give a more reliable figure than to take the average of ten readings at any one wave length.

The spectrophotometric method, provided a spectrophotometer possessing the precision of the Purdue instrument is used, is capable of giving results for iron in ores which will be within ±0.30 per cent of the values given by the dichromate titration method and many will be within ±0.10 per cent. Results may be duplicated on the same sample with a precision of about ±0.10 to ±0.20 per cent.

TABLE II. COMPARISON OF PERCENTAGE ERRORS IN IRON AND COPPER DETERMINATIONS

	Percentage Error		
	Maximum	Minimum	Average
Iron, 12 samples	0.65	0.00	0.20
Copper, 11 samples	1.98	0.00	0.69

In Table II is shown a comparison of the reliability of the results for iron, on a percentage basis, with those previously obtained by the writer (*4*) for copper ores, where the actual percentages were much lower. The figures indicate that the spectrophotometric method involving the use of the molecular extinction coefficient can be satisfactorily extended to the determination of iron where the percentage of the constituent determined is relatively high.

Summary

The spectrophotometric method employing the molecular extinction coefficient has been applied to the determination of iron in ores. Although the iron content is relatively high, the percentage error is smaller than when similar transmittancy measurements were made on the copper-ammonia system where the copper content was relatively very low.

The spectrophotometric method gives results for iron in ores which check well with those given by the dichromate method.

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Determination of Acetylmethylcarbinol

Effect on Certain Analytical Procedures

A. F. LANGLYKKE AND W. H. PETERSON, University of Wisconsin, Madison, Wis.

SINCE acetylmethylcarbinol is found in varying quantities among the fermentation products of a large variety of bacterial cultures and enzymatic preparations (*3, 8, 11, 13, 14, 15*), a knowledge of its characteristics and its effect on various analytical procedures is particularly desirable. Because of the nature of the compound it reacts in many of the common analytical procedures, causing error in the final results. Thus, because of its reducing properties it interferes in the common methods for the determination of reducing sugars. Since it is volatile it appears in distillates and causes error in the analyses for volatile products. It reacts with alkaline iodine and therefore interferes with the ordinary method for the determination of acetone (*6*). It gives rise to acid products on oxidation and in this way interferes with methods for the determination of alcohol which depend on oxidation.

The acetylmethylcarbinol used for these experiments was obtained from the Lucidol Corporation, Buffalo, N. Y. The liquid material was crystallized by holding for one month at a temperature of approximately −10° C. The material so obtained was of a mushy consistency and showed a yellow coloration and pungent odor which were removed by washing several times with ether. The resulting white crystalline powder gave a melting point which varied from 84° to 125° C., depending on the rate at which the oil bath was heated. Statements in the literature indicate that the melting point of dimeric acetylmethylcarbinol is rather irregular and varies with the method of crystallization (*1, 2*).

Determination of the molecular weight of the crystalline material by elevation of the boiling point indicated an initial value of about 160 which dropped to 101 after boiling for 25 minutes because of conversion to the monomer. Other investigators (*1, 2*) have found molecular weights for acetylmethylcarbinol dimers which ranged from 170 to 195 as compared to the theoretical value of 176.

Reaction with Alkaline Iodine

Since acetylmethylcarbinol contains the acetyl group (CH_3CO—) directly linked to carbon, it will react with alkaline iodine to form iodoform (*5*). To verify the nature of the reaction between acetylmethylcarbinol and iodine, aliquots of standard aqueous solutions of the compound (containing 3 to 10 mg.) were made alkaline with 1 *N* sodium hydroxide and standard iodine solution was added. Iodoform was precipitated and, after standing for 10 to 15 minutes, the excess iodine was liberated by the addition of 1 *N* sulfuric acid and titrated. The averages of a large number of determinations are presented in Table I. The iodine used approaches six atoms per molecule of iodoform, which supports the following formulation for the reaction:

$$3I_2 + 6NaOH = 3NaOI + 3NaI + 3H_2O$$
$$CH_3CHOHCOCH_3 + 3NaOI = CHI_3 + CH_3CHOHCOONa + 2NaOH$$

The iodoform isolated from one reaction sublimed at 118° to 119° C. as compared to the reported value of 119° (*9*). Lactic acid was recovered from the products of the reaction by extraction of the acidified solution with ether. From the ether extract the zinc salt was prepared and analyzed. The water of crystallization was determined by drying to a constant weight at 110° C., the zinc content by ignition, and lactic

TABLE I. REACTION OF ACETYLMETHYLCARBINOL WITH ALKALINE IODINE

Sample	0.1 N I_2 per Mg. $CH_3CHOH-COCH_3$ Cc.	Atoms I per Molecule $CH_3CHOH-COCH_3$	Per Cent of Theory
Crystals washed with ether	0.671	5.91	98.5
Ether-washed product recrystallized from acetone	0.672	5.92	98.7
Theoretical for reaction	0.681	6.00	100.0

TABLE II. VOLATILITY OF ACETYLMETHYLCARBINOL IN AQUEOUS SOLUTION

Fractions, Percentage of Total Distilled	Acetylmethylcarbinol in Distillate—Concentration of Solution: 0.005%	0.02%	0.072%	0.10%	Av.
	%	%	%	%	%
25	31.8	32.1	31.5	..	31.8
50	59.7	60.3	59.6	59.2	59.7
75	83.7	83.9	83.1	..	83.6

acid by the method of Friedemann and Graeser (*4*) with the following results:

Calcd. for $Zn(C_3H_5O_3)\cdot 3H_2O$:H_2O 18.2. Found: 18.0
Calcd. for $Zn(C_3H_5O_3)$: Zn 26.9, $CH_3CHOHCOOH$ 74.0. Found: Zn 29.0, $CH_3CHOHCOOH$ 73.9.

The data show that the product isolated was nearly pure inactive zinc lactate containing three molecules of water of crystallization. The high zinc content is probably due to the presence of small amounts of excess zinc—e. g., zinc hydroxide—which raise the zinc value but have no appreciable effect on the other analyses.

Volatility

The rate of distillation of acetylmethylcarbinol from aqueous solution was determined by a modification of the method of Virtanen and Pulkki (*18*). In order to prevent condensation and reflux, an insulating hood made from a large metal can was used to cover the distillation flask and in order to ensure uniform heating, a metal shield was used to protect the Bunsen burner. In the experiments 200 cc. of solution were distilled and three 50-cc. fractions were collected. These fractions as well as the residue were analyzed by application of the iodoform reaction. The percentage of acetylmethylcarbinol in the distillates was found to be independent of the rate of distillation when the precautions to prevent reflux were observed. The data for the distillation of solutions varying in concentration from 0.005 to 0.1 per cent are presented in Table II.

As shown by the data, the distribution of acetylmethylcarbinol during distillation is constant and independent of the concentration of the solution. A quantitative expression for the volatility may be calculated by application of the formula used by Virtanen and Pulkki (*18*):

$$k = \frac{\log y_1 - \log y_2}{\log x_1 - \log x_2}$$

In this equation y_1 represents the amount of volatile compound in the solution at the beginning of distillation and y_2 the amount at the end, while x_1 represents the amount of water at the beginning and x_2 the amount at the end. The values for the distillation constant, k, as calculated from the average figures for the first, second, and third fractions (Table II) were 1.330, 1.297, and 1.298. On comparison with the figures of Virtanen and Pulkki (*18*) it is seen that acetylmethylcarbinol is a little more volatile than propionic acid (k = 1.239 to 1.245) and less volatile than butyric acid (k = 1.940 to 2.035).

In the determination of acetone in a fermented medium containing acetylmethylcarbinol, a correction must be made which depends on the concentration of acetylmethylcarbinol and the volume of distillate collected. If 25 per cent of the liquid is distilled off, approximately 32 per cent of the acetylmethylcarbinol will be contained in the distillate. For other volumes distilled, the correction may be applied most conveniently by plotting a curve using the values of Table II, or the correction may be made by calculation using the equation

$$\log \frac{y_1}{y_2} = k \log \frac{x_1}{x_2}$$

When 35 per cent of the liquid is distilled, $\frac{x_1}{x_2} = \frac{100}{65}$, and setting k equal to 1.31 the value for y_2, the amount of acetylmethylcarbinol remaining undistilled, is found to be 56.8 per cent. Therefore, 43.2 per cent of the acetylmethylcarbinol will be found in the distillate.

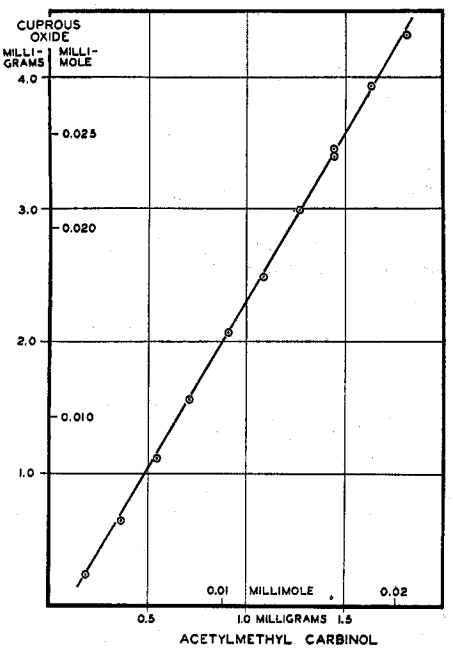

FIGURE 1. CUPROUS OXIDE PRODUCED IN REDUCTION OF SHAFFER-HARTMANN SUGAR REAGENT BY ACETYLMETHYLCARBINOL

Reaction with Oxidizing Reagents

In order to determine the corrections to be applied in the analyses for reducing sugar, two methods for the determination of glucose were investigated. These were the Shaffer-Hartmann method as modified by Stiles, Peterson, and Fred (*17*), which depends on the reduction of copper and iodometric determination of the cuprous oxide produced, and the Hagedorn-Jensen method (*7*), which depends on the reduction of potassium ferricyanide to ferrocyanide and titration of the excess ferricyanide with dilute standard thiosulfate solution. In each case varying amounts of an aqueous solution of acetylmethylcarbinol were submitted to the procedure used in the determination of the reducing sugars and the extent of reduction of the reagent was determined. The results are shown graphically in Figures 1 and 2.

REDUCTION OF CUPRIC SULFATE. According to Kling (*12*), acetylmethylcarbinol is oxidized by alkaline cupric oxide

to acetic acid, but such a result was not obtained in these experiments. Stahly and Werkman (*16*) report that 3.01 grams of cuprous oxide are produced from each gram of acetylmethylcarbinol under the conditions of the Munson-Walker method. In the copper-reduction method used, the tubes are only loosely stoppered to prevent oxidation by the air and loss of volatile products is not prevented. It is also possible that the 15-minute reaction period is not long enough for complete reaction. For these reasons the amount of copper reduced does not bear a stoichiometric relation to the amount of acetylmethylcarbinol in the sample, although there is a regular relation between these two variables.

If acetylmethylcarbinol is oxidized to diacetyl, two hydrogen equivalents per mole are required, while for the oxidation to acetic acid four hydrogen equivalents are necessary. As calculated from the graph of Figure 1, 2.95 hydrogen equivalents are actually used in the oxidation, which shows that only about one-half of the diacetyl formed is further oxidized to acetic acid. The points described a straight line, which does not, however, pass through the origin. This apparent anomaly can be explained only on the assumption that a constant small amount of acetylmethylcarbinol escapes oxidation, or that a constant small amount of diacetyl is lost through evaporation.

Reduction of Ferricyanide. As in the reduction of copper, the reduction of ferricyanide proceeds without complete conversion of the acetylmethylcarbinol to acetic acid. In this case 2.67 hydrogen equivalents are required, although more variation was observed in this reaction. This is shown by the fact that the individual points on the graph of Figure 2 do not correspond so well to a straight line as in the case of the reduction of copper.

Oxidation with Acid Potassium Dichromate

In the determination of butyl and ethyl alcohols by the method of Johnson (*10*), a distillate containing the neutral volatile products from a bacterial fermentation is oxidized by means of acid potassium dichromate solution and the acid resulting from the oxidation is removed by distillation. Two fractions are collected and the content of butyl and ethyl alcohols in the original sample is calculated from the titrations of these fractions. Since acetylmethylcarbinol yields acid products on oxidation, it is of interest to know what effect this compound will exert in the alcohol method.

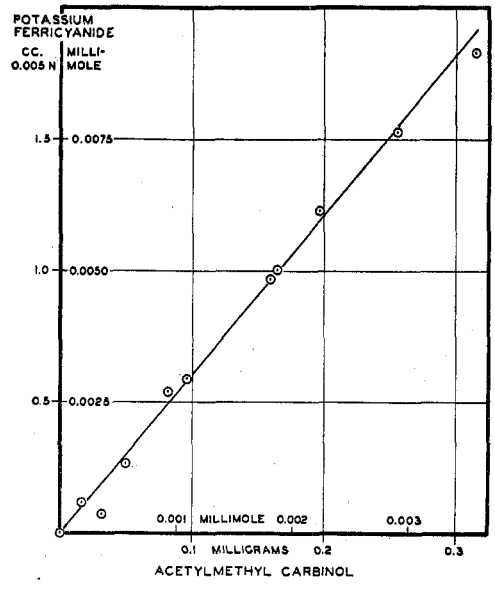

Figure 2. Reduction of Potassium Ferricyanide (Hagedorn-Jensen Reagent) by Acetylmethylcarbinol

Table III. Effect on Johnson Method for Butyl and Ethyl Alcohols

Acetylmethylcarbinol Sample	Alcohol Indicated: Actual, Butyl alcohol	Actual, Ethyl alcohol	Theoretical[a], Butyl alcohol	Theoretical[a], Ethyl alcohol
Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*
5	−0.01	5.26	0	5.23
10	0.14	10.48	0	10.46
15	0.14	15.87	0	15.69
Av. (per mg. acetoin)	0.007	1.053	0	1.046

[a] Based on assumption that one mole of acetylmethylcarbinol is equivalent to two moles of ethyl alcohol.

Samples of 5, 10, and 15 mg. of acetylmethylcarbinol in aqueous solution were analyzed according to the procedure for the determination of alcohols by the Johnson method (*10*). In Table III the results obtained are expressed as milligrams of butyl and ethyl alcohols. The oxidation of acetylmethylcarbinol by the acid dichromate yields acetic acid, and therefore for purposes of correcting any alcohol determinations one mole of acetylmethylcarbinol corresponds to two moles of ethyl alcohol.

Determination of Acetylmethylcarbinol

Since acetylmethylcarbinol reacts quantitatively with alkaline iodine reagent and since the carbinol is distilled from aqueous solution at a definite rate which is independent of the concentration, it was possible to develop a method for its determination in which these properties were applied. Attempts to separate the carbinol from the fermented medium were unsuccessful, as neither ether extraction nor constant volume distillation gave a quantitative recovery of the compound.

In order to apply the iodoform reaction directly to the determination of acetylmethylcarbinol it is necessary to eliminate from the sample any other compounds which will react with the iodine reagent. Acetone is commonly present in large excess over the acetylmethylcarbinol content in some fermentations, but this compound is practically completely eliminated by distillation of half the volume of an aqueous solution. Ethyl alcohol in appreciable concentration will also react with alkaline iodine, although this reaction is very incomplete at room temperature and in the short reaction time used. However, it is also completely eliminated by half distillation. Butylene glycol is also likely to occur in association with acetylmethylcarbinol in fermentations and will react with alkaline iodine in 15 minutes at room temperature to produce an apparent acetylmethylcarbinol content equivalent to 10 per cent of its own weight. However, because of the nonvolatility of this compound, the error resulting by analysis of the third quarter of distillate is negligible.

By far the most convenient method for the determination of acetylmethylcarbinol involves direct distillation from the fermentation mixture, and by this method quantitative results could be obtained. The disadvantage of direct distillation from the culture medium lies in the fact that many of the media are prone to foam badly during distillation. However, many ordinary media may be directly distilled in the fractional distillation procedure and certain procedures may be applied to minimize foaming when this characteristic is displayed.

It was found that a mineral salts–peptone-glucose medium which had been fermented by *A. aerogenes* in the presence of an excess of calcium carbonate could not be distilled, because of excessive foaming. To correct this condition the culture was acidified with sulfuric acid, heated just to boiling and, after cooling, filtered by suction through an asbestos mat.

TABLE IV. DIRECT DETERMINATION OF ACETYLMETHYLCARBINOL BY FRACTIONAL DISTILLATION OF CULTURE

Sample	Acetylmethylcarbinol Found	Added	Found (after addition)	Recovery of Added Acetylmethylcarbinol	
	Mg./100 cc.	*Mg./100 cc.*	*Mg./100 cc.*	*Mg./100 cc.*	%
Sterile salts–peptone medium	..	49.2	50.3	50.3	102.1
	..	21.0	21.4	21.4	101.9
Cl. polymyxa in 6% corn mash	40.3	49.6	89.5	49.2	99.2
A. aerogenes in salts-peptone medium	29.1	37.8	67.9	38.8	102.7
A. aerogenes in salts-peptone medium (excess $CaCO_3$)	39.4	44.0	85.1	45.7	103.9
A. aerogenes in salts-peptone medium	18.2	10.0	28.1	9.9	99.0
Cl. acetobutylicum in 6% corn mash	37.4	49.8	87.1	49.7	99.8

The resulting product showed considerable less tendency to foam, but as an added precaution the sample was diluted to four times the volume before applying the distillation procedure. By this procedure quantitative results could be obtained.

For the results reported in Table IV the following general procedure was used:

Enough of the culture to give 100 cc. of clear filtrate is filtered through a fluted filter and 100 cc. of the clear filtrate are then pipetted into the 300-cc. distillation flask which contains 6 or 8 glass beads to prevent bumping. The flask is directly connected to a short condenser by means of a ground-glass joint and is supported by a ring bearing a wire gauze with an asbestos mat center. The insulating hood is placed over the flask, and heating is started with a burner which is protected by a shield so as to guarantee uniform heating. The rate of heating is so adjusted that the distillate which collects is cold and the medium under distillation boils evenly. A fraction of exactly 50 cc. is collected in a volumetric flask and may either be discarded or used in the determination of the more volatile products of the fermentation. A second fraction of exactly 25 cc. is then collected and analyzed by the iodoform reaction.

The contents of the 25-cc. volumetric flask are added to 15 cc. of 1 *N* sodium hydroxide in a 150-cc. Erlenmeyer flask. The volumetric flask is rinsed with 3 portions of a few cubic centimeters of water and the rinsings are added to the sample in the Erlenmeyer. Then while the flask is constantly shaken in one hand, exactly 5 cc. of 0.2 *N* iodine are added by rapid dropping from a pipet held in the other. The precipitation of iodoform begins almost immediately and after 10 minutes the reaction is complete. After standing for 10 to 15 minutes out of direct sunlight, 20 cc. of 1 *N* sulfuric acid are run into the flask and the liberated iodine is titrated immediately to the starch end point with standard 0.1 *N* sodium thiosulfate solution. A semimicroburet which may be read accurately to 0.01 cc. is used. A blank for the purpose of standardizing the iodine solution is run on 5 cc. of the iodine solution, with distilled water replacing the sample in the ordinary procedure.

Since one mole of acetylmethylcarbinol is equivalent to 6 atoms of iodine, one millimole (or 88 mg.) is equivalent to 60 cc. of 0.1 *N* iodine. Then, for each cc. of 0.1 *N* iodine used in the iodoform reaction there are 1.467 mg. of acetylmethylcarbinol in the sample. According to Table II, 23.9 per cent of the acetylmethylcarbinol in the sample appears in the third quarter of distillate. Therefore, to obtain the total acetylmethylcarbinol content of the 100-cc. sample distilled, the content of the third quarter of distillate is divided by the factor 0.239.

The results reported in Table IV were obtained on a variety of cultures as shown. The salts-peptone medium used consisted of 0.07 per cent dibasic ammonium phosphate, 0.5 per cent peptone, 0.1 per cent asparagine, and tap water. In the case of the fermentations 3 per cent of glucose was added. The corn mash was made up with 6 per cent of corn meal in tap water. In the second column of Table IV are given the concentrations of acetylmethylcarbinol as determined directly on the culture by the procedure outlined. Acetylmethylcarbinol was then added in the amounts given in the third column and the content of the samples so prepared was again determined with the results expressed in the fourth column. In the fifth and sixth columns are entered the recoveries of the added acetylmethylcarbinol as obtained from the difference between the determinations before and after the addition of a known quantity of acetylmethylcarbinol. The data reported are the averages of triplicate analyses. The deviation of individual results from the average ranged from 0 to 4.1 per cent and averaged 1.13 per cent. The average error in the recovery of added acetylmethylcarbinol was 2.2 per cent.

The recoveries are in general a little high, and the average recovery is about 101.2 per cent. Therefore, a somewhat higher degree of accuracy may be obtained if the recovery is determined separately for each different medium used, as this value will vary somewhat with the nature of the solution under analysis. Thus, if instead of using the value of 23.9 per cent recovery as determined for aqueous solutions a corrected value of 23.6 per cent is used, the errors in the determinations reported would vary from −2.2 to +2.7 per cent.

Summary

Because of the unique nature of the compound, acetylmethylcarbinol affects a variety of common analytical procedures. Methods for the correction of acetone, alcohol, and reducing sugar determinations in the presence of acetylmethylcarbinol are described. Based on its volatility and iodoform reaction, a method for its quantitative determination has been developed.

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