used to reduce the molybdate complex, *e.g.* exposure to hydrogen sulfide, ultraviolet radiation[5], spraying with $SnCl_2$ in dilute HCl[6], or spraying with 10 % aqueous ascorbic acid followed by heating at 37° for 5 min[7]. Color formation with ascorbic acid is immediate for P_1 and PP_1 but slower for sulfate. Reduction with H_2S gives blue spots for PP_1 and sulfate which can be distinguished in the presence of each other; the former is ultramarine while the latter is cobalt blue. Sulfate migrates at a distinctly different rate than P_1 and PP_1 in other solvent systems which have been used to separate P_1 and PP_1, *e.g.* isopropanol–isobutanol–water–ammonia (40:20:39:1, v/v/v/v)[4,8], isopropanol–ammonia–water (70:10:20, v/v/v)[9], and *tert.*-butanol–water–formic acid (80:20:5, v/v/v)[5].

It should be noted that this same problem is encountered in the paper electrophoresis of solutions containing these ions if the electropherograms are sprayed with the HANES-ISHERWOOD reagent. On the other hand, using the LOWRY-LÓPEZ procedure[7] for the quantitative determination of phosphate, following digestion with sulfuric acid, 0.5 μmole of magnesium sulfate or sodium sulfate did not give a detectable blue color (measured at 675 mμ) (0.01 μmole P_1 can be readily detected by this procedure).

Acknowledgement

This work was supported in part by a Public Health Service research career program award 1-K3-GM-22,684-01 from the National Institutes of Health.

Genetics Foundation, The University of Texas, Austin, Texas (U.S.A.) J. M. LAGOWSKI

1 J. P. EBEL, *Mikrochim. Acta*, (1954) 679.
2 C. S. HANES AND F. A. ISHERWOOD, *Nature*, 164 (1949) 1107.
3 H. HETTLER, *J. Chromatog.*, 1 (1958) 389.
4 K. GASSNER, *Mikrochim. Acta*, (1957) 594.
5 J. CROWTHER, *Anal. Chem.*, 26 (1954) 1383.
6 D. N. BERNHART AND W. B. CHESS, *Anal. Chem.*, 31 (1959) 1026.
7 S. P. COLOWICK AND N. O. KAPLAN (Editors), *Methods in Enzymology*, Vol. III, Academic Press, New York, 1957, p. 845.
8 E. KARL-KROUPA, *Anal. Chem.*, 28 (1956) 1091.
9 R. W. CHAMBERS AND H. G. KHORANA, *J. Am. Chem. Soc.*, 80 (1958) 3749.

Received May 3rd, 1965

Equilibrium separation of glucose, galacturonic acid, and sulfuric acid with a strongly basic anion exchange resin

Following acid hydrolysis of plant cell wall polymers, the strong acid used for hydrolysis must be separated from the rest of the mixture before colorimetric analysis of the sugar fraction or further chromatography. $Ba(OH)_2$ is often recommended for this step but did not give reproducible results in our hands.

This report describes a separation of a strong acid (1 *N* H_2SO_4), weak acids, and sugars by equilibrium column chromatography on a strongly basic anion exchange resin. This technique is defined in Dowex: *Ion Exchange*[1] as a means of separating strong and weak acids, due to an equilibrium of sulfate and bisulfate anions. To our knowledge, it has not previously been reported as a means of separating weak acids from sugars.

Experimental

The strong basic anion exchange resin was prepared in the sulfate form by the following steps:

(1) Suspend the resin in NaCl and pour a 0.9 cm (I.D.) by 20 cm column.

(2) Wash resin with distilled water until the eluate is chloride free by $AgNO_3$ test.

(3) Wash the resin with 5 % H_2SO_4 until eluate is chloride free. The flow should be controlled so that 30–60 min are required for the step.

(4) Wash with distilled water until eluate is sulfate-free by tropeolin OO of thymol blue color indicator tests. $Ba(OH)_2$ may be used but it is not as sensitive.

(5) Add sample.

(6) Develop column with distilled water (2 ml/min).

Qualitative evaluation of the column was done primarily with color indicators. Either methyl red or bromocresol green was used to detect galacturonic acid and either thymol blue or tropeolin OO to detect sulfuric acid.

The NELSON-SOMOGYI method[2] was used to quantitate the sugar and uronic acid data.

Results

The NELSON-SOMOGYI method of analysis was equally satisfactory for both galacturonic acid and glucose; but separate standard curves must be used for each compound.

A 50–100 mesh resin charged with 5 % sulfuric acid provided good separation and recovery over a range of values (Table I) from 1.66 to 13.30 mg of glucose and

TABLE I

RECOVERY OF GLUCOSE AND GALACTURONATE FROM 50–100 MESH RESIN CHARGED WITH 5 % SULFURIC ACID

Glucose		*Galacturonate*	
mg applied	*% recovered*	*mg applied*	*% recovered*
1.66	102.1	1.66	94.0
3.33	101.7	3.33	99.3
6.65	101.1	6.65	98.3
13.30	92.5	13.30	123.0

galacturonate. 13.3 mg seems to be the upper limit for this size column. The pattern of separation for the 1.66 mg sample is shown in Fig. 1. Xylose, ribose, and galactose were eluted in the same fractions as glucose.

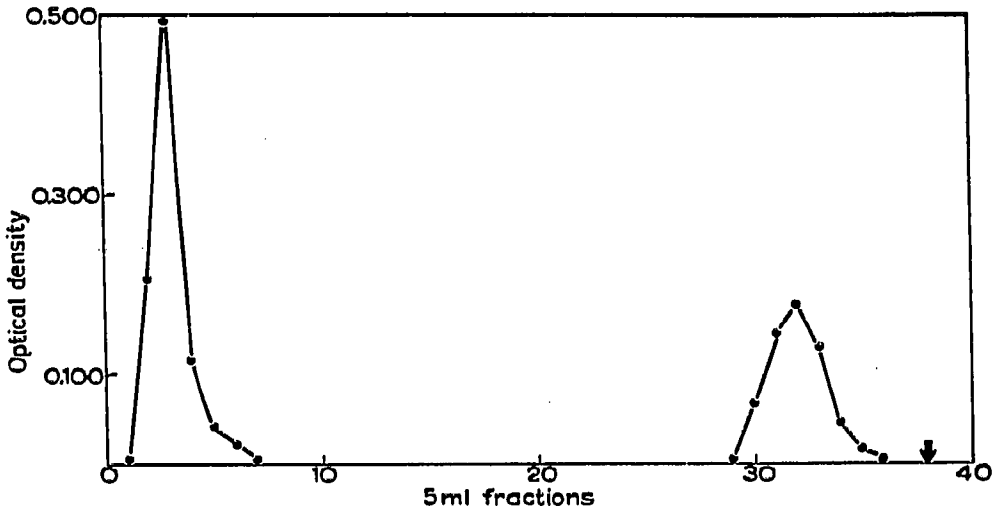

Fig. 1. Pattern of separation of 1.66 mg of glucose (fractions 1–7), 1.66 mg of galacturonic acid (29–36), and sulfuric acid (arrow, fraction 38).

Smaller mesh resin was partially effective up to 3.33 mg, but above this concentration the sugar and uronic acid peaks overlapped. Resins from several sources were checked, but no consistent difference could be detected.

Decreasing the strength of the sulfuric acid in the charge solution increased the distance between each compound (Table II). Although this is helpful in gaining complete separation, it greatly increases the volume of water per sample.

TABLE II

EFFECT OF VARIOUS CONCENTRATIONS OF SULFURIC ACID IN CHARGE SOLUTION ON DEVELOPMENT OF COLUMN

% *Acid*	*Fraction*		
	Glucose	*Galacturonate*	*Sulfate*
5.0	1–10	17–36	> 37
3.0	1–7	15–36	>> 40
1.0	1–7	18–31	>> 45
0.5	1–7	34–45	> 60

Pectin extracts of citrus rind have been satisfactorily separated on the column. Quantitation of the galacturonate agreed with carbazole determinations on non-fractionated extracts.

University of California,
Riverside, Calif. (U.S.A.)

L. N. LEWIS
C. W. COGGINS, JR.
J. C. F. KNAPP

1 Dowex, *Ion Exchange*, The Dow Chemical Company, Midland, Michigan.
2 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375.

Received April 20th, 1965