CHROM. 4910

A COMPARISON OF THIN-LAYER CHROMATOGRAPHIC ADSORBENTS, SUPPORTS AND DEVELOPING UNITS

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(Received July 1st, 1970)

SUMMARY

Development of available silica gel adsorbents applied on glass plates, plastic films or glass fibers, yields reliable chromatographic data regardless of whether a glass tank, a sandwich unit, or a Gelman chamber is used. Depending on the type of sample and the user's need the presented data serve as a guide for the selection of a suitable system for a particular use.

INTRODUCTION

Those who use thin-layer chromatography are periodically importunated to discard the adsorbents they are currently using. They are urged to try a new, more versatile adsorbent, which, if used, allegedly will yield better chromatograms. In addition, they continually debate the choice of a developing unit and the optimal conditions for development of the chromatogram. Much of the data which follow were collected so that reasonable answers to these three questions could be obtained. Acid and basic drugs were chromatographed using many different adsorbents, developing units, and conditions for development. The data collected in these experiments were tabulated and are the basis for the subsequent discussion and conclusions.

EXPERIMENTAL

Equipment

Developing units. The following units were employed: (a) Developing tanks: 21 × 11 × 21 cm, glass, with ground glass lip and matching lid (hereafter referred to as *tanks*). (b) Chromatogram developing apparatus: sandwich unit introduced by the Eastman Kodak Co. (hereafter referred to as *trough*). (c) ITLC chromatography chamber: chromatographic chamber developed by the Gelman Instrument Company (hereafter referred to as *chamber*).

Plate-coating unit: A Desaga apparatus designed to coat five 20 × 20 cm plates was used.

Chemicals

All chemicals used for developing solutions and spray reagents were analytical grade. All drugs were obtained from cooperating drug manufacturers and were pharmaceutical grade chemicals.

Developing systems. The following systems were applied: (a) chloroform–acetone (9:1), (b) Davidow's, *i.e.* ethyl acetate–methanol–conc. ammonium hydroxide (85:10:5), (c) CBD, *i.e.* cyclohexane–benzene–diethylamine (75:15:10), and (d) 95 % ethanol.

Drug reference solutions. The acids were prepared as follows: 40 mg of the free acid were dissolved in 20 ml of absolute ethanol (sodium diphenylhydantoin being the only exception). For the preparation of the bases 40 mg of the salt were dissolved in 20 ml of absolute ethanol. If the salt was insoluble in ethanol, then the sample was dissolved in a minimal volume of 0.1 *N* sulfuric acid. This solution was then diluted to 20 ml with absolute ethanol.

Detection reagents. For the preparation of mercuric sulfate 5 g of mercuric oxide were suspended in 100 ml of distilled water. To this 20 ml of concentrated sulfuric acid were added with continuous mixing. The resulting mixture was cooled and diluted to 250 ml with distilled water.

The Dragendorff reagent consisted of 10 ml of a solution A, 10 ml of a solution B, 20 ml of glacial acetic acid and 100 ml of water. For the preparation of solution A 2.125 g of bismuth subnitrate were dissolved in 25 ml of glacial acetic acid and 100 ml of water; for solution B, 50 g of potassium iodide were dissolved in 125 ml of water.

The sodium nitrite reagent was prepared by dissolving 5 g in 100 ml of water.

Adsorbents. The adsorbents applied were: pre-coated glass plates, supplied by Brinkmann Instruments, Inc., Laboratory Products Department, Corning Glass Works, and Analtech, Inc.; pre-coated films, supplied by Brinkmann Instruments, Inc. and Eastman Kodak, Inc.; impregnated glass fiber, type SA, SAF of Gelman Instrument Co.; and glass-coated plates prepared in the laboratory from the following silica gel powders: Merck G, Corning C_3 (with binder), Corning C_5 (without binder), Merck GF, Merck HF, Silicar 7G, Silicar 7GF, Silicar 4GF, Camag GF, Whatman G41, Anasil B, Anasil S and Adsorbosil-2.

Procedure

Preparation of glass-coated plates

20 g of adsorbent were suspended in 50 ml of water and stirred until the gel appeared to be setting. Using a Desaga apparatus, this slurry was applied to five 20 × 20 cm glass plates. The plates were allowed to set at room temperature. After the coating had set, the plates were stored in a constant temperature (70° F) and constant humidity (40 %) room. Prior to sample application the plates were scribed (Chemical Rubber Company Scriber, Catalog No. 8705/975).

Spotting

All chromatograms were spotted with 3–4 μl of sample to give a 7.5-mm spot.

TABLE I

$R_F \times 100$ VALUES OF ACID AND NEUTRAL DRUGS — DEVELOPMENT IN SATURATED TANKS

	Amobarbital	*Phenobarbital*	*Pentobarbital*	*Secobarbital*	*Diphenyl-hydantoin*	*Glutethimide*	*Range*	*Median*
	Solvent: chloroform–acetone							
Pre-coated plates								
Brinkmann	18	14	21	24	12	41	29	20
Corning	20	13	20	25	16	42	29	20
Analtech	34	24	33	37	21	52	31	34
Self-coated plates								
Merck G	29	21	31	33	17	47	30	30
Corning C_3	20	12	19	25	15	44	32	20
Corning C_5	25	15	24	28	15	40	25	25
Films								
Kodak	33	24	36	36	20	49	29	35
Brinkmann	25	17	26	30	14	44	30	26
Sheet								
Gelman SA	65	53	63	69	46	78	32	64
	Solvent: Davidow's							
Pre-coated plates								
Brinkmann	43	20	46	47	45	76	52	46
Corning	56	37	62	69	69	89	52	66
Analtech	51	30	55	66	54	84	54	55
Self-coated plates								
Merck	55	32	58	59	57	84	52	58
Corning C_3	52	26	53	60	60	86	60	57
Corning C_5	61	33	65	66	66	92	59	66
Films								
Kodak	66	43	67	67	66	80	37	67
Brinkmann	50	20	58	67	63	94	74	63
Sheet								
Gelman SA	69	53	73	73	70	100	47	72

TABLE II

R_F × 100 VALUES OF BASIC DRUGS — DEVELOPMENT IN SATURATED TANKS

	Chlor-cyclizine	*Metha-phenyline*	*Quinine*	*Prop-oxyphene*	*Carbin-oxamine*	*Phenir-amine*
	Solvent: Davidow's					
Pre-coated plates						
Brinkmann	64	57	67	71	55	50
Corning	84	71	84	93	68	59
Self-coated plates						
Merck G	78	81	76	84	60	61
Corning C_3	57	100	83	100	89	87
Films						
Kodak	66	66	56	69	64	62
Brinkmann	86	85	70	91	69	68
Sheet						
Gelman SA	88	90	71	92	81	79
	Solvent: CBD					
Pre-coated plates						
Brinkmann	41	47	03	32	30	26
Corning	40	46	02	57	20	24
Analtech	67	51	03	63	25	42
Self-coated plates						
Merck	59	63	03	70	40	50
Corning C_3	56	60	02	63	37	36
Corning C_5	60	63	02	82	38	52
Films						
Kodak	61	62	16	66	52	57
Brinkmann	57	59	02	73	37	46
Sheet						
Gelman SA	74	76	10	81	57	70

TABLE III

R_F × 100 VALUES OF BASIC DRUGS ON VARIOUS ADSORBENTS — DEVELOPMENT IN SATURATED TANKS

Solvent: 95% ethanol.

	Antistine	*Cyclome-thycaine*	*Clemizole*	*Covatin*	*Chlorcy-clizine*	*Meclizine*	*Diatrine*	*Quinine*
Self-coated plates								
Merck G	14	19	61	35	31	72	10	22
Merck GF	11	14	58	25	21	65	15	20
Merck HF	13	15	57	27	22	68	15	26
Silicar 7G	32	25	58	46	32	74	40	26
Silicar 7GF	64	27	65	46	35	76	13	26
Silicar 4GF	63	34	64	64	61	78	58	18
Camag GF	11	10	46	26	17	58	18	33
Whatman G41	11	13	51	26	21	61	22	18
Anasil B	02	04	71	32	31	74	24	24
Anasil S	02	03	71	29	28	75	22	24
Adsorbosil-2	17	37	58	38	32	68	33	38
Sheet								
Gelman SA	13	18	75	43	43	80	15	31
Film								
Kodak	27	37	63	49	43	86	15	35

Clemizole	*Methamino-diazepoxide*	*Acetyl-phenazine*	*Carphenazine*	*Desmethyl-imipramine*	*Range*	*Median*
70	81	52	58	66	31	64
92	89	80	73	85	33	83
81	89	56	61	67	35	71
57	87	71	82	82	43	82
67	66	50	53	53	17	64
89	66	60	77	77	45	77
87	84	65	69	73	21	81
30	02	03	03	18	49	18
22	01	00	01	22	57	20
37	01	03	04	24	66	24
42	07	05	07	32	67	42
31	03	02	02	20	70	31
45	01	04	04	27	83	27
52	11	21	26	43	51	46
40	02	03	05	24	73	21
63	08	09	12	38	74	48

Pipradol	*Amol-anone*	*Azacy-clonone*	*Propoxy-phene*	*Levallor-phan*	*Benzocaine*	*Pyrathi-azine*	*Range*	*Median*
61	52	10	44	40	71	18	62	40
25	36	14	31	30	65	12	54	23
33	40	13	33	30	67	10	58	27
66	56	58	54	40	74	25	49	48
66	58	64	58	45	74	30	63	52
72	71	71	64	54	78	35	60	64
22	33	30	31	24	55	12	48	25
23	36	31	32	28	60	13	50	27
07	47	03	28	34	nd	05	72	26
nd	47	02	26	31	nd	08	73	25
60	48	55	48	53	66	29	51	48
79	57	08	49	44	77	22	72	44
49	57	23	54	44	52	27	71	43

Development

Tanks. "Saturated" means that two 4 × 20 cm blotter pads were placed at each end of the tank. Of the developing solution 100 ml were poured over the pads and into the bottom of the tank 1 h prior to developing the chromatogram. "Non-saturated" means that no blotter pads were present in the tank. The chromatogram was developed by placing it in the tank immediately after the solvent had been added.

Trough. The chromatogram was spotted and placed between the glass plates. The ground glass edges of the plates were coated with a thin film of petrolatum prior to use. The plates were then fastened together and the sandwich unit was inserted through the slit in the cover of the trough which had been filled with 100 ml of the developing solution.

Chamber. "Non-equilibrated" means that the chromatogram was developed immediately. With the chamber in an upright position, 25 ml of developing solvent were placed over the saturation pad in the center compartment of the chamber. Another 60 ml were placed in the bottom compartment. The spotted chromatogram was then placed in the chamber, the cover glass placed in position and the glass cover was sealed with Scotch tape. (Sheets and films were held in place by magnetic clips.) "Equilibrated" means that the chromatogram was placed in the chamber for 1 h prior to development of the chromatogram. During this period the chamber was horizontal, the developing solvent was in the bottom well and the pad was saturated with this solvent. The spotted chromatogram was placed in the chamber so that the solvent did not come into contact with the support. (Sheets and film were held in place by magnetic clips.) The chamber was closed with its glass cover and sealed with Scotch tape. After 1 h the chamber was placed in an upright position allowing the solvent to come into contact with the adsorbent. The chamber was secured in this upright position by the support rods of the unit.

RESULTS

The following tabulations summarize the results of many chromatographic determinations made to explore the effects of adsorbents, supports, developing chambers and operating conditions on the separation of acidic and basic drugs. Each datum represents the average of three replicate determinations. These were within ± 0.05 of the average. All R_F values whose difference was less than 0.05 were considered to be the same.

The "range" and "median" were determined for each chromatogram. "Range" refers to the difference in the R_F values between the lowest and highest spots. "Median" refers to the median R_F value. The "range" is used to assess the resolution of the system and the "median" the relative position of the band of substances on the chromatogram.

To evaluate the influence of adsorbents and supports on R_F values, the data presented in Tables I–III were accumulated.

Using different silica gel adsorbents, chromatographic data were obtained for non-basic drugs. These were developed in saturated tanks using chloroform–acetone or Davidow's solvent (Table I). In each of these developing solvents all the silica gel plates gave similar ranges and medians with the exception of the lower median obtained on one plate in Davidow's solvent. In chloroform–acetone, the ranges and

TABLE IV

$R_F \times$ 100 VALUES OF ACID AND NEUTRAL DRUGS — DEVELOPING UNITS AND CONDITIONS OF DEVELOPMENT

	Amobarbital	*Phenobarbital*	*Pentobarbital*	*Secobarbital*	*Diphenyl-hydantoin*	*Glutethimide*	*Range*	*Median*
	Solvent: chloroform–acetone							
Saturated tanks								
Merck G							30	30
Gelman SA	See Table I						32	64
Kodak film							29	35
Unsaturated tanks								
Merck G	74	62	79	84	52	89	37	77
Gelman SA	79	61	81	86	49	92	41	83
Kodak film	76	51	75	78	62	83	32	76
Gelman chamber — non-equilibrated								
Merck G	54	36	53	62	29	73	44	54
Gelman SA	62	51	61	66	44	74	30	62
Kodak film	50	38	49	51	33	62	29	49
Gelman chamber — equilibrated								
Merck G	37	28	38	44	25	58	33	38
Gelman SA	62	52	61	66	47	75	28	62
Kodak film	37	26	38	42	26	50	24	38
Kodak trough								
Kodak film	55	41	53	56	33	77	44	54
	Solvent: Davidow's							
Saturated tanks[a]								
Merck G							52	58
Gelman SA							47	70
Kodak film							37	67

[a] Data for other than saturated tanks are not recorded because they gave R_F values which precluded determining valid range and median values.

medians on the two films were comparable to those on the plates. In Davidow's system both films gave medians comparable to those on the plates but the Brinkmann film gave the best resolution of any of the adsorbents studied. In both systems the ranges on the Gelman SA sheets were comparable to those on the silica gel plates, but the medians were higher.

Some basic compounds were developed in saturated tanks using Davidow's and

TABLE V

$R_F \times 100$ VALUES OF BASIC DRUGS — DEVELOPING UNITS AND CONDITIONS OF DEVELOPMENT

	Chlor-cyclizine	*Metha-phenylene*	*Quinine*	*Prop-oxyphene*	*Carbin-oxamine*	*Phenir-amine*
	Solvent: Davidow's					
Saturated tanks						
Merck G						
Gelman SA	See Table II					
Kodak film						
Unsaturated tanks						
Merck G	93	94	67	97	84	87
Gelman SA	93	87	49	92	71	73
Kodak film	90	91	86	92	91	90
Gelman chamber — non-equilibrated						
Merck G	85	82	56	94	74	66
Gelman SA	87	85	66	93	77	75
Kodak Film	73	74	60	74	72	71
Gelman chamber — equilibrated						
Merck G	85	88	59	91	71	67
Gelman SA	76	77	58	84	69	66
Kodak film	72	73	59	75	75	63
Kodak trough						
Kodak film	40	42	53	97	64	65
	Solvent: CBD					
Saturated tanks						
Merck G						
Gelman SA	See Table II					
Kodak film						
Unsaturated tanks						
Merck G	77	70	02	90	39	54
Gelman SA	81	74	06	85	53	65
Kodak film	80	84	04	91	56	70
Gelman chamber — non-equilibrated						
Merck G	54	56	06	80	38	49
Gelman SA	67	61	05	72	45	56
Kodak film	61	61	09	61	52	57
Gelman chamber — equilibrated						
Merck G	43	44	03	61	21	31
Gelman SA	58	54	04	63	41	49
Kodak film	54	55	08	51	40	46
Kodak trough						
Kodak film	87	88	13	88	61	67

CBD solvents (Table II). For plates developed in Davidow's solvent the best resolution was obtained on the Corning C_3 adsorbent. In the CBD solvent, the Corning C_5 was best. In both solvents the Brinkmann plate gave the lowest medians. The ranges and the medians on the Brinkmann film in both solvents were higher than those on the Brinkmann plate. In both solvents the Kodak film had the smallest range and in CBD it had the highest median. The range and median on the Gelman SA sheet in

Clemizole	*Methamino-diazepoxide*	*Acetyl-phenazine*	*Carphenazine*	*Desmethyl-imipramine*	*Range*	*Median*
					35	71
					21	81
					19	75
97	79	51	56	62	46	87
84	82	46	54	59	47	82
90	89	77	81	84	15	90
90	92	47	56	57	47	81
92	86	61	68	68	32	82
76	66	57	61	63	21	72
85	86	49	55	54	42	71
80	80	57	62	62	27	76
69	77	55	56	67	22	72
93	96	54	60	57	44	65
					67	42
					74	48
					51	46
52	62	05	04	28	88	52
65	64	08	11	44	75	65
59	78	04	11	37	86	62
45	—	07	08	29	74	42
51	60	07	09	32	67	56
48	59	16	18	34	52	52
40	35	03	05	15	58	26
47	—	08	09	34	54	47
40	43	10	13	26	47	45
60	72	18	22	46	75	61

Davidow's system were about the same as those of the Kodak film. In CBD the range on the sheet was similar to that on the plates but the median was higher than those on any plate or film.

Other basic drugs were developed in saturated tanks using 95% ethanol (Table III). In this solvent the best resolution was obtained on the Anasil B and S plates and the Gelman SA sheet. The median was highest on Silicar 4GF but varied considerably on all the other adsorbents.

Using plates, films, and sheets, the influence of varying developing conditions on R_F values was investigated (Tables IV–VI). To facilitate the evaluation of these data they were collated and summarized in Table VII.

For each developing solvent, the ranges and medians on plates, sheets and films were usually higher in unsaturated tanks than in saturated tanks. In chambers after equilibration development of plates with the CBD, Davidow or chloroform–acetone system gave R_F values which were lower than those obtained following immediate development. In these three solvents, sheets and films gave the same R_F values when they were developed either immediately or after 1 h of equilibration. This same phenomenon pertained to all chromatograms developed in 95% ethanol, whatever the supporting material was.

In tanks, saturated or unsaturated, the ranges on sheets and plates were generally comparable to and higher than those on the films. The highest median values were usually obtained on the sheets. In chambers, the ranges on sheets and films were less than those obtained on plates. Those on the film were generally slightly lower than

TABLE VI

$R_F \times 100$ VALUES OF OTHER BASIC DRUGS — DEVELOPING UNITS AND CONDITIONS OF DEVELOPMENT
Solvent: 95% ethanol.

	Antistine	*Cyclomethycaine*	*Clemizole*	*Covatin*	*Chlorcyclizine*	*Meclizine*	*Diatrine*	*Quinine*
Saturated tank								
Merck G Gelman SA Kodak film	See Table I							
Unsaturated tank								
Merck G	13	29	66	43	31	73	21	31
Gelman SA	13	17	74	42	42	81	13	28
Kodak film	32	37	71	54	41	83	15	28
Gelman chamber — non-equilibrated								
Merck G	12	22	65	33	29	75	24	25
Gelman SA	13	24	73	40	35	81	24	29
Kodak film	26	49	68	58	52	70	50	43
Gelman chamber — equilibrated								
Merck G	13	21	65	34	25	72	09	23
Gelman SA	08	22	74	43	48	80	08	30
Kodak film	30	44	62	52	46	65	45	39
Kodak trough								
Kodak film	39	64	97	79	73	95	71	62

those on the sheet. Median values were generally highest on the SA sheet. With the exception of Davidow's system, ranges on the film developed in the trough were usually comparable to those on plates and sheets developed in unsaturated tanks and, therefore, higher than those obtained in chambers, equilibrated or non-equilibrated. However, the medians on the films were higher. Following development of Kodak film in the trough, resolution was comparable to that obtained on plates or sheets in saturated tanks.

Whichever adsorbent and support was used, it took longer to develop the chromatogram in unsaturated tanks and in a sandwich cell than in the saturated tank or in a chamber[1]. The time required for a 10-cm development in each of the solvent systems used is given in Table VIII.

DISCUSSION OF RESULTS

Available silica gel adsorbents applied on glass plates, plastic films or glass fibers yield reliable chromatographic data regardless of the developing unit used. In a given laboratory with controlled humidity and temperature the R_F value can be reproduced[2] to within ± 0.05. No one adsorbent was optimal for all applications and no generalization as to selection for a particular application seems appropriate.

In saturated tanks, all the plates gave comparable ranges and medians. Supporting a given adsorbent on a film rather than on a plate resulted in comparable ranges but higher medians. The highest medians on all supports were those obtained

Pipradol	*Amol-anone*	*Azacy-clonone*	*Propoxy-phene*	*Levallor-phan*	*Benzocaine*	*Pyrathi-azine*	*Range*	*Median*
							62	40
							72	44
62	54	19	46	31	75	21	62	43
75	57	08	46	41	76	23	73	42
14	61	23	52	43	29	57	68	41
60	56	10	42	41	73	16	65	33
41	54	13	49	44	81	22	68	41
55	63	22	51	43	67	60	48	51
59	54	14	39	41	70	17	63	34
40	58	07	53	43	81	23	74	43
50	59	26	53	33	63	53	45	53
76	92	33	85	61	98	85	65	76

TABLE VII

DEVELOPING UNITS AND CONDITIONS OF DEVELOPMENT — SUMMARY OF DATA IN TABLES IV–VI

R = range; M = median.

	Tank				*Chamber*				*Trough*	
	Unsaturated		*Saturated*		*Non-equilibrated*		*Equilibrated*			
	R	*M*	*R*	*M*	*R*	*M*	*R*	*M*	*R*	*M*
Acids developed in chloroform–acetone										
Plate	37	77	30	30	44	54	33	38	—	—
Film	32	76	29	35	29	49	24	38	44	54
Sheet	41	83	32	64	30	62	28	62	—	—
Acids developed in Davidow's system										
Plate	—	—	52	58	—	—	—	—	—	—
Film	—	—	37	67	—	—	—	—	—	—
Sheet	—	—	47	72	—	—	—	—	—	—
Bases developed in Davidow's system										
Plate	46	87	35	71	47	81	42	71	—	—
Film	15	90	17	64	21	72	22	72	44	65
Sheet	47	82	21	81	32	82	27	76	—	—
Bases developed in CBD										
Plate	88	52	67	42	74	42	58	26	—	—
Film	86	62	51	46	52	52	47	45	75	61
Sheet	75	65	74	48	67	51	59	47	—	—
Bases developed in 95% ethanol										
Plate	62	43	62	40	65	33	63	34	—	—
Film	68	41	71	43	48	51	45	53	65	76
Sheet	73	42	72	44	68	41	74	43	—	—

TABLE VIII

TIME FOR 10-CM DEVELOPMENT

	Solvent system			
	Chloroform–acetone (9 : 1)	*CBD*	*Davidow's*	*95% ethanol*
Plate				
Unsaturated tank	33	43	35	63
Saturated tank	19	37	26	48
Sheet				
Unsaturated tank	46	55	43	106
Saturated tank	25	27	30	75
Film				
Unsaturated tank	50	55	65	108
Saturated tank	35	35	48	102
Plate				
Non-equilibrated chamber	34	28	35	60
Equilibrated chamber	25	28	23	42
Sheet				
Non-equilibrated chamber	33	32	30	80
Equilibrated chamber	30	30	26	70
Film				
Non-equilibrated chamber	48	48	47	110
Equilibrated chamber	41	45	40	104
Film				
Trough	55	50	50	103

on the sheets. The ranges on the sheets were equivalent to those on plates and films.

For a particular application, one adsorbent may be selected because its range or median is desirable. If extracts of normal biological samples are known to contain "artifacts" whose R_F values are very low and very high, a suitable system can be selected whose median is between these values. This selection would simplify the interpretation of the results because the spots due to the artifacts would not be in the same region as those due to any drugs.

The three developing units gave satisfactory data. Other reports also indicate that the development of drugs in unsaturated tanks gives a higher median and greater resolution than does the development in saturated tanks[3-5]. For plates, films, and sheets, development of drugs in a chamber generally results in lower medians and ranges than those obtained in unsaturated tanks. In the chambers, the lower ranges and medians obtained following immediate development indicate that the adsorbent is more saturated under these conditions than it is in an unsaturated tank, whereas after 1 h of equilibration in the chamber results are comparable to those obtained in a saturated tank.

Although originally designed for sheets, the sealed Gelman chamber was found to be satisfactory for developing plates and films. In this chamber the SA sheet and Kodak film are apparently rapidly saturated because equilibrating the units in the chamber for 1 h gave R_F data identical to those obtained when the chromatograms were developed immediately.

ACKNOWLEDGEMENTS

The authors thank HALLE LANDESMAN for her technical assistance. This work was partially supported by United States Public Health Service Grants GM-09863-07 and GM-01784-02.

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