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ION-EXCHANGE CHROMATOGRAPHY OF SUGARS, SUGAR ALCOHOLS, AND SUGAR ACIDS USING U.V. SPECTROMETRY FOR DIRECT DETECTION

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ABSTRACT

Sugars, sugar alcohols, and sugar acids, after separation on an anion-exchange column, can be detected by using the strong absorption at $\lambda \sim 200$ nm. The absorption depends strongly on the type of eluant. The sensitivity of u.v. detection is superior to that using chromic acid by a factor of 2–30, but inferior to that for sugars using the orcinol reagent by a factor of ~ 100.

INTRODUCTION

Ion-exchange chromatography is a well-known technique for the separation of sugars, sugar alcohols, and sugar acids, and we have applied the method for the routine analysis of mixtures of (*1*) glucose, fructose, and mannose obtained from the isomerization of glucose[1]; (*2*) glucose, fructose, glucitol, and mannitol obtained from the simultaneous isomerization and catalytic hydrogenation of glucose[2]; and (*3*) gluconic acid, glucaric acid, and degradation products obtained from the catalytic oxidation of gluconic acid[3]. The detection of the eluted components is usually effected chemically with the orcinol reagent for sugars, and chromic acid for sugar alcohols and sugar acids.

Recently, we have become interested in non-destructive systems of detection. Refractometry[4] and u.v. spectroscopy[5] ($\lambda \sim 192$ nm) have been used for the direct detection of sugars. We now report an application of the latter method to sugars, sugar alcohols, and sugar acids, after their separation by elution from an anion-exchange column with inorganic salt solutions.

EXPERIMENTAL

The analytical system was based on the Technicon Auto-Analyser. A 25-cm Aminex A-25 column was used, with a Perkin–Elmer LC-55 spectrophotometer; the remaining components of the analytical system have been described before[1].

The outlet of the column was connected to the spectrophotometer by a capillary. A double detection was usually applied, first with the spectrophotometer, and then

chemically (15 g of $K_2Cr_2O_7$/l, 40% v/v H_2SO_4) with a Technicon Single Channel colorimeter. A comparison of the two methods was thus effected.

The following conditions were used for analyses of the mixtures (*1*) glucose, fructose, glucitol, and mannitol: eluant 0.09M H_3BO_3 + 0.09M $Na_2B_4O_7$ at 0.85 ml/min and 65°; (*2*) glucose, fructose, and mannose: eluant 0.19M H_3BO_3 + 0.01M $Na_2B_4O_7$ + 0.025M NaCl at 0.85 ml/min and 75°; (*3*) gluconic acid and glucaric acid: eluant 0.16M Na_2SO_4 at 0.85 ml/min and 70°.

For some components, an absorption spectrum was also recorded with a Cary 14 scanning spectrophotometer. Each of the components was dissolved in its eluant, and the eluant was used as a reference. The absorption spectra of the various eluants were also recorded, with water as the reference. The peak areas of the components were expressed in E.t units, where E is the extinction and t is the time in min. This method provides a direct comparison of the different analytical systems and the two detection methods.

RESULTS AND DISCUSSION

The results obtained with the scanning spectrophotometer are presented in Fig. 1. The absorption spectra of glucose in the eluants 1–3 (lines *A*) illustrate the marked dependence of the spectra on the composition of the medium. This variation may reflect the effect of complex formation between glucose and boric acid. The spectra of the other components show similar qualitative behaviour. Significant absorption occurs at ~200 nm, and the intensity depends strongly on the type of eluant. In selecting the most suitable wavelength, account must be taken of the

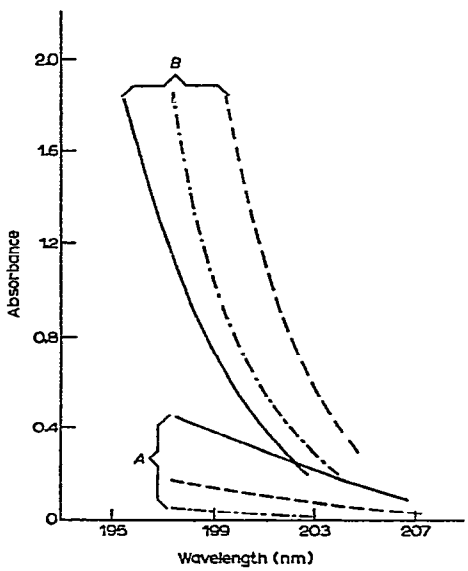

Fig. 1. *A*, Absorption spectra of glucose in eluants 1 (——, 4 mmol/l), 2 (----, 5 mmol/l), and 3 (·····, 20 mmol/l); *B*, absorption spectra of eluants.

absorptions of the eluant and the components to be detected, and the noise level of the detector. The lines *B* in Fig. 1 show the absorption spectra of the various eluants.

The remaining data were obtained by connecting the LC-55 spectrophotometer to each of the three analytical systems. Absorptions were determined by recording fixed-wavelength chromatograms under steady chromatographic conditions. A plot of extinction against wavelength for glucose, mannose, glucitol, and mannitol is shown in Fig. 2 for eluant 1. The on-line noise level of the LC-55 spectrophotometer as a function of the wavelength is given in Fig. 3. At lower wavelengths, the noise level strongly increases. Although Fig. 2 indicates a wavelength of ~195 nm to be optimal, the eluant absorption (Fig. 1) and the noise level (Fig. 3) led to the selection of somewhat higher wavelengths, namely, eluant 1, 197 nm; eluant 2, 199 nm; and eluant 3, 201 nm. Fig. 4 shows a chromatogram of a glucose–fructose–glucitol–mannitol mixture (0.6 μmol of each component, eluant 1). Clearly, the detection using u.v. absorption is stronger than that with chromic acid, particularly for the sugar alcohols. Peak-broadening in the chemical detection was <5% and therefore need not be considered in comparing these signals. By successive injection of various amounts (up to 1 μmol) of the components, calibration lines were obtained and, as for chemical detection[2], a linear relation between amount injected and peak area was obtained. The resolution of the various components, especially glucose and mannitol, is markedly improved by decreasing the amounts injected (*cf.* Ref. 2).

A chromatogram of a glucose–fructose–mannose mixture (u.v. detection, 0.3 μmol of each component) is shown in Fig. 5. A chemical detection was not applied since, using orcinol, the signals were too strong for the amounts injected. Clearly, for this mixture, chemical detection is superior to that based on u.v. absorption. A marked

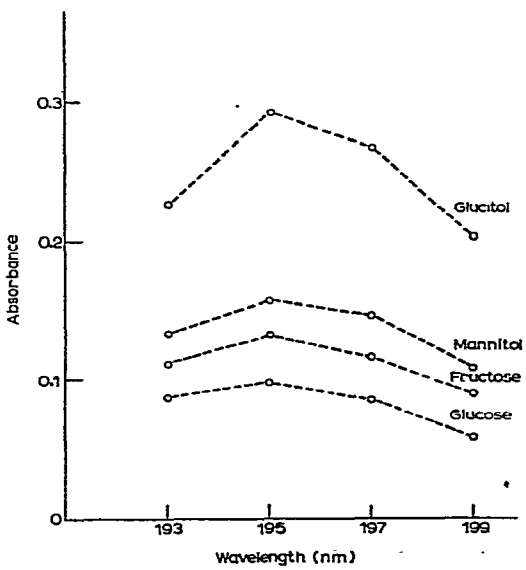

Fig. 2. Absorbance as a function of wavelength for eluant 1; 2 μmol of each component.

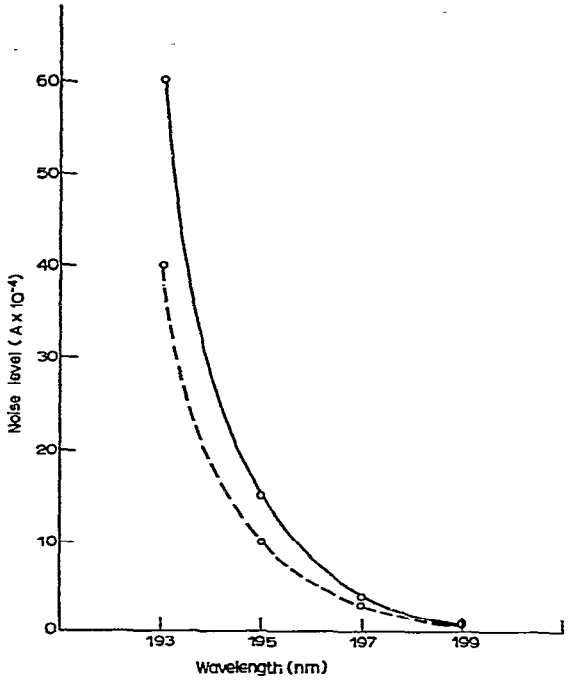

Fig. 3. Noise level of the Perkin–Elmer LC-55 spectrophotometer as a function of wavelength: ——, damp 1"; ----, damp 2".

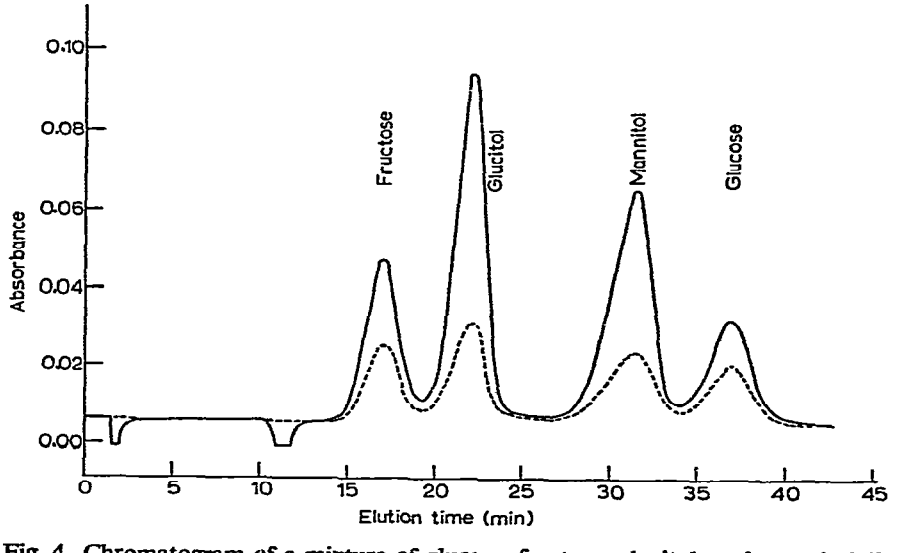

Fig. 4. Chromatogram of a mixture of glucose, fructose, glucitol, and mannitol (0.6 μmol of each component, eluant 1); detection by u.v. absorbance at 197 nm (——), and with chromic acid at 600 nm (----).

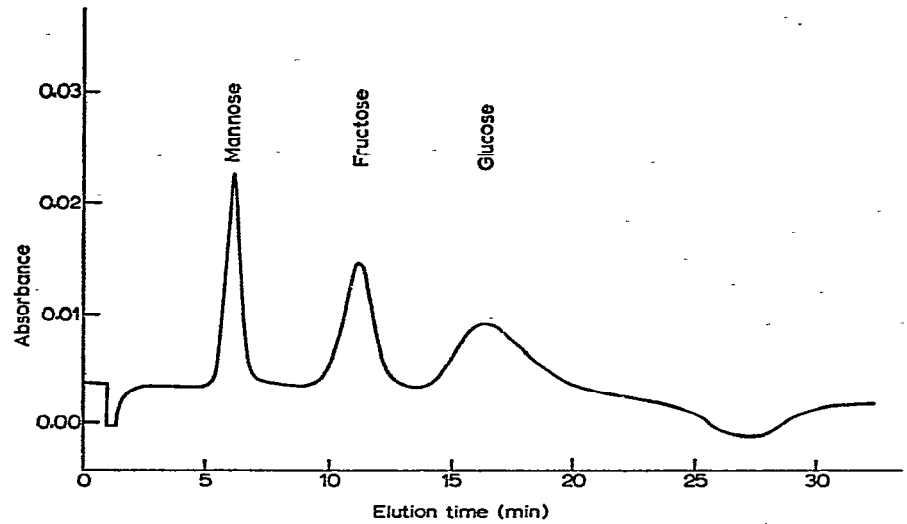

Fig. 5. Chromatogram of a mixture of glucose, fructose, and mannose (0.3 μmol of each component, eluant 2); detection at 199 nm.

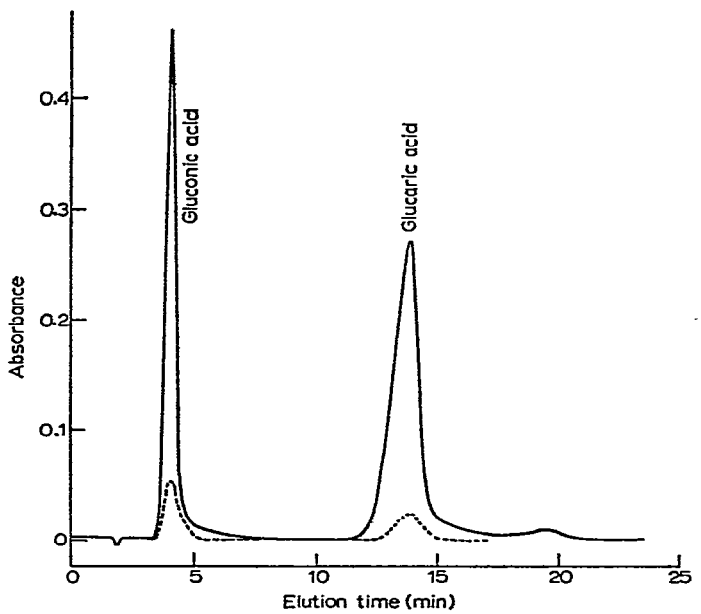

Fig. 6. Chromatogram of a mixture of gluconic acid and glucaric acid (0.5 μmol of each component, eluant 3); detection by u.v. absorption at 201 nm (——), and with chromic acid at 600 nm (----).

decrease in resolution was found at the relatively high injection amounts necessary for u.v. detection, which is an additional disadvantage. Below 0.5 μmol of each component, there was a linear relationship between amount injected and peak area. A

non-linear relationship has been found for orcinol detection[1]. Our present experiments suggest that this result should be ascribed to the detection part of the analytical system.

Fig. 6 shows a chromatogram of a gluconic acid–glucaric acid mixture (0.5 μmol of each component). The u.v. detection yields much stronger signals than that with chromic acid, and a linear relationship was obtained between injection amount and peak area up to 0.5 μmol of each component.

The signal ratios for detection using u.v. absorption and chromic acid are given in Table I. These ratios were corrected for the difference in cell length (spectrophotometer, 6 mm; colorimeter, 15 mm). As the noise level of the spectrophotometer and the colorimeter are almost identical ($1–3 \times 10^{-4}$E), the data in Table I can be used to compare the two detection methods. The sugar acids and the sugar alcohols produce much stronger signals in u.v. detection than in detection with chromic acid. The sugars also give stronger signals in u.v. detection, but the signal ratio depends significantly on the eluant composition. An additional example is shown in Fig. 1, where glucose in a Na_2SO_4 solution gives rise to a very low signal.

For our analytical systems, u.v. detection is more favourable than chemical detection if chromic acid is the reagent (*i.e.*, for sugar alcohols and sugar acids), and the orcinol reagent is superior if sugars have to be analysed. Apart from the sensitivity, the simplicity and reliability of u.v. detection are important advantages.

TABLE I

SIGNAL RATIOS FOR THE DIRECT DETECTION WITH A SPECTROPHOTOMETER AND THE CHEMICAL DETECTION WITH CHROMIC ACID

	Eluant 1	*Eluant 2*	*Eluant 3*
Mannose		1.6	
Fructose	5.2	1.7	
Glucose	4.0	2.6	
Glucitol	8.6		
Mannitol	8.3		
Gluconic acid			14.3
Glucaric acid			29.8

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