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Note

Application of a sintered silica gel plate to the thin-layer chromatography of carbohydrates

NORIKO IIZIMA, MICHIO FUJIHARA and TERUKAZU NAGUMO

Department of Biophysics, School of Hygienic Sciences, Kitasato University, 1-15-1 Kitasato, Sagamihara-shi, Kanagawa 228 (Japan)

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Thin-layer chromatography (TLC) using silica gel coatings is an essential technique in carbohydrate analysis. The adsorbent is coated on glass plates and this form of chromatoplate is commercially available. For a satisfactory separation of carbohydrates, the silica gel has to be impregnated with inorganic salts[1] either by mixing the gel with the salts before coating the adsorbent on glass plates or by immersing the pre-coated plates in the salt solutions for a long time (15–20 h)[2]. Disadvantages of these plates are that each plate can be used only once and that the impregnation procedures are troublesome and time-consuming.

Recently, Okamura and Kadono[3] prepared a sintered silica gel plate by fixing the adsorbent mixed with glass powder to a soda-lime glass plate by heating at high temperatures, and applied it to TLC of alkaloids, food preservatives, amino acids, steroids, etc. The plate could be used repeatedly after cleaning with a chromic acid–sulphuric acid mixture. Itoh *et al.*[4] investigated the application of the sintered plate to analysis of lipids, and indicated that the plate was very sturdy and could be immersed without special precautions in a number of lipid reaction mixtures such as Sudan black staining solution. It was suggested that if the plate could be applied to TLC analysis of carbohydrates, many advantages could be expected, such as a simple impregnation of the plate with inorganic or organic salts, high reproducibility of TLC data through repeated use of the same plate and simpler chromatographic procedure. However, there have been few reports of the TLC analysis of carbohydrates on such plates.

We have now investigated the application of the sintered plate to TLC of carbohydrates in order to determine the optimum conditions for such analyses. Our results show that the sintered plate gives much improved analyses compared with coated plates.

MATERIALS AND METHODS

The solvents methanol, ethanol, isopropanol, *n*-butanol, acetone, phenol and pyridine were used after distillation. Other chemicals used were of analytical grade. Mono-, di- and trisaccharides, uronic acids and amino sugars used were of the highest purity commercially available. Purified D-mannuronic acid and L-guluronic acid were prepared from commercial alginic acid by the method of Haug and Larsen[5].

Sintered plates (200 × 50 mm, Replate 50, 0.2 mm silica gel layer) were purchased from Yamato Scientific (Tokyo, Japan). The plates were reconditioned after use according to the method of Itoh *et al.*[4]. The plates were immersed overnight in chromic acid–sulphuric acid (saturated aqueous $K_2Cr_2O_7$ solution–concentrated H_2SO_4, 1:1, v/v) and then washed in tap water for 2 h. The plates were washed three or four times with distilled water and dried in the air.

The silica gel plates were impregnated with an inorganic or organic salt solution of required concentration by the following procedure. Fifteen or sixteen plates were placed in a square polystyrol tank (200 × 75 mm, height 60 mm) in a vertical position. The salt solution was then carefully poured into the tank until the plates were covered. The plates were allowed to stand in the tank for 1 min, 5 min, 74 min or 14 h, then removed and air-dried in a vertical position. The impregnated plates were activated at 105°C for 1 h and cooled in a desiccator before use.

For comparison, non-impregnated plates were used after activation under the same conditions.

For chromatography, aqueous 2% carbohydrate solutions were employed. Fifty nanolitres of a solution containing one carbohydrate or a mixture of carbohydrates were applied to the plates with a glass capillary which had been calibrated volumetrically. The plates were developed by the ascending technique in a closed glass tank. The following solvent systems were employed (all proportions are v/v):

(1) Acetone–phenol–water (8:1:1)
(2) Isopropanol–methanol–water (16:1:3)
(3) Phenol–water (5:1)
(4) *n*-Butanol–water (9:1)
(5) Isopropanol–water (17:3)
(6) *n*-Butanol–pyridine–water (4:5:3)
(7) *n*-Butanol–pyridine–water (6:4:3)
(8) *n*-Butanol–pyridine–water (8:4:3)
(9) *n*-Butanol–acetone–phenol–water (3:12:3:2)
(10) *n*-Butanol–ethanol–water (2:1:1)
(11) *n*-Butanol–ethanol–water (16:1:3)
(12) *n*-Butanol–ethanol–water (14:3:3)
(13) *n*-Butanol–acetone–water (4:5:1)
(14) *n*-Butanol–pyridine–benzene–water (5:3:1:2)
(15) *n*-Butanol–isopropanol–water (3:5:2)
(16) *n*-Butanol–methanol–water (16:1:3)
(17) Isopropanol–acetone–0.1 *M* phosphoric acid (2:1:1)
(18) *n*-Butanol–ethanol–0.1 *M* phosphoric acid (1:10:5)
(19) Isopropanol–methanol–0.1 *N* hydrochloric acid (7:1:3)
(20) Ethanol–phenol–pyridine–0.1 *M* phosphoric acid (5:1:1:2)

Carbohydrates were detected with diphenylamine–aniline–phosphoric acid reagent (at 85 °C for 10 min)[6].

RESULTS AND DISCUSSION

Carbohydrates could not be separated on non-impregnated, sintered plates. All the spots obtained were observed at the front of each solvent. Thus, in an attempt to separate carbohydrates, the sintered plates were impregnated overnight (15–20 h) with inorganic or organic salt solutions by the method of Hansen[2].

Attempts with 0.01–0.5 *M* solutions of $Na_2B_4O_7$, H_3BO_3 and sodium acetate were unsuccessful. Many of the spots obtained were elongated and no separation of the carbohydrates was observed. When sodium mono- and dihydrogen phosphates, or bicarbonate, were employed as the impregnating salts, satisfactory circular spots were obtained and a good separation of the carbohydrates was achieved.

Although impregnation of the plates with salts was found to yield carbohydrate separations, the time required for the treatment (15–20 h) was too long. Therefore, in an attempt to shorten the time required for impregnation, the relationship between the separation of monosaccharides and this time was investigated under conditions in which all monosaccharides tested were completely separated: solvent system, 13; impregnating salt, 0.3 *M* Na_2HPO_4. As shown in Table I, there was no relationship between the English impregnation time and the separation of carbohydrates. For each impregnation time, a good separation was achieved and the R_F values were also fairly reproducible. It was found that satisfactory results could be obtained by treating the plates with the impregnating salts for only a few minutes. Consequently, further chromatography was performed with impregnation times of 5 min.

TABLE I

EFFECT OF IMPREGNATION TIME ON R_F VALUES OF MONOSACCHARIDES ON SINTERED SILICA GEL PLATES IMPREGNATED WITH 0.3 *M* Na_2HPO_4

Solvent system, 13. R_F values are expressed as the averaged value ± the standard deviation.

Carbohydrates	*Impregnation time*		
	14 h	*74 min*	*1 min*
Rhamnose	0.73 ± 0.03	0.78 ± 0.01	0.74 ± 0.00
Fucose	0.54 ± 0.02	0.55 ± 0.02	0.54 ± 0.02
Ribose	0.40 ± 0.03	0.41 ± 0.01	0.43 ± 0.02
Xylose	0.34 ± 0.03	0.36 ± 0.01	0.37 ± 0.01
Arabinose	0.24 ± 0.02	0.25 ± 0.01	0.26 ± 0.01
Mannose	0.17 ± 0.01	0.18 ± 0.01	0.20 ± 0.01
Glucose	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
Galactose	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.01

Tables II–IV show the best results obtained for carbohydrate separation under the conditions described above. Impregnation with sodium mono- and dihydrogen phosphates yielded separations of mono-, di- and trisaccharides and uronic acids. The effective concentrations of the salts were 0.3–0.5 *M* and 0.1–0.3 *M* for NaH_2PO_4 and Na_2HPO_4, respectively. Impregnation with sodium bicarbonate was also effective in separation of mono- and oligosaccharides in the salt concentration range of 0.2–0.4 M, but separation of uronic acid was not observed in the presence of the salt at all concentrations tested. Amino sugars could not be separated satisfactorily under any conditions. The best separations were obtained on plates impregnated with 0.1 and 0.3 *M* Na_2HPO_4 and 0.5 *M* NaH_2PO_4 for monosaccharides, and with 0.3 *M* NaH_2PO_4 for uronic acids and oligosaccharides, respectively.

With regard to the solvent systems, a fairly satisfactory separation was achieved for monosaccharides in the following systems (in addition to those listed

in the tables): 4, 7, 10, 11, 12, 15, 16 (0.3 *M* NaH_2PO_4); 4, 6, 7, 10, 12, 15, 16 (0.5 *M* NaH_2PO_4); 5, 7, 8, 14 (0.1 *M* Na_2HPO_4); 1, 2, 3, 5, 6, 9 (0.3 *M* Na_2HPO_4); 8 (0.2 *M* $NaHCO_3$) and 7 (0.4 *M* $NaHCO_3$). With these systems, more than six monosaccharides in a mixture containing eight samples were satisfactorily separated. For oligosaccharide separation, good results were also obtained with solvent system 7 (0.5 *M* NaH_2PO_4, 0.1 *M* Na_2HPO_4 and 0.4 *M* $NaHCO_3$). Uronic acids could be separated satisfactorily with only acid solvent systems 17–20.

In order to examine whether the sintered plates could be used repeatedly, monosaccharide separation was studied on a plate impregnated with 0.3 *M* Na_2HPO_4. The solvent system 13 was employed. After the completion of each TLC analysis,

TABLE II

TLC OF MONOSACCHARIDES AND THEIR MIXTURES ON SINTERED SILICA GEL PLATES

R_F values are given. P = Partial separation; S = satisfactory separation of mixtures.

Carbohydrates	*Plates impregnated with*							
	Na_2HPO_4			*NaH_2PO_4*			*$NaHCO_3$*	
	0.1 M	*0.1 M*	*0.3 M*	*0.3 M*	*0.5 M*	*0.5 M*	*0.2 M*	*0.4 M*
Solvent No.	*2*	*9*	*13*	*13*	*2*	*9*	*12*	*6*
Rhamnose	0.88	0.75	0.74	0.82	0.89	0.61	0.61	0.83
Fucose	0.73	0.61	0.54	0.61	0.72	0.40	0.46	0.61
Ribose	0.62	0.51	0.43	0.51	0.57	0.29	0.38	0.58
Xylose	0.65	0.45	0.37	0.53	0.62	0.25	0.38	0.58
Arabinose	0.51	0.34	0.26	0.38	0.45	0.17	0.29	0.44
Mannose	0.48	0.25	0.20	0.35	0.48	0.13	0.29	0.45
Glucose	0.36	0.16	0.12	0.26	0.37	0.08	0.22	0.39
Galactose	0.29	0.13	0.09	0.17	0.26	0.05	0.18	0.31
Monosaccharide mixtures	S	S	S	P	S	S	P	P

TABLE III

TLC OF URONIC ACIDS AND THEIR MIXTURES ON SINTERED SILICA GEL PLATES

Details as in Table II.

Carbohydrates	*Plates impregnated with*			
	0.3 M NaH_2PO_4			*0.3 M Na_2HPO_4**
Solvent No.	*18*	*19*	*20*	*17*
Gulurone	0.95	0.95	0.89	—
Glucurone	0.89	0.87	0.83	—
Mannurone	0.85	0.75	0.75	—
Mannuronic acid	0.63	0.49	0.44	0.21
Glucuronic acid	0.55	0.40	0.36	0.14
Guluronic acid	0.49	0.37	0.23	0.08
Galacturonic acid	0.37	0.27	0.18	0.04
Uronic acid mixtures	S	S	S	S

* Samples were chromatographed after each uronic acid lactone was completely transformed into the acid form by keeping each sample solution at pH 8.0 and room temperature for 30 min.

TABLE IV

TLC OF OLIGOSACCHARIDES AND THEIR MIXTURES ON SINTERED SILICA GEL PLATES

Details as in Table II.

Carbohydrates	*Plates impregnated with*						
	Na_2HPO_4	*NaH_2PO_4*			*$NaHCO_3$*		
	0.1 M	*0.3 M*	*0.3 M*	*0.5 M*	*0.2 M*	*0.2 M*	*0.4 M*
Solvent No.	*2*	*2*	*15*	*2*	*6*	*12*	*2*
Sucrose	0.60	0.58	0.38	0.47	0.67	0.29	0.39
Maltose	0.48	0.45	0.27	0.34	0.64	0.23	0.33
Lactose	0.43	0.31	0.19	0.22	0.54	0.19	0.27
Melezitose	0.47	0.50	0.25	0.36	0.56	0.21	0.27
Raffinose	0.24	0.24	0.13	0.16	0.43	0.12	0.15
Oligosaccharide mixtures	P	S	P	S	S	P	P

the plate was reconditioned with chromic acid–sulphuric acid, then impregnated with the salt, activated and used for the next chromatography. The procedure was repeated fourteen times. As shown in Table V, satisfactory separation was obtained in each TLC analysis. The R_F value of each monosaccharide was constant, within experimental error, throughout all the analyses. This shows that the plate can be used repeatedly at least twelve times. Further use of the plate gave poor results because small amounts of silica gel fell off the glass plate. However, these results suggest that the sintered plates may be used more than twelve times if suitable precautions are taken to prevent loss of silica gel.

In this study sintered silica gel plates have been shown to be effective in TLC of carbohydrates if the plates are suitably impregnated, especially with sodium mono- and dihydrogen phosphates. Ovodov *et al.*[7] systematically analyzed the effects

TABLE V

EFFECT OF REPEATED TLC USING THE SAME SINTERED SILICA GEL PLATES ON R_F VALUES OF MONOSACCHARIDES

Chromatography was performed on plates impregnated with 0.3 *M* Na_2HPO_4 and in solvent system 13. A monosaccharide mixture was used as the sample. Each plate was used repeatedly until the fourteenth analysis. R_F values of monosaccharides obtained with six plates in each run were averaged. The results are expressed as the averaged R_F value ± the standard deviation.

Carbohydrates	*No. of analysis*			
	2nd	*4th*	*8th*	*12th*
Rhamnose	0.76 ± 0.02	0.74 ± 0.01	0.71 ± 0.02	0.81 ± 0.02
Fucose	0.53 ± 0.01	0.51 ± 0.03	0.52 ± 0.02	0.56 ± 0.02
Ribose	0.38 ± 0.02	0.37 ± 0.02	0.38 ± 0.02	0.42 ± 0.01
Xylose	0.33 ± 0.02	0.31 ± 0.02	0.32 ± 0.02	0.35 ± 0.02
Arabinose	0.23 ± 0.01	0.22 ± 0.02	0.23 ± 0.02	0.25 ± 0.01
Mannose	0.17 ± 0.01	0.16 ± 0.01	0.18 ± 0.02	0.18 ± 0.01
Glucose	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.02	0.11 ± 0.01
Galactose	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.02	0.08 ± 0.01

of the type of impregnating salt and its concentration on the chromatographic behaviour of certain carbohydrates in a number of solvents, and showed that a satisfactory separation can be obtained on silica gel impregnated with phosphates. For monosaccharides and uronic acids, the best phosphate concentrations were between 0.2 and 0.3 *M*, and for oligosaccharides between 0.05 and 0.1 *M*. Hansen[2] separated mono-, di- and trisaccharides on silica gel impregnated with 0.5 *M* sodium dihydrogen phosphate. Our results obtained on sintered silica gel plates are consistent with those of the above authors, although the phosphate concentrations which have the best separations were different from those found in the present study. These results suggest that phosphate may be essential for a satisfactory separation of carbohydrates on silica gel, except for amino sugars.

The present results show that the TLC analysis of carbohydrates on sintered silica gel plates can be performed more simply and more economically than that on other types of silica gel plates, because of the simple impregnation of the sintered plates with salts, the low impregnation time required for a satisfactory separation and because the plates can be used repeatedly if they are reconditioned with chromic acid–sulphuric acid. Sintered plates may also be useful for quantitative thin-layer chromatographic analysis, because the results are highly reproducible even after repeated use of the plates.

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