DETERMINATION OF KETOSES BY OXIDATION WITH CERIC PERCHLORATE AND ABSORPTION OF CARBON DIOXIDE

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AN ABSTRACT

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ABSTRACT

A method for determining ketoses has been developed which depends on the oxidation of these substances by ceric perchlorate followed by absorption of the evolved carbon dioxide on Ascarite.

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INTRODUCTION

The various ceric salts and their oxidizing properties have been known for a long time. Since 1860 occasional suggestions have been made for their use in analytical procedures.

In 1861 Lange (1) recognized the oxidizing properties of ceric sulfate and suggested its use as a volumetric reagent. Sonnenshein (2) in 1870 recommended it instead permanganate for the titration of iron. In 1899 A. Job (3) mentioned the stability and strong oxidizing properties of acid solutions of ceric salts. He noticed that there was possible a substitution of ceric salts for permanganate in cases where the latter is not applicable, as in the analysis of oxalchlorides.

The use of ceric sulfate as a quantitative oxidizing agent was first suggested by Barbieri (4) in 1905. Barbieri described two procedures for determination of nitrous acid. One was a direct titration of nitrite with ceric sulfate in which the end point was determined by a disappearance of the yellow color of ceric ion. In the second procedure excess ceric sulfate was used and the unreduced ceric ion was determined iodometrically. Nitrite solution could contain nitrates and ceric sulfate other rare earths without causing any difficulty in these determinations. Barbieri also noted that hydrazine and hydroxylamine reduce ceric compounds in cold solution.

Sommer and Pincas (5) found that ceric sulfate as well as other ceric salts could be used for quantitative oxidation of hydrazoic acid In this work Sommer and Pincas treated hydrazoic acid in neutral or acidic solution with an excess of ceric salt and then measured the evolved nitrogen gas. The amount of nitrogen evolved corresponds to the reaction:

$$2\ Ce^{+4} + 2HN_3 = 3\ N_2 + 2\ Ce^{+3} + 2\ H^+$$

More recently martin (6) used ceric sulfate for oxidation of hydrazine. The reagent was added in excess and then back-titrated iodometrically. Martin reported that the precision obtained in this determination lies within 0.1 per cent.

Benrath and Ruland (7) tried the ceric oxidation of various organic compounds, such as hydrazine, tartaric and oxalic acids, anthracene, hydroxylamine as well as thiosulfate, sulfurous acid and hypophosphorous acid. Someya (7) titrated ceric salt potentiometrically with standard ferrous sulfate, while VanName and Fenwick (7) developed graphs to describe the potentiometric titration of ceric sulfate with titanous sulfate.

Application of ceric oxidimetry to determination of organic substances has been studied by a number of investigators. Willard and Young (8) applied oxidation by ceric ion to determination of organic acids, such as maleic and tartaric acids. Similarly, Furman and Wallace (9) determined hydroquinone by ceric oxidation, Chapin (10) p-aminophenol, and White (11) a variety of organic compounds.

Smith and his co-workers (12, 13) made a research into the theory of oxidizing action and developed better quantitative methods for oxidation of a number of organic compounds including alcohols and carbohydrates. Sharma (14) rechecked previous studies of carbohydrates and organic acids and developed better techniques for quantitative oxidations.

The rather late development of ceric oxidimetry can be attributed to early difficulties in determining the end-points of these reactions, and until the potentiometric method for an end point was developed, any extensive work in this field was impossible. Thus, most of the early titrations with ceric sulfate employed potentiometric determinations of end points. However, in 1931 Walden, Hammet and Chapman (7) found that ferrous-o-phenanthroline complex ion, now known as ferroin, could serve as a reversible oxidation-reduction indicator of high potential. In its reduced form the ferrous o-phenanthroline complex possesses a deep red color, while the oxidized form is pale blue. The ferric complex was found to be resistant to acids and to action by permanganate, ceric or dichromate ions in acid solution. In 1934 Hammet, Walden and Emonds (7) in their study of high potential indicators prepared derivatives of phenanthroline and diphenylamine. They found that nitrophenanthroline ferrous ion, called nitroferroin, has a potential of 1.25 volts and is more stable to acids than ferroin, which possesses a potential of 1.14 volts. The discovery of these indicators made possible simple visual determination of end points in ceric oxidimetry. Some of the advantages of ceric sulfate and other ceric salts can be summarised as follows:

1. Ceric salts are easily prepared.
2. Solutions of ceric salts can be easily and accurately standardized against readily available primary standards under a wide variety of experimental conditions.
3. Solutions of ceric salts are stable even on boiling (15). (Ceric perchlorate solution was found to be rather sensitive to light).

4. Solutions of ceric salts can be used in excess for oxidation in hot or cold solutions, then back-titrated in a sulfuric, perchloric or nitric acid medium with a standard reducing agent.
5. Ceric ion has a rapid and positive oxidizing action.
6. Oxidations by ceric ion can be adapted to a number of different indicators. They can be followed also by potentiometric techniques.

The use of nitrato and perchlorato cerate anions as a rapid qualitative test for alcoholic hydroxyl group was developed by Duke and Smith (16). This test was based on the intense red color given by hexanitrato potassium or ammonium cerate upon solution in alcohols. The color suggested the possibility of the reaction of an alcohol with the complex nitrato cerate ion. The same type of results were obtained with hexaperchlorato cerate ions, except that the color produced in some cases was less permanent than with the nitrato cerate test solutions. Alcohols having up to ten carbon atoms, hydroxycarboxylic acids, and carbohydrates (such as glucose, sucrose and dextrins) gave positive tests. In the case of carbohydrates the color produced was rapidly lost as the result of oxidation.

Willard and Young (17), in their determination of hydroxy carboxylic acids with permanganate found that there is a slow and variable oxidation of formic acid, which was an intermediate product. Then they confirmed Benrath and Ruland's observation that formic acid was not oxidized with ceric sulfate even on boiling. Therefore, theoretically, in oxidation of tartaric acid by ceric sulfate six equivalents of oxygen would be required per molecule of tartaric acid if it is oxidized to formic acid only. If oxidation proceeds completely to carbon dioxide and water 10 equivalents of oxygen would be required. But Willard and Young found that, under conditions employed by them, the tartaric acid required 7.2 equivalents of oxygen per molecule. Similar observations were obtained with glycolic, malic and malonic acids. No explanation was offered for the results.

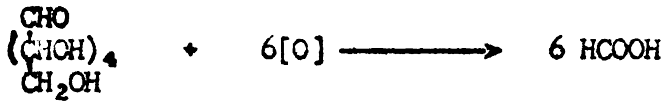
Sharma (18) repeated Willard and Young's work and obtained similar results. Sharma attempted to explain his results in the following manner: "If the organic acid decomposed via formic acid only, then the above observations suggest that although formic acid is not oxidized appreciably when present alone, yet its oxidation is induced by the energy evolved in the oxidation of other organic molecules.... Another plausible explanation might be that a portion of the organic acids is not oxidized via formic acid, but by some other mechanism in which its oxidation proceeds completely to carbon dioxide and water."

In reinvestigating the oxidation of formic acid with ceric sulfate, Sharma found that a slight amount of reagent was always consumed by formic acid. Thus it seemed that ceric sulfate can oxidize the formic acid but at a much slower rate. An increase in acid concentration seemed to increase the oxidation of formic acid. However, oxidation seemed to be complete in 50 minutes in the presence of 66 per cent (by volume) of sulfuric acid in the reaction mixture.

Thus Sharma showed that formic acid can be oxidized quantitatively by ceric sulfate in the presence of concentrated sulfuric acid. Sharma also showed that, by increasing the concentration of sulfuric acid, maleic, fumaric, benzoic, phthalic and salicylic acids can be oxidized with ceric sulfate completely to carbon dioxide and water, while succinic and acetic acids are not oxidized at all.

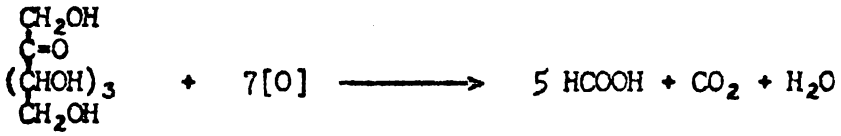
Sharma and Mehrotra (18) confirmed Smith and Duke's (19) observation that glycerol is oxidized to formic acid not only with ceric perchlorate but also with cerate sulfate. Glycerol is not oxidized to tartronic acid, as was believed by Cathill and Atkins (20). But the cerate formic acid oxidation still disturbed Sharma and his co-workers and again they repeated the experiments with pure ceric sulfate (21). Then they found that the oxidation was negligible. The impurity catalyzing the reaction in the other experiments was found to be chromium, while iron, manganese, silver, osmium and other rare-earth elements showed no appreciable catalytic influence.

The action of ceric perchlorate or ceric sulfate on reducing sugars was also studied. It was found that sugars containing aldehyde group are oxidized to formic acid only:



$$\begin{array}{l}\mathrm{CHO}\\(\mathrm{CHOH})_4\\\mathrm{CH_2OH}\end{array} + 6[O] \longrightarrow 6\ \mathrm{HCOOH}$$

The ketonic sugars also have formic acid as an end product, but in addition, the ketonic group is oxidized to carbon dioxide.



$$\begin{array}{l}\mathrm{CH_2OH}\\\mathrm{C{=}O}\\(\mathrm{CHOH})_3\\\mathrm{CH_2OH}\end{array} + 7[O] \longrightarrow 5\ \mathrm{HCOOH} + \mathrm{CO_2} + \mathrm{H_2O}$$

Smith and Duke (22) on treating glucose with ceric perchlorate found the oxidation to formic acid was complete in 45 minutes and A. A. Forist and J. C. Speck, Jr. (23) by two other procedures found all sugars tested, with the exception of DL-glyceraldehyde, to be completely oxidized within one hour. Sharma (14) studied the action of ceric sulfate on reducing sugars and obtained the following results.

Observations with Glucose

Volume of glucose solution (ml.)	0.145N $Ce(SO_4)_2$ added (ml.)	Refluxing time (min.)	$Ce(SO_4)_2$ consumed (ml.)	Equivalents of oxygen consumed	Glucose found (g.)	Glucose present (g.)
2	5	5	2.42	10.52	.00527	.00600
2	5	10	2.68	11.65	.00583	.00600
2	5	15	2.74	11.97	.00597	.00600
2	5	30	2.73	11.91	.00595	.00600
2	5	60	2.74	11.97	.00597	.00600
2	5	120	2.74	11.97	.00597	.00600
3	5	10	3.81	11.05	.00829	.00900
3	5	15	4.03	11.73	.00877	.00900
3	5	20	4.11	11.98	.00895	.00900

The above data show that glucose is oxidized to formic acid within 15 minutes if about 100 per cent excess of ceric sulfate is present. The time required for complete reaction increases if the excess of ceric sulfate is less.

Observations with Fructose

Volume of fructose solution (ml.)	0.145 N $Ce(SO_4)_2$ added (ml.)	Refluxing time (min.)	$Ce(SO_4)_2$ consumed (ml.)	Equivalents of oxygen consumed	Fructose found (g.)	Fructose present (g.)
2	5	10	3.065	12.82	.00572	.00626
2	5	15	3.355	14.04	.00626	.00626
2	5	30	3.34	13.97	.00623	.00626
2	5	60	3.34	13.97	.00623	.00626
2	5	120	3.355	14.04	.00626	.00626
1	5	10	1.666	13.94	.00311	.00313
1	5	15	1.676	14.02	.00313	.00313

The results in the above table show that 14 equivalents of oxygen are consumed per mole of fructose oxidized. Carbon dioxide was detected as one of the products of the reaction.

This ability to produce carbon dioxide from the ketonic group on the oxidation with the cerate reagent has found an application in tracer studies also. M. Calvin and his co-workers (24) in their study of path of carbon in photosynthesis used this method of oxidation of carbonyl carbon of a ketose to carbon dioxide which was precipitated out and counted as barium carbonate.

In our study an attempt was made to develop a method for a quantitative determination of detoses present by measuring the amount of carbon dioxide absorbed on Ascarite.

EXPERIMENTAL

Apparatus.

The apparatus for carbon dioxide absorption on semi-micro scale was set up as shown in Figure 1. The vessels were connected with impregnated rubber tubing which was made in the following way: Rubber tubing of medium thickness was cut into pieces of 3-4 inches in length and placed in a suction flask containing melted wax. Then the wax was heated in a boiling water bath and the rubber placed in it for 30 minutes, continuing the heating under reduced pressure until no more air bubbles could be seen coming out of the rubber tubing. This step was repeated several times.

Materials.

The following reagents were used in these experiments:

0.5 M Ceric perchlorate solution in 6 N perchloric acid (G. F. Smith Chemical Co. reagent)

Ethylene glycol

0.18 N and 0.2 N sodium oxalate in 0.1 M perchloric acid

Nitro-ferroin indicator, 0.025M

0.28 N ceric perchlorate in 4 M perchloric acid

0.01 to 0.03 N perchlorate cerate in 2 M perchloric acid

Formic acid

D-Fructose

D-Sorbose

Dihydroxyacetone

L-Arabinose

Ascarite

Magnesium perchlorate (anhydrous)

Concentrated sulfuric acid

Nitrogen gas

Original Oxidation Procedure.

The apparatus was set up as shown in Figure 1 and the following oxidation procedure was carried out: About 0.5 millimole of fructose (or other sugar) was weighed accurately on an analytical balance and placed in the reaction vessel. It was dissolved in approximately 1 ml. of water. Then the Ascarite-filled absorption tube was wiped with a damp cloth, dried with a clean, dry cheese cloth, allowed to equilibrate next to the analytical balance for 5 minutes and weighed. Afterwards it was connected back into the system.

The stopcocks were opened to allow the nitrogen to pass through the system and the flow of nitrogen was adjusted to 5 ml. per minute as measured by the displacement of water. Then about 20 ml. of approximately 0.5M ceric perchlorate reagent was slowly introduced through the side arm of the vessel by the use of a syringe. The reaction was allowed to proceed for 3 hours. Then the gas flow was stopped, the Ascarite tube containing the absorbed carbon dioxide was cleaned in the same manner as described above and weighed. The Ascarite tube was then reconnected and the system swept with nitrogen for an additional 30 minutes. The weighing procedure was repeated. The additional sweepings and weighings were made for several times, but the constant weight was never obtained.

The same procedure was repeated with L-arabinose and formic acid.

Determination of the Rate of Formic Acid Oxidation.

The procedure of Forist and Speck (23) was followed with the following exceptions.

Method I

To 88 mg. of formic acid placed in a dark flask and kept at 30°, 50 ml. of approximately 0.28N ceric perchlorate reagent was added. Ten-ml. aliquots were removed at intervals during the oxidation and added to 15 ml. of 0.18M sodium oxalate. The excess of oxalate was titrated with 0.03N ceric perchlorate reagent to a nitro-ferroin end point.

- The same procedure was repeated for the oxidation at 20°C.

Method II

To 88 mg. of formic acid in a dark flask at 20°, 25 ml. of approximately 0.5M ceric perchlorate was added. One-ml. aliquots were removed from time to time and added to 3 ml. of 0.2N sodium oxalate. The excess of oxalate was titrated with 0.03N ceric perchlorate to a nitro-ferroin end point.

Revised Oxidation Procedure.

Since the oxidation of fructose was found to be complete in 15 minutes when it was performed in the same way (Method I) as the formic acid oxidation at 20° (see Table III), the following oxidation procedure was developed:

To a reaction vessel kept at 20°C. a weighed amount of D-fructose was added and dissolved in 1 ml. of water. The Ascarite tube was cleaned and weighed as described previously. The system was connected together and the flow of nitrogen adjusted to 4-5 ml. per minute as measured by the displacement of water. Then about 18 ml. of 0.5M ceric perchlorate reagent was slowly introduced into the reaction vessel and the reaction allowed to proceed for 15 minutes. At the end of this time 7-8 ml. of ethylene glycol was added to stop the reaction and the nitrogen flow was adjusted to 7-8 ml. per minute. The sweeping was continued for 2.25 hours and the Ascarite tube was cleaned and weighed. Additional sweepingsof 15 minutes were made and the tube was reweighed. If the increase of weight was not greater than 0.5 mg. the sweeping was considered complete.

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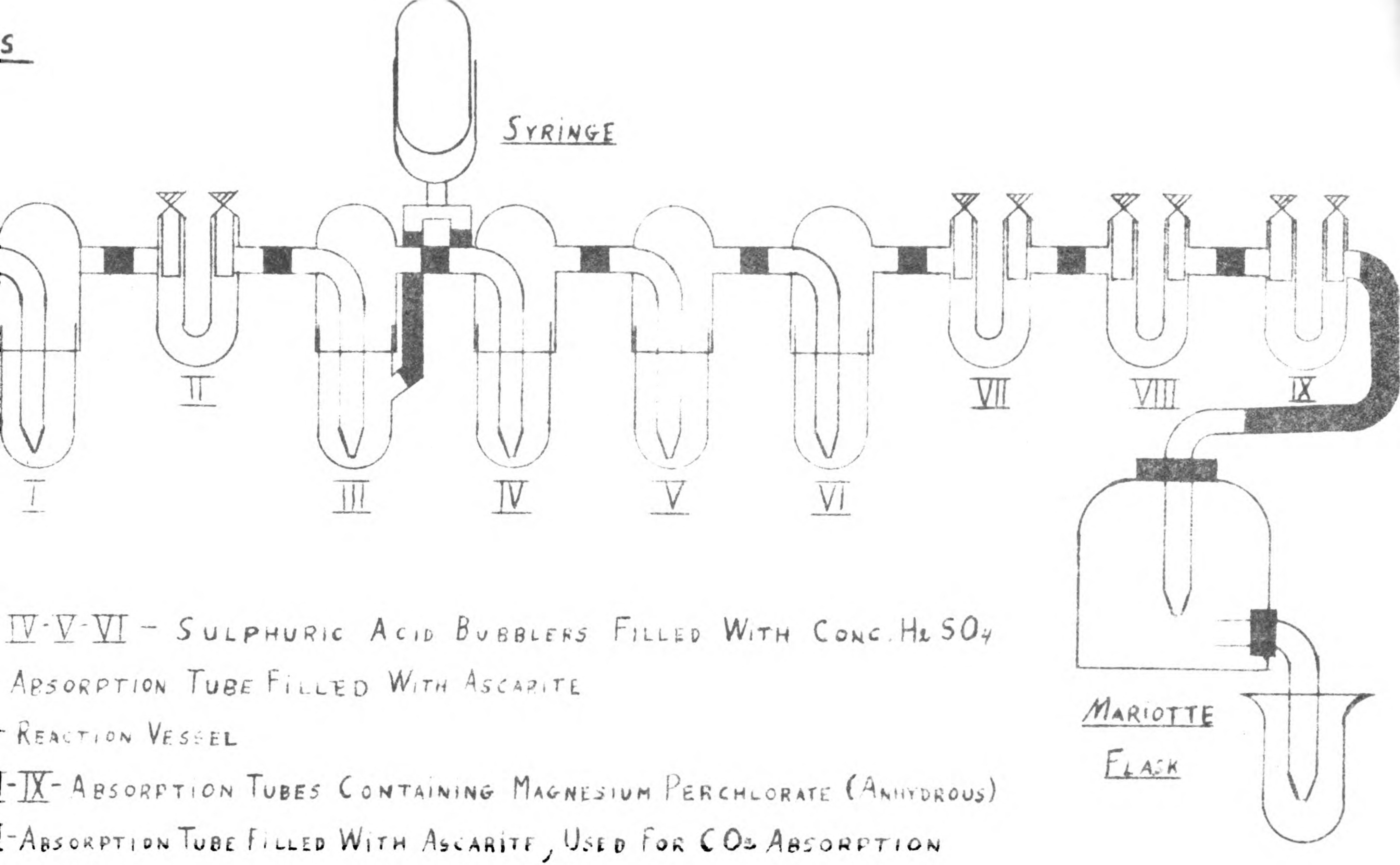

IV-V-VI - SULPHURIC ACID BUBBLERS FILLED WITH CONC. H_2SO_4

ABSORPTION TUBE FILLED WITH ASCARITE

REACTION VESSEL

I-IX- ABSORPTION TUBES CONTAINING MAGNESIUM PERCHLORATE (ANHYDROUS)

I-ABSORPTION TUBE FILLED WITH ASCARITE, USED FOR CO_2 ABSORPTION

FIGURE-I

RESULTS AND DISCUSSION

The results in the original oxidation procedure were always high, as can be seen in Table I. Attempts to get better results were made by shortening sweeping periods, but still about 110-118 per cent of the theoretical quantity of carbon dioxide was obtained. Even with arabinose there was an increase in weight in the Ascarite-filled tube. The possibility of the increase due to moisture was discounted due to the presence of three tubes filled with concentrated sulfuric acid and a U-tube filled with anhydrous magnesium perchlorate preceding the Ascarite-filled tube. Another possibility was the oxidation of the formic acid which is formed during the oxidation of the sugars. Therefore, the oxidation of formic acid was tested under these conditions; the results can be seen in Tables II and III.

TABLE I

Original Oxidation Procedure

Sugar	Amount of Sugar Oxidized mg.	Reaction Time minutes	Amount of CO_2 Absorbed Found, mg.	Calculated, mg.
D-Fructose	89.8	3	26.7	21.9
	90.0	3	23.5	22.0
	94.8	3	24.6	23.2
	90.2	2 1/2	26.9	22.1
	90.1	2 1/2	25.1	22.0
L-Arabinose	67.4	2 1/2	7.5	0.0
	91.1	2 1/2	7.9	0.0
Formic acid	88.0	3	1.6	

0.5M ceric perchlorate used for oxidation.

TABLE II

Oxidation of Formic Acid by Method I

Time minutes	Mg. of Formic Acid Oxidized at 30°	20°
0	6.20; 5.85; 7.70	0.00
15	10.35; 10.90; 12.65	1.95
30	12.65; 14.95; 17.25	4.30
60	17.25; 20.70; 19.55	6.10

0.28M ceric perchlorate was used for oxidation
88 mg. of formic acid was used for oxidation.

TABLE III

Oxidation of Formic Acid by Method II

Time minutes	Mg. of Formic Acid Oxidized at 20°
0	0.00
5	0.75; 0.50
10	1.50; 1.50
15	2.00; 2.25
20	3.00; 3.50
25	3.50; 3.75
30	4.50; 4.50
40	5.00; 5.50
50	6.00; 5.50
60	6.25; 6.00

0.5M ceric perchlorate used for oxidation.
88 mg. of formic acid was oxidized.

As can be seen from the results a considerable amount of formic acid is oxidized during a 1-hour period at room temperature, while only a limited oxidation occurs at 20°C.

Since complete oxidation of sugar can be obtained in 15 minutes with a weaker solution of ceric perchlorate (Table IV), the addition of ethylene glycol was introduced in the revision of the original oxidation procedure. The purpose of ethylene glycol was to react with the excess of ceric perchlorate present. It was noticed that during the addition of the ceric perchlorate reagent to the sugar, or on addition of ethylene glycol to the ceric perchlorate, the solution would turn dark red, and the color faded quickly with sugars while it lasted longer with ethylene glycol. This type of observation was made by Duke and Smith (15) also.

Results of the revised oxidation procedure are given in Table V.

The low results of sorbose and dihydroxy acetone were due to poor samples. Probably the materials were hydrated. Similar low results were obtained with these substances by Forist and Speck's (23) method also.

TABLE IV

Oxidation of Fructose at 20° by Method I

Time minutes	Mg. of Fructose Oxidized
1	75.27; 62.25; 67.50
15	96.05; 95.25; 98.25
30	98.58; 97.50; 99.75
60	101.10; 101.25; 102.75

90 mg. of Fructose was oxidized.
.28M ceric perchlorate used for oxidation.

TABLE V

Revised Oxidation Procedure for Ketoses

Sugar	Amount Oxidized Mg.	Theoretical Yield calculated mg.	Actual Yield mg.
Fructose	90.6	22.2	22.4
	90.3	22.1	24.6
	91.6	22.4	22.4
	91.6	22.4	22.7
	90.8	22.2	21.3
	90.0	22.0	21.8
	90.8	22.2	19.3
	93.2	22.8	19.8
Sorbose	92.2	22.5	17.7
	92.0	22.5	17.8
Dihydroxy acetone	92.0	45.0	37.6
	90.3	44.1	37.0

Constant temperature bath at 20° was employed.
0.5M ceric perchlorate used in oxidation.
Ethylene glycol was added after 15 minutes of reaction.
Total sweeping time was 2 1/2 hours.

SUMMARY

The history of the development of ceric oxidimetry was traced.

The determination of ketoses by the absorption of carbon dioxide was attempted and a method for ceric perchlorate oxidation was developed. Fructose, sorbose and dihydroxy acdtone were oxidized by this method.

The rate of formic acid oxidation was checked and it was found that there is considerable amount of oxidation at room temperature at high ceric perchlorate concentrations, while only slow oxidation occurred at 20°C.

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