THIN-LAYER CHROMATOGRAPHY ON MICROCRYSTALLINE CELLULOSE*

M. L. WOLFROM, D. L. PATIN AND ROSA M. DE LEDERKREMER
Department of Chemistry, The Ohio State University, Columbus, Ohio (U.S.A.)
(Received July 16th, 1964)

INTRODUCTION

Silica gel has been widely used in the thin-layer chromatography, both qualitative and isolative, of amino acids, sugars and sugar derivatives[1,2]. The calcium sulfate binder required in this technique presents difficulties in isolative work. Paper chromatographic techniques are well established for such separations, for many of which, particularly the isolative, much time, even days, is required.

The use of cellulose spread on thin-layer plates has no doubt appealed to researchers for some time and although limited success has been recorded, the method has not been widely adopted. SCHWEIGER[3] has used "Cellulose MN 300" to separate some free sugars and alduronic acids. WOLLENWEBER[4] investigated amino acids and RANDERATH AND STRUCK[5] nucleic acid bases and nucleosides. RANDERATH[6], in comparing paper and cellulose layers, found the latter gave equal separations with more distinct zones (nucleic acid bases and nucleotides). Binders have been necessary in some cases and the layers spread were not very uniform.

In this laboratory we have prepared thin-layer plates from a commercial product known as "Avirin" ("Avicel") which is prepared by acid treatment of cellulose and represents the microcrystalline fraction of that material.

EXPERIMENTAL

Adsorbent

"Avirin" is a microcrystalline cellulose obtained from the Avicel Sales Division of American Viscose Co., Marcus Hook, Pa. Avicel is the pharmaceutical grade of the same material. We found that this grade possessed no advantage over our samples of Avirin, but either grade is satisfactory.

Preparation of chromatoplates

The microcrystalline cellulose ("Avirin" or "Avicel", 100 g) is blended in a Waring blender for 15–45 sec with 430 ml of water (the amount may vary with the particular lot) and glass plates (20 × 20 × 0.4 cm), which must be very clean, were coated with a layer 1.0 mm thick by means of a Desaga applicator. The 1.0 mm plates were found to be most advantageous for both analytical and preparative work. Plates spread only to a thickness of 0.25 mm did not show good performance. Slight differences

* Preliminary communication: *Chem. Ind.* (London), (1964) 1065.

with time of blending have been noted but the criterion for good smooth plates is that the blend "peaks" somewhat when poured in the applicator and the plates should be spread by drawing the applicator quite slowly, in contrast to the usual fairly rapid spreading with silica gel.

The plates are dried overnight at room temperature or for 30–60 min at 80° until the surface moisture has been released. The plates "set up" sufficiently after 30 min, and then are separated from one another, after which they may be stored for long periods of time stacked together in no special location or container much as one would store paper itself. The dried plates may be touched and written on without crumbling. They need not be desiccated or specially activated.

The compounds were dissolved in water or methanol according to their solubility and applied to the chromatoplates with a melting point capillary which had been drawn to a fine tip.

Developers

The solvent systems utilized were (A)[7] pyridine–ethyl acetate–acetic acid–water (5:5:1:3); (B) butanol–acetic acid–water (3:1:1); (C)[8] butanone–water azeotrope; (D)[9] ethyl acetate–acetic acid–formic acid–water (18:3:1:4).

Development of chromatoplates

The plates were developed in glass jars (29.5 × 27 × 10 cm) containing the solvent system to a depth of about 0.5 cm, and saturated by lining the walls with filter paper. The solvent was allowed to ascend to a height of 11–17 cm. The time of development varied from 1 to 3 h depending on the solvent. Solvent D gave the fastest and solvent B the slowest development.

Where the R_F values are low, as are most of those in Table II, a simple alternative to a commercial continuous flow apparatus may be used for better separations. A piece of Whatman No. 3 paper is attached to the top of the thin-layer plate to extend down 3 cm and is folded over the top behind the face of the plate (3–10 cm) by means of a rubber band. No problems are encountered with the cellulose crumbling under these conditions.

Spray reagents

Silver nitrate–sodium hydroxide[10] was used, followed by spraying with dilute sodium thiosulfate solution, which provided permanent zone location without heating.

In addition, the following were also used: aniline hydrogen phthalate[11], triphenyltetrazolium chloride (Ref. 1, p. 101), alkaline permanganate[12] (1 % potassium permanganate in 10 % aqueous sodium hydroxide) and 0.2 % ninhydrin in 95 % ethanol[13].

After development, the plates were dried with hot air and sprayed with the selected reagent. The free sugars and methyl glycosides were detectable, without heating, by the silver nitrate reagent while the hydroxy acids and lactones were likewise detectable, at room temperature, by the permanganate reagent. The saccharide methyl ethers were detected with the aniline phthalate or the triphenyltetrazolium reagent after heating for 5–10 min at 90–100°. The amino sugars and amino acids showed after heating for 5 min at 80° with the ninhydrin reagent. The N-

acetylamino sugars were not detected with this reagent but were shown after spraying the same plate with the silver nitrate reagent. The zones obtained were generally compact and showed little tendency to streak.

The results of the qualitative investigations are summarized in Tables I to VI.

Seminicro preparative separation

The chromatographic plate was prepared as described above. D-Galactose, 0.025 g, and D-xylose, 0.025 g, were dissolved in a small amount of water and the solution was applied to the plate by means of a sample applicator[14]. The plate was developed with solvent A. After development, the plate was dried and covered with a plastic shield to leave a 0.5-cm strip in the middle and 0.7-cm strip on each side of the plate. It was then sprayed with the silver nitrate reagent, and the zones were located and marked with a pencil. The individual bands were removed and the sugar was eluted by adding 30 ml of water. The cellulose was separated by centrifugation. This operation was repeated three times; the third eluate showed a negative reaction toward Fehling's solution. The combined eluates from each zone were evaporated under reduced pressure to 10 ml. Each fraction proved to be homogeneous by qualitative thin-layer chromatography as described above. The sugar content of a 2 ml aliquot was determined with sodium hypoiodite[15]. Found: D-galactose 0.020 g, D-xylose 0.019 g. From a 5 ml aliquot the sugars were recovered as the respective phenylosazones in good yields.

RESULTS AND DISCUSSION

"Avirin", a microcrystalline cellulose ("Avicel" is the pharmaceutical grade) produced by the American Viscose Corporation, has been found to be very useful for the thin-layer chromatography of water-soluble substances such as free sugars, glycosides, methyl ethers, hydroxy acids, lactones, amino sugars, and amino acids. We have found that Avirin cellulose in general gives more effective separations than does silica gel. For example, of the pentoses which SMITH and co-workers[2] examined, two ran the same and the third only 0.02 of an R_F unit different. In our Table I (with Solvent A) corresponding R_F values are 0.46, 0.52 and 0.59 for L-arabinose, D-xylose, and D-ribose. The R_F values for a number of monosaccharides and oligosaccharides are listed in Table I. It can be noted therein that D-glucose and D-galactose are separable in less than two hours of development time whereas on papergrams days are required to effect such a separation.

All the solvent systems we tried that have been previously reported for paper chromatography could be satisfactorily used for the thin-layer chromatography on Avirin cellulose; from these, four solvent systems were chosen as the best. The FISCHER-NEBEL[7] solvent (A) proved to be the most versatile and was found to be suitable for the separation of amino sugars and derivatives (Table II). Solvent A was also used for the preparative separation of a mixture of D-galactose and D-xylose as well as for the qualitative separation of methyl glycosides (Table III). In the latter case, the two anomeric pairs tried were separable in 2 h. Solvent C, butanone–water azeotrope, reported by SMITH and co-workers[8] as suitable for the separation of methylated sugars on paper, gave very good results on Avirin cellulose (Table IV). With these substances, an admixture of four (Table IV) was separated

TABLE I

R_F VALUES FOR SOME SACCHARIDES

Substance	R_F	
	Solvent A *	*Solvent B* **
L-Arabinose	0.46	0.31
D-Ribose	0.59	0.39
D-Xylose	0.52	0.33
D-Galactose	0.36	0.21
D-Glucose	0.39	0.25
D-Mannose	0.44	0.30
D-Fructose	—	0.29
L-Rhamnose	0.60	0.46
Cellobiose	0.25	0.13
Maltose	0.29	0.15
Maltotriose	—	0.07

* Solvent front: 13.3 cm; time: 105 min.
** Solvent front: 14.2 cm; time: 180 min.

TABLE II

R_F VALUES FOR AMINO SUGARS AND AMINO SUGAR DERIVATIVES IN SOLVENT A*

Substance	R_F
1,2-Diamino-1,2-dideoxy-D-glucitol dihydrobromide	0.11
1,2-Diamino-1,2-dideoxy-D-mannitol dihydrochloride	0.12
2-Amino-2-deoxy-D-galactose hydrochloride	0.18
2-Amino-2-deoxy-D-glucose hydrochloride	0.22
3-Amino-3-deoxy-D-mannose hydrochloride	0.24
2-Amino-2-deoxy-D-ribose hydrochloride	0.25
2-Amino-2-deoxy-D-lyxose hydrochloride	0.28
2-Amino-2-deoxy-L-xylose hydrochloride	0.31
1,2-Diacetamido-1 2-dideoxy-D-glucitol	0.38
2-Acetamido-2-deoxy-D-glucose	0.54
2-Acetamido-2-deoxy-D-glucose diethyl dithioacetal	0.85

* Solvent front: 15.5 cm; time: 115 min.

TABLE III

R_F VALUES FOR SOME METHYL GLYCOSIDES IN SOLVENT A*

Substance	R_F
Methyl β-D-arabinopyranoside	0.65
Methyl α-D-lyxopyranoside	0.75
Methyl β-D-xylopyranoside	0.70
Methyl α-D-glucopyranoside	0.57
Methyl β-D-glucopyranoside	0.61
Methyl α-D-galactopyranoside	0.54
Methyl β-D-galactopyranoside	0.56
Methyl β-D-galactofuranoside	0.72
Methyl β-cellobioside	0.44

* Solvent front: 15.6 cm; time: 120 min.

TABLE IV

R_F VALUES FOR SOME METHYL ETHERS OF SACCHARIDES (SOLVENT C*)

Substance	R_F
3-O-Methyl-D-galactose	0.02
4-O-Methyl-D-galactose**	0.02
3-O-Methyl-D-glucose	0.04
2,6-Di-O-methyl-D-galactose	0.15
2,4-Di-O-methyl-D-galactose**	0.23
2,4,6-Tri-O-methyl-D-galactopyranose**	0.48
2,3,6-Tri-O-methyl-D-mannose	0.57
2,3,6-Tri-O-methyl-D-glucose	0.60
Tetra-O-methyl-D-galactopyranose**	0.78
Tetra-O-methyl-D-fructopyranose	0.82
Tetra-O-methyl-D-glucopyranose	0.86

* Solvent front: 17.1 cm; time: 120 min.
** Separated in admixture and R_F values found to be the same as listed.

TABLE V

R_F VALUES FOR SOME HYDROXY ACIDS AND LACTONES IN SOLVENT D*

Compound	R_F
D-Arabinonic acid	0.16
Calcium D-erythronate	0.24
Glyoxylic acid	0.30
L-Erythraric [(+)-tartaric] acid	0.34
D-Arabinono-1,4-lactone	0.40
D-Erythrono-1,4-lactone	0.54
Oxalic acid	0.65

* Solvent front: 11.5 cm; time: 60 min.

TABLE VI

R_F VALUES FOR SOME AMINO ACIDS IN SOLVENT B*

Substance	R_F
Histidine	0.09
Lysine	0.10
Arginine	0.12
Serine	0.22
Threonine	0.28
Valine	0.51
Phenylalanine	0.61
Isoleucine	0.61
Leucine	0.64

* Solvent front: 16.7 cm; time: 180 min.

and the R_F values in the admixture were found to be identical with those found singly. Solvent D, ethyl acetate–acetic acid–formic acid–water (18:3:1:4), used by JONES AND WISE[9] for the separation of uronic acids on paper, has been used for the separation of hydroxy acids, lactones and aldonic acids (Table V). Paper chromatography has been an important tool in the investigation of amino acids; we found that Avirin cellulose could be used for this purpose (Table VI). The data of this table show that leucine and isoleucine are separable and that valine is separable from either.

The same spray reagents used on papergrams could be employed to detect the compounds on the thin-layer Avirin plates. Thus, alkaline silver nitrate[10] was used for the unsubstituted sugars and the methyl glycosides, aniline hydrogen phthalate[11] or triphenyltetrazolium chloride[1] for the methyl ethers of sugars, alkaline potassium permanganate[12] for acids and lactones and ninhydrin[13] for the amino sugars and amino acids. The N-acetylhexosamines were detected with the silver nitrate reagent.

A mixture of 0.025 g each of D-galactose and D-xylose was resolved on a plate with solvent A, the zones located and eluted as described in the experimental section. Estimation of the sugars with sodium hypoiodite[15] showed good recovery. From an aliquot the sugars were isolated as the respective phenylosazones in good yield.

The microcrystalline cellulose ("Avirin" or "Avicel") is much superior, in our experience, to other forms of cellulose, for thin-layer chromatography of water-soluble sugars, sugar derivatives, and amino acids. This material slurries nicely and spreads evenly. The cellulose adheres so firmly that the plates may be stacked on one another, written on, and stored without special precautions. The surface need not be specially activated. The advantages over silica gel are quite distinct and especially so with preparative separations. Their cost is about one-tenth that of silica gel G. These plates have good capacity, may be loaded with the aid of a heat gun and the material has no tendency to crumble (at 0.5 to 1.0 mm thickness), which is often the case with silica gel. When carrying out preparative work with silica gel on small amounts of natural products (especially where elution is necessary with polar solvents), one encounters contaminating colloidal silica gel and binder with the isolated zones even after extensive removal attempts.

This method of chromatography opens up the vast effective literature of papergram work while providing the ease and speed of the thin-layer technique. The zones obtained are generally very compact and show little streaking tendencies. It should readily be possible to quantitize the chromatograms by one or more of the methods established for papergrams, such as by using the proper colorimetric method for locating, removing, and eluting the zone material. The method should be well adapted for rapid undergraduate type experiments as well as for the research laboratory.

ACKNOWLEDGEMENT

Supported by the Pioneering Research Program of The Institute of Paper Chemistry (IPC Project 2150; OSURF Proj. 1353).

SUMMARY

"Avirin", or "Avicel", a microcrystalline form of cellulose, has been used with success in this laboratory for thin-layer chromatography. The material is employed

without a binder and has, in this laboratory, essentially completely displaced the papergram because of its speed, quality of separation, and the ease with which preparative plates may be run. In all cases tried it has been found that the papergram solvent systems and spray reagents are directly transferable to the new system, a finding which opens up the vast papergram literature to this type of thin-layer chromatography. By all comparisons, where both systems can be used, the method has proved superior, for technical and economic reasons, to that employing silica gel.

REFERENCES

1 J. M. BOBBITT, *Thin Layer Chromatography*, Reinhold, New York, 1963.
2 G. W. HAY, B. A. LEWIS AND F. SMITH, *J. Chromatog.*, 11 (1963) 479.
3 A. SCHWEIGER, *J. Chromatog.*, 9 (1962) 374.
4 P. WOLLENWEBER, *J. Chromatog.*, 9 (1962) 369.
5 K. RANDERATH AND H. STRUCK, *J. Chromatog.*, 6 (1961) 365.
6 K. RANDERATH, *Biochem. Biophys. Res. Commun.*, 6 (1961/1962) 452.
7 F. G. FISCHER AND H. J. NEBEL, *Z. Physiol. Chem.*, 302 (1955) 10.
8 L. BOGGS, L. S. CUENDET, I. EHRENTHAL, R. KOCH AND F. SMITH, *Nature*, 166 (1950) 520.
9 J. K. N. JONES AND L. E. WISE, *J. Chem. Soc.*, (1952) 3389.
10 W. E. TREVELYAN, D. P. PROCTER AND J. S. HARRISON, *Nature*, 166 (1950) 444.
11 S. M. PARTRIDGE, *Nature*, 164 (1949) 443.
12 W. H. MCNEELY, W. W. BINKLEY AND M. L. WOLFROM, *J. Am. Chem. Soc.*, 67 (1945) 527.
13 A. R. PATTON AND P. CHISM, *Anal. Chem.*, 23 (1951) 1683.
14 M. E. MORGAN, *J. Chromatog.*, 9 (1962) 379.
15 E. L. HIRST, L. HOUGH AND J. K. N. JONES, *J. Chem. Soc.*, (1949) 928.