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THIN-LAYER CHROMATOGRAPHY ON ALUMINA–SINTERED GLASS PLATES

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SUMMARY

A new alumina pre-coated medium for thin-layer chromatography, which has rigid alumina layer coherency, was prepared. Its chromatographic characteristics were compared with those of commercially available alumina pre-coated plates and sheets.

INTRODUCTION

The advantages of using thin-layer adsorbents pre-coated on rigid and flexible supports has profoundly affected the application of thin-layer chromatography in separation chemistry. In a previous paper[1], we reported the preparation of a new pre-coated plate made of a mixture of silica gel and fused glass powder, for thin-layer chromatography.

We have now developed an alumina–sintered glass plate, which consists of a 200-μm layer of a mixture of alumina and fused glass powder bound to a 1.3-mm-thick glass support. The separation of various classes of compounds on the alumina–sintered glass plates was compared with that on other commercially available pre-coated plates and sheets.

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 prepared had a particle size as fine as that of alumina for TLC use.

*Preparation of alumina–sintered glass plates**. A mixture of 1 part of alumina for TLC and 1–6 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 obtained was spread on soda-lime glass plates in the usual manner and air-dried, and the layer was then heated in an electric furnace at 450–750° for several minutes to yield an alumina–fused glass layer (layer thickness

* Sintered glass plates are commercially available under the name of REPLATE® (Yamoto Scientific Co., Ltd., Nihonbashi Honcho 2-9, Chuoh-ku, Tokyo, Japan).

ca. 200 μm). It is essential that the glass powder is fused without melting the alumina, so as to protect the chromatographic activity of the layer.

Development

All chromatographic experiments, whether on plates or sheets, were carried out in a cylindrical or rectangular chromatographic chamber containing a saturated atmosphere of the developing solvent. For repeated use, the sprayed sintered glass plates were soaked in a cleaning solution such as chromic acid, concentrated nitric acid or an organic solvent, washed with running water and re-activated by heating them at 110° for 30–60 min.

Detection

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

Alkaloids were detected by spraying with Dragendorff reagent[2] or by UV-irradiation in the presence of mixed fluorescent materials[3] with an improved light source, giving coloured quenching spots.

RESULTS AND DISCUSSION

Solvent migration 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 alumina were used, and were re-activated prior to use. Table I shows the migration times of solvents of different polarities. The rates on the sintered glass plate are slightly faster that those on the polyester

TABLE I

SOLVENT MIGRATION RATE ON PRE-COATED ALUMINA 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*)	*TCI Spotfilm* *** (*100 μm*)
n-Hexane	30	73	30
Carbon tetrachloride	29	93	47
Benzene	27	82	26
Chloroform	25	78	34
Diethyl ether	18	53	20
Ethyl acetate	20	61	28
Acetone	17	47	13
Methanol	30	96	30

* Merck aluminium oxide neutral (Type T)–sintered glass powder (1:3).
** Merck glass plate, aluminium oxide F_{254} (Type T).
*** Tokyo Chemical Industry alumina F polyester sheet.

sheet and much faster than those on the glass plate. This difference can be attributed to the unique formation of capillaries[4] in the sintered glass plate.

Separation of organic compounds on alumina–sintered glass plates

Separation of azo test dyes. Table II and Fig. 1 show the separation of a test dye mixture containing azobenzene, sudan yellow and *p*-aminoazobenzene on three pre-coated materials. The hR_F values obtained for azo test dyes on the sintered glass plate were higher than those on the glass plate and polyester sheet, which indicates that the sintered glass plate is essentially different from the other two

TABLE II

hR_F VALUES OF AZO DYES ON ALUMINA PRE-COATED PLATES

Solvent: carbon tetrachloride. Results are mean values from five runs on different plates. The standard deviation of each hR_F value was less than 3.

Azo dye	*Sintered glass plate (200 μm)*	*Merck glass plate (250 μm)*	*TCI polyester sheet (100 μm)*
p-Aminoazobenzene	16	2	4
Sudan yellow	52	17	30
Azobenzene	65	40	50

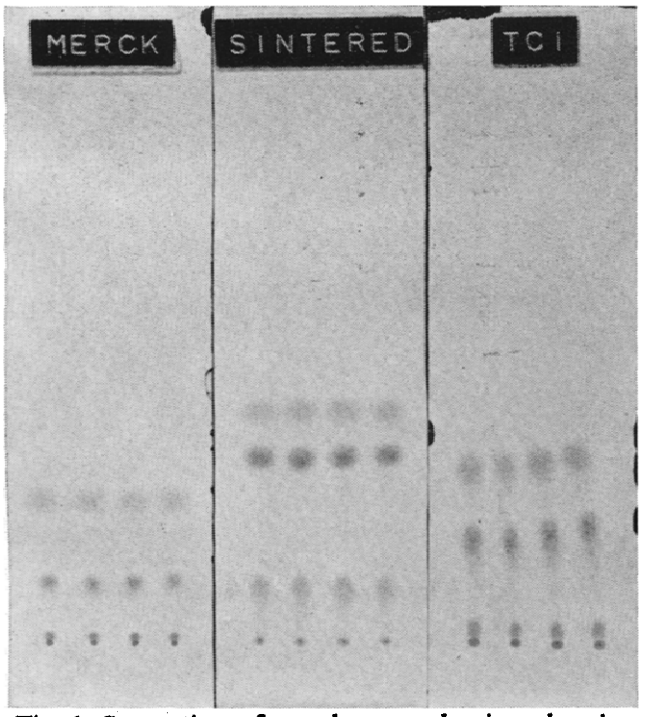

Fig. 1. Separation of azo dyes on alumina glass bases and polyester base. Left to right: Merck pre-coated glass plate; sintered plate; TCI Spotfilm. Solvent: carbon tetrachloride. Dyes separated in order of decreasing hR_F values: azobenzene, sudan yellow and *p*-aminoazobenzene.

TABLE III

hR_F VALUES OF ALKALOIDS ON TWO TYPES OF ALUMINA PLATE

Thin-layer: (A) alumina–sintered glass powder (1:4) (10% of a mixed fluorescent substance[3] was added to each layer; the alumina was Merck aluminium oxide neutral (Type T)); (B) alumina +10% gypsum. Solvent: benzene–chloroform–diethylamine (9:4:1). Detection: fluorescent colour in UV light followed by spraying with Dragendorff reagent. *hR_F* values are mean values from five runs.

Compound	*hR_F value of alkaloid*		*Detection colour*[3]
	A	*B*	
Codeine	54	50	Reddish violet
Thebaine	82	90	Bright blue
Atropine	49	55	Violet
Yohimbine	44	43	Blue
Reserpine	71	68	Reddish violet
Ergotamine	28	23	Blue
Brucine	67	71	Blue
Strychnine	72	73	Violet
Nicotine	80	82	Reddish violet
Quinine	55	46	Reddish violet
Cinchonine	57	59	Blue
Aconitine	73	78	Reddish violet
Emetine	76	75	Violet
Caffeine	48	59	Red

thin-layer materials in the layer components when used either with or without prior activation. This effect is attributed to the weaker chromatographic activity of the sintered glass plate.

Other examples which show that the sintered glass plates are as efficient as conventional glass plates involve the separation of alkaloids, steroids and sterols.

Separation of alkaloids. A number of alkaloids were separated on the sintered glass plates and on home-made glass plates coated to a wet thickness of 300 μm. Both plates contained 10% of a mixed fluorescent substance[3] in alumina. Table III gives the hR_F values of the alkaloids. In the separation of alkaloids, the hR_F values of each compound were similar on the two types of thin-layer plate.

Separation of steroids. Sapogenins, sterols and cardiac glycosides and genins were studied and Tables IV and V (and also Tables VII and VIII) show the separation of these compounds on an alumina–sintered glass plate.

Reproducibility of separation on alumina–sintered glass plates

The sintered glass plates can be used repeatedly as described previously[1], and Table VI gives the reproducibility of the hR_F values of alkaloids on alumina–sintered glass plates that were used repeatedly. With alumina–sintered glass plates which were different from silica gel–sintered glass plates[1], it was observed that as the number of recoveries carried out increased, each alkaloid tended towards the higher hR_F value gradually. This effect is attributed to the decrease in the chromatographic properties of the alumina–sintered glass plates after cleaning with a solution

TABLE IV

hR_F VALUES OF VARIOUS STEROIDAL SAPOGENINS ON ALUMINA-SINTERED GLASS PLATES

Detection with concentrated sulphuric acid. Solvents: I= benzene–ethanol (17:1); II= benzene–ethyl acetate (4:1); III= chloroform–acetone (9:1); IV= chloroform–ethanol (9:1); V= chloroform–methanol (9:1). Results are mean values from five runs on different plates. The standard deviation of each hR_F value was less than 3.

Compound	*hR_F value of sapogenin*					*Colour with sulphuric acid*
	I	*II*	*III*	*IV*	*V*	
Chiapagenin	69	11	41	39	69	Brown
Isochiapagenin	69	10	41	40	68	Brown
Chlorogenin	46	1	7	7	48	Green
β-Chlorogenin	60	3	15	13	56	Blue
Convallamarogenin	77	20	50	42	77	Brown
Digitogenin	18	0	0	0	48	Violet
Diosgenin	73	40	61	60	85	Green
Diotigenin	4	0	0	0	24	Violet
Isodiotigenin	3	0	0	0	23	Violet
Gentrogenin	80	20	70	62	82	Yellow
Gitogenin	28	1	6	5	56	Purple
Neogitogenin	26	1	6	5	58	Purple
Hecogenin	76	21	59	58	81	Yellow
Isonarthogenin	74	8	40	34	61	Greenish yellow
Kogagenin	2	0	0	0	16	Brown
Pennogenin	75	18	56	55	81	Yellow
Smilagenin	83	19	80	75	85	Green
Tigogenin	77	41	67	67	83	Green
Neotigogenin	76	41	66	65	81	Green
Tokorogenin	6	0	0	0	30	Purple
1α-Tokorogenin	9	0	1	0	33	Brown
Yonogenin	34	1	9	6	54	Brownish violet
Neoyonogenin	38	1	8	7	55	Brown

such as chromic acid mixture or 60% nitric acid (Table VI). Table VII shows another example of reproducibility in the separation of cardiac glycosides and genins. In general, the standard deviation in R_F values can be controlled to within less than 0.05 unit when sufficient care is taken with the factors that affect the reproducibility of separation. With the alumina–sintered glass plate, the variation in the standard deviation was found to be less than 0.04 unit.

Comparison of separation behaviour on alumina–sintered glass plates with behaviour on other plates

Table VIII gives a comparison of the separation characteristics of cardiac glycosides and genins and alkaloids on the alumina–sintered glass plate with those on other alumina–Kieselguhr and alumina–gypsum plates. The results given in Table VIII indicate that the separation characteristics of the alumina–sintered glass plate are similar to those of mixed layers of alumina and Kieselguhr.

TABLE V

hR$_F$ VALUES OF STEROLS ON ALUMINA-SINTERED GLASS PLATES

Detection with concentrated sulphuric acid. Solvents: I= benzene–acetone (4:1); II= chloroform–ethanol (50:1); III= chloroform; IV= *n*-hexane–diethyl ether (5:1); V= *n*-hexane–diethyl ether (3:1); VI= *n*-hexane–diethyl ether (1:1); VII= *n*-hexane–ethyl acetate–acetic acid (180:30:1). Solvent migration rates: I= 20, II= 25, III= 21, IV= 15, V= 15, VI= 15, VII= 25 min per 10 cm. Results are mean values from five runs on different plates. The standard deviation of each *hR*$_F$ value was less than 3.

Compound	*hR*$_F$ *value of sterol*						
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*
Cholesterol	57	66	28	5	8	27	21
Δ^2-Cholestenol	79	95	94	73	73	72	91
3-Ethyl-Δ^2-cholestenol	80	96	95	73	75	74	91
Ergosterol	63	72	35	6	9	30	23
2α,3α-Oxidocholestane	82	98	92	66	67	74	87
2β,3β-Oxidocholestane	85	98	94	71	72	76	88
Cholestanol	69	70	28	7	12	37	27
5α-Hydroxycholestanol	29	24	5	0	1	3	4
6β-Hydroxycholestanol	70	14	0	0	0	1	6
β-Sitosterol	75	73	29	9	13	38	20
Stigmasterol	70	77	33	10	14	37	27
Δ^7-Stigmastenol	89	79	36	9	12	36	26
$\Delta^{8(14)}$-Stigmastenol	88	76	34	8	10	34	25
Δ^{22}-Stigmastenol	70	77	35	11	14	34	30
Stigmastanol	69	78	36	9	14	35	29
Lanosterol	76	89	58	22	27	53	54

TABLE VI

REPRODUCIBILITY OF *hR*$_F$ VALUES OF SOME ALKALOIDS ON ALUMINA-SINTERED GLASS PLATES

Thin-layer: Merck aluminium oxide neutral (Type T)–sintered glass powder (1:4). Solvent: benzene–chloroform–diethylamine (36:8:1). Detection: Dragendorff reagent[2]. Values given are mean *hR*$_F$ values from five runs on different plates.

Compound	*Number of recoveries*				*Home-made plate* *
	1–32	*33–52*	*53–77*	*78–100*	
Quinine	25±3	34±3	31±3	34±5	22
Codeine	42±4	54±4	51±6	57±6	15
Brucine	59±4	67±2	66±2	70±3	15
Thebaine	80±2	81±1	82±2	86±2	50

* Merck aluminium oxide neutral (Type T)+10% gypsum.

TABLE VII

REPRODUCIBILITY OF hR_F VALUES OF CARDIAC GLYCOSIDES AND GENINS ON ALUMINA–SINTERED GLASS PLATES

Thin-layer: Merck aluminium oxide neutral (Type T)–sintered glass powder. Solvents: chloroform–methanol (10:1) for cardiac glycosides and ethyl acetate–acetone (3:1) for cardiac genins. Detection: concentrated sulphuric acid.

Plate	*hR_F value of compound*			*Run number*
	Gitoxin	*Digoxin*	*Digitoxin*	
Alumina–glass powder (1:4)	50 ± 1	57 ± 3	70 ± 1	5
Home-made plate *	35	45	48	1
	Gitoxigenin	*Digoxigenin*	*Digitoxigenin*	
Alumina–glass powder (1:3)	31 ± 4	46 ± 4	69 ± 3	17
Home-made plate *	15	32	57	1

* Merck aluminium oxide neutral (Type T)+10% gypsum.

TABLE VIII

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

Compound	*Solvent*	*hR_F value of compound*					
		Alumina–10% gypsum*	*Alumina*–sintered glass (1:1)*	*Alumina*–Kieselguhr***			
				1:1	*2:1*	*3:1*	*5:1*
Cardiac glycosides							
Gitoxin	Chloroform–methanol (10:1)	30	50	59	50	47	43
Digoxin		45	57	60	55	50	47
Digitoxin		48	70	71	64	56	53
Cardiac genins							
Gitoxigenin	Ethyl acetate–acetone (3:2)	15	31	68	56	50	38
Digoxigenin		32	46	75	74	60	44
Digitoxigenin		57	69	86	78	74	69
Alkaloids							
Quinine	Benzene–chloroform–diethylamine (36:8:1)	2	25	21t***	21t	12	–
Codeine		15	42	26t	26t	17	–
Brucine		15	59	54	37	34	–
Thebaine		50	80	75t	60	60	–

* Merck aluminium oxide neutral (Type T).
** Wako Kieselguhr B-O.
*** t = tailing spot.

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REFERENCES

1 T. Okumura, T. Kadono and M. Nakatani, *J. Chromatogr.*, 74 (1972) 73.
2 R. Munier and M. Macheboeuf, *Bull. Soc. Chim. Biol.*, 33 (1961) 846.
3 Z. Tamura, *Talanta*, 19 (1972) 573.
4 T. Okumura and T. Kadono, *Yakugaku Zasshi (J. Pharm. Soc. Jap.)*, 92 (1972) 1370.