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SINTERED THIN-LAYER CHROMATOGRAPHY*

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CONTENTS

1. INTRODUCTION

During the past 15 years, thin-layer chromatography (TLC) has established itself as one of the most powerful, exact and useful tools for experimental chemists. The techniques involved are relatively simple and applicable to the separation of both volatile and non-volatile substances, and the equipment required is inexpensive. TLC

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offers, in many instances, almost the only practical solution to difficult separation and purification problems.

Recently, a number of authors have reviewed the newer developments in TLC[1–5], including the combination of TLC with other techniques, for example vapour-phase chromatography.

Various kinds of pre-coated plates and sheets are now commercially available[6,7]. However, these plates become charred when sprayed with corrosive reagents or heated at high temperatures, owing to the presence of organic binders[8] such as starch, poly(vinyl alcohol), polymethacrylates and polyolefins, which are added to the thin layer to confer good abrasion resistance.

The author has devised sintered-glass TLC plates, which are acid-resistant pre-coated thin-layer plates containing inorganic adsorbents such as silica gel, alumina, Kieselguhr, Florisil, titania and magnesia, fixed with sintered soda-lime or borosilicate glass. These sintered-glass plates are highly porous and allow the developing solvents to penetrate quickly, showing that the nature of the binder does not affect the adsorption process that occurs on the surface of the adsorbents. They contain no organic binders and do not become charred under drastic conditions, such as heating at high temperatures after spraying with corrosive reagents, *e.g.*, concentraded sulphuric acid, chromic acid mixtures, trichloroacetic acid and antimony trichloride. The sprayed plates can be regenerated readily by soaking in cleaning solutions, washing with water and reactivation by heating. The author subsequently prepared polyolefin sintered sheets using, in addition to the inorganic adsorbents mentioned above, organic ion-exchange resins, cellulose ion exchangers, synthetic porous polymers, dextran gels and cellulose as the adsorbent, and microparticulate polyolefin as the binding agent. Further, in an attempt to study quantitative thin-layer stick chromatography aided by flame-ionization detection scanning, the author prepared various kinds of sintered-glass rods using silica gel and alumina as the adsorbent and soda-lime, borosilicate and ceramic glass powder as the binder. Thin-layer chromatography performed using these sintered plates, sheets and rods is termed here "sintered thin-layer chromatography".

This review describes the methods of preparing various kinds of sintered plates, sheets and rods, and presents the results of chromatographic separations on these materials of a variety of organic and inorganic compounds such as lipids, steroids, alkaloids, vitamins, amino acids, peptides, sugars, antibiotics, catecholamines, barbiturates, polychlorinated biphenyls, pesticides, tranquillizers, food dyes, organic phosphates, sulphates, nitrates, metal ions, halogen ions and oxyanions. Based on scanning electron microscopic data, the mechanism of the "welding" between the adsorbent, binder and support in the process of the formation of sintered thin layers is discussed. Also presented are data obtained from thermal analysis [thermogravimetric–differential thermal analysis (TG–DTA) and thermogravimetric–differential scanning calorimetry (TG–DSC)], scanning electron microscopy and the determination of various physical properties of the adsorbents, showing the thermal stability of porous inorganic and organic adsorbents under the welding conditions, that is, heating at 400–770° for several minutes.

2. FUNDAMENTAL STUDIES ON SINTERED THIN-LAYER CHROMATOGRAPHY

2.1. Preparation of and thin-layer chromatography on sintered-glass plates with porous inorganic adsorbents

2.1.1. Materials and apparatus

2.1.1.1. Glass powder[9]. Broken soda-lime glass or borosilicate glass was ground in a ball mill, screened with a 200-mesh sieve and fractionated by sedimentation in water. This glass powder had a particle size of 1–10 μm, which is slightly smaller than that of silica gel for TLC use.

2.1.1.2. Preparation of various sintered-glass plates.

2.1.1.2.1. Silica gel[10]. A mixture of 1 part of silica gel for TLC and 2–5 parts of the prepared glass powder was suspended in a solvent such as benzene, chloroform, acetone, ethyl acetate, methanol, ethanol or water. Of these solvents, acetone was the most effective for the preparation of porous sintered plates[11]. The slurry was spread on a soda-lime glass plate in the usual manner and air dried. The layer was then heated in an electric furnace at 470–770° for several minutes to obtain a silica gel–sintered glass layer. The glass powder had to be sintered without melting the silica gel in order to protect the chromatographic activity of the layer.

2.1.1.2.2. Alumina[12] *and Kieselguhr*[13]. A mixture of 1 part of alumina or Kieselguhr for TLC and 1–6 parts of the glass powder was suspended in the solvents mentioned above. The welding procedure was the same as with the silica gel sintered plate.

2.1.1.2.3. Florisil, titania, zinc oxide and magnesia[14]. For the preparation of Florisil, titania and zinc oxide sintered plates, mixtures of 1 part of the adsorbent and 3 parts of the glass powder were used. For the magnesia sintered plate, a mixture of 1 part of magnesia and 4 parts of the glass powder was used.

2.1.2. Development procedure for thin-layer chromatographic separation

All chromatographic experiments, whether on plates or sheets, were carried out in a cylindrical or rectangular chromatographic chamber with an atmosphere saturated with the solvent. For repeated use of plates other than those of zinc oxide and magnesia, the developed plates were soaked in cleaning solutions such as a chromic acid mixture, concentrated nitric acid or organic solvents, then washed with running water and reactivated by heating at 110° for 30–60 min. Sprayed plates were heated at 400–450° for 20–30 min in an electric furnace to burn off organic compounds, including visible dyes and fluorescent or UV-absorbing substances.

2.1.3. Detection

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

2.1.4. Relationship between compound mobility and the mixing ratio of adsorbent to glass powder

Table I shows the relationship between estrogen mobility and the mixing ratio of silica gel to glass powder. A ratio of 1:2 to 1:4 allowed excellent separations.

TABLE 1

hR_F VALUES OF ESTROGENS ON SILICA GEL SINTERED PLATE

Ratio of silica gel to glass powder	hR_F *value**		
	Estriol	*Estradiol*	*Estrone*
1:1	67	55	9
1:2	74	59	9
1:3	76	60	9
1:4	78	63	10
1:7	83	72	14
1:10	88	76	17
1:20	94	88	36
1:20**	79	66	8
Silica gel	46	30	10

* Mean value of five runs, using benzene–ethyl acetate (4:1) and conc. H_2SO_4.
** Benzene–ethyl acetate (2:1).

Similar mixing ratios gave the best separation of alkaloids on alumina sintered plates, as shown in Table 2. With Kieselguhr sintered plates, ratios of 1:1 to 1:6 gave good results with azo dyes (Table 3).

TABLE 2

hR_F VALUES OF ALKALOIDS ON ALUMINA SINTERED PLATE

Ratio of alumina to glass powder	hR_F *value**						
	Codeine	*Yohimbine*	*Reserpine*	*Ergotamine*	*Quinine*	*Cinchonine*	*Emetine*
1:2	32	32	59	24	44	45	72
1:3	50	50	58	31	51	57	90
1:4	54	44	71	28	55	57	76
1:10	76	67	86	81	75	82**	Front
1:15	82	73**	91	93	75	85**	90
1:20	83	73**	88	—**	79	86**	89
1:20	84	77**	87	—**	87	95**	90
Alumina***	50	43	68	23	46	59	75

* Mean value of five runs, using benzene–chloroform–diethylamine (9:4:1) and Dragendorff reagent.
** Tailing spot.
*** Merck aluminium oxide (Type T).

2.1.5. Reproducibility of separation on sintered plates

Many factors affect the reproducibility of R_F values in TLC[15–18] including the nature of the adsorbent, the thickness and activity of the adsorbent layer, the quality of the solvent and the degree of solvent saturation in the chromatographic chambers. Recycling the sintered thin layers eliminates factors such as the nature of the adsorbent and the thickness and activity of the layer. Table 4 gives the reproducibility of hR_F values ($R_F \times 100$ values) of steroids on silica gel sintered plates that were used repeatedly.

TABLE 3

hR_F VALUES OF AZO DYES ON KIESELGUHR SINTERED PLATE

Ratio of Kieselguhr to glass powder	hR_F *value**			
	Sudan Red G	*Sudan Yellow*	*Butter Yellow*	*p-Methoxy-azobenzene*
1:1	10	50	66	88
1:2	12	57	73	90
1:3	14	60	75	89
1:4	12	59	75	90
1:5	11	56	75	90
1:6	12	57	75	90
1:8	13	59	78	91
1:10	15	65	81	92
1:20	18	70	84	94
Kieselguhr**	43***	86**	Front	Front

* Mean value of five runs, using cyclohexane.
** Wako Kieselguhr B-O.
*** Tailing spot.

TABLE 4

REPRODUCIBILITY OF hR_F VALUES ON SILICA GEL SINTERED PLATES

Adsorbent	hR_F *values**			n**	*Detection*	*Cleaning solution*
	Estriol	*Estradiol*	*Estrone*			
Silica gel–sintered glass powder:						
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
Silica gel (laboratory prepared)***	4 ± 1	22 ± 3	40 ± 3	5	Sulphuric acid	None
Silica gel (fast running)§	8 ± 0	54 ± 3	67 ± 3	5	Sulphuric acid	None
	Cortisone§§	*Testosterone*§§	*Progesterone*§§			
Silica gel–sintered glass powder (1:3)	36 ± 4	61 ± 2	76 ± 2	50	Sulphuric acid	Chromic acid mixture
Silica gel (laboratory prepared)***	20 ± 4	48 ± 4	68 ± 3	5	Sulphuric acid	None

* Benzene–ethyl acetate (2:1).
** n = Number of runs on the same plate in the case of the sintered plate. In the case of laboratory-prepared or silica gel fast-running plates, five different plates were used.
*** Merck silica gel H or HF.
§ Merck aluminium sheet, silica gel–Kieselguhr.
§§ Chloroform–acetone (4:1).

TABLE 5

REPRODUCIBILITY OF hR_F VALUES ON ALUMINA SINTERED PLATE

Alkaloid	*Number of recovery**				*Laboratory-prepared*
	1–32	*33–52*	*53–77*	*78–100*	
Quinine	25 ± 3	34 ± 3	31 ± 3	31 ± 3	2
Codeine	42 ± 4	54 ± 4	51 ± 6	51 ± 6	15
Brucine	59 ± 4	67 ± 2	66 ± 2	66 ± 2	15
Thebaine	80 ± 2	81 ± 1	82 ± 2	82 ± 2	50

* Mean value of five different plates, using benzene–chloroform–diethylamine (9:2:0.25) and Dragendorff reagent.

** Merck aluminium oxide (Type T).

Tables 5 and 6 indicate the reproducibility of the hR_F values of alkaloids on alumina sintered plates and those of sugars on Kieselguhr sintered plates, respectively. In general, the standard deviation of R_F values can be controlled to within less than 0.05 when sufficient care is taken with the factors that affect the reproducibility of separation. With the author's sintered plates, the variation in the standard deviation was less than 0.04. For comparison, hR_F values on laboratory-prepared and other pre-coated silica gel plates are shown in Table 4.

TABLE 6

REPRODUCIBILITY OF hR_F VALUES ON KIESELGUHR SINTERED PLATE

Sugar	*hR_F value**			
	*Sintered***			*Laboratory-prepared****
	1	*2*	*3*	
Rhamnose	81 ± 3	81 ± 2	82 ± 2	77
Glucose	41 ± 5	47 ± 5	49 ± 4	43
Lactose	24 ± 6	25 ± 4	27 ± 5	21
Digitoxose		Origin		Origin
Digitalose		83 ± 1		84
Digitoxose		91 ± 2		96

* Buffer, 0.02 *M* sodium acetate; solvent, ethyl acetate–isopropanol–water (65:24 12); detection, conc. H_2SO_4.

** Mean value of ten runs on the sample plate.

*** Wako Kieselguhr B-10.

Tables 7, 8 and 9 compare the separation characteristics of estrogens, cardiac glycosides and azo dyes on silica gel, alumina and Kieselguhr sintered plates with those on other laboratory-prepared and pre-coated plates. It can be seen that the hR_F value of each compound on the sintered plate is greater than that on the other types of plates, the difference (ΔhR_F) between estradiol and estriol on the silica gel sintered plate (Table 7) is greater than those on the other plates, and ΔhR_F between estrone and estriol on the sintered plate is less than those on the other plates. The separation characteristics on the author's plates were similar to those on a mixed layer of silica gel–Kieselguhr or alumina–Kieselguhr (Tables 7 and 8). This indicates that the sintered plate is essentially different from the conventional TLC plates.

TABLE 7

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**			*Development rate (min per 10 cm)*
	Estriol	*Estradiol*	*Estrone*	
Silica gel:				
Laboratory-prepared	4 ± 1 $\Delta 18$	22 ± 3 $\Delta 18$	40 ± 3	17
Pre-coated**	4 ± 1 $\Delta 32$	36 ± 2 $\Delta 15$	51 ± 1	20
Silica gel–Kieselguhr (1:1):				
Laboratory-prepared	8 ± 2 $\Delta 44$	52 ± 2 $\Delta 15$	67 ± 2	20
Pre-coated***	11 ± 2 $\Delta 51$	62 ± 2 $\Delta 13$	75 ± 3	12
Silica gel–glass powder (1:4):				
Laboratory-prepared	4 ± 1 $\Delta 51$	55 ± 1 $\Delta 16$	71 ± 2	20
Sintered	11 ± 1 $\Delta 47$	58 ± 1 $\Delta 15$	73 ± 1	15

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

TABLE 8

COMPARISON OF SEPARATION BEHAVIOUR ON SINTERED ALUMINA, ALUMINA–KIESELGUHR AND ALUMINA–GYPSUM PLATES

Alumina = Merck aluminium oxide neutral (Type T); Kieselguhr = Merck Kieselguhr G.

Compound	*hR_F value*					
	Alumina–10% gypsum	*Sintered alumina (1:4)*	*Alumina–Kieselguhr*			
			1:1	*2:1*	*3:1*	*5:1*
*Cardiac glycoside**						
Gitoxin	30	50	59	50	47	43
Digoxin	45	57	60	55	50	47
Digitoxin	48	70	71	64	56	53
*Cardiac genin***						
Gitoxigenin	15	31	68	56	50	38
Digoxigenin	32	46	75	74	60	44
Digitoxigenin	57	69	86	78	74	69
Alkaloids						
Quinine	2	25	21‡	21‡	12	—
Codeine	15	42	26‡	26‡	17	—
Brucine	15	59	54	37	34	—
Thebaine	50	80	75‡	60	60	—

* Chloroform–methanol (10:1).
** Ethyl acetate–acetone (3:2).
*** Benzene–chloroform–diethylamine (9:2:0.25).
‡ Tailing spot.

Compound mobility can be controlled by adding sintered glass powder to the adsorbents. By using these sintered plates containing silica gel, alumina, Kieselguhr and other porous metal oxides, the author separated various organic compounds, namely, lipids, steroids, alkaloids, vitamins, amino acids and sugars[9,19], peptides and food dyes[20], polychlorinated biphenyls[13], pesticides[14], antibiotics, catecholamines and barbiturates[21], and organic phosphates, sulphates and nitrates[22].

TABLE 9

COMPARISON OF SEPARATION BEHAVIOUR ON SINTERED KIESELGUHR AND KIESELGUHR–GYPSUM PLATES

Thin layer: 1 = Kieselguhr–sintered glass powder (1:3); 2 = Kieselguhr + 10% (Wako) or 15% (Merck) gypsum. Solvent: Cyclohexane.

Azo dye	hR_F *value**			
	*Merck***		*Wako*	
	1	*2*	*1*	*2*
Sudan Red G	17	10	14	4
Sudan Yellow	66	43	60	29
Butter Yellow	77	64	75	44
p-Methoxyazobenzene	94	86	89	73
Sudan I	77	75	71	65
Sudan II	74	72	67	63
Sudan III	16	17	17	10
Sudan IV	20	18	17	17

* Mean value of five runs; the standard deviation for each hR_F value was less than 2. Development rate: 30 min per 10 cm.

** Slightly tailing spots.

2.2. Preparation of and thin-layer chromatography on sintered sheets of organic adsorbents[20,23]

In this instance, microparticulate polyolefins were used instead of glass powder as the binder. The adsorbents were silica gel, alumina, Kieselguhr, Florisil, synthetic porous polymers, ion-exchange resins, cellulose ion exchangers, dextran gels and cellulose.

2.2.1. Microparticulate polyolefins

Commercially available polyethylene or polypropylene (about 20 mesh) powder was recrystallized according to a known procedure[24] from an aromatic solvent such as benzene, toluene or xylene to give microparticulate material (particle size 10 μm).

2.2.2. Preparation of various polyolefin sintered sheets

A mixture of 1–5 parts of adsorbent and 1 part of microparticulate polyolefin was suspended in acetone and the slurry was spread on glass or plastic supports in the usual manner. After drying in air, the layer was heated in an electric oven at 100–180° for 10–30 min. Next, the sintered plates were soaked in a lipophilic solvent such as benzene, chloroform, diethyl ether or ethyl acetate at room temperature for several minutes. In this process, the polyolefin sintered layers peeled off from the glass or plastic supporting plates. The sintered sheets prepared in this way are self-supporting, and have satisfactory adsorption, partition and ion-exchange functions. Table 10 gives the conditions for the preparation of various polyolefin sintered sheets. Using these sheets, the author has successfully separated various organic compounds: peptides on carboxymethylcellulose sintered sheets, nucleotides on polyethyleneimine-cellulose sintered sheets and food dyes on cellulose sintered sheets. Table 11 shows

TABLE 10

PREPARATION OF VARIOUS POLYOLEFIN SINTERED PLATES

Adsorbent	*Welding conditions*		*Adsorbent to polyolefin ratio*
	Temperature (°C)	*Time (min)*	
Silica gel	180	10	2:1
Alumina	180	10	3:1
Kieselguhr	180	10	2:1
Florisil	180	10	2:1
Polyamide	120	30	1:1
Amberlite XAD	180	10	2:1
Amberlite GC-120*	140	10	3:1
Amberlite CG-50**	140	10	3:1
Amberlite CG-400***	180	10	3:1
Lewatit MP 7080§	180	10	3:1
CM-cellulose**	160	20	5:1
PEI-cellulose§	160	10	5:1
ECTEOLA-cellulose§	160	10	5:1
Cellulose	160	10	5:1

* Strong acid cation exchanger.
** Weak acid cation exchanger.
*** Strong base anion exchanger.
§ Weak acid anion exchanger.

TABLE 11

TLC OF ORGANIC COMPOUNDS ON POROUS POLYMER SINTERED SHEETS

Porous polymer	*Compound*	*hR_F value*	*Solvent***	*Development time (min)*	*Detection*
Polyamide	*Nitrophenols*		BA401	20	Visible
	2,6-Dinitrophenol	25			
	2,5-Dinitrophenol	50			
	Dns-amino acids		BA91	16	UV (365 nm), visible
	His	14			
	Gly	30			
	Lys	55			
	Pro	80			
XAD-2	*vitamins*		Ethanol	29	$SbCl_3$
	A pal	17			
	A ace	34			
	D_2*	70			
	D_3*	75			
XAD-4	*vitamins*		Ethanol	34	$SbCl_3$
	A pal	6			
	A ace	13			
	D_2*	36			
	D_3*	42			

* With $AgNO_3$ impregnation.
** BA401 = benzene–acetone (40:1); BA91 = benzene–acetone (9:1).

the separation of nitrophenols and Dns-amino acids on polyamide sintered sheets and of fat-soluble vitamins on porous polymer (XAD-2, XAD-4) sintered sheets.

Table 12 shows the TLC analysis of 17 free amino acids on a strongly acidic cation-exchange resin (Amberlite CG-120) sintered sheet. The system of a strongly acidic cation exchanger and citrate buffer was better than that of a strongly basic anion exchanger (Amberlite CG-400) and pyridine–acetic acid–water.

The sintered sheet looks like a filter-paper for paper chromatography, but is mechanically stable and acid-resistant. Hence it is also suitable for the separation of radioactive compounds.

TABLE 12

TLC OF AMINO ACIDS ON ION-EXCHANGE RESIN SINTERED SHEETS

Amino acid	*hR_F value*	
	*Amberlite CG-120**	*Amberlite CG-400***
Arg	10	65
Trp	18	51
His	19	71
Lys	25	81
Phe	39	72
Tyr	45	72
Pro	53	72
β-Ala	64	85
Ala	85	86
Gly	84	88
Ser	90	90
Cys	91	90
$GluNH_2$	95	75
$AspNH_2$	96	71
Asp	95	70
Glu	95	76
Tau	100	83

* Eluent: citrate buffer (pH 5.30), Na^+ 0.35 *M*, citrate 0.117 *M*.
** Eluent: pyridine–acetic acid–water (1:10:100) (pH 3.45).

2.3. Consideration of the "welding" mechanism

At the beginning of this work, the author was not certain whether solid welding would occur among heterogeneous substances such as adsorbent, binder and support, because their coefficients of expansion (α) differ greatly from one another. For example, the coefficient of expansion of soda-lime glass powder ($\alpha = 92 \cdot 10^{-7}$ cm/cm·°C) is about 17 times greater than that of silica gel ($\alpha = 5.4 \cdot 10^{-7}$ cm/cm·°C). However, solid welding of these substances did occur to yield highly porous thin layers with intact chromatographic activity, as shown in Tables 1, 2 and 3.

The author has tried to clarify the mechanism of welding of sintered plates containing silica gel[25], alumina[26] and Kieselguhr[27] by means of scanning electron microscopy (SEM). Figs. 1, 2 and 3 are scanning electron micrographs showing the surfaces of silica gel, alumina and Kieselguhr sintered glass plates, respectively. The larger particles are silica gel, alumina and Kieselguhr, and they are not fused. The

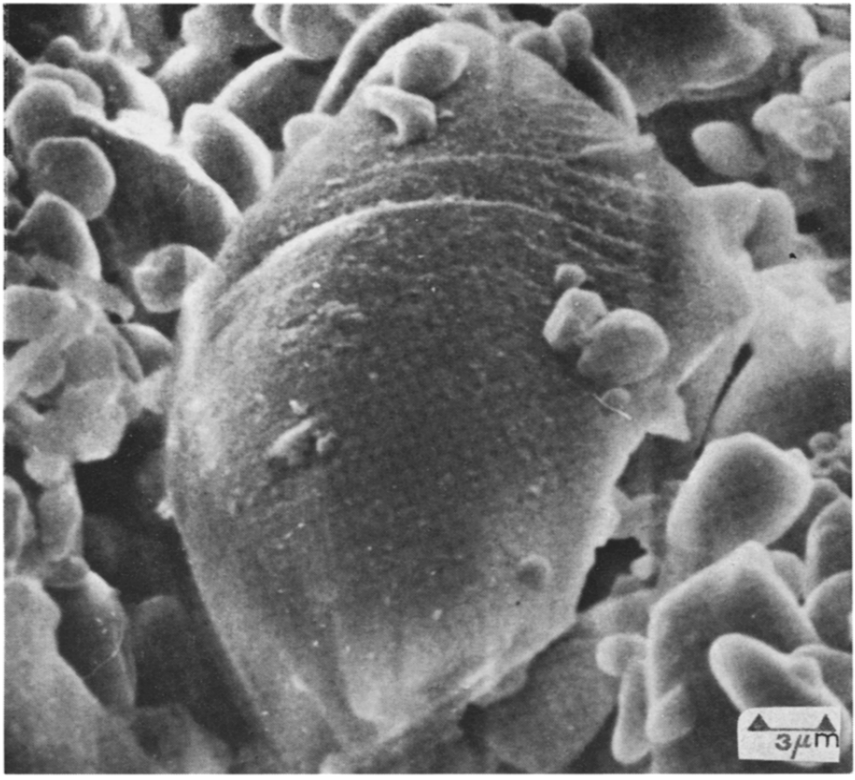

Fig. 1. SEM of silica gel sintered plate surface.

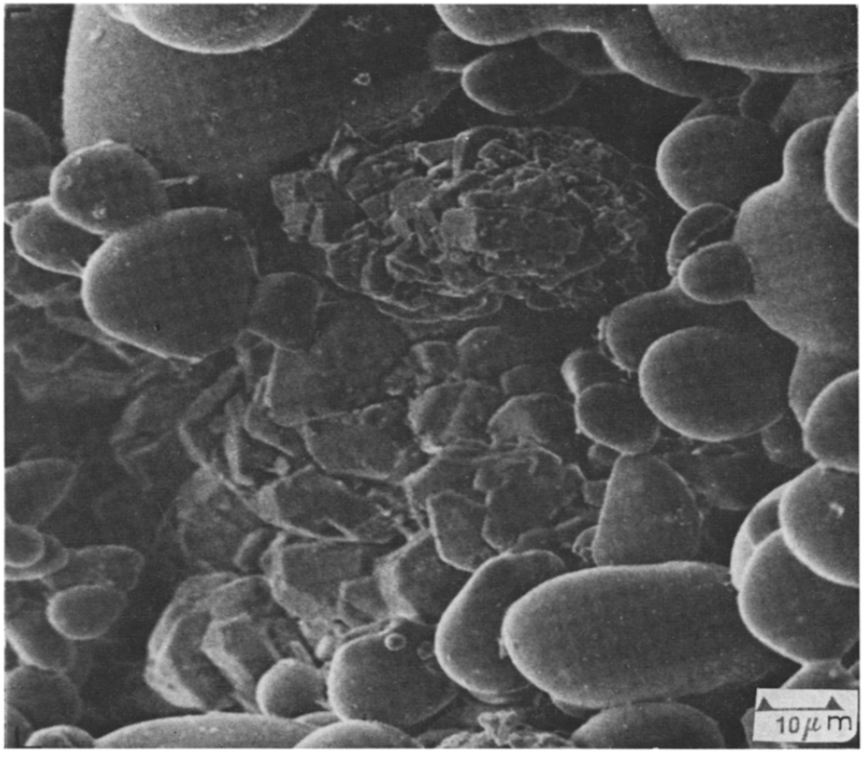

Fig. 2. SEM of alumina sintered plate surface.

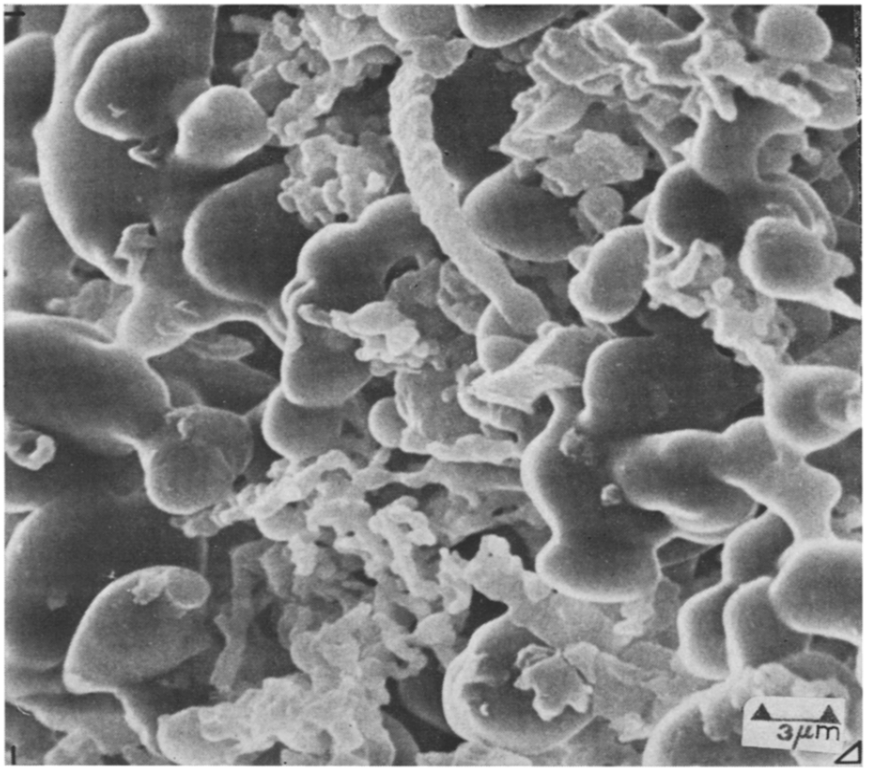

Fig. 3. SEM of Kieselguhr sintered plate surface.

smaller particles are sintered glass powder. Figs. 4, 5 and 6 show cross-sectional views of these three kinds of sintered plates. The lower layer is the glass supporting plate, and large adsorbent particles and small sintered glass particles are seen in the upper layer.

Thus, as shown in the schematic structure of the sintered glass plate (Fig. 7), these adsorbent particles are fixed, without any damage to their surface structures, in the three-dimensional space formed by the sintered-glass powder and the glass supporting plate. The sintered-glass powder plays a binding role between the adsorbent and the glass plate.

2.3.1. Influence of pH of glass binders on formation of sintered thin layers

Unlike soda-lime glass, borosilicate glass failed to give homogeneous thin-layers. As shown in Fig. 8, Stahl's test dyes behaved normally on the soda-lime glass sintered plate (left), but they migrated abnormally on the borosilicate glass sintered plate (right). The author thought that this phenomenon was due to the lower pH of borosilicate glass. A 10% aqueous suspension of borosilicate glass had a pH of 9.05 and that of soda-lime glass a pH of 10.35.

Fig. 9 shows the scanning electron micrograph of the homogeneous layer of silica gel on the soda-lime glass sintered plate, and Fig. 10 shows that of the hetero-

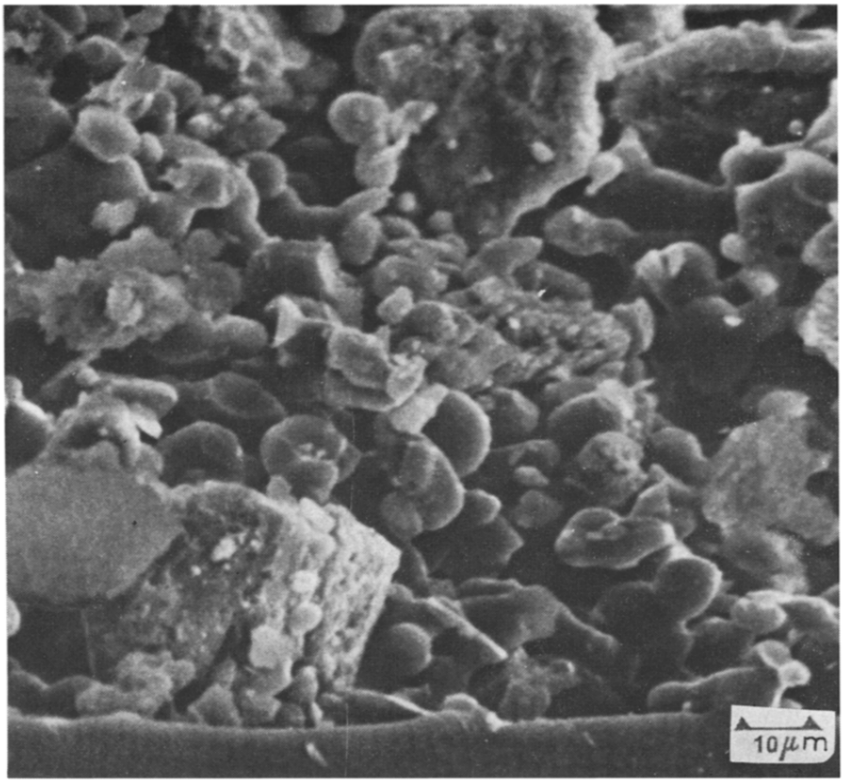

Fig. 4. SEM of silica gel sintered plate cross-section.

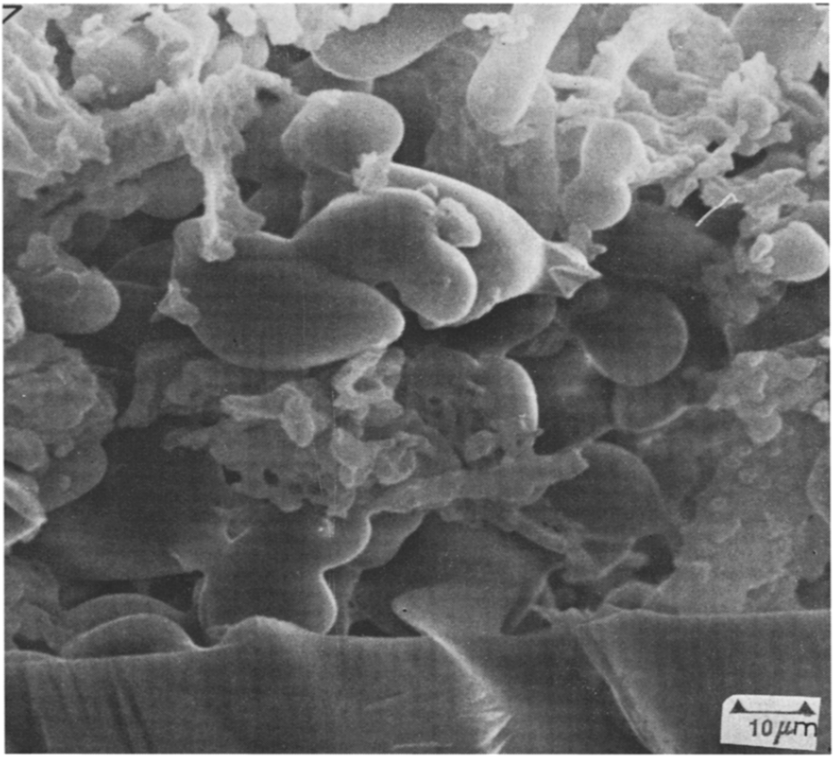

Fig. 5. SEM of alumina sintered plate cross-section.

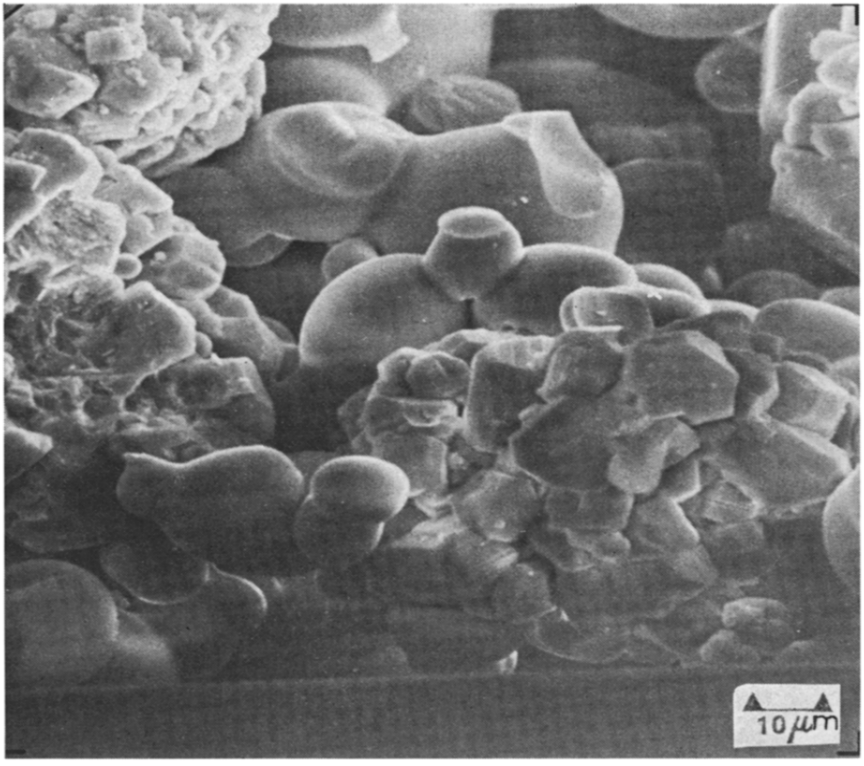

Fig. 6. SEM of Kieselguhr sintered plate cross-section.

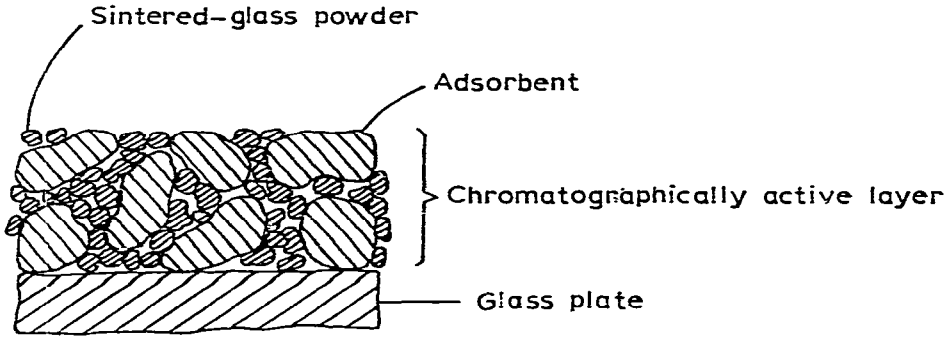

Fig. 7. Schematic diagram of structure of sintered plate.

geneous layer of silica gel on the borosilicate glass sintered plate, in which large cracks are visible.

In order to explain the different results obtained with soda-lime and borosilicate glass, their sedimentation volumes were measured. Table 13 indicates that the sedimentation volume of borosilicate glass increases about 2–2.8-fold on addition of a basic flocculant such as sodium methoxide and ammonia solution. Investigation of

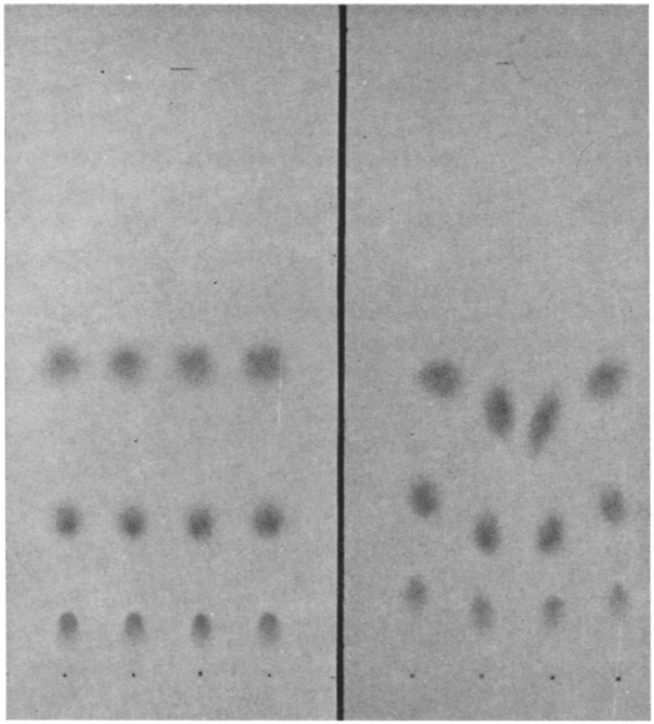

Fig. 8. TLC separation of Stahl's test dyes on silica gel sintered plate. Left, soda-lime glass; right, borosilicate glass. Dispersion medium: acetone.

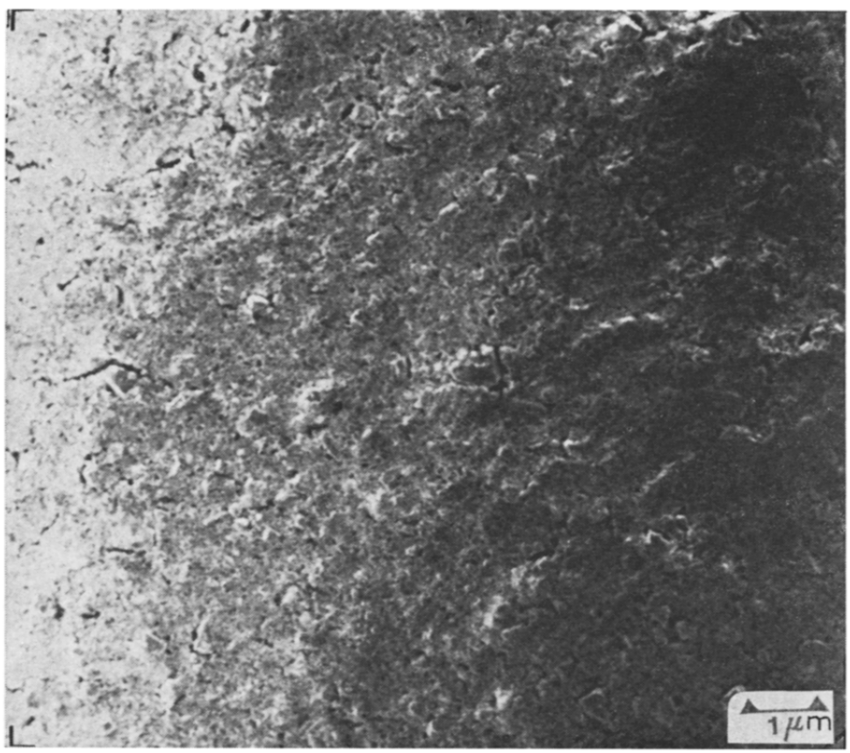

Fig. 9. SEM of silica gel–soda lime glass sintered plate.

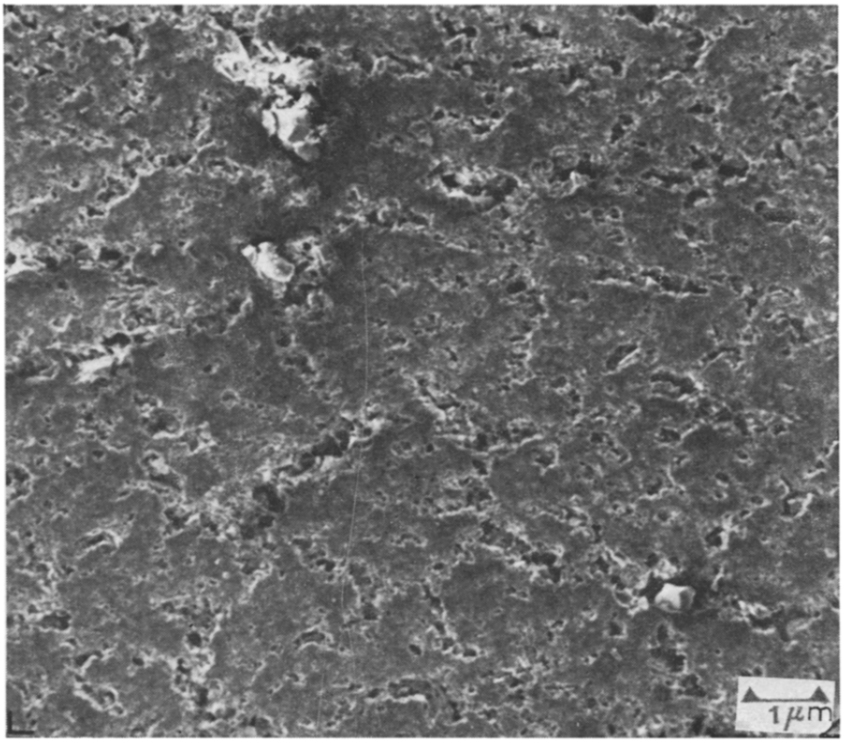

Fig. 10. SEM of silica gel–borosilicate glass sintered plate.

TABLE 13

SEDIMENTATION VOLUMES (V_s) OF SILICA GEL AND GLASS POWDERS USED FOR SINTERED PLATES

Powder	V_s *(ml)* *		V_s *with* $NaOCH_3$ / V_s *without* $NaOCH_3$
	With $NaOCH_3$ **	*Without* $NaOCH_3$	
Merck silica gel H***	15.8	15.2	1.04
Soda-lime glass***	10.7	10.3	1.04
Borosilicate glass***	11.0	4.0	2.75
Silica gel H–soda-lime glass mixture (1:3)§	10.8	9.0	1.20
Silica gel–borosilicate glass mixture (1:3)§	13.8	7.0	1.97

* Left to settle overnight.
** 2% (w/w) $NaOCH_3$ was added.
*** Six grams each suspended in 15 ml of acetone.
§ A mixture of 1.5 g and 4.5 g, respectively, suspended in 15 ml of acetone.

the relationship between the amount of basic flocculant added and the pH of sintered borosilicate glass–silica gel plates showed that the addition of 2–3% of sodium methoxide was necessary in order to prepare plates with the same pH as that of a soda-lime glass sintered plate (Table 14). As shown in Table 15, the addition of

TABLE 14

pH VALUES OF SILICA GEL–BOROSILICATE GLASS SINTERED PLATES

$NaOCH_3$ (%, w/w)	*pH value*	
	*pH meter**	*pH indicator***
0	7.14	5.6
0.1	7.87	5.8
0.3	7.98	6.2
0.5	8.22	6.4
1	8.45	6.8
2	8.70	7.2
3	9.31	8.4
4	9.51	8.6
5	9.57	8.6
Silica gel–soda-lime sintered plate	8.83	8.4

* Measured with a Hitachi-Horiba F-5 pH meter.
** Measured with a Nishicator[28].

TABLE 15

hR_F VALUES OF STEROIDAL HORMONES ON SILICA GEL–BOROSILICATE GLASS SINTERED PLATES TREATED WITH SODIUM METHOXIDE

Solvent, chloroform–methanol (10:1); detection, conc. sulphuric acid.

$NaOCH_3$ (%, w/w)	*hR_F value*			*Separation*
	Cortisone	*Testosterone*	*Progesterone*	
0	50	70	80	Irregular
0.1	53	73	83	Sharp
0.3	58	75	83	Sharp
0.5	57	77	87	Sharp
1	50	66	71	Sharp
2	62	71	76	Slightly leading
3	75	80	85	Leading
4	89	89	89	Leading
5	90	90	90	Leading

0.1–1% of sodium methoxide to the borosilicate glass gave normal and sharp separation of steroidal hormones. Sodium methoxide-treated borosilicate sintered plates also allowed the normal separation of estrogens, alkaloids (silica gel, Tables 16 and 17), azo dyes (alumina) and polychlorinated biphenyls (PCBs, Kieselguhr)[11].

Figs. 11 and 12 show the schematic sedimentation structure of mixtures of silica gel and borosilicate glass powder. When a mixture of silica gel and borosilicate glass powder is dispersed on a supporting plate without the addition of the basic flocculant, closely packed sedimentation results, as shown in Fig. 11a. After sintering, this closely packed layer changes into a cracked surface (Fig. 11b). The heterogeneous sintered thin layer thus formed results in the abnormal separation of various organic compounds. On the other hand, when a mixture of silica gel and borosilicate glass powder is dispersed after addition of the basic flocculant, loosely packed sedimentation is obtained, as shown in Fig. 12a. After sintering, this loosely packed layer

TABLE 16

hR_F VALUES OF ESTROGENS ON SILICA GEL–BOROSILICATE GLASS SINTERED PLATES TREATED WITH SODIUM METHOXIDE

Solvent, benzene–ethyl acetate (2:1); detection, iodine vapour.

$NaOCH_3$ (%, w/w)	hR_F value			Separation
	Estriol	*Estradiol*	*Estrone*	
0	9	46	60	Irregular
0.1	10	53	67	Sharp
0.3	10	53	66	Sharp
0.5	8	54	65	Sharp
1	9	57	68	Sharp
2	9	65	74	Slightly leading
3	13	78	84	Leading
4	26	93	97	Leading
5	31	85	90	Leading

TABLE 17

hR_F VALUES OF ALKALOIDS ON SILICA GEL–BOROSILICATE GLASS SINTERED PLATES TREATED WITH SODIUM METHOXIDE

Solvent, chloroform–diethylamine (30:1); detection, Dragendorff reagent.

$NaOCH_3$ (%, w/w)	hR_F value				Separation
	Quinine	*Codeine*	*Brucine*	*Thebaine*	
0	10	29	35	39	Irregular
0.1	10	32	37	43	Sharp
0.3	12	37	45	50	Sharp
0.5	10	35	43	48	Sharp
1	15	38	46	57	Sharp
2	16	45	50	65	Slightly leading
3	50	74	80	87	Leading
4	80	90	95	100	Leading
5	88	97	100	100	Leading

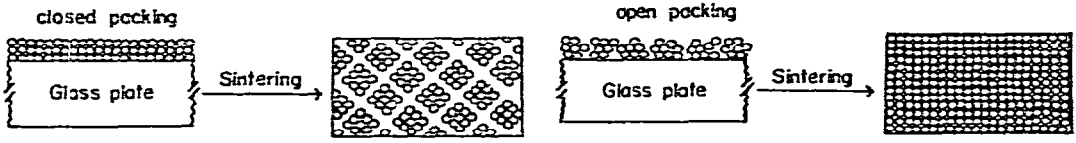

Fig. 11. Schematic diagram of structure of silica gel and borosilicate glass powder before and after sintering.

Fig. 12. Schematic diagram of structure of silica gel and borosilicate glass powder with basic flocculant before and after sintering.

changes into a homogeneous surface without cracks (Fig. 12b) which enables normal chromatographic separation. Of the several solvents tested, acetone was the most effective for increasing the sedimentation volume. The author also prepared homogeneous borosilicate alumina and Kieselguhr sintered glass plates[11].

2.4. Thermal stabilities of adsorbents

Sintering of silica gel begins at 600–700° (ref. 29). However, in the preparation of sintered plates, the heating period is short and sintering of silica gel can be avoided. There is no example in the literature of the heating at high temperatures of silica gel for TLC, which has physical properties[30] such as a specific surface area of about 400 m^2/g, a specific pore volume of about 0.7–0.8 ml/g and a particle size distribution of 33.7% at 6 μm, 1.9% at 6–30 μm and 64.4% at 30–66 μm. Therefore, heat treatment of the silica gel was carried out under mild conditions, *i.e.*, at 470–770° for 2–7 min.

Table 18 presents some of the physical properties of silica gel for TLC. Before heat treatment, its specific surface area was about 400–500 m^2/g, specific pore volume 1.1–1.2 ml/g and particle size distribution 10–60 μm. After heating to 770°, these values remained the same. However, heating at 1000° for 30 min caused sintering of the silica gel powder, as indicated by the lower physical constants.

TABLE 18

PHYSICAL PROPERTIES OF SILICA GEL HEATED AT NORMAL PRESSURE
Merck silica gel H.

Treatment		*Specific surface area (m^2/g)*		*Specific pore volume*** *(ml/g)*
Temperature (°C)	*Time (min)*	N_2*	N_2/He**	
Not heated		420	494	1.21
470	7	419	497	1.15
570	7	415	481	1.14
670	7	413	479	0.95
770	7	396	482	0.94
870	7	361	426	0.92
1000	7	197	116	0.43
1000	30	80	22	0.26

* Measured by BET method.
** Measured by continuous flow method.
*** Calculated from (1/particle density) − (1/true density).

Sintered plates were prepared from silica gel heated to 770° and tested with the separation of mixtures such as estrogens and Stahl's azo dyes. Tables 19 and 20 show the constancy of the R_F values and the good separation of the test mixtures, indicating that sintering of the silica gel did not occur on heating to 770° (ref. 31). However, extreme tailing was observed in the separation of the test mixtures when silica gel powder heated at 1000° for 30 min was used. Fig. 13 is a scanning electron micrograph showing the sintering of this type of silica gel.

Tables 21 and 22 present the data on heat treatment of alumina[32] and Kieselguhr[33] for TLC, respectively. Tables 23 and 24 show the influence of the heat treatment on the TLC separation of alkaloids on the alumina and those of PCBs on the Kieselguhr, indicating that heating of these two kinds of adsorbents at 1000° for 30 min did not alter their physical properties or chromatographic activities, in contrast to the results with silica gel.

TABLE 19

hR_F VALUES OF ESTROGENS ON HEATED SILICA GEL LAYERS

Solvent, benzene–ethyl acetate (2:1); detection, conc. sulphuric acid. Merck silica gel H.

Treatment		*hR_F value*			*Separation*	*Development rate (min per 10 cm)*
Temperature (°C)	*Time (min)*	*Estriol*	*Estradiol*	*Estrone*		
Not heated		4	41	59	Sharp	13
470	7	4	40	57	Sharp	13
570	7	4	39	57	Sharp	13
570	7	5	40	57	Sharp	11
770	7	5	36	53	Sharp	12
870	7	5	43	59	Sharp	11
1000	7	7	53	67	Slight tailing	11
1000	30	—	—	—	Marked tailing	8

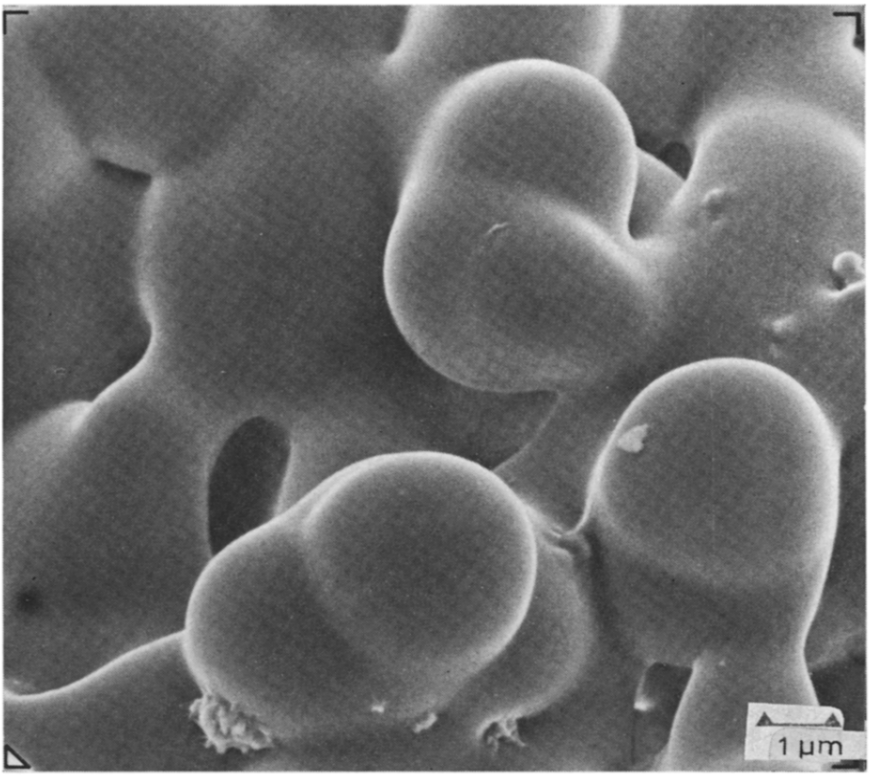

Fig. 13. SEM of silica gel heated at 1000° for 30 min.

Of the adsorbents silica gel, alumina and Kieselguhr, alumina was the most thermostable and Kieselguhr was more stable than silica gel. The author also investigated the thermal stabilities of other inorganic adsorbents[14], such as Florisil, titania, magnesia and zinc oxide, and various kinds of organic adsorbents used for the preparation of sintered sheets[20,23], employing SEM, X-ray diffraction analysis and thermal analysis (TG–DTA and TG–DSC).

TABLE 20

hR_F VALUES OF STAHL'S DYES ON HEATED SILICA GEL LAYERS

Solvent, benzene. Adsorbent, Merck silica gel H.

Treatment		*hR_F value*			*Separation*	*Development time (min per 10 cm)*
Temperature (°C)	*Time (min)*	*Indophenol*	*Sudan Red G*	*Butter Yellow*		
Not heated		4	12	37	Sharp	14
470	7	4	11	34	Sharp	13
570	7	3	10	31	Sharp	14
670	7	2	9	29	Sharp	14
770	7	2	8	27	Sharp	12
870	7	2	8	28	Sharp	13
1000	7	5	20	47	Slight tailing	13
1000	30	Tailing spot from original point to solvent front				7

TABLE 21

PHYSICAL PROPERTIES OF ALUMINA HEATED AT NORMAL PRESSURE

Merck aluminium oxide neutral (Type T).

Treatment		*Specific surface area (m^2/g)*		*Specific pore volume*** (ml/g)*
Temperature (°C)	*Time (min)*	*N_2**	*N_2/He***	
Not heated		103	107	0.51
470	7	102	108	0.51
570	7	102	105	0.52
670	7	104	95	0.49
770	7	96	96	0.51
870	7	90	100	0.50
1000	7	78	98	0.50
1000	30	65	66	0.50

* Measured by BET method.

** Measured by continuous flow method.

*** Calculated from (1/particle density) — (1/true density).

TABLE 22

PHYSICAL PROPERTIES OF KIESELGUHR HEATED AT NORMAL PRESSURE

Wako Kieselguhr B-0.

Treatment		*Specific surface area* (m^2/g)*	*True specific gravity**	*Particle specific gravity**	*Mean particle diameter** (μm)
Temperature (°C)	*Time (min)*				
Not heated		3.38	2.24	2.02	1.77
470	7	3.60	2.24	2.07	1.66
570	7	3.60	2.15	2.07	1.67
670	7	3.64	2.14	2.09	1.65
770	7	3.62	2.17	2.07	1.66
870	7	3.64	2.21	2.10	1.65
1000	7	3.87	2.23	2.20	1.55
1000	30	3.76	2.26	2.22	1.60

* Measured by air permeability method.

TABLE 23

hR_F VALUES OF ALKALOIDS ON HEATED ALUMINA LAYERS

Solvent, benzene–chloroform–diethylamine (9:4:1); detection, Dragendorff reagent. Merck aluminium oxide neutral (Type T).

Treatment		*hR_F value**			*Separation*	*Development rate (min per 10 cm)*
Temperature (°C)	*Time (min)*	*Quinine and codeine*	*Brucine*	*Thebaine*		
Not heated		25	51	73	Sharp	23
470	7	27	57	77	Sharp	18
570	7	29	53	75	Sharp	20
670	7	29	55	75	Sharp	19
770	7	31	54	74	Sharp	16
870	7	30	58	78	Sharp	17
1000	7	34	60	78	Sharp	16
1000	30	53	71	85	Sharp	15

* Mean values of three runs.

TABLE 24

hR_F VALUES OF PCBs ON HEATED KIESELGUHR LAYERS

Treatment		*DCB*	*Kanechlor 600*			*Separation*	*Development rate (min per 10 cm)*
Temperature (°C)	*Time (min)*		*Spot A*	*Spot B*	*Spot C*		
Not heated		7	21	28	36	Sharp	42
470	7	9	27	35	45	Sharp	15
570	7	7	24	30	39	Sharp	14
670	7	10	28	35	45	Sharp	14
770	7	8	25	32	41	Sharp	14
870	7	9	25	33	42	Sharp	14
1000	7	9	28	36	44	Sharp	14
1000	30	9	27	37	46	Sharp	11

3. NOVEL DETECTION METHODS FOR SINTERED THIN-LAYER CHROMATOGRAPHY

3.1. Fluorescence quenching detection[34]

There are many inorganic phosphors for fluorescence quenching detection, such as zinc orthosilicate, zinc sulphide, calcium tungstate, strontium pyrophosphate and yttrium vanadate. However, they are not acid-resistant, except for yttrium vanadate[35]. Acid lability is unfavourable in TLC, because solvent systems containing strong acids are sometimes chosen as the mobile phase and spraying with concentrated sulphuric acid is a routine process in TLC. These acid-labile inorganic phosphors are dissolved or decomposed during the development or detection process.

To resolve this difficulty, the author used partially crystallized fluorescent glass, namely zinc silicate, calcium tungstate and cadmium borate glasses with partially crystallized structures, in place of the above inorganic phosphors. Partially crystallized glass is prepared by fusing a mixture of silica, basic zinc carbonate,

manganese dioxide, sodium nitrate, lead tetroxide, sodium fluorosilicate and aluminium hydroxide at 1000–1500° for 1–3 h in an electric furnace (Fig. 14a), then inducing partial crystallization on the surface by heating this glass again at 900–1100° for 30–40 min, as shown in Fig. 14b. On prolonged heating, the partially crystallized glass melted again, as shown in Fig. 14c.

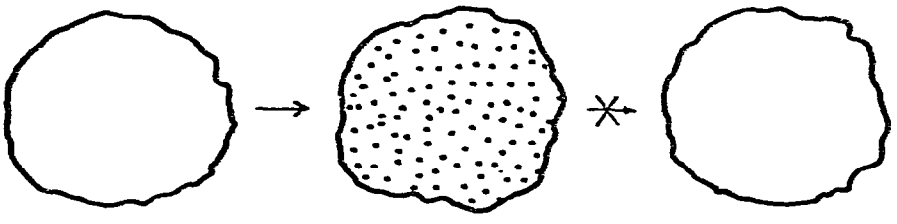

Fig. 14. Crystallized fluorescent glass.

Table 25 presents the chemical compositions of the three kinds of fluorescent glass. The X-ray diffraction analysis data shown in Fig. 15 offered proof of the partially crystallized structure of zinc silicate fluorescent glass, a scanning electron micrograph of which is shown in Fig. 16. The crude crystallized fluorescent glass thus obtained was ground in a ball mill and fractionated by water sedimentation. In this way, a fine powder of fluorescent glass with a particle distribution of 1–20 μm was obtained. Table 26 shows the properties of three kinds of fluorescent crystallized glass and some of the fluorescent materials, which indicate that in their fluorescent properties zinc silicate glass and calcium tungstate glass correspond to zinc silicate phosphor and strontium pyrophosphate phosphor, respectively.

TABLE 25

CHEMICAL COMPOSITIONS OF FLUORESCENT GLASSES

Fluorescent glass	*Component (%, w/w)*									
	SiO_2	Na_2O	Al_2O_3	CaO	ZnO	WO_3	B_2O_3	CdO	MnO	PbO
Zn_2SiO_4/Mn	60.0	10.0	3.7	—	26.0	—	—	—	0.3	—
$CaWO_4$/Mn, Pb	56.0	8.0	—	24.0	—	10.7	—	—	0.3	1.0
$Cd_2B_2O_5$/Mn	10.0	—	2.0	—	—	—	26.0	61.7	0.3	—

Using crystallized fluorescent glass, the author prepared acid-resistant fluorescent sintered plates, for example fluorescent sintered-glass–silica gel plates, by the procedure shown in Table 27. With these plates, it was possible to separate and detect minute amounts of, for example, steroidal hormones, water-soluble vitamins and inorganic cations, without spraying with a detection reagent.

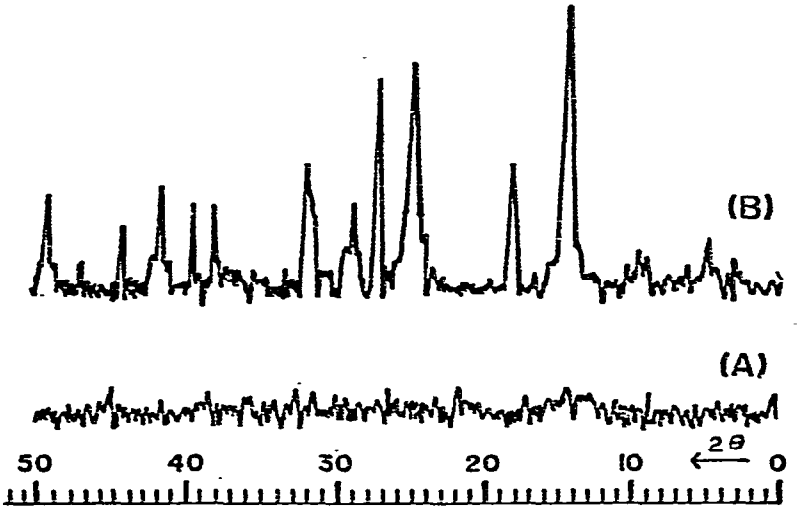

Fig. 15. X-Ray diffraction patterns of Zn_2SiO_4/Mn fluorescent glass: Cu Kα radiation. (A) Before crystallization; (B) after crystallization.

TABLE 26

PROPERTIES OF FLUORESCENT SUBSTANCES

Fluorescent substance	*Fluorescent properties*			
	*Excitation (max.) (nm)**	*Emission (max.) (nm)**	*Colour*	*Intensity*
Glass				
Zn_2SiO_4/Mn	276	527	Green	Strong
$CaWO_4$/Mn, Pb	247	445	Blue-violet	Strong
$Cd_2B_2O_5$/Mn	258	608	Orange-red	Medium
Uranium	282	518	Yellow	Strong
Lead	328	432	Blue-white	Medium
Phosphor				
Zn_2SiO_4/Mn	284	528	Green	Strong
$Sr_2P_2O_7$/Sn	266	454	Blue	Strong
YVO_4/Eu	330	619**	Red	Strong

* Uncorrected.
** Main peak wavelength.

TABLE 27

PREPARATION OF FOUR KINDS OF FLUORESCENT SINTERED PLATES

Sintered plate	*Fluorescent glass*	*Ratio of silica gel* to fluorescent glass to UV-absorbing glass (w/w/w)*
A	Zn_2SiO_4/Mn	2:1:6
B	$CaWO_4$/Mn, Pb	2:1:6
C	$Cd_2B_2O_5$/Mn	2:2:3
D**	Zn_2SiO_4/Mn-$CaWO_4$/Mn, Pb-YVO_4/Eu***	1:0.4:3

* Merck silica gel H (Type 60).
** Mixed fluorescent sintered plate.
*** Toshiba phosphor (SPD-373B) instead of $Cd_2B_2O_5$/Mn glass.

Table 28 shows the hR_F values and detection limits of steroidal hormones. The separation was excellent and the detection limit was nearly equal to that obtained with a Merck pre-coated silica gel plate.

TABLE 28

TLC OF STEROIDAL HORMONES ON ZINC SILICATE–FLUORESCENT SILICA GEL SINTERED PLATE

The limit of detection was 0.05 μg in each instance. Detection: UV (254 nm), fluorescence quenching spot.

n*	hR_F value**		
	Cortisone	*Testosterone*	*Progesterone*
1	9	40	65
2	8	42	68
3	9	42	68
4	11	44	70
5	9	45	71
$\bar{x} \pm$ s.d.***	9 ± 1	43 ± 2	68 ± 2
Merck silica gel pre-coated glass plate	5	28	52

* Recycled by soaking in chromic acid mixture after spraying with sulphuric acid.
** Solvent: chloroform–acetone (9:1).
*** Mean ± standard deviation.

Table 29 presents the hR_F values and fluorescent quenching colours of water-soluble vitamins using the mixed fluorescent[35] silica gel sintered plates. Table 30 shows the successful separation of lead, tin, chromium, nickel, copper(II), iron(III) and bismuth cations. These cations can be located rapidly by the fluorescence quenching method even after development in solvent systems containing strong acids such as nitric and hydrochloric acids. The author confirmed that the fluorescent plates could be used repeatedly by dipping in concentrated nitric acid or chromic acid mixture, without any change in their fluorescent and chromatographic properties.

In the preparation of the acid-resistant sintered plates, a "UV-rays" glass (borosilicate glass) was used instead of soda-lime glass[36], because the former is

TABLE 29

TLC OF WATER-SOLUBLE VITAMINS ON MIXED FLUORESCENT SILICA GEL SINTERED PLATE

n	*Thiamine*		*Riboflavin*		*Nicotinamide*	
	hR_F*	*Fluorescent colour***	hR_F*	*Fluorescent colour***	hR_F*	*Fluorescent colour***
1	16	Red	25	Yellow	76	Reddish violet
2	11	Red	23	Yellow	69	Reddish violet
3	13	Red	20	Yellow	68	Reddish violet
$\bar{x} \pm$ s.d.****	13 ± 3		23 ± 3		71 ± 3	

* Solvent: acetone–water (9:1).
** Detection: fluorescence quenching spot observed by continuous UV wavelength.
*** Mean ± standard deviation.

Fig. 16. SEM of Zn_2SiO_4/Mn fluorescent glass. Left, before crystallization; right, after crystallization.

TABLE 30

TLC OF INORGANIC CATIONS ON ZINC SILICATE–FLUORESCENT SILICA GEL SINTERED PLATE

Sample size: 20 μg per spot of each cation. Solvent: 2 *N* nitric acid–2 *N* hydrochloric acid–*n*-butanol (2:1:6, upper layer). Development time: 90 min per 10 cm. Detection: UV (254 nm) quenching spot.

	Inorganic cation						
	Pb^{2+}	Sn^{2+}	Cr^{6+}	Ni^{2+}	Cu^{2+}	Fe^{3+}	Bi^{3+}
hR_F value	0	5	28	40	60	67	82

more sensitive than the latter to fluorescent quenching detection. Soda-lime glass absorbs UV light in the region below 300 nm. Fig. 17 compares the UV transmittance of four kinds of glasses, and Table 31 compares the detection limits between soda-lime and "UV-rays" glass plates. The author also confirmed the thermal stability of phosphors such as zinc orthosilicate, strontium pyrophosphate and yttrium vanadate under the welding conditions by determining their physical properties and fluorescent characteristics using SEM and thermal analysis[36].

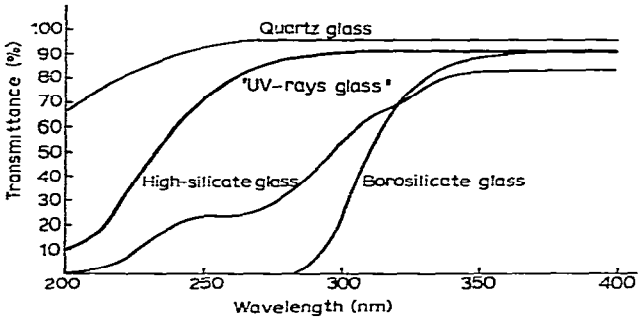

Fig. 17. Ultraviolet transmittance of various glasses. Data provided by Akagawa Glass Works and Kinmon Glass Works, Osaka, Japan.

TABLE 31

COMPARISON OF DETECTION LIMITS OF ORGANIC COMPOUNDS BETWEEN "UV-RAYS" AND SODA-LIME GLASSES AS THE BINDER

Fluorescent sintered plate	*Glass*	*Compound*	*Detection limit (μg)* *
Silica gel	"UV-rays"	Testosterone	0.05
	Soda-lime		0.2
Alumina	"UV-rays"	Thebaine	0.5
	Soda-lime		1
Kieselguhr	"UV-rays"	PCB	0.2
	Soda-lime		1

* UV quenching spot on the fluorescent background of the plate.

3.2. Flame-ionization detection scanning[37,38]

Hydrogen flame-ionization detection (FID) scanning[39], when applied to TLC, is a versatile and effective method for the quantitative determination of thermo-labile or non-volatile organic compounds, which are not detectable by gas chromatography.

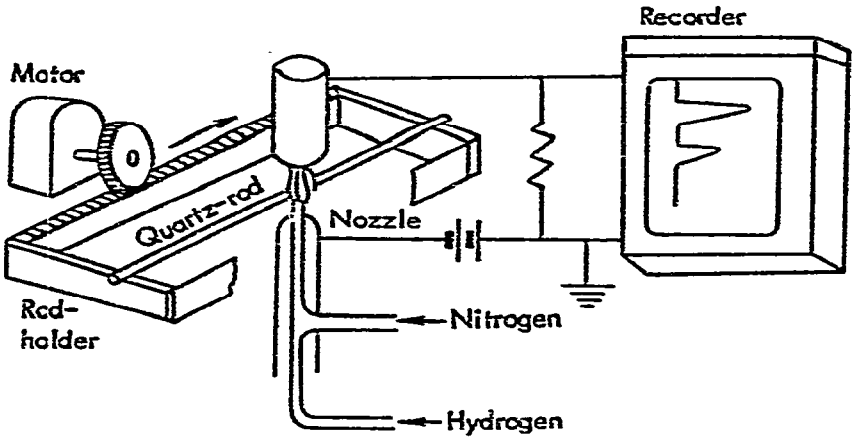

Fig. 18. Schematic diagram of flame ionization detection of TLC spots.

TABLE 32

RESPONSE CHARACTERISTICS OF SILICA GEL SINTERED RODS

Merck silica gel H–sintered glass powder (1:2). Layer thickness: 50 μm.

Sintered glass	*Glass composition (%, w/w)*						*Baseline noise* or residual response (mm)*
	SiO_2	Na_2O	PbO	B_2O_3	Al_2O_3	*Others*	
Lead silicate	66.0	3.0	30.0	—	—	1.0	62
Soda-lime	71.6	13.3	—	—	1.0	14.1	66
Borosilicate**	73.3	7.3	—	15.1	2.8	1.5	5
Borosilicate***	80.5	3.8	—	12.9	2.2	0.6	3
		ZrO_2	As_2O_3	Sb_2O_3			
Ceramic	69.4	3.0	0.5	0.5	21.8	4.8	2
Laboratory-prepared	Merck silica gel H–alumina sol (20:0.2)						2

* Maximum height from baseline.
** Akagawa Z.
*** Pyrex.

Fig. 18 gives a schematic diagram of FID scanning. The author successfully prepared silica gel– and alumina–quartz sintered rods of standard quality for use in this method. Ceramic glass was selected as a binder because it has a low baseline noise response, as shown in Table 32.

Lead silicate glass, soda-lime glass and certain borosilicate glasses showed unfavourable baseline responses to the FID, which might have resulted from the flame reaction between the FID and the glass components, disodium oxide and lead oxide. For flame-ionization detection, the author devised a compact cubic developing chamber (165 mm long × 85 mm wide × 13 mm deep). This chamber was usually lined with the silica gel sintered plates for saturation with the solvent vapour. With sintered ceramic glass–silica gel–quartz rods, a developing chamber and an FID scanner, the author was able to separate and detect minute amounts of various organic compounds such as lipids, sulphonamides, alkaloids, amino acids, water-

soluble vitamins, pesticides, polychlorinated biphenyls (PCBs), cardiac glycosides and genins, estrogens, progestins, androgens and corticoids.

The silica gel and alumina sintered rods are thermo-stable, acid resistant and can be used repeatedly without reactivation after processing in a hydrogen FID.

Fig. 19 shows the TLC separation of lipids on sintered ceramic glass–silica gel rods as located with the FID scanner. Using silica gel–sintered quartz rods, the quantitative determination of neutral lipids was achieved. The reproducibility of the response towards lipids was good. The coefficient of variation was less than 5% at three weight ratios (Table 33).

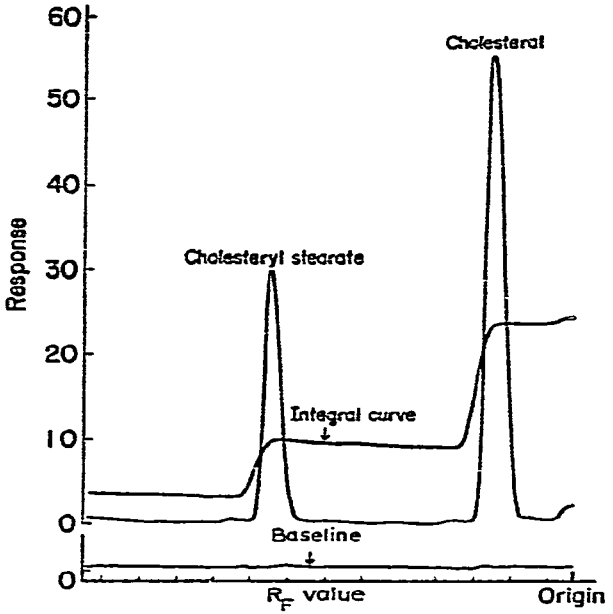

Fig. 19. TLC separation of lipids on silica gel sintered rod with FID scanner. Binder: glass ceramic.

Fig. 20 illustrates the TLC separation and determination of sulphanilic acid and sulphonamides. Alumina–sintered ceramic rods are useful for the separation and quantitation of silica gel-labile compounds.

The sintered rods, when used together with FID equipment, can facilitate both qualitative and quantitative analyses of lipids in biological fluids[40,41], heavy oil fractions in the petroleum industry[42], complex organic components in higher plants[43,44] and conjugated bile acids in bear gall[45].

4. THEORETICAL CONSIDERATIONS ON SINTERED THIN-LAYER CHROMATOGRAPHY

4.1. Inorganic anions

Thus far, qualitative analyses of inorganic anions by paper chromatography[46] and TLC[47,48] have been reported. However, the detection of the separated anions

TABLE 33

REPRODUCIBILITY OF RESPONSE TOWARDS LIPIDS ON SILICA GEL SINTERED RODS WITH FID SCANNER

Merck silica gel H–sintered glass ceramic powder (1:4), developed with *n*-hexane–diethyl ether–acetic acid (180:30:1). Sample: mixture of 1.03 mg of cholesterol and 0.98 mg of cholesteryl stearate was dissolved in 200 μl of tetrahydrofuran.

Sample size (μg)	*Run No.**	*Cholesterol*		*Cholesteryl stearate*	
		Peak area		*Peak area*	
		mm²	*%*	*mm²*	*%*
5	1	17.5	60.8	11.3	39.2
	2	18.2	60.1	12.1	39.9
	3	16.8	59.0	11.7	41.0
	4	15.0	61.7	9.3	38.3
	5	16.3	59.4	10.9	40.6
	$\bar{x} \pm \sigma$	17 ± 1	60 ± 1	11 ± 1	40 ± 1
	C.V.		1.7		2.5
10	1	39.4	57.1	29.6	42.9
	2	41.8	55.3	32.5	43.7
	3	41.5	60.1	27.6	39.9
	4	40.2	61.9	24.8	38.1
	5	43.8	58.2	31.4	41.8
	$\bar{x} \pm \sigma$	41 ± 2	56 ± 2	30 ± 3	41 ± 2
	C.V.		3.6		4.4
15	1	83.5	60.5	54.5	39.5
	2	84.2	57.3	62.5	42.7
	3	76.0	58.9	53.0	41.1
	4	78.0	57.3	58.0	42.6
	5	80.9	59.6	54.7	40.4
	$\bar{x} \pm \sigma$	80 ± 3	59 ± 1	57 ± 1	44 ± 2
	C.V.		1.7		2.5

* $\bar{x} \pm \sigma$ = mean ± standard deviation. C.V. = coefficient of variation.

with various colour reagents was troublesome. Some anions (iodide, bromide, chloride, thiocyanate, thiosulphate and sulphite) are known to quench the fluorescence of quinine, fluorescein and eosin, and oxyanions such as iodate, bromate and nitrate have a quenching effect on the fluorescence of anthranilic acid and naphthol[49]. Nishikawa *et al.*[50] have applied the fluorescence characteristics of morin–metal complexes to the fluorimetry of metal ions.

The author has established a simple and rapid method of detecting inorganic anions using the fluorescent aluminium–morin complex[51]. 29 inorganic anions were developed on paper and cellulose thin layers and located under UV light at 365 nm after spraying with this complex. Of these anions, 25, such as halides, halogenoxy acids, sulphur-containing anions, cyanoferrates, chromates, nitrite, nitrate, phosphate, tungstate, molybdate and vanadates, showed fluorescence quenching on the chromatograms. The remaining anions (arsenite, selenite and selenate) did not show a substantial quenching effect. Tables 34 and 35 show the separation and detection of these inorganic anions. The fluorescence quenching effect of the anions could be classified into three groups: strong (violet colour: iodo- and bromoxy

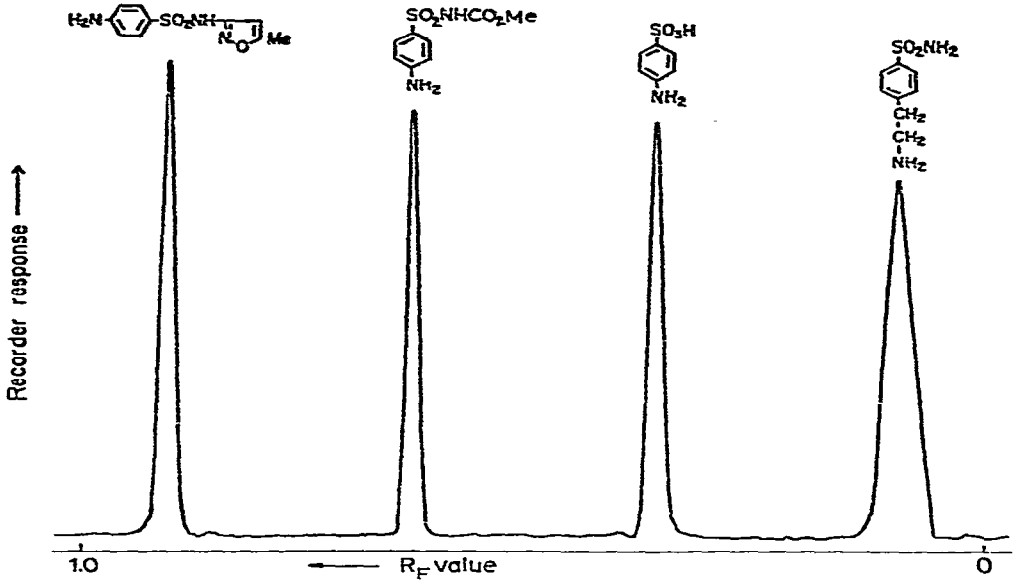

Fig. 20. TLC separation of sulphanilic acid and sulphonamides on silica gel sintered rods with FID scanner. Developer: *n*-butanol–ethanol–0.1 *N* acetic acid (3:1:1).

TABLE 34

QUALITATIVE ANALYSIS OF INORGANIC ANIONS

Anion	*Cation*	*hR_F value of anion**			*Fluorescent colour***	*Detection*
		TLC		*PPC*		
F^-	Na^+	0		0	Blue	$AgNO_3$–UV (254 nm)
Cl^-	K^+	38		45	Blue	
Br^-	Na^+	60		57	Blue	
I^-	K^+	77	78***	72	Blue	
IO_3^-	Na^+	13	30***	16	Violet	KI–HCl
IO_3^-	K^+	14		19	Violet	
IO_4^-	Na^+	12	31***	24	Violet	
BrO_3^-	K^+	43		57	Violet	
ClO_2^-	Na^+	35		45	Yellow	
ClO_3^-	Na^+	63		70	Yellow	
ClO_4^-	Na^+	80		78	Yellow	
ClO_4^-	K^+	78		70	Yellow	
S^{2-}	Na^+	10	33§	11	Blue	$AgNO_3$
$S_2O_3^{2-}$	Na^+	8	30§	9	Blue	$AgNO_3$
SCN^-	K^+	85		70	Yellow	$Fe(NO_3)_3$–HNO_3

* Solvent system: 28% ammonia solution–acetone–*n*-butanol (60:130:30). TLC: Merck precoated cellulose plate; 90 min per 10 cm. PPC: Toyo filter-paper No. 51A; 180 min per 20 cm. Sample size: 10 $\mu g/\mu l$.

** Under UV irradiation at 365 nm after spraying with 0.3 m*M* aluminium isopropoxide and 1 m*M* morin in ethanol.

*** Dioxane–water (3:2); 90 min per 10 cm.

§ Acetone–28% ammonia solution (3:2); 80 min per 10 cm.

TABLE 35

QUALITATIVE ANALYSIS OF INORGANIC ANIONS

Anion	*Cation*	*hR_F value of anion**		*Fluorescent colour***	*Detection*
		TLC	*PPC*		
$Fe(CN)_6^{4-}$	K^+	2	3	Violet	$Fe(NO_3)_3$–HNO_3
$Fe(CN)_6^{3-}$	K^+	32	20	Violet	$Fe(NO_3)_3$–HNO_3
CrO_4^{2-}	K^+	10***	—	Violet	$Pb(OAc)_2$
CrO_7^{2-}	K^+	68***	—	Violet	$Pb(OAc)_2$
NO_2^-	Na^+	55	62	Violet	Sulphanilic acid, etc.
NO_3^-	Na^+	60	72	Violet	Sulphanilic acid, etc.
WO_4^{2-}	Na^+	44‡	—	Yellow	$AgNO_3$
MoO_4^{2-}	Na^+	36‡	—	Green	$AgNO_3$
VO_3^-	Na^+	20‡	—	Blue	Diaminobenzidine–KI–$SnCl_2$–HCl
VO_4^{3-}	Na^+	16‡	—	Blue	Diaminobenzidine–KI–$SnCl_2$–HCl
AsO_2^-	Na^+	43***	—	Weak	Dithizone–HCl
AsO_4^{3-}	Na^+	42***	—	Weak	Dithiozne–HCl
SeO_3^{2-}	Na^+	29***	—	Weak	$SnCl_2$–HCl
SeO_4^{2-}	Na^+	30***	—	Weak	$SnCl_2$–HCl

* Solvent system, TLC plate, PPC filter-paper and sample size as in Table 34.
** As in Table 34.
*** Acetone–acetic acid–water (20:1:10); 120 min per 10 cm.
‡ As in Table 34..

acids, sulphite, sulphate, cyanoferrates, chromates, nitrite, nitrate and phosphate), medium (blue colour: halide, sulphide and vanadates) and weak (yellow colour: chlorooxy acids, thiocyanate and tungstate).

The mechanism of the fluorescence quenching caused by these inorganic anions might involve a reaction that converts the aluminium–morin complex into non-fluorescent aluminium salts:

$$Al(C_{15}H_9O_7)_3 + 6X^- \rightarrow AlX_6^{3-} + 3C_{15}H_{10}O_7 \quad (1)$$

fluorescent non-fluorescent

where X is a monodentate ligand.

In the separation of these inorganic anions, iodoxy, sulphate, silicate, borate, phosphate and fluoride were strongly adsorbed on paper or cellulose thin layers. Chloroxy, chlorite, nitrate, nitrite, chloride and bromide were moderately adsorbed on the stationary phase. Perchlorate and iodide were weakly adsorbed on the stationary phase when the basic, polar mobile phase 28% ammonia solution–acetone–*n*-butanol was used.

4.2. Organic compounds

4.2.1. Steroidal sapogenins[52]

There are many steroidal sapogenins of natural origin[53] with the general structure shown in Fig. 21. The author conducted systematic TLC analysis of 40 kinds of steroidal sapogenins from higher plants such as *Dioscores*, *Heloniopsis*, *Metanarthecium*, *Smilax*, *Rhodea*, *Convallaria* and *Digitalis* (Table 36).

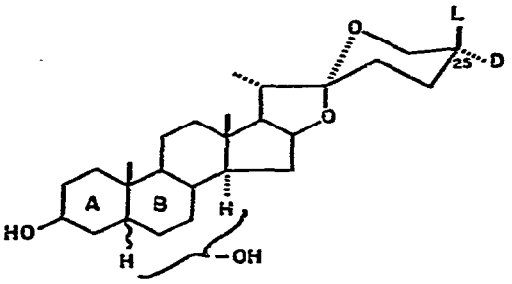

Fig. 21. Structure of steroidal sapogenins.

TABLE 36

STEROIDAL SAPOGENINS SEPARATED BY TLC ON SINTERED PLATES

Compound	*M.p.(°C)*	*A/B ring*	C_{25}*-Me*	*OH*	*Plant source*
Chiapagenin	249–251	Δ^5	L	3β,12β	*Dioscorea chiapasensis*
Isochiapagenin	233–235	Δ^5	D	3β,12β	*Dioscorea chiapasensis*
Chlorogenin	265–266	*trans*	D	3β,6α	Synthetic from laxogenin
β-Chlorgenin	240–242	*trans*	L	3β,6β	Synthetic from laxogenin
Convallamarogenin	253–258	*cis*	$=CH_2$	1β,3β	*Convallaria majalis*
Digitogenin	296	*trans*	D	2α,3β,15β	*Digitalis purpurea*
Diosgenin	203–206	Δ^5	D	3β	*Dioscorea tokoro*
Diotigenin	281–282	*cis*	L	2β,3α,4β	*Dioscorea tenuipes*
Isodiotigenin	279–281	*cis*	D	2β,3α,4β	*Dioscorea tokoro*
Gentrogenin	213–214	Δ^5	D	3β-12-oxo	*Heloniopsis orientalis*
Gitogenin	272	*trans*	D	2α,3β	*Digitalis purpurea*
Neogitogenin	250–254	*trans*	L	2α,3β	*Anemarrhena asphodeloids*
Hecogenin	260	*trans*	D	3β,12-oxo	*Agave rigida* var. *sisalana*
Heloniogenin	212–213	Δ^5	D	3β,12α	*Heloniopsis orientalis*
Kitigenin	298	*cis*	D	1β,3β,4β,5β	*Reinechea cornea*
Kogagenin	310	*cis*	D	1β,2β,3α,5β	*Dioscorea tokoro*
Kryptogenin	189	Δ^5	—	3β,16,22-diox	*Heloniopsis orientalis*
Laxogenin	210–212	*trans*	D	3β,6-oxo	*Smilax sieboldii*
Luvigenin	183–185	—	D	Aromatic A ring 4-Me	*Metanarthecium luteo-viride*
Markogenin	149–151	*cis*	L	2β,3β	*Anemarrhena asphodeloides*
Metagenin	264–265	*cis*	D	2β,3β,11α	*Metanarthecium luteo-viride*
Meteogenin	157–158	—	D	Aromatic A ring 1-Me,11α	*Metanarthecium luteo-viride*
Narthogenin	214–216	Δ^5	L	3β,27	*Metanarthecium luteo-viride*
Isonarthogenin	238–240	Δ^5	D	3β,27	*Metanarthecium luteo-viride*
Pennogenin	234–235	Δ^5	D	3β,17α	*Heloniopsis orientalis*
Rhodeasapogenin	284–285	*cis*	L	1β,3β	*Rohdea japonica*
Isorhodeasapogenin	240–241	*cis*	D	1β,3β	*Convallaria keiskei*
Ruscogenin	204–206	Δ^5	D	1β,3β	*Ruscus aculeatus*
Sarsasapogenin	198	*cis*	L	3β	*Asparagus cochinchinensis*
Smilagenin	183–188	*cis*	D	3β	*Allium grayi*
Tigogenin	205	*trans*	D	3β	*Digitalis purpurea*
Neotigogenin	198–199	*trans*	L	3β	*Smilax sieboldii*
Tokorogenin	266–268	*cis*	D	1β,2β,3α	*Dioscorea tokoro*
1α-Tokorogenin	218–219	*cis*	D	1α,2β,3α	Synthetic from kogagenin
Neotokorogenin	266–269	*cis*	L	1α,2β,3α	*Dioscorea tenuipes*
Yonogenin	242–243	*cis*	D	2β,3α	*Dioscorea tokoro*
Neoyonogenin	198–199	*cis*	D	2β,3α	*Dioscorea tenuipes*

TABLE 37

TLC SEPARATION OF STEROIDAL SAPOGENIN ISOMERS ON SILICA GEL AND ALUMINA SINTERED PLATES

A/B cis-, trans- isomers		*hR_F value**							
Compound	*cis/trans*	*Silica gel*				*Alumina*			
		Sintered		*Merck*		*Sintered*		*Merck*	
Smilagenin	*cis*	72		39		85		63	
Tigogenin	*trans*	63	*Δ9*	30	*Δ9*	71	*Δ14*	52	*Δ11*
Sarsasapogenin	*cis*	71		37		87		63	
Neotigogenin	*trans*	63	*Δ8*	30	*Δ7*	79	*Δ8*	54	*Δ9*
2α-Samogenin	*cis*	21		9		27		17	
Gitogenin	*trans*	30	*Δ9*	10	*Δ1*	40	*Δ13*	25	*Δ8*

* Benzene–ethanol (17:1). Distance travelled: 15 cm. Detection: conc. H_2SO_4. *Δ* = Difference.

Table 37 shows the excellent resolution of stereoisomeric pairs of sapogenins. They differ from each other in the A and B ring junction: 2α-samogenin (an A/B *cis*-isomer) and gitogenin (an A/B *trans*-isomer). Smilagenin and tigogenin, and sarsasapogenin and neotigigenin, are also A/B *cis*- and *trans*-isomers, respectively. As shown in Table 38, a good resolution was also obtained with sapogenins differing in the configuration of the hydroxyl group, such as chlorogenin (an equatorial 6α-hydroxyl compound) and β-chlorogenin (an axial 6β-hydroxyl compound) and tokorogenin (an equatorial 1β-hydroxyl compound) and 1α-tokorogenin (an axial 1α-hydroxyl compound). On the other hand, separation was not possible of diastereomeric pairs differing in the configuration at the C_{25} methyl group, that is, D- and L-isomers, such as gitogenin and neogitogenin, smilagenin and sarsasapogenin, tigogenin and neotigogenin, tokorogenin and neotokorogenin, and yonogenin and neoyonogenin (Table 39). As shown in Table 38, however, clear separations were obtained with

TABLE 38

TLC SEPARATION OF STEROIDAL SAPOGENIN ISOMERS ON SILICA GEL AND ALUMINA SINTERED PLATES

Axial equatorial epimers	*hR_F value**							
	Silica gel				*Alumina*			
	Sintered		*Merck*		*Sintered*		*Merck*	
Chlorogenin (6α-OH, eq.)	21		5		23		11	
β-Chlorogenin (6β-OH, ax.)	27	*Δ6*	7	*Δ2*	32	*Δ9*	15	*Δ4*
Tokorogenin (1β-OH, eq.)	13		5		11		3	
1α-Tokorogenin (1α-OH, ax.)	18	*Δ5*	7	*Δ2*	15	*Δ4*	4	*Δ1*
Narthogenin (27-OH, ax.)**	60		8		53		6	
Isonarthogenin (27-OH, eq.)**	55	*Δ5*	7	*Δ1*	46	*Δ7*	5	*Δ1*

* Benzene–ethanol (17:1). Distance travelled: 15 cm. Detection: conc. H_2SO_4. *Δ* = Difference.
** Chloroform–acetone (9:1).

TABLE 39

TLC OF STEROIDAL SAPOGENIN C_{25}-METHYL ISOMERS ON SILICA GEL AND ALUMINA SINTERED PLATES

Diastereomer	*hR_F value**			
	Sintered	*Silica gel*	*Sintered*	*Alumina*
	*A***	*B****	*A***	*B****
Chiapagenin (L)	19	43	15	53
Isochiapagenin (D)	17	41	15	52
Diotigenin (L)	75§	6	35§	0
Isodiotigenin (D)	76§	6	36§	0
Gitogenin (D)	6	20	2	9
Neogitogenin (L)	6	20	2	9
Rhodeasapogenin (L)	20	40	20	43
Isorhodeasapogenin (D)	21	42	21	45
Sarsasapogenin (L)	50	78	64	74
Smilagenin (D)	47	80	65	74
Tigogenin (D)	32	76	50	68
Neotigogenin (L)	32	74	50	68
Tokorogenin (D)	81§	9	44§	0
Neotokorogenin (L)	82§	9	44§	0
Yonogenin (D)	4	25	2	15
Neoyonogenin (L)	4	25	2	15

* Distance travelled: 15 cm per 2 h. Detection: conc. H_2SO_4.
** Solvent A: benzene–ethanol (17:1).
*** Solvent B: chloroform–acetone (9:1).
§ Solvent C: chloroform–methanol (9:1).

diastereomers with a hydroxymethyl group instead of a methyl substituent at C_{25}, that is, narthogenin (L-form) and isonarthogenin (D-form), which resisted separation on laboratory-prepared plates and commercial pre-coated plates. This result indicates that the polarity of the substituent attached to the steroidal sapogenin nucleus influences the resolution of the diastereoisomers. Table 40 shows the excellent reproducibility of separations with various polar sapogenins.

TABLE 40

REPRODUCIBILITY OF hR_F VALUES OF STEROIDAL SAPOGENINS ON SILICA GEL SINTERED PLATES

Solvent: chloroform–acetone (9:1). Detection: cinnaminaldehyde–antimony richloride.

Compound	*Run No.*										$\bar{x} \pm \sigma$*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	
Luvangenin	81	83	82	83	87	85	78	87	80	85	83 ± 3
Diosgenin	59	62	62	62	66	65	58	66	60	65	63 ± 3
Pennogenin	49	52	52	52	56	49	56	56	53	55	53 ± 5
Hecogenin	35	42	42	42	46	46	40	45	41	44	43 ± 3
Chiapagenin	25	26	26	26	28	28	24	27	25	26	26 ± 1
Yonogenin	17	22	23	21	22	22	19	21	19	22	21 ± 2
Gitogenin	13	19	19	19	20	18	16	17	17	19	18 ± 2
Kogagenin	1	1	1	1	2	1	2	1	1	2	1 ± 0

* Mean ± standard deviation.

The application of sintered TLC to crude extracts containing steroidal sapogenins from higher plants revealed the presence of diotigenin, tokorogenin and yonogenin in the aerial part of *Dioscorea tokoro*, tokorogenin and diosgenin in its rhizome, diotigenin, yonogenin and sterol in its seedlings, ruscogenin and sterol in *Ophyopogon japonicus*, and diotigenin, tokorogenin, yonogenin, sterol and chlorophylls in *Dioscorea tenuipes*[54]. These components had not been separated by paper chromatography or TLC on laboratory-prepared and pre-coated plates.

4.2.2. Organic phosphates, sulphates and nitrates[22,55]

Sulphate[56,57] and phosphate[58,59] esters of organic compounds are often extracted and isolated from natural products and biological materials. Paper chromatography[60], TLC[61], column chromatography[62] and, recently, high-performance liquid chromatography[63] of these organic esters have been reported.

4.2.2.1. Steroidal sulphates and nitrates. Cholesteryl-3-O-sulphate [mobile phase: (a) *n*-butanol–acetic acid–water (3:1:1) and (b) benzene–acetone (9:1)] and dehydroisoandrosterone sulphate [mobile phase: (a) and (c) chloroform–acetone (4:1)] were chosen as test compounds for silica gel sintered TLC. Both esters moved from the original point in the acidic mobile phase (a), but not in the neutral mobile phases (b) and (c), presumably owing to strong adsorption on to the silica gel thin layer containing soda-lime glass powder. The hR_F values were small compared with those of the free steroids [cholesterol, 70 in (a), 43 in (b); sulphate, 54 in (a), 0 in (b); dehydroisoandrosterone, 73 in (a), 54 in (c); sulphate, 37 in (a), 0 in (c)].

6-Nitroandrostenediol diacetate, a steroidal nitrate, moved smoothly even in the neutral mobile phase benzene–ethyl acetate (4:1). Its hR_F value (55) was slightly lower than that of the steroid itself (61).

4.2.2.2. Sugar phosphates and sulphates. Table 41 shows the hR_F values of hexose phosphates and free hexoses on silica gel sintered plates and Merck silica gel

TABLE 41

TLC OF SUGAR PHOSPHATES ON SILICA GEL PLATES

*Hexose phosphate**	*hR_F value***			
	*REPLATE****	*ΔhR_F*	*MS*[‡]	*ΔhR_F*
Aldose:				
Glucose	73 ± 3		30 ± 1	
Glucose-1-phosphate	50 ± 2	23	9 ± 2	21
Glucose-6-phosphate	55 ± 3	18	10 ± 1	20
Ketose:				
Fructose	73 ± 2		33 ± 1	
Fructose-1-phosphate	51 ± 2	22	10 ± 1	23
Fructose-6-phosphate	59 ± 4	14	12 ± 1	21
Fructose-1,6-diphosphate	35 ± 3	38	5 ± 2	28

* Products of Wako Chemicals.

** Developer, BAW311; detection, Al–morin reagent followed by conc. sulphuric acid; development rate per 10 cm, 2.0 h (REPLATE), 2.5 h (Merck silica gel–glass plate); $n = 5$.

*** REPLATE: trade-name of silica gel sintered plate distributed by Yamamoto Scientific (Tokyo, Japan).

‡ Merck silica gel–glass plate.

glass plates. The hR_F values on REPLATEs were larger than those on the Merck plates. The difference in hR_F values (ΔhR_F, 38) between fructose and its 1,6-diphosphate on the REPLATE was almost twice that (22, 14) between fructose and its 1- or 6-monophosphate, showing the additivity of ΔhR_F values for mono- and diphosphates.

Table 42 gives the hR_F values of three kinds of hexoses and their sulphates on REPLATEs. Additivity of ΔhR_F values was also observed, as with hexose phosphates.

TABLE 42

TLC OF SUGAR SULPHATES ON SILICA GEL PLATES

*Hexose sulphate**	hR_F *value***			
	*REPLATE****	ΔhR_F	*MS*§	ΔhR_F
Aldose				
Glucose	72		27	
Glucose-6-sulphate	62	10	17	10
Glucose-1,6-disulphate	55	17	10	17
Galactose	72		28	
Galactose-6-sulphate	61	11	16	12
Galactose-1,6-disulphate	51	21	10	18
Ketose				
Fructose	71		32	
Fructose-6-sulphate	63	8	21	11
Fructose-1,6-disulphate	56	15	12	9

* Synthesized by Soda's method[64].
** As in Table 41.
*** As in Table 41.
§ As in Table 41.

4.2.2.3. Adenosine phosphates. Table 43 shows the results of the TLC separation of adenosine 5'-mono-, -di- and -triphosphates (AMP, ADP and ATP) on polyethyleneimine (PEI)–cellulose–polyethylene sintered sheets[20]. The hR_F values of adenosine phosphates decrease as the number of phosphate groups increases, in a similar

TABLE 43

TLC OF NUCLEOTIDES ON PEI–CELLULOSE SINTERED SHEET*

The cellulose sintered sheet was impregnated with 1% PEI (mol. wt. 30,000–40,000) hydrochloride aqueous solution. Detection: UV (254 nm) and Al–morin.

Compound	hR_F *value*	*Developer**
Adenosine-5'-monophosphate	55 (ΔhR_F 25)	1.0 *M* LiCl
Adenosine-5'-diphosphate	30 (ΔhR_F 23)	1.0 *M* LiCl
Adenosine-5'-triphosphate	7	1.0 *M* LiCl

* Development rate: 60 min per 10 cm.

manner to the steroidal sulphates mentioned above. As PEI–cellulose is an anion exchanger, sorption of the phosphate anion in the substrate on the stationary phase (weakly basic cation ) is more evident than in the partition chromatography of the inorganic and organic anions cited above.

5. CONCLUSION

The finding of unexpected stability of inorganic and organic adsorbents under "welding" conditions led the author to the preparation of various sintered plates, rods and sheets. The TLC separation data for a variety of compounds presented here show that parallelism exists between mobilities on sintered plates and those on laboratory-prepared and pre-coated plates, indicating that the nature of the binder does not affect the adsorption process that occurs on the surface of adsorbents. In general, sintered plates and rods require developing solvents of slightly lower polarity to give mobilities comparable to those on laboratory-prepared and pre-coated plates. It is noteworthy that sintered plates almost invariably allow satisfactory separations of polar substances such as steroidal sapogenin isomers, which resist separation on laboratory-prepared or pre-coated plates. Organic phosphates and sulphates also gave clear separations only on the sintered plates, leading to the additivity rules for their differences in hR_F values.

The excellent stability of the sintered plates, rods and sheets towards abrasion

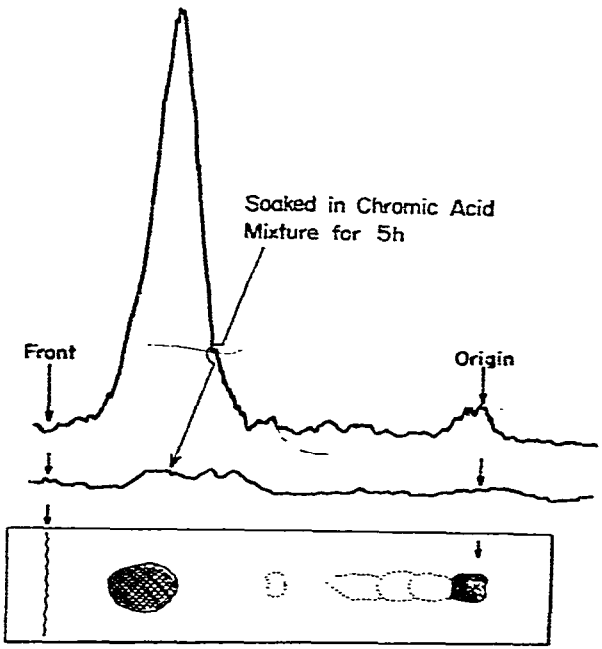

Fig. 22. Autoradiograph of ^{3}H-labelled steroid on silica gel sintered plate[9].

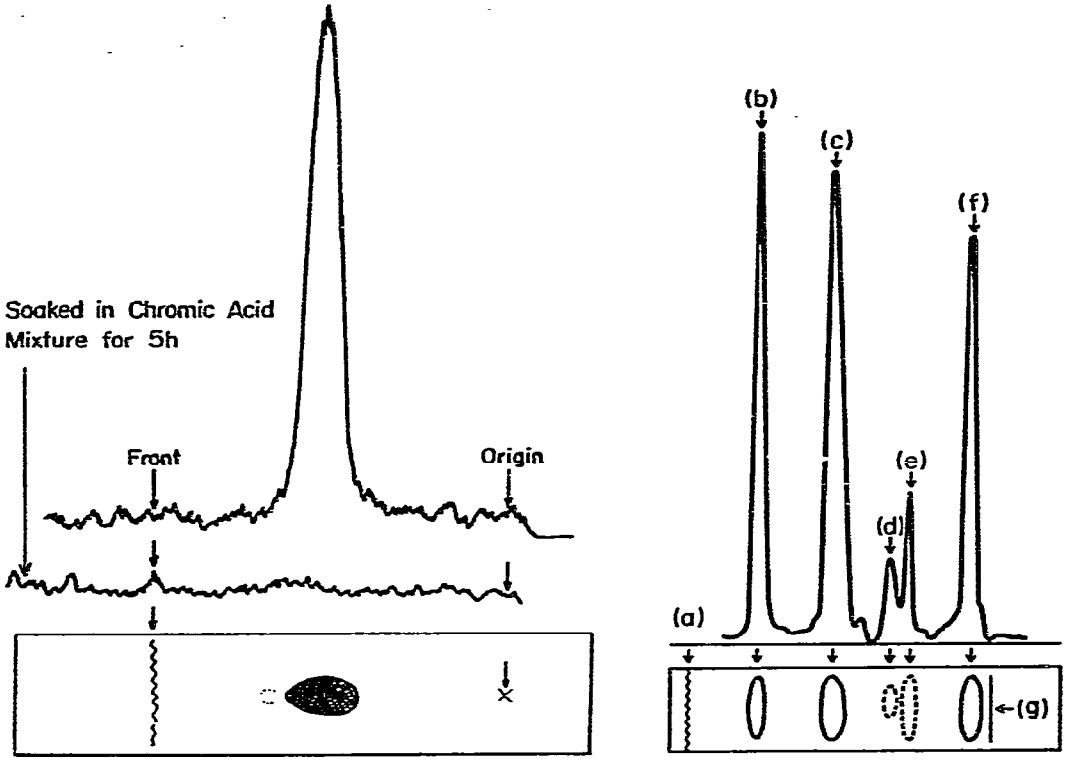

Fig. 23. Autoradiograph of ^{14}C-labelled steroidal mixtures on silica gel sintered plate[9].

Fig. 24. Thin-layer densitometry of lipids with silica gel sintered plate[9,65]. (a) Front; (b) cholesteryl ester; (c) triglyceride; (d) diglyceride; (e) monoglyceride; (f) phospholipids; (g) origin.

heat and acids resulted in a superior reproducibility of the separation of various compounds and resolved some difficulties in current TLC techniques. For example, one can easily perform the separation and determination of radioactive compounds, the thin-layer densitometry of biological materials and the bioautography of antibiotics. Figs. 22 and 23 show autoradiograms of ^{3}H- and ^{14}C-radioactive steroids, and Fig. 24 shows the quantitative determination of serum lipids[66]. Fig. 25 compares a bioautogram on a silica gel sintered plate and that on a laboratory-prepared plate, showing the superiority of the sintered plate in the separation and detection of inhibition zones

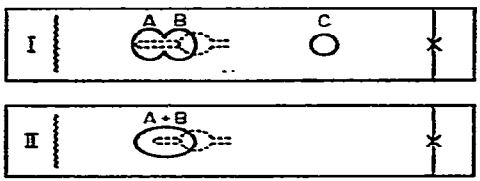

Fig. 25. Comparison of bioautography test with silica gel sintered and laboratory-prepared plates for antibacterial screening of antibiotics against *Bacillus subtilis* PCI 219. Plate I, sintered plate F; plate II, home-made plate GF. A, B and C: antibacterial spots. From ref. 9.

given by antibiotic substances. Further, the sintered plates, rods and sheets should be very useful for reversed-phase TLC, thin-layer electrophoresis, TLC with flame-ionization detection[67–69] and, probably, for many other purposes in the vast field of chromatographic analysis.

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7. SUMMARY

A new type of thin-layer chromatographic (TLC) plates, "sintered-glass thin-layer plates", was prepared, the layer of which consists of silica gel, alumina, Kieselguhr or another porous adsorbent, fixed with sintered glass.

These sintered plates are mechanically stable, heat- and acid resistant, and can be used in the same way as the usual laboratory-prepared or commercial TLC plates. It appears that the nature of the binder for the adsorbent particles does not essentially affect the separation process that occurs on the surface of the adsorbents. The sintered plates do not contain organic binders and are resistant to, *e.g.*, heating after being sprayed with corrosive reagents. The developed sintered plates can be regenerated readily by soaking in cleaning solutions, washing with water and reactivating by heating. The reproducibility of chromatographic separations is further improved by recycled use of the sintered plates. It was also discovered that TLC using sintered rods with flame-ionization detection is very useful for qualitative and quantitative analyses of organic compounds. The method of preparation of the sintered plates and rods, and chromatographic separation data of various organic and inorganic compounds on these sintered materials, are presented. "Welding" mechanisms among adsorbent, binder and support, and thermal stability of the adsorbent at "welding" conditions are discussed.

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