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**Note**

# Ion-exchange chromatography of the main reaction products of the catalytic oxidation of D-glucose and D-gluconic acid

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The catalytic oxidation of carbohydrates over platinum-on-carbon has been widely studied[1,2], but quantitative and even qualitative analytical data are often lacking. The products formed from D-glucose are D-fructose, D-mannose, D-gluconic acid, D-glucuronic acid, L-guluronic acid, D-glucaric acid, $C_1$–$C_5$ aldonic acids, $C_2$–$C_5$ aldaric acids, and 2- and 5-ketogluconic acids. With the exception of D-glucuronic acid, all the foregoing acids are formed from D-gluconic acid. D-Gluconic acid, L-guluronic acid, and D-glucuronic acid are the main (70–100%) reaction products of the oxidation of D-glucose, whereas L-guluronic acid and D-glucaric acid are the main (60–70%) oxidation products of the oxidation of D-gluconic acid[3,4].

Recently, we reported on the separation (during ~15 min) of the $C_1$–$C_5$ aldonic acids and aldaric acids by isotachophoresis[4]. Glucaric acid could also be determined by this system, but gluconic acid and the alduronic acids were not separated. However, isotachophoresis cannot be automated easily. Moreover, neutral sugars cannot be analysed by this technique, but can be determined by ion-exchange chromatography with boric acid as eluant[5].

Samuelson *et al.* have reported the analysis of aldonic acids[6–11], alduronic acids[7–9], and aldaric acids[12–16] by chromatography on a strongly basic anion-exchange resin (Dowex-1 X8), with elution by aqueous solutions of acetate, sulphate, phosphate, or borate. Reported separations have usually involved mixtures of only one or two classes of these compounds. We now report a procedure for the analysis of mixtures of glucose, gluconic acid, guluronic acid, glucuronic acid, and glucaric acid by ion-exchange chromatography.

Fig. 1 depicts a chromatogram of a mixture of D-glucose, D-gluconic acid, and D-glucaric acid (1 μmol of each). A good separation was obtained in ~35 min, which decreased to ~20 min in continuous automatic analysis. Fig. 2 gives the calibration curves, which are non-linear; sample volumes of 10 μl of various concentrations were

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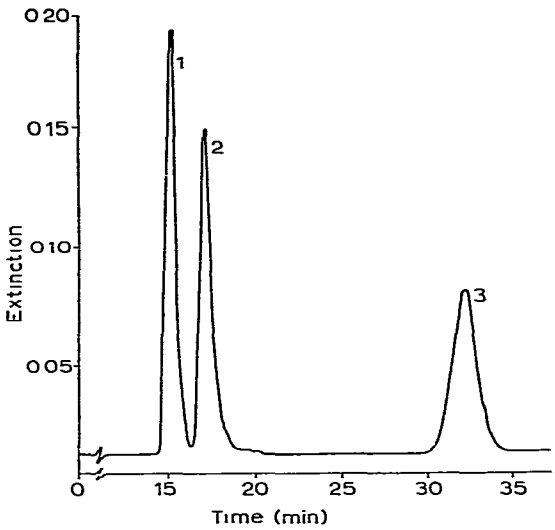


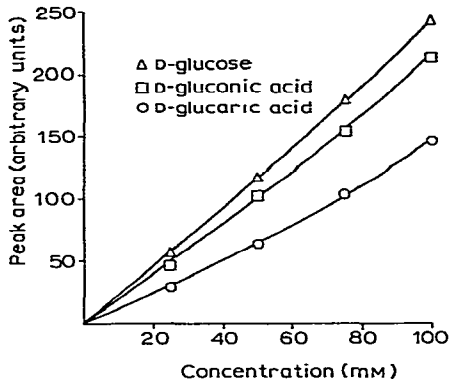


Fig. 1 (left). Chromatogram of a mixture of D-glucose (1), D-gluconic acid (2), and D-glucaric acid (3). See Experimental for details.

Fig. 2 (right). Calibration curves for D-glucose, D-gluconic acid, and D-glucaric acid.

used. The deviation from linearity can be ascribed to the formation of carbon dioxide during the oxidation with chromic acid, which leads to an increase of the length of the gas bubbles.

The concentration of the various components can be calculated conventionally from the peak areas. Fructose, mannose, and glucose were eluted simultaneously under the standard analytical conditions (see Experimental), but appreciable amounts of fructose and mannose were obtained only under conditions of high pH and temperature.

Fig. 3 depicts a chromatogram of a sample from an oxidation of D-gluconic acid. In addition to gluconic acid and glucaric acid, there are several other components. The 2- and 5-ketogluconic acids, which were eluted after gluconic acid and were usually present in low concentration, were not quantified in routine analyses. Eluted after glucaric acid were xylaric acid + arabinaric acid, tartaric acid, and tartronic acid. No signal was obtained for oxalic acid. The low concentrations of these aldaric acids were measured more conveniently by isotachophoresis than by ion-exchange chromatography. Erythronic acid and arabinonic acid, which were eluted shortly after gluconic acid, were usually present in low concentration and did not interfere with the quantification of gluconic acid. Glycolic acid and glyceric acid, also present in low concentration, were eluted between gluconic acid and glucaric acid. L-Guluronic acid, an intermediate in the oxidation of D-gluconic acid to D-glucaric acid, was eluted almost simultaneously with gluconic acid, but could be separated therefrom by elution with sodium acetate[8,9].

Orcinol reacts with uronic acids, but not with aldonic acids and aldaric acids,

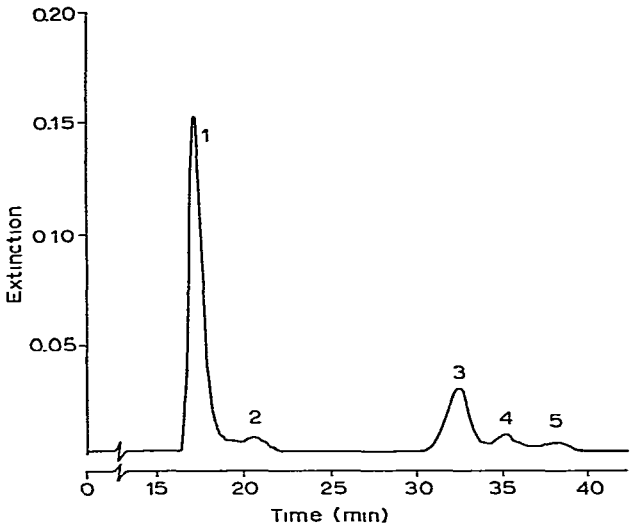


Fig. 3. Chromatogram of the oxidation products of D-gluconic acid: gluconic acid + guluronic acid (1), ketogluconic acids (2), glucaric acid (3), xylaric acid + arabinaric acid (4), and tartaric acid (5). See Experimental for details

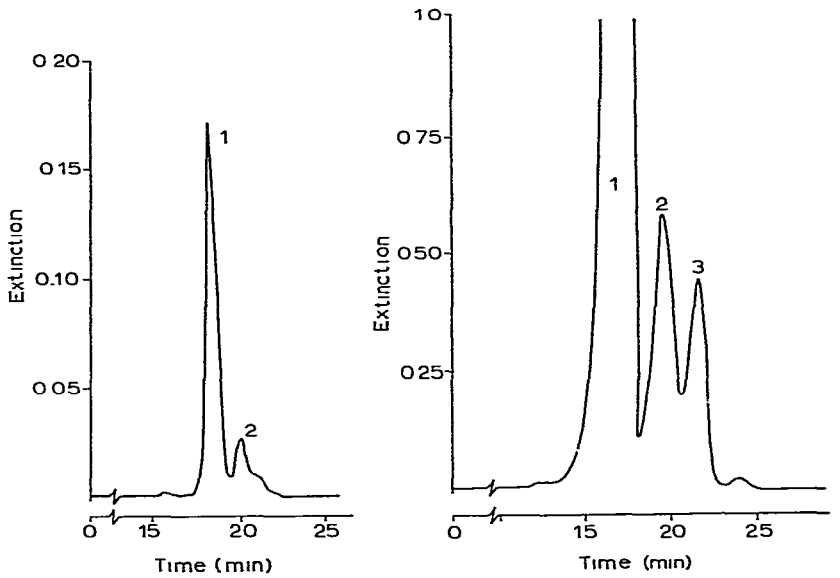


Fig. 4 (left). Chromatogram of the oxidation products of D-gluconic acid when detected with orcinol: guluronic acid (1) and 5-ketogluconic acid (2). Elution at 0.6 ml/min. See Experimental for other details.

Fig. 5 (right). Chromatogram of the catalytic oxidation products of D-glucose: glucose (1), guluronic acid (2), and glucuronic acid (3). Elution with 0.1M NaOAc at 0 6 ml/min, and detection with orcinol See Experimental for other details.

and can be used for the analysis of guluronic acid in the presence of gluconic acid. Fig. 4 depicts a separation of guluronic and 5-ketogluconic acids, based on detection with orcinol, using the same sample as that used to obtain Fig. 3. The eluant flow was reduced to 0.6 ml/min, otherwise the conditions were those given in the Experimental section.

In the oxidation of D-glucose, L-guluronic acid and D-glucuronic acid are the main side-products. The uronic acids are separated from glucose, but the separation of guluronic acid and glucuronic acid was very poor. A better result was obtained by elution with sodium acetate (Fig. 5).

In the routine automatic analysis, four standard samples of different concentrations, each containing the components of interest, were used; after each 15 reaction

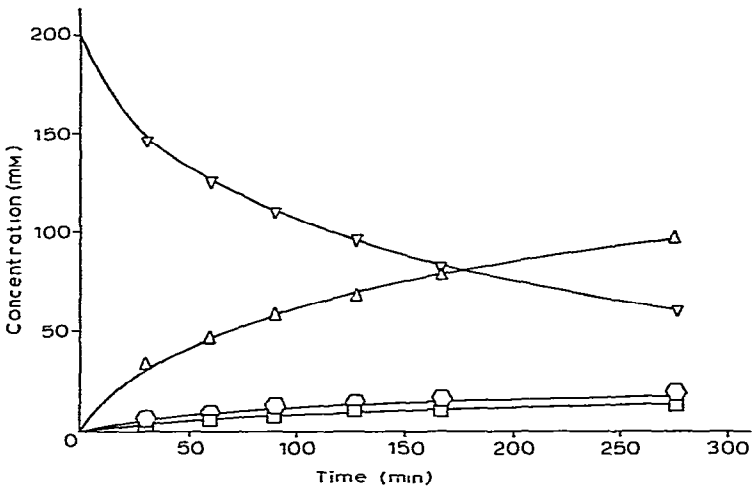


Fig. 6. Oxidation of D-glucose: glucose (—▽—), gluconic acid (—△—), guluronic acid (—□—), and glucuronic acid (—◇—).

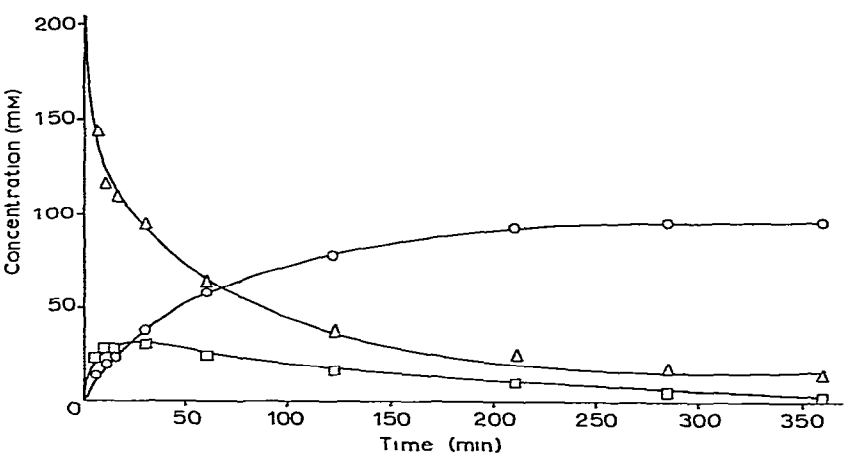


Fig. 7 Oxidation of D-gluconic acid: gluconic acid (—△—), guluronic acid (—□—), and glucaric acid (—○—).

samples, the standard samples were injected. Good reproducibility was obtained: the deviation in peak area between standard samples during 24 h was $<5\%$.

The foregoing procedures were used for the routine analysis of the main reaction products in the catalytic oxidation of D-glucose and D-gluconic acid. Typical results are given in Figs. 6 and 7. The reaction conditions are given in the Experimental. Considerable amounts of guluronic and glucuronic acids were obtained in the oxidation of D-glucose (Fig. 6). The formation of uronic acids is in contrast with previously published data[1,2,17] and may be ascribed to the analytical systems used. For example, De Wilt[17] used g.l.c., which probably failed to separate the uronic acids from glucose and gluconic acid. The oxidation of gluconic acid to glucaric acid proceeds[4] *via* guluronic acid; side-products are formed from gluconic acid and glucaric acid. A discussion of the influence of the reaction conditions on the yield of glucaric acid and the product distribution has been discussed[4]. A new reaction model for the oxidation of glucose and gluconic acid, in which the interaction between oxygen and the catalyst plays a dominating role, will be published elsewhere.

## EXPERIMENTAL

A Technicon Auto-Analyser was used, for which details have been given[18]. Detection was effected with either chromic acid (40% $H_2SO_4$, 60 g of $K_2Cr_2O_7$/litre) or orcinol reagent (70% $H_2SO_4$, 1 g of orcinol/litre). Gluconic acid and glucaric acid may be detected on the basis of u.v. absorption[19]. Colorimetric detection was used in the present study, because it permits the determination of certain components without a complete chromatographic separation.

*Analytical conditions.* — The following parameters were used for the analysis of mixtures of glucose, gluconic acid, and glucaric acid on a column (25 cm × 4 mm) of Aminex A-27 at 75°: eluant, 0.16M $Na_2SO_4$ at 0.7 ml/min; injection volume, 10 $\mu$l; reaction time, 10 min; detection with chromic acid.

*Reaction conditions*[4]. — Oxygenated (1 litre/min) 0.2M solutions of D-glucose or D-gluconic acid at pH 9 were kept at 55° in the presence of 5% platinum-on-carbon (2 and 20 g, respectively); reaction volume, 500 ml.

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