
THE WET CHEMICAL OXIDATION OF ORGANIC COMPOSITIONS

Employing
PERCHLORIC ACID

WITH — or — WITHOUT
added

HNO_3 — H_5IO_6 — H_2SO_4

By

G. Frederick Smith, Ph.D.
Professor of Chemistry Emeritus
University of Illinois, Urbana



"The Liquid Fire Reactions."

Methods for the Subsequent Micro-, or Macro, Determination
of Associated Metallic and Non-Metallic Elements

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THE G. FREDERICK SMITH CHEMICAL CO. INC.
867 McKinley Ave., Columbus, Ohio 43223, P.O. Box 23344 U.S.A.

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PREFACE

The first major study employing perchloric acid in the wet oxidation procedures for the destructive oxidation of organic structures of a general applicability was that of Doctor M. Ernest Kahane in France. Most of Kahane's procedures involved biological motivations. The objective was the elimination of proteins, mostly in large-sized portions of animal organisms, preparatory to the determination of minor elements present with emphasis on toxicological investigations. Other important destructive oxidations were also studied and a rather extensive series of procedures, widely varying in applicability, were originated and are a valuable addition to analytical methodology.

The first important procedure employing an oxygen donor acid in the wet oxidation of organic compositions was the familiar Kjeldahl amino nitrogen reaction employing hot concentrated sulfuric acid. Hundreds of published studies of this procedure which introduced modifications to serve as attempted improvements are a part of the past published work in this reaction procedure.

During the past 25 years, the preparatory elimination of organic compositions, with subsequent determination of trace elements as well as major metallic natural or added components present, has been predominantly those methods which involve perchloric acid as oxygen donor. Nitric acid, sulfuric acid and periodic acid have been employed as secondary oxygen donors to accompany perchloric acid as reaction modulating additives.

Wet chemical oxidation of organic compositions have rightfully displaced dry ignition in air at high temperatures to a predominant extent, (the dry ashing processes), for reasons that have been scientifically justified due to inherent errors not duplicated employing the procedures herein described.

Many millions of routine analytical control analyses are being relentlessly employed annually, involving sample preparation following wet oxidation procedures to replace dry ashing techniques. The fields of agricultural feed studies, leather tanning determinations of chromium and other associated elements, food trace element contaminants including wines, beer, and spiritus liquors, dairy products in control investigations involving shelf life deterioration, as well as wet oxidations in the field of paper and wood pulp manufacture, etc., are representative. Such procedures are a part of the extensive

investigations applied continuously and results reported in current chemical publications.

While the present review as compiled is by no means exhaustively treated herein, the coverage is materially representative of the present status of the most important developments. It will be noticed that the emphasis is placed upon work published from the Chemical Laboratories of the University of Illinois. This is the result of placing emphasis justifiably, it would seem, even chauvanistically. This we are reluctant to disavow.

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1965

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SECTION I

PERCHLORIC, NITRIC AND PERIODIC ACIDS AS OXYGEN DONORS

All the principle ingredients of naturally occurring or synthetic organic samples for oxidation, alcohols, cellulose, sugars, and proteins, are often mineralized, (but only partially), using nitric acid alone as oxygen donor. Reaction is often initiated at ordinary temperatures. The oxidation accelerates as the temperature is increased to the boiling point of 66 to 67 percent acid concentration, (app. 120.5°C) the $\text{HNO}_3\text{--H}_2\text{O}$ azeotrope. Reaction intensity is indicated by the evolution of brown fumes of oxides of nitrogen. Since complete oxidation by nitric acid is generally not attained, an added oxygen donor acid is simultaneously employed. For this reason mixed nitric and perchloric acids are chosen as reactants. With partial but incomplete oxidation provided by nitric acid, the excess is distilled out, and at 140° to 200° the perchloric acid at steady step-wise increase in potential, completes the reaction.

Such $\text{HNO}_3 + \text{HClO}_4$ oxidations of organic compositions are known as "*liquid fire reactions*."

Periodic acid when added to oxidations, thus employing H_5IO_6 and HClO_4 , replaces the effectiveness of nitric acid at much lower temperatures. By employing these two oxygen donor acids we designate the procedure as "*the periodic acid liquid fire reaction*."

In the periodic acid liquid fire reaction the periodic acid serves two important functions. At moderate temperature, generally less than 100°, the periodic acid is reduced to iodic acid. The alcohols, sugars, cellulose and proteins being oxidized are first fragmented following the famous Malaprade reaction to form new entities of lesser molecular weight. There then follows oxidation with further reduction of periodic to iodic acid. The reaction is often quite exothermic. The periodic acid is added in minor amounts. As the reaction temperature is gradually elevated from 140° to 200° the perchloric acid completes the oxidation. If the iodic acid, (being a poor oxygen donor), retained by the wet ash resulting is objectionable, it may be readily removed as iodine by the addition of sulfurous acid and digestion. In the initial reaction of the periodic acid liquid fire reaction, also, iodine is evolved. By the procedures employing these oxygen donors, organic compositions of a wide variety may be efficiently and rapidly wet ashed.

Organic compositions such as those principally protein, collagen, wool, foods and feeds, leather, coal, and synthetic fibers, including fat-free dairy products, etc., are successfully oxidized employing either the plain or periodic acid liquid fire reaction.

Following the principles involved in the use of the liquid fire reactions, some analysts, possibly apprehensive of uncontrolled reaction rates, specify stepwise acid digestion. First digestion using nitric acid individually is employed. When its effect is as complete as possible, perchloric acid is then added and the temperature taken into the 140°-200° range to complete the oxidation. This two-stage technique is quite unnecessary as will become obvious as indicated by the following disclosures.

In the case of using either the nitric or periodic acid liquid fire reagents as oxygen donors their effective oxidation is complete when the temperature is elevated to 140°. Excess iodine from the periodic acid then is completely removed with conversion of iodine to iodic acid, and nitric acid to nitrogen oxide. Above 140°C and at increasing temperatures, the perchloric acid strength at 50 percent concentration and at 150°C comes into reactions of oxidation. At this point all easily oxidized organic matter has been eliminated. The more oxidation resistant organic matter is to be destroyed by perchloric acid digestion. Over the temperature range 140° to 200° and at perchloric acid concentration increasing from 50 percent to 72.5 percent the available oxidation potential increases from approximately 0.85 volts stepwise to approximately 2.0 volts. Here further concentration of perchloric acid ends due to the formation of the perchloric acid-water azeotropic composition. All the most oxidation resistant organic matter has thus been slowly, relentlessly and quietly removed. The wet ash acid residue is thus made ready for determination of the elements retained therein.

Periodic acid, besides being a high potential oxygen donor, serves, (as cited above), another valuable purpose. Following the famous Malaprade reaction organic structures such as sugars, starches, and proteins, have been broken down into degradation products which are more readily oxidizable. This Malaprade reaction takes place at ordinary or slightly elevated temperatures. By the combined action of perchloric plus periodic acid organic compositions in general are oxidized at temperatures less than 100°. These oxidations are in most cases markedly exothermic.

Among the many valuable properties of perchloric acid as applied to analytical reactions of a wide variety of applications, there are two which prove of great value in the wet oxidation of organic compositions. First of these provides for the dissolution of a wide variety of organic materials. Cellulose, starch, proteins and sugars are readily soluble in perchloric acid. Unlike the sulfuric acid oxidation of such entities at higher temperatures there is no carbonization. The solvent promoting properties of perchloric acid provides mostly clear solutions throughout their oxidation. Such condition is continuous over the temperature range up to the boiling point of the perchloric acid-water azeotropic composition at approximately 200°C. The value

of this dissolution of organic compositions, provided by perchloric acid as oxygen donor, cannot be over-estimated. It is of outstanding significance.

The second and most valuable property of perchloric acid as oxygen donor is not duplicated by any of the other oxygen donor acids, chloric, nitric, periodic, chlorous acid (HClO_2), or hypochlorous acid (HClO) or their salts in acid media. This perchloric acid property of relinquishing its high oxygen content at increasing oxidation potential only in small increasing increments with increase in concentration and boiling point is not duplicated by any of these other cited oxygen donors. The availability of its oxygen is a fixed function of acid concentration and the temperature at which each acid concentration is controlled. By regulating the acid concentration at its boiling point, (under reflux to keep acid concentration constant), any oxidation potential magnitude between 0.85 to 2.0 volts can be realized and statically controlled. This property is alien to all the oxygen donor acids previously cited.

Perchloric acid up to 50% concentration is not an oxygen donor at temperatures up to 100° or even higher. Iron under these conditions dissolves with the evolution of hydrogen and the formation of ferrous perchlorate. Organic compositions such as cellulose dissolve in the acid but are immune to attack in oxidation of carbon to dioxide. Metallic zinc under these conditions dissolves with the evolution of hydrogen. Ferric perchlorate in 50% HClO_4 solution may be quantitatively reduced to ferrous perchlorate employing the Jones zinc reductor.

It is thus evident that boiling 50% perchloric acid, or lesser concentrations, is not oxidizing in reactivity.

Above 50% concentration, (controlled at any specific acid concentration by reflux of vapor condensate), and at the resultant boiling temperature, perchloric acid becomes an oxygen donor. As the acid concentration is increased in small increments the available oxygen increases in intensity as a practically straight line function of increased acid concentration. For example a perchloric acid solution of cellulose begins to be converted to carbon dioxide at 50% acid strength and at 140°. This oxidation is rate controlled and accelerates up to an acid strength of 65% at its boiling point. (And at 70% acid concentration and at 200°C this reaction results in violent intensity.)

As a result of the reactivity outlined in the previous paragraph, one technique in the wet oxidation of organic compositions, (later to be illustrated), has been devised employing boiling, controlled strength perchloric acid as the only oxygen donor.

Many perchloric acid wet oxidation procedures are favorably modulated

in reaction time by the presence of traces of vanadium as reaction catalyst. The basis of such catalyzed oxidations will be subsequently illustrated.

As demonstrated by Pijck, Gillis, and Hoste and reprinted in the second section of this brochure dry ashing by ignition in air at 400° to 900°C results in material loss of the elements to be determined. Wet ashing procedures employing reaction mixtures of oxygen donors less specific than perchloric acid is obviously inefficient. It thus is essential that routine control laboratories familiarize their personnel with the chemical properties of perchloric acid, a subject which is in general avoided in the university chemical training curricula. There are a multiplicity of resultant rewards. Many hundreds of thousands of individual determinations in macro- and micro-magnitude, determinations of the various metallic and non-metallic elements, are annually applied involving previously eliminated organic interfering major constituents. Many industrial control laboratories are involved representing various industries: manufacturers of foods and feeds, the leather tanning industry, pulp and paper, medicinals and pharmaceutical laboratories, the wine industry control laboratories, etc. Proper, precise, and time-saving factors are worthy of any innovations that might be provided. Perchloric acid applications to the problems involved are indeed most rewarding.

In addition to the wet ashing methods of the Liquid Fire Reaction, the Periodic Acid Liquid Fire Reaction, and Perchloric Acid Statically Controlled Graded Potential Reactions, four other methods of importance are hereinafter described and experimentally demonstrated. Three of these employ such oxygen donor reagents as mixed $\text{HClO}_4 + \text{H}_2\text{SO}_4$, two methods of two stage mineralization, preliminarily by H_2SO_4 treatment and subsequently by the addition of HClO_4 , and finally a general procedure employing $\text{HCl} + \text{HNO}_3 + \text{NH}_4\text{ClO}_4$ with HClO_4 generated *in situ* particularly well suited to the wet ashing of larger weight organic composition samples. Mixed $\text{HClO}_4 + \text{H}_2\text{SO}_4$ as oxygen donors involve available oxidation potentials of greater magnitude than that provided by the boiling $\text{HClO}_4 - \text{H}_2\text{O}$ azeotropic composition.

Reactions to be subsequently demonstrated place special emphasis on time-saving innovations. Wet chemical digestions in multiple sampling may be simultaneously implemented in the use of well ventilated fume hoods. Individual determinations may be carried out in the use of laboratory work benches without inconvenience from evolved noxious fumes. Reaction apparatus for use in both cases are to be described. Reactions performed with maximum speed may be just as effectively carried out with less rapid increase in the rate of the increased temperature applied. For analysts in fear of uncontrolled reaction rates, such applications may be preferred. It must

be added that implication that the methods involved are of possible hazard is far more common than can be justified.

As for sample weights, it is seldom necessary to employ more than one or two grams. Means are available for the determination of trace element quantities in the wet ash acid residue which are more than adequate. These include extraction processes using immiscible organic solvents, spectrophotometric evaluation, or micro redox titration and other methods. If the required amounts of mineral acids specified seem to be large, (10-20 ml), this is not an acute complication. Such reagents required are commercially available in more than satisfactory purity. The A.C.S. specifications call for 70% perchloric acid having no more than 0.003% of non-volatile impurities. Perchloric acid, (70-72%), is commercially available with non-volatile impurities of only 15 parts per million, with iron, phosphorus, arsenic and nitrogenous ingredients essentially absent.

The presence of perchloric acid in the wet oxidation reaction residue if undesirable can be eliminated by the addition of sulfuric acid and digestion to volatilize the unwanted acid. It is to be noted that for most elements to be determined perchloric acid involves no interference. Sulfur as sulfate may be isolated as barium sulfate without interference. Phosphorus may be isolated as the familiar yellow polymolybdenum complex in the presence of perchloric acid. Iron may be likewise spectrophotometrically evaluated following one of the ferroin reactions such as those involving bathophenanthroline or tripyridyl triazine. Arsenic may be determined by micro-titration in perchloric acid solution employing perchloratoceric acid as titrant easily with accuracy in the few parts per million concentration. Calcium oxalate may be similarly evaluated after precipitation and filtration from a perchloric acid solution. Chromium if present is retained quantitatively as chromic acid and readily evaluated by numerous available procedures.

It may be accurately stated that the examination of the perchloric acid residue from wet oxidation acid residues justifies a more representative number of macro and trace quantity determinations than could be provided by the use of any other mineral acid.

On behalf of the many applicable properties provided by perchloric acid for the purpose at hand, it might be remarked that if one intends to drive a 3 inch diameter stake 60 inches long into the ground it would be folly to attempt it using a tack hammer in place of 20 pound maul.

Concluding Commentary. Teflon (polytetrafluoro-ethylene), Durco (Duriron), the diamond, and nicotinic acid are entirely immune from attack by boiling 72.5 percent perchloric acid at the temperature 203°C. Recovery

of diamond dust from the salvaged stumps of grinding wheels depends upon the use of the liquid fire reaction to destroy the organic bonding material of the material to be salvaged. A patented process for the manufacture of nicotinic acid involves the oxidation of quinoline in which perchloric acid plus H_2SO_4 serves as oxygen donor. Teflon lined tubing, gasket designs and "O" rings of teflon are invaluable adjuncts as applied by the manufacturer of perchloric acid. Stainless steel beakers and dishes if lined with teflon could be used for reactions with hot concentrated perchloric acid. If you wish to examine wool fabric suspected of adulteration by cotton, test by applying 70 percent perchloric acid to the fabric at ambient temperature. The cotton is immediately dissolved and the wool not affected.

SECTION II

WET CHEMICAL ASHING PROCEDURES VERSUS DRY IGNITION TECHNIQUES

The importance of sample preparation in the determination of trace or macro-quantities of metallic and non-metallic elements in organic compositions, both natural and synthetic, is widely acknowledged. The subject is vital in biochemical investigations. Feeds, foods, beverages and medicinals represent major fields in trace contamination influences necessitating analytical examination. Forensic investigations are involved. Fuels, rubber, leather and synthetic fibers are also involved. In total, many millions of such analyses following mineralization of the sample are annually processed.

The preliminary elimination of organic matter with quantitative retention of metals and non-metals in the residue, introduces problems of many controversial facets. The problems involved have been extensively investigated and documented.

The choice of either wet chemical oxidation at low temperatures 120-220°C or dry ashing procedures at 400° to 900°C in air is involved. Methods are promoted which are hybrid procedures involving both chemical oxygen donors and simultaneous ignition in air.

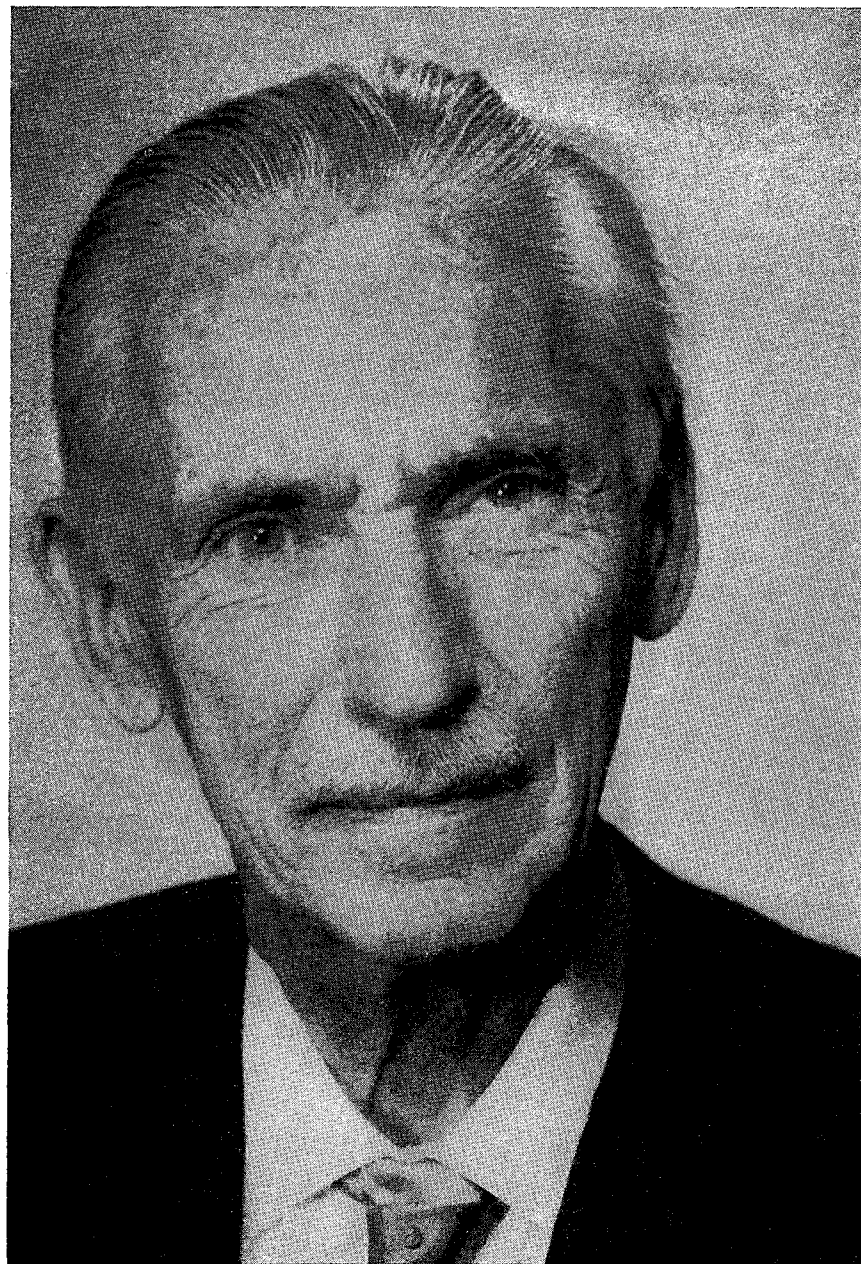
Wet chemical oxidations at moderate temperatures employ HNO_3 , HClO_4 , H_2SO_4 and H_5IO_6 or various combinations of the same which provide requisite available oxygen *in situ* at 120-220°C or above. Atmospheric oxygen is involved in plain dry reaction ignitions at elevated temperatures 450-900°C. The hybrid procedure involves HNO_3 plus dry ashing proceeds at moderately elevated temperatures (300-350°C).

The determination of the metals and non-metals following all of the available methods in the elimination of the primary organic matter of the sample involve the following chemical and physical chemical techniques:

- (a) Gravimetric, volumetric oxidation-reduction applications, precipitation, electro-deposition or precipitation carrier base isolation.
- (b) Spectrophotometric determination following chelation with or without solvent extraction.
- (c) Spectrographic determination of wet and dry mineralizations.
- (d) Radioactivation analyses applied both to wet and dry mineralization.

The major importance of these disciplines is attested by the extensive reference experimentation which has been documented on the subject. In all its aspects controversy is involved in these studies. To cover the subject adequately and exhaustively is beyond the scope of this review. Reference only to a selected few of the important contributions will be cited.

From a fair appraisal of the over-all conclusions it must be said that the



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destruction of organic composition by oxidation by the wet method is rapidly taking precedence over all other procedures. To account for this conclusion, the studies involving radioactivation analyses play an important role. Following these fully tested procedures in all types of mineralization wet chemical oxidations employing perchloric acid as oxygen donor have proven to be the favorite procedure. Such perchloric acid wet ashing methods are less time consuming, provide outstandingly accurate trace element and macro-magnitude contamination evaluations and are adaptable to a wider variety of sample types than any other general procedure.

The study of both dry ashing and wet chemical oxidation of the destruction of organic compositions and the subject of procedural losses of elements to be determined has been documented by J. Pijck, J. Gillis, and J. Hoste (1). This referee testing followed the methods of radioactivation analyses. The metals involved were As, Sb, Cr, Ca, Au, Fe, Cu, Hg, Pb, Mn, Mo, V, Ag and Zn.

Representative organic compositions were adulterated by the addition of the proper radioactive isotopic metals. The samples were then dry ashed at temperatures of 400°C to 900°C and the extent of the recovery followed by the appropriate radioactivation techniques. The same samples were similarly adulterated and subjected to wet oxidation employing a mixture of $\text{HNO}_3 + \text{HClO}_4 + \text{H}_2\text{SO}_4$ 3:1:1 by volume of the 66, 70, and 96% acid strengths, respectively, as oxygen donors. Again recoveries were determined by the same radioactivation techniques.

The results of these investigations using the additive radio isotopes of the metals listed above can be summarized as follows:

"Dry destruction (1) by ignition is to be rejected as a general method as many of the metals investigated are lost to a considerable extent, even at relatively low temperatures of 500-550°C. With the liquid destruction mixture ($\text{HNO}_3\text{-HClO}_4\text{-H}_2\text{SO}_4$) complete recovery can be obtained for all of the elements investigated, excepting Sb, As, Au, Fe and Hg. These can be quantitatively recovered if an efficient reflux condenser is used during mineralization.

A number of possibly interfering (n,p) and (n, α) reactions due to fast neutrons were investigated, namely Zn^{64} (n,p) Cu^{64} ; Fe^{54} (n, α) Cr^{51} ; Ni^{58} (n,p) Co^{58} .

Interference was proved to be less than 1 percent when irradiated in BRL at a flux of $5 \times 10^{11} \text{ n/cm}^2 \text{ sec}$.

Finally trace quantities of the elements Cu, Cr, Zn, and Co were determined in blood serum applying radioactivation studies. The authenticity of the findings was then checked for duplicate findings using the most delicate wet chemical procedures of spectrophotometry. The latter technique was used



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when applicable, employing extraction procedures to augment specificity and selectivity in the 25 to 250 gamma range.

The results of an exhaustive investigation by Pijck, Gillis, and Hoste (1,2) involved the trace element determination of the 15 elements listed in Table 1. The determinations were made applying the procedures of radioactivation analysis, the isotope used being indicated.

TABLE 1. MINERALIZATION BY DRY ASHING
(% of tracer recovery—number of analyses in parentheses)
(Radioactivation Evaluation)

Element with Atomic Number	Temperature-Time of Ignition in Hours at °C % Recovery			
	400° 24 hours	500° 12 hours	700° 6 hours	900° 3 hours
Sb ¹²³	67% (4)	81% (4)	35% (4)	9% (4)
As ⁷⁶	23 (3)	0 (6)	0 (6)	0 (6)
Cr ⁵¹	99 (6)	99 (6)	86 (6)	56 (6)
Co ⁶⁰	98 (6)	75 (5)	67 (6)	30 (6)
Au ¹⁹⁸	19 (4)	0 (5)	0 (5)	0 (4)
Fe ⁵⁹	86 (4)	81 (4)	52 (5)	27 (3)
Cu ⁶⁴	100 (5)	98 (4)	87 (4)	58 (5)
Hg ²⁰³	1 (5)	0 (4)	0 (4)	0 (4)
Pb ²¹⁰	103 (3)	70 (3)	32 (3)	13 (8)
Mn ⁵⁶	99 (4)	96 (4)	85 (4)	79 (4)
Mo ⁹⁹	100 (4)	97 (3)	85 (3)	13 (3)
V ⁴⁸	102 (5)	99 (5)	70 (5)	60 (5)
Ag ¹¹⁰	65 (4)	67 (4)	45 (4)	21 (4)
Zn ⁶⁵	100 (4)	98 (6)	69 (6)	30 (6)

From an examination of the data of Table 1 Pijck, Gillis and Hoste stated (1): "It appears that for most of these elements the losses are considerable, even at temperatures which are relatively low. These losses must be due to absorption on the porcelain ware (Hg, Pb, Cu, Zn) or by volatilization (Fe, As, Sb, Au, Hg)."

MINERALIZATION BY WET CHEMICAL OXIDATION USING MIXED HNO₃-HClO₄-H₂SO₄

The same type samples which had been subjected to dry ash mineralization were seeded using the same elements employed as shown in Table 1 and recovery determined employing again radioactivation. Samples of 500 milligrams of dry powdered vegetable matter, 5 milliliters of whole blood, 25 ml of urine and 2.5 g of muscular tissue were wet ashed in 50 ml micro-Kjeldahl flasks. The oxygen donor mixed acids consisted of 5 ml of a mixture of 66% HNO₃, 96% H₂SO₄ and 70% HClO₄ proportioned 3:1:1. The



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duration of oxidation was 30 minutes to 120 minutes with no attempt to use minimum elapsed time in reaction.

"This mixture (1) of rapid total destruction of a wide variety of materials was without resultant danger."

The procedures in activation analysis are described in detail by Gordon (3), and Meinke (4,5).

"Employing radio-active tracers permits obtaining in a relatively simple manner the necessary data concerning the status of a number of trace elements during the mineralization of organic substrates. Actually in measuring activities before and after the destruction it is easy to determine the extent of eventual losses" (1). The procedures employed are described by Pijck, Gillis, and Hoste's contribution (2), in greater detail than their report reference (1).

TABLE 2. MINERALIZATION OF BLOOD, URINE, POWDERED VEGETABLES, AND MUSCULAR TISSUES EMPLOYING THE WET OXIDATION OXYGEN DONORS, $\text{HNO}_3\text{-HClO}_4\text{-H}_2\text{SO}_4$

(Determination by radioactivation evaluation. The same tagged tracer elements as were used in Table 1 were again employed. Duplicate test numbers in parentheses.)

Element	Blood	Urine	Powdered Vegetable	Muscular Tissue	Applying Reflux Digestion
Sb	99.4 (4)	95 (5)	95 (5)	94 (5)	101 (4)
As	93 (6)	94 (4)	95 (6)	93 (5)	101 (4)
Cr	100 (6)	100 (6)	101 (6)	100 (6)	
Co	100 (11)	100 (6)	101 (6)	99 (6)	
Au	77 (4)	100 (6)	77 (6)	65 (4)	101 (4)
Fe	98 (17)	92 (5)	95 (6)	85 (6)	100 (12)
Cu	102 (5)	101 (5)	102 (5)	102 (5)	
Hg	24 (5)	87 (5)	45 (4)	30 (6)	100 (4)
Pb	100 (3)	101 (4)	101 (4)	101 (4)	
Mn	99 (5)	94 (5)	98 (4)	100 (5)	
Mo	101 (5)	101 (6)	101 (5)	100 (5)	
V	100 (5)	100 (5)	100 (5)	100 (5)	
Ag	100 (6)	101 (6)	100 (5)	100 (6)	
Zn	99 (12)	101 (6)	99 (5)	100 (6)	

By examination of the data of Table 2 following the wet oxidation procedure employing mixed $\text{HNO}_3\text{-HClO}_4\text{-H}_2\text{SO}_4$ it is observed that only five of the 14 showed loss of metallic trace elements. Of these five all were quantitatively recovered by wet digestion under total reflux.*

*In the wet oxidations of Table 2 the mineralization employing either the Liquid Fire Reaction, Section (5), or the Periodic Acid Liquid Fire Reaction, Section (6), in both cases with no H_2SO_4 added, the destruction of the organic substrates would have

been just as complete. In such case it is certain that iron (as well as copper) would have been totally recovered as demonstrated in wine analyses, see page 66. By the presence of H_2SO_4 in the acid mixture employed, as recorded in Table 2, it is probable that the reduction product of H_2SO_4 , namely SO_2 , formed in its oxygen donor reactivity, has contributed in the small loss of iron found in case the oxidations were not carried out under total reflux of condensed distillate from the boiling acid mixture. At any event the use of the Bethge apparatus for the digestions of Table 2 provides for the total recovery of the trace elements listed. See apparatus assembly, page 20.

Special attention to the trace element determination of Cu, Cr, Zn, and Co in serum was also documented employing radioactivation analysis by Pijck, Gillis and Hoste (1). Results duplicating those of Table 2 were also obtained.

A hybrid wet-dry ashing procedure for the mineralization of organic materials has been investigated by Middleton and Stuckey (6). This procedure involves dry ashing with small and repeated additions of 66%, or fuming nitric acid. This method is much more time consuming, and is subject to the usual shortcomings of dry ashing. It has been adapted to large sample weights, with only the advantage that much less nitric acid is required than in wet ashing. Baking at 300-350°C is requisite.

An extensive review evaluation program as referee analyst was provided by Gorsuch (7). The wet oxidation by $\text{HNO}_3 + \text{H}_2\text{SO}_4$, $\text{HNO}_3 + \text{HClO}_4$, $\text{HNO}_3 + \text{HClO}_4 + \text{H}_2\text{SO}_4$ as well as by the Middleton and Stuckey procedure and three dry ashing procedures employing additives were tested. The elements investigated were Pb, Zn, Se, Cu, Co, Ag, Cd, Sb, Cr, Mo, Sr and Fe. Gorsuch recommended the use of the Bethge apparatus, Figure 2, page 20. The general conclusions by Gorsuch (7) paralleled those of Pijck, Gillis and Hoste (1,2). Comparative results for the recovery of all metals above listed were tabulated by Gorsuch following all the reaction procedures of both wet and dry ashing.

Mineralization following dry ashing procedures has been documented by R. E. Thiers (8) of the Biophysics Research Laboratory, Harvard Medical School, Peter Bent Brigham Hospital, Boston, and this exhaustive study of dry ashing mineralization is full of vital information on the subject. The metal traces determined were evaluated following spectroscopic examination.

Literature Cited

1. J. Pijck, J. Gillis, and J. Hoste, "The Determination of Cu, Cr, Zn, and Co, in Serum by Radioactivation," *International Journal of Applied Radiation and Isotopes*, **10**, 149-57 (1961) (Pergamon Press Ltd.).
2. J. Pijck, J. Gillis, and J. Hoste. Proceedings of the International Symposium on Micro Chemistry, Birmingham, 1958, p. 48, Pergamon Press, London (1958).
3. C. L. Gorman, *Anal. Chem.*, **26**, 176 (1954).
4. W. W. Meinke, *ibid.*, **28**, 736 (1956).
5. W. W. Meinke, *ibid.*, **30**, 686 (1958).
6. G. Middleton and R. E. Stuckey, *Analyst*, **79**, 138 (1954).
7. T. T. Gorsuch, *ibid.*, **84**, 135 (1959).
8. R. E. Thiers (D. Glick, Editor). "Methods of Biochemical Analysis," Interscience Publishers Inc., New York and London, Vol. V, p. 284 (1957).

Summary Comments

To even one who, by the nature of his research might be said to have a decided prejudice in favor of wet or dry mineralization of organic compositions, the conclusion to be inevitably accepted is that wet ashing procedures only are to be fully trusted. By all the types of estimation of the trace elements involved, spectrophotometric, spectroscopic, or radioactivation, if following dry ashing the elements so evaluated, no matter how expertly this recovery is done, the studies are suspect. Mineralization without loss, and recovery of the trace elements with great accuracy, must be of tandem efficiency.

The studies of Pijck, Gillis, and Hoste have been subjected to criticism on the grounds that the radioactive tracer elements were additives to the samples mineralized and not originally incorporated in the matrix of the samples serving as substrates. It is a fair assumption that this criticism is invalid.

The various trends of errors resulting in loss of elements sought following dry ashing techniques have been identified by Thiers (8) including the influence of temperature upon ignition, type of containers employed and the prevention of accidental contamination.

The most frequent criticism of wet ashing procedures is that considerable amounts of oxygen donor chemicals are required and sample weights curtailed in magnitude. These oxygen donor acids (HNO_3 , HClO_4 , H_2SO_4 , H_5IO_6) are commercially available in an extremely high degree of purity, the most important of these being perchloric acid. It is interesting to know that 70 percent acid is available which has been double vacuum distilled the second purification employing vycor ware and ampule storage in vycor. Even reagent quality perchloric acid of 70-72 percent strength is available in any quantity demanded which has but a maximum of 15 parts per million non-volatile impurities. Equally pure HNO_3 , H_2SO_4 or H_5IO_6 are made commercially available.

The question of hazard involved in wet chemical mineralization using perchloric acid, which is frequently cited, indeed has been effectively countermanded. The answer to the problem of hazard is to be found in study of the basic properties of perchloric acid in training programs and guarded experimental intuition and procedural control in practical experimentation. This is the proper answer to this over-stressed hazard citation.

The whole subject of this section of this review reminds one of the ancient oriental adage namely "never descend into a deep well suspended from a rotten rope."

SECTION III

WET OXIDATION OF ORGANIC MATTER EMPLOYING PERCHLORIC ACID AT GRADED POTENTIALS AND CONTROLLED TEMPERATURES

Introduction. The oxygen donor properties of boiling solutions of perchloric acid do not function to any appreciable extent below an acid strength of 50 percent at boiling point of approximately 145 to 150°C. At acid concentrations greater than 50 percent up to 66 percent, and over the boiling temperature range 150° to 175°, the available oxidation potential increases as a straight line function as may be experimentally substantiated. Above 66 percent acid concentrations and up to 72.5 percent the increase in available oxidation potential rapidly accelerates to values estimated to be approximately 2.0 volts. In the latter range the destructive oxidation of such organic compositions as cellulose or cellulose rich organic compositions as dried feed stock, hay, alfalfa, raffia, etc. may react with violence.

The individual oxygen donor reactivity of perchloric acid when applied over the 60 to 66 percent concentration and over the temperature range 160 to 175° may be applied without the slightest hazard in the destruction of organic entities provided digestion apparatus is suitably available to maintain the temperature applied. This is done by conducting such reactions under reflux of the digestion vapor phase. The selected acid content of the reacting media is thus rigidly maintained. For such reactions the required time for completion may be favorably lowered by the addition of 5 to 10 milligrams of vanadium as catalyst.

Since this method of destructive oxidation of organic compositions involves only perchloric acid as oxygen donor, such applications will be first illustrated, to be followed by disclosure of mixed acid attack to broaden the field of practical procedures.

The present Section 3 of this brochure has been documented by publication in *Analytica Chimica Acta* (1). This research was carried out at the Chemical Laboratories of The University of Illinois. The literature publication is herein reprinted with some modifications of additions and omissions. By proper selection of the best suited perchloric acid concentration, hazard free, complete destruction of organic compositions can be provided. Reaction times in general range from 5 to 30 minutes. Total recovery of either trace or macro amounts of metallic and non-metallic elements present is then evaluated in the perchloric acid residue. The determination of calcium, manganese, the alkali metals or phosphorus in dried feed items in animal husbandry and plant pathology investigations make wide use of these wet oxida-

tion procedures in the control laboratories of agricultural chemistry. Determination of cobalt is also an important plant trace element determination.

New Objectives in the Wet Oxidation of Organic Matter Employing Boiling Controlled Concentrations of Perchloric Acid

Generalities. Popular use of perchloric acid in the wet oxidation of organic matter has been somewhat retarded in routine applications by apprehension that such procedures involve violent reaction hazards. The vast number of routine control laboratory analyses of this type now performed daily in a number of chemical industries, in reality discount this viewpoint of accompanying hazards. This Section 3 has been designed to demonstrate that the redox potential of perchloric acid as individual oxidant can be effectively controlled. This completely eliminates the possibility of hazard in the rapid destruction of organic matter of selected type samples. The recovery of the elements to be determined in the wet ash residue is quantitative. The process is not complicated due to the presence of excess perchloric acid in the carbon free completed digestion. Controlled oxidation potentials are maintained and their magnitude adapted to the requirements in each particular case. All possible reaction hazard is thus eliminated. These oxidative reaction intensities of perchloric acid are constant and static through digestion under refluxing conditions. Sample types experimentally explored include cellulose, proteins, and carbohydrates or products predominantly of this composition.

The Variation of Boiling Point of 50 to 70 Percent Perchloric Acid

The perchloric acid-water azeotrope (72.5 percent HClO_4) has an oxygen content of 46.4 percent. Most of this oxygen content is made available at its boiling point. With perchloric acid constituting lower hydrate forms ($2\text{HClO}_4 \cdot 5\text{H}_2\text{O}$, $\text{HClO}_4 \cdot 3\text{H}_2\text{O}$, and $2\text{HClO}_4 \cdot 7\text{H}_2\text{O}$) the available oxygen decreases and the boiling point lowers. At concentrations below 50 percent, aqueous perchloric acid at its boiling point is practically zero in oxygen availability.

By control of acid concentration at selected levels, the redox potentials may be simultaneously graded and controlled. The negligible oxidation potential of boiling perchloric acid at 50 percent concentration may be slowly and steadily increased as the acid concentration is augmented. At 70 to 72.5 percent boiling solutions of perchloric acid make oxygen more readily available. In the presence of readily oxidizable materials such solutions may promote uncontrolled reaction intensities. Employing controlled perchloric acid concentrations, reactions of oxidation may be regulated to give a wide variety of oxidation potentials. Reaction intensities from 0.8 to 0.85 volts for boiling 50 percent perchloric acid, with all gradations at ascending values to 2.0

volts for boiling 73.6 percent acid strength, (the exact $\text{HClO}_4 \cdot 2\text{H}_2\text{O}$ hydrate), may be precisely established.

Apparatus design to maintain selected concentrations of perchloric acid is available. Controlled acid concentration results from the use of the reflux condenser principle. Acid concentration is thus unaltered throughout a given period of reaction. Means are provided also for increasing the active acid concentration at any time interval. Such conditions are provided through apparatus design permitting withdrawal of accumulated reflux during a given time interval. By such operation the perchloric acid concentration in any reaction may be progressively increased over the range of 50 to 72.5 percent.

Practical oxidation reaction intensities illustrative of graded potential control may be informative.

1. Hypophosphorous acid is not oxidized by boiling 30 percent perchloric acid. Its oxidation is instantaneous and intensely violent with hot 72.5 percent perchloric acid.
2. Boiling 50 percent perchloric acid converts Fe^{++} to Fe^{+++} at very slow rate, (90% oxidation approximately in a 10-minute interval). Under these conditions the redox potential is estimated to be 0.8 to 0.85 volts.
3. Boiling 50 percent perchloric acid does not oxidize VO_2^{++} to VO_3^- . Thus the redox potential is indicated to be less than 1.0 to 1.1 volts.
4. Boiling 57.5 percent perchloric acid slowly oxidizes the vanadyl ion to vanadate. The redox potential is thus approximately 1.3 to 1.4 volts.
5. Boiling 60 percent perchloric acid readily oxidizes the vanadyl ion to vanadate. The oxidation potential required is approximately 1.45 to 1.5 volts.

A convenient and sufficiently accurate method for the approximate determination of the concentration of perchloric acid in aqueous solutions is by the determination of the boiling point. The increase of the boiling point as a function of the acid concentration is given in the plot of data in Figure 1 over the range 56 to 68 percent covering the range important in wet oxidations employing perchloric acid alone as oxygen donor. The data shown in Figure 1 are tabulated in Table 1.

The acid concentrations given in Table 1 were determined by titration with standard sodium hydroxide (average of duplicate determinations). The standard alkali was evaluated by the use of the exact dihydrate of perchloric acid, ($\text{HClO}_4 \cdot 2\text{H}_2\text{O}$), as primary standard. Accurately weighed samples of this standard in small glass stoppered vials were placed in beakers, 150-200 ml of water added and titrated by the alkali employing bromthymol

blue as indicator. By this procedure stock solutions of varying concentrations of perchloric acid were established in acid content.

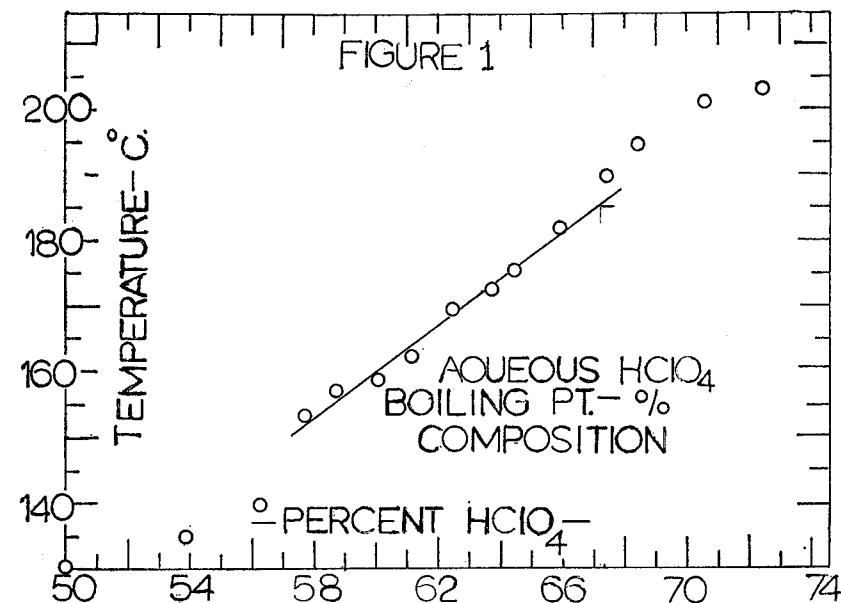


TABLE 1. THE BOILING POINT OF VARIOUS CONCENTRATIONS OF AQUEOUS PERCHLORIC ACID

Acid Conc. %	B.p. °C	Acid Conc. %	B.p. °C	Acid Conc. %	B.p. °C
56.3	140	59.2	157	64.5	175
57.7	154	60.1	160	66.0	182
58.8	157	62.6	170	67.5	190
				68.4	195

The boiling points of the stock solutions of Table 1 and Figure 1 were determined using a 125-ml Erlenmeyer flask surmounted by a 20-cm reflux condenser. The union between flask and condenser was made using a 24/40 ground glass combination. A 20-ml sample of acid was added. Carborundum boiling chips prevented superheating. The thermometer was suspended within the condenser and its mercury well completely immersed in the boiling acid.

Pycnometer determination of the Sp.Gr. at $25^\circ/4^\circ$ of perchloric acid over the range 56 to 68 percent acid is given in Table 2. At $15^\circ/4^\circ$ these values are increased approximately one hundredth unit. The use of a hydrometer would be sufficiently accurate. It will be observed that, over the range of acid concentrations involved, the increase in specific gravity with increase

in concentration is a linear function. This is also true of the increase in boiling point, (Figure 1), with increase in acid strength.

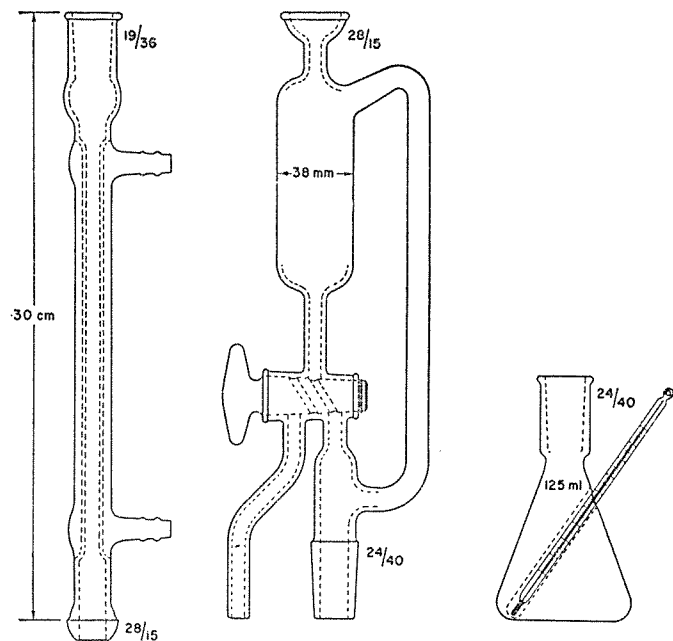


Figure 2.—Bethge Digestion Apparatus.

TABLE 2.

SPECIFIC GRAVITY OF 56 TO 68 PERCENT PERCHLORIC ACID

Wt. % Acid	Sp.Gr. 25°/4°	Wt. % Acid	Sp.Gr. 25°/4°	Wt. % Acid	Sp.Gr. 25°/4°
56	1.470	60	1.524	64	1.578
57	1.483	61	1.537	65	1.591
58	1.497	62	1.551	66	1.605
59	1.510	63	1.564	68	1.632

Apparatus Design and Manipulation

For individual reactions on an open laboratory work bench a slightly modified Bethge apparatus is employed. A line drawing is shown in Figure 2.

In place of the insealed thermometer well as shown, a 10/30 ground glass thermometer receiver is an improved alteration. The thermometer is inserted employing a teflon adapter for improved contact of its mercury well with the

bottom of the reaction Erlenmeyer flask. A photo of the assembled apparatus is shown in Figure 3.

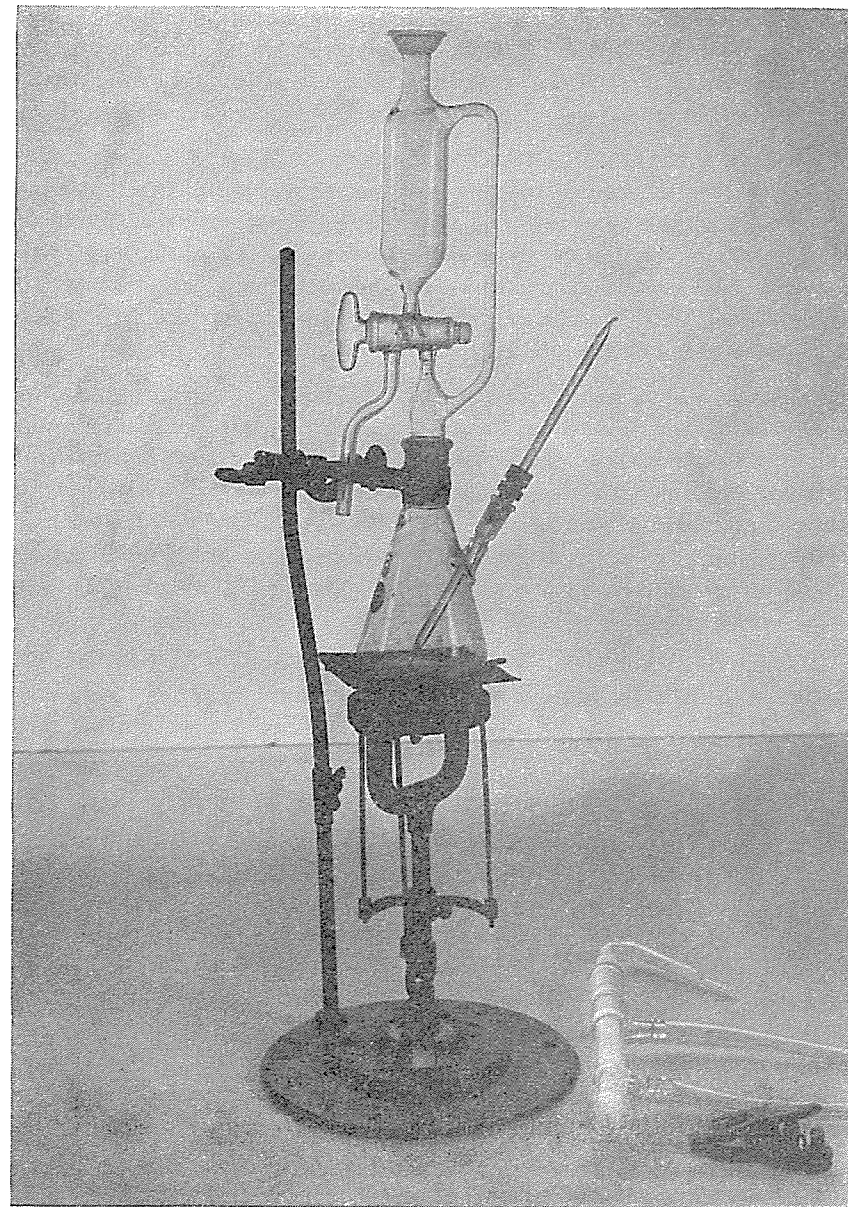


Figure 3.—Bethge Reaction Apparatus and Modified Rogers Ring Burner

In place of the modified Rogers ring burner shown in Figure 3, a ring stand or tripod support for wire gauze mounting of the reaction flask may be substituted. Gas flame heating may be replaced by the use of a small, ring stand supported, electric hot plate. The apparatus should be positioned adjacent to a laboratory sink for disposal of condenser water. Multiple reaction assemblies may be employed in a laboratory fume hood and condenser cooling water passed simultaneously from condenser to adjacent condenser thence to the drain for disposal.

Vapors from the boiling reaction acid may pass either of two routes into the combination air condenser and water cooled condenser surmounting the same. By use of selective positions of the 3-way stopcock at the base of the air-cooled condenser the collection of condensate may pass through the side arm duct. With the 3-way stopcock open the vapors pass directly into the air condenser and thus the condensate continually returns to the reaction flask.

Any reflux not collected in the air-cooled section is collected in the surmounting water-cooled condenser and returned to the stopcock terminated, air-cooled condenser and back into the reaction flask. The ball and socket standard ground joint between the water-cooled and air-cooled condensers provides for its ready removal in disassembling. It is firmly held in place without clamp support employing a customary double "U" spring clamp support, (see Figure 3).

A single ring stand clamp firmly attached to the neck of the reaction flask, together with the support by the 24/40 ground joint connecting the reaction flask and air condenser, obviates further clamp supports to hold the three piece apparatus assembly firmly in place.

With the stopcock adjusted to return the reflux condensate to the reaction flask, the starting concentration of boiling perchloric acid may be maintained throughout any given reaction period.

Closing the stopcock permits collection of any desired measure of condensate with accompanying increase in reactant acid concentration. The extent of this adjustment can be followed by thermometer observation, or the volume of condensate collected can be determined after removal, then the air condenser stopcock can be readjusted to again return condensate into the reaction flask.

By such manipulation the perchloric acid concentration may be augmented stepwise over the effective oxidation range 50 to 72.5 percent. If desired, the withdrawn condensate may be returned to the reaction flask through the opened stopcock of the air-cooled condenser and the original reaction conditions restored.

Reaction Characteristics

The destructive oxidation of cellulose serves admirably as a characteristic reaction illustration.

TABLE 3. GRADED POTENTIAL OXIDATION OF CELLULOSE,
(S AND S ASHLESS PAPER), OR RAFFIA
20.0 ml HClO_4 - 0.01 g NH_4VO_3
CATALYST — BOILING TEMPERATURE

Sample 0.8 g	HClO_4 , Conc. %	Time for Complete Oxidation, Minutes	Remarks
Cellulose	50.0	375	Reaction still incomplete
Cellulose	57.7	60	Excess foaming
Cellulose	60.1	52	No excess foaming
Cellulose	62.6	45	—
Cellulose	64.0	17	—
Cellulose	66.0	9	—
Raffia	64.5	18	Same type oxidation as
Raffia	66.0	7	pure cellulose

From the examination of the data of Table 3 it will be observed that the oxidation potential of 50 to 60 percent perchloric acid at boiling point 132 to 160°C is not sufficient to rapidly oxidize the chocolate brown degradation products initially formed as the acid attains the boiling temperature. Without the use of vanadium as catalyst, even at 60 percent acid concentration the oxidation rate is prohibitively slow. At concentrations between 62.5 to 65.0 percent the potential has sufficiently augmented to bring about rapid oxidation, ($2\text{HClO}_4 \cdot 7\text{H}_2\text{O}$ to $\text{HClO}_4 \cdot 3\text{H}_2\text{O}$ hydrate range). At 66 percent acid strength the reaction time is decreased so that to exceed this acid concentration would permit the reaction rate to get out of hand. At 72.5 percent acid concentration the oxidation would occur with violence.

The destruction of samples such as raffia, dried hay or straw or other types consisting predominantly of cellulose would be predicted to follow trends as those indicated in Table 3.

Differential Oxidations Employing Perchloric Acid

A favorable property of perchloric acid as a graded potential oxidant previously demonstrated, consists in the fact that it is a good solvent for cellulose, sugars of all types, as well as protein predominant compositions such as leather, collagen, wool, etc. This has the advantage that the organic matter to be oxidized is instantly and thoroughly dispersed in the oxidation medium even before a temperature is attained which produces fragmentation of the sample. This at the boiling temperature produces a semi-charred carbonaceous accumulation during the subsequent complete oxidation. Flocks of fluffy car-

bonaceous degradation matter will result. These, while dark colored, are far removed from the character of reaction products which result from the substitution of sulfuric acid for perchloric acid as oxygen donor. These flocks of carbonaceous compositions are much more extended in active surface and are of such density that they are easily suspended in the reaction medium which has a density of less than 1.60.

A very great advantage provided by perchloric acid as oxidant also consists in the ready solubility of such elements as calcium as perchlorate (rather than as sulfate in the Kjeldahl oxidations), and other similarly insoluble sulfates. Phosphorus, if present, is quantitatively retained after oxidation from lower valence states. All metallic elements, even if detectably volatile under ordinary conditions are retained through the influence of reflux return to the reaction medium. Sulfur at oxidation state less than that of sulfate is not quantitatively oxidized to sulfate by perchloric acid. (This is not the case in procedures of oxidation subsequently to be described.)

Complex organic compositions may be fractionally destroyed. An example is that of the destructive decomposition of wool. By control of the perchloric acid concentration the oxidation of organic matter can be regulated in such manner as to leave unoxidized an oily or fat-like oxidation resisting material which continues to be volatilized and condensed out of the cooled distillate. This product resists oxidation until in contact with boiling perchloric acid of increased concentration. Its isolation in the digestion as above described can be provided by withdrawing the condensate and replacing it by an equal amount of water to maintain an unincreased oxidation potential throughout the digestion.

The digestions as described, besides requiring no fume hood for work in the open laboratory, also provide for the removal of most of the excess perchloric acid preliminary to the determination of ingredients retained in the wet ash residue. Although such digestions involve considerable ($\frac{1}{2}$ to 1 inch) foam formation during the early stages of the reactions, which cause carbon flocks to attach themselves to the inner walls of the reaction flask, their removal is not troublesome. When the principle reaction of oxidation is complete at a fixed perchloric acid concentration, the reflux return to the reaction flask is cut off and the acid rapidly concentrates, (2 to 4 min.), to the composition of 72.5 percent at which concentration the vapor phase is rapidly reactive in the oxidation of the foam deposited carbon flocks.

Graded Potential Oxidation of Wool

A gram sample of wool yarn was digested in 20 ml of 64.5 percent perchloric acid using 0.01 g of NH_4VO_3 as catalyst. Reaction temperature

control was provided by withdrawal of collected samples of distillate. The results are given in the data of Table 4.

TABLE 4. GRADED POTENTIAL OXIDATION OF WOOL

Reaction Time, Min.*	HClO_4 Conc. %	Temp. °C	Reaction Color	Remarks
3	64.5	175	Dark red brown	No foaming
5	64.5	175	Dark red brown	—
10	64.5	175	Light red brown	—
20	64.5	175	Orange	Oily condensate
24	66.0	185	Light yellow	—
26	66.0	185	Light yellow	No oily condensate
28	66.0	185	Light green	Returned collected concentrate
30	68.0	193	Light yellow	Reaction complete

*Reflux condensate collected at 22 and at 28 minutes reaction time.

From an examination of the data of Table 4 attention is called to the fact that at no stage of the digestion is there any char formation, foaming or formation of insoluble products. The destruction of organic matter is not complete at acid concentrations of 64.5 percent. An oily fraction continues to pass into the condenser mechanism and return to the reaction flask. As indicated, 66 percent acid at boiling point 185°C is effective in the oxidation of this oily decomposition product. Its oxidation is noticeably exothermic and rapid. The isolated reflux condensate is shown to have a small amount of organic matter contamination and upon return to the reaction flask this is indicated by color change from light yellow, (due to oxidized vanadium catalyst), to light green. Final complete oxidation is accompanied by the return of the vanadate yellow color.

This reaction was duplicated starting with 61.0 percent perchloric acid and at 165°C and allowed to proceed 8 minutes. Reflux was cut off in one minute intervals, for periods of 1 minute duration, followed by 1 minute boiling. By this operation the oxidation was complete in 15 minutes. By this same procedure and allowing the reaction to progress until the color is reddish-orange then increasing the acid concentration from 65 percent to 66-68 percent through retention of refluxing condensate, the following types of samples, (gram samples), dried beef, longhorn cheese, dried apricot and vegetable tanned leather, were wet ashed. The times of complete reaction were respectively 17, 20, 26, and 26 minutes.

Not all types of organic matter require less than 70-72.5 percent perchloric acid for their quiet oxidation. Oxalic acid may be quietly and safely oxidized by boiling 70-72.5 percent perchloric acid. Formates likewise may be oxidized. Subsequent material is devoted to this subject. Also the mechanism of reactivity due to the use of vanadium as catalyst in speeding up such digestions is elucidated.

The Perchloric Acid Oxidation Potentials at Concentrations Greater than 72.5 Percent

A subsequent discussion of oxidation resistant organic compositions such as fecal material makes use of the 72.5 to 85 percent range of acid strength. The increased perchloric acid strength is provided *in situ* by employing mixed perchloric and sulfuric acid as combined oxygen donors. At various proportional parts of such reaction mixtures the strength of perchloric acid made operative can be readily adjusted. The advantage consists in making available necessarily increased oxidation potential as may be dictated by the particular organic compositions to be wet oxidized.

For oxidations at 60.0 to 68.0 percent perchloric acid, vanadium generally serves as the best catalyst. For reactions at 68 to 72.5 percent perchloric acid concentrations, either small amounts of vanadium or chromium are effective. For oxidations at higher acid strength than 72.5 percent, molybdenum may be effective as catalyst. In all these cases the reaction of oxidation takes place in the absence of a catalyst at the expenditure of increased reaction time.

Threshold Perchloric Acid Oxidation Potential

The oxidation of a number of types of organic compositions is not practical employing perchloric acid alone as a graded potential oxygen donor. The range of acid concentration and temperature within which a rapid, smooth and controlled rate of oxidation is provided is too narrow to be trustworthy. Over such a threshold acid concentration, by a narrow range of perchloric acid concentration, the rate of oxidation becomes, if not violent, none the less uncontrolled.

An example of this type reaction is the destructive wet oxidation of rubber.

A one-gram sample of pure gum rubber in contact with 62.5 percent perchloric acid at its boiling point, (170°C), was virtually unattacked.

By duplicate attempt employing 64.5 percent perchloric acid at 175° it required 24 hours for complete oxidation.

In the use of 66 percent acid at 184°, while being noticeably reactive, there was indication that the oxidation to completion would be too time consuming to be practical. From a 20-ml sample of acid after one-hour reaction on 1 gram of rubber at 184° a 0.5-ml portion of distillation reflux was isolated. At this point the still remaining, partially oxidized rubber caught fire within the reaction flask and the remaining unoxidized organic matter was completely carbonized in a time interval of approximately 5 seconds.

The use of vanadium as catalyst under these conditions is without effect.

Samples such as fats and lecithin or oils in general are in the same group including rubber. In all these cases, (narrow threshold oxidation conditions, which prevent smooth, effective and wide range application of definitely adjusted, boiling perchloric acid concentrations), the use of methods to be later described serve admirably in practical, experimentally modified procedures. For example, the wet oxidation of vulcanized rubber in determination of sulfur is a well known procedure employing the oxidation procedure involving mixed nitric and perchloric acids, "the liquid fire reaction", later to be fully detailed and experimentally demonstrated.

The attractive feature in the case of the use of graded potential perchloric acid oxidations is that the operations involved require little manipulative attention for their wide range of possible application.

Generalizations

The author has yet to encounter a sample of coal, anthracite or bituminous, involving any hazard when digested in boiling 70 percent perchloric acid. In the wet oxidation of coal the most important mixture of oxygen donor acids is that of periodic-perchloric acid. This procedure forms the basis of a separate section of this procedural review.

"Small amounts of copper, (less than 1 ppm), are known to promote formation of rancidity in dairy products and vegetable fats, (2), and contamination must be guarded against during processing, packaging and storage . . . Paper and paper board may contain upwards of 20 ppm of copper. In the manufacture of specialty papers—i.e. butter wrap and ice cream cartons—the copper content must be carefully controlled and the tolerance for the metal is very low. . . ."

Borchard and Butler (2) found that dry ashing of paper resulted in variable results in the copper determination. Wet ashing of paper using graded potential control with perchloric acid digestion might be preferred in the determination of copper in paper following the procedure employed by Borchard and Butler, ($\text{HNO}_3 + \text{HClO}_4$ digestion). The influence of the presence of trace amounts of copper, (more than 2 ppm), in the field of wine processing, will be subsequently treated in this review.

While the number of illustrations of the destruction of organic matter cited in the present section is limited, the number of possible applications for this procedure may easily be applied following trial and experimentation into many further applications, i.e. in the field of trace element determinations in the field of animal feeds such as dried forage crops, hay, alfalfa, and similar products. In such tested application let the principles illustrated in this section be the guide and let experiment decide.

Literature Cited

1. "Wet Oxidation of Organic Matter Employing Perchloric Acid at Graded Potentials and Controlled Temperatures." G. Frederick Smith, *Anal. Chim. Acta*, **17**, 175 (1957), in English.
2. "Determination of Trace Amounts of Copper. Application of the Bathocuproine Reagent to Pulp, Paper and Pulping Liquors." Leroy Borchard and John P. Butler, *Anal. Chem.*, **29**, 414-19 (1957).

SECTION IV

THE DESTRUCTIVE OXIDATION OF OXALIC AND FORMIC ACID EMPLOYING HOT CONCENTRATED PERCHLORIC ACID. CATALYSIS INFLUENCE OF MILLIGRAM ADDITIONS OF VANADIUM.

In the process of the wet oxidation of complex organic compositions it is postulated that the stepwise degradation mechanism involves, in many cases, the intermediate formation of oxalic and formic acids (1).

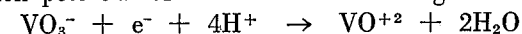
Since the boiling azeotrope of $\text{HClO}_4 \cdot 2\text{H}_2\text{O}$ serves at 203°C to quietly and smoothly destroy oxalates and formates with the liberation of carbon dioxide, the study of this special oxidation is appropriate. The perchloric acid content of its water azeotrope is 72.5 percent.

For such destructive oxidations the use of milligram amounts of vanadium as catalyst serves to accelerate reaction rates. Milligram quantities of chromium (III) serves as indicator for complete oxidation. Approximately 70 percent, boiling perchloric acid instantly oxidizes the green Cr(III) ion to orange chromic oxide, (CrO_3) , only after complete removal of organic compositions. Chromic acid is soluble in 70 percent perchloric acid when hot and quantitatively insoluble in the same acid when at ordinary temperatures.

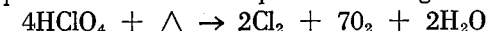
The destructive oxidation of oxalic or formic acid, both avid reducing agents, in the presence of boiling 72.5 percent perchloric acid might logically be anticipated to take place with explosive violence. Such is not the case. This fact establishes one of the most unusual properties of boiling concentrated perchloric acid as an oxygen donor.

The Mechanism of the Catalytic Action of Vanadium In the Wet Oxidation of Organic Compositions

The oxidation potential of vanadic acid following the reaction,



has a value of approximately 1.45 volts in mineral acid solutions. This value is exceeded in magnitude by 0.4 to 0.5 volts by the oxidation potential of the boiling water azeotropic composition of perchloric acid. For this reason, the reaction of perchloric acid in the presence of organic compositions,



may be superseded by the reaction of the vanadate as oxidant. The vanadyl ion resulting is then reoxidized to vanadic acid. It thus serves as an effective catalyst. Hot concentrated perchloric acid then serves to reoxidize the vanadyl ion to V(V) and continue this favorable oxidation mechanism. In

the absence of vanadium the oxidation by hot concentrated perchloric acid serves equally well in the conversion of such products as oxalic and formic acid to carbon dioxide but at a materially less rapid reaction rate.

This situation is dependent upon the vanadic-vanadyl redox potential being high enough for its individual effect to take place before that of hot concentrated perchloric acid comes into play. The two oxygen donors vanadic and perchloric acid may be effective simultaneously. Provided, therefore, that the oxidation of V(IV) to V(V) is a more rapid reaction at a given temperature and concentration of perchloric acid than is the oxidation of a given organic composition through the action of perchloric acid, a small amount of vanadium as catalyst is operationally rationalized. Also V^{+5} must oxidize original material or it will have no effect.

The Oxidation of Oxalic Acid by Hot Concentrated Perchloric Acid Alone and With Vanadium as Catalyst

Apparatus Design. A convenient assembly of apparatus for processing oxidations employing hot concentrated perchloric acid in the open laboratory with disposal of evolved, noxious gaseous reaction products is shown in Figure 4.

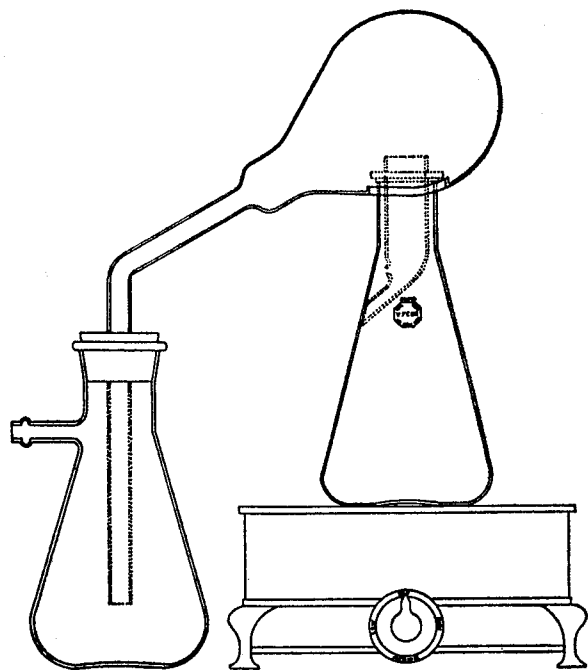


Figure 4.—Oxidation Reactor and Fume Erradicator Assembly.

A 250 to 500 ml Vycor Erlenmeyer flask is employed as reaction container. (A Pyrex Erlenmeyer flask may be substituted). Heat is provided by an electric hot plate which may be replaced by the use of a tripod and wire gauze support for the reaction flask heated by a gas flame. Loss of reaction products through spray formation is prevented by the use of a refluxing still head with tubular opening at the bottom end to vent evolved gaseous reaction products. The return flow of condensed vapors collected are returned from the refluxing still head to the reaction flask down the side to prevent "spitting" when dilute acid contacts the hot concentrated perchloric acid in the reaction flask. Carborundum boiling chips are used to prevent bumping.

The evolved gaseous reaction products are removed through the fume eradicator surmounting the reaction flask and refluxing still head. The aspirator bottle charged with sodium hydroxide solution serves as a scrubber. It is attached to a water aspirator through the use of a flexible tube. A withdrawal of fumes is thus provided due to intake air entering the fume eradicator through its internally flanged opening which effectively prevents condensation on the inner walls of the modified Kjeldahl flask. The fume eradicator may be placed in position with ease and may be readily removed and set aside at will, being supported by the weight of the aspirator bottle and its scrubbing, alkaline solution absorber.

If desired this apparatus assembly may be used within a fume hood without employing a fume eradicator. The apparatus so far illustrated, (Figures 2 and 4, pages 20 and 30) are adequate for all type reactions employed in the various wet oxidation procedures described in the present review.

Experimental. Concentrated perchloric acid 70-72.5 percent (20 ml) serves as oxygen donor. Four milligrams of $K_2Cr_2O_7$ are added as indicator. The acid is heated to boiling, (195-203°C), and after momentarily removing the fume eradicator and refluxing still head 5 grams of crystalline oxalic acid, ($H_2C_2O_4 \cdot 2H_2O$), are added and the apparatus quickly reassembled. The time required for complete oxidation is then noted. The Cr(VI) is at the start immediately reduced to Cr(III). On complete oxidation of the oxalic acid the light green tint is instantly converted to orange.

The same reaction procedure is repeated with the addition of 0.15 to 0.75 milligrams of vanadium (NH_4VO_3) in gradually increasing amounts to individual reactions. The results from six digestions of 5-gram samples of oxalic acid are recorded in Table 5.

TABLE 5. THE OXIDATION OF OXALIC ACID BY 70 PERCENT PERCHLORIC ACID AT 195-200°C WITH AND WITHOUT VANADIUM AS CATALYST

Oxalic acid 5 g $\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$. 20 ml of 70% HClO_4 . 10 mg $\text{K}_2\text{Cr}_2\text{O}_7$ as indicator. Vanadium added as NH_4VO_3 in 70% HClO_4 . (0.1 ml = 0.15 mg of vanadium).

Vanadium added in mg	0.0	0.15	0.30	0.45	0.60	0.75
Reaction time in minutes	9.0	5.75	4.25	3.25	1.75	1.15

The data of Table 5 are graphically outlined in Figure 5.

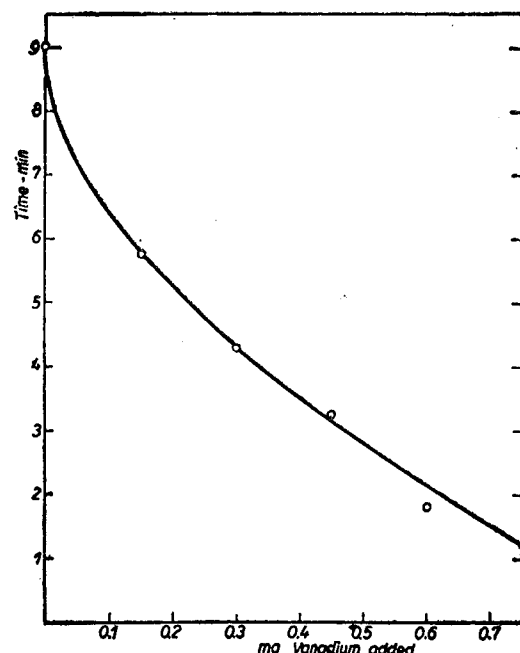
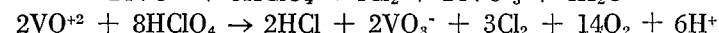
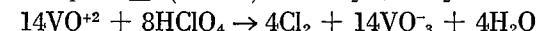
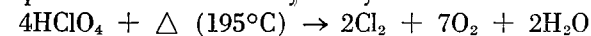


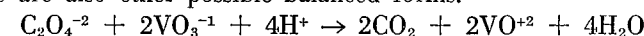
Figure 5.—Vanadium Catalysis in the Oxidation of Oxalic Acid.

The data of Table 5 indicate that the catalysis due to vanadium results from the rapid reaction $\text{VO}_3^- + e^- + 4\text{H}^+ \rightleftharpoons \text{VO}^{+2} + 2\text{H}_2\text{O}$ in alternate directions. The reaction of the oxidation of oxalic acid by hot concentrated perchloric acid is not catalyzed by the Cr(VI) - Cr(III) couple as test demonstrated with and without added chromium. Therefore, for the reactions of Table 5 chromium serves only as an indicator. Thus the oxidation of oxalic acid requires a potential greater than the Cr(VI) - Cr(III) couple and less than that of the V(V) - V(IV) couple. Since vanadium serves effectively as catalyst, vanadate must oxidize oxalate and perchloric acid must oxidize vanadyl much more rapidly than perchloric acid of the same strength and potential oxidizes oxalate.

The reactions accompanying the oxidation of oxalic acid by boiling 70-72.5 percent perchloric acid as catalyzed by vanadium are:



There are also other possible balanced forms.



The Oxidation of Formic Acid by Hot Concentrated Perchloric Acid

Mixtures of glacial acetic acid and 70-72.5% perchloric acid may be heated to boiling without reaction. The acetic acid may be separated from the perchloric acid completely by distillation. From this observation it might be logically predicted that formic acid would likewise resist oxidation. Such is not the case. A sample of crystalline sodium formate when added to 70 percent perchloric acid at 195°C results in its rapid oxidation to CO_2 with a gush of gas evolution.

For the purpose of estimation of the time factor the reactants are best mixed at ordinary temperature and then brought to 195°C on the hot plate. Chromium as indicator was again used. Its indicator effect differs from its reaction in the case of the oxidation of oxalic acid. The Cr(VI) is immediately reduced to Cr(III) which then evidently forms a complex with formic acid which is colorless in low concentrations and faint blue in higher amounts. After all the uncomplexed formic acid has been oxidized, a slow oxidation of the chromium-formate complex begins with the formation of the green chromic ion. This first formation of a green color indicates the practically complete oxidation of formic acid. Approximately 15 minutes is required for the formation of red chromic acid with the oxidation of the last trace of formic acid complexed with chromium.

Experimentally, 5 g of anhydrous, crystalline sodium formate was placed in a 500-ml Vycor flask using the apparatus of Figure 4 page 30 and 20 mg of $\text{K}_2\text{Cr}_2\text{O}_7$ added. After the addition of 20 ml of 70 percent perchloric acid, the flask and contents were placed on a 195° hot plate and allowed to react until the first green color appeared. The time required for the flask and contents to attain 195° was found to be 5 min. At this point the reaction of oxidation becomes rapid as indicated by the rapid evolution of carbon dioxide. The reaction mixture, at first colorless, turns green in 13 minutes. This marks completion of the oxidation except for the small amount of formate held in its complex with chromium.

A repetition of the test oxidation with 0.75 mg of vanadium as catalyst gave duplicate experimental results. Allowing five minutes heating interval to attain

a reaction temperature of 195°, which marks the approximate point of initial reaction, the time required for complete oxidation is 8 minutes. Neither vanadium nor chromium serves as a catalyst for this oxidation. The reaction temperature at the completion of the oxidation was found to be approximately 210° as a result of the accumulation of sodium perchlorate as solute from the sodium formate originally added.

The oxidation of either oxalic or formic acids, as above described, involves only clear solutions throughout the complete oxidation. No dark decomposition products are formed.

Both tartaric and citric acids may be oxidized in the manner designated for the oxidation of oxalic and formic acids, but in such cases there are formed chocolate brown degradation products before reaction is complete. It is possible in these cases that digestions under reflux at lower perchloric concentration, as outlined in Section 3 above, could be carried out without the formation of dark soluble reaction products forming. However, as will be demonstrated in Section 5, other innovations solve this problem.

Formaldehyde is not oxidized smoothly by hot concentrated, (70-72.5 percent), perchloric acid. The problem involved is likewise adequately obviated as provided for in the procedures to be described in Sections 5 and 6 to follow.

Organic compositions such as aliphatic alcohols should not be subjected to oxidation with hot concentrated perchloric acid. Under these conditions the perchloric acid is a powerful dehydrating medium. Hence alcohols are converted into perchlorate esters. These perchlorate esters, as exemplified by ethyl perchlorate, are explosive under heat and under shock. Here again the oxidation of organic compositions containing ethyl alcohol present no problem as is to be demonstrated in the procedures outlined in Sections 5 and 6 to follow.

In the oxidations experimentally demonstrated in this section of classification, it can be readily recognized that for rapid, quiet, and complete oxidation of organic compositions in general, a distinct advantage accrues from processes that result in degradative fragmentation of complex organic molecules into simple organic entities such as oxalates or formates which can be, as just demonstrated, more readily destroyed. As will be again pointed out in subsequent demonstrations, such oxygen donors as periodic acid, (and to a lesser extent nitric acid), provide similar fragmentations. It cannot be overstressed that since concentrated perchloric acid is outstanding as a solvent for many and varied types of organic structures a big advantage is found in its use as oxygen donor. The reaction media, completely foreign to such wet ashing procedures as Kjeldahl destruction of organic compositions, is thus homogeneous throughout.

Many properties of perchloric acid in addition to those demonstrated as our review progresses, are operative and are priceless in advantageous utility. It remains for those seeking mastery involving the mineralization of organic compositions to make an exhaustive study of these almost limitless advantages provided as a result of the use of perchloric acid as oxygen donor.

It is evident that almost no effort is put forward in educational seats of chemical learning to train analytical, inorganic, organic, agricultural and biological students in the properties and advantages in the use of perchloric acid. It is not a sufficient dodge or a wise limitation to cancel out such training under the implication that perchlorate chemistry is fraught with excessive hazard. In truth, its most valuable asset is, (in reality), its remarkable freedom from hazard due to its equally remarkable reaction stability under use by anyone versed in its basic chemical properties. As this review continues to develop, these truths become ever more evident.

Theory and knowledge guide. Experimentation decides.

Lethargy and indifference can develop into an unproductive award.

Literature Cited

1. The Author, "The Wet Ashing of Organic Matter Employing Hot Concentrated Perchloric Acid. The Liquid Fire Reaction." *Anal. Chim. Acta.*, 5, May (1953).

THE LIQUID FIRE REACTION. MIXED CONCENTRATED NITRIC AND PERCHLORIC ACIDS AS OXYGEN DONORS.

Nitric acid at its azeotropic composition, (32% H_2O - 68% HNO_3), is an avid oxygen donor. There is an active oxidation of organic compositions in its attack even at ambient temperatures. As the temperature is increased up to 120.5°C , the azeotropic boiling point, its oxidation of organic compositions intensifies. The rate of reaction is indicated by increasing evolutions of reddish brown nitrogen oxides. It is probable that to a marked extent in this process complex organic structures are fragmented to form simpler entities. However, complete oxidations are not provided even by extended reaction time intervals. Hence the use of nitric acid alone is not completely effective. Its use is none the less quite a *sine qua non*. It is indeed a most effective preliminary reaction innovation.

Concentrated perchloric acid, (the HClO_4 -water azeotrope, strength of acid 72.5%), or reagent strength 70-71% is added to the nitric acid. The strength of nitric acid is thus augmented to values much increased due to the dehydration reaction provided by the perchloric acid. The perchloric acid is correspondingly lowered in strength. Its ability to promote dissolution of organic compositions is still retained, a most effective reaction adjunct.

Over the temperature range, ambient to 120.5° , nitric acid completes its reactivity and its excess is distilled from the reaction mixture over the increase, 120 to 130° . At this point the perchloric acid first exhibits weak oxidation potentials. Continued application of heat steadily effects concentration over the HClO_4 strength 50 to 72.5% concluding in elevation at the HClO_4 - H_2O azeotropic strength of 72.5 percent at 203°C . The oxidation potential gradually and relentlessly increases from approximately 0.85 to 2.0 volts. The intensity of destructive oxidation of organic compositions is thus provided stepwise in an ideally effective series of successive destructive potentials. The rate at which heat is applied may be retarded as desired at the expense of reaction time involved. There is no advantage to carrying out the reaction in two-stage modification.

As a result a wide variety of naturally occurring and synthetic organic compounds are completely oxidized to carbon dioxide. These types include cellulose and starches, proteins, carbohydrates, alcohols, leather, wool, fat-free meats such as liver, kidney and lung tissues, dried foods such as casein, non-fat milk solids, wines and cordials, medical catgut sutures and fruit preserves. Reaction is smooth, hazard-free and requires minimum reaction periods.

The wet ash acid residue from these oxidations retains all metallic im-

purities quantitatively as soluble perchlorates, all of which are adequately soluble, except insoluble silica, in 72.5% perchloric acid. Sulfur and phosphorus at all stages of oxidation are completely recovered as sulfate and phosphate. Amino nitrogen is almost all retained as ammonium perchlorate. Some cases (consult Section 1) require reaction accompanied by reflux retention of volatile vapors, otherwise traces of metallic entities could be lost. By excluding sulfuric acid from these oxidations (as was used in Section 1) iron and arsenic are not lost from the wet ash acid residue. Nitric acid and perchloric acid are commercially available of very high purity. 70-72% perchloric acid of reagent purity is available having only 10 to 15 ppm non-volatile impurities. Methods are available for the determination of wet ash recoveries in perchloric acid for both micro and macro ranges of elements to be evaluated and sample sizes need not generally exceed one gram for examination. Analytical recovery procedures in the determination of the elements sought are not interfered with in general by the presence of perchloric acid. If required, sulfuric acid may be added and by digestion the perchloric acid boiled out, leaving a sulfuric acid wet ashing recovery of elements sought.

For such is the versatility of wet oxidations of organic compositions employing the liquid fire reaction. They are hazard free, rapid in operation, broad in application and their routine applicability provides for millions of individual determinations in many branches of quantitative analytical chemical fields.*

*The first demonstration of the liquid fire reaction called to the attention of the author of this review was carried out by Professor H. H. Willard in his research laboratory at the University of Michigan in 1920. A 2-inch pyrex test tube about 8 inches long served as reaction vessel. About 15 milliliters of perchloric acid, 70% adulterated by the addition of 2 to 3 ml of reagent nitric acid was added. A one-gram weight of ashless filter paper was torn into bits and placed in this reaction mixture. A Bunsen burner flame served as the source of heat. The professor held the test tube and contents in the full heat of the gas flame appropriately continuously agitating its contents. In one minute there was a test tube full of brown fumes of nitric oxides followed by white fumes of boiling perchloric acid and the reaction was complete with no discoloration of the reacting acids. The oxidation was accomplished so rapidly that the test tube was held in the bare hand at the top by the manipulator with no discomfort. The professor's grin was provocative as he wryly remarked with a twinkle of satisfaction in his eyes, "now that is the procedure for the recovery of trace precipitates on filter papers for subsequent examination."

The Oxidation of Tartaric Acid by Concentrated Perchloric Acid and as Modified Employing the Liquid Fire Reaction

A sample of ammonium tartrate weighing one gram was added to a 20-ml sample of 70% perchloric acid in a 500-ml Erlenmeyer flask employing the

assembled apparatus of Figure 4, page 30. A 20-mg portion of $K_2Cr_2O_7$ was added and the reactants heated from ordinary temperature on a 195°C hot plate. At four minutes the solution was green and at 8-10 minutes it was chocolate brown with evolution of CO_2 . There was some carbon char formation. At 27 minutes the reactants were green following the apparent complete oxidation. In 15 additional minutes the solution was orange colored with CrO_3 formation. Evidently the complex formation between the chromic ion and tartaric acid is very stable and slow to oxidize to CrO_3 as was the case in the oxidation of formic acid previously described in Section IV, page 33.

The reaction was repeated with the single modification that 1.5 mg of vanadium was added. At six minutes digestion the solution became straw colored and then chocolate brown. From 6 to 16 minutes CO_2 was evolved but no char resulted. The solution was then green after all organic matter was destroyed. It then required but 3 minutes digestion until the solution was orange from CrO_3 formation.

Upon repeating the same reaction conditions as just described but with the addition of 2 ml of concentrated nitric acid it was found that at 4-6 minutes heating there was CO_2 evolution with minimal evidence of brown fumes being evolved. The reacting flask contents were without dark color or char formation. After 7 minutes the solution was green and all organic matter was destroyed. At 15 minutes the solution was orange in color from CrO_3 formation.

From these reactions it is seen that oxidation of tartaric acid by hot concentrated perchloric acid results in no uncontrolled reaction rates. Vanadium serves as an efficient catalyst in reducing reaction time to half that required in its absence. It also prevents char formation. The best combination of reactants is nitric mixed with perchloric acid and added vanadium as catalyst. This combination prevents char formation and the development of brown soluble reaction products with further shortening of reaction time. The next test was carried out with the substitution of tartaric acid for ammonium tartrate.

A four-gram sample of crystalline tartaric acid was allowed to react with 20 ml of 70% perchloric acid to which was added 6 ml of 68% nitric acid and 1.5 mg of vanadium as catalyst. At 195°C for 4 minutes no appreciable brown fumes were evolved or CO_2 and the reacting solution was colorless. At 7 minutes brown fumes were evolved and the flask contents green. At a little past 11 minutes an exothermic reaction set in with vigorous evolution of CO_2 and the solution was light canary yellow. At 11.5 minutes the exothermic reaction was at its maximum and there were copious white fumes of perchloric acid formed. At 12 minutes the solution turned orange from light green $Cr(III)$ conversion to $Cr(VI)$.

The Oxidation of Citric Acid by Hot Concentrated Perchloric Acid and by the Liquid Fire Reaction

The procedures just above described were repeated in the case of the oxidation of citric acid. The reactants were 20 ml of 70% perchloric acid with 20 mg of $K_2Cr_2O_7$ and 1.5 mg of vanadium added. At 195° in 4 minutes the solution was green with CO_2 being evolved at 6 minutes. At 10 minutes the solution was chocolate brown with 12 mm of foam resulting from CO_2 liberation and some char formation. At 15 minutes the solution was again green. At 18 minutes it was orange from CrO_3 formation.

The oxidation reaction was twice repeated, first with 1.5 then 3.0 mg of vanadium. In each case 2 ml of reagent nitric acid was added. After 4 minutes the solution was colorless with evolved brown fumes and brisk formation of CO_2 . After 5 minutes the mixture was straw colored and by exothermic reaction it at once turned green. With practically all organic matter oxidized it required 20 additional minutes to bring about oxidation to form CrO_3 .

From the last two digestions it is seen that nitric acid prevents the oxidation passing through the chocolate brown stage, (partial carbonization or caramelization). Vanadium is effective as catalyst.

A 4-gram sample of citric acid was digested on a 195° hot plate in 20 ml of 70% perchloric acid plus 10 ml of 68% nitric acid. 20 mg of $K_2Cr_2O_7$ served as indicator and 1.5 mg of vanadium as catalyst. At 3.5 minutes there was no apparent oxidation and the flask contents colorless. At 4 minutes reaction is first initiated and the solution straw colored due to nitric acid with some evolution of CO_2 . At 5 to 6 minutes copious NO_2 fumes appeared along with brisk CO_2 evolution. The 7 minute digestion brought on an exothermic reaction rate. At 10 minutes the solution was green and apparently all citric acid oxidized. It required an additional 11 minutes to oxidize the chromium complex of citric acid to form CrO_3 .

Since oxidation of organic compositions in the presence of aliphatic alcohols is not safe employing perchloric acid alone it is imperative to employ the liquid fire reaction modification of oxidation with its added nitric acid. The alcohols are rapidly oxidized by the nitric acid at temperatures much below the temperature of 120°C. Below this temperature the perchloric acid of the mixed nitric plus perchloric acid is not an oxygen donor. Aldehydes, if present, react in the same manner. The application of the liquid fire reaction in the wet oxidation of either ordinary or fortified wines will be subsequently described. Wine wet ashing in the case of routine laboratory control accounts for countless numbers of routine winery processing operations in the determination of trace amounts of iron and of copper. Above 5 ppm iron content, wine cannot be bottled for sale because higher iron content results in "iron casse", (hydrolytic formation of turbidities due to iron).

More than 2 ppm of copper in wine causes off flavors upon storage. For these reasons of importance, the liquid fire reaction in the wet oxidation of wine is taken up in a later section of the present review.

Concentrated Perchloric Acid as a Premium Reactant in the Dissolution of Organic Compositions

Organic compositions such as those predominantly composed of cellulose, polyhydric alcohols, sugars, collagen, protein and many other types are in general best suited to oxidation applying the liquid fire reaction. A major advantage consists in the ready dissolution of such products without any destructive degradation producing charring and often even no discoloration. This property is outstanding and cannot be over-valued. Such solutions in strong perchloric acid have this property because at ordinary temperatures the acid has no oxygen donor properties. Pyrogallol acid may be dissolved in 72.5 percent perchloric acid out of contact with air with no oxidation such as would be immediately the result upon contact with reagent nitric acid. The nitric acid dilution factor in its mixtures with 70-72.5 percent perchloric acid to form the liquid fire reaction composition, as pointed out previously, further retards oxidative processes by perchloric acid until temperatures above 130°C are attained. In accord with these considerations organic compositions upon application of the liquid fire reaction, generally provide clear solutions throughout the entire heat span, ambient to 203°C. This remarkable advantage cannot be overestimated in value when associated with the liquid fire oxidations of a wide variety of samples, as will hereinafter be demonstrated in the present review section.

The Oxidation of Sulfur Employing Hot Concentrated Perchloric Acid

Concentrated perchloric acid, (the water-perchloric acid azeotropic composition 72.5% acid at boiling point 203°), oxidizes sulfur smoothly and is hazard free. If a sample of 1-2 grams of sulfur is added to a sample of 15-20 ml of this boiling perchloric acid the sulfur melts and floats on the surface of the boiling acid. It is rapidly oxidized to volatile or gaseous products with only approximately 25% or less of the sulfur being retained as sulfuric acid.

The oxidation of organic compounds by the persulfate ion, ($S_2O_8^{2-}$), in warm dilute sulfuric acid as catalyzed by the presence of silver, has been described (1) by Feigl and Schäffer. In the decomposition of cupferrates, nickel dioxime, tannates, oxinates, methylene blue, and acid base indicators among other organic materials, the organic matter is efficiently destroyed. By this process certain of the organo-metallic precipitates (such as Co(III)- α -nitroso- β -naphthalate) are not destroyed and in the case of the thio-compounds elemental sulfur is formed. By the perchloric acid decomposition of sulfur-

containing organics, no elemental sulfur can form. If the liquid fire reaction is applied all the sulfur present is retained in the wet ash residue as recovered sulfuric acid. This principle has been applied to the solution of copper sulfide ores (2). If the sulfide ore is treated with concentrated nitric acid to provide dissolution there is a formation of elemental sulfur. The solution of the ore by nitric acid treatment is far more time consuming.

In applications of the liquid fire reaction sulfur, if present, is retained in the wet ash residue as sulfuric acid. Phosphorus in its lower valence states is likewise completely oxidized to phosphoric acid and completely recovered. The phosphoric acid may then be quantitatively determined by conversion to the familiar "yellow precipitate" in the presence of perchloric acid with no attendant complications, or it may be otherwise determined spectrophotometrically. Arsenic is retained quantitatively in the liquid fire oxidation wet ash residue. Its determination in the case of a wide variety of pharmaceutical preparations following wet ashing of such organic arsenicals provides a ready means of evaluation.

Practical Applications in the Wet Oxidation of Medical Chromacized Catgut Sutures

The liquid fire reaction in the determination of chromium in chromacized medical catgut (3). The chromium content of medical catgut employed in internal operative surgery governs the time interval required for sutures involved to be absorbed and eliminated. The preparation of such medical catgut calls for control of the chromium content.

Destruction of organic matter in chromacized catgut. Catgut sutures of varying degrees of chromacization were cut into 6 mm lengths and dried to constant weight at 80°C and stored in weighing bottles in preparation for mineralization to eliminate organic matter in the recovery of their chromium in the liquid fire reaction wet ash residue.

Weighed samples of 1 to 2 grams were transferred to 500 ml Vycor Erlenmeyer flasks and then using the apparatus assembly illustrated in Figure 4, page 30, were digested using varying proportions of 70-72% perchloric acid and 68% nitric acid. The most satisfactory mixture was found to be 20 ml of perchloric acid and 6.5-7.0 ml of nitric acid.

The sample, a short time after heating begins, is completely dissolved. In 5-6 minutes the nitric acid reaction, accompanied by the production of heavy brown fumes, is complete. The excess of nitric acid is then volatilized at temperatures 120-130°. During and at the end of the nitric acid reaction the solution is green. As the temperature increases to 140° through 160° to 180° a vigorous reaction takes place, with the copious formation of gaseous reaction products but with little or no foam formation. If the rate of temperature increase is not too high, the reacting ingredients maintain an almost

colorless status. In 8 to 13 minutes the decomposition of organic matter is complete, as shown by the production of an orange color as the chromium content of the sample is completely oxidized to chromic oxide.

The sample is kept in contact with the heating device for 2 to 3 minutes after the appearance of the chromic acid color at 70-72.5% perchloric acid concentration and boiling point of approximately 200°. The flask containing the wet ash residue is quickly removed from the heat source and plunged immediately with a swirling motion of the flask contents into a bath of ice water for a 5-10 second cooling period. (A Vycor flask is better able than is a Pyrex flask to resist the heat lowering shock effect but both types have been used successfully.) CrO_3 is quantitatively insoluble in 70% HClO_4 at ordinary temperatures.

The wet ash residue is then quickly diluted by the addition of 60-70 ml of cold water rinsing the removed refluxing still head in the process. A boiling chip of carborundum is added and the diluted wet ash solution boiled for 1-2 minutes to remove the small amount of chlorine formed during the process. The solution is then diluted to 200 ml with cold water, a drop of 0.025 M ferroin sulfate indicator is added and the chromic acid titrated using 0.033 N ferrous sulfate. The first production of a pink cast to the solution, from the first drop of excess reductant, indicates the completion of reaction. One ml of 0.033 N ferrous sulfate solution corresponds to 1.1112 mg of CrO_3 .

The results from a series of analyses are given in Table 6.

TABLE 6. MINERALIZATION OF CHROMACIZED CATGUT AND DETERMINATION OF CHROMIC OXIDE

20 ml of 70-72% HClO_4 plus 7.0 ml of 68% HNO_3 per sample. HNO_3 reaction time 5-6 minutes. Hot plate temperature 210-225°C. Final reaction temperature 200°C.

Sample No.	Weight in Grams	Reaction Time - Min.	0.033 N Fe^{++} Required - ML.	CrO_3 %	Average % CrO_3
1	2.000	16	25.15	1.40	1.40±0.01
	2.000	13	25.30	1.41	
	2.000	9	25.04	1.39	
2	2.000	11	31.55	1.75	1.75
	2.000	9	31.42	1.75	
3	2.000	11	31.90	1.78	1.76
	2.000	10	30.60	1.74	
4	2.000	11	31.70	1.76	1.76
	2.000	13	31.60	1.76	
5	2.000	10	41.32	2.30	2.30±0.03
	2.000	10	41.60	2.31	
	2.000	9	41.87	2.33	
	2.000	10	41.08	2.28	
6	1.002	15	14.65	1.62	1.62±0.01
	1.006	15	14.72	1.63	
	1.012	13	14.51	1.61	
7	1.0037	15	16.03	1.78	1.79±0.01
	1.0017	14	16.19	1.80	

THE LIQUID FIRE REACTION IN THE WET OXIDATION OF BLOOD FIBRIN (3)

Clinical studies of trace elemental blood metallic and non-metallic determinations are of great importance in blood therapy and forensic chemical investigations.

Destruction of blood fibrin by wet oxidation employing either hot concentrated perchloric acid or by application of the liquid fire reaction.

The apparatus assembly is the same as that employed in the reactions for the determination, (Figure 4), of chromium in medical catgut immediately above. Blood fibrin from beef blood dehydration serves as the test sample.

The oxidation is complete, smooth and rapid employing perchloric acid alone as oxygen donor, (60 percent perchloric acid). One milligram of vanadium was tested as catalyst. The sample and perchloric acid is heated on an electric hot plate set to give a maximum temperature of 210-215°C. The blood fibrin is completely soluble in the acid giving a deep purple color. After 3 minutes heating the color alters into a chocolate brown, accompanied by noticeable carbonization. With 10 minutes digestion there is a generous foaming reaction as evolved gases are removed together with fumes of the boiling perchloric acid. The reacting products rapidly cause the color to turn light brown. In 5 additional minutes the oxidation reaction is complete and the wet ash residue colorless, or at most a faint yellow from the presence of a little chlorine. The use of vanadium as catalyst was of no great influence. The volume of 60% perchloric acid used should be 5-6 ml per gram of blood fibrin or a minimum of 10-15 ml to provide enough to cover the bottom of the digestion flask. Results are given in Table 7. Following this procedure one applies 60% perchloric acid which is slowly concentrated to 70-72.5 percent with a steady, relentless crescendo in applied oxidation potential. If desired, the rate of heat application may be retarded which decreases the rate at which the available oxidation potential is made operative. Such modification promotes oxidation without the production of brown reaction products and partial carbonization.

The oxidation of blood fibrin in the application of the liquid fire reaction is more rapid. No carbonization of the organic matter results. By this method a preliminary vigorous oxidation by the nitric acid at temperatures up to 120°C occurs, accompanied by copious brown fumes of nitric oxide. During this stage the frothing may amount to 2.5-5 cms (1 to 2 inches). After 3 to 4 minutes the nitric acid reaction is complete. After an additional 5 minutes the excess nitric acid has been volatilized. The solution then rapidly turns chocolate brown and foaming of 35 mm magnitude

(1.5 inch) sets in and fumes of hot perchloric acid appear. The color of the reacting solution rapidly becomes lighter, CO_2 is evolved and in 15 to 18 minutes the reaction is complete. No catalyst should be employed. Results are given in the data of Table 7.

TABLE 7. WET OXIDATION OF BLOOD FIBRIN USING PERCHLORIC ACID ALONE AND BY USE OF THE LIQUID FIRE SYSTEM IN DESTRUCTION OF ORGANIC MATTER

Sample g.	70-72% HClO_4 ML.	68% HNO_3 ML.	Reaction Time of HNO_3 Minutes	Total Reaction Time Minutes
3	10	10	7	13
4	10	10	5	13
5	12.5	17.5	7	14
6	15	20	5	20
7	15	20	6	20
8	15	25	7	33
9	15	25	7	16
10	17.5	30	8	16
60% HClO_4				
1	15	Absent	—	18
2	15	"	—	15
3	21	"	—	13
4	21	"	—	18
4	25	"	—	21
5	30	"	—	19

Determination of Iron in Blood Fibrin

The wet oxidation of the blood fibrin samples of 1 to 5 grams employing the liquid fire reaction is carried out as previously described. The wet ash residue to which is added a few ml of sulfuric acid is then continued in digestion until all perchloric acid is evolved. The reaction flask is cooled and the solution is diluted to 150 ml. The ferric iron is then reduced to ferrous sulfate by addition of 1 ml of 10% hydroxylamine hydrochloride. A solution of dilute aqueous ammonia is added to give a pH indicated by Congo red paper's acid-base transition, (pH 4 to 4.5). Five milliliters of a saturated solution of bathophenanthroline, (4,7-diphenyl-1,10-phenanthroline), to chelate with the ferrous iron are added. The solution thus resulting may then be extracted employing amyl alcohol and the iron determined spectrophotometrically. Details of this and other applicable spectrophotometric determinations are outlined in detail in the review brochure (4) prepared by Harvey Diehl and the author and made available at no charge by the publishers of the present review. The blood fibrin used for the experiments of Table 7 was found to contain 70 parts per million of iron.

The determination of chromium in leather by wet oxidation can be best

carried out by a liquid fire reaction with added sulfuric acid, which discussion has its proper place in the Section VIII to be subsequently taken up.

For the remainder of the present book section the application of the liquid fire reaction in the wet oxidation of a wide variety of organic compositions, both naturally occurring or of synthetic origin will be illustrated with the results given in tabular form.

The Wet Oxidation of Proteins. Generalizations.

Proteins in general constitute a large group of organic matter of animal origin. Examples are lean beef, casein, albumin, keratin, haemoglobin, elastin, and collagen. The liquid fire reaction serves well in such cases. Proteins contain 50-55% of carbon, 15-17.5% of nitrogen, 19-24% of oxygen, hydrogen 6.5-7.5% and up to 5% of sulfur and in some cases phosphorus. They contain a major proportion of carbon as the carbonyl group. Smooth oxidation takes place with both concentrated nitric acid and perchloric acid when hot and concentrated. Sulfur and phosphorus, if present, are quantitatively retained in protein wet chemical oxidation following their oxidation to sulfate by hot concentrated nitric acid and in the case of phosphorus by hot concentrated perchloric acid. Arsenic, if present, is retained in the wet ash quantitatively only when the wet oxidation takes place under reflux. The Behge apparatus, Figure 2, page 20, rather than the apparatus of Figure 4, page 30 is used in case arsenic is to be determined.

As a typical example of wet chemical oxidation of protein the popular food product dried beef was selected for study. The results are given in the data of Table 8.

TABLE 8. THE OXIDATION OF DRIED BEEF BY USE OF THE LIQUID FIRE REACTION*

(All Tests Included 20 mg of $K_2Cr_2O_7$ As Final Oxidation Indicator)

Sample g.	68% HNO_3 ml.	70% $HClO_4$ ml.	V Added mg.	Maximum Hot Plate Temp. °C	HNO_3 Reac- tion Time Minutes	$HClO_4$ Reac- tion Time Minutes	Remarks
5.0	10	15	9	235	8	9	Vigorous HNO_3 reaction. Much foaming. Exothermic $HClO_4$ reaction. $HClO_4$ reaction stage with green solution only.
10.0	10	15	None	215	15	16	Same as above. Lower hot plate temperature. Reaction mixture took on dark brown color.
10.0	15	25	9	235	3.5	9.5	Reaction mixture dark brown then light brown then green. Some char formation. Last stage exothermic.
8.5	15	25	9	215	13	13	Same as the above except no char formation.

*The reactions of this table were carried out on abnormally large samples using high hot plate temperatures to demonstrate controlled reactions under such conditions. The same procedure at lower hot plate temperatures might be used to advantage in such wet ashing reactions.

From examination of the data of Table 8 it is observed that the hot concentrated nitric acid is capable of oxidizing much of the material. Its destructive action leaves much organic matter following evolution of excess nitric acid. As the reaction temperature mounts to $140-150^\circ$ the perchloric acid completes the reaction stepwise with increasing exothermic reaction attack. Often after partial oxidation in the last stage, by perchloric acid activity, the solution in the digestion flask darkens in color. In this case one adds a small amount, often as little as 1-2 ml of concentrated nitric acid. At $160-170^\circ$ this will immediately decolorize the solution. With the perchloric acid at increase in temperature to $178-180^\circ$, the final oxidation results as an exothermic last stage. For this intermittent addition of nitric acid the apparatus duplicating that of Figure 6 with modified fume eradicator shown is convenient.

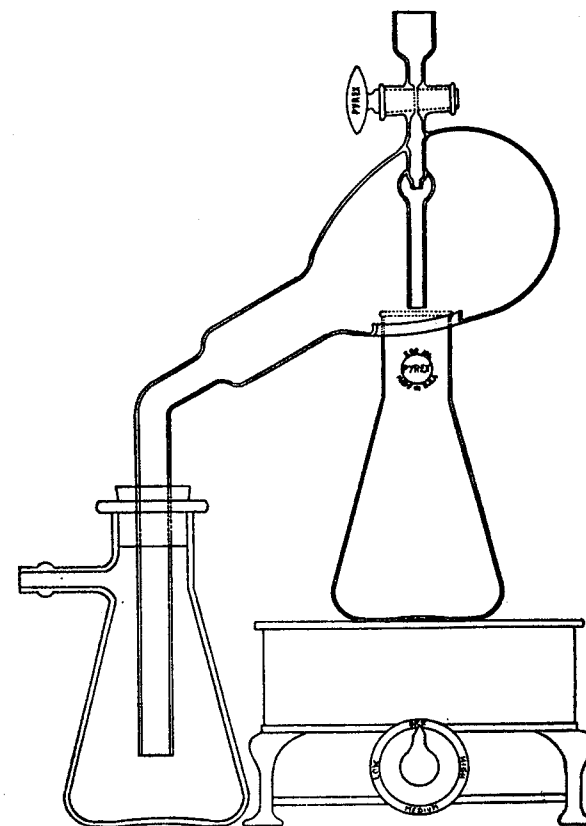


Figure 6.—Modified Fume Eradicator Digestion Assembly

The best reaction conditions in the application of the liquid fire reaction are those which result in complete oxidation without formation of dark reaction products which give the flask reaction contents a dark chocolate color. The use of vanadium as catalyst often prevents development of these chocolate brown reaction developments. The slower increase of temperature often provides the same results.

TABLE 9. THE OXIDATION OF ANIMAL PROTEIN BY HOT CONCENTRATED PERCHLORIC ACID OR THE LIQUID FIRE REACTION

(20 mg of $K_2Cr_2O_7$ added as indicator)

Sample Type	Weight g.	HNO_3 68% ml.	$HClO_4$ 70% ml.	Vanadium Added mg.	Hot Plate Temp. $^{\circ}C$	Time of Reaction HNO_3 min. $HClO_4$ min.	$HClO_4$ Reaction Color	Remarks
Egg White (Hard Boiled)	5.0	10	15	9	235	3.0	6.0	Green
	5.0	10	15	None	230	7.0	32.0	Green
Hippuric Acid	1.0	None	20 (60%)	None	210	—	38.0	Chocolate
	5.0	10	15	None	235	10.0	17.0	Green
Salmon Fillet (Fresh Frozen)	1.0	10	15	9	235	10.0	12.5	Green
	12.5	15	15	None	230	12.0	23.0	Chocolate
	12.5	15	15	9	215	7.5	24.0	Chocolate
	12.5	15	25	None	215	15.0	17.5	Chocolate
	12.5	30	25	None	215	32.0	88.0	Chocolate
	12.5	15	15	None	230	15.0	17.0	Chocolate
Halibut Fillet (Fresh Frozen)								

Smooth reaction throughout
No pronounced exothermic effect.
Omission of HNO_3 modifies reaction unfavorably.
Hippuric acid forms C_6H_5COOH and NH_2CH_2COOH upon acidification.
Smooth vigorous reaction over all.
Reaction too vigorous. Fire resulted.
Exothermic reaction at 32.5 min. Color from brown to green.
Reaction time consuming. Very smooth throughout.
Smooth vigorous reaction throughout.

Sample Type	Weight g.	HNO ₃ 68% ml.	HClO ₄ 70% ml.	Vanadium Added mg.	Hot Plate Temp. °C	Time of Reaction HNO ₃ min. HClO ₄ min.	HClO ₄ Reaction Color	Remarks
Gelatin	3.0	15	15	None	235	12.0 4.0	Chocolate	Addition of vanadium beneficial.
Casein	3.0	10	15	9	235	5.0 6.5	Green	
	2.0	10	15	None	215	13.0 17.0	Chocolate	Vanadium added prevents dark reaction products
	2.0	10	15	0.75	215	13.0 9.0	Green	
Oyster (Fresh)	10.0	10	15	None	215	12.0 16.0	Green	HClO ₄ reaction exothermic
Egg Yolk (Hard Boiled)	3.0	10	15	None	230	10.0 19	Chocolate	
	3.0	20	15	None	230	22.0 9	Chocolate	
	3.0	15	30	None	230	15.0 4	Chocolate	Vanadium effectively oxidizes dark products
	3.0	10	15	9	235	3.0 3	Chocolate	
Wool Yarn	3.0	10	15	None	230	20.5 20.5	Green	Vanadium speeds reaction at low temperature
	3.0	10	15	0.75	215	4.5 10.0	Chocolate	
Pork Rib Bone	3.0	10	15	None	230	8.5 27.6	Green	HClO ₄ markedly exothermic
Fresh Shrimp	5.5	10	15	None	235	15.5 15.5	Chocolate	No catalytic effect using vanadium
	6.5	10	15	9	235		Chocolate	

From an examination of the data of Table 9 it is seen that the boiling nitric acid provides for oxidation of a significant part of the organic matter. After its removal at 120 to 130° the gradual elevation of the temperature represents the second stage of reaction. The extent and increasing intensity of the perchloric acid oxidation stage is indicated by its exothermic trend. Lower hot plate temperature would be appropriate at the expense of elapsed time for the completed oxidation. The addition of vanadium as catalyst again, (as found in the data of Table 8), has in some cases a beneficial effect. In other cases its use may bring about too rapid reaction conditions. In this case lower hot plate temperatures should be employed.

It may be said that in these reaction studies, (Tables 8 and 9), the liquid fire reaction which was applied produced short reaction times for complete oxidations. Less intense heat applications at the expense of reaction time may be preferable in case uncontrolled reaction rates are feared.

The Oxidation of Cellulose Based Organic Compositions

The wet oxidation of hay, alfalfa, grain, tobacco, wood, straw and cotton represent sample types. Perchloric acid may be employed for their oxidation at 60-67 percent concentration and under reflux employing the Bethge apparatus, (as in Section 3). Controlled reaction time may be shortened for this type material and a wider variety of such cellulose rich products may be accommodated by the use of the liquid fire reaction in their mineralization. Mineralization of hay, grain and animal feed for the determination of P, Ca, Mg, and the alkali metals is a most important consideration in the studies of animal husbandry. The use of the liquid fire reaction has been adopted widely in this field of studies.

Illustrative reactions are given in the data of Table 10.

TABLE 10. THE LIQUID FIRE REACTION IN OXIDATION OF
CELLULOSE RICH COMPOSITIONS
(INCLUDING GLYCEROL AND SUGAR)
(Indicator $K_2Cr_2O_7$ added)

Sample Type	Weight g.	HNO_3 68% ml.	$HClO_4$ 70% ml.	Vanadium Added mg.	Hot Plate Temp. $^{\circ}C$	HNO_3 min.	Time of Reaction $HClO_4$ min.	$HClO_4$ Reaction Color	Remarks
Rhubard (Dried)	5.0	10	15	None	235	9.5	23.5	Chocolate Green	Energetic HNO_3 reaction. Catalytic vanadium reaction effective
	5.0	10	15	9	235	4.5	9.0		
Tobacco	3.0	10	15	None	235	8.5	11.5	Light Brown	Some char formation Less char formation No char formation
	3.0	15	15	None	235	14.0	8.5	Light Brown	
	3.0	15	15	9	235	6.5	12.5	Green	
Cellulose	1.0	10	15	None	230	5.0	12.0	Green	HNO_3 reaction predominates
Glycerol	1.0	10	15	None	230	3.0	17.0	Colorless	HNO_3 reaction predominates
Cane Sugar	1.0	10	15	None	230	5.0	17.0	Colorless	HNO_3 reaction predominates
Dried Grass	3.0	10	15	None	230	8.0	19.5	Dark Brown	Both HNO_3 and $HClO_4$ react vigorously

TABLE 11. THE LIQUID FIRE REACTION AS APPLIED TO
NYLON AND ION EXCHANGE RESINS
($K_2Cr_2O_7$ added as indicator)

Sample	Weight g.	HNO_3 68% ml.	$HClO_4$ 70% ml.	Hot Plate Temp. $^{\circ}C$	HNO_3 Effect min.	Time of Completion	$HClO_4$ Color Development	Remarks
Dowex 50	1	10	15	230	—	20	Colorless	No Evolution of NO_2
IR-120	2	5	10	215	—	14	Dark Brown	No Evolution of NO_2 , Foaming
Nylon	2	None	15	215	—	37	Colorless	Rapid
Nylon	2	10	15	215	10	38	Dark Brown	Rapid CO_2 evolution from both HNO_3 and $HClO_4$ reaction

The Destructive Oxidation of Nylon and Ion Exchange Resins.

The application of the liquid fire reaction is given in the data of Table 11.

The liquid fire reaction was found to be effective in the destructive oxidation of nutmeg, olive oil, caraway seed, pecan nuts, ground mustard, ground pepper, ginger, tea, coffee and cinnamon. Details are given in Table 11 in the literature references cited as at the conclusion of this section.

The liquid fire reaction does not apply to the wet ashing of any material not miscible in mixed $\text{HNO}_3 + \text{HClO}_4$, such products as butter, fuel oil or paraffin oil. The wet oxidation of these organics will be subsequently treated in the following section of this review.

Literature Cited

- (1) F. Feigl and A. Schaeffer, *Anal. Chim. Acta.*, **4**, 458 (1950)
- (2) Harvey Diehl and G. Frederick Smith, "Quantitative Analysis," John Wiley 1952 page 304
- (3) G. Frederick Smith, *Ind. Eng. Chem. Anal. Ed.*, **18**, 157 (1946)
- (4) Harvey Diehl and G. Frederick Smith, "The Iron Reagents, Bathophenanthroline, 2,4,6-Tripyridyl-s-Triazine, Phenyl-2-pyridyl Ketoxime," The G. Frederick Smith Chemical Company 1960. (Revised and extended in the 2nd Edition 1965).

SECTION VI

GENERAL PROCEDURE IN THE LOW-TEMPERATURE WET OXIDATION OF ORGANIC COMPOSITIONS PERCHLORIC AND PERIODIC ACIDS AS OXIDANTS "THE PERIODIC ACID LIQUID FIRE REACTION"*

Introduction

Perchloric acid is the most adaptable oxidant in general use for the wet oxidation of organic compositions. With added nitric acid, these combined oxidants serve to constitute the "liquid fire reactions" (1). These mixed reactants are of extensive general applicability. Mixtures of sulfuric and perchloric acids have also been employed (2, 3). The highest oxidation potentials available are thus provided. A wide variety of wet oxidations result from the individual use of perchloric acid (4). For such reactions the perchloric acid is employed at controlled concentrations and temperatures.

In all these wet oxidation procedures, conditions are defined, and suitable apparatus has been designed, to ensure hazard-free reaction kinetics.

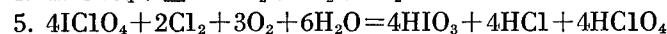
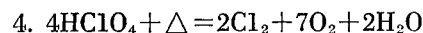
Employing periodic acid in preferential applications, as a substitute for nitric acid, has marked advantages. This modification effects improvements in extensive general applicability. Lower reaction temperatures may be employed. Periodic acid serves not only as oxidant, but contributes to the ready fragmentation of large molecular species, by degradation, to smaller organic entities. The effective oxidation potentials cover the range 1.6 to 2.0 V. The present contribution describes the new procedure, designated "the periodic acid liquid fire reaction."

*The origination of the periodic acid liquid fire reaction resulted from the author's experimentation at the University of Illinois. This study has involved additional research here in the development of a new and novel manufacturing procedure in the synthesis of iodic acid. This procedure involves the vapor phase oxidation of sublimed iodine and the fumes provided by boiling 70-72.5 per cent perchloric acid (5). Both the periodic acid liquid fire reaction now being described and the synthesis of iodic acid were checked out in the laboratory of Professor Harvey Diehl and his graduate students at Iowa State University. Published work on the synthesis of iodic acid by Hayes, Diehl and the author is now in press. Professor Harvey Diehl's checking of the procedure in wet oxidations employing the periodic liquid fire reaction is the basis of this booklet's Section 7 (6).

Reactions Involved

The following reactions are fairly well established and appear to apply concurrently in the reaction mixture:

1. $2\text{H}_5\text{IO}_6 + (\text{HClO}_4 + \Delta) = 2\text{HIO}_3 + \text{O}_2 + 4\text{H}_2\text{O}$
2. $4\text{HIO}_3 + (\text{HClO}_4 + \Delta) = 2\text{I}_2 + 5\text{O}_2 + 2\text{H}_2\text{O}$
3. $\text{HIO}_3 + 2\text{I}_2 + 5\text{HClO}_4 + 3\text{H}_2\text{O} = 5\text{IClO}_4 + 3\text{H}_2\text{O}$



The effective degradation of the large complex molecules of organic compositions by periodic acid, to form fragmentation products of lesser molecule weight, is of major importance to the reaction mechanism. Such reactions are extensively applied in a wide variety of reactions of practical analytical determinations and studies of related structural significance. The extent to which these reaction types contribute to the wet oxidation reactions being described, is not well established. The reactions in strong perchloric acid, at moderately elevated temperatures (150-200°), are undoubtedly much more extensive and complicated than those which apply in neutral or moderately acid solutions. Evidence will be submitted which demonstrates the reality and extent of the periodic acid fragmentation reactions which contribute effectiveness to the periodic acid liquid fire reactions.

In the reactions stated above the oxidants simultaneously operative are periodic, iodic and perchloric acids, iodine and iodine perchlorate, oxygen and chlorine.

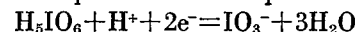
Reaction (1), the thermal dissociation of periodic acid in hot concentrated perchloric acid, is relatively slow. Tests in boiling 70% perchloric acid indicate that 3 to 5 min are required. In lesser strength acid, a longer interval is required. In the wet oxidation mixture, therefore, the periodic acid is available for a sufficient period to provide both for fragmentation and oxidation.

Reactions (2) and (3) occur simultaneously at low temperature (40-70°), in the presence of organic matter dispersed in concentrated (60-70%) perchloric acid. These reactions are markedly exothermic. Perchloric acid concentrations should be selected which minimize reaction (2) to such rates that reaction (3) keeps pace with reaction (2). Both iodine and iodine perchlorate may be evolved. They may be retained by the use of a reaction apparatus of the vapor refluxing principle.

Reaction (4) increases in rate as the perchloric acid concentration is augmented from 60 to 72.5% and at its boiling point.

Reaction (5) is slow, but intensifies with increases under the same conditions as reaction (4). Iodine is oxidized to iodic acid, following the immediate formation of iodine perchlorate, under the same conditions. Total destruction of organic matter often occurs before reactions (4) and (5) have become jointly complete.

The standard reduction potential of the periodic-iodic acid couple (7)



has been estimated to be 1.6 V. The oxidizing power of perchloric acid depends upon its concentration and temperature. Exact measurements of its

reduction potential have not, and perhaps cannot, be made. Even the exact nature of the reduction products is not known with certainty. On the basis of its effects at different concentrations approximate values can be assigned. Thus, since in 58 to 60% perchloric acid at 150° to 160° ferrous iron is oxidized to ferric, the potential is estimated to be 0.8 V. For progressively increasing concentrations, from 60 to 72.5%, the boiling temperature increases in almost direct proportion (to 203° at 760 mm for the 72.5% water-perchloric acid azeotrope), and the oxidizing power rises markedly and approximately in direct proportion, reaching an estimated value of 2.0 V.

In the periodic acid-perchloric acid mixture, the former brings about the initial reaction in oxidation of organic compositions at moderate temperature and with controlled intensity. Fragmentation of organic compositions by periodic acid is well known. It reacts rapidly upon organic material bearing hydroxyl or amino groups on adjacent carbon atoms, aldehydes and ketones being assumed to be hydrated. Degradation products, formic acid and probably oxalic acid among other simple formulations, are then readily oxidized to carbon dioxide through the medium of hot concentrated perchloric acid.

Fragmentation is more extensive in the reactions at hand owing to the presence of concentrated perchloric acid. As the temperature increases following initiation of oxidation by periodic acid, which is exothermic, the perchloric acid provides more extensive fragmentation before final conversion of carbon to carbon dioxide. The perchloric acid has an additional function. It effects dissolution of organic matter of a wide range of structures, both in hot or cold concentrated acid, without being accompanied by char formation. The periodic acid liquid fire reactions are thus seen to have many unusually effective reaction advantages.

Experimental

Reagents

Periodic Acid: Colorless, crystalline, non-hygroscopic, reagent grade para-periodic acid, H_5IO_6 . Free adsorbed water, at 85°, not more than 0.3%. Non-volatile after ignition, not more than 0.01%. Assay 99.98%. Sulfate and heavy metal free. Commercially available.

Perchloric acid: Reagent grade vacuum distilled. Assay 66.0, 68.0, 70, and 72%. Non-volatile matter, as sulfate, not in excess of 0.003%. Sulfate, chloride, phosphate and iron free. Intermediate concentrations are obtained by blending. Commercially available.

Vanadium: Used in mg amounts as catalyst. NH_4VO_3 or V_2O_5 , reagent grade.

Apparatus

The most suitable apparatus has been previously described and its design designated by line drawings, Figs. 2, 3, 4 pages 20, 21 and 30. The Bethge apparatus serves best for general use. The apparatus employed in the liquid fire reactions may serve for many applications of the new periodic acid liquid fire reaction, for example in the determination of heavy metal trace elements in the wet ash residue. Vycor reaction flasks are preferable but Pyrex glass may be substituted. The Bethge apparatus provides for reflux retention of volatile products. This maintains a fixed concentration of the original perchloric acid employed. The complete retention of volatilized spray formation is assured. By use

of the three-way stopcock of the Bethge apparatus, an increase in the concentration of perchloric acid wet ash residue is made operative.

Oxidation of cellulose

To 1.0 g cellulose, in the 250-ml conical flask of the Bethge apparatus, was added 15.0 ml of 68% perchloric acid and 1.5 g of para-periodic acid. The mixture (both reactants cellulose and periodic acid, being soluble in the perchloric acid) was heated gently to initiate the reaction, which began at 40 to 50°. The reaction was markedly exothermic and, in 15 to 20 sec after initiation, the temperature rose to 100 to 110° without further heating. Heat was again applied and the oxidation of the cellulose was complete in 2 to 5 min. Continued heating to the boiling point completed the conversion of the iodine perchlorate formed to iodic acid. The latter operation is favored by cutting off the return of condensate. The course of the temperature during the operation is shown graphically in Fig. 7.

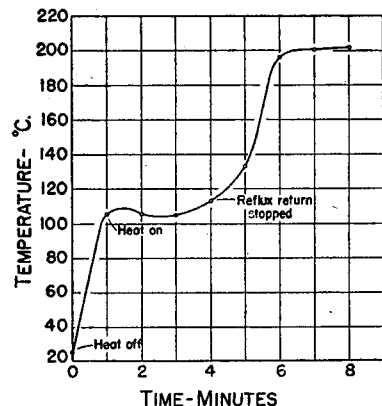


FIG. 7.—Cellulose oxidation.

Oxidation of wool

Wool yarn was subjected to the periodic-perchloric acid treatment under the same conditions given above for cellulose. Wool is not measurably attacked by concentrated perchloric acid when cold, but it is dissolved when the acid is heated. The initial reaction is exothermic, but to a lesser degree than with cellulose. Little or no iodine was evolved. Oxidation was complete in 5 min, the final temperature being 170°. The course of the reaction temperature effects is shown in Fig. 8.

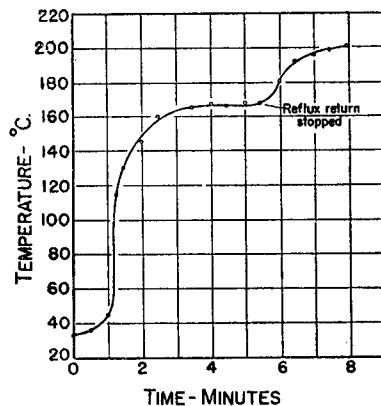


FIG. 8.—Wool oxidation.

Oxidation of sucrose

Cane sugar is soluble, without charring, in concentrated perchloric acid. Its oxidation under conditions identical with those given above for cellulose follows. The reaction began at about 40° and was markedly exothermic. Iodine was liberated, then oxidized to iodine perchlorate. Oxidation was complete in 5 or 6 min, the final temperature being 190°, with most of the time taken to effect oxidation of the iodine perchlorate to iodic acid. The results are shown graphically in Fig. 9.

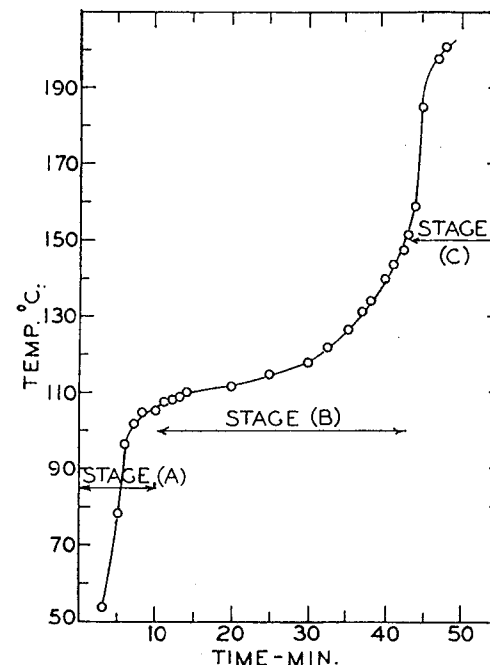


FIG. 9.—Sucrose oxidation.

Oxidation of vegetable and chrome tanned leather

Vegetable tanned leather was treated in duplication of conditions given for the oxidation of cellulose. The initial reaction occurred at approximately 40° and the reaction was complete in 14 to 15 min, the final temperature being 175°. In the initial reaction considerable iodine was formed, soon to be converted to iodine perchlorate. The results are shown in Fig. 10. Chrome tanned leather is often fortified by incorporation of such additives as paraffin and barium sulfate. The presence of 3 to 6% of chromium in the tanning process also increases the difficulty in its wet oxidation. The same method of attack was applied to a sample of such type chrome tanned leather as that applied to vegetable tanned leather, except that 2 mg of vanadium was added as catalyst. At the initial reaction temperature, 50°, the temperature rose rapidly to 150° in 4 min. At 5 min the solution of wet ash acids has a green color, with considerable turbidity, and oxidation was essentially complete with the flask walls coated with paraffin still unoxidized. After 9 min the reflux was cut off and at 13 min and 198° the reaction mixture was orange colored from dissolved chromic acid, indicating complete removal of organic matter. The boiling wet ash solution was clear but, upon cooling, a precipitate of barium sulfate was present.

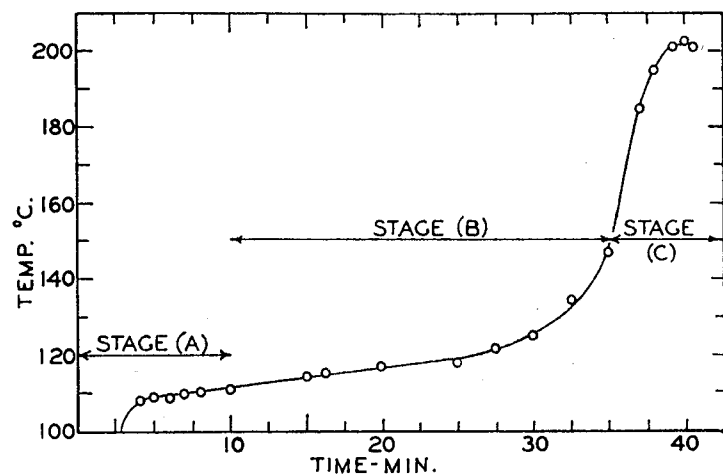


FIG. 10.—Vegetable-tanned leather oxidation.

Wet oxidation of various types of coal

The wet oxidation of soft coal (high and low V.C.M.) has recently been described. The major objective of the present study was to provide conditions of wet oxidation suited to the subsequent determination of arsenic. It was postulated that the periodic acid liquid fire reaction would provide more suitable reaction conditions than in the previously cited method. This was found to be the case. In the previous study, the wet oxidation procedure developed the condition that the volatile combustible matter of coal was evolved as in the case of the regularly applied V.C.M. determination in the standard (proximate analysis) analytical routine. By the use of the present procedure the volatile combustible matter in coal is oxidized rather than volatilized. In addition, the presence of periodic and iodic acids, as well as iodine perchlorate, ensures more favorable conditions for retention of arsenic in the wet ash residue.

Coal samples of all types may be boiled in contact with 70% perchloric acid for periods of 1 to 3 hr before complete oxidation results. The presence of a few mg of chromium shortens the required boiling period to 30 min. The addition of 1 or 2 mg of vanadium in place of chromium catalytically shortens the required boiling period to 15 min. The reaction mechanism accounting for this valuable catalyst influence has been previously described.

The time required for the oxidation of coal by a new procedure varies to a large extent with the initial concentration of perchloric acid. Thus, for anthracite (see Fig. 11, a 1 hr digestion was required using 70.0% perchloric acid concentration, but only 20 min using 71.28% concentration. With either concentration of perchloric acid, the initial reaction set in at 30 to 40° and was exothermic. For the lower acid concentration the formation of iodine perchlorate was very pronounced; at the higher concentration little was formed and its conversion to iodic acid was rapid. The results for the oxidation of anthracite coal are shown graphically in Fig. 11.

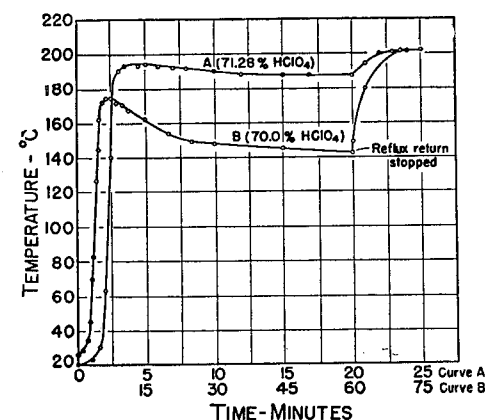


FIG. 11.—Anthracite coal oxidation.

Soft coals containing a moderate amount of volatile combustible matter, such as Pocahontas coal, require less concentrated perchloric acid for their rapid oxidation. Considerable foam, a layer 25 to 40 mm thick, was formed but did not interfere. Relatively little iodine was formed. Starting with 69.5% perchloric acid, the reaction was practically complete in 5 min and assuredly complete in 10 min at 200°; with 69%, the time was about doubled. The results are shown in Fig. 12.

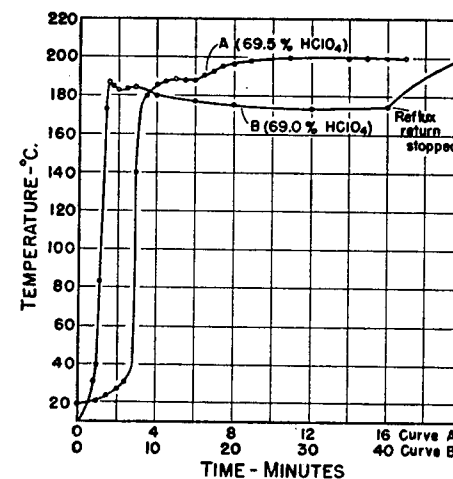


FIG. 12.—Pocahontas coal oxidation.

Bituminous coal containing high volatile combustible matter, such as the Orient coal of Southern Illinois, was oxidized expeditiously with high initial concentrations of perchloric acid, 70.0 and 71.28%. Considerable foam, a layer of approximately 50 mm thick, was present during the early stages of the reaction. Some iodine was formed in the 70%

perchloric acid digestion but little or none in the 71.28% mixture. The effect of the initial acid concentration on the time required is shown in Fig. 13.

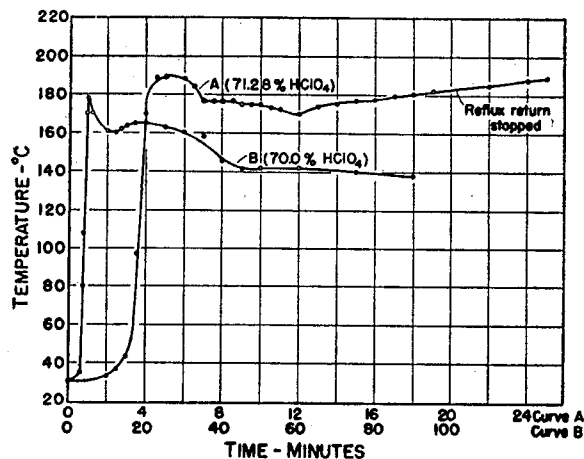


Figure 13.—Illinois Orient Coal Oxidation.

As was expected, the ash remaining following the periodic-perchloric acid digestion of coal was less than that obtained by dry ashing: Pocahontas 6.83 and 9.72%, respectively; Orient coal 4.60 and 7.50%, respectively.

Oxidation of adipic acid

Because of the use of adipic acid in Nylon synthesis its wet oxidation was studied. The Bethge apparatus reactants were: 1.0 g of adipic acid, 15.0 ml of 65.5% perchloric acid, 1.5 g of para-periodic acid, both with and without vanadium. The results were as follows:

1. Without vanadium, the reaction was smooth and complete in 10 min. The maximum temperature was 162°. The evolution of iodine was excessive for best conditions.
2. With 1.0 mg of vanadium added, the reaction was smooth, more rapid and the evolution of iodine retarded in favor of iodine perchlorate. The oxidation was complete in 8 min at a maximum temperature of 159°.
3. With 2.0 mg of vanadium added, the best conditions were attained. The reaction was complete in 6 min and the maximum temperature was 157°.

The last of these conditions was applied to the oxidation of Nylon with the exception that the perchloric acid concentration was augmented.

Oxidation of nylon

For Nylon oxidation (polymerized adipic acid and hexamethylenediamine), the use of 69.5% perchloric acid was found to favor iodine perchlorate formation with little iodine. The data for this oxidation is given in Fig. 14. Nylon is seen to be completely oxidized at a maximum temperature of 145°. The ashed acid residue, upon cooling, deposited ammonium perchlorate. The wet ash was soluble in water, except for its titanium dioxide when used as additive.

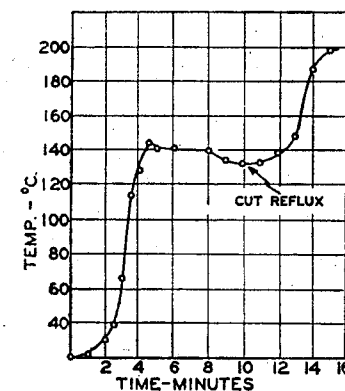


FIG. 14.—Nylon oxidation.

Oxidation of uric acid

Uric acid was investigated as an organic type involving heterocyclic ring nitrogen structure. Also, because it is a component of urine and of many types of fecal matter. One gram of uric acid with 1.5 g of periodic acid, 15.0 ml of 65.5% perchloric acid and 2 mg of vanadium, was digested in the Bethge apparatus. The initial exothermic reaction was at 45-50°, evolving a modest amount of iodine, followed by iodine perchlorate. Reaction was complete after 5 to 6 min at a maximum temperature of 150°. The cold wet ash residue deposited ammonium perchlorate and was completely soluble upon dilution with water.

Oxidation of methyl methacrylate or methyl methacrylate-polymer P-1 and lucite

Methyl methacrylate is miscible with 68.5% perchloric acid. It is smoothly oxidized by the use of the periodic acid liquid fire reaction. Low temperature treatment retards foam formation. Vanadium, as catalyst, is effective. One gram samples were oxidized in 15 ml of perchloric acid in 17 min without vanadium. Ten minutes were required with 2 mg of catalyst. 1.5 g of para-periodic acid constitutes a generous excess. The polymer of methyl methacrylate, du Pont P-1, in the presence of vanadium, was similarly oxidized in 16 to 17 min. Lucite was similarly oxidized in the same time interval. The production of iodine perchlorate as the reduction product of periodic acid predominated. At a lesser perchloric acid concentration iodine was formed initially. The oxidation of polymerized methyl methacrylate is shown in Fig. 15.

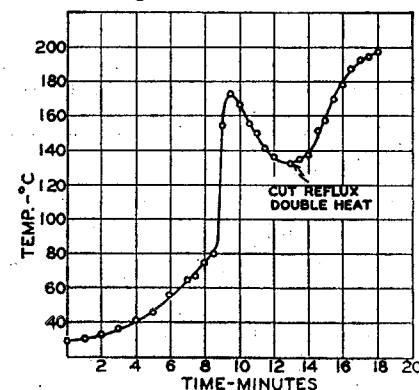


FIG. 15.—Methyl methacrylate oxidation.

As shown in Fig. 15 reaction is initiated exothermally at 80°. It would appear that, in all these reactions, the oxidation follows the same course as for methyl methacrylate. The initial exothermic reaction is followed by a second stage of oxidation contributed by perchloric acid. This latter oxidation accounts for the catalysis by vanadium.

Iodine perchlorate as the reduction product of periodic acid

A 60-g sample of the volatile orange colored reduction product from the periodic acid oxidation of organic matter was collected for examination of its composition. Upon distillation the sample dissociated materially to liberate iodine. The most stable fraction had a constant boiling point of 112.5°. This fraction was subjected to analysis for iodine by titration of its solution in excess potassium iodide using sodium thiosulfate. A sample weight of 0.5261 g required 26.60 ml of 0.0930*N* thiosulfate, corresponding to 0.5770 g of $I(ClO_4)_3$. The problem was not studied further. The conclusion was postulated that the reduction products of periodic acid in the oxidation of organic matter consists of iodine monoperchlorate which dissociates to liberate iodine, thus accounting for random ratios of iodine to the perchloric acid anions. In the reactions as described, iodine and iodine perchlorate are oxidized to iodic acid by boiling with concentrated perchloric acid.

Polyethylene oxidation

Polyethylene is well known to be non-reactive in contact with hot concentrated perchloric and periodic acids. The periodic acid liquid fire reaction could thus be predicted to be ineffective. This was experimentally substantiated. Evidence was produced that oxidation was progressing due to the formation of iodine perchlorate and by the exothermic reaction properties, but the time that would be required for complete oxidation would be excessive.

The rapid oxidation of polyethylene is provided by boiling 70-72% perchloric acid with added ceric ion. This reaction at 200°, is rapid (10 to 15 min), and the wet ash residue is completely soluble when hot and, after dilution, when cold. The high oxidation equivalent weight of cerium requires the use of considerable amounts. These tests indicate that polyethylene requires the application of 1.9 to 2.0 V for rapid destructive oxidation.

Procedural treatment of residual wet ash solution

If the removal of iodic acid from the wet ash solution is desirable, this is accomplished by its dilution on addition of an equal portion of water followed by a stream of sulfur dioxide from a small, lecture demonstration, pressure bottle. Boiling will remove the iodine, leaving a perchloric acid solution. If perchloric acid is objectionable, the addition of sulfuric acid and boiling to expel it may be easily provided. In many procedures for determination of wet ash concentrates neither iodic nor perchloric acids are interferences. An excess of sulfur dioxide causes the formation of hydriodic acid. It is, therefore, an advantage to add the sulfur dioxide to the boiling, diluted wet ash residue. Small amounts of sulfuric acid are formed by the sulfur dioxide reduction of iodic acid.

Advantages in The Application of The Periodic Acid Liquid Fire Reaction

The new procedure, herein described, is the most widely applicable of any process which may be applied in wet ashing techniques. It is seen to apply to the rapid wet oxidation of cellulose, sugars and other polyhydric organic compositions, proteins and protein rich materials.

The special feature of these reactions is the much lower temperature required.

The most important reaction property is that of degradation reactions of heavy molecular weight species to produce smaller fragments which are readily destroyed through contact with concentrated perchloric acid. The

effective catalysis of the oxidation by very small amounts of vanadium is thus made operative to a greater extent.

The oxidation of coal at the lowest temperature which is effective in complete removal of organic matter is an attractive procedure in preparation for the determination of arsenic.

The special property of periodic acid in fragmentation of polymeric compositions has made the wet oxidation of such products as nylon, methyl methacrylate polymers and other comparable products easily operative.

Owing to the use of low strength perchloric acid it is postulated that all amino nitrogen of organic compositions is retained in the wet ash digestion residue. This assumption is now being investigated.

The wet oxidation of cellulose type products such as dried feeds, which is a much employed procedure in the determination of calcium, magnesium, sulfur, phosphorus and the alkali metals as applied routinely by the animal nutritionists using the nitric acid liquid fire reaction almost entirely, may now be preferentially replaced with gains in many considerations.

The periodic acid wet oxidation procedure has been applied to cellophane, Dacron and Orlon fabrics, dried clover, corn leaves and ground corn stalks, honey, dried beef, Lucite, Du Pont Mylar, and starch, with equal success. Many other applications are deemed attractive.

Because of the use of much less highly concentrated perchloric acid in the reactions described, and the need for only lower temperatures of reaction, there is no tendency to encounter uncontrolled reaction rates.

Literature Cited

1. The Author, *Anal. Chim. Acta.*, **8**, 397 (1953)
2. The Author, and Harvey Diehl, *Talanta*, **3**, 41 (1959)
3. Harvey Diehl and The Author, *ibid.*, **2**, 209 (1959)
4. The Author *Anal. Chim. Acta.*, **17**, 175 (1957)
5. In Press
6. The Author and Harvey Diehl, *Talanta*, **4**, 185 (1960)
7. W. H. Latimer, "Oxidation Potentials," Prentice Hall Inc., 2nd Ed., 1952 p. 66

The Determination of Iron and of Copper in Wine. The Application of The Liquid Fire Reaction Followed by Spectrophotometric Determinations.

(1,10-phenanthroline and 4,7-diphenyl-1,10-phenanthroline in the determination of iron and 4,7-diphenyl-2,9-dimethyl-1,10-phenanthroline in the determination of copper).

Introduction

The determination of iron in wine is a requisite routine plant control analysis. A wine processing procedure which leaves the bottled wine free from iron in excess of 5-10 parts per million is followed. Bottled wine having iron in excess of this limit promotes "iron casse" (turbidity due to formation of insoluble hydrolytic iron compounds). Such turbid conditions downgrade wine in quality and in resultant consumer acceptance.

The determination of trace contamination by copper in wine is of greater significance. Copper content in excess of 1-2 parts per million contributes to the development of "off flavors" during short periods of shelf life. In the case of dairy products (such as milk and butter) this quality impairment is termed "a tallowy flavor."

Procedures for the determination of iron and of copper in wine, at present preferred, involve "wet ashing" in the preparation of the sample for determination of iron and of copper. All organic matter (alcohol, sugars, coloring matter, etc.) of the wine sample are destructively oxidized and removed by this process. Two reaction techniques have been practically applied in the wet ashing procedure. The preferred reaction is the nitric-perchloric acid digestion. This process has been applied subsequent to wet ashing employing mixed perchloric and sulfuric acid, (the Saywell and Cunningham process (1)). The liquid fire reaction employed by Banick and Smith (2) is a much to be preferred wet oxidation procedure. With this latter procedure being used as control, the *in situ* determination of iron and of copper in wine was then developed (2) as a procedure which did not involve the preliminary wet ashing of the sample.

In the published procedure of Deibner and Bouzique (3), a wet ashing procedure employing hydrogen peroxide, which is more time consuming and less effective than either the sulfuric-perchloric acid or liquid fire reaction, was used in the destructive oxidation of the wine sample. Deibner and Bouzique failed to cite the work of Saywell and Cunningham which employed

1,10-phenanthroline as color reagent. Copper was not investigated by Saywell and Cunningham.

As an improvement over the use of 1,10-phenanthroline in the spectrophotometric determination of iron, Banick and Smith substituted the doubly sensitive, 4,7-diphenyl-1,10-phenanthroline described originally by Smith, McCurdy, and Diehl (4). The trivial name adopted for this new chelation reagent is bathophenanthroline, a specific ferroin reactant for the determination of iron in the presence of copper.

The determination of copper follows the use of another sensitive and specific ferroin reacting organic reagent, (Smith and Wilkins (5)), 4,7-diphenyl-2,9-dimethyl-1,10-phenanthroline for which the trivial name bathocuproine is used. This is a copper chelation reagent used for the spectrophotometric determination of copper and which is copper specific, (unreactive with ferrous iron), and of high molecular extinction coefficient. Both these reactants may be used following wet oxidation of the wine sample. Their use in the *in situ* determination of iron and of copper is an advantageous innovation. Its application saves the need for wet ashing of the wine and has been found to be of equal accuracy in the determination of trace amounts of iron and copper.

The Determination of Iron and of Copper in Wine. The Method of Saywell and Cunningham.

The first procedure employing wet oxidation of dried fruits preparatory to the determination of iron was that of Leavell and Ellis (6). The sample, (0.5 g), was suspended in a mixture of concentrated sulfuric acid, (3 ml), to which had been added 0.5 ml of 60 percent perchloric acid using a small Kjeldahl flask as container. The reaction was heated until the organic matter was well carbonized. After cooling, additional 0.5 ml portions of 60% perchloric acid were added, with digestion followed by cooling and repetition of the procedure until the reaction mixture is free from carbonaceous reaction products.

Such sample treatment required 30 minutes to 120 minutes. Milk powder, blood fibrin as well as dried fruits could thus be wet chemically oxidized. Leavell and Ellis, following the preparation of the sample in elimination of organic matter, determined iron by the mercaptoacetic acid colorimetric method.

This was a major improvement over the Stugart method of dry ashing and thiocyanate color estimation (7).

Saywell and Cunningham (1) developed an improved procedure for the determination of iron in fruit juices and wine. Employing the wet ashing

procedure of Leavell and Ellis (6), the estimation of iron content was provided by the chelation of ferrous iron with 1,10-phenanthroline. This procedure was accurate down to one part of iron in 10 million parts of sample.

"As the iron in the strongly acid solution remaining from the wet-ashing is in the ferric condition, reduction to the ferrous condition before the addition of o-phenanthroline is necessary. . . . Iron-free hydroxylamine hydrochloride has been adopted."

"Pyrex test tubes 25x150 mm, are calibrated to contain 10 and 15 ml. Using such tubes the entire procedure of wet ashing, development of color, and dilution may be carried out in the same tube without possible error of transfer and at a considerable saving of time. Iron-free concentrated sulfuric acid, 60 or 70 per cent perchloric acid, 10 per cent aqueous hydroxylamine hydrochloride solution, 1.5 percent solution of o-phenanthroline in ethyl alcohol, and concentrated ammonium hydroxide are required. Congo red paper also is used."

"A primary standard iron solution is prepared by dissolving 1 gram of pure iron, G. Frederick Smith Chem. Co. Item No. 226, in dilute sulfuric acid and concentrated nitric acid. Oxides of nitrogen should be expelled by boiling to fumes of sulfur trioxide. The solution is then diluted to 1 liter. Other standard solutions may be prepared by diluting 20, 10, and 5 ml. of primary standard to 1 liter each, these standards contained 20, 10, and 5 ppm of iron respectively. Further standards may be prepared as desired. All standards should be made acid with sulfuric acid."

Procedure

"Samples of 2 ml. of a fruit juice or wine are digested with 1 ml. of concentrated sulfuric acid and 0.7 to 1 ml of 60 to 70 per cent perchloric acid in the Pyrex test tubes over a free flame. It is convenient to hold the tube in a wire clamp and agitate contents vigorously during the heating, thereby greatly increasing the rate of heating and digestion. Digestion to a clear water-white liquor will occur ordinarily in 2 to 10 minutes. If combustion is not complete in this time an additional 0.5 to 1 ml of perchloric acid should be used and digestion continued. When digestion is completed, 1 ml of distilled water is added. This is followed by the addition of 1 ml of the 10 percent hydroxylamine hydrochloride solution, shaking, and the addition of 0.5 ml of the 1.5 percent o-phenanthroline solution. Concentrated ammonium hydroxide (about 2 ml) is added until a light red or pink color develops, a small piece of congo red indicator is dropped into the solution, and the addition of ammonium hydroxide is continued until the Congo red paper is distinctly pink. In the meantime the full color of the ferrous o-phenanthroline complex will have developed. The solution is then diluted with distilled water to the graduation mark on the test tube indicating a volume of 10 ml and thoroughly mixed. If the color intensity of this solution is greater than that of the highest standard available, the unknown solution may be diluted to the 15 ml graduation mark and appropriate calculation made. Determinations may conveniently be made on a series of samples, and the reagents added consecutively from measuring pipets or burets."

"Standards should be prepared by the same procedure. A blank determination on the distilled water and reagents should also be made. For purposes of comparison with a colorimeter, standards containing 2.5, 5.0, 7.5, 10, and 15 ppm, respectively, have been convenient. For routine plant purposes where many determinations are being made, much time may be saved by having permanent standards of 1, 2, 4, 6, 8, 10, 12, and 15 ppm, respectively, in a rack so that it is only necessary to compare the solution in two similar

tubes. The iron content can be easily estimated to within 1 ppm or better in this manner. . . ."

"A group of four wines of varying sugar and alcohol contents were taken for duplicate analysis by each of four analysts. The data are presented in Table 1.

Samples for Analysis

TABLE 1. IRON CONTENT OF WINES

Analyst	Iron Content			
	Muscat ppm	Haute Sautern ppm	Dry Sautern ppm	Claret ppm
A	2.4	7.6	7.4	5.9
	2.4	7.7	7.4	6.0
B	2.3	7.7	7.3	5.8
	2.4	7.7	7.4	6.0
C	2.4	8.0	7.5	6.0
	2.3	7.9	7.3	6.0
D	2.4	7.7	7.5	6.0
	2.5	7.6	7.5	5.9

"Another group of six wines were analyzed in duplicate for iron by the mercaptoacetic acid, Stugart and o-phenanthroline methods. The data are given in Table 2.

TABLE 2. IRON CONTENT OF WINES

Method	Iron Content					
	Muscat ppm	Sherry ppm	Port ppm	Sautern ppm	Riesling ppm	Claret ppm
Stugart	3.3	3.1	3.5	4.8	7.4	3.9
	3.1	3.5	3.2	4.9	7.8	3.8
Mercaptoacetic acid	3.2	2.8	3.1	5.4	7.1	3.7
	3.4	2.9	3.3	5.0	7.8	4.7
o-phenanthroline	3.4	3.3	3.2	5.2	7.4	3.9
	3.5	3.3	3.2	5.3	7.3	4.0

Resume Commentary

The choice of mixed perchloric and sulfuric acids by Leavell and Ellis (6), and by Saywell and Cunningham (1) for the wet ashing of wine, fruit juices and dried fruit was an innovation that served its purpose. However, this wet ashing method is a precision time saver when compared with the dry ashing process of Stugart (7), but not to be preferred over the liquid fire reaction. The principle effect of the hot concentrated sulfuric acid is the decomposition of organic matter and the black formation of carbon-char degradation products. The perchloric acid is added to complete the conver-

sion of the charred carbon to carbon dioxide. This is an unnecessarily time consuming process, not particularly well adapted to the wet ashing of wine.

Since the principle components of wine to be eliminated are sugar and alcohol, the liquid fire reaction for wet ashing is much to be preferred. The digestion with nitric acid as oxygen donor in the absence of perchloric acid swiftly eliminates both alcohol and sugar by removal of these ingredients at temperatures below those at which the hot perchloric acid subsequently completes the removal of organic matter. Instead of the reaction of mixed perchloric and sulfuric acids in wet ashing which promotes char formation and requires stepwise addition of perchloric acid, the liquid fire reaction as a substitute completes the wet oxidation in one operational digestion in a very few (2-3) minutes. The nitric acid excess is volatilized away and if desired a little sulfuric acid may be added after wet oxidation is complete and the excess perchloric acid expelled from the wet ash residue.

As will be subsequently demonstrated, the perchloric-sulfuric acid wet oxidation procedure has its valuable utility; nonetheless, it is not the proper choice for the wet oxidation of wine, fruit juices, dried fruits, dried milk powder and similar organic compositions. The Saywell and Cunningham analysis of wine could just as effectively apply to the determination of iron in wine if the liquid fire reaction of $\text{HNO}_3 + \text{HClO}_4$ had been applied.

The use of the liquid fire reaction in the wet oxidation of wine is demonstrated in the following reprinted data (2) from the published work of Banick and Smith.

The In Situ Determination of Iron and of Copper in Wine With control analyses applying the $\text{HNO}_3 + \text{HClO}_4$ liquid fire reaction

The samples for analysis were all of Continental European vintage, three white wines and two red wines. The various wine samples are listed in Table 3.

TABLE 3. WINE SAMPLE IDENTIFICATION AND TYPE

Sample no.	Type	Trade name	Winery	Origin	Alcohol content vol. %	Wt. of 5 ml sample g
1	Red	Famosa Sherry	Manual Fernandez	Spain	19.5	4.984
2	White	Graacher Muenzlay (1949)	Max Huesgen	Germany	11.5	4.972
3	White	Sauternes (1952)	Lolivier Freres	France	13.0	5.027
4	Red	Moulin-A-Vent (1952)	F. Hasenklever	France	12.0	4.926
5	White	Binger-Babbel-Lieschen (1952)	Heinrich Jung	Germany	10.5	4.982

The samples of wine listed in Table 3 were purchased from the Great Lakes Wine Company of Chicago, Illinois (Permit No. 9-1-87), importers.

Pertinent Reference Physical Constants of Wine Samples

Data relative to the pH of the various wine samples as sampled, and after buffer solution was added, together with the weight of a pipetted 5-ml sample are given in Table 4.

Reagents Required

Perchloric acid. Double distilled 70-72% is preferred. Single vacuum distilled acid may be employed.

Nitric, sulfuric and hydrochloric acids. Reagent grade acids (67%, 95% and 37% strength respectively).

Water. The best sample of de-ionized water should be employed and for a series of determinations a large enough supply should be at hand to insure comparable blank corrections.

TABLE 4. PHYSICAL CONSTANTS OF WINE SAMPLES
(ORIGINAL pH, BUFFERED pH AND SAMPLE WEIGHT)

Wine sample no.	Sample pH	Sample buffered in equal volumes. Buffer pH = 4.0	Sample buffered in equal volumes. Buffer pH = 7.1	Wt. of 5.00 ml. sample g
1	3.5	4.3	5.9	4.984
2	3.1	4.1	5.7	4.972
3	3.6	4.1	5.7	5.027
4	3.4	4.0	5.7	4.926
5	3.5	4.0	5.7	4.982

Buffer solutions. For pH 4.0 an acetic acid-sodium acetate buffer is employed. For the buffer (pH 7.1), an ammonium acetate buffer is suitable.

Ethyl alcohol. Reagent 95% ethanol.

Copper sulfate solution. A solution standardized to contain 0.1277 mg of copper per gram of solution. Weight burets were employed in sampling this solution. Other values may be employed of comparable concentration.

Isoamyl alcohol. Reagent grade redistilled.

Hydroxylamine hydrochloride. Best reagent grades tested to prove their limited amount of iron impurities which seem always to be present to a greater or less degree. Both 10% and 20% by weight aqueous solutions are prepared.

1,10-Phenanthroline, aqueous solution. Mol. wt. = 198 as the monohydrate. Prepare a 0.01M solution in de-ionized water.

Bathophenanthroline (4,7-diphenyl-1,10-phenanthroline). Mol. wt. = 332. Prepare a 0.01M solution in ethyl alcohol.

Bathocuproine (4,7-diphenyl-2,9-dimethyl-1,10-phenanthroline). Mol. wt. = 360. Prepare a 0.001M solution in ethyl alcohol.

Apparatus Required

For wet ashing procedure it is always preferable to employ Vycor digestion flasks. The perchloric acid-water azeotrope has a boiling point of approximately 203°C. at atmospheric pressure. Rapid cooling of pyrex flasks from this temperature to 15 or 20°C sometimes may cause too great heat strain and result in fractured flasks. It is not a requisite to employ Vycor reaction flasks; however, it is not only a wise precaution but makes possible chilling of such flasks from 200°C by immersion at once under running cold tap water or preferably by immersion in a bath of ice-water with swirling motion for rapid cooling. See the apparatus drawing Figure 4 page 30.

Transfer pipets are suitable for the sampling of the wine for analysis as well as for the addition of reagents during the progress of the reactions. Individual pipets used exclusively for separate reagents should be provided.

Weight burets should be employed for the addition of standard copper sulfate solution in the determination of the blank as well as the wine copper plus blank determination.

The stock units represented by the standard type of 60 ml. separatory funnel are less convenient in application because of the diameter of the opening just above the delivery stopcock. Using pyrex separatory funnels it is preferred to remove the stock stopcock immediately above its union with the bowl of the apparatus and to attach in its place a micro-stopcock having a 0.5 mm bore, a two cm wide barrel and 3 mm bore side terminals. A group of such modified separatory funnels with supporting rack are much to be desired. A photo reproduction is shown in Fig. 16.

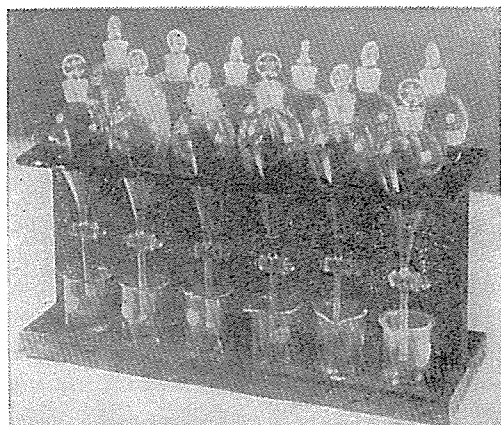


Figure 16.—Imiscible Solvent Extraction Apparatus.

The wet ashing apparatus assembly Figure 4, page 30 is recommended and precludes the use of a fume hood which is a worthwhile innovation. A fume hood may be employed in its stead but the refluxing still head is still requisite.

The process may be carried out as described employing a Cary Model 11 recording instrument in place of the Beckman DU apparatus.

Control Analytical Procedure In The Reference Analyses For Copper And Iron In Wine

Determination of iron

A modification of the procedure of Saywell (1) was used utilizing nitric-perchloric acid wet oxidation and by spectrophotometric estimation of iron using 1,10-phenanthroline as color producing ligand. The Saywell procedure was modified only in the use of 5 ml of wine in place of 1-2 ml sample. In addition the ferroin reaction complex of iron(II) was determined employing a Beckman D.U. spectrophotometer and a 512 m μ light source. Both these alterations were used in the interest of accuracy. The molecular extinction coefficient applied was 11,500 with cell cuvettes of 1 cm light path. The calculation involves the formulation:

$$\text{optical density} = \xi cl$$

where (ξ) is the molecular extinction coefficient, (c) is the concentration of iron in gram atoms per liter and (l) is the light path in cm. By reference to a graph previously prepared relating optical density as abscissa and parts per million of iron as ordinate the calculation may be simplified.

A 5 ml sample of wine is transferred to a 250 ml Vycor Erlenmeyer flask containing 10 ml of reagent nitric acid and 5 ml of 70% perchloric acid. The digestion apparatus employed is that described by the author, whereby a fume hood is not required. After the initial reaction has subsided, heat is applied from a medium heat electric hot plate, with the refluxing still head and fume eradicator in place and well vented by the use of a water aspirator.

When all nitric acid excess has been expelled and dense fumes of perchloric acid are evolved, discontinue the digestion. Remove the reaction flask and partially cool the contents under a stream of running tap water. Rinse and remove the refluxing still head and transfer the contents of the flask quantitatively to a 100 ml Pyrex beaker and add 2 drops of reagent sulfuric acid.

Provide the beaker with a cover glass using twin cover glass hooks. By use of a moderately heated hot plate in a fume hood, evaporate the sample to near dryness. Dissolve the beaker contents after the addition of 3 drops of reagent hydrochloric acid and 1 ml of water. Add 1 ml of reagent hydroxylamine hydrochloride and warm to insure complete solution and reduction of ferric iron. Buffer the solution by the addition of 5 ml of buffer (pH 4.0). Add 1 ml of 0.01M aqueous 1,10-phenanthroline solution to form the colored Fe(II) complex. Transfer the solution thus prepared to a 10 ml glass stoppered volumetric flask and dilute to volume with de-ionized water. The spectrophotometric evaluation of iron as above outlined completes the determination. A blank correction is applied using a correction for reagent iron content following the same procedure throughout, without the addition of the wine sample. Water is used as reference for 100% transmission in the spectrophotometric evaluation. (The reference cell in the case of bathophenanthroline and bathocuproine chelation and spectrophotometric determination is one composed of isoamyl alcohol and ethyl alcohol in the proportions 6 volumes of the former to 4 volumes of the latter.)

Determination of copper

The blank determinations employed were found to be small but measurable. Because of the very low copper content of the wines it was thought to be preferable to add copper to the sample for analysis. This seeding technique is often used in gravimetric and radiochemical processes and serves as a check on the accuracy of the method and the reliability of the results.

The wet ashing procedure just previously described in the determination of iron is followed in its entirety up to the point at which the evaporated solution of the wet ashing residue is nearly dry and free from excess perchloric acid. The remainder of the procedure in the determination of copper is as follows:

Take up the nearly dry beaker contents by the addition of 3 drops of reagent hydrochloric acid and 2 ml of water. Heat gently to promote complete solution and transfer the dissolved contents of the beaker quantitatively to a 60 ml glass stoppered separatory funnel. Add 5 ml of buffer solution (pH 7.1), and a weighed amount of copper sulfate solution, 2 ml of 10% hydroxylamine hydrochloride solution and 2 ml of 0.005M batho-

cuproine. If not at this point a total of approximately 20 ml in volume, add water to obtain this total volume. Add 6 ml of isoamyl alcohol.

Shake the stoppered flask contents 60 seconds and then allow the two liquid phases to separate during 5 minutes. Draw off the lower aqueous layer and transfer the alcohol layer quantitatively into a 10 ml volumetric flask and dilute to the mark by use of ethyl alcohol. Determine the absorbancy employing a Beckman D.U. spectrophotometer set at 479 $m\mu$ wavelength. A blank determination on reagents completes the procedure. The molecular extinction coefficient applied is 14,200.

The *in situ* Determination of Iron by Direct Color Formation and Immiscible Solvent Extraction After Bathophenanthroline Chelation

Determination of iron in red wines

The wine sample reaction reagents are as follows (use a 100 ml beaker in sampling and preliminary reactions):

- 5.00 ml of wine. (Three ml may be used for iron content in excess of 10 ppm)
- 5.0 ml of buffer solution (pH 4)
- 2.0 ml of 10% aqueous hydroxylamine hydrochloride.
- 1.0 ml of 0.01M bathophenanthroline solution.
- 2.0 ml of 95% ethanol.

The reaction mixture is now approximately 25% ethanol by volume. Heat the contents of the beaker to a gentle boil and boil for one minute. Transfer the contents of the beaker while still hot to a 60 ml glass stoppered separatory funnel. Add 6 ml of isoamyl alcohol and shake during 30 seconds. Allow the two liquid phases to separate for 3 minutes and draw off the lower aqueous layer. The beaker used for the pre-digestion is now rinsed with 1 ml of 95% ethanol, and 2-5 ml portions of water, transferring the rinsings to the separatory funnel containing the alcoholic extract of the reaction mixture. Add 10 ml of buffer solution (pH 4.0), and shake the contents of the separatory funnel 30 seconds. Allow the two liquid phases to separate 5 minutes and draw off the lower aqueous layer. The alcohol phase is transferred quantitatively to a 10 ml graduated flask and diluted to the mark with 95% ethanol.

The absorbancy is measured as previously described employing a light source of wave-length 533 $m\mu$. The molecular extinction coefficient used in the calculation is 22,400. A blank correction to correct for contamination of reagents is applied as before.

Determination of iron in white wines

For this procedure the reagents employed are the same as those listed for the red wine procedure with added 2 ml of ethanol omitted.

The wine sample and reagents in a 100 ml beaker are heated gently to boiling and boiled for 60 seconds. The beaker contents are allowed to cool. Transfer the beaker contents into a 60 ml separatory funnel rinsing the beaker first with 1 ml of 95% ethanol and 2-5 ml portions of water. Add 6 ml of isoamyl alcohol. Shake the flask for 2 min to establish equilibrium and allow the two liquid phases to separate 5 min. The aqueous layer is then drawn off and the alcohol layer transferred to a 10 ml graduated flask and diluted to the mark with 95% ethanol. Determine the absorbancy as before at 533 $m\mu$ and calculate the iron using 22,400 for the molecular extinction coefficient. Again apply a reagent blank correction.

The *in situ* Determination of Copper by Direct Color Formation and Immiscible Solvent Extraction After Bathocuproine Chelation

Determination of copper in red and white wines

The wine sample reaction ingredients are placed in a 60 ml glass stoppered separatory funnel in the order given below:

- 5.00 ml of wine
- 5.0 ml of buffer (pH 4.0)
- 0.2 ml of standard CuSO_4 solution (wt. buret sampling)
- 2.0 ml of 10% hydroxylamine hydrochloride solution
- 2.0 ml of bathocuproine solution

The contents of the separatory funnel are shaken for 2 min and the two liquid phases are allowed to separate. The aqueous layer is then drawn off. Add 5 ml of buffer (pH 7.1), and 5 ml of water. Again shake for 1 min and allow 5 min for phase separation.

The aqueous layer is again drawn off and the alcohol layer transferred quantitatively to a 10 ml volumetric flask and diluted to volume with 95% ethanol. Absorbancy is then determined using a light source of 479 $m\mu$. The calculation is again made employing 14,200 as the molecular extinction coefficient. A blank determination is made to correct for reagent contamination.

For the highest possible accuracy in the *in situ* determination of iron and copper the solution used as reference for 100% transmission should be prepared as follows: Extract an analysis size sample of the wine with 6 ml isoamyl alcohol. Draw off the lower phase. Transfer the alcohol layer to a 10-ml volumetric flask and dilute to volume with 95% ethanol. Spectrophotometric standardization using this solution compensates for any extracted color not due to the Fe(II) or Cu(I) complex of the reacting ligand.

The spectrophotometric reference solution just described was used in obtaining the results shown in Tables 6 and 7. If the above described spectrophotometric reference solution was not used, the determination of iron and copper was in error by approximately 1% for all the wines analyzed. The Red Burgundy wine was an exception. Failure to use the compensating spectrophotometric reference solution would lead to errors of approximately 5% total for both iron and copper.

The results in the determination of iron and of copper in wine sample number four (red wine), are given in Tables 5 and 6 for the purpose of illustrating multiple duplicate analyses and variation in results of individual replicas of the same sample.

TABLE 5. DUPLICATE DETERMINATION OF IRON IN SAMPLE NUMBER FOUR, FRENCH VINTAGE RED WINE

Wet ashing procedure 1,10-phenanthroline chelation			Direct extraction 4,7-diphenyl-1,10-phenanthroline chelation		
Sample wt. g	Absorbancy at 512 mμ (blank corrected)	Fe found p.p.m.	Sample wt. g	Absorbancy at 533 mμ (blank corrected)	Fe found p.p.m.
4.93	0.807	8.20	2.96	0.993	8.37
4.93	0.808	8.16	2.96	0.983	8.28
4.93	0.833	8.33	2.96	0.973	8.19
2.96	0.505	8.28	2.96	0.970	8.18
2.96	0.497	8.15	2.96	0.979	8.25
		Av. 8.22			Av. 8.25

From examination of the data of Table 5 it will be found that the results in the two general procedures, the one with wet ashing of the wine sample for analysis, compared with the newly described procedure of *in situ* determination, are directly comparable in accuracy. The range of determined values in each case is 0.18 p.p.m., 0.19 p.p.m., respectively, and the average deviation is similarly comparable at 0.064 p.p.m. and 0.058 p.p.m., approximately 2 parts per hundred of the total iron found.

TABLE 6. DUPLICATE DETERMINATIONS OF COPPER IN SAMPLE NUMBER FOUR, FRENCH VINTAGE RED WINE 4,7-DIPHENYL-2,9-DIMETHYL-1,10-PHENANTHROLINE (BATHOCUPROINE) CHELATION

Wet ashing procedure					Direct extraction				
Sample wt.	Copper added	Copper found	Copper of sample		Sample	Copper added	Copper found	Copper of sample	
g	μg	μg	μg	p.p.m.	g	μg	μg	μg	p.p.m.
2.96	19.44	21.24	1.80	0.61	2.96	17.11	18.95	1.84	0.62
2.96	34.04	36.02	1.98	0.67	2.96	15.81	17.64	1.83	0.62
2.96	35.96	37.86	1.90	0.64	2.96	16.00	17.95	1.95	0.66
					2.96	17.83	19.75	1.92	0.65
					2.96	16.17	17.95	1.78	0.60
				Av. 0.64					Av. 0.63

From examination of the data of Table 6 it will be observed that the results in the two general procedures, wet ashing and direct chelation and extraction give comparable results. The range of values is respectively 0.18 and 0.17 micrograms or 0.06 parts per million. The corresponding average deviations are 0.03 p.p.m. and 0.02 p.p.m.

Complete Tabulation of Results for Five Individual Wine Samples

In condensed form tabulation the results from the analyses of five wine samples following both the wet ashing procedure and the *in situ* direct che-

lation, extraction and spectrophotometric evaluation are given in the data of Table 7.

Commentary Observations

It is probable that companion 1,10-phenanthroline copper specific organic ligands could be used to replace bathocuproine in the procedure herein described. One such probable substitute is cuproine (2,2'-biquinoline). The substitution of another organic ligand for bathophenanthroline in the iron determination is more restricted, since a ligand which forms a suitable iron complex must either not form a copper (I) complex at pH 4, or the complex, if formed, must be either colorless or not extracted by the immiscible solvent used. The similar use of 1,10-phenanthroline is thus inapplicable. For comparable precision in the determination of copper, if cuproine is to be employed, the magnitude of the wine sample treated would of necessity be increased.

The wet ashing procedure in the preparation of the wine samples for analysis, as described, provides proof that in such procedure no copper is lost by possible volatilization, since the same copper found in direct chelation and extraction duplicated that found when wet ashing was employed. This has also been found to be the case in the determination of copper in paper by J. P. Butler (9) who has substantiated that in such method, HNO_3 - HClO_4 wet ashing procedure in preparation of paper and paper pulp for analyses (as well as the use of bathocuproine as color producing ligand), is highly to be desired.

No hazardous reactions were encountered during the study just recorded and the only complication brought about through the use of the wet ashing procedure described is, that a fume hood is required for conducting the evaporations involved.

TABLE 7. TABULAR RESULTS IN THE DETERMINATION OF IRON AND OF COPPER IN WINE (FOR DETAILED INDIVIDUAL DETERMINATIONS SEE TABLE 3)

Wet ashing procedure $\text{HNO}_3 + \text{HClO}_4$. *In situ* chelation and direct extraction

Wine sample number	Number of replicas	Iron found p.p.m.	Average deviation p.p.m.	Copper found p.p.m.	Average deviation p.p.m.	Number of replicas	Iron found p.p.m.	Average deviation p.p.m.	Copper found p.p.m.	Average deviation p.p.m.
1	5 and 5	3.94	0.038	1.70	0.036	4 and 5	4.00	0.015	1.66	0.016
2	8 and 5	6.03	0.070	2.05	0.003	5 and 5	6.08	0.024	2.03	0.010
3	5 and 5	7.08	0.052	0.76	0.018	5 and 5	6.95	0.040	0.77	0.014
4	5 and 3	8.22	0.064	0.64	0.030	5 and 5	8.25	0.058	0.63	0.020
5	8 and 5	5.01	0.036	1.21	0.024	5 and 4	5.04	0.036	1.20	0.008

Conclusions

A new procedure in the determination of iron and of copper in wine has been devised. The process may be applied to both red and white wines of 10-20% alcohol content. The procedure does not require the preliminary treatment of the sample to destroy its organic matter by either dry ashing or wet ashing procedures. This time-consuming operation is eliminated by use of a direct treatment of the wine by suitable reactants, namely 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline), and 4,7-diphenyl-2,9-dimethyl-1,10-phenanthroline (bathocuproine). Chelation by the former with ferrous iron results in the formation of the corresponding tris-ligand-ferrous complex cation which may be extracted quantitatively from the wine employing isoamyl alcohol. The latter reagent similarly reacts to form a bis-ligand cation with the cuprous ion and may be similarly extracted. The spectrophotometric estimation of both iron and copper is thus provided for. Both chelation reagents are of high molecular absorptivity at wave-lengths of maximum absorption 533 $m\mu$ and 479 $m\mu$, the former at 22,400 and the latter at 14,200 ξ value. This high sensitivity is particularly suited to the accurate determination of the low concentration of iron and of copper ordinarily present in wine of commerce, the influence of which upon quality and shelf life is of prime importance. The new method saves time-consuming operations previously required for the same determinations in preliminary treatment without loss in either accuracy or specificity. The new spectrophotometric applications are provided by new organic reagents which are commercially available and few new types of manipulative equipment are required.

Literature Cited

1. L. G. Saywell and B. B. Cunningham, *Ind. Eng. Chem., Anal., Ed.*, **9**, 66 (1937).
2. W. W. Banick and G. Frederick Smith, *Anal. Chim. Acta*, **16**, 464 (1957).
3. L. Deibner and H. Bouzique, *Mikrochim. Acta*, **5**, 501 (1954).
4. G. Frederick Smith, W. H. McCurdy and H. C. Diehl, *Analyst*, **77**, 418 (1952).
5. G. Frederick Smith and D. H. Wilkins, *Anal. Chem.*, **25**, 510 (1953).
6. G. Leavell and N. R. Ellis, *Ind. Eng. Chem., Anal. Ed.*, **6**, 46 (1934).
7. R. Stugart, *Ind. Eng. Chem., Anal. Ed.*, **3**, 390 (1931).
8. C. Elliott and J. W. Robinson, *Anal. Chim. Acta*, **13**, 235 (1955).
9. J. P. Butler, *Anal. Chem.*, **29**, 414 (1957).

SECTION VIII

REACTIONS INVOLVING PERCHLORIC ACID AS OXYGEN DONOR AND DEHYDRATING AGENT

The use of concentrated 56-68 percent perchloric acid at its boiling point in the wet oxidation of organic compositions has been described in Section III. The use of perchloric acid in the determination of chromium by oxidation from Cr(III) to Cr(VI) as originated by Willard and Gibson (1) accounts for the greatest consumption, industry wide, of the acid. This analysis of stainless steel for chromium is practically exclusively used routinely by the steel works control laboratories. Another important use of perchloric acid involves the routine control method for the determination of chromium in leather or chromacized medical catgut previously detailed in this review, Section V, page 41. The wet chemical oxidation of organic compositions employing perchloric acid accounts for its use annually in many hundreds of thousands of routine control laboratory procedures on the subject of the present review.

The use of concentrated boiling perchloric acid as a dehydrating agent in the determination of silicon in iron, steel, and ferro-alloys is almost universal in such industry's control laboratories. Its application to the determination of silica following the Willard and Cake (2) method is applied to control analytical work of the lime and cement industry. The most valuable property provided by perchloric acid in the determination of silicon and silica consists in the fact that its metallic salts are all soluble in the concentrated acid as well as in dilute acid solution or water. This fact accounts for its outstanding adoption in the dehydration of silicic acid. With complete dehydration of silicic acid in a single operation not handicapped by the necessity to provide a second dehydration with intermediate filtration, this application of perchloric acid has practically no other procedural competing substitute method, including hydrochloric acid and the troublesome two stage baking to dryness technique.

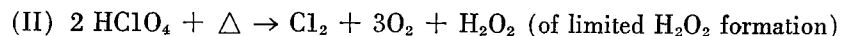
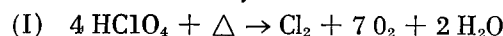
The fact that perchloric acid is a good solvent for organic compositions without char formation even when used in concentrated form at elevated temperatures has been repeatedly stressed in this review. This property coupled with the possibility to grade potential magnitudes over a wide range (0.85 to 2.0 volts) by control of perchloric acid concentration and temperature has been shown to be of particular advantage in previously detailed applications. Means are readily at hand to provide much higher potential reaction conditions by applying perchloric acid in the 73.6-85% concentration range. This provides for the wet oxidation of organic matter such

as fecal matter and forms of carbon such as decolorizing carbon or gas mask cannister carbon. Means are also provided for the completion of wet oxidation reactions which involve extensive production of precipitated carbon followed by their oxidation with perchloric acid concentrations stronger than the azeotropic composition of 72.5 percent.

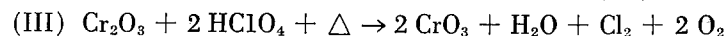
There is here reason to discuss the important oxygen donor properties of hot concentrated perchloric acid from the viewpoint of possible influence of side reactions. Some of the conclusions may be controversial but are believed by the author to be sound. The ideas expressed have been documented (3).

We are now concerned with the oxygen donor intensity of perchloric acid of the concentration range 72.5 percent and up to 85 percent and higher strengths.

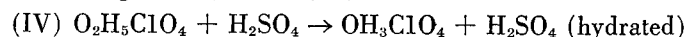
Hot concentrated perchloric acid makes oxygen available in accord with reaction I and secondary reaction II.



Both reactions (I) and (II) are intensified in proportion as the concentration increases. The available oxidation potential thus provided is greater than 2.0 volts. An illustrative reaction employing 72.5 to 100% perchloric acid is that of the oxidation of Cr(III) to CrO₃, reaction (III).



The *in situ* concentration of 72.5 percent perchloric acid follows the addition of concentrated sulfuric acid to form higher strength perchloric acid according to the reaction (IV).



Thus 73.6 percent perchloric acid is strengthened to 85 percent acid and may be carried up further depending upon the use of fuming sulfuric acid as additive.

Since reaction (III) is basic to the determination of chromium by the Willard and Gibson method (1), it is of great interest that Lundell, Hoffman, and Bright (4) in their tests declared reaction III to be reversible to the extent of 0.5 percent. It has been conclusively proven, however, that since reaction (II) indicates that a trace of hydrogen peroxide accompanies reaction (I), if means are not provided to eliminate the effect of this H₂O₂, reaction (II) could account for incomplete oxidation of chromium. The means to prevent low oxidation of Cr(III) to CrO₃ due to H₂O₂ is to provide rapid chilling of the reaction from approximately 200 degrees to ordinary temperatures. This may readily be accomplished using an ice-water slurry into which the reaction container of hot acid is immersed with swirling

of the flask contents. By this means the oxidation of chromium has been proven to be complete. No literature refutation of this remedy has been reported.

The influence of hydrogen peroxide in the reduction of CrO₃ to Cr(III) can be easily substantiated by its addition to boiling perchloric acid solutions of CrO₃.

The Oxidation of Chromium (III) to Chromic Acid (CrO₃) as Influenced by the Increasing Concentration of Boiling Perchloric Acid of 70 to 85 Percent Strength and Above

Two hundred milligrams of chromic oxide (Cr₂O₃) were treated by dissolution in 50 ml of OH₃ClO₄ (84.79 percent HClO₄) and heated to 168°C. The reaction components (CrO₃+HClO₄) were allowed to cool. Red crystals of chromic acid (CrO₃), insoluble in 70 percent acid or higher strength, appear and the reaction *supernatant* acid was colorless. Upon standing 12 hours at room temperature the acid became colored owing to the presence of green chromic perchlorate. Reheating of the reaction components gave the same results by duplicate treatment.

The oxidation of chromic oxide (Cr₂O₃) was made using increasing strengths of perchloric acid provided by the addition of sulfuric acid (15% fuming H₂SO₄ + 72% HClO₄). The results are given in Table 1. Parallel experiments were made replacing chromium (III) by small amounts of manganous perchlorate (MnClO₄·6H₂O). The manganese was momentarily oxidized to manganate of permanganate purple and the reaction rapidly reversed as shown by the immediate decolorization due to hydrogen peroxide formed simultaneously following reaction (II).

TABLE 1. SEE REFERENCE (4).

THE OXIDATION OF Cr(III) AND Mn(II) BY CONCENTRATED PERCHLORIC ACID

H ₂ SO ₄ 15% Fuming ml	HClO ₄ 72% ml	Temp. Rise from 25°C in °C	Cr ₂ O ₃ Oxidation to CrO ₃ in °C	Mn(II) Oxidation to Mn(VI) or MnO ₄ ⁻ in °C
10	10	82.0	136	150
12	8.0	84.4	128	137
13.3	6.6	86.5	120	128
15.0	5.0	88.4	119	125
10.0(95%)	10	40.0	168	—

The temperature attained upon mixing the acids of Table 1 is an indication of the extent to which the perchloric acid has been dehydrated. By using 75 percent sulfuric acid in a mixture with 72 percent perchloric acid there results no appreciable heat evolution. The temperature after mixing was ob-

served in a 30-ml Dewar test tube, unsilvered and the temperature maximum observed using a small Anschütz thermometer.

It will be observed that the dehydration of perchloric acid by the sulfuric acid in all cases was equivalent at least to the formation of 85% perchloric acid since the temperature at which chromium was oxidized is equal to or less than that required by 84.79 percent perchloric acid as previously noted. The greater the dehydration and increase in perchloric acid strength, the lower is the temperature of Cr (III) oxidation to Cr (VI) as well as the oxidation of Mn(II) to the higher valence state. These observations indicate that the oxidation potentials provided by the perchloric acid of strength up to and beyond 85 per cent exceed that of boiling 70-72.5% acid and exceed 2.0 volts in magnitude. In all cases cited in Table 1 the oxidation of chromium was clearly reversed to an increasing extent at higher strength HClO_4 as shown by the rate of color change, (orange to green), upon allowing the oxidized solutions to cool and stand. In the case of the higher concentrations of perchloric acid the reduction of Cr(VI) to Cr(III) was complete in a few hours. This indicates the magnitude of reaction (II) as governed by perchloric acid concentration.

If the dehydration of perchloric acid by added fuming sulfuric acid is objectionable, a substitute reaction condition to replace sulfuric acid is the addition of anhydrous magnesium perchlorate to 70-72.5 per cent perchloric acid. Both mixtures of fuming sulfuric acid and perchloric acid and solutions made from added anhydrous magnesium perchlorate to concentrated perchloric acid have been applied to the preparation of anhydrous perchloric acid (5).

From the previous material of this section, one would tend to predict that the use of mixed sulfuric and perchloric acid would provide oxidation potentials too high to be of service following controlled oxidation of organic compositions. But by careful investigation of conditions with confirming reactions, this combination of perchloric and sulfuric acids will be shown to constitute another basic technique in the wet oxidation of organic compositions in Sections 9 and 10 of this review.

¹H. H. Willard and R. C. Gibson, *Ind. Eng. Chem. Anal. Ed.*, **3**, 88 (1931)

²H. H. Willard and W. E. Cake, *J. Am. Chem. Soc.*, **42**, 2646 (1920)

³G. Frederick Smith, *Ind. Eng. Chem. Anal. Ed.*, **6**, 229 (1934)

⁴Ludell, Hoffman and Bright, "Chemical Analysis of Iron and Steel" John Wiley and Sons Inc., New York, 1931.

⁵The Author. *Talanta*, **7**, 212 (1961)

SECTION IX

WET OXIDATION OF ORGANIC MATTER EMPLOYING MIXED PERCHLORIC AND SULFURIC ACIDS AT CONTROLLED TEMPERATURES AND GRADED HIGH POTENTIALS

Introduction

For the wet ashing methods requiring the application of the highest available oxidation potential, the use of mixed perchloric and sulfuric acids is required. The present section deals with this procedure. The variable factors governing the magnitude of oxidation potential attainable are given and illustrative procedures in wet oxidation of organic compositions most resistant to oxidation are provided (1).

The application of mixed perchloric and sulfuric acids in the wet oxidation of feeds and feces has been extensively employed by Bolin and Stamberg. Molybdenum was added as catalyst (2). This process has been adapted to the determination of Eden's Indicator Method for evaluation of "digestive coefficient" using chromic oxide (Cr_2O_3) as indicator. This procedure is widely used in animal feeding experiments.

By the use of mixed perchloric and sulfuric acids, the effective concentration of perchloric acid made available is from 73.6 to more than 84.79% ($\text{O}_2\text{H}_5\text{ClO}_4$ to $\text{OH}_3\text{ClO}_4 + \text{HClO}_4$ anhydrous), or intermediate concentrations. By this procedure the presence of sulfuric acid causes the rate of oxidation of organic matter to be tempered by its dilution effect. The dehydration of perchloric acid by sulfuric acid accounts for the increase in concentration of perchloric acid.

For such mixed acids, by carrying out reactions under reflux, any desirable oxidation potential provided by the selected proportioning of the acids chosen may be maintained.

By the use of vanadium in milligram amounts the time interval for a given wet ashing oxidation may be shortened. Apparatus design and manipulation during a given digestion governs further reaction control of both available perchloric acid concentration and temperatures involved.

The digestion apparatus best suited to these reactions is the Bethge assembly illustrated in Figure 2, page 20.

Previous Applications of Perchloric-Sulfuric Acids in the Destruction of Organic Compositions

An early use of hot concentrated sulfuric acid in the destruction of organic compositions using perchloric acid as accelerator was that of Gau-

duchon-Truchot in a "Contribution to the Study of the Kjeldahl Process Employing Perchloric Acid for the determination of amino nitrogen (3)." The boiling solution of concentrated sulfuric acid and the resulting black carbonized and caramelized organic material was treated dropwise by the addition of perchloric acid. After each small increment of perchloric acid is added to the boiling mixture at 250-275°C, a small increment of the black carbon laden reaction mixture is at once consumed. Continuing this process with time intervals of short duration between perchloric acid additions, the reaction finally clears in color. When the last drop of perchloric acid required to clear up the dark color of the reacting mixture is added, removal of organic matter is complete. The rest of the determination of amino nitrogen then follows the usual Kjeldahl nitrogen determination. Complete recovery of nitrogen was demonstrated. This procedure was obviously tedious requiring too much close attention by the analyst, but was found to be attractive when applied to the micro-determination of amino nitrogen by Wm. H. Taylor, Jr. (4).

This procedure type, (dropwise addition of HClO_4 as accelerator), has been extensively applied by M. Oosting (5) as a general wet ashing procedure. The organic composition was heated in contact with concentrated sulfuric acid and then oxidized by the dropwise addition of perchloric acid. Added perchloric acid was left in contact with the organic matter until consumed before additional dropwise addition of oxidant. Chromium as $\text{K}_2\text{Cr}_2\text{O}_7$, vanadium as NH_4VO_3 and selenium dioxide in 5 mg amounts were used as catalyst. Vanadium was found to be the most effective of the three.

The Oxidation of Darco Decolorizing Carbon and Coconut Charcoal

A sample of coconut charcoal (1 g) of 2-3 mm particle size was placed in a 125-ml Bethge apparatus flask and 4-5 mg of ammonium vanadate added. Fifteen milliliters of a mixture of equal parts by volume of 93.6% sulfuric acid and 69.6% perchloric acid were employed as oxidant. The air and water-cooled reflux condenser parts of the digestion apparatus were put in place and the heat applied. In 4 minutes the reaction temperature was 209°C and the mixture still orange in color due to the pervanadic acid formed. The particles of carbon were not being oxidized at this temperature. At 5 minutes and 213°C, the carbon particles began to disintegrate and oxidize as indicated by the reduction of the pervanadic acid and the black color of the reacting solution. At 12 minutes the reaction mixture was yellow in tint and at 15 minutes the reaction was complete and its color orange, due to reoxidized vanadium, indicating complete destruction of carbon. There was no acid insoluble residue.

The same reaction conditions, in the oxidation of Darco decolorizing carbon, (Atlas Powder Co., in one-gram samples, required only 9 minutes digestion for complete oxidation. The Darco oxidation was accompanied by 30-35 mm of foam formation and the reaction produced considerable white fumes emitted by the water condenser. The foam quickly subsided and no insoluble matter was evident in the cold, diluted, wet ash residue.

The Wet Oxidation of Coal, Anthracite or Bituminous, of High and of Low Volatile Combustible Matter

The determination of arsenic in coal is of particular importance in the case of English mined coal. The wet ashing of coal by the periodic acid liquid fire reaction has been previously dealt with in Section 5. The determination of arsenic is provided for by its complete retention in such wet ash residues by usual methods. The presence of complex heterocyclic ring nitrogen compounds requires oxidation potentials of high intensity. The wet oxidation of coal is thus a matter of interest following the use of mixed perchloric and sulfuric acids.

For such wet oxidation of coal a two-stage reaction is involved. First the volatile combustible matter, (the VCM of the proximate analysis of coal), is oxidized. The required oxidation potential is not excessive for this stage of the reaction. The second stage of oxidation demands a much higher oxidation potential. Foam formation accompanies the first stage but not the second stage. The first stage is markedly exothermic and must be temperature controlled. The second stage is carried out at higher temperature and vanadium is added as catalyst.

The procedure found best suited for the wet oxidation of coal is as follows:

The Bethge digestion apparatus with 125-ml reaction flask is suited to the oxidation of one-gram samples. The first stage reaction employs 2.5 ml of 96.3% sulfuric acid and 7.5 ml of 69.6% perchloric acid. No vanadium as catalyst.

Heat is applied and in less than 2 minutes the temperature is 80 to 85°. At this point the heat is discontinued and the resulting exothermic reaction carries the reaction temperature to 180-185°. The foam formation at this stage is from a small amount (15 mm) for anthracite coal to 50 mm for bituminous high VCM type coal. After a 5-minute interval in reaction the temperature begins to drop slowly. A 5-ml portion of sulfuric acid, in which 5-6 mg of NH_4VO_3 is dissolved, is then added through the water condenser and the heat again applied. The completion of the digestion follows in 15-20 minutes for anthracite, 13 to 14 minutes for Pocahontas coal and 9 to 10 minutes with high volatile Orient (Southern Illinois) coal.

A feature of the wet oxidation reaction with equal parts by volume of sulfuric and perchloric acids, (effective perchloric acid concentrations of 90-100%) is the formation of decomposition products, which during the early stages of the oxidation issue as white fumes out the top of the water condenser. These are due to the dissociation of perchloric acid into chlorine, oxides of chlorine and oxygen. No indication of an oily reflux of decomposition products from the coal is noted by examination of the inner walls of the air condenser. This seems to indicate no loss of volatile combustible matter through the water condenser.

Wet Oxidation of Coal as a Single Stage Reaction

The oxidation of coal may be applied as a single stage reaction. The mixed sulfuric and perchloric acids of the same strength and proportions previously used with added vanadium catalyst serve to oxidize 2 g of Orient high V.C.M. coal using a 500-ml Bethge apparatus reaction flask.

The temperature upon digestion 2 minutes was 66°. White fumes filled the reaction flask. After 3 minutes the temperature was 110° and rapidly increasing. At 4 minutes the temperature was 225° and there was 50 mm of foam over the boiling reacting acids. At 5 minutes the foam had subsided and the reacting acids turned the colors black to red brown and the temperature had fallen to 202°. At 8 minutes and at 203° the reaction mixture was yellow. With the reflux return to the reaction flask cut off, the temperature increased to 210° at the 14-minute interval and the oxidation was complete.

The fumes escaping from the water condenser in this reaction were collected in a dry-ice, acetone trap. There was thus collected 2.35 g of condensate. There remained, after warming to room temperature 1.05 g of a yellow oil. This constituted the unoxidized volatile combustible matter from the coal oxidation. The reflux collected in the air condenser had a volume of approximately 3.0 ml.

Essentially the same results were attained with 12.5 ml of sulfuric acid plus 15 ml of perchloric acid in the oxidation of the same coal. In this case the exothermic reaction carried the temperature to 202° in 7 minutes. At this point the reflux return was cut off and the reaction complete after 30 minutes, with other results the same.

A 3-g sample of anthracite coal was similarly oxidized using 18 ml of sulfuric acid and 15 ml of perchloric acid, again with 2-3 mg of vanadium catalyst. In this case there was no appreciable foam formation. The exothermic reaction carried the temperature to 208° at the eight-minute interval. The return of reflux was cut off at 25 minutes and the reaction complete at

50 minutes. The cold trap condensate was 2.35 g reduced to approximately one gram at room temperature, with loss of high volatile matter.

Under the same conditions a 2-g sample of Pocahontas coal gave the same type results, except that the starting acid mixture was 10 ml of sulfuric acid and 15 ml of perchloric acid. The reaction produced 15 mm of foam at the 5-minute interval at 224°, indicating again a markedly high exothermic reaction. At 13 minutes, the reflux return was cut off and the reaction complete in 30 minutes. The reflux collected in the air condenser had a volume of 4.5 ml. The cold trap condensate again was approximately 1 ml after warming to ambient temperature.

The determination of arsenic in coal, following procedures described for its proximate analysis, presents the problem of possible loss of arsenic by either volatilization or in the acid insoluble ash. The same problems exist in a wet oxidation procedure which employs nitric-sulfuric acids as reactants. By reasonable prediction, no arsenic will be lost by volatilization in wet ashing because of the high potential provided to maintain the arsenic in its higher valence state.

Pre-Digestion by Sulfuric Acid Followed by Final Oxidation with Added Perchloric Acid

As previously shown, carbon as coconut charcoal or Darco decolorizing carbon may be wet ashed employing boiling mixed sulfuric and perchloric acids with added vanadium catalyst. With the two concentrated acids, in equal volume mixtures, an effective perchloric acid strength of 90-100% is provided.

This suggests a preliminary digestion with concentrated sulfuric acid. By the carbonization of the sample, (as preliminary Kjeldahl type introductory process), the subsequent addition of perchloric acid completes the oxidation. Such a procedure is more time-consuming but has the advantage that foam formation is avoided.

The wet oxidation of ion-exchange resin IR-120 was employed to demonstrate this type reaction.

The Bethge apparatus with 500 ml reaction flask was charged by the addition of 2 g of sample, 2 to 3 mg of vanadium and 15 ml of concentrated sulfuric acid. A 60-minute digestion period was employed for this reaction (at 15 minutes 180°, at 30 minutes 185° and at 60 minutes 220°). The inner walls of the reaction flask at this point were well spattered with charred sample, reactants and the solution carbon laden, and jet black.

At this point there was added (by pouring through the water condenser) 5 ml of 70% perchloric acid.

In 2 to 3 minutes the temperature rose to 230° and then receded to 210°. The inner walls of the reaction flask were completely clear of black deposit. The reaction of oxidation was complete in 10 minutes of the second stage at a temperature of 203°. The cooled acid residue was turbid but dilution with an equal volume of water gave a clear solution.

This procedure was applied with equally good results to dried whole milk powder. It constitutes a general method of wet ashing processes.

The Wet Oxidation of Wool at High Oxidation Potential

The wet oxidation of wool may be carried out by one of three different procedures:

1. By applying the liquid fire reaction ($\text{HNO}_3 + \text{HClO}_4$).
2. By reaction with 65.5% boiling perchloric acid with vanadium as catalyst (see Section III).
3. By digestion with equal volumes of mixed 96.3% sulfuric acid and 69.6% perchloric acid in presence of vanadium as catalyst. This procedure is the most rapid of the three. It is demonstrated as follows:

The Bethge apparatus (250-ml flask) was charged with 1 g of wool yarn, 2-3 mg of vanadium as NH_4VO_3 and 15 ml of equal parts by volume of concentrated sulfuric acid and 69.6% perchloric acid.

The digestion was at low heat. At 2.5 minutes the sample was completely in solution. At 3.5 minutes the temperature was 165° with foam formation of 10 mm. At 4 minutes the temperature was 200°C with 25 mm of foam formation and the color changed from brown to green. At 4.5 minutes and 205° the foam subsided. At 5 minutes the temperature was the same and the reacting solution yellow. The oxidation was complete in 6 minutes at 203° and orange in color from pervanadic acid as indicator. The cool reaction mixture deposited crystals of ammonium perchlorate but gave a clear solution after dilution.

The Wet Oxidation of Organic Materials Requiring High Oxidation Potentials

The wet oxidation of quinoline serves as an example. A 2-ml sample (2.2 g) was digested in the Bethge apparatus using a 250-ml flask. The oxidant was 15 ml of equal parts by volume of concentrated sulfuric acid and 96.6% perchloric acid with 2 mg of vanadium as catalyst (effective HClO_4 concentration approaching 100% strength). With 4 minutes heating the

temperature was 190° and vigorous reaction set in, the temperature after 5 minutes was 214° accompanied by the formation of 25 mm of foam. At 6 minutes the temperature was 225° and the solution changed from dark brown to red brown. At 8 minutes the temperature fell to 218° and the reactants were yellow in color with no foaming. At 10 minutes the temperature was 225° and reaction complete as indicated by the pervanadic acid color.

The wet ash residue was evaporated to remove excess acid. After removal of perchloric followed by sulfuric acid, there remained, upon cooling a white crystalline residue soluble in water and strongly acid in reaction. A sample of this residue was neutralized to pH 5.5 and copper sulfate added. A blue insoluble precipitate was obtained indicating the reaction product to be nicotinic acid.

Such oxidation of quinoline has been employed for the patented procedure in the manufacture of nicotinic acid.

The Wet Oxidation of 8-Hydroxyquinoline

The same reaction oxidant mixture, as employed in the oxidation of quinoline, was applied. The wet ash residue was evaporated to remove the excess mixed acids and the catalyst and a small amount of ammonium sulfate and ammonium vanadate only remained.

The oxidation of both ring structures of "Oxine" was in this case complete as distinct from the partial oxidation of quinoline to nicotinic acid.

A one-gram sample of Snyder's reagent, (4,7-hydroxy-1,10-phenanthroline), was oxidized under the same conditions as for "Oxine" and quinoline. The reaction was complete in 10 minutes and the wet ash residue upon evaporation to remove excess acids showed recovery only of the vanadium catalyst. This oxidation was accomplished without any carbonization or formation of dark reaction solutions.

Wet Oxidation of Synthetic Fabric Materials

This type oxidation is difficult to carry out employing procedures other than in the application of high potential oxygen donors. The mixed acids used were equal parts by volume of 96.3% sulfuric and 69.6% perchloric acid with 1 to 2 mg of vanadium as catalyst. The Bethge digestion apparatus with 250-ml reaction flask was employed at moderate heat. The results are given in Table 1.

TABLE 1. THE WET OXIDATION OF NYLON, DACRON AND ORLON (Read Horizontally)

Time min.	1	1.5	2.0	2.5	3.0	3.5	4.0	5.0	6.0	7.0	8.0
Nylon	54°	—	66	90	123	194	213	213	210*	—	—
Dacron	47°	54	65	80	105	165	209	209	210	211	211*
Orlon	41°	150	192	218	218	215	214	214*	—	—	—

*Oxidation complete.

All three materials were exothermic in reaction to a marked degree. No carbon degradation products were formed. Foam formation was pronounced with Nylon and Orlon, but normal with Dacron. The residual ash solution acid was clear in each case when hot. When cool a precipitate of ammonium perchlorate formed in the case of Nylon and Orlon but not Dacron. Upon dilution with water, all gave clear solutions except Orlon. With Orlon the oxidation was most rapid and the exothermic nature of the reaction the most pronounced.

The Wet Ashing of Rubber

For analytical determinations of rubber additives, excluding sulfur, pre-digesting with hot concentrated sulfuric acid is a recommended procedure.

A 1-gram sample of pure gum rubber, finely divided, was placed in a 250-ml Bethge apparatus flask. Concentrated sulfuric acid, (7.5 ml), was added and 1-2 mg of NH_4VO_3 . The pre-digestion period was 10 minutes at 200°. The inner walls of the reaction flask were coated black from the sprayed reaction products, the solution was black. Sulfur dioxide was emitted at the top of the water condenser.

This reaction mixture was cooled to 40° and 7.5 ml of 69.6% perchloric acid added by pouring through the water condenser. Heating for 3 minutes brought the reaction to 100° and oxidation was initiated with the formation of 50 mm of foam. At 5 minutes the reaction temperature was 200° and oxidation vigorous. At 8 minutes the solution was dark brown and at 215°. In the reaction period 8 to 20 minutes the temperature fell to 188°, the solution changed from brown, through red, then yellow, and finally to orange, to indicate completion of oxidation.

The wet ash was clear when hot, turbid when cold and clear again with dilution.

Concluding Remarks

For organic compositions requiring the highest oxidation potentials such as coal, ion-exchange resins, alkaloids, heterocyclic nitrogen compounds, decolorizing carbon and gas mask coconut charcoal, phenanthrolines, quinoline

and structures such as caffeine, the use of mixed perchloric acid and sulfuric acid represents a very important operative technique. The use of vanadium as catalyst is of material influence along with the dilution effect of sulfuric acid in smoothing and tempering reaction conditions.

In establishing correct procedures for this, as for any of the wide variety of individual wet oxidation procedures available, no hazard need be encountered. Test any new procedure with 50-100 mgs of sample. Then increase sample weight to 200-300 mg samples or larger. The use of a scheme to protect the operator, such as a transparent reaction flask shield, is not an admission of possible ensuing violent reaction, but is rather a sensible precaution. This is, after all, a correct procedure with many reactions in which perchloric acid is not involved.

Literature Cited

- (1) Harvey Diehl and G. Frederick Smith, *Talanta*, **2**, 209 (1950).
- (2) D. W. Bolin and O. A. Stamberg, *Ind. Eng. Chem., Anal. Ed.*, **16**, 345 (1944).
- (3) Henriette Gauduchon-Truchot, *Imprimerie Henry* (Ph.D. thesis) Rue Pascal, Paris, 1936.
- (4) Wm. M. Taylor, Jr., Dissertation, 1941, University of Illinois.
- (5) M. Oosting, Ph.D. Dissertation, Delft, Holland (1958) (in Dutch).

SECTION X

The Wet Oxidation of Bone and Bone Marrow Digestion with 100 Percent Sulfuric Acid Followed by the Addition of 73.6 Percent Perchloric Acid, (Dioxonium Perchlorate $O_2H_5ClO_4$)

Introduction

The wet oxidation of bone as sampled, without other treatment, in preparation for the trace element determination of metallic components, presents unusual operational prerequisites. The fat-laden, bonding fibrin of the bone structure and of the bone marrow, tallowy fat content, especially lecithin, is not effectively oxidized by digestion in the presence of concentrated nitric acid. The fats involved do not suffer dissolution in the perchloric acid employed in the application of the liquid fire reaction. Hence their destructive oxidation is not favorable through oxidation by the perchloric acid left in the last stages of the liquid fire reaction procedure.

The regular procedures of wet ashing require modification. For bone tissue and bone marrow a two-stage oxidation reaction is requisite.

A preliminary digestive oxidation employing 100 percent sulfuric acid is necessarily applied. The thoroughly carbonized sample thus treated, (giving a reaction resulting in complete char formation), is subjected to a second stage digestion after lowering the reaction temperature materially, (300-325°C to 195°), by the addition of 73.6 percent perchloric acid and completion of the digestion at 200-210° to rapidly oxidize the black suspended carbonaceous char left after the first stage digestion is complete. A milligram of vanadium is added as catalyst. This process is not at a disadvantage from uncontrolled reaction conditions.

The Present Study

The first stage of the procedure, that of hot concentrated sulfuric acid, involves the decomposition of organic matter to form carbon or degradation carbonaceous products suspended in or soluble in hot concentrated sulfuric acid. 100 percent sulfuric acid for this purpose has a 20-degree higher boiling point than reagent 96-97 percent acid and is hence a more thorough degradation reaction promoter at more abbreviated digestion time intervals. The second stage of reaction follows the lowering of the temperature to approximately 195° from approximately 320°. By the addition of concentrated perchloric acid and further digestion the destructive oxidation of precipitated carbon and acid soluble degradation products of the first stage treat-

ment are rapidly wet oxidized. The mineral components such as lead or beryllium and calcium (or phosphorus) of the bone are thus retained in the acid residue from the digestion. The literature reference to this procedure described by the author and Harvey Diehl is cited for this type oxidation (2).

Experimental Procedure

Reagents employed

100% sulfuric acid: Made by mixing 96-97% (reagent grade) sulfuric acid with the necessary amount of 15-18% fuming sulfuric acid. A weight of 337.5 g of reagent grade acid is added to 453 g of the oleum. By analysis the resulting acid was found to be 99.5% H_2SO_4 . M.p. 10.5°C. Sp.Gr. (25°/4°) = 1.837. For preparation of 100% acid, employing oleum of different SO_3 content, the following formula, as devised by Gerster(1), is applicable.

$$X = 100(b - a) / (a - c)$$

where X = quantity of sulfuric acid to be added to 100 parts of oleum.

a = total SO_3 per 100 parts of the acid desired,

b = total SO_3 per 100 parts of the original oleum,

c = total SO_3 per 100 parts of the acid used for dilution.

If the SO_3 content of the oleum is not known a close approximation to the desired 100% sulfuric acid can be made by determining the boiling point of a given reagent grade acid after the addition of increasing amounts of oleum. Sulfuric acid of 100% H_2SO_4 content has a boiling point of 325° at approximately 750 mm atmospheric pressure. By examination of the results in Fig. 19, this procedure is seen to be less accurate because of the limited increase in boiling point with increase concentration of SO_3 .

Dioxonium perchlorate (73.6% perchloric acid) Made by vacuum distillation of 72.5% acid (the perchloric acid-water azeotrope) at 2-7 mm pressure.

Vanadium catalyst: Ammonium vanadate or vanadic oxide.

Apparatus assembly

The apparatus, as originated by Bethge, and employed in previous procedures in wet oxidations, was again employed. See Figure 2, page 20. To provide for the disposal of evolved SO_2 from the oxidation of organic matter by sulfuric acid, the top terminal of the water cooled condenser is fitted with an adaptor. A small-bore rubber tube conducts away the SO_2 which is vented into the sink drain for absorption in running water. The Bethge apparatus, manipulations, and manner of use, have been previously described.

Test sample selection

Small diameter fore-leg bone of young beef carcass was cut into 5 mm thick discs. No attempt was made to remove adhering cartilage. The bone centre was left intact. These bone slices were then diced into fragments of 10-mm maximum dimension.

Bone samples from beef of varying ages were not tested. The presence of soft marrow in bone from very young animals would react towards boiling 100% sulfuric acid with similar formation of precipitated carbon and soluble carbon degradation products. The second stage of oxidation, following the addition of perchloric acid, would not be altered materially in carbonaceous residue.

Digestion of sample

Three samples, 0.5, 1.6, and 3 grams, were consecutively wet ashed. The larger the sample taken the lower the boiling temperature becomes for a given volume of sulfuric acid in the first stage of oxidation. This is due to dilution by the resulting reaction products. For the larger sample weights the volume of sulfuric acid should be increased. Otherwise the boiling temperature falls materially below 325°.

The sulfuric acid digestion is complete when SO_2 is no longer evolved. Further digestion for the first stage reaction is then of little value. The heating is then dis-

continued and the reaction temperature allowed to fall to approximately 200°. The second stage of oxidation, following the addition of 73.6% perchloric acid, is rapid and exothermic.

The results of the digestions are given in Tables 1, 2 and 3.

Reaction Characteristics

100 per cent sulfuric acid is employed in the first stage destructive carbonization reaction to shorten the required reaction time. The boiling temperature should be in the range 315° to 325°. Increase in sample weight requires increase in sulfuric acid. Reaction degradation products lower the boiling temperature. This is illustrated by the results in Tables 1, 2 and 3.

The use of 100% H₂SO₄ prevents formation of insolubles in the wet ash residue. The use of lesser concentrations causes precipitation.

Boiling 100% H₂SO₄ requires 300 minutes for the complete wet ashing of 0.5 g of chrome tanned leather, and 240 minutes for 1 g of cellulose at 325°.

TABLE 1. WET OXIDATION OF 0.5-G SAMPLE OF BONE

10 ml of 100% H₂SO₄ plus 1.0-2.0 mg of vanadium in first stage reaction.

5 ml of 73.6% HClO₄ for the second stage of digestion.

Bethge apparatus with 250-ml reaction flask and SO₂ disposal vent.

100% H ₂ SO ₄ digestion			73.6% HClO ₄ digestion		
Reaction time, min	Temp, degrees C	Reaction effects	Reaction time, min	Temp, degrees C	Remarks
Start	28		21		
3	210	Reactants darken	22	195	HClO ₄ , 73.6% added
4	240	Minimum side wall deposit	23	160	Reaction exothermic
				201	Reaction complete
5	306	Solution dark chocolate color	24	210	Solution clear
6	313	Solution in ebullition			
7	318}	Exothermic reaction			
8	323}				
10	317}	Exothermic reaction			
12	318}	completed			
15	319	Discontinued heating			

In the preliminary oxidation period some volatile reaction product, which escapes through the SO₂ reaction drain tube, is evolved in the wet ashing

of bone. The product condenses to a fluffy white, water-insoluble material of undetermined structure.

By elevating the temperature slowly during sulfuric acid digestion, cleaner reaction-flask walls result. This advantage is minor. Boiling 100% sulfuric acid refluxes these black deposited products down the flask walls rapidly. This stage of reaction demands no attention.

If desired, the addition of 73.6% perchloric acid may be made in 1-ml portions. By this modification less acid is often required. Little additional reaction time is thus consumed.

Other Applicable Reactions

Biological tissues such as heart, lung, liver, hair collagen or fat-free protein structures (or blood fibrin) may be wet ashed without complications following procedures previously described. Fat-laden materials require the procedure as described because their perchloric acid degradation products, or their original structure, makes them acid-immiscible. Neither, in this

TABLE 2. WET OXIDATION OF 1.6-G SAMPLE OF BONE

10 ml of 100% H₂SO₄ plus 1.2 mg of vanadium in first-stage reaction.

5 ml of 73.6% HClO₄ for the second stage of digestion.

Bethge apparatus with 250-ml reaction flask and SO₂ disposal vent.

100% H ₂ SO ₄ digestion			73.6% HClO ₄ digestion		
Reaction time, min	Temp, degrees C	Reaction effects	Reaction time, min	Temp, degrees C	Remarks
Start	26		18	210	73.6% HClO ₄ added
4	250	Reactants black	19	178	Heat continued
5	266	Reaction refluxing	20	205	Solution red-brown
6	272	Inside flask walls clearing	21	209	Reaction exothermic
8	288	Exothermic effect shown	22	211	Turbid solution
					White deposit
10	283	Exothermic reaction over	23	214	Reaction complete
12	284				
14	285	SO ₂ evolution over			
15	286	Discontinue heating			

TABLE 3. WET OXIDATION OF 3.0-G SAMPLE OF BONE

15 ml of 100% H_2SO_4 plus 1-2 mg of vanadium in first-stage reaction.

5 ml of 73.6% HClO_4 for the second stage of digestion.

Bethge apparatus with 250-ml reaction flask and SO_2 disposal vent.

100% H_2SO_4 digestion			73.6% HClO_4 digestion		Remarks
Reaction time, min	Temp, degrees C	Reaction effects	Reaction time, min	Temp, degrees C	
Start	27		18	222	
1	50	Solution dark brown	19	196	73.6% HClO_4 added
2	165	Slight foaming	19.5	180	Heat continued
3	215	Solution black	20	205	Solution red-brown
5	268	Solution refluxing	21	213	Reaction exothermic
8	295	Inside flask walls clear	22.5	215	No turbidity formed
10	312		23	215	Reaction complete
12	322				
15	323	Discontinued heating			

case, is the presence of nitric acid a solution to the problem. Such two-phase systems, with fats supernatant in contact with boiling perchloric acid and its fumes, do not oxidize smoothly. Fires are ordinarily produced. Fumes of boiling perchloric acid in the presence of volatilized fat may lead to uncontrolled reaction kinetics. The use of boiling 100% sulfuric acid in a single stage reaction obviates this complication.

It is reasonable to apply the method as described to the wet ashing of cream, butter, cheese, lecithin or egg yolk.

Literature Cited

- (1) M. Gerster, *Chem. Ziet.*, **11**, 209 (1887)
- (2) G. Frederick Smith and Harvey Diehl, *Talanta* **3**, 41 (1959).

SECTION XI

WET OXIDATION OF ORGANIC COMPOSITIONS

Mixed Nitric and Hydrochloric Acid with Ammonium

Perchlorate as Oxygen Donors.

Introduction

The wet oxidation of samples of organic compositions employing perchloric acid and its mixtures with other mineral acids, or by use of perchloric acid alone at graded selected oxidation potentials, present a wide variety of systems for selection as best adapted to any type sample. Some procedures of more than one type of oxidation, previously experimentally demonstrated, may be applied to the same oxidations of the individual samples of organic composition as a matter of preferential choice.

The present section (1) is devoted to the experimental demonstration of yet another generally applicable procedure. It substitutes for methods which have been previously developed, particularly those of the liquid fire reaction, Section 5, and the periodic liquid fire reaction, Section 6, in most of their range of oxidation of variable type organic, natural and synthetic, compositions.

The new procedure requires a much more leisurely reaction period to provide conditions applicable to the digestion of larger sample weights. In this new procedure the perchloric acid as chief oxygen donor is generated *in situ*, the reaction components are diluted with added water, and such modifications may prove more attractive in that they seem far removed from any precautions fostered by the operator's thought of violent reactions.

Experimental

Reagents

Concentrated (67-68%) nitric and (37-38%) hydrochloric acids

Ammonium perchlorate (99.5%). As produced in multiple tonnage for use in the solid rocket fuel program. Recrystallization purification is recommended.

Apparatus

The Bethge apparatus is requisite. See Figure 2, page 20. By its use reaction conditions are best controlled. Heat is provided using a ring burner and modified nichrome wire gauze. A modified Rogers ring burner with micro control of gas-fuel intake is preferred. An electric hot plate or a glass fabric heating mantle with voltage control may be substituted. Rate of energy input should be of such magnitude as to bring the reaction temperature to no more than 100° during the first 5 min. Adjustment of the thermometer insert is provided by a teflon adapter as shown.

Results and Discussion

Reaction characteristics

The reactions involved fall under three stages:

(A) Initial conversion of ammonium perchlorate to perchloric acid. The reaction with mixed hydrochloric and nitric acid converts the ammonium ion to nitrous oxide and water. An amount of water approximately equal to the combined mixed acid volumes is added to the sampling mixture. Reaction time is 10 min at a maximum of 110°. Heat is applied at such magnitude to attain 90° to 100° in not less than 5 min. Reaction conditions involve a mildly exothermic condition and the reaction is conducted under reflux of the condensed vapor phase. In general, the organic compositions being oxidized are dissolved by the three mixed acids present during stage (A).

(B) Adjustment is provided to retain condensate during the remainder of the digestion. The hydrochloric acid has been consumed. The dilute solution of mixed nitric and perchloric acids gradually concentrates over the temperature range 110° to 150°. The reaction is exothermic to a greater or less extent depending upon the nature of the organic material being oxidized. During this stage the nitric acid undergoes gradual uniform increase in concentration. The oxidation potential of the nitric acid increases gradually to its maximum value at 150°. All organic matter capable of destruction by nitric acid has been reacted upon and excess nitric acid has been collected in the condensate. Little reactivity from perchloric acid has, as yet, been applied. Stage (B) requires from 30 to 40 min at the same application of heat applied throughout the three stages (A), (B) and (C). Variation in time during stage (B) is governed by the exothermic reaction provided by nitric acid oxidation.

(C) The final stage completes the oxidation following the limited destruction of organic matter provided by nitric acid and elimination of its excess at 140° to 150°. At 150° the first influence of perchloric acid as oxygen donor becomes effective. As the temperature advances because of condensation increase, the oxidation potential steadily and uniformly increases from approximately 0.8 to 2.0 V. The perchloric acid concentration increases from 50% at 150° to 72.5% at 203°. The reaction over this range becomes increasingly exothermic. At 203° the distillate and residual acid in the reaction flask is at the water-perchloric acid azeotropic composition. The extent of the exothermic reaction depends upon the nature of the organic material being oxidized. Stage (C) requires 10 min, in general. Total reaction time, over-all, is approximately 60 min.

Reaction advantages

Previously described wet oxidation procedures have one common ob-

jective, that of controlled oxidation at minimum time intervals. All types of organic compositions may be oxidized by one to three of the various applicable procedures. In general, the products oxidized involve little or no carbonization. Degradation products, as well as the original compositions, are usually all in homogeneous solution throughout the entire digestion. Such reactions, if accompanied by the formation of dark brown or black reaction products, are still reaction rate controlled. However, in general, sample weights are limited to the 1 to 2 g magnitude by previously outlined methods.

The present work involves no reactions resulting in dark or black reaction media. Samples of 5 g are applicable. Conditions are adjusted to require longer reaction periods. Accordingly, the full possible effect of nitric acid as oxygen donor is provided. The oxidation of organic matter is thus as complete as possible before the perchloric acid oxidation begins. By following the reaction temperature during stage (b), the mildly exothermic nitric acid oxidation indicates the extent to which the removal of organic matter is progressing. Adjustment in the volume of nitric acid employed may then be made. Perchloric acid reaction in stage (c) may thus be favorably minimized.

The entire new procedure requires little operator attention. The apparatus involved is readily assembled and dismantled. It does not require a fume hood. The use of the new procedure encourages those reluctant to apply perchloric acid in wet oxidations, in fear of uncontrolled reaction intensities, to adopt the procedure in routine operations. In the present studies, no protective reaction screen was employed in any test oxidation.

Graphic representation of data

Three distinct types of organic compositions for wet oxidation include those which are predominantly cellulose, sugars or proteins. The time-temperature recording of the progress of the oxidation is the most informative technique serving to characterize each type of reaction. Employing the Bethge apparatus (Fig. 3, page 21), the oxidation of 2 g of tuna fish is characterized in Fig. 17. Because of the small weight of sample the reaction flask employed is 250 ml in volume. The oxidation is, for this reason, complete in 26 min. Stage (A) in Fig. 17 is complete in 6 min, and from 4 to 6 min no temperature rise indicates the conversion of ammonium perchlorate to perchloric acid. The sample during this period has been completely dissolved. At this point the return of reflux to the reaction flask is discontinued. For the following 16 min the stage (B) reaction of nitric acid is operative. Over the period 6 to 14 min the temperature elevation is slow and uniform indicating no appreciable exothermic nitric acid oxidation. Over the reaction period

15 to 22 min the oxidation is progressing at an increased tempo, as shown by the materially increased slope of the time-temperature plot. At 22 min the temperature has advanced to 150°. All excess nitric acid has now left the reaction flask to be collected in the condenser system. The effective oxidation period, 22 to 26 min, involves the reaction of perchloric acid as oxygen donor, stage (C). At 150° the perchloric acid concentration is approximately 50%. Here there comes into play a gradual increase in oxidation potential accompanied by a material exothermic reaction complete in 4 min. The completion of oxidation (as indicated by the oxidation of the chromium indicator, Cr^{III} to Cr^{VI} at 202°) indicates a perchloric acid

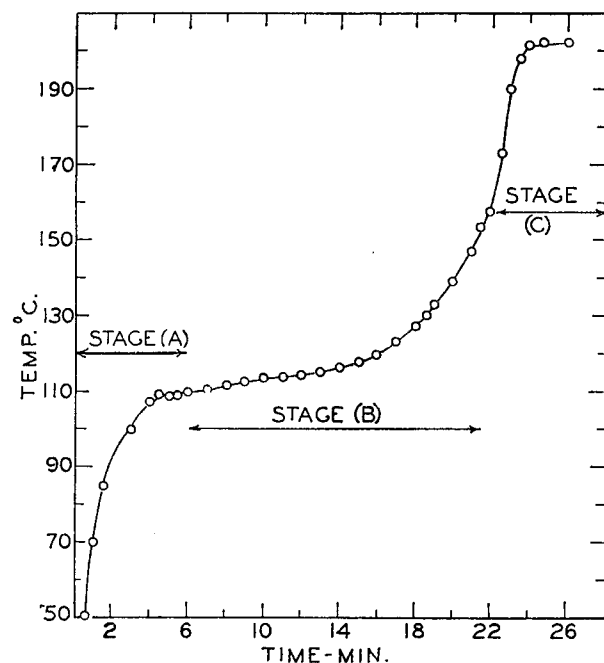


Fig. 17.—Oxidation of tuna fish [2 g of sample; 250-ml reaction flask; 10 ml of HCl; 17.6 ml of HNO_3 ; 21.5 g of NH_4ClO_4 ; 26 ml of H_2O ; chromium indicator; 43 ml of distillate; 21.75 g (13ml) of residue in HClO_4 flask].

concentration of 72.5%.* During the entire reaction [stages (A), (B) and (C)] no alteration of heat applied has been made. Stage (C) owes its temperature rise almost entirely to its exothermic properties.

At the completion of the oxidation the condensate may be removed, the air and water-cooled condenser rinsed and the distillation continued with elevation of temperature input. By this means excess perchloric acid can

* 1-2 mg of potassium dichromate are included in the reaction mixture. As long as any organic matter is present, the chromium is held in the green trivalent state. When all traces of organic matter are oxidized, the chromium is immediately oxidized to the orange hexavalent state.

be removed from the reaction flask. Any silica originally present in the sample is now dehydrated and insoluble. The material in the reaction flask can now be diluted with water, filtered and made ready for determination of metallic and non-metallic minor or major components originally present in the organic compositions of the sample. The flask contents, after completion of the oxidation, described by the results of Fig. 17, weighed 21.75 g or approximately 13 ml of perchloric acid. The distillate was 43 ml.

Non-condensed volatile products may be conducted from the top of the water-cooled condenser by flexible tubing into the sink drain, thus avoiding, by the use of the Bethge apparatus, the need for a fume hood in which to perform the oxidation.

Oxidation of 5-g samples of organic compositions

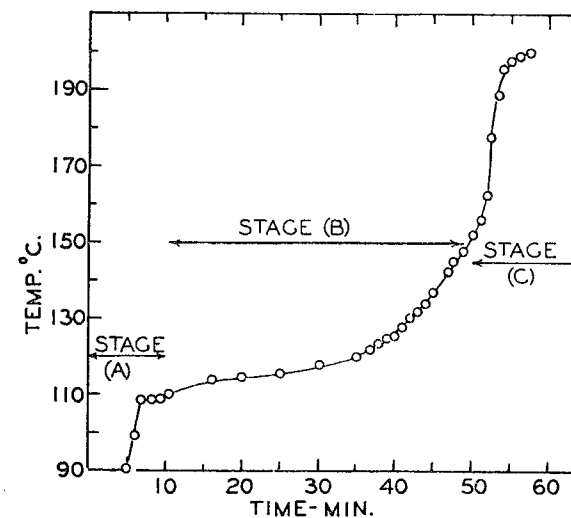


Fig. 18.—Oxidation of dried beef [5 g of sample; 1000-ml reaction flask; 15 ml of HCl; 26.5 ml HNO_3 ; 32 g of NH_4ClO_4 ; 39 ml of H_2O ; chromium indicator; 67 ml of distillate; 33.6 g (20 ml) of residue in HClO_4 flask].

The oxidation of 5 g of dried beef is characterized in Fig. 18. A 1000-ml flask with the Bethge apparatus was employed. As well as using a 5-g sample, the reactants were augmented and a resultant 60-min reaction time was required. As could be predicted, the general profile of the graphical recording of data duplicates that of Fig. 17 for the reason that both organic compositions oxidized are proteins (distillate: 67 ml; perchloric acid remaining in the reaction flask: 33.6 g or 20 ml).

The oxidation of 5 g of vegetable tanned leather is graphically recorded

in Fig. 19. The reaction time is shown as 41 min under the same conditions as for an equal sample of dried beef. In the case of the leather it is observed

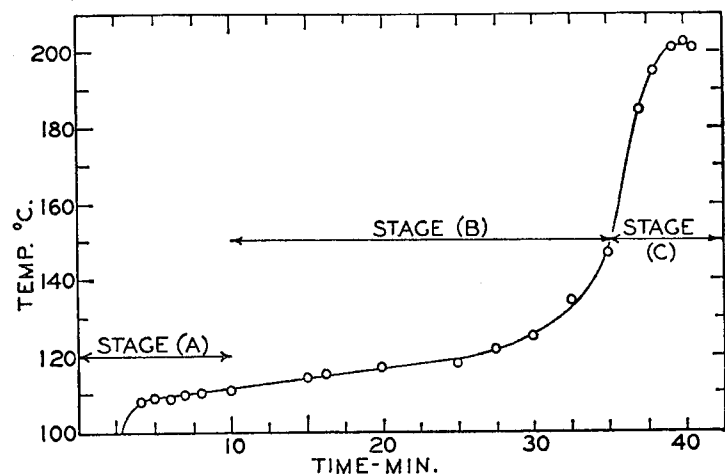


Fig. 19.—Oxidation of leather [5 g of sample; 500 or 1000-ml reaction flask; 15 ml of HCl; 35 ml of HNO_3 ; 32 g of NH_4ClO_4 ; 39 ml H_2O ; chromium indicator; 71 ml of distillate; 29 g (17.4 ml) of residue in HClO_4 flask].

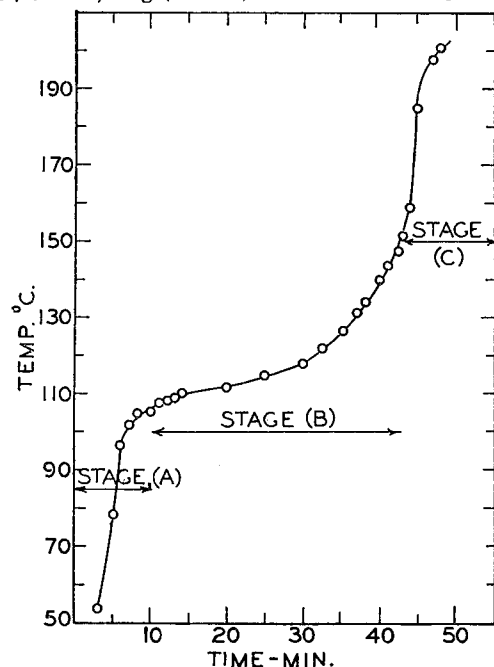


Fig. 20.—Oxidation of sugar [5 g of sample; 1000-ml reaction flask; 15 ml of HCl; 35 ml of HNO_3 ; 32 g of NH_4ClO_4 ; 39 ml of H_2O ; chromium indicator; 70 ml of distillate; 35.3 g (21.1 ml) of residue in HClO_4 flask].

that the oxidation is less in the nitric acid stage (B) and the burden of reactivity is placed upon the perchloric acid stage (C). With the same sample weight, the distillate was 71 ml and the perchloric acid in the reaction flask 29 g or 17.4 ml. The same amounts of mixed reactants were employed in the reactions shown in Figs. 18 and 19. An additional 2.6 ml of perchloric acid was consumed.

The oxidation of 5 g of cane sugar is shown in the graph of results in Fig. 20. The results indicate that the oxygen donor contribution of both nitric acid and perchloric acid are approximately equal.

The oxidation of 5 g of raffia and 5 g of cinnamon bark (principally both cellulose) is depicted in Figs. 21 and 22. As would be predicted, the results indicate almost duplicate reaction characteristics. Subsequent to the reaction with nitric acid in stage (B), the exothermic nature of the perchloric acid oxidation is more pronounced than that of either the oxidation of samples essentially sugar or protein in composition.

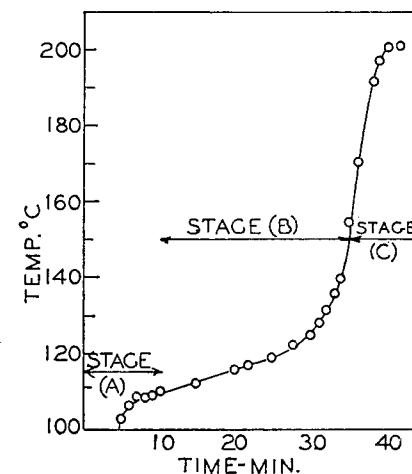


Fig. 21 — Oxidation of raffia [5 g of sample; 1000-ml reaction flask; 15 ml of HCl; 35 ml of HNO_3 ; 32 g of NH_4ClO_4 ; 39 ml of H_2O ; chromium indicator; 71 ml of distillate; 29 g (17.4 ml) of residue in HClO_4 flask].

Reaction commentary

Nitrous oxide formed in reaction stage (A), being soluble in water and many organic compositions, especially in the case of cellulose-laden materials, promotes foam formation. Such foams are very light and can be counteracted by increasing the flask volume of the Bethge apparatus.

The oxidants described in Figs. 17 to 22 may be well conditioned by employing modified amounts of reactants to suit any particular type of routine plant control oxidation samples. By trial, for example, the volume of nitric acid may be reduced to such favorable volume, provided there results no

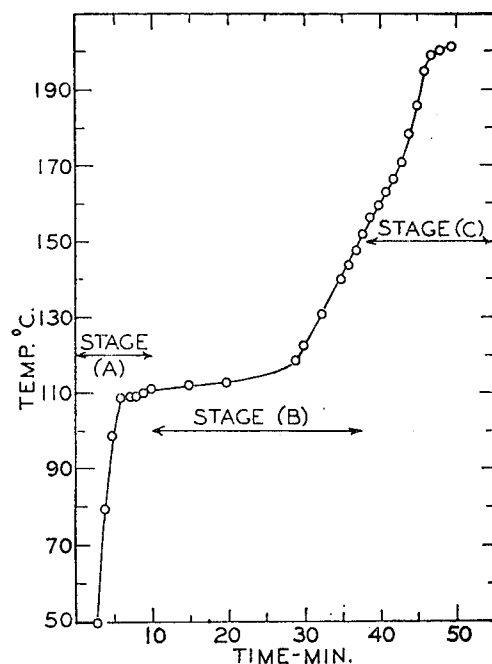


Fig. 22 — Oxidation of cinnamon bark [5 g of sample; 1000-ml reaction flask; 15 ml of HCl; 35 ml of HNO₃; 32 g of NH₄ClO₄; 39 ml of H₂O; chromium indicator; 73 ml of distillate].

dark brown or black degradation reaction products. The time required may thus be favorably reduced for a given application.

Sufficient perchloric acid should be obtained to prevent dry spots from forming on the inside bottom of the reaction flask. By withdrawing distillate from the apparatus condenser system, and rinsing with distilled water at the completion of oxidations, one may collect by further distillation the bulk of the perchloric acid formed previously.

Organic compositions containing siliceous materials, in boiling 72.5% perchloric acid, precipitate insoluble SiO₂ quantitatively. In general, perchloric acid is a preferred medium for final determination of metallic and

non-metallic residual isolated and retained elements. If for any reason a sulfuric acid solution is preferred, it may be added to the perchloric acid solution and the latter displaced by volatilization upon fuming at 180-190°.

Forty test oxidations of a wide variety of organic compositions have been carried out. Analysts, formerly apprehensive of methods previously described in this review, may well make "assurance doubly sure" through the use of the present disclosures.

Literature Cited

- (1) G. Frederick Smith. "Wet Oxidation of Organic Compositions. Mixed Nitric and Hydrochloric Acids With Ammonium Perchlorate as Oxygen Donors," *Talanta*, 11, 633-40 (1964).

Since the writing of this monograph, the following article has appeared:

Wet ashing of coal with perchloric acid mixed with periodic acid for the determination of sulphur and other constituents. Gerald I. Spielholz and Harvey Diehl *Talanta* **13**, 7, 991-1002 (1966)