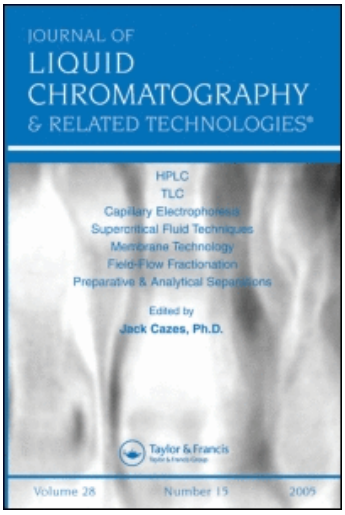


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# Determination of Carbohydrates by Anion Exchange Chromatography with Pulsed Amperometric Detection

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# DETERMINATION OF CARBOHYDRATES BY ANION EXCHANGE CHROMATOGRAPHY WITH PULSED AMPEROMETRIC DETECTION

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## ABSTRACT

Carbohydrates such as sugar alcohols, monosaccharides, disaccharides, and other oligosaccharides are separated as anions by ion exchange chromatography with a sodium hydroxide eluent. Retention time and selectivity are controlled by varying eluent strength and column temperature. The carbohydrates are detected by oxidation at a gold electrode. A repeating sequence of three potentials electrochemically cleans the electrode surface of oxidation products and other interfering species. Detection limits are as low as 30 ppb for sugar alcohols and monosaccharides, and about 100 ppb for oligosaccharides. Other species containing CHOH groups can also be detected, such as alcohols and glycols.

## INTRODUCTION

The determination of carbohydrates by chromatography has been hampered by two factors: the lack of a suitable high performance separation method and the inability to detect the carbohydrates at low levels. A number of separation methods have been used. Carbohydrates can be determined by gas chromatography, however, their non-volatile nature requires a time consuming derivitization procedure (1). The most common method is the use of high capacity strongly acidic cation exchange columns in the metal form using water as the eluent (2). Calcium is the most common metal, although silver and lead have also been used.



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These columns suffer from several drawbacks. First, the organic acids present in many samples elute metals from the cation exchange resin. This results in resin bed shrinkage and requires frequent regeneration of the column back to the original metal form. Second, these columns exhibit poor selectivity for higher oligosaccharides. Low crosslink resin is required for resolution of even the lower oligosaccharides such as DP2 through DP4. The resin exhibits high compressibility, making it unsuitable for high flow rates and rapid analysis. Third, high column temperatures (85°C) are needed for optimum column performance. These high temperatures present a severe test of the performance of the detector, particularly for a refractive index detector. Finally, since only water or water organic solvent mixtures can be used as an eluent, the methods of retention and selectivity control left open to the chromatographer are severely limited.



A second major method of carbohydrate analysis involves the use of micro-particulate silica with an amino bonded phase (3). While this system successfully separates mono and disaccharides, the higher oligosaccharides (DP3-DP10) are eluted as broad peaks and require extended analysis times. Furthermore, amino stationary phases are subject to reaction with aldehydes or ketones in samples (frequently present in foods and beverages) to form a Schiff's base. Because of this, amino bonded phases tend to exhibit reduced lifetimes with certain samples.

A third method is the separation of sugars via their borate complexes (4). While the selectivity of these systems are superior to the above two methods, the kinetics of the complex formation process is slow, thus requiring low flow rates and long analysis times.

We report here an alternative to the above method; the direct separation of carbohydrates by anion exchange. Because carbohydrates have pK values ranging from 12 to 14 (5), retention is possible on a strongly basic hydroxide form anion exchange column with highly alkaline eluents. Such a system is previously

unreported, although retention of methylglycosides on a strongly basic hydroxide form anion exchange column has been reported (6).

Due to the absence of a strongly absorbing UV chromophore, carbohydrates are usually detected by a refractive index detector. R.I. detectors have low sensitivity and are difficult to use. Low UV detection (< 200 nm) has been employed, but the need for ultra clean eluents and interference problems have limited its utility.

The detection method reported here uses an electrochemical detector which supplies a triple potential sequence to a gold working electrode. Both potentiometric and single potential (D.C.) amperometric detectors have been used to detect carbohydrates (7). Potentiometric detectors suffer from slow response times. Single potential oxidation of sugars causes the electrode surface to become contaminated by the products of the reaction, thus rendering the detector unusable. Triple pulse amperometry of sugars was first reported by Hughes and Johnson using a platinum electrode (8). Although the detection method is successful, the potential at which the oxidation current is measured (E1,-0.40V) is sufficiently negative to cause the reduction of oxygen. Gold was investigated as an alternative working electrode material.



## EXPERIMENTAL

All chromatography was performed on a Dionex System 2011 (P/N 35295) Ion Chromatograph. The sample loop size was 50 µL and the eluent flow rate 1.0 mL/min. The detector was a breadboard version of a Dionex IonChrom™/Pulsed Amperometric Detector (P/N 35227) consisting of an amperometric flow-through cell and a potentiostat. The cell is a thin layer design with a gold working electrode, a silver/silver chloride reference electrode, and a glassy carbon counterelectrode. The potentiostat applies a series of up to three potentials (E1, E2, E3) in a repeating waveform. The pulse durations (t1, t2, t3) are selectable by the user.

The cell current is measured only during E1 for 16.67 ms (to cancel 60 hz line noise). A 40 ms delay following the potential step allows charging current to decay.

The anion exchange column used was a prototype HPIC-AS6 column (P/N 35391). The ion exchange resin used in the column consisted of 10 micron substrate coated with a monolayer of anion exchange latex similar to that used in other HPIC anion Ion Chromatography columns.

The eluent consisted of NaOH in deionized water (0.15 N). 0.2 M $CH_3CO_2Na$ was added as a pusher to elute oligosaccharides. Eluents were prepared from carbonate free 50% NaOH solutions and protected from carbon dioxide contamination with Ascarite traps. Sugar samples were purchased from Pfanstiehl Laboratories (Waukegan, IL) and Sigma Chemicals Co., St. Louis, MO. The maltose oligomers were a generous gift of the A.E. Staley Company.



## RESULTS & DISCUSSION

### Cyclic Voltammetry of Glucose

The choice of potentials and pulse durations for chromatographic detection is most easily determined from voltammetric information. Accordingly, the cyclic voltammetry of sugars on gold was studied and is illustrated in Figure 1 by glucose. The dashed line is a background scan of the 0.1 N NaOH supporting electrolyte. It shows irreversible oxidation of gold beginning at approximately 0.25 V. The reduction of the surface oxide back to gold is shown as a cathodic peak at approximately 0.1 V.

With glucose added to the solution, oxidation begins at -0.5 V on the positive going scan. The current rises slightly and remains unchanged until it rises at -0.15 V towards a peak at 0.26 V. On the reverse scan, the current actually reverses from cathodic to anodic at the onset of the gold oxide reduction. This implies that glucose oxidation which occurs at the bare gold surface is inhibited upon the formation of gold oxide. As soon as the reduction of gold oxide back to gold begins,

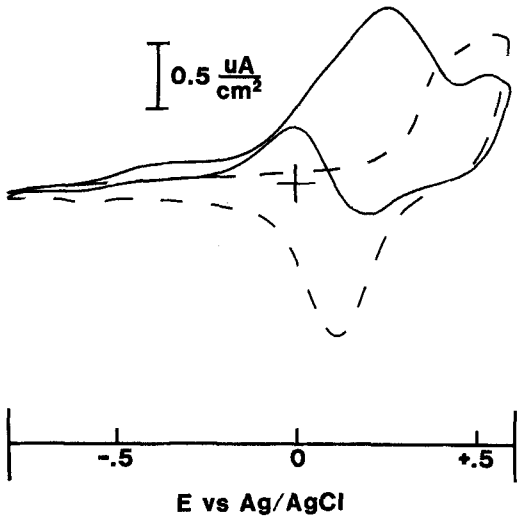


Figure 1. Cyclic voltammetry of 0.92 mM (166 ppm) glucose on a gold working electrode. Dashed line is 0.10 M NaOH supporting electrolyte. 0.20 V/sec sweep rate.



oxidation of glucose that had diffused toward the electrode while the surface was covered with oxide takes place.

Cyclic voltammetry of the sugar alcohols xylitol and sorbitol and the disaccharide sucrose were also studied. They were similar to glucose, except the height of the anodic peak at 0 V on the negative going scan was larger with the sugar alcohols and smaller with sucrose.

## Choice of Applied Potentials

Single potential (D.C.) amperometry with $E_{app}$ set to 0.1 V was attempted. 0.1 V was chosen because at this potential, there should be a large fraction of unoxidized gold on the electrode surface. Although detection was possible, the response decreased rapidly as products from the oxidation reaction deactivated the surface. A large improvement in reproducibility was obtained by pulsing the applied potential from -0.8 V to 0.2 V and measuring the oxidation current at 0.2 V. The negative potential apparently serves as a

cleaning step, ensuring the reduction of gold oxide back to gold. Also, the magnitude of the sugar oxidation current from a voltage pulse is greater than that caused by the hydrodynamics of flow when only a single potential is used. The use of both negative and positive cleaning potentials was found to provide the most reproducible chromatograms, as well as minimum interference from other species in the samples. The following potentials and times (shown in Figure 2) have been chosen as optimum for carbohydrate determination: E1: 0.20 V, 60 ms; E2: 0.60 V, 60 ms; E3: -0.80 V, 240 ms.

The signal-to-noise ratio is affected the most by the choice of the detection potential, E1. The peak heights for three sugars and the background current are shown in Figure 3. The large increase in background current from 0.2 V to 0.4 V is accompanied by a 4X increase in noise. The optimum value for E1 is 0.2 V. Due to the similarity of the electrochemistry of the different sugars, this value may be optimum for most, if not all, sugars.

Small (0.1 V) changes in E2 and E3, as well as their durations, have only a minor effect on response.



## Chromatography

One of the main advantages of the anion exchange chromatography of carbohydrates as compared to metal form cation exchange is the ability to influence retention time and retention order. The primary operating variables are eluent strength and column temperature. The separation of a mixture of sugar alcohols and saccharides is shown in Figure 4. The addition of acetate ion results in reduction in the K' of all solutes. This enables the elution of the oligosaccharides DP2 through 10, shown in Figure 5.

In general, the observed anion exchange affinity follows the trend: sugar alcohols < monosaccharides < disaccharides < oligosaccharides, although in some cases overlap is observed in the elution order of mixtures of disaccharides and oligosaccharides. For a homologous series of carbohydrates, retention

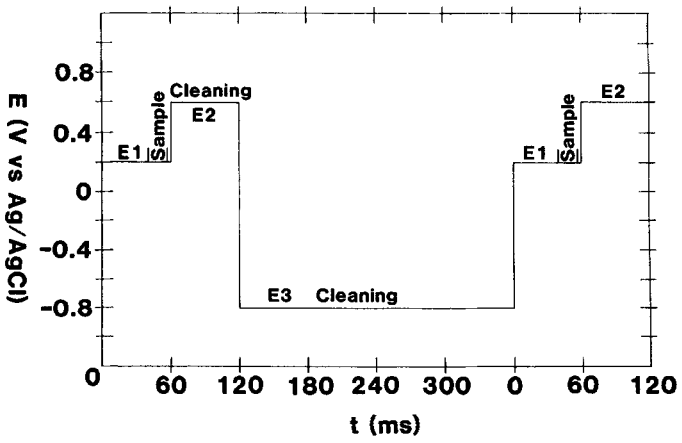


Figure 2. Triple potential program applied to the gold working electrode for the detection of carbohydrates. Oxidation current is sampled from 40 to 56.7 ms after the beginning of E1.



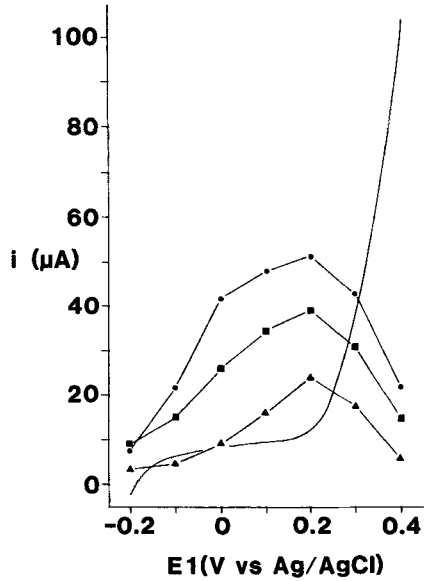


Figure 3. $i_p$ as a function of E1 for 100 ppm xylitol (dots), 100 ppm glucose (squares), and 316 ppm sucrose (triangles). Background current as a function of E1 is also shown.

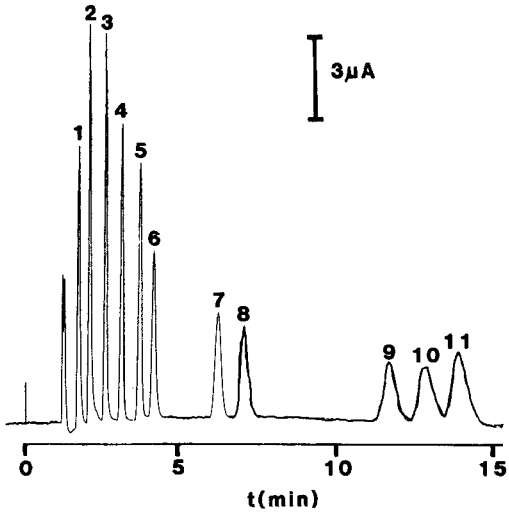


Figure 4. Separation of sugar alcohols and saccharides. Listed in order, they are: 25 ppm xylitol (1); 50 ppm sorbitol (2), rhamnose (3), arabinose (4), glucose (5), fructose (6), lactose (7); 100 ppm sucrose (8), raffinose (9), stachyose (10); 150 ppm maltose (11). 0.15 M NaOH eluent at 36°C.

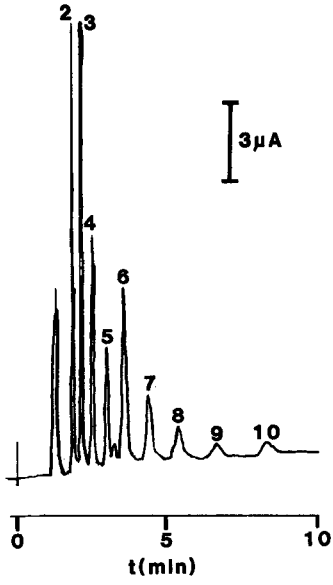


Figure 5. Separation of oligomers of maltose, DP 2 (maltose) through DP 10 (maltodecose). 0.2 M NaOH, 0.2 M $CH_3CO_2Na$ eluent at 34°C.

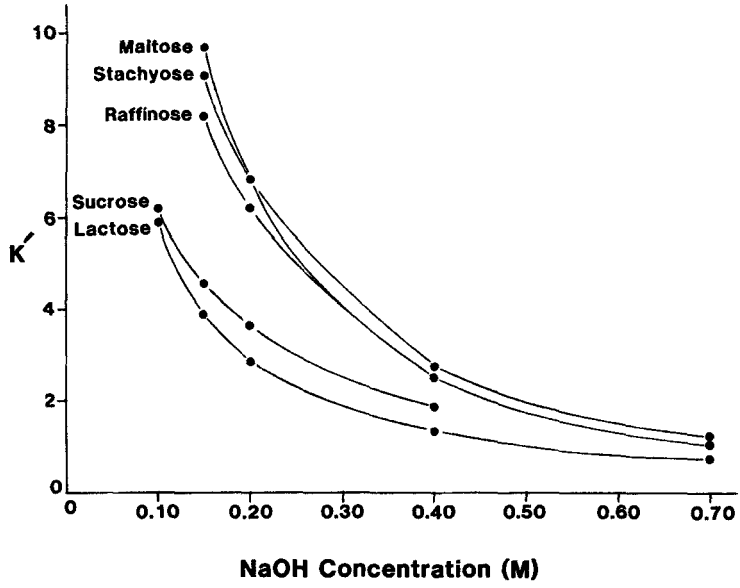


Figure 6. Capacity factor versus NaOH concentration. Column temperature: 34°C; sample concentrations: 100 ppm each.



increases as the degree of polymerization (DP) increases. Selectivity changes are often observed as the hydroxide ion concentration is varied. Typical selectivity changes observed for oligosaccharides are illustrated in Figure 6. For the oligosaccharides shown, optimum selectivity is observed at 0.150 M NaOH. Similar chromatographic behavior is observed for monosaccharides and sugar alcohols. While in some cases these selectivity changes may be used to advantage, in general hydroxide ion concentration is not a useful method for controlling selectivity. Often, the desired selectivity for a particular mixture of carbohydrates is only observed at K' values which are unacceptably large or small.

The hydroxide ion concentration has a significant effect on the observed efficiency for a particular solute. It is generally observed that column efficiencies increase as the

hydroxide ion concentration is increased. For glucose, efficiency nearly doubles when hydroxide ion concentration is increased from 0.10 M NaOH to 1.0 M NaOH.

A second variable useful for control of selectivity and retention is column temperature. Retention decreases for all solutes as the temperature increases. The degree to which temperature alters retention is loosely related to the carbohydrate size; the observed magnitude of the temperature effect being: oligosaccharides > disaccharides > sugar alcohols > monosaccharides. This can be used to advantage for adjusting the elution order for a given mixture of carbohydrates. The effect of temperature on the selectivity observed for some common carbohydrates is shown in Figure 7. Note that an elution order reversal is observed for maltose (a disaccharide) and stachyose (a tetrasaccharide) as the temperature is increased.



Three factors serve to limit the effective upper range of temperature in the retention of sugars on hydroxide form anion exchange columns:

(1) Tailing and secondary peaks are observed for some carbohydrates (particularly glucose and mannose) when the temperature of the column exceeds 45°C. This is probably caused by rearrangement about the anomeric carbon atom, the Lobry de Bruyn-Van Ekenstein transformation.
(2) Efficiencies tend to decrease when temperatures exceed 45°C.
(3) Anion exchange materials in the hydroxide form are subject to Hoffman degradation at elevated temperatures.

Because of the above, columns are normally operated at temperatures between 20°C and 45°C.

A third variable for control of selectivity and retention is the addition of anions other than hydroxide to the eluent. The addition of other anions is sometimes desirable due to the fact that high levels of NaOH (>0.2 M) can result in increased detector noise as well as peak assymetry for the sugar

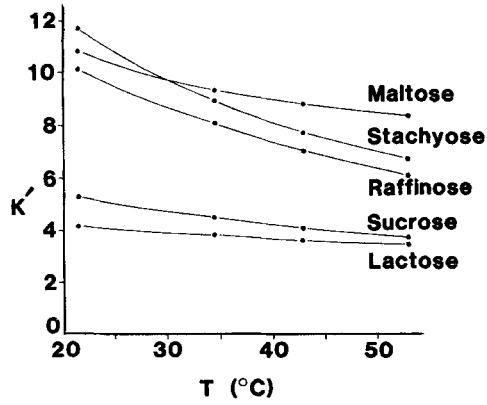


Figure 7. Capacity factor versus temperature. Eluent: 0.15 M NaOH; sample concentrations: 100 ppm each.



alcohols. Acetate, carbonate, nitrate, and sulfate have been evaluated as eluent additives. In our work, acetate was found to be the preferred eluent additive due to the fact that its affinity for the anion exchange resin is similar to that of hydroxide. The other anions were found to be compatible with the detector but their high affinity for the anion exchange resin resulted in a substantial reduction of the column loading capacity.

Because of the elution power of carbonate, it is important that carbonate be excluded from the eluent system. This is particularly true for eluent systems used for the analysis of sugar alcohols and monosaccharides. Proper precautionary measures include the use of an Ascarite trap on the eluent bottle and ASMT Class I deionized water when making up eluents.

## Sensitivity, Linearity and Reproducibility

The detection limits for the carbohydrates shown in Figure 4 range from 30 ppb for the sugar alcohols and monosaccharides to 100 ppb for the oligosaccharides. Plots of the log of peak height vs the log of concentration are linear up to several hundred to 1,000 ppm, where overloading of the capacity of the

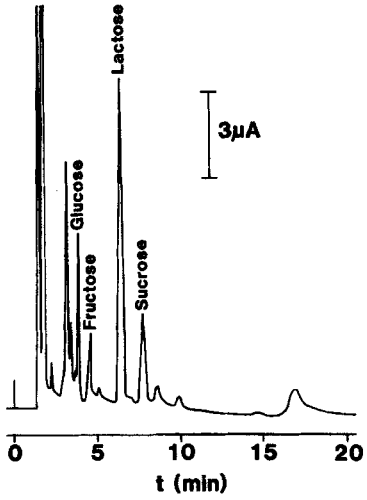


Figure 8. Extract from flavored potato chips. Lactose concentration in the extract is 70 ppm.



column occurs. Although the log/log plots are linear, the slopes for several sugars are: xylitol, 1.07; glucose, 1.03; sucrose, 1.16. The near linearity of $i_p$ *vs* C plots, rather than $1/i_p$ *vs* 1/C as on platinum (8) implies that adsorption of carbohydrate molecules during t3 (E3 = -0.80 V) is not part of the reaction mechanism.

Reproducibility of peak heights for multiple injections of a single sample is generally better than 1%. Peak heights have a tendency to increase with time, beginning with the time at which the working electrode was cleaned. This increase has been observed to be approximately 5 to 15% after 8 hours of operation. Errors in concentration measurements can be minimized by the frequent use of standards.

## CONCLUSION

The anion exchange separation of carbohydrates with alkaline eluent systems provides a powerful new tool in the analysis of carbohydrates. The combination of this chromatographic system

with triple potential detection provides a highly selective and sensitive method particularly well suited to complex samples. For example, the determination of lactose in flavored potato chips, shown in Figure 8, was easily accomplished even though the solution to be analyzed contained a high concentration of potentially interfering salts.

The triple potential detection method can also be used to determine other species containing the alcohol functional group. Using different chromatographic conditions, simple alcohols and glycols have been detected. NOTE: The analytical methods described in this paper are the subject of pending patents.

## ACKNOWLEDGMENTS

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