THIN-LAYER CHROMATOGRAPHY OF CARBOHYDRATES

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The first attempts to separate sugar mixtures by thin-layer chromatography were made in 1961[1-4] and papers concerning this problem have continued to appear. Silica gel G[5,6], kieselguhr[7], gypsum[8], cellulose powder[9] as well as silica gel and/or kieselguhr impregnated with boric acid, sodium tetraborate, bisulfite and acetate[1-4,6,10-12] have been used on chromatoplates for the separation of carbohydrates and many different solvents were employed as developers. However thin-layer chromatography of carbohydrates did not gain general acceptance in research work. Poor separation of some of the more common sugars and the low capacity of the chromatoplates are two disadvantages. Therefore at present paper chromatography is usually used in most laboratories for sugar separations. Recently thin-layer chromatography on silica gel impregnated with sodium dihydrogen phosphate or sodium monohydrogen phosphate in phosphoric acid was successfully used[13,14]. Our data also suggest excellent results in this case.

The present paper studies the effect of (a) the type and concentration of the impregnating salts, (b) the total sugar concentration in mixtures and (c) the solvents and temperature on sugar separations on impregnated silica gel.

EXPERIMENTAL

Preparation of the chromatoplates

35 g of silica gel "KSK" (200 mesh and more), washed free from impurities before use, were mixed with 2.5 g of gypsum in 100 ml of the inorganic salt solution of the required concentration. The suspension obtained was applied to the glass plates at a thickness of about 0.5 mm. These plates are allowed to stand for 24 h at room temperature and then used for chromatography.

Procedure

For chromatography 1% sugar solutions in 70% aqueous ethanol were employed. An aliquot containing a suitable quantity of the pure sugars or their mixtures was applied to the plates with a micro-pipette. The plates were developed by the ascending technique. The following solvent systems were employed:

(1) *n*-Butanol–acetone–water (4:5:1, v/v),
(2) *n*-Propanol–water (85:15, v/v),
(3) *n*-Butanol–pyridine–water (8:4:3, v/v),

(4) Ethyl acetate–*n*-propanol–water (4:1:2, v/v),
(5) Ethyl acetate–acetic acid–water (6:3:2, v/v),
(6) *n*-Butanol–ethanol–water (2:1:1, v/v),
(7) Ethyl acetate–pyridine–water (2:1:2, v/v),
(8) *n*-Butanol–methanol–water (5:3:1, v/v),
(9) *n*-Propanol–pyridine–water (5:3:2, v/v),
(10) *n*-Butanol–ethanol–0.1 *M* phosphoric acid (1:10:5, v/v),
(11) *n*-Butanol–ethanol–0.1 *N* hydrochloric acid (1:10:5, v/v),
(12) Ethanol–formic acid–water (5:1:1, v/v),
(13) Ethyl acetate–pyridine–water (20:13:15, v/v).

The detection reagents used were as follows: (1) conc. sulfuric acid at 110°/15 min, (2) aniline hydrogen phthalate at 105°/15 min and (3) Tollens' reagent.

RESULTS AND DISCUSSION

Attempts to separate sugars on silica gel in the presence of 0.01–0.3 *M* solutions of KCl, $NaNO_3$, K_2SO_4, KCNS, Na_2CO_3, Na_3PO_4 and $ZnCl_2$ were unsuccessful. All the spots obtained were shown to have an unsatisfactory elongated form and no separation of the sugars was observed. Nearly circular spots were obtained when sodium mono- and dihydrogen phosphate, bicarbonate and tungstate and disodium phenyl phosphate were employed as additives to the silica gel. In a number of cases a good sugar separation was achieved. Impregnation of silica gel with sodium bisulfite, acetate and tetraborate or boric acid gave rise to satisfactory spots and good separation of the sugars only when there were low sugar concentrations (5–30 μg) in the mixture. Higher sugar concentrations were found to lead to elongated spots.

Tables I–VI illustrates the best results obtained for sugar separation. For comparison the results obtained on pure silica gel are given in certain cases. As may be seen from Tables I and II higher R_F values and unsatisfactory separation were ob-

TABLE I

CHROMATOGRAPHY OF MONOSACCHARIDES AND THEIR MIXTURES ON SILICA GEL THIN-LAYERS*

Compounds	*Pure silica gel*		*Silica gel impregnated with*								
			0.3 M Na_2HPO_4		*0.4 M* $NaHCO_3$		*0.2 M AcONa*		*0.15 M* $NaHSO_3$		*0.05 M* Na_2PhPO_4
Solvents	*1*	*5*	*1*	*3*	*1*	*2*	*6*	*8*	*1*	*2*	*1*
Galactose	65	50	12	17	26	18	17	11	51	22	39
Glucose	65	59	13	21	32	23	26	19	51	23	47
Mannose	66	63	19	29	33	26	29	20	53	24	50
Arabinose	66	59	20	30	33	22	30	20	53	24	49
Xylose	68	66	27	44	37	32	31	29	53	27	57
Fucose	68	64	30	43	—	—	31	—	54	—	54
Rhamnose	72	73	49	60	45	39	37	46	55	54	63
Monosaccharide mixtures	N	P	S	S	P	P	P	P	N	P	P

* $R_F \times 100$ values are given. The total sugar concentration in mixtures was not more than 400–500 μg. N = No separation of mixtures; P = partial separation of mixtures; S = satisfactory separation of mixtures.

TABLE II

CHROMATOGRAPHY OF OLIGOSACCHARIDES AND THEIR MIXTURES ON SILICA GEL THIN LAYERS*

Compounds	*Pure silica gel*	*Silica gel impregnated with*				
		0.3 M Na_2HPO_4			*0.15 M $NaHSO_3$*	*0.05 M Na_2PhPO_4*
Solvent No.	*1*	*1*	*10*	*11*	*1*	*1*
Sucrose	53	Remains	42	53	35	40
Cellobiose	45	at the	39	49	29	33
Maltose	45	start	35	47	29	34
Trehalose	45		31	45	27	31
Raffinose	32		29	40	12	24
Oligosaccharide mixtures	N		S	P	P	S

* See footnote to Table I.

TABLE III

CHROMATOGRAPHY OF URONIC ACIDS AND THEIR MIXTURES ON SILICA GEL THIN LAYERS*

Compounds	*Silica gel impregnated with 0.3 M Na_2HPO_4*	
Solvent No.	*10*	*11*
Galacturonic acid	8	16
Glucuronic acid	27	26
Mannuronic acid	23	33
Glucuron	50	55
Mannuron	36	46
Uronic acid mixtures	S	S

* See footnote to Table I.

TABLE IV

CHROMATOGRAPHY OF MONOSACCHARIDES AND THEIR MIXTURES ON SILICA GEL THIN LAYERS IMPREGNATED WITH SODIUM DIHYDROGEN PHOSPHATE*

Compounds	*Molarity of sodium dihydrogen phosphate*																	
	0.3										*0.2*				*0.07*			
Solvent No.	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*1*	*3*	*5*	*6*	*1*	*3*	*5*	*6*
Galactose	13	19	12	01	13	11	09	18	—	45	15	14	16	15	34	37	36	33
Glucose	19	20	16	03	15	15	14	22	—	52	23	26	16	25	42	48	38	40
Mannose	25	23	23	04	19	19	15	26	40	57	29	31	19	31	46	56	39	47
Arabinose	26	20	22	05	23	16	17	24	40	55	30	29	20	28	45	55	41	44
Xylose	41	33	34	07	27	27	23	40	51	64	44	46	27	38	55	64	48	53
Fucose	36	—	—	—	—	30	35	50	66	65	42	51	27	38	49	68	47	49
Rhamnose	61	53	59	28	47	48	51	66	71	79	57	61	39	56	57	73	55	58
Monosaccharide mixtures	S	P	S	S	S	S	S	S	P	P	S	S	P	S	P	P	P	P

* See footnote to Table I.

TABLE V

CHROMATOGRAPHY OF OLIGOSACCHARIDES AND THEIR MIXTURES ON SILICA GEL THIN LAYERS IMPREGNATED WITH SODIUM DIHYDROGEN PHOSPHATE*

Compounds	*Molarity of sodium dihydrogen phosphate*				
	0.3	*0.2*		*0.07*	
Solvent No.	*1*	*1*	*6*	*1*	*1a***
Sucrose	Remain on the start	19	10	39	64
Cellobiose		11	17	31	59
Maltose		12	18	30	56
Trehalose		06	00	23	48
Raffinose		00	00	14	39
Oligosaccharide mixtures	N	P	P	S	S

* See footnote to Table I.
** 1a = Twofold development with solvent system 1.

TABLE VI

CHROMATOGRAPHY OF URONIC ACIDS AND THEIR MIXTURES ON SILICA GEL THIN LAYERS IMPREGNATED WITH SODIUM DIHYDROGEN PHOSPHATE*

Compounds	*Molarity of sodium dihydrogen phosphate*						
	0.3			*0.2*		*0.07*	
Solvent No.	*10*	*11*	*12*	*10*	*11*	*10*	*11*
Galacturonic acid	14	28	39	16	15	51	30
Glucuronic acid	37	41	46	35	29	60	34
Mannuronic acid	42	45	52	41	37	60	42
Glucuron	51	—	56	47	42	70	50
Mannuron	57	69	62	67	63	71	52
Uronic acid mixtures	S	S	P	S	S	P	P

* See footnote to Table I.

served in this case. Tables I–III demonstrate that satisfactory separation of carbohydrates was obtained on silica gel impregnated with 0.3 *M* sodium monohydrogen phosphate. The best separation was observed on silica gel in the presence of sodium dihydrogen phosphate. 0.07, 0.2, 0.3 and 0.6 *M* solutions of sodium dihydrogen phosphate were used to determine the optimal impregnating concentration. The best results for monosaccharides were obtained on silica gel impregnated with 0.2 or 0.3 *M* solutions of inorganic salts (Tables IV and VI). Thin-layer chromatography of oligosaccharides was better when using silica gel in the presence of 0.05–0.1 *M* salt solutions (Tables II and V). There was no separation on silica gel impregnated with 0.6 *M* salt solutions.

Sugar acid mixtures are known to separate only on silica gel in the presence of acid solvent systems[15], as a rule the separation being poor because of unsatisfactory elongated spot forms. However the application of silica gel impregnated with sodium mono- and dihydrogen phosphate has resulted in satisfactory separation. The best results are observed when an 0.2 *M* salt solution is employed (Tables III and VI). The determination of the capacity of silica gel thin layer impregnated with 0.3 *M*

dium dihydrogen phosphate demonstrated that sugars gave well shaped spots ithout "tailing") at a concentration of not more than 400 μg. A good separation of gar mixtures occurred when the total sugar concentration was not more than o–500 μg. Unlike the case of paper chromatography, separation of carbohydrates thin layers of silica gel in the presence of sodium mono- and dihydrogen phosate is considerably less influenced by inorganic salt impurities which are often ntained in the hydrolysates of carbohydrate derivatives. In our institute the above ocedure was successfully used in studying the hydrolysates of various polysaccharis and glycosides. This shows that the above method may be widely used in research oblems. Chromatography at elevated temperatures (30–50°) increases the R_F lues by 5–15 % and slightly influences the quality of the separation.

A great number of reagents was employed for detection. The most convenient es are those given in the experimental section. The detection limit was 0.5 μg. satisfactory separation of sugar alcohols was also observed on silica gel impregnated ith sodium mono- and dihydrogen phosphate; however, spot detection was difficult.

Impregnation of silica gel with inorganic salts seems to decrease sugar adsorpon on silica gel and results in well shaped spots. Sugar solubility in inorganic salt olutions is known to increase with the increase of salt concentration within certain mits. This leads to higher solubility of the sugar in the stationary phase, to decrease R_F values and consequently to better separation. As may be seen from Tables -VI acid salts of strong bases as well as weak or medium acids are the most suitable r silica gel impregnation.

JMMARY

The separation of carbohydrates on thin layers of silica gel is due first of all the type and quantity of impregnating salt.

The results of chromatography of carbohydrates on thin layers of silica gel in e presence of sodium mono- and dihydrogen phosphate are often better than those paper chromatography (*e.g.* a good separation of complex mixtures, less sensivity to inorganic impurities, and faster separation).

The present method can be recommended for the study of the monosaccharide mposition of oligo- and polysaccharides.

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