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COUPLED LAYERS: A NEW TECHNIQUE FOR THE TWO-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY OF CARBOHYDRATES

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SUMMARY

A new method for the thin-layer chromatography of carbohydrates is described. The chromatoplate is made of two adjacent layers, each of which exhibits different chromatographic properties and each of which has an intrinsic function in the individual development. This technique, which we call "coupled-layer chromatography," exploits two different chromatographic principles on the same chromatoplate for the separation of a class of substances.

The number and variety of sugars separated is the greatest yet achieved with a simple two-dimensional development. With the use of appropriate solvents and impregnants, the number of sugars separated might even be increased.

INTRODUCTION

The present work is part of our extensive investigations on the thin-layer chromatography (TLC) of carbohydrates.

In the first paper[1], we considered the behaviour of carbohydrates on silica gel impregnated with boric acid, where the migration of the sugar–borate complexes was so solvent-dependent that it was possible to set up a two-dimensional separation of twenty sugars.

Our next studies dealt with new and interesting applications of the TLC of carbohydrates[2–4], but with the use of other impregnants the solvent-dependence exhibited by boric acid impregnation was lost and no further two-dimensional chromatography was possible[5,6].

In all of these studies, the oligosaccharides were the most difficult to separate. It is evident that the chromatographic relevance of the steric orientation of the OH groups is less important in the molecule of an oligosaccharide than it is in the molecule of a monosaccharide. Moreover, because of their physical properties, the migration of

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oligosaccharides, with very few exceptions, is much slower than that of the monosaccharides, and is therefore limited to a small area of the chromatogram.

We tried to solve this problem with tungstic and molybdic acid impregnation and we succeeded in separating twelve oligosaccharides, of which nine were disaccharides, but three developments were necessary and improved separation of the oligosaccharides was made at the expense of the monosaccharides[7].

In conclusion, it was the slowness of the oligosaccharide migration that created the main obstacle to good separations of a complex mixture of carbohydrates containing a high number of oligosaccharides. It was therefore necessary to find some new impregnant, technique or chromatographic material in order to make further progress.

The solution that we present in this paper is simple and efficient and should facilitate chromatographic research in other areas as well. The imaginative researcher should easily be able to adapt the basic procedure to other problems.

EXPERIMENTAL

Preparation of the chromatoplates

Any spreader can be adjusted in a few minutes to prepare "coupled-layer" chromatoplates[8–13]. A 3-mm thick Plexiglas septum was fastened to the trough walls of the spreader with Scotch tape so as to divide the trough into two sections of 4.2 cm and 14.5 cm.

Two separate slurries of Silica Gel G were prepared, rapidly mixed and each was immediately added to one of the two sections of the spreader. Twenty grams of Silica Gel G plus 33.3 ml of the selected impregnant solution were poured into the narrow section; 40 g of Silica Gel G and 66.6 ml of the selected impregnant solution were poured into the wider section.

Adsorbents and impregnants should be weighed and measured in advance, since all mixing, pouring and spreading must take no more than 2 min, otherwise the binder will start to harden. This is the most difficult step in the preparation of the chromatoplates. When dry, these chromatoplates have a smooth, uniform surface with a barely visible line dividing the adjacent layers. Throughout the present work, we refer to this line as "the dividing line."

*Impregnants**

Impregnant P: sodium tetraborate, aqueous solution, 0.132 *M* (solution A); boric acid, aqueous solution, 0.204 *M* (solution B); sodium tungstate, aqueous solution, 0.06 *M* (solution C). Mix equal volumes of the three solutions.

Impregnant Q: boric acid, aqueous solution, 0.036 *M*.

Impregnant R: sodium acetate, aqueous solution, 0.24 *M*.

Impregnant S: monosodium phosphate, aqueous solution, 0.24 *M*.

Impregnant T: molybdic acid, saturated aqueous solution.

* Impregnants A–O were reported in a previous paper[7].

*Solvents**

13: *n*-Butanol–ethyl acetate–isopropanol–acetic acid–water (35:100:60:35:30).
15: *n*-Propanol–water (85:153).
29: Ethyl acetate–isopropanol–water (100:60:30).
32: Ethyl acetate–acetic–acid–methanol–water (60:15:15:10).
36: Acetone–water (9:1).
37: Acetone–water–chloroform–methanol (8:0.5:1:1).
54: Ethyl acetate–isopropanol–water (2:2:1).
106: Isopropanol–*n*-propanol–water (42:42:15).
108: Methyl acetate–isopropanol–water (2:2:1).

Samples

Monosaccharides

Aldoses

(1) Glycoaldehyde
(2) Glyceraldehyde
(3) Erythrose
(4) Xylose
(5) Lyxose
(6) Ribose
(7) Arabinose
(8) Glucose
(9) Mannose
(10) Galactose

Ketoses

(11) Dihydroxyacetone
(12) D-Ribulose
(13) Fructose
(14) Sorbose
(15) Tagatose
(16) Mannoheptulose
(17) Sedoheptulose

2-Deoxy sugars and methyloses

(18) 2-Deoxy-D-ribose
(19) 2-Deoxy-D-glucose
(20) 2-Deoxy-D-galactose
(21) 6-Deoxy-L-mannose (L-Rhamnose)
(22) 6-Deoxy-L-galactose (L-Fucose)
(23) 2,6-Deoxyallose (digitoxose)

Methyl derivatives

(24) β-Methyl-D-xyloside
(25) α-Methyl-D-mannoside
(26) β-Methyl-D-glucoside
(27) β-Methyl-D-arabinoside

Oligosaccharides

Disaccharides

(28) Sucrose
(29) Maltose
(30) Trehalose
(31) Cellobiose
(32) Turanose
(33) Lactose
(34) Lactulose
(35) Melibiose
(36) Palatinose
(37) Isomaltose

* For all solvents not reported here, see ref. 7.

Trisaccharides
(38) Melezitose
(39) Raffinose

Tetrasaccharide
(40) Stachyose

Two-dimensional chromatography

A mixture of 10 mg of each of the sugars selected was dissolved in 2 ml of distilled water. One microlitre of the solution, containing 5 μg of each sugar, was applied with a micropipette on to the narrow layer, about 1 cm from the line dividing the two layers and about 2 cm from the lower edge of the plate.

The chromatoplate was then placed in the tank containing the solvent system in such a position as to have the solvent flow parallel to the dividing line. In this position, the first chromatographic development takes place only on the narrow layer.

The walls of the tank were lined with filter-paper that was impregnated with the developing solvent.

After the first development, the plates were dried in an oven at 55° or at room temperature (see RESULTS AND DISCUSSION) until the odour of the solvent could no longer be detected (do not dry the plates in the oven for more than 3 h).

The second development was then carried out at right angles to the first. The mixture which had been partially separated on the narrow layer was thus definitively resolved on the wider layer.

Detection of spots

After development, the chromatoplate was dried in an oven at 110° until the odour of the solvent could no longer be detected (*ca.* 0.5 h). The heated chromatoplate was then sprayed with a freshly prepared solution of 20 mg of naphthoresorcinol, 10 ml of ethanol and 0.5 ml of concentrated H_2SO_4 and the plates were reheated for 3–5 min at 110° to produce the characteristic colours.

The white background darkens overnight and the colours fade away at room temperature. A glass plate placed over the chromatogram preserves the original colours of the spots and the background for many days.

Glycoaldehyde gave only dimly coloured spots, and results for this sugar have been omitted from the figures and tables.

Resolutions obtained with the above impregnants and solvents in the two runs are given in Table I.

RESULTS AND DISCUSSION

During our previous work, we observed that the migration of sugars on silica gel impregnated with monosodium phosphate[6], sodium acetate[6], sodium tungstate/phosphoric acid or molybdic acid[7] tended to be inversely proportional to molecular weight, that is, the partition coefficient of the sugar in the mobile phase diminished proportionally to the increasing number of OH groups (Fig. 1).

Boric acid impregnation, on the other hand, produced a wide variety of migration patterns, each of which was peculiar to the solvent used (Fig. 2).

The point of departure of the present work is a new impregnant (P) whose effect on sugar migration places it in an entirely different category. Impregnant P is charac-

TABLE I

RESOLUTIONS OBTAINED WITH DIFFERENT IMPREGNANTS AND SOLVENTS

Impregnants		*Solvents*		*Resolution*	*Notes*
Narrow layer	*Wide layer*	*First run*	*Second run*		
P	Q	108	13	Very good	High R_F values. Deoxy sugars and methylglycosides sharply defined
P	Q	108	32	Very good	
P	Q	54	13	Very good	R_F lower than P–Q 108/32 or P–Q 108/13
P	Q	54	32	Excellent	
P	Q	106	13	Excellent	
P	Q	106	32	Excellent	
P	R	54	13	Very good	
P	R	54	32	Very good	
P	R	106	13	Very good	
P	R	106	32	Very good	
P	R	108	32	Very good	
P	R	108	36	No resolution	Slow-running sugars tend to remain inside the P layer
P	R	108	37	No resolution	
P	S	108	36	No resolution	
P	S	108	32	Fair	Chromatograms not reproducible
P	S	108	37	No resolution	
R	P	36	108	Excellent	Most oligosaccharides show double image
R	P	37	108	Very good	
S	P	37	108	Very good	Chromatograms not reproducible
S	P	36	108	Very good	
T	Q	15	32	Good	Sugars run according to molecular weight
T	Q	29	13	Good	
T	R	15	37	No resolution	
T	R	29	36	Fair	
T	R	29	37	Fair	
T	R	15	36	Fair	
T	R	15	32	Poor	
T	R	29	32	Poor	
T	S	15	36	Good	
T	S	15	37	Fair	
T	S	29	36	Good	
T	S	29	37	Good	
S	T	36	15	No resolution	
S	T	37	15	No resolution	
S	T	36	29	Poor	
S	T	37	29	Poor	
R	T	37	29	Fair	
R	T	37	15	Fair	
R	T	36	29	Good	
R	T	36	15	Fair	
R	T	32	15	No resolution	
R	T	32	29	No resolution	

terized by complete lack of sensitivity towards distinctions between mono- and oligosaccharides. Many monosaccharides, in fact, migrate less than di- and trisaccharides on silica gel prepared with this impregnant (Fig. 3).

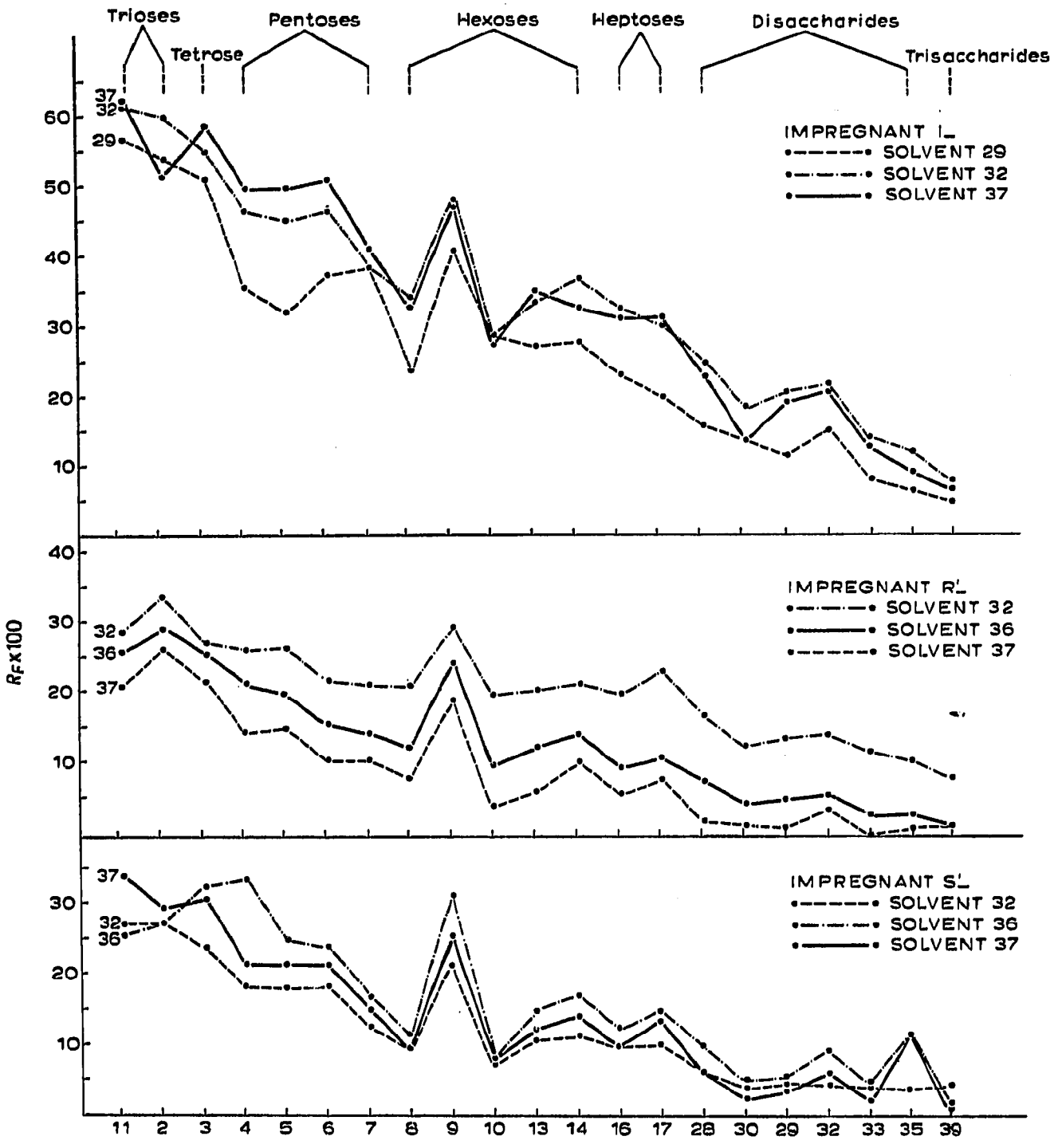

Fig. 1. Plots of $R_F \times 100$ values of carbohydrates on impregnants I (sodium tungstate, 0.05 *M*–phosphoric acid, 0.2 *M*), R′ (sodium acetate, 0.2 *M*) and S′ (monosodium phosphate, 0.2 *M*) with various solvents. The numerals correspond to the sugars enumerated in the EXPERIMENTAL section.

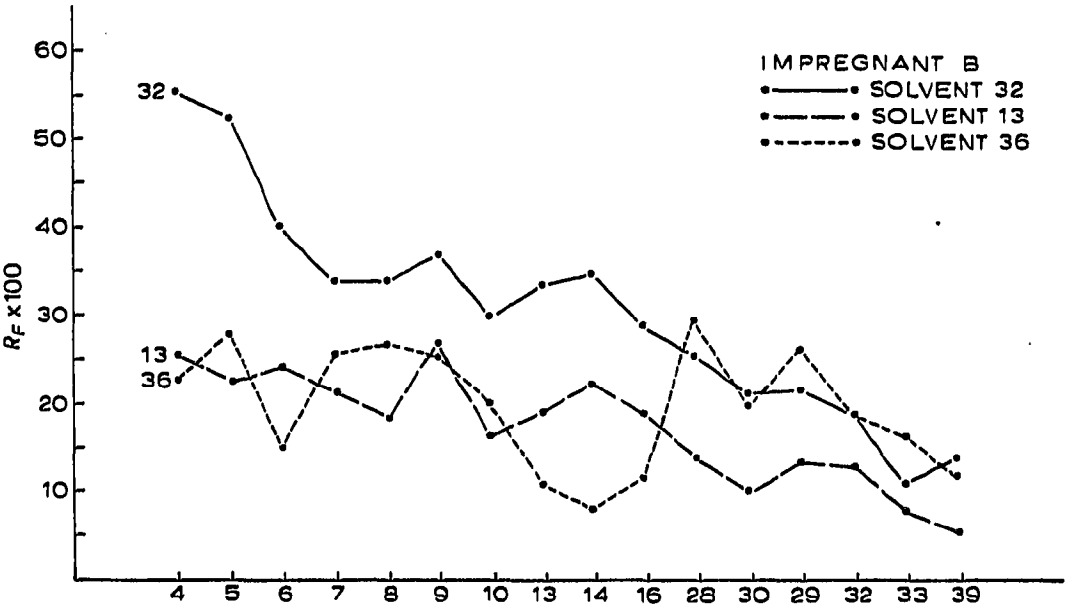

Fig. 2. Plot of $R_F \times 100$ values of carbohydrates on impregnant B (boric acid, 0.03 M).

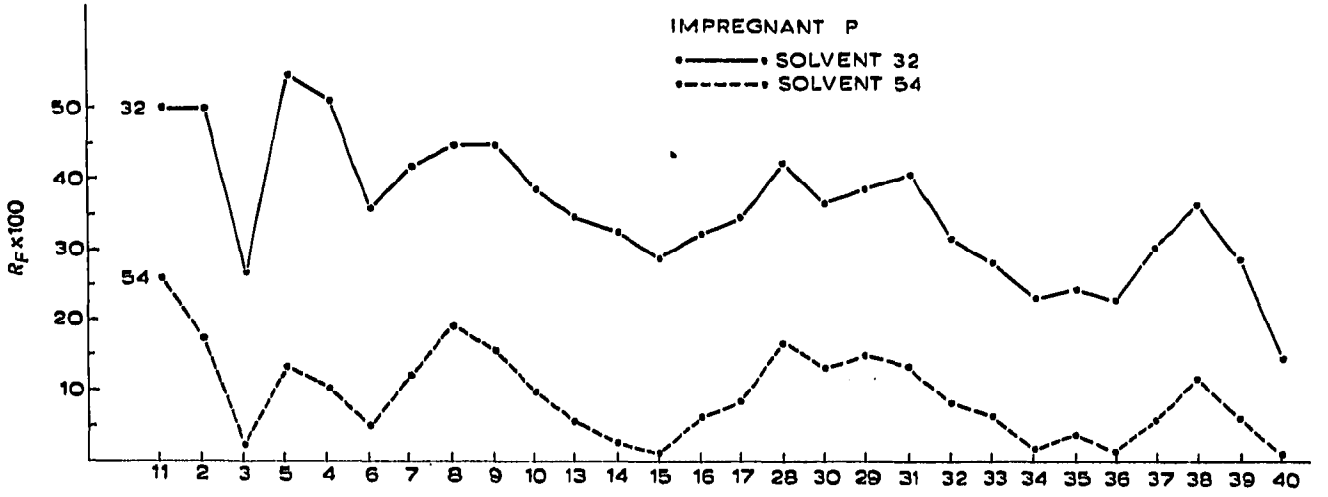

Fig. 3. Plot of $R_F \times 100$ values of carbohydrates on impregnant P with two substantially different solvents.

Only methylglycosides show high R_F values, probably because of their inability to complex with borate ions, as reported by MACPHERSON AND PERCIVAL[14].

The reasons for the unusual behaviour of sugars on impregnant P are not clearly understood, but if a typical TLC migration pattern of various carbohydrates on impregnant P is compared with an anion-exchange chromatogram[15] of the borate complexes of the same carbohydrate, the R_F values of the former appear strikingly similar to the order of elution of the latter. We therefore suggest that ion exchange could somehow be involved in chromatography with impregnant P. This would also explain why a small sugar, such as erythrose, has a lower R_F value than any of the di- and trisaccharides examined.

However, despite the exceptional chromatographic properties exhibited by

impregnant P, it was immediately apparent that, even if a two-dimensional technique were used, this support could not be applied to the separation of a rich carbohydrate mixture. In this medium, sugars lack the solvent-dependence that is typical of impregnant B (boric acid, 0.03 *M*).

The problem was to combine the chromatographic behaviour of impregnant P with the known properties of the group of impregnants that resolve carbohydrates according to their molecular weight (*i.e.*, impregnants R, S, T, etc.) so that we could fully exploit the advantages of both systems of separation.

We solved the problem by preparing the chromatoplate with two differently impregnated, adjacent slurries, one for the first development, and the other for the second. The second development was carried out at right angles to the first. The results are illustrated in Figs. 4–7.

A dual-layer technique had already been described in the literature. KIRCHNER[16] applied it to the separation of bergamot oil; he used magnesium silicate and silicic acid as the two layers, but both of these materials utilized only one chromatographic principle, *viz.*, adsorption. KRAFFCZYK *et al.*[17] juxtaposed an ion-exchange resin layer and a cellulose layer, but only the latter was used for chromatography. The ion-exchange layer was used only to de-salt.

BERGER and co-workers[8–13] thoroughly investigated multiple-layer chromatoplates. By using supports with diverse chromatographic properties, they separated widely different substances. However, the present work exploits for the first time the high potential of the technique for the separation of a class of substances such as carbohydrates.

The solvents selected for the present study were chosen on the basis of their performance in our previous work. The requirements were: sharp spots, good resolution, a tendency to give medium-to-high R_F values, a short development time and, whenever possible, a capacity to separate mono- from oligosaccharides.

Acetic acid should not be used in the first solvent, no matter which impregnant is used for the first development, because it impairs the second separation; when impregnant P is used in the first run, however, the solvent for the second run must contain acetic acid, otherwise the spots have a tendency to remain within the boundaries of the layer prepared with impregnant P.

Similar requirements had to be satisfied in our work on the TLC of carbohydrates on silica gel with boric acid impregnation[1], but the parallel chromatographic behaviour was probably due to a different set of physicochemical causes. Fig. 8, for example, shows what happens when acetic acid is introduced into the developing solvent. While impregnant P is virtually unaffected, the migration pattern on impregnant Q is drastically altered and the R_F values tend to descend in proportion to the increasing molecular weight of the sugar sequence.

The behaviour of impregnant P is unusual. Most solvent mixtures do not produce significant shifts in its usual pattern, whether or not they contain acetic acid, but exceptions to this rule are illustrated in Fig. 9, where isopropanol is substituted by acetic acid in increasing amounts. Solvent 32 has no effect on impregnant P, as shown in Fig. 8, despite the fact it contains 15% of acetic acid. But although solvent 54A, shown in Fig. 9, contains only 8% of acetic acid, the impregnant begins to lose its properties, and the sugars tend to migrate proportionally to their decreasing molecular weight.

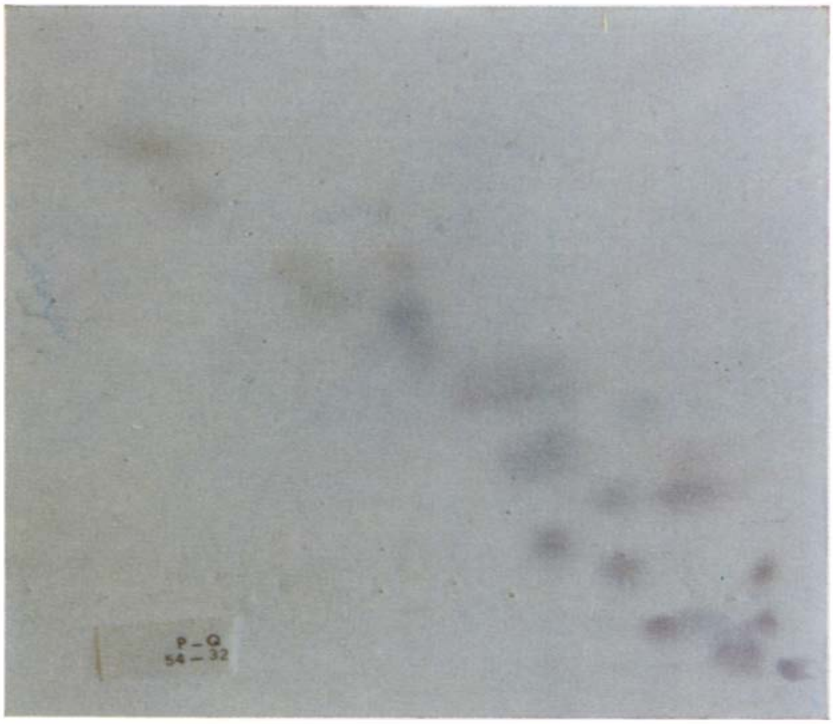

Fig. 4. Coupled-layer chromatogram of a carbohydrate mixture. Impregnation P–Q; solvents 54/32.

Fig. 5. Coupled-layer chromatogram of a carbohydrate mixture. Impregnation P–Q; solvents 106/13.

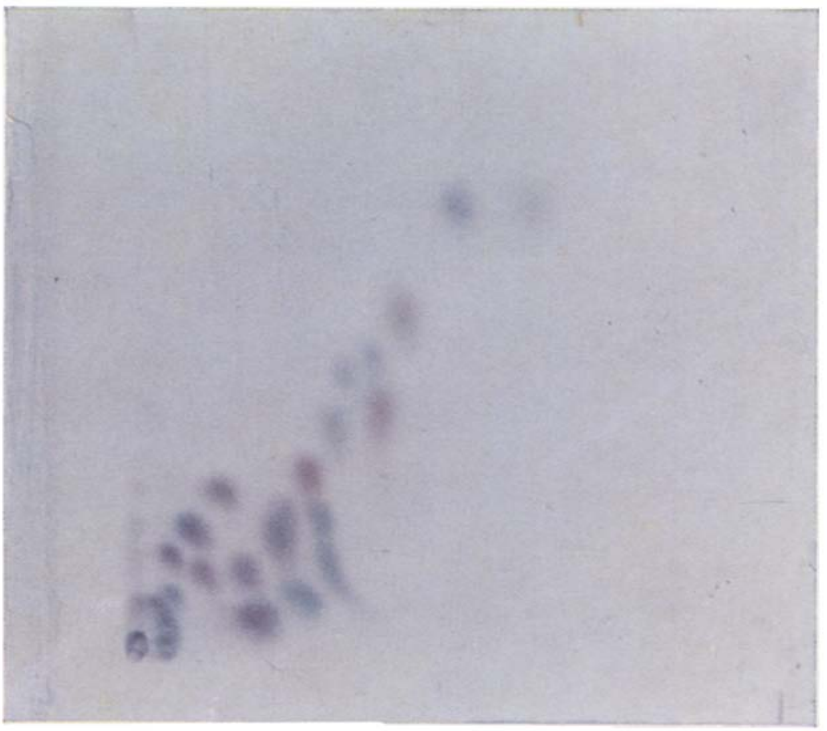

Fig. 6. Coupled-layer chromatogram of a carbohydrate mixture. Impregnation P–R; solvents 54/13.

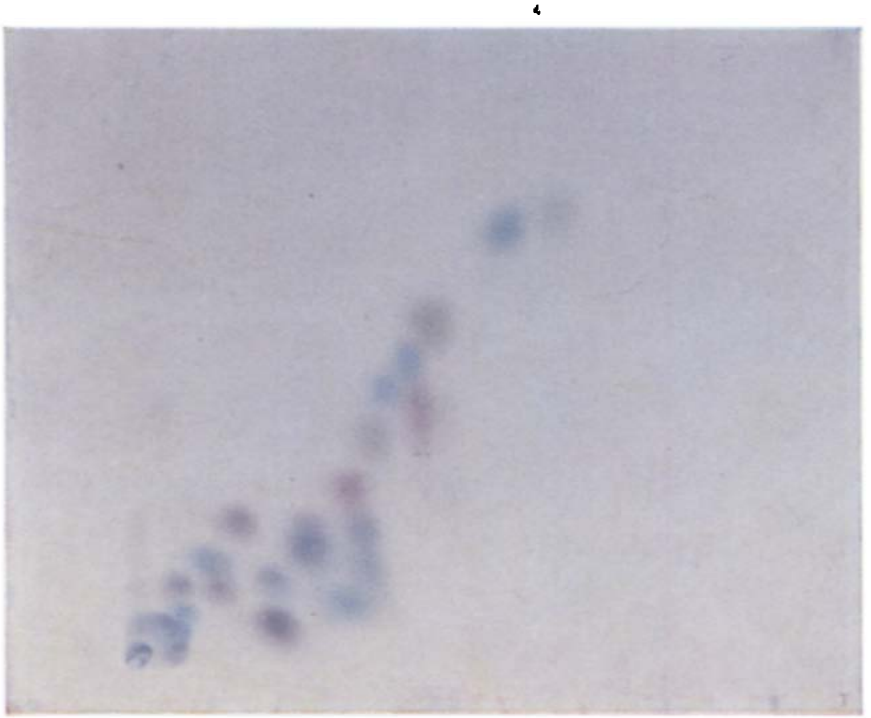

Fig. 7. Coupled-layer chromatogram of a carbohydrate mixture. Impregnation P–R; solvents 54/32.

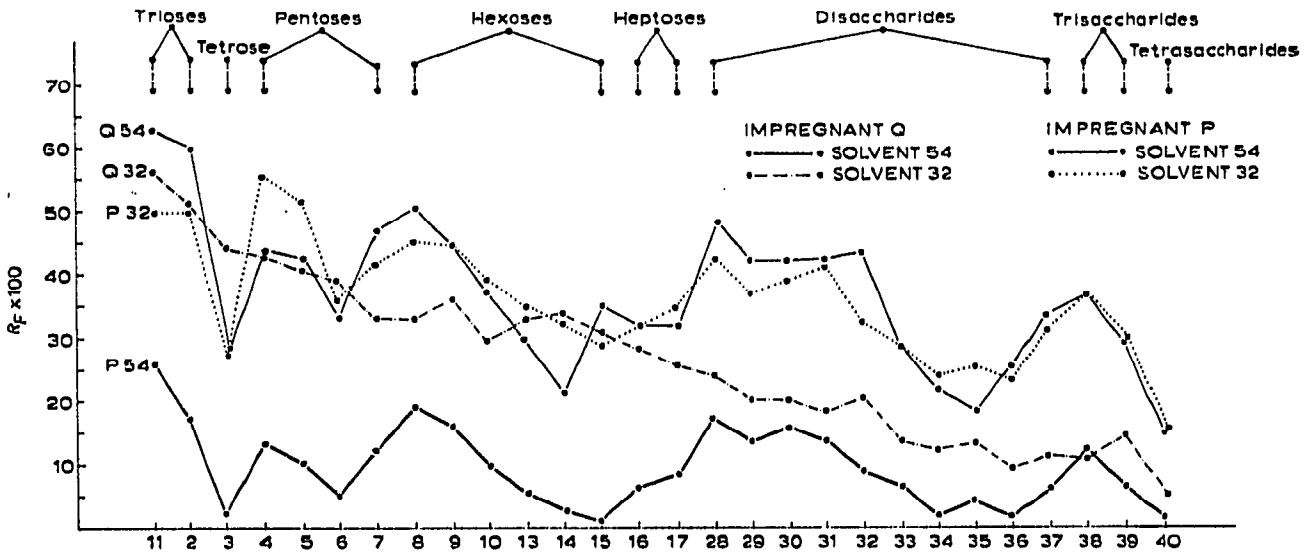

Fig. 8. Plot of R_F × 100 values of carbohydrates on impregnants P and Q with solvents 32 and 54. Solvent 32 contains 15% of acetic acid.

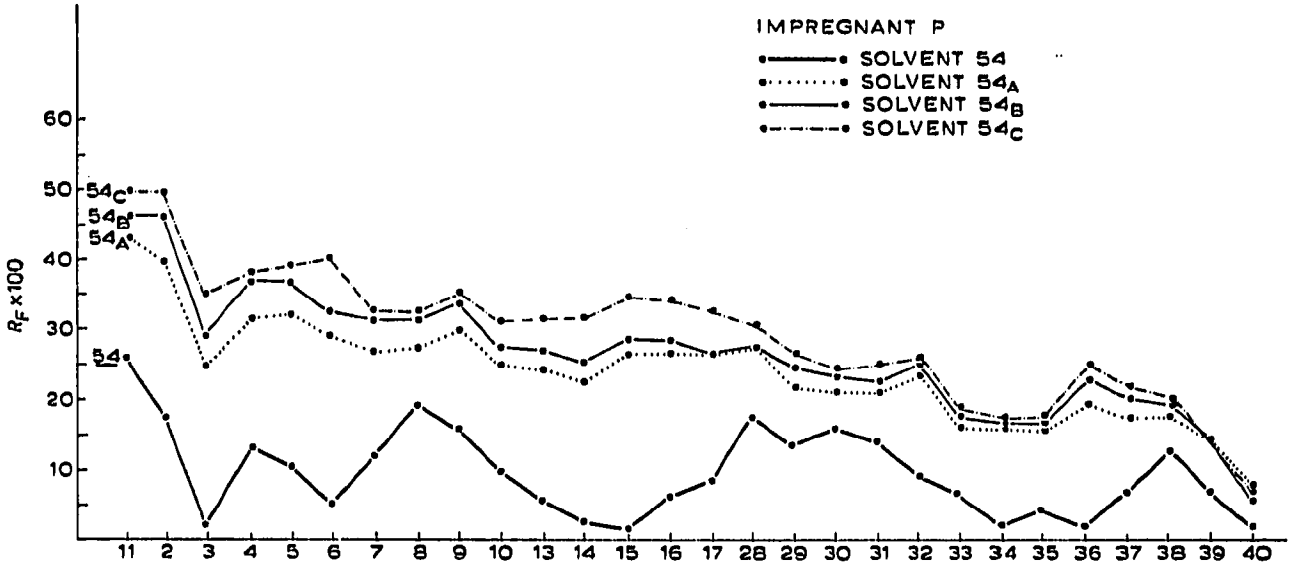

Fig. 9. Plot of R_F × 100 values of carbohydrates on impregnant P with isopropanol replaced by increasing gradients of acetic acid. Solvent 54, ethyl acetate–isopropanol–water (40:40:20); 54A, ethyl acetate–isopropanol–water–acetic acid (40:32:20:8); 54B, ethyl acetate–isopropanol–water–acetic acid (40:24:20:16); 54C, ethyl acetate–isopropanol–water–acetic acid (40:16:20:24).

Fig. 10 shows the effect of the substitution of acetic acid on the R_F values of specific sugars. The upper half of the chromatoplate seems unaffected, but the lower half changes considerably: turanose, which has a much shorter migration than sucrose, moves up to the same level as the latter. Palatinose and mannose, on the other hand, move up, but the distance between them does not change. Raffinose and fructose, which are poorly resolved, separate very well. The same holds true of the melibiose–glucose pair.

We are continuing to investigate the parameters that regulate the behaviour of impregnant P.

The slurry for the preparation of the chromatoplates should be thicker than usual so as to avoid uneven reciprocal diffusion along the dividing line between the two adjacent layers. Thus, the ratio of impregnant solution to silica gel used was 5:3 instead of the usual 2:1, but the impregnant contained a proportionally higher salt molarity in order to maintain the same final concentration.

The width of the layer used for the first development was fixed at 45 mm; this width, however, should be varied according to the specific requirements of the researcher, as we have observed that these variations either increase or decrease the resolution in specific regions of the chromatoplate.

Despite some very good results, we cannot recommend the use of impregnant S because of its erratic behaviour along the dividing line and with respect to the migration of the solvent. It is almost impossible to obtain two identical chromatograms with this impregnant. In any event, impregnant S should be used only for the wider layer (second run).

Some chromatoplates with P impregnation on the narrow layer presented an interesting phenomenon. Instead of the two spots that should have appeared in the glyceraldehyde and dihydroxyacetone positions, four were observed, arranged at the four corners of an imaginary rectangle. This split is an instance of double isomerization, the proof of which can be seen in the chromatograms in Figs. 11 and 12. Here the chromatoplates were prepared with three layers: two narrow ones with P impregnation and a wide one with Q impregnation. The single sugars and a mixture of both were deposed in such a way as to "witness" each single development. After the runs, the following was found: (a) the first development did not produce isomerization; (b) if heated to 50–60° between the two runs, the chromatoplate showed double isomerization (Fig. 11); (c) if dried at room temperature between the runs, the chromatoplate did not show isomerization (Fig. 12).

The cause of isomerization through the enediol (Lobry de Bruyn–Van Ekenstein transformation) is the alkalinity of impregnant P (pH 8.9).

P–Q impregnation

The use of impregnants P and Q gave the results shown in Table II.

TABLE II

RESULTS OBTAINED WITH IMPREGNANTS P AND Q

Impregnant		*Solvent*		*Spots*		
Narrow layer	*Wide layer*	*First run*	*Second run*	*Resolution*[a]	*Shape*[b]	*No. of sugars separated*
P	Q	108	13	+ +	BC	26
P	Q	108	32	+ +	AB	34
P	Q	54	13	+ +	A	28
P	Q	54	32	+ + +	A	31
P	Q	106	13	+ + +	B	28
P	Q	106	32	+ + +	B	28

[a] + + = good; + + + = very good.
[b] A = sharply defined; B = slightly diffused; C = diffused and/or tailed.

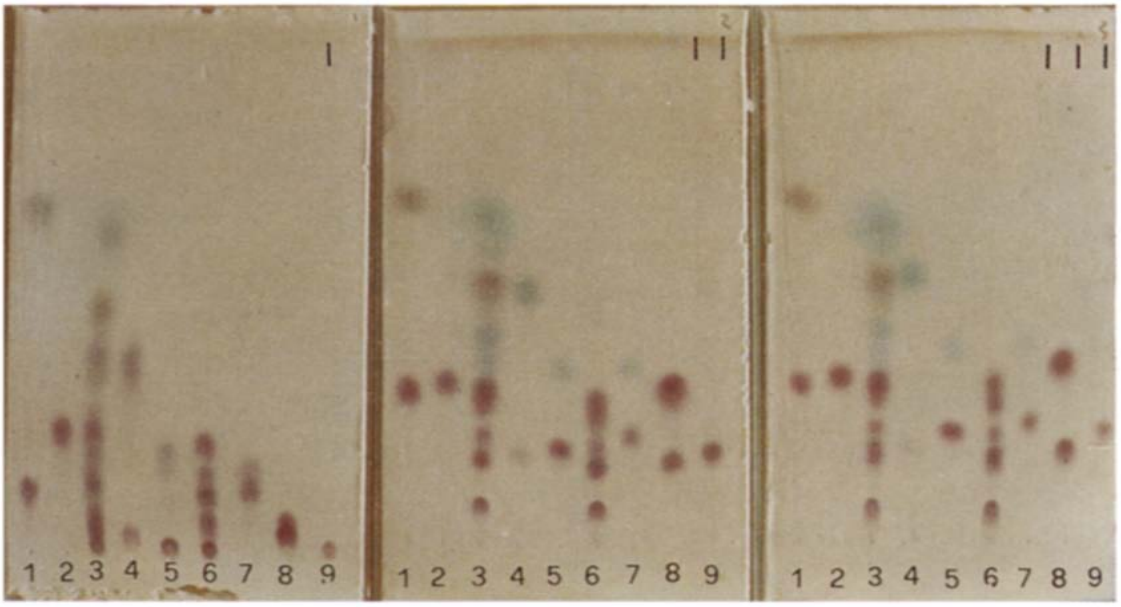

Fig. 10. Chromatograms on impregnant P with increasing amounts of acetic acid added to solvent 54. I, solvent 54; II, solvent 54A; III, solvent 54C. 1 = Turanose and digitoxose; 2 = sucrose; 3 = sugar mixture; 4 = melezitose and rhamnose; 5 = palatinose and mannose; 6 = oligosaccharide mixture; 7 = melibiose and glucose; 8 = raffinose and fructose; 9 = lactulose.

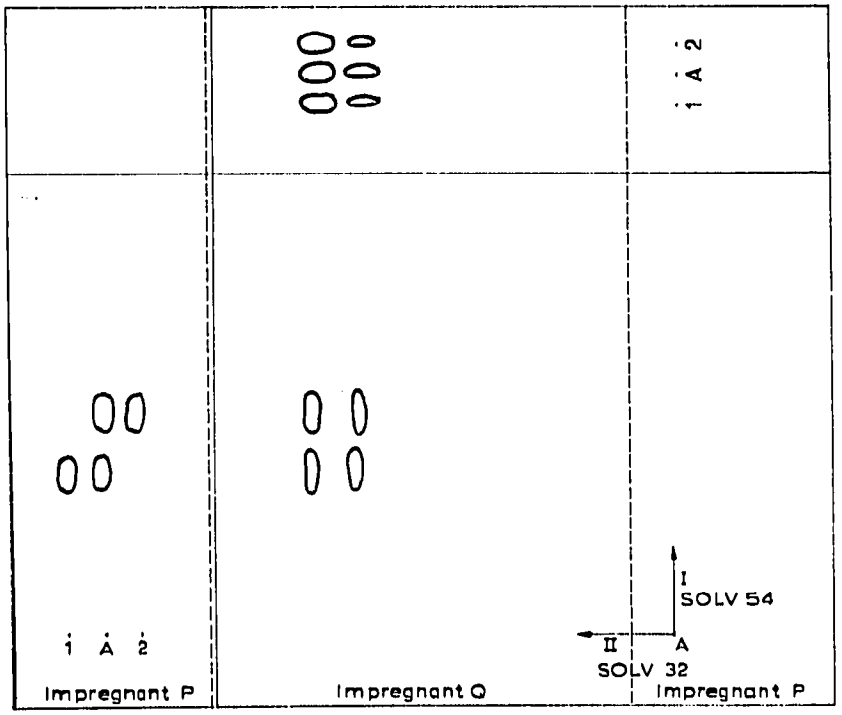

Fig. 11. Chromatogram of glyceraldehyde and dihydroxyacetone showing isomerization. The plate was heated to 55° after the first run. 1 = glyceraldehyde; 2 = dihydroxyacetone; A = mixture of both sugars.

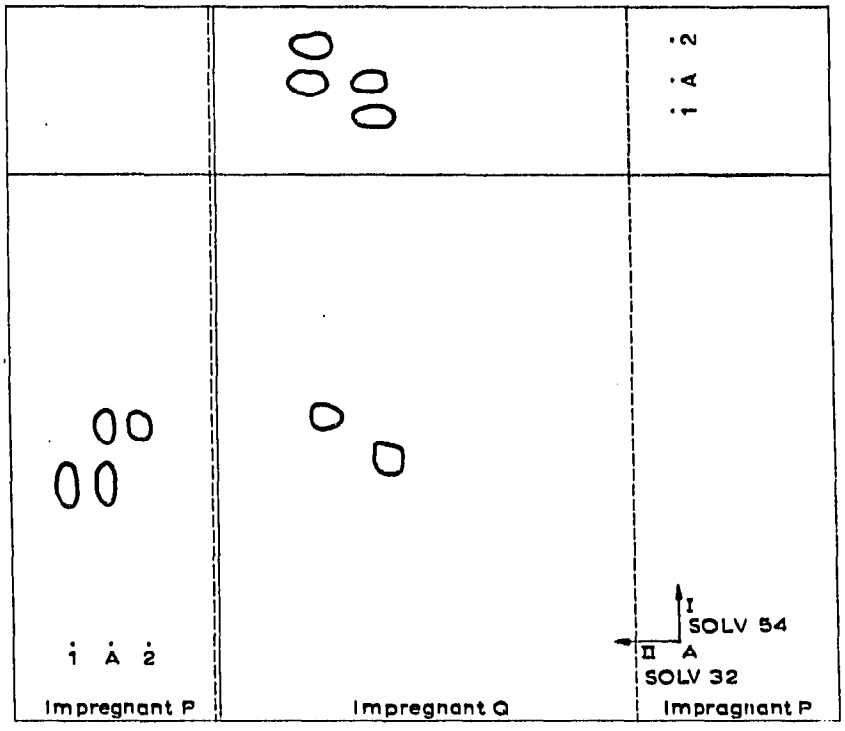

Fig. 12. Chromatogram of glyceraldehyde and dihydroxyacetone dried at room temperature after the first run. No isomerization occurred.

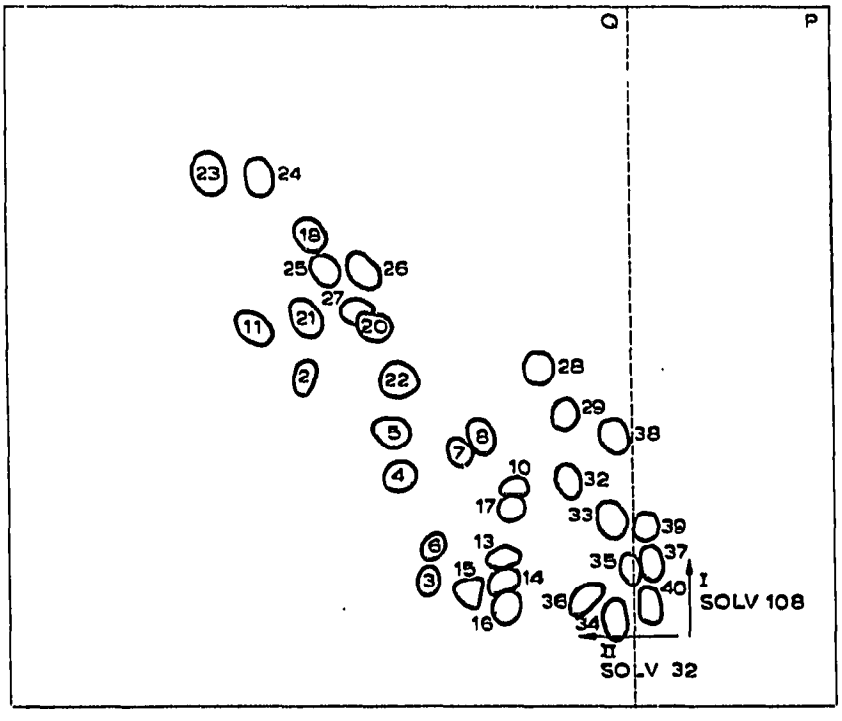

Fig. 13. Coupled-layer chromatogram of carbohydrates. Impregnation P–Q; solvents 108/32. 2 = Glyceraldehyde; 3 = erythrose; 4 = xylose; 5 = lyxose; 6 = ribose; 7 = arabinose; 8 = glucose; 10 = galactose; 11 = dihydroxyacetone; 13 = fructose; 14 = sorbose; 15 = tagatose; 16 = mannoheptulose; 17 = sedoheptulose; 18 = 2-deoxyribose; 20 = 2-deoxygalactose; 21 = rhamnose; 22 = fucose; 23 = digitoxose; 24 = β-methylxyloside; 25 = α-methylmannoside; 26 = β-methylglucoside; 27 = β-methylarabinoside; 28 = sucrose; 29 = maltose; 32 = turanose; 33 = lactose; 34 = lactulose; 35 = melibiose; 36 = palatinose; 37 = isomaltose; 38 = melezitose; 39 = raffinose; 40 = stachyose.

Solvent 108 produced higher R_F values than solvent 54. The resolution of the fast-running sugars was diminished, but in the region including most of the hexoses resolution was accentuated.

Solvent 106 gave good general resolution, but the oligosaccharide region was not well resolved and the running time was about 8 h.

The two solvents used for the second run were almost equivalent: solvent 13 resolved the fast-running spots well, whereas solvent 32 gave better resolution with the slow-running sugars.

The carbohydrates that this chromatoplate did not separate were glucose, mannose and maltose–trehalose–cellobiose.

Typical chromatograms are shown in Figs. 13 and 14.

Inversion of the order of two layers is not practical, since the first developments would have to be made with a solvent system containing acetic acid, which would completely deactivate impregnant P.

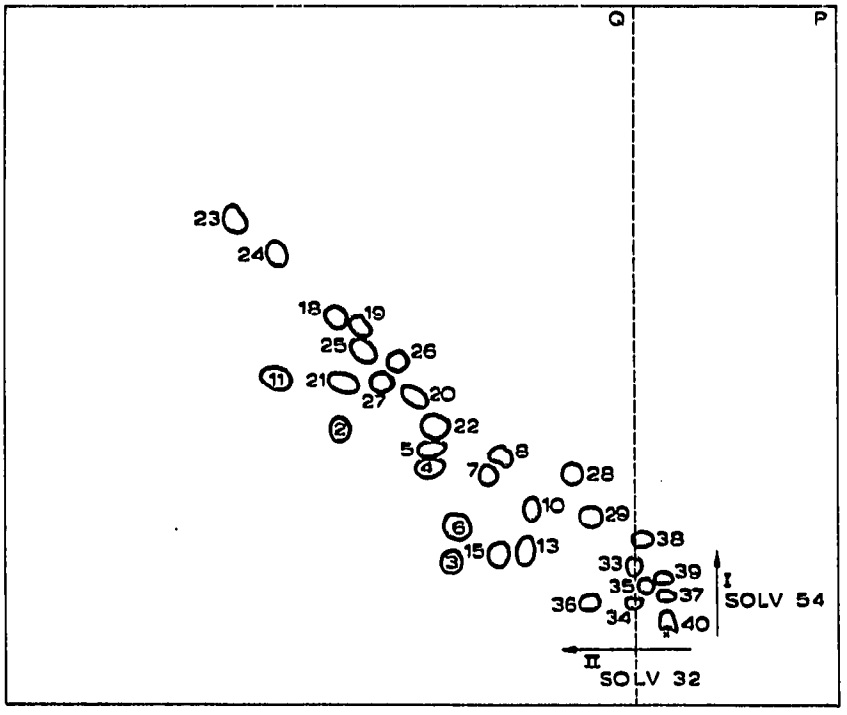

Fig. 14. Coupled-layer chromatogram of carbohydrates. Impregnation P–Q; solvents 54/32. 2 = Glyceraldehyde; 3 = erythrose; 4 = xylose; 5 = lyxose; 6 = ribose; 7 = arabinose; 8 = glucose; 10 = galactose; 11 = dihydroxyacetone; 13 = fructose; 15 = tagatose; 18 = 2-deoxyribose; 19 = 2-deoxyglucose; 20 = 2-deoxygalactose; 21 = rhamnose; 22 = fucose; 23 = digitoxose; 24 = β-methylxyloside; 25 = α-methylmannoside; 26 = β-methylglucoside; 27 = β-methylarabinoside; 28 = sucrose; 29 = maltose; 33 = lactose; 34 = lactulose; 35 = melibiose; 36 = palatinose; 37 = isomaltose; 38 = melezitose; 39 = raffinose; 40 = stachyose.

P–R impregnation

The use of impregnants P and R gave the results shown in Table III.

In general, these layers gave good results and the preparation of the chromatoplates was easy. There was no diffusion of the two impregnants along the "dividing line".

TABLE III

RESULTS OBTAINED WITH IMPREGNANTS P AND R

Impregnant		*Solvent*		*Spots*		
Narrow layer	*Wide layer*	*First run*	*Second run*	*Resolution*[a]	*Shape*[b]	*No. of sugars separated*
P	R	54	13	++	B	32
P	R	54	32	++	A	31
P	R	106	13	++	B	24
P	R	106	32	++	B	26
P	R	108	32	++	AB	32
P	R	108	13	++	BC	25

[a] ++ = good.
[b] A = sharply defined; B = slightly diffused; C = diffused and/or tailed.

Impregnant R gave good resolution even with solvents that did not contain acetic acid. It was therefore possible to invert the two layers.

The carbohydrates that this chromatoplate did not resolve were maltose–trehalose, arabinose–glucose and fructose–mannoheptulose–galactose–sedoheptulose.

Typical chromatograms are shown in Figs. 15 and 16.

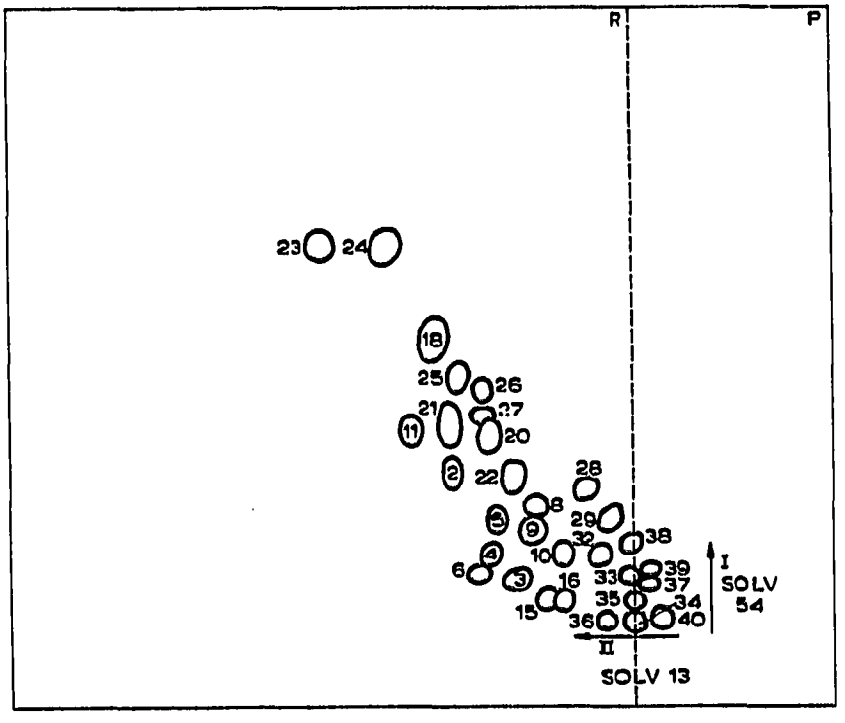

Fig. 15. Coupled-layer chromatogram of carbohydrates. Impregnation P–R; solvents 54/13. 2 = Glyceraldehyde; 3 = erythrose; 4 = xylose; 5 = lyxose; 6 = ribose; 8 = glucose; 9 = mannose; 10 = galactose; 11 = dihydroxyacetone; 15 = tagatose; 16 = mannoheptulose; 18 = 2-deoxyribose; 20 = 2-deoxygalactose; 21 = rhamnose; 22 = fucose; 23 = digitoxose; 24 = β-methylxyloside; 25 = α-methylmannoside; 26 = β-methylglucoside; 27 = β-methylarabinoside; 28 = sucrose; 29 = maltose; 32 = turanose; 33 = lactose; 34 = lactulose; 35 = melibiose; 36 = palatinose; 37 = isomaltose; 38 = melezitose; 39 = raffinose; 40 = stachyose.

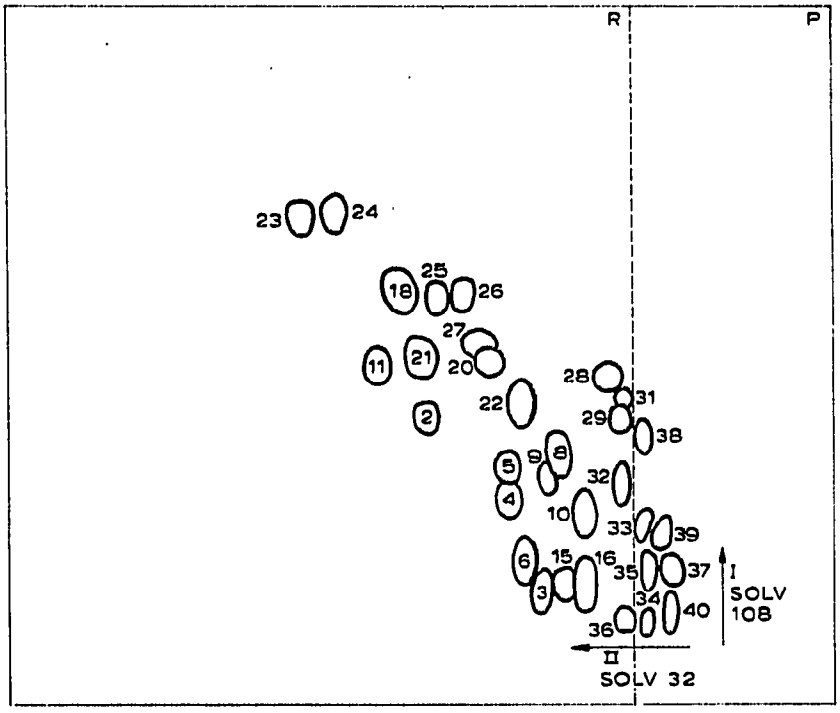

Fig. 16. Coupled-layer chromatogram of carbohydrates. Impregnation P–R; solvents 108/32. 2 = Glyceraldehyde; 3 = erythrose; 4 = xylose; 5 = lyxose; 6 = ribose; 8 = glucose; 9 = mannose; 10 = galactose; 11 = dihydroxyacetone; 15 = tagatose; 16 = mannoheptulose; 18 = 2-deoxyribose; 20 = 2-deoxygalactose; 21 = rhamnose; 22 = fucose; 23 = digitoxose; 24 = β-methylxyloside; 25 = α-methylmannoside; 26 = β-methylglucoside; 27 = β-methylarabinoside; 28 = sucrose; 29 = maltose; 31 = cellobiose; 32 = turanose; 33 = lactose; 34 = lactulose; 35 = melibiose; 36 = palatinose; 37 = isomaltose; 38 = melezitose; 39 = raffinose; 40 = stachyose.

R–P impregnation

The use of impregnants R and P gave the results shown in Table IV.

TABLE IV

RESULTS OBTAINED WITH IMPREGNANTS R AND P

Impregnant		*Solvent*		*Spots*		
Narrow layer	*Wide layer*	*First run*	*Second run*	*Resolution*[a]	*Shape*[b]	*No. of sugars separated*
R	P	37	108	++	A	28
R	P	36	108	+++	AB	34

[a] ++ = good; +++ = very good.
[b] A = sharply defined; B = slightly diffused.

Good results were obtained with solvents 37 (Fig. 17) and 36 (Fig. 18) in the first run. These two solvents also had the advantage of moving at very fast rates (1½ h for solvent 36 and 1 h for solvent 37).

A strange phenomenon was observed when solvent systems 36/108 were used: many oligosaccharides appeared as two spots, one of which was smaller and lighter than the other. We do not know what causes this phenomenon, or why it occurs only in this instance.

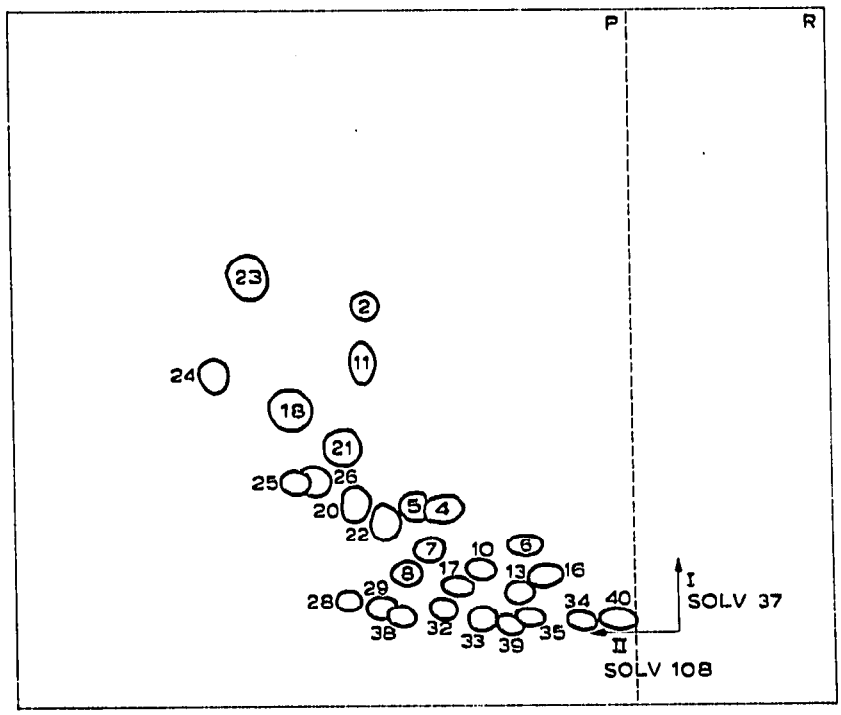

Fig. 17. Coupled-layer chromatogram of carbohydrates. Impregnation R–P; solvents 37/108. 2 = Glyceraldehyde; 4 = xylose; 5 = lyxose; 6 = ribose; 7 = arabinose; 8 = glucose; 10 = galactose; 11 = dihydroxyacetone; 13 = fructose; 16 = mannoheptulose; 17 = sedoheptulose; 18 = 2-deoxyribose; 20 = 2-deoxygalactose; 21 = rhamnose; 22 = fucose; 23 = digitoxose; 24 = β-methylxyloside; 25 = α-methylmannoside; 26 = β-methylglucoside; 28 = sucrose; 29 = maltose; 32 = turanose; 33 = lactose; 34 = lactulose; 35 = melibiose; 38 = melezitose; 39 = raffinose; 40 = stachyose.

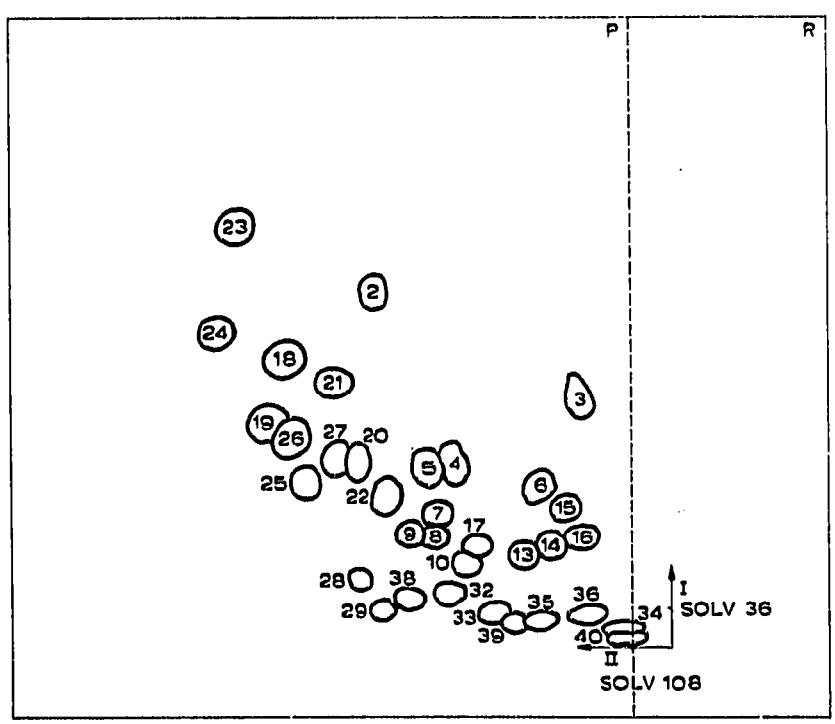

Fig. 18. Coupled-layer chromatogram of carbohydrates. Impregnation R–P; solvents 36/108. 2 = Glyceraldehyde; 3 = erythrose; 4 = xylose; 5 = lyxose; 6 = ribose; 7 = arabinose; 8 = glucose; 9 = mannose; 10 = galactose; 13 = fructose; 14 = sorbose; 15 = tagatose; 16 = mannoheptulose; 17 = sedoheptulose; 18 = 2-deoxyribose; 19 = 2-deoxyglucose; 20 = 2-deoxygalactose; 21 = rhamnose; 22 = fucose; 23 = digitoxose; 24 = β-methylxyloside; 25 = α-methylmannoside; 26 = β-methylglucoside; 27 = β-methylarabinoside; 28 = sucrose; 29 = maltose; 32 = turanose; 33 = lactose; 34 = lactulose; 35 = melibiose; 36 = palatinose; 38 = melezitose; 39 = raffinose; 40 = stachyose.

T–Q, T–R, T–S, S–T, and R–T impregnation

The use of these impregnants gave the results shown in Table V.

TABLE V

RESULTS OBTAINED WITH IMPREGNANTS T, Q, R AND S

Impregnant		*Solvent*		*Spots*		
Narrow layer	*Wide layer*	*First run*	*Second run*	*Resolution*[a]	*Shape*[b]	*No. of sugars separated*
T	Q	15	32	++	A	22
T	Q	29	13	+	A	20
T	R	29	36	++	B	20
T	R	29	37	++	A	22
T	R	15	36	+	AB	22
T	R	15	32	++	C	21
T	R	29	32	+	C	19
T	S	15	36	++	B	19
T	S	15	37	++	BC	19
T	S	29	36	++	A	21
T	S	29	37	++	A	22
S	T	36	29	+	BC	19
S	T	37	29	+	BC	19
R	T	37	29	+	B	21
R	T	37	15	+	A	22
R	T	36	29	+	B	23
R	T	36	15	+	A	21

[a] + = poor; ++ = good.
[b] A = sharply defined; B = slightly diffused; C = diffused and/or tailed.

Although this group of chromatoplates did not contain impregnant P, the results were interesting. This is a good example of the versatility of the technique.

Some chromatoplates, notably R–T, 36/29; T–S, 29/36 (Fig. 19) and T–S, 29/37 gave excellent resolution and succeeded in separating more than twenty carbohydrates.

Solvent 15 has the disadvantage of moving at an extremely slow rate.

CONCLUSION

The technique described in this paper permits the separation of the largest number of carbohydrates ever achieved on a single thin-layer chromatogram, yet the time necessary for separation is within the limits of normal two-dimensional chromatography.

One of the most important features of this chromatographic process is the successful resolution on the same chromatoplate of -oses (from trioses to heptoses) as well as di-, tri- and tetrasaccharides, deoxy sugars and methylglycosides.

The high resolution obtained with this procedure is illustrated by the separation on a single chromatogram of twelve oligosaccharides, nine of which (sucrose, maltose, cellobiose, turanose, lactose, isomaltose, lactulose, melibiose, palatinose) are disaccharides.

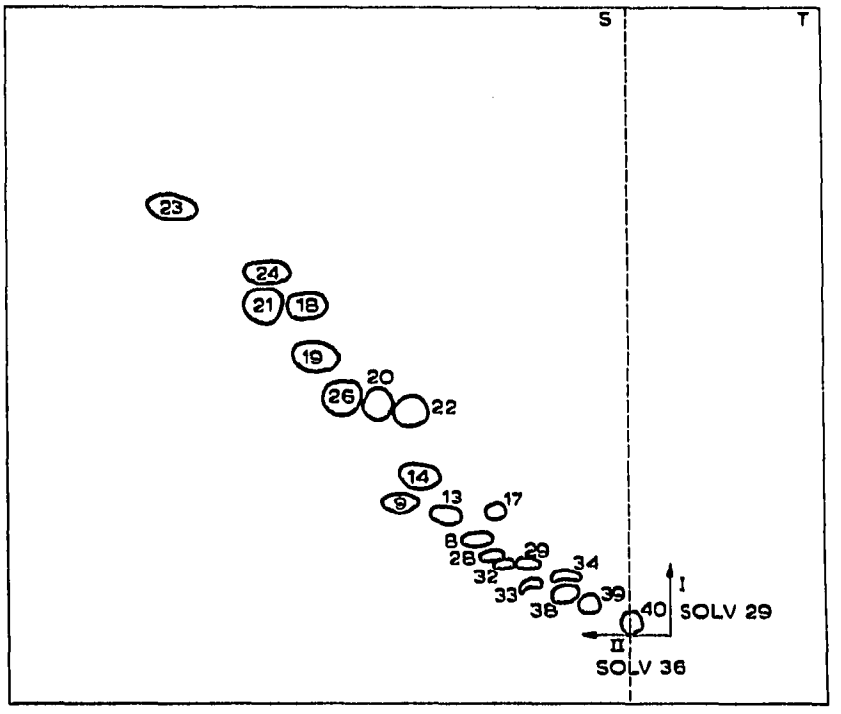

Fig. 19. Coupled-layer chromatogram of carbohydrates. Impregnation T–S; solvents 29/36. 8 = Glucose; 9 = mannose; 13 = fructose; 14 = sorbose; 17 = sedoheptulose; 18 = 2-deoxyribose; 19 = 2-deoxyglucose; 20 = 2-deoxygalactose; 21 = rhamnose; 22 = fucose; 23 = digitoxose; 24 = β-methylxyloside; 26 = β-methylglucoside; 28 = sucrose; 29 = maltose; 32 = turanose; 33 = lactose; 34 = lactulose; 38 = melezitose; 39 = raffinose; 40 = stachyose.

The high number, diversity and fine resolution of the carbohydrates separated was made possible only by the application of the principle of "coupled-layer chromatography", and we believe that better results can be achieved. New chromatographic materials, impregnants and solvents must still be investigated.

The versatility of the "coupled-layer" technique is such that it will surely be applied to the separation of many groups of substances of chemical, biological and pharmaceutical interest. This procedure should therefore facilitate research in areas such as biochemistry, the chemistry of natural substances, phytochemistry and physiology.

By varying the parameters used, it becomes possible to separate not only groups of chemically analogous molecules, such as carbohydrates or amino acids, but also families of substances that may occur together in nature but that are chromatographically unrelated, for example, cardiac glycosides and genines, or purines, pyrimidines and nucleosides.

We have already successfully applied this technique to the separation of free anthraquinones, aloin-like glycosides and anthraquinone glycosides present in plant extracts and pharmaceutical preparations.

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