

# SOME FACTORS AFFECTING THE LANE AND EYNON TITRATION METHOD FOR DETERMINING REDUCING SUGARS IN SUGAR PRODUCTS

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## Abstract

The effects of (a) method of standardisation of Fehling's solution, (b) rate of boiling and (c) method of removal of calcium from solutions prior to titration by the method of Lane and Eynon were investigated. It was established that the method of standardisation should be uniform and similar to the working conditions used, that the rate of boiling influences the titre and that either EDTA or potassium oxalate may be used to remove the interference of calcium but that EDTA is preferred. The quantity of EDTA to be used for various sugar products was also investigated and recommendations made.

## Introduction

The titration method of Lane and Eynon<sup>1</sup> is used extensively in the cane sugar industry to determine the reducing sugars content of a wide range of sugar products<sup>2, 3, 4, 5</sup>. The method is especially suited to solutions containing between 0,15 and 2,35% reducing sugars<sup>6</sup> and products such as juice, syrup, massecuites and molasses can easily be analysed. There are, however, certain aspects regarding this method which have a significant influence on the values obtained, viz standardisation of Fehling's solution, rate and temperature of boiling and the method of removal of calcium from the sugar solution prior to titration, etc.<sup>6, 7, 8, 9</sup>.

This paper investigates and quantifies some of these aspects and suggests ways of overcoming problems caused by them, not only in the determination of invert in molasses but also in other sugar factory products.

## Analytical Procedure

### (a) Influence of rate of boiling on titration.

In order to quantify the influence of the rate of boiling, various molasses solutions were analysed using both the constant volume<sup>7</sup> and the classical<sup>2</sup> methods. The results are given in Table 1 and show that the percentage reducing sugars found varies with the rate of boiling. This confirms earlier findings mentioned during the discussion of Subjects 7, 8 and 14 in the Proceedings of ICUMSA<sup>6, 11</sup>, that the Lane and Eynon method is strongly dependent upon the boiling conditions of the solution.

### (b) Standardisation of Fehling's solution.

In the South African sugar industry a "Fehling's Factor" is used for the correction of titres<sup>2</sup> i.e. instead of preparing Fehling's solutions so that 24,8 cm<sup>3</sup> of 0,5% standard invert solution reacts with 25,0 cm<sup>3</sup> of mixed Fehling's solution, a factor is calculated from the titre of 0,5% standard invert obtained, e.g. titre of 0,5% standard invert obtained = 24,60 cm<sup>3</sup>

$$\begin{aligned} &= \frac{24,8}{24,60} \\ \text{then, factor} &= \frac{24,60}{24,8} \\ &= 1,010. \end{aligned}$$

TABLE 1

Influence of rate of boiling on the percentage of reducing sugars

Sample	Constant Volume Method			
	Time taken to reach boiling point			
	1 min	1,5 min	2 min	2,5 min
1	13,53	13,44	13,33	13,27
2	16,64	16,50	16,42	16,26
3	17,48	17,38	17,16	16,97

Sample	Classical Method			
	Time taken to reach boiling point			
	1 min	1,5 min	2 min	2,5 min
4	17,31	17,13	16,81	16,55
5	18,97	18,78	18,39	18,04
6	14,13	14,03	13,83	13,66

When in sample analysis a titre of say 22,75 cm<sup>3</sup> is found, the corrected titre will be 22,75 x 1,010 = 22,98 cm<sup>3</sup>, which is then used to obtain mg RS/100 cm<sup>3</sup> from the Lane and Eynon table. Using the same Fehling's solution in different modifications of the Lane and Eynon titration<sup>1</sup> it has been established that for each specific method a separate standardisation is necessary (see Table 2). It is best, therefore, to dispense with "factors" and adjust the Fehling's solution to the exact concentration for the method employed. Furthermore, it is necessary to standardise the method of preparing the mixed Fehling's solution because 10 cm<sup>3</sup> A + 10 cm<sup>3</sup> B and 20 cm<sup>3</sup> of mixed Fehling's use different volumes of standard invert solution. Some laboratories, e.g. South African<sup>2</sup>, use 25 cm<sup>3</sup> mixed Fehling's and 0,5% standard invert for standardisation when in fact their working method for analysis uses only 10 cm<sup>3</sup> of mixed Fehling's and a sample concentration closer to 0,25% invert.

TABLE 2

Standardisation of Fehling's Solution

Method of Standardisation	Volume Fehlings (cm <sup>3</sup> )	Titre against 0,5% inv. (cm <sup>3</sup> )	Titre against 0,25% inv. (cm <sup>3</sup> )	Factor
Const. volume	25 mixed	24,60	—	1,010
Const. volume	10 mixed	10,70	—	0,929
Const. volume	5 + 5	10,72	—	0,928
Classical	25 mixed	24,37	—	1,020
Const. volume	20 mixed	—	39,85	1,003
Const. volume	10 + 10	—	40,00	1,000
Const. volume	10 mixed	—	21,35	0,936
Const. volume	5 + 5	—	21,49	0,931
Classical	10 + 10	—	39,65	1,009

(c) *Potassium oxalate and EDTA as decalcifying agents.*

Calcium must be removed from the titrating solution prior to the determination of reducing sugars by the Lane and Eynon method<sup>6, 7</sup> due to the reaction of calcium with reducing sugars, and while potassium oxalate has been used in the past, the time-consuming filtration of calcium oxalate has been replaced by the addition of EDTA which forms a complex with the calcium<sup>8</sup>. In order to test these two methods, various cane final molasses were analysed using both oxalate and EDTA applying them in both the classical<sup>2</sup> and constant volume methods<sup>7</sup>. Since molasses is unhomogeneous and therefore difficult to sub-sample into continually representative samples, one stock solution was prepared and four aliquots taken to give the dilution concentration required for the determination. This eliminated sampling errors and provided a common base line from which the comparisons could more easily be made. Two of these portions were treated with potassium oxalate and two with EDTA and, of these pairs, one was used for each of the classical<sup>2</sup> and constant volume<sup>7</sup> methods of titration.

Procedure of analysis for the constant volume method was based on the UMTC procedure<sup>7</sup> using 0,25 g of potassium oxalate/1 g of molasses, 4 cm<sup>3</sup> of 4% EDTA/1 g of molasses and 10 cm<sup>3</sup> Fehling's A plus 10 cm<sup>3</sup> Fehling's B in all titrations. Procedure for the titration of the classical method<sup>2</sup> was performed as described in the Laboratory Manual for South African Sugar Factories<sup>2</sup>.

Analytical procedures for preparation and analysis of samples were as follows :

A sample of 25,00 g of molasses was quantitatively transferred to a 200 cm<sup>3</sup> volumetric flask with distilled water, dissolved, made to the mark and shaken thoroughly. Two aliquots (50 cm<sup>3</sup>) of this stock solution

were pipetted into two 500 cm<sup>3</sup> volumetric flasks (1 and 2) and two aliquots of 20 cm<sup>3</sup> into two further 500 cm<sup>3</sup> volumetric flasks (3 and 4). Flask (1) was made to the mark with water and transferred to a 600 cm<sup>3</sup> Erlenmeyer flask. The stoppered flask was heated to 40°C on a water bath and 1,56 g potassium oxalate (equivalent to 0,25 g potassium oxalate/1 g molasses) added. The flask was shaken vigorously and left to cool to room temperature whereupon the solution was filtered through Whatman No. 1 filter paper using 4 g acid washed Kieselguhr and rejecting the first 40 cm<sup>3</sup> of filtrate. The remainder of the filtrate was used for reducing sugars determination against 10 cm<sup>3</sup> Fehling's A plus 10 cm<sup>3</sup> Fehling's B solution. Flask (2) was made to the mark after addition of 25 cm<sup>3</sup> of 4% EDTA (equivalent to 4 cm<sup>3</sup> of 4% EDTA/1 g molasses) and used for reducing sugars determination against 10 cm<sup>3</sup> Fehling's A plus 10 cm<sup>3</sup> Fehling's B solution.

Flasks (3) and (4) were used for inversion by adding 200 cm<sup>3</sup> of distilled water, heating to exactly 60°C in a water bath set at 66°C and adding 20 cm<sup>3</sup> of Jackson and Gillis<sup>10</sup> hydrochloric acid, leaving in the water bath for 2 minutes and setting aside at room temperature for 30 minutes. After neutralisation with 4 M sodium hydroxide solution (using phenolphthalein as indicator), flask (3) was made to the mark with distilled water, treated with 0,62 g potassium oxalate (equivalent to 0,25 g potassium oxalate/1 g molasses) as described for flask (1), while 10 cm<sup>3</sup> of 4% EDTA (equivalent to 4 cm<sup>3</sup> of 4% EDTA/1 g molasses) were added to flask (4) and made to the mark. These solutions were used for the determination of total invert against 10 cm<sup>3</sup> of Fehling's A plus 10 cm<sup>3</sup> of Fehling's B solution. The results of these analyses are given in Table 3 and the statistical analyses of the results are given in Table 4. Columns (g) - (j) and (a) - (j) appear to be on the

TABLE 3  
Comparison between the Classical and Constant Volume Method using both Oxalate and EDTA for sequestering calcium

Sample	Classical Method						Constant Volume Method					
	Potassium Oxalate			EDTA			Potassium Oxalate			EDTA		
	a	b	c	d	e	f	g	h	i	j	k	l
% RS	% Total Inv.	% Sucrose	% RS	% Total Inv.	% Sucrose	% RS	% Total Inv.	% Sucrose	% RS	% Total Inv.	% Sucrose	
1	16,87	49,48	30,98	16,76	50,28	31,84	16,97	49,50	30,90	16,81	50,53	31,86
	16,98	50,10	31,46	16,82	50,43	31,93	17,02	50,06	31,39	16,69	50,19	31,83
2	19,92	53,75	32,14	19,87	54,19	32,60	19,86	53,76	32,20	19,72	54,17	32,73
	19,88	54,16	32,57	19,97	54,04	32,37	19,83	53,94	32,40	19,70	53,79	32,39
3	22,22	54,14	30,32	22,04	55,43	31,72	22,21	54,05	30,25	22,01	55,52	31,83
	22,36	54,91	30,92	22,12	55,40	31,62	22,15	54,98	31,19	22,01	55,56	31,87
4	18,27	50,71	30,82	18,12	50,43	30,69	18,26	50,86	30,97	18,11	50,35	30,63
	18,21	50,66	30,83	18,08	50,45	30,75	18,26	50,53	30,66	18,16	50,38	30,61
5	19,11	54,38	33,51	18,92	54,68	33,97	19,24	54,38	33,38	18,96	54,53	33,79
	19,14	54,57	33,66	19,04	54,53	33,72	19,22	54,47	33,49	18,97	54,42	33,68
6	17,18	49,86	31,05	16,86	49,65	31,15	17,34	49,65	30,70	16,99	49,75	31,12
	17,32	49,90	30,95	16,98	49,15	30,56	17,45	50,02	30,94	17,05	49,12	30,47
7	20,73	51,48	29,21	20,71	51,11	29,16	20,74	51,41	29,14	20,71	51,65	29,38
	20,95	52,15	29,64	20,81	52,19	29,81	20,81	51,98	29,61	20,66	52,15	29,92
8	18,74	49,92	29,62	18,58	51,41	31,19	18,73	50,02	29,73	18,48	51,65	31,51
	18,70	51,32	30,99	18,71	50,96	30,64	18,70	51,48	31,14	18,56	50,83	30,66
9	17,95	51,95	32,30	17,82	51,81	32,29	18,04	51,98	32,24	17,78	51,79	32,31
	18,01	51,79	32,09	17,78	51,91	32,42	18,00	51,88	32,19	17,74	51,79	32,35

**TABLE 4**  
Statistical Analysis (t-test) performed on the Data in Table 2

	Comparison	Average Difference	SD	Calculated t	t17; 95%	Significance
(a) Different reagents, same method . . . . .	a — d	+ 0,142	0,107	1,32	2,11	NS
	b — e	+ 0,173	0,570	0,30	2,11	NS
	c — f	- 0,298	0,552	0,54	2,11	NS
	g — j	+ 0,207	0,097	2,14	2,11	S
	h — k	- 0,179	0,665	0,27	2,11	NS
	i — l	- 0,357	0,612	0,58	2,11	NS
(b) Different methods, same reagent . . . . .	a — g	- 0,016	0,095	0,17	2,11	NS
	b — h	+ 1,560	0,120	0,13	2,11	NS
	f — l	- 0,029	0,139	0,21	2,11	NS
	e — k	- 0,010	0,157	0,06	2,11	NS
	d — j	+ 0,049	0,102	0,48	2,11	NS
	c — i	+ 0,030	0,149	0,20	2,11	NS
(c) Different reagents and different methods . . . . .	c — l	- 0,327	0,633	0,52	2,11	NS
	b — k	- 0,163	0,661	0,25	2,11	NS
	f — i	+ 0,328	0,545	0,60	2,11	NS
	e — h	+ 0,189	0,579	0,33	2,11	NS
	d — g	- 0,158	0,162	0,98	2,11	NS
	a — j	+ 0,191	0,089	2,14	2,11	S

**TABLE 5**  
Addition of EDTA solution before and after inversion of Molasses Solution

Sample	R S, %	Total Invert, %		Sucrose, %	
		EDTA Before Inv.	EDTA After Inv.	EDTA Before Inv.	EDTA After Inv.
1	23,40	54,21	54,04	29,17	29,11
2	16,63	49,55	49,39	31,27	31,12
3	22,24	53,52	53,52	29,72	29,72
4	18,13	52,16	51,96	32,33	32,14
5	17,88	50,16	50,24	30,67	30,74
6	19,53	49,03	49,03	28,02	28,02
7	24,10	53,97	53,90	28,38	28,31
8	22,61	56,68	56,48	32,37	32,18
9	20,47	55,44	55,65	33,22	33,42
10	16,95	55,80	56,19	36,91	37,28
11	28,73	59,56	59,34	29,28	29,07
12	22,05	52,09	51,97	28,53	28,42
13	13,77	47,68	47,55	32,21	32,09
14	17,16	51,15	51,15	32,29	32,29
15	18,35	50,54	49,98	30,58	30,05
16	18,43	50,95	51,08	30,89	31,02
17	21,13	53,45	53,45	30,70	30,70
18	21,65	52,68	52,48	29,48	29,29
19	23,14	55,91	55,91	31,13	31,13
20	22,25	52,68	52,68	28,91	28,91
21	20,25	51,22	51,22	29,42	29,42
SD		0,190		0,179	
Calculated t		0,306		0,280	
Table value t 20,95%		2,086		2,086	

border line of significance and it seems that statistically there is no difference between methods employed or reagents used. Furthermore, the constant volume method<sup>7</sup>, having the more dilute solution, provides a clearer end point.

(c) *Addition of 4% EDTA as calcium sequestrant.*

When determining total invert, EDTA solution is usually added to the prepared molasses solution after inversion. In practice it is preferable to add EDTA to the original molasses stock solution to eliminate further preparation of the sub-samples pipetted for reducing sugars and total

invert determinations. In order to investigate differences in adding EDTA before and after inversion with Jackson and Gillis hydrochloric acid<sup>10</sup>, analyses were done using the classical Lane and Eynon method<sup>2</sup> with additions of 4% EDTA (4 cm<sup>3</sup> EDTA per 1 g of molasses). The results listed in Table 5 are averages of duplicate titrations not differing by more than 0,1 cm<sup>3</sup>. From these results it is concluded that EDTA solution may be added before or after inversion and it is recommended that this addition of EDTA be made to the original stock solution of molasses.

(d) *The influence of excess EDTA in the Lane and Eynon titration.*

From results reported in Table 6, it was confirmed that an excess of EDTA in solution containing the reducing sugars should be avoided<sup>8</sup>.

**TABLE 6**  
Effect of excess 4% EDTA solution on the determination of reducing sugars using standard invert solution\*

Sample	cm <sup>3</sup> of 4% EDTA added					
	0	2,5	5	10	15	20
Standard Invert Sol.	202,60	202,60	201,80	201,00	200,00	199,40

\* Results expressed as mg RS/100 cm<sup>3</sup> of prepared solution.

These results also show that a maximum excess of 5 cm<sup>3</sup> of 4% EDTA solution is tolerable, and that a greater amount of EDTA significantly suppresses the amount of reducing sugars found.

(e) *Application of Lane and Eynon method in analysis of various sugar products for reducing sugars.*

The Lane and Eynon<sup>1</sup> method was used to determine the reducing sugars in various sugar products using different quantities of 4% EDTA as calcium sequestrant. These samples were collected from both diffuser and milling factories in different geographical areas of the South African sugar belt.

(i) *Final molasses.*

The already internationally established amount of 4 cm<sup>3</sup> of 4% EDTA solution per 1 g of sample to be used was confirmed<sup>7</sup>.

(ii) *A- and B- molasses.*

The results for A- and B- molasses are given in Table VII where the values of reducing sugars obtained without EDTA addition are represented as 100% and those obtained after addition of 4% EDTA are expressed as percentages of them.

From the average values reported in Table 7 (see also Figure 1) a volume of 2 cm<sup>3</sup> of 4% EDTA solution per 1 g of A- and B- molasses is sufficient to render all reducing sugars available for determination.

(iii) *A-, B- and C- massecuites.*

The results obtained for A-, B- and C- massecuites are given in Table 7 (see also Figure 1) and show that a volume of 1 cm<sup>3</sup> of 4% EDTA per 1 g of sample will be sufficient to render all reducing sugars available.

(iv) *Mixed juice, clarified juice and syrup.*

The results obtained for mixed juice and clarified juice are given in Table 8 while those for syrup are given in Table 9 (see also Figure 1) and show that for mixed and clear juices a volume of 10 cm<sup>3</sup> of 4% EDTA per 50 g of sample will be sufficient to render all reducing sugars available, and for syrup 5 cm<sup>3</sup> of 4% EDTA per 8,33 g of sample.

**Conclusions**

When the titration method of Lane and Eynon is used to determine the reducing sugars content of sugar products, certain important preliminary measures must be taken :

- (a) Several procedures state that the solution to be titrated should come to the boil in 2 to 2,5 minutes. Since there is a significant difference in titre over an interval of only half a minute, it is important therefore that the time taken to reach boiling point is standardised.
- (b) Preparation of mixed Fehling's solution (10 cm<sup>3</sup> A + 10 cm<sup>3</sup> B or 20 cm<sup>3</sup> A and B) and the concentration

**TABLE 7**  
The effect of 4% EDTA Solution on the reducing sugars in molasses and massecuites (zero addition = 100%)

Sample	cm <sup>3</sup> of 4% EDTA added per 1g sample						
	0	1,0	2,0	3,0	4,0	5,0	6,0
<b>A-molasses</b>							
Number of samples ..	6	6	6	6	6	5	—
Range .. .. .	100,0	100,4 — 102,1	100,0 — 102,0	99,2 — 100,7	98,4 — 99,7	98,0 — 99,2	—
Average .. .. .	100,0	101,0	100,8	100,0	99,2	98,8	—
<b>B-molasses</b>							
Number of samples ..	6	6	6	6	6	6	6
Range .. .. .	100,0	100,2 — 102,2	100,3 — 103,1	99,9 — 103,0	99,6 — 102,4	99,4 — 102,1	99,0 — 101,0
Average .. .. .	100,0	100,8	100,9	100,8	100,5	100,2	99,7
<b>A-massecuite</b>							
Number of samples ..	5	5	5	5	5	4	—
Range .. .. .	100,0	100,4 — 102,3	99,1 — 101,0	98,3 — 100,0	97,5 — 99,2	97,5 — 99,0	—
Average .. .. .	100,0	101,4	100,2	99,4	98,6	98,1	—
<b>B-massecuite</b>							
Number of samples ..	6	6	6	6	5	5	6
Range .. .. .	100,0	100,6 — 105,0	98,9 — 101,1	98,7 — 101,0	98,5 — 100,2	98,1 — 100,2	97,7 — 100,2
Average .. .. .	100,0	102,4	100,3	100,1	99,7	99,2	98,7
<b>C-massecuite</b>							
Number of samples ..	6	6	6	6	6	6	6
Range .. .. .	100,0	100,0 — 101,3	99,8 — 102,0	99,3 — 102,2	98,9 — 101,7	98,9 — 100,9	98,9 — 100,2
Average .. .. .	100,0	100,5	100,6	100,5	100,3	100,0	99,7

**TABLE 8**  
The effect of 4% EDTA Solution on the reducing sugars in juices (zero addition = 100%)

Sample	cm <sup>3</sup> of 4% EDTA added per 50 g of sample							
	0	5	10	15	20	40	50	60
<b>Mixed juice</b>								
Number of samples ..	16	12	16	15	12	5	—	—
Range .. .. .	100,0	100,0 — 107,1	99,8 — 107,1	99,5 — 102,0	96,8 — 103,0	97,8 — 101,3	—	—
Average .. .. .	100,0	102,2	101,6	100,9	100,2	99,4	—	—
<b>Clarified juice</b>								
Number of samples ..	14	7	14	5	14	14	8	1
Range .. .. .	100,0	100,0 — 101,4	100,0 — 102,8	100,0 — 101,2	99,7 — 102,8	97,2 — 105,6	94,7 — 100,8	99,1
Average .. .. .	100,0	100,8	101,2	100,8	101,0	101,0	97,9	99,1

**TABLE 9**  
**The effect of 4% EDTA Solution on the reducing sugars in syrup**  
 (zero addition = 100%)

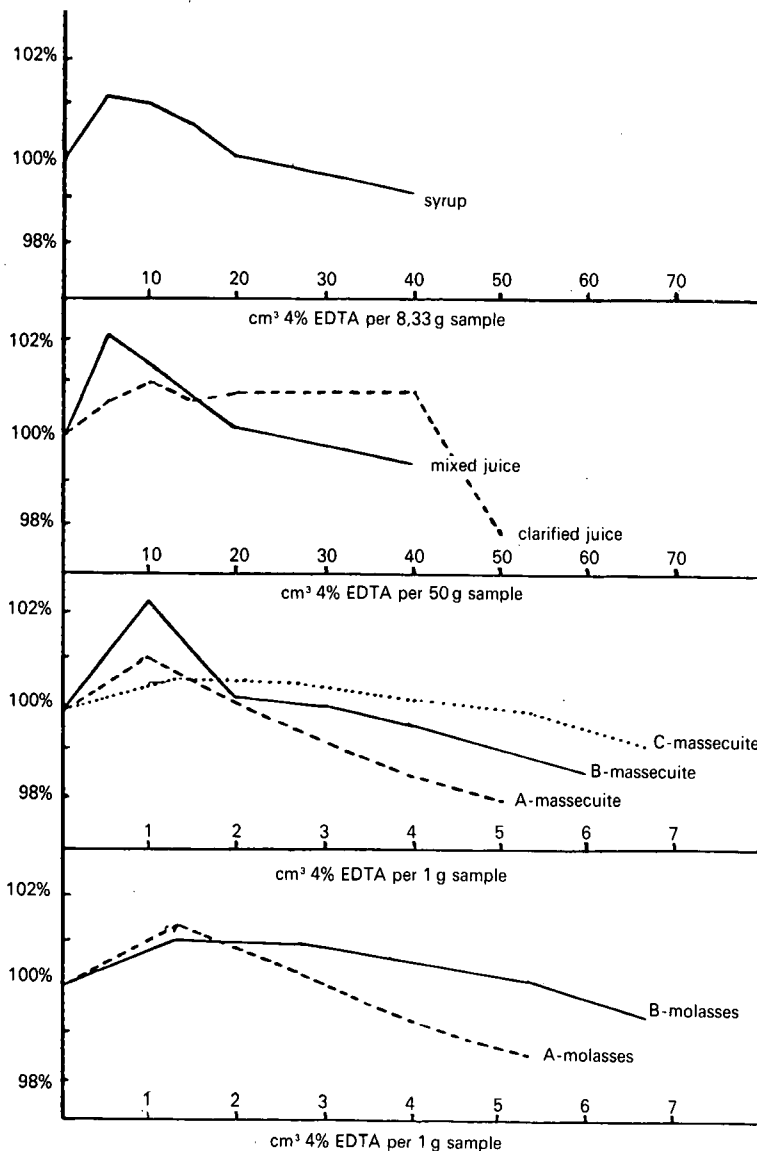
Sample	cm <sup>3</sup> of EDTA added per 8,33 g of sample					
	0	5	10	15	20	40
Syrup						
Number of samples ..	14	12	14	9	14	12
Range .. .. .	100,0	99,8 — 103,0	100,0 — 102,9	99,5 — 102,1	98,6 — 101,2	97,4 — 101,0
Average .. .. .	100,0	101,4	101,2	100,8	100,1	99,3

of standard inverts (0,25% rather than 0,5%) solution must be standardised.

- (c) The use of EDTA rather than potassium oxalate is preferred as calcium sequestrant because it eliminates the heating, filtration and subsequent evaporation of the solution.
- (d) An excess of EDTA should be avoided and recommendations are made for quantities to be used when analysing mixed and clarified juices, syrup, massecuites and molasses.

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**FIGURE 1** The effect of 4% EDTA on reducing sugars (zero addition — 100%).