49	26	5.7	52	27	5.6
5	15	0.167				56	33	10.9
5	14	0.179	50	32	11.7			
8	20	0.200	53	30	9.8	57	39	9.8
8.5	20	0.213				59	39	12.5
10	20	0.250	62	40	11.5	56	28	13.7

vertical movement. Results from two rectangles run in this same series of experiments were included at the top of Table VI showing 0.000 taper. These values coincide very well with results from similar runs listed in Table I at 3 cm with green color mixture. The slight taper at 0.125 apparently does not influence $R_F \times 100$ values achieved for orange and red spots from the red mixture. The pyramids with taper at 0.143 and above, all show $R_F \times 100$ values well below those recorded for rectangles for the same spotting mixtures in previous sections of this report. At taper of 0.143 and above the red color mixture migrations are grouped within a small range at 52 to 59 or 27 to 39 for the orange and red spots respectively. In this group of panels the figures do not show progressive change with increased taper but suggest a rather all or none depression above 0.143 to 0.250 taper. Also, the effect of taper appears overemphasized at 0.143 taper, which might be due to the fact that the front distance is 50 % of that desired. Another uncontrolled variant is the base dimension which may influence the role of taper by altering the actual distance of the migrating spot from the tapered panel edges.

TABLE VII

INFLUENCE OF TAPER ON $R_F \times 100$ FOR PYRAMIDS WITH 9 cm BASE

Taper	*Green color mixture*		*Front (cm)*	*Time (min)*	*Average front rate (mm/min)*
	Blue spot	*Yellow spot*			
0	71.2	50.8	9.3	161	0.58
0	73.5	54.6	9.4	161	0.58
0.1	71.0	53.4	10.3	151	0.68
0.15	70.0	52.4	10.3	151	0.68
0.20	66.5	47.2	10.5	143	0.73
0.25	66.0	47.2	10.6	143	0.74
0.30	62.0	42.0	10.0	126	0.79
0.35	56.3	38.7	12.2	126	0.97
0.40	58.8	40.0	9.4	103	0.91
0.45	51.0	33.0	10.0	103	0.97
0.90	50.0	31.0	10.0	92	1.09

In a separate series of experiments studies were made of the influence of taper on $R_F \times 100$ values with constant base and front distance. The taper was achieved by changing the altitude. A preliminary series suggested that the effect of taper was constant above 0.1. Panels were prepared with pyramids having 9 cm bases at the level of the spot and continuing down for 1 inch to the bottom. At spot level the panels were cut to form pyramids with varied altitudes which provided tapers of 0.1 through 0.45. This was the limit achievable with a 9 cm base because the altitude for this taper is 10 cm, the desired front distance. An additional panel was added, having a pyramid base of 18 cm with an altitude of 10 cm to determine the influence of a taper of 0.90. Although this panel showed results consistent with the others, it was not included in the regression equation data following.

Table VII lists the $R_F \times 100$ values of ten panels with a base of 9 cm, and one, the bottom one, with a base of 18 cm and taper of 0.90. The two top 0 taper panels were 9 cm by 20 cm. These were run in pairs starting from the top with the last 18 cm base panel alone. Panels were cut to taper starting 1 in. from the bottom. Some were not pyramid shaped because the point would lie above the 20 cm panel limit. This was not a factor in taper considerations because all solvent development was terminated at approximately 10 cm. With two panels of different taper in a chamber together, the effect of taper on solvent rate of travel is apparent by different front distances for the same time interval.

The average rate of front travel listed in Table VII shows that the solvent achieves its 10 cm distance at a markedly increased rate as the taper is increased. There is a 170 % increase in average rate of front migration over the range of taper of o to 0.45 for pyramids with 9 cm measurements. The last case of 18 cm base pyramid showed further rate increase along with greater taper of 0.90.

The $R_F \times 100$ values for tapers from 0.15 up show progressive decrease for both blue and yellow migrating spots. This decrease was studied by plotting the reciprocal of the taper as independent variable on the abscissas and the $R_F \times 100$ achieved as the dependent variable on rectangular coordinate graph paper (see Fig. 2). To these plotted points regression lines were fitted according to the method of least squares[8] and the regression established parallelism with slopes of 3.93 and 3.94 for blue and yellow migrating spots respectively. The alterations of $R_F \times 100$ values are equivalent for both migrating spots.

With tapers less than 0.15, including rectangles, the $R_F \times 100$ remained constant for all migrating spots studied.

The regression line does not go through the origin because it applies only to data of a useful range of tapers.

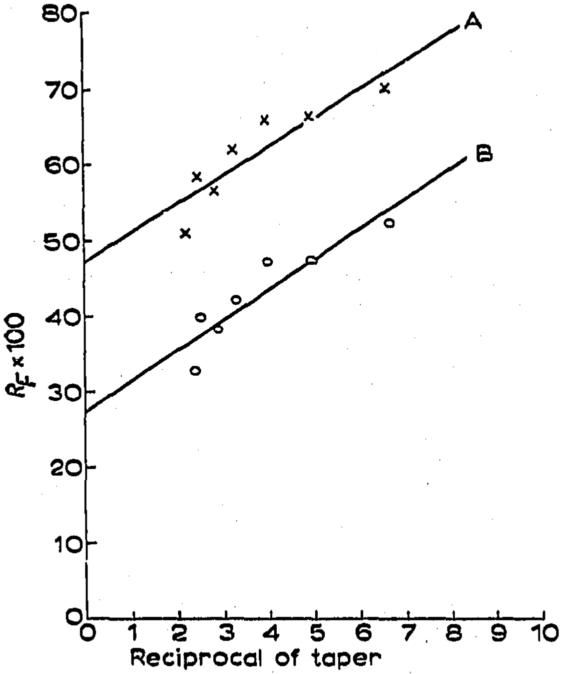

Fig. 2. Regression plots with lines fitted according to the least squares technique. Equations in the slope intercept form are: (A) Upper blue spots, $y = 46.6 + 3.93X$, slope $= 3.93$; (B) Lower yellow spots, $y = 28 + 3.94X$, slope $= 3.94$. Rectangular coordinate system.

Tapered curves

The preceding studies with tapered panels show that the progressively diminishing adsorptive surface enhances solvent front progress without correspondingly increasing advancement of centered migrating spots. This suggests that solvent flow proceeds at a faster pace at the outer edges of tapered panels than at the center. This amounts to a tendency of the solvent to bypass the centered spots or at least an enrichment of solvent flow in paths other than through the spot. According to the regression study preceding, this adds an increment to the solvent front distance proportional to the taper of the panel. In the course of these experiments, it was observed that the solvent front consistently moved ahead at the tapered edges to

TABLE VIII

TAPERED CURVES AND THE LATERAL DISTANCES (mm) ACHIEVED BY MIGRATING SPOTS

Curve radius (cm)	*Green color mixture*			*Red color mixture*		
	Lateral distance (mm)		*Front (cm)*	*Lateral distance (mm)*		*Front (cm)*
	Blue spot	*Yellow spot*		*Orange spot*	*Red spot*	
8*	20	11	19.2	22	11	18.9
6*	15	6	14.1	18	9	14.5
5*	14	8	11.5	10	1	10.2
5**				20	13	10.8
6**	29	12	15.0			

* Straight approach.
** Flared approach.

take on a concave form with the center remaining lowest. Also, the appearance of the migrating spots on the pyramid panels shows that solvent flowing on either side at a faster rate than through it decreases the tendency of the spot to spread laterally as it ascends.

It was of interest to determine if the independence of solvent movement lateral to the migrating spot could be utilized to influence its movement. Tapered curves were made to provide a path for a migrating spot which would provide a progressively decreasing front path and determine panel shapes which would influence migrating spots to move laterally. These panels had an outer curve of 8, 6, or 5 cm. A hemidisc of one of these radii was scribed above a horizontal axis in the silica gel thin layer. A second curve was scribed centered on the same horizontal axis half way between the center of the large arc and the circumference, horizontal axis intersection. This provided a 1/2 crescent-like form. To complete the panel, a straight approach was made by projecting a vertical line 1 inch down from the center of the large arc and another line from the point of meeting at its right circumference with the horizontal. This type panel is illustrated in Fig. 1G. Color mixture was spotted midway between the center and right border on the horizontal axis. The solvent proceeded uncurved up the 1-in. approach until it reached the dried spot. Further progress of solvent and migrating spot were on the curved panel and both followed this general path.

The results for eight panels, listed in Table VIII, show that all 4 migrating spots were moved in a path directed toward traversing the curve of the panel. The lateral distances in the direction of the curvature achieved by migrating spots were measured by their perpendicular distance from a vertical line through the center of the original spotted position on the panel. The solvent front distance was the linear distance from the original dried spot along a centered curve to the front. Examination of the upper three panels for either green or red color mixture trials disclosed that lateral progress was slight. In contrast to these, it was markedly higher, compared for comparable solvent front distance, for panels having flared approaches. In these the bottom panel edges were both tapered outward on the 1 in. lower area of the panel which contacted the solvent in the reservoir.

In the two flared panels the lateral movement of migrating spots more nearly

TABLE IX

TAPERED CURVES AND THEIR EFFECT ON $R_F \times 100$

Curve radius (cm)	*Green color mixture*			*Red color mixture*		
	$R_F \times 100$		*Front (cm)*	*$R_F \times 100$*		*Front (cm)*
	Blue spot	*Yellow spot*		*Orange spot*	*Red spot*	
8	37	27	19.2	37	25	18.9
control	53	30	9.8	56	28	9.8
6	38	23	14.1	41	26	14.5
control	50	32	11.7	57	33	10.9
5	36	23	11.5	40	25	10.2
control	49	26	5.7	52	27	5.6
Mean tapered curve	37	24.3		39.3	25.2	
Mean pyramid control	50	29		55	29.3	
Pyramid increase	+26%	+17%		+29%	+14%	

follow the centered path of the crescent curve. The 6 cm curve with tapered approach produced 100% increase in lateral displacement of both migrating spots blue and yellow over corresponding spots on the 6 cm curve with straight approach. Migrating spots on straight approach curves moved higher vertically and were less influenced by panel shape.

The tapered curve experiments have shown that it is possible to influence the change of path of migrating spots from a vertical direction and that the change can be enhanced by influencing solvent flow lateral to the spots. The $R_F \times 100$ values of the migrating spots on tapered curves and pyramids of similar base and linear path dimensions are listed in Table IX. The pyramid, called *control*, was spotted and developed together with the crescent curve panel. Trimix solvent was used. Green and red mixtures were developed in separate chambers.

The $R_F \times 100$ values were lower for curved panels than for pyramid controls. The average of the blue spots was 26% higher, the yellow spots averaged 17%, the orange 29%, and the red 14% higher. Another important difference between control and curve panels was the markedly higher linear front distance in the case of the curved panel. This value was often 100% greater for the curve.

The dimensions of the pyramids were of the order of magnitude to show that taper exerted marked depression of $R_F \times 100$ values. These values were around 50 in the case of the blue migrating spot corresponding to values obtained with pyramids of taper of 0.9 in Table VII. The $R_F \times 100$ values were markedly depressed below this value in case of the curves, showing that more than taper effect is involved.

A promising explanation is associated with the marked difference in path length of the inner and outer borders of the crescent curves. The inner curve is the arc of a circle of radius 1/2 the radius of the outer circle; consequently, the hemicircumference of the inner curve is one half that of the outer curve and the front movement on the inner curve has half the distance to go. For a given time period the solvent traverses twice the fraction of its total path on the inner curve than on the outer curve. This

TABLE X

THE EFFECT OF HEMIDISCS ON $R_F \times 100$

Color mixture: red in each case.

Outer curve radius (cm)	*Hemidiscs*			*Control 1 cm strips*		
	$R_F \times 100$		*Front (cm)*	*$R_F \times 100$*		*Front (cm)*
	Orange spot	*Red spot*		*Orange spot*	*Red spot*	
4	29	14	11.3	65	40	5.8
4	29	14	11.4			
4	26	13	12.0	63	32	5.9
4	28	14	12.0			
4	29	17	12.1	69	40	5.7
4	32	16	11.8			
Mean and standard error	29 ± (1.0)	14.7	11.8	65.6	37.6	5.8
7 cm	33	18	16.5			
% Increase over 4 cm hemidiscs	12.1 %	18.3 %	28.5 %			

leads to a marked differential of solvent front with a rapid pile up on the inner curve to one side of the migrating spot and a lag on the outer curve edge. This produces an enhanced solvent front movement or an increased bypass of migrating spot by solvent front on the inner curve side.

Observation of the experiment in progress discloses that there is a swollen solvent front on the short curve side of the migrating spots which, besides extending front distance, tends to displace migrating spots above a path centered over the course of the crescent curve.

Hemidiscs

In a series of hemidisc panels the $R_F \times 100$ values were depressed to an extent similar to that encountered in tapered curves. These were panels prepared by scribing a half circle on the silica gel layer of a 20 × 20 cm panel. A horizontal line was drawn from the center left to the circumference and 1-in. vertical lines were drawn perpendicular from the center down 1 in., and from the right circumference limit down. This resulted in a hemidisc with a 1-in. by 1-radius rectangular approach panel below the right half (see Fig. 1H). This was similar to the tapered curve or crescent panels described in a preceding section, except these did not have the semicircle cut out on the left half. In contrast to the crescent curve panels, these hemidisc panels were spotted with color mixture, red, off center at the right horizontal axis 0.5 mm left of the right circumference, placing it 1 in. from the bottom edge. In all cases the migrating spots remained on the adsorbent without accumulating or spilling over the outer edge. The faster orange migrating spot sometimes centered a little closer than 0.5 mm to the outer curve but the slower red spot consistently remained at the same distance from the outer curve as spotted, as it progressed around the course of the curve.

Table X lists the $R_F \times 100$ values for the two spots from the red color mixture. The solvent used was trimix. Two sizes hemidiscs were used. Six were made with an outer curve radius of 4 cm and one was made with an outer curve radius of 7 cm. The

TABLE XI

WIDE PANEL, CURVED BORDER AND ITS EFFECT ON LINEAR $R_F \times 100$

Color mixture: red in each case.

Half circle radius (cm)	*Wide panel curved border*			*Control 1 cm strips*		
	$R_F \times 100$		*Front (cm)*	*$R_F \times 100$*		*Front (cm)*
	Orange spot	*Red spot*		*Orange spot*	*Red spot*	
1.25	71	48	10.4	86	57	10.2
1.50	66	49.5	10.7			
2	61	37	9.5	78	47	10.6
3	60	44	9.0			

smaller panels were developed two in a chamber (listed together in Table X), along with a 1 cm wide vertical strip control with color spot centered 0.5 mm from both edges and 1 in. from the bottom. Development time was 55 minutes.

Linear $R_F \times 100$ values were consistently similar for the six different 4 cm hemidiscs, at approximately 40% of the corresponding measurements for control strips. The mean $R_F \times 100$ figure of 29 was 44.7 % of the mean 65.6 value for control, while the mean of 14.7 was 39.2 % of the control red migrating spot. The 7 cm hemidisc $R_F \times 100$ values were low also.

These low $R_F \times 100$ values probably reflect an effect similar to that encountered in the crescent panels recorded in Table X. The $R_F \times 100$ were even lower for the hemidiscs. The solvent front had a tendency to fan out and swell out below migrating spots; while it lagged at the right as it wheeled along the long outer edge of the disc. The front was measured by its linear distance along a curve passing through the migrating spots, and 0.5 mm from the outer edge. This same path served as linear distance for spot migration distance.

Wide panel, curved border

Some panels were prepared starting 4 cm wide at the bottom with straight vertical rise for 1 in. Starting at 1 in., these panels were extended in curved projections to the right in a series of vertically extending 1/2 circle projections in forming a scalloped border. In 4 panels the projecting 1/2 circles had radii of 1.25, 1.50, 2, and 3 cm repectively. The projections had the same size repeated at constant intervals over the 20 cm right side and they were spaced at 0.5, 1, 2, and 4 cm intervals, corresponding to the order of size of projections (see Fig. 1 I).

Red color mixture was spotted 1 in. from the bottom of the panel 0.5 mm from the right edge. Two of these four panels, 1.25 and 1.50 cm circle border, were developed in trimix solvent in one chamber along with a 1 cm control strip for 177 minutes. The remaining two, along with another control strip, were similarly developed in a separate chamber over the same time period. The measurements from these experiments are listed in Table XI.

The panels with projections along the right edge produce depression of $R_F \times 100$ values calculated for all of the migrating spots, as shown by comparison with corresponding control values. This depression effect is due to a combination of two things.

As the solvent front ascends and encounters a projection on the right, the rate of ascension markedly decreases as laterial flow tends to cover the projecting panel to the right. Further ascension of the front brings it past the mid region, widest projecting point, and the front now tends to increase ascension as the projecting adsorbent layer diminishes. This upper portion of the projection is much like the border of the pyramid which tends to increase rate of solvent front movement as the adsorbent surface diminishes.

The trailing migrating spot is spread out laterally toward the center of the projection in a relatively slow phase as it ascends, compared to the front. This results in a greater disparity of relative position of front and spot in their final positions at the end of development. At this stage, in every case the solvent front has gone through more of the fast decreasing layer phase than the trailing migrating spots, with a resulting greater disparity of linear distance covered than is the case for straight controls.

DISCUSSION

The results section listed experimental evidence that solvent fronts and migrating spots may be induced to vary their direction of travel on thin-layer chromatography panels by altering the shape of the adsorbent panel. Various shapes were tried, using green or red color mixes, each having 2 visible migrating spots—blue and yellow, and orange, and red, repectively, in order of fastest to slowest migration rates.

Little or no effect on migration rates of the color spots were encountered in thin strips, angled strips from 75° to 15°, reversing angled strips, curved strips, or multiple curved strips. A slight depression of $R_F \times 100$ was encountered in curved strips associated with differential rates of solvent front on the two sides of the migrating color spots. This slight depression in multiple curve strips was less consistent, possibly because solvent flow differential on either side of migrating spots was not consistently the same. Flow on the left could be faster than on the right for one interval of time, and then the reverse would be the case for another interval. This alternating influence of front migration rate seemed to diminish the tendency for the $R_F \times 100$ values to vary from control vertical strips.

In contrast to these, the pyramids, tapered curves, hemidiscs, and wide-panel curved border panels all displayed marked depression of $R_F \times 100$ values. This depression of migration in these several type panels could always be associated with altered rate of solvent front movement of areas lateral to the migrating spots. Observation of the migrating solvent front on pyramid panels disclosed that the front tended to pile up along the ascending tapered edges in proportion to the amount of taper. Regression plots of reciprocal of taper as abscissas, and $R_F \times 100$ as ordinate, disclosed good correlation between these values. The regression lines were parallel for the two visible migrating spots from one color mixture, showing that both spots are influenced to the same degree by the taper.

Depression of $R_F \times 100$ was greater for tapered, crescent-shaped curves than that encountered with pyramids with comparable taper. This was probably due to the marked differential between solvent front flow along the edges of the panel. The solvent front traversed the inner curve edge much faster, relatively speaking, because the actual linear distance amounted to 1/2 that of the outer curve. This added to the effect of taper, cited for the pyramid, produced a piling up of solvent on the inner curve, and an obvious lag of solvent front on the slower outer curve. The final position of the migrated spot and solvent front showed that there was a tendency of solvent to bypass the migrating spot by an amount greater than could be accounted for by taper alone.

The migrating spots on tapered curves tended to be displaced slightly above a path centered between the outer and inner curves as it progressed in the general path of the curve. Improvement toward following a centered path was encountered when tapered approaches to these panels were made so that the rate of solvent flow on the larger outer curve was enhanced relative to flow on the inner curve. The best result was encountered when the approach was flared out on the large curve side and flared in, partly cut away, on the small curve side. These two changes of approach did much toward increasing the movement of migrating spots along a path centered in the tapered curve.

Spots very near the outer curve of hemidiscs followed this curvature very well. They did not tend to migrate toward the outer edge and appeared to remain concentrated as they progressed around the disc. The $R_F \times 100$ values were markedly depressed in a manner like that encountered in tapered curves, except the taper influence of the shorter inner curve was absent. Instead, the solvent tended to swell out laterally over the central portions of the hemidisc and get ahead of the migrating spots which remained near the outer curve. Flow of solvent front along the outer edge of the larger curve remained slow and contributed to the lag in colored spot movement.

The migrating colored spots were depressed in their progress on wide panels with curved borders, as shown by marked depression of linear $R_F \times 100$ values. This depression increased as the area of these projections increased. It was noted that, as the solvent progressed laterally into a border projection, the ascending progress was lessened, and when the vertical midpoint of the lateral projection was reached, the front ascension rate began increasing as the area decreased, similar to the taper effect. In this later phase the solvent would tend to speed ahead of the migrating spot which is bypassed by the faster moving lateral column of solvent. The overall effect is to decrease the $R_F \times 100$ value measured after the solvent development is stopped.

The overall view of the experiments reported in this paper provides basic information about the behavior of migrating spots carried by solvent systems over adsorbent media. These experiments are related to the problems of explaining variations between laboratories, in recorded $R_F \times 100$ values which are often considered to be due to differences in coating materials. Some differences may be caused by variations in adsorbent layers which alter solvent flow by undetected gradients in adsorbent particles, or their arrangement which results in a channeling of solvent and solute in a pathway other than vertical. This project has disclosed controlling factors in the progress of migrating solutes on thin-layer chromatograms. Exploration was undertaken to show some ways in which the path of a migrating spot could be directed, either by limiting the pathway physically as on narrow strips angled and curved, or by influencing the rate of flow of solvent front lateral to the moving solute as on tapered curves with selectively tapered approach panels. The studies with pyramid shapes of varied taper show how the separations may be speeded up by shortening the period for solvent front achievement of preset front distance. The experiments show this is at the expense of distance accomplished by migrating spot, which is inversely proportional to the amount of taper of the pyramid. It was observed that the pyramid form influenced the concentration of migrating spot which had less tendency to spread laterally on this than on rectangular panels. These pyramid studies contrast with the interesting studies of REINDELL AND HOPPE[2] in which they demonstrated the slower travel of solvent fronts with increased lateral spread and decreased vertical spread of migrating solute spot on panels with inverted pyramid shape.

Together, the studies of inverted pyramid of REINDELL AND HOPPE and upright pyramid have suggested the probable future development of practical methods for shaping the migrating solute spots and enhancing detectability and quantitation.

Studies with pyramids, tapered curves, hemidiscs, and wide panels with curved border all demonstrate that means can be found to alter rate relationship between solvent front and migrating solute. It is interesting to note that in all cited instances of depressed $R_F \times 100$ values, there were associated observations that the final measured front distance used to compute $R_F \times 100$ values could have been increased by more rapid advance of solvent front lateral to the front which originally passed through the migrating solute. Had it been possible to measure this small increment of front at its final compared position, it is probable that the $R_F \times 100$ would have been close to the usual value measured in ascension thin-layer chromatography. This was supported by the experiments in which solutes were directed over a variety of curves in narrow strip panels in which the $R_F \times 100$ was essentially unaltered. Experiments to reinfore this contention are under consideration.

SUMMARY

Examinations were conducted of the tendency of migrating substances to ascend on thin strips of varying widths, to follow: 1 cm strips at varying angles above the horizontal compared with the vertical and with reversal of angle of solvent flow, varying degrees of curvature in a single narrow arc or in a series of ascending arcs producing reversal of lateral direction, progress up the center of a pyramid panel, follow the outer curve of a hemidisc, the center pathway of a tapered curve with pyramid control panels, and to course along the curved lateral border of wide panels.

It was established that solute migrations could be directed along a wide variety of paths without alteration of rate factor, provided the shape of the panel was designed so the solvent front of areas lateral to the front passing through the advancing solute did not advance at a faster rate.

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