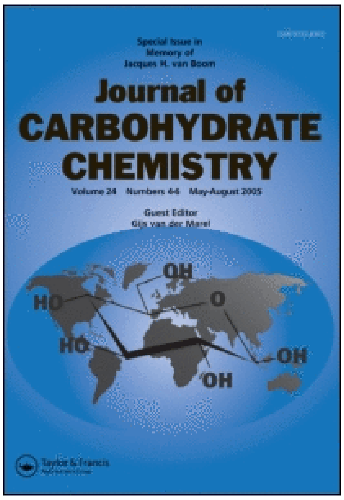

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THE MEASUREMENT OF DEXTRAN IN RAW SUGARS USING ^{1}H NMR[1]

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ABSTRACT

In the cane sugar industry the purchase price of raw cane sugar, the product of sugar cane processing, is determined by polarimetric measurement of sucrose content in raw sugar solutions, expressed as Pol. Raw sugar generally contains more than 96 % sucrose, but also contains other saccharides and non-sugars which can contribute to Pol. Dextrans, one class of polysaccharides often found in raw sugar, effect an increase in Pol and interfere with subsequent refining. The U.S. sugar refining industry can impose a penalty on the raw sugar purchase price for high dextran content. While there are several wet chemical methods for the determination of dextran in raw sugar, the results of these analyses are rarely in agreement. The existing wet chemical methods for the determination of dextran in raw cane sugar are reviewed and the results of these wet chemical analyses are compared with the results obtained from the physical measurement of dextran in raw sugar by ^{1}H NMR spectroscopy.

INTRODUCTION

Dextrans are a class of extracellular microbial polysaccharides consisting of a backbone of α-D-glucopyranosyl residues with (1→6) linkages. Naturally occurring dextrans usually contain (1→3) branch points and sometimes (1→2) or (1→4) branch points.[2]

Some dextran forming microorganisms produce several distinctly different α-glucans. For example, *Streptococcus cricetus* strain AHT produces three different types of α-glucans, *viz.*, two water soluble, highly branched dextrans, one type with (1→3) linkages only at branch points, and the other with some linear (1→3) linkages, and a water insoluble, relatively linear, predominately (1→3) linked α-glucan.[3]

The degree of branching of dextrans depends on the microbial source and varies widely among species. For example, while the dextran produced by *Betacoccus arabinosaceous* has a unit chain length of only six or seven α-(1→6) linked glucosyl residues and is highly branched, the dextran produced by *Leuconostoc mesenteroides* B-512F may have a unit chain length of greater than 10,000 residues with less than 5% branching.[2] The degree of branching also varies among strains within species. For example, the water soluble, high molecular weight dextran (> 10,000 residue unit chain length) from *Leuconostoc mesenteroides* NRRC B-512F (ATCC 10830a) consists of 95% (1→6) linked α-D-glucopyranosyl residues with 5% α-(1→3) linked D-glucosyl or isomaltosyl side chains. However, *L. mesenteroides* NRRC 523 (ATCC 14935) predominately produces a lower molecular weight, water insoluble dextran which consists of only 66% (1→6) linkages with 24% (1→3) and 10% (1→4) branch linkages.[4] It may also be infered from the data of Wilham, *et al.*[5] that in a single bacterial strain the degree of branching of the polydisperse dextrans may vary across the molecular weight range, with higher probability of branching in the lower molecular weight fractions.

Infections of sugar cane and cane milling or sugar processing streams with dextran forming bacteria can cause loss of sucrose to dextran, with concurrent increase in viscosity that leads to reduced recovery of sucrose and reduced factory efficiency. Furthermore, dextrans and α-glucan oligosaccharides (the products of dextran hydrolysis) are dextrorotary and their presence in raw sugar or cane juice at the point of sale increases the polarimetric determination of sucrose content.

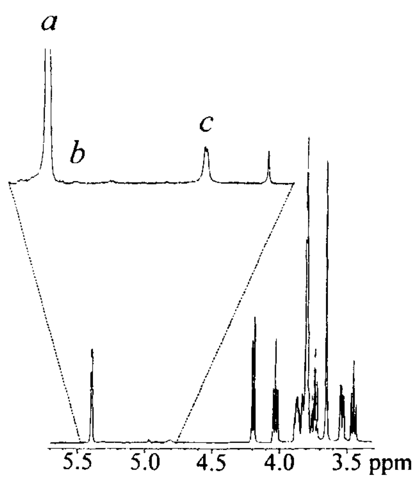
Review of methods. Currently, two methods for the analysis of dextran in raw sugar are employed in the sugar industry. The more commonly used haze assay[6] involves enzymic removal of high molecular weight starch, ion-exchange removal of inorganic salts, precipitation of proteins with trichloroacetic acid and measurement of turbidity of a 50% aqueous ethanol solution of the sugar. The haze assay is not sensitive at low dextran concentrations and is specific for high molecular weight, relatively linear dextran (*i.e.*, dextran that precipitates in 50% aqueous ethanol). The haze assay was recently accepted by the

International Commission for Uniform Methods of Sugar Analysis (ICUMSA) for the measurement of dextran in raw sugars. The second method is an official method of the AOAC,[7] and is commonly referred to as the Roberts' copper method. The AOAC method involves quantitative precipitation of total polysaccharides in raw sugars in 80% aqueous ethanol. The precipitate is redissolved and selective precipitation of dextrans in alkaline copper solution is followed by colorimetric determination of sugars with the phenol-sulfuric acid reagent. While the haze assay is selective for high molecular weight dextran, the AOAC method appears to determine a wide molecular weight range. Hence, the AOAC method results are usually significantly higher than those of the haze assay. In addition to the total dextran, the copper precipitate of the AOAC method may contain 1 to 4% non-dextran polysaccharides, as does the alcohol precipitate in the haze assay.

Enzymic methods for the analysis of dextrans in sugar have been developed since 1974,[8] but as routine analyses these methods are technically difficult and time consuming. Recently, Galea, *et al.*[9-11] have reported the development of an enzyme (dextranase)-HPLC analysis for dextran in raw sugars; their method requires a minimum of two days per batch of samples, but is proposed as a reference method (rather than a routine analysis) by which currently favored methods (*viz.*, AOAC and haze methods) could be compared.[11]

The enzyme-HPLC method involves quantitative precipitation of total polysaccharides in 80% aqueous ethanol, digestion of the precipitate with dextranase from *Chaetomium gracile*, and HPLC analysis of the isomaltose product of dextranase hydrolysis. There is no doubt that the enzyme is specific for dextran; dextranase from *C. gracile* catalyses the hydrolysis of α-(1→6)-D-Glc*p* glycosidic linkages, and produces predominately isomaltose from linear dextrans.[12] However, the calculation of dextran concentration in the raw sugar is based on a dextran to isomaltose conversion factor. This conversion factor is determined by the action of the dextranase on Pharmacia T-series dextrans (Pharmacia Fine Chemicals) and cane dextran purified by precipitation in 50% aqueous ethanol. These dextran standards are comparatively linear with a narrow molecular weight ranges. Hence, the method fails to take into account the polydispersivity and heterogeneity of naturally occurring dextrans (*i.e.*, the method underestimates dextrans with higher branching frequencies). Consequently, the enzyme-HPLC analysis of dextran in raw sugars cannot be called a reference method.

This paper reports our attempts to develop a reference method for the analysis of dextran in raw sugars that is based on a physical measurement of dextran (using ^{1}H NMR) with little wet chemical preparation of the raw sugar sample.



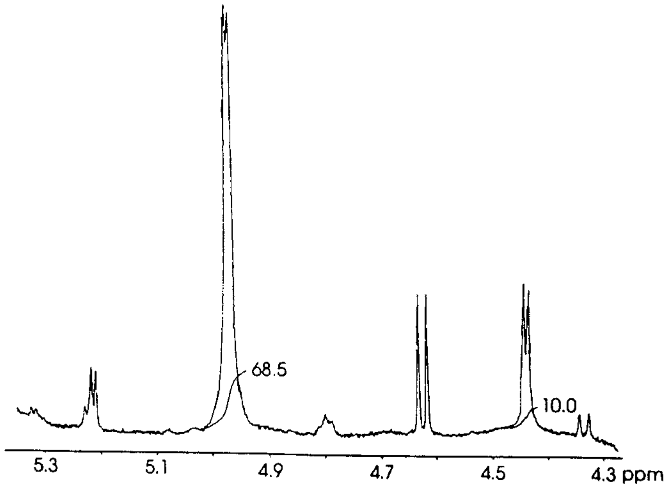
a - 5.40 ppm, d, $J = 3.64$ Hz (sucrose, C1-H of glucose moiety)
b - 5.33 ppm, d, $J = 3.92$ Hz (dextran, C1-H of α-(1→3) linked D-Glc*p*)
c - 4.98 ppm, d, $J = 3.41$ Hz (dextran, C1-H of α-(1→6) linked D-Glc*p*)

Fig. 1. ^{1}H NMR spectrum of a raw sugar sample in D_2O at 500 Mhz.

RESULTS AND DISCUSSION

Figure 1 shows an ^{1}H NMR spectrum of a raw sugar sample (designated as sample 1 below). The dominant features of this spectrum are the proton signals of sucrose, but other signals from the minor components of raw sugar are also present. The ^{1}H NMR spectrum of sucrose has been fully assigned.[13] For the purpose of this study we are interested in the signals from *ca.* 4.4 to 5.7 ppm (*i.e.*, anomeric proton region). The region of the spectrum expanded in Figure 1 shows two doublet signals, 4.98 ppm, $J = 3.41$ Hz and 5.33 ppm, $J = 3.92$ Hz that can be assigned to the anomeric protons of dextran (based on ^{1}H NMR spectra of pure dextrans and dextran spiking of raw sugars); the δ 4.98 ppm signal is from C1-H of α-(1→6) linked D-Glc*p* of dextran while the δ 5.33 ppm signal is from C1-H of α-(1→3) linked D-Glc*p* of dextran.

Direct integration of the small dextran peaks in the ^{1}H NMR spectrum of this raw sugar is not possible. However, after concentration of the medium and high molecular weight components (> 10,000 Da) by membrane filtration the dextran peaks in the ^{1}H NMR of this concentrate can be reliably integrated.



4.46 ppm, d, J =8. 30 Hz (stractan, C1-H of β-(1→3) linked D-Gal*p*)
4.98 ppm, d, J = 3.41 Hz (dextran, C1-H of α-(1→6) linked D-Glc*p*)

Fig. 2. **The expanded ^{1}H NMR spectrum of the high molecular weight fraction of a dextran/stractan spiked raw sugar sample in D_2O at 500 Mhz.**

The quantitative dextran determination involves the use of a polysaccharide internal standard so that dextran concentration in the high molecular weight fraction can be related to dextran concentration in the raw sugar. Stractan (Champion International Corp.) was chosen as an internal standard since it has a molecular weight >10,000 Da, it is water soluble and has no ^{1}H NMR signals that overlap in the anomeric proton region with either dextran or sucrose. Stractan, a polysaccharide from western larch (*Larix occidentalis*), is composed of D-galactose and L-arabinose in a ratio of 6:1. The β-(1→3)-D-Gal*p* backbone of stractan has side chains of *ca.* two aldose units (containing mostly β-(1→3)-L-Ara*f* and β-(1→6)-D-Gal*p* and some β-(1→6)-L-Ara*f*; the disaccharide side chains are (1→6) linked to the β-(1→3)-D-Gal*p* backbone). The ^{1}H NMR spectrum of stractan shows a major signal in the anomeric proton region (4.46 ppm, d, J= 8.3 Hz) due to the C1-H of β-(1→3) linked D-gal*p*; other minor peaks do not overlap with the dextran anomeric proton signals.

Four raw sugar sample were dissolved in aqueous solutions of dextran and stractan; the resulting solutions contained 2000 ppm stractan (based on raw sugar), and either 1000 or 2000 or 4000 ppm added dextran (based on raw sugar). After concentration by membrane

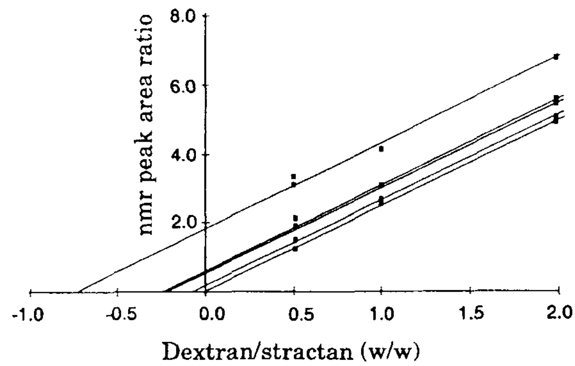


Fig. 3. NMR peak area ratios versus added dextran/stractan weight ratios in spiked raw sugars.

filtration and exchange in D_2O, the ^1H NMR spectra of the high molecular weight fractions were obtained at 500 Mhz. Figure 2 is an example spectrum that is expanded to show the anomeric proton signals of interest.

The peak area ratios of the anomeric proton signals of dextran (4.98 ppm) and stractan (4.46 ppm) when plotted against the weight ratios of added dextran/stractan for the four samples are a series of parallel lines (see Figure 3). The integration results obtained from the stock solutions of dextran and stractan without raw sugar are shown in Figure 3 by a fifth parallel line that intersects with the origin; this base line represents the data that would be obtained from a raw sugar with no dextran. The four lines from dextran/stractan spiked raw sugar samples are offset from the base line by the initial amounts of dextran in the raw sugars. The dextran concentrations in the raw sugars can be calculated by extrapolation of each raw sugar sample line to the weight ratio axis; the negative values represent the amount of dextran initially present in the raw sugar.

The results of the determination of dextran concentration in the four raw sugars by our ^1H NMR method are compared to the results of the two favored wet chemical methods in Table 1. It would appear from this preliminary comparison of methods that, at least in these four samples, the haze method underestimates dextran content in raw sugars, and that the AOAC method is in good agreement with the ^1H NMR method.

Obviously, the 500 Mhz NMR instrument, operator, and maintenance costs mitigate against routine use of this method in the sugar industry. We do not suggest that this ^1H NMR

Table 1. Comparative study of methods for determination of dextran in raw sugars.

Sample number	AOAC method (ppm)	Haze method (ppm)	^{1}H NMR method (ppm)
1	1551	1058	1568
2	557	218	594
3	430	173	483
4	156	83	136

method should replace existing methods for the determination of dextran in raw sugars. It has been developed as a reference method so that the other commonly used analytical methods can be compared in this paper and in a more comprehensive study that is still in progress.

EXPERIMENTAL

General methods. The raw sugars were from the library of sugars at the Sugar Processing Research Institute and are representative of the range of dextran concentrations found in raw sugars. The stractan grade 2 (Champion International Corp.) was a gift from S. Vercellotti (V-Labs, Inc, Covington, LA). Pharmacia T2000 dextran was used as a standard in all spiking experiments. All other chemicals were analytical grade.

Raw sugar dextran analysis by ^{1}H NMR. Raw sugars (2.0 g) were each dissolved in stock aqueous solutions of stractan (0.08 gL^{-1})/dextran (0.08, 0.16 and 0.32 gL^{-1}) mixtures so that the final volume was 50 mL. The high molecular weight fractions of an aliquot (20 mL) of these raw sugar solutions were prepared by membrane filtration (Centriprep 10 Concentrator, Amicon; 10,000 Da cut off): centrifugation in the Centriprep 10 reduces the 20 mL of raw sugar solution to a final volume 2 mL while retaining the high molecular weight material. The high molecular weight fractions were concentrated to dryness and pre-exchanged with D_2O four times. The ^{1}H NMR spectra were recorded at 500 Mhz using a GE Omega series spectrometer. Sixty-four acquisitions procuced spectra of suitable quality for integration (good signal to noise ratio).

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