THE QUEST TO ELIMINATE THE USE OF LEAD IN THE LABORATORY: PART 2

By A. DUNSMORE
Sugar Milling Research Institute, Durban

Abstract

In continuing the search to eliminate the use of toxic reagents in the laboratories of the South African sugar industry, the results of evaluating a high wavelength polarimeter which operates at a wavelength of 882 nanometers and is thus able to read darkly coloured solutions are presented. No clarification agent is required. The solutions, although dark in colour, must not contain any turbidity so it is essential to have fast, effective filtration. With juices and the extract from the direct analysis of cane there is good agreement between the pol values using this instrument and those obtained using lead clarification and a conventional polarimeter. With more concentrated factory products the agreement is poorer probably because the high colour of these products often dictates that if they are not clarified then they must be diluted more than usual to enable adequate light transmission.

Introduction

This report contains the results of evaluating a high wavelength polarimeter, which has a light source at a wavelength in the near infrared portion of the light spectrum, in an attempt to eliminate the use of toxic reagents in the laboratories of the South African sugar industry. The light source of the Polartronic NIR has a wavelength of 882,6 nm compared with the 589,44 nm of the Polartronic Universal and the object of operating at this high wavelength is to facilitate the reading of darkly coloured solutions of sugar products without having to clarify them first with toxic reagents such as basic lead acetate powder.

Trials at the SMRI

Linearity

The Australian Standard K-157 of 1968 states that "the deviation from the true value should not be more than 0,03°Z over the range zero to 120°Z".

Five colourless sugar solution replicates at each concentration of ca. 20, 40, 60, 80, 100 and 120°Z were prepared from a first boiling refined sugar and distilled water and two more sets of five solutions each were prepared using the same first boiling refined sugar dissolved in distilled water to which Carameline dye had been added. One of these sets was amber in colour and similar to the colour of the filtrate of a 1:10 solution of final molasses which had been clarified with basic lead acetate powder, while the second set was dark brown and resembled the colour of a 1:25 solution of final molasses which had been filtered but not clarified with lead. The true values of these solutions were calculated from the mass, corrected for Karl Fischer moisture, and the volume of the volumetric flask. All of these solutions were read five times on the instrument and the averages of the five readings obtained were compared with the calculated values. The results of all three sets are presented in Table 1.

With the exception of the 120°Z point, the differences of the colourless solutions, although biased, are within the Australian specification. The differences in the amber solutions are slightly less than the colourless ones but a bias is still evident. The differences between the readings obtained and calculated for the solutions between 20 and 80°Z of the dark solutions also show bias but are slightly lower than those of the amber solutions. There is, however, a larger variation from the true value for the 100 and 120°Z solutions. With the exception of the 120°Z samples of the colourless solutions and the 100 and 120°Z samples of the dark solutions, the instrument complies with the specification although there is a consistent bias in that the readings are lower than the calculated values. In addition, the differences on the whole become smaller as the colour increases.

Table 1

Polarimeter readings, found and calculated, of colourless and dyed pure sucrose solutions

	Average value found (°Z)	Average value calculated (°Z)	Found–calculated (°Z)
Colourless			
20°Z	20,195	20,216	-0,021
40°Z	40,446	40,473	-0,027
60°Z	60,135	60,159	-0,024
80°Z	80,187	80,200	-0,012
100°Z	100,444	100,440	0,004
120°Z	119,929	119,963	-0,034
Amber			
20°Z	20,376	20,393	-0,017
40°Z	40,798	40,814	0,016
60°Z	60,060	60,067	-0,007
80°Z	80,310	80,324	-0,014
100°Z	100,190	100,204	-0,014
120°Z	120,668	120,697	-0,029
Dark			
20°Z	20,284	20,292	-0,012
40°Z	40,178	40,186	-0,008
60°Z	60,230	60,238	-0,008
80°Z	80,536	80,659	-0,012
100°Z	100,584	100,629	-0,033
120°Z	120,810	120,863	-0,053

Mixed juice

Weekly samples of mixed juice from all factories were obtained from the SASA Cane Testing Service (CTS) GC laboratory at Mount Edgecombe where pol, brix and sugars by gas chromatography are determined routinely. The brix values of these juices were determined in the usual way by filtering them through Whatman No. 6 filter papers with Celite 577 filter aid. The remaining portions of the filtrates were used to determine the saccharimeter reading on the Polartronic NIR. At the same time portions of the juices were clarified with basic lead acetate powder and filtered through Whatman No. 91 filter papers and these filtrates were read on both the Polartronic Universal and NIR instruments. The brix and pol results were compared with those found originally by the CTS staff and the agreement was found to be very good. Filtrates from clarification with basic lead acetate powder were also read on the NIR at the

SMRI. All the pol results are listed in Table 2 where column A contains the original results found by the CTS, B has the results of the juices after lead clarification and C lists the results obtained after filtration only.

Table 2

Comparative pol results on mixed juice

Factory	Polarisation °Z				
	CTS	NIR clarified	NIR filtered	Differences	
	A	B	C	C - A	C - B
ML	10,12	10,15	10,08	-0,04	-0,07
PG	10,35	10,36	10,32	-0,03	-0,04
UF	10,62	10,71	10,67	0,05	-0,04
FX	10,52	10,57	10,53	0,01	-0,04
AK	10,78	10,80	10,78	0,00	-0,02
DL	11,35	11,42	11,34	-0,01	-0,08
MS	11,17	11,19	11,18	0,01	-0,01
ME	12,68	12,72	12,69	0,01	-0,03
GD	11,24	11,25	11,22	-0,02	-0,03
GH	10,95	11,02	10,94	-0,01	-0,08
NB	13,32	13,38	13,33	0,01	-0,05
UC	14,22	14,25	14,21	-0,01	-0,04
IL	11,52	11,58	11,55	0,03	-0,03
SZ	11,12	11,16	11,10	-0,02	-0,06

The filtration rates using Whatman No. 6 paper were very slow, the samples from some factories taking a few hours to collect sufficient filtrate with which to measure the brix and the polarimeter reading with the flow through cell. It was therefore decided to repeat the exercise using a medium fast filter paper, Whatman No. 540, and a fast one, Whatman No. 91, together with Celite 577 filter aid. The Whatman No. 540 paper produced filtrates which were not absolutely clear and the readings were sometimes unsteady. The juices with high suspended solids still took a long time to filter although the resulting filtrates were much clearer than the faster filtering juices. The Whatman No. 91 paper which is used routinely in the determination of pol filtered quickly but the filtrates were cloudy and impossible to read. Readings were, however, obtainable on the two or three samples which, because of high suspended solids, filtered slowly but gave clear filtrates.

Final molasses

Samples of final molasses are diluted on a 1:5 mass/mass basis and then filtered through a Whatman No. 6 filter paper with 2 g of Celite 577 filter aid for the routine determination of brix. This 1:5 solution is then further diluted volume/volume to give a 1:10 solution which is clarified with basic lead acetate powder for the determination of pol.

A sample of final molasses was diluted 1:5 and then subdivided into two portions. The first portion was filtered through a Whatman No. 6 filter paper with Celite 577 and then diluted to give solutions of 1:10, 1:20 and 1:40 while the second portion was diluted first and then filtered with the same type of paper and Celite. All of these solutions were read on the Polartronic NIR and the results, together with the original pol obtained using basic lead acetate, are in Table 3. This table also shows the results obtained by calculating the final pol by different methods. In method A the pol values of the dilute solutions were obtained from Schmitz table and then multiplied by the dilution factor. In method B the readings were multiplied by the dilution factor to bring them to the original 1:5 dilution on which the brix is read. The pol from Schmitz table was found and the result finally multiplied by five. The latter is the method by which calculations are usually done and duplicate pol results should agree within 0,2°Z (Mellet *et al.*, 1982). The solutions which were diluted and then filtered were not as clear as those which were filtered first and then diluted. The NIR readings on the 1:10 dilutions were not steady.

Table 3

Comparative pol °Z values of final molasses using different dilutions

Dilution	Filtered then diluted		Diluted then filtered	
	Calculation A *	Calculation B **	Calculation A	Calculation B
1:10	22,81	22,13	22,89	22,21
1:20	23,25	22,21	23,96	22,89
1:40	23,59	22,35	24,21	22,94
Pol obtained using basic lead acetate = 25,1				

* Both refractometer and polarimeter readings were used to find the pol from Schmitz table. This pol was then multiplied by the dilution factor.

** The polarimeter readings were multiplied by the dilution factor to bring them to the equivalent of a 1:5 dilution. The pol was taken from Schmitz table and that figure was multiplied by 5.

It was decided to analyse weekly molasses samples by preparing 1:5 dilutions and sub-sampling into two portions. One portion was centrifuged at high speed and the other was filtered through a Whatman No. 6 paper with Celite 577. The treated sub-samples were then diluted further to 1:25 and the results were compared with the GC sucrose and the pol determined by clarifying with basic lead acetate powder. The results are in Table 4.

Centrifuging the 1:5 diluted molasses was much less time consuming than waiting several hours for sufficient sample to filter and all of the relevant comparisons except four are within the ± 0,2°Z tolerance allowed for duplicate pol results.

Trials by the Cane Testing Service

Illovo laboratory

The instrument was installed in the CTS laboratory at IL where several tests were conducted on the extract from the Direct Analysis of Cane (DAC) and mixed juice. Since preliminary tests at the SMRI showed the instrument to be very sensitive to turbidity and slow to filter mixed juice samples, trials were done using varying amounts of Celite 577 with Whatman Nos. 91 and 540 filter papers. The outcome of this was that 10 g of filter aid gave filtrates which were clear enough to give steady readings on the instrument. The results for mixed juice and DAC extract are listed in Table 5.

The filtrates from both the mixed juice and DAC extract were clearer when using the Whatman No. 540 filter papers than with the No. 91 papers. When using the Whatman No. 540 paper, duplicate samples of 200 cm³ were filtered and the time taken to collect 100 cm³ of each filtrate was, on average, about five minutes faster than the conventional lead filtration.

These preliminary studies in the factory situation indicate that 10 g of Celite 577 added to 200 cm³ DAC extract or mixed juice and filtered through a Whatman No. 540 paper produces a filtrate of sufficient clarity to read on the Polartronic NIR. In most cases the results compared favourably with those performed in the standard manner using basic lead acetate.

GC laboratory at Mount Edgecombe

The filter papers used in this exercise were S&S (Schleischer and Schull) No. 287, MN (Macherey-Nagel) pre-coated and Whatman No. 540 together with 5 and 10 g of Celite 577 in 150 cm³ mixed juice. The S&S No. 287 paper proved to be the best filter medium in both speed of filtration and agreement with the lead pol read on the conventional Polartronic Universal. The results are presented in Table 6. In addition it was found that 5 g Celite/150 cm³ sample gave good results. The results with the Whatman No. 540 paper were disappointing as the LED on the instrument flashed with nearly all the samples which indicates that insufficient light is falling on the photomultiplier. This was probably due to dark/turbid filtrates. The differences between comparative instrument readings when converted to pol, however, are minimal.

Table 4

Comparative pol values on South African weekly and Simunye monthly final molasses samples

Factory	Sucrose by GC	1:10 Clarified with basic lead acetate powder	1:5 filtered		1:5 centrifuged
			1:25 calculation A*	1:25 calculation B*	1:25 calculation A
ML	30,1	27,60	24,32	23,14	23,94
PG	29,9	27,68	23,93	22,76	23,74
UF	33,1	32,57	29,78	28,30	29,91
FX	31,3	30,10	27,84	26,43	27,71
AK	31,0	29,71	26,50	25,15	26,36
DL	31,8	31,19	28,04	26,64	27,66
MS	32,5	31,40	28,43	27,00	28,23
ME	29,6	28,41	26,70	25,39	27,21
GD	33,0	31,67	28,57	27,16	28,56
GH	34,4	34,09	31,20	29,63	31,13
NB	29,0	26,83	26,27	25,06	26,34
UC	29,0	24,97	22,92	21,83	22,79
IL	31,9	31,49	31,33	29,79	31,91
SZ	30,4	28,89	27,93	26,56	27,99
SM	32,1	26,34	22,18	21,03	22,12

* The notes at the end of Table 3 also apply here.

Table 5

Comparative pol values for mixed juice and DAC extract using different filter papers at IL

Sample	Mixed juice			DAC extract		
	Lead pol °Z	NIR pol °Z		Lead pol °Z	NIR pol °Z	
	Whatman No. 91	Whatman No. 91	Whatman No. 540	Whatman No. 91	Whatman No. 91	Whatman No. 540
A	11,35	–	11,07	4,52	4,50	4,50
B	11,01	10,96	11,00	5,87	5,85	5,87
C	8,52	8,52	8,55	5,06	5,06	5,10
D	9,90	9,90	9,92	–	–	–
E	10,14	10,10	10,14	–	–	–

Table 6

Comparative instrument readings of mixed juice using various filter papers in the GC laboratory at ME

Sample	S&S 287		MN pre-coated		Whatman No. 540		Lead pol Whatman No. 91
	5 g per 150 cm³	10 g per 150 cm³	5 g per 150 cm³	10 g per 150 cm³	5 g per 150 cm³	10 g per 150 cm³	
1	–	39,35	–	39,35	–	–	39,48
2	–	44,79	–	44,76*	–	–	44,78
3	37,49	37,45*	37,47	37,50*	–	–	37,48
4	44,35	44,38	44,36	44,36	–	–	44,36
5	47,04	47,02	47,03	47,06	–	–	47,05
6	45,41	45,40	45,43	45,43	n/r*	45,57*	45,48
7	38,59	38,61*	38,57	39,61*	38,84*	38,61*	38,62
8	40,21	40,23	40,18	40,23	40,21	40,17*	40,22
9	36,37*	36,45	36,43*	36,92*	36,86*	n/r*	36,42
10	41,68	41,66	41,64	41,65	41,84	41,72*	41,73

* flashing LED
– no filtration done
n/r no reading obtainable

Trials at the South African Sugar Terminals

The instrument was transported to the South African Sugar Terminals (SAST) where various filter papers supplied by the SMRI were tested for clarity and rate of filtration. In addition, different types of sugars were used to compare the pol values determined in the usual manner with basic lead acetate solution and read on the Polartronic Universal polarimeter with filtered solutions read on the Polartronic NIR polarimeter. Since some of the sugars were very dark in colour, a few were analysed using a half normal solution (13 g sugar in 100 cm^3) as well as a normal (26 g in 100 cm^3) solution.

Varying amounts of Celite 577 (1, 2, 3, 4, 5, 6 and 7 g) were used with several types of filter paper, viz. MN 615, MN 616, MN 619, MN 713, MN 692/70 and the standard Whatman No. 91. Of these the MN 692/70 and the Whatman No. 91 filtered well taking about eight minutes to filter, but in spite of up to 7 g of filter aid being used, there was always a slight haze in the filtrate. In subsequent tests 3,5 g of Celite 577 and a Whatman No. 91 paper were used.

Triplicate analyses of different sugars were undertaken firstly using basic lead acetate solution read on the Polartronic Universal and secondly using normal as well as half normal solutions filtered and read on the Polartronic NIR. The values obtained from the NIR instrument of normal sugar solutions were lower than those of the half normal sugar solutions but the latter values agreed well with those from lead clarification. The differences in the results for the normal solution could be due to the slight haziness in the filtrates and the greater discrepancies found for the low pol sugars could be due to a combination of the haze and the darker colour of the solutions.

Two sets of ten replicates each of six sugars (normal solutions) were prepared. One set was clarified with basic lead acetate polution and read on the Polartronic Universal while the other was filtered and read on the Polartronic NIR. The mean differences and standard deviations are listed in Table 7 which shows that the replication (SD = ca. 0,015) was very good but the average mean difference of the lead - NIR (0,107) was not.

Table 7

Mean differences and standard deviations of the replicate analysis of various sugars

Sugar	Standard deviations		Means		
	Lead	NIR	Lead	NIR	Lead - NIR
VHP 1	0,011	0,025	99,537	99,413	0,124
VHP 2	0,016	0,016	99,520	99,417	0,103
LP 1	0,011	0,015	97,758	97,652	0,106
LP 2	0,016	0,021	97,806	97,690	0,116
Swaziland	0,017	0,013	98,720	98,637	0,083
Zimbabwe	0,018	0,009	98,669	98,560	0,109
Average	0,015	0,016	—	—	0,107

It is concluded that turbidity affects the pol value of sugars as found by NIR but this can be offset by using a half normal solution.

Conclusions

- The Polartronic NIR can cope with darkly coloured solutions but not with turbidity.
- Centrifugation at high speed is faster than filtration and gives comparable results.
- There is good agreement between lead and NIR pol values of mixed juice and DAC extract.
- Other factory products have to be diluted and the agreement is poorer.
- Final molasses samples have to be diluted 1:25 before they can be read and different methods of calculation give different results.
- As far as mixed juice is concerned, filtration through S&S 287 filter papers with 5 g Celite 577 appears to give the clearest filtrates.
- The agreement between the high wavelength polarimeter and the normal instrument for very high pol (VHP) raw sugar is within the analytical tolerance of ± 0,03°Z (Mellet *et al.*, 1982) but the difference becomes greater as the pol of the sugar becomes lower. This could be due to the presence of turbidity combined with the dark colour. A half normal solution gives better results on dark sugars.
- This instrument could be used in the CTS laboratories for the analysis of DAC extract and for mixed juice. As far as the mill laboratories are concerned, however, higher brix products will have to be diluted further in order to obtain stable readings, but the results from these dilute solutions will be different from those analysed in the normal manner. Furthermore, the filtrates from lead clarification can be read on the Polartronic NIR but the results will be biased and pol/sucrose ratios will be different.

Acknowledgements

The author wishes to thank the management and staff of the South African Sugar Association's Cane Testing Service in Durban for permission to use the results of their tests and IL, the GC laboratory at ME and the South African Sugar Terminals for their co-operation and analytical work. Also acknowledged are Schmidt and Haensch who, together with Labotec (SA), made the instrument available for the tests.

REFERENCES

Mellet, P, Lionnet, GRE, Kimmerling, ZJ and Bennett, PJ (1982). Standards for the analytical precision of sugar and molasses analyses. *Proc S Afr Sug Technol Ass* 56: 55-57.