A critical study of the Detection and Estimation of Impurities in Foodstuffs with special reference to Sugars.

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LIST OF PRINCIPAL ABBREVIATIONS USED IN THE REFERENCES.

	Abbreviated Title.	Full Title.
-1-	Annalen. . . .	Justus Liebig's Annalen der Chemie.
-2-	Ann. Chim. . . .	Annales de Chemie.
-3-	Ber.	Berichte der deutschen chemischen Gesellschaft.
-4-	Biochem. J. . .	The Biochemical Journal.
-5-	Biochem. Z. . .	Biochemische Zeitschrift.
-6-	Br. Chem. Abs. . .	British Chemical Abstracts (Section A).
-7-	C.A.	Chemical Abstracts (American).
-8-	Chem. News. . . .	The Chemical News (now discontinued).
-9-	Chem. Zentr. . . .	Chemisches Zentralblatt.
-10-	Compt. rend. . . .	Comptes rendus hebdomadaires des Séances de l'Académie des Sciences.
-11-	Helv. Chim. Acta.	Helvetica Chimica Acta.
-12-	I.S.J.	International Sugar Journal.
-13-	J. Am. C.S. . . .	Journal of the American Chemical Society.
-14-	J.C.S.	Journal of the Chemical Society.
-15-	J.I.E.C.	Industrial and Engineering Chemistry.
-16-	J.I.E.C.(anal.) Ed..	Industrial and Engineering Chemistry: Analytical Edition.
-17-	J. Pharm.	Journal de Pharmacie et de Chimie.
-18-	J. pr. Chem. . . .	Journal für praktische Chemie.
-19-	J.S.C.I.	Journal of the Society of Chemical Industry
-20-	Pharm. Weekblad.	Pharmaceutisch Weekblad.
-21-	Z. anal. Chem. . .	Zeitschrift für analytische Chemie.
-22-	Z. angew. Chem . .	Zeitschrift für angewandte Chemie (now Angewandte Chemie).
-23-	Z. anorg. Chem.	Zeitschrift für anorganische und allgemeine Chemie.

DEFINITIONS OF TECHNICAL TERMS USED:

% ASH: This indicates percentage total mineral matter left on ignition of a sample in a muffle furnace.

BRIX° The Brix hydrometer is calibrated to give directly the percentage of sucrose in a sugar solution in which it is immersed. Degrees Brix are equivalent to % sucrose only when the solution contains no other solute than pure sucrose. If impurities are present the °Brix is higher than the sucrose per cent.

LIQUOR The term liquor is generally used to refer to a sugar solution from which no sugar has been removed by crystallisation.

DIRECT POLARISATION This indicates approximately the percentage of (pure) sucrose in a sugar or sugar solution.

PURITY The amount of sucrose expressed as a percentage of the total solid substances present in a sample of sugar, juice, syrup, molasses, is known as the purity of the solution.

RAW WASHINGS This is the syrup obtained by centrifugal separation of a mixture ("magma") of raw sugar crystals and water. The

surface layer of the crystals which contains the bulk of the impurities is thus partially dissolved, the sugar obtained from the centrifugals being termed "washed" sugar.

MASSECUITE The term means literally a "cooked mass" and refers to the mixture of crystals and mother-liquid resulting from boiling of sugar liquors in a vacuum pan.

MOLASSES This is the syrup spun off in centrifugals from the final massecuite: the impurities present are in sufficient amount to make it uneconomical to recover further sugar from this molasses by ordinary means.

CRYSTALLISER SUGAR The sugar obtained by spinning in a centrifugal a massecuite boiled mainly from raw washings.

"BUFFERING" Resistance to change in pH value on the addition of acid or alkali. Usually caused by the presence of salts of weak acids and bases.

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P A R T I.

THE OCCURRENCE OF IMPURITIES IN VARIOUS SUGAR PRODUCTS.

In this introductory section, various impurities in sugar products are discussed, and a survey of the relative literature is made: in some cases, experimental work by the author is described.

THE PROCESS OF SUGAR MANUFACTURE.

(a) RAW CANE SUGARS:- Raw cane sugars are made by the following processes -

(1) Defecation.

(2) Sulphitation.

(3) Carbonatation.

In the simple defecation process lime is added to the extracted juice. The precipitate produced is settled and the clear juice drawn off and concentrated to syrup. This syrup is boiled to grain, and the crystals separated by centrifugals, the mother-liquor being worked up with fresh syrup until sugar will no longer crystallise from it, when it is discarded as molasses.

The sulphitation and carbonatation processes are similar to those described later under "Direct Process" sugars, but the purification for raw sugars is not taken so far.

Raw beet sugars are generally made by sulphitation or carbonatation processes. The bulk of beet sugar is produced white for direct consumption.

(b) DIRECT PROCESS SUGAR: The term "Direct process" or "Plantation white" sugar is applied to sugars which are made directly from the juice of the cane or beet by some chemical process generally (1) sulphitation or

(2) carbonatation

(1) SULPHITATION: Lime is added to the raw juice and sulphur dioxide (produced by burning sulphur) is passed in, forming insoluble calcium sulphite. This precipitate adsorbs at its surface a proportion of the colloidal impurities of the juice. Separation of the precipitate is effected by decantation or by filtration. The sulphur dioxide lessens the colour of the juice by its bleaching (reducing) action. The sulphitation process sugars are characterised by loss of their whiteness during storage, due to re-oxidation of part of the bleached colouring matter. Such sugars are not suitable for direct home consumption, being usually refined by animal charcoal in the refineries. These sugars are principally made in Java, Natal, Mauritius, Mexico, and Louisiana.

(2) CARBONATATION: In this process, which is applied to both cane and beet juices, much more lime is added initially. Carbon dioxide is

passed through the limed juice, and the granular precipitate of calcium carbonate carries down with it a large proportion of the juice impurities. Filtration follows, and is succeeded by treatment with more carbon dioxide and further filtration. Finally sulphur dioxide is passed in, after concentration of the juice to syrup.

Carbonatation sugars are superior to sulphitation sugars, but may also lose their whiteness during storage; they are principally made in Java and Natal and in most beet growing countries.

(c) REFINED SUGARS: In the refining of raw sugars for the production of first quality granulated sugar it is necessary to adopt a process involving the use of carbon, either as animal charcoal or as "activated carbon." The activated carbons have not come into general use in the refining industry. They have excellent decolorising properties, but in the amounts used they do not remove ash to any great extent. With animal charcoal, ash is very easily removed in addition to adsorption of colouring matters.

DIFFERENCES BETWEEN DIRECT PROCESS and REFINED SUGARS.

1. Direct process sugars darken on storage by re-oxidation of colouring matters which are merely bleached and not removed by the process.
2. Direct consumption sugars are not sterile and may contain mould spores, being thus unsuitable for the canning industries, since fermentation may arise with consequent disruption of the container.
3. On boiling solutions of direct process cane and beet sugars an albuminous scum rises, which is objectionable for the making of confections.
4. Sulphitation sugars are often mixed with ultramarine to neutralise any yellow tint. This may cause the formation of sulphuretted hydrogen by action with the acid in the sugar.
5. Direct process beet sugars have an alkaline reaction (due to potassium carbonate). This alkalinity is not readily tolerated by children. This alkaline reaction may also prevent "gelling" in the manufacture of jams and jellies.

All the above undesirable properties are caused by the incomplete removal of impurities, principally colloids, by the direct process of manufacture. By the use of animal charcoal or carbon in refining these impurities are adsorbed and removed.

IMPURITIES IN WHITE SUGARS.

The impurities in a refined white sugar as it leaves the final drier in the refinery are ash or mineral matter, reducing sugars, and other organic matter.

During storage in bags in a warehouse further impurities may be gathered by the sugar, these being mainly dust from the air, fibre from the bags, and water, which is absorbed until an equilibrium has been reached with the atmosphere.

The purity of an average white sugar is seldom below 99.8% sucrose (by polarisation method), and normally 99.9% sucrose.

The estimation of the other constituents is thus difficult since they are present in such small amounts, but estimation is necessary for several reasons, detailed below.

SIGNIFICANCE OF IMPURITIES.

IRON:- Excessive iron occurring in the sugar will give it a greyish appearance which lessens its value on the market, sugar being normally valued by its whiteness, since other factors (e.g. polarisation) are usually equal.

HYGROSCOPIC SALTS: The presence of deliquescent salts such as magnesium chloride will affect the deterioration of the sugar in storage. Hygroscopic compounds may also occur due to the use of excess of lime in the refining process, whereby lime-glucose decomposition products are formed which absorb water readily.

DIRT, FIBRE, etc. This must be kept to the minimum always, for hygienic reasons. In special cases it must be eliminated as completely as possible - e.g. in the sweetening of condensed milk where particles of dust or fibre in the final product would be objectionable. White sugars for such purposes are tested for dirt by dissolving 200 gms. in water and filtering the solution through a cotton wool pad about 3 cms. in diameter. The dirt which collects on the pad is compared with standards.

OTHER ORGANIC MATTER: Traces of organic matter may cause development of odour during storage, thus betaine in beet sugars gives rise to the characteristic "beet" smell after long storage of these sugars. The presence of these nitrogenous bodies may be used, in fact, to distinguish between white cane and beet sugars (by nesslerising). The ash content may be used as a basis in the chemical control of refineries.

REDUCING SUGARS:- Estimation of reducing sugars will

—DIRT·TESTS·ON·VARIOUS·SUGARS—

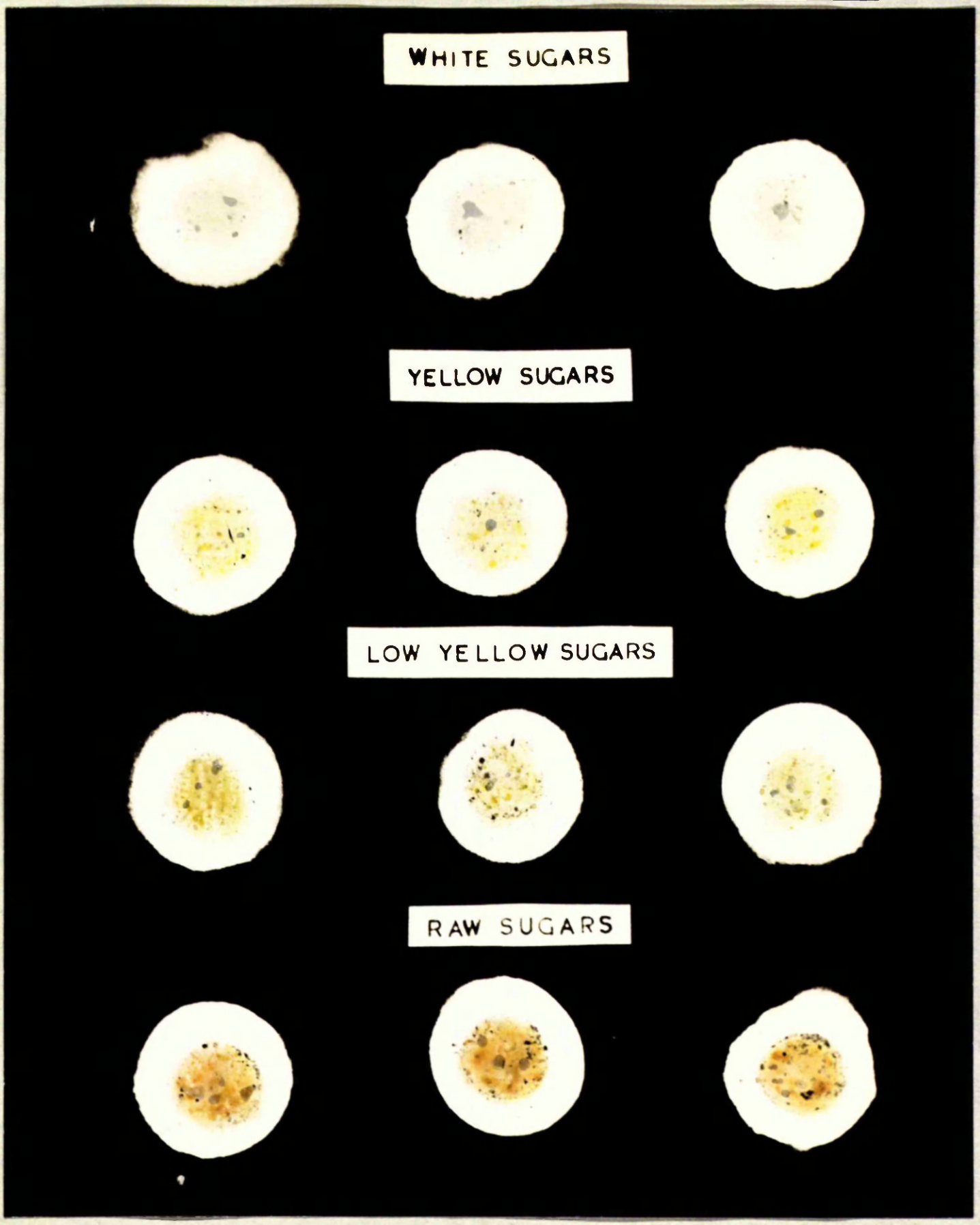


FIG.1

give information regarding the purity of a sugar, particularly with lower grade products. Ash and reducing sugar amounts are generally of the same order, i.e. it is unusual to find a low reducing sugar content with a high ash content. The amount of reducing sugars in white sugars is also an index of the extent to which inversion has taken place during the manufacturing process.

WATER:- It is well known that dry sugar will absorb water until equilibrium is attained with the humidity present in the surrounding atmosphere. When the water content is high the sugar is more liable to deteriorate due to the activity of micro-organisms.

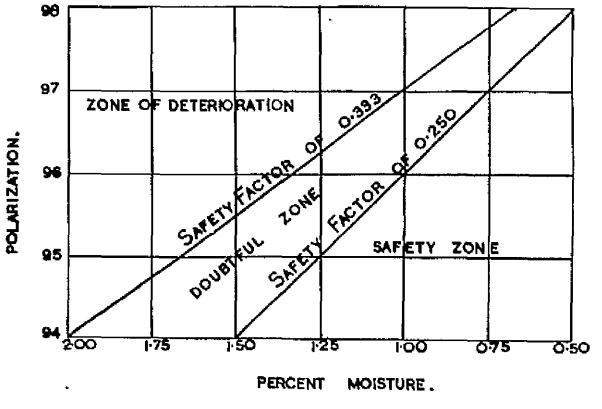
For raw sugars a tentative figure known as a "factor of safety" has been put forward by the Colonial Sugar Refining Co. of Australia. This may be expressed: when $\left(\frac{\text{Water}}{100 - \text{polarisation}}\right) < 0.333$, the sugar will not deteriorate. The exact value of the factor is connected with the concentration at which activity begins, and a different factor will obtain dependent on whether micro-organisms present in the sugar are bacteria, yeasts, or moulds. The production of large-grained sugars with surface area at a minimum will lessen moisture absorption. It is apparent that even with highly refined white sugars the estimation of water is important, and this importance increases with lower quality products, especially in buying and selling.

SAFETY FACTOR:- Many investigators have studied this question of deterioration, and they agree that the original factor is too high for many sugars, and that to cover all cases for positive safety it should be expressed:-

$$\left(\frac{\text{Moisture}}{100-\text{Polarisation}}\right) = 0.25 \text{ or less.}$$

The chart shown below is useful for determining the keeping quality of a sugar.

EXAMPLE:- A sugar has a direct polarisation of 96.0 and a moisture content of 0.90 per cent. It is within the "safety zone". If the moisture were 1.06 per cent for the same polarisation it would fall in the "doubtful zone," whereas a moisture of 1.37 per cent at 96.0 polarisation would definitely mark the sugar as likely to deteriorate.



'SAFETY FACTOR' FOR RAW SUGARS.

FIG. 2

ABSORPTION OF WATER BY WHITE SUGARS.

10 grams of each sugar weighed into a Petri dish of 10 cms. diameter and exposed beside dishes containing water under bell jars.

SUGAR	REDUCING SUGARS %	ASH %	ORIGINAL WATER %	AFTER 18 HOURS WATER %	AFTER 66 HOURS WATER %
GRANULATED 1	.007	.010	.020	.100	1.55
-do- 2	.012	.011	.010	.100	1.53
-do- 3	.016	.019	.018	.170	1.61
-do- 4	.020	.018	.014	.200	1.66
Z GRANULATED	.046	.042	.080	.560	2.10

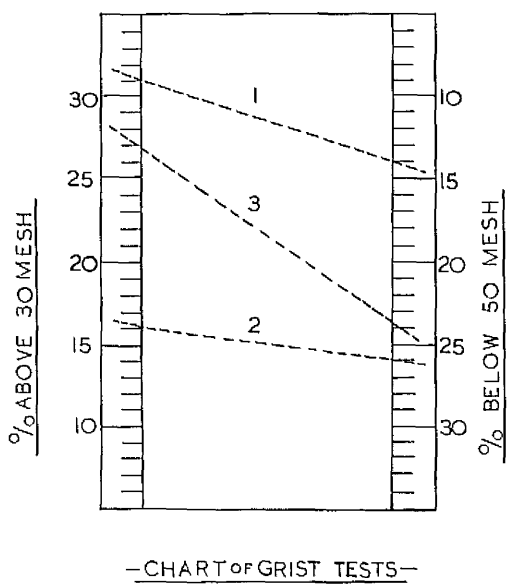
SCREEN ANALYSIS OF ABOVE SUGARS.

SUGAR	SIEVES - MESH PER LINEAR INCH						
	20	25	30	Passing 30	40	50	Passing 50
GRANULATED 1	22.5	24.3	21.8	31.4	-	-	-
-do- 2	10.6	33.4	33.7	22.3	-	-	-
-do- 3	-	-	25.0	-	58.5	13.5	3.0
-do- 4	-	-	25.0	-	51.0	13.0	11.0
Z GRANULATED.	-	-	30.0	-	62.0	6.0	2.0

GRIST TESTS OF SUGARS.

Grain analysis is useful in controlling the operations in the vacuum pans: it is essential to keep the sugar within reasonable limits of grain size. The graphic method of reporting the tests is shown in the accompanying figure. The significant figures are the amount of sugar retained on a screen of 30 mesh per linear inch (directly proportional to the coarseness of grain) and the amount of sugar passing through a 50 mesh screen (inversely proportional to the coarseness of grain). By representing the vertical scales as shown, one direct and one inverse, results may be recorded as in lines 1, 2, and 3.

Line 1 shows 31 per cent of coarse grain (above 30) and 16 per cent of fine grain (below 50): the sugar is thus a rather coarse grade of fine granulated, true "coarse granulated" being much larger. Sugar 2 has 16 per cent on 30 mesh and 26 per cent through 50 mesh, and is an "extra fine" granulated. Sugar 3 has 27 per cent on 30 mesh and 24 per cent through 50, and is therefore a badly boiled sugar, containing much coarse and much fine grain mixed.



-CHART OF GRIST TESTS-

FIG. 3

ANALYSIS OF SUGARS.

The results below were obtained by the author in a sugar refinery and represent some five hundred analyses made over a period of three years.

GRADE 1. GRANULATED WHITE SUGAR.

YEAR & SERIES	REDUCING SUGARS Per Cent			A S H Per Cent			W A T E R Per Cent		
	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
1.	.012	.050	.025	.007	.014	.010	.010	.050	.039
2.	.008	.030	.017	.003	.015	.009	.025	.060	.041
3.	.008	.031	.017	.005	.024	.011	.036	.080	.052

Reducing sugars estimations were made by Ost's method; ash determined by a conductivity method; water percentages by drying 10 grams in a steam oven.

The figures shown in the next table illustrate the increase in impurities as the various sugars decrease in purity - the process of refining sugar being essentially one of fractional crystallisation on the large scale, after the initial chemical purification has been accomplished.

LIMITS FOUND FOR WHITE SUGARS.

GRADE of SUGAR	NORMAL		ALLOWABLE MAXIMUM		SUM OF ALLOWABLE MAXIMA
	Red. Sugars %	Ash %	Red. Sugars %	Ash %	
Crystal 1	.004	.006	.010	.010	.020
-do- 2	.005	.008	.010	.010	.020
Grade 1 Granulated.	.006	.009	.014	.014	.028
Grade 2 Granulated.	.011	.011	.015	.015	.030
Grade 3 Granulated.	.014	.017	.020	.020	.040
Grade 4 Granulated	.015	.020	.025	.025	.050
Manufacturing crystals	.015	.015	.030	.030	.060
Off-colour	.016	.020	.025	.025	.050
Cubes 1	.005	.020	.010	.030	.040
Cubes 2	.005	.025	.010	.040	.050
Castor sugar	.010	.020	.015	.030	.045
Z Granulated	.030	.040	.050	.070	.120

Methods of analysis - Ost's method for reducing sugars, conductivity method for ash.

Using the above methods of analysis, the following results were obtained for Beet-sugars from seven different factories:-

	A S H	RED.SUGARS
FACTORY	Per Cent	Per Cent
A	.017	.001
B	.012	.005
C	.017	.001
D	.018	.005
E	.011	.001
F	.024	.001
G	.021	.001

Beet sugars contain very little reducing sugar. This is primarily the case since the sugar beetroot contains only a very small amount of reducing sugar, (about 0.010 per cent) whereas the sugar cane contains from 0.4 to 2 per cent. Furthermore, the pH value is kept well in the alkaline range during beet sugar manufacture, while in the cane-sugar process it is often just at the neutral point, or even in the acid range.

DETERMINATION OF ASH OR MINERAL MATTER IN SUGAR PRODUCTS.

For many years the direct incineration method of determining ash was considered the only accurate method, the method involving addition of sulphuric acid ("sulphated ash") being regarded suitable only for routine work.

Brown and Gamble ("Facts about sugar" Dec. 15th 1923), published results showing the sulphated ash method to be the more reliable determination. It is now accepted that neither method gives an absolute measure of the salts present, but that the sulphated ash gives results capable of replication.

Withrow and Jamieson (J.I.E.C. 15, 4, 1923) found that instead of the normal 10 per cent deduction from the result to allow for the sulphation, 33.25 per cent was the lowest deduction for the sugar used in their work. They found porcelain crucibles suitable for ash work, but obtained low results with fused silica crucibles.

The introduction of electrometric ash methods has shortened the time required for ash estimation. These methods are described in Spencer and Meade's "Handbook for cane sugar manufacturers", p. 270, (John Wiley, 1929,) and will not be discussed here.

COMPOSITION OF ASH OR MINERAL MATTER.

The ash of sugars can be analysed quantitatively very completely, but these analyses do not indicate the combinations originally present in the sugar, because of pyrolytic changes during the process of ashing. Sulphates and chlorides, for example, may be lost in part, sulphates being reduced to sulphites or sulphides, while chlorides may be oxidised to chlorine or displaced by less volatile acidic radicles.

In most cases nitrates, nitrites and ammonium salts will be driven off completely.

Carbonates occurring in the ash are formed partly by the carbon dioxide produced by the burning of the sugar. Phosphorus and sulphur in organic combination may be lost, or converted into inorganic compounds.

So far no methods have been evolved for determining in white sugars such organic non-sugars as the saponins, individual proteins, gums, waxes, fats and oils, and polyphenolic compounds, all of which have been shown to be present and probably exert influence on the behaviour of sucrose during heating.

T O T A L A S H.

The total ash in sugars should be as low as possible. The constituents of the total ash vary considerably; average analyses are shown in the subjoined table.

	(1)	(2)	(3)
CONSTITUENT	Sulphated Raw Cane Sugar Ash	Cane Sugar Ash.	Beet Sugar Ash.
Silica	6.84	8.29	1.78
K_2O	17.54	28.79	34.19
Na_2O	16.85	0.87	11.12
CaO	12.38	8.83	3.60
MgO	0.52	2.73	0.16
Al_2O_3	6.49	6.90	0.28
Fe_2O_3	0.41		
Cl	2.77		
SO_3	26.87	43.65	48.85
Loss on ignition 1.75% carbon 8.05% CO_2	9.80	-	-
	100.47	100.06	99.98
Less O ≡ Cl	.62		
	99.85		

(1) Average of Raw Sugar samples from a Refinery over one year.

(2) & (3) From "Sugar Growing & Refining" Lock, Wigner, & Harland, Spon. (1885).

ANALYSES OF THE ASH (SULPHATED) OF VARIOUS RAW SUGARS.

Sugar Origin.	% Ash on Dry.	% on Ash			
		CaO	Fe_2O_3 & Al_2O_3	SiO_2	P_2O_5
Australia	0.23	12.96	11.42	30.20	5.01
Argentine	0.51	15.88	6.54	8.27	4.34
Brazil	0.51	17.63	9.58	30.15	1.71
Demarara	0.46	15.67	23.63	15.12	2.11
Natal	0.43	13.79	15.68	46.20	1.63
Peru	0.90	12.38	8.69	19.33	3.90

The phosphate content is of primary importance to refiners. Sugars low in phosphate are notoriously difficult to filter. Phosphate may be added artificially, in fact, to help the filtering qualities.

The silica is generally inocuous, but may cause filtration trouble if colloidal. The calcium oxide in raw cane sugars varies from 12 to 14 per cent. This is important later on in process, as soluble calcium salts are positive molasses-formers, so that excess of these salts will diminish the yields of white sugar. Thus a 4th syrup may contain 2 per cent of ash and 25 per cent of this ash may be calcium (calculated as oxide). The decalcification of sugar liquors by adding sodium carbonate is not easy, since excess of soda is not recoverable, and seems to combine with or destroy the levulose.

The above analyses were made by the Author.

APPROXIMATE SULPHATED ASH CONTENT OF VARIOUS RAW CANE SUGARS.

SUGAR ORIGIN	% ASH on DRY.
AUSTRALIA	0.2 - 0.5
BARBADOS	0.6 - 0.7
BRAZIL	0.3 - 0.8
BRITISH WEST INDIES	0.5 - 1.0
CUBA	0.5 - 0.8
DEMERARA	0.5 - 0.8
JAMAICA	0.5 - 1.0
JAVA	0.1 - 0.3
MAURITIUS	0.05 - 0.15
MOZAMBIQUE	1.0 - 1.30
NATAL	0.2 - 1.0
PERU	0.45- 0.75
SAN DOMINGO	0.5 - 0.8

These figures were extracted by the author from results obtained by him over a period of some three years, The number of samples ranged from over 100 in the case of Cuban sugars to at least 10 in the case of sugars less frequently imported

TYPICAL FIGURES FOR CALCIUM AND FOR ALKALIS IN REFINERY AFTER-PRODUCT SUGARS ARE SHOWN HERE:-

REFINERY YELLOWS AND LOW PRODUCT SUGARS:-

No.	Total Ash	%CaO in Ash	%(Na2O) (K2O) in Ash
(1)	0.47	4.0	61. 8
(2)	1.12	1.8	45. 1
(3)	1.26	4.8	52. 8
(4)	1.62	1.5	41. 8
(5)	0.69	23.5	21. 4
(6)	0.66	15.0	35. 6

EXPERIMENTAL WORK ON DETERMINATION OF ASH IN RAW SUGAR.

5 gms. peruvian raw sugar used in each test.
Moisture per cent in sugar - 1.27 per cent.

SERIES I.

VESSEL	SUBSTANCE ADDED	% ASH I	% ASH II.	% ASH MINUS 1/10 I.	% ASH MINUS 1/10 II.	MEAN %
Silica	Nil	0.540	0.552	-	-	0.516
Silica	0.5 cc. H_2SO_4	0.700	0.682	0.630	0.614	0.622
Platinum	Nil	0.594	0.600	-	-	0.597
Platinum	I cc. H_2SO_4	0.780	0.756	0.702	0.682	0.692
Platinum	6.5 gms. Quartz Sand	0.524	0.574	-		0.549
Platinum	2 gms. Vaseline	0.650	0.610	0.630 *	0.591	0.611
Porcelain	Nil	0.610	0.630	-	-	0.620
Porcelain	I cc. H_2SO_4	0.730	0.718	0.657	0.646	0.652

* Corrected for ash in vaseline.

The mean results shown are minus onetenth where sulphuric acid has been used. Concentrated Sulphuric Acid (1.84 sp. gravity) was used.

The present writer found a progressive loss in weights with fused silica crucibles. Starting with two new silica crucibles, six estimations of ash were made with each, using 5 gms. of Peruvian raw sugar and 1.0 cc. of 50 per cent sulphuric acid.

Heating	Crucible I Loss in weight gms.	Crucible II Loss in weight gms.
1	0.0012	o.0074
2	0.0014	0.0063
3	0.0013	0.0032
4	0.0016	0.0030
5	0.0009	0.0045
6	0.0010	0.0027

These decreases appear to be due at first to attack of the fused surface of the new crucible. In later use the loss decreases, possibly since there is then some gain as well by the crucibles by absorption of salts from the ash.

ASH ESTIMATIONS - SERIES II.

5 gms. same Peruvian Raw Sugar (1.27% moisture) used in each test.

EFFECT OF VARYING QUANTITY OF SULPHURIC ACID

VESSEL	SULPHURIC ACID ADDED	% ASH	% ASH MINUS 1/10
Platinum	I cc. 10%	0.700	0.630
"	5 ccs.10%	0.800	0.720
"	I cc. 50%	0.782	0.703
"	2 ccs.50%	0.789	0.710
"	I cc.concentrated	0.780	0.702
"	1.5 ccs. "	0.782	0.703
"	2.0	0.786	0.707
Electro-metric Ash	---	0.610	--

DISCUSSION OF RESULTS OF ASH DETERMINATION:

The silica crucible with the sulphation method appears to give low results, this being due probably to the etching of the crucible by the hot acid. The direct method without sulphating appears to give low and variable results; all the sulphated ashes are high, some deduction being necessary.

The whole series illustrates the relative inaccuracy of the incineration method.

A control test was made by burning off 100 gms. of the same Peruvian Raw Sugar in a large platinum vessel in instalments using 20 ccs. of sulphuric acid. The result obtained was 0.690% ash. (- ‰).

From these tests it would seem that an adequate quantity of sulphuric acid must be added, and that the most consistent results are those using platinum dishes.

The incinerations were carried out in all cases in an electrically heated muffle furnace at a temperature not exceeding 750°F.

OCCURRENCE OF HEAVIER METAIS IN SUGAR PRODUCTS.

The metals most commonly tested for in sugars are copper, lead, tin and arsenic. Results obtained by the author are shown here.

	ARSENIC (Marsh's Test)		
SAMPLE	PARTS per MILLION OBSERVED STAIN Approx.	P.p.m. As corrected for blank	% As
Peruvian Raw Sugar	1	0.9	0.00009
Cuban Raw Sugar	1.5	1.4	0.00014
Mauritius Raw Sugar	0.5	0.4	0.00004
Yellow Refined Sugar	0.5	0.4	0.00004
Refinery Molasses	0.1	0.0	Nil.
Direct Process Beet Sugar	0.7	0.6	0.00006

The blank showed 1/10 part per million. The results above were obtained by comparison with standard stains in the usual way.

The results obtained here are all below the Government limit for solid foodstuffs ($\frac{1}{100}$ grain per pound).

COPPER AND TIN: The presence of copper and tin in sugar products is purely adventitious, and is generally due to the storage of the product (usually syrup) in metal containers. Copper may be acquired by boiling of the products in vacuum pans made of this metal. Results obtained by the author for golden syrup are shown below.

GOLDEN SYRUPS TESTED AFTER LYING FOR 1 YEAR IN TINS.

SAMPLE	PARTS PER MILLION		
	COPPER	TIN	IRON
1	1.5	0.3	15
2	0.6	1.7	12
3	1.7	1.5	25

The above results show that iron is the preponderating heavy metal in the sugar product examined.

The copper was estimated by sodium diethyldithiocarbamate (Callan and Henderson, Analyst, 54, 650 (1929) the iron being first removed by double precipitation as hydroxide, and the copper then precipitated as sulphide from a solution at pH 3.0.

The tin was determined by a modified form of the molybdenum blue method recommended by Feigl (Chem. Ztg. 47, 561, 1923). This modification is described by Strafford (Institute of Chemistry lecture, 1933, p.25)

Manufacturers of food products have long recognised that some commercial white sugars when heated, withstand elevated temperatures with less decomposition or with less formation of invert sugar than others. Similarly one of two sugars may be more susceptible to fermentation than the other, although they may be indistinguishable when examined for ash, invert sugar, sucrose, pH, colour, or turbidity.

This difference of behaviour can only be attributed to the presence of minute quantities of impurities which have either been considered as not worthy of estimation, or else incapable of determination.

A study of several such impurities has been made by the Carbohydrate Division of the Bureau of Chemistry and Soils, U.S.A.

The main results obtained in this work are summarised on the following pages.

I. NITROGEN IN WHITE SUGARS.

J.I.E.C.

Ambler & Byall Vol.4, No.1 (1932) P.34.

SUGAR	TOTAL NITRO-GEN P.p.m.	PROTEIN N P.p.m.	AMINO ACID N. P. p.m.	N as AMMONIA	N as NITRATE	N as NITRITE
1	35	0.3	3.5	0	4	0.004
2	45	3.9	4.0	0	8	0.004
3	5	0	0	1	5	0
4	10	1.0	5.5	2	4	0.014
5	35	0.8	5.5.	0	8	0.024
6	64	1.5	1.0	2	30	0.000
Max.	186	2.1	10.0	12	12	0.001
Min.	4	0.0	0.0	1.0	2.0	0.0

(2) SILICA and P_2O_5 in WHITE SUGARS.

Ambler & Byall J.I.E.C. Anal.Ed. Vol.4 No.3 (1932) p.325.

SAMPLE	SILICA P.p.m.	ORGANIC P_2O_5 P.p.m.	INORGANIC P_2O_5 P.p.m.
1	8.7	0.0	0.0
2	29.0	0.8	0.3
3	21.8	1.1	trace
4	45.1	1.1	trace
5	38.5	0.5	0.1
6	9.4	0.6	trace
7	8.0	0.6	trace
8	53.0	0.3	0.3
9	29.0	1.1	trace
10	52.3	1.1	0.0

Phosphates are present in the juices of the sugar cane and of the sugar beet, and become lime salts during the manufacturing process. A small quantity of these calcium phosphates dissolves in the liquors and may occur in the final white sugar as shown above.

Organic phosphorus compounds such as lecithin and the nucleic acids also occur in sugar juices and their decomposition products (e.g. calcium glycero-phosphate) may also be detected in the final stages of sugar manufacture.

(3) CHLORIDES IN WHITE SUGARS (by Direct Determination)
Ambler & Byall, J.I.E.C. (Anal.Ed.)Vol4 No.4 (1932) p.379.

(By titration with 0.02 N. Silver Nitrate).

SUGAR	Cl- P.p.m.	SUGAR	Cl- P.p.m.
1	7.8	11	33.3
2	1.4	12	27.7
3	40.4	13	0.0
4	17.7	14	5.0
5	33.3	15	22.7
6	0.7	16	39.0
7	31.9	17	44.0
8	14.2	18	31.9
9	16.3	19	20.6
10	2.1	20	10.6

(4) SULPHATES, SULPHITES and ALDEHYDE-SULPHITES IN WHITE SUGARS.

Ambler, Snider & Byall (J.I.E.C. Vol.3 No.3 Anal.Ed.)

Sulphates occur in cane and beet sugar juices in amounts varying annually, and also varying with the soil type and climatic conditions. The amount of sulphate in the final products is also influenced by sulphates from the process chemicals and from the water used. The soluble alkali sulphates may be occluded in the crystals.

SULPHUR TRIOXIDE AND SULPHUR DIOXIDE IN WHITE SUGARS.

BEET SUGARS DIRECT PROCESS	ASH	SO_3	SO_2	
			INORGANIC	ORGANIC
	P.p.m.	P.p.m.	P.p.m.	P.p.m.
1	0.014	0.0	10.2	1.2
2	0.019	22.3	38.4	3.1
3	0.254	1188.4	46.1	6.0
4	0.092	270.1	29.4	4.6
5	0.041	145.8	18.0	0.4
6	0.009	0.0	6.1	0.9
CANE SUGARS DIRECT PROCESS				
1	0.061	249.9	29.9	2.9
2	0.012	24.0	0.9	0.0
3	0.042	44.6	1.3	2.1
4	0.015	19.0	2.2	0.8

SULPHUR TRIOXIDE AND SULPHUR DIOXIDE IN WHITE SUGARS.

REFINED CANE SUGARS.	ASH P.p.m.	SO_3 P.p.m.	SO_2 INORGANIC P.p.m.	SO_2 ORGANIC P.p.m.
1	0.004	0.0	0.6	0.0
2	0.005	0.0	0.5	0.1
3	0.006	0.0	0.4	0.0
4	0.003	0.0	0.4	0.0
5	0.010	0.0	0.6	0.0

The small amount of inorganic SO_2 indicated for refined white cane sugars is very probably not actually SO_2 but another iodine absorbing impurity. Ambler, Snider & Byall suggest that these oxidisable substances may be polyphenols or tannins which are known oxygen absorbents. Their presence in refined sugar may be due to their partial non-elimination during refining, or to the formation of polyphenols from the sugar by condensation or rearrangement during the refining process.

(5) LABILE ORGANIC SULPHUR (as Cystine) in WHITE SUGARS.

Cystine is a common cleavage product of proteids, so derivatives of this sulphur compound may be expected in the cane and beet juices, and conceivably in the final white sugar. The accompanying table shows the amounts found by Ambler (J.I.E.C. Anal. Ed. Vol.3 No.3 p.341).

LABILE ORGANIC SULPHUR AS CYSTINE.

DIRECT PROCESS BEET SUGARS	Sample	1	2	3	4	5	6	7
	P.p.m.	0.8	1.5	1.1	0.8	0.8	1.6	2.9
DIRECT PROCESS CANE SUGARS	Sample	1	2	3				
	P.p.m.	3.4	0.8	0.4				
REFINED CANE SUGARS	Sample	1	2	3				
	P.p.m.	1.4	trace	0.8				

DISTRIBUTION OF IMPURITIES IN THE SUGAR CRYSTAL.

Keane, Ambler & Byall, (J.I.E.C. 27, 30-3 1935). show that over 50 per cent of the ash, sulphates, chlorides, sodium, potassium, and total nitrogen is located in the outer 5 per cent of the crystal, whereas, colour, calcium, and sulphites are more uniformly distributed throughout the whole crystal. Screened sugars (between 30 and 50 mesh) from various sources, were mingled at room temperature with pure sugar solutions of 60 to 66.5 per cent concentration, centrifuged and washed. Analyses were made before and after mingling. A few of the results before and after mingling are shown below.

ANALYTICAL DATA ON ORIGINAL AND TREATED SUGARS.

SUGAR:-	Q	Z	A A
Per Cent SUGAR Removed by MINGLING.	10.0	6.7	6.3
COLOUR			
Original)	54	42	-
Treated)P.p.m.	48	36	-
% Removed	11	14	-
ASH			
Original)	268	260	133
Treated)P.p.m.	98	103	43
% Removed	63	60	68
SO_3			
Original)	15	23	15
Treated)P.p.m.	4	6	2
% Removed	73	74	87
SO_2			
Original)	15	27	24
Treated)P.p.m.	12	22	16
% Removed	20	18	33
CaO			
Original)	30	7	9
Treated)P.p.m.	16	6	3
% Removed	47	14	67
Na_2O			
Original)	7	20	1
Treated)P.p.m.	1	4	1
% Removed	86	80	0
K_2O			
Original)	105	110	32
Treated)P.p.m.	41	41	9
% Removed	61	63	72
Cl			
Original)	8	10	7
Treated)P.p.m.	2	4	4
% Removed	75	60	43
TOTAL N.			
Original)	-	28	15
Treated)P.p.m.	-	11	7
% Removed	-	61	53

It is to be expected that the outer shell of the crystals will contain more impurities than the interior, since these outside layers are formed during boiling from sugar liquor which is becoming progressively of higher non-sugar content.

AUTO-INVERSION OF SUCROSE: Experiments by Ambler and Byall (J.I.E.C. Anal. Ed. Vol.7 No.3 p.168, 1935) have shown that the quantity of hydrogen ions liberated from sucrose is apparently enhanced by elevation of temperature and this causes the so-called "auto-inversion" of pure sucrose by heat. The ionisation of water is also increased at higher temperatures, but this factor is of minor significance in comparison with the increased ionisation of sucrose.

Increase of ash in a sugar increases its resistance to inversion, thus sugars with ash of about 0.010 per cent or less may undergo much greater inversion than sugars of higher ash content. As little as 0.010 per cent of ash may reduce considerably the degree of auto-inversion of the sucrose.

DECOMPOSITION OF INVERT SUGAR: When heated alone or with very dilute or weak acids both the sugars comprising invert sugar pass with little discoloration into a series of anhydrides ranging from simple anhydrides such as glucosan, levulosan and w-oxymethyl furfural through reversion products

of the oligosaccharide type to highly polymerised, more completely dehydrated products of colloidal nature.

In alkaline solution, however, both dextrose and levulose undergo deep-seated and complex isomerisations and degradations accompanied by caramelisation and oxidation. These degradation products, especially those containing an aldehydic carbonyl group, undergo further polymerisations and condensations in alkaline media, forming highly coloured colloidal matter.

The nitrogenous non-sugars react readily with invert sugar to form the intensely coloured melanoidins of Maillard (Ann.Chim.5, 258, 1916).

The presence of iron salts causes the formation of deeply coloured complex compounds. Iron has long been recognised as an extremely objectionable impurity which should be eliminated from sugars as far as possible. The occurrence of iron in sugars is discussed in a later section of this work.

OTHER ORGANIC MATTER IN SUGAR PRODUCTS:-

The organic matter other than sugars which is present in a material such as raw sugar is of widely varying composition. Some of the constituents of this organic matter are often referred to as "gums" by which are meant those substances precipitated by alcohol from an acid solution of the sugar product. The remaining constituents are divided between salts of organic acids, nitrogenous bodies, caramel, and other products of decomposition of sucrose, dextrose and levulose. The composition of the other organic matter is therefore obviously extremely complex.

The gummy matters interfere with crystallisation, while proteins induce fermentation. Hazewinkel (Archief (Java) 1910, 18, p.44) mentions an instance of very high gum content in the sugar juices causing extremely slow boiling in the vacuum pans with copious formation of false grain.

The percentage of crude gums in a raw sugar is known to affect its working qualities in the refinery: there may be considerable financial loss occasioned by slow filtration and crystallisation due to high gums content.

DETERMINATION OF GUMS.

The average density of the sample solution should be arranged at approximately 50 per cent solids. For samples of high gums content 10 ccs. is taken corresponding to 5 gms. of the original product, and for samples of low gums content 20 ccs. are pipetted, equivalent to 10 gms. of the sugar product.

The sample is placed in a 250 ccs. beaker and 0.5 cc. of concentrated hydrochloric acid added for the 10 ccs. sample, or twice this amount for the 20 cccs. sample. Alcohol of 93 to 96 per cent strength is now added from a slow delivery pipette with vigorous agitation, using 50 ccs. for the 10 ccs. sample or 100 ccs. for the 20 ccs. sample.

The precipitate is allowed to settle for 15 minutes and then filtered, avoiding slow filtration. When the last drop has filtered through, the precipitate is washed with alcohol of the same strength as that used for precipitation. A Gooch crucible with asbestos mat is preferable for filtration. The precipitate is dried at 100 - 105°C. for one hour, cooled and weighed. The organic matter is burned off in a muffle furnace at low red heat, and the crucible again cooled and weighed. The difference between the weight after drying and the weight after ignition represents the dried gums. The results of the determination are dependent upon many factors; strict adherance to the technique and method are essential for comparable results. (Ruff and Withrow J.I.E.C. Vol.4 No.12 1922)

EXPERIMENTAL: (1) The following data was obtained by the author with three samples of refinery molasses, using the method just described. All analyses are shown as per cent on dry material.

CONSTITUENT	MOLASSES FROM REFINING OF:-		
	CUBA RAW SUGAR	REFINED RAW SUGAR	NATAL RAW SUGAR
GUMS	0.79	1.71	1.52
ASH (Sulphated)	5.02	6.57	5.15
SILICA in ASH	7.92	9.11	2.95
ALBUMINOIDS by KJELDAHL PROCESS	1.18	1.35	1.23

(2) The following table shows the gums content of various refinery products.

No.	SAMPLE	NORMAL BRIX°	PURITY or DIRECT POLARISATION	% GUMS on DRY	pH	% ASH on DRY
1.	Peru Raw Sugar	1.31% water	Pol. 96.2	0.28	5.6	0.63
2.	Raw Washings	65.0	81.0	1.35	4.7	3.25
3.	Washed Sugar	3.76% water	98.7	0.16	7.2	0.18
4.	Raw Massecuite	95.0	74.0	1.59	6.9	3.42
5.	Crystalliser Sugar	1.0% water	98.6	0.14	6.2	0.37
6.	Molasses	85.0	51.0	2.35	4.5	5.50
7.	Filtered Raw Liquor	63.0	97.4	0.37	7.6	0.37
8.	Refined Liquor	62.5	99.2	0.02	7.1	0.17
9.	Yellow Syrup	80.0	71.6	0.77	5.3	3.96
10.	Yellow Sugar	3.3% water	Pol. 87.3	0.25	6.0	1.55

NOTES ON THE GUMS CONTENT OF THE ABOVE PRODUCTS.

The results for refinery products are shown in order of progression of the raw sugar through the refining process. It will be observed that there is not complete elimination of gummy matter by the defecation process or by the filtration through animal charcoal which results in refined liquor.

pH VALUE.

This was determined by an Aten Quinhydrone Electrode (Aten and van Gimmeken) (Recueil Travaux Chimiques des Pays Bas 1925, p.937; 1926, p.753 and p. 792). This type has a gold-quinhydrone electrode, and porous alundum joints between the two half cells and KCl. Bridge., Readings were corrected for temperature in all cases. Frequent checks were made colorimetrically, and by means of the Harrison glass electrode. (G.B. Harrison, J.C.S. 1930 p.1528)

All sugars were made up in 50 per cent solutions for pH determination. In the table below some of the check figures for Standard Buffer solutions are shown.

COMPARISON OF STANDARD BUFFER SOLUTIONS BY

(a) ATEN QUINHYDRONE ELECTRODE

(b) HARRISON GLASS ELECTRODE

(c) COLORIMETRIC METHODS

(1) B.D.H. Capillator
(2) Hellige Comparator.

Solution	(Calculated) pH.	Quinhydrone pH.	Harrison pH.	Capillator pH.	Hellige pH.
Di-Sodium Hydrogen Phosphate & Citric Acid O.I.M.	3.0	3.09	3.16	3.2	3.2
-do-	4.0	4.08	4.02	4.3	4.3
-do-	5.0	4.87	5.11	5.3	5.2
-do-	6.0	6.11	6.06	6.0	6.1
-do-	7.0	7.19	7.19	7.2	7.3
-do-	8.0	7.99	8.00	8.1	7.9
Sodium Acetate & HCl.	1.0	0.86	-	-	-
-do-	2.0	1.79	-	-	-
Sodium Carbonate & HCl.	10.17	10.03	-	-	-
	10.68	10.96	-	-	-
	10.36	10.51	-	-	-

The Quinhydrone electrode is usually not employed above 8-9 pH, as considerable errors may occur due mainly to potential drift. The colorimetric method is often limited by the number of indicators at hand, the greatest accuracy generally being obtainable where a value occurs at the middle of a range, or where two indicators overlap at an end of their ranges.

pH values of refined cane sugars.

Sugars representing the full range of white refined products from a sugar refinery were tested for pH value by the four methods below.

GRADE OF SUGAR	Quin-hydrone Electrode	Harri-son Glass Electrode	B.D.H. Capill-ator	Hellige Compar-ator.
Crystal 1.	6.71	6.76	6.9	6.8
Crystal 2.	6.80	6.93	6.9	6.8
Grade 1. Granu-lated	6.83	6.80	6.7	6.8
-do- 2. -do-	6.59	6.67	6.7	6.8
-do- 3. -do-	6.60	6.62	6.4	6.5
-do- 4. -do-	6.56	6.52	6.5	6.5
Manufacturing Crystals	6.53	6.59	6.6	6.6
Off-Colour	6.37	6.33	6.4	6.3
Cubes 1.	6.57	6.59	6.6	6.6
Cubes 2.	6.52	6.61	6.6	6.6
Castor sugar	6.88	6.92	6.8	7.0
Z Granulated	6.26	6.28	6.3	6.4

These values are all on the acid side, but are not dangerously acid. The "off-colour" sugar would probably deteriorate more quickly than the others.

pH VALUES IN A SUGAR REFINERY

(Blowski and Holven "Sugar" Feb., 1927).

PRODUCT	pH.
Unfiltered Raw Liquor	7.35
Filtered -do-	7. 2
Av. of liquids char	7. 0
Refined liquor after filtration (animal)	(7. 0 to
Refined liquor after filtration (charcoal)	(6. 3
Sweet water from filtration (animal)	(5. 5 to
Sweet water from filtration (charcoal)	(5.75
No.1 remelt massecuite	6. 9
No.1 -do- syrup	6. 4
No.2 -do- -do-	5.95
No.1 -do- sugar	6.65
No.2 -do- -do-	6.20
No.3 -do- -do-	5.35

Only a very narrow range of pH is permissible in refining cane sugar. If the alkalinity is excessive, reducing-sugars are decomposed with the formation of objectionable colouring matters, and of organic acids which may result in the original acidity of the material being restored, and in any case will leave melassigenic lime salts in process, thus diminishing the yield of crystallisable sugar. On the other hand, if not enough lime is used, acidity may cause considerable inversion of sucrose with resulting loss of sugar yield.

pH VALUES in BEET SUGAR FACTORIES OF FRANCE

SEASON 1927-28. E. Saillard.

PRODUCT	pH	SUGAR %
Raw Diffusion Juice	5.00	13.40
Battery waste pulp	6.05	0.18
Pulp press water	3.48	0.36
1st Carbonatation Juice	10.70	11.46
2nd -do- -do-	9.00	13.44
Juice before Sulphitation	8.47	52. 6
-do- after -do-	7.95	52. 0
1st Massecuite	8.77	77.2
1st Wash Syrup	8.80	63. 4
1st Green Syrup	8.70	64. 0
2nd Massecuite	8.30	68. 0
Molasses	8.28	51. 9
2nd Sugar	8.00	94. 9
Final white sugar	7.50	99. 9

The values are chiefly in the alkaline range, and the final sugar is distinctly above neutral-point.

P A R T 2.

THE DETERMINATION OF SULPHUR DIOXIDE IN SUGAR PRODUCTS.

The presence of sulphur dioxide in sugar products became important when a legal limit was prescribed in 1926. In this section of the work, experiments on the principal methods for determining sulphur dioxide are recorded.

DETERMINATION OF SULPHUR DIOXIDE IN SUGAR PRODUCTS.

The total free and combined sulphur dioxide (SO_2) in sugars must not exceed 70 parts per million (or 0.007 per cent) according to a British Public Health Regulation which came into force on January 1st, 1927.

This directed attention to the determination of SO_2 in sugars, and many methods were put forward, chief among which were -

1. The Distillation Method (Monier Williams, Public Health Report No.3, 1927),
2. The Stain Method (I.S.J. 1926, 644),
3. The Direct Titration Method (Ripper, J. Prakt. Chem. 2, 46, 428 (1892), and
4. Direct Precipitation as Barium Sulphate (Blarez and Tourrou, J. Pharm. Chim. 9.533, 1899).

At the eighth session of the International Commission for Uniform Methods of sugar Analysis held in 1932 (I.S.J. 1933, p.62) it was resolved that the direct titration method with iodine was satisfactory for routine testing, but that for exact determinations the sulphur dioxide should be distilled from the acidified solution after expulsion of the air with carbon dioxide; the distillate should be collected in a dilute solution of hydrogen peroxide, bromine water, or iodine solution, or in sodium bicarbonate

solution with subsequent oxidation, the sulphuric acid formed being estimated gravimetrically.

Many criticisms have been made of the methods for the determination of sulphur dioxide in food products. Thus the Stain Method is stated to have the disadvantage of being not specific for sulphur dioxide, many other sulphur-containing compounds reacting in a similar manner. The distillation method has also been criticised on the grounds that the oxidation procedure which forms part of the method is not limited to sulphur dioxide, as other compounds respond to the oxidation treatment.

Sulphur dioxide reacts with many of the products with which it comes into contact, and causes an immediate loss of preservative, partly by oxidation and partly by direct combination with the food material. This missing proportion may or may not reappear during the estimation. Some foodstuffs show the presence of sulphur dioxide when none has actually been added, others, which originally gave no test for sulphur dioxide will show appreciable amounts on standing. The direct titration method with iodine is affected by any other substance present which reduces iodine.

It is therefore difficult to find a method which is satisfactory with all samples examined.

APPLICATION TO SUGAR PRODUCTS:

Pure sucrose is stated not to combine with sulphur dioxide and to be negatively catalytic in action, retarding oxidation of the sugar by air.

Commercial sugars may contain iodine absorbing substances other than sulphite in amounts sufficient to interfere with direct titration.

With raw sugars, the amount of sulphur dioxide present is very variable and normally depends on the process of clarification used in the raw factory and on how efficiently this process is controlled.

Coghill (I.S.J. 1930, p.359) shows a range of SO_2 content for 104 samples of South African raw sugars of 14 to 288 p.p.m. for the iodine titration method, and of 4 to 370 p.p.m. for the distillation method. In the case of a Natal sugar made by the carbonatation process iodine titration gave 13.3 parts per million, and distillation 5.5 p.p.m. It was demonstrated that some iodine-reducing substance other than sulphur dioxide was present, by absorbing the distillation products by the Monier-Williams Method (I.S.J. 1927, 571).

These iodine-absorbing impurities are probably constituents of the organic matter present in the raw sugars e.g. volatile aldehydes and ketonic acids.

EXAMINATION AND COMPARISON OF METHOD FOR THE DETERMINATION of SULPHUR DIOXIDE IN SUGAR PRODUCTS.

The methods of distillation and direct titration, and the Stain method were investigated.

1. STAIN METHOD: The apparatus used was a modified form of that applied in the laboratories of Tate and Lyle Ltd., and described by Ogilvie (I.S.J. (1927) 305, 331, 371). The test consists of reduction of sulphite to sulphuretted hydrogen by means of nascent hydrogen generated from zinc and acid. The mixed gases are passed through lead acetate paper and the stain produced on the paper is compared with standard stains for evaluation of the sulphite content expressed as parts SO_2 per million.

APPARATUS: A flat bottomed flask of 400 ccs. capacity is provided with a two-holed cork carrying a tap-funnel and an ordinary funnel. To the ordinary funnel a metal clamping device is attached by means of which a filter paper is tightly fixed over the mouth of the funnel. A cotton wool plug is fitted in the stem of the funnel.

REAGENTS: Arsenic-free "Analar" Zinc (washed with arsenic-free hydrochloric acid), anhydrous "Analar" sodium sulphite, and dilute arsenic-free hydrochloric acid (1:2 water) are required.

STAIN PAPERS: Whatman No.1 Filter papers, 9 cms. diameter are soaked in a 25 per cent solution of neutral lead acetate, dried slightly in air, and kept moist in a desiocator over wet pumice.

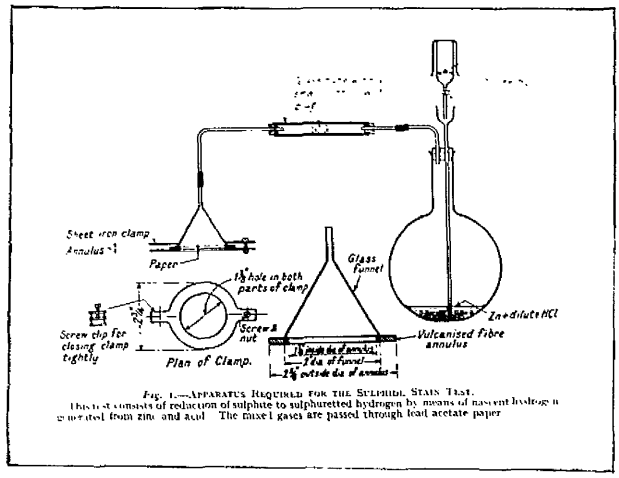


Fig. 1.—Apparatus Required for the Sulphuretted Stain Test.
This test consists of reduction of sulphate to sulphuretted hydrogen by means of nascent hydrogen generated from zinc and acid. The mixed gases are passed through lead acetate paper

FIG. 4

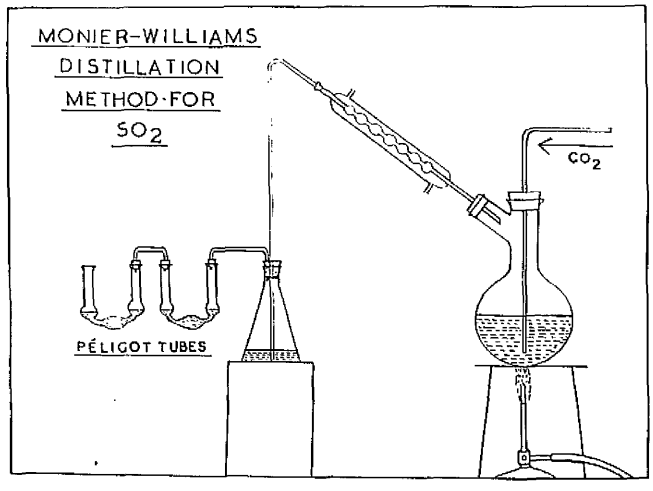


FIG. 5

PROCEDURE: In the flask are placed 50 gms. of zinc, 5 gms. of the sugar product under examination and 5 ccs. of water. The tap funnel is charged with 50 ccs. of the dilute hydrochloric acid, and the other funnel is fitted with a lead acetate paper. The hydrochloric acid is now slowly run in, and the gas evolved is allowed to pass through the lead acetate paper for 30 minutes. The stain produced is then compared with standard stains. These standards are replaced monthly.

BLANK TEST: The above procedure is followed without sample. The zinc is rarely free from sulphur and the preliminary wash with dilute acid is to remove this sulphur.

PREPARATION OF STANDARD STAINS:

Sodium Sulphite Solution:- 0.06 gm. of "Analar" sodium sulphite is added to a sugar solution made by dissolving 300 gms. of pure sucrose in 300 ccs. of distilled water. The strength of this sulphite solution is checked by addition of 4 ccs. of standard iodine solution, titrating the excess against standard thio-sulphate solution. The sulphite solution must be diluted again for direct use.

Standard stains are prepared representing from 1 to 70 parts of SO_2 per million.

NOTES: Sulphides, if present can be distinguished by replacing the zinc by marble.

The distillation test is normally only resorted to when the stain test shows SO_2 content of the sample to be above the limit of 70 parts per million.

RESULTS OF DTERMINATIONS OF SULPHUR DIOXIDE IN SUGAR PRODUCTS.

No.	SAMPLE	gms. TAKEN	SO_2 PARTS PER MILLION
1.	Mauritius raw sugar	2.5	36
2.	San Domingo " "	5.0	below 1
3.	Java " "	5.0	below 1
4.	Australian " "	15.0	10
5.	Cuba I. " "	5.0	13
6.	Natal " "	1.5	60
7.	Peru " "	5.0	10
8.	Cuba II. " "	5.0	13
9.	Canadian Yellow "	5.0	2
10.	British " "	100.0	below .
11.	Beet White Sugar I.	20.0	5
12.	Beet White Sugar II.	5.0	16
13.	Beet White Sugar III.	5.0	17
14.	Beet White Sugar IV.	5.0	15
15.	Beet White Sugar V.	5.0	18
16.	Beet 2nd Product Sugar	5.0	19
17.	Golden Syrup I.	5.0	10
18.	Golden Syrup II.	5.0	7
19.	Refinery Molasses I.	20.0	15
20.	" " II.	5.0	29
21.	Liquid Glucose	0.5	700
22.	American Cane Syrup	5.0	24
23.	Refined White Sugar	100.0	1/20
24.	Refinery Yellow Syrup	5.0	20

NOTE ON THE STAIN METHOD: The clamp must be screwed very tight on the filter paper to avoid leakage. Leaks are shown sometimes on the final paper by a stain round the edges. Similarly the papers should be examined for perforations or thin spots since the gas will take the line of least resistance.

TESTS ON THE STAIN METHOD:

EXPERIMENT 1.

(a) The stain method was applied using 5 gms. of Mauritius Raw Sugar. Result:-
SO_2 = 36 parts per million.

(b) A new paper was inserted and the test continued for half-an-hour. A second stain was obtained. Result:-
SO_2 = 20 parts per million.

(c) A third paper was inserted and the contents of the flask boiled for half-an-hour under reflux. A third stain darker than in B was obtained. Result:-
SO_2 = 25 parts per million.

(d) A fourth paper was inserted and the apparatus left overnight, the flask being allowed to cool. No stain was obtained.

EXPERIMENT 2. A test was run as follows:-

The zinc and distilled water were brought to the boil and 5 gms. of the Mauritius Raw Sugar washed into the flask with boiling water. The

apparatus was now assembled and the acid added, the test being continued in the normal way, allowing the flask to cool.

A fresh paper was inserted at the end of half-an-hour and the test continued for a further half hour.

Result:- 1st Half Hour SO_2 = 36 parts per million.

2nd " " SO_2 = 20 " " "

The results of these experiments demonstrate the need for standardisation of time and conditions in the stain test.

DIRECT TITRATION METHOD FOR SO_2 IN SUGARS.

PROCEDURE: 20 gms. of the test sample are dissolved in water and 50 ccs. of $\frac{N}{100}$ iodine solution added. The excess iodine is titrated with $\frac{N}{100}$ Thiosulphate using fresh starch indicator.

NOTE: When the titration is complete it is best to check it by bringing back the blue colour by addition of $\frac{N}{100}$ iodine, noting the amount required.

The $\frac{N}{100}$ solutions are best made up each day by dilution of accurately standardised $\frac{N}{10}$ solutions.

The amount of $\frac{N}{100}$ iodine added initially can be graded to suit the approximate sulphur dioxide content of the sample being tested.

SULPHUR DIOXIDE CONTENT OF SUGARS

BY DIRECT TITRATION METHOD

Using 20 gms. of sample

No.	SAMPLE	SO_2 PARTS per MILLION	PARTS PER MILLION STAIN METHOD	DIFFERENCE p.p.m.
1.	Mauritius Raw Sugar	64	36	28
2.	Natal " "	83	60	23
3.	Peru " "	112	10	110
4.	Cuba I. " "	27	17	10
5.	Beet White Sugar III	41	15	26
6.	" " " IV	34	18	16
7.	" " " V	34	17	17
8.	Beet and Product Sugar	101	19	82
9.	Golden Syrup I.	63	10	53
10.	Liquid Glucose *	720	700	20

* 1 gm. of sample taken.

DISCUSSION:

It is apparent from these contrasted results that the direct titration method gives higher results than the stain method, and that there is no uniformity in the differences between the two methods. The direct titration method is difficult to use where the sample is coloured.

The best application of direct titration would be as a sorting-out test, since if the sulphur dioxide found by direct titration is below the permissible limit it would be safe generally to assume that the content by other methods of estimation would also be below the limit.

The liquid glucose does not appear to contain any other iodine-absorbing substance, but the error of direct titration is considerable with the other products.

THE DISTILLATION METHOD FOR ESTIMATION OF SULPHUR DIOXIDE IN SUGARS.

The method due to Monier-Williams (loc. cit.) was used and is here described.

APPARATUS: A round bottomed flask of 1500 ccs. capacity provided with two necks is connected with a sloping reflex condenser which leads to a 200 ccs. conical flask followed by two Péligot tubes. The tube which enters the conical flask reaches right to the bottom of the flask. The conical flask contains 10 ccs. of pure ten volume (3 per cent) hydrogen peroxide, free from sulphuric acid. The first Péligot tube also contains 10 ccs. of hydrogen peroxide, and the second contains 5 ccs. of a mixture of hydrogen peroxide and barium chloride acidified with hydrochloric acid. This second tube acts as a guard tube for the other two: any sulphur dioxide reaching it will produce turbidity.

PROCEDURE: The apparatus is connected up and 500 ccs. of distilled water and 20 ccs. of hydrochloric acid are placed in the flask. This solution is boiled for a short time in a current of pure CO_2 to remove air, the CO_2 being led in through the second neck of the flask. The sample is now introduced through the neck of the flask. The mixture is then boiled for one hour in a slow current

of carbon dioxide. Just before the end of the distillation the flow of water in the condenser is stopped, which causes any sulphur dioxide retained by condensed moisture in the delivery tube to be driven over into the receiver.

The connecting tubes are washed down with a little water and the contents of the first Péligot tube transferred to the conical flask. The liquid, which will amount to about 50 ccs., is then titrated in the cold against $\frac{N}{10}$ sodium hydroxide solution using bromphenol blue (pH range 2.8 to 4.6) as indicator. If desired the titration result may be checked by a gravimetric determination as barium sulphate.

COMPARISON OF SULPHUR DIOXIDE RESULTS BY 3 METHODS.

No.	SAMPLE	SULPHUR DIOXIDE PARTS PER MILLION		
		DISTILLATION.	STAIN METHOD.	DIRECT TITRATION.
1.	Mauritius Raw Sugar	37	36	64
2.	Natal " "	56	60	83
3.	Peru " "	17	10	112
4.	Beet White Sugar III.	16	15	41
5.	Golden Syrup I.	15	10	63
6.	British Yellow Sugar	8	Below 1	-

If the distillation results are accepted as correct it is seen that the Stain Method has given nearly correct results for each sample examined. The direct titration method is least accurate. In connection with the direct titration procedure it should be observed that in general the "combined" sulphite contained in the sample must first be decomposed by preliminary digestion with cold sodium hydroxide followed by acidification and immediate direct titration. This combined sulphite may exist in aldehydic, ketonic, or ester form, and it is very probable that such stable sulphite compounds with sugars and starch and perhaps cellulose, are quite inert physiologically. This is supported by the known fact that they no longer inhibit mould-growth.

DISTILLATION RESULTS USING VARIOUS OXIDISING AGENTS.

Distillation tests were made on Mauritius raw sugar using the following oxidising agents:-

(a) Iodine

(b) Bromine water

(c) Hydrogen Peroxide

(d) Sodium perborate.

(a) IODINE: The Monier-Williams Apparatus was used with 500 gms. of Mauritius Sugar, the distillate being led into a solution of 0.5 gm. of iodine and 0.75 gm. potassium iodide in 100 ccs. distilled water. The sulphate was precipitated in presence of the iodine solution and estimated gravimetrically.

(b) BROMINE WATER: As in (a), receiving the distillate in a flask containing 50 ccs. of bromine water. The bromine water was boiled off and the sulphate estimated gravimetrically.

(c) As in (a), but 50 ccs. of hydrogen peroxide solution, containing 10 ccs. of 100 volume hydrogen peroxide was used as oxidising agent.

(d) As in (a), using sodium perborate in place of the iodine.

NOTE: All reagents were tested for sulphate, and where found (hydrogen peroxide and sodium perborate) a blank test was applied and appropriate corrections made.

RESULTS: MAURITIUS RAW SUGAR.

No.	REAGENT	SULPHUR DIOXIDE PARTS PER MILLION.
1.	Iodine	38
2.	Bromine Water	26
3.	Hydrogen Peroxide	37
4.	Sodium Perborate	41

The results for this sugar by the stain method was 36 p.p.m. and by direct titration 64 p.p.m. The bromine water method has given an inexplicably low result.

SUMMARY: The three principal methods of determining sulphur dioxide in sugars have been examined and compared and results are shown for various sugar products. The distillation method (using hydrogen peroxide as oxidising agent) need only be used when the sulphur dioxide found is close to or above the maximum amount legally allowed, since results by the stain and direct titration methods normally appear to be high.

P A R T 3.

THE DETERMINATION OF REDUCING SUGARS.

The electrometric and the methylene blue volumetric methods of determining reducing sugars have been examined. A permanent sintered glass cell devised by the author is described. A study has been made of various factors affecting reducing sugar determinations.

THE DETERMINATION OF REDUCING SUGARS.

GENERAL INTRODUCTION.

LITERATURE:-

The literature relating to the determination of reducing sugars is very voluminous, and the methods proposed are more varied and have been subject to more revision than probably any other study in sugar analysis.

Many of these modifications are described by Browne, (Handbook of Sugar Analysis, John Wiley & Sons, 1912), and some of the methods are described in detail in the present work.

SMOLENSKI'S CHART:-

A chart drawn up by K. Smolenski of the Polish Sugar Institute is shown on page 74 with the appropriate references. This chart was presented at the sixth session of the International Commission for Uniform Methods of Sugar Analysis held in London in 1936. It outlines the principles of the chief methods designed for use in the chemical control of sugar manufacture.

In general the methods of this chart were not put forward as suitable especially for the estimation of small amounts of reducing sugars such as found for example in biochemical analysis. As a result a distinct

set of methods is found in this type of work.

REDUCTION METHODS:-

The principal chemical methods for determining reducing sugars are based upon the property which all aldehydes and ketones possess of reducing alkaline solutions of certain metallic salts.

Trommer (Ann.39, 360,) in 1841 first introduced alkaline copper sulphate as a reagent to distinguish dextrose from sucrose, and his method was improved by Barreswill in 1844 (J.Pharm [3] 6, 301) by the addition of potassium tartrate which greatly increased the stability of the reagent by preventing precipitation of cupric hydroxide.

In 1848, Fehling [Ann. 72, 106, 1849: 106, 75,1858] introduced a quantitative method based on Barreswill's improved reagent, and gave stoichiometrical equivalents between copper and dextrose.

OTHER METHODS:-

There are other methods for estimating invert sugar. One of them is based on the reaction of Iodine with reducing sugars. Dextrose and Levulose take up definite quantities of Iodine thus:-

$$C_6H_{12}O_6 + 4KOH + 3I = C_5H_{10}O_5CO_2K + 3KI + 3H_2O$$

The excess of Iodine is titrated with N/10 thiosulphate, 1 cc. of which equals 0.009 g. of glucose. The

method is not applicable when these sugars are to be estimated in presence of excess of sucrose.

Oxidising methods employing potassium permanganate, chromic acid, silver salts or bromine have not been brought to the stage of practicability. The oxidation process cannot be sharply limited to a definite phase in the decomposition of, say, dextrose, the sugar generally being transformed into an indefinite mixture. The ideal reagent would be one which changed glucose to another definite substance.

TWO TYPES OF METHOD:-

It will be seen from Smolenski's chart that methods may be divided into two classes - volumetric and gravimetric.

In both cases, despite numerous attempts to improve upon the original proposals (e.g. of Fehling and Sohxlet), there is still no method which will give perfect quantitative results in the analysis of mixtures of common sugars.

REDUCTION OF COPPER SALTS:-

The methods in general use to-day employ alkaline cupric solutions almost exclusively, reactions in the case of other metals such as mercury or silver being seldom quantitative.

Even with copper solutions, the oxidation of say,

dextrose, is not governed by a definite stoichiometric reaction, and the ratio of 1 molecule of dextrose to 5 of copper which was regarded as constant by Fehling was shown by Sohxlet in 1878 [J.Prakt.Chem[2] 21, 227] to vary according to the excess of copper present during the reaction.

FRAGMENTATION:-

These tests and determinations are always conducted in strongly alkaline solution where the sugar molecules are split into fragments which are active reducing agents.

Hence reducing power is actually a measure of the extent of fragmentation under a given set of conditions.

This explains why glucose reduces approximately four times as much of a cupric salt as would be calculated if the reaction were a simple oxidation of the carbonyl group to carboxyl. It also explains why isomeric sugars under parallel conditions have different reducing powers.

Accurate determinations depend upon reproducing the same conditions of temperature, time, concentration, etc., during the reduction.

REDUCING POWER:-

The reducing power of different sugars is not the same; for example:-

0.50	gm.	of	invert-sugar	reduces	101.2	ccs.	of	Fehling's solution.
0.50	"	"	glucose	"	105.2	"	"	"
0.50	"	"	lactose	"	74.0	"	"	"
0.50	"	"	fructose	"	97.2	"	"	"

The reducing sugars present in sugar factory products consist generally of more or less equal amounts of dextrose and levulose.

The Fehling-Sohxlet solution is always standardised under exactly the same conditions as those of the test, against a neutral standard invert sugar solution.

ALKALINITY OF REAGENT:-

The alkalinity of the copper reagent is a most important factor as it greatly influences the behaviour of the reagent towards reducing sugars, and towards any sucrose which may be present with it. The copper reagents thus fall into two groups, those of high alkalinity and those of low alkalinity. Saillard has shown that the higher the alkalinity, the more rapid is the rate of reduction, hence it could be said that it is better to use copper reagents of high alkalinity. However, in sugar products there is always sucrose present which may amount to hundreds of times the quantity of invert-sugar, and this in presence of alkali also causes appreciable reduction of cupric copper to cuprous. The higher the alkalinity

the greater the extent of reduction. For this reason alkali carbonates and bicarbonates are substituted for the hydroxides in many copper reagents and in these the action of the alkali on sucrose is very small.

It is considered by Quisumbing & Thomas (J.Am.C.S. P.1503 (1921). that sodium hydroxide in Fehling's solution gives a more satisfactory precipitation than when potassium hydroxide or the carbonates are used.

When sucrose is present in a solution along with invert-sugar, the caustic alkali of the copper solution attacks the sucrose and reducing substances are formed which reduce the solution themselves. This action decreases with increase of concentration of the sucrose on account of the simultaneously increasing viscosity of the solution which tends to immobilise the hydroxide ions.

TEMPERATURE OF REDUCTION:-

The higher the temperature of the reduction, the greater its rate, but at the same time the greater is the action of the alkali on the sucrose. To minimise this action of alkali many of the methods use a reaction temperature lower than the boiling point of the reaction mixture, generally the boiling

point of water, and Saillard's method which uses a solution of high alkalinity, completes reduction at a temperature of 62°. Thus, according to the temperature of the experiment, the methods may be further divided into two groups - those in which the reduction is carried out at the boiling point of the reaction mixture and those in which a lower temperature is used.

MODE OF HEATING.

COMPARISON OF LANE AND EYNON'S METHOD WITH THE METHOD OF HEATING IN A WATER-BATH.

Ofner (I.S.J. 1926 p.618) makes the following objections to the volumetric method where the reaction liquid is boiled :-

(1) The oxidation is inconstant in presence of sucrose.

(2) Deviations of procedure markedly affect the results.

(3) Reduction varies greatly with rate of boiling.

(4) There may be great variation in the time taken to come to boiling point.

(5) Certain impurities influence the cuprous oxide separation.

E. Saillard makes this remark "One can reach boiling-point, or boil, in a manner which varies considerably". R.F. Jackson (I.S.J. p.444, 1929) states "Volumetric methods have one chief fault - the reaction time must of necessity vary while the end-point is being sought".

Lane and Eynon (I.S.J. p.559, 1926), give their objections to water bath heating in these words "In a volumetric method in which Fehling's solution is titrated to exhaustion , the risk of back-oxidation seems to make water bath heating unsuitable for accurate work".

OTHER FACTORS IN REACTION:-

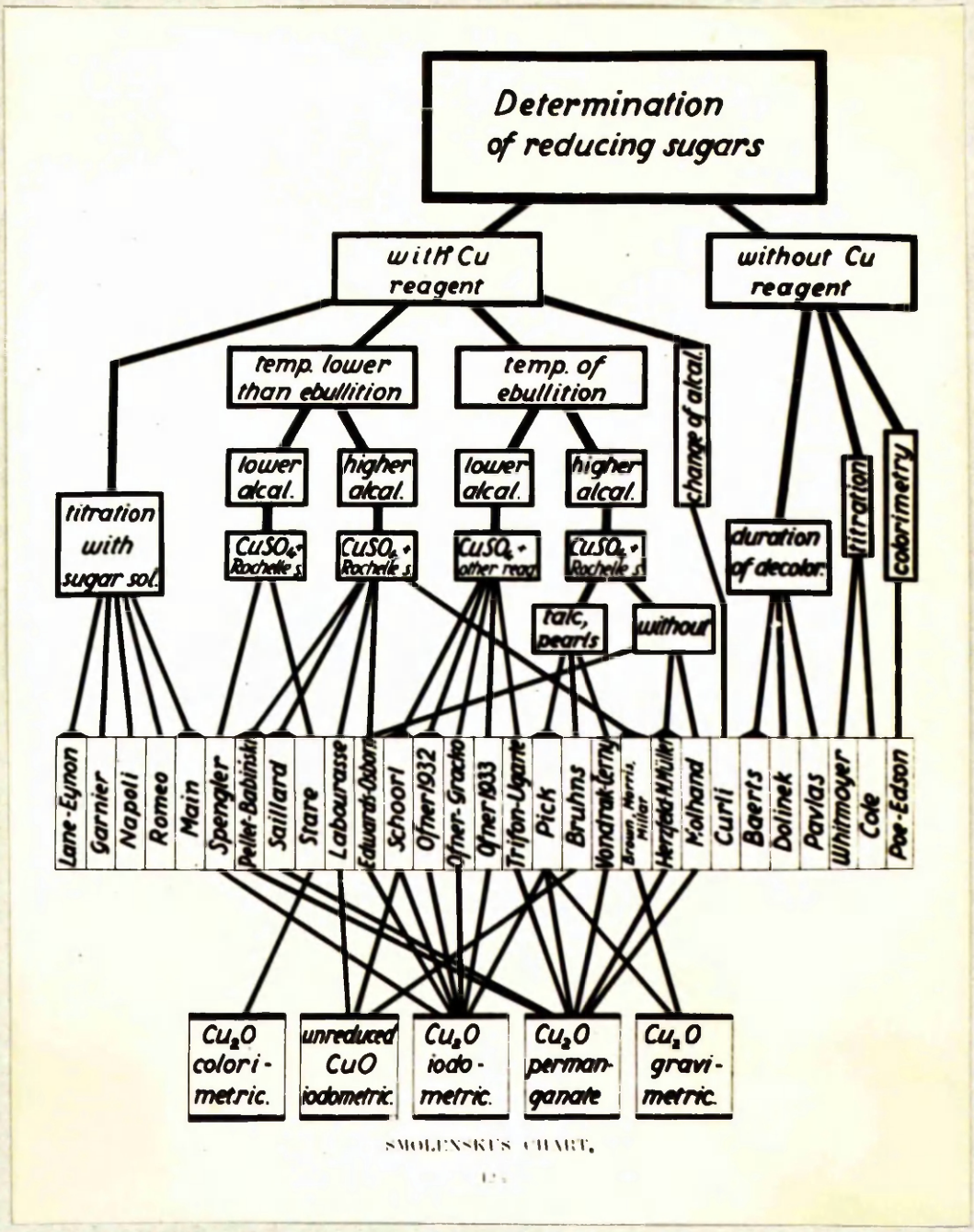
The time of heating is an important factor and must be accurately controlled. The heating time may be divided into the time necessary to bring the solution to boiling point, and the time of actual boiling required for completion of the reaction.

Other minor factors which affect the results are the shape of the flask used, the nature of its surface, and the use of materials such as pumice powder or talc, used to help even ebullition, and prevent super-heating.

The relative convenience of the method must also be considered - for example whether it employs a one-solution or two-solution reagent, and whether the reagents require frequent standardisation.

Back-oxidation may cause error if the temperature of heating is below boiling-point, or where boiling is required, if the boiling be interrupted.

The chemicals used in the reagent should be the purest obtainable.



SMOLENSKI'S CHART.

FIG. 6

REFERENCES TO METHODS NAMED IN SMOLENSKI'S CHART OPPOSITE (IN ORDER FROM LEFT TO RIGHT.)

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INCIDENCE OF ERROR IN REDUCING SUGARS ESTIMATIONS.

Consideration of the controlling factors described shows that each factor is a potential source of error.

The chief errors involved are :-

(1) Reducing action of sucrose.

(2) Auto-reduction of the copper solution.

(3) Lack of exact control of temperature (a) Barometric pressure.
(b) Super heating.

In addition certain other factors must be controlled such as :-

(1) Degree of Dilution of solution.

(2) Time of boiling or heating.

(3) Surface area of Solution.

Empirical tables have been compiled relating to titrations at various concentrations, and allowing for the reducing action of sucrose under the strictly standardised conditions of the given method (e.g. Brown, Morris & Millar J.Chem.Soc.1897, 71, 281; Munson & Walker, J.Am.Chem. Soc. 1906, 28, 665; Lane & Eynon, (J.S.C.I.1923,34T, 143T & 463T.)

It is apparent that the conditions of analysis must be exactly complied with, since any change may render the tables useless. The reactions involved are so sensitive that the amount of copper reduced

is influenced to a great extent by the excess of copper remaining in solution, as well as by the above factors.

With pure sugars such as dextrose and invert sugar, the error introduced by slight deviations from the standard procedure is not great, but with impure sugar solutions such as molasses, the error caused may be considerable.

DILUTION ERROR:-

The same degree of dilution should be maintained for the mixed copper reagent in all experiments.

Sohxlet found that 0.5 gm. of dextrose reduced 105.2 ccs. of Fehling's Solution when undiluted and only 101.1 ccs. when diluted with 4 parts of water: similar results were obtained with other sugars.

Such a difference might produce a variation of several per cent in the reducing sugar value.

It is also evident that to obtain the most concordant results, the sugar solution should be diluted to contain approximately the same percentage of reducing sugars - i.e., for a given volume of Fehling's solution, the titration figures for different samples should be within narrow limits, for example 30 to 35 ccs.

That this error due to dilution with water is not fully appreciated in the sugar industry has been

apparent to the author for some time. Thus in one sugar refinery dilution of the 10 ccs. mixed Fehling's solution with an indefinite amount of water (approx. 20-30 ccs.) was commonly practised. In another refinery, 15 ccs. of water was used with 10 ccs. mixed Fehling's solution and 2.5 ccs. of Fehling's solution was used in titrating refined liquors of low reducing sugar content. In yet another refinery, the Lane and Eynon method was used without accurate timing and for the purer products only, the outside indication method with ferrocyanide being used for dark solutions.

A fourth refinery has adapted the Lane and Eynon method with dilution as follows:- The usual incremental titration is made, and from the number of ccs. used, the amount of water is calculated which is necessary to make the total volume 70 ccs. at the end of the titration. Thus if the preliminary titration is 30 ccs. 40 ccs. of distilled water is added in the cold before proceeding with the final titration.

This variation of procedure is bound to cause variable results from one laboratory to another.

In a paper by Whaley in "The Planter and Sugar Manufacturer" Vol.LXXX. No.3.Jan.1928, dilution of ten ccs. of mixed copper solution to "about 100 mils" is recommended. For "such material as raw sugars the desired result may be obtained by a larger dilution,

say to 150 mils, while with blends containing honey, glucose--- a smaller volume can be maintained".

Fischer and Hooker (Journal of Laboratory and Clinical Medicine 1918, 3, no.6) point out that "the different colours observed in the reduction of Fehling's solution by reducing substances are nothing more than colour changes coincident with a gradual increase in the size of the copper oxide particles".

Addition of a hydrophilic colloid to the solution will much delay the rate at which the various colours are obtained, and in fact, the copper oxide may be stabilised in any of its various states of sub-division by adjusting the concentration of the solution.

"The scientific basis of the old trick of diluting heavily the material to be examined whenever questionable reductions are obtained is easily seen. Dilution not only dilutes the highly concentrated reducing substance, but more important, it reduces the stabilising colloids to a point where their powers in this direction are largely lost".

GENERAL INTRODUCTION TO TYPICAL METHODS:-

Volumetric:- Sohxlet heated Fehling's solution in an open dish and added sugar solution until all the copper was reduced. Greater accuracy was made possible by using a closed vessel. The change from blue to colourless in the supernatant liquid was the

end-point. Later potassium ferrocyanide was used as an external indicator to find the end-point.

These methods were only moderately accurate because of the somewhat indefinite end-point, and, with ferrocyanide, inaccuracies due to the time required to filter off a portion of the solution for the end-point test.

The methylene blue modification due to Lane & Eynon J.S.C.I.(ibid)was developed in 1923 to remedy these defects. This method uses methylene blue as an internal indicator, the blue dyestuff being reduced to the colourless leuco compound when it has reached a characteristic potential. The end-point is very sharp with pure or relatively pure sugar solutions, but as it is sometimes obscured by the cuprous oxide precipitate in the boiling solution a little practice is necessary to distinguish it with certainty. Occasionally with dark solutions the end-point is difficult to see, particularly when the sugar solution is gummy or colloidal - e.g. molasses. In addition the method is usually limited to the hours of daylight, as the colour change is not clear by artificial light.

To overcome these troubles, the electrometric method due to Tryller [Zeitsch.Spiritus.ind.52,p.27,1929] [Saint, I.S.J.p.353 (1932)], has been adopted by the Queensland Bureau of Sugar Experiment Stations [Laboratory Manual, 1934].

C.R.von Stieglitz and L.C. Home (Proc. Queensland Soc. Sugar Cane Techn.1936, 101) have published results showing very close agreement between the methylene blue and the electrometric methods.

More recently de Whalley [Int.Sug.J.1939,P.312.] has described a modification of the electrometric method heating in a water bath at 80 °C in place of boiling the solution.

It is clear that a method of analysis dependent upon oxidation/reduction potential such as is that of estimating reducing sugars can be performed by any method which affords:-

(1) A colour-change in the system. (colorimetric)

(2) A possibility of measuring the potential at the end point. (potentiometric titration).

(3) Reversal of the sign of the charge at the end point. (electrometric "null-point" method).

(4) A Precipitation. In this case the estimation may be completed colorimetrically, volumetrically or gravimetrically or by any other means which measures the change.

Method (2) has been described by Britton & Phillips (Analyst 65, 18,1940) for the potentiometric titration of glucose.

The precipitation method has been extensively used, the general procedure being to boil a known volume of sugar solution with excess of copper solution, collect

the cuprouss oxide precipitated and estimate the amount of copper by one of several methods.

In the following pages, the method of Lane and Eynon and the electrometric method are described. The conditions of testing observed in the experimental work on these two methods are also described.

CONDITIONS OF TESTING OBSERVED IN EXPERIMENTAL WORK ON REDUCING SUGARS.

(1) The various pipettes used were standardised, and were the same ones throughout these tests.

(2) For Lane & Eynon's method:- 3 drops of methylene blue were used in all cases.

(3) The standard b[illegible]s provided with a capillary jet, [illegible]wance was made for drainage.

(4) Distilled water was used in all tests.

(5) The boiling-point was taken as the point when bubbles appeared at the edge of the solution in the flask in addition to those first appearing in the centre.

(6) "Analar" reagents were employed throughout.

(7) Each result is the mean of at least 3 titrations.

(8) The gas flame was regulated by a water manometer in all the experiments.

(9) All filtrations were made in 250 ccs. round "Monax" resistance glass flasks with flat bottom.

(10) The flask was not removed from the flame during any titration: asbestos covered wire gauze was used.

(11) The copper solution was measured always from a micro-burette; the alkaline tartrate solution was pipetted.

(12) All titrations were performed in daylight, but not in strong sunlight.

LANE AND EYNON'S METHOD FOR REDUCING SUGARS.

Reagents:-

Methylene Blue:- 1 gram of methylene blue dissolved in distilled water and diluted to 100 ccs. The methylene blue should be the best biological stain quality.

FEHLING-SOHXLET SOLUTION:-

[A] Copper Sulphate Solution:- 34.639 gms. of "Analar" (analytical reagent) $CuSO_4.5H_2O$ dissolved in distilled water and made to 500 ccs.

[B] Alkaline Tartrate Solution:-

173 gms. "Analar" Rochelle salt

50 gms. "Analar" Sodium Hydroxide

dissolved in distilled water and diluted to 500 ccs.

The solutions A and B are kept separately and mixed in equal proportions immediately before use.

STANDARD INVERT SOLUTION:-

9.5 gms. of the purest obtainable refined sugar is dissolved in distilled water, 5 ccs. of concentrated Hydrochloric acid (sp.gr.1.19) added, and the solution diluted to about 100 ccs. The solution is allowed to stand for 2 or 3 days if the temperature is over 20°C., or a week if the temperature is lower. It is then diluted to 1 litre without neutralising and transferred to a stock-bottle. The solution is sufficiently acid to arrest development of micro-organisms and will keep for several months. If a mycelial growth does develop

it may be filtered off: the concentration of the filtrate remaining unaffected.

100 ccs. of this solution contains 1 gram of Invert sugar.

STANDARDISATION OF THE FEHLING-SOHXLET SOLUTION:-

50 ccs. of the Standard Invert Solution are neutralised with caustic soda, and made to 1 litre with water. This solution is titrated as described below under "Analysis" taking the mean result of several titrations; 25.64 ccs. of the invert-sugar solution should be required. The copper solution is adjusted to correct strength but the alkali solution need not be adjusted if it has been made up strictly according to directions.

Apparatus:- Accurately graduated burettes were used for measuring the copper and preparedsugar solutions. A standardised 5 ccs. pipette was used for the alkali solution. The tests were made in 250 ccs. round flasks with flat bottoms,of thin Bohemian glass. An asbestos coated wire gauze was used with an ordinary Bunsen flame. The boiling was timed in all cases by a stop-clock. The burette was fitted with a twice bent jet of capillary tubing (see photograph page 99).

Analysis:- Incremental Test:- A preliminary test is first made following in general the directions given below. In this test, a considerable portion of the sugar solution is added before boiling, and the methylene blue is not added until near the end-point.

Exactly 10 ccs. of the mixed Fehling-Sohxlet solution are delivered into the dry boiling flask. No water should be added, as dilution will give erroneous results. Within 1 cc. of the required amount of sugar solution is now added, the solution brought to the boil, and boiled for exactly 2 minutes. 3 drops of methylene blue solution are now added without touching the neck of the flask. The timing is started as soon as bubbles appear at the edges of the solution as well as in the centre. Without removing from the heat or interrupting the boiling, the sugar solution is cautiously added from the burette until the end-point is reached. This should not take over a minute making the total boiling-time 3 minutes. After each addition of the sugar solution, the flask should be given a rotary movement without removing from the flame. The solution is kept boiling vigorously throughout the test to keep the flask free from air. A very slight contact with air will re-oxidise the methylene blue and give erroneous results. A little vaseline may be rubbed in the neck of the flask when solutions froth excessively.

End-point:- When the methylene blue is added it colours the contents of the flask a deep blue or violet which begins to fade when all but a small amount of the copper has been reduced. As the blue fades, the

yellow colour of most sugar solutions will give the solution a greenish tint. This indicates a very close approach to the end-point and two or three drops more are usually sufficient. The final disappearance of the blue can best be seen at the edge of the solution. With practice this can be determined so exactly that the accuracy with which the solutions are made up and measured out is the limiting factor in the precision of the test.

Calculation of Results:- The factor corresponding to the number of ccs. used is looked up under the column corresponding to the sucrose concentration of the prepared samples, interpolating if necessary, between the columns. Dividing this factor by the volume of solution used, and multiplying by 100 gives the milligrams of invert sugar in 100 ccs. of the prepared sample. The per cent of reducing sugars in the original sample is calculated from the amount of sample weighed for the original prepared solution.

Preparation of Sample:- The sucrose content of the sample must be known as this is used in calculating the results. It should not exceed 5 grams per 100 ccs. in the prepared sample. The concentration of the reducing sugars should be such that from 15 to 50 ccs. of the solution are required for the titration. Preferably the amount should be from 25 to 40 ccs. Thus, for

molasses 10 grams is a suitable amount to weigh, this being diluted with water, clarified with 3 ccs. of neutral lead acetate solution (10 per cent), diluted to 250 ccs. and filtered: 50 ccs. of the filtrate is deleaded with solid potassium oxalate and diluted to 250 ccs. after filtering.

Tables of Factors:- A portion of Lane and Eynon's table has been interpolated for simplification of the calculations necessary in the experimental section of this work. These interpolated tables are shown here:-

Lane & Eynon's Table Interpolated

(showing milligrams Invert Sugar).

10 ccs. - Fehling-Sohxlet Solution/gms. Sucrose per 100 ccs. sugar solution

ccs. sugar solution	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
15	50.5	50.44	50.38	50.32	50.26	50.20	50.14	50.08	50.02	49.96	49.9
16	50.6	50.54	50.48	50.42	50.36	50.30	50.24	50.18	50.12	50.06	50.0
17	50.7	50.64	50.58	50.52	50.46	50.40	50.34	50.28	50.22	50.16	50.1
18	50.8	50.73	50.66	50.59	50.52	50.45	50.38	50.31	50.24	50.17	50.1
19	50.8	50.74	50.68	50.62	50.56	50.50	50.44	50.38	50.32	50.26	50.2
20	50.9	50.83	50.76	50.69	50.62	50.55	50.48	50.41	50.34	50.27	50.2
21	51.0	50.92	50.84	50.76	50.68	50.60	50.52	50.44	50.36	50.28	50.2
22	51.0	50.93	50.86	50.79	50.72	50.65	50.58	50.51	50.44	50.37	50.3
23	51.1	51.02	50.94	50.86	50.78	50.70	50.62	50.54	50.46	50.38	50.3
24	51.2	51.11	51.02	50.93	50.84	50.75	50.66	50.77	50.48	50.39	50.3
25	51.2	51.12	51.04	50.96	50.88	50.80	50.72	50.64	50.56	50.48	50.4
26	51.3	51.21	51.12	51.03	50.94	50.85	50.76	50.67	50.58	50.49	50.4
27	51.4	51.30	51.20	51.10	51.00	50.90	50.80	50.70	50.60	50.50	50.4
28	51.4	51.31	51.22	51.13	51.04	50.95	50.86	50.77	50.68	50.59	50.5
29	51.5	51.40	51.30	51.20	51.10	51.00	50.90	50.80	50.70	50.60	50.5
30	51.5	51.40	51.30	51.20	51.10	51.00	50.90	50.80	50.70	50.60	50.5
31	51.6	51.50	51.40	51.30	51.20	51.10	51.00	50.90	50.80	50.70	50.6
32	51.6	51.50	51.40	51.30	51.20	51.10	51.00	50.90	50.80	50.70	50.6
33	51.7	51.59	51.48	51.37	51.26	51.15	51.04	50.93	50.82	50.71	50.6
34	51.7	51.59	51.48	51.37	51.26	51.15	51.04	50.93	50.82	50.71	50.6
35	51.8	51.69	51.58	51.47	51.36	51.25	51.14	51.03	50.92	50.81	50.7
36	51.8	51.69	51.58	51.47	51.36	51.25	51.14	51.03	50.92	50.81	50.7
37	51.9	51.78	51.66	51.54	51.42	51.30	51.18	51.06	50.94	50.82	50.7
38	51.9	51.78	51.66	51.54	51.42	51.30	51.18	51.06	50.94	50.82	50.7
39	52.0	51.88	51.76	51.64	51.52	51.40	51.28	51.16	51.04	50.92	50.8
40	52.0	51.88	51.76	51.64	51.52	51.40	51.28	51.16	51.04	50.92	50.8
41	52.1	51.97	51.84	51.71	51.58	51.45	51.32	51.19	51.06	50.93	50.8
42	52.1	51.97	51.84	51.71	51.58	51.45	51.32	51.19	51.06	50.93	50.8
43	52.2	52.06	51.92	51.78	51.64	51.50	51.36	51.22	51.08	50.94	50.8
44	52.2	52.07	51.94	51.81	51.68	51.55	51.42	51.29	51.16	51.03	50.9
45	52.3	52.16	52.02	51.88	51.74	51.60	51.46	51.32	51.18	51.04	50.9
46	52.3	52.16	52.02	51.88	51.74	51.60	51.46	51.32	51.18	51.04	50.9
47	52.4	52.25	52.10	51.95	51.80	51.65	51.50	51.35	51.20	51.05	50.9
48	52.4	52.25	52.10	51.95	51.80	51.65	51.50	51.35	51.20	51.05	50.9
49	52.5	52.35	52.20	52.05	51.90	51.75	51.60	51.45	51.30	51.15	51.0
50	52.5	52.35	52.20	52.05	51.90	51.75	51.60	51.45	51.30	51.15	51.0

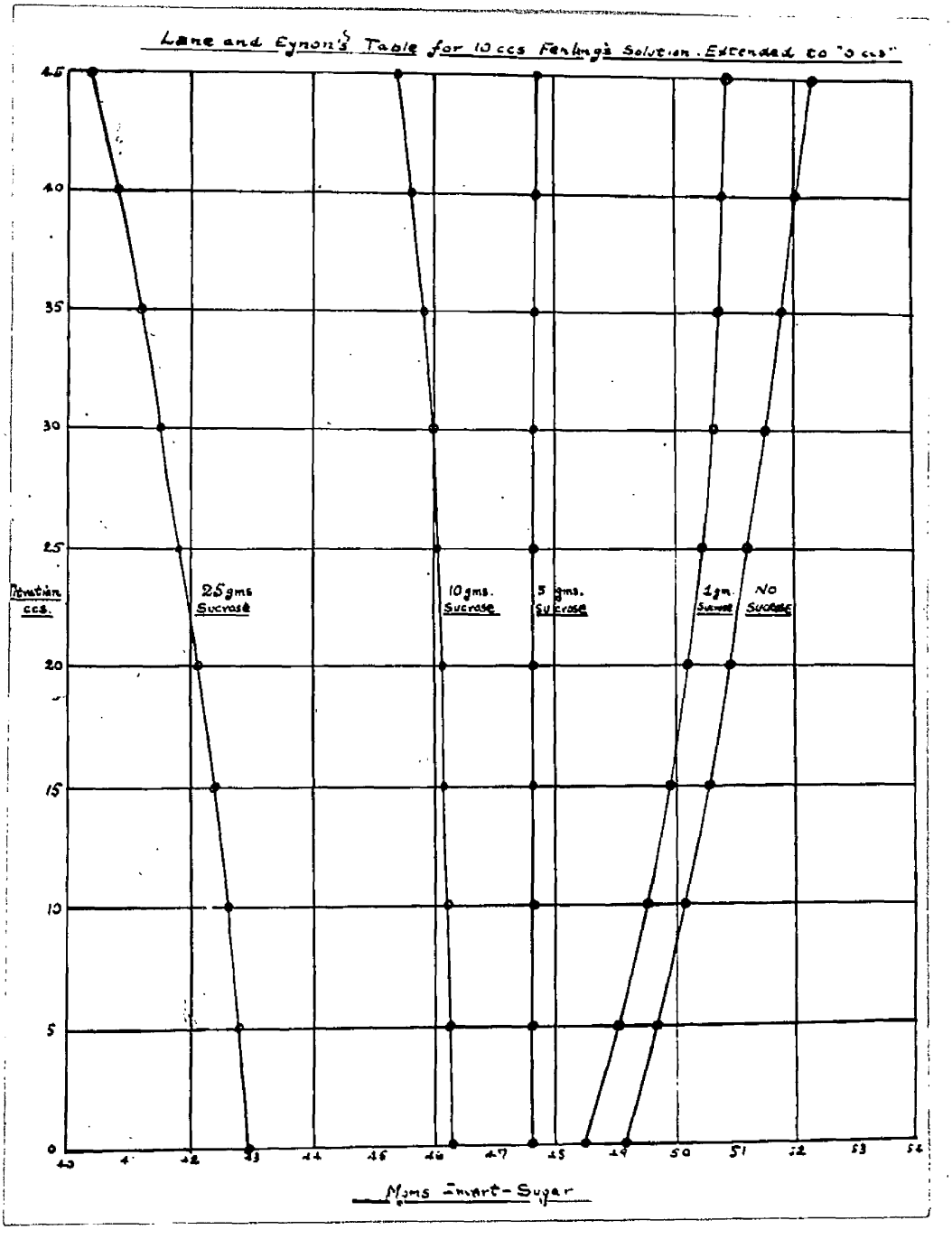
Lane and Eynon's Table for 10 ccs Fehling's Solution. Extended to "0 ccs"
45
40
35
30
25
20
15
10
5
0
Titration ccs.
25gms Sucrose
10gms. Sucrose
5 gms. Sucrose
1gm. Sucrose
No Sucrose
40
41
42
43
44
46
46
47
48
49
50
51
52
53
54
Mgms Invert-Sugar

FIG. 7

Lane and Eynon's Table for Molasses: 0.3 gm. sucrose present per 100 ccs. 1% Solution.

Ccs. sugar solution	Mgms. I.S.	ccs. sugar solution	Mgms. I.S.	ccs. sugar solution	Mgms. I.S.	ccs. sugar solution	Mgms. I.S.	ccs. sugar solution	Mgms. I.S.
15.0	335.5	22.0	230.9	29.0	176.6	36.0	143.0	43.0	120.4
.2	331.4	.2	229.0	.2	175.4	.2	142.3	.2	119.9
.4	327.4	.4	227.0	.4	174.2	.4	141.5	.4	119.3
.6	323.3	.6	225.1	.6	173.1	.6	140.8	.6	118.8
.8	319.3	.8	223.1	.8	171.9	.8	140.0	.8	118.2
16.0	315.2	23.0	221.2	30.0	170.7	37.0	139.3	44.0	117.7
.2	311.6	.2	219.4	.2	169.7	.2	138.6	.2	117.2
.4	308.0	.4	217.6	.4	168.6	.4	137.8	.4	116.7
.6	304.5	.6	215.8	.6	167.6	.6	137.1	.6	116.3
.8	300.9	.8	214.0	.8	166.5	.8	136.3	.8	115.8
17.0	297.3	24.0	212.2	31.0	165.5	38.0	135.6	45.0	115.3
.2	294.0	.2	210.6	.2	164.5	.2	134.9	.2	114.8
.4	290.7	.4	209.0	.4	163.4	.4	134.3	.4	114.3
.6	287.5	.6	207.4	.6	162.4	.6	133.6	.6	113.8
.8	284.3	.8	205.8	.8	161.3	.8	133.0	.8	113.3
18.0	281.0	25.0	204.2	32.0	160.3	39.0	132.3	46.0	112.8
.2	278.1	.2	202.7	.2	159.4	.2	131.6	.2	112.3
.4	275.2	.4	201.1	.4	158.5	.4	131.0	.4	111.9
.6	272.3	.6	199.6	.6	157.5	.6	130.3	.6	111.4
.8	269.4	.8	198.0	.8	156.6	.8	129.7	.8	111.0
19.0	266.5	26.0	196.5	33.0	155.7	40.0	129.0	47.0	110.5
.2	263.9	.2	195.0	.2	154.8	.2	128.4	.2	110.0
.4	261.3	.4	193.6	.4	153.9	.4	127.8	.4	109.6
.6	258.7	.6	192.1	.6	152.9	.6	127.3	.6	109.1
.8	256.1	.8	190.7	.8	152.0	.8	126.7	.8	108.7
20.0	253.5	27.0	189.2	34.0	151.1	41.0	126.1	48.0	108.2
.2	251.1	.2	187.9	.2	150.3	.2	125.5	.2	107.8
.4	248.8	.4	186.6	.4	149.5	.4	124.9	.4	107.4
.6	246.0	.6	185.2	.6	148.7	.6	124.3	.6	107.0
.8	244.1	.8	183.9	.8	147.9	.8	123.7	.8	106.6
21.0	241.7	28.0	182.6	35.0	147.1	42.0	123.1	49.0	106.2
.2	239.5	.2	181.4	.2	146.3	.2	122.6	.2	105.8
.4	237.4	.4	180.2	.4	145.5	.4	122.0	.4	105.4
.6	235.2	.6	179.0	.6	144.6	.6	121.5	.6	104.9
.8	233.1	.8	177.8	.8	143.8	.8	120.9	.8	104.5

Note on the preceding table:-

The milligrams of invert sugar per 100 ccs. shown in this table serve to calculate the reducing sugars percentage in a cane sugar molasses containing approximately 30 per cent of sucrose: this is a good average sucrose content for molasses.

The table refers to a 1 per cent solution of molasses, conveniently made by weighing 5 grams of the sample and diluting to 50 ccs.

The milligrams of invert sugar per 100 ccs. divided by 10 give the per cent reducing sugars directly for a one per cent solution.

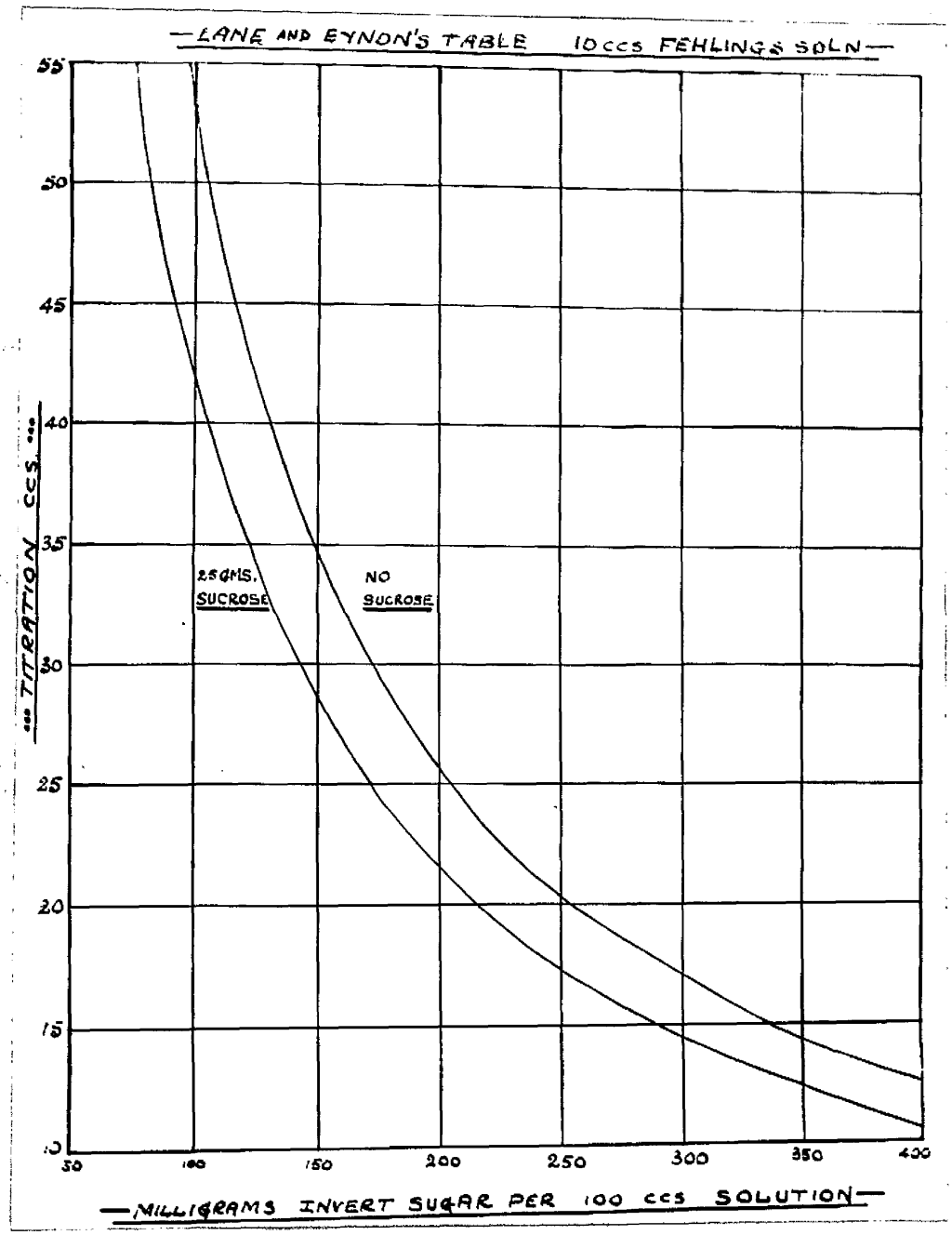
—LANE AND EYNON'S TABLE 10 CCS FEHLING'S SOLN—
55
50
45
40
35
30
25
20
15
10
TITRATION CCS.
25 GMS. SUCROSE
NO SUCROSE
50
100
150
200
250
300
350
400
—MILLIGRAMS INVERT SUGAR PER 100 CCS SOLUTION—

FIG. 8

EXAMPLE OF CALCULATION FOR LANE & EYNON'S METHOD.

West Indian Molasses.

Total solids	= 80.62%	(Refractometric)
Ash	= 9.36%	(Sulphated)
Sucrose	= 36.65%	(Dry lead method)
"	= 36.50	(Wet lead method).

The solution was prepared according to Lane & Eynon's directions viz:-

13 gms. molasses made to 250 ccs, clarifying with neutral lead acetate solution and deleading by solid potassium oxalate solution. 50 ccs. of this clarified solution was then made to 250 ccs. This solution now contained 1.04 gms. of the original molasses.

Titration Figures.

	(1) Incremental	(2)	(3)	(4)
ccs.	29.3	30.2	30.1	30.2

Lane and Eynon Calculation (continued)

Factor $\frac{24.8}{26.2}$ $\therefore$ $30.2 \times \frac{24.8}{26.2} = 28.6$ ccs.

From Lane & Eynon's Table:-

ccs.	mgms. invert sugar no sucrose	mgms. invert sugar 1 gm. sucrose 100 ccs.
28	183.7	180.2
29	177.6	174.1
	6.1	6.1

.6 x 6.1 = 3.66 mgms.
i.e. 3.7 "

179.5
176.0
3.5

Taking means

ccs.	No sucrose	1g. 100 ccs.
28.6	179.5	176.0mgms.

.5 x .38 = 1.3

Solution contains 1.04 gms. of molasses per 100 ccs. of 36.5% sucrose content, equal to 0.38 gm. sucrose per 100 ccs.

$\therefore$ Solution contains (179.5 - 1.3) = 178.2 mgms. invert sugar per 100 ccs.

$\therefore$ Per cent invert sugar $= \left(\frac{178.2}{1.04} \times 100\right) = 17.13\%$

Potentiometric determination of Reducing Sugar.
H. Tryller (Z. Spiritus. ind., 1929, 52, 27-28).
Abstracted in J.S.C.I., 1929, 48, p.223B. (J.H.Lane).

In the determination of reducing sugars by titration against Fehling's solution, the end-point is ascertained electrically by the vanishing of potential difference between two thick copper wires (electrodes) one of which is immersed directly in the boiling reaction liquid, and the other is enclosed in a tube containing a similar solution free from copper, closed at the bottom by a porous plug, and also immersed in the reaction liquid.

The free electrode and the tube containing the enclosed one pass through the stopper of the reaction flask, which also has an opening for admission of sugar solution from a burette and for escape of steam. The two electrodes are wired to a galvanometer. As the end point of the reaction is approached the galvanometer deflection approaches zero and becomes extremely sensitive to slight changes of copper-content of the reaction liquid, so that the end-point can be easily ascertained to within 0.1 cc. of sugar solution.

This method has been put into use by Messrs.S.J.Saint chemist to the Department of Agriculture, Barbardos,

and R.R. Follett-Smith, Chemist-Ecologist to the Department of Agriculture, British Guiana. It is stated to yield accurate results and to be particularly useful for highly coloured solutions such as molasses. Details of the method are given in the Int.Sug.J.,34, 353 (1932).

Electrometric method of Tryller, modified by Saintet al

Reagents:- (a) Fehling-Sohxlet Solution:- Solutions A & B, prepared as described under the methylene blue method.

(b) Sodium sulphate solution for cell:- This is hereafter called "cell-solution". 39.415 gms. of anhydrous sodium sulphate dissolved in water and made to 1 litre.

Apparatus.

(1) An electrode of thick copper wire (about 14 s.w.g.)

(2) A cell of wide (8 mms. internal diameter) pyrex glass tubing with a plug of plaster of Paris. The plug is about 8 mms. long and is made from a slurry of plaster rather than a paste. It is washed in the cell solution and stored in the cell solution when not in use.

(3) A dead beat, moving-coil galvanometer with central zero, provided with press-key.

(4) 250 ccs. pyrex round bottomed flask and the usual burettes, etc.

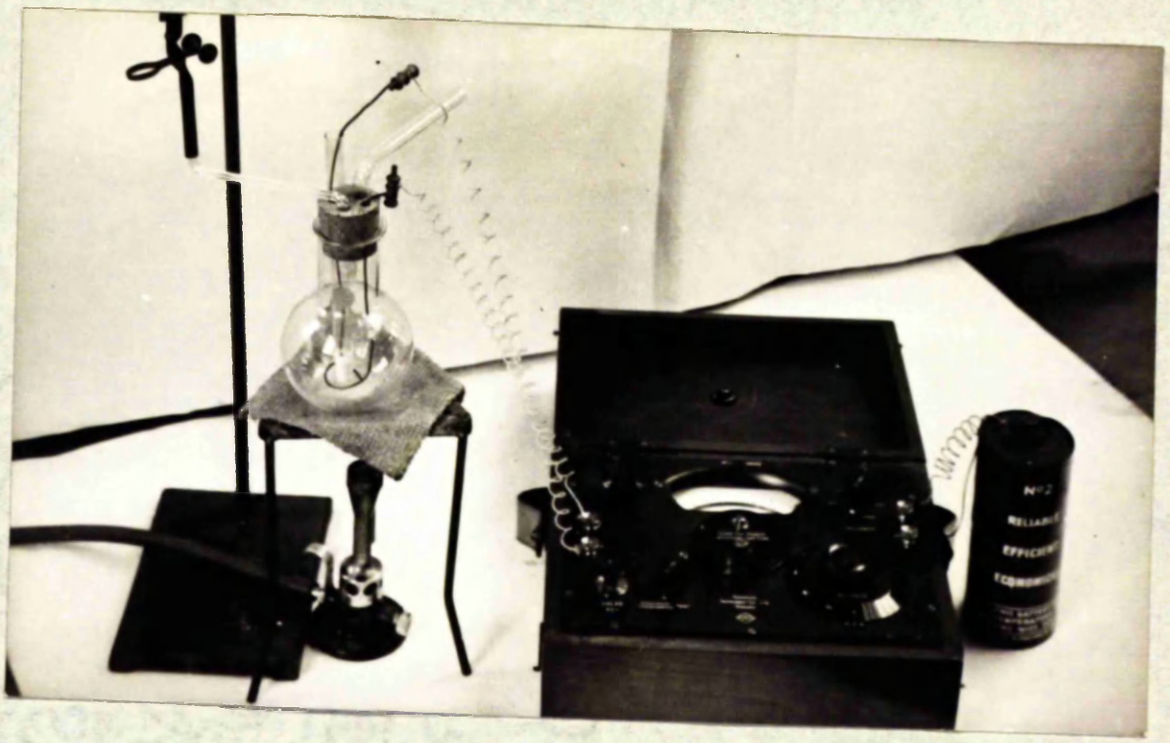

FIG.9

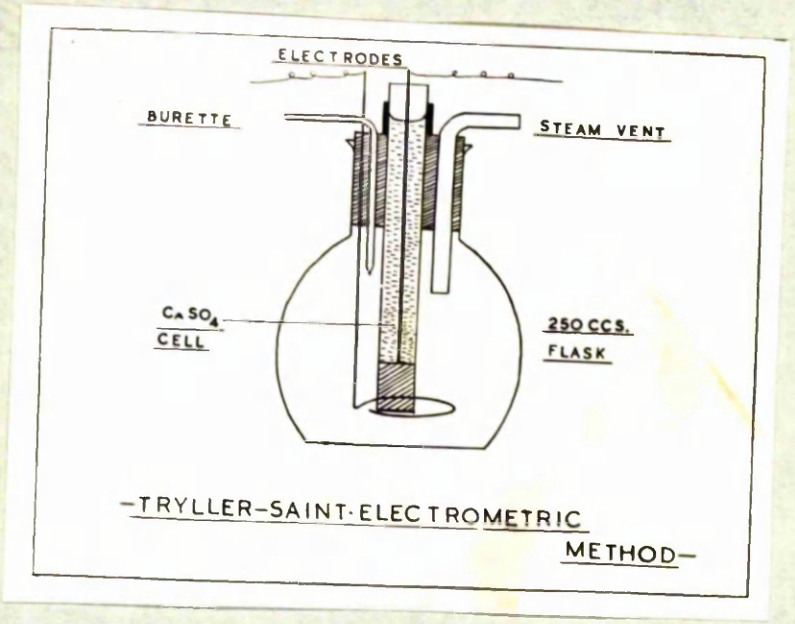
ELECTRODES
BURETTE
STEAM VENT
$CaSO_4$
CELL
250 CCS.
FLASK
—TRYLLER—SAINT-ELECTROMETRIC
METHOD—

FIG.10

Method:- The galvanometer is wired directly to the electrodes through the press key.

The pyrex flask is fitted with a cork which holds the copper wire electrode, the burette tip, the porous cell and the steam-vent.

The cell solution is made by mixing 5 ccs. of the above sodium sulphate solution, 5 ccs. of Fehling-Sohxlet solution B, and 40 ccs. of water. This may be made up and stored for short periods, the liquid in the cell being changed every 6 determinations.

The plaster of Paris plug lasts for about 25-30 determinations, after which the sensitivity is reduced and another cell is used. The cell is easily changed if the hole in the cork is made a sliding fit, then placing a rubber collar round the top and adjusting it to fix the plug at a convenient depth in the solution. An asbestos sheet is fitted over the neck of the flask to deflect the heat and facilitate manipulation.

In making a determination the standard method of Lane and Eynon is followed as far as the end of the two minutes boiling:- i.e., when the methylene blue should be added. At this stage the switch is depressed and if the galvanometer needle moves to one side, sugar solution is added drop by drop.

The swing of the needle gets less and less, and finally one drop will send it over to the other side of the zero point. It has been found that at the end-point

it is better to wait 3 to 5 seconds between the addition of one drop and the next, as at this stage there appears to be a slight lag before the system attains equilibrium.

EXPERIMENTAL WORK.

The experimental work on reducing sugar estimations by the Lane-Eynon and electrometric methods may be divided into six sections:-

1. Check on Lane and Eynon's tables of factors; and preparation and standardisation of Fehling-Sohxlet solution.
2. The effect of dilution of Fehling-Sohxlet solution (a) on standard invert sugar solutions before titrating. (b) on syrup and molasses.
3. The development of a permanent cell for the electrometric method (tests with silica, alundum and kieselguhr cells).
4. The construction of tables and graphs showing the divergencies from Lane & Eynon's tables caused by (a) dilution (b) use of permanent cells in the electrometric method.
5. Checks on all above methods using various types of molasses.
6. Various tests: (a) Various experiments with molasses.
   (b) Tests on boiling times.
   (c) Effect of shape and size of flasks used.

SECTION (1):- Preparation and standardisation of Fehling's solution.

4.5 litres of each solution (Fehling's solution A & B) were prepared by Lane and Eynon's directions.

Standard invert solution was prepared and the Fehling's solution was standardised with the following results (each result being the mean of at least three titrations).

Standard invert solution mgms. per 100 ccs.	ccs. Fehling solution	ccs. water added	Titration ccs.
500	25	0	24.80 X.
500	20	0	19.77
200	10	0	25.50
200	10	20	26.10
200	10	40	26.60
200	15	0	38.23
200	15	20	38.98
200	15	40	39.60
200	20	0	50.25
200	20	20	51.03
200	20	40	51.60

X. This Fehling's solution was exactly correct for use in Lane and Eynon's method, since Lane and Eynon give 24.80 ccs. as the correct figure for 10 ccs. solution.

SECTION (1):- Check on Lane and Eynon's Tables of Factors.

Zerban and Wiley of the New York Sugar Trade Laboratory (J.I.EC.Anal.Ed. Vol.6.No.5. P.355(1934) checked Lane & Eynon's figures for invert sugar in presence of sucrose and state "The factors for invert sugar are generally a little higher than those given by Lane & Eynon. This is no doubt due to small differences in manipulation, and each analyst should check these values under his own individual conditions, or construct his own tables of factors.

In confirmation of a statement made by Lane & Eynon that in the presence of a large excess of sucrose small variations in the details of the procedure have a much greater effect than in the absence of sucrose, it is found that the precision is not quite as high as when reducing sugars alone are present, but it is nevertheless satisfactory for routine analyses of raw sugars."

Lane and Eynon's tables have been checked by the author; some results are shown below. As with Zerban and Wiley's check, the results vary slightly from those found by Lane and Eynon.

EXPERIMENTAL CHECK ON LANE & EYNON'S TABLE.

Gms. Invert Sugar per 100 ccs.

s. - o- r O s.	0.1 gm.			0.15 gms.			0.20 gms.			0.4 gms.		
	Titration ccs.	Mgms. L & E	Mgms found	Titration ccs.	Mgms. L & E	Mgms found	Titration ccs.	Mgms L & E	Mgms found	Titration ccs.	Mgms L & E	mgms Found
0	53.08	52.7	53.08	35.10	51.80	52.65	25.50	51.25	51.00	12.70	50.36	5080
1	52.52	52.65	52.52	34.00	50.60	51.00	25.05	50.40	50.10	-	-	-
5	48.00	47.7	48.00	31.90	47.70	47.85	23.98	47.60	47.96	-	-	-
0	45.15	45.4	45.15	30.50	45.95	45.75	23.11	46.10	46.22	-	-	-
5	41.63	41.75	41.63	28.00	42.70	42.00	21.56	43.15	43.12	-	-	-

Differences from Lane and Eynon Mgms.

0	+ 0.38	+ 0.85	- 0.25	+ 0.44
1	- 0.13	+ 0.40	- 0.30	-
5	+ 0.30	+ 0.15	+ 0.36	-
0	- 0.25	- 0.20	+ 0.12	-
5	- 0.12	- 0.70	- 0.03	-

SECTION (2):- Examination of the effect of dilution on the determination of reducing sugars.

It occurred to the author that the differences caused by a certain dilution might be advantageously formulated into a table based on the Lane & Eynon table for different concentrations of sucrose and invert-sugar.

It would seem that dilution must permit of easier recognition of the end-point, otherwise it would not be so widely practised. This problem of dilution occurs with the electrometric method also. The original Tryller Method as modified by Saint uses 40 ccs. of water. Stieglitz and Home recommend 20 ccs. of water for titrations of 15 to 30 ccs. and 30 ccs. where the titration figure is above 30 ccs.

EXPERIMENTAL WORK:- Tests were made using the standard Lane & Eynon procedure to establish the error due to dilution with varying amounts of water. A further series of experiments with standard invert sugar solution in presence of different concentrations of sucrose enabled tables to be constructed for dilution with 20 ccs. and 40 ccs. of water.

It was found that the dilution with water lessened super heating and consequent "bumping" during boiling. Super heating is eliminated in the electrometric method by the presence of the calcium sulphate or silica cell in the solution.

SECTION (2):- Dilution of Fehling's solution in Lane and Eynon's method: titration of Standard Invert Sugar Solution.

Experiment (1):- Standard Invert Sugar Solution was prepared according to Lane & Eynon's directions.

Titrations were made with 10 ccs. and with 20 ccs. Fehling's solution with varying amounts of water added. At least 3 titrations were made in each case.

(a) Using 10 ccs. Fehling's Solution:-

ccs. water added.	Av. Titration ccs.	Increase in titration ccs.	Total increase	Factor of Fehling's solution. gms. Invert-Sugar per cc.
0	24.40	-	-	.00488
10	24.80	0.40	0.40	.00496
20	25.10	0.30	0.70	.00502
30	25.40	0.30	1.00	.00508
40	25.65	0.25	1.25	.00513
50	26.00	0.35	1.60	.00520
60	26.30	0.30	1.90	.00526

(b) Using 20 ccs. Fehling's Solution:-

0	48.25	-	-	.004825
10	48.40	0.15	0.15	.004840
20	48.60	0.20	0.35	.004860
30	48.90	0.30	0.65	.004890
40	49.25	0.35	1.00	.004925
50	49.45	0.20	1.20	.004945
60	50.00	0.55	1.75	.00500

Result:- The more water added, the greater the equivalent weight of invert sugar per cc. of Fehling's solution.

Dilution of Fehling's Solution in Lane & Eynon's method.

Experiment (2):- Titration of Dextrose:-

Dextrose solution 2.5 gms./litre. The moisture content of this sample of Dextrose was 1.06 per cent.

Fehling solution 20 ccs. ≡ .1000 gm. Invert Sugar
≡ .09595 gm. Dextrose.

ccs. Fehling's solution.	ccs. Distilled water added.	Titration ccs. Av.	Per cent Dextrose
20	0	39.15	98.03
20	20	39.40	97.41
20	40	40.15	95.52

$$\% \text{ Dextrose} = \left(\frac{.09595 \times 1000 \times 100}{2.5 \times 39.15}\right) = 98.02\%$$

Estimation of Reducing Sugars: effect of Dilution.

West Indian Molasses.

In this series of tests in addition to the effect of added water, the influence of the method of defecation of the molasses solution was observed.

Experiment (3):- Decalcification only. 5 gms. molasses made to 500 ccs. De-calcified with dry potassium oxalate and filtered with Kieselguhr.
10 ccs. Fehling's solution used in all tests.

Test No.	ccs. Distilled water added.	% Reducing Sugars.
(1)	0	17.42
(2)	20	16.99
(3)	40	17.12
(4)	Electrometric alundum 40 ccs. cell.	17.11

Experiment (4):- Lead Acetate Defecation. 5 gms. molasses made to 500 ccs; / with 9.5 ccs. of 10% neutral lead acetate added and filtered with Kieselguhr. Now added dry A.R. potassium oxalate and refiltered with Kieselguhr.

Test No.	ccs. Distilled water added.	Av. Titration ccs.	10 ccs. Fehling factor	% Reducing sugars.
(5)	0	28.25	.0488	17.28
(6)	40	29.83	.0513	17.20
(7)	Electrometric alundum 40 ccs.	29.80	.0506	16.98

EFFECT OF DEFECATION ON MOLASSES

REDUCING SUGARS ESTIMATIONS.

Experiment (5):- West Indian Molasses.

Test No.	ccs. Distilled water added	No defecation % Red. sugars. 5 gms/500 ccs.	Oxalate only % Red. sugars. 7.5 gms/500 ccs.	Oxalate only % Red. sugars. 3.75 gms/500 ccs.
8	0	16.92	17.68	17.00
9	20	-	-	17.57
10	40	17.10	18.19	17.83
11	Electro-metric alundum	17.60	18.09	17.79
12	Munson & Walker's method	16.86	-	-
13	$KMnO_4$ method	16.92	-	-
14	Thiosul-phate method	16.82	-	-

Summary of Experiments (3) to (5):-

No.	ccs.water added	No defecation	Oxalate only	Lead acetate and oxalate.
	0	16.92	17.42	17.28
	20	-	16.99	17.20
	40	17.10	17.12	16.98

The agreement in this particular case is remarkably good: this molasses was not a difficult one to titrate - i.e., it did not appear to contain excess colloidal matter or calcium.

These results should be compared with those given in Section (6)a for molasses of different origin.

ESTIMATION OF REDUCING SUGARS:- Effect of dilution.

Experiment (6):- Test with Golden Syrup using dilution water: 6 gms. weighed in 2 litres: no defecation. All tests using 10 ccs. Fehling's solution.

(a) Lane & Eynon.

Test No.	ccs. Distilled water added	Av. Titration ccs.	10 ccs. Fehling Factor	% Reducing Sugars	Difference from (1)
(1)	0	34.13	.0488	47.65	-
(2)	10	34.50	.0496	47.93	+0.28
(3)	20	34.95	.0502	47.86	+0.21
(4)	30	35.18	.0508	48.15	+0.50
(5)	40	35.48	.0513	48.20	+0.55
(6)	50	36.10	.0520	48.12	+0.48
(7)	60	36.45	.0526	48.07	+0.42

(b) Electrometric.

	cell.	Dilutions.				
(8)	$CaSo_4$	0	No result	-	-	-
(9)	"	10	34.78	.0498	47.73	0.08
(10)	"	20	35.10	.0502	47.67	0.02
(11)	Alun-dum.	40	35.20	.0506	47.92	0.27

It was noteworthy that with no added water the galvanometer did not reach zero in test No. 8: this is the concentration effect appearing: the method is essentially the employment of a concentration cell.

It was found very necessary to avoid leaving the cells in contact with Fehling's B any longer than essential. The immersed electrometric cell stops super heating and bumping - i.e., it acts like a porous chip.

SECTION (3):-

THE USE OF SINTERED GLASS CELLS IN PLACE OF THE CALCIUM SULPHATE CELLS.

The calcium sulphate cells as used in Tryller's method are not permanent, and require a certain amount of attention to keep them in good order.

Saint recommends storing the cells in "cell solution", but the alkali of the cell solution soon attacks the calcium sulphate. The calcium sulphate is more or less attacked by the hot alkaline solution during the boiling. A blue deposit eventually appears inside the cell (by diffusion of the copper). The bottom likewise usually becomes coated with cuprous oxide, and it is suspected that this may have a catalytic action upon the reduction. These effects are minimised in the sintered cells because of the lower surface presented, and the action of the alkaline solution on the sintered portion is not noticeably great.

It was thought that a sintered glass cell would be suitable for the purpose, and cells were made using various materials.

The best results were obtained with Pyrex glass and silica: alundum was not so satisfactory probably on account of the amphoteric nature of alumina.

The ideal construction was one which did not allow liquid to drip through the cell but allowed electrical contact through the porous end without dripping.

This kept diffusion to a minimum.

It was found that the cells could be cleaned with acid, and that their efficiency was unimpaired two years after the first use.

Details are given here of the method of making the cells.

Preparation of Sintered Cells:-

A piece of glass tubing 0.8 mm. bore by 1 cm. external diameter was cut into lengths of about 35 cms. Each length was drawn out in the middle and cut, thus leaving a tapered end on each final piece of approximately 3 mms. external diameter. The end was rounded off to leave merely a pin-point aperture. Different mixtures were put into each prepared tube, as below, to fill the tapered portion, tapping the mixture well into place. The whole tapered portion was now heated red-hot in the Bunsen flame. This usually sealed the end off. Heating was continued until the whole mass had sintered - about 1 to 2 minutes. After cooling, the tip of the 3 mm. portion was snipped off, and the end rounded off in the flame.

Mixtures:- (next page).

Mixtures:-

(1)	1 : 1 of powdered glass and coarse metallurgical alundum refractory powder.
(2)	1 : 1 of powdered glass and fine metallurgical alundum refractory powder.
(3)	1 : 1 of powdered glass and pure anhydrous alumina.
(4)	2 : 1 of powdered glass and pure anhydrous alumina.
(5) (a)(b)(c)	3 : 1 of powdered glass and pure anhydrous alumina (3 tubes prepared).
(6)	4 : 1 of powdered glass and pure anhydrous alumina.

Several cells with plugs of the Tryller-Saint type were made up using pastes made of the fine and coarse alundum refractory powders respectively. These hardened in a day or two, but flaked considerably on rubbing. They were placed in a steam-oven for 2 days but still flaked, and were therefore discarded.

Filtration tests on prepared cells:-

5 ccs. of water placed in each tube and also ina similar tube plugged with calcium sulphate, and left over small measuring cylinders for 68 hours (week-end).

Results:-

(1) (2) (3) (4) all water through.

(5) (a) no water through.

(b) all water through.

(c) 1 cc. water (approx) left in tube.

$CaSo_4$ plug - no water through.

After testing with water these cells were tested with the galvanometer and standard invert solution in the determination of invert sugar. They were found to vary considerably in performance, and also cracked rather badly in most cases, on changing the cell in the hot solution.

It was decided to make up a fresh set of cells, employing Pyrex glass throughout, and grading the powdered constituents for size.

Preparation of Pyrex Sintered Cells:-

(1) Six cells were prepared using 1 : 1 SiO_2 : Pyrex glass mixture and pyrex glass tubing. These were found to be insufficiently strong mechanically. The pyrex tubing was drawn out in the hottest part of the Bunsen flame, and the sealing-off was done in the blow pipe flame. Four of the cells survived and leaked water slowly - about 2 ccs. in 1½ hours for 3 of them and 5 ccs. in 1½ hours for the other.

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(2) Six cells were made with 0.5 mm. bore pyrex tubing, using a 2 : 1 pyrex silica mixture and leaving shorter ends than usual. These were sound mechanically, but leaked water extremely slowly.

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(3) Three cells were prepared with 4 : 1 pyrex to kieselguhr mixture. These cells were strong and appeared satisfactory for rate of leakage.

The grain size of the pyrex glass used was that retained by a 60 mesh sieve and passed by a 30 mesh sieve.

An alternative method of preparing Sintered Glass Cells:-

An alternative method of preparing a sintered glass cell is to blow a small bulb on the end of a piece of pyrex glass tubing of about 5 mms. diameter. The bulb is quarter filled with powdered pyrex glass, passing 60 mesh and retained by 90 mesh sieve. The tube is transferred to a muffle furnace at low red heat and left for about a quarter of an hour.

On cooling, the bulb is filled with dilute sulphuric acid, and the base outside is allowed to dip into hydrofluoric acid. The two liquids are connected in series with a dry battery and galvanometer and the acid is allowed to act on the glass until a current will pass.

Adaptation of sintered junction for pH work.

This type of sintered junction has also been adapted with the author's help as a standard Normal Calomel half cell for pH work. Such a cell would be useful for taking the pH values of pastes and similar products where an ordinary liquid junction would be undesirable. The half-cell prepared behaved very satisfactorily.

Preparation of $CaSo_4$/glass Sintered Cells:-

Pyrex glass was ground in a mortar and sieved, using the portion passing a 30 mesh and retained on a 60 mesh sieve.

(1) Three cells (A, B and C), were prepared using this glass and $CaSo_4$ in equal amounts, the glass being fused as for the previous cells.

These cells were tested with Fehling's solution using 40 ccs. cell solution, and gave moderately good deflections, but on the whole rather too low. With A cell the deflection was off the galvanometer scale; B and C cells gave about 40 scale divisions.

(2) Two cells (D and E) were made as above using 2 parts of calcium sulphate to 1 part of 30/60 pyrex glass. These were tested as above and both gave deflections well off the galvanometer scale (over 200 millivolts for No. 4.)

All 5 cells were quite sound mechanically.

The above cells are hereafter called "sealed calcium sulphate cells".

Comparative Tests of various calcium sulphate, sealed calcium sulphate, alundum and silica cells.

The test solution used contained 10 gms. sucrose and 0.15 gm. of Invert Sugar per 100 ccs.

10 ccs. Fehling's solution used:-

Method & cell used	ccs. water added	Av. Titration ccs.	Equivalent mgms. Invert Sugar
methylene blue	0	30.35	46.00
Electrometric 2 : 1 silica cell			
No. 1	40	31.40	47.10
2	40	31.55	47.33
3	40	31.35	47.03
4	40	31.15	46.73
5	40	31.42	47.13
6	40	31.25	46.88
Sealed $CaSo_4$			
A	40	31.35	47.03
13	40	31.75	47.63
14	40	31.65	47.48
15	40	31.62	47.43
Ordinary $CaSo_4$			
No. 1	40	31.65	47.48
No. 3	40	31.75	47.63
Alundum	40	31.05	46.58
Kieselguhr	40	31.50	47.24

It will be observed that there is not more than ordinary experimental difference in the titrations: the milligrams of invert sugar show comparatively close agreement from cell to cell. The silica cells were eventually selected, as giving good results with sound mechanical strength.

Experiments on Electrometric method for Estimation of Reducing Sugars: Alundum cell and sintered Filter-stick.

Tryller's method as applied by Saint to sugar products was used, employing (a) the $CaSo_4$ cells as in original method (b) a porous alundum disc fused into glass. The effect of dilution was tried.

Example:- When 40 ccs. water was added to the titration flask - the Na_2So_4 solution put in the $CaSo_4$ cell was diluted to a similar concentration.

Each result shown average of at least 3 titrations.

No.	ccs. Fehling's solution.	ccs. Distilled water added.	$CaSo_4$ cell	Alundum cell.
(1)	10	0	25.1	24.7
(2)	10	10	24.9	24.9
(3)	10	20	25.1	25.2
(4)	10	30	25.0	25.4
(5)	10	40	25.0	25.3

It was observed that the alundum cell was much more porous than the $CaSo_4$ cells hence diffusion was more rapid. This cell was supplied for pH work by Poulenc Frères, Paris and was in fact a ready-made porous cell which was adapted for this work.

Similar tests were carried out using a Jena sintered glass micro filter-stick but this was also

found to be too porous and to present too much surface causing cuprous oxide to deposit. In general these were much less convenient than the pointed type of sintered cell (see photograph).

—SINTERED·CELLS—

FIG.11

A — SILICA CELL

B — SEALED $CaSO_4$ CELL

C — SINTERED FILTER-STICK

D — KIESELGUHR CELL

E — ALUNDUM CELL

Section (4). Preparation of Tables and Graphs for (a) diluted Fehling-Sohxlet solution.

(b) use of permanent electrometric cells.

(c) use of Tryller-Saint calcium sulphate cells.

Experimental:- Two series of tests were carried out:-

(1) Titration of standard invert solutions of varying concentration (a) in absence of sucrose.

(b) in presence of 1, 5, 10 & 25 gms. of sucrose.

These titrations were made (1) using Lane & Eynon's method with no dilution and with 20 and 40 ccs. dilution, and (2) using silica, alundum, calcium sulphate, and kieselguhr cells. The calcium sulphate cell was the normal Tryller-Saint cell, and not the sealed calcium sulphate cell.

(2) A second series of tests similar to those in (1) using one calcium sulphate cell and one silica cell throughout. This series served as a check on series (1).

The results of these tests were plotted, and from the graphs tables were constructed which are analogous to those prepared by Lane and Eynon. These tables cover the use of the Tryller calcium sulphate cells, the permanent silica cells, and the use of 20 ccs. and of 40 ccs. dilution water in the ordinary methylene blue procedure.

Summary of Results:-

Dilution:- The tables presented will enable a better

end-point to be obtained in Lane and Eynon's volumetric process, by allowing dilution with either 20 or 40 ccs. of water.

Permanent Cells:- The author hopes that the use of permanent silica cells will extend the usefulness of the electrometric method of determining reducing sugars, which already has the advantage for routine work of being independent of daylight, since a colour change is not the criterion of end-point.

It is recommended that the electrometric method be used for routine work, the Lane-Eynon process being generally more exact.

Differences due to method used:- The differences in the milligrams of invert-sugar shown by the various methods are summarised below.

Milligrams Invert-Sugar:		No sucrose present			
Titration ccs.	Lane & Eynon ordinary	$CaSo_4$ cell	SiO_2 cell	Lane & Eynon 20 ccs. water	Lane & Eynon 40 ccs. water
15	50.5	51.8	51.8	52.2	53.5
20	50.9	52.7	52.4	52.7	53.9
25	51.2	53.4	52.8	53.2	54.3
30	51.5	53.8	53.1	53.6	54.7
35	51.8	54.2	53.4	54.0	55.0
40	52.0	54.5	53.7	54.1	55.3
45	52.3	54.7	54.0	54.3	55.5
50	52.5	54.9	54.2	54.4	55.7

These results show that a considerable effect is produced by water: with the calcium sulphate and silica cells the higher figures are due to the cell concentration also.

The silica cells used in this and all subsequent work were of the 1 : 1 silica pyrex glass type.

Comparison of Methylene Blue and Electrometric Reducing sugar methods:-

(1) The reading on the galvanometer can be altered by varying the distance between the electrodes, so this distance must be unaltered throughout the course of one experiment. It would probably be better to have this distance permanently fixed.

(2) It was found that variations in total length of leads and between the length of leads did not materially affect the results obtained. The leads were kept of the same length throughout these experiments.

(3) It was found that Tryller's method definitely indicates the approaching end-point and of course there is no trouble in this respect with methylene blue addition.

(4) Bright sunshine is about as bad as poor light or artificial light for viewing the end-point with methylene blue.

(5) With the electrometric method, the boiling comes to full pitch almost at once - i.e., it is easier to detect the exact point of full ebullition than in the methylene blue method.

(6) There is difficulty sometimes in getting the last tinge of purple to disappear in the Lane & Eynon method.

(7) The electrometric method takes slightly longer per individual titration, but the results appear to be more reproducible than for the methylene blue method.

Series (1):- Titration of Standard Invert solution in absence of Sucrose.

Method	ccs. water added	Av. Titration ccs.	mgms. Invert Sugar	Av. Titration ccs.	mgms. Invert Sugar	Av. Titration ccs.	mgms. Invert Sugar	Av. Titration ccs.	mgms. Invert Sugar
(a) Methylene Blue	0	12.70	50.80	53.08	53.08	35.10	52.65	25.50	51.00
	20	13.00	52.00	54.40	54.40	35.97	53.96	26.10	52.20
	40	13.32	53.28	54.44	54.44	36.73	55.10	26.70	53.40
	50	-	-	-	-	36.88	-	-	-
(b) Electrometric.									
1 : 1 silica	40	13.15	52.60	Poor result	-	36.05	54.08	26.16	52.32
Alundum	40	13.14	52.56	Poor result	-	36.03	-	26.12	52.24
2 : 1 silica	40	13.45	53.80	53.98	53.98	36.15	-	25.99	51.98
$CaSo_4$	40	13.10	52.40	54.98	54.98	35.98	53.97	26.26	52.52
Kiesel-guhr	40	13.60	54.40	54.11	54.11	35.88	-	26.07	52.52
		No Sucrose. solution contains 0.40 gm. Invert sugar per 100 ccs.		No Sucrose. solution contains 0.10 gm. Invert sugar per 100 ccs.		No Sucrose. solution contains 0.15 gm. Invert sugar per 100 ccs.		No Sucrose. solution contains 0.20 gm. Invert sugar per 100 ccs.	

The above figures show how dependent the Lane and Eynon method is on the exact volume of liquid present in the flask during the standard titration.

Series (1):- Titration of Standard Invert Solution in presence of sucrose.

0.2 gm. Invert Sugar per 100 ccs.

Method	5 gms. Sucrose present. ccs. water added.	Av. Titration ccs.	mgms. Invert Sugar	10 gms. Sucrose present ccs. water added	Av. Titration ccs.	mgms. Invert Sugar
(a) Methylene Blue	0	23.98	47.96	0	23.11	46.22
	20	24.69	49.38	20	23.56	47.12
	40	25.32	50.64	40	24.10	48.20
(b) Electrometric						
1 : 1 silica	40	24.50	49.00	40	23.60	47.20
alundum	40	24.50	49.00	40	23.50	47.00
2 : 1 silica	40	24.40	48.80	40	23.50	47.00
$CaSo_4$	40	24.50	49.00	40	23.74	47.48
Kieselguhr	40	24.68	49.36	40	23.30	46.60

0.2 gms. Invert Sugar per 100 ccs.

Method	25 gms. Sucrose present ccs. water added	Av. Titration ccs.	mgms. Invert Sugar
(a) Methylene Blue	0	21.56	43.12
	10	21.93	43.86
	20	22.16	44.32
	40	22.60	45.20
(b) Electrometric			
1 : 1 silica	40	22.10	44.20
alundum	40	21.80	43.60
2 : 1 silica	40	22.10	44.20
CaSo	40	22.00	44.00
Kieselguhr	40	21.85	43.70

Series (1):- Titration of Standard Invert Solution in presence of Sucrose.

Method	ccs. water added	Av. Titration ccs.	mgms. Invert Sugar	Av. Titration ccs.	mgms. Invert Sugar	Av. Titration ccs.	mgms. Invert Sugar
Methylene Blue	0	28.00	42.00	30.50	45.75	52.52	52.52
	20	29.35	44.03	31.80	47.70		
	40	29.95	44.93	32.35	48.53		
Electrometric							
1 : 1 silica	40	29.29	43.94	-	-		
Alundum	40	-	-	-	-		
2 : 1 silica	40	28.95	43.23	32.20	48.30		
$CaSo_4$	40	28.95	43.23	31.70	47.55		
Kieselguhr	40	-	-	-	-		
		25 gms. sucrose per 100 ccs. 0.15 gm. Invert sugar per 100 ccs.		10 gms. sucrose per 100 ccs. 0.15 gm. Invert sugar per per 100 ccs.		1 gm. sucrose per 100 ccs. 0.1 gm. Invert sugar per per 100 ccs.	

The colloidal orange colour of the Cu_2O is intensified when the concentration is as high as 25 gms. sucrose.

Mgms. Invert Solution: calculated thus:-

Titration 28 ccs. but 1 cc. solu. cont. .0015 gm. Invert sugar. ∴ equivalent mgms. Invert solu.

$= 1000 \times 28 \times .0015 = 42.00$

That is, equivalent to 10 ccs. Fehling's solution for the given conditions.

Series (1):- Titration of Standard Invert Solution in presence of sucrose.

For 10 ccs. Fehling's solution.

Method	ccs. water added.	Av. Titration ccs.	Equivalent mgms. Invert sugar.
Methylene Blue	0	25.05	50.10
	10	25.45	50.90
	20	25.62	51.24
	30	25.95	51.90
	40	26.17	52.34
	50	26.50	53.00
Electrometric			
1 : 1 silica	40	25.55	51.10
Alundum	40	25.67	51.34
2 : 1 silica	40	Poor result	-
$CaSo_4$	40	25.95	51.90
Kieselguhr	40	25.50	51.00
2 : 1 silica	20	25.40	50.80
Kieselguhr	20	25.80 Poor result	-

The above Test solution contained 1 gm. sucrose and 0.2 gm. of Invert sugar per 100 ccs.

Tests with Standard Invert Solution.

Series (2)

Gms. Invert Sugar per 100 ccs.	Gms. Sucrose per 100 ccs.	$CaSO_4$ Cell Titration ccs.	$CaSO_4$ Cell Mgms. Invert Sugar	SiO_2 Cell Titration ccs.	SiO_2 Cell Mgms. Invert Sugar	Approx. mgms. from L. & E. table
0.25	0	21.06	52.65	21.00	52.50	51.0
	1	20.60	51.50	20.44	51.10	50.2
	5	20.13	50.30	19.60	49.00	47.6
	10	19.18	47.95	18.90	47.25	46.1
	25	18.15	45.38	17.90	44.75	43.3
0.125	0	43.69	54.60	42.73	53.81	52.2
	1	41.80	52.25	40.95	51.19	50.8
	5	39.12	48.90	38.35	47.94	47.7
	10	37.15	46.44	36.75	45.94	45.7
	25	34.45	43.07	34.08	42.60	42.2
0.100	0	55.00	55.00	54.45	54.45	52.8
	1	52.30	52.30	52.00	52.00	51.1
	5	48.75	48.75	48.45	48.45	47.7
	10	46.20	46.20	45.65	45.65	45.4
	25	42.45	42.45	41.50	41.50	41.7
0.20	0	26.80	53.60	26.33	52.66	51.4
	1	26.00	52.00	25.60	51.20	50.4
	5	24.85	49.70	24.53	49.06	47.6
	10	23.88	47.76	23.10	46.20	46.1
	25	22.35	44.70	21.70	43.40	43.1

Graphs were drawn using the above data, and from the plotted curves, tables were constructed. These are recorded on the following pages.

Invert Sugar Table for 10 ccs. Fehling's solution for use with Calcium Sulphate Cells.

Results shown are for series I & II Calcium Sulphate Tests.

	I	II	I	II	I	II
Titration ccs.	0 gms. Sucrose mgms Invert Sugar		1 gm. Sucrose mgms Invert Sugar		5 gms. Sucrose mgms Invert Sugar	
15	51.83	52.5	51.00	51.00	51.10	50.05
16	52.05		51.10		50.90	
17	52.20		51.2		50.75	
18	52.40		51.3		50.60	
19	52.55		51.4		50.40	
20	52.72	52.80	51.50	51.48	50.30	49.60
21	52.85		51.60		50.15	
22	53.00		51.65		50.00	
23	53.10		51.70		49.90	
24	53.23		51.80		49.80	
25	53.35	53.30	51.90	51.84	49.65	49.28
26	53.45		52.00		49.55	
27	53.58		52.00		49.45	
28	53.65		52.10		49.40	
29	53.72		52.10		49.30	
30	53.82	53.62	52.20	52.10	49.27	49.00
31	53.90		52.20		49.20	
32	54.00		52.25		49.20	
33	54.10		52.30		49.15	
34	54.17		52.30		49.10	
35	54.22	54.02	52.30	52.30	49.10	48.75
36	54.30		52.30		49.00	
37	54.35		52.35		49.00	
38	54.40		52.35		48.95	
39	54.45		52.40		48.90	
40	54.50	54.33	52.40	52.46	48.90	48.60
41	54.55		52.40		48.85	
42	54.60		52.40		48.80	
43	54.60		52.40		48.80	
44	54.65		52.40		48.80	
45	54.70	54.60	52.40	52.60	48.75	48.55
46	54.70		52.40		48.70	
47	54.80		52.40		48.70	
48	54.80		52.40		48.70	
49	54.80		52.40		48.70	
50	54.85	54.75	52.40	52.65	48.70	48.50

Invert Sugar Table for Calcium Sulphate Cells (continued).

Series I & II.

	I	II	I	II	I
Titration ccs.	10 gms. Sucrose gms. Invert Sugar		25 gms. Sucrose gms. Invert Sugar		0.3 gms. Sucrose
15	48.75	48.20	45.80	44.95	51.55
16	48.60		45.60		51.73
17	48.40		45.45		51.87
18	48.25		45.30		52.03
19	48.15		45.15		52.17
20	48.00	47.70	45.00	44.20	52.31
21	47.90		44.85		52.43
22	47.75		44.70		52.55
23	47.65		44.55		52.63
24	47.55		44.40		52.75
25	47.45	47.42	44.25	43.58	52.87
26	47.35		44.10		52.97
27	47.25		44.00		53.05
28	47.20		43.85		53.13
29	47.10		43.70		53.18
30	47.00	47.00	43.58	43.18	53.28
31	46.90		43.45		53.33
32	46.80		43.35		53.42
33	46.70		43.25		53.50
34	46.65		43.15		53.55
35	46.55	46.73	43.02	42.90	53.58
36	46.50		42.95		53.63
37	46.55		42.85		53.68
38	46.40		42.80		53.72
39	46.35		42.70		53.77
40	46.30	46.47	42.60	42.62	53.82
41	46.30		42.55		53.83
42	46.25		42.50		53.87
43	46.25		42.40		53.87
44	46.20		42.35		53.90
45	46.15	46.27	42.25	42.38	53.93
46	46.10		42.20		53.93
47	46.10		42.10		54.00
48	46.10		41.90		54.00
49	46.10		42.00		54.00
50	46.10	46.20	42.00	42.20	54.03

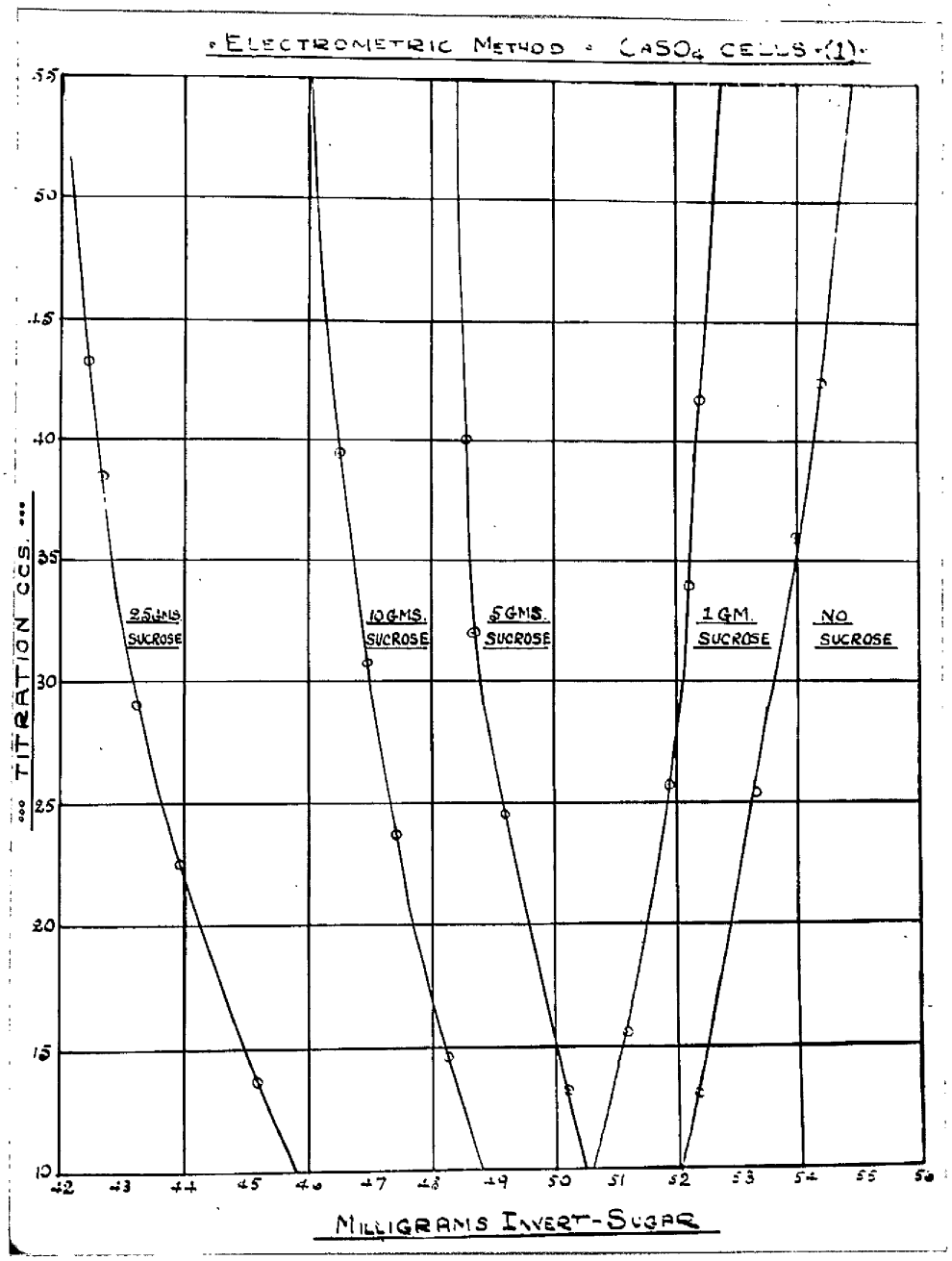

ELECTROMETRIC METHOD - CaSO4 CELLS (1).
TITRATION CCS.
55
50
45
40
35
30
25
20
15
10
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
2.5 GMS. SUCROSE
10 GMS. SUCROSE
5 GMS. SUCROSE
1 GM. SUCROSE
NO SUCROSE
MILLIGRAMS INVERT-SUGAR

FIG. 12

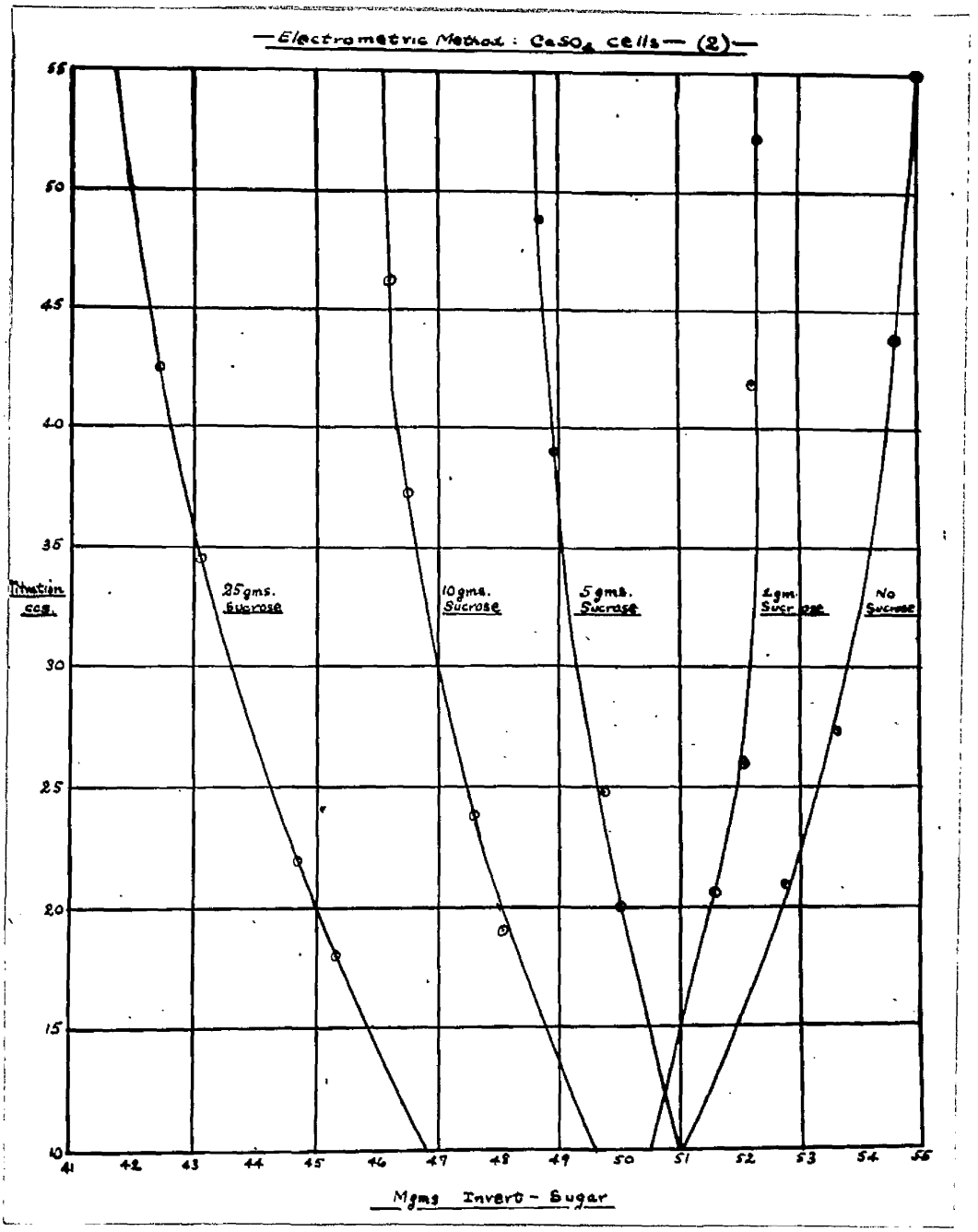

—Electrometric Method : CaSO4 cells— (2)—
55
50
45
40
35
30
25
20
15
10
Titration cc's.
25gms. Sucrose
10gms. Sucrose
5gms. Sucrose
2gm. Sucrose
No Sucrose
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
Mgms Invert - Sugar

FIG.13

Invert Sugar Table for Silica Cells.

10 ccs. Fehling's Solution.

Titration ccs.	0 gms. Sucrose	1 gm. Sucrose	5 gms. Sucrose	10 gms. Sucrose	25 gms. Sucrose	0.3 gms. Sucrose.
	Mgms. Invert Sugar					
15	51.80	50.50	49.10	47.10	45.00	51.37
16	51.90	50.60	49.10	47.00	44.70	51.47
17	52.05	50.70	49.10	46.90	44.50	51.60
18	52.15	50.80	49.05	46.75	44.30	51.70
19	52.25	50.90	49.05	46.65	44.00	51.80
20	52.40	50.95	49.00	46.55	43.80	51.92
21	52.50	51.00	49.00	46.45	43.60	52.00
22	52.60	51.05	49.00	46.35	43.40	52.08
23	52.65	51.10	49.00	46.30	43.25	52.13
24	52.75	51.20	48.95	46.20	43.15	52.23
25	52.80	51.25	48.95	46.20	43.10	52.28
26	52.85	51.30	48.90	46.15	43.00	52.33
27	52.90	51.35	48.90	46.15	42.90	52.38
28	53.00	51.40	48.90	46.10	42.80	52.47
29	53.05	51.40	48.85	46.10	42.70	52.53
30	53.10	51.45	48.85	46.10	42.60	52.55
31	53.20	51.50	48.80	46.05	42.55	52.63
32	53.25	51.50	48.80	46.00	42.45	52.67
33	53.30	51.50	48.80	46.00	42.40	52.70
34	53.35	51.55	48.75	46.00	42.30	52.75
35	53.40	51.60	48.75	46.00	42.20	52.80
36	53.50	51.60	48.70	45.95	42.00	52.87
37	53.55	51.65	48.70	45.95	41.90	52.92
38	53.60	51.70	48.70	45.90	41.85	52.97
39	53.65	51.75	48.65	45.90	41.75	53.02
40	53.70	51.80	48.65	45.85	41.65	53.07
41	53.70	51.80	48.60	45.80	41.55	53.07
42	53.75	51.85	48.60	45.80	41.50	53.12
43	53.85	51.85	48.60	45.80	41.40	53.18
44	53.90	51.90	48.55	45.75	41.30	53.23
45	53.95	51.90	48.55	45.70	41.20	53.27
46	54.00	51.90	48.50	45.70	41.15	53.30
47	54.05	51.95	48.50	45.65	41.10	53.35
48	54.10	51.95	48.50	45.60	41.00	53.38
49	54.15	52.00	48.50	45.60	40.95	53.43
50	54.20	52.00	48.45	45.55	40.90	53.47

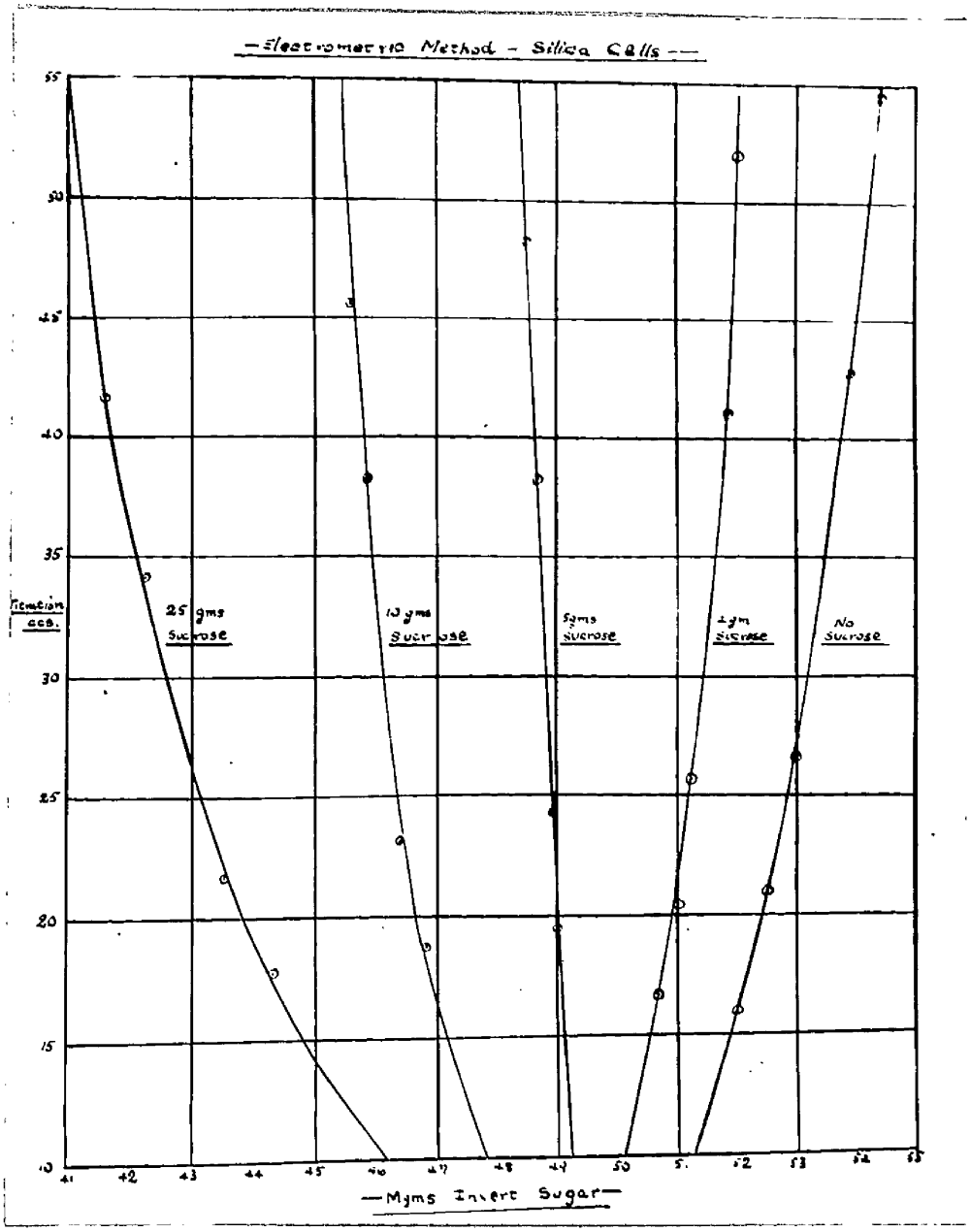

—Electrometric Method - Silica Cells—
55
50
45
40
35
Rotation
ccs.
30
25
20
15
10
25 gms
Sucrose
10 gms
Sucrose
5gms
Sucrose
1gm
Sucrose
No
Sucrose
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
—Mgms Invert Sugar—

FIG. 14

Invert Sugar Table for 10 ccs. Fehling's Solution

by Lane & Eynon's method: 20 ccs. Dilution Water added.

Titration ccs.	mgms. Invert Sugar					
	No Sucrose	1 gm. Sucrose	5 gms. Sucrose	10 gms. Sucrose	25 gms. Sucrose	0.3 gms. Sucrose
15	52.20	50.30	49.40	47.65	44.90	51.57
16	52.30	50.40	49.40	47.60	44.80	51.67
17	52.40	50.50	49.40	47.50	44.75	51.77
18	52.50	50.60	49.40	47.45	44.65	51.87
19	52.60	50.70	49.40	47.40	44.55	51.97
20	52.70	50.80	49.35	47.30	44.50	52.07
21	52.80	50.90	49.35	47.25	44.40	52.17
22	52.90	51.00	49.35	47.20	44.30	52.27
23	53.00	51.10	49.35	47.10	44.25	52.37
24	53.05	51.20	49.35	47.05	44.20	52.43
25	53.15	51.30	49.35	47.00	44.20	52.53
26	53.25	51.35	49.35	46.90	44.15	52.62
27	53.35	51.40	49.35	46.85	44.15	52.70
28	53.40	51.45	49.35	46.80	44.10	52.75
29	53.50	51.50	49.35	46.75	44.05	52.87
30	53.60	51.60	49.30	46.65	44.00	52.93
31	53.65	51.65	49.30	46.60	43.95	52.98
32	53.70	51.75	49.30	46.55	43.95	53.05
33	53.80	51.80	49.30	46.50	43.90	53.13
34	53.85	51.90	49.30	46.45	43.85	53.20
35	53.95	52.00	49.30	46.40	43.80	53.30
36	54.00	52.05	49.30	46.40	43.75	53.35
37	54.00	52.10	49.30	46.35	43.75	53.37
38	54.05	52.20	49.30	46.35	43.70	53.43
39	54.10	52.25	49.30	46.30	43.65	53.48
40	54.10	52.30	49.30	46.25	43.65	53.50
41	54.15	52.30	49.25	46.20	43.65	53.53
42	54.20	52.35	49.25	46.20	43.60	53.58
43	54.20	52.35	49.25	46.15	43.60	53.58
44	54.25	52.40	49.25	46.15	43.60	53.63
45	54.25	52.40	49.25	46.15	43.60	53.63
46	54.30	52.45	49.25	46.10	43.55	53.68
47	54.30	52.45	49.25	46.10	43.55	53.68
48	54.35	52.50	49.25	46.10	43.55	53.73
49	54.35	52.50	49.25	46.05	43.55	53.73
50	54.40	52.55	49.25	46.05	43.50	53.78

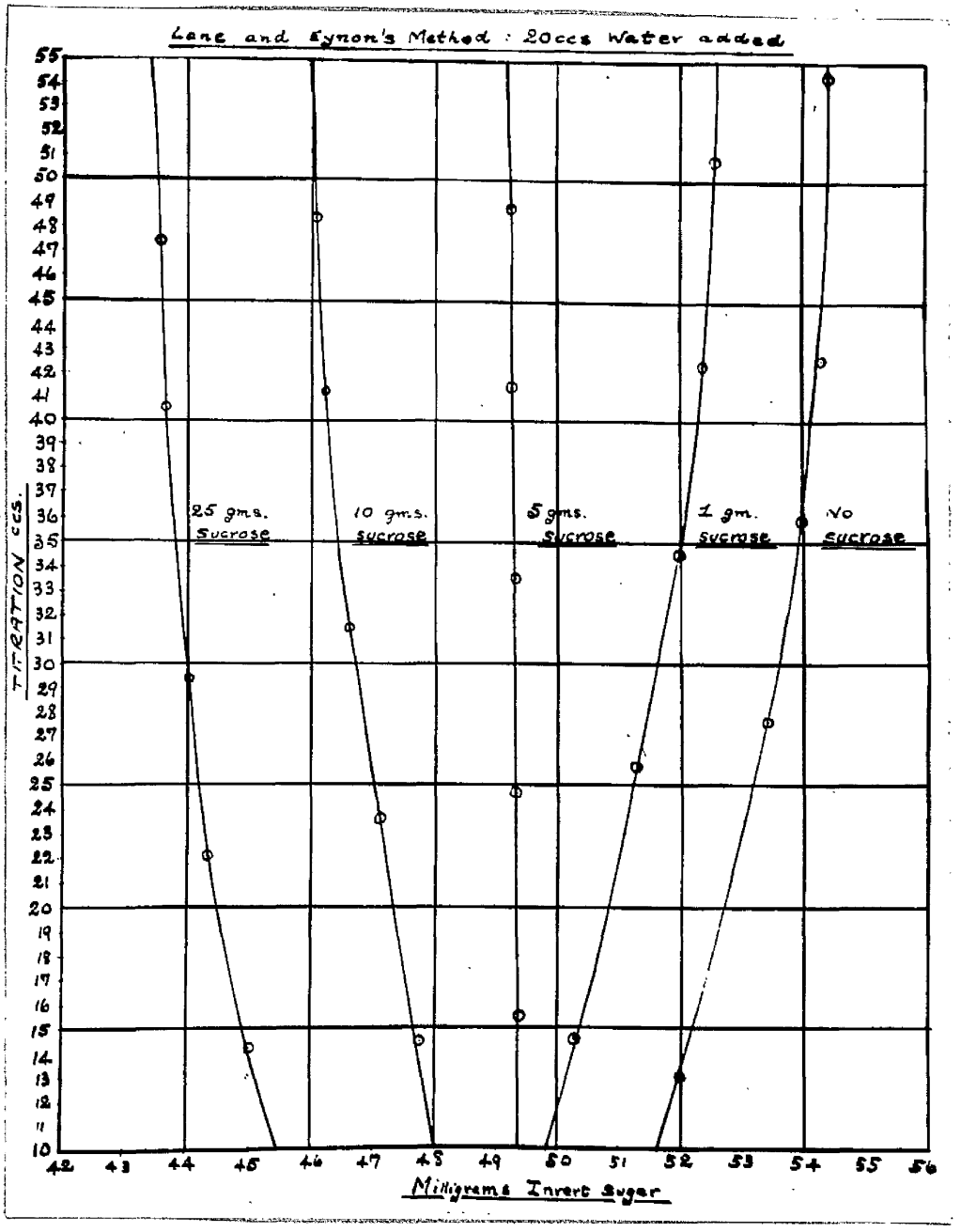

Lane and Eynon's Method : 20ccs Water added
TITRATION ccs.
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
25 gms. Sucrose
10 gms. sucrose
5 gms. Sucrose
1 gm. sucrose
No Sucrose
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
Milligrams Invert Sugar

FIG.15

Invert-Sugar Table for 10 ccs. Fehling's Solution

by Lane & Eynon's method: 40 ccs. dilution water.

Titration ccs.	mgms. Invert Sugar					
	No Sucrose	1 gm. Sucrose	5 gms. Sucrose	10 gms. Sucrose	25 gms. Sucrose	0.3 gms. Sucrose.
15	53.45	51.35	50.65	48.50	45.70	52.75
16	53.50	51.45	50.65	48.45	45.65	52.82
17	53.60	51.55	50.65	48.40	45.60	52.92
18	53.70	51.65	50.65	48.40	45.50	53.02
19	53.80	51.70	50.65	48.35	45.45	53.10
20	53.90	51.80	50.65	48.35	45.40	53.20
21	54.00	51.90	50.65	48.30	45.35	53.30
22	54.10	52.00	50.65	48.25	45.30	53.40
23	54.15	52.10	50.65	48.25	45.25	53.47
24	54.20	52.20	50.65	48.20	45.20	53.53
25	54.30	52.30	50.65	48.15	45.15	53.63
26	54.40	52.35	50.65	48.10	45.10	53.72
27	54.50	52.45	50.65	48.05	45.05	53.82
28	54.55	52.50	50.65	48.00	45.00	53.87
29	54.60	52.60	50.65	48.00	45.00	53.93
30	54.65	52.65	50.65	47.95	44.95	53.98
31	54.70	52.70	50.65	47.90	44.90	54.03
32	54.80	52.75	50.65	47.85	44.85	54.12
33	54.85	52.80	50.65	47.80	44.80	54.17
34	54.90	52.90	50.65	47.75	44.75	54.22
35	54.95	52.90	50.65	47.70	44.70	54.27
36	55.00	53.00	50.65	47.70	44.65	54.33
37	55.05	53.00	50.65	47.65	44.60	54.37
38	55.10	53.05	50.70	47.60	44.60	54.42
39	55.20	53.10	50.70	47.60	44.60	54.50
40	55.25	53.15	50.70	47.60	44.60	54.55
41	55.30	53.20	50.70	47.55	44.55	55.60
42	55.35	53.25	50.70	47.55	44.55	55.65
43	55.40	53.30	50.70	47.50	44.50	55.70
44	55.45	53.35	50.70	47.45	44.50	55.75
45	55.50	53.40	50.70	47.45	44.45	55.80
46	55.55	53.40	50.70	47.40	44.45	54.83
47	55.60	53.45	50.70	47.40	44.40	54.88
48	55.60	53.50	50.70	47.40	44.40	54.90
49	55.65	53.55	50.70	47.40	44.40	54.95
50	55.70	53.60	50.70	47.40	44.40	55.00

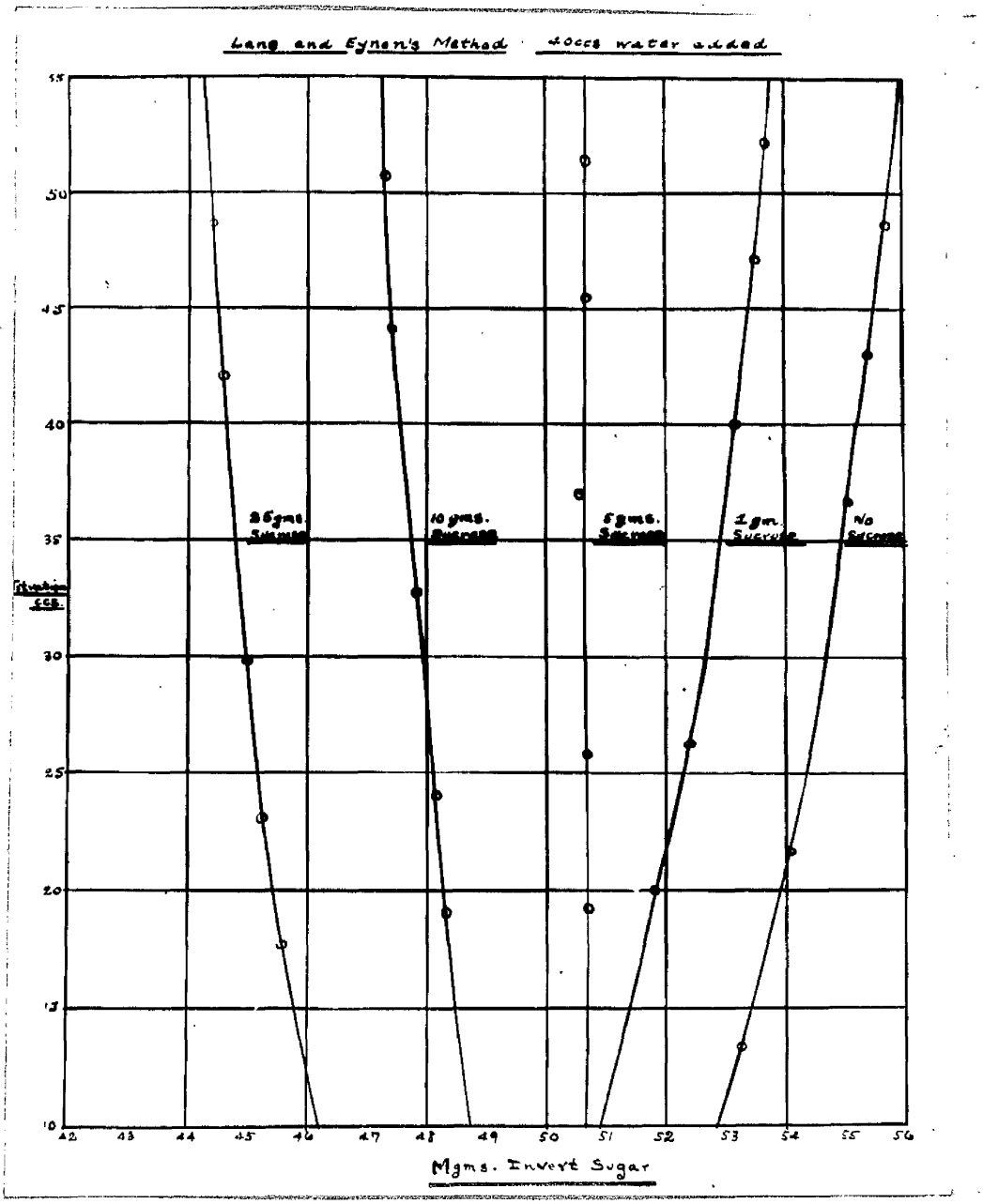

Lane and Eynon's Method
40ccs water added
55
50
45
40
35
30
25
20
15
10
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
Mgms. Invert Sugar
2.5gms. Sucrose
10 gms. Sucrose
5 gms. Sucrose
1 gm. Sucrose
No Sucrose

FIG.16

Table Showing Milligrams Invert-Sugar per 100 ccs. for Electrometric Method using Calcium Sulphate and Silica Cells.

Titration ccs.	No Sucrose	1 gm. Sucrose	5 gms. Sucrose	10 gms. Sucrose	25 gms. Sucrose.
		Calcium Sulphate Cells.			
15	345.5	340	334	325	305
20	264	258	252	240	225
25	213	208	199	190	177
30	180	174	164	157	145
35	155	149	140	133	123
40	136	131	122	116	107
45	122	116	108	103	94
50	110	105	97	92	84
		Silica Cells.			
15	345	337	327	313	300
20	262	255	245	233	219
25	211	205	196	185	172
30	177	172	163	154	142
35	152.5	147	139	131	121
40	134	129.5	122	115	104
45	120	115	108	102	92
50	108	104	97	91	82

Table Showing Milligrams Invert-Sugar per 100 ccs. for Lane & Eynon Method with 20 ccs. & 40 ccs. dilution water.

Titration ccs.	No Sucrose	1 gm. Sucrose	5 gms. Sucrose	10 gms. Sucrose	25 gms. Sucrose
	Lane & Eynon Method: 20 ccs. Water added.				
15	348	335	329	318	299
20	263.5	254	247	236.5	222.5
25	213	205	197	188	177
30	179	172	164	155.5	147
35	154	149	141	132.5	125
40	135	131	123	116	109
45	121	116	109	103	97
50	109	105	98.5	92	87
	Lane & Eynon Method: 40 ccs. Water added.				
15	356	342	338	323	305
20	269.5	259	253	242	227
25	217	209	203	193	181
30	182	175.5	169	160	150
35	157	151	145	136	128
40	138	143	127	119	111.5
45	123	119	113	106	99
50	112	107	101	95	89

SECTION (5):- TESTS ON MOLASSES BY VARIOUS METHODS OF DETERMINING REDUCING SUGARS.

The main work in this section was done by taking three dilutions of each of five samples of different molasses and estimating their reducing sugar content by the following five methods:-

(1) Lane and Eynon method: no water added

(2) " " " " : 20 ccs. water added

(3) " " " " : 40 ccs. " "

(4) Electrometric " : $CaSO_4$ cell } 20 and
(5) " " silica " } 40 ccs.

An average of three titrations was taken as the final figure in each case, and the results were calculated from the appropriate tables: the method of calculation is shown in detail.

The molasses solutions were defecated with normal neutral lead acetate solution and potassium oxalate.

In the final tabulation of results the check figures shown in columns (6) (7) (8) and (9) are due to N. Venkatayya B.A.,A.R.T.C., to whom acknowledgment is made.

The object of taking different dilutions was to observe the effect of concentration: each dilution meant a different titration result - that is, a different volume of boiling liquid, which it was thought would mean a change in the reducing sugar

percentage found, since the conditions of precipitation of copper oxide would vary with each dilution. Several other experiments on molasses are recorded in section (6).

A table is shown which recommends amounts of molasses to be taken in order to bring the titration range between 30 and 35 ccs.

Tests on Molasses using varying concentrations.

All defecated by Normal lead acetate & potassium oxalate.

Molasses.	% solm. of Molasses.	No water Titn. ccs.	No water % R.S.	20 ccs. water Titn. ccs.	20 ccs. water % R.S.	40 ccs. water. Titn. ccs.	40 ccs. water. % R.S.
Java	0.50	43.90	23.67	-	-	-	-
% Sucrose = 33.3	0.60	36.60	23.52	-	-	-	-
Ash=10.86%	0.65	34.40	23.04	-	-	-	-
Hawaii	0.75	40.55	17.02	41.70	51.80* 16.56	42.68	51.86* 16.20
% Sucrose = 30.0	1.00	30.22	16.95	30.98	51.30 16.56	31.60	51.34 16.25
ash=9.75%	1.50	19.73	17.09	20.55	50.62 16.43	21.05	50.64 16.04
Cuba	0.75	44.40	15.60	44.75	51.95 15.47	45.95	51.96 15.08
% Sucrose = 31.59	1.00	33.20	15.45	33.85	51.30 15.16	33.75	51.30 15.20
Ash=8.99%	1.50	21.42	15.76	22.29	50.71 15.17	22.96	50.72 14.72
Antigua	0.50	40.55	25.60	42.03	51.92 25.31	42.59	51.97 24.41
% Sucrose = 28.65 % Ash	0.75	26.80	25.46	28.02	51.21 24.36	28.65	51.26 23.85
= 10.12%	1.00	19.80	25.61	20.80	50.76 24.40	21.20	50.78 23.95
Egyptian	0.75	42.20	16.37	42.01	51.79 16.44	43.90	51.89 15.76
% Sucrose = 32.01 % Ash	1.00	30.30	16.90	31.10	51.28 16.49	31.75	51.28 16.15
= 13.41	1.50	20.65	16.33	21.10	50.62 16.00	21.60	50.64 15.63

*Milligrams invert sugar found.

Tests on Molasses using varying concentrations.

All defecated by Normal lead acetate & potassium oxalate.

		$CaSO_4$ 20ccs. water	$CaSO_4$ 40ccs. water		Silica 20ccs. water	Silica 40 ccs. water.	
Molasses.	% soln. of Molasses.	Titn. ccs.	Titn. ccs.	% R.S.	Titn. ccs.	Titn. ccs.	% R.S.
Java % Sucrose = 33.3 Ash=10.86%	0.50	-	45.90	23.67	-	47.80	22.48
	0.60	-	38.55	23.44	-	37.75	23.48
	0.65	35.90	36.20	22.90	35.40	37.20	21.98
Hawaii % Sucrose = 30.0 Ash=9.75%	0.75	42.18	42.73	16.88	40.78	40.58	17.50
	1.00	31.10	30.65	17.40	30.09	29.83	17.63
	1.50	20.75	20.25	17.18	20.10	19.90	17.33
Cuba % Sucrose = 31.59 Ash=8.99%	0.75	45.50	45.55	15.85	44.73	44.68	15.95
	1.00	33.95	33.95	15.79	34.95	32.98	15.99
	1.50	22.35	22.44	15.57	21.85	21.61	15.27
Antigua % Sucrose = 28.65% Ash = 10.12%	0.50	40.95	41.10	26.40	41.10	40.75	26.22
	0.75	27.65	27.70	25.65	28.00	27.68	25.34
	1.00	20.80	20.80	25.23	20.18	20.19	25.75
Egyptian % Sucrose = 32.01% Ash = 13.41	0.75	43.39	43.45	16.60	43.10	41.85	16.97
	1.00	32.15	32.20	16.60	31.70	31.29	16.84
	1.50	20.88	20.95	16.65	22.05	20.40	16.90

Estimation of Reducing Sugars in Molasses by Various Methods.

Molasses.	% Sucrose	% Ash	% solu. of Molasses.	(1) Lane & Eynon no water	(2) Lane & Eynon 20 ccs. water added.	(3) Lane & Eynon 40 ccs. water added.	(4) Electro metric $CaSO_4$	(5) Electro metric silica
(1) Java	33.30	10.86	0.50	23.67	-	-	23.67	22.48
			0.60	23.52	-	-	23.44	23.48
			0.65	23.04	-	-	22.90	21.98
(2) Hawaii	30.00	9.75	0.75	17.02	17.19	17.14	16.88	17.50
			1.00	16.95	17.10	17.11	17.40	17.63
			1.50	17.09	16.84	16.81	17.18	17.33
(3) Cuba	31.59	8.99	0.75	15.60	16.03	15.97	15.85	15.95
			1.00	15.45	15.72	16.07	15.79	15.99
			1.50	15.76	15.56	15.42	15.57	15.27
(4) Antigua	28.65	10.12	0.50	25.60	25.66	25.89	26.40	26.22
			0.75	25.46	25.22	25.20	25.65	25.34
			1.00	25.61	25.19	25.20	25.23	25.75
(5) Egypt	32.01	13.41	0.75	16.37	17.06	16.69	16.60	16.97
			1.00	16.90	16.92	17.04	16.60	16.84
			1.50	16.33	16.40	16.37	16.65	16.90

Estimation of Reducing Sugars in Molasses by Various Methods (continued).

	(6)	(7)	(8)	(9)
Molasses	Lane & Eynon no water Check.	Munson & Walker method Check.	$KmnO_4$ Method Check.	Low's Thiosulphate method Check.
(1) Java	23.40 - -	By Cu_2O 23.17 By CuO 22.91 -	23.70 - -	22.80 - -
(2) Hawaii	17.28 - -	By Cu_2O 18.52 By CuO 17.46 -	17.82 - -	17.25 - -
(3) Cuba	15.60 - -	By Cu_2O 16.31 By CuO 15.79 -	15.79 - -	15.74 - -
(4) Antigua	25.26 - -	By Cu_2O 25.51 By CuO 24.72 -	24.96 - -	24.39 - -
(5) Egypt	16.50 - -	By Cu_2O 17.05 By CuO 16.51 -	16.72 - -	16.53 - -

Table showing method of calculation of per cent reducing sugars in molasses.

This shows results for silica cells by the electrometric method: the milligrams of invert sugar are taken from the appropriate table.

Molasses	% solu. concentration	Titration ccs.	Mgms. invert sugar from table.	% red. sugars	sucrose conc^{n}. gms. per 100 ccs.	Interpolation from table.		
Java	0.50	47.80	53.72	22.48	0.17	54.05	51.95	2.10
						54.10	51.95	2.15
	0.60	37.75	53.20	23.48	0.20	53.55	51.65	1.90
						53.60	51.70	1.90
	0.65	37.20	53.14	21.98	0.22	53.55	51.65	1.90
						53.60	51.70	1.90
Hawaii	0.75	40.58	53.26	17.50	0.23	53.70	51.80	1.90
						53.70	51.80	1.90
	1.00	29.83	52.59	17.63	0.30	53.05	51.40	1.65
						53.10	51.45	1.65
	1.50	19.90	51.74	17.33	0.45	52.25	50.90	1.35
						52.40	50.95	1.45
Cuba	0.75	44.68	53.46	15.95	0.24	53.90	51.90	2.00
						53.95	51.90	1.95
	1.00	32.98	52.72	15.99	0.32	53.25	51.50	1.75
						53.30	51.50	1.80
	1.50	21.61	51.84	15.27	0.47	52.50	51.00	1.50
						52.60	51.05	1.55
Antigua	0.50	40.75	53.43	26.22	0.14	53.70	51.80	1.90
						53.70	51.80	1.90
	0.75	27.68	52.63	25.34	0.21	52.90	51.35	1.55
						53.00	51.40	1.60
	1.00	20.19	52.00	25.75	0.29	52.40	50.95	1.45
						52.50	51.00	1.50
Egyptian	0.75	41.85	53.28	16.97	0.24	53.70	51.80	1.90
						53.75	51.85	1.90
	1.00	31.29	52.67	16.84	0.32	53.20	51.50	1.70
						53.25	51.50	1.75
	1.50	20.40	51.73	16.90	0.48	52.40	50.95	1.45
						52.50	51.00	1.50

Table showing concentration of molasses solutions to bring titration value in range from 30 to 35 ccs. using Lane and Eynon's 1.04 per cent molasses solution.

If Preliminary Titration is - ccs.	Make this per cent solution.	Dilution.
15	0.52	50 ccs. to 100 ccs.
20	0.624	60 " to 100 "
25	0.780	75 " to 100 "
30	-	-
35	-	-
40	1.39	Weigh 6.95 gms. to 500ccs. Dry-deleading.
45	1.56	Weigh 7.80 gms. to 500 "
50	1.73	Weigh 8.65 gms. to 500 "

This table is designed to minimise the effect of dilution by arranging to have the titration value at 30 to 35 ccs.

SECTION (6)

In this section the following work is recorded:-

(a) Various experiments with molasses.

(b) Result of variation of boiling time in reducing-sugar estimations.

(c) Effect of the shape and size of flasks used in reducing-sugar determinations.

(a) EXPERIMENT (1):- Effect of concentration with the incremental method.

Increment as amount of solution added in the cold is increased:- Antigua molasses: Lane & Eynon method.

Titration No.	ccs. added before boiling.	Titration ccs.	
(1)	35.0	37.85	
(2)	37.0	38.25	
(3)	38.0	38.80	mean taken as
(4)	38.5	38.95	38.88 ccs.

This increment seems to be more pronounced where the titration figure is high e.g. round about 40 ccs.

This result raises the question - How much of the supposed "action of sucrose" on reduction of copper solutions is really due to the concentration and viscosity of the sugar solution being tested?

The results obtained here would suggest that one incremental titration may not be enough in Lane and Eynon's method. If the titration value in No. 2 titration had been accepted, there would obviously have been a relatively large error in the result as compared with the mean of titrations Nos. (3) and (4).

(a) EXPERIMENT (2):- Effect of small titration figures.

Antigua Molasses:-

% Sucrose = 28.65

% Ash = 10.12

		% Reducing Sugars			
		(a) using 1% solu.		(b) using 0.5% solu.	
Method	Details	Av. Titration ccs.	% Reducing Sugars.	Av. Titration ccs.	% Reducing sugars.
Lane & Eynon ordinary	No water	18.48	26.40	38.88	25.10
	40 ccs. water	19.98	25.66	40.05	25.62
Electro-metric.	Alundum	19.20	26.04	40.20	25.18
	1 : 1 silica	19.45	25.96	39.06	25.86
	$CaSO_4$	19.43	26.04	39.33	25.42
		Av. of all	26.02	-	25.44

Results by other methods of Estimation:-

(1) Munson & Walker's method (by Cu2O) = 25.51
(2) " " " (by Cu2O corrected) = 24.69
(3) " " " by CuO = 24.72
(4) By $KMnO_4$ method = 24.96
(5) By Low's Thiosulphate method = 24.39

Mean of methods 2 to 5 = 24.69

The results with a 1 per cent solution giving titrations below 20 ccs. are apparently too high. This points to the necessity for taking a concentration which will give titration figures of 30 to 35 ccs.

(a) EXPERIMENT (3):- INTRODUCTION.

The effect of Calcium Salts on Reducing Sugar Determinations.

Eynon and Lane (loc. cit.) have shown that the presence of calcium salts causes low results in reducing sugar estimations. They recommend that potassium oxalate be used as a decalcifying and deleading agent. Meade and Harris (J.I.E.C., 8, p.507) obtained higher results when using potassium oxalate, as did Norris & Brodie (Hawaiian Planter's Record, Vol.17, p.311). These differences were attributed by them to a reducing action of potassium oxalate on Fehling's solution.

Cook and McAllep (Ibid. Vol.32, p.142) showed that the presence of potassium oxalate or disodium phosphate does not affect the reducing power of pure solutions. They also demonstrated that in the presence of neutral lead acetate either a deficiency or an excess of potassium oxalate leaves lead and lime in solution and causes low results: in the absence of lead an excess of potassium oxalate does not affect the results.

EXPERIMENTAL:- Some experiments made by the author on molasses using oxalate alone and lead acetate followed by oxalate defecation are reported on the following pages.

(a) EXPERIMENT (3):- Effect of method of defecation

Java Molasses:-

I. Lead acetate and oxalate treatment.

5 gms. washed into 500 ccs. flask, 9.5 ccs. of neutral lead acetate added, made to volume and filtered using Kieselguhr. Then added solid potassium oxalate and refiltered with Kieselguhr. Filtration was very rapid.

II. Oxalate only.

5 gms. in 500 ccs. Solid potassium oxalate added and filtered using Kieselguhr.

Test No.	Method	ccs. water added.	% Reducing Sugars. I.	II.
(1)	Lane and Eynon	0	23.70	24.04
(2)	" " "	20	23.85	24.15
(3)	" " "	40	23.60	23.96
(4)	Electrometric silica cell	40	23.50	23.90
(5)	" $CaSO_4$ cell	40	23.70	23.90
(6)	" alundum cell	40	23.25	23.18
Hawaiian Molasses.	Methods of treatment I and II exactly as for above Java sample.			
(1)	Lane and Eynon	0	16.95	17.23
(2)	" "	20	17.10	17.45
(3)	" "	40	17.11	17.18
(4)	Electrometric silica cell	40	17.23	17.55
(5)	" $CaSO_4$ cell	40	17.40	17.85
(6)	" Alundum cell	40	17.51	18.28

Results for Java and Hawaiian Molasses by other methods.

No.	Method	Java	Hawaiian
(1)	Lane and Eynon without any treatment	22.65	16.88
(2)	Electrometric: silica no treatment	22.60	16.96
(3)	Munson and Walker	23.17	18.65
(4)	Permanganate	23.70	17.82
(5)	Low's Thiosulphate method	22.80	17.25

SUMMARY:-

The effect of calcium salts in causing low results reported for the Lane and Eynon method, has been confirmed for the Lane and Eynon and Electrometric methods. With neutral lead acetate defecation followed by decalcification, the results on the whole were very slightly lower than when oxalate alone was used. This points to the precipitation of reducing non-sugars by the lead acetate. The highest results were those for oxalate treatment alone, where the reducing non-sugars remained, but the depressing effect of calcium salts was removed.

(b) Tests on Boiling Times.

A set of ordinary flat bottomed flasks and a set of pyrex flasks were procured and each flask was weighed.

Ordinary set 250 ccs.capacity.		Pyrex set 250 ccs.	
Flask No.	Gms.	Flask No.	Gms.
1	49.35	1P	82.25
2	45.55	2P	92.00
3	48.45	3P	82.50
4	48.85	4P	86.35
5	36.95	5P	96.35
6	55.45	6P	87.15
7	49.90	7P	91.95
8	45.65		
9	59.05		
10	46.60		

Flask Dimensions.

Diameter at neck	2.1 cms.	3.5 cms.
Length of neck	8.5 cms.	5.3 cms.
Diameter of bottom	4.0 cms.	4.0 cms.
Total height	16.8 cms.	13.0 cms.
Max.Diameter of bulb	7.8 cms.	8.4 cms.

From above weights it is seen that the glass of these flasks must have varied greatly in thickness from flask to flask.

(b) Tests on Boiling Times.

250 ccs. Flasks of ordinary and Pyrex series tested.

Test Solution:-

125 gms. Sucrose + 100 ccs. standard invert solution made to 500 ccs. 100 ccs. standard invert solution contains 1 gm. of invert sugar.

Thus concentration of Test Solution was:-

(25 gms. Sucrose, 0.2 gm. Invert Sugar) Per 100 ccs.

Test No.	Flask No.	Flame inches	Fehling's solution ccs.	ccs. solution added	Time to boil Mins.	Time to boil secs.	Titration ccs.	ccs. water added.
(1)	1	6	10	26.0	3	25	out	0
(2)	3P	6	10	24.0	2	55	out	0
(3)	5	6	10	15.0	2	10	20.65	0
(4)	4	6	10	20.0	2	55	21.10	0
(5)	2P	6	10	20.0	2	40	21.02	0
(6)	6	8	10	20.0	2	05	20.92	0
(7)	1	8	10	20.0	1	55	21.05	0
(8)	1P	8	10	20.0	1	50	21.00	0
(9)	9	4	10	20.0	5	00	20.90	0
(10)	10	4	10	20.0	4	15	21.10	0
(11)	17P	9	10	20.0	1	35	21.40	0
(12)	8	9	10	20.0	1	15	21.50	0

(b) Tests on Boiling Times (contd.)

Test No.	Flask No.	Flame inches	Fehling's solution ccs.	ccs. solution added	Time to boil Mins.	secs.	Titration ccs.	ccs. water added.
(13)	3	9	10	20.0	2	15	22.22	20
(14)	7P	9	10	21.5	2	25	22.30	20
(15)	7	9	10	22.0	3	05	22.50	40
(16)	2P	9	10	22.0	2	50	22.90	40
(17)	3	9	10	22.0	3	00	22.85	40
(18)	4P	4	10	22.0	5	15	22.00	20
(19)	7	4	10	20.0	5	10	22.20	20
(20)	1	4	10	22.0	7	15	22.60	40

Results of Tests on boiling times and on Flask shape and size.

In these tests the time of boiling was varied in two ways

(1) by adjusting the size of flame.

(2) by adding water before heating.

This made a range of "times to boil" from 1 minute 15 seconds to 7 minutes 15 seconds. The variations in volume used for titration was from 20.65 ccs. to 21.5 ccs. without added water, rising to 22.90 ccs. with 40 ccs. added water.

The differences obtained show that the time of boiling is certainly very important, and so also is the total volume in the flask, since this is the most important factor in the time taken to boil.

Recommendation:-

It is recommended therefore that flasks of uniform size, shape, and thickness of glass should be selected for the volumetric estimation of reducing sugars, and that the amount of liquid in the flask should be adjusted to give approximately the same volume for each titration.

In order to get a good end-point with the methylene blue procedure it is further recommended that a volume of either 20 or 40 ccs. distilled water be selected for addition before heating, and that the titration should be arranged so that 30 to 35 ccs. of test solution are used.

The flasks preferred by the author are round flat-bottomed Pyrex flasks of 250 ccs. nominal capacity and of weight 85 to 95 gms.

(C) Effect of Varying Shape and Size of Boiling Flasks used for Lane & Eynon's Method.

Test Solution:- Concentration per 100 ccs.

25 gms. Sucrose

7" Flame throughout. 0.2 gm. Invert Sugar.

10 ccs. Fehling's Solution used in each case & 3 Titrations performed.

20 ccs. of Test solution was added to each flask before heating.

Test No.	Particulars of flask	Nominal capacity ccs.	Actual capacity to neck ccs.	Diameter of Neck cms.	Length of neck cms.	Diameter bottom cms.	Height cms.	Greatest diameter cms.	Av. Titration ccs.
(1)	Flat Bottomed Round Resistance Moncrieffs.	250	255	2.1	8.5	4.0	16.8	7.8	21.10
(2)	Conical Flask Moncrieffs	500	500	2.7	3.0	7.0	16.5	9.8	20.89
(3)	Conical Pyrex	250	290	2.5	3.0	6.0	13.5	8.4	21.12
(4)	Conical Bohemian glass.	200	230	2.1	2.3	4.6	13.0	7.3	21.20
(5)	Conical Moncrieffs	100	105	1.8	2.4	4.5	11.0	5.5	21.20
(6)	Conical Moncrieffs	50	50	1.8	3.1	3.3	9.0	4.6	21.00
(7)	Flat Bottomed Pyrex wide mouth	250	280	3.5	5.3	4.0	13.0	8.4	21.00

The results obtained in the above tests for titration ccs. would seem to show that the effects of kind of glass and surface exposed have been exaggerated. There is apparently very little difference between using a 50 ccs. flask and a 500 ccs. flask. It is obviously preferable to use one size and shape of flask for standard purposes.

In concluding this section it should be mentioned that the determination of reducing sugars forms subject 4 of the research agenda of the International Commission for Uniform Methods of Sugar Analysis under the Presidency of Doctor Frederick Bates, U.S. Bureau of Standards.

The author is an associate referee for Subject 4: he hopes to present the results here recorded at the Tenth Session of the Commission.

P A R T 4.

THE DETERMINATION OF REDUCING SUGARS IN WHITE SUGARS.

In this part of the work, the determination of reducing sugars in white sugars has been made by several methods. The methods have been compared, using specially purified sucrose, samples of white sugar, and invert-sugar solutions of known concentration.

THE DETERMINATION OF REDUCING SUGARS IN WHITE SUGARS.

Until fairly recently no attention had been paid to this question: even now some refiners are reluctant to adopt control of reducing sugars in the final product.

The importance of analysis of white sugars has been shown in Part 1 of this work, and there is need for an accurate method of determining small amounts of reducing sugars in presence of a large excess of sucrose.

Seven methods which have been put forward are here described. The methods have been compared, and their advantages and defects are discussed.

The introduction to part 3 (page 66) describing the important factors in reducing sugar estimations, may be referred to as having obvious connection with the methods now detailed.

INTRODUCTION TO THE METHODS:

In the following experimental work, several known methods of determining small quantities of reducing sugars in white sugars were investigated.

1. Saillard's method, copper reagent of high alkalinity; reaction carried out at a lower temperature than the boiling point of water, cuprous oxide estimated by Bertrand's permanganate method.

2. Berlin Institute and 3. Ofner's method, iodometric, reduced copper being determined.

4. Luff-Schoorl, estimation made iodometrically through the unreduced copper.

5. Ost's method, copper reagent made with Potassium bicarbonate and carbonate; cuprous oxide estimated by Bertrand's permanganate method.

6. Main's method, using "L.F.S." solution, volumetric, methylene blue as internal indicator.

SAILLARD'S METHOD: The copper reagent contains 65 g. NaOH/Litre otherwise it is prepared like Fehling-Sohxlet solution. A mixture of 20 ccs of this reagent and 50 ccs of sugar solution is heated in a conical flask in a water bath at 63 - 64 °C for 22 minutes and the Cu_2O ascertained by Bertrand's method in exactly the same way as in Ost's method. A table given oovers a range of 0 - 80 mg. of invert sugar and 0 - 8.15 g. of sucrose in the 50 cc used for the test. The acid ferric sulphate used for the dis-solution of Cu_2O contains ferric sulphate 50 g. and H_2SO_4 200 g/L (Betterave et Sucrerie de Betterave, 1923, Vol. 1. p. 137).

Example of Application of SAILLARD'S METHOD.

Determination of Invert Sugar in "Ely" Beet

Molasses:- 13 g. of molasses plus 25 ml. of a 10 per cent neutral lead acetate solution was made to 250 ml. filtered, 50 ml. of filtrate plus 5 ml. of a 10 per cent potassium oxalate solution and a little alumina cream was made to 100 ml. and filtered. 50 ml. of the filtrate was used for the estimation.

0.9 cc. of .5% $KMnO_4$ was used = 9 mg. of copper

= 4.68 mg. of Invert (table)

Per cent of invert sugar = $\left(\frac{.00468}{1.3} \times 100\right)$ = .36

In the same sample, invert sugar was estimated by Ofner's method = .677 per cent and by the electrometric method = .665 per cent.

This method is very good for large percentages of invert sugar, as in cane molasses and raw sugars, but is not suitable for white sugars, as seen below:-

	Std. 118	Castor	Castor purified by double purification with alcohol.
Saillard's	.000	.0136	.000
Ofner's	.0015	.009	.000

DESCRIPTION OF THE METHODS OF OFNER, BERLIN INSTITUTE, LUFF SCHOORL, OST and MAIN WITH EXAMPLES OF EACH.

1. OFNER'S METHOD: In Herzfeld's Method, the considerable reducing action of sucrose necessitates very careful observance of the operating conditions, such as the time taken to bring to boiling, the duration of boiling etc. Ofner recommends the replacement of Soxhlet's solution by a copper reagent on which sucrose has no action. Such solutions can be used for all kinds of sugar products irrespective of their sucrose content, no tables being necessary to work out the results.

Method of 1932 as adopted by the Czechoslovakian Sugar Industry:- The copper reagent is prepared as follows - 5 g. of $CuSO_4$ .5 H_2O. 10 g. anhydrous Na_2CO_3. 300 g. Rochelle's salt 50 g. of Na_2HPO_4 .12 H_2O. the constituents dissolved mainly in the cold, but heating for two hours on a water bath to destroy the spores is recommended. When cool and made up to volume it is shaken with a little active carbon or kieselguhr and filtered after standing for some time.

The Iodine solution contains 4.0995 g. of 1/litre

The thiosulphate " " 8.0165 g. of $Na_2S_2O_3.5H_2O$/L

Iodine is soluble in Water only to the extent of one part in 7000 parts of water, consequently the above solution could not be prepared with iodine alone. 2.6 g. KI plus the iodine is dissolved in a little water and made to volume. (KI plus I_2 = KI_3).
The iodine solution for the Berlin Institute Method was also made with KI.

PROCEDURE: 50 ccs. of this solution and 50 ccs of the sugar solution containing 10 g. or less of the sample is brought to boiling in 4 - 5 minutes in a 250 ccs. spherical flask (Ofner recommends 300 cc. Brlenmeyer flask) and kept in gentle ebullition for exactly five minutes, after which it is rapidly cooled under the tap without motion, the liquid acidified with 15 ml. of N HCl. treated at once with excess of .0323 N.1 solution and excess of iodine is titrated with 0.0323 N thiosulphate solution, using starch indicator.

CALCULATION: OFNER'S METHOD.

Each cc. of iodine consumed equals 1 mg. of invert sugar so that .006357 g. of copper =.00310 g. of invert. One molecule of invert sugar $C_6H_{12}O_6$ reduces 6.2 CuO to cuprous oxide; a correction of 1 cc. of iodine is made for the reducing action of 10 g. of sucrose.

EXAMPLE:

In a test 20 ccs. of .0323 N.1 was added and 14.2 ccs. of 0.0323 thiosulphate were required for the excess of iodine.

Find the percentage of invert sugar.

Iodine consumed = (20.0 - 14.2) = 5.8 ccs.

Correction for reducing action of 10 g. of sucrose = 1.0 ccs.

(5.8 - 1.0) - 4.8 ccs. of .0323 N1 = 4.8 mg. of invert; percentage of invert sugar = $\left(\frac{.0048}{10} \times 100\right)$ = .048

(The International Sugar Journal, Jan.1937, p.14S).

Berlin Institute Method.

The copper reagent is prepared as follows (Muller's Solution)

35 g. of $CuSO_4$ $5H_2O$ dissolved in 400 ccs. of boiling water. In another vessel 175 g. of Rochelle Salt, together with 68 g. of anhydrous Ns_2CO_3 are dissolved and made to 500 ccs. After cooling the two solutions are mixed, made to 1 litre, shaken with 1 - 2 teaspoonfuls of active carbon, and after standing for several hours filtered through a hardened filter paper.

PROCEDURE: BERLIN INSTITUTE METHOD.

The sugar solution is not defecated. 10 g. or lesser quantity of sugar to contain less than 30 mg. of invert sugar is dissolved in 100 ccs. of distilled water, mixed with 10 ccs. of Mueller's solution in a 250 cc. spherical flask (Method recommends 300 ml. Erlenmeyer flask) and heated in a water bath boiling so vigorously that the immersion of the flask does not stop ebullition, for exactly 10 minutes, after which the flask is cooled without shaking under the tap, treated with 5 ccs. of 5 N acetic acid (tartaric acid may be used), excess of N/30 I solution added shaken so that the precipitate may dissolve and the excess determined by titration with N/30 thiosulphate, a few ccs. of 1 - 2 per cent starch solution being used as indicator.

Calculation. The volume of iodine consumed is corrected as follows:-

(1) Correction made occasionally using distilled water instead of the sugar solution; found to be 0.2 cc.

(2) Correction for the iodine consumption of 10 g. of sucrose found by repeating the test with the exception that no heating is applied, generally 0.1 cc. though may be as high as .7 cc.

(3) 2 cc. of N/30 I for the reducing action of 10 g. of sucrose.

After the corrections have been made, each cc. of N/30 iodine equals 1 mg. of invert sugar.

EXAMPLE: BERLIN INSTITUTE METHOD:

In a test carried out in the hot 20 ccs. of iodine was added and 12.0 ccs. of thiosulphate used in back titration. In the experiment carried out in the cold 5 ccs. of N/30 I was added and 4.3 ccs. of thiosulphate used in back titration.

CORRECTIONS:- In an experiment using only 100 ccs. of distilled water plus 10 ccs. of Muller's solution 5 ccs. of N/30 iodine solution were added and 4.8 ccs. of thiosulphate used in back titration. Find the percentage of invert sugar if the reducing action of 10 g. of sucrose equals 2.0 ccs.

Iodine consumed in test = (20 - 12.0) = 8.0 ccs.

(1) Correction for iodine consumption of 10 g. of sucrose (5 - 4.3) = .7 ccs.

(2) -do- for action of distilled water (5 - 4.8) = .2 ccs.

(3) -do- reducing action of 10 g. of sucrose = 2

(8 - .7 - .2 - 2.0) = 5.1 ccs. = 5.1 mg. of invert sugar.

$$\text{Percentage of invert sugar} = \frac{(.0051 \times 100)}{(10)}$$

$$= .051.$$

Luff-Schoorl Method.

The copper reagent is prepared as follows:-

17.3 g. of $CuSO_4\ 5H_2O$ and 115 g. of citric acid crystals dissolved in 200 ccs. of water by gentle heating. To this solution, when cool, is added with shaking, a solution containing 185.3 g. of anhydrous Na_2CO_3 in about 500 ccs. of water in small quantities at a time so that solution may take place. After cooling, the solution is made to one litre, shaken with 2 g. of washed and ignited kieselguhr and filtered by suction. The alkalinity of the solution should be 1.78 N (by phenolphthalein) and should be controlled.

Raw sugars are defecated with neutral lead acetate and excess lead removed by phosphate-oxalate mixture.

PROCEDURE - LUFF-SCHOORL METHOD:

0.5 g. or a smaller quantity of sugar to contain less than 45 mg. of invert sugar is dissolved in 25 ccs. of distilled water, and mixed with 25 ccs. of the copper reagent in a 250 ccs. spherical flask, a little pumice powder being added to lessen super-heating.

The solution is heated under reflux while resting on wire gauze which in turn rests on an asbestos card having a central hole of 6.5 oms. diameter. The liquid is brought to boiling in three minutes (using a Méker Burner) and boiling continued for five minutes. The flask is then cooled under the tap and 15 ccs. of 20 per cent KI (iodate free) solution added, followed by 15 ccs. of 25 per cent H_2SO_4 which is added slowly to avoid loss by effervescence. The iodine liberated is then titrated with N/10 $Na_2S_2O_3$ $5H_2O$. 1 cc. of a one per cent starch solution is added as indicator near the end of the titration. The H_2SO_4 should be free from iron to avoid after-blueing. The end point of the reaction is shown by the disappearance of blue and appearance of a cream coloration.

A blank determination is made using 25 ccs. of distilled water, this determining the amount of copper in the reagent. The difference between the colume of N/10 thiosulphate used in the blank and that used in the actual test is referred to in Luff Schoorl's Table and the invert sugar determined. Since the copper reagent contains 17.3 g. of $CuSO_4$ per litre, the volume of N/10 thiosulphate used in the blank theoretically should be $\frac{(.0173 \times 25)}{(.24971)}$

= 17.32 ccs.

(The mean of a number of blanks carried out at different times was 17.35 ccs.)

LUFF SCHOORL'S TABLE.

Showing Milligrams of Invert-Sugar.

cc.N/10 Thiosulphate.	No Sucrose	1.25 g.	2.5 g.	5 g. of Sucrose.
0.0	0.00 mg.	-	-	-
0.4	1.40	1.00 mg.	0.60 mg.	0.15 mg.
0.5	1.75	1.30	0.90	0.45
1.0	3.50	2.90	2.50	2.00
2.0	6.55	5.90	5.50	5.00
4.0	12.55	11.90	11.50	11.00
6.0	18.60	18.00	17.50	17.00
8.0	24.70	24.10	23.50	23.00
10.0	30.85	30.25	29.75	29.05
12.0	37.10	36.55	36.25	35.30
14.0	43.50	42.95	42.50	41.70
16.0	50.20	49.65	49.15	48.30

(From the International Sugar Journal, Jan. 1937, p.145. International commission for uniform methods of Sugar Analysis).

Example of Calculation:- In a blank test carried out the iodine liberated equalled 17.35 ccs. N/10 thiosulphate; in a test using 5 g. of sugar the iodine liberated equalled 16.7 ccs. N/10 thiosulphate. Find the percentage of invert sugar.

Iodine consumed = (17.35 - 16.7) = 0.65 ccs. which from the Luff Schoorl's Table is seen to correspond to 0.45 $\left(\frac{.15}{15} \times 1.55\right)$ = .915 mg. of invert. Percentage of invert sugar $= \left(\frac{.00915}{5} \times 100\right) = .0183$

This method estimates the invert sugar by determining the amount of unreduced copper.

$$2CuSO_4 + 9\ KI = Cu_2I_2 + I_2 + 2K_2SO_4$$

or $2Cu + 41 - Cu_2I_2 + I_2$

For this reaction to be complete the potassium iodide used must be ten times that required theoretically. (Clowes and Coleman, Quantitative Chemical Analysis p.191 Churchill, 1931.) The amount of KI solution used in the Luff Schoorl Method justifies this.

Ost's Method.

The copper reagent is prepared as follows:- 250 g. K_2CO_3 and 100 g. $KHCO_3$ is dissolved in about 700 ml. of water in a litre flask by heating in a water bath to 70 - 80° C. cooled and a solution containing 15.7 g. of $CuSO_4$ is added to it in small amounts at a time, meanwhile agitating. It is next made to mark at the proper temperature, well shaken, and passed through a hardened filter paper. This is called the normal solution. A fifth normal solution is prepared from this, which serves for the examination of refined products, by mixing one volume of the normal solution with four of a solution containing 250 g. of K_2CO_3 and 100 g. of $KHCO_3$ per litre. This is more sensitive than solutions of higher copper content and attacks sucrose less.

Procedure. Ost's Method.

In the determination of invert sugar in white sugars 75 ccs. of N/5 solution is mixed with 50 ccs. of an aqueous solution containing 10 grams of the sample of white sugar in a 250 ccs. spherical flask and heated in a vigorously boiling water for exactly 15 minutes, after which it is filtered on a Gooch crucible covered with asbestos, under vacuum, the cuprous oxide removed to the filter by hot water, and thoroughly washed by hot water; next it is dissolved in 70 ccs. of acid ferric sulphate (50 g. of ferric sulphate and 200 g. of H_2SO_4 per litre), the filtrate being received in another clean flask together with washings; the ferrous iron produced is titrated with 0.5 per cent $KMnO_4$ (this concentration of $KMnO_4$ is chosen, so that one ml. of it equals 10 mg. of copper for $\left(\frac{5}{3.16} \times .006357\right)$ equals .01004 g.) using a microburette, which reads the volume to one fiftieth of a cc. The following reactions take place:- (1) $C_6H_{12}O_6$ + approximately $(6\ CuO \rightarrow 3Cu_2O)$;

(2) $Cu_2O + 5\ Fe_2(SO_4)_3 + H_2SO_4 = 2\ Fe\ SO_4 + 2CuSO_4 + H_2O$

(3) $10\ FeSO_4 + 2\ KMnO_4 + 8\ H_2SO_4 = 5\ Fe_2(SO_4)_3 + K_2SO_4 + 2MnSO_4 + 8H_2O$

(Schwartz Method). This method is known to give accurate results while being less tedious than the gravimetric method. The percentage of invert sugar is found from the weight of invert sugar formed from Ost's Table.

OST'S TABLE for N/5 SOLUTION.

Invert Sugar.	Cu.	Invert Sugar.	Cu.	Invert Sugar.	Cu.
1 mg.	9.0 mg.	8 mg.	28.0mg.	15 mg.	45.3 mg.
2	11.8	9	30.6	16	47.6
3	14.6	10	33.1	17	49.9
4	17.4	11	35.6	18	52.1.
5	20.1	12	38.1	19	54.3
6	22.8	13	40.5	20	56.5
7	25.4	14	42.9	21	58.6

(Zeitschrift der Vereins der Deutscher Zuckerindustrie,1919) No. 765 p. 403 - 443).

The normal solution contains .004 g. of Cu per cc. The N/5 solution contains .0008 g. of Cu per cc. The sugar solution prepared for analysis should contain less than 21 mg. of invert sugar.

EXAMPLE OF CALCULATION. 1.94 ccs. of .95 x .5 per cent $KMnO_4$ was found equivalent to the ferrous iron produced by dissolving the precipitated cuprous oxide in acid ferric sulphate. Find the percentage of Invert sugar.

1.94 ccs. of .95 x .5 per cent $KMnO_4$

= 1.84 ccs. of .5% $KMnO_4$

= 18.4 mg. of copper.

from Ost's Table this corresponds to ($4 \div \frac{10}{27} = 4.43$mg) of invert sugar.

Percentage of Invert Sugar = $\frac{.00443}{10} \times 100 \div 0.0443$.

For Ost's method a blank test ought to be carried out using 25 ccs. of distilled water. Such tests were carried out and always less than 0.05 cc. of permanganate was required. The correction was ignored as in many blank determinations no permanganate was found necessary.

OST'S METHOD.

Test on white sugar containing known amounts of Invert Sugar, using "Fifth Normal" Ost's Solution: 10 gms. crystal sugar(best grade)used in each test and invert sugar solution added in quantities corresponding to the under-noted milligrams.

Milligrams Invert Sugar.	ccs. $KMnO_4$ Required	Corrected for Blank	Equivalent Milligrams Copper.	Milligrams from Ost's Table.
5	2.26	2.11	21.1	20.1
5	2.17	2.02	20.2	20.1
10	3.50	3.35	33.5	33.1
10	3.48	3.33	33.3	33.1
15	4.70	4.55	45.5	45.3
15	4.71	4.56	45.6	45.3
20	5.65	5.50	55.0	56.5
20	5.67	5.52	55.2	56.5

Micro-burette used: Blank = 0.15 cc.

The above results show slight deviations from those given by Ost. In general the agreement is good.

CAUSE OF LOW RESULTS IN PERMANGANATE METHOD.

Low results are due to re-oxidation of the ferrous sulphate when the cuprous oxide is dissolved in a mixture of ferric sulphate and sulphuric acid. If the Cu_2O is first dissolved in ferric sulphate or ferric alum solution, and the sulphuric acid is not added until immediately before the titration with permanganate, correct results are obtained.

Vide:

Schoorl and Regenbogen:

Z. ver. deut. Zuckerind. 67. 563. (1917)

Bruhns: Contr. Zuckerind. 38. 1018. (1930)

Pick: Z. Zuckerind Cechoslovak Rep.,

49, 235, (1924/5)

MAIN'S METHOD.

This is a volumetric method using methylene blue as internal indicator. For larger percentage of invert sugar Sohxlet's modification of Fehling's solution is used as the copper reagent, but for the estimation of small proportions of invert sugar in presence of large amounts of sucrose, a special copper reagent named "L.F.S." Solution is prepared containing potassium ferrocyanide.

(1) 173 g. of Rochelle salt, 40 g. of NaOH are dissolved in water also 14.647 g. $K_4Fe(CN)_6$ dissolved in distilled water, the two solutions made up to 250 ccs., after mixing.

(2) 17.315 g. of $CuSO_4 . 5H_2O$ made to 250 ccs.

(3) 100 g. of NaCl made to 500 ccs. (5N solution).

Equal volumes of (1) and (2) is known as "L.F.S. solution". For purposes of determination of small percentages of invert sugar, equal volumes of L.F.S. and solution (3) are mixed; this is known as the "extra alkaline L.F.S," 1 cc. of which equals .0025 g. of invert sugar.

The solution is standardized against standard invert sugar solution.

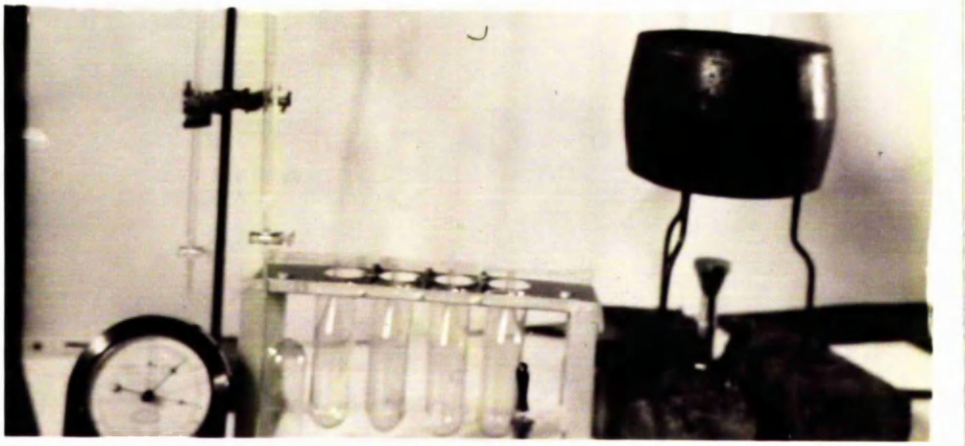


FIG. 17

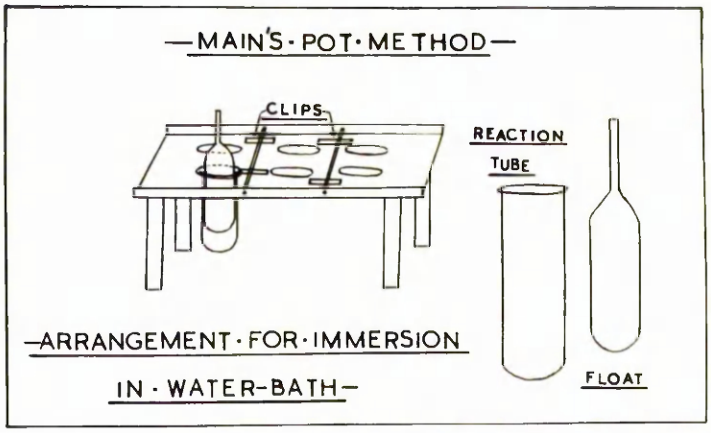

—MAIN'S · POT · METHOD—
CLIPS
REACTION TUBE
FLOAT
—ARRANGEMENT · FOR · IMMERSION IN · WATER-BATH—

FIG. 18

APPARATUS.

The apparatus consists of tubes 15 0 mm. long, 38 mm. in diameter, provided with floats of the same form which make a sliding fit in the other tubes. The ends of the floats are drawn out to a taper making the total length 170 ccs. The water bath is an ordinary cylindrical pot of three gallons capacity and is filled with water to within 5 c.m. of the top.

The tubes can be fitted in and locked to a rack which can rest on the pot.

PROCEDURE.

A twenty per cent or thirty per cent solution of the sugar to be examined is prepared, the concentration chosen being according to the invert sugar concentration present. Gradually increasing volumes of this solution are placed in adjacent tubes in the rack, the volumes differing by equal increments. A definite volume of the extra alkaline L.F.S. (1 or 2 ml. according to the concentration of the invert sugar present) is added to each tube together with two drops of a ten per cent.solution of methylene blue which serves as indicator. The tubes are well shaken to mix the contents.

The floats are placed in position, pushed down and allowed to rise by buoyancy, the purpose being to eliminate air bubbles. The tubes are then locked to the rack and the rack placed in the boiling pot for exactly five or ten minutes depending on the concentration of invert sugar present, at the end of which time the rack is taken out of the bath and the colours observed. The correct volume of the sugar solution which reduces completely the volume of the L.F.S. under the given conditions will lie between the volume of the last sugar solution which appears blue after the test and the volume of the first sugar solution which will be colourless at the end of the test. If the limits were not found in the first test, a second test should be done. In subsequent tests the intervals will be lessened between the found limits and so on until the required volume of the sugar solution is determined down to a tenth of a ml.

Results are found from a Table given by the author of the method.

The potassium ferrocyanide makes the L.F.S. exceedingly sensitive; it is to be present in the quantitative amount required. It reacts with the cuprous oxide as it is formed, thus removing the red coloration that tends

to mask the end point of the reaction as indicated by methylene blue. The end point is shown by the sudden disappearance of the blue colour.

$$2Cu_2O + K_4 Fe(CN)_6 + 2H_2O = Cu_4Fe(CN)_6\ 4KOH$$

(The International Sugar Journal, June, 1932.)

EXAMPLE.

With a twenty per cent, solution of sugar, 1cc of extra-alkaline L.F.S., the volume of solutions used in test (1) were 15 ml. 20 ml. 25 m.l. 30 ml. After five minutes of heating first was blue and other colourless. In a second test volumes of the solution used were 16 ml. 17 ml. 18 m.l 19 ml, under the same conditions as before; after five minutes of heating, the first two tubes were blue, last two tubes were colourless. In a third test the volumes of the same sugar solution used were 17.1, 17.3, 17.5 and 17.7. ml. under the same conditions and it was found that the first tube showed a trace of blue while others were all colourless.

The required volume was taken equal to $\frac{17.1 + 17.3}{2}$

$= 17.2$ ml.

From Main's Table this was found to correspond to 0.0363 per cent. of invert sugar.

PART OF MAIN'S TABLE.

Sucrose Grams per Litre	20	30
L.F.S. Extra Alkaline. ccs.	1	1
Time of heating (Minutes)	5	10
ccs. of Solution required for reduction.	% Invert Sugar	% Invert Sugar
15	.0265	.0085
16	.0251	.0071
17	.0229	.0060
18	.0208	.0052
19	.0191	.0045
20	.0177	.004
21	.0166	.0035
22	.0158	.0031
23	.0151	.0028
24	.0145	.0025
25	.0139	. -
26	.0133	.002
27	.0128	-
28	.0123	.0015
30	.0144	.001
32	.0105	-
35	.0094	-

(From the International Sugar Journal, June 1932).

NOTES ON MAIN'S POT METHOD:

The use of a boiling water bath as a source of heat is not new. It was proposed many years ago by Reischauer and Kruis (Oest Ungar Zeitsch Zuckerind 12, p.254) who used a fixed volume of sugar solution and increasing volumes of Fehling's solution and immersed the reaction tubes in a bath of boiling water for 20 minutes (see Browne's "Handbook of Sugar Analysis" (1912) p.398, where it is remarked that "the large amount of labour and time necessary for completing a determination has been, however, a serious obstacle against the general use of the method.")

J.S. Mann adopted the same principle, but used increasing volume of Fehling solution. This method was tried by Main but it was found that the back-oxidation of the cuprous oxide and the methylene blue indicator made the end-point uncertain. After a number of experiments floats were devised which simply but effectively excluded the air from the solution during heating. The end-points were now very definite.

These tubes should be of nearly the same weight and size, and as thin as practicable, since thin glass allows of more rapid heating of the liquid. Those used in this work were made of pyrex glass. The floats are similar tubes making a sliding fit

in the reaction tubes.

MAIN'S POT METHOD:- Standardisation of the L.F.S. extra alkaline solution.

36.5, 37, 37.5, 38, 38.5 mls. of Standard Invert Sugar Solution containing 0.025 gms. "invert sugar" / 100 mls.,were added to each of 5 tubes, 4 ml. of the extra alkaline L.F.S. solution added and then two drops of a 1 per cent methylene blue solution, the floats inserted and the tubes immersed in the boiling bath for exactly five minutes. It was found that 38 mls. and lower were still blue while the 38.5 ml. was discoloured. On further experiment -

38.1, 38.2, 38.3, 38.4 } mls. were employed and it was found that 38.3 mls. were just required for complete discolouration. On repeating this range the result obtained was 38.2 ml. so the mean 38.25 mls. was taken as the required amount of Standard Invert Sugar Solution required for complete reduction. This gives a normality of $\frac{38.2}{37} = 1.0338$ for the L.F.S. i.e. -

$1.0338 \times X = 4$

$X = \frac{4}{1.0338}$

$= 3.8$ mls.

where X = amount of L.F.S. extra alkaline solution required to give a standard solution.

In order to test this normality figure 36.9 37.0 and 37.1 mls. of the Standard Invert Sugar Solution containing 0.025 gms./100 ml. were placed in each of three tubes and the experiment carried out as before except that 3.87 mls. of Fehling's extra alkaline L.F.S.

solution were added, and the tube immersed in the boiling water for exactly five minutes. It was found that 37.0 mls. were just sufficient to cause decolorisation, and this figure is in agreement with the required amount of Standard Invert Sugar Solution required.

EXPERIMENTAL.

1. With sugar purified by crystallising out from an aqueous solution by addition of absolute alcohol. A super-saturated solution of the purest available cane sugar was prepared and filtered: specially purified absolute alcohol was added and the sugar that separated spun off in a centrifugal. A portion of this was dried in the air and then in a vacuum oven at a temperature of 40°C. and was labelled "sugar purified by single precipitation with alcohol." The other portion was again dissolved in water, precipitated in the same way as before, spun off in a centrifugal, air dried and dried finally in a vacuum oven at 40°C. for two hours. Sugar purified by double precipitation with alcohol should contain no invert sugar: sucrose prepared in the above way is used for standardizing saccharimeters.

The methods for purification of the alcohol and sucrose are here given in detail:-

PREPARATION OF PURE SUCROSE.

PREPARATION OF ALCOHOL: The alcohol used for the precipitation of pure sugar should be highly purified with respect to acids or aldehydes. It is not essential that it be dry or free from other members of the alcohol group. The method of purification here described meets these requirements.

Dissolve 1.5 gms. of silver nitrate in 3 ccs. of water, add to a litre of 95% alcohol in a glass stoppered cylinder and shake.

Dissolve 3 gms. of potassium hydroxide in 10 ccs. of warm alcohol and after cooling pour slowly into the alcoholic silver nitrate solution. Do not shake. Allow to stand overnight. Siphon off and distil.

PURIFICATION OF SUGAR: Prepare a saturated solution of sugar in distilled water, never allowing the temperature to exceed 60°C. Filter through a sintered glass funnel or asbestos under vacuum. The solution should be brilliantly clear. Keep in a bath at 60°C and add enough of the prepared and wormed alcohol to precipitate the bulk of the sugar. Filter through a lintless and thoroughly washed piece of linen in a Buchner funnel. Never let solutions touch metal, paper, or be contaminated by dust. Keep all vessels covered. Wash crystals with a little warmed prepared alcohol, then again dissolve in distilled water at 60°C. making a saturated soln. precipitating and then filtering through the re-washed linen as before.

Dry crystals at not over 40°C. in dust-free air, then reduce to a moderately fine powder: do not screen, and dry in a layer not more than $\frac{1}{8}$" thick over freshly burned lime, preferably in a vacuum. Keep in a large weighing bottle and determine amounts taken by difference in wts. of bottle.

Determine moisture in a sample by drying four hours at 70°C in a vacuum. Correct weight of portions taken for the moisture contained.

The original sugar and the two purified samples were tested by the following methods:-

METHOD	ORIGINAL SUGAR.	Purified by SINGLE PRECIP-ITATION BY ALCOHOL.	Purified by DOUBLE PRECIP-ITATION BY ALCOHOL.
1. Saillard	-	.000	.000
2. Ofner	.011	.0015	.000
3. Berlin Institute	.026	.019	.003
4. Luff Schoorl	-	.001	.0000
5. Ost	-	.000	.0000
6. Main	-	-	.0005

The results for the sugar purified by single precipitation by alcohol are high; this may be due to traces of alcohol still present in the sugar which exert reducing actions on the copper reagent.

2. With White Sugar. The percentage of invert sugar was determined in a number of samples of white sugars by the five methods of Ofner, Berlin Institute, Luff Schoorl, Ost and Main. The results are shown in the following Table. Results by De Whalley's method, described later, are included for comparison.

COMPARISON OF METHODS FOR DETERMINATION OF REDUCING SUGARS IN WHITE SUGARS.

(% REDUCING SUGARS)

SAMPLE	BERLIN INSTITUTE	LUFF-SCHOORL	MAIN	OFNER	OST	DE WHALLEY
A	.009	.007	.001	.010	.004	.006
B	.021	.002	.001	.008	.005	.006
C	.014	.002	.001	.008	.005	.006
D	.020	.002	.003	.016	.005	.006
E	.017	.003	.002	.013	.004	.005
F	.019	.002	.001	.013	.005	.005
G	.015	.002	.004	.014	.004	.004
H	.033	.015	.006	.020	.015	over
I	.052	.033	.034	.043	.036	-
J	.039	.015	.009	.028	.017	over
K	.051	.018	.036	.048	.045	-
L	.015	.002	.001	.008	.008	.006

The sugars analysed represented direct process sugars, refined cane sugars, and carbonatation beet sugars.

3. Tests carried out with Solutions of Invert Sugar.

PREPARATION OF STANDARD INVERT SOLUTION:- 9.5 g. of pure sucrose is dissolved in about 75 ccs. of water in a 100 ccs. flask and 5 cc. of HCl of 1.19 specific gravity is added and the flask allowed to stand for one week at 14 - 15°C. It is then transferred with rinsings to a litre flask, made to volume and stored. Solution contains $\left(9.5 \times \frac{100}{95}\right)$ or 10 grams of invert sugar per litre. It keeps for months without change.

In a series of tests neutral invert sugar solutions of different concentrations were used, the invert sugar in 50 ccs. being determined by the five methods. For example, to prepare an invert sugar solution which contains 25 mg. of invert in 50 ccs. 50 ccs. of the stock solution was taken, neutralised with ten per cent caustic soda using litmus as indicator and diluted to one litre. 50 ccs. of this solution were used for the test except in the case of Luff Schoorl's method where 25 ccs. were used.

RESULTS.

mg. of Invert Per 50 ml.	Ofner.	Berlin Institute.	Luff Schoorl.	Ost	Main
5	5.45	4.5	4.55	3.9	4.94
10	10.95	10.0	9.42	7.23	9.93
12.5	13.5	12.35	11.57	10.28	12.38
15	16.25	15.05	13.7	11.92	15.0
20	21.0	20.08	18.8	16.35	20.05
25	26.35	25.32	23.58	21.14	25.08
Average 14.58	15.58	14.57	13.60	11.80	14.57

Average of the determination by different methods for each concentration.

Mg. of Invert per 50 ml.	Average
5	4.67
10	9.51
12.5	12.01
15	14.38
20	19.25
25	24.3

4. DISCUSSION OF RESULTS:- From the experiments done with sugar purified by double precipitation by alcohol, assuming that the sugar was finally free from Invert sugar, it is seen that in all the methods the copper reduced by the action of sucrose on the reagent has agreed well with what had been allowed for the respective methods, except in the case of Berlin Institute Method where the reducing action of 10 g. of sucrose has been found higher than the equivalent of 2.0 ml. of N/30 iodine (what is allowed) by 0.3 ml.

OFNER'S AND BERLIN INSTITUTE METHODS: Results for the white sugars by the Ofner's and Berlin Institute Methods are fairly comparable, though in any particular case the result recorded for the Berlin Institute Method is higher than that for Ofner's Method. In the case of invert sugar solutions, the opposite is true, but in this case it must be noted that the concentration of invert is higher. However, in the case of Berlin Institute Method, the results obtained for invert sugar solution are in close agreement with the actual values, which is evidence in favour of the accuracy of the method. It must be observed that the concentration of invert sugar in the invert sugar

solutions used are higher than in the solutions of white sugars.

For the invert sugar solutions, results by Ofner's Method are higher than those of Main's and Berlin Institute's by 1 mg.

The accuracy of the two methods appears about equal.

For each method, if the sugar is weighed accurately and volumes measured to 0.1 ml. concordant results can be obtained for the same sugar to .001 per cent. In many duplicate determinations, this has been found to be true.

COMPARISON WITH OTHER METHODS

LUFF SCHOORL METHOD:

The Luff Schoorl Method is not as accurate as the two preceding methods for the following reasons:-

1. Invert Sugar is determined in half as much the quantity of sugar as in other methods (5 gr.).
2. Result is found from titration using N/10 thiosulphate, whereas in the above two methods answers are calculated from readings in terms of N/30 solutions.
3. The method does not determine the reduced copper directly but by the difference of two estimations, one the total copper and

the other the unreduced copper and therefore, the result will contain the errors of both estimations.

The range of invert sugar determination is wider in this method; the method was principally designed for estimation of larger quantities of invert sugar. Invert sugar can be estimated by the method to 0.03 per cent.

With the Luff Schoorl Method, the results found for the white sugars are not concordant. The amount of Invert Sugar in white sugar corresponds to less than 0.5 ml. of the thiosulphate solution, hence the difficulty of obtaining accuracy.

OST'S METHOD:

In Ost's Method, a mirco-burette was used for the titrations. The permanganate method of ascertaining the copper (Bertrand's Method) has been criticised in that some of the ferrous sulphate will be retained by the asbestos. Neglecting this, invert sugar can be determined to .002 per cent. It is possible to obtain concordant results by this method.

It must be pointed out that solutions which contain a certain quantity of invert sugar plus a large quantity of sucrose differ from solutions which contain a certain quantity of invert sugar with an equal quantity of sucrose, because the greater viscosity due to the excess sucrose in the first case will interfere with the formation of cuprous oxide.

Results given for invert sugar solutions with Ost's Method have been found from Ost's Table. They are lower than the quantities of invert sugar known to be present in the solutions. If it be assumed that six groups of CuO are reduced per invert sugar group, results calculated with the aid of this assumption agree more closely with the actual values as shown below:-

mg. of invert present per 50 ml.	Result found from Ost's Table.	Result by the above assumption.
5	3.9	4.64 mg.
10	7.23	9.35
12.5	10.28	13.0
15	11.92	14.96
20	16.35	19.8
25	21.14	24.9

DEFECTS OF THE OFNER & BERLIN INSTITUTE METHODS.

These two iodimetric procedures have the following disadvantages:-

1. They require the use of several solutions and the manipulation is too complicated for routine work.
2. The colour of the copper reagent interferes with the starch indicator change at the end-point.
3. The correct amount of sample to be taken for an estimation is difficult to gauge, since there is no indication until the end of the test as to whether or not too much invert sugar has been present.
4. The application of so many corrections unduly complicates the methods.
5. The necessity for maintenance of standard iodine and thiosulphate solutions is a great disadvantage: the copper reagent itself does not keep very well, requiring occasional blank tests: most potassium iodide contains some iodate, which causes after-blueing.
6. Any increase in accuracy obtainable does not seem to justify the extra time and labour involved.

MAIN'S METHOD:

This method is recognised to be exceedingly accurate, determining the percentage of invert sugar in white sugars to .001 per cent. The method is employed by the London refiners.

The average results for Luff Schoorl Method and Main's Method are of the same order of magnitude. The individual results, however, do differ considerably in many cases. The results shown for the invert sugar solutions by Main's Method closely agree with those of the Berlin Institute and the actual results.

The foregoing comparisons have shown that the results for white sugars and invert sugar solutions are not comparable. This is because the concentration of invert sugar in a reaction mixture consisting of white sugars is much less than in a reaction mixture containing the chosen concentration of invert sugars.

Further, the results for invert sugar alone are in closer agreement than those for white sugars. Similarly, whenever the percentage of invert sugar in the sugar is higher the relative difference of the determination by the different methods is less. These facts show that as the quantity of invert sugar increases, the results found by the different methods approach one another more closely.

CONCLUSIONS:

The results obtained by the Berlin Institute Method are the highest, the next highest being those by Ofner's Method.

It can be seen that as the precentage of invert sugar present increases, the results by the different methods approach each other more closely.

The time required for a single estimation by the different methods is :-

METHOD	TIME IN MINUTES
Luff Schoorl's	18
Ofner	20
Berlin Institute	20
Ost	15
Main	20 to 50

These times assume that water baths etc., are all ready. With a series of tests it was found that the time per estimation was reduced considerably but not equally for all methods. Ost's Method is probably the quickest where many estimations are necessary.

SUMMARY.

The Berlin Institute Method and Ofner's Method appear to give results which are too high when the reducing sugar content of a white sugar is below 0.010 per cent: it is significant that the lowest percentage obtained by either method was 0.008 per cent.

On the other hand, the Luff Schoorl Method and Main's Method seem to give results which are too low when the reducing sugar content is below 0.010 per cent.

The methods of Ost and De Whalley are apparently most satisfactory at below 0.010 per cent reducing sugars. De Whalley's method has the disadvantage that 0.020 per cent of invert sugar completely discharges the methylene blue colour and it is not possible to change the weight taken without altering the whole procedure.

Above 0.010 per cent reducing sugars the results by all methods tend to come closer together.

RECOMMENDATION: Taking all considerations into account the author would recommend the use of either the Ost or the Main Method for the estimation of small amounts of reducing sugars in white sugars.

Method for the Determination of Invert Sugar in Refined White Sugars.

by H.C.S. de Whalley. [I.S.J. Aug. 1937, p.300.]

Solutions Required:-

(1) 0.20% Methylene Blue

(2) 3N.NaOH (between 2.90 & 3.10 $\frac{N}{1}$)

Details of Method:- 7 gms. of ground sugar sample are weighed out (to within 0.05 gm.), poured into a clean drained test-tube 6" x $\frac{3}{4}$" (of 9.4 to 9.6 gms. wt.), and to it added:-

(6 ccs. distilled water
1 cc. of methylene blue solution
1 cc. of NaOH solution) from micro-burette.

The mixture, stoppered by a rubber bung, is shaken vigorously for 15 seconds, cork removed, and the tube immersed in a boiling water bath for 120 seconds. It is then removed and compared with standard tubes: 5 seconds or less is required to match it. The gas is controlled by a manometer; 3.5"-3.75" of water.

Standard tubes:- Ammoniacal copper solutions.

Basis = 19.5 gms. $CuSO_4 .5H_2O$ in boiled distilled water made to 500 ccs.

Invert Standard %	Copper Solution ccs.	.88 Ammonia ccs.
0.001	40.00	10
0.002	24.60	10
0.003	16.40	10
0.004	10.66	10
0.005	7.18	10
0.006	4.92	10
0.007	2.97	10
0.008	2.26	10
0.009	1.74	10
0.010	1.33	10
0.015	0.50	10

Each made to 50 ccs. with boiled distilled water and sealed in a glass tube.

Ammonia 32.9% NH_4OH by Titration.

DE WHALLEY'S METHYLENE BLUE METHOD FOR REDUCING SUGARS IN WHITE SUGAR.

EXPERIMENTAL:- (a) WHITE SUGARS:-

The white sugars tested by other methods of determining reducing sugars were used for this method. The results obtained are shown in the comparative table on page **202.**

(b) WITH SODIUM CARBONATE IN PLACE OF SODIUM HYDROXIDE.

Tests were made using 1 cc. of a 3 Normal solution of sodium carbonate and 1 cc. of the usual methylene blue solution: other details were as given for the standard method, i.e., 7 gms. sugar weighed, etc.

No.	White Sugar	Minutes boiling	% Red. Sugars	% Reducing Sugars using NaOH with 2 minutes boiling.	Remarks.
(1)	E	2	below .001		It is apparent that the time of boiling when sodium carbonate is used as alkali in this method, must be increased to 5 minutes.
(2)	"	4	.0035		
(3)	"	4.5	.004		
(4)	"	5	.004	.0045	
(5)	"	5	.004		
(6)	"	7	.005		
(7)	"	10	.007		
(8)	C	5	.005	.0060	
(9)	"	5	.005		
(10)	Pure Sucrose	5	.0035	.0028	
(11)	"	5	.0035		

(c) EFFECT OF TIME OF BOILING:-

Using the standard method (sodium hydroxide alkali), the time of boiling was varied.

No.	White Sugar	Minutes Boiling	% Red. Sugars	Remarks.
(1)	E	1	.0035	These results show the
(2)	"	1	.0030	
(3)	"	2	.0045	
(4)	"	2	.0045	necessity for accurate
(5)	"	2.5	.0065	
(6)	"	3	.015	
(7)	"	3	Colourless	timing in this method.

(d) VARIATION OF WEIGHT OF SUGAR TAKEN:-

2 minutes boiling.

No.	White Sugar	Gms. taken	% Red. Sugars	Equivalent to Standard No.
(1)	E	1	.002	2
(2)	"	2	.002	2
(3)	"	3	.003	3
(4)	"	3.5	.003	3
(5)	"	4	.0035)	between 3
(6)	"	5	.0035)	and 4
(7)	"	6	.005	5
(8)	"	7	.005	5

These experiments show that the weight taken need not be weighed very accurately. The percentages shown above have not been calculated from the weight used, but have been deduced from the equivalence with the prepared standards.

(e) RECOVERY OF DEXTROSE ADDED TO WHITE SUGAR OF KNOWN REDUCING SUGARS CONTENT.

Dextrose containing 0.38 per cent of moisture was used: 1.004 gms. were made up to 500 ccs.

No.	White Sugar gms.	Gms. Dextrose added.	Total Reducing Sugars present	Reducing Sugars found.	Remarks.
(1)	7	.001	.006	.005	The recovery of dextrose is satisfactory except in No. 6 test, where it is low.
(2)	7	.002	.007	.006	
(3)	7	.003	.008	.0075	
(4)	7	.004	.009	.008	
(5)	7	.005	.010	.009	
(6)	7	.010	.015	.012	

(f) VARIATION OF METHYLENE BLUE STRENGTH:-

No.	White Sugar	Methylene Blue %	ccs. Methylene Blue	% Red. Sugars	Remarks.
(1)	E	0.1	1	.0045	These results show that there is little difference produced by alteration of the methylene blue strength.
(2)	"	0.1	1	.0045	
(3)	"	0.2	1	.005	
(4)	"	0.2	1	.005	
(5)	"	0.4	1	.0055	
(6)	"	0.4	1	.0055	

De Whalley's Method for Reducing Sugars.

The use of the Photoelectric Absorptiometer in place of standards.

The standards described by De Whalley were examined in the Absorptiometer, and a curve constructed from the results obtained.

This curve requires only one standard solution to be available when using De Whalley's method.

Ammoniacal cupric sulphate solutions have been examined photoelectrically by Yoe and Crumpler (J.I.E.C., Anal.Ed., Vol. 7. No. 4. (1935)).

The readings obtained by the author are shown below.

No.	Cuprammonium sulphate solution ccs. per 100 ccs.	Scale readings.	Corresponding Reducing sugars %
1.	0.25	Between 3.0 & ∞	.030
2.	0.50		.015
3.	1.33	2.60	.010
4.	1.74	2.55	.009
5.	2.26	2.40	.008
6.	2.97	2.27	.007
7.	4.92	2.05	.006
8.	7.18	1.65	.005
9.	10.66	1.17	.004
10.	16.40	0.767	.003
11.	24.60	0.046	.002

DE WHALLEY'S METHOD FOR REDUCING SUGARS.

DISCUSSION:- It would appear from the results with sodium carbonate in place of sodium hydroxide that this replacement does not affect the accuracy of the method, and allows of more latitude in regard to time of boiling.

The main defect of the method is its limited range: since 0.02 per cent of reducing sugars completely discharges the blue colour, the test appears to be too delicate for commercial use. It is accurate, however, within its range. The use of methylene blue in this method is an interesting application of an oxidation/reduction indicator.

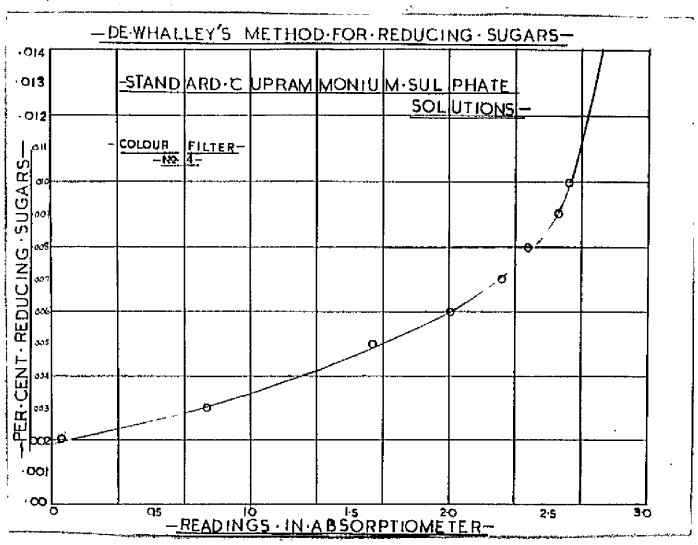


FIG.19

APPENDIX. 1. LAW OF REDUCING ACTION OF SUGARS.

The reducing action of a mono-saccharide upon Fehling's solution may be expressed in general terms as follows:- If for the first minute quantity, s. of a given sugar a definite amount c. of copper is reduced, then for any multiple n of s the weight of copper would be nc if the same concentration of copper was maintained. The latter consideration is, however, never realized in practice and with continuous removal of copper from solution the value nc becomes $nc - (n - 1 + n - 2 + n - 3 \ldots m - n)k$. When working with weighable quantities of sugar, this expression should be modified to -

$nc - (n - 1)\,d - (n - 2 + n - 3 + \ldots + n - n)k$ in which d is the difference between the weight of copper for the first two members of the series s and 2s. The values of d and k are found empirically and knowing these it is possible to construct tables for any of the reducing sugars.

Calculation of reduction tables for reducing power of different sugars is usually made by the method of least squares according to the general formula $y = A + Bx \pm C x^2$ in which x is the mg. of copper reduced by y mg. of sugar, A, B and C are constants. Having determined the values of x for 10 or more values of y the calculation of A, B and C is done as usual.

(From C.A. Browne, A Handbook of Sugar Analysis, p.401).

APPENDIX II.

An Account of the Nature of Ofner's and Berlin Institute's Solutions and the Original Experiments carried out with them by their respective authors, due to J.H. LANE.

The action of Fehling's solution on sucrose is considerable. Ofner's solution has very little action on sucrose. In the Czechoslovakian Sugar Institute some 464 samples of raw beet sugar were tested and their invert sugar content determined by the following five methods. The results show:-

By Herzfeld's Method	0.0397	per cent average
" Pick's "	0.0577	" " "
" Vondrak-Czerny Method	0.0581	" " "
" Ofner's Method	0.0617	" " "
" Ofner's short Method	0.0645	" " "

The results as found by Ofner's method are higher than those found with other methods.

The Berlin Institute Method was developed at the Berlin Institute, using Muller's solution, with beet sugars. The method was applied to a number of first product beet sugars; the results were in the range .015 - .034 per cent. When known quantities of invert sugar were added to the quantities already present, after the determination of the quantities present, the results agree as follows:-

INVERT SUGAR ADDED.	FOUND
0.050 per cent	0.052 per cent
0.100 " "	0.105 " "
0.200 " "	0.220 " "
0.500 " "	0.534 " "

Effect of the various conditions on the reducing action of invert sugar and sucrose. The reducing action of these were compared under the conditions of boiling water bath heating for various periods, with excess of alkaline copper solution of equal copper content but different alkalinities, ranging from that of Fehling's solution which at the ten fold dilution had pH = 13, to that of a solution having pH = 9, it was found that the lower the pH the slower the rate of the reduction of sucrose. The invert sugar showed on the contrary a rapid rise to a practically constant value. The higher the alkalinity of the reaction mixture, the lower was the constant value, but the more rapidly was it attained. At pH 10 - 11 (that of Muller's solution) or more the constant was reached in ten minutes.

With Fehling's solution, the constant value corresponded to the reduction of about five equivalents of copper per hexose; with Muller's solution to 6 and with a solution of about the same alkalinity as Ofner's solution (pH = 9) it corresponded to about seven equivalents of copper but required forty-five minutes to reach constancy.

P A R T 5.

THE COLORIMETRIC DETERMINATION OF IRON IN SUGARS.

The problem of the occurrence of iron in sugar products is here discussed. Fifteen colorimetric methods of determining iron have been examined with particular regard to iron in sugars, and for several of the methods graphs are given for use with the Photoelectric Absorptiometer.

IRON IN SUGARS.

The presence of iron in both cane and beet sugar products has long been known.

The colour of raw cane juice is largely derived from iron compounds of polyphenols. Steuerwald (Archief voor de Java Suikerindustrie, 1911, 19, 1543: I.S.J. (1912) 53) has suggested that the compound Saccharetin isolated by him from the sugar cane, may be the cause of the intense colour produced by the presence of iron, since saccharetin forms an intensely dark compound with iron.

L. Briant in his "Textbook for Brewers" (1911) states that not more than 0.005% of Fe_2O_3 should be present in sugars used for the best quality brewing products, and that 0.025% Fe_2O_3 should be considered the maximum amount allowable in any case.

Zscheye (Centr. Zuckerind 32, 1336, 1924) asks why certain raw beet sugars cannot be washed white, and finds a cause in the formation of iron glucinate in the second carbonatation of the juice.

COLORATION OF SUGARS BY IRON SALTS: Mere traces of iron compounds impart a dark tint to sucrose crystals formed in such solutions. Thus La Bastide mentions a case of yellow coloured sugar obtained from syrup which had been filtered over sand containing iron (Archief 1903, 954).

Stolle determined the solubility of iron salts in

sucrose solutions, and stated that although the solubilities were very low, the amounts were more than sufficient to impart a yellow or red colour to the sucrose solution.

IRON SALTS SOLUBILITY IN SUGARS PER LITRE AT 17.5°C.

Per Cent Sucrose.	Ferric Hydroxide	Ferric Oxide	Ferroso-Ferric Oxide.
10	3.4	1.4	10.3
30	2.3	1.4	12.4
50	2.3	0.8	14.5

IRON DISSOLVED FROM FACTORY EQUIPMENT: Geerligs ("Cane sugar and its Manufacture" Norman Rodger, London, 1924, p.270) remarks that sugar from the commencement of a cane-milling season contains much more iron than the sugar produced after the rust in the tanks and pipes has been washed away.

IRON INTRODUCED FROM PROCESS MATERIALS: In refining, iron may be introduced into the sugar liquors from the animal charcoal used. This initially contains about 0.2 to 0.3 per cent of iron which is probably derived from nails etc., mixed with the bones. The iron in animal charcoal increases with use of the charcoal, by adsorption from sugar liquors, and from abrasion by machinery, and may reach a point where the bone-char becomes saturated with iron and loses its power of removing iron from sugar solution.

Tucker ("Manual of Sugar Analysis" Van Nostrand,

1890 p.305) mentions an increase of iron in animal charcoal from 0.18 per cent to 0.36 per cent during 282 days of use.

COLORATION DUE TO TANNINS AND POLYPHENOLS:

Schneller has proved that the addition of ferric salts causes very little coloration in solutions of pure sucrose and glucose (Heriot "Manufacture of Sugar from the Cane and Beet" Longmans 1920) He attributes the dark colour of juices containing much iron to the simultaneous presence of tannins and polyphenols, which combine with the iron. It is known that tea infusion turns dark when made with sugar or water containing more iron than normal, and the above statement is analogous to this.

Reducing agents cause only a temporary bleaching of this dark colour: the darkening of direct process sugars is due to re-oxidation of traces of phenol-iron compounds.

Iron in the cane-juice can be minimised by tapping the cane "low" - that is removing more of the tannin containing upper joints of the plant before milling.

IRON DERIVED FROM PROCESS MATERIALS.

IRON IN WATER: Iron is one of the most frequent constituents of potable water. Thrash & Beale (Water and Water Supplies, Churchill 1925, P.160) state there shou should not be more than 0.5 part per million of iron as carbonate present in a potable water. Water for brewing purposes must be practically free from iron, and it follows that sugars for brewing must also be iron-free. If more than 1 part per million of iron is present in water a deposit of the oxycarbonate results.

In the average sugar refinery process about 3 tons of water is used per ton of sugar melted, of which 2 tons are used at the boilers. Thus a refinery melting 200 tons of sugar per day will use 200 tons of water and if the water contains 0.00014% iron, this will mean approximately 6.3 lbs. of iron per day introduced in this way.

IRON IN DEFECATING CHEMICALS: The average per cent of iron in the lime used is about 0.2 and in calcium phosphate ("superphosphate") about 0.5. The amounts of iron introduced to process from these are small since only about 2 cwts. each of lime and phosphate are used per 200 tons of sugar melted. The blue used in the final stages of refining to neutralise any yellow tint in the sugar also contains iron. Analyses

made by the author showed the following amounts of iron on a dry basis in various types of sugar blue. Indanthrene blue paste 1.01 per cent:ultramarine blue 0.097 per cent:"Sumazine" blue (sodium chloride base) 0.019 per cent: Perline blue .005 per cent. The quantity of blue used per ton of sugar is extremely small, so that there is not much danger from this direction: it must be remembered, however, that this is a direct addition to the nearly finished product.

BALANCE OF IRON IN A SUGAR REFINERY:

In the following flow sheet of a sugar refinery process the amounts of iron found by the author in typical average products are shown (in brackets) following each product. The quantities of sugar products in each stage represent average yields for good working. (The sucrose lost in manufacture is about 0.7 to 1.0 per cent.)

The figures show that the bulk of the iron is washed into the syrup (raw washings), during the preliminary mingling and centrifuging of the raw sugar. The balance is by no means exact, owing mainly to the sampling difficulties in following one batch of sugar through the process. The balance relates to a melt of Natal sugar.

It will be noted that there is only about 5 pounds of iron in circulation per 100 tons of raw sugar.

The animal charcoal appears to adsorb about half of the total iron present, and most of that left goes out in the lower product sugar and golden syrup.

The house syrup is of pH 5.9 and is normally stored in iron tanks in a hot atmosphere where some solution of the tank material takes place. It is known that such tanks wear thin in the course of some years.

The rise in the iron content of the raw liquor is probably due to melting the raw sugar in filter washing water and to gain of iron from process materials added.

All analyses were made by the sulphide colorimetric method.

IRON BALANCE IN A SUGAR REFINERY FOR 100 DRY TONS NATAL RAW SUGAR.

PRODUCT	% Fe on dry	Tons of Product On Dry	Lbs. of Fe On Dry
Raw Sugar	0.0021	100	4.70
Raw washings	0.0180	10	4.03
Crystalliser Sugar	0.0035	6	0.47
Molasses	0.0270	4	2.42
Washed Sugar	0.0003	90	0.60
Raw Liquor	0.0014	95.7	3.00
Filtered Raw Liquor	0.0005	95.5	1.07
Refined Liquor	0.0003	94.6	0.64
Standard White Sugar	0.00016	89.3	0.32
Yellow Sugar	0.0030	3.0	0.20
House Syrup	0.0270	2.0	1.21
Golden Syrup	0.0020	1.9	0.084
Filter Cake	0.0180	5.0	2.02

IRON IN RAW SUGARS.

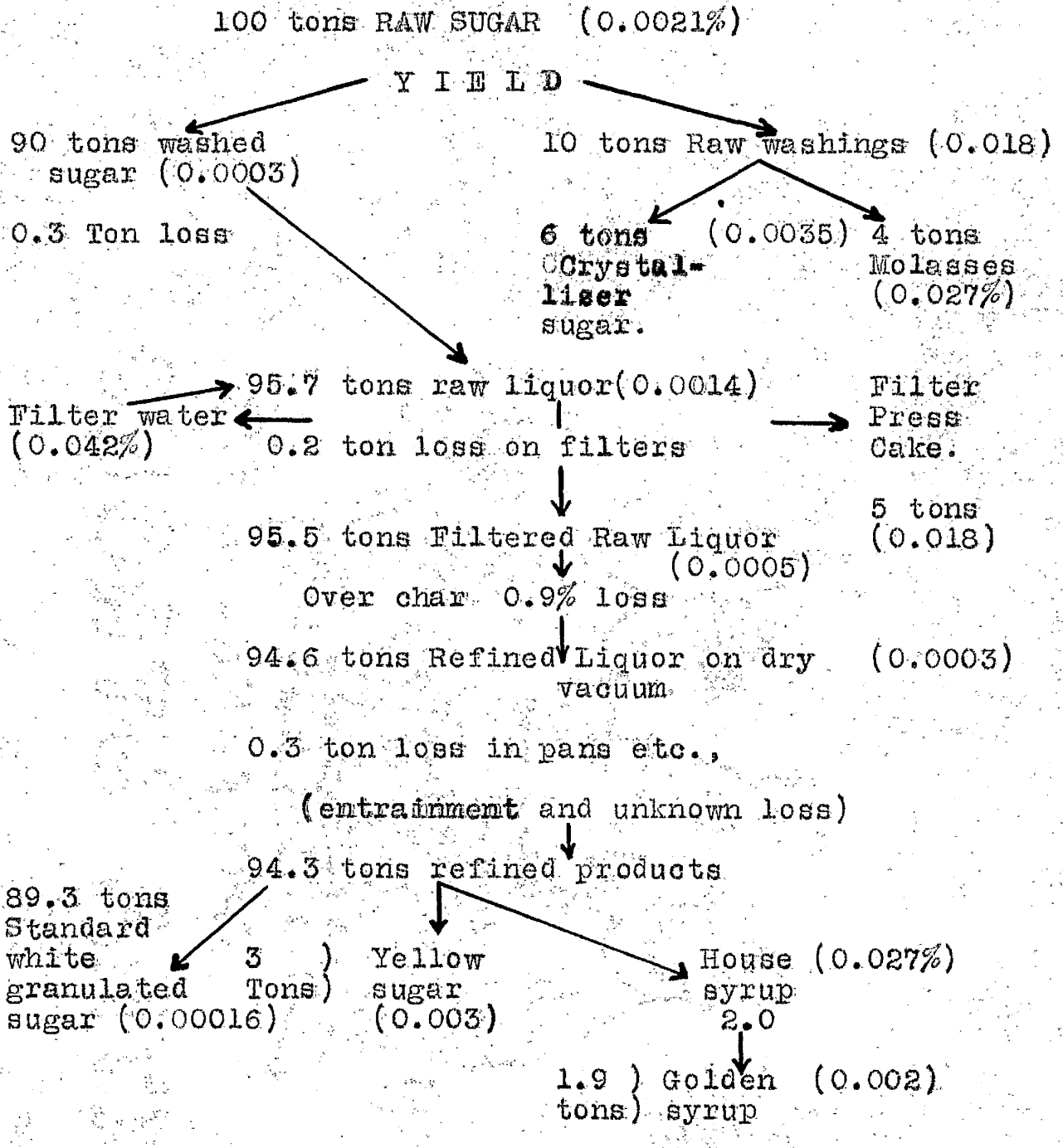
By Sulphide Colorimetric Method:

Analyses made by Author.

All results on Dry Basis.

ORIGIN	PARTS per MILLION Fe	% Fe
Australia	10	0.001
Cuba	8	0.0008
Java	8	0.0008
Mauritius	5	0.0005
Natal	21	0.0021
Peru	6	0.0006
San Domingo	7	0.0007

FLOW-SHEET OF SUGAR REFINING PROCESS (ON DRY BASIS)



All Fe results calculated on dry basis, and shown in parenthesis.

The Colorimetric Determination of Iron.

The colorimetric determination of iron has engaged the attention of many chemists on account of the numerous difficulties encountered.

In 1885 Thomson (J.C.S. 1885, 47, 49) using the well-known thiocyanate reaction claimed a sensitivity such that 1 part of iron in 50,000,000 parts of water could be detected. This reaction had been used quantitatively as early as 1837.(H.Ossian Pharm.central blatt, 1837, 205).

Most of the methods for iron are of more recent development, and many of the older methods have been greatly improved in the last twenty or thirty years. The voluminous literature relating to tests for iron requires much careful sifting before the best method available can be selected for a given purpose.

The great sensitivity of some of the methods is almost an embarrassment and special precautions have to be taken to avoid impurities in the reagents used.

The method most generally employed for sugar products is the Sulphide colorimetric method described by Eastick, Ogilvie, and Linfield [Int.Sugar Jl.14, 428 (1912)].

This is an adaptation of the sulphide process due to Winkler (Z.Ang.Chemie, 141 and 550, 1902): it is

claimed that it may be used in presence of the sugar for certain products, particularly the light coloured juices and sugars of the refinery.

The author used this method for some years in refinery work, and was never entirely satisfied with the matching of the standard tints with those produced in the sample. In addition, the preparation of ammonium monosulphide is troublesome, and it must be freshly made for each series of estimations. It seemed desirable to investigate various methods of estimating iron to discover if possible an alternative procedure which could also be used in presence of sugar. It is obvious that a method which is not interfered with by the presence of sugar would be simpler and more rapid, since it would eliminate the tedious ashing of the material.

It is also apparent that where colour is already present in the sample, great accuracy could not be expected.

Requirements of a Colorimetric Method.

The conditions which should exist in a good colorimetric procedure are here enumerated (abstracted from J.H. Yoe "Photometric Chemical Analysis" Vol. I. John Wiley 1928, p.5 et seq).

(1) Colour produced by the reagent must be characteristic of the test substance.

(2) Colour produced by reagent and test substance should be the only colour present in the solution.

(3) Colour produced by the reagent must be reasonably permanent.

(4) Neither colour nor intensity of colour produced by test substance and reagent must be affected by electrolytes likely to be present - i.e. pH may have to be adjusted carefully before comparing.

(5) Colour-intensity should vary directly with the concentration of the test-substance.

(6) Colour should be easy to distinguish and match - e.g. blue : red : green.

(7) Method should be rapid, accurate, and sensitive.

Object of present work:- With the above considerations in mind, tests were made with several colorimetric methods of determining iron with the object of finding a method which might be an improvement on the existing sulphide procedure.

Quantitative Colorimetric Analysis.

The basic principle of colorimetric measurements is the comparison under standardised conditions of the colour produced by the substance in unknown amount with the same colour produced by a known amount of the material being examined.

There are four general methods of making this comparison:-

(1) The Standard Series Method:-

The test solution is made to a definite volume, and its colour compared with a series of standards similarly prepared. In certain cases permanent standards may be made up from inorganic salts.

---

(2) The Duplication Method:- A standard solution of the substance under examination is added to the reagent until the colour produced matches that of the unknown sample in the same volume of solution. This is less accurate than method (1).

---

(3) The Dilution Method:-

The sample and standard are observed horizontally through Nessler tubes of the same diameter, and the stronger one is diluted until the colours match. The relative concentrations of original solutions are then proportional to the heights of the matched solutions in the tubes.

---

(4) The Method of Balancing:-

The height of the liquid in one tube is adjusted so that when both tubes are observed vertically the colour intensities in the two tubes are equal.

If the concentration in one tube be known, that in the other is calculated from the respective lengths of the two columns of liquid.

In practice these concentrations are not exactly in inverse proportion to the lengths, partly since monochromatic light is not employed, and partly because of chemical changes which may occur on dilution - e.g. increase in degree of ionisation.

For accurate work by this method the solutions compared should not differ greatly in concentration, and an empirical calibration curve should be constructed.

Other Methods:-

In the Lovibond Tintometer and the Hellige comparator the colour of a solution is matched against coloured glasses which are standardised to show parts per million of the substance being estimated.

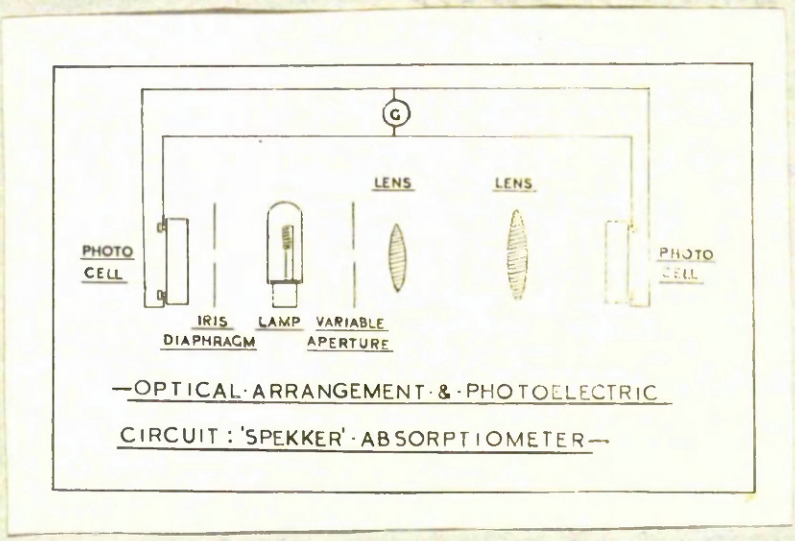
By the use of photo-electric cells combined with suitable light filters it is possible to attain high sensitivity and in many cases a straight line calibration curve in agreement with the Lambert-Beer Law. A colorimetric determination by this means is thus a precise physical measurement independent of the judgment and idiosyncrasies of the human eye.

THE SPEKKER PHOTOELECTRIC ABSORPTIOMETER.

A number of different systems may be, and are, used in "photoelectric colorimeters." In some systems no provision is made for eliminating the effects of fluctuations in the voltage supply to the lamp; consequently it must either be run from some special, stabilised circuit, or else the liability to small unrecognised errors arising out of voltage fluctuations must be accepted. The system used in the Spekker Absorptiometer eliminates the effect of these fluctuations and thus allows the instrument to be run from the mains supply.

In other systems, although provision is made for the elimination of the effects of mains fluctuation, the reproducibility of the readings depends on certain assumptions concerning the intensity-current characteristic of the photocell used, and these assumptions may be invalidated by changes occurring as the cell ages. In the Spekker Absorptiometer the arrangements are such that the readings are independent of any assumption concerning the linearity of the response of the cell which is used merely as an indicator in a null method.

FIG. 20



—OPTICAL ARRANGEMENT & PHOTOELECTRIC CIRCUIT : 'SPEKKER' ABSORPTIOMETER—

FIG. 21

The chief features of the Spekker Photoelectric Absorptiometer may be summarised as follows:

(1) It is run directly from the electric mains supply - no batteries are required.

(2) The readings are independent of fluctuations in the mains supply.

(3) The photocells are of the "rectifier" (or "Sperrschicht") type, and are extremely robust and durable.

(4) The instrument readings are not affected by variations in the sensitivity of the cell or of the galvanometer, since a null method is employed.

(5) The galvanometer which indicates the photo-electric current is a robust pointer instrument, and is used as a null indicater.

(6) Readings can be taken with as little as 7c.c of liquid.

(7) Although the instrument embodies a properly designed optical system there are few optical surfaces to be kept clean.

(8) The calibration does not depend on the use of wedges, which are not readily made accurately and usually show individual variations.

(9) Independence of the visual judgment of the observer.

(10) Freedom from the necessity of having a standard solution against which to compare every test solution (as is necessary with visual "colorimeters"). Standard solutions for a given test are prepared and measured on first use of the instrument, and need be repeated only after long intervals. (Some workers nevertheless prefer to make a standard solution with each batch of test solutions as a check on their routine).

PERCENTAGE TRANSMISSION FIGURES OF FILTER SET H.455

λ	Red No. 1	Orange No. 4	Green No. 5	Blue-Green No. 6	Blue No. 7
.4	-	-	-	36.7	18.4
.42	-	-	-	46.1	26.0
.44	-	-	-	54.8	28.1
.46	-	-	0.9	59.5	23.1
.48	-	-	9.2	61.0	9.2
.50	-	0.4	26.0	60.0	0.9
.52	-	3.7	41.2	54.2	-
.54	-	15.9	41.2	36.7	-
.56	-	47.3	26.0	23.1	-
.58	0.9	75.8	11.6	13.0	-
.60	15.9	81.4	4.6	5.8	-
.62	75.8	83.3	1.3	2.3	-
.64	93.5	84.0	0.6	0.8	-
.66	93.5	84.6	0.3	0.2	-
.68	93.5	85.1	0.2	-	-
.70	93.5	85.7	0.1	-	0.4
.72	93.5	86.0	-	-	0.2
.74	93.5	86.3	-	-	1.2

These are average figures and do not necessarily hold to within 2.3% for any particular filter.

BEER-LAMBERT LAW is obeyed by solutions in which no alteration in colour is caused by chemical changes occurring on diluting the solution.

STATED MATHEMATICALLY: $I_o = I \times 10^{ecd}$

Where I_o = Intensity of the incident light

I " " " emergent light

c = concentration of coloured substance

d = the thickness of solution

e = the molecular extinction co-efficient for a given wave-length of light.

or $\frac{(\text{Log } I_o)}{(\text{Log } I)}$ = absorption density

= A.D. = Δ_c = ecd.

So for light of a given wave-length the absorption density for a given thickness of solution is directly proportional to the concentration of the coloured substance.

For $\Delta c = ecd$ & $\Delta c_1 = ec_1d$

$$\therefore \frac{c}{c_1} = \frac{\Delta c}{\Delta c_1}$$

N. Strafford, Analyst, Vol.61, 1936, p.170.

Sensitivity of Tests:-

The following definitions of terms used in expressing the results of micro-chemical qualitative tests ("spot-tests") are extracted from an Institute of Chemistry lecture by Briscoe and Matthews (1934).

In defining the sensitivity of a test several terms are used:-

(1) The Limit of Identification is the smallest amount recognisable, usually expressed in micro-grammes or gamma, one gamma being the thousandth part of a milligramme or one millionth of a gram (0.000001 gm).

(2) The Concentration Limit is the greatest dilution in which the test gives positive results: it is expressed as a ratio of the substance to the solvent.

(3) The Proportion Limit... meaning the least proportion of "X" which can be detected by the test in the presence of another specified constituent Y.

These values are by no means fixed quantities for a given test, but are largely dependent on the method of carrying it out.

PUBLISHED SENSITIVITIES OF TESTS FOR IRON.

Method	Limit of Identification. γ	Concentration Limit.	Reference
1. Acetyl-acetone	3.0	-	Combes(loc.cit.)
2. Alloxantin	-	1 in 2 million	"Few tenths of a mgm per litre"
3. Ammonium sulphide	-	1 in 2,000,000	Eastick et al (loc. cit.)
4. Dimethylgloxime	0.04	1 : 125,000	Feigl, P.93
5. 2 : 4 Di-nitroso resorcinol	-	3.5 parts per million	Nichols & Cooper (loc. cit.)
6. 7-Iodo 8-Hydroxy Quinoline 5-Sulphonic Acid	-	1 part per million	Yoe (loc. cit.)
7. Potassium Ferricyanide	-	10 parts per million	Methods of water analysis (loc. cit.)
8. Potassium Ferrocyanide	0.1	1 in 1 million	Feigl, P.93 1 in 13,000,000 (Bailey "Brewer's Analyst" Kegan Paul 1907).
9. Pyramidon	20.0	-	H. van Urk (loc. cit.)
10. Pyrocatechin	-	1 in 161,300	Bernouilli (loc. cit.)
11. Salicylic Acid	10.0	-	Scott "Standard Method of Chem. Analysis" P.223.
12. Sulphosalicylic Acid	10.0	-	Lorber (loc.cit.
13. Ammonium Thiocyanate	0.25	1 in 200,000	Feigl, P.93.
14. Thioglycollic Acid	2.5	1 in 10 million	Leavell & Ellis J.I.E.C.Anal.Ed. Vol.6.P.46(1934).
15. $\alpha\ \alpha'$ Dipyridyl	0.03	1 in 1,660,000	Feigl, P.93

METHODS INVESTIGATED:-

The methods selected for study were chosen as being representative, but they by no means cover all the methods which have been proposed.

An extensive bibliography of iron methods is given by Yoe (loc. cit. p.613). (see Bibliography).

It was planned to observe the following data for each method:-

(1) Colour grading of standards.

(2) Fading of standards.

(3) Time taken to develop maximum colour.

(4) Effect of pH value, adjusting by buffer solutions.

(5) Effect of white sugars in various concentrations.

(6) Effect of raw sugars.

(7) Sensitivity of method.

The question of interfering substances other than sugar, was not dealt with, as this already has been established for many of the methods.

A short description of each method is given, with details of the experiments made.

The methods examined are summarised on the next two pages.

SELECTED METHODS FOR COLORIMETRIC DETERMINATION OF IRON.

Method	Reference	Reagents required
1. Acetyl-Acetone	Combes, Comptes Rendu, 105, 868 (1887)	0.5 per cent acetyl acetone in water or weak alcohol. Red colour with ferric salts.
2. Alloxantin	G. Deniges Comptes Rendu 1925, 180, 519. J.C.S. 1925, 128A, ii, 441.	Alkaline Tartrate solution: Alloxantin. Blue colour with ferric salts.
3. Ammonium Sulphide	L.W. Winkler Z. Anal. Chem. 41, 550 (1902).	Fresh ammonium sulphide solution. Ferrous salts give a brown colour.
4. Di-Methyl Glyoxime	Tschugaeff and Orelkin Z. anorg. Chem. 89, 401. (1914).	Dimethylglyoxime: sat. in 95 per cent alcohol. Red colour with ferrous salts.
5. 2:4 Di-Nitroso Resorcinol	Nichols & Cooper J.Am.C.S. 47, 1268 (1925).	Hot saturated solution of reagent. Olive-green colour with ferrous iron.
6. 7-Iodo 8-Hydroxy Quinoline 5-Sulphonic Acid	J.H. Yoe J.Am.C.S. 54, 4139, 1932	Aqueous solution of reagent gives a stable green colour with ferric iron.
7. Potassium Ferricyanide	Methods of water analysis American Public Health Association, New York, 6th Edition 1925, P.49.	Freshly prepared potassium ferricyanide solution: 0.5 per cent. Blue colour (Turnbull's blue) with ferrous iron.

METHODS FOR IRON (continued).

Method	Reference	Reagents required
8. Potassium Ferrocyanide	T. Carnelley, Chem. News. 30, 257 (1874).	0.5 per cent solution of potassium ferrocyanide. Blue colour with ferric salts.
9. Pyramidon	H. van Urk (Pharm Weekblad, 63, 1121, 1926.	1 per cent Pyramidon solution. Light mauve colour with ferric salts.
10. Pyrocatechin (Catechol)	A. Bernouilli Helv. Chim. Acta 9, 827 (1926)	1 per cent solution in water. Dark green colour produced.
11. Salicylic Acid	A. Vogel. Chem. Zentr. 375 (1876)	Saturated solution of salicylic acid (0.15 gm. per 100 ccs. at 15°C. Red colour with ferric iron.
12. Sulpho-salicylic Acid	L. Lorber. Biochem. Z., 181, 391 1927.	20 per cent sulpho-salicylic acid. 10 per cent ammonia. Yellow colour with total iron.
13. Ammonium Thiocyanate	H. Stokes and J.R. Cain. J.Am.Chem. Soc. 29, 409 (1907) (extended study)	4 per cent solution of potassium thiocyanate. Red colour with ferric iron.
14. Thioglycollic Acid	R. Lyons. J.Am.C.S. 49, 1916-20 (1927)	4 ccs. Thioglycollic acid added to 8 ccs. conc. ammonia in 50 ccs water. Red or purple colour with ferric salts in alkaline solution.
15. $\alpha\alpha'$ Di-pyridyl	F.Blau. Mh.chem. 19, 647, (1898)	2 per cent solution in dilute HCl or in alcohol. Deep red colour with ferrous salts.

EXPERIMENTAL APPARATUS:-

The observations of colour gradings were made in Nessler glasses, placed in stands having a white glass base.

For accurate matching of sample with standards a stand was designed and constructed which held four Nessler glasses in pairs. A prism with eyepiece served to bring the fields of the sample and standard together. The arrangement was modelled on the Lovibond or Hellige type of pH comparator; a photograph of this apparatus is shown on page 251.

This was found to be very useful, and permitted of very close matching.

The pipettes and flasks used were re-calibrated at 15°C.

As far as possible all colour matchings were made with the same intensity of light.

STANDARD IRON SOLUTIONS:-

A list of the standard iron solutions recommended for the various methods is here given, with an account of an experiment carried out on the hydrolysis of iron solutions on standing.

STANDARD IRON SOLUTIONS.

Method	Reagent	Gms. per litre.	Fe content per cc. gm.
Dimethylglyoxime Acetyl Acetone Potassium Ferro-cyanide	Ferrous ammonium sulphate $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$	0.1404	0.00002
Ammonium Sulphide	Ferrous sulphate $FeSO_4 \cdot 7H_2O$ in 50-60% sucrose solution	10.0	0.002
Potassium Ferricyanide	Ferrous ammonium sulphate	0.7022	0.0001
Sulpho-salicylic acid	Ferrous sulphate or Ferrous ammonium sulphate	0.4978 0.7022	0.0001 0.0001
Salicylic Acid	Ferric ammonium sulphate $Fe_2(SO_4)_3.(NH_4)_2SO_4.24H_2O$	0.0864	0.00001
Thioglycollic acid	Iron wire	1.0	0.001
Thiocyanate	Ferric potassium sulphate $Fe_2(SO_4)_3.K_2SO_4 \cdot 24H_2O$	0.315	0.005

In all iron solutions it is advisable to add sulphuric acid to prevent hydrolysis of the dissolved iron salts to insoluble basix compounds.

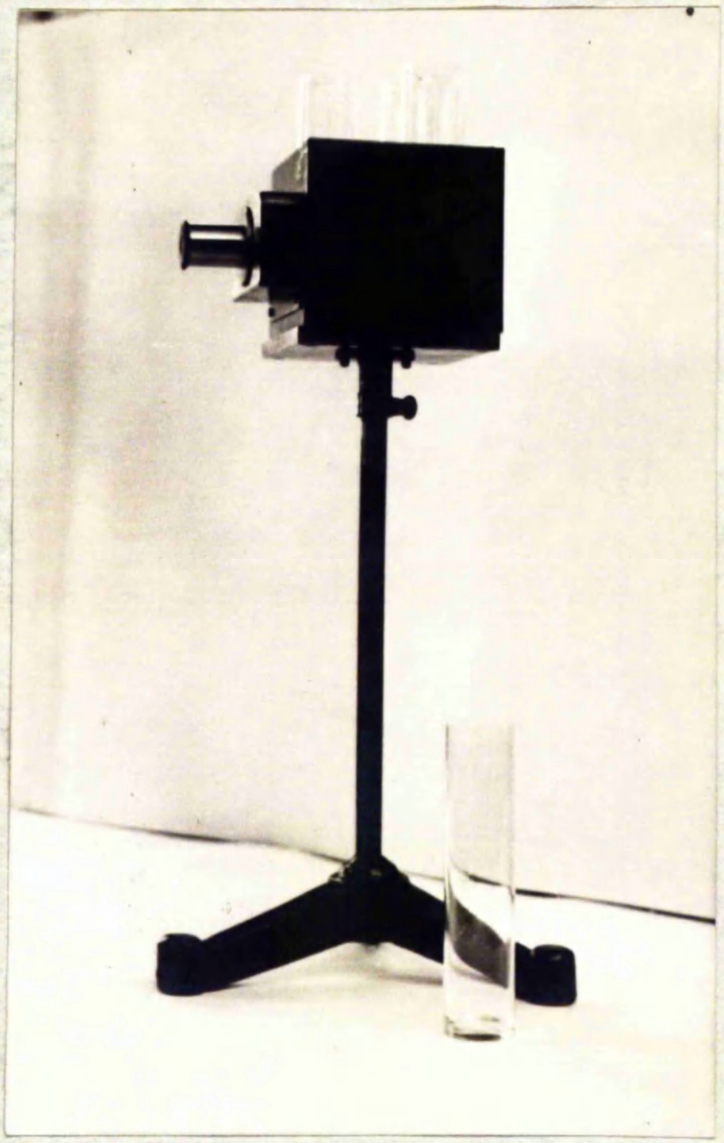


FIG.22

STANDARD BUFFER SOLUTIONS.

In connection with the use of buffers, two sets of standard buffer solutions, having the following composition, were made up and standardised by the glass and quinhydrone electrodes.

SERIES I.		SERIES II.	
*p*H	Composition	*p*H	Composition
1.	HCl / KCl	-	-
2.	HCl / KCl	-	-
3.	NaOH / Phthalate	-	-
4.	" "	4	Acetic acid/sodium acetate
5.	NaOH / KH_2PO_4	5	" "
6.	" "	6	" "
7.	" "	7	Borax/boric acid/NaCl
8.	" "	8	" " "
9.	NaOH / Boric acid	9	" " "
10.	" "	-	-
11.	HCl / Na_2CO_3	-	-

References:- I. Julius Grant "measurement of H-ion concentration". Longmans, 1930, p.142.

II. Lowry & Sugden "Class-book of Physical Chemistry," McMillan, 1930, p.375.

EFFECT OF pH on the HYDROLYSIS OF STANDARD IRON SOLUTIONS.

Solutions were prepared containing 10 ccs. of standard iron solution and 90 ccs. of standard buffer solutions. These were left standing for 3 months (July-September) and examined.

No.	pH	Iron conc^{n}. per cc. solution	Buffer solution	Result after 3 months
1.	1.	0.0001 gm.	KCl + HCl	Colourless: no hydrolysis
2.	2.	0.0001	KCl + HCl	" " "
3.	3.	0.0001	Succinic acid + borax	Basic compound precipitated
4.	4.	0.0001	Acetic acid + sodium acetate	Brown colour: no precipitate
5.	5.	0.0001	" "	Basic compound precipitated: more than in 3.
6.	6.	0.0001	" "	Basic compound precipitated: more than in 5.
7.	-	0.0001	No Buffer	Hydrolysis: Yellow precipitate.
8.	-	0.0001	0.10 cc. conc. H_2SO_4	Colourless: no precipitate.

It is evident that at 1 or 2 pH the hydrolysis is supressed sufficiently to give the solution good keeping properties. It is recommended that standard iron solutions for pH work be made up in this way with standard buffer solution of 1 pH. This will be found advantageous where a reagent sensitive to hydrogen ion concentration is being used. The usual haphazard addition of acid to give iron solutions stability will be avoided.

DETERMINATION OF IRON.

Method (1):- Using Acetyl Acetone: $CH_3.CO.CH_2.CO.CH_3$

Acetyl acetone gives a red coloration with ferric salts by formation of ferric acetyl-acetone due to the replacement of enolic hydrogen. Acetyl acetone is a colourless liquid: b.pt. 137°C.

Reagents:- Acetyl-acetone - 0.50 per cent solution is made by diluting freshly distilled acetyl acetone with water or dilute alcohol.

Standard Iron Solution:- 1 cc. equals 0.00002 gm. of iron.

Procedure:- The sample taken should contain 0.00005 to 0.00006 gm. Fe. It is placed in a Nessler tube and 2 ccs. of the acetyl acetone reagent added. Standard tubes containing known amounts of iron plus 2 ccs. of reagent are matched with the sample.

Notes:- The smallest amount of Iron which can be detected is given by Lyons (loc.cit.) as 0.000003 gm. Nitrogen oxides give a brown colour and must be removed. Alkali and acids destroy or change the colour. The colour is stated to be quite permanent, and is only slightly altered by strong sunlight. The colour does not vary uniformly with the height of the column of liquid, so the method of balancing cannot be used in comparing sample

and standards. The amount of free acid present must be the same in sample and standards.

In aqueous solution the ferric acetyl-acetone complex is only slightly ionised, but it hydrolyses to ferric hydroxide.

METHOD (1) ACETYL ACETONE:- EXPERIMENTAL.

(1) Test Limits:- Standards were prepared using a ferric alum solution and a 0.5 per cent solution of acetyl acetone. 2 ccs. of reagent used in each case.

No.	Gms. Fe present in 50 ccs.	Parts per million.	γ Fe in 50 ccs.	Remarks.
(1)	0.00001	0.2	10	Almost white: yellow compared with water.
(2)	0.00002	0.4	20	Pale yellow.
(3)	0.00003	0.6	30	Slightly darker yellow.
(4)	0.00004	0.8	40	Yellowish pink.
(5)	0.00005	1.0	50	" "
(6)	0.00006	1.2	60	" "
(7)	0.00010	2.0	100	Salmon pink.

Each sample was made to 50 ccs. in a Nessler glass.

Standard number seven was still of the same colour after three days. Below 0.00005 gm. of Iron the colour was not distinctly pink and this would appear to be the lower limit of the test for quantitative work.

(2) Effect of Buffer Solutions: optimum pH.

Nessler glasses were prepared using carefully standardised standard buffer solutions and adding to these 5 ccs. of the acetyl acetone reagent and 0.00005 gm. of Fe in each case.

(2).

EFFECT OF BUFFER SOLUTIONS ON ACETYL-ACETONE REACTION WITH IRON.

No.	pH of Buffer solution	Remarks.	pH of solution after mixing.
1	1.0	Pink	1.2
2	2.0	Orange Pink	2.0
3	3.0	Yellowish-orange-pink	2.8
4	4.0	Orange-yellow	3.7
5	5.0	Deep yellow	4.9
6	6.0	Pale yellow	5.8
7	7.0	Paler than (6)	6.75
8	8.0	" " (7)	7.6
9	9.0	" " (8)	8.9
10	11.0	Almost colourless	10.8
11	5 ccs. 0.2N HCl added.	Deep Pink	Below 1.0
12	No Buffer (blank).	Dirty orange-pink.	3.3

The distilled water used had a pH value of 6.7, and the iron solution as prepared showed pH 1.6.

There was no evidence of fading after 3 hours. pH is evidently important here: it should be 1.0 pH or below, and always in the acid range.

ACETYL-ACETONE:- (3) EFFECT OF SUCROSE ON COLOURS PRODUCED.

Standards were made with the addition of varying amounts of sucrose. The iron present was 0.00005 gm. for each tube, with 2 ccs. acetyl acetone reagent added.

No.	Gms. Sucrose	γ Fe in 50 ccs.	Remarks.
1	0.625	50	Colour gradation was good
2	1.25	50	but the colour in the blank
3	2.50	50	(No.6) was distinctly darker
4	5.0	50	than even No. 1. The solutions
5	10.0	50	containing 5 and 10 gms. of
6	Blank	50	sucrose were colourless.

The above test shows that acetyl acetone is useless in presence of sucrose.

(4) ESTIMATION OF IRON IN ASH OF SUGAR PRODUCTS USING ACETYL-ACETONE.

No.	Sample	Per cent shown on original product. % ASH (1)	% ASH (2)	% IRON (1)	% IRON (2)
1	Egyptian molasses	13.49	13.33	0.0022	0.0028
2	Natal molasses	11.84	12.03	0.032	0.018
3	Java Raw sugar	0.54	0.55	0.0010	0.0013
4	Demerara Raw sugar	0.37	0.36	0.0008	0.0010

(1) and (2) are duplicates.

Determination of Iron by Acetyl-acetone.

Readings in Spekker Photoelectric Absorptiometer.

No.	Fe P.P.M.	γ Fe in 50 ccs.	Mean Scale Readings.
1	9	450	standard
2	8	400	.026
3	7	350	.031
4	6	300	.038
5	5	250	.043
6	4	200	.050
7	3	150	.060
8	2	100	.070
9	1	50	.086
10	0.2	10	.100

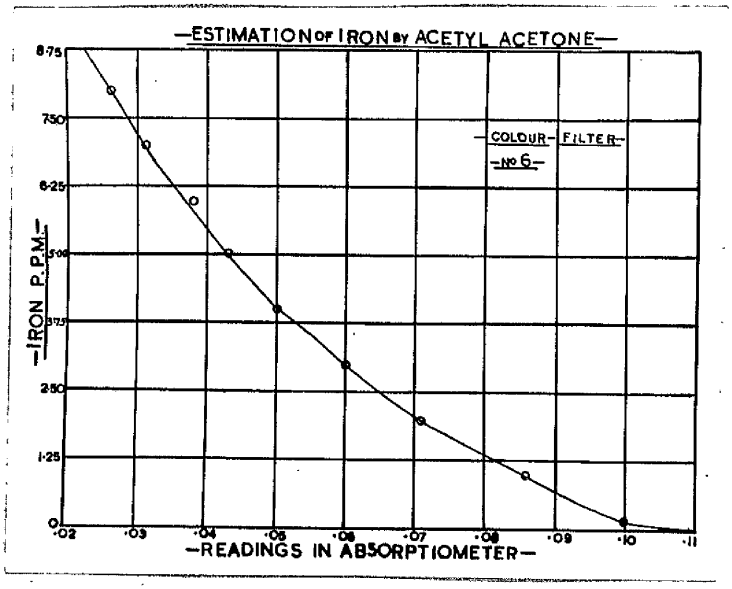


FIG. 23

ACETYL ACETONE - GENERAL CONCLUSIONS.

The results for iron in the ash of sugar products are satisfactory. It was observed during these tests that the reagent did not deteriorate on standing for over three months: freshly prepared and three months old solutions gave the same results.

The method cannot be used in presence of sugar; the limit for quantitative estimation appears to be 0.00005 gm. Iron, and the pH value is preferably kept below 1.0 for the best development of colour.

Note:- ɣ Gamma is one millionth part of a gram, equivalent to 0.000001 gm. Thus in the above experiments 0.00005 gm. of iron represents 50 gamms and also is 1 part per million since it was made up to 50 ccs.

DETERMINATION OF IRON:-

Method (2):- Using Alloxantin. $C_8H_6O_8N_4.2H_2O$.

Alloxantin gives a blue colour with ferric salts in alkaline tartrate solution. The sensitivity is said to be 1 part per million. A violet blue precipitate is given with Baryta - water.

Reagents:- Alloxantin. 0.1 gm. dissolved in 10 ccs. of $N/_1$ caustic soda. If rose coloured it is boiled and then cooled.

Standard Iron Solution:- A ferric solution is selected.

Procedure:- The colour produced in the sample tube is matched with standards. The blue colour is distinct when 1 cc. of the alloxantin reagent is added to 2 ccs. of a solution containing 0.001 gm. of Ferric Iron per litre. The lowest limit of sensitivity is a few tenths of a milligram per litre (Denigès).

Tartaric and citric acids which hinder the reaction of iron with salicylic acid, thiocyanate or ferrocyanide do not seem to interfere here, and even appear favourable.

METHOD (2):- ALLOXANTIN EXPERIMENTAL.

(1) Test Limits:- 1 gm. of reagent was dissolved and made up to 100 ccs. with $\frac{N}{1}$ caustic soda. The colour of the solution was a very pale green, and the salt was difficult to dissolve.

Standards were made up with varying Iron content, using 5 ccs. of the alloxantin reagent and making to 50 ccs. in Nessler glasses.

No.	gms. Fe present in 50 ccs.	Parts per million	γ Fe in 50 ccs.	Remarks.
(1)	0.000002	0.04	2	Very little difference between these: nevertheless detectable.
(2)	0.000005	0.1	5	
(3)	0.00001	0.2	10	
(4)	0.00002	0.4	20	Sharply distinct from No. 3.
(5)	0.00003	0.6	30	Colours well graded.
(6)	0.00004	0.8	40	
(7)	0.00005	1.0	50	
(8)	0.00010	2.0	100	

The colours appeared to be fairly permanent; there was no fading after 2 hours. The real blue colour only commences at 20 gamma, and the sensitivity can be taken as commencing at this figure.

(2) Optimum pH:- Standards made up with Buffer solutions of known pH with 0.00005 gm Iron and 5 ccs. of saturated alloxantin reagent without caustic soda, present in each case.

(2) EFFECT OF BUFFER SOLUTIONS ON ALLOXANTIN REACTION WITH IRON.

No.	pH of Buffer solution	Remarks.	pH after mixing.
(1)	2.0	Colourless	2.1
(2)	4.0	"	4.05
(3)	6.0	"	6.15
(4)	8.0	Yellow-green	8.0
(5)	9.0	Green	9.0
(6)	11.0	Dark green	11.0
(7)	No Buffer (blank)		-

Next morning all the tubes were identical in colour, numbers (5) and (6) being slightly more yellow than the others; the general colour was a dark green.

This reagent appears to be much better in the alkaline range, the development of colour being hindered by acidity. In above experiments the colour developed more freely on standing.

(3) Effect of Sucrose on colours produced:-

Varying amounts of sucrose were added to Nessler glasses each containing 0.0001 gm of Iron. After solution was complete, 5 cc. of the alloxantin reagent were pipetted in, and the effect observed.

No.	gms. sucrose	γ Fe in 50 ccs.	Remarks.
(1)	5.0	100	In order of amount of sugar present as far as colour went - the more sugar the less colour.
(2)	2.5	100	
(3)	1.25	100	
(4)	5.0	Nil	Colourless - no reaction.

The colours changed in presence of sugar from the rich blue with a tinge of purple found in the original standard solutions to a faded reddish purple. Evidently this reagent is useless in presence of sugar and no further tests were made.

DETERMINATION OF IRON.

Method (3):- Using Ammonium Sulphide.

Description of the Sulphide Colorimetric Method:- (Eastick: Ogilvie & Linfield).

Stock Solution of Iron:- 10 gms. of pure ferrous sulphate $FeSO_4.7H_2O$ are dissolved in a 50-60 per cent sucrose solution, with addition of a few drops of sulphuric acid, and made to 1 litre. The acid should be largely diluted with water before addition.

This stock solution is diluted with water as required - e.g. 10 ccs. to 100 ccs, and 50 ccs. of this solution to 500 ccs., this giving a solution containing 0.000002 gm. Fe per cc.

Determination of Iron:- For light coloured products 3 to 10 gms. of the sample, depending on the amount of iron present, are dissolved in water in a Nessler cylinder and made up to 50 ccs.

Into a series of Nessler tubes of the same height and diameter as that used for the sample are placed increasing amounts of the standard iron solution, and the volumes are completed to 50 ccs.

To each cylinder 2 ccs. of freshly prepared ammonium monosulphide solution are now added and the contents stirred. After 10 minutes the cylinder containing the sample is matched with one of the standards. Both

then contain the same quantity of iron, that is, the quantity of iron in the sugar used. The cylinders should stand on white glass in making the comparison.

The sulphide is prepared by saturating ammonium hydroxide with sulphuretted hydrogen, and then adding an equal volume of ammonium hydroxide.

In the case of dark sugars, the material is incinerated with the addition of iron-free sulphuric acid, burning at the lowest possible temperature. The ash is dissolved in a minimum quantity of iron-free hydrochloric acid, and the iron is estimated in this solution as described above.

The advantages claimed for this method are:-

(1) Incineration is not necessary except for dark coloured products.
Note:- The ferrocyanide method for iron is stated by Bailey [Brewer's Analyst, Kegan Paul, 1907] to be capable of application in presence of sugar, provided the colour of the sample is not dark. The ferrocyanide method is described later in this section.

(2) The colloidal ferrous sulphide flocculates very slowly.

(3) The state of oxidation of the iron is immaterial.

(4) The maximum colour intensity is quickly reached.

(5) The tints of standard and sample are usually identical.

METHOD (3):- AMMONIUM/SULPHIDE EXPERIMENTAL.

(1) Test Limits:- It was found that with white sugars the lowermost limit of detection was 0.1 part per million.

Standards:- The standard $FeSO_4$ solution in sucrose was diluted so that 1 cc. of it contained 0.000005 gm. of iron per cc. (= 5 gamma). Standards were prepared as usual in Nessler tubes by taking 1, 2, 4, 6, 10, 15 and 20 ccs. of the iron solution (thus ranging from 5 to 100 gamma), and adding 1 cc. of freshly prepared ammonium sulphide and making up to 50 ccs. Colours from very faint blue to deep green-blue were obtained in successive concentrations.

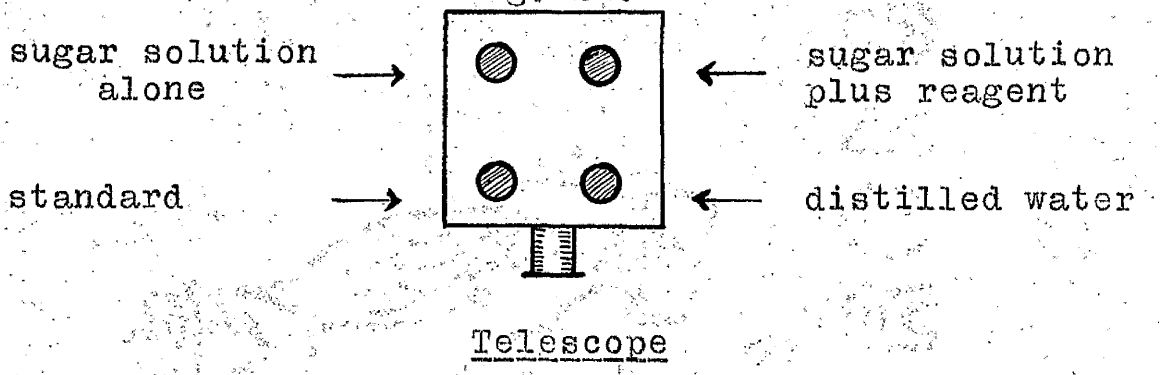
Raw Sugars:- 25 gms. of raw sugar was dissolved, made to 200 ccs, and filtered using Kieselguhr. 10 ccs. of this filtrate representing 1.25 gms. of sugar were pipetted into a Nessler glass, 1 cc. of the ammonium sulphide added, volume made to 50 ccs, and the tube matched against the standards.

On addition of the ammonium sulphide the already yellow or brown colour of the sugar solution was intensified without producing any appreciable blue tint. The colour-matching in the comparator could only be done very roughly as the tints of sample and standards were not identical.

Tests using 5 ccs. of the sugar solution were likewise unsatisfactory, except where the raw sugar was fairly light in colour.

COMPARATOR:- The arrangement in the comparator is here shown:-

Fig. 24.



Four Nessler glasses were arranged as shown above, the yellow colour of the sugar solution being thus common to both sides of the comparator.

When left overnight the standards darkened considerably and unevenly.

AMMONIUM SULPHIDE:- IRON IN SUGARS BY DIRECT METHOD:-

Sugar	Amount taken	% Iron	% Iron by ashing
West Indies	10 ccs.	0.0028	0.0020
San Domingo	10 ccs.	0.0040	0.0031
Natal	10 ccs.	0.0032	0.0040
Demerara	10 ccs.	0.0016	0.0023
Java	10 ccs.	0.0012	(0.0013 (0.0019
Mauritius	10 ccs.	0.0020	0.0016
White Beet	10 gms.	0.0002	0.00009

AMMONIUM SULPHIDE METHOD APPLIED TO ASH OF SUGAR PRODUCTS.

The ash was determined in duplicate by sulphation and incineration in the usual way. The ash was dissolved in 5 ccs. of concentrated hydrochloric acid, boiled for 5 minutes, filtered, neutralised, and made up to 250 ccs.

10, 20 or 40 ccs. of the ash solution were pipetted into the Nessler tube and the reagent added. The tubes were then matched with standards.

No.	Sample	% Ash minus 1/10	% Iron
1	Egyptian Molasses	13.33 13.49	0.081 0.013
2	Natal Molasses	12.03 11.84	0.0086 0.0150
3	Java Raw Sugar	0.55 0.54	0.0013 0.0019
4	Demerara Raw Sugar	0.36 0.37	0.0024 0.0022
5	West Indies Raw Sugar	0.48 0.48	0.0023 0.0019
6	San Domingo Raw Sugar	0.46 0.49	0.0026 0.0036

No.	Sample	% Ash minus 1/10	% Iron
7	Natal Raw Sugar	0.37 0.39	0.0039 0.0041
8	Mauritius Raw Sugar	0.34 0.35	0.0012 0.0020
9	White Beet Sugar	0.018 0.019	0.00007 0.00011

Discussion:- The results for raw sugars are much more consistent than those for molasses.

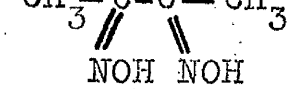
The agreement between the direct estimations and the ashing results is good except in the case of the white sugar where the ash method result is much lower, probably due to volatilisation of salts in the burning-off.

In the case of the two molasses samples, the agreement between the duplicates is extremely poor.

The colour due to the ammonium sulphide was found to require at least 20 to 25 minutes for full development. It was found essential to prepare the reagent on the day of use: with even faintly yellow ammonium sulphide a much brighter green colour is produced, and the proper gradations of colour do not occur.

With white sugars the method is better than with raw sugars, but it seems inadvisable to use it in presence of even moderately dark colours.

DETERMINATION OF IRON.

Method (4):- Using Dimethylglyoxime 

Dimethylglyoxime in alcoholic solution added to a solution containing ferrous iron produces a bright red colour due to $Fe(C_4H_7N_2O_2)_2$, one of the oximino hydrogen atoms being replaced by an equivalent of iron, followed by co-ordination. The iron is conveniently reduced by solid hydrazine sulphate.

Reagents:- Dimethylglyoxime - A saturated solution in 95 per cent ethyl alcohol is used.

Ammonia - 25 per cent solution of redistilled ammonia.

Standard Iron Solution - Prepared from ferrous ammonium sulphate: 1 cc. equals 0.00002 gm. Fe.

Procedure - The iron content of the sample should be 0.01 to 0.06 gm. per litre. Take 50 ccs. of the sample, add 1 gm. of hydrazine sulphate, 5 ccs. of the Dimethyl-glyoxime solution and heat to boiling. Add 10 ccs. of 25 per cent ammonia solution, continue boiling for half a minute, cool rapidly and dilute to 100 ccs. for comparison. The colour matching may be made by any of the usual methods.

Notes:- The minimum amount which can be detected is less than 0.00005 milligram. Magnesium may interfere and the method cannot be used in the presence of relatively large amounts of aluminium or zinc.

METHOD (4):- DIMETHYLGLYOXIME - EXPERIMENTAL.

(1) Test Limits:- Using standard ferrous iron solutions a faint pink colour was obtained with 1 part of iron in 5 millions.

(2) Effect of different Buffer solutions:- 10 ccs. of stock solution was taken in a boiling tube, and 0.2 gm. of hydrazine sulphate was added, followed by 5 ccs. of the saturated alcoholic solution of dimethylglyoxime,

The solution was made to about 40 ccs. with the required buffer solution, heated to boiling, cooled rapidly, washed into a Nessler glass and made up to 50 ccs.

The buffers used were of pH 1, 2, 3, 4, 5, 6, 7, 8, 9 and 11. No colour appeared in the acid range: with pH 9 and 11 a faint yellowish tinge appeared.

(3) With Ammonia:- Prepared solutions as above using 10 ccs. of 25 per cent ammonia in place of the buffer. Boiled for half a minute, cooled quickly, and made to 50 ccs in a Nessler glass. A bright yellow colour appeared which faded gradually to pale orange in a few minutes. The solutions were made containing varying amounts of iron, and the results are shown in the table below.

The colour for each amount was a bright red of different intensity, but this faded to the pale orange colour on standing, when a gradation of colours could be seen. This gradation was not entirely regular, the 40 gamma tube being deeper in colour than the 50 gamma.

A minimum of about 15 minutes appeared necessary before judging the colour.

In this method much seems to depend on the mode of heating before adding the ammonia.

If heated just to boiling-point a pure pink colour develops, but if it is boiled even for a second or two before adding ammonia a yellowish pink colour is obtained which is much lighter than the pink colour. The method appears to be neither very convenient nor very reliable, but is sensitive.

With sugar solutions immediate fading took place, and the colours were very much lighter than those obtained in absence of sucrose.

No.	gms. Fe present in 50 ccs.	γ Fe in 50 ccs.	Parts per million	Remarks.
1	0.0000025	2.5	0.05	Faintly pink.
2	0.000005	5.0	0.10	Fairly well graded - yellowish pink shades.
3	0.000020	20.0	0.40	
4	0.000040	40.0	0.80	
5	0.000050	50.0	1.00	Deep pink.
6	0.00010	100.0	2.00	Deep pink.

The alkalinity necessary is apparently outside the pH range.

The need for boiling is the worst feature of the method. It is valueless in presence of sugar.

DETERMINATION OF IRON.

Method (5):- Using Di-Nitroso Resorcinol $C_6H_2O_2(NOH)2.H_2O$

Dinitroso resorcinol gives an olive-green colour with solutions containing ferric iron.

Reagent:- Di-Nitroso-resorcinol solution:- this is prepared by adding excess to boiling water and filtering. The solution is made just previous to use.

Procedure:- The ferric hydroxide precipitated from the sample is dissolved in the least possible quantity of hydrochloric acid and nearly neutralised with sodium hydroxide; 2 to 4 ccs. of the reagent is added, keeping hot while making test, and a few crystals of sodium acetate are dropped in.

Iron gives a green precipitate or colour.

The solution must not be strongly acid.

Notes:- The method has been only qualitatively examined by Nichols and Cooper (J.Am.C.S. 47, 1268, 1925).

Solutions in 50 per cent acetic acid or 50 per cent alcohol were not so sensitive as the aqueous solution.

It was found that the sodium acetate coagulates the precipitate and darkens the colour.

The sensitivity appears to be about 3.5 parts per million.

Method (5):- Di-Nitroso Resorcinol - Experimental.

(1) Reagent:- The sample of the solid reagent obtained was a dark brown colour and on boiling with water gave an opaque brown solution. On boiling with decolorising carbon and filtering, there was no change in the colour. It was therefore accepted that this dark brown colour was the natural colour of the product: this compound is described in Heilbron's "Dictionary of Organic Compounds", as occurring in bronze leaflets.

With 2-4 ccs. of the reagent and standard iron solutions, the colours were much too brown to detect differences in the green tints. About 3 drops gave the required olive green colour.

Solubility:- The compound was practically insoluble in most of the ordinary solvents - ethyl alcohol, ethyl acetate, ether, toluene, chloroform, anyl alcohol. With ether and iron solution a deep green tint was left in the water layer - the excess of reagent remaining in the ether layer. In methyl alcohol there was slight solubility.

10 drops of reagent with 0.00025 gm. of Iron solution made to 50 ccs. in a Nessler glass gave a fine olive green colour, but this took several minutes to develop. Addition of solid sodium acetate intensified this colour - making it a "bluer" green.

(2) Test Limits:- Preliminary experiments using 3 drops of the reagent with amounts of iron ranging from 5 to 100 gamma gave yellow colours in the Nessler glasses with poor gradation of tint. These colours were intensified by adding solid sodium acetate which also gave better grading. After standing overnight these standards were much greener, but there was still poor grading. Experiments were now made using buffer solutions as detailed below (section 3) and 3.0 was selected as the optimum pH (using sodium hydroxide-phthalate buffer solution).

Test Limits using 3 drops reagent at 3.0 pH.

No.	gms. Fe present in 50 ccs.	Parts per million	γ Fe in 50 ccs.	Remarks.
1	0.000005	0.10	5	The solutions were made
2	0.000010	0.20	10	up with 3.0 pH Buffer
3	0.000015	0.30	15	solution, 3 drops of
4	0.000020	0.40	20	reagent and 3 drops of
5	0.000025	0.50	25	saturated sodium acetate
6	0.000050	1.0	50	solution being added.
7	0.000075	1.5	75	The acetate solution was
8	0.00010	2.0	100	added last. The colour grading was not very marked after 10 minutes. Left overnight.

Next morning there colours were extremely well graded - the 2 parts per million tube was almost too dark for good comparison. It is evident that this development of colour is very slow, which would be a bad feature in colorimetric analysis.

(3) Effect of Buffer Solutions:- Optimum pH.

Tests were made as with other iron methods, using standard buffer solutions ranging from 1 to 11 pH. It was found that borate buffer solutions interfered giving a blue colour instead of the blue-green.

Effect of Buffer Solutions.

Solutions containing 1 p.p.m. of iron were treated with 3 drops of reagent, 3 drops of saturated sodium acetate solution, and made to 50 ccs. with standard buffer solutions. The colours obtained after 1½ hours are described below.

The buffers used were free from borate.

No.	Buffer Solution pH	Remarks.
1	1	Lighter than (3)
2	2	Olive-green
3	3	Deep greenish blue - best colour.
4	4	Same tint as (3) : slightly lighter.
5	5	Olive green - paler than (2).
6	6	Pale greenish-yellow.
7	7	Paler greenish yellow than (6)
8	8	More yellow than (7).
9	9	Light yellow.
10	11	Deep yellow.
11	Blank	Deep olive-green-deeper than (2).

These results show that di-nitroso-resorcinol has almost the properties of an indicator. The colours intensify very slowly, so that standards and test samples should be made up at the same time, as the time factor is obviously very important here. The amount of sodium acetate added to intensify must also be carefully controlled.

(4) Effect on Sugar Solutions:-

In the presence of white sugars there was no fading of colour, but rather intensification.

A raw sugar (San Domingo) was used for estimation of iron by this method, and gave a result of 0.0006 per cent iron as against 0.0036 by other methods; using double the quantity of sugar 0.0007 per cent was obtained. This discrepancy appears to rule out the method for quantitative work under the above conditions.

DETERMINATION OF IRON.

Method (6):- Using 7-Iodo-8-Hydroxy-Quinoline.

5-Sulphonic Acid.

$C_9H_4N.OH.I.SO_3H$. ("Ferron").

This reagent in aqueous solution gives a green colour with ferric iron which is very stable to light.

Procedure:- 5 drops of a 0.2 per cent solution of the reagent is added to the solution under test, which is then made up to 50 ccs., and matched against standards prepared in the usual way from a standard ferric iron solution.

Notes:- The sensitivity is about 1 part per million. The colour is destroyed by strong acids or bases: pH 2.5 is an optimum region.

The method may be used in presence of all metals with colourless ions: only cupric ions interfere unless the green colour is masked by the presence of excess of other coloured ions.

With a concentration of about 1 in 10 millions a greenish yellow colour is obtained. With higher concentrations a full green colour develops which is almost indefinitely stable.

pH Range:- In inorganic combination iron hydrolyses very slowly at about pH3, but at pH5 and above more rapid precipitation occurs. With the above reagent more acid solutions turn blue-green, and above 3.2pH a yellowish colour appears.

Method (6):- 7-Iodo-8-Hydroxy-Quinoline 5-Sulphonic Acid.

Experimental:-

(1) Test Limits:- The reagent in 0.2 per cent aqueous solution is a bright golden yellow colour. Standard iron solutions were prepared as below.

No.	gms. Fe present in 50 ccs.	Parts per million	γ Fe in 50 ccs.	Remarks.
1	0.000001	0.02	1	The colours were well graded
2	0.000002	0.04	2	from Nos. 4 to 9 but very
3	0.000004	0.08	4	much alike below No.4,
4	0.000005	0.1	5	tending more to yellow as
5	0.00001	0.2	10	the iron decreased.
6	0.00002	0.4	20	The limit of sensitivity is
7	0.00003	0.6	30	thus about 5 gamma, and the
8	0.00004	0.8	40	limit of concentration 0.1
9	0.00005	1.0	50	part per million under these conditions.

(2) Optimum pH:- using 0.00001 gm. Fe in each tube and 5 drops reagent.

No.	pH of Buffer Solution.	Remarks.
1	1	Very pale green
2	2	Darker green
3	3	almost identical: maximum intensity
4	4	of green.
5	5	Slightly more yellow than Nos. 3 and 4.
6	6	Much paler yellow-green
7	7	Almost colourless
8	8	Less colour than No. 7
9	9	Colourless
10	11	Colourless.

Optimum pH continued:-

This reagent is evidently very sensitive. The limits of the range are 2 to 4 pH.

(3) Effect of sugar on colours produced:-

(a) Raw Sugar:- I. Without added Iron:-

Solutions were prepared containing 1.25, 2.5 and 5.0 gms. of San Domingo Raw Sugar, with 5 drops of reagent and 2 drops of $\frac{N}{10}$ HCl to adjust to correct pH range. These solutions were found to be without any green tint, the yellow of the sugar completely masking any colour developed by the reagent with the iron originally present in the sugar.

II. With Iron Added:-

This was further tested by adding 20 ccs. of iron solution to 1.25, 2.5 and 5.0 gms. of the above raw sugar. On adding the reagent the colour of all three was less than the 1 cc. standard for ion, which conclusively showed that the method was valueless in presence of even moderately coloured sugars.

(b) White Sugars:-

No.	gms. Fe present in 50 ccs.	gms. Sucrose	Remarks.
1	0.0001	1.25	Identical with 4
2	0.0001	2.50	Very slightly darker than standard.
3	0.0001	5.00	Practically equal to standard.
4	0.0001	nil.	Standard, a more vivid green than others.

White sugars do not seem to have much effect here: the reagent would probably be satisfactory for white sugars without ashing, provided the pH is carefully controlled.

Tests were now made comparing the method with the ammonium sulphide and sulphosalicylic acid methods, all estimations being made in presence of white sugar.

No.	White Sugar	% IRON		
		Ferron	Sulphide	Sulpho salicylic
1	A	.00012	.00007	.00010
2	B	.00010	.00011	.00014
3	C	.00008	.00009	.00012
4	D	.00021	.00028	.00027

These results agree remarkably well, much better in fact than many of the comparative results shown for raw sugars at the end of this section.

(c) With ashed sugar products:-

Various ashed products were tested by this method, adjusting the pH value to the correct range by $\frac{N}{10}$ HCl. Duplicate results are shown here:-

No.	Sample	% IRON					
		Ferron		Sulphide		Sulpho salicylic	
		(1)	(2)	(1)	(2)	(1)	(2)
1	Egyptian molasses	.020	.011	.081	.013	0.108	.005
2	Natal molasses	.009	.012	.009	.015	.005	.013
3	San Domingo Raw Sugar	.0006	.0004	.0014	.0008	.0014	.0015

These results show large differences: those for Natal molasses and the sugar are fairly good, but the Egyptian molasses figures vary so much that it is probable that some other constituent in this particular ash is interfering.

DETERMINATION OF IRON.

Method (7):- Using Potassium Ferricyanide $K_3Fe(CN)_6$

Solutions of Ferrous salts give with ferricyanide a blue colour due to the formation of Turnbull's Blue. This is indistinguishable in colour from Prussian Blue (Method 8).

$$3FeCl_2 + 2K_3Fe(CN)_6 \longrightarrow Fe_3(Fe(CN)_6)_2 + 6KCl.$$

Reagents:- Potassium ferricyanide -

A freshly prepared aqueous solution: 0.5 gm. per 100 ccs. is used. Sulphuric acid - a 6 normal solution of iron-free acid is required.

Standard iron solution - freshly prepared ferrous solution: 0.1 mgm. per cc.

Procedure:- To 50 ccs. of the sample solution 10 ccs. of the acid is added, filtering if necessary: 15 ccs. of the ferricyanide reagent is now added and volume made to 100 ccs. in a Nessler glass. The test solution is compared at once with freshly made ferrous iron standards.

Notes:- The comparison of the colour developed in both sample and standards must be made in matched Nessler tubes in the presence of equivalent concentrations of acids, immediately after the reagent is mixed with the solutions. The colour is deepened by an excess of the reagent, and diminished by an excess of acid. It fades rapidly on standing. The sensitivity is stated to be about 10 parts per million,

Method (7):- Potassium ferricyanide - Experimental.

(1) Test Limits:- Ferrous iron standards were made up, adding to each 5 ccs. of 6 N sulphuric acid followed by 7.5 ccs. of 0.5% freshly prepared ferricyanide solution.

No.	gms. Fe present in 50 ccs.	γ Fe in 50 ccs.	Parts per million	Remarks.
1	0.0000025	2.5	0.05	Yellow colours were obtained except with No. 11 which was perceptibly blue: this appears to be the smallest amount detectable. On standing overnight, there was a slight indistinct gradation in the colours which were now pale greenish-yellow. No. 11 was distinctly blue, but the colouring matter had separated as a precipitate on the bottom of the tube.
2	0.0000050	5.0	0.10	
3	0.0000075	7.5	0.15	
4	0.000010	10.0	0.20	
5	0.0000125	12.5	0.25	
6	0.00002	20	0.40	
7	0.000025	25	0.50	
8	0.00003	30	0.60	
9	0.00004	40	0.80	
10	0.00005	50	1.00	
11	0.000175	175	3.50	

This appears to fix the lowest limit of concentration at 3.5 parts per million of iron.

The colours obtained were not satisfactory, and necessitated immediate comparison. The fact that reagent has to be freshly made each time is also a disadvantage. The colloidal precipitate appears to flocculate more easily than that produced by ferrocyanide.

(2) With White Sugars:- With white sugars no apparent fading occurred, but on account of the low sensitivity no further tests were made with this reagent.

DETERMINATION OF IRON.

Method (8):- Using Potassium Ferrocyanide.

Potassium ferrocyanide produces an intense blue colour (Prussian Blue) with Ferric Salts by forming ferric ferrocyanide

$$4FeCl_3 + 3K_4Fe(CN)_6 \longrightarrow Fe_4\left[Fe(CN)_6\right]_3 + 12KCl$$

Reagents:- Potassium Ferrocyanide:- used in 0.5 per cent solution.

Standard Iron Solution:- A ferrous solution is prepared the iron being oxidised by permanganate: 1 cc. equals 0.00002 gm. Fe.

Procedure:-

The solution to be tested should contain approximately 0.002 gm. of Iron per litre.

The estimation is made in Nessler glasses as usual by comparison of the sample with standards.

For total iron, the solution is acidified with sulphuric acid and treated with permanganate to oxidise the ferrous iron; afterwards the colour is matched as above. The methods of dilution or balancing may also be employed. It is best to allow the tubes to stand for 15 minutes before comparison.

Notes:-

The test is inferior in point of delicacy to that

with potassium thiocyanate.

The colour produced varies from blue to pale green according to the nature and amount of the acid present, and the full intensity of the colour is not always produced immediately the reagent is added. Gum ghatti or gum arabic solution can be used to stabilise the suspension. Permanent methylene blue standards can be used.

Method (8):- Potassium Ferrocyanide - Experimental.

(1) Test Limits:- Standards were made using the oxidised standard iron solution and 5 ccs. of reagent followed by sulphuric acid.

No.	gms. Fe present in 50 ccs.	γ Fe in 50 ccs.	Parts per million.	Acid concentration	Remarks.
1	0.0000005	0.5	0.01	0.24N	Nos. 1, 2 and 3 were colourless. Blue colours appeared at once in Nos. 4, 5 and 6.
2	0.00000025	2.5	0.05	0.24N	
3	0.000005	5	0.1	0.24N	
4	0.00001	10	0.2	0.24N	Very faintly blue
5	0.000025	25	0.5	"	Blue
6	0.0001	100	2.0	"	Deeper blue

This fixes a limit at 0.2 part per million or 1 part in 5 millions. The order of addition is very important - the acid must always be added last-otherwise no colour develops. On standing 72 hours, no Prussian Blue had precipitated: the colours had changed to a slightly greener blue.

(2) Optimum pH:- Effect of Buffer Solutions.

Standards were prepared with buffer solutions ranging from 1 to 11 pH, using 0.00001 gm. Fe (or 10 gamma) in each tube. After standing for 15 minutes, the colours were well graded from 1 pH which was darkest. Tubes at 2 and 3 pH were also blue, but only about half

so intense. At 4 pH the tint was a pale greenish-yellow, and 7, 8, 9, 10 and 11 pH were identical in tint. Apparently this reagent must be used at 1.0 pH or below.

(b) Effect of Acid Concentration:-

No.	gms. Fe present in 50 ccs.	N Acid concentration.	Remarks.
1	0.0001	0.06	Blue colours appeared
2	0.0001	0.12	immediately. Nos. 2, 3 and 4
3	0.0001	0.24	were the most intense: No.1
4	0.0001	0.48	was not quite so blue.
5	0.0001	0.60	Nos. 3, 4, 5 and 6 were almost
6	0.0001	1.20	identical. Optimum acid concentration is 0.1 to 0.25 normal.

The acid must be added after the reagent. On standing for 72 hours 4, 5 and 6 had precipitated Prussian Blue: Nos. 2 and 3 were unaltered.

(3) Effect of white sugars on colours produced:-

Preliminary tests showed that there was a good grading of colour with sugar present, but there was definite fading of colour from the colours of the standards made with sugar. It seemed probable that if the standard iron solution were made with sugar present, the Prussian Blue method would be satisfactory in presence of sucrose. The following table shows

results obtained with white sugars compared with results by the sulphosalicylic acid method and the sulphide method:-

No.	Sugar	% Fe by Ferro-cyanide	% Fe by Sulpho-salicylic	% Fe by Sulphide
1	A	.00008	.00010	.00007
2	B	.00009	.00014	.00011
3	C	.00011	.00012	.00009
4	D	.00021	.00027	.00028

(4) Iron in Raw Sugars:-

It was found impossible to get a match of the colours when raw sugars were tried without ashing. The yellow of the sugar made the blue colour appear greener and no proper matching was obtainable with the comparator.

With ashing of the raw sugars the following results were obtained:-

No.	Raw Sugar	% IRON		
		Ferrocyanide	Sulphosalicylic	"Ferron"
1	Cuban	.0006	.0006	.0002
2	San Domingo	.0011	.0014	.0003
3	Java	.0004	.0007	.0001
4	Peru	.0008	.0010	.0002
5	Natal	.0013	.0012	.00045

These results are comparable for the sulphosalicylic and ferrocyanide methods, but low for all the samples by the "Ferron" method.

---

(5) Graph by Photometric Absorptiometer:-

The ferrocyanide standards were read in a Hilger "Spekker" absorptiometer and a curve drawn. The darkest colour was selected and the remaining standards compared with it. This curve renders the standards superfluous - only the one darkest solution need be prepared, and the test sample is compared with it, the percentage iron being found from the graph.

Note:-

One disadvantage found with the ferrocyanide method is the difficulty of removing the Prussian Blue colour from the Nessler glasses, especially if the blue has precipitated.

Determination of Iron by Potassium Ferrocyanide.

Readings in Spekker Photoelectric Absorptiometer.

No.	Fe P.P.M.	γ Fe in 50 ccs.	Mean Scale Readings.
1	3.0	150	standard
2	2.5	125	0.058
3	2.0	100	0.110
4	1.5	75	0.151
5	1.0	50	0.196
6	0.8	40	0.212
7	0.6	30	0.232
8	0.5	25	0.245
9	0.25	12.5	0.275

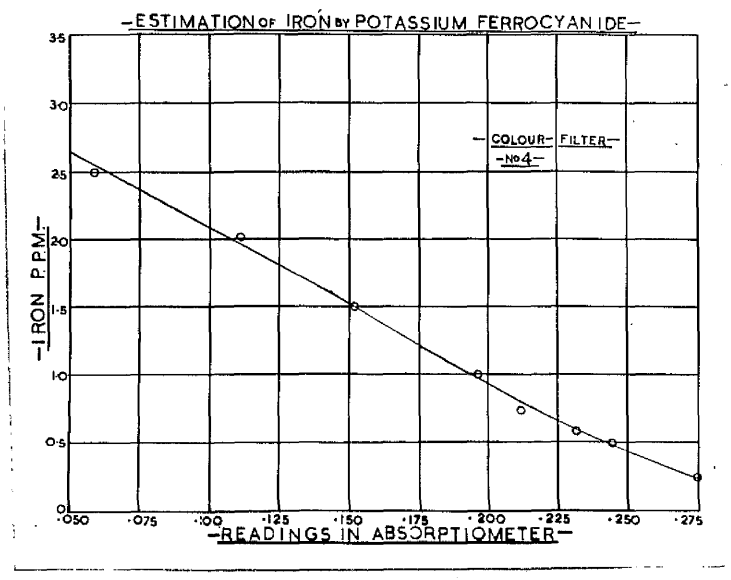
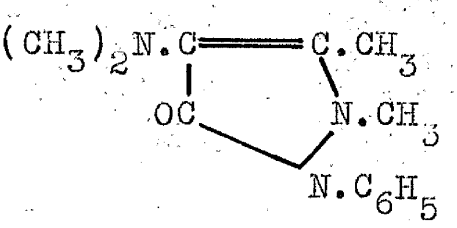


FIG. 25

DETERMINATION OF IRON.

Method (9):- Using Pyramidon:-



Pyramidon (4-Dimethylaminoantipyrine), produces a blueish-violet colour with ferric salts in acid solution.

Reagent:- A 1 per cent solution of pyramidon is used, with a ferric standard iron solution.

Procedure:- The sample is matched with the standard tubes in the usual way. The most suitable working range is from 0.0005 to 0.003 gms. per litre. In dilute sulphuric acid solution at 0.2 normal and beyond acid concentration has little influence: but below this acidity interference occurs.

Experimental:-

(1) Test Limits:- Standards were prepared using standard ferric iron solution and making up with 4.0 pH buffer solution: 2 ccs. of reagent were added to each tube.

No.	gms. Fe present in 50 ccs.	Parts per million	γ Fe in 50 ccs.	Remarks.
1	0.00001	0.2	10	The colour gradation was good. No.3 seems about the minimum amount which is detectable i.e., 0.6 part per million or 30 gamma. This limit agrees well with the 0.0005 gm per litre above which is 0.5 part per million.
2	0.00002	0.4	20	
3	0.00003	0.6	30	
4	0.00004	0.8	40	
5	0.00005	1.0	50	
6	0.00006	1.2	60	

(2) Effect of Buffer Solutions on Pyramidon Colours:-

(a) A test range of buffer solutions from pH 1.0 to pH 11.0 showed that the pH must be on the acid side. The glasses at 7 to 11 pH were colourless, and at pH 6 the solution was just coloured. The four tubes from 2 to 5 pH were of equal intensity, and the colour was slightly more intense at 1 pH.

(b) A test made 5½ months later with the same pyramidon solution gave good results when made up with 2.0 pH buffer solution. The colours were a bright blueish-purple. There was very marked fading in 1 hour: next morning all tubes were colourless.

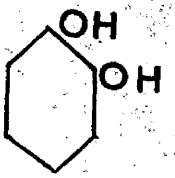
The optimum pH is about 1.0.

(3). Effect of Sugars:- Tests made with raw sugars showed that the colour of the sugar completely masked the delicate mauve colour of the pyramidon-iron complex. With white sugars there was no change in the colour. This method is therefore suitable for use with white sugars

Summary:- Pyramidon gives strong blueish-purple tints with ferric iron at an optimum of 1.0 pH, and the method may be used for the direct estimation of iron in white sugars. Results obtained for white sugars A.B.C.D. were 0.0001; 0.0001; 0.0001, and 0.00025 respectively.

The method is not suitable for use with raw sugars: its chief defect is the relatively rapid fading of the tints produced.

DETERMINATION OF IRON.



Method (10):- Pyrocatechin or Catechol

Pyrocatechin (1:2-Dihydroxy-benzene) gives with ferric salts a dark green colour. This reaction apparently depends on the phenolic properties of pyrocatechin. The colour is stated to be a brilliant violet in faintly alkaline solution.

Reagent:- A 1 per cent solution in water, and a standard ferric iron solution are used. The solubility of the reagent is 80 parts to 100 water at 15°C.

Procedure:- Bernouilli (loc. cit.) used a special sliding-gauge colorimeter, and obtained results accurate to 0.5 per cent at a concentration of 0.0062 gm. per litre (i.e. 1 part in 161,300).

The author employed the usual matching method.

Experimental:-

(1) Test Limits:- Standards were made using ferric iron solution and adding 5 ccs. of a 1 per cent pyrocatechin solution to each tube. There was a good grading of colour with standards ranging from 5 to 100 gamma. The minimum detectable appears to be about 0.6 parts per million; at 2 parts per million the colour is a distinct green, and this would be the limit for quantitative work. The colours had changed considerably after 1 hour, but the fading is not immediate. One of the chief troubles with this method is the darkening of the pyrocatechin

by oxidation, both in the solid state and in solution.

(2) Effect of Buffer Solutions:- A range from 1 to 11 pH was tried out with pyrocatechin. Solutions buffered at 1, 2, 3 and 4 pH were practically colourless, and solutions buffered at 6, 7, 8 and 9 pH were in shades of violet. The 11 pH solution turned a very dark brown. Only at 5 pH was there a green colour.

The reagent appears to be extremely sensitive to pH, which is a disadvantage. The iron present in 50 ccs. was 0.00001 gm. with each buffer solution.

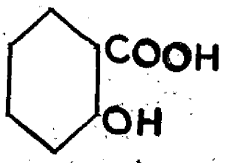
---

(3) Effect of Sugars:- With raw sugars the colours could not be matched: with white sugars the tendency was for the colour to be a truer green, this colour then fading rather quickly to brown and then to colourless. No quantitative work was possible owing to this fading.

Summary:-

This reagent is too sensitive to influences such as pH, light, and oxidation, to allow of its general use for determining iron. The violet colours in alkaline solution were very faint.

DETERMINATION OF IRON.



Method (11):- Salicylic Acid.

Salicylic acid (ortho-hydroxybenzoic acid) gives a reddish-violet colour with ferric salts. Solutions of ferrous iron remain colourless.

Reagents:- Salicylic acid - a saturated solution is used. The reagent is filtered and the clear solution used.

Standard Iron Solution:- ferric solution used containing 0.00001 gm. Iron per cc.

The solubility of salicylic acid is 0.15 gms. per 100 ccs. at 15°C.

Procedure:- The solution to be tested should contain between 0.00001 and 0.0002 gm. Iron. The method of matching standards cannot be used since the standards fade fairly rapidly in the light. It is necessary to add the salicylic acid to the sample and to the standard at the same time and to compare them at once. The method of balancing or method of dilution gives good results.

The sample is placed in a Nessler glass and 5 ccs. of the salicylic acid reagent added. The standard iron solution is added from a burette to 5 ccs. of the reagent in another Nessler tube, until the colour of the standard matches the sample. A plunger is used to stir the liquids.

Note:- Phosphates, thiosulphates, sulphites, fluorides, bisulphites and free mineral acids interfere. Organic matter should be absent.

As little as 0.00001 gm. ferric iron can be detected.

Experimental:-

(1) Test Limits:- Standards were made up with ferric iron solution, adding 5 ccs. of the salicylic acid solution. There was a distinct pink colour at 60 gamma grading down quite sharply to 10 gamma, but not below this. The effective limit of detection can thus be taken as 0.20 part per million (i.e., 0.00001 gm. Fe in 50 ccs. solution). Fairly rapid fading of standards took place.

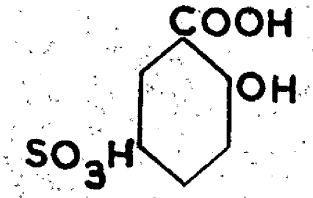
(2) Effect of Buffer Solutions:- With buffers ranging from 1 to 9 pH it was found that the optimum pH lay between 3 and 5 pH. At 6 pH the pink colour was very light and at 7, 8 and 9 pH the solutions were colourless. A pH of 4.0 was selected as giving the best development of colour.

(3) Effect of Sugar:- With raw sugars the pink colour was masked by the sugar colour. With white sugars it was found that even 1 gm. of white sugar caused lightening of the pink colour, and this fading was intensified as more sugar was added.

Summary:-

(1) The colour develops best at 4 pH.
(2) Rapid fading of standards occurs.
(3) With sugars fading renders the method useless.
(4) The limit of detection is 0.20 part per million, with the best working range for quantitative work commencing at about 1 part per million.
(5) On the whole, not a good method.

DETERMINATION OF IRON.



Method (12):- Sulphosalicylic acid (2-Hydroxy 5-sulphobenzoic acid, $C_7H_6O_6S$) gives a rose-red coloration with ferric iron in acid solutions. In alkaline solutions both ferric and ferrous iron react giving a golden-yellow colour.

Reagents:- Sulphosalicylic acid:- 20 gms. in 100 ccs. distilled water. Ammonia:- 10 per cent aqueous solution.

Procedure:- To the test solution 2 ccs. of the sulphosalicylic acid reagent is added, followed after mixing by 2 ccs. of the ammonia solution. A golden yellow colour develops in presence of iron (ferric and ferrous). The order of addition must be adhered to. The test solution is then matched with standards similarly prepared.

Notes:- The colour is influenced by hydrion manganese, and organic substances. The influence of manganese is difficult to eliminate: disturbance by the other factors can be prevented by the use of citrate buffers and colour filters (F.Alten, et al, Z.Amorg. Chemie, 215, 81-91,1933).

The method is stated to be sensitive to 0.2 mgm. of Fe (Lappin & Kill, Z. Hyg. 112, 719, 1931).

For ferric iron the tint gradation and sensitiveness are very similar to the thiocyanate reaction with iron.

Sodium, potassium, lithium, ammonium, calcium, magnesium, zinc, aluminium, chloride, bromide, iodide, nitrate, sulphate and phosphate do not interfere either in acid or in alkaline solution. Manganese interferes in alkaline solution by precipitation of the hydroxide. Nitrite interferes in the ferric estimation in acid solution (Lappin and Kill, Ibid).

Experimental:- Sulphosalicylic Acid.

(1) Limits of Test:- Standards were prepared using Iron concentrations ranging from 1 gamma to 100 gamma. A fine golden yellow colour was produced, the grading being very distinct. It was found that 1 gamma represented the lowermost limit of detection, the best working limit being with 5 or 10 gamma as the lower limit for quantitative work.

(2) Effect of Buffer Solutions:- With a range of buffer solutions from 1 to 11 pH the yellow colour was produced only at 9, 10 and 11 pH. It is most important to add the buffer solution last, otherwise the colour does not develop properly. Later work with sugar solutions showed that even at 11 pH the alkalinity was not high enough, and it was found necessary to go back to the full ammonia addition. The reagent gives the rose colour with ferric iron alone in acid solution, and/must the solution be decidedly alkaline to give the golden-yellow tint with total iron.

(3) The Effect of White Sugars:-

Weighed quantities of white sugar varying from 0.5 to 10 gms. were dissolved in Nessler glasses in distilled water and then each was treated with 2 ccs. of the sulpho-salicylic reagent followed by 2 ccs. of ammonia, and made up to 50 ccs.

On comparing the colours produced, with standard iron solutions similarly prepared the following results were obtained:-

	(1)	(2)	(3)	(4)	(5)	(6)
gms. sugar taken	0.5	1.0	2.0	3.0	4.0	5.0
Per cent iron	0.00008	0.00008	0.0007	0.00009	0.00010	0.0001[illegible]2

By ashing the per cent obtained was 0.00005

By the direct sulphide method the per cent obtained was 0.000015 (using 5 gms.).

There was no appearance of fading or lightening of colour, and the reagent appeared to be entirely satisfactory in presence of white sugar. The variation in the results obtained above is no doubt due to the small quantity taken in No. (1) as compared with ten times the amount taken in No. 6.

(4) The Effect of Raw Sugars:-

It was found that the determination of iron could be carried out in raw sugars of moderate colour by sulpho-salicylic acid, and that the results were more reliable

than those by the ashing method. These results compared well with the results of other methods of estimating iron. Some tests were carried out to find the effect of ammonia on the raw sugar alone.

Effect of Ammonia on Colour of Raw Sugars:-

The darkening effect of alkalis on the colouring-matter of sugars is well known. It is important therefore to know how this affects the colour produced in the colorimetric estimation of iron by sulphosalicylic acid where ammonia is added.

Test (1):- 25 gms. of Demerara Raw Sugar were dissolved in water made up to 200 ccs. and filtered using Kieselguhr. Ten ccs. of the filtered solution were taken in each of seven Nessler tubes. To five of these were added varying amounts of standard iron solution. To one of the two remaining ammonia alone was added, and the other one was made up to 50 ccs. with water alone. Iron standards were also prepared in the usual way.

(a) Sugar and Ammonia alone as blank.

No.	Gms. Fe added to 50 ccs.	Gms. raw sugar present	ccs. 20% sulphosalicylic reagent added.	ccs. 10% Ammonia	Gms. Fe found in 50 ccs.
1	Nil	1.25	2	2	0.0000025
2	0.000010	1.25	2	2	0.000005
3	0.000025	1.25	2	2	0.000015
4	0.000050	1.25	2	2	0.000045
5	0.000075	1.25	2	2	0.000080

(b) 1.25 gms. of raw sugar alone in blank.

No.	Gms. Fe added to 50 ccs.	Gms. raw sugar present	ccs. 20% sulphosalicylic reagent added.	ccs.10% Ammonia	Gms. Fe found in 50 ccs.
1	Nil	1.25	2	2	0.00005
2	0.000010	1.25	2	2	0.00006
3	0.000025	1.25	2	2	0.00008
4	0.000050	1.25	2	2	0.00010

From No. 1 (b) per cent iron in this sugar is 0.004. This compares with 0.0036 obtained by the sulphide method.

The results in (a) show slightly less iron found present than has been added.

An obvious explanation of this result lies in the fact that whereas in the blank 2 ccs. of free ammonia has been added, a part at least of the ammonia in the sample tube has neutralised the sulphosalicylic acid, forming presumably ammonium sulphosalicylate.

Titration showed:-

25 ccs. of 20 per cent sulphosalicylic acid required 20 ccs. of the 10 per cent ammonia to produce a yellow colour. By titration against standard acid and alkali the strength of the 20 per cent sulphosalicylic acid was 1.472 Normal, and the 10 per cent ammonia was 1.712 Normal.

Yellow Sulphosalicylate Mixture:-

The mixture produced by titrating 20 ccs. of the 10 per cent ammonia against 25 ccs. of sulphosalicylic acid until yellow colour appeared was found electrometrically to have a pH of 7.2. An attempt was made to use this solution for the colorimetric work, but it was found that the alkalinity was not enough to overcome the natural acidity of the sugars being tested. A further attempt to stabilise the yellow colour by using buffers of 8 and 9 pH. (Borax and Boric acid and sodium chloride) gave a maximum intensity of yellow-colour only after half-an-hour. It would be possible, however, to keep this reagent ready-mixed with the ammonia. It seems obvious that the alkalinity must be high to obtain the correct golden-yellow colour.

Effect of Salts of Heavy Metals on Sulphosalicylic Acid determination of Iron.

Solutions of metallic salts were made up containing 0.000005 gm. per cc. of Manganese, Chromium, Copper, Nickel and Lead:- 5, 10 and 20 ccs. of each solution was treated with an equal quantity of iron in a Nessler glass and compared with a similar tube containing an equivalent amount of iron alone. Two ccs. of the reagent were used in each tube followed by 2 ccs. of 10% ammonia. It was found that these metal salts had practically no effect on the golden-yellow colour, although a very small difference

was detected with 20 ccs. of Manganese, the intensity being slightly greater in presence of the foreign salt.

Determination of Iron after adding known amounts of Iron to Raw Sugars.

To 25 gms. of each sugar of known iron content, varying amounts of iron were added, the solution being then made to 200 ccs. and filtered using Kieselguhr. 10 ccs. of filtrate used in each test.

		A	B	C	D	
No.	Sugar	Gms. Iron Added	Per cent Iron Added	% Total Iron Found	% Iron in Sugar alone	Original % Iron Found in Sugar	Difference of C and D.
1.	San Domingo	0.0009	0.0036	.0084	.0048	.0036	.0012
		0.0015	0.0060	.0092	.0032	.0036	.0004
		0.0020	0.0080	.0115	.0035	.0036	.0001
2.	Cuban	0.0009	0.0036	.0046	.0010	.0008	.0002
		0.0015	0.0060	.0065	.0005	.0008	.0003
		0.0020	0.0080	.0088	.0008	.0008	Nil
3.	Java	0.0009	0.0036	.0046	.0010	.0007	.0003
		0.0015	0.0060	.0071	.0011	.0007	.0004
		0.0020	0.0080	.0086	.0006	.0007	.0001

These results show that the sulphosalicylic acid method is reliable in the presence of sugar of moderate colour, since the recovery of the added iron is reasonably good, and probably within the limits of accuracy of a colorimetric method.

Results of Iron Determinations by Sulphosalicylic Acid and Ammonium Sulphide Methods.

No.	Sample	% Fe: Sulphide Method		% Fe: Sulphosalicylic Method	
		Direct	Ashed	Direct	Ashed
1	Molasses Egyptian	-	0.013 0.081	-	0.108 0.005
2	Natal	-	0.0086 0.0150	-	0.005 0.013
3	Raw Sugars				
	San Domingo	0.0040	0.0026 0.0036	0.0036 0.0036	0.0014 0.0015
4	Java	0.0012	0.0013 0.0019	0.0009 0.0011	0.0013 0.0013
5	Demerara	0.0016	0.0024 0.0022	0.0020 0.0023	0.0016 0.0022
6	West Indies	0.0028	0.0023 0.0019	0.0020 0.0025	0.0016 0.0018
7	Natal	0.0032	0.0039 0.0041	0.0012 0.0018	0.0007 0.0014
8	Mauritius	0.0020	0.0012 0.0020	0.0014 0.0015	0.0009 0.0010
9	White Sugars White Beet	0.0002	0.00007 0.00011	0.0002 0.0002	0.00007 0.00007

It was found here that 5 to 10 minutes sufficed to develop the maximum intensity of yellow with the sulphosalicylic acid. With ammonium sulphide 20 to 25 minutes elapsed before the maximum colour appeared.

On analysing the results it appears that there is as much likelihood of error in the ashed samples as in those where estimation was direct.

In practice, these estimations are seldom done in duplicate: the duplicate results here reported show large variations for the molasses samples.

The raw sugars results also vary considerably, but are probably as accurate without ashing as with ashing.

Results with Spekker Photoelectric Absorptiometer for Sulphosalicylic acid method for Iron.

Conditions:- A ferrous standard iron solution was used 1 cc. ≡0.0001 gm. Iron containing sulphuric acid to prevent hydrolysis. 2 ccs. of 20 per cent sulphosalicylic acid and 10 ccs. of 10% ammonia were added in that order, to Nessler tubes containing 0.5 to 11 ccs. of the standard iron solutions. Readings were made in the Absorptiometer in 1 cm. cells using colour-filter No. 5 and taking the darkest tube as standard.

No.	ccs. Iron Solution	Gms. Fe present in 50 ccs.	γ Fe per cc.	Parts per million	Reading in Photometer
1.	11	0.0011	22	22	Standard
2.	10	0.0010	20	20	0.035
3.	9	0.0009	18	18	0.043
4.	8	0.0008	16	16	0.060
5.	7	0.0007	14	14	0.070
6.	6	0.0006	12	12	0.088
7.	5	0.0005	10	10	0.103
8.	4	0.0004	8	8	0.125
9.	3	0.0003	6	6	0.140
10.	2	0.0002	4	4	0.165
11.	1	0.0001	2	2	0.185
12.	0.5	0.00005	1	1	0.200

The larger amount of ammonia was used to neutralise the excess of acid found necessary to stabilise the ferrous standard solution. It will be seen from the accompanying curve that the sulphosalicylic acid colours with iron obey the Beer-Lambert law fairly well. This graph enables iron to be estimated using only one standard (containing 22 parts per million of Iron).

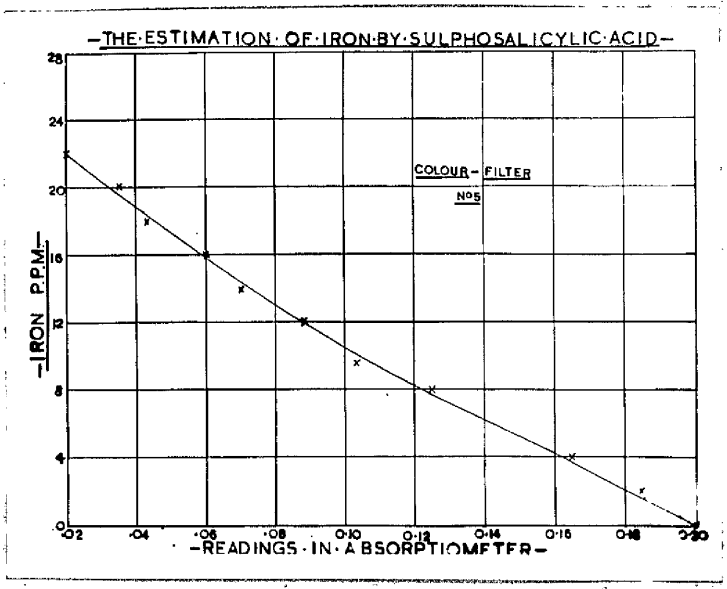

—THE ESTIMATION OF IRON BY SULPHOSALICYLIC ACID—
—IRON P.P.M.—
28
24
20
16
12
8
4
0
COLOUR—FILTER
NO5
·02
·04
·06
·08
0·10
0·12
0·14
0·16
0·18
0·20
—READINGS IN ABSORPTIOMETER—

FIG.26

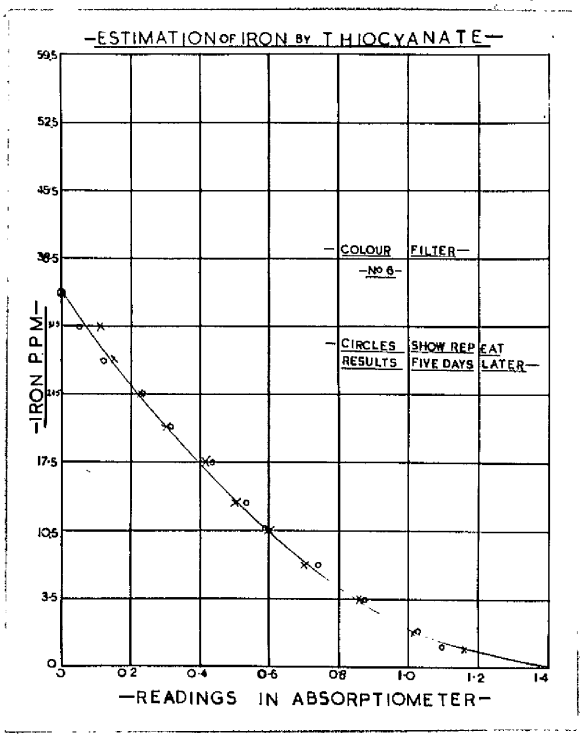

—ESTIMATION OF IRON BY THIOCYANATE—
—IRON P.P.M.—
59·5
52·5
45·5
38·5
31·5
24·5
17·5
10·5
3·5
0
COLOUR FILTER—
—NO 6—
CIRCLES SHOW REPEAT
RESULTS FIVE DAYS LATER—
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
—READINGS IN ABSORPTIOMETER—

FIG.27

Examination of a modification of the Sulphosalicylic Acid Method.

After the above work had been completed the author's attention was drawn to a paper by Alten, Weiland, and Hille Z. Anorg. Chemie, 215, 81 (1933) modifying the sulphosalicylic acid method for iron. Details are given below:-

Process of Estimation:- Pipette the test-solution into a 100 ccs. flask and mix with 10 ccs. of $\frac{N}{1}$ NH_4Cl, 2 ccs. of sulphosalicylic acid, and 20 ccs. of $\frac{M}{10}$ sodium citrate. Now add $\frac{N}{1}$ NaOH from a burette: the initial red colour changes to yellow-red, becoming brighter; with very small amounts of iron it may disappear completely.

The caustic soda is now added drop by drop until the yellow colour reaches its maximum intensity, when 10 ccs. of $\frac{N}{1}$ NaOH and 20 ccs. of borate buffer solution of pH 12 are added and the solution made up to the mark. After 3 hours the reading is taken in a colorimeter through a blue or violet filter. The colour is more yellow with decrease of hydrion and changes to a golden-yellow at pH 7.9 or thereabouts. If Manganese is responsible for half or more of the colour visible, re-estimate in acid solution.

Reagents:- N NH_4Cl :- 53.5 gms. per litre

$\frac{N}{10}$ Disodium citrate:- Dissolve 21.01 gms. of citric acid in 200 ccs. of normal sodium hydroxide and make up to 1 litre.

Borate Buffer Solution 12 pH:- Dissolve 12.04 gms. of boric acid in 100 ccs. of CO_2- free normal NaOH and made to 1 litre. 5.4 parts of $\frac{N}{10}$ NaOH and 4.6 parts of the borate solution give 12.0 pH buffer solution.

Experimental:- This method was tried out with sugars. The colour gradation in the standards was found to be inferior to that with no buffer present. The exact point when the addition of NaOH should be stopped is very difficult to judge, and the three hours delay before reading is a disadvantage. For sugars, where no interference of heavy metals is likely to occur, the method appears to be too cumbersome for use. Several white and raw sugars were tested, and gave good agreement with the results obtained without buffering.

DETERMINATION OF IRON.

Method (13):- Ammonium Thiocyanate:- A red colour is produced when a thiocyanate solution is added to a solution containing ferric ions. The colour intensity is extremely dependent upon the composition of the solution and is by no means proportional to the concentration. The red colour is due to the undissociated salt and to its double compounds, the ionised salt being colourless. The red coloration fades slowly on exposure to bright diffused daylight.

Reagents:- Ammonium Thiocyanate:- 7.612 gms. of the pure salt made to 100 ccs. with distilled water: this gives a molar solution.

A standard ferric solution is used.

Procedure:- The sample solution is treated with 5 ccs. of the reagent solution and matched with standard solutions similarly prepared, in Nessler glasses.

Notes:- The colour is probably due to a complex salt which ferric thiocyanate forms with the alkali salt: to the potassium complex salt the formula $Fe(CNS)_3 . 9KCNS$ has been attributed. More recently the formula $Fe\left[Fe(CNS)_4\right]$ has been given to this complex. (McAlpine and Soule, "Qualitative Chemical Analysis," (Chapman and Hall, 1933), p.319). Many substances interfere markedly with the reaction, the action being so pronounced that it may be impossible to produce a colour even in the presence of considerable

quantities of iron. Improvement is obtained by extraction of the ferric thiocyanate with solvents such as amyl alcohol or ether. N. Strafford in an Institute of Chemistry Lecture, (1933) said "the method requires so many precautions that in view of more reliable methods, it needs no further consideration". Stokes and Cain (loc.cit.) who investigated the method very fully, stated:- "the intensity of the colour is so influenced by the nature and concentration of the substances present that unless the test solution and the standard solution with which it is compared have identical composition and concentration, results varying many hundred or even thousand per cent from the truth, may be obtained".

Experimental:- The presence of white sugars was found to cause fading with this reagent. With raw sugars the pink colour was completely obscured by the yellow colour of the sugar. No further tests were made with sugars, but a series of readings was taken in the photoelectric absorptiometer.

Thiocyanate readings with the Photoelectric Absorptiometer.

Conditions:- A ferric standard solution was used containing 0.0001745 gm. Fe per cc. Two ccs. of $\frac{N}{1}$ ammonium thiocyanate were added and the volume made up to 50 ccs., reading immediately in the photometer in a 1 cm. cell using No. 6 filter, and taking the darkest tube as standard.

No.	ccs. Iron Solution	Gms. Fe present in 50 ccs.	γ Fe per cc. or parts per million	Readings in Photometer	Readings repeated with same solutions.
1	11	0.00192	38.0	Standard	Standard
2	10	0.00175	35.0	0.115	0.06
3	9	0.00157	31.4	0.150	0.13
4	8	0.00140	27.9	0.230	0.235
5	7	0.00122	24.4	0.300	0.31
6	6	0.00105	20.9	0.410	0.42
7	5	0.00087	17.5	0.495	0.54
8	4	0.00070	14.0	0.590	0.60
9	3	0.00052	10.4	0.698	0.74
10	2	0.00035	7.0	0.860	0.88
11	1	0.00017	3.49	1.05	1.03
12	0.5	0.000087	1.74	1.16	1.10

The line plotted on the graph from the above figures shows that the Beer-Lambert law is not obeyed: this may be due to the fading which inevitably occurs with thiocyanate during the reading of a series of solutions.

It is noteworthy that the curve approximates more to a straight line when the readings were repeated after some days had elapsed.

DETERMINATION OF IRON.

Method (14):- Using Thioglycollic Acid

(Mercapto-acetic acid $HS.CH_2.COOH$, or $C_2H_4O_2S$)

With ferric salts, thioglycollic acid gives a transient blue colour due to ferric thioglycollate.

Excess of the reagent reduces this compound to ferrous thioglycollate which is colourless in neutral or acid solution. On making alkaline, however, an intense purple colour appears caused by formation of the coloured anion $Fe\left[(S.CH_2.COO)_2\right]^{--}$

This reagent is used to determine total iron, since ferric salts are reduced to the ferrous state by the reagent itself, without need for prior reduction. Oxidising agents should be avoided since they tend to prevent this reduction, and also may oxidise the reagent to Dithioglycollic acid which gives no colour either with the ferric or ferrous iron. The purple colour is unstable in the presence of fairly small amounts of strong alkalis, and therefore the use of ammonia is recommended.

Reagents:- Thioglycollic acid solution:- prepared by the addition of 4 ccs. of thioglycollic acid to a solution of 8 ccs. of concentrated ammonia in 50 ccs. of water.

Standard iron solution - a ferric solution is prepared from iron wire.

Procedure:- The test solution in a Nessler glass is treated with 1 cc. of the thioglycollic acid reagent, and then made strongly alkaline by the addition of 1-2 ccs. of concentrated ammonia. It is now made up to volume, and compared with standards similarly prepared.

Notes:- A comparatively large excess of ammonia has no appreciable effect on the colour produced by a given amount of iron.

The preparation with ammonia reduces the unpleasant odour of the reagent.

The standards may fade in an hour, but even 24 hours later the colour can be restored by shaking.

Interference:- Chlorides, sulphates, flourides, and phosphates have no effect; sodium silicate has a bleaching action on the colour. Copper salts cause a slow fading, negligible when only small amounts are present.

With manganese salts a brown colour develops at first, but is reduced by the reagent on standing.

Nickel gives a reddish-brown colour which may mask the iron colour; cobalt interferes seriously, giving a deep yellowish brown colour. Chromium gives a pink-purplish-red colour and must be separated from the iron. With aluminium present, iron can be determined if the aluminium is kept in solution by addition of excess tartaric acid.

Thioglycollic acid is not very satisfactory as a spot test for detecting iron owing to interference by the above metals but is suitable for quantitative work in their absence.

The reagent will give an immediate coloration with one part in five million parts of solution, and will give a colour on standing for a few minutes in a solution containing one part of iron in ten million.

Experimental:- Thioglycollic Acid.

(1) Test Limits:- using 5 drops (0.25 ccs.) reagent and 2 ccs. of 10% ammonia ($\frac{v}{v}$).

Series(1).

No.	Gms. Fe present in 50 ccs.	γ Fe in 50 ccs.	Parts per million	Remarks.
1	0.000005	5	0.1	The colour grading was
2	0.000010	10	0.2	immediately very distinct:
3	0.000015	15	0.3	after standing overnight the
4	0.000020	20	0.4	colours were still distinctly
5	0.000025	25	0.5	graded but showed a little
6	0.000050	50	1.0	intensification on shaking.

Series (2). As in Series (1) using lower concentrations of iron and 0.5 cc. of Thioglycollic reagent.

No.	Gms. Fe present in 50 ccs.	γ Fe in 50 ccs.	Parts per million	Remarks.
1	0.000001	1	0.02	Nos. 3, 4 and 5 were
2	0.000002	2	0.04	distinctly pink. No. 2
3	0.000003	3	0.06	appeared pink only when
4	0.000004	4	0.08	compared with the blank and
5	0.000005	5	0.1	No. 3. No. 1 was
6	Nil	-	-	practically equal to the blank tube. No. 6 blank.

Left overnight: the colours next morning were still well graded, the pinks being slightly duller.

(2) Effect of Buffer Solutions:-

Solutions were prepared by taking 10 ccs. of standard iron solution containing 50 gamma of iron in 50 ccs. adding 0.25 cc. of thioglycollic reagent, and making up to 50 ccs. with standard buffer solutions. No colour was developed below 7.4 pH (in the completed tube). The use of ammonia in making up the reagent originally, brought up the pH values of the final tubes. Above 7.4 pH the colour was fully restored on shaking after 24 hours. The reaction appears to be more satisfactory well on the alkaline side. The odour of the reagent is not objectionable when well masked by ammonia.

(3) Effect of Concentration of Ammonia:-

10 ccs. of standard iron solution containing 50 gamma of iron were placed in each of 15 Nessler glasses followed by 0.25 cc. of the thioglycollic reagent and then by the under-noted quantities of concentrated (0.88 sp.gr.) ammonia:- 0.05; 0.1; 0.2; 0.4; 0.6; 0.8; 1, 2, 4, 6, 8, 10, 20, 30, 40 ccs.

In each case a bright pink colour was obtained initially, which faded to pale orange on standing. The intensity of colour was equal in the tubes up to 20 ccs. of conc, ammonia: after this concentration there was discharge of colour. On leaving overnight the colours disappeared and did not re-appear on shaking. This indicated that too little of the reagent was present.

(4) Effect of Concentration of Thioglycollic Acid:-

(a) 1 cc. of reagent with 1.5 ccs. of conc. ammonia and 50 gamma of iron were made to 50 ccs. in a Nessler tube and left overnight: the colour was permanent throughout.

(b) Using 1.5 ccs. of conc. ammonia and 50 gamma of iron tubes were made up with 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0 ccs. of the thioglycollic reagent. After 0.6 ccs, the colour intensity was not altered by additional reagent, but below 0.6 cc. the pink tints were progressively weaker and less stable on standing.

(c) With 1 cc. of thioglycollic reagent, bright and stable colours were obtained when the ammonia was varied from 0.2 to 20 ccs.

Conclusions:- 1.5 ccs. of conc. ammonia and 1 cc. of thioglycollic acid reagent are the optimum amounts to use.

(5) Effect on Sugars:-

With white sugars there was no fading of the pink colour with 5 gms. of white sugar present, but with yellow or raw sugars the least trace of yellow masked the pink colour completely and rendered comparison impossible.

The method appears suitable for determination of iron in white sugars, but cannot be applied in presence of coloured sugars.

(6) Determination of Iron in Ashed Sugar Products: Comparison of Thioglycollic Acid Method with Sulphide and Sulphosalicylic Acid Methods.

No.	Sample	% IRON		
		By Thioglycollic Acid	By Ammonium Sulphide	By Sulphosalicylic Acid.
1	Egyptian Molasses	0.022 0.020	0.013 0.081	0.108 0.005
2	Natal Molasses	0.021 0.040	0.0086 0.0150	0.005 0.013
3	Raw Sugars San Domingo	0.0017 0.0019	0.0026 0.0036	0.0014 0.0015
4	Java	0.0009 0.0009	0.0013 0.0019	0.0013 0.0013
5	Demerara	0.0011 0.0008	0.0024 0.0022	0.0016 0.0022
6	West Indies	0.0014 0.0014	0.0023 0.0019	0.0016 0.0018
7	Natal	0.0022 0.0017	0.0039 0.0041	0.0007 0.0014
8	Mauritius	0.0010 0.0010	0.0012 0.0020	0.0009 0.0010

The results are shown in duplicate. The same order of results obtains for the three methods in the case of raw sugars, but the molasses figures are very widely divergent.

Determination of Iron by Thioglycollic Acid

Readings in Spekker Photoelectric Absorptiometer.

No.	Fe P.P.M.	γ Fe in 50 ccs.	Mean Scale Readings.
1	3.0	150	standard
2	2.5	125	0.050
3	2.0	100	0.077
4	1.5	75	0.106
5	1.2	60	0.124
6	1.0	50	0.132
7	0.7	35	0.150
8	0.5	25	0.163
9	0.3	15	0.174
10	0.1	5	0.185

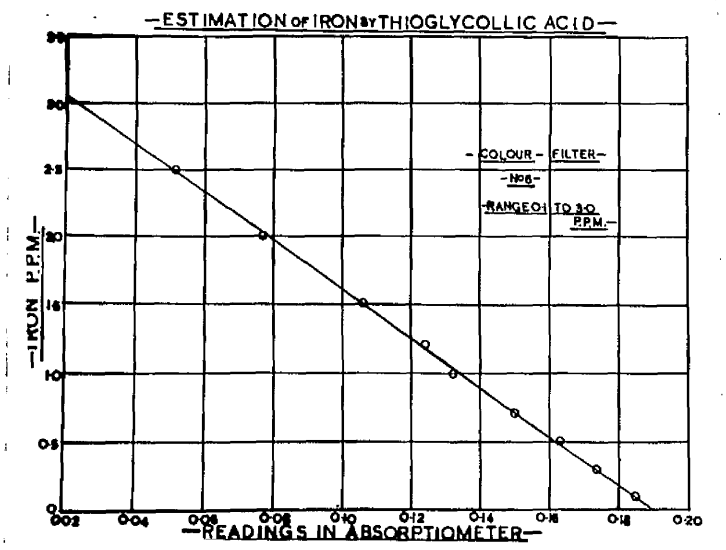
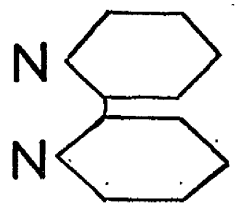

—ESTIMATION of IRON by THIOGLYCOLLIC ACID—
—COLOUR - FILTER—
—NO6—
—RANGE 0·1 TO 3·0 P.P.M.—
—IRON P.P.M.—
2·5
2·0
1·5
1·0
0·5
0
0·02
0·04
0·06
0·08
0·10
0·12
0·14
0·16
0·18
0·20
—READINGS IN ABSORPTIOMETER—

FIG. 28

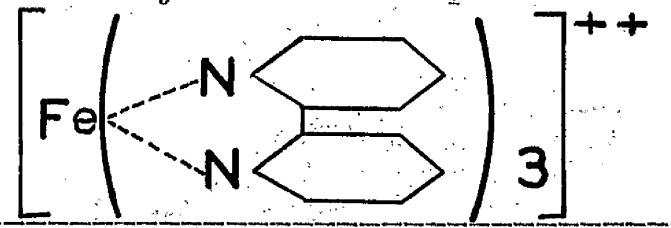
DETERMINATION OF IRON.



Method (15):- Using

$C_{10}H_8N_2$ $\alpha\,\alpha'$ Dipyridyl

Ferrous salts react in solutions of mineral acids with this organic base to give a soluble deep red complex cation ; this is very stable and possesses the co-ordination formula



Ferric salts do not react under the same conditions. Other ammine-forming metallic ions in acid solution also react with the reagent but only give weak colours.

See Feigl and Hamburg, Z. Analyt. Ch. 86, 7, (1931) also W.D. McFarlane J.I.E.C. Anal. Ed. Vol. 8, p.124, (1936). For preparation of $\alpha\,\alpha'$ Dipyridyl see F. Blau, Berichte, 21, 1077, (1888), and Hein and Retter, Berichte 61, 1790, (1928)

Reagents:- $\alpha\,\alpha'$ Dipyridyl is used as a 2 per cent solution in hydrochloric acid or in alcohol. A ferrous iron standard solution may be employed. McFarlane (loc. cit.) uses artificial standards (made from cobalt nitrate) in a photo-electric colorimeter.

Procedure:- The sample under test is treated with 1 cc. of the Dipyridyl reagent, and then 0.25 cc. of 0.004 N Titanous chloride solution or a tiny fragment of solid sodium

thiosulphate is added to reduce any ferric iron to the ferrous condition. The solution is diluted to 100 ccs. with acetate buffer solution of pH 4.7 (made by mixing equal parts of 0.2 N acetic acid and 0.2N.NaOH) and compared with standards prepared in the same way.

Notes:- The ferrous dipyridyl compound reaches its maximum intensity of colour in a few minutes when the pH is above 3.5.

The method is most satisfactory when the range is 0.25 to 3.0 gamma per cc.

Experimental:-

(1) Test Limits:- A 1 per cent solution of the reagent in alcohol was used. Standards were made containing from 1 to 25 gamma of ferrous iron in 50 ccs., with 0.5 cc. of the reagent added to each tube.

The colours produced varied from very pale pink to pinkish red. They intensified to a maximum in 10 minutes, and became duller after about 2 hours without showing serious fading. The colours were then indefinitely stable. With 0.0002 gm. of iron (1 part in 250,000) a very bright deep red colour was produced. The lowest amount with which a pink colour could be discerned was 0.04 gamma per cc.

(2) Effect of Buffer Solutions:- A series of tests showed that the maximum colour intensity persisted between 3.5 and 8.5 pH this confirming the limits mentioned by Snell ["Colorimetric methods" Vol. I. p.310]. Outwith these limits the colour was not so pronounced.

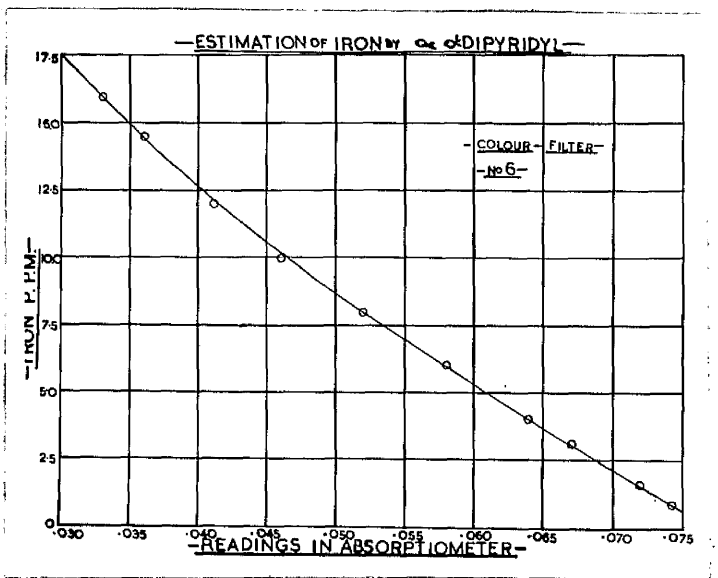

—ESTIMATION OF IRON BY α α' DIPYRIDYL—
—COLOUR—FILTER—
—No 6—
—IRON P.P.M.—
17·5
15·0
12·5
10·0
7·5
5·0
2·5
0
·030
·035
·040
·045
·050
·055
·060
·065
·070
·075
—READINGS IN ABSORPTIOMETER—

FIG.29

(3) Effect of Sugars:- With white sugars there was no change in the colour with 5 grams of sugar added. Raw sugars of even light yellow colour masked the red completely, giving at best a dirty orange colour.

(4) Readings in "Spekker" Photoelectric Absorptiometer:- Using a 1% alcoholic solution of $\alpha\,\alpha'$ Dipyridyl: 0.5 ccs. of this reagent added to each Nessler glass. The colours produced varied from very pale pink to pinkish-red, and intensified slightly on standing reaching a maximum after about 10 minutes. Slow fading occurred after two hours.

No.	Fe. P.P.M.	Mean Scale Readings.
1	0.8	.074
2	1.6	.072
3	2.4	.069
4	3.2	.067
5	4.0	.064
6	6.0	.058
7	8.0	.052
8	10.0	.046
9	12.0	.041
10	16.0	.033
11	20.0	standard

These results were plotted graphically, the line obtained being very nearly straight, showing that the Beer-Lambert law is followed by this reaction for the limits taken.

SUMMARY OF RESULTS

FOR THE COLORIMETRIC DETERMINATION OF IRON.

No.	Method	Optimum Reaction At	Fading of Standards	With White Sugars	With Raw Sugars
1	Acetyl-acetone	1.0 pH or below	Do not fade	Useless	Useless
2	Alloxantin	11 pH or above	Do not fade	Useless	Useless
3	Ammonium Sulphide	High alkalinity	Darken on standing	Fair	Fair
4	Dimethylglyoxime	High alkalinity	Fade slowly	Useless	Useless
5	Dinitroso-resorcinol	3 pH	Intensify on standing	Useless	Useless
6	"Ferron"	2 to 4 pH	Stable	Good	Useless
7	K. Ferricyanide	High acidity	Fade rapidly	No fading	Useless
8	K. Ferrocyanide	0.1 to 0.25 Normal acidity	Intensify on long standing	Slight fading	Useless
9	Pyramidon	1.0 pH 0.2N acid	Fade slowly	Good	Useless
10	Pyrocatechin	5 pH green 11 pH violet	Fade slowly	Useless	Useless
11	Salicylic acid	4 pH	Fade rapidly	Useless	Useless
12	Sulphosalicylic acid	High alkalinity	Do not fade	Good	Good
13	Ammonium Thiocyanate	pH 5 to 7	Fade rapidly	Useless	Useless
14	Thioglycollic acid	High alkalinity	Do not fade	Good	Useless
15	$\alpha\,\alpha'$ Dipyridyl	3.5 to 8.5 pH	Very slow fading	Good	Useless

SUMMARY OF RESULTS FOR THE COLORIMETRIC DETERMINATION OF IRON.

Method	Test Limits Parts per million	Time for Development of full colour minutes.
1. Acetyl acetone	1.0	Immediate
2. Alloxantin	0.4	Immediate
3. Ammonium Sulphide	0.1	20-25
4. Dimethylglyoxime	0.2	15
5. 2:4 Dinitroso resorcinol	0.1	Very slow
6. 7-Iodo 8-Hydroxy Quinoline 5-Sulphonic Acid	0.1	Immediate
7. Potassium Ferricyanide	3.5	Immediate
8. Potassium Ferrocyanide	0.2	5-10
9. Pyramidon	0.6	Immediate
10. Pyrocatechin	0.6	Immediate
11. Salicylic Acid	0.6	Immediate
12. Sulphosalicylic Acid	0.02	Immediate
13. Ammonium Thiocyanate	0.02	Immediate
14. Thioglycollic Acid	0.06	Immediate
15. α α' Dipyridyl	0.04	10

DISCUSSION:- It is evident from the tabulated results that the methods most suitable for determining iron in the presence of sucrose are those employing (1) "Ferron", (2) Pyramidon, (3) Sulphosalicylic Acid, (4) Thioglycollic Acid, (5) $\alpha\alpha'$ Dipyridyl, (6) Potassium Ferrocyanide, and (7) Ammonium Sulphide.

Of these methods, the Sulphosalicylic procedure appears to be the most satisfactory, since it can be used also in the presence of moderately coloured sugars. It has also the advantage of low cost: some of the reagents are expensive, e.g. $\alpha\alpha'$ Dipyridyl costs 24/- per gram at present, and this hinders its general use.

RECOMMENDATION:- The Sulphosalicylic Acid method for determining iron appears to offer advantages over any other method tried, and it is recommended as suitable for use with sugar products either with or without incineration.

The reactions of organic compounds with ferric chloride.

A. Reddish coloration or precipitate:- given by almost all simple carboxylic acids.

B. Intense yellow coloration:- given by non-phenolic alpha-hydroxy acids.

C. Green blue or violet colorations:- given by most phenols and phenolic compounds (some in alcoholic solution only); Keto-enolic esters, and similar compounds.

The permanence of the colours varies greatly; some persist indefinitely, some disappear only after several minutes, while others change in tint almost immediately. In some cases, phenols give colorations in alcoholic solution, but none in water solution.

Pyrocatechin, acetyl-acetone, salicylic and sulphosalicylic acids are examples of the above classes of compounds which have been adapted to the colorimetric estimation of iron.

Tests were made with a few compounds in an endeavour to adapt them for quantitative use.

With thymol the colour produced was transient and not very deep even with large amounts of iron: with buffer solutions and with ammonia, sodium carbonate, and sodium

hydroxide respectively, no improvement resulted. The solubility of thymol is only 0.3 part per 100 of water at 15°C, and this rendered it unsuitable, since with alcoholic solution precipitation occurred on addition to the iron solution.

With vanillin (solubility 1 part per 100 of water at 15°C) and salol (insoluble in water), no better results were got. The salol dissolved in alcohol and ether, gave a faint violet colour with 10 gamma of iron, and with methyl salicylate in the same solvent a faint pink colour resulted with the same quantity of iron. Evidently the insolubility in water is a drawback to the use of these compounds for the colorimetric detection of iron.

In general, ferrous salts react with a group containing enolisable hydrogen such as .CO.Ċ:N.OH in an open chain, whereas the combination of an alcoholic group with an oxime, which is specific for cuprous copper does not react with ferrous iron. (see Dubsky & Langer, Br. Chem. Abstr., AI, 638, Dec. 1938).

The rate of fading of the colours produced is generally dependent on the nature of the groups present in the compound formed, and how prone such groups may be to oxidise, e.g. with the $\alpha\alpha'$ Dipyridyl compound there is literally nothing present to oxidise.

Other Tests for Iron.

Some methods of detecting iron are here listed: these were not investigated in this work.

1. Determination of iron by a colorimetric method using O-phenanthroline. Saywell and Cunningham, J.I.E.C., Anal. Ed. Vol. 9. No. 2 (1937).
2. Formaldoxime gives a violet red colour with iron in several minutes (Deniges C.A., 927, (1933)
3. Determination of Iron by Iso-nitroso-acetophenone (Krohnke, Gas u. Wasserfach 70, 510, (1927); Brit. Chem. Abs., 2, B, 542 (1927).
4. Determination of Iron by the colour of ferric chloride itself. J. Hostetter (J. Am. C.S., 41, 1531, (1919).
5. Chromotropic acid (1, 8 Dihydroxy naphthalene, 3, 6 disulphonic acid) is stated by Gutzeit to give a coloration with iron salts.
6. Di-isonitroso acetone gives a blue colour with ferrous salts (Dubsky and Kuvas, Chem. Listy. 23, 496, (1929); Br. Chem. Abs., 4, A, 1414, (1929).

PROFESSOR J. R. CURRIE, M.A., M.D., D.P.H
Clerk of Senate

The University, Glasgow.

30th May, 1940.

Dear Sir,

The Secretary of the University Court has passed to me you letter intimating your willingness to examine the thesis of **Thomas J. Mitchell, A.R.T.C.,A.I.C.,** for the degree of Ph.D.

I send herewith copy of the Regulations for the degree, or which I have marked portions for your particular attention. I may add that the Ph.D. degree is designed to occupy a lower position than the D.Sc. degree, and to be granted on research c under supervision at a comparatively early stage in a candidate career. At the same time a candidate must furnish a clear acc of a genuine piece of research which has required for its execu tion both training and originality of treatment.

I am also sending the thesis, which please return, along wi your report, at your earliest convenience, and, if possible, not later than **12th June, (failing that date 25th June,) 1940.**

Kindly acknowledge receipt.

The Faculty of Science requests that, as Additional Examin you should, in your report, make a definite recommendation for its guidance:

(a) that the candidate be approved for the degree without fur conditions; or

(b) that the candidate be approved for the degree subject to amendment of the thesis or to further examination on th subject-matter thereof; or

(c) that the candidate be not approved for the degree.

Yours faithfully,

J.R. CURRIE,

Clerk of Senate.

Encls.

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History & Ph. survey
1850 ... 1925

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