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THIN-LAYER CHROMATOGRAPHY ON SILICA GEL-SINTERED PLATES

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SUMMARY

A new pre-coated plate for thin-layer chromatography, made from silica gel and fused glass powder mixtures, has been prepared. This sintered thin-layer plate contains no organic binders and is mechanically stable, heat-stable and acid-resistant, and can be used repeatedly by soaking the chromatogram in cleaning solutions with no decrease in its chromatographic activity.

INTRODUCTION

Thin-layer chromatography (TLC) is a very simple and convenient separation technique. It permits rapid development and spraying with corrosive reagents, and various kinds of pre-coated plates and sheets are commercially available[1,2]. These plates, however, become charred when sprayed with corrosive reagents and heated above 130°, owing to the presence of organic binders such as starch, polyvinyl alcohol and polyolefines, which are used to give good abrasion-resistance to the carriers.

We have now devised an acid-resistant pre-coated plate, namely, a silica gel–sintered glass plate. The adsorbent layer of this plate consists of silica gel for TLC and finely powdered sintered glass as a binding agent. It is highly porous and allows the developing solvents to penetrate quickly. This sintered plate can be used in the same way as the usual thin-layer plates. It appears from our experiences that the nature of the binder for the silica gel particles does not essentially affect the adsorption process that occurs on the surface of the silica gel. The new plate contains no organic binders and is not charred under any drastic conditions, *i.e.*, by heating after spraying corrosive reagents such as concentrated sulphuric acid, chromic acid mixtures, trichloroacetic acid and antimony trichloride. The developed sintered plate can be regenerated readily by soaking it in cleaning solutions, washing it with water and reactivating it. Moreover, the use of a sintered glass plate combines the ease of handling paper chromatography and the sharpness of separation (of spots) bij the usual TLC procedures. Thus, impregnation by soaking with buffer solutions such as sodium acetate, boric acid and sodium bisulphite permits the separation of sugars, and soaking with lipophilic reagents such as liquid paraffin, undecane, tetradecane and silicone oil enables reversed-phase partition chromatography to be carried out.

EXPERIMENTAL

Materials and apparatus

Glass powder. A broken soda-lime glass plate was ground in a ball mill, screened with a 200-mesh sieve and fractionated by sedimentation in water. The glass powder thus obtained had a particle size slightly smaller than that of silica gel for TLC.

Preparation of silica gel sintered plates. A mixture of one part of silica gel for TLC and two to five parts of the glass powder prepared as above was suspended in a solvent such as benzene, chloroform, acetone, ethyl acetate, methanol, ethanol or water. The slurry was spread on soda-lime glass plates in the usual manner and air-dried. The layer was then heated in an electric furnace at 450–750° for several minutes to yield a silica gel–fused glass layer (layer thickness 200 μm). It is essential to fuse the glass powder without melting the silica gel, so as to protect the chromatographic activity of the layer.

Development

All chromatographic experiments, whether on plates or on sheets, were carried out in a cylindrical or rectangular chromatographic chamber with a saturated atmosphere. For repeated use, the sprayed sintered plates were soaked in cleaning solutions such as chromic acid mixture, concentrated nitric acid or organic solvents, washed with running water and reactivated by heating them at 110° for 30–60 min.

It is conventional also to heat the chromatographic plates at 400–450° for 20–30 min in an electric furnace during recovery and reactivation in order to burn off the combustible compounds such as visible dyes and fluorescent or UV-absorbing substances.

Detection

The spots on the developed chromatogram on the sintered plate can be made visible by most of the techniques used in conventional TLC, especially by spraying the sintered plate with concentrated sulphuric acid, followed by heating at above 130° for general detection.

Dragendorff reagent[4] and ninhydrin were used for making the spots of alkaloids and amino acids visible. Alkaloids and water-soluble vitamins were detected by UV-irradiation in the presence of mixed fluorescent materials[5] with an improved light source, giving coloured quenching spots.

RESULTS AND DISCUSSION

Solvent travel rate

Initial comparisons of the rates of migration of various solvents through the thin-layer adsorbent were investigated. In these experiments, conventional thin-layer pre-coated plates and sheets of silica gel were used. These were reactivated prior to use. Table I shows the migration times of solvents of different polarities. It can be seen that as the polarity of the solvent decreases or increases, the solvent travel rate decreases. The rates on the sintered plate are slightly faster than those on the other plate and the sheet. This may be attributed to the unique capillary formation in the sintered glass plate.

TABLE I

SOLVENT TRAVEL RATE ON PRE-COATED SILICA GEL PLATES

Mean values of five runs; time in minutes required to travel 12 cm at room temperature (25°).

Solvent	*Sintered glass plate (200 μm)*	*Merck glass plate (250 μm)*	*Eastman chromagram sheet (100 μm)*
Cyclohexane	24	42	35
Carbon tetrachloride	23	42	35
Benzene	19	27	21
Chloroform	18	26	23
Diethyl ether	14	17	15
Ethyl acetate	16	18	17
Acetone	12	16	14
Methanol	18	39	30

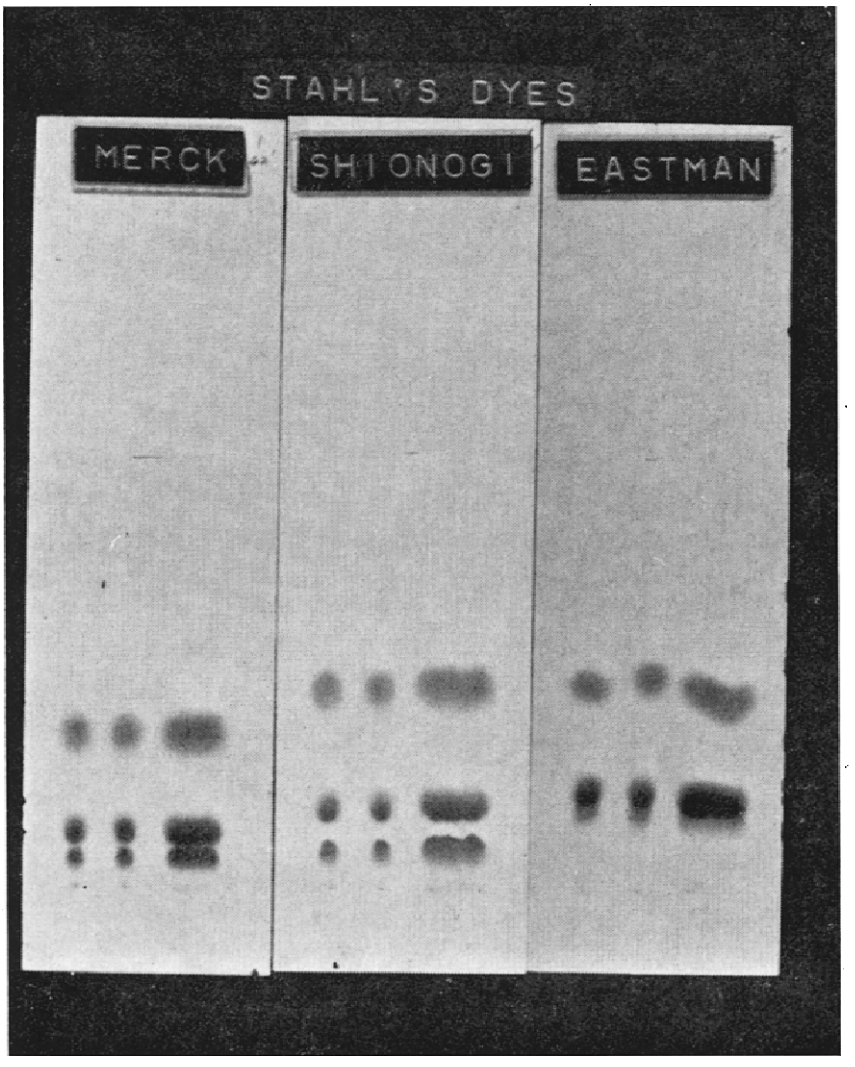

Fig. 1. Separation of Stahl's dyes on glass bases and polyester base. Left to right: Merck pre-coated glass plate, sintered plate, and Eastman chromagram sheet. Solvent: benzene. Dyes separated in order of decreasing hR_F values: Butter Yellow, Sudan Red G and Indophenol.

TABLE II

hR_F VALUES OF VARIOUS CLASSES OF STEROIDS ON SILICA GEL–SINTERED PLATES DEVELOPED WITH VARIOUS SOLVENTS

Detection with concentrated sulphuric acid (heated at over 130° for several minutes). Solvents: I = chloroform–acetone (4:1); II = chloroform–acetone (9:1); III = benzene–chloroform–acetone (7:2:1); IV = chloroform–ethyl acetate (4:1); V = benzene–ethyl acetate (2:1); VI = benzene–methanol (9:1); VII = benzene–methanol (5:1) VIII = benzene-methanol (10:1); IX = chloroform–acetic acid (9:1); X = chloroform–methanol (9:1); XI = chloroform–methanol (4:1); XII = chloroform–acetone (3:2); XIII = ethyl acetate; XIV = ethyl acetate–acetone (3:1). The values shown are means from five different plates. The standard deviation of each hR_F value was less than 3. The silica gel on the sintered plate was Merck Silica Gel H.

Compound	*hR_F values of steroids*											
	Sintered plate						*Merck glass plate*					
Estrogens	*I*	*II*	*III*	*IV*	*V*	*VI*	*I*	*II*	*III*	*IV*	*V*	*VI*
Estriol	18	10	1	9	9	22	7	3	0	3	2	3
Estradiol	61	54	19	52	52	44	38	26	10	26	27	17
Estrone	72	67	34	66	65	61	51	42	20	43	42	33
Androgens	*II*	*IV*	*VII*				*II*	*IV*	*VII*			
Testosterone	48	41	60				28	22	34			
Adrenosterone	50	42	61				32	27	38			
17α-Methyl-testosterone	52	42	67				33	24	47			
Dehydroisoandrosterone	54	50	63				33	30	39			
Corticoids	*I*	*VIII*	*VII*	*IX*			*I*	*VIII*	*VII*	*IX*		
Hydrocortisone	14	10	51	13			10	13	34	8		
Cortisone	26	17	55	24			19	16	37	17		
Desoxycortisone	41	31	63	42			35	22	44	28		
	X	*XI*	*VII*	*XII*			*X*	*XI*	*VII*	*XII*		
Predonisolone	37	64	27	45			25	50	20	24		
Betamethasone	42	67	26	55			28	51	21	31		
Dexamethasone	45	67	27	56			30	51	23	31		
Predonisone	54	71	31	55			36	56	25	33		
Progestins	*I*	*III*	*IX*				*I*	*III*	*IX*			
11α-Hydroxy-progesterone	42	5	37				21	3	31			
17α-Hydroxy-progesterone	67	18	64				41	9	51			
Progesterone	76	37	77				54	19	64			
Cardiac glycosides	*XIII*	*X*	*I*				*XIII*	*X*	*I*			
Gitoxin	18	31	2				4	27	0			
Digoxin	23	28	4				8	27	1			
Digitoxin	34	42	8				14	34	2			
Strospeside	31	38	14				12	29	5			
Cardiac genins	*XIV*	*II*	*VI*				*XIV*	*II*	*VI*			
Gitoxigenin	50	13	25				38	2	12			
Digoxigenin	53	19	14				39	3	7			
Digitoxigenin	67	48	40				49	14	22			

Separation of various organic compounds on silica gel–sintered plates

Separation of Stahl's test dyes. Fig. 1 shows a separation of a test dye mixture containing Butter Yellow, Sudan Red G and indophenol on three pre-coated materials. The hR_F values obtained for Stahl's test dyes on the sintered plate were slightly higher than those on the Merck glass plates. This indicates that the sintered plate is essentially different from the other two thin-layer materials in the layer components when used either with or without activation. This is attributed to the weaker chromatographic acitivity of the sintered plate.

Other examples which show that the sintered plates are as efficient as conventional glass plates involve the separation of steroids, akaloids, water-soluble vitamins, amino acids and sugars.

Separation of steroids. The following compounds were studied: estrogens, androgens, progestins, corticoids and cardiac glycosides and genins. Table II shows the separation of these compounds on a silica gel–sintered plate.

The results show that the hR_F value of each compound on the sintered plate is slightly higher than that on the Merck glass plate. The same tendency was also observed with other classes of compounds. This may be attributed to the weaker chromatographic activity of the sintered plate, as in the case of Stahl's test dyes.

Separation of alkaloids. A number of alkaloids were separated on the sintered plates and on home-made glass plates coated to a wet thickness of 300 μm. Both plates contained 10 % of a mixed fluorescent substance[5] in silica gel. Table III indicates hR_F values of the alkaloids. In the separation of alkaloids, the hR_F value of each compound was similar on the two types of thin-layer plate.

Separation of water-soluble vitamins. Various vitamins of the B group and vitamin C were separated with acetone–water on silica gel plates containing 10 % of the

TABLE III

hR_F VALUES OF VARIOUS ALKALOIDS ON SILICA GEL–SINTERED PLATES

Detection with Dragendorff reagent. Solvent: chloroform–diethylamine (9:1). Results are mean values from five different plates. The standard deviation of each hR_F value was less than 3.

Compound	*hR_F values of alkaloids*		*Fluorescent colour[b] observed*
	Sintered plate[a]	*Home-made plate[b]*	
Codeine	55	55	Reddish violet
Thebaine	79	65	Bright blue
Atropine	56	42	Violet
Yohimbine	60	71	Blue
Reserpine	84	55	Reddish violet
Ergotamine	84	67	Blue
Brucine	46	40	Blue
Strychnine	61	55	Violet
Quinine	30	16	Reddish violet
Cinchonine	45	35	Blue
Aconitine	83	64	Reddish violet
Emetine	84	67	Violet
Benzocaine	84	80	Violet
Caffeine	80	77	Red

[a] Merck Silica Gel H.
[b] Merck Silica Gel G.

mixed fluorescent substance. For detection, the developed and dried chromatograms were inspected under an improved light source[5]. Water-soluble vitamins can be recognized as coloured quenching spots against a white fluorescent background. Table IV shows hR_F values of the vitamins on the sintered plates and the home-made plates. The hR_F values of the vitamins on the sintered plates were slightly higher than those on the home-made plates.

Separation of amino acids. Because of the polar character of amino acids, the use of polar solvent systems was necessary. Usually the developing solvent contains water, so in this case we are dealing with partition chromatography rather than absorption chromatography. Amino acids were separated by developing with an *n*-pro-

TABLE IV

hR_F VALUES OF WATER-SOLUBLE VITAMINS ON SILICA GEL–SINTERED PLATES

Solvent: acetone–water (9:1). Results are mean values from five different plates.

Compound	*hR_F values of the vitamins*		*Fluorescent colour[b] observed*
	Sintered plate[a]	*Home-made plate[b]*	
Thiamine	20	4	Violet
Cyanocobalamine	1	1	Red
Riboflavine	47	48	Yellow
Nicotinamide	72	59	Reddish violet
Pyridoxine	67	39	Violet
Nicotinic acid	19	11	Reddish violet
Ascorbic acid	31	7	Violet

[a] Merck Silica Gel H + 10% mixed fluorescent substance.
[b] Merck Silica Gel G + 10% mixed fluorescent substance.

TABLE V

hR_F VALUES OF AMINO ACIDS ON SILICA GEL–SINTERED PLATES

Detection with 0.3% ninhydrin–methanol solution. Solvent: *n*-propanol–14% ammonia solution (4:1). Results are mean values from five different plates. The standard deviation in each hR_F value was less than 3.

Compound	*hR_F values of amino acids*	
	Sintered plate[a]	*Merck glass plate[b]*
Arginine	27	13
Lysine	30	14
Ornithine	31	14
Glutamine	45	30
Alanine	47	27
Cysteine	55	32
Serine	55	34
Glycine	56	34
Valine	70	57
Methionine	71	53
Leucine	73	60
Isoleucine	73	57

[a] Developing rate 60 min per 10 cm.
[b] Developing rate 180 min per 10 cm.

panol–ammonia mixture on silica gel-sintered plates and Merck glass plates. Table V indicates the hR_F values of free amino acids on the sintered and pre-coated glass plates.

Fig. 2 shows chromatograms of amino acids on the two types of thin-layer sup-

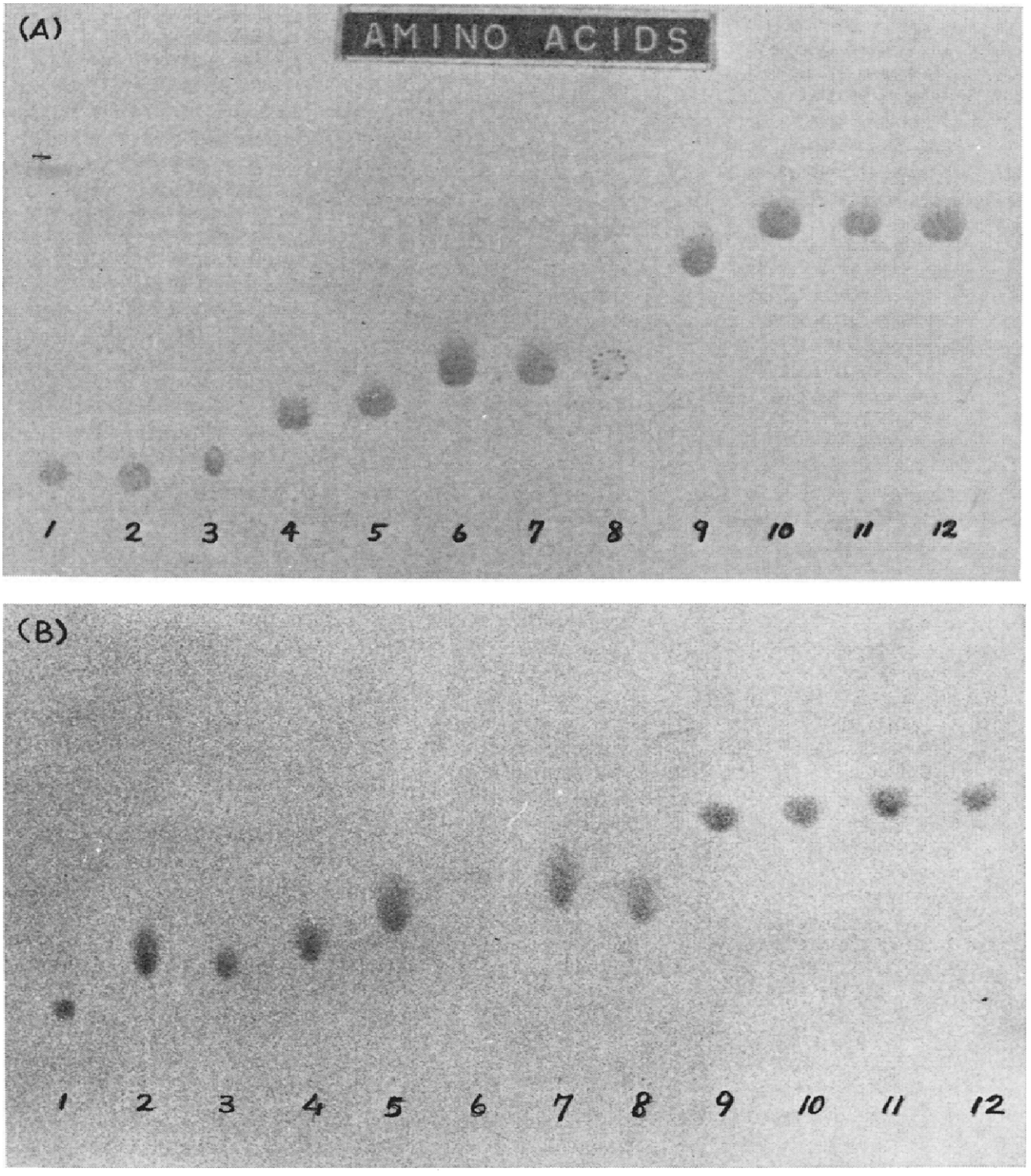

Fig. 2. Separation of amino acids on the two types of thin-layer support. (A) Silica gel–sintered plate. (B) Merck silica gel glass plate. Amino acids: 1 = arginine, 2 = lysine, 3 = ornithine, 4 = glutamine, 5 = alanine, 6 = cysteine, 7 = serine, 8 = glycine, 9 = valine, 10 = methionine, 11 = leucine, 12 = isoleucine.

ports. In this solvent system, the hR_F values for amino acids were not the same on the two supports. However, the separations obtained were very similar. In any event, the examples serve to illustrate the usefulness of this material for separation of various classes of organic compounds.

Separation of sugars. Owing to their high polarity, sugars have a high solubility in water and a low solubility in less polar organic solvents. These characteristics led us to select polar solvent systems for TLC and to include water in the solvent. Water in the solvent saturates the stationary phase, so in this instance also we are generally dealing with partition chromatography. For the separation of sugars, many methods have been investigated. We separated sugars on layers of silica gel impregnated with 0.1 *M* sodium bisulphite solution. The sodium bisulphite-impregnated home-made plates were prepared according to the following procedure. A slurry of 20 g of "Kieselgel G nach Stahl" in 40 ml of 0.1 *M* sodium bisulphite solution was spread in the usual manner on the glass plates to give a thickness of about 250 μm. These plates were allowed to stand for 30 min at room temperature, and then dried in an oven at 110–120° by ADACHI's procedure[6]. On the other hand, the silica gel–sintered plates were soaked in 0.1 *M* sodium bisulphite solution for 30 min and then dried in an oven at 110° for 1 h. Because of the mechanical stability of the sintered plate, the impregnation by soaking was carried out conveniently without damage in the thin-layer. Table VI lists hR_F values of sugars on the two types of thin-layer plate.

In the separation of sugars, the hR_F values of each sugar were similar with the two types of plate.

TABLE VI

hR_F VALUES OF VARIOUS SUGARS ON SILICA GEL–SINTERED PLATES

Detection with *o*-aminodiphenyl–orthophosphoric acid reagent or concentrated sulphuric acid; the latter is recommended. Solvent: *n*-propanol–water (17:3). Results are mean values from five different plates. The standard deviation of each hR_F value was less than 2.

Compound	*hR_F values of sugars*	
	Sintered plate[a]	*Home-made plate*[b]
Diginose	0	0
Raffinose	17	22
Lactose	28	29
Maltose	41	38
Galactose	46	40
Glucose	53	47
Mannose	57	50
Xylose	62	57
Digitoxose	65	64
Rhamnose	67	62
Digitalose	68	53

[a] Merck Silica Gel H. Developing rate 120 min per 13 cm.
[b] Merck Silica Gel G. Developing rate 180 min per 13 cm.

Reproducibility of separation on silica gel–sintered plates

There are many factors that control the reproducibility of R_F values in TLC[7–10], including the nature of the adsorbent, the thickness and activity of the plate, the quality of the solvent and the saturation of the solvent in the chromatographic

TABLE VII

REPRODUCIBILITY OF hR_F VALUES ON SILICA GEL–SINTERED PLATES

Silica gel–sintered glass powder	*hR_F values[a] of estrogens*			*n*[b]	*Detection*	*Cleaning solution*
	Estriol	*Estradiol*	*Estrone*			
1:2	8 ± 1	51 ± 2	65 ± 2	50	Sulphuric acid	Chromic acid mixture
1:3	10 ± 1	55 ± 2	70 ± 3	50	Sulphuric acid	Chromic acid mixture
1:4	11 ± 1	59 ± 2	73 ± 1	50	Sulphuric acid	Chromic acid mixture
1:4	8 ± 0	54 ± 2	67 ± 1	10	Ceric sulphate	Chromic acid mixture
1:4	8 ± 1	54 ± 1	68 ± 1	10	Sulphuric acid	Conc. nitric acid
Home-made[c]	4 ± 1	22 ± 3	40 ± 3	5	Sulphuric acid	None
Silica gel, fast-running[d]	8 ± 0	54 ± 3	67 ± 3	5	Sulphuric acid	None
	hR_F values[e] of other steroids					
	Cortisone	*Testosterone*	*Progesterone*			
1:3	36 ± 4	61 ± 2	76 ± 3	50	Sulphuric acid	Chromic acid mixture
Home-made[c]	20 ± 4	48 ± 4	68 ± 3	5	Sulphuric acid	None

[a] Benzene–ethyl acetate (2:1).
[b] n = Number of runs on the same plate in the case of the sintered plate. In the case of home-made or silica gel fast-running plates, five different plates were used.
[c] Merck Silica Gel H or HF.
[d] Merck aluminium sheet, silica gel–Kieselguhr.
[e] Chloroform–acetone (4:1).

chamber. It can be expected that reproducible R_F values will be obtained by eliminating those factors which affect them, such as the nature of the adsorbent and the activity of the layer. Our sintered plate can be used repeatedly as described above. Table VII gives the reproducibility of hR_F values of steroids on silica gel-sintered plates that were used repeatedly. In general, the standard deviation in R_F values can be controlled to within less than 0.05 when sufficient care is taken of the factors that affect the reproducibility of separation. With our sintered plate, the variation of the standard deviation was found to be less than 0.04. Table VII also shows hR_F values on home-made and other pre-coated silica gel plates for comparison with those on the sintered plate.

Comparison of separation behaviour on silica gel–sintered plates with behaviour on other plates

Table VIII gives a comparison of the separation characteristics of estrogens on the silica gel-sintered plate with those on other home-made and pre-coated plates. The results given in Table VIII indicate that the hR_F value of each estrogen on the sintered plate is greater than that on the other two types of plate, that the difference in hR_F values (ΔhR_F) between estradiol and estriol on the sintered plate is greater than those on the other two plates, and that ΔhR_F between estrone and estradiol on the sintered plate are less than those on the other two plates. The separation characteristic of our plate was similar to that of a mixed layer of silica gel and Kieselguhr.

TABLE VIII

hR_F VALUES OF ESTROGENS ON VARIOUS SILICA GEL PLATES

Detection with concentrated sulphuric acid. Solvent: benzene–ethyl acetate (2:1).

Silica gel	*hR_F values*[a] *of estrogens*						*Developing rate (min per 10 cm)*
	Estriol		*Estradiol*		*Estrone*		
Silica gel							
Home-made	4 ± 1	Δ18	22 ± 3	Δ18	40 ± 3		17
Pre-coated[b]	4 ± 1	Δ32	36 ± 2	Δ15	51 ± 1		20
Silica gel–Kieselguhr (1:1)							
Home-made	8 ± 2	Δ44	52 ± 2	Δ15	67 ± 2		20
Pre-coated[c]	11 ± 2	Δ51	62 ± 2	Δ13	75 ± 3		12
Silica gel–glass powder (1:4)							
Home-made	4 ± 1	Δ51	55 ± 1	Δ16	71 ± 2		20
Sintered	11 ± 1	Δ47	58 ± 1	Δ15	73 ± 1		15

[a] Results are mean values from five different plates; Δ = differences in *hR_F* values.
[b] Merck pre-coated TLC plate, silica gel.
[c] Merck aluminium sheet, silica gel–Kieselguhr.

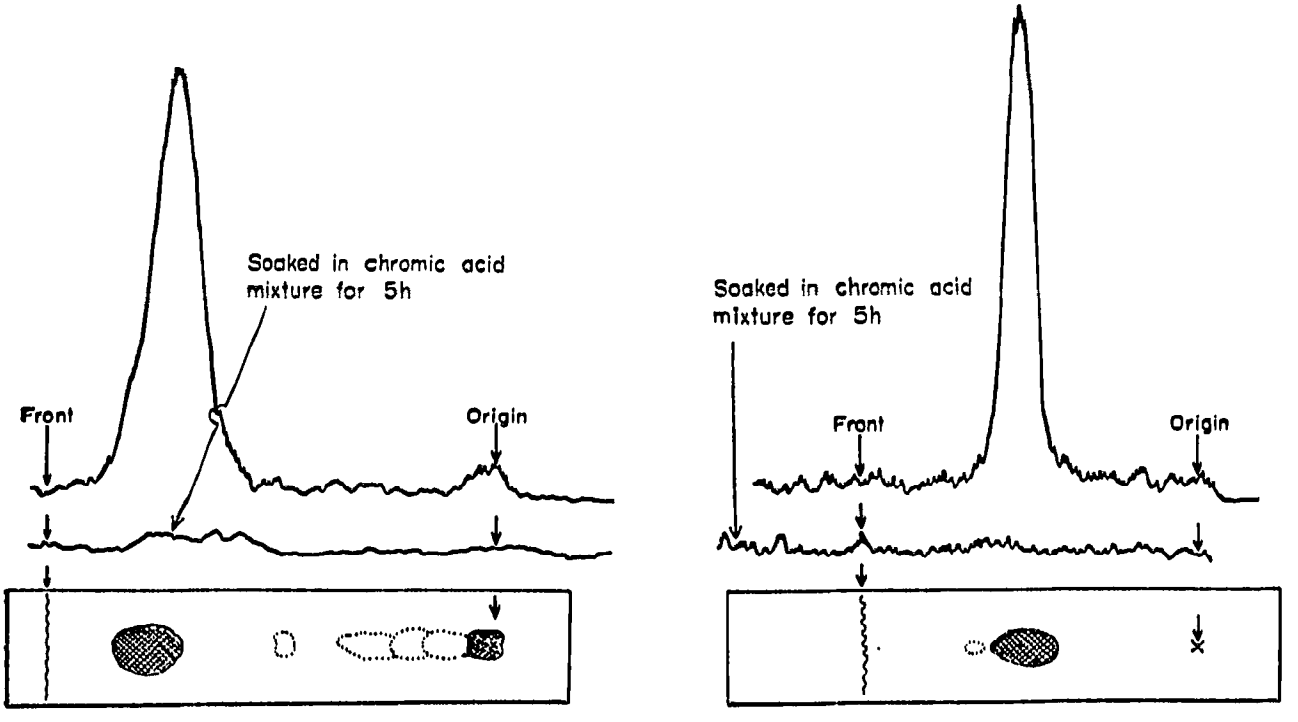

Fig. 3. Autoradiograph of ^{14}C-labelled steroidal mixtures on silica gel–sintered plate.

Fig. 4. Autoradiograph of ^{3}H-labelled steroid on silica gel–sintered plate.

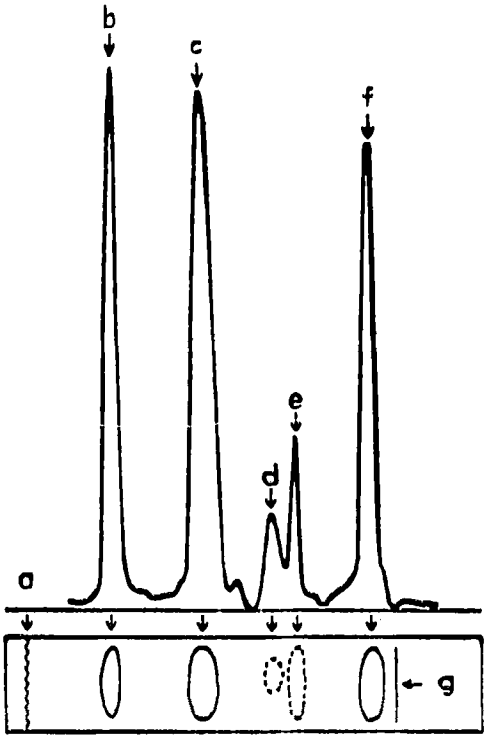

Fig. 5. Thin-layer densitometry of lipids with silica gel–sintered plate. (a) Front; (b) cholesteryl ester; (c) triglyceride; (d) diglyceride; (e) monoglyceride; (f) cholesterol; (g) origin.

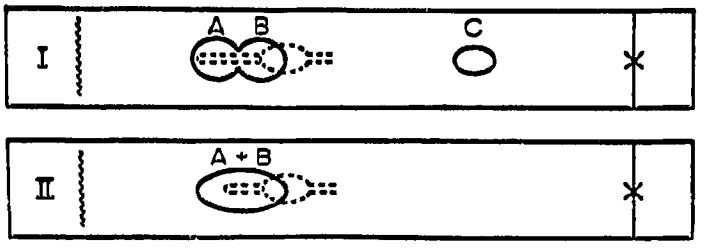

Fig. 6. Bioautography test with silica gel–sintered and home-made plates for antibacterial screening of antibiotics against *Bacillus subtilis* PCI 219. Plate I: sintered plate F. Plate II: home-made plate GF. As shown, direct contact of silica gel–sintered plate with test medium permits the separation of spots A and B, and reveals the presence of another spot, C.

Use of sintered plates for various analytical purposes

The characteristics of this sintered plate are mechanical stability (good abrasion resistance), heat stability, acid resistance and suitability for repeated use. These properties permit, besides its general use, the use of the plate for the separation of radioactive compounds (*e.g.*, autoradiography of ^{14}C- or ^{3}H-labelled steroids, Figs. 3 and 4), thin-layer densitometry (*e.g.*, semi-quantitative determination of serum lipids[11], Fig. 5), clinical analysis (lipids, sugars, amino acids and steroids, etc.), reversed-phase TLC (*e.g.*, separation of polychlorinated biphenyls), bioautography (*e.g.*, Fig. 6), thin-layer electrophoresis (TLE) and, probably, for various other purposes in the vast field of chromatographic analysis.

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