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THIN-LAYER CHROMATOGRAPHY OF CARBOHYDRATES ON SILICA GEL IMPREGNATED WITH SODIUM ACETATE, MONOSODIUM PHOSPHATE AND DISODIUM PHOSPHATE

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SUMMARY

The chromatographic behavior of carbohydrates on silica gel plates impregnated with sodium acetate, monosodium phosphate and disodium phosphate is described and discussed.

The study shows that bidimensional thin-layer chromatography of carbohydrates with the above impregnants is not feasible, but that excellent monodimensional separations of up to ten sugars can be achieved with the sodium acetate and monosodium phosphate impregnants.

An investigation is specifically devoted to the setting up of a monodimensional chromatogram which separates the most important carbohydrates (biologically) in an 8-hour run.

INTRODUCTION

In a previous paper we explored the limitations and potentiality of thin-layer chromatography of carbohydrates on silica gel impregnated with boric acid. The present work is a study of the chromatographic behavior of sugars on silica gel impregnated with sodium acetate, monosodium phosphate and disodium phosphate.

EXPERIMENTAL

Preparation of the chromatoplates

30 g of silica gel, TLC grade, with binder (Fluka G) was mixed with each of the following solutions:

(a) Sodium acetate, 0.2 *M*,
(b) Monosodium phosphate, 0.2 *M*,
(c) Disodium phosphate, 0.2 *M*.

* Managing Director of the Istituto di Puericultura.

The suspensions thus obtained were applied in the usual manner in an 0.3 mm layer to the glass plates. These plates were dried for 24 hours at room temperature and then activated for 1 hour at 110°.

Samples

The following pure sugar samples were used

Monosaccharides	*Oligosaccharides*	*α-Methyl-derivatives*
Glyceraldehyde	Sucrose	α-Methyl-D-glucoside
Dihydroxyacetone	Turanose	α-Methyl-D-mannoside
Erythrose	Maltose	
Ribose	Lactose	
Arabinose	Trehalose	
Xylose	Melibiose	
Lyxose	Raffinose	
Fucose		
Rhamnose		
Glucose		
Galactose		
Mannose		
Levulose		
Sorbose		
Mannoheptulose		
Sedoheptulose		

Solvent systems

The following solvent systems were employed:

(1) *n*-Butanol–boric acid, 0.03 *M* (9:1)
(2) *n*-Butanol–acetone–water (4:5:1)
(3) *n*-Butanol-acetic acid–water (4:1:5)
(4) *n*-Butanol–pyridine–water (8:4:3)
(5) *n*-Butanol–pyridine–acetone–boric acid, 0.03 *M* (60:3:20:17)
(6) *n*-Butanol–isopropanol–water (3:5:2)
(7) *n*-Butanol–ethanol–water (2:1:1)
(8) *n*-Butanol–ethanol–phosphoric acid, 0.1 *M* (1:10:5)
(9) *n*-Butanol–ethanol–hydrochloric acid, 0.1 *N* (1:10:5)
(10) *n*-Butanol–methanol–water (5:3:1)
(11) *n*-Butanol–ethyl acetate–boric acid, 0.03 *M* (70:15:15)
(12) *n*-Butanol–ethyl acetate–pyridine–water (2:3:2:3)
(13) *n*-Butanol–ethyl acetate–isopropanol–acetic acid–water (35:100:60:35:30)
(14) *n*-Butanol–ethyl acetate–isopropanol–acetic acid–water (3:10:6:6:3)
(15) *n*-Propanol–water (85:15)
(16) *n*-Propanol–acetic acid–water (4:1:5)
(17) *n*-Propanol–acetic acid–boric acid, 0.03 *M* (4:1:5)
(18) *n*-Propanol–pyridine–water (5:3:2)
(19) *n*-Propanol–ethyl acetate–water (1:4:2)
(20) Ethyl acetate–acetic acid–water (6:3:2)
(21) Ethyl acetate–pyridine–water (2:1:2)

(22) Ethyl acetate–pyridine–water (3:1:2)
(23) Ethyl acetate–pyridine–water (3:3:2)
(24) Ethyl acetate–pyridine–water (4:3:2)
(25) Ethyl acetate–pyridine–boric acid, 0.03 *M* (2:1:2)
(26) Ethyl acetate–acetic acid–boric acid, 0.03 *M* (3:1:3)
(27) Ethyl acetate–isopropanol–water (65:22:11)
(28) Ethyl acetate–isopropanol–acetic acid–water (100:60:35:30)
(29) Ethyl acetate–isopropanol–water (100:60:30)
(30) *n*-Butanol–methanol–boric acid, 0.03 *M* (5:3:1)
(31) *n*-Butanol–acetic acid–water (5:4:1)
(32) Ethyl acetate–acetic acid–methanol–water (60:15:15:10)
(33) Isopropanol–ethyl acetate–water (7:1:2)
(34) Chloroform–methanol (6:4)
(35) Isopropanol–water (4:1)
(36) Acetone–water (9:1)
(37) Acetone–water–chloroform–methanol (8:0.5:1:1)
(38) Methanol–chloroform–acetone–ammonia (28 Bè) (5:2:3:2)
(39) Methanol–chloroform–ammonia (28 Bè) (6:4:0.7)
(40) *n*-Butanol–ethyl ether–water (4:5:1)
(41) *n*-Butanol–ethyl acetate–isopropanol–water (200:100:70:35)
(42) *n*-Butanol–ethyl acetate–isopropanol–water (35:100:60:30)

Monodimensional chromatography

10 mg of each sugar were dissolved in 2 ml distilled water. One μl of each solution (= 5 μg of carbohydrate) was applied to the chromatoplate with a micropipette. The chromatoplate was then placed in a tank containing the developing solvent system, the walls being lined with filter paper impregnated with the developing solvent. The plates were so slightly tilted that they were practically upright. The room temperature was kept at 22°.

Detection of the spots

After development, the chromatoplate was dried at room temperature for a short time. It was then heated in an oven at 110° until the odor of the solvents could no longer be detected (*ca.* 1 hour). The heated chromatoplate was then sprayed with a freshly prepared solution of 20 mg of naphthoresorcinol, 10 ml of ethanol and 0.2–0.4 ml of conc. H_2SO_4. (The sodium acetate impregnated plates needed 0.4 ml of H_2SO_4 for good color development). After a few minutes at room temperature, vivid spots appeared against a white or slightly tinted background. The white background darkened if the chromatoplates were overheated after spraying. The colors of the spots have already been described in a preceding study[1], where they were developed on boric acid impregnated plates. These colors proved to be equally vivid on the monosodium phosphate impregnated plates, but the sodium acetate impregnation caused the shades to vary a little (they tended slightly toward an overall brownish tint). Disodium phosphate impregnation caused a deadening of the colors coupled with a darkening of the background.

Tables I, II and III show the approximate R_F values (× 100) of the carbohydrates used, relative to each solvent reported.

TABLE I

R_F VALUES (× 100) ON PLATES IMPREGNATED WITH SODIUM ACETATE

Carbohydrates	*Solvent system*																	
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
Glyceraldehyde	8	53	60	54	20	32	55	55	64	55	19	47	48	45	52	84	68	74
Dihydroxyacetone	22	53	63	58	0	35	41	60	69	46	21	55	43	51	42	84	66	64
Erythrose	16	44	66	49	0	29	40	64	70	46	16	58	23	49	45	84	68	60
Ribose	8	29	55	38	23	21	35	66	67	32	7	36	29	47	30	77	59	55
Arabinose	7	25	60	15	21	20	31	59	65	32	4	30	25	38	27	68	58	51
Xylose	13	36	58	47	26	29	37	71	71	43	9	55	32	52	39	81	65	63
Lyxose	13	34	66	44	27	28	36	66	71	42	8	46	31	42	37	79	59	63
Fucose	10	32	56	41	25	25	37	69	69	39	7	46	28	48	35	81	63	58
Rhamnose	21	47	59	58	38	36	43	66	73	50	16	56	40	6	47	81	66	69
Glucose	5	22	66	30	18	21	31	66	68	35	3	33	25	36	30	74	60	58
Galactose	3	19	55	25	13	16	31	62	67	29	2	31	23	29	24	77	67	55
Mannose	20	43	66	55	37	36	43	71	70	51	16	51	39	46	46	79	63	69
Levulose	4	23	55	32	18	20	33	59	68	31	3	33	24	42	28	77	66	52
Sorbose	7	26	58	33	20	23	33	63	73	36	4	33	26	43	31	74	62	55
Mannoheptulose	4	20	60	30	18	21	31	64	67	33	2	27	25	39	29	72	59	58
Sedoheptulose	5	21	60	26	19	18	31	55	71	28	3	25	23	35	25	76	60	56
Sucrose	2	17	55	24	12	16	32	68	72	27	2	27	21	38	24	81	66	51
Turanose	1	13	60	23	12	15	27	63	68	25	1	27	20	31	22	71	58	53
Maltose	1	13	55	20	10	13	26	63	70	23	1	25	19	35	20	57	58	46
Lactose	0	9	55	18	5	8	21	52	67	16	0	22	15	27	14	77	58	36
Trehalose	2	12	69	18	13	15	26	66	71	24	2	20	19	25	20	74	56	48
Melibiose	0	8	58	14	5	10	21	53	67	16	0	22	15	23	14	71	56	42
Raffinose	0	4	55	15	3	3	15	59	68	10	0	21	11	21	9	71	55	33
α-Methyl-D-glucoside	15	37	55	44	27	31	41	70	74	45	12	33	34	51	41	81	66	63
α-Methyl-D-mannoside	8	26	55	34	20	24	33	70	74	34	5	31	27	42	34	75	63	58
Shape of spots	*A*	*B*	*AB*	*B*	*D*	*B*	*D*	*D*	*D*	*B*	*A*	*E*	*A*	*B*	*A*	*E*	*E*	*B*

The shape of the spots in each solvent used is represented in Tables I–III by the letters A–E which are defined below:

A = sharply-defined and well-rounded spots;

B = slightly diffuse, but well-rounded spots;

C = well-defined, but deformed spots;

D = diffuse spots;

E = diffuse and tailed spots.

Solvents producing "A" and "B" spots are, naturally, the best, but "C" spots are often also acceptable.

DISCUSSION

The choice of 0.2 *M* solutions was based on the work of OVODOV *et al.*[2], which showed that best results are obtained with 0.2–0.3 *M* impregnation.

The thickness of the layer was left at 0.33 mm, as in our preceding study[3], for the reasons stated in that paper.

The spots were usually sharply defined when sodium acetate and monosodium phosphate were used for impregnation, but in the second case, they were more

19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
55	57	38	44	55	31	43	60	33	38	29	54	64	67	57	41	41	58	52	99	55	25	47	37
45	54	32	42	50	11	39	71	37	54	22	55	49	57	54	41	47	51	41	99	49	15	30	25
47	50	34	5	47	23	38	73	19	34	30	56	46	54	51	44	45	51	43	80	51	9	23	19
56	43	27	20	50	15	40	54	12	42	20	42	45	44	38	8	38	31	21	33	8	4	14	14
46	35	25	25	27	16	28	47	5	37	13	41	37	43	39	17	35	29	21	36	18	3	9	10
37	48	32	24	49	18	39	47	7	45	21	52	51	52	50	24	46	43	29	42	30	4	19	19
48	46	30	16	45	24	25	73	9	44	21	52	43	53	49	22	44	40	30	39	25	4	17	16
47	42	29	31	45	19	41	53	8	46	18	48	49	50	43	20	42	36	26	36	29	3	14	16
54	56	36	29	64	22	41	58	18	54	33	5	57	63	60	33	53	5	42	49	41	9	27	27
47	34	29	14	34	13	25	71	4	37	10	44	37	43	43	11	37	25	16	16	13	0	9	9
54	31	26	16	16	10	39	43	4	35	9	37	36	40	32	8	34	20	9	16	5	0	7	8
49	53	25	35	58	30	35	74	17	5	30	58	48	60	57	47	51	49	39	47	41	7	24	26
56	36	27	18	41	14	35	55	7	37	13	39	39	42	37	7	37	25	13	17	6	2	9	10
38	40	29	21	45	16	39	43	5	38	12	46	42	43	43	14	41	29	21	25	15	3	11	11
46	32	27	14	32	7	14	66	3	36	10	42	36	41	41	4	36	20	12	10	12	0	9	9
46	29	27	25	22	14	32	46	4	34	10	37	31	48	34	16	31	23	17	19	14	3	8	8
52	24	25	14	33	13	50	38	2	33	8	35	32	35	32	4	36	16	5	7	3	0	5	7
45	19	25	7	15	8	18	63	1	29	6	34	25	30	35	0	30	13	9	6	11	0	5	6
47	20	24	18	18	9	41	35	0	30	7	25	30	29	26	4	29	11	3	6	2	0	4	5
45	14	23	13	20	8	39	27	0	23	3	18	21	25	16	0	22	7	1	4	0	0	2	4
44	16	23	18	18	9	28	55	1	26	17	34	19	26	33	5	30	10	4	5	3	0	4	5
38	14	26	15	14	8	16	55	0	22	2	23	20	23	26	0	20	7	3	4	0	0	3	3
44	8	22	13	27	4	32	25	0	17	2	6	15	18	15	0	21	3	4	0	0	0	0	2
40	43	30	32	58	25	41	47	9	44	19	51	42	50	52	30	51	33	46	47	34	3	16	16
40	34	30	27	56	23	40	44	4	37	13	47	39	42	43	12	43	26	19	18	9	3	11	11
D	B	CD	CD	CD	CD	CD	CD	A	A	A	A	B	A	BC	E	B	A	B	E	E	A	A	C

rounded and less diffuse (OVODOV *et al.* reported similar results). With disodium phosphate impregnation, the spots were generally well shaped, but color development was poor. Apart from the tendency of the background to darken when heated, many sugars (even though we used 5-μg quantities) were difficult to detect with the naphthoresorcinol reagent.

The migration ratio of each carbohydrate tended to be independent of the solvent system used with all three impregnants, and we observed that, with very few exceptions, R_F values obtained with the disodium phosphate impregnant were lower than the corresponding R_F's obtained with monosodium phosphate, but that the general pattern of spots with these two impregnants was very similar. Exceptions to this pattern of independence of the solvent used occurred only when boric acid was incorporated into the system.

Common characteristics of all three types of impregnation were:

(a) The R_F values of the following 11 carbohydrates (from bottom to top) were in ascending progression: raffinose, lactose, maltose, sucrose, galactose, levulose, ribose, fucose, xylose, rhamnose and glyceraldehyde.

(b) Oligosaccharides tended to have the same migration rates, especially the triad, raffinose, trehalose and melibiose, which was extremely difficult to separate.

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	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
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Dihydroxyacetone	22	53	63	58	0	35	41	60	69	46	21	55	43	51	42	84	66	64
Erythrose	16	44	66	49	0	29	40	64	70	46	16	58	23	49	45	84	68	60
Ribose	8	29	55	38	23	21	35	66	67	32	7	36	29	47	30	77	59	55
Arabinose	7	25	60	15	21	20	31	59	65	32	4	30	25	38	27	68	58	51
Xylose	13	36	58	47	26	29	37	71	71	43	9	55	32	52	39	81	65	63
Lyxose	13	34	66	44	27	28	36	66	71	42	8	46	31	42	37	79	59	63
Fucose	10	32	56	41	25	25	37	69	69	39	7	46	28	48	35	81	63	58
Rhamnose	21	47	59	58	38	36	43	66	73	50	16	56	40	6	47	81	66	69
Glucose	5	22	66	30	18	21	31	66	68	35	3	33	25	36	30	74	60	58
Galactose	3	19	55	25	13	16	31	62	67	29	2	31	23	29	24	77	67	55
Mannose	20	43	66	55	37	36	43	71	70	51	16	51	39	46	46	79	63	69
Levulose	4	23	55	32	18	20	33	59	68	31	3	33	24	42	28	77	66	52
Sorbose	7	26	58	33	20	23	33	63	73	36	4	33	26	43	31	74	62	55
Mannoheptulose	4	20	60	30	18	21	31	64	67	33	2	27	25	39	29	72	59	58
Sedoheptulose	5	21	60	26	19	18	31	55	71	28	3	25	23	35	25	76	60	56
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Turanose	1	13	60	23	12	15	27	63	68	25	1	27	20	31	22	71	58	53
Maltose	1	13	55	20	10	13	26	63	70	23	1	25	19	35	20	57	58	46
Lactose	0	9	55	18	5	8	21	52	67	16	0	22	15	27	14	77	58	36
Trehalose	2	12	69	18	13	15	26	66	71	24	2	20	19	25	20	74	56	48
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α-Methyl-D-mannoside	8	26	55	34	20	24	33	70	74	34	5	31	27	42	34	75	63	58
Shape of spots	*A*	*B*	*AB*	*B*	*D*	*B*	*D*	*D*	*D*	*B*	*A*	*E*	*A*	*B*	*A*	*E*	*E*	*B*

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30	31	44	39	67	41	35	38	38	52	55	64	67	55	66	58	62	55	59	99	48	21	62	54
28	46	44	28	75	60	43	37	49	57	60	54	51	55	66	55	54	52	68	89	41	14	44	48
29	38	45	38	70	57	23	37	36	55	47	53	51	47	67	48	56	65	61	82	23	13	41	42
26	27	27	25	71	49	32	40	16	35	26	47	41	36	54	33	49	49	43	37	27	9	28	27
24	19	27	11	35	33	26	33	7	22	12	42	31	24	43	30	41	33	30	41	20	13	13	12
29	27	38	13	70	43	33	40	13	35	24	52	43	37	58	37	51	67	43	38	30	10	25	25
26	26	26	15	62	46	28	37	14	35	23	49	40	36	59	36	47	52	43	34	26	10	26	24
26	24	30	14	67	43	32	40	11	31	20	51	40	35	58	38	52	49	42	41	27	9	24	23
29	37	41	33	85	58	34	40	32	52	50	60	52	51	68	47	62	68	57	40	41	11	45	47
25	14	25	9	40	28	24	36	4	18	10	40	30	20	41	22	35	24	20	13	12	8	10	9
30	11	23	7	39	25	23	40	3	13	8	31	28	16	36	16	30	16	15	18	11	5	10	7
27	37	30	33	81	62	34	38	26	49	46	55	49	47	71	49	59	63	52	38	35	13	41	40
31	16	24	11	53	34	26	40	7	20	13	40	33	24	41	22	41	31	25	36	15	7	16	14
23	20	30	9	50	29	26	35	6	23	13	42	37	26	45	26	44	35	29	19	17	14	13	12
24	14	23	9	45	29	24	37	5	18	10	42	31	21	45	20	37	27	21	11	10	4	13	11
22	16	30	9	46	30	25	34	5	19	10	42	29	21	41	27	40	32	26	16	17	13	10	10
30	9	21	7	50	25	24	41	2	10	5	35	25	13	45	9	42	19	13	13	5	5	8	6
24	8	22	7	48	25	25	37	2	9	5	36	23	10	41	8	36	20	13	6	4	5	6	5
30	7	19	6	41	18	23	42	2	6	4	30	24	9	35	7	29	11	8	9	3	3	5	3
30	4	16	7	27	14	21	44	0	4	3	20	18	9	24	5	16	6	5	4	2	1	3	2
23	7	25	9	31	16	21	36	0	6	3	38	22	8	31	4	30	10	5	4	2	10	2	2
23	5	15	6	21	10	24	37	0	3	2	15	19	8	15	3	11	22	2	3	1	0	1	1
30	2	14	7	20	9	21	35	0	1	2	13	15	8	19	2	9	2	3	4	0	0	2	0
26	31	37	11	54	33	27	35	12	37	22	46	47	36	61	44	60	57	43	36	40	13	24	26
24	19	33	11	53	27	26	36	6	20	14	41	31	20	45	23	46	31	24	13	14	14	13	12
D	C	CD	CD	CD	CD	CD	BD	A	A	A	A	C	A	B	E	A	A	A	E	B	A	A	A

monosodium phosphate, solvent 5 (which contains boric acid) separated raffinose and trehalose, as well as the triad, dihydroxyacetone–erythrose–glyceraldehyde. On the other hand, solvent 5 was only able to separate dihydroxyacetone from glyceraldehyde when sodium acetate was used for impregnation. Again, with the sodium acetate impregnation, solvent 1 (contains boric acid) separated dihydroxyacetone, erythrose and glyceraldehyde.

There was no solvent, in conjunction with any type of impregnation, which succeeded in separating all the components of the two triads (raffinose–melibiose–trehalose and dihydroxyacetone–erythrose–glyceraldehyde). To sum up, the most difficult problem to solve is the separation, on the same plate, of oligosaccharides and the low molecular weight trioses and tetroses. The best separations were always in the pentose–hexose range.

The monosaccharide quartet, arabinose–sedoheptulose–sorbose–α-methyl-D-mannoside, was impossible to split into more than two components with the monosodium or disodium phosphate impregnants. Sodium acetate impregnation, however, resulted in solvents 4, 8 and 20 separating the group into three spots, while solvent 23 separated all four sugars. Unfortunately, the spots obtained with this last-mentioned solvent were badly-shaped and diffuse.

TABLE III

R_F VALUES (× 100) ON PLATES IMPREGNATED WITH DISODIUM PHOSPHATE

Carbohydrates	*Solvent system*																	
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
Glyceraldehyde	30	66	55	41	55	59	63	71	71	67	52	49	47	55	63	87	78	84
Dihydroxyacetone	29	62	43	30	43	45	41	66	66	52	28	41	41	49	46	89	76	76
Erythrose	30	56	44	30	38	45	42	67	66	51	27	44	35	47	48	87	77	78
Ribose	7	34	42	23	24	30	27	59	57	30	6	30	23	33	28	92	67	60
Arabinose	7	20	34	21	13	20	22	57	53	24	5	22	14	20	25	89	69	52
Xylose	10	35	38	27	26	32	29	64	65	38	8	41	23	34	31	92	74	64
Lyxose	14	38	35	22	26	35	30	66	66	41	10	49	22	33	34	89	74	67
Fucose	13	35	38	24	24	31	30	64	64	38	10	31	23	32	30	92	71	67
Rhamnose	31	59	41	33	49	52	45	71	71	60	27	44	40	51	52	88	76	84
Glucose	5	14	35	15	11	19	20	56	55	20	3	38	11	16	18	90	73	42
Galactose	3	9	44	12	9	13	18	51	47	15	2	24	8	14	13	91	71	33
Mannose	27	56	36	32	45	50	47	72	75	55	25	38	40	51	49	89	74	82
Levulose	4	19	43	19	9	15	22	56	55	23	2	27	14	22	20	90	74	47
Sorbose	3	21	36	18	7	23	23	63	62	29	2	24	16	22	24	91	69	54
Mannoheptulose	4	16	35	16	8	21	21	59	59	24	2	29	12	19	20	90	73	47
Sedoheptulose	5	21	34	19	13	20	23	63	59	26	3	22	14	19	24	92	69	56
Sucrose	2	10	45	11	9	14	15	62	58	15	1	19	7	12	15	89	73	51
Turanose	2	11	35	13	8	16	16	62	62	17	2	25	7	10	15	91	73	52
Maltose	0	7	46	7	5	9	11	58	53	11	0	14	5	8	11	87	73	49
Lactose	0	4	47	5	4	7	9	54	48	9	0	14	3	5	8	88	73	39
Trehalose	1	5	34	7	5	9	13	56	53	10	1	19	4	7	21	88	66	34
Melibiose	0	2	35	3	3	4	9	44	42	4	0	19	2	4	4	88	71	18
Raffinose	0	1	47	2	2	3	6	51	48	3	0	15	2	2	4	87	71	22
α-Methyl-D-glucoside	16	46	38	30	33	41	35	76	73	49	18	27	29	40	46	88	74	79
α-Methyl-D-mannoside	7	19	40	18	15	24	24	63	62	25	6	26	15	19	25	90	70	52
Shape of spots	*B*	*B*	*B*	*B*	*D*	*B*	*D*	*D*	*D*	*B*	*A*	*E*	*B*	*B*	*A*	*E*	*E*	*A*

Solvent systems containing pyridine usually made good separations, but resulted in tailed and diffuse spots.

Bidimensional chromatography

Despite our efforts to test all possible combinations of solvents, we were unable to obtain a chromatogram that came even near the results achieved in our previous work with boric acid as impregnant[3].

The pattern of spots was always a broad, confused and diffused line. With some solvent systems, a number of sugars could be separated (eleven to fifteen), but the effort was not worth it, since the spots were always diffused and almost the same number of sugars could be separated in a monodimensional run. The addition of boric acid to the second solvent of the bidimensional development slightly improved separations. Thus, we added boric acid to many of the solvents, but found that the overall improvement was very slight. We finally concluded that only the borate complex of the carbohydrates can be separated bidimensionally on silica gel, and that successful separation in this case occurs only when no large amounts of other ions are present.

In the case of boric acid impregnation, a unique migration pattern in each

19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
42	30	37	23	41	58	24	52	26	44	29	44	67	47	66	30	52	35	54	84	41	10	55	46
41	42	34	16	48	58	18	48	45	41	47	58	48	54	58	22	41	44	63	84	40	20	38	48
41	38	35	20	44	55	20	47	29	33	44	57	48	55	59	25	52	46	48	42	21	20	35	41
42	24	22	1	43	41	21	54	11	29	26	27	37	35	45	3	38	18	22	20	13	5	16	22
39	14	16	5	15	27	17	41	5	20	14	21	30	25	35	5	26	18	11	32	13	20	9	12
41	24	30	4	35	41	23	53	9	28	24	36	41	38	52	7	43	32	24	37	20	3	16	20
36	24	23	7	34	44	17	49	11	27	26	38	40	39	52	11	44	38	27	30	22	19	19	23
41	23	23	6	41	41	21	53	8	27	22	32	39	36	49	4	40	19	22	25	19	3	15	20
41	36	33	16	53	68	23	50	29	44	29	42	51	39	67	27	52	32	53	41	32	11	38	43
36	12	19	3	21	23	20	49	3	16	10	27	28	21	34	2	26	11	7	10	7	16	7	8
43	8	15	2	33	22	18	54	2	14	8	13	23	16	27	0	19	7	3	10	6	0	4	5
40	38	22	18	53	64	23	48	26	44	47	53	50	53	63	16	58	5	41	36	30	20	33	40
44	14	17	6	35	27	20	53	4	21	14	21	29	24	37	1	28	13	9	14	8	2	9	11
39	15	21	3	27	30	19	41	5	20	13	29	35	29	41	5	34	20	12	25	9	20	10	12
35	12	19	3	28	24	19	49	3	19	11	21	30	23	38	2	31	11	7	9	4	13	8	9
38	19	19	3	23	32	18	41	4	18	11	24	28	24	36	7	27	20	13	14	10	20	9	11
41	8	15	2	45	25	17	56	2	11	6	13	20	13	33	0	27	5	2	5	3	0	3	5
34	8	16	1	27	23	20	51	2	10	5	15	23	12	35	0	27	10	2	4	2	12	4	5
40	6	13	1	13	14	14	55	0	7	3	9	19	11	23	0	19	2	2	3	2	0	1	3
38	5	10	2	9	7	16	56	0	3	2	5	16	9	18	0	13	0	1	2	1	0	0	2
38	16	12	3	13	14	17	47	0	5	4	8	24	7	24	0	16	3	1	3	1	20	2	2
32	5	7	1	7	7	18	52	0	3	2	2	20	8	12	0	7	0	0	1	0	0	0	0
33	3	8	1	18	6	14	55	0	1	1	0	13	9	11	0	9	0	0	0	0	0	0	0
40	30	30	5	31	32	21	43	12	33	26	48	45	43	59	25	55	47	33	36	33	20	23	29
40	15	27	5	28	30	20	41	5	18	14	30	32	25	42	4	37	22	8	13	7	20	10	12
D	*C*	*CD*	*CD*	*CD*	*CD*	*CD*	*CD*	*AB*	*A*	*A*	*A*	*C*	*A*	*B*	*E*	*A*	*A*	*A*	*E*	*B*	*B*	*A*	*A*

solvent system was apparent, but such was not the case with the sodium acetate, monosodium phosphate and disodium phosphate impregnants. This is, naturally, the basic obstacle to successful bidimensional chromatography.

Monodimensional chromatography

The sodium acetate and monosodium phosphate impregnants, however, have a great advantage: they can separate sharply different mixtures (according to the solvent used) of up to 9–10 carbohydrates on a monodimensional run.

Figs. 1 and 2 show some examples of separations on a standard 18 cm run.

The data in Tables I, II and III can of course be used for selecting the conditions for a given carbohydrate mixture.

We wish to point out that with monosodium phosphate impregnation, solvent 37 was successful in separating a mixture of sugars containing glucose, levulose, ribose and other biologically important carbohydrates. The following 10 sugars were separated on a 33 cm run: raffinose, lactose, maltose, galactose, sucrose, glucose, levulose, arabinose, ribose and rhamnose. This finding means that only one, rather than two, standard carbohydrate mixtures are necessary for clinical applications. In a preceding paper, which described a monodimensional technique for the qualitative and semi-

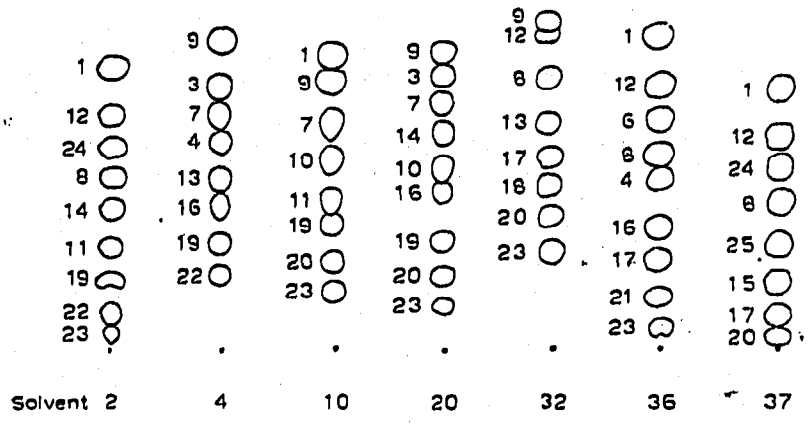

Fig. 1. Separation of carbohydrate mixtures on silica gel impregnated with 0.2 *M* sodium acetate. Carbohydrates: 1 = glyceraldehyde; 2 = dihydroxyacetone; 3 = erythrose; 4 = ribose; 5 = arabinose; 6 = xylose; 7 = lyxose; 8 = fucose; 9 = rhamnose; 10 = glucose; 11 = galactose; 12 = mannose; 13 = levulose; 14 = sorbose; 15 = mannoheptulose; 16 = sedoheptulose; 17 = sucrose; 18 = turanose; 19 = maltose; 20 = lactose; 21 = trehalose; 22 = melibiose; 23 = raffinose; 24 = α-methyl-D-glucoside; 25 = α-methyl-D-mannoside.

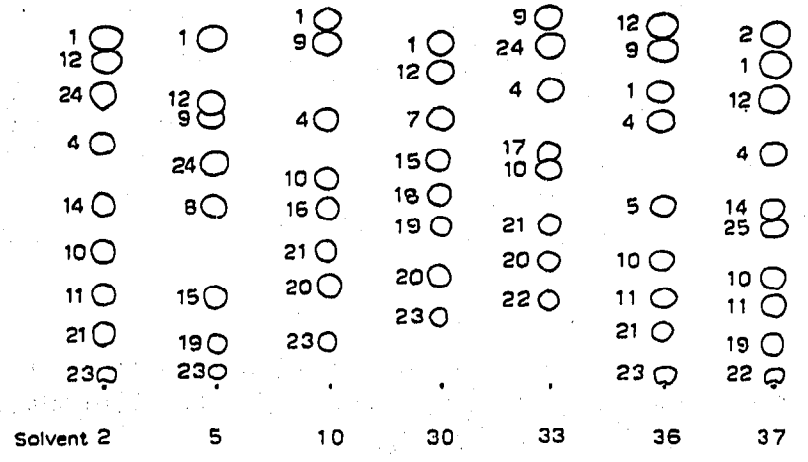

Fig. 2. Separation of carbohydrate mixtures on silica gel impregnated with 0.2 *M* monosodium phosphate. For numbering of carbohydrates see Fig. 1.

quantitative analysis of carbohydrates commonly found in biological fluids[1], we were forced to propose a separate glucose standard in addition to the standard carbohydrate mixture, since glucose bridged the ribose and levulose spots.

Fig. 3 shows how clearly solvent 37 achieves the separation of the above-mentioned sugars. The time required for this run was exactly 8 hours.

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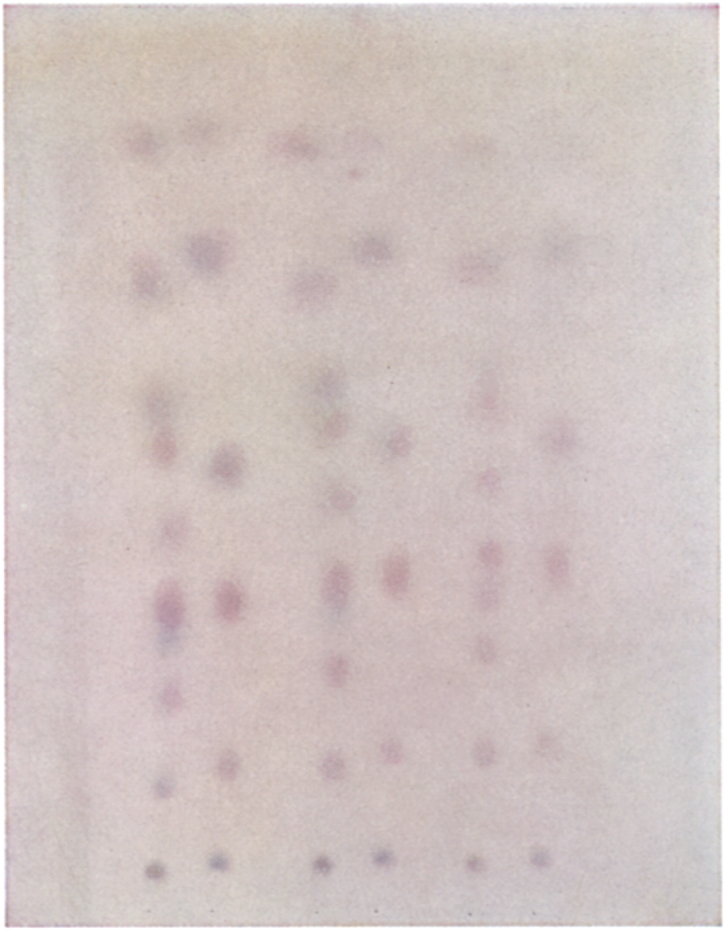

Fig. 3. Monodimensional chromatography of two carbohydrate mixtures on a 20 × 35 cm plate. Adsorbent: Silica Gel G with 0.2 *M* monosodium phosphate impregnant. Solvent system: acetone–water–chloroform–methanol (8:0.5:1:1). 1, 3 and 5 = 8, 4 and 2 μg per spot respectively of (from bottom to top): raffinose, lactose, maltose, galactose, sucrose, glucose, levulose, arabinose, ribose, rhamnose. 2, 4 and 6 = 8, 4 and 2 μg per spot respectively of (from bottom to top): melibiose, trehalose, turanose, mannoheptulose, lyxose and dihydroxyacetone.