Journal of Chromatography, 515 (1990) 205–212
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 1727

Porous glass sheets for use in thin-layer chromatography

MASANORI YOSHIOKA*, HIROKO ARAKI and MIWAKO KOBAYASHI
Faculty of Pharmaceutical Sciences, Setsunan University, 45–1, Nagaotoge-cho, Hirakata, Osaka, 573-01 (Japan)
FUMIKO KANEUCHI, MADOKA SEKI and TADASHI MIYAZAKI
Japan Spectroscopic Co., Ltd. (JASCO), 2967–5, Ishikawa-cho, Hachioji, Tokyo, 192 (Japan)
and
TAKESHI UTSUKI, TAKAO YAGINUMA and MASAAKI NAKANO
Ise Chemical Industries Co., Ltd., 2–7–12, Yaesu, Chuo-ku, Tokyo, 104 (Japan)

ABSTRACT

Porous glass was made from a mixture of 45–70% SiO_2, 8–30% B_2O_3, 8–25% CaO, 5–15% Al_2O_3, 3–8% Na_2O + 1–5% K_2O and 0–8% MgO. The mixture was heated at 600–850°C for 20 h and cut into square sheets of 5 cm × 5 cm × 0.5 mm. Each sheet was leached with 1 *M* hydrochloric acid at 80–90°C for 4–16 h to make it porous. The surface of each sheet was examined with a scanning electron micrograph. The various sheets with pore diameters from 110 to 1200 nm were developed with a few common solvents for thin-layer chromatography (TLC). The larger the pore diameters, the shorter were the developing times with the solvents. A sheet of 700 nm pore diameter required only a few minutes and showed good separations, and was adopted in the subsequent TLC study.

Twenty Dns-amino acids were applied to the sheet and detected by their fluorescence at picomole levels. Free amino acids were also applied and derivatized to fluorescent compounds with fluorescamine, and were detected at 100-pmol levels. Inorganic cations and anions at concentrations down to nanomole levels were applied and sensitively detected by colour reactions.

The fluorescence spectrum of DNS-alanine on the sheet was measured directly. A Fourier transform IR spectrum of phenacetin *in situ* on the sheet was obtained, and was reliable in the region of wavenumber from 1550 to 4000 cm^{-1}. The sheet is very stable towards strong acids, alkalis and mechanical scratching. It has no supporting matrix, although the conventional thin layers have, and showed a sharp front line without a necklace effect.

INTRODUCTION

In thin-layer chromatography (TLC), originated by Stahl[1,2], fine particles of silica gel, several tens of micrometres in diameter, are coated in a layer less than 1 mm

0021-9673/90/$03.50

thick on a supporting matrix such as glass or plastics. In place of silica gel, alumina crystalline cellulose and polyamide can be used. Recently, these particles have been made much smaller and refined to increase the resolution in so-called high-performance TLC.

These thin layers are very good for separation, but several problems arise when other functions need to be improved. Various kinds of binders are necessary to coat the particles on the matrix and, even if the particles are completely coated with the binders, they are apt to scrape during handling, especially spotting. Separations are sometimes affected by the boundary between the thin layer and its supporting matrix. When a separated zone is preparatively extracted with a solvent, fine powders from the particles remain in the extract. When the zone is measured spectrophotometrically *in situ*, it is difficult to obtain a detailed spectrum owing to the high background of the surface of the layer.

In this study, we aimed not only to solve the above problems, but also to prepare more technologically advanced thin layers. Recently, porous glass has been produced and used in high-performance liquid chromatography[3] and we thought that a porous glass sheet could act both as the separation layer and the supporting matrix in TLC. Sheets of various diameters were prepared and examined for their versatility. Separation profiles were determined for 5-dimethylaminonaphthalene-1-sulphonyl (Dns) amino acids, free amino acids and inorganic ions. For their detection, we examined several new methods such as chemical reactions, direct measurements of fluorescence and Fourier transform (FT) IR spectrometry on the sheets.

EXPERIMENTAL

Reagents

Dns-Amino acids were obtained from Pierce (Rockford, IL, U.S.A.). Twenty L-amino acids were obtained from Wako (Osaka, Japan). Glycine, cysteine, histidine, arginine and lysine were in the hydrochloride form. Inorganic ions in the following forms were also obtained from Wako: $MgCl_2 \cdot 6H_2O$, $AlCl_3 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, VCl_3, $CrCl_3 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, $FeCl_2 \cdot nH_2O$, $FeCl_3 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $ZnCl_2$, $GeCl_4$, As_2O_3, $SeCl_4$, $SrCl_2 \cdot 6H_2O$, $YCl_3 \cdot 6H_2O$, $ZrCl_2O \cdot 8H_2O$, $PbCl_2$, $AgNO_3$, $CdCl_2 \cdot 2.5H_2O$, $InCl_3 \cdot 4H_2O$, $SnCl_2 \cdot 2H_2O$, $SbCl_3$, $TeCl_4$, $CeCl_3 \cdot 7H_2O$, $TbCl_3 \cdot xH_2O$, $YbCl_3 \cdot 6H_2O$, $IrCl_4$, $H_2PtCl_6 \cdot 6H_2O$, $HAuCl_4 \cdot 4H_2O$, $HgCl_2$, TlCl, $PbCl_2$, $BiCl_3$, NaF, NaCl, KI, $KBrO_3$, KIO_3, $K_2B_4O_7 \cdot 4H_2O$, $Na_2C_2O_4$, $NaNO_2$, KNO_3, $Na_3PO_4 \cdot 12H_2O$, Na_2SO_4, $Na_2S_2O_3 \cdot 5H_2O$, NaSCN, K_2CrO_4, $K_2Cr_2O_7$, $K_4Fe(CN)_6 \cdot 3H_2O$, $K_3Fe(CN)_6$ and Na_2CO_3. Fluorescamine was obtained from Nippon Roche (Kamakura, Japan). Dithizone and 8-hydroxyquinoline were obtained from Wako.

Reagent solutions

For spraying the sheets, solutions of 0.05% dithizone in chloroform, 1% 8-hydroxyquinoline in methanol, 25% ammonia, 0.1% silver nitrate and $7.5 \cdot 10^{-5}$ *M* fluorescamine in acetone were prepared.

Preparation of new glass sheets

The glass consisted of 45–70% SiO_2, 8–30% B_2O_3, 8–25% CaO, 5–15% Al_2O_3,

3–8% Na_2O + 1–5% K_2O and 0–8% MgO. The mixture was heated at 600–800°C for 20 h to effect a phase separation and the product was cut into square sheets of 5 cm × 5 cm × 0.5 mm. Each sheet was leached with 1 *M* hydrochloric acid at 80–90°C for 4–16 h to etch the B_2O_3 phase to make it porous, washed with water and dried.

TLC

For spotting sample solutions, a 1-μl glass capillary (32 mm × 0.1 mm I.D.) (Microcaps) was cut to one tenth of the original length, *i.e.*, 3.2 mm long. The small tip of the capillary was connected with a silicon tube of 0.5 mm I.D. The tip was dipped into the sample solution and 0.1 μl was sucked into it. This solution was applied to the sheet by pushing the silicon tube, dried and developed with a solvent. Dns-Amino acids were dissolved in 95% ethanol to give $1 \cdot 10^{-6}$ *M* solutions. The Dns-amino acids were located by irradiation with a UV lamp at 366 nm. The fluorescence spectrum of Dns-Ala, 7 μl of a solution of which was spotted on the sheet, was measured with an FP-770 fluorescence spectrophotometer (JASCO).

Amino acids were dissolved in a small aliquot of 0.1 *M* hydrochloric acid and diluted with water to make $1 \cdot 10^{-3}$ *M* solutions. The glass sheet used for the free amino acids was reheated at 600°C for 5 h to change the silanol groups to siloxanes. For detection, the developed sheet was immersed in a fluorescamine solution in acetone.

Each inorganic cation was dissolved in 0.1 *M* hydrochloric acid and diluted with water to 0.01 *M*. For detection, the developed sheet was sprayed with 1% 8-hydroxyquinoline or 0.05% dithizone.

Each inorganic anion was dissolved in 5% sodium carbonate solution to make 0.05–1 *M* solutions. For detection, the sheet was sprayed with 0.1% silver nitrate solution and irradiated with the UV lamp.

Phenacetin was dissolved in chloroform to make a 0.1 *M* solution and 7 μl of the solution were spotted on the sheet and dried. An FT-IR spectrum of the spot was measured *in situ* with a Model 8000 FT-IR spectrometer (JASCO).

RESULTS

An electron micrograph of a glass sheet is shown in Fig. 1. The pore edge of diameter 1000 nm was so thin and sharp that it was fragile in comparison with that of 500 nm. The larger the pore sizes, the shorter were the developing times with normal solvents, as shown in Fig. 2. The sheet of pore size 700 nm took only a few minutes, showed good separations and was adopted in the subsequent TLC studies. The sheet was further cut into half to make the developing time shorter and more economical. The development was carried out in a small sample bottle in a few minutes.

The separation of twenty Dns-amino acids was tried with two solvents as shown in Fig. 3. Their fluorescences were seen at the same places on both sides of the sheet. It should be possible to identify an unknown sample by locating it at the same place on the front side as for a standard on the other side. It was also possible to obtain a fluorescence spectrum of Dns-Ala on the sheet, as shown in Fig. 4. The spectrum almost corresponded to that taken in a solvent.

Twenty free amino acids were separated as shown in Fig. 5. The separations of structurally related compounds were dependent on their sizes, as shown by Gly, Ala and Val.

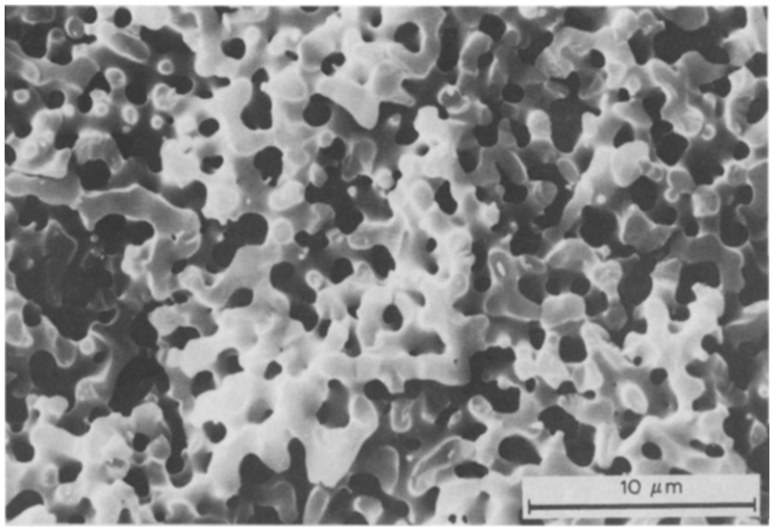


Fig. 1. Electron micrograph of glass sheet (700 nm).

A good FT-IR spectrum of the thin layer was obtained, as shown in Fig. 6. The spectrum was reliable for wavenumbers in the range above 1550 cm^{-1}.

The cations were well separated by two solvent systems, as shown in Fig. 7. The visible colours or the fluorescence were almost the same as those in conventional TLC using a crystalline cellulose or silica gel. However, no shrinkage of the sheet with strong acid was observed.

The sheets for the anions were developed with two solvent systems as shown in Fig. 8. The colours of the spots obtained with 0.1% silver nitrate solution and UV irradiation were sensitive and clear. This kind of UV irradiation effect was not found on silica gel plates. The separation on the sheet was characteristic, as shown in Fig. 9, where a small difference in the solvent composition led to a large difference in the separation.

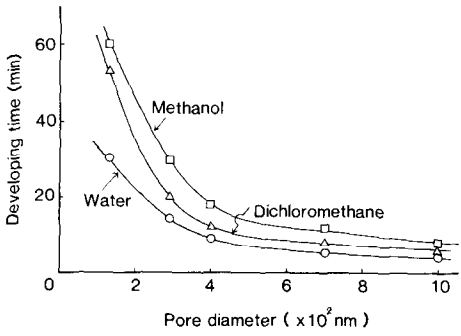


Fig. 2. Relationship between pore size and developing time. The sheets were developed to 4.5 cm with the three solvents indicated.

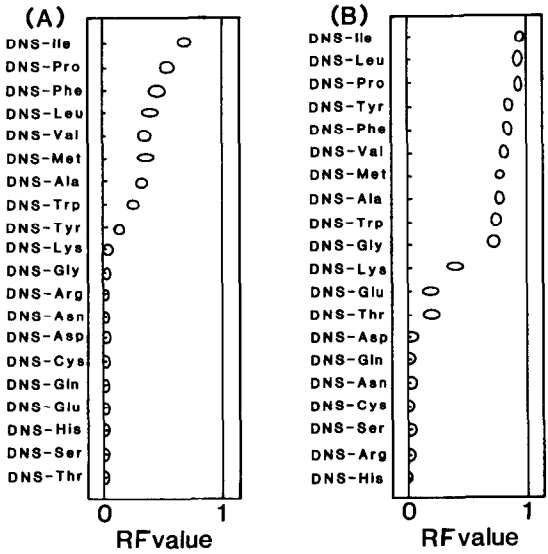


Fig. 3. Chromatograms of twenty Dns (DNS)-amino acids on a sheet of pore size 700 nm developed with (A) chloroform and (B) benzene–acetic acid (19:1, v/v).

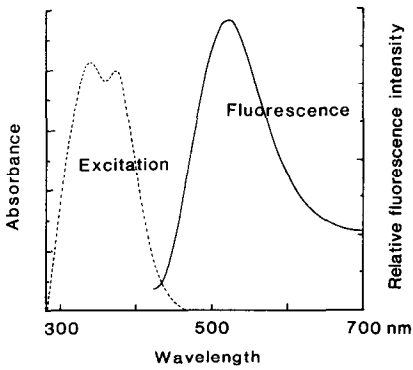


Fig. 4. Excitation and fluorescence spectra of Dns-Ala on a sheet.

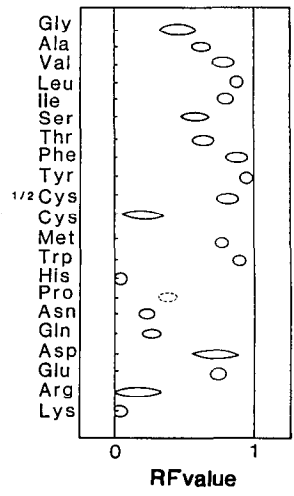


Fig. 5. Chromatogram of 100 pmol of amino acids on a reheated sheet (2.5 cm × 5 cm) developed with *n*-butanol–acetic acid–water (10:1:1, v/v/v) and detected with fluorescamine. Pro (dotted circle) was detected with ninhydrin.

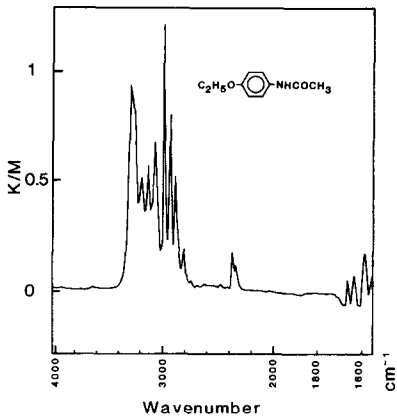
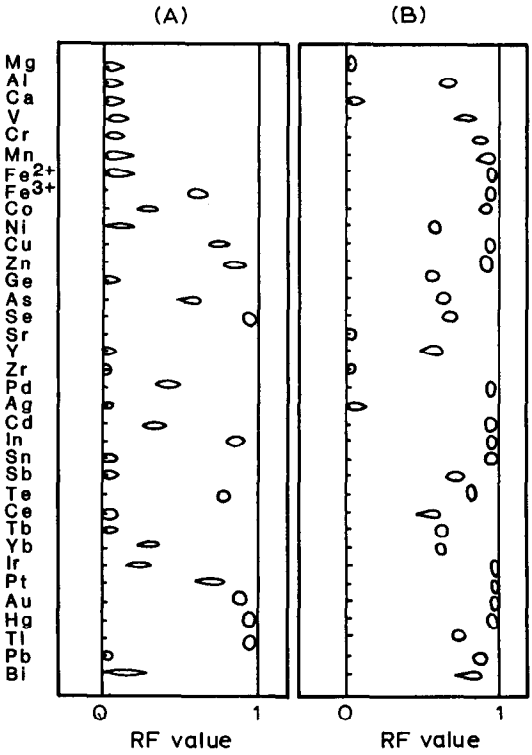
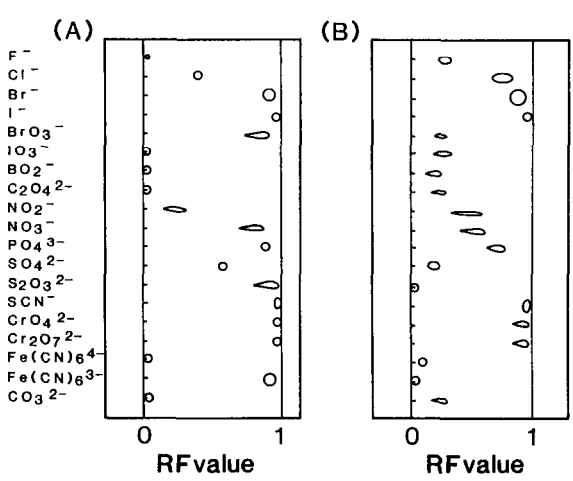


Fig. 6. FT-IR spectrum of 0.7 μg of phenacetin on the sheet. K/M means the Kubelka–Munk format.



Dithizone	Oxine	Oxine upon UV
	Yellow	Yellowish green
	Yellow	Yellowish green
	Light yellow	Yellow
Brown		Dark
Reddish orange		Dark
Pink	Yellow	Dark
Brown	Grey	Dark
Brown	Grey	Dark
Purple	Yellow	Dark
Purple	Yellow	Dark
Brown	Yellow	
Reddish purple	Light yellow	Orange
	Light yellow	Orange
Brown		
Yellow	Light yellow	Yellow
		White
	Yellow	Brown
	Yellow	Orange
Green		Yellow
Reddish orange	Brown	Dark
Orange	Ligth yellow	Yellow
Pink		
Purple	Yellow	Orange
Orange		
Orange		Dark
Yellowish brown		Dark
	Yellow	Dark
	Yellow	Dark
Brown	Brown	Dark
Reddish orange	Brown	Dark
Orangish brown	Brown	Dark
Orange	Yellow	Dark brown
		Dark
Reddish orange		
Pink	Yellow	Dark

Fig. 7. Chromatograms of metal ions developed with (A) *n*-butanol–benzene–1 *M* HNO_3–1 *M* HCl (75:69:4:2, v/v) and (B) acetone–3 *M* HCl (99:1, v/v). Colours with dithizone, oxine and oxine upon UV are given at the right-hand side.



$AgNO_3$	$AgNO_3$ upon UV
	Dark
Grey	Dark
Grey	Dark
Grey	Dark
Grey	
	Dark
	Dark
Grey	Dark
	Dark
Grey	Dark
Bluish grey	Dark
Grey	
Brown	Dark
Brown	Dark
Reddish brown	Dark
Reddish brown	Dark
Yellow(A).Grey(B)	Dark
Orange brown	Dark
Orange	

Fig. 8. Chromatograms of anions developed with (A) acetone–water (96:4, v/v) and (B) *n*-butanol saturated with water. Colours with $AgNO_3$ and $AgNO_3$ upon UV are given at the right-hand side.

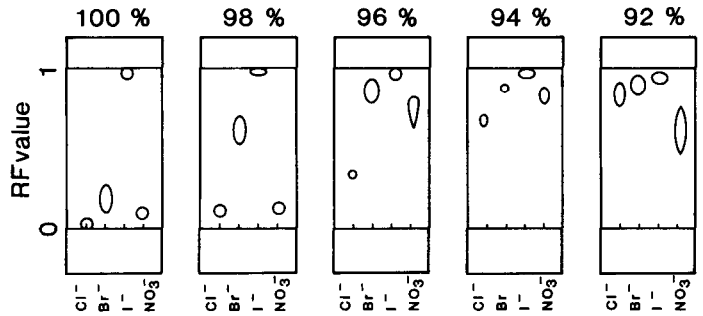


Fig. 9. Chromatograms of anions developed with various concentrations of acetone in water.

DISCUSSION

Satisfactory TLC of representative compounds was achieved on the porous glass sheets. The spots appear broader than those on conventional plates. The separation characteristics on the sheets such as the R_F values and the broadening did not vary much with the pore diameters, although the broadening on the sheet of pore size 700 nm was larger.

The principle of the separation has not been elucidated. However, the higher the R_F values, the larger were the molecular weights of the related compounds, as shown in the chromatograms for the halogens (Fig. 8), amino acids (Fig. 5) and Dns-amino acids (Fig. 3) such as Leu, Val, Ala and Gly. In addition, silanols on the sheet and interactions between the solutes and the solvent seem to influence the separation.

It is possible to measure the fluorescence and FT-IR spectra *in situ* on the sheet. The spectrum is influenced by the environment of the solute on the surface. For identification, the spectrum of a standard must be taken on the same sheet. The

application is still limited, since at wavenumbers below 1550 cm^{-1} the influence of silanol groups is so great that correction with the background is impossible. Previously, FT-IR spectra were measured by Chalmers *et al.*[4] after the sample had been extracted with a solvent from the zone on the layer. With the present type of sheet, extraction is unnecessary and the recovery therefore need not be considered. Zuber *et al.*[5] measured FT-IR spectra *in situ* on a silica gel plate, but below 1550 cm^{-1} the background due to silanols was high and above 1550 cm^{-1} the influence of the surface was too high to obtain clear signals. In this respect the glass sheet is superior.

It is possible to achieve fine separations by using more developing solvents and to detect nanomole levels of inorganic cation except Mg^{2+} and Ca^{2+} at the 10-nmol level. For the anions, 10-nmol levels are also detectable. These sensitivities are higher than those reported by Nakamura and Tamura[6], who detected these ions on a thin layer containing a mixture of fluorescent compounds, but not those of specific colour reactions for the respective ions. The sensitivities for the fluorescence of the Dns-amino acids and fluorescamine derivatives on the sheet are 1000 times higher than those for the above colour reactions and slightly higher than those for fluorescence reported by Spivak *et al.*[7] and Nakamura and Pisano[8], judging from the detection limits given.

The sheet is mechanically strong compared with conventional thin layers of powders coated on the matrix. It is also more stable against acids and alkalis so that shrinkage of the sheet does not occur during the separations of inorganic compounds and detection. The detection on the sheet is based completely on "dry" chemistry, as the spot is developed, dried and reacted with a spray reagent. The volume per gram of the sheet is 0.39 ml/g, making a large specific surface area of 6.6 m^2/g, which accelerates the reduction of Ag^+ on the UV irradiation as shown in Fig. 8. The sheet whould be useful for the study of this kind of dry chemistry. The equivalence of the front and rear sides of the sheets is another merit. The boundary between the thin layer and the matrix gives rise to a necklace effect of the front of the developing solvent in conventional TLC, but on the glass sheet the front line is straight and does not show such an effect.

It is easy to cut the sheet with a cutter and to extract the separated zone using a solvent, as the surface of the glass is so strong that no powder is introduced into the extracted residue. The spectra measured *in situ* on the sheet show fine detail. The glass sheets under investigation are expensive and it is undesirable to dispose of them like conventional plates. The sheets should be washable with strong acids and organic solvents and hence can be reused. With further development the high performance and function of these sheets should lead to ideal thin layers for TLC.

REFERENCES

1 E. Stahl, *Pharmazie*, 11 (1956) 633.
2 E. Stahl, *Arch. Pharm.*, 292 (1959) 411.
3 Y. Matsushima, Y. Nagata, K. Takakusagi, M. Niyomura and N. Takai, *J. Chromatogr.*, 332 (1985) 265.
4 J. M. Chalmers, M. W. Mackenzie and J. L. Sharp, *Anal. Chem.*, 59 (1987) 415.
5 G. E. Zuber, R. J. Warren, P. P. Begosh and E. L. O'Donnell, *Anal. Chem.*, 56 (1984) 2935.
6 H. Nakamura and Z. Tamura, *Bunseki Kagaku*, 22 (1973) 1356.
7 V. A. Spivak, V. M. Orlov, V. V. Shcherbukhin and Ya. M. Varshavsky, *Anal. Biochem.*, 35 (1970) 227.
8 N. Nakamura and J. J. Pisano, *J. Chromatogr.*, 121 (1976) 79.