

A DEXTRAN TEST FOR VHP SUGARS

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Abstract

The monoclonal antibody (MCA) SucroTest is a commercially available kit (Midland Research Laboratories Inc) for the specific analysis of dextran in juices and sugar. The method is simple and quick and is based on the selective and specific reaction between the antibody and its antigen (in this case dextran). The resultant insoluble complex causes turbidity which is measured after a set time using a nephelometer and single point calibration against a commercial high molecular weight dextran standard.

Very high pol (VHP) sugars from the South African Sugar Terminal (SAST) were analysed using the MCA-SucroTest. Repeatability was slightly worse than that of the haze method. The correlation between the MCA and haze method was good. Although the MCA results were, on average, 20% higher than the haze data, the ease and speed of the analysis lends itself to the application of a screening and rejection protocol for VHP sugars.

Keywords: dextran, analysis, nephelometry, immunology, antibody

Background

The immunological assay for dextran is based on the ability of an antibody to react specifically with dextran. The dextran-antibody complex forms a detectable turbidity which can be measured in a turbidity meter (nephelometer). It is important that the antibody is in excess to prevent redissolution of the dextran-antibody complex. As a result of the specificity of this immunological assay it is usually possible to measure dextran directly without prior separation from the large amount of sucrose in the sample matrix.

A drawback to wider application of immunological techniques to the analysis of dextran has been the inconsistent supply of the antibody reagent (in particular monoclonal sera). This was one of the problem areas addressed by Day and co-workers at Louisiana State University (LSU) (Day and Plhak, 1998). In collaboration with Midland Research Laboratories they undertook a programme to develop a simple, readily available, portable and reliable assay system for determining dextran in all sugar streams. Some of the teething problems encountered during commercialisation of the procedure have been described by Day *et al* (2001).

A kit incorporating recent modifications to the instrument (e.g. extended NTU range, monoclonal antibody) was loaned to the SMRI by Aqua Centre (South Africa). The kit consists of a hand-held nephelometer, turbidity and dextran standards and includes antibody reagent. The nephelometer range is 0 to 200 Nephelometric units (NTU) covering a dextran range of 0 to 500 mg/L. The latest version of the analytical procedure [Version 4.3 (3/07/03, Anon, 2003)] was used to evaluate the usefulness of the unit for analysing dextran in very high pol (VHP) sugar.

Experimental

The analytical procedure supplied with the MCA dextran kit was followed (Anon, 2003). VHP sugar samples produced during the 2004/2005 season and haze dextran (Anon, 1994) results were supplied by the South African Sugar Terminal (SAST).

Results and discussion

The experimental repeatability of the method was determined by analysing eight VHP sugar samples on two different days under similar conditions. The haze dextran range of the sugars was 34 to 534 mg/kg (Table 1).

Table 1. Repeatability of VHP sugars using the MCA dextran procedure.

Sample	Haze dextran (mg/kg)	MCA dextran (mg/kg)		Rsd* (%)	Horwitz ratio
		1st value	2nd value		
1	34	81	117	25.8	4.5
2	59	128	153	12.7	2.4
3	60	191	203	4.4	0.9
4	126	263	281	4.7	1.0
5	268	381	407	4.6	1.0
6	261	408	430	3.6	0.8
7	480	543	625	10.0	2.3
8	534	871	825	3.8	0.9

*rsd = relative standard deviation

When evaluating method uncertainty ICUMSA recommends the use of the Horwitz formula which takes into account the close correlation between the relative standard deviation (rsd) and the concentration of the analyte (Godshall, 2002). The calculated Horwitz ratio compares the experimentally derived relative standard deviation (rsd) to the theoretical rsd and should generally not exceed a value of 2. The repeatability of the MCA method was found to be acceptable in the range of about 140 to 850 mg/kg (corresponding to a haze dextran range of 60 to 530 mg/kg) with Horwitz ratios ranging from 0.8 to 2.4. An F-test confirmed that the values showed no significant difference at a 10% confidence level ($P=0.10$). The absolute difference between two readings applicable to the range 140 to 850 mg/kg (MCA dextran) is 54 mg/kg.

Fifteen VHP sugar samples with known haze dextran values were prepared and analysed for MCA dextran. The MCA values were plotted against the haze dextran values (Figure 1) and correlation data obtained through linear regression ($MCA=1.2 \times \text{Haze} + 50$, $r=0.99$, $n=15$). The correlations compared favourably with previously published data (Curtin and McCowage, 1986; Sarkar *et al.*, 1991; Day *et al.*, 2001; Saska *et al.*, 2002).

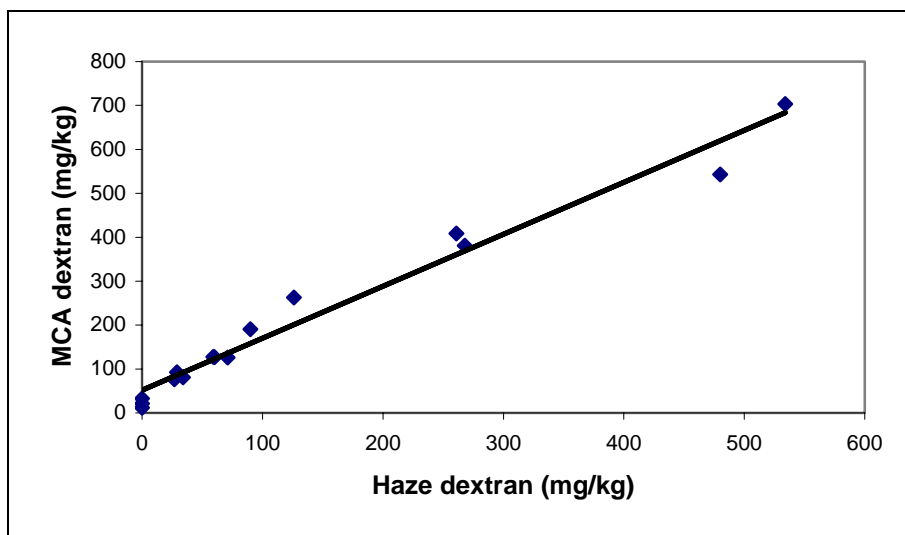


Figure 1. Haze and MCA dextran of VHP sugars.

Other considerations

This study was limited to VHP sugars and a brief look at some refined sugars (Morel du Boil and Schoonees, 2005). Godshall *et al.* (2004) compared the MCA, Roberts and haze procedures for white sugar analysis. In principle, the procedure should be applicable to all factory products with no sample preparation other than dilution. However, lower molecular weight (LMW) dextrans (40 000 to 100 000 daltons) will be included in the MCA method (Curtin and McCowage, 1986) and so lower purity products containing proportionately more LMW dextran, will give poorer agreement than the haze method.

A US sugar refinery claims to have reduced analytical costs by carrying out haze dextran analyses only on incoming sugars that produced high dextran results with the antibody test (Day *et al.*, 2001). Data from the monitoring of raw sugar factories (mixed juice, clear juice, syrup, A-massequite, sugar) as well as from juice deterioration trials have been published (Day *et al.*, 2002; Rauh *et al.*, 2003). However, the ease with which samples can be membrane-filtered (at 0.45 μm) may be a limiting factor, necessitating excessive dilution. The instrument tolerance of ± 0.1 NTU under the assay conditions corresponds to about 1 mg/l in solution, whilst the experimental repeatability translates to 1.5 NTU. Provided that, after dilution, turbidity readings are of the order of 2 NTU, acceptable results should be possible. By way of example, a juice sample diluted to 5°Bx and giving a turbidity reading of 2 NTU would contain 400 mg dextran/kg Bx.

One vial of freeze-dried antibody is sufficient for six tests including one standardisation test per vial. The test kit contains 20 vials which is sufficient to analyse 100 samples.

The MCA dextran test procedure is straightforward and precise results should be obtainable by a person with experience in basic analytical techniques. One set of samples (six tests) can be analysed in under two hours including the preparation of samples, standard and buffer solutions and the antibody reagent.

Conclusions

The antibody method was found to be repeatable using a standard ICUMSA evaluation procedure (Horwitz ratio) normally used for inter-laboratory comparisons. The method repeatability, i.e. the absolute difference between two values for the same sample, is 54 mg/kg for VHP sugars in the range 140 to 850 mg/kg. This is only slightly worse than the ICUMSA haze repeatability of 40 mg/kg.

The correlation between haze dextran and MCA dextran for VHP sugars is good ($r=0.99$) and compares favourably with recent data from the USA. In general MCA values are higher than Haze values by about 20%.

The MCA analysis for dextran provides an alternative measurement for dextran on VHP sugar. Results are available sooner and the procedure is simpler than the haze procedure and so the technique could be used, for example, to screen incoming sugar. Although the analytical cost is higher than the haze procedure, its ease and speed lends itself to quality monitoring systems.

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