ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editor

# Power Is Not the Only Aspect

IT IS quite understandable that the greatest emphasis at the first international conference on the peaceful uses of atomic energy staged at Geneva is on power. Certainly, the most immediate need in power-starved areas of the world is a reasonably economical means of providing energy in large quantities.

It is this basic essential to modern living that unquestionably is the nucleus for further improving the health and wealth of millions of now underprivileged peoples. The truth of this statement is evident in every history book. Once relatively cheap power becomes available, agriculture and industry begin to expand and existing material resources can be exploited efficiently.

We trust, however, that at the scientific and technological deliberations at the Geneva Conference the new tracer techniques which the successful splitting of the atom made possible, will not be neglected in favor of the more spectacular power aspects.

Analysts today have at their command new tools for research and the maintenance of closer and more uniform quality control in many fields heretofore not available to them. These should be exploited to the fullest extent in the shortest possible time. We have, of course, followed these developments and many papers have appeared in this journal over the past decade. Nevertheless, we were somewhat surprised at the variety of uses of isotopes in analysis and control work as reported at the recent Birmingham, England, meeting of the Society of Chemical Industry by G. B. Cook of the Isotope Division of the British Atomic Energy Research Establishment at Harwell.

As we listened to Dr. Cook present his paper on "The Use of Radioactive Isotopes for Continuous Control in Industry," we were thoroughly convinced that while much progress in this direction has been made to date, we are merely on the threshold of much greater things. Incidentally, our readers will be very much interested to know that Harwell is shipping more radioactive isotopes for analytical and control experimental purposes than any other agency in the world.

Analysts who turn to this field of specialization will, we believe, find it particularly challenging and the ultimate rewards in the way of scientific achievement will be especially gratifying.

Research analysts most fortunately have gained a very enviable reputation over the past couple of decades for their pioneering into brand new channels of research. As a result, the classical gravimetric and volumetric methods so long considered to be the only tools of analysts have been supplemented, and in many instances superseded, by speedier and more accurate techniques. We are most confident that the opportunities offered by the now wide availability of radioisotopes will not go unheeded by the men and women who today direct important research in all forms and fields of analysis.

# Defining Analytical Chemistry

IN THE February 1954 issue of ANALYTICAL CHEMISTRY we made the following comment, which caused considerable discussion among some of our most loyal and consistent readers:

> While on the subject of names, we wonder sometimes if eventually we in this country and our professional associates abroad will come to the conclusion that the term "analytical chemistry" is too restrictive in the sense that analysts now employ many physical as well as chemical techniques in their work...

The gist of most of the comment we received was to the effect that our so-called "definition" was still much too restrictive, and we frankly plead guilty.

One very well informed reader wrote to us to say that there have been as important extensions on the biological side as on the physical side, so that the term of "analytical chemistry" as now used is restrictive in both directions.

Our reader from abroad, A. L. Bacharach of Glaxo Laboratories, Ltd., Greenford, Middlesex, England, then summarizes his views in the following vein:

> Whether anything should be done about the terms "chemical analysis," "analytical chemistry," and suggested alternatives, I am not at all sure, though I am pretty certain that nothing can or will be. Fortunately, on both sides of the Atlantic people know pretty well nowadays what is meant by chemical analysis and how wide a sphere of activities its practice covers, so that a little terminological imprecision probably does not do much harm.

Dr. Bacharach certainly makes some good points. It is very evident that the field of analytical chemistry is expanding its boundaries in many diverse directions. To attempt to coin an all-inclusive descriptive title is difficult and, perhaps, at the moment an unnecessary task to undertake when there are so many more important things awaiting accomplishment.

We must confess, however, that the subject of semantics intrigues and frequently infuriates us—this latter happens when we listen to individuals (frequently chemists) who would convey the impression that the word "research" should not be associated with the term "analytical chemist." The real analytical chemist *is* most certainly carrying on research, even though, perhaps, the objective of such research is not the synthesis of some new compound.

# Polarography with a Mercury Pool Cathode in Stirred Solutions

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**As part of a program for the development of procedures for determination of trace components, attempts were made to increase the sensitivity of polarographic methods. A large mercury pool cathode in a stirred solution was found to be adaptable to such work. Because of the low charging current of this electrode, it is possible to obtain polarograms of solutions in the micromolar range. The polarograms have the same shape as those obtained with a dropping mercury electrode but have no maxima, and the current fluctuations caused by the drop growth are absent. Studies were made of ion-metal, ion-amalgam, ion-ion, and molecule-molecule reductions. A rapid, simple method requiring no special apparatus is proposed for the determination of the number of electrons involved in polarographic reductions.**

IT IS known that diffusion currents measured with a large electrode in stirred solutions are proportional to the concentration of the species being oxidized or reduced (*2*). Except in amperometric titrations, few applications of this fact have been used in the solution of analytical problems. Lee (*3*) devised an electrode, consisting of a small amount of mercury rotated at high speed in a grooved Bakelite rotor, that extended the range of polarographic analysis. Marple and Rogers (*4*) replaced the dropping mercury electrode with a stationary mercury-plated platinum wire to obtain greater sensitivity. Streuli and Cooke (*6, 7*) proposed the use of a quiet mercury pool for increased sensitivity. Because of the low ratio of charging current to diffusion current at a mercury pool electrode, it was possible to analyze more dilute solutions than was possible with the dropping mercury electrode. Stirring the solution above the electrode caused an increase in the diffusion current without substantially increasing the charging current. This paper is concerned with the study of this arrangement.

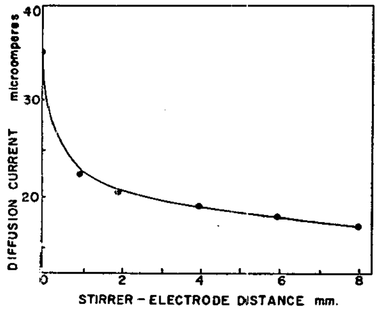

**Figure 1. Effect of stirrer position**

## APPARATUS AND REAGENTS

The electrolysis cell used in this work was the same as one previously described (*6*), except that a glass stirrer was added. This stirrer was fabricated from precision ground glass rod and was driven at the desired speed by a synchronous motor. The bearing for the glass shaft was constructed of polytetrafluoroethylene (Teflon) ground to a 24/40 taper to fit the cell. The mercury cathode had an area of about 3 sq. cm., and used 3 ml. of mercury per run. The glass vessel containing the pool was treated with an organic silicon compound (Desicote) to increase the reproducibility of the mercury-solution interface (*6*). Water at 25.0° C. was circulated through the external jacket. Seaford-grade nitrogen (impurities $<0.1\%$) was bubbled through the sintered-glass disk at the bottom of the cell to remove dissolved oxygen before the addition of the mercury. In order to shorten the time necessary for deaeration, the solution to be analyzed was made 5% with respect to isopropyl alcohol. The currents were determined by measuring the voltage drop across standard resistances with a Leeds & Northrup recorder, which was equipped with an adjustable range and adjustable zero (1-second full scale). The polarizing unit was a 100-ohm, 25-turn potentiometer which could be driven over a wide range of scanning rates. No provisions were made for damping the current fluctuations, and all the polarograms were obtained with only the natural period of the recorder limiting the pen movement.

The chemicals used as supporting electrolytes were found to require purification with the exception of the potassium hydroxide. The potassium chloride, potassium nitrate, and sodium acetate were recrystallized from reagent grade materials. The acetic acid, ammonium hydroxide, hydrochloric acid, and ethylenediamine were redistilled.

## PROCEDURE

Twenty-five milliliters of the solution to be analyzed were placed in the cell and nitrogen was passed through the sintered disk for 2 minutes. The desired potential was applied, and 3 ml. of mercury were added through the side arm. Lower, more uniform backgrounds were obtained if the nitrogen was then bubbled through the mercury pool for an additional 5 minutes. The stirrer was rotated at 540 r.p.m. and the polarization rate was 100 mv. per minute except where noted. The diffusion current was measured by subtracting the extrapolated value of the residual current from the total current. The half-wave potentials were corrected for the voltage drop across the cell, which was calculated from the alternating current resistance.

## EFFECT OF CELL GEOMETRY AND STIRRING RATE

The geometry of the cell greatly influenced the magnitude of the diffusion current. The effect of changing the distance between

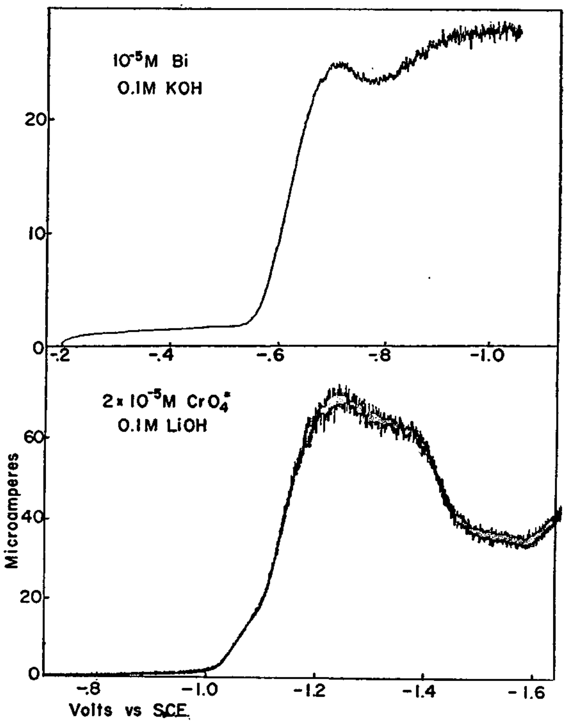

**Figure 2. Anomalous polarograms**

the stirrer and the electrode surface is seen in Figure 1. The highest sensitivity was obtained when the stirrer just touched the mercury surface, but under these conditions the results were erratic and a small change in position introduced a relatively large error. At distances greater than 2 mm., the slope of the curve was more gradual. The stirrer was therefore fixed permanently 6 mm. from the mercury surface, at which point a change of 1 mm. introduced a 3% change in diffusion current.

The design of the stirring propeller and the effect of its rotation were also studied. The variation of diffusion current with stirring speed was nearly linear up to 1200 r.p.m. Stirrers of various designs had different efficiencies, but in each case the currents varied linearly with increasing rotation.

## RATE OF CONCENTRATION DECREASE

The rate at which the concentration of a reducible species is decreased during electrolysis is inversely proportional to the ratio of solution volume to electrode area, and to the thickness of the diffusion layer. With the dropping mercury electrode, the decrease in concentration during the electrolysis is usually negligible. At the stirred mercury pool the ratio of electrode area to volume is much greater, and the diffusion layer is smaller These factors, which are responsible for the increased sensitivity, also caused a more rapid decrease in the limiting current. This did not prove experimentally troublesome. When a polarogram was recorded and the voltage maintained at some value corresponding to a point on the current plateau, the wave height decreased approximately 20% in 10 minutes. The recorded values were 20% for cadmium, 18% for chromium, and 24% for nickel.

## EFFECT OF POLARIZATION RATE

The shapes of polarograms recorded at the dropping mercury electrode are independent of the polarization rate, but those recorded at a quiet mercury pool electrode are greatly dependent on the rate of voltage scanning. Polarograms obtained at the stirred pool electrode were found to be independent of the scanning rate from 20 to 400 mv. per minute. The residual currents were smoother at lower scanning rates, but the effect was not great. The charging current would be smaller at lower polarization rates, and any solid impurities present on the surface of the electrode would be reduced with less distortion of the residual current curve. Wave heights were slightly smaller at lower scanning rates because of the decrease in concentration by electrolysis during the longer time necessary to reach the plateau. The diffusion current for a $10^{-5}M$ solution of benzil was 14.4 μa. at 100 mv. per minute and 13.8 μa. at 20 mv. per minute.

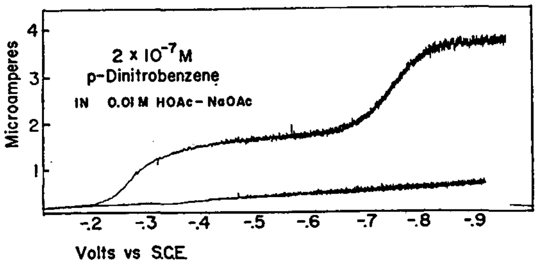

**Figure 3. Polarogram of *p*-dinitrobenzene**

When polarograms were obtained by manually increasing the voltage in discrete steps, equilibrium current values were quickly reached except at concentrations below $2 \times 10^{-6}M$. In this range a steady value was obtained in about 15 seconds.

## RESULTS

Polarograms of ten different reducible species chosen to represent a variety of different types of electrolytic processes were recorded using the mercury pool electrode: cadium, copper, lead, thallous, cobaltic, chromate, nickelous ions, azobenzene, benzil, and *p*-dinitrobenzene. No maxima were obtained in any of the reductions, although in some cases anomalous, unexplained peaks were obtained (Figure 2). The principal disadvantage of the method is perhaps the loss of useful voltage range because of the increased sensitivity of the electrode to the discharge of hydrogen (*3*, *6*). To check the long-range reproducibility, polarograms of two different species were recorded periodically over an interval of 1 month. The average deviation of 17 runs on $10^{-5}M$ *o*-nitrotoluene was 2.4%, while 13 polarograms of $10^{-5}M$ cadmium ion gave an average deviation of 3.1%. No trends were noted in either case.

The sensitivity of the proposed electrode was about 300 times greater than the conventional dropping mercury electrode. Factors such as the ratio of diffusion current to background and the slope of the residual current curve enter into such a comparison. In all cases, the supporting electrolyte concentration was 0.1*M*. It was felt that this presented a realistic approach to the application of the method to practical analytical problems, as excess salts are usually present. If circumstances allow the use of a more dilute supporting electrolyte, the residual current can be de-

**Table I. Tabulation of Results**

| | 0.1*M* NaOAc–0.1*M* HOAc | | | | | | 0.1*M* KOH, $CrO_4^{--}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Benzil | | | *p*-Dinitrobenzene | | | | | |
| Concn. | *Id*[a] | *Id/c*[b] | $E_{1/2}$[c] | *Id*[a] | *Id/c*[b] | $E_{1/2}$[c] | *Id*[a] | *Id/c*[b] | $E_{1/2}$[c] |
| $5 \times 10^{-7}$ | ... | ... | ... | 5.83 | 11.65 | −0.27<br>−0.73 | ... | .. | ... |
| $1 \times 10^{-6}$ | 1.51 | 1.51 | −0.55 | 11.10 | 11.10 | −0.26<br>−0.73 | ... | .. | ... |
| $2 \times 10^{-6}$ | 2.92 | 1.46 | −0.55 | 22.00 | 11.00 | −0.27<br>−0.73 | 11.6 | 5.80 | −1.21 |
| $5 \times 10^{-6}$ | 7.50 | 1.50 | −0.55 | 53.4 | 10.68 | −0.29<br>−0.77 | 26.1 | 5.21 | −1.21 |
| $1 \times 10^{-5}$ | 14.1 | 1.41 | −0.56 | 102.0 | 10.20 | −0.31<br>−0.79 | 49.5 | 4.95 | −1.22 |
| $2 \times 10^{-5}$ | 27.2 | 1.36 | −0.58 | 194.6 | 9.73 | −0.32<br>−0.87 | 93.0 | 4.65 | −1.23 |
| $5 \times 10^{-5}$ | 65.5 | 1.31 | −0.60 | 433.0 | 8.66 | −0.39<br>−0.96 | 220.5 | 4.41 | −1.27 |
| | 0.1*M* $NH_4OH$ + 0.1*M* $NH_4Cl$ | | | | | | 0.1*M* $KNO_3$ + 0.05*M* Ethylenediamine, $Cu^{++}$ | | |
| | Cd$^{++}$ | | | Azobenzene | | | | | |
| $1 \times 10^{-6}$ | 1.71 | 1.71 | −0.66 | 1.80 | 1.80 | −0.46 | 1.84 | 1.84 | −0.45 |
| $2 \times 10^{-6}$ | 3.50 | 1.75 | −0.66 | 3.80 | 1.90 | −0.46 | 3.68 | 1.84 | −0.45 |
| $5 \times 10^{-6}$ | 9.55 | 1.91 | −0.67 | 9.50 | 1.90 | −0.48 | 10.3 | 2.05 | −0.49 |
| $1 \times 10^{-5}$ | 18.8 | 1.88 | −0.66 | 19.0 | 1.90 | −0.50 | 21.1 | 2.11 | −0.50 |
| $2 \times 10^{-5}$ | 38.2 | 1.91 | −0.68 | 34.2 | 1.71 | −0.52 | 43.8 | 2.19 | −0.51 |
| $5 \times 10^{-5}$ | 90.0 | 1.80 | −0.70 | 81.5 | 1.63 | −0.54 | 99.5 | 1.99 | −0.52 |
| | 0.1*M* $NH_4OH$–0.1*M* $NH_4Cl$, $Co(NH_3)_6Cl_3$ | | | 0.1*M* NaK Tartrate, $Pb^{++}$ | | | 0.1*M* KCl, $Tl^{+}$ | | |
| $1 \times 10^{-6}$ | 0.86 | 0.86 | −0.24 | 2.12 | 2.12 | −0.47 | 1.92 | 1.92 | −0.44 |
| $2 \times 10^{-6}$ | 1.64 | 0.82 | −0.25 | 4.14 | 2.07 | −0.47 | 3.74 | 1.87 | −0.44 |
| $5 \times 10^{-6}$ | 4.15 | 0.83 | −0.25 | 10.4 | 2.07 | −0.48 | 9.15 | 1.83 | −0.45 |
| $1 \times 10^{-5}$ | 9.00 | 0.90 | −0.27 | 20.9 | 2.09 | −0.49 | 17.7 | 1.77 | −0.44 |
| $2 \times 10^{-5}$ | 17.6 | 0.88 | −0.28 | 40.4 | 2.02 | −0.53 | 36.0 | 1.80 | −0.45 |
| $5 \times 10^{-5}$ | 44.0 | 0.88 | −0.32 | 100.0 | 2.00 | −0.54 | 90.5 | 1.81 | −0.44 |

[a] *Id* expressed in microamperes.
[b] *Id/c* expressed in amperes per mole per liter.
[c] $E_{1/2}$ expressed as volts *vs.* *S.C.E.*

creased. Figure 3 shows a polarogram of a solution of $2 \times 10^{-7}M$ *p*-dinitrobenzene and the residual current background using $0.01M$ supporting electrolyte.

The results obtained are summarized in Tables I and II. Cadmium, copper, lead, and thallium were chosen as examples of reductions of ion to amalgam. Tables I and II show that the half-wave potentials of these ions are almost independent of concentration and agree reasonably well with the potentials obtained at the quiet mercury pool and the dropping mercury electrode. There is a small, though distinct, shift in half-wave potential toward more negative values in the case of cadmium, lead, and copper. The shift of the half-wave potentials to more negative values than those obtained with the dropping mercury electrode can be explained by the accumulation of the metals in the mercury (*3*). The diffusion currents are proportional to concentration over the range studied. The departure from linearity was 4.0% for cadmium, 4.5% for copper, 3.1% for lead, and 2.7% for thallium. Polarograms for the reduction of lead ion were not reproducible when the lead was a cation. Under such circumstances, $10^{-6}M$ solutions had deviations as high as 50% because of the pronounced memory effect of the cell which defied even prolonged rinsings. By using a tartrate medium, in which the lead is in the form of an anion, this difficulty was eliminated.

Studies of the reduction of nickelous ion at the mercury pool electrode revealed that the reproducibility of the diffusion currents was poor. Variations as large as 20% rendered this method of doubtful analytical value. The deposited metal lowered the hydrogen overvoltage of the mercury by about 0.3 volt, which is in agreement with previous observations (*5*). In addition, the diffusion current was found to be dependent on the concentration of the supporting electrolyte. A tenfold decrease in the concentration of the supporting electrolyte caused a 25% decrease in wave height.

Two examples of ion-ion reductions were $CrO_4^{--} \rightarrow Cr^{+++}$ and $Co^{+++} \rightarrow Co^{++}$. In both cases the half-wave potentials were shifted to more negative values than are obtained at the dropping mercury electrode. There seems to be no thermodynamic explanation for this shift of half-wave potentials for ion-ion reductions.

**Table II. Reduction Potentials at Various Electrodes**

| | Stirred Pool, $E_{1/2}$, Concn. $10^{-5}M$ | Quiet Pool, Half-Peak Potential, Concn. $10^{-5}M$ | Dropping Electrode, $E_{1/2}$ |
|---|---|---|---|
| $Cd^{++}$ | −0.62 | −0.66 | −0.60 |
| $Tl^{+}$ | −0.45 | −0.42 | −0.46 |
| $Pb^{++}$ | −0.49 | −0.48 | −0.50 |
| $Co^{+++}$ | −0.25 | −0.33 | −0.18 |
| $CrO_4^{--}$ | −1.22 | −1.07 | −0.85 |
| Azobenzene | −0.67 | −0.67 | −0.65 |
| Benzil | −0.56 | −0.50 | −0.55 |
| *p*-Dinitrobenzene | −0.31 | −0.30 | −0.19 |
| | −0.79 | −0.78 | −0.43 |

Three organic compounds were studied as examples of molecule-molecule reductions. Azobenzene, benzil, and *p*-dinitrobenzene yielded well defined polarograms (Tables I and II). With increasing concentration the half-wave potentials shifted to more negative voltages and the $I_d/c$ ratio decreased. This was characteristic of other organic compounds that have been subsequently studied.

### REVERSE POLAROGRAMS

In conventional polarography there is no difference between polarograms recorded from negative to positive potentials and those recorded from positive to negative potentials. With the mercury pool electrode such is not always the case. The reason for this is twofold: The electrode is not renewed, thereby allowing metals to accumulate in the mercury, and the electrode area is so large that appreciable reduction can occur. The latter effect allows the accumulation of comparatively large quantities of the reduced product.

A polarogram of $Cd^{++}$ was recorded, and the applied potential was allowed to remain at a point on the plateau portion of the curve until about 10% of the total cadmium was deposited. The voltage was then scanned toward positive values (Figure 4). As the ratio of the volume of solution to the volume of mercury was about 10, the concentration of cadmium in both phases was approximately equal. The reverse polarogram, under these conditions, showed an anodic branch corresponding to the electrolytic decomposition of the cadmium amalgam. The limiting anodic current falls off rapidly because of the rapid removal of cadmium from the amalgam. This rate is much higher than the corresponding depletion of ions from solution because of the larger ratio of area to volume of the mercury phase.

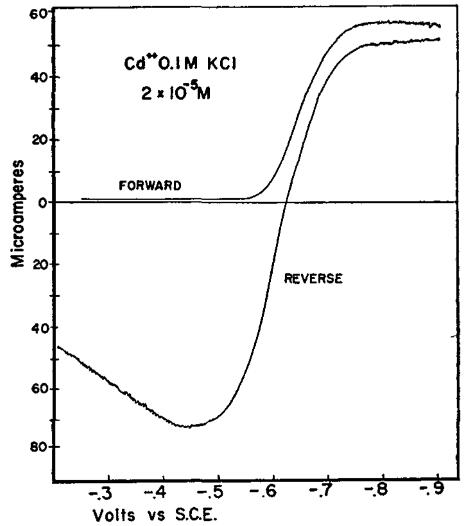

**Figure 4. Forward and reverse polarograms of cadmium**

When a similar experiment was carried out by depositing nickelous ion in the mercury, the reverse polarogram had no distinct anodic wave. There was only a small diffuse wave at a more positive potential than the cathodic half-wave value. The nickel could not be oxidized at potentials at which the mercury was not decomposed. Such passivity is characteristic of nickel.

The reduction of chromate was also found to be irreversible, as no anodic oxidation of the reduced product occurred. The reverse polarograms in such cases were similar to those obtained by forward scanning. Similar curves would be obtained whenever the reduced species is not capable of reoxidation. When the reduction product is a soluble, oxidizable species, another form of reverse polarogram is obtained. Azobenzene, for example, gives a reversible polarogram in a supporting electrolyte of $0.1M$ lithium hydroxide as evidenced by the curves in Figure 5. In this case, reduction was allowed to proceed until the diffusion current was about one half of its initial value, which corresponds to equimolar amounts of azobenzene and hydrazobenzene. The lower polarogram was then recorded by scanning toward positive voltages. The reduction product was hydrazobenzene, since an equimolar mixture of azobenzene and hydrazobenzene gave an identical polarogram. With azobenzene in a $0.1M$ ammonium hydroxide–ammonium chloride supporting electrolyte, different results were obtained. The reverse polarogram indicated that the cathodic reduction of azobenzene and the anodic oxidation of hydrazobenzene occurred at substantially different potentials. The normal polarogram of this reduction and the polarogram after reducing 50% of the original material are shown in Figure 5. This type of curve indicates that the system is thermodynamically irreversible. An identical polarogram was obtained for an equimolar mixture of azobenzene and hydrazobenzene in the same medium. These reverse polarograms are useful in the interpretation of the reduction process and give information as to the nature and identity of the products formed.

### REDUCTION OF MIXTURES OF CADMIUM AND THALLIUM IONS

It was important to know if the accumulation of one metal in the mercury electrode would affect the reduction of a metal with a more negative half-wave potential. To evaluate this effect, polarograms of mixtures of cadmium and thallium were obtained. The value of $I_d/c$ for the cadmium was 2.07 amperes per mole in mixtures containing up to five times as much thallium. This is in good agreement with the constant obtained for cadmium alone and indicates that the previously discharged metal has no noticeable effect on the height of the cadmium wave over the range studied.

### MEASUREMENT OF VALUES OF *n*

The usual procedure for the determination of the number of electrons in a polarographic process involves the reduction of a known amount of material and a measurement of the number of coulombs consumed. The reduction is allowed to proceed until the diffusion current is a small percentage of the original value. The current during this electrolysis is integrated by means of a coulometer in series with the cell. A method was used in this work which eliminates the coulometer and the exhaustive electrolysis.

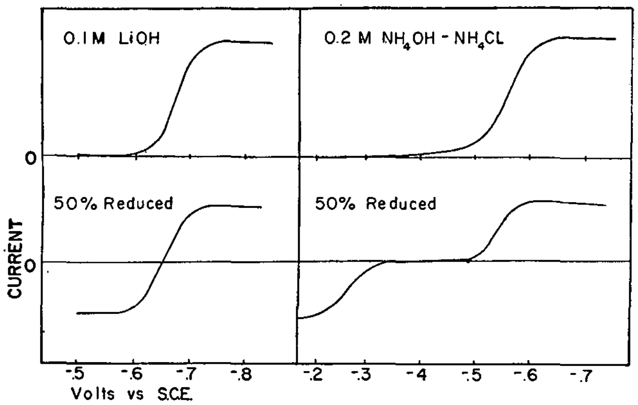

**Figure 5. Polarograms of azobenzene**

Although the principles underlying this procedure are known, experimental difficulties have mitigated against its use (*1*). The values of *n* obtained in this work are in good agreement with theory (Table III). The fact that low concentrations are used may have contributed to the success of this modified procedure.

**Table III. Values of *n* for Various Reductions**

| | Value Obtained | Value Expected | Error, % |
|---|---|---|---|
| Thallium | 0.99 | 1 | 1.0 |
| Cadmium | 1.91 | 2 | 4.5 |
| Copper | 2.06 | 2 | 3.0 |
| Lead | 2.02 | 2 | 1.0 |
| Chromate | 2.88 | 3 | 4.0 |
| Azobenzene | 1.94 | 2 | 3.0 |
| Benzil | 1.97 | 2 | 1.5 |
| Indium | 3.01 | 3 | 0.3 |
| *o*-Dinitrobenzene | 11.75 | 12 | 2.1 |
| | | Average | 2.3 |

In a diffusion-controlled process at constant potential, the value of the current at time *t* after the initiation of the electrolysis is:

$$i = i_0 e^{-kt} \qquad (1)$$

where $i_0$ is the current at $t = 0$, and $k$ is a constant. By measuring the diffusion current for a few time intervals, a straight line can be obtained when the results are plotted on semilog paper. The slope of this line is the constant $k$. The area under the current-time curve, from $t = 0$ to $t = \infty$, represents the number of coulombs which would be involved in the entire process if it had been carried to completion.

$$\text{Total coulombs} = i_0 \int_{t=0}^{t=\infty} e^{-kt}dt \qquad (2)$$

Evaluation of this integral gives the equation

$$\text{Total coulombs} = \frac{i_0}{k}$$

Knowing the initial concentration, current and slope $k$, the value of $n$ can be calculated. The value $i_0$ is difficult to measure experimentally. To avoid this problem, the currents in the beginning of the electrolysis are ignored and only enough points are plotted to characterize the slope of the line. The value of $i_0$ is then determined by extrapolation of the straight line to zero time. A coulometer is not needed and prolonged electrolysis is unnecessary. The values of $n$ obtained agree with the expected figures within 2.3%.

### ANOMALOUS RESULTS

The polarographic waves for some reductions had unexplainable characteristics. Benzil, in lithium hydroxide, had a small prewave which did not appear when potassium hydroxide was used. A more striking difference between these two electrolytes is illustrated in the polarogram for the reduction of chromate in $0.1M$ lithium hydroxide (Figure 2). When $0.1M$ potassium hydroxide was used, a normal polarogram was obtained. Further differences in the alkali hydroxides were found in the reduction of chromate at a quiet mercury pool. Different waves were obtained in supporting electrolytes of lithium, sodium, and potassium hydroxides. Bismuth also yielded an abnormally shaped polarogram (Figure 2). The discontinuity in this wave was not caused by an adsorbed compound since the shape of the polarogram was unaffected at higher rates of voltage polarization. If this peak were caused by adsorption, or the presence of a solid substance on the electrode surface, it would be more pronounced at higher scanning rates.

### ACKNOWLEDGMENT

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# Effect of Cell Circuit Resistance in Polarography with Stationary and Dropping Electrodes

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**Ohmic drop of several hundredths of a volt, due largely to cell resistance, can change the voltage scanning rate enough to produce a marked effect on the polarographic current at a stationary electrode. By an electromechanical method of instantaneous resistance compensation it is shown that this effect accounts for most of the variation in the ratio of maximum current to concentration observed in the oxidation of organic sulfides. The compensation system is applicable also to dropping or rotating electrodes.**

THE polarographic current at a stationary electrode depends upon the rate of change of potential with time (*1–6*). In previous work in this laboratory (*4*) a distortion of wave form for the oxidation of organic sulfides at a cylindrical electrode was observed as the concentration increased, even after subtraction of *iR* drop in the cell circuit from the apparent applied voltage. Adsorption was suggested as a possible cause of this effect. In the present work an explanation is sought in the variation of $dE/dt$ with the cell current by virtue of the ohmic drop in the cell and current-measuring resistor.

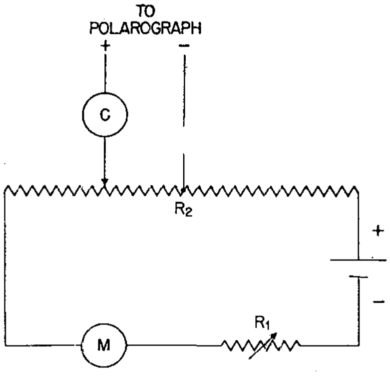

**Figure 1. Resistance compensator circuit**

This problem of ohmic drop has been discussed by Delahay (*3*) and Randles (*5*), and Snowden and Page (*7*) incorporated the feature of compensation for the measuring resistor in their oscillographic circuits. An arrangement described here provides instantaneous compensation for both cell and measuring resistor during the recording of a polarogram. This device is applicable also to dropping and rotating electrodes and should be especially useful in organic solutions, where high cell resistances are frequently encountered.

### EXPERIMENTAL

Current-voltage curves were recorded on a Sargent Model XXI polarograph on a 2.00-volt span without damping. Details of procedure for diethyl sulfide are given in a previous paper (*4*). The same platinum wire electrode was used. In addition, a few comparison curves were obtained for reduction of cadmium ion at the dropping mercury electrode in potassium chloride solution. A balancing decade capacitor was used in measurement of cell resistances on a 1000-cycle conductance bridge.

The resistance compensator circuit is shown in Figure 1. Current from a 1.5-volt dry cell is controlled by resistor $R_1$, variable from zero to 5000 ohms, and measured on milliammeter *M*, which introduced an additional 20 to 80 ohms. $R_2$, a ten-turn 9.52-ohm Helipot with center tap, was mounted on the polarograph recorder in the position provided for a drum-type slidewire, so that the sliding contact is operated by the pen drive mechanism. In this way, a corrective voltage is added in series with the cell, *C*, which is at all times equal and opposite to the *iR* drop in the cell and current-measuring resistor. The effective potential difference between the electrodes is then the linear function of time supplied by the bridge of the polarograph. By means of an adjustable shaft coupling, the center position on $R_2$ is set to correspond to any selected zero current position on the chart. Thus, compensation is achieved for positive and negative cell currents. Setting of the compensator current is determined by the expression $i_{\text{comp}} = SDR$, where *S* is the sensitivity, current per unit pen deflection, at which the recording is made, *D* is the pen deflection per ohm of $R_2$, and *R* is the resistance of the cell plus the measuring resistor. The size of the slidewire cable drum attached to the Helipot was such that $D = 85.5$ mm. per ohm.

The influence of resistances larger than those normally found in the cell circuit was determined by addition of resistors in series with the cell.

### RESULTS AND DISCUSSION

The effect of ohmic drop in 5 m*M* diethyl sulfide solution, at relatively high cell current, is shown in Figure 2, where the ab-

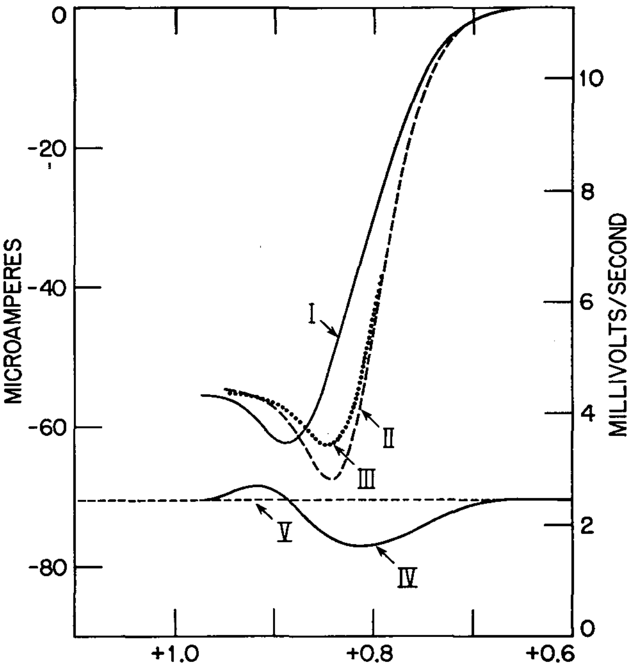

**Figure 2. Effect of ohmic drop in 5 m*M* diethyl sulfide in methanolic 0.1*M* HCl**

*R* is 670 ohms
I. Uncompensated
II. Compensated
III. *iR* subtracted
IV. $dE/dt$, uncompensated
V. $dE/dt$, compensated

scissa represents apparent potential as given by the time coordinate of the chart. The measured resistance of the cell was 650 ohms; recordings of curves I and II were made with the minimum cell circuit resistance at suitable current sensitivity. The uncompensated polarogram, when plotted as *i/c vs. E*, is similar to that obtained with a 1 m*M* solution and 5100-ohm series resistance. In the absence of an extra series resistor the greatest *iR* drop in the 1 m*M* solution is 10 mv., and even in this instance the change by compensation is significant. The maximum compensated current in Figure 2 occurs at a potential 0.02 volt more anodic than that of the corresponding 1 m*M* curve. Subtraction of *iR* along the uncompensated polarogram yields the dotted curve, III. This type of correction neglects the difference in current at any corrected potential but apparently permits fairly accurate location of the peak on the potential scale. The pronounced deviation of *dE/dt* during recording of a large uncompensated current is seen in curve IV, Figure 2. The horizontal dashed line, V, represents the constant voltage scanning rate of the polarograph.

Maximum current-concentration ratios with and without resistance compensation are plotted against diethyl sulfide concentration in Figure 3, together with data from a previous investigation using the same electrode. The ordinates are on a large scale. The ratios were computed after subtraction of residual currents. Up to a concentration of 2.5 m*M*, ohmic drop accounts satisfactorily for the variation of $-i_{max.}/c$ with concentration; at 5 m*M*, it appears to account for about 75% of the decrease from the limiting value. A 25% change in compensator current, corresponding to a 10-mv. increment at the peak, was found to change $-i_{max.}/c$ in the 5 m*M* solution by one or two tenths of a unit on the scale of Figure 3. The uncertainty in the alternating current measurement of cell resistance was of the order of 5%. The present compensation method is exact only if the effective cell resistance is a constant, independent of current, and if all of the polarization occurs at one electrode. The possibility remains that some other complication, such as adsorption on the electrode surface during the passage of current, may contribute to the small slope of the compensated curve of Figure 3, but such an effect is a minor one compared to that of the estimated ohmic drop. A very satisfactory correction is obtained in this case by use of an approximate equation given by Delahay (*3*): $(i_{\max.})_v = (i_{\max.})_{v'} (1 + Ri_{\max.}/\Delta E)^{1/2}$, where $(i_{\max.})_v$ is the peak current at constant *dE/dt*, $(i_{max.})_{v'}$ is the value in the presence of *iR* drop, and $\Delta E$ is the voltage interval on the ascending branch of the wave.

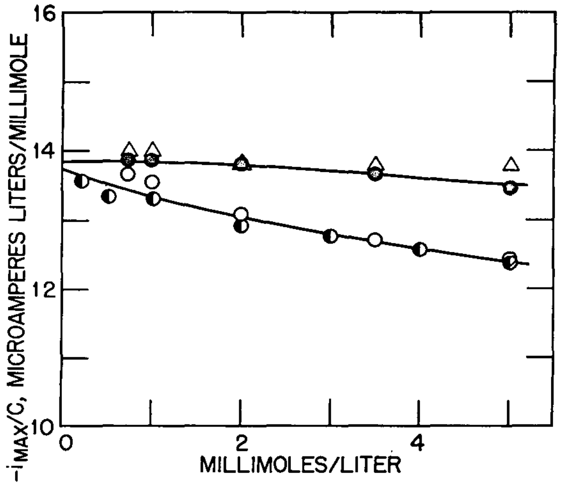

**Figure 3. Effect of ohmic drop on current-concentration ratio for diethyl sulfide**

○. Uncompensated, this investigation
⊖. Compensated, this investigation
◐. Uncompensated, previous investigation (*4*)
△. Delahay equation (*3*)

These results re-emphasize the importance of cell circuit resistance in the case of stationary electrodes. The automatic compensator appears to retain the essential shape of the polarogram, a critical point in the study of electrode processes by the voltage scanning technique (*2*). It also yields a simpler current-concentration relationship for analytical purposes.

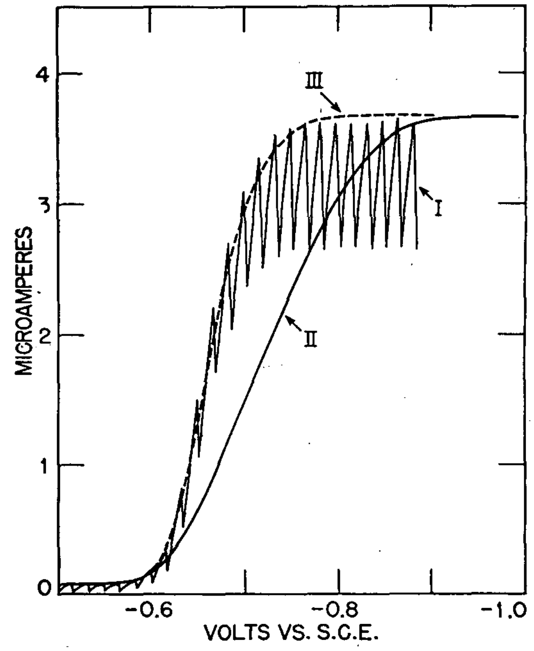

**Figure 4. Application of resistance compensator to dropping mercury electrode**

With 0.5 m*M* $CdCl_2$ in 1*M* KCl containing 0.01% gelatin
I. *R* is 160 ohms, uncompensated
II. *R* is 30,600 ohms, uncompensated
III. *R* is 30,600 ohms, compensated
II, III. Drawn through maximum drop currents

**Application to Other Electrode Types.** Although an adequate correction for cell resistance usually can be made arithmetically on polarograms at dropping or rotating electrodes, an instrumental method is convenient. Figure 4 shows the action of the compensator circuit on a cadmium wave in which a 30,000-ohm series resistor was present. This value was chosen for illustrative purposes, and use of a cell of such high resistance is not recommended.

## ACKNOWLEDGMENT

The writer wishes to thank Carl P. Tyler for aid with the experimental work.

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# Rapid Gas Analyzer Using Ionization by Alpha Particles

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**A method and apparatus are presented for analysis of a flowing or quiescent gaseous mixture, by means of ionization of the mixture using alpha particles from polonium in an aged radium D source.**

**By utilizing a proper combination of applied voltage and electrode spacing in the ionized mixture, ionization currents of the order of $10^{-8}$ amp. are obtained. The current depends in its precise value on the composition of the gas (binary, ternary, and possibly more components) at constant temperature and pressure. Primary calibration is necessary with recalibration or compensation at intervals, because of radioactive decay of the source (half life, 22 years). Electrical output may be recorded by a variety of devices or used in control systems. Binary systems: $H_2$—$N_2$, $H_2$—$C_2H_4$, $C_2H_4$—$C_2H_6$ and $N_2$—$CO_2$, and the ternary: $N_2$—$H_2$—$C_2H_6$ have been analyzed satisfactorily. In principle, gases of different molecular, atomic, or electronic structure should be capable of differentiation, examples of exceptions thus being ortho $H_2$-para $H_2$ and $H_2$—$D_2$.**

**Theoretical time of response is of the order of $10^{-3}$ second; precision of analysis in the prototype apparatus was about 0.2 to 0.3 mole % for binary mixtures. The device is safe and relatively inexpensive.**

IN THE course of an investigation on the application of frequency response techniques to the measurement of diffusional processes in catalyst particles, which is reported elsewhere (*2*), the need arose for a direct recording, rapid, precise, and relatively inexpensive gas analyzer capable of giving the instantaneous composition of a binary gas mixture. It was also necessary that the instrument be capable of insertion directly into the gases under study, and that it interfere with the flow of these gases as little as possible.

Various well known methods of gas analysis were investigated and were found to fulfill only a few of the above requirements. High response speed ruled out thermal conductivity cells, as a response time of 1 to $^1/_{50}$ second was deemed necessary. Refractometry, while rapid, was found unsatisfactory because of the necessity for a gas depth much greater than would be available, and infrared or ultraviolet absorption techniques were found to be excessively costly for the purpose in hand. Electronic problems, involved in developing methods for measuring the time of traverse of sound for a distance as small as the width of the gas stream, proved to be insurmountable at any reasonable cost. The search for a new method of gas analysis was initiated, and led to the development of the alpha particle gas analyzer. Further experiments were carried out to determine whether the gas analyzer could be adapted to analysis of ternary mixtures.

The device depends on the measurement of the rate of production of ions in a gas by alpha particles. The form of the apparatus is primarily for binary gas mixtures, or for mixtures behaving as binary mixtures; however, one ternary mixture has been analyzed and adaptation to use with more than three components appears possible. For all systems a primary calibration is necessary, with recalibration at intervals because of the decay of the alpha particle source. The device permits analysis of almost any binary mixture which can be analyzed by means of thermal conductivity cells, with but few exceptions as noted below. In contrast, the applicability of the analyzer to multicomponent mixtures is more restricted by the relative ionization properties of the pure components in question. Precision, while not equal at present to commercially available thermal conductivity cells, is satisfactory for many uses, amounting to 0.2 or 0.3 mole %. High response speed and low cost requirements, as well as the other requirements, are all fulfilled by this instrument. The output may be recorded by a variety of recording devices or used in control systems.

## THEORY AND DESIGN CONSIDERATIONS

If an alpha particle source is placed in a closed gas-filled chamber, the number of ions produced per unit time in the chamber depends, in general, on the nature of the gas for a particular source. These ions render the gas conductive, so that if a pair of electrodes appropriately spaced is placed in the chamber and a voltage is applied to the electrodes, a current flows across the conductive gas and through the circuit external to the chamber. For a binary mixture of gases at constant temperature and pressure, the rate of production of ions by the alpha particles is a function of gas composition only. If the voltage impressed upon the electrodes is maintained constant, then the current produced is also a function of gas composition only, and measurement and recording of this current make possible the measurement and recording of the gas composition, providing that the device is first calibrated with known gas mixtures.

Similar considerations hold for a ternary or more complex mixture of gases, except that for a given spacing of the elctrodes in the chamber and fixed impressed voltage the current is not a unique function of composition. This difficulty can be overcome, in the case of a ternary mixture, by submitting the gas mixture to irradiation by alpha particles in a second chamber having a different spacing of electrodes and/or a different applied voltage. After appropriate calibration with known mixtures, the two independent measurements of ionization current yield an analysis. For a *C* component mixture *C*-1 independent measurements are necessary.

Ionization chambers containing electrodes and a gas are well known devices used for the detection of ionizing radiations. The present gas analyzer chamber differs from such an ionization chamber, in that the gas analyzer chamber contains in addition a fixed known source of ionizing radiations and properly spaced electrodes, and that the gas analyzer is used to determine the

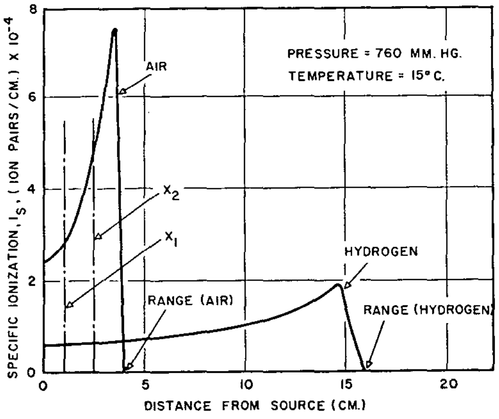

**Figure 1. Specific ionization curves for alpha particles from polonium in air and hydrogen**

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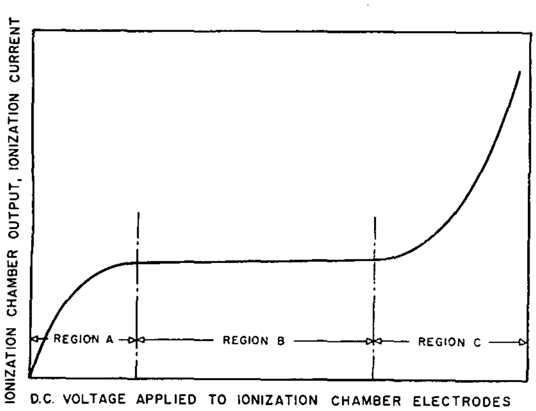

**Figure 2. Typical ionization chamber output-applied voltage curve**

composition of the gas within it. Certain operating characteristics of the gas analyzer are similar to those of ionization chambers; however, some discussion of these characteristics, as well as of the characteristics of ionizing radiations in general, are necessary here. Fuller treatment of these subjects is available (*1, 6–8*).

Alpha particles cause ionization in gases by a variety of mechanisms. Whatever the mechanism, for every positive charge created a negative charge is also created, and such pairs of charges are called "ion pairs." The number of ion pairs, which a particle produces in a gas, depends on the energy of the particle. As a given particle loses energy in traversing a gas, the particle's ionizing power is a function of the distance it has traveled in the gas, as well as of its initial energy. Ionizing power of an alpha particle at a particular distance from its source is often expressed in terms of the specific ionization it produces at that point. Specific ionization is the number of ion pairs produced per unit of path traversed by an alpha particle. Characteristic curves of specific ionization as a function of distance from the source are shown in Figure 1 for alpha particles from polonium in air and in hydrogen. The curve for air was calculated from a curve for alpha particles from radium C in air as given by Lind (*8*), and the curve for hydrogen was calculated from the data of Hatfield, Lockenvitz, and Young (*4*).

The point at which specific ionization becomes zero is the range of the alpha particles. Ranges of alpha particles from a given source in different gases generally differ widely. Range is usually expressed in terms of relative stopping power, *S*, which is the ratio of the range in air at some standard condition to that in the gas of interest.

The area under a specific ionization curve is the total number of ion pairs produced by an alpha particle. This total ionization is expressed frequently as total relative ionization, the ratio of the total ionization in the gas in question to that in air at standard temperature and pressure. Total relative ionization does not change much from gas to gas, being approximately 1.0 for gases such as hydrogen, nitrogen, oxygen, ammonia, and carbon dioxide. A high total relative ionization is exemplified by that of *n*-pentane which is 1.35. An analytical chamber designed to measure total ionization is not capable, therefore, of distinguishing very clearly between many of the common gases. Furthermore, the analytical cell has to be at least as large as the maximum range encountered in use with any gas mixture. An analytical cell has to be several centimeters in diameter, and holdup in it is so large for many applications that rapid changes in concentration are averaged out. Such a cell does not give satisfactory performance.

The analytical cell for binary mixtures is so constructed that its important dimensions are smaller than the minimum range of the alpha particles for all the gas mixtures of interest (Figure 1).

If the electrodes are placed at $x_1$ and $x_2$ in such a manner that only those ions formed between them are collected, then the ionization current produced when air is in the cell is considerably greater than when hydrogen is in the cell, as can be seen by comparing the areas under the air and hydrogen curves between $x_1$ and $x_2$. The two gases are now easily distinguished from each other. It is this latter design which is used in the present binary apparatus.

For a constant gas composition, the ionization current produced is a function both of the rate of production of ions and of the voltage applied to the electrodes. A typical curve of cell output or ionization current against applied voltage is shown in Figure 2. Three regions are readily distinguished.

Region *A* represents that region in which the applied voltage is so low that the ions move slowly enough to permit considerable recombination before they are collected on the electrodes. As the applied voltage is increased, the speed of the ions increases, more ions reach the electrodes before recombining, and cell output current increases with increasing voltage.

Region *B* represents a range of voltage over which virtually all of the ions produced reach the electrodes. In this region the output current is almost constant, regardless of the applied voltage.

In region *C*, the applied voltage accelerates the ions to the point at which the ions themselves have sufficient energy to produce additional ions, so that the output current increases rapidly with increasing applied voltage.

Region *B* is the region in which it is most desirable to operate an analytical cell when analyzing binary gas mixtures, as no great precautions need be taken to regulate the applied voltage. The shape of the output curve varies as the nature of the gas in the cell is varied, and also as the size and configuration of the cell and electrodes are varied. For a given cell, it is necessary to locate region *B* for each pure component in the mixture to be used, and then to apply a voltage within this region for the best operation of the cell.

For the analysis of ternary mixtures two independent measurements of ionization current (from two different cells, for example) are needed. Some of the considerations pertaining to the operation of a cell in the analysis of binary mixtures can no longer apply to both of the cells used in analyzing a ternary mixture, however. This fact can best be demonstrated through a consideration of the theoretical expressions for the output current, for cells operated in the manner recommended for the analysis of binary mixtures, Equations 9 and 11. These equations are derived as follows:

Using an empirical expression relating the velocity of an alpha particle to the distance the particle has traveled from its source, and assuming that the intensity of ionization at any point is proportional to the rate of loss of kinetic energy by the alpha particle, the following expression for the specific ionization curve has been obtained (*1*)

$$I_s = a(L - z)^{-1/3} \tag{1}$$

where $I_s$ is the specific ionization, $L$ is the range of the alpha particle in the gas in question, $z$ is the distance from the source, and $a$ is a proportionality constant. The total ionization, $I$, is the integral of $I_s$ from $z = 0$ to $z = L$, so that, from Equation 1,

$$I = \frac{3}{2} aL^{2/3} \tag{2}$$

For an analytical cell whose important dimension, $l$, is less than $L$, and in which the electric field is such that all ions formed are collected, the output current, $i_o$, from the cell is given by the expression

$$i_o = k\int_0^l I_s dz \tag{3}$$

where $k$ is a constant dependent on the rate of emission of alpha particles from the source. From Equations 1, 2, and 3 the following expression is obtained

$$i_o = kI\left[1 - \left(1 - \frac{l}{L}\right)^{2/3}\right] \tag{4}$$

For $l << L$, Equation 4 may be simplified to the approximate relationship

$$i_o = \frac{kIl}{L} \tag{5}$$

from the expansion of Equation 4 by the binomial theorem.

The relative stopping power of a gas is given by

$$S = \frac{L_a}{L} \tag{6}$$

where $L_a$ is the range in air at S.T.P. Therefore,

$$i_o = cS, \tag{7}$$

where $c$ is substituted for the constant, $\frac{kIl}{L_a}$.

The relative stopping power, $S_m$, of a mixture of two gases has been found (*3*) to be given by

$$S_m = S_Ax_A + S_Bx_B \tag{8}$$

where $x_A$ and $x_B$ are the mole fractions of gases $A$ and $B$ with relative stopping powers $S_A$ and $S_B$, respectively. Equations 7 and 8 then yield the relationship

$$i_m = c\,(S_A - S_B) \times x_A + cS_B \tag{9}$$

for the output current, $i_m$, for a binary gas mixture. Equation 9 shows that for a given cell and source, operating as described and with $l << L$, the output current is a unique and linear function of the composition of a binary gas mixture. For a ternary mixture, it is assumed that an extension of Equation 8 holds, as follows

$$S_m = S_Ax_A + S_Bx_B + S_Cx_C \tag{10}$$

which, when substituted into Equation 7 yields the relationship

$$i_m = c(S_A - S_B)x_A + c(S_C - S_B)x_C + cS_B \tag{11}$$

The cell output current is no longer uniquely determined by composition, and if the ionization currents for a given ternary gas system are determined from two cells, both of which have important dimensions less than the minimum range of alpha particles in all the mixtures of the system, and both of which have an applied voltage in region $B$ for all mixtures, the ratio of these currents is the same for all mixtures. An analysis under these conditions is impossible since the two measurements are not independent. Therefore, necessary operation of at least one of the two cells in such a way as to make the measurements independent (or, so that Equation 11 no longer holds) is necessary. For example, operation in a manner such that the rate of recombination of ion pairs becomes important in determining the output current of one of the cells, gives the desired effect.

Recombination of ions may become important in several circumstances. When the cell is operated in region $A$ as illustrated in Figure 2, one such circumstance is present. Another may occur in a cell of large radius in which the nonuniformity of the electric field in the end regions may lead to weak field areas where recombination can take place. Both effects are probably present in the ternary analysis system described here. In any event, the necessary independent measurements were obtained using a small cell operated in region $B$, together with a cell whose important dimensions were larger than the minimum range of alpha particles in the mixtures operated in region $A$ for two of the three pure components. Operation in region $A$ requires control of the voltage impressed on the cell.

The possibility of using radiations other than alpha particles was not overlooked. Alpha particles have several outstanding advantages, however. Alpha particles present little or no safety problem by themselves. Their very short ranges in air, amounting to only a few centimeters, makes the shielding problem simple. The wall thickness of the apparatus in which alpha particles are used generally is sufficiently large to provide complete shielding. Physical contact with alpha emitters should always be avoided.

Another major advantage of alpha particles over other types of radiations is that with their combination of a short range, high field, and high energies, large output currents are obtained. The problem of measuring the output is thus greatly simplified. With the present apparatus, output ionization currents of the order of $10^{-8}$ amp. are obtained, which are large compared to currents generally encountered in radiation studies.

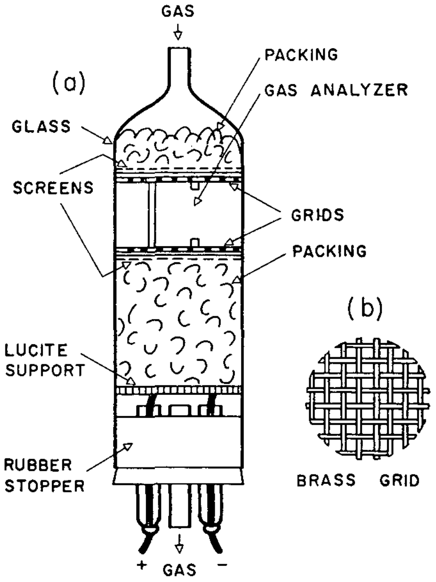

**Figure 3.** ***a*, Test packed tube assembly gas analyzer in place; *b*, brass packing support grid**

Safety considerations mentioned above are applicable only to alpha particle sources producing no radiations other than alpha particles, and producing no gaseous radioactive products. The desirable features of an alpha emitter are, then, that it emit only alpha particles, or that other radiations be of very low intensity; that it have not too short a half life in order to avoid the necessity of frequent recalibration of the analytical cell; that it be nonvolatile solid, yielding only nonvolatile solids as decay products and that the specific rate of alpha particle emission be high. This last requirement follows from the fact that the per cent standard deviation of the total emission rate is inversely proportional to the square root of the total emission rate. As a high standard deviation due to a low total emission rate causes a correspondingly high variance in the output current, precision of the analytical cell is impaired considerably by too low a rate. Furthermore, the specific emission rate, or rate per unit mass, must be high in order not to have to use too bulky an alpha particle source to achieve a high total emission rate.

Only one source, radium D, has been found which combines all of the desirable characteristics. While radium D is not an alpha emitter, its decay leads to the production of polonium by the following process:

$$\text{RaD} \xrightarrow[22y]{\beta} \text{RaE} \xrightarrow[5d]{\beta} \text{Po} \xrightarrow[140\ d]{\alpha} \text{Pb}^{206}$$

Polonium is the alpha emitter. Its half life is only 140 days so that its use by itself would necessitate too frequent recalibration. Radium D has a half life of 22 years, however, and thus provides an almost constant source of polonium. The alpha particle emission rate of the above source decreases only about 1.0% in 4 months.

Radium D and radium E decays are accompanied by the emission of beta rays so weak that 0.5 mm. of brass is more than enough shielding (*9*). The analytical cell and the container in which it is located will serve as shielding in this case. All of the substances are nonvolatile solids, the final product being stable lead. The beta rays emitted have such slight ionizing power compared with the alpha rays that their separate effect on cell output may be ignored.

Radium D, an isotope of lead, melts at 327.5° C. If analysis at higher temperatures is desired, a high melting point salt of radium D could be used as the source.

One general consideration remaining in connection with the use of ionizing radiations in general and alpha particles in particular is the possibility of chemical effects on the systems being analyzed. Such effects do exist, and Lind (*8*) presents a number of reactions actually observed. Hydrocarbons tend both to decrease and increase in molecular weight when subjected to alpha particles. In fact almost any reactive system undergoes change. Efficiencies are low, however, and reaction rates are negligible for most purposes. While hydrogen and oxygen react very rapidly in an eudiometer in which the reaction is initiated by an electric spark, mixtures of about 13 cc. in volume of the gases, acted on by 0.13 mc., take from 7 to 19 days for complete reaction (*8*). The only effect that slow reactions of this sort might have is the slow fouling of the radium D surface with liquid or solid higher hydrocarbons. No such effect was noted while using ethylene and ethane, although prolonged use of the gas analyzer might make it apparent.

## APPARATUS FOR BINARY MIXTURES

**Flow System.** The two gases used in an analysis experiment pass from their cylinders, through the usual diaphragm-type regulators, through needle valves, through capillary flow meters, to a mixing point. The mixed gases then pass into the test packed section containing the analyzer cell (Figure 3). A static manometer provides for the measurement of gas pressure above the packed section.

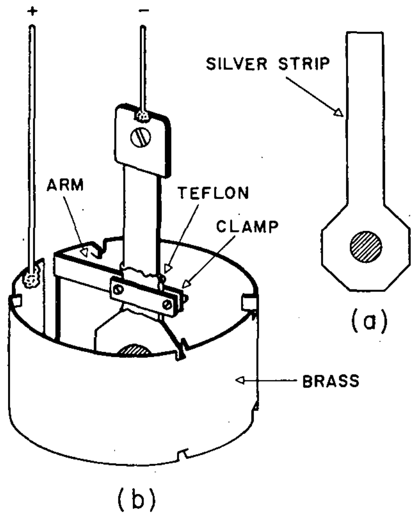

**Figure 4. *a*, Radium D source on silver strip; *b*, assembled gas analyzer chamber**

**Alpha Particle Source.** Figure 4, *a*, shows the radium D source on a strip of silver. The silver is approximately 3 cm. long and 1 cm. wide at the widest part. In a single analytical cell two such strips are used, clamped together as shown in Figure 4, *b*. Two strips are used, as it was possible to place radium D on only one side of a strip, but it was desired to irradiate all the gas in the gas analyzer chamber.

Radium D was obtained on the silver supporting strips from the Canadian Radium and Uranium Corp., 630 Fifth Ave., New York, N. Y. The radium D was rolled onto the silver, a process used instead of electroplating. Not only does radium D have the properties of lead, making electroplating difficult, but also an electroplated layer of radium D which contains little polonium. This requires approximately 2 years for a usable alpha particle activity to build up. By means of the rolling process, it is possible to place already aged radium D on the silver, a source ready for immediate use.

The amount of radium D on each strip is sufficient to give a total emission rate of alpha particles equivalent to 0.25 mc. approximately.

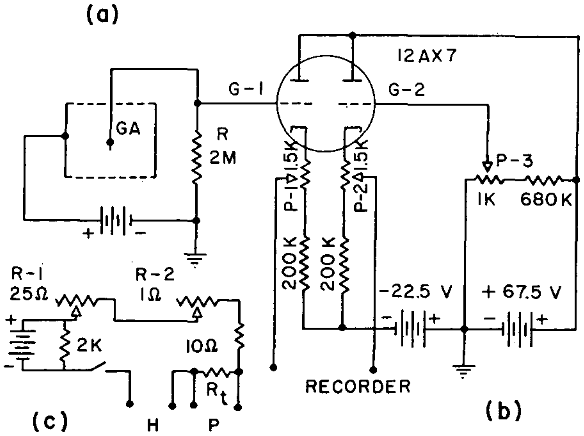

**Figure 5. *a*, Gas analyzer chamber (GA) and gas analyzer chamber circuit; *b*, cathode follower circuit; *c*, supply circuit for 12AX7 tube heaters**

**Analyzer Chamber.** The analyzer chamber is a cylinder of drawn brass (Figure 4, *b*). The cylinder is not completely closed in order that it can be forced into a tube of slightly smaller diameter as the chamber is designed to be inserted into a packed tube.

In Figure 4, *b*, the two silver strips are clamped to the arm in the center of the chamber so that the radium D sources are exposed to the gases in both halves of the chamber when in operation. The strips are wrapped in Teflon tape before mounting to insulate the strips electrically from the supporting arm and the chamber.

Two grids (Figure 3, *b*) are mounted loosely at each end of the chamber to serve as packing supports. These grids are made of brass, and are not attached to the chamber. When the whole assembly was placed in the packed tube (Figure 3, *a*) the packing and the walls of the tube held the chamber and the grids in place. Circular pieces of 80-mesh screen were placed above and below the whole assembly inside the tube to prevent the packing from plugging the holes in the grid.

A piece of wire soldered to the brass cylinder, and another clamped to the silver strips (Figure 4, *b*), served as electrical connections to the external circuit (Figure 5, *a*). Thus, the cylinder wall, grids, screens, and supporting arm constitute one electrode, and the radium D and silver strips the other. Referring to Figure 1, the first electrode would correspond approximately with $x_2$, and the second with $x_1$ placed at the source. The radius of the cylinder is the maximum distance between electrodes. This distance, 1.4 cm., is considerably smaller than the minimum range encountered in the investigation (2.4 cm. for carbon dioxide).

The entire experimental test assembly is shown in Figure 3. The packing used was 3-mm. glass beads. The two electrical leads were brought out through separate tubes, and the tubes were sealed with de Khotinsky cement for gas tightness. A schematic representation of the assembly, including a portion of the external circuit, is shown in Figure 5, *a*. The positive terminal of the applied voltage source used, represented by a battery, was connected to the brass cylinder, *GA*. The ionization current then flowed from the cylinder, through the gas, to the silver strips, through the resistor, *R*, and back to the voltage source, completing the circuit. The voltage drop thus created across *R* was then measured and recorded.

**Measuring and Recording.** The voltage drop across resistor *R* (Figure 5, *a*) was made the input to the cathode follower circuit (Figure 5, *b*). The output from the cathode follower was then recorded directly. The recorder used was a Leeds and Northrup Speedomax, Model 60,000. It is possible to use recorders of many types, both recording potentiometers such as the one used here, and recording microammeters, with some modifications in the cathode follower circuit.

The cathode follower circuit is necessary, for although its output voltage is slightly lower than its input voltage, the impedance of the output is low compared to that of the input, and the input to the recorder employed is a low impedance input. Direct recording of the voltage drop across the 2-megohm resistor, *R*, is possible, but the response time of the recorder becomes 2 minutes instead of its usual 2 seconds. The cathode follower circuit thus preserves the low response time of the recorder.

The cathode follower is also a power amplifier, an attribute which makes it a useful circuit if a recording microammeter is to be used. The cathode follower circuit is commonly used in vacuum tube voltmeters. More discussion of this circuit is available in Hill (*5*) or other electronics texts.

With a cell output of about $10^{-8}$ amp. and resistance *R* of about 2 megohms, the input to the cathode follower is of the order of 20 mv., which is within the range of the recorder. The cathode follower of Figure 5, *b*, actually consists of two cathode followers. One has as its input the voltage drop across *R*, and the other has as its input the bias of grid $G_2$, which can be set arbitrarily by means of $P_3$. The net output of this total circuit is the voltage difference between $P_1$ and $P_2$, which is the difference in output of the two separate cathode followers. This output becomes the input to the recorder.

This arrangement makes possible a shift in zero point in the cathode follower, itself, while maintaining the zero point of the recorder fixed. When first constructed, $P_1$ and $P_2$ were simply junction points. The 12AX7 tubes were found to be sufficiently out of balance. Some tubes tried permitted adequate control of the zero point by means of $P_3$, whereas others did not. Therefore, $P_1$ and $P_2$ were installed to act as a rough adjustment, $P_3$ then acting as a fine adjustment.

In operation the output of the cathode follower circuits was found to be very sensitive to the tube heater voltage. This difficulty was corrected by using a tube heater voltage supply circuit (Figure 5, *c*). Rheostats $R_1$ and $R_2$ serve as coarse and fine setting devices, respectively. The steadiness of the supply can be checked by measuring the voltage drop in resistor $R_t$, in series with the tube heater. This drop equals 10.130 mv. if the supply voltage to the tube heater is 6.28 volts, and any deviation from 10.130 mv. could be corrected by means of $R_1$ and $R_2$. The voltage drop in $R_t$ was measured by means of a Leeds and Northrup Type K-2 potentiometer. The 2K resistor prevents polarization of the batteries by always permitting a small current withdrawal.

With the exception of the sensitivity of the cathode followers to the tube heater voltage, these circuits are very stable. Stability is another inherent advantage of cathode followers in general, making their use preferable, whenever possible, to direct current amplifiers.

**Analytical Cell Voltage Supply.** Almost any direct current voltage supply capable of providing a voltage in the neighborhood of 500 volts is usable with the gas analyzer, and, as mentioned earlier when operating in Region *B* (Figure 2), accurate regulation of the supply voltage is relatively unimportant. A pile of B-batteries was used in series giving a source capable of a maximum output of 900 volts. Power requirements for the source are negligible.

## APPARATUS FOR TERNARY MIXTURES

**Flow System.** The three gases used in an analysis experiment flow from their pressure regulators, through needle valves, through calibrated rotameters to a mixing point. The mixed gases then flow through the large cell and then through the small cell.

**Alpha Particle Source.** The source in the small cell is the same as that used in the binary experiments. For the large cell, an improved form was used. The radium D was rolled in a narrow band along one edge of a rectangle and the rectangle formed into a 3-mm. diameter cylinder with axis perpendicular to the radium D band. Thus, irradiation of all the gas in the analyzer is possible using a single 0.5-mc. source.

**Analyzer Chamber.** The small chamber is the same 1.4-cm. radius cylinder used in the binary experiments. The large chamber is a similar cylinder having a radius of 3.5 cm. and length of 4.0 cm. The source is mounted as shown in Figure 4, *b*. No packing was used around the large cell.

Electrical connections to the external circuit for the large cell are the same as those for the small cell. The cell voltages were supplied from B-battery packs.

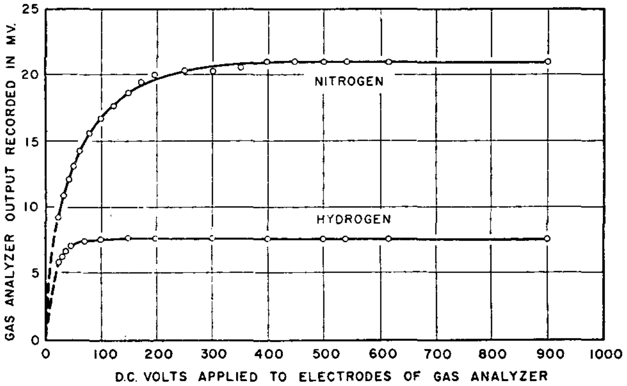

**Figure 6. Characteristic output–applied voltage curves for gas analyzer**

With 2.8-cm. inside diameter chamber for nitrogen and hydrogen

**Measuring Circuit.** The resistor *R*, Figure 5, *a*, is replaced by a 100-megohm resistor for the ternary experiments and a switch provided so that the current from either cell can flow through it to ground. The voltage drop across the resistor was made the input to a Keithly Instrument Co. Model 200, vacuum tube electrometer. This instrument is ideally suited to steady state measurements of ionization current since it has an input impedance of the order of 100-million megohms. The cathode follower and recorder used in the binary experiments were dispensed with since no permanent record was needed. The voltage drop across the 100-megohm resistor varies from 0.3 to 4.4 volts for the gas system used, a range that is easily handled by the electrometer with its full-scale ranges of 2 and 20 volts.

### NOMENCLATURE

$I$ = total ionization, ion pairs
$I_s$ = specific ionization, ion pairs/cm.
$I_t$ = total ionization, relative to that of air
$i_m$ = output current for gas mixture, amp.
$i_o$ = output current for pure gas, amp.
$L$ = range of alpha particle, cm.
$L_a$ = range of alpha particle in air at S.T.P., cm.
$l$ = important cell dimension, cm.
$P_o$ = standard pressure
$P_1$ = pressure at which $v_1$ measured
$P_x$ = pressure at which $v_x$ measured
$R_o$ = $v_{xo} - v_{1o}$
$S$ = stopping power of pure gas, relative to that of air
$S_m$ = stopping power of gas mixture, relative to that of air
$T_o$ = standard temperature
$T_1$ = temperature at which $v_1$ measured
$T_x$ = temperature at which $v_x$ measured
$U$ = velocity of alpha particle, cm./sec.
$v_1$ = output voltage for gas of lower $I_tS$ value, at $P_1$ and $T_1$, mv.

$v_x$ = output voltage for gas mixture of mole-fraction $x$, at $P_x$
$v_{1o}$ = output voltage for gas of lower $I_tS$ value, at $P_o$ and $T_o$, mv.
$v_{xo}$ = output voltage for gas mixture of mole-fraction $x$, at $P_o$ and $T_o$, mv.
$x$ = mole fraction
$z$ = distance from source, cm.
$a$, $c$, $k$ = constants

### PROCEDURE WITH BINARY MIXTURES

The investigation of characteristics of the gas analyzer as used for binary mixtures included the following:

Determination of the characteristic curve of output *vs.* voltage for the analyzer chamber, with pure gases in the chamber;

Effect of density and flow rate of pure gases on the analyzer output;

Determination of the calibration curves for several binary gas systems;

Determination of the reproducibility of the calibration curves, and determination of the precision of the analyzer; and

Response time of the gas analyzer.

Gas systems used were: hydrogen-nitrogen, hydrogen-ethylene, ethylene-ethane, and carbon dioxide–nitrogen. The system hydrogen-nitrogen happened to be of particular interest and was therefore used for most of the tests. Tests 1, 2, 4, and 5 were performed using that system primarily, so that sufficient information on the operating characteristics of the analyzer could be obtained. Calibration curves were obtained for the other systems as an illustration of the more general applicability of the analyzer.

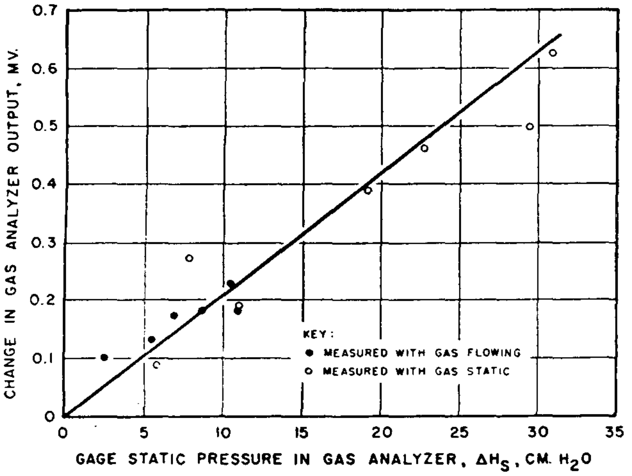

**Figure 7. Effect of gas pressure on output of gas analyzer**

With 2.8-cm. inside diameter chamber

**Operating Curves.** The characteristic operating curves of the analyzer are shown in Figure 6 for hydrogen and for nitrogen. These curves were obtained by setting the tube heater voltage at the constant, standard value, and then, with the pure gas in question flowing steadily through the analyzer, the output was read for various applied voltages. The highest voltage that was available for this test was 900 volts; thus, the full extent of the operating range was not determined. The threshold for hydrogen was approximately 70 volts, and for nitrogen approximately 300 volts. The remainder of the tests was then made at an applied voltage of 400 volts.

**Effects of Pressure and Flow Rate.** These were investigated using pure nitrogen in the gas analyzer. Small changes in output were noted as the flow rate was varied. These changes were found to be linear functions of the changes in static pressure in the cell brought about by the changing flow rate. Experiments were carried out under conditions of no flow, varying the gas pressure in the analyzer and packed section, and reading the corresponding output. Figure 7 shows the change in output of the gas analyzer chamber from the output at zero static gage pressure plotted against the static gage pressure in the chamber, for experiments conducted under conditions both of flow and no flow. The flow itself has no effect, while pressure does have an effect. That flow has no effect is reasonable, because the rate of motion of the ions to the electrodes is very rapid compared to the flow of the gases, thus the sweeping away of the ions by the gases before collection on the electrode is a negligible effect.

**Table I. Comparison of Total Output for Various Pure Gases with Properties of Gases**

| Gas | Total Output, Mv. | Relative Stopping Power, $S^a$ | Total Relative Ionization, $I_t^a$ | $I_tS$ | Mol. Wt. |
|---|---|---|---|---|---|
| $H_2$ | 7.6 | 0.24 | 1.00 | 0.24 | 2 |
| $N_2$ | 21.4 | 0.99 | 0.96 | 0.95 | 28 |
| $CO_2$ | 26.2 | 1.51 | 1.03 | 1.52 | 44 |
| $C_2H_4$ | 32.4 | 1.35 | 1.22 | 1.65 | 28 |
| $C_2H_6$ | 39.6 | 1.51 | 1.30 | 1.97 | 30 |

[a] Data for $S$ and $I_t$ are from Lind (*8*).

To compute ranges from $S$-values, the range in air of an alpha particle from polonium, energy 5.2 m.e.v., is approximately 3.9 cm. at 1.0 atm. and 15° C.

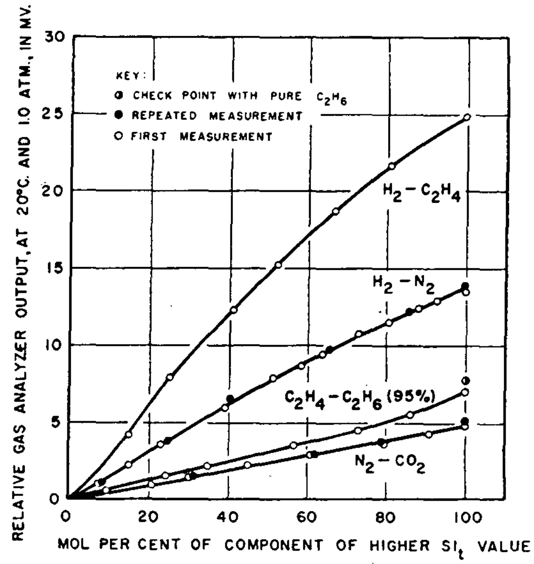

**Figure 8. Calibration curves for binary mixtures, 2.8-cm. inside diameter chamber**

(See Table I for $SI_t$ values)

Changes in density, whether caused by pressure or temperature should have an effect on the rate of formation of ions and therefore on the analyzer output. This effect (Figure 7) is directly proportional to pressure which corresponds to a direct proportionality to density. From the last proportionality, Equation 12 was derived for the correction of results to a standard temperature and pressure

$$R_o = v_{xo} - v_{1o} = R_x \left[\frac{P_oT_x}{T_oP_x}\right] + v_{1o}\left[\frac{P_1T_x - P_xT_1}{P_xT_1}\right] \qquad (12)$$

**Calibration Curves.** For all systems studied, the calibration curves are shown in Figure 8. The ordinate is the output from the cathode follower in millivolts for the gas mixture over and above that for the pure component of lesser $I_tS$ in the mixture (see Table I), corrected for pressure and temperature to 20° C. and 1.0 atm. In order to obtain the total output for the system ethylene-ethane at any particular concentration—for example, in Figure 8 the ordinate for that system at the desired concentration is added to the ordinate for pure ethylene from the curve for hydrogen-ethylene. To this value add the output for pure hydrogen taken from Figure 6 at an applied voltage of 400 volts.

This value, multiplied by 0.507 gives the current in amperes × $10^{-9}$ which would flow through the analyzer at 20° C. and 1.0 atm., when the particular mixture of ethylene-ethane is in the analyzer. The calibration curves are plotted as they are because the final output is in millivolts rather than amperes, and because the subtractions resulting in the ordinate of Figure 8 are performed electronically by varying the zero point of the cathode follower output. The figure 0.507 follows the characteristics of this particular cathode-follower circuit and the value of $R$ of 2.0 megohms, Figure 5, *a*. Total outputs in millivolts are listed in Table I for pure components.

From Figure 8, this analytical cell can be used satisfactorily with the above systems. If for any mixture the range of output is not sufficient to permit a desirable precision of measurement, the value of $R$, Figure 5, *a*, may be increased resulting in a directly proportional increase in cathode follower output. There is some limitation to the size of $R$ due to the existence of an inherent positive grid current in most vacuum tubes. This difficulty may be overcome by the use of electrometer tubes of very low positive grid current, or by redesign of the cathode follower. Many different circuits are also available for the measurement of ionization currents, so that application of the technique to any given case is a relatively simple matter.

**Reproducibility of Analyzer.** This is best illustrated by the calibration curve for hydrogen-nitrogen, Figure 8. This curve contains data taken at intervals of 11 days. Graphically, the reproducibility appears to be good. The nitrogen–carbon dioxide data were taken within the same day.

**Precision.** Precision was obtained numerically as follows: With pure hydrogen in the analyzer, the zero point of the readings was set to correspond to approximately zero reading on the recorder. This reading was recorded, the hydrogen was turned off, and nitrogen turned on. The output for nitrogen in the analyzer was read and from it was subtracted the reading for hydrogen. This result was corrected for temperature and pressure by means of Equation 12. Another value was obtained in the same manner, but with a different zero point to avoid human error. This procedure was repeated seven times on different dates, yielding a series of values for the difference in output for pure hydrogen and nitrogen at 20° C. and 1.0 atm. The standard deviation of these values was ±0.0219 mv. The average difference in output between hydrogen and nitrogen for these runs was 13.764 mv., so that the standard deviation would give a standard deviation in concentration terms of ±0.16 mole %.

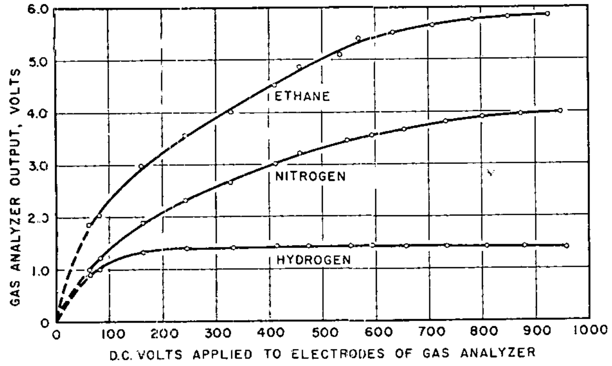

**Figure 9. Characteristic output–applied voltage curves for gas analyzer**

With 7.0-cm. inside diameter chamber for ethane, nitrogen, and hydrogen

**Response Time.** Response time was investigated briefly, experimentally, and theoretically. The recorded response time for a substitution of one gas for another in the cell was compared with the time calculated from the flow rate of the new, incoming gas. The two times were generally found to be within about 0.1 second of each other, except at very high flow rates such that the calculated response time was less than 2 seconds, which is the minimum response time of the recorder used.

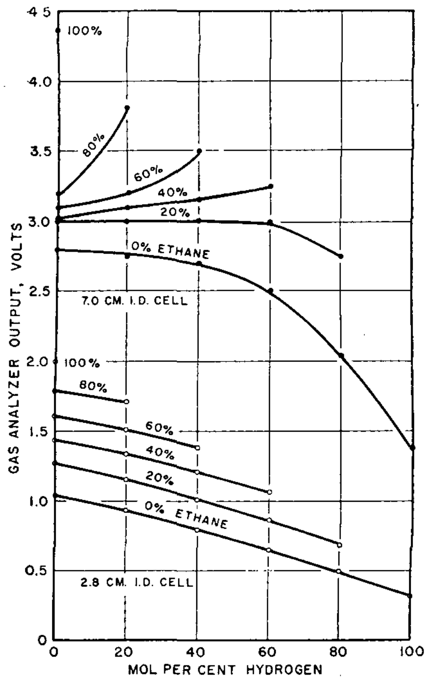

**Figure 10. Calibration curves for the ternary mixture: ethane, nitrogen, and hydrogen**

Outputs of 7.0- and 2.8-cm. inside diameter chambers for various mole percentage mixtures

Theoretically, estimation of the response time to the analyzer is possible. Korff (7) gives a formula,

$$v = \frac{kE}{p} \tag{13}$$

in which $v$ is the velocity of an ion in a gas in cm. per second, $E$ is the average field in volts per cm., $p$ is the gas pressure in atmospheres, and $k$ is the ionic mobility in cm. per second volt per cm. at unit pressure. For positive ions in air, values for $k$ of about 1.35 have been found, while in hydrogen $k$ is about 6. A conservative value for the hydrogen-nitrogen system would be 1.4, over the whole range of concentration. Equation 13 applies to a cylindrical ionization chamber with a wire in the center, one electrode being the cylinder and the other the wire. The gas analyzer approximates this case. The time of collection for a cell 1.4 cm. in radius with an applied voltage of 400 volts is about 3.5 × $10^{-3}$ second.

## PROCEDURE WITH TERNARY MIXTURE

Only two of the characteristics of the gas analyzer as used for ternary mixtures were determined: These were the curves of output *vs.* applied voltage for each of the three pure gases measured in the large chamber and the calibration curves for the ternary mixture. The gas system was hydrogen-nitrogen-ethane.

**Operating Curves.** These curves of the 3.5-cm. radius chamber for hydrogen, nitrogen, and ethane are shown in Figure 9. They were obtained by reading the output of the cell directly from the Keithly vacuum tube electrometer as voltage drop across 100 megohms while the applied voltage was varied. The effect of the larger cell radius is to decrease the average field strength from that for the results shown in Figure 6, and thus to require higher applied voltages before the threshold is reached. A measurement of the operating curve for ethane in the cell of Figure 6 gave a threshold of 400 volts instead of near 1000 volts in the large cell. Further tests were made at an applied voltage of 450 volts for both cells.

**Calibration Curves.** The calibration curves for the system studied are shown in Figures 10 and 11. Measurements were carried out in a few hours of the same day and no corrections of the ordinate of Figure 10 for temperature and pressure were made. Since the 100-megohm resistor is the only element through which the cell current flows, the ordinate also reads directly in amp. × $10^{-8}$. Output of the 2.8 cm. inside diameter cell is approximately linear, as predicted by Equation 11. However, the output of the 7.0 cm. inside diameter cell is highly nonlinear. The output is seen to increase with increasing hydrogen content for mixtures of more than 20% ethane. Recombination of ion pairs is undoubtedly a factor causing this reversal of slope.

A cross plot of these data is shown in Figure 11. Figure 11 indicates that very good analytical precision can be obtained for mixtures of high ethane content, and reasonable precision can be expected for mixtures of high hydrogen content. The area of least precision is along the ethane-nitrogen axis.

Through comparisons of slopes in Figures 9 and 11, an estimate of the required regulation of cell voltage for analysis is obtained. On the average, for the present cell and ternary system a change of 1 volt in applied voltage corresponds to a composition change of 0.85 mole %.

## DISCUSSION

Although Equations 9 and 11 indicate the general form of calibration curves for small cells operated in the flat region of the characteristic curves, calibration curves for any given combination of source, chamber, and gases must be determined experimentally. Geometric factors in the comparatively short cylindrical cells are not covered by the simple equations. Another more fundamental problem is self-absorption for any readily available source. Self-absorption is the effect of the source material absorbing energy from the alpha particles produced within it. Particles produced within the source reach the gas phase with reduced energies, if they reach the gas phase at all. The spectrum of alpha particle energies so produced depends on very small irregularities in the thickness and distribution of the source material on its support. No two sources produced by present methods probably give analyzers identical characteristics. This can be ameliorated somewhat by adjustment of the external resistors, *R* (Figure 5, *a*) in order to make the total range of two analyzers equal, but some difference in the form of the curves still remains. A prediction of the nature of curves for large cells operated in the rising portion of the characteristic curves is rendered improbable by the present lack of knowledge about the kinds of ions formed and their rates of recombination. A further complication is the strong effect of trace impurities on recombination rates.

As far as the applicability of the analyzer to various binary gas systems is concerned, almost any two gases of different molecular, atomic, or electronic structure should have different specific ionizations, at least over some portion of the range of the alpha particles in them. One would not expect to be able to distinguish between ortho and para hydrogen, or between hydrogen and deuterium, by this method, whereas thermal conductivity methods do make the distinction. Hatfield, Lockenvitz, and Young (*4*) showed that hydrogen and deuterium are identical in specific ionization throughout the whole range of alpha particles in them.

If information concerning total ionization and stopping power is available, their product should be a good index of distinguishability, as it is proportional to the average specific ionization for a given gas. These three numbers are given for the pure components used in Table I. Comparison with the total outputs for

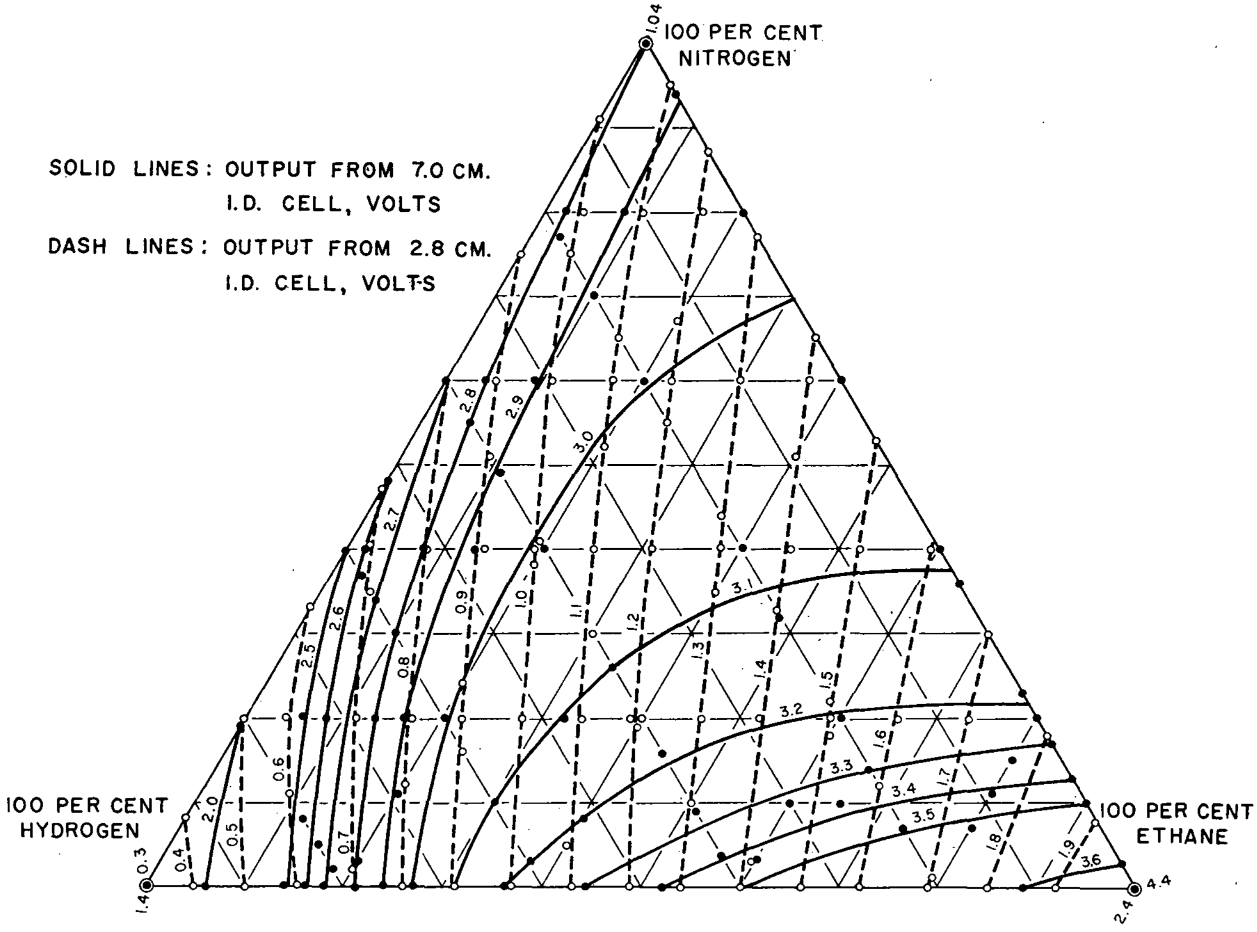

**Figure 11. Calibration curves for the ternary mixture: ethane, nitrogen, and hydrogen**

Lines of constant output for 7.0- and 2.8-cm. inside diameter chamber each component shows that the last named product is a good index, and that stopping power itself is not too bad, while total ionization is too indeterminate. Molecular weight, also shown, is not a reliable index.

A general statement as to the applicability to ternary or more complex mixtures is not possible. The problem faced is the experimental one of determining the proper electrode spacing and operating voltages to give a workable system. For example, an attempt was made to obtain calibration curves for the hydrogen-ethylene-ethane system. The result, at the one combination of conditions tried, was a series of parallel lines of constant output for both small and large cells on a triangular diagram. Changes in conditions would probably have made an analysis possible. In general, any given ternary system precision varies with composition. This limitation is not serious unless the whole range of composition is being measured.

The form of analyzer used in the above investigation was developed for a specific purpose—namely, to fit into a packed tube and to interfere as little as possible with the flow through the tube. Many other arrangements are possible. For example, a small cell for the measurement of instantaneous point concentration could be made using a source on a fine needle, surrounded closely by a grid of fine wire—the source being one electrode and the grid the other. A similar grid arrangement might be used for ternary analysis. A series of chambers irradiated by the same source, but separated by grids would serve to provide the necessary two independent conditions of measurement.

For pure gases or for gases of fixed composition, the analyzer can serve very well as an instantaneous density meter, and at constant temperature, the analyzer becomes a pressure gage. Rapid fluctuations in pressure or density of a gas may be measured without the inertial effects encountered in most other systems.

The gas analyzer was satisfactory for the original service intended. Random noise in the electrical output, owing to randomness of alpha particle emission by the source and to trickle currents flowing through the insulation from the positive to the negative electrode, was damped by the Speedomax recorder. Suitable filter circuits for the removal of this noise have been included in the latest installation which utilizes a cathode-ray oscillograph as the recording device. Trickle currents can be minimized by using as insulation, two thicknesses of Teflon or other insulating material with grounded metal foil. Such a piece of foil would act as a guard ring, a device much used in ionization chambers, and would conduct trickle currents to ground.

Electrical disturbances in the vicinity of the analyzer from closing switches, operating fluorescent lamps, or the sparks of truck motors caused sudden and violent changes in the recorded output, fortunately of short duration. This difficulty was eliminated by shielding the apparatus containing the analyzer, using shielded wire for all connections, and grounding the entire supporting rack on which the apparatus was erected. The output from the cells, as indicated on the Keithly electrometer, was very stable even without complete shielding; the slow response of the electrometer served to damp out the effects of local disturbances.

## ACKNOWLEDGMENT

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# Identification of Amines from X-Ray Powder Diffraction Diagrams of Their Hydrochloride Derivatives

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**The present investigation was prompted by the need for a simple foolproof procedure for identifying milligram quantities of amines. The hydrochloride derivative was chosen because of the simplicity of the procedure for preparing derivatives of volatile amines, the quantitative nature of the reaction, the absence of side reactions, and the ease of removing the excess reagent used. The procedures for preparing the derivatives and x-ray specimens are described. The interplanar spacings, *d*, and the relative intensities, $I/I_1$, are tabulated for the diffraction maxima of 52 aliphatic and aromatic amines.**

X-RAY powder diffraction techniques have been used successfully in the identification of several classes of organic compounds (*2, 3, 5, 7–10*). The x-ray method of identification is rapidly becoming more popular because of the ease and positive nature of the identification which can be made using a single derivative. The working time required is not much more than that required to obtain a melting point. In addition, derivatives can be made on small quantities of sample, the product can usually be identified without purification, and a permanent record of the nature of the material is obtained.

It is generally accepted that the ideal derivative for identification by melting point should be crystalline, be easily purified, and have a sharp melting point. The yield of the reaction should be high, and the melting points of the various products should differ enough to permit identification. DeLange and Houtman (*3*) have discussed the disadvantages encountered in identifying derivatives by using melting points.

The hydrochloride salts do not appear to be too popular as derivatives for identifying amines by the melting point method. This is due primarily to the hygroscopic nature of some of the products and the fact that the melting points of amine hydrochlorides are considered poor for identification purposes (*14*).

For identification of derivatives by x-ray diffraction, however, it is only necessary that the derivatives be crystalline enough to give a diffraction diagram. Ideally the derivatives should be easily prepared in high yields, and the reagent used should be capable of being readily removed.

The hydrochloride derivatives of amines fulfill all these conditions. The hygroscopic nature of some of these hydrochloride derivatives presents little difficulty when samples are prepared in the proper manner.

Gould and Gross (*5*) have previously identified amines by x-ray powder patterns of their chloroplatinates. This is an excellent method for identifying submilligram quantities of volatile amines by taking advantage of the gain in weight upon conversion to the chloroplatinate. The hydrochloride derivatives, on the other hand, are suitable for identifying milligram quantities of amines. However, an identification can be made with as little as 0.1 mg. of the derivative. The hydrochloride derivative is easily and simply prepared. There are no side reactions to interfere with the identification of the amine and the excess reagent is completely removed by volatilization. The hydrochloride derivative has an advantage over the chloroplatinate derivative in that the former salts absorb less x-radiation, permitting the use of thicker specimens without dilution. The diffraction patterns can, therefore, be recorded using the usual capillary specimen tubes and commercially available diffraction cameras. In general, many of the hydrochloride derivatives exhibit a simple diffraction pattern at angles which are relatively wide for organic compounds. This facilitates their identification.

## EXPERIMENTAL

The following procedure was found to be generally applicable for the preparation of the amine hydrochloride derivatives that were made for this study.

A few drops of the amine or aqueous amine solution is added to a small Petri dish containing 10 ml. of 1*N* hydrochloric acid. The resulting solution is stirred and evaporated to dryness in a 110° C. oven. The crystalline product is dissolved in anhydrous ethyl alcohol. The solution is filtered and again evaporated to dryness in a watch glass.

The crystalline hydrochloride is then ground to a fine powder in an agate mortar and packed in a cellulose acetate tube. If only a small amount of derivative is recovered, it may be scraped into a pile on the watch glass and packed into the cellulose acetate tube without grinding. The cellulose acetate tubes are made according to the procedure described by Fricke and others (*4*). X-ray diffraction diagrams were obtained with a Philips 114.59-mm. powder camera using copper K$\alpha$ radiation from a Philips x-ray generator operated at 35 kv. and 20 ma. Exposures varied from 1.5 to 2 hours using Kodak No-Screen x-ray film and the recommended developing procedure.

In some cases, the hydrochlorides are hygroscopic, and in such instances it was necessary to use a few simple precautions in preparing the samples. Satisfactory results were usually obtained by keeping the crystals in a 70° C. oven and removing them intermittently for grinding. After the sample was ground sufficiently, it was quickly packed into a cellulose acetate tube; the open end of the tube was sealed by dipping it into melted paraffin wax. The diffraction diagram should be taken immediately.

Some of the amine hydrochlorides of low molecular weight exist as a melt in the 110° C. oven. In such cases these salts are crystallized in a 70° C. oven.

Tributylamine and triamylamine hydrochlorides are liquid at 70° C. and must be removed from the oven and crystallized, using agitation and cooling with dry ice.

Table I. Amines Studied

| Amine | Source | Nature of Hydrochloride |
|---|---|---|
| Methyl | Rohm & Haas | Hygroscopic |
| Dimethyl | Eastman | Very hygroscopic, liquid at 110° C. |
| Trimethyl | Commercial Solvents | Very hygroscopic |
| Ethyl | Sharples (70% soln.) | Very hygroscopic, liquid at 110° C. |
| Diethyl | Sharples (98% soln.) | Very slightly hygroscopic |
| Triethyl | Sharples (tech.) | |
| 2-Aminoethanol | Paragon | Hygroscopic, liquid at 110° C. |
| 2,2′-Iminodiethanol | Eastman | Noncrystalline |
| 2,2′,2″-Nitrilotriethanol | Fisher Scientific | |
| Propyl | Sharples (tech.) | Hygroscopic |
| Dipropyl | Sharples (tech.) | |
| Tripropyl | Eastman | |
| Isopropyl | Eastman | Hygroscopic |
| Diisopropyl | Commercial Solvents | Hydrous and anhydrous forms |
| Butyl | Sharples (94% soln.) | |
| Dibutyl | Sharples (98% soln.) | |
| Tributyl | Sharples (tech.) | Very hygroscopic, liquid at 70° C. |
| Isobutyl | Sharples (tech.) | |
| Diisobutyl | Sharples | |
| *sec*-Butyl | Eastman | |
| Di-*sec*-Butyl | Sharples (tech.) | |
| *tert*-Butyl | Rohm & Haas | |
| Amyl | Sharples (90% soln.) | Slightly hygroscopic |
| Diamyl | Sharples (99% soln.) | Insoluble in dilute HCl |
| Triamyl | Eastman | Liquid at 70° C. |
| Isoamyl | Eastman | |
| Diisoamyl | Eastman | Insoluble in dilute HCl |
| Triisoamyl | Eastman | Insoluble in dilute HCl, partially liquid at 110° C. |
| *sec*-Amyl | Sharples (tech.) | |
| *tert*-Amyl | Sharples (tech.) | Hydrous and anhydrous forms |
| Hexyl | Eastman | |
| Heptyl | Eastman | |
| Octyl | Sharples | |
| 1,1,3,3-Tetramethylbutyl | Rohm & Haas | |
| Ethylenediamine | Carbide and Carbon | Insoluble in ethyl alcohol |
| 1,3-Propanediamine | Sharples (tech.) | |
| 1,3-Butanediamine | Sharples (tech.) | |
| 1,4-Butane diamine | Du Pont | |
| 1,6-Hexanediamine | Biological Laboratories | |
| Aniline | Baker and Adamson | |
| Diphenylamine | Eastman | |
| Benzyl | Sharples (tech.) | |
| Dibenzyl | Sharples | Insoluble in dilute HCl |
| Tribenzyl | Eastman | |
| Cyclohexyl | Monsanto | |
| Dicyclohexyl | Monsanto | |
| Morpholine | Carbide and Carbon | |
| Piperazine | Carbide and Carbon | |
| Piperidine | Eastman (98% soln.) | Slightly hygroscopic |
| Pyridine | Baker and Adamson | Very hygroscopic, liquid at 110° C. |
| Quinoline | Eastman | Noncrystalline |
| *o*-Toluidine | Baker and Adamson | |
| *m*-Toluidine | Eastman | |
| *p*-Toluidine | Baker and Adamson | |

The amines studied and their sources are given in Table I. The amines were not purified but were used as supplied. Some of the characteristics of the hydrochloride derivatives are also noted.

The diameters of the diffraction maxima were measured with a Philips line-measuring device. Using the 114.59-mm. camera, 2 mm. measured on the film is equivalent to an angle of 1° $\theta$ in the Bragg formula, where $\lambda = 2d\sin\theta$. The interplanar spacing values, *d*, were then determined using the charts prepared by Parrish and Irwin (*12*) for copper K$\alpha$ radiation ($\lambda = 1.5418$ A.).

The interplanar spacings, measured in Angstrom units, for the amine hydrochlorides studied are listed in Table II together with the visually estimated relative intensities, $I/I_1$. The relative intensities of the diffraction maxima were determined using photographic standards as recommended by Hanawalt, Rinn, and Frevel (*6*).

Of the 54 amines studied only 2,2′-iminodiethanol and quinoline failed to form crystalline hydrochlorides from dilute aqueous hydrochloric acid solutions. Three amines formed poorly crystalline hydrochlorides: tributylamine, triamylamine, and pyridine. Although these amine hydrochlorides gave poor diffraction diagrams, they were nevertheless suitable for identification purposes.

Diisopropylamine hydrochloride gave two slightly different diffraction diagrams, depending upon the conditions of preparation. One of these is apparently due to a hydrated form which

## Table II. X-Ray Diffraction Data for Amine Hydrochlorides

Methylamine

| $d$ | $I/I_1$ |
|---|---|
| 5.06 | 0.46 |
| 3.89 | 0.25 |
| 3.27 | 1.00 |
| 3.03 | 0.84 |
| 2.83 | 0.15 |
| 2.60 | 0.25 |
| 2.53 | 0.15 |
| 2.40 | 0.15 |
| 2.18 | 0.25 |
| 2.14 | 0.25 |
| 1.98 | 0.15 |
| 1.94 | 0.15 |
| 1.80 | 0.25 |
| 1.63 | 0.15 |
| 1.57 | 0.15 |
| 1.53 | 0.15 |

Dimethylamine

| $d$ | $I/I_1$ |
|---|---|
| 4.98 | 0.69 |
| 3.64 | 1.00 |
| 3.35 | 0.04 |
| 3.10 | 0.04 |
| 2.94 | 0.46 |
| 2.78 | 0.29 |
| 2.58 | 0.20 |
| 2.29 | 0.07 |
| 2.17 | 0.07 |
| 1.89 | 0.04 |

Trimethylamine

| $d$ | $I/I_1$ |
|---|---|
| 6.14 | 0.09 |
| 4.61 | 0.79 |
| 3.52 | 1.00 |
| 3.05 | 0.05 |
| 2.91 | 0.43 |
| 2.75 | 0.09 |
| 2.69 | 0.18 |
| 2.49 | 0.09 |

Ethylamine

| $d$ | $I/I_1$ |
|---|---|
| 8.26 | 0.35 |
| 4.07 | 0.08 |
| 3.88 | 0.35 |
| 3.39 | 1.00 |
| 3.25 | 0.08 |
| 3.14 | 0.05 |
| 3.01 | 0.65 |
| 2.82 | 0.05 |
| 2.74 | 0.05 |
| 2.65 | 0.15 |
| 2.40 | 0.15 |
| 2.37 | 0.15 |
| 2.15 | 0.05 |
| 2.13 | 0.08 |
| 2.03 | 0.05 |
| 1.88 | 0.05 |
| 1.80 | 0.08 |
| 1.69 | 0.05 |
| 1.63 | 0.05 |
| 1.50 | 0.08 |

Diethylamine

| $d$ | $I/I_1$ |
|---|---|
| 6.54 | 0.32 |
| 4.77 | 0.19 |
| 3.67 | 1.00 |
| 3.33 | 0.25 |
| 3.19 | 0.25 |
| 2.62 | 0.19 |
| 2.44 | 0.09 |
| 2.40 | 0.06 |
| 2.32 | 0.06 |
| 2.26 | 0.09 |
| 2.04 | 0.09 |
| 1.87 | 0.02 |
| 1.83 | 0.03 |
| 1.76 | 0.03 |
| 1.73 | 0.06 |
| 1.65 | 0.03 |

Triethylamine

| $d$ | $I/I_1$ |
|---|---|
| 7.25 | 1.00 |
| 5.07 | 0.22 |
| 4.18 | 0.33 |
| 3.64 | 0.22 |
| 3.54 | 1.00 |
| 3.22 | 1.00 |
| 2.70 | 0.22 |
| 2.54 | 0.15 |
| 2.41 | 0.03 |
| 2.24 | 0.03 |
| 2.17 | 0.03 |
| 2.09 | 0.08 |
| 1.99 | 0.05 |
| 1.93 | 0.05 |
| 1.79 | 0.08 |
| 1.75 | 0.03 |
| 1.72 | 0.03 |
| 1.63 | 0.05 |
| 1.58 | 0.03 |
| 1.53 | 0.05 |
| 1.44 | 0.05 |
| 1.39 | 0.02 |
| 1.26 | 0.03 |

2-Aminoethano

| $d$ | $I/I_1$ |
|---|---|
| 7.08 | 0.35 |
| 3.66 | 0.24 |
| 3.54 | 0.82 |
| 3.44 | 1.00 |
| 3.20 | 1.00 |
| 2.89 | 0.35 |
| 2.74 | 0.15 |
| 2.59 | 0.49 |
| 2.47 | 0.24 |
| 2.32 | 0.15 |
| 2.14 | 0.04 |
| 2.09 | 0.15 |
| 2.02 | 0.08 |
| 1.92 | 0.08 |
| 1.82 | 0.15 |
| 1.59 | 0.15 |
| 1.46 | 0.08 |

2,2′,2″-Nitrilotriethanol

| $d$ | $I/I_1$ |
|---|---|
| 6.08 | 0.18 |
| 4.48 | 0.58 |
| 4.15 | 0.15 |
| 3.83 | 0.18 |
| 3.63 | 0.03 |
| 3.42 | 1.00 |
| 3.04 | 0.22 |
| 2.80 | 0.46 |
| 2.67 | 0.15 |
| 2.45 | 0.18 |
| 2.38 | 0.02 |
| 2.24 | 0.02 |
| 2.18 | 0.08 |
| 2.07 | 0.15 |
| 2.02 | 0.05 |
| 1.93 | 0.08 |
| 1.88 | 0.11 |
| 1.82 | 0.05 |
| 1.75 | 0.05 |
| 1.70 | 0.03 |
| 1.64 | 0.03 |
| 1.50 | 0.02 |
| 1.45 | 0.03 |
| 1.40 | 0.03 |
| 1.34 | 0.02 |
| 1.30 | 0.02 |
| 1.27 | 0.02 |
| 1.21 | 0.02 |

Propylamine

| $d$ | $I/I_1$ |
|---|---|
| 7.42 | 0.24 |
| 4.78 | 0.24 |
| 4.42 | 0.15 |
| 3.79 | 1.00 |
| 3.12 | 1.00 |
| 2.86 | 0.35 |
| 2.62 | 0.05 |
| 2.38 | 0.15 |
| 2.21 | 0.24 |
| 2.12 | 0.15 |
| 1.91 | 0.24 |
| 1.74 | 0.07 |

Dipropylamine

| $d$ | $I/I_1$ |
|---|---|
| 9.25 | 0.46 |
| 4.98 | 0.22 |
| 4.61 | 0.02 |
| 3.94 | 0.27 |
| 3.78 | 0.01 |
| 3.64 | 1.00 |
| 3.51 | 0.01 |
| 3.38 | 0.22 |
| 2.86 | 0.05 |
| 2.57 | 0.11 |
| 2.48 | 0.08 |
| 2.35 | 0.01 |
| 2.24 | 0.02 |
| 2.14 | 0.05 |
| 1.95 | 0.02 |
| 1.82 | 0.02 |
| 1.79 | 0.02 |
| 1.62 | 0.01 |
| 1.60 | 0.01 |

Tripropylamine

| $d$ | $I/I_1$ |
|---|---|
| 8.60 | 0.31 |
| 5.46 | 0.19 |
| 4.97 | 0.19 |
| 4.31 | 0.19 |
| 4.07 | 0.31 |
| 3.66 | 0.09 |
| 3.52 | 1.00 |
| 3.25 | 0.13 |
| 2.95 | 0.09 |
| 2.87 | 0.13 |
| 2.72 | 0.09 |
| 2.39 | 0.06 |
| 2.24 | 0.03 |
| 1.89 | 0.03 |
| 1.76 | 0.03 |
| 1.66 | 0.03 |

Isopropylamine

| $d$ | $I/I_1$ |
|---|---|
| 9.20 | 0.29 |
| 6.48 | 0.29 |
| 4.85 | 0.09 |
| 4.60 | 1.00 |
| 4.10 | 0.18 |
| 3.87 | 0.05 |
| 3.70 | 0.79 |
| 3.56 | 0.43 |
| 3.41 | 0.05 |
| 3.22 | 0.05 |
| 3.04 | 0.05 |
| 2.89 | 0.59 |
| 2.75 | 0.05 |
| 2.53 | 0.18 |
| 2.33 | 0.05 |
| 2.28 | 0.29 |
| 2.21 | 0.05 |
| 2.15 | 0.05 |
| 2.09 | 0.18 |

Diisopropylamine

| $d$ | $I/I_1$ |
|---|---|
| 7.05 | 1.00 |
| 5.25 | 0.41 |
| 5.03 | 0.41 |
| 4.18 | 0.04 |
| 4.00 | 1.00 |
| 3.84 | 0.69 |
| 3.68 | 0.06 |
| 3.46 | 0.84 |
| 3.23 | 0.84 |
| 3.01 | 0.13 |
| 2.88 | 0.20 |
| 2.77 | 0.20 |
| 2.72 | 0.13 |
| 2.52 | 0.13 |
| 2.48 | 0.20 |
| 2.37 | 0.06 |
| 2.25 | 0.06 |
| 2.18 | 0.13 |
| 2.11 | 0.06 |
| 2.01 | 0.06 |
| 1.91 | 0.06 |
| 1.84 | 0.06 |
| 1.81 | 0.04 |
| 1.77 | 0.04 |
| 1.75 | 0.06 |
| 1.65 | 0.04 |
| 1.55 | 0.04 |
| 1.34 | 0.04 |

Butylamine

| $d$ | $I/I_1$ |
|---|---|
| 14.38 | 0.59 |
| (14.98) | |
| 7.42 | 0.05 |
| 4.77 | 0.49 |
| 4.17 | 0.49 |
| 3.56 | 1.00 |
| 3.46 | 0.14 |
| 2.99 | 0.05 |
| 2.88 | 0.05 |
| 2.58 | 0.05 |
| 2.49 | 0.14 |

Dibutylamine

| $d$ | $I/I_1$ |
|---|---|
| 11.41 | 0.79 |
| (11.56) | |
| 5.71 | 0.02 |
| 5.01 | 0.54 |
| 4.27 | 0.46 |
| 3.76 | 0.02 |
| 3.63 | 1.00 |
| 3.46 | 0.38 |
| 3.06 | 0.06 |
| 2.77 | 0.02 |
| 2.62 | 0.02 |
| 2.56 | 0.09 |
| 2.49 | 0.09 |
| 2.34 | 0.02 |
| 2.19 | 0.03 |
| 2.05 | 0.02 |
| 1.80 | 0.02 |
| 1.61 | 0.02 |

Tributylamine

| $d$ | $I/I_1$ |
|---|---|
| 11.27 | 1.00 |
| (11.34) | |
| 6.74 | 0.19 |
| 5.71 | 0.19 |
| 4.38 | 0.31 |
| 3.95 | 0.63 |
| 3.69 | 0.31 |

Isobutylamine

| $d$ | $I/I_1$ |
|---|---|
| 11.75 | 0.19 |
| (11.91) | |
| 5.95 | 0.09 |
| 5.09 | 0.09 |
| 4.89 | 0.09 |
| 4.08 | 0.38 |
| 3.89 | 1.00 |
| 3.70 | 0.32 |
| 3.25 | 0.09 |
| 2.96 | 0.19 |
| 2.78 | 0.09 |
| 2.68 | 0.09 |
| 2.62 | 0.09 |
| 2.45 | 0.06 |
| 2.37 | 0.09 |
| 2.30 | 0.09 |
| 2.23 | 0.06 |
| 2.12 | 0.09 |
| 1.93 | 0.03 |
| 1.86 | 0.06 |
| 1.70 | 0.06 |
| 1.62 | 0.03 |
| 1.49 | 0.06 |

Diisobutylamine

| $d$ | $I/I_1$ |
|---|---|
| 10.06 | 1.00 |
| 5.30 | 0.09 |
| 4.98 | 0.43 |
| 4.72 | 0.43 |
| 4.26 | 0.29 |
| 4.07 | 0.29 |
| 3.75 | 1.00 |
| 3.51 | 0.59 |
| 3.37 | 0.18 |
| 3.01 | 0.29 |
| 2.74 | 0.09 |
| 2.65 | 0.09 |
| 2.56 | 0.18 |
| 2.48 | 0.18 |
| 2.41 | 0.05 |
| 2.29 | 0.09 |
| 2.07 | 0.09 |
| 1.86 | 0.09 |

*sec*-Butylamine

| $d$ | $I/I_1$ |
|---|---|
| 9.10 | 0.31 |
| 5.17 | 0.19 |
| 4.60 | 0.19 |
| 4.17 | 0.06 |
| 3.74 | 1.00 |
| 3.48 | 0.31 |
| 3.06 | 0.25 |
| 2.90 | 0.09 |
| 2.62 | 0.06 |
| 2.53 | 0.19 |
| 2.37 | 0.06 |
| 2.23 | 0.03 |
| 2.15 | 0.06 |
| 2.10 | 0.13 |
| 1.99 | 0.03 |
| 1.83 | 0.03 |
| 1.76 | 0.03 |
| 1.64 | 0.03 |
| 1.51 | 0.03 |
| 1.40 | 0.03 |

Di-*sec*-Butylamine

| $d$ | $I/I_1$ |
|---|---|
| 8.69 | 1.00 |
| 7.82 | 0.20 |
| 6.66 | 0.20 |
| 5.35 | 0.84 |
| 4.24 | 0.20 |
| 4.06 | 1.00 |
| 3.89 | 0.30 |
| 3.81 | 0.30 |
| 3.68 | 0.20 |
| 3.55 | 0.54 |
| 3.43 | 0.20 |
| 3.26 | 0.30 |
| 3.16 | 0.20 |
| 3.06 | 0.30 |
| 2.80 | 0.06 |
| 2.73 | 0.06 |
| 2.68 | 0.06 |
| 2.59 | 0.12 |
| 2.48 | 0.06 |
| 2.37 | 0.12 |
| 2.21 | 0.06 |
| 2.09 | 0.06 |

*tert*-Butylamine

| $d$ | $I/I_1$ |
|---|---|
| 9.05 | 0.21 |
| 6.40 | 0.14 |
| 5.94 | 0.14 |
| 5.17 | 0.60 |
| 4.51 | 1.00 |
| 4.37 | 1.00 |
| 4.04 | 0.29 |
| 3.92 | 0.29 |
| 3.67 | 0.49 |
| 3.31 | 0.14 |
| 3.14 | 0.21 |
| 2.99 | 0.14 |
| 2.86 | 0.39 |
| 2.74 | 0.39 |
| 2.51 | 0.39 |
| 2.42 | 0.29 |
| 2.29 | 0.09 |
| 2.18 | 0.14 |
| 2.13 | 0.04 |
| 1.77 | 0.14 |

Amylamine

| $d$ | $I/I_1$ |
|---|---|
| 15.77 | 0.84 |
| (16.67) | |
| 4.87 | 0.70 |
| 4.34 | 0.25 |
| 4.13 | 0.17 |
| 3.74 | 0.17 |
| 3.60 | 1.00 |
| 3.52 | 0.34 |
| 3.33 | 0.05 |
| 3.22 | 0.05 |
| 3.03 | 0.05 |
| 2.72 | 0.15 |
| 2.53 | 0.25 |
| 2.44 | 0.05 |
| 2.18 | 0.05 |
| 2.10 | 0.05 |

Diamylamine

| $d$ | $I/I_1$ |
|---|---|
| 13.05 | 0.84 |
| (13.38) | |
| 5.07 | 0.69 |
| 4.44 | 0.54 |
| 3.64 | 1.00 |
| 3.51 | 0.41 |
| 3.19 | 0.13 |
| 2.81 | 0.06 |
| 2.58 | 0.13 |
| 2.52 | 0.13 |
| 2.40 | 0.06 |
| 2.23 | 0.06 |
| 2.12 | 0.04 |
| 1.81 | 0.04 |

Triamylamine

| $d$ | $I/I_1$ |
|---|---|
| 12.58 | 1.00 |
| (12.85) | |
| 8.50 | 0.09 |
| 7.82 | 0.09 |
| 4.78 | 1.00 |
| 4.32 | 0.72 |
| 3.94 | 0.15 |
| 3.79 | 0.15 |
| 3.64 | 0.49 |
| 3.43 | 0.31 |
| 3.07 | 0.15 |
| 2.83 | 0.31 |
| 2.41 | 0.09 |
| 2.32 | 0.09 |

Isoamylamine

| $d$ | $I/I_1$ |
|---|---|
| 14.32 | 1.00 |
| (14.86) | |
| 7.32 | 0.07 |
| 5.37 | 0.82 |
| 5.05 | 0.15 |
| 4.56 | 0.49 |
| 4.18 | 0.65 |
| 3.86 | 0.82 |
| 3.71 | 0.35 |
| 3.56 | 1.00 |
| 3.38 | 0.82 |
| 3.11 | 0.65 |
| 2.87 | 0.82 |
| 2.61 | 0.15 |
| 2.48 | 0.15 |
| 2.32 | 0.07 |
| 2.19 | 0.07 |
| 2.13 | 0.35 |
| 1.87 | 0.07 |
| 1.70 | 0.15 |

Diisoamylamine

| $d$ | $I/I_1$ |
|---|---|
| 12.42 | 0.64 |
| (12.63) | |
| 5.22 | 0.31 |
| 4.86 | 0.31 |
| 4.58 | 0.06 |
| 4.40 | 0.38 |
| 4.20 | 0.06 |
| 3.89 | 0.19 |
| 3.62 | 1.00 |
| 3.47 | 0.06 |
| 3.33 | 0.25 |
| 3.16 | 0.06 |
| 2.97 | 0.09 |
| 2.75 | 0.02 |
| 2.66 | 0.19 |
| 2.61 | 0.09 |
| 2.45 | 0.06 |
| 2.24 | 0.09 |
| 2.04 | 0.06 |
| 1.81 | 0.03 |
| 1.67 | 0.06 |

Triisoamylamine

| $d$ | $I/I_1$ |
|---|---|
| 11.94 | 1.00 |
| (12.14) | |
| 9.64 | 0.29 |
| 8.00 | 0.14 |
| 7.01 | 0.09 |
| 6.01 | 0.49 |
| 5.53 | 0.14 |
| 4.96 | 1.00 |
| 4.48 | 0.29 |
| 4.35 | 1.00 |
| 3.98 | 0.29 |
| 3.78 | 0.04 |
| 3.66 | 0.21 |
| 3.51 | 0.49 |
| 3.35 | 0.21 |
| 3.19 | 0.14 |
| 3.09 | 0.49 |
| 3.00 | 0.39 |
| 2.91 | 0.14 |
| 2.82 | 0.09 |
| 2.71 | 0.14 |
| 2.62 | 0.04 |
| 2.55 | 0.21 |
| 2.44 | 0.09 |
| 2.32 | 0.09 |
| 2.23 | 0.09 |
| 2.13 | 0.04 |
| 1.75 | 0.09 |
| 1.69 | 0.09 |
| 1.65 | 0.04 |
| 1.42 | 0.09 |

**Table II** (*Continued*)

*sec*-Amylamine

| *d* | $I/I$ |
|---|---|
| 10.57 | 0.79 |
| 7.55 | 0.09 |
| 5.01 | 0.18 |
| 4.75 | 0.09 |
| 3.76 | 1.00 |
| 3.63 | 0.38 |
| 3.52 | 0.09 |
| 2.95 | 0.13 |
| 2.65 | 0.13 |
| 2.50 | 0.03 |
| 2.32 | 0.09 |
| 2.14 | 0.13 |
| 2.08 | 0.03 |
| 1.99 | 0.03 |
| 1.67 | 0.03 |
| 1.59 | 0.03 |

*tert*-Amylamine

| *d* | $I/I$ |
|---|---|
| 13.58 | 0.04 |
| (13.92) | |
| 11.34 | 0.09 |
| (11.51) | |
| 9.38 | 0.29 |
| 6.92 | 0.21 |
| 6.39 | 0.04 |
| 5.20 | 1.00 |
| 4.50 | 0.14 |
| 4.17 | 0.09 |
| 3.98 | 0.09 |
| 3.81 | 0.71 |
| 3.62 | 0.71 |
| 3.39 | 0.04 |
| 3.23 | 0.04 |
| 3.07 | 0.04 |
| 2.95 | 0.14 |
| 2.88 | 0.14 |
| 2.67 | 0.21 |
| 2.57 | 0.04 |
| 2.47 | 0.09 |
| 2.36 | 0.04 |
| 2.24 | 0.09 |
| 2.13 | 0.09 |
| 1.79 | 0.09 |

Hexylamine

| *d* | $I/I$ |
|---|---|
| 19.31 | 1.00 |
| 4.86 | 0.84 |
| 4.46 | 0.13 |
| 3.97 | 0.41 |
| 3.52 | 1.00 |
| 3.23 | 0.04 |
| 3.06 | 0.04 |
| 2.86 | 0.04 |
| 2.60 | 0.06 |
| 2.49 | 0.20 |
| 2.40 | 0.06 |
| 2.18 | 0.04 |
| 2.11 | 0.06 |

Heptylamine

| *d* | $I/I$ |
|---|---|
| 21.80 | 1.00 |
| 7.24 | 0.02 |
| 5.43 | 0.12 |
| 4.96 | 0.32 |
| 4.73 | 0.24 |
| 4.34 | 0.24 |
| 3.89 | 0.24 |
| 3.66 | 0.02 |
| 3.50 | 1.00 |
| 3.37 | 0.02 |
| 3.09 | 0.04 |
| 2.97 | 0.02 |
| 2.71 | 0.02 |
| 2.48 | 0.17 |
| 2.16 | 0.02 |
| 2.09 | 0.02 |

Octylamine

| *d* | $I/I$ |
|---|---|
| 25.32 | 1.00 |
| 8.50 | 0.20 |
| 6.37 | 0.13 |
| 4.72 | 0.54 |
| 4.48 | 0.54 |
| 4.21 | 0.84 |
| 3.95 | 0.06 |
| 3.49 | 0.04 |
| 3.34 | 1.00 |
| 2.56 | 0.06 |
| 2.35 | 0.20 |
| 1.66 | 0.06 |
| 1.50 | 0.06 |

1,1,3,3-Tetramethylbutylamine

| *d* | $I/I_1$ |
|---|---|
| 13.58 | 0.84 |
| (13.92) | |
| 9.10 | 0.20 |
| 7.89 | 0.06 |
| 6.99 | 0.06 |
| 6.43 | 0.13 |
| 5.88 | 1.00 |
| 4.55 | 1.00 |
| 3.46 | 0.41 |
| 3.21 | 0.20 |
| 3.01 | 0.20 |
| 2.88 | 0.41 |
| 2.81 | 0.13 |
| 2.52 | 0.20 |
| 2.27 | 0.04 |
| 1.80 | 0.06 |

Ethylenediamine

| *d* | $I/I_1$ |
|---|---|
| 3.72 | 0.13 |
| 3.48 | 0.69 |
| 3.35 | 0.54 |
| 3.26 | 0.54 |
| 2.99 | 1.00 |
| 2.84 | 0.04 |
| 2.72 | 0.20 |
| 2.63 | 0.41 |
| 2.51 | 0.06 |
| 2.46 | 0.13 |
| 2.41 | 0.06 |
| 2.35 | 0.20 |
| 2.22 | 0.04 |
| 2.15 | 0.04 |
| 2.09 | 0.20 |
| 2.05 | 0.13 |
| 1.88 | 0.20 |
| 1.78 | 0.20 |
| 1.70 | 0.13 |
| 1.64 | 0.13 |
| 1.60 | 0.29 |
| 1.50 | 0.06 |

1,3-Propanediamine

| *d* | $I/I_1$ |
|---|---|
| 5.27 | 0.18 |
| 4.72 | 0.09 |
| 4.21 | 0.79 |
| 3.80 | 0.79 |
| 3.62 | 1.00 |
| 3.33 | 0.43 |
| 3.12 | 0.43 |
| 3.02 | 0.18 |
| 2.93 | 0.29 |
| 2.84 | 0.18 |
| 2.62 | 0.29 |
| 2.50 | 0.18 |
| 2.45 | 0.29 |
| 2.37 | 0.29 |
| 2.29 | 0.09 |
| 2.20 | 0.18 |
| 1.96 | 0.05 |
| 1.82 | 0.09 |
| 1.73 | 0.18 |

1,3-Butanediamine

| *d* | $I/I_1$ |
|---|---|
| 9.29 | 0.37 |
| 6.85 | 0.07 |
| 5.53 | 0.12 |
| 4.98 | 0.37 |
| 4.54 | 0.54 |
| 3.81 | 0.54 |
| 3.67 | 0.75 |
| 3.51 | 0.37 |
| 3.34 | 0.23 |
| 3.12 | 1.00 |
| 2.99 | 1.00 |
| 2.87 | 0.23 |
| 2.78 | 0.37 |
| 2.55 | 0.12 |
| 2.34 | 0.23 |
| 2.29 | 0.23 |
| 2.24 | 0.23 |
| 2.16 | 0.23 |
| 2.01 | 0.12 |
| 1.85 | 0.12 |
| 1.77 | 0.12 |
| 1.69 | 0.23 |
| 1.62 | 0.12 |
| 1.55 | 0.07 |
| 1.46 | 0.12 |
| 1.43 | 0.12 |

1,4-Butanediamine

| *d* | $I/I_1$ |
|---|---|
| 6.61 | 0.09 |
| 4.55 | 0.09 |
| 4.03 | 0.43 |
| 3.89 | 0.43 |
| 3.72 | 0.59 |
| 3.57 | 0.43 |
| 3.29 | 1.00 |
| 3.17 | 0.59 |
| 2.99 | 0.59 |
| 2.69 | 0.29 |
| 2.55 | 0.18 |
| 2.47 | 0.29 |
| 2.38 | 0.59 |
| 2.31 | 0.18 |
| 2.20 | 0.18 |
| 2.15 | 0.18 |
| 2.08 | 0.09 |
| 2.00 | 0.09 |
| 1.93 | 0.06 |
| 1.79 | 0.18 |
| 1.75 | 0.06 |
| 1.70 | 0.06 |
| 1.65 | 0.06 |
| 1.50 | 0.09 |
| 1.41 | 0.06 |

1,6-Hexanediamine

| *d* | $I/I_1$ |
|---|---|
| 10.50 | 0.21 |
| 4.62 | 0.09 |
| 4.39 | 0.04 |
| 4.21 | 0.29 |
| 3.97 | 0.21 |
| 3.80 | 0.14 |
| 3.47 | 1.00 |
| 3.32 | 0.09 |
| 3.24 | 0.09 |
| 3.12 | 0.21 |
| 3.05 | 0.14 |
| 2.78 | 0.14 |
| 2.63 | 0.09 |
| 2.54 | 0.09 |
| 2.42 | 0.04 |
| 2.29 | 0.09 |
| 2.19 | 0.09 |
| 1.96 | 0.04 |
| 1.92 | 0.09 |
| 1.74 | 0.04 |
| 1.65 | 0.09 |

Aniline

| *d* | $I/I_1$ |
|---|---|
| 7.83 | 0.17 |
| 4.45 | 0.07 |
| 4.24 | 1.00 |
| 4.06 | 0.37 |
| 3.90 | 0.07 |
| 3.60 | 0.17 |
| 3.48 | 0.07 |
| 3.35 | 0.17 |
| 3.26 | 0.17 |
| 3.16 | 0.17 |
| 3.02 | 0.07 |
| 2.70 | 0.07 |
| 2.60 | 0.07 |
| 2.53 | 0.37 |
| 2.42 | 0.07 |
| 2.22 | 0.04 |
| 2.14 | 0.04 |
| 2.05 | 0.07 |
| 1.93 | 0.07 |
| 1.87 | 0.04 |
| 1.82 | 0.07 |
| 1.73 | 0.04 |
| 1.66 | 0.04 |
| 1.63 | 0.07 |
| 1.55 | 0.04 |

Diphenylamine

| *d* | $I/I_1$ |
|---|---|
| 8.10 | 0.05 |
| 7.31 | 0.17 |
| 6.55 | 0.17 |
| 5.82 | 0.17 |
| 5.47 | 0.25 |
| 5.12 | 0.17 |
| 4.53 | 0.11 |
| 4.12 | 1.00 |
| 3.94 | 0.84 |
| 3.65 | 0.34 |
| 3.49 | 0.25 |
| 3.36 | 0.11 |
| 3.20 | 0.34 |
| 3.05 | 0.11 |
| 2.91 | 0.17 |
| 2.74 | 0.25 |
| 2.60 | 0.05 |
| 2.46 | 0.05 |
| 2.36 | 0.11 |
| 2.28 | 0.17 |
| 2.19 | 0.01 |
| 2.10 | 0.05 |
| 2.01 | 0.01 |
| 1.93 | 0.01 |
| 1.83 | 0.05 |
| 1.69 | 0.01 |

Benzylamine

| *d* | $I/I_1$ |
|---|---|
| 14.14 | 0.54 |
| (14.75) | |
| 5.68 | 0.84 |
| 5.25 | 0.29 |
| 4.84 | 0.20 |
| 4.30 | 1.00 |
| 4.08 | 0.29 |
| 3.82 | 0.06 |
| 3.54 | 0.41 |
| 3.38 | 0.41 |
| 3.25 | 1.00 |
| 3.15 | 0.20 |
| 2.94 | 0.54 |
| 2.83 | 0.54 |
| 2.62 | 0.06 |
| 2.45 | 0.13 |
| 2.27 | 0.06 |
| 2.08 | 0.41 |
| 1.99 | 0.06 |
| 1.90 | 0.06 |
| 1.77 | 0.13 |
| 1.73 | 0.13 |
| 1.69 | 0.04 |
| 1.47 | 0.13 |

Dibenzylamine

| *d* | $I/I_1$ |
|---|---|
| 14.26 | 0.25 |
| (14.93) | |
| 11.97 | 0.70 |
| (12.25) | |
| 5.69 | 0.17 |
| 5.18 | 1.00 |
| 4.79 | 0.03 |
| 4.51 | 1.00 |
| 4.28 | 0.58 |
| 4.00 | 0.70 |
| 3.60 | 0.70 |
| 3.51 | 0.70 |
| 3.31 | 0.70 |
| 3.13 | 0.70 |
| 2.94 | 0.17 |
| 2.82 | 0.17 |
| 2.58 | 0.34 |
| 2.46 | 0.11 |
| 2.16 | 0.03 |
| 2.09 | 0.17 |
| 2.00 | 0.17 |
| 1.91 | 0.03 |
| 1.83 | 0.17 |

Tribenzylamine

| *d* | $I/I_1$ |
|---|---|
| 7.80 | 1.00 |
| 6.05 | 0.85 |
| 4.79 | 1.00 |
| 4.51 | 0.49 |
| 4.08 | 0.49 |
| 3.89 | 1.00 |
| 3.29 | 0.85 |
| 3.16 | 0.03 |
| 3.03 | 0.39 |
| 2.95 | 0.29 |
| 2.82 | 0.39 |
| 2.61 | 0.21 |
| 2.52 | 0.21 |
| 2.39 | 0.21 |
| 2.27 | 0.21 |
| 2.17 | 0.14 |
| 2.11 | 0.04 |
| 2.02 | 0.04 |
| 1.97 | 0.29 |
| 1.86 | 0.29 |
| 1.80 | 0.14 |
| 1.71 | 0.04 |
| 1.68 | 0.09 |
| 1.65 | 0.03 |
| 1.61 | 0.03 |
| 1.48 | 0.03 |
| 1.41 | 0.03 |

Cyclohexylamine

| *d* | $I/I_1$ |
|---|---|
| 11.41 | 0.13 |
| (11.51) | |
| 7.24 | 0.09 |
| 5.72 | 0.13 |
| 5.22 | 0.64 |
| 4.92 | 0.19 |
| 4.67 | 0.38 |
| 4.32 | 0.09 |
| 4.10 | 0.64 |
| 3.94 | 0.03 |
| 3.78 | 1.00 |
| 3.59 | 0.09 |
| 3.35 | 0.19 |
| 2.97 | 0.31 |
| 2.79 | 0.25 |
| 2.69 | 0.09 |
| 2.58 | 0.13 |
| 2.43 | 0.03 |
| 2.33 | 0.19 |
| 2.22 | 0.19 |

Cyclohexylamine (*Contd.*)

| *d* | $I/I_1$ |
|---|---|
| 2.13 | 0.03 |
| 2.05 | 0.06 |
| 1.92 | 0.03 |
| 1.85 | 0.06 |
| 1.79 | 0.03 |
| 1.75 | 0.06 |
| 1.66 | 0.03 |
| 1.60 | 0.03 |
| 1.49 | 0.03 |
| 1.44 | 0.03 |

Dicyclohexylamine

| *d* | $I/I_1$ |
|---|---|
| 10.04 | 0.54 |
| 5.66 | 0.29 |
| 5.37 | 0.04 |
| 4.90 | 0.54 |
| 4.60 | 0.20 |
| 4.42 | 0.13 |
| 3.97 | 1.00 |
| 3.80 | 0.41 |
| 3.65 | 0.04 |
| 3.53 | 0.06 |
| 3.35 | 0.04 |
| 3.18 | 0.20 |
| 2.98 | 0.20 |
| 2.64 | 0.20 |
| 2.57 | 0.13 |
| 2.46 | 0.04 |
| 2.32 | 0.04 |
| 2.23 | 0.06 |
| 1.88 | 0.04 |
| 1.77 | 0.06 |

Morpholine

| *d* | $I/I_1$ |
|---|---|
| 5.34 | 0.15 |
| 4.74 | 1.00 |
| 4.20 | 1.00 |
| 3.78 | 0.82 |
| 3.62 | 0.03 |
| 3.45 | 1.00 |
| 3.29 | 0.24 |
| 3.01 | 0.15 |
| 2.83 | 1.00 |
| 2.70 | 0.24 |
| 2.60 | 0.15 |
| 2.46 | 0.35 |
| 2.34 | 0.24 |
| 2.20 | 0.15 |
| 2.14 | 0.15 |
| 2.08 | 0.03 |
| 1.93 | 0.07 |
| 1.88 | 0.15 |
| 1.80 | 0.15 |
| 1.70 | 0.15 |
| 1.67 | 0.24 |

Piperazine

| *d* | $I/I_1$ |
|---|---|
| 5.32 | 1.00 |
| 4.62 | 0.52 |
| 3.81 | 0.79 |
| 3.65 | 0.79 |
| 3.42 | 0.22 |
| 3.17 | 1.00 |
| 3.09 | 0.35 |
| 2.97 | 1.00 |
| 2.88 | 0.22 |
| 2.68 | 0.35 |
| 2.58 | 1.00 |
| 2.51 | 0.22 |
| 2.45 | 0.22 |
| 2.32 | 0.52 |
| 2.23 | 0.11 |
| 2.09 | 0.22 |
| 2.02 | 0.22 |
| 1.91 | 0.11 |
| 1.78 | 0.22 |
| 1.71 | 0.11 |
| 1.67 | 0.11 |
| 1.62 | 0.11 |
| 1.41 | 0.11 |

Piperidine

| *d* | $I/I_1$ |
|---|---|
| 9.91 | 0.15 |
| 4.92 | 1.00 |
| 4.39 | 0.15 |
| 4.08 | 0.15 |
| 3.70 | 0.84 |
| 3.47 | 0.17 |
| 3.30 | 0.17 |
| 3.15 | 0.15 |
| 2.96 | 0.34 |
| 2.86 | 0.48 |
| 2.73 | 0.17 |
| 2.54 | 0.05 |
| 2.46 | 0.17 |
| 2.38 | 0.05 |
| 2.26 | 0.03 |
| 2.20 | 0.17 |
| 2.14 | 0.03 |

Piperidine (*Contd.*)

| *d* | $I/I_1$ |
|---|---|
| 2.00 | 0.05 |
| 1.86 | 0.03 |
| 1.82 | 0.03 |
| 1.74 | 0.15 |
| 1.71 | 0.15 |
| 1.64 | 0.15 |

Pyridine

| *d* | $I/I_1$ |
|---|---|
| 7.70 | 0.23 |
| 5.56 | 0.12 |
| 4.07 | 0.37 |
| 3.83 | 0.23 |
| 3.62 | 1.00 |
| 3.47 | 0.07 |
| 3.11 | 0.12 |
| 2.98 | 0.12 |
| 2.80 | 0.07 |
| 2.56 | 0.12 |
| 2.44 | 0.07 |
| 2.23 | 0.07 |

*o*-Toluidine

| *d* | $I/I_1$ |
|---|---|
| 7.99 | 0.09 |
| 5.87 | 0.59 |
| 5.43 | 0.18 |
| 5.12 | 0.43 |
| 4.45 | 0.79 |
| 3.96 | 0.05 |
| 3.83 | 1.00 |
| 3.61 | 0.18 |
| 3.46 | 0.79 |
| 3.33 | 1.00 |
| 3.13 | 0.29 |
| 2.94 | 0.18 |
| 2.86 | 0.18 |
| 2.72 | 0.18 |
| 2.60 | 0.18 |
| 2.54 | 0.79 |
| 2.41 | 0.05 |
| 2.30 | 0.29 |
| 2.22 | 0.09 |
| 2.17 | 0.05 |
| 2.10 | 0.05 |
| 1.99 | 0.05 |
| 1.87 | 0.05 |
| 1.86 | 0.05 |
| 1.80 | 0.05 |
| 1.70 | 0.09 |
| 1.67 | 0.05 |
| 1.64 | 0.09 |

*m*-Toluidine

| *d* | $I/I_1$ |
|---|---|
| 12.42 | 0.58 |
| (12.45) | |
| 6.74 | 0.46 |
| 6.22 | 0.01 |
| 4.93 | 0.11 |
| 4.17 | 0.17 |
| 3.98 | 0.11 |
| 3.79 | 0.17 |
| 3.57 | 1.00 |
| 3.37 | 0.25 |
| 3.30 | 0.05 |
| 2.91 | 0.25 |
| 2.81 | 0.34 |
| 2.69 | 0.05 |
| 2.52 | 0.11 |
| 2.46 | 0.17 |
| 2.24 | 0.05 |
| 2.00 | 0.11 |
| 1.78 | 0.11 |

*p*-Toluidine

| *d* | $I/I_1$ |
|---|---|
| 7.29 | 0.07 |
| 6.40 | 0.54 |
| 5.95 | 0.07 |
| 4.79 | 0.75 |
| 4.53 | 0.07 |
| 4.29 | 0.75 |
| 4.02 | 1.00 |
| 3.86 | 0.23 |
| 3.54 | 0.37 |
| 3.32 | 0.54 |
| 3.12 | 0.54 |
| 2.92 | 0.23 |
| 2.79 | 0.23 |
| 2.73 | 0.23 |
| 2.61 | 0.23 |
| 2.56 | 0.12 |
| 2.47 | 0.07 |
| 2.41 | 0.07 |
| 2.34 | 0.07 |
| 2.27 | 0.12 |
| 2.12 | 0.07 |
| 1.83 | 0.12 |

occurs when the hydrochloride is not completely dry. *tert*-Amylamine hydrochloride likewise produces two slightly different diffraction diagrams. In each case the interplanar spacings (Table II) are given for the anhydrous form which is prepared by drying at 110° C.

The problem of encountering polymorphic forms can be eliminated by crystallizing all products from the same solvent. Alcohol is generally used. However, crystallization from chloroform removes interference which may be due to ammonia and the formation of ammonium chloride, as this salt is insoluble in chloroform.

It will be noted (Table II) that two values are given for spacings longer than 11 A. The first value is obtained following the experimental procedure given above. The second value (in parenthesis) is the more accurate of the two and has been determined by replacing the exit tube of the camera with a small cork. Because of the design of the entrance and exit tubes used in the x-ray camera, there is a small blind area around the 0° and 180° film holes as described by Parrish and Cisney (*11*). This results in an error in measuring the *d* values in these regions. The use of the cork permits the beams diffracted at low angles to be recorded unaffected, although more air scattering occurs.

**Table III. Long Spacings of Alkylamine Hydrochlorides**

| Primary Amines | | Secondary Amines | | Tertiary Amines | |
|---|---|---|---|---|---|
| Amine HCl | *d* | Amine HCl | *d* | Amine HCl | *d* |
| Methyl | 5.06 | Dimethyl | 4.98 | Trimethyl | 5.95 |
| Ethyl | 8.26 | Diethyl | 6.54 | Triethyl | 7.29 |
| Propyl | 7.42 | Dipropyl | 9.25 | Tripropyl | 8.65 |
| Butyl | 14.98 | Dibutyl | 11.56 | Tributyl | 11.34 |
| Amyl | 16.67 | Diamyl | 13.38 | Triamyl | 12.86 |
| Hexyl | 19.31 | | | | |
| Heptyl | 21.80 | | | | |
| Octyl | 25.32 | | | | |
| Isopropyl | 9.20 | Diisopropyl | 7.05 | | |
| Isobutyl | 11.91 | Diisobutyl | 10.10 | | |
| Isoamyl | 14.86 | Diisoamyl | 12.63 | | |

The long spacings for hexylamine, heptylamine, and octylamine were determined on a flat cassette-type long spacing camera, as these spacings were too long to be recorded with the Philips equipment.

The intensities of many of the long spacings are estimated, because this spacing usually shows some degree of preferred orientation. Long spacing measurements, made with the equipment described, are not as accurate as measurements of diffraction maxima occurring at wider angles.

The long spacings of some of the alkylamine hydrochlorides have been tabulated in Table III. In general, the long spacing increases with chain length within the classes of normal primary, secondary, and tertiary amines and for primary and secondary isoalkylamines.

In identifying an unknown amine hydrochloride the interplanar spacings and relative intensities given in Table II can be used. However, it is usually more satisfactory to identify the unknown by a direct comparison with a set of standard films or charts. For rapid identification one simply has to measure the longest spacing, which in many cases is one of the most intense. The appropriate standards with similar long spacings can then be selected from the file of diagrams of known amine hydrochlorides. Comparison with these standards will give a positive identification.

Binary mixtures of amines can be readily identified provided the minor component is present in sufficient quantity to give a diffraction pattern. In some cases even ternary mixtures are identifiable.

## APPLICATIONS

The method described should be of general use in the qualitative identification of organic amines.

It has particular utility in the identification of aliphatic amines separated in micro quantities from fabricated products. The procedure has been used by Brock and Louth (*1*) in the identification of amines which are used in compounding rubber, latex products, and rubber cements. Rubber samples containing as little as 5 mg. of cyclohexylamine have been analyzed and the amine successfully identified by the procedure described. Once the amines are identified, they can be determined quantitatively by the nonaqueous titration method of Pifer and Wollish (*13*). The quantitative determination of amines in fabricated products such as rubber vulcanizates, however, is dependent upon the efficiency of the extraction and separation procedures used.

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# Analytical Chemistry of Titanium Alloys

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**The rapid growth of the titanium industry has resulted in many new methods for the analysis of titanium alloys. This paper is written to correlate these methods. A general survey of the analytical chemistry of titanium alloys is made including methods of dissolving the sample, complexation, separations, and application of instrumental methods of analysis. A survey of the methods used for the determination of many elements in titanium and titanium alloys is included. The elements covered are aluminum, boron, calcium, carbon, chloride, chromium, cobalt, copper, hydrogen, iron, magnesium, manganese, molybdenum, nickel, niobium, nitrogen, oxygen, phosphorus, silicon, silver, sulfur, tantalum, tin, titanium, tungsten, vanadium, and zirconium. Sampling procedures are discussed.**

IT IS the purpose of this paper to discuss the analytical chemistry of titanium alloys and correlate the many new methods that have been published recently on the analysis of titanium and titanium alloys.

## METHODS OF SOLUTION

Of great importance in the study of an alloy are the methods that can be used to bring the alloy into solution. The most rapid solvent for titanium and its alloys is hydrofluoric acid, a mixture of hydrofluoric and nitric acids, a mixture of hydrofluoric and sulfuric acids, or a mixture of hydrofluoric and hydrochloric acids. By the application of heat, titanium and titanium alloys dissolve fairly readily in hydrochloric acid (over the range of about $12N$ to $3N$), sulfuric acid (over the range of about $18N$ to $3N$), phosphoric acid (concentrated), and fluoboric acid. Phosphoric acid is not recommended as a solvent, because a mass of titanium phosphate precipitates on dilution with water. Nitric acid, a mixture of nitric and hydrochloric acids, or a mixture of nitric and sulfuric acids hardly attacks titanium or titanium alloys because of the passivating action of the nitric acid. Hydrogen peroxide will hinder attack of titanium and its alloys by sulfuric, hydrochloric, or phosphoric acids. Sulfurous acid and organic acids (acetic, oxalic, formic, tartaric, or citric) have little action on titanium and its alloys. The same is true of ammonium hydroxide or sodium hydroxide. Perchloric acid is not a very useful solvent for titanium or titanium alloys because of the precipitation of metatitanic acid. Fusion with bisulfate alone attacks titanium and titanium alloys very slowly. A 0.2-gram sample can be dissolved using 15 to 25 grams of potassium bisulfate and 10 drops of sulfuric acid (*61*).

A fused mixture of sodium carbonate and sodium nitrate attacks titanium and its alloys with sputtering and the production of sparks. Titanium, or titanium alloys, will not be attacked by mercury or mercury salts (a method of attack used for aluminum alloys). Titanium and its alloys are not attacked by chlorine, bromine, chlorine water, or bromine water at room temperature. However, if the metal is heated to red heat in a tube, chlorine or bromine will readily attack it. Dry hydrogen chloride gas will also attack titanium and its alloys under these conditions. Titanium powder was analyzed by first igniting it in air and then fusing the resultant titanium dioxide with sodium carbonate (*12*).

In dissolving titanium or titanium alloys consideration must be given to the fact that under certain conditions the loss of certain elements is possible. Titanium itself may volatilize slightly from a hydrofluoric acid solution if sulfuric acid is not present (*38*). Phosphorus may be lost as phosphine and sulfur may be lost as hydrogen sulfide unless an oxidizing attack is used. In determining silicon in titanium or titanium alloys there does not seem to be any danger of losing silicon as silane when the sample is dissolved in a nonoxidizing acid. In this respect titanium and its alloys resemble steels and differ from aluminum alloys.

## COMPLEXATION

Titanium shows a strong tendency to hydrolyze unless a moderate amount of acid or a complexing agent such as fluoride, citrate, or tartrate is present. For complexing titanium, citrate is preferred to tartrate because of the danger of precipitating insoluble titanium tartrate. Titanium forms peculiar complexes with some metals. For instance, it is impossible to hydrolyze niobium and tantalum from a solution containing a larger amount of titanium, because of the complexes between titanium and niobium, and titanium and tantalum (*54, 58*). Tungsten cannot be separated from large amounts of titanium by cinchonine because of a complex between titanium and tungsten (*3, 14*). Calcium will not precipitate from an alcoholic solution if large amounts of titanium are present (*16*).

## SEPARATIONS

Methods of separations are important in the analysis of an alloy. A most useful separation of titanium from such elements as aluminum, magnesium, and calcium is a cupferron precipitation, or a cupferron precipitation followed by a chloroform extraction. A review paper has appeared which contains much information applicable to the use of cupferron for separations in the analysis of titanium alloys (*34*). Sulfide separations are very useful for titanium alloys. Copper, tin, molybdenum, and silver can be separated from titanium by a sulfide precipitation from an acidic medium, while iron, cobalt, and nickel can be separated from titanium by a sulfide precipitation in an alkaline citrate medium. A sodium hydroxide precipitation has been used to separate titanium from chromium, molybdenum, tungsten, and vanadium (*61*). According to investigations in this laboratory ammoniacal precipitations are not successful in the separation of boron, calcium, molybdenum, or tungsten from large amounts of titanium.

Extraction techniques are useful for the analysis of titanium and its alloys. Extraction of the cupferron complex of titanium with chloroform has already been mentioned. Molybdenum (*21*) and iron (*51*) can be determined in titanium and titanium alloys after extraction of the chloride complexes with ether. Molybdenum and tungsten can be separated from titanium by extracting the dithiol complexes with amyl acetate (*59*). Molybdenum can be separated from titanium by extracting the thiocyanate complex with butyl acetate (*64*).

The usual distillation methods have been used in the determination of such elements as boron, sulfur, and nitrogen in titanium and titanium alloys. Distillation of tin from a hydrobromic or hydrochloric acid solution with or without sulfuric or perchloric acid was not too successful in this laboratory when 0.5 gram or more of titanium was present. It is not feasible to volatilize chromium as chromyl chloride from a fuming perchloric acid solution when a large amount of titanium is present.

Paper chromatographic separations have been used in the separation of tin, iron, and molybdenum from titanium prior to the determination of these elements (*41*). Ion exchange has been used to separate titanium from boron in the determination of boron in titanium and titanium alloys (*50*).

## USE OF INSTRUMENTAL METHODS OF ANALYSIS

Instrumental methods are being much used in the analysis of titanium and titanium alloys. Colorimetric methods for many elements have been developed. Potentiometric methods are regularly used for the determination of chromium and vanadium in titanium and titanium alloys. Polarographic methods have been proposed for the determination in titanium and titanium alloys of molybdenum (*21*), aluminum (*44*), cobalt, copper, nickel, manganese, and chromium (*43*), and chromium and iron (*33*). An amperometric method for determining vanadium in titanium has been proposed (*33*). Spectrographic methods are frequently used for the analysis of titanium and its alloys (*1, 5, 8, 36, 55, 66*). Fluorescence methods have not been employed for the analysis of titanium and titanium alloys. X-ray fluorescence has been suggested as a means for analyzing titanium alloys (*9*), but it is not known to the present authors whether the method is being used industrially.

As far as is known, the flame photometer has not been applied to the analysis of titanium and titanium alloys. According to investigations in this laboratory, the direct determination of calcium and magnesium in titanium or titanium alloys by means of the flame photometer is not feasible. Neutron activation methods for the determination of chloride, tungsten, oxygen, and other elements in titanium and titanium alloys have been suggested (*5*). However, the methods have not proved useful industrially, because they require the use of a high neutron source (pile). A mass spectrometer method for the determination of oxygen in titanium alloys has been suggested (*6, 40*). In this method a known amount of oxygen-18 is added to the metal, and the new ratio of oxygen-18 to oxygen-16 determined by the mass spectrometer, after extraction of most of the oxygen as carbon monoxide by a vacuum fusion procedure. The method is of great interest, but has not been used industrially. Electron diffraction techniques have been used to study oxide coatings on titanium and its alloys (*37*), and to study the effect of polishing operations (*10*). X-ray diffraction techniques have been used to study transformation kinetics of titanium systems (*11*).

## DETERMINATION OF ELEMENTS FOUND IN TITANIUM AND TITANIUM ALLOYS

**Aluminum.** Aluminum has been determined gravimetrically after a cupferron precipitation and chloroform extraction, using ammonia (*63*) or 8-quinolinol (*31, 35, 57, 64*) for the final gravimetric separation. It has been determined colorimetrically with aluminon after a cupferron precipitation (*22*), or a cupferron precipitation and a chloroform extraction (*30*). The most reliable and accurate method for the determination of small amounts of aluminum in titanium or titanium alloys is the polarographic procedure (*44*). In this method the aluminum is separated from the titanium by a cupferron precipitation, or a cupferron precipitation and chloroform extraction, eriochrome violet dye is added, and the solution polarographed. The wave which is due to the reduction of the aluminum–azo dye complex occurs at −0.44 volt *vs.* S.C.E. The method is applicable to the range of 0.005 to 10% aluminum. The 8-quinolinol gravimetric method following cupferron precipitation and chloroform extraction is recommended by the Panel on Methods of Analysis of the Metallurgical Advisory Committee on Titanium.

**Boron.** Boron has been determined in titanium and titanium alloys colorimetrically with dianthramide after prior distillation of the boron as methyl borate (*23*). It has also been determined volumetrically after separation of the titanium by ion exchange (*50*). The small amount of titanium not removed by ion exchange is eliminated by a calcium carbonate precipitation (*50*).

**Calcium.** Calcium has been determined in titanium by precipitation as calcium sulfate from a methanol medium after a prior separation of the titanium by a cupferron precipitation and a chloroform extraction (*16*). Another proposed method is to precipitate the calcium and magnesium together as the mixed phosphates, and then separate the calcium from the magnesium by the use of an alcohol medium (*64*).

**Carbon.** Carbon is usually determined in titanium and titanium alloys by combustion in oxygen (*57, 63, 64*). Various combustion methods are being investigated by the Panel on Methods of Analysis. According to investigations in this laboratory complete combustion of the titanium and carbon will be obtained by using granulated lead as a flux. A colorimetric method applicable to the range of 0.02 to 0.7% carbon in titanium metal has been proposed (*26*). In this method the sample is dissolved in fluoboric acid, and nitric acid is added. The yellow nitrated organic complex is then measured with a spectrophotometer.

**Chloride.** Chloride is ordinarily found only in sponge titanium, a form of titanium manufactured by reacting magnesium with titanium tetrachloride. Chloride in titanium is determined by precipitation as silver chloride and weighing as such (*62, 63*). It can also be determined colorimetrically by dissolving the silver chloride precipitate in ammonium hydroxide and adding sodium sulfide to produce an amber color (*19*).

**Chromium.** Chromium in titanium and titanium alloys has been determined by volumetric and potentiometric means after oxidation to the sexivalent state (*57, 63*). The oxidation is best carried out in a moderately strong sulfuric acid medium, using ammonium persulfate and potassium permanganate in the presence of silver nitrate. A correction must be made for vanadium in the potentiometric method if this element is present. The hot perchloric acid oxidation method seems to give fairly reliable results for chromium in titanium and titanium alloys, even though the titanium is precipitated during the fuming. Chromium in titanium and titanium alloys has been determined colorimetrically with diphenylcarbazide (*48*). Vanadium, molybdenum, and tungsten do not interfere with this colorimetric procedure. Chromium after oxidation to the sexivalent state has been determined polarographically in titanium and titanium alloys, using an alkaline (*44*), or alkaline cyanide medium (*33*). The volumetric method and the diphenylcarbazide colorimetric method are recommended by the Panel on Methods of Analysis.

**Cobalt.** Cobalt has been determined gravimetrically with 1-nitroso 2-naphthol after first separating the cobalt by a sulfide precipitation in an alkaline citrate medium (*31*). Cobalt has been determined directly in titanium and titanium alloys by nitroso-R salt in the presence of citric acid with the addition of nitric acid, if iron is present (*31*). A polarographic procedure has been proposed for determining cobalt, together with copper and nickel, by use of a pyridine–pyridinium chloride solution after hydrolysis of most of the titanium from a perchloric acid solution (*43*).

**Copper.** Copper in titanium and titanium alloys can be determined by electrolysis after dissolving the sample in a mixture of fluoboric and nitric acids (*4*). A prior separation of the copper with hydrogen sulfide from an acidic citrate solution is sometimes desirable (*31*). A direct method for copper in titanium, using dithiocarbamate in an ammoniacal citrate medium, has been suggested (*31*). Copper has been determined polarographically from a pyridine–pyridinium chloride solution (*43*). Neocuproine is ideally suited for the colorimetric determination of copper in titanium (*39*). It should be borne in mind, in working with titanium alloys containing copper, that the titanous ion will reduce copper to the metallic state.

**Hydrogen.** Hydrogen has been determined in titanium and titanium alloys by vacuum fusion methods (*60, 67*), by igniting in oxygen in the presence of lead as a flux and collecting the water formed (*25*), by hot extraction (*68*), and by an equilibrium pressure method (*42*). Various methods for hydrogen in titanium and titanium alloys are being evaluated in a program sponsored by Wright Air Development Center.

**Iron.** Iron is best determined in titanium and titanium alloys colorimetrically. The thiocyanate method (*28, 35, 57, 62, 63*) and *o*-phenanthroline methods (*51*) are directly applicable. For the determination of small amounts of iron in titanium alloys containing large amounts of alloying elements a prior extraction of the iron by ether is recommended (*51*). Iron has been determined volumetrically after a prior separation of the iron as the sulfide from an alkaline citrate medium (*31, 63*). The direct determination of iron in titanium and its alloys by reduction with hydrogen sulfide or sulfur dioxide in acidic solution is not feasible, because the titanium hydrolyzes when the solution is heated. Polarographic determination of iron in titanium and titanium alloys by use of an alkaline citrate medium or alkaline cyanide medium has been proposed (*33*). Iron in titanium and titanium alloys can be separated from the titanium by paper chromatography, and the iron determined polarographically from an oxalate medium, or colorimetrically with *o*-phenanthroline (*41*). The direct colorimetric method using *o*-phenanthroline and a volumetric method after prior separation of the iron as the sulfide are recommended by the Panel on Methods of Analysis.

**Magnesium.** Magnesium has been determined gravimetrically with 8-quinolinol after an alkaline peroxide separation of the magnesium from the bulk of the titanium (*62*). Another procedure calls for a double precipitation of the magnesium as magnesium ammonium phosphate from an ammoniacal citrate medium (*15*). Magnesium in titanium has also been determined by precipitating as magnesium ammonium phosphate after a cupferron precipitation and chloroform extraction (*30*). A procedure has been proposed in which the magnesium is determined colorimetrically with titan yellow or Solochrome Cyanin R200 after a cupferron precipitation and chloroform extraction of the titanium (*13*).

**Manganese.** Manganese can be determined in titanium without difficulty. The usual persulfate oxidation-arsenite reduction and bismuthate methods give excellent results (*31*). The colorimetric determination using periodate is readily applicable (*28, 62, 63*). Manganese can be determined polarographically in titanium alloys after hydrolysis of the titanium by barium carbonate (*43*). The persulfate oxidation–arsenite reduction method and the periodate colorimetric method are recommended by the Panel on Methods of Analysis.

**Molybdenum.** Molybdenum in titanium alloys can be determined gravimetrically by precipitating the sulfide from a hydrofluoric-sulfuric acid medium and igniting to the oxide (*47*). In developing this method an exhaustive study was made of conditions for obtaining quantitative precipitation of molybdenum sulfide. A very useful method for the determination of 0.06 to 10% molybdenum in titanium is a direct colorimetric method proposed by Sam Vigo of Watertown Arsenal (*64*). In this method a 50-mg. sample is dissolved in hydrofluoric acid, and the solution evaporated to fumes of perchloric acid. Ferric perchlorate solution, stannous chloride, and thiocyanate are then added and the red color is developed. Moderate amounts of tungsten interfere with the method, if the molybdenum content of the sample is less than 1% (*64*).

The butyl acetate extraction method of the thiocyanate complex is satisfactory for titanium and titanium alloys (*64*). Molybdenum in titanium and titanium alloys has been determined as the dithiol complex after extraction with amyl acetate (*59*). A polarographic method, especially useful for small amounts of molybdenum in titanium and titanium alloys, has been proposed (*21*). In this method the molybdenum is extracted with ether from a sulfuric-hydrochloric acid solution, and the molybdenum polarographed from a perchloric acid solution. Determination of the molybdenum polarographically from a perchloric acid solution, or colorimetrically with thiocyanate after a prior separation of the molybdenum from titanium by paper chromatography, has been proposed (*41*). The sulfide separation method and the direct thiocyanate colorimetric method are recommended by the Panel on Methods of Analysis.

**Nickel.** Nickel can be determined colorimetrically in titanium and titanium alloys with dimethylglyoxime without any separations, using an ammoniacal citrate medium (*31, 64*). For large amounts of nickel, a sulfide separation of the nickel from an alkaline citrate medium, followed by the gravimetric determination of nickel as nickel dimethylglyoxime, is recommended (*31*). Nickel in titanium and titanium alloys can be determined polarographically from a pyridine–pyridinium chloride medium (*43*).

**Niobium.** Niobium can be determined colorimetrically with thiocyanate (yellow color) after prior separation of the niobium from the bulk of the titanium by the use of tannin (*54*). A hydrofluoric-nitric acid medium containing boric acid is used for the tannin precipitation. Considerable work was done to establish conditions for the quantitative precipitation of the niobium in the presence of large amounts of titanium (*54*). Tantalum can interfere with the colorimetric procedure for niobium. For titanium alloys containing less than 1% of niobium the amount of tantalum that may be present without interfering is 0.5%. For alloys containing more than 1% of niobium the amount of tantalum that may be present is 0.4 the amount of niobium present (*54*). Molybdenum and up to 1% of tungsten do not interfere with the colorimetric method for niobium in titanium.

**Nitrogen.** According to data assembled by a task force sponsored by the Watertown Arsenal, the determination of nitrogen in titanium and titanium alloys offers little difficulty. Hydrochloric (*63*), sulfuric (*57, 62*) or fluoboric acid can be used to dissolve the sample. A little hydrofluoric acid does no harm. It is not necessary to oxidize the titanium, or to evaporate to fumes of sulfuric acid, or to digest with selenium. In the determination of nitrogen in titanium metal (not alloys) direct nesslerization without distillation has been used (*31*). For such a procedure, of course, the titanium must be in the quadrivalent state. Vacuum fusion methods do not completely recover nitrogen from titanium. A method has been proposed for determining nitrogen in an alloy containing 90% of copper and 10% of titanium in which the sample is dissolved in perchloric acid (*27*). Nitrogen in titanium nitride has been determined after dissolving the sample with 15 grams of potassium bisulfate, 30 ml. of sulfuric acid, and 1 ml. of 1.2% of selenium oxychloride solution (*56*).

**Oxygen.** The determination of oxygen in titanium and titanium alloys is of considerable importance. Vacuum fusion methods (*63, 67*) and chlorination procedures have been used for this determination. In the latter methods the titanium is treated with chlorine (*29*) or hydrogen chloride (*7*) in a hot tube. The titanium volatilizes as the chloride, and the oxygen remains behind as titanium dioxide. Recently an entirely new method for the determination of oxygen in titanium and titanium alloys, a bromination-carbon reduction method, has been developed in this laboratory (*24*). The method has been in use in this laboratory for several months, has proved reliable and accurate, and has cut costs to a fraction of what they were previously. The vacuum fusion method and various halogen methods using chlorine, bromine, and bromine trifluoride are at present being investigated by the Panel on Methods of Analysis.

**Phosphorus.** Volumetric, or gravimetric methods for the determination of phosphorus in titanium or titanium alloys are not reliable (*20*). An accurate colorimetric method has been proposed (*20*). In this method the sample is dissolved in hydrofluoric and nitric acids, the solution evaporated to fumes of sulfuric acid, and fluoride added to complex the titanium. The phosphorus is then determined by the molybdenum blue method.

**Silicon.** Silicon can be determined gravimetrically in titanium and titanium alloys by dissolving the sample in sulfuric acid, adding hydrogen peroxide, and evaporating to fumes of sulfuric acid to form silicic acid (*63*). Hydrochloric acid or perchloric acid evaporation methods cannot be used, because of the precipitation of metatitanic acid. Molybdenum-blue colorimetric methods have been proposed (*12, 18*). The Panel on Methods of Analysis is investigating gravimetric and colorimetric methods.

**Silver.** The determination of silver offers no problem. It is precipitated as silver chloride from a sulfuric acid medium, the precipitate dissolved in ammonia, and reprecipitated (*31*). When present in larger amounts, a prior separation of the silver as the sulfide from an acidic solution may be desirable (*31*).

**Sulfur.** Sulfur in titanium and titanium alloys can be determined by dissolving the material in hydrochloric acid, driving over the hydrogen sulfide into an ammoniacal solution containing cadmium, and titrating with standard potassium iodide-iodate solution (*17*). Another method is to ignite the sample in oxygen and titrate the sulfur dioxide formed with standard potassium iodide-iodate solution (*17*). Both of these methods are somewhat empirical and require the use of standard samples (as with other metals). Sulfur is not often found in titanium.

**Tantalum.** For the determination of tantalum in titanium and titanium alloys, the tantalum is separated completely from the titanium by three tannin precipitations and is then determined colorimetrically with pyrogallol in a bisulfate oxalate medium (*53*). The tantalum must be completely separated from the titanium because the latter also gives a yellow color with pyrogallol. In developing the method it was found that niobium does not interfere with the tantalum color. However, more than 0.0025 gram of niobium interferes by causing titanium to be dragged down by the tannin precipitate (*53*). Evidently it is much easier to separate tantalum from titanium by the use of tannin, than niobium from titanium.

**Tin.** Tin in titanium and titanium alloys has been determined by iodometric titration after a sulfide separation (*49*). Recently a direct method applicable to commercial tin–titanium alloys has been developed (*32*). In this method the sample is dissolved in fluoboric acid and the tin reduced with iron. Tin has been determined polarographically from a sulfuric–hydrochloric acid–ammonium chloride medium after separating the tin from the titanium by paper chromatography (*41*). The direct iodometric titration method is recommended by the Panel on Methods of Analysis.

**Titanium.** Titanium in titanium and titanium alloys is usually determined by difference. However, its actual determination is sometimes performed. A method has been proposed for the determination of titanium in titanium metal, in which the titanium is reduced with a Jones reductor and the titanous ion titrated with standard ferric alum solution in the presence of thiocyanate as an indicator (*61*). Interference from chromium, molybdenum, tungsten, and vanadium can be eliminated by a sodium hydroxide precipitation (*61*). Titanium has been accurately determined in titanium and titanium alloys colorimetrically by the hydrogen peroxide color using the differential colorimetry technique (*45, 46*).

**Tungsten.** Tungsten can be determined by precipitating with $\alpha$-benzoinoxime in the presence of molybdenum as a gathering agent, separating the tungsten with cinchonine, and igniting to the oxide (*14*). It has also been determined by precipitating with $\alpha$-benzoinoxime in the absence of molybdenum, and then correcting for the occluded titanium (*57*). A very reliable direct colorimetric method applicable to the determination of small amounts of tungsten in titanium has been proposed (*52*). In this method the yellow tungsten thiocyanate color is developed in the presence of the blue titanous ion, using zinc as a reducing agent. The color is then read with a spectrophotometer (*52*). A colorimetric method in which the dithiol complex of tungsten is extracted with amyl acetate has been used for the determination of tungsten in titanium (*59*). A hydroquinone colorimetric method following an $\alpha$-benzoinoxime precipitation has been recommended (*39*).

**Vanadium.** Vanadium has been determined potentiometrically intitanium and titanium alloys with ferrous ammonium sulfate, after oxidation with persulfate in the absence of silver nitrate (*57, 63*). An amperometric method for vanadium in titanium alloys in which the oxidized vanadium is titrated at 0.65 volt *vs.* S.C.E. with ferrous solution has been proposed (*33*). The volumetric method using sodium diphenylbenzidene sulfonate as an indicator is recommended by the Panel on Methods of Analysis.

**Zirconium.** A method has been proposed by Sam Tour and Co. for determining 0.1 to 10% zirconium in titanium and titanium alloys using mandelic acid (*65*). In this method a 1- to 5-gram sample is dissolved in 200 to 400 ml. of concentrated hydrochloric acid, the solution diluted one third with water, and filtered. To the filtrate 50 ml. of 16% mandelic acid solution is added, and the solution digested for 30 to 60 minutes. The zirconium mandelate is filtered and washed back into the beaker. Sodium hydroxide solution is added to break up the mandelate complex, and the zirconium hydroxide precipitate is then redissolved by adding hydrochloric acid. To this solution the mandelic acid solution is added, and the precipitate is filtered, and ignited to the oxide.

## SAMPLING

Thompson (*62*) and Corbett (*31*) have pointed out the difficulties encountered in obtaining representative samples of titanium sponge. Small particles give different analyses than large particles (*31*). The usual method for sampling sponge is crushing, riffling, and quartering (*2*). A better sampling procedure is riffling, followed by compressing and drilling, and riffling of the drillings (*62*). Thompson (*62*) used a 10-gram sample and took aliquots for the different determinations.

Sampling of other types of titanium besides sponge offers no special difficulties. No conclusive data are available concerning segregation in cast titanium or titanium alloys now being produced. Some titanium alloys made according to early mill practices were prone to segregation. However, the use of consumable electrodes and double melting has greatly reduced this danger of segregation. Titanium and titanium alloys that have been heat treated develop a surface skin of oxide. This coating must be removed in any sampling procedure.

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# Quantitative Infrared Analysis of Condensed Phosphates

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**Methods are presented for the quantitative analysis of condensed phosphates in the form of dry powders. The use of the potassium bromide pressed disk technique is demonstrated with a number of mixtures. The commercial sodium triphosphate which occurs in two crystalline phases ($Na_5P_3O_{10}I$ and $Na_5P_3O_{10}II$), sometimes is contaminated with small amounts of sodium pyrophosphate ($Na_4P_2O_7$) and sodium trimetaphosphate ($Na_3P_3O_9$). In this four-component mixture the amounts of Phase I, pyrophosphate and trimetaphosphate, can be determined with accuracy within ±0.5% over the range of concentration occurring in commercial material. The infrared method of analysis compares very favorably with existing x-ray and chemical methods in regard to speed and accuracy, and should be readily applicable to any phosphate mixture in which the individual components give reasonably strong and nonoverlapping absorption maxima.**

RECENT infrared investigations (*2, 3*) of dry powdered salts of phosphorus oxyacids have shown they possess absorption bands characteristic of the type of anion present. In many cases mixtures of these compounds give nonoverlapping absorptions of sufficient intensity to enable quantitative analysis to be undertaken.

Semiquantitative analysis of minerals and inorganic salts, using the method of sedimentation of finely powdered sample on rock salt plates, has been described by Hunt, Wisherd, and Bonham (*6*). The recent introduction of the potassium bromide pressed disk technique (*5, 12, 13*) has removed many of the difficulties associated with this former method, and the precise quantitative analysis of powder mixtures is made practicable. This paper describes the development and application of the potassium bromide disk method to the analysis of condensed phosphate mixtures.

## APPARATUS AND TECHNIQUE

The absorption spectra were recorded on a Perkin-Elmer Model 21C double-beam instrument. Slit widths were 83 microns at 8.0 microns and 287 at 14 microns, and the scanning speed was 2 minutes per micron. The potassium bromide disks were made with a hydraulic press and die similar to that described by Schiedt (*12*). The die was constructed from 60-ton steel and was kept in a desiccator when not in use to avoid corrosion. Clear disks, 12 mm. in diameter and 1 mm. thick, were obtained from the powder under water pump vacuum using a pressure of 7 tons per sq. cm. for 3 minutes. These disks were mounted in a special frame and placed just behind the entrance window to the spectrometer.

To avoid the complicating effects of hydration, much of the sample preparation, prior to insertion in the press, was carried out in a specially constructed dry box. The box (about 6 × 6 inches) was made of metal with large Perspex windows, and contained a slotted platform to hold a small agate mortar in addition to a tray of phosphorus pentoxide. A single rubber glove enabled the necessary mixing and grinding operations to be carried out while the small volume of the box permitted rapid dehydration of the interior. Less than 20% relative humidity was necessary for satisfactory results.

## MATERIALS

Potassium Bromide. Analar potassium bromide was ground to pass 250-mesh sieve and dried at 120° C. for several days. The stock material was kept in the oven.

Sodium Triphosphate Hexahydrate. Sodium trimetaphosphate was hydrolyzed in dilute sodium hydroxide solution, and recrystallized by precipitation from aqueous solution with ethyl alcohol.

Sodium Triphosphate, Phase II. Pure hexahydrate was heated at 300° to 350° C. for 24 hours.

Sodium Triphosphate, Phase I. Pure hexahydrate was heated at 500° to 550° C. for 24 hours, then chilled. (No change was evident in the infrared spectrum after heating an additional 24 hours.)

Sodium Pyrophosphate. Disodium hydrogen phosphate (Analar) was heated at 600° C.

Sodium Trimetaphosphate. Disodium hydrogen phosphate (Analar) was heated at 530° C.

Sodium Metaphosphate Kurrol Salt. Melted sodium trimetaphosphate was cooled slowly and seeded at 600° to 550° C. with some preformed crystals.

X-ray powder photographs (taken with a focusing camera) of the above materials were found to be identical with those used as standards in these laboratories.

## EXPERIMENTAL PROCEDURE

The method adopted for the preparation of the potassium bromide disks was as follows:

> About 1 gram of the required phosphate mixture was first weighed out from materials which had been dried previously and ground to a particle size of less than 5 microns. Then 0.0040 gram of the mixture and 1.000 gram of potassium bromide were put in a small agate mortar and placed in the dry box as quickly as possible. This was followed by hand grinding for 15 minutes to ensure thorough dispersion of the sample in the potassium bromide. The mixture was then removed from the dry box and 0.400 gram of powder weighed out and placed in the die. After 2 minutes under vacuum the full pressure was applied to produce the disks.

All operations must be carried out in as dry conditions as possible. Unless these precautions were taken, moisture was absorbed rapidly by the potassium bromide, and in some cases by the sample material itself—e.g., sodium triphosphate I which changed to the hexahydrate. Excessive absorption of moisture produced disks of variable opacity which gave an uncertain background level to the spectra and impaired reproducibility of results. Some hydrated products—e.g., sodium triphosphate hexahydrate—produced interfering absorption peaks.

The concentration of phosphate mixture used was found to give slightly cloudy disks with some mixtures. Although clear disks could be obtained by using less of the phosphate mixture in the potassium bromide, the loss in sensitivity was not counterbalanced by any significant gain in reproducibility.

## RESULTS AND DISCUSSION

Figure 1 shows the absorption spectra of the various phosphates recorded over the range of 7 to 15 microns. In order to obtain maximum sensitivity the strongest nonoverlapping peaks were chosen for analysis according to the scheme indicated in Figure 2. Calibration curves were prepared for the binary mixtures, using samples of known composition to cover the required concentration ranges.

**Sodium Triphosphate II–Sodium Triphosphate I.** The calibration curve for this mixture is shown in Figure 3, where the difference in absorbance between 12.55 and 14.15 microns is plotted against the amount of Phase I present. Below 15% level of Phase I, the absorption at 14.15 microns did not appear as a definite peak, but rather as a lowering of the base line between the Phase II absorptions at 13.61 and 15.02 microns. The calibration points lie almost on a straight line with the maximum deviation representing an error of 1% of Phase I, while the majority of the points lie within 0.5%.

**Sodium Triphosphate II–Sodium Pyrophosphate.** In Figure 4 the difference in absorbance between wave lengths of 9.68 and 9.75 microns is plotted against concentration of pyrophosphate. The points on this graph again lie almost on a straight line with the maximum deviation representing an error of 0.75%, whereas the majority of the points do not deviate more than 0.5%.

**Sodium Triphosphate II–Sodium Trimetaphosphate.** The absorption owing to small quantities of trimetaphosphate occurred as a kink at about 13.00 microns on the side of the triphosphate peak at 13.61 microns, and the calibration curve for these mixtures is also shown in Figure 4. In practice, however, a reliable estimate of the trimetaphosphate content could be obtained merely by observation of the shape of the indentation near 13.00 microns.

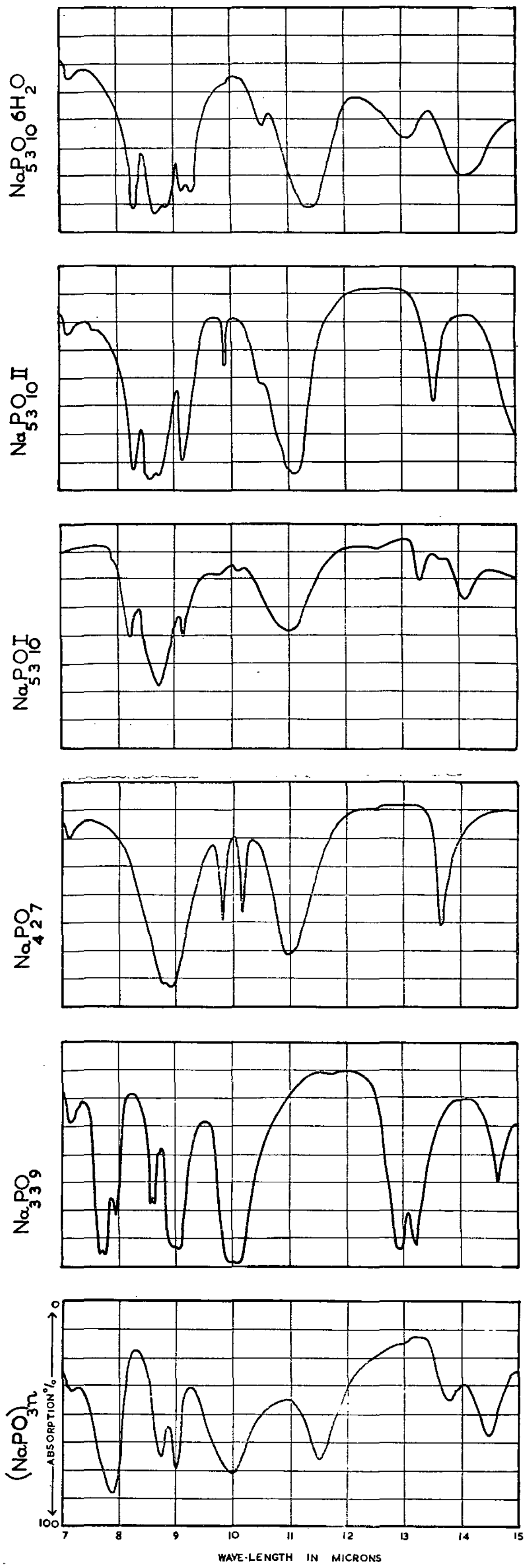

Figure 1. Infrared absorption spectra of condensed sodium phosphates

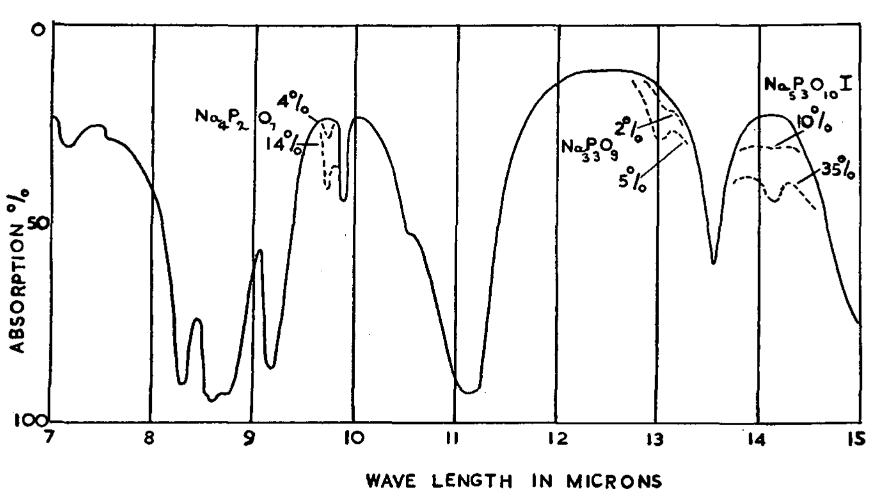

**Figure 2. Analysis of four-component mixture showing absorptions superimposed on spectrum of $Na_5P_3O_{10}II$**

**Sodium Metaphosphate–Sodium Trimetaphosphate.** Figure 5 shows the calibration curves for small amounts of trimetaphosphate in long-chain metaphosphate, and for trimetaphosphate containing up to 25% of long chain metaphosphate. Relatively strong absorption peaks are used and the calibration points show smaller deviation than those in Figures 3 and 4.

**Sodium Trimetaphosphate–Sodium Pyrophosphate.** Curves are shown in Figure 6, *A*, for mixtures containing 0 to 35% of trimetaphosphate, using the strong absorption at 12.97 microns, and Figure 6, *B*, for mixtures containing 20 to 95% trimetaphosphate utilizing the weaker absorption at 14.56 microns. The 12.97-micron absorption gives a steeper slope than that at 14.56, consequently the former is suitable for more accurate estimations at low concentrations.

**Sodium Triphosphate II–Sodium Triphosphate I–Sodium Pyrophosphate–Sodium Trimetaphosphate.** Analysis of the four-component system can be carried out from the spectrum of a single disk, by measurement of the absorbance at the points used for the binary mixtures (Figure 2). At these points, background interference effects from the presence of the other components are very small over the concentration ranges considered.

Table I shows the results obtained from a series of samples of known composition, using the calibration curves prepared for the binary mixtures. The quantity of Phase II was in each case determined by difference. The average discrepancy of the 40 determinations is 0.5%, whereas the mean value taken from two disks is about 0.33%. Because a strong absorption is available for measuring the trimetaphosphate content, its determination is more accurate than that of the other components which rely on much weaker absorption peaks. For the most accurate results, choice of as strong a peak as possible and measurements taken from several disks are desirable. In this way the amount of each component can be estimated to within ±0.5%. The calibration curves of the other binary mixtures, shown in Figures 5 and 6, indicate a similar or better accuracy is attainable. Absorption curves repeated with the same disk indicated the amount of error probably introduced by nonreproducibility of the spectrometer was less than 0.1%.

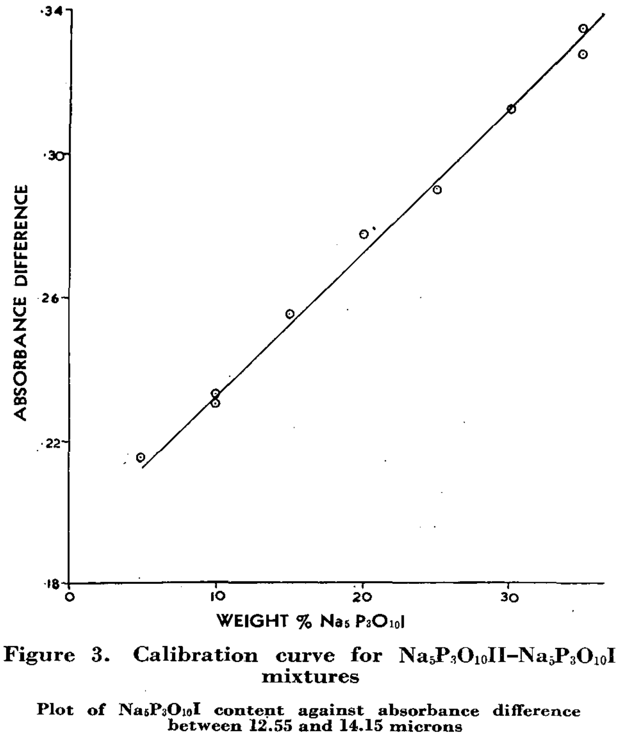

**Figure 3. Calibration curve for $Na_5P_3O_{10}II$–$Na_5P_3O_{10}I$ mixtures**

Plot of $Na_5P_3O_{10}I$ content against absorbance difference between 12.55 and 14.15 microns

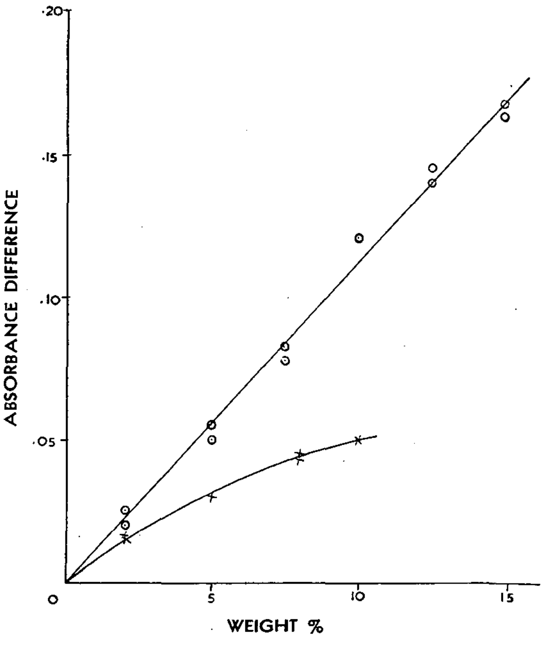

**Figure 4. Calibration curves for $Na_5P_3O_{10}II$–$Na_4P_2O_7$ and $Na_5P_3O_{10}II$–$Na_3P_3O_9$ mixtures**

Plot of $Na_4P_2O_7$ content against absorbance difference between 9.68 and 9.75 microns, and of $Na_3P_3O_9$ content against estimated absorbance of $Na_3P_3O_9$ peak at 13.00 microns

⊙. $Na_4P_2O_7$
×. $Na_3P_3O_9$

Each disk should be produced under as standard conditions as possible. This applies particularly to the elimination of water (especially in mixtures containing triphosphate Phase I) and the dispersion of the sample in the potassium bromide before pressing. Complete elimination of water from the disks was difficult. Care-

Table I. Analysis of Triphosphate Mixtures of Known Composition

| | Per Cent | | | |
|---|---|---|---|---|
| | $Na_5P_3O_{10}$II | $Na_5P_3O_{10}$I | $Na_4P_2O_7$ | $Na_3P_3O_9$ |
| Actual | 90.3 | 9.0 | 0.7 | 0 |
| Found | 89.9 | 9.0 | 1.1 | 0 |
| Actual | 83.4 | 4.2 | 9.8 | 2.6 |
| Found | 83.3 | 4.7 | 9.0 | 3.0 |
| Actual | 71.3 | 20.4 | 1.5 | 6.8 |
| Found | | | | |
| Disk 1 | 69.5 | 21.3 | 2.2 | 7.0 |
| Disk 2 | 72.4 | 19.0 | 1.6 | 7.0 |
| Mean | 71.0 | 20.1 | 1.9 | 7.0 |
| Actual | 80.6 | 10.1 | 5.1 | 4.2 |
| Found | | | | |
| Disk 1 | 80.9 | 8.5 | 6.1 | 4.5 |
| Disk 2 | 80.2 | 9.7 | 6.1 | 4.0 |
| Mean | 80.5 | 9.1 | 6.1 | 4.3 |
| Actual | 81.4 | 13.2 | 3.7 | 1.7 |
| Found | | | | |
| Disk 1 | 80.9 | 13.8 | 3.3 | 2.0 |
| Disk 2 | 82.4 | 13.8 | 2.8 | 1.0 |
| Mean | 81.6 | 13.8 | 3.1 | 1.5 |
| Actual | 90.0 | 3.0 | 3.0 | 4.0 |
| Found | | | | |
| Disk 1 | 89.5 | 3.5 | 2.8 | 4.2 |
| Disk 2 | 90.4 | 2.0 | 3.6 | 4.0 |
| Mean | 90.0 | 2.7 | 3.2 | 4.1 |
| Average discrepancy (means only) | | 0.55 | 0.55 | 0.15 |

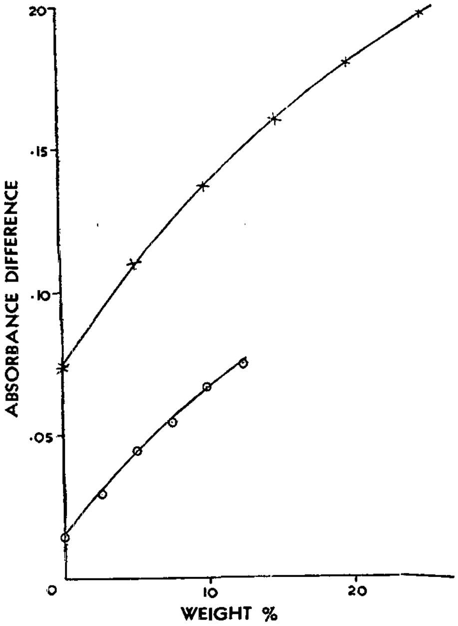

Figure 5. Calibration curves for $(NaPO_3)_n$–$Na_3P_3O_9$ mixtures

Plot of $Na_3P_3O_9$ content against absorbance difference between 12.65 and 12.97 microns, and of $(NaPO_3)_n$ content against absorbance difference between 11.47 and 12.13 microns

O. $Na_3P_3O_9$
X. $(NaPO_3)_n$

fully prepared disks always showed a small absorption near 3 microns. However, if the amount of water held in the disk mixture was kept below a certain limit, indicated by the clarity of the disks, and at a reasonably constant level, as ensured by this technique, the reproducibility of the method was not affected seriously. All instrument settings should be kept constant throughout the series of experiments. A small variation in the amplifier gain setting was found to have a significant effect on the slope of the calibration curves.

Several possible ways for improving the technique are evident.

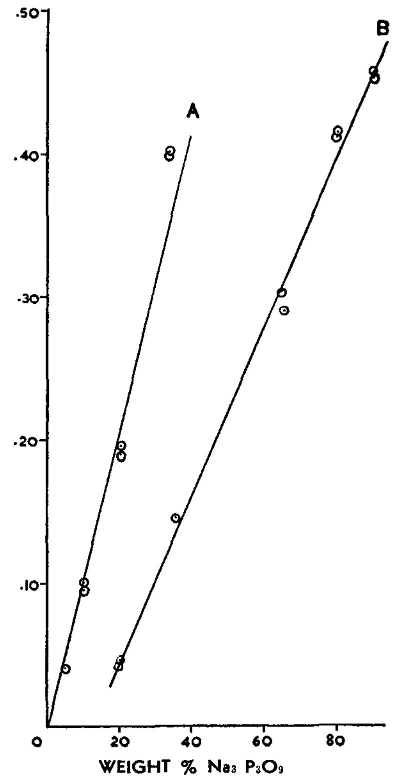

Figure 6. Calibration curves for $Na_4P_2O_7$–$Na_3P_3O_9$ mixtures

Plot of $Na_3P_3O_9$ content against
*A.* Absorbance difference between 12.50 and 12.97 microns
*B.* Absorbance difference between 14.15 and 14.56 microns

A more rapid and thorough mixing of the sample with the potassium bromide may be accomplished by the use of a small mechanically driven ball mill. The entire disk preparation including the weighings can be carried out in a large dry box, and may be essential with salts of a very hygroscopic nature. When less hygroscopic materials were used—e.g., as in the case of the mixtures in Figures 5 and 6—the exclusion of water from the disk mixture was not so critical. A less hygroscopic disk material—e.g., potassium chloride—may be more useful.

Application of the disk method to the analysis of sodium triphosphate hexahydrate and other hydrated salts has not yet been tried. In this case errors might arise from partial dehydration of the sample in the vacuum press, differential dehydration of the components in the mixture, or even salt double decomposition with the potassium bromide (*5*).

The pressed disk infrared technique has several advantages over other methods for the analysis of condensed phosphates. Chemical (*1, 9, 11, 14*) and chromatographic (*4*) methods are time-consuming and insensitive to differences of crystalline form. The infrared method is comparatively rapid, because the spectra of three or four disks from an unknown sample, can be obtained in less than 1 hour, and only a very small quantity of sample (0.05 gram) is needed.

Infrared, x-ray diffraction (*7, 10*) and temperature rise (*8*) methods are sensitive to differences of crystalline form, but the last has so far only found application to sodium triphosphate mixtures containing Phases I and II.

The estimation of amorphous phases may be possible by the infrared method, but no attempt has been made to record the spectra of any of these. (Small amounts of amorphous material may sometimes be present in commercial triphosphate.)

Geiger counter x-ray techniques (*7*) applied to the analysis of commercial triphosphate are quicker and more sensitive than photographic methods (*10*), but the infrared method appears to be at least as rapid and as accurate.

ACKNOWLEDGMENT

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# Spectrometric Determination of Rhenium and Its Separation from Molybdenum

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**The hydrazine reduction of perrhenate in hydrochloric acid forms hexachlororhenate(IV) ion. The strong absorption of this ion at 281.5 m$\mu$ permits its spectrophotometric measurement in the presence of equal amounts of molybdenum. Larger quantities of molybdenum may be removed by the ether extraction of its cupferrate. Less than 2 hours are required for the separation of 0.1 mg. of rhenium from 0.4 gram of molybdenum and determination of the rhenium with an accuracy of ±2%.**

IN ITS natural occurrences, rhenium is always a minor constituent and is usually associated with larger quantities of molybdenum. Gravimetric determinations of perrhenate as the nitron (*4*) or tetraphenylarsonium (*16*) salts are applicable only to materials of high rhenium content because of the significant solubility of these precipitation forms. The more sensitive, colorimetric measurement of rhenium as a thiocyanate complex has been extensively investigated (*7, 8, 10*), but is subject to several serious limitations. The color formation is a function of time, temperature, and reagent concentrations; it is very difficult to provide for the quantitative exclusion of molybdenum which is a serious interference.

Separations of molybdenum from rhenium by $\alpha$-benzoinoxime precipitation of the molybdenum (*12*), the distillation of perrhenic acid (*6*), the selective reduction of molybdenum and its extraction in the presence of thiocyanate (*9*), acid sulfide concentration of the rhenium (*5*), chloroform extraction of tetraphenylarsonium perrhenate (*15*), ether extraction of molybdenum with ethyl xanthate (*11*), and other methods (*2, 13, 14*) have been advocated. Each of these techniques has its own particular merits and limitations, but most of these procedures are tedious; some are limited to low ratios of molybdenum to rhenium, and some introduce reagents which would be incompatible with suitable measuring steps.

The rhenium(IV) hexahalide complexes exhibit strong absorption in the ultraviolet region. Their formation, using nonabsorbing reagents, seemed to promise a useful measuring technique. While perrhenate does not form a cupferron salt, the cupferrate complex of molybdenum is quantitatively extractable with organic solvents (*3*). The aim of the present study has been to combine these two techniques into a useful procedure for the determination of rhenium, applicable in the presence of molybdenum.

## EXPERIMENTAL

**Apparatus.** Spectrophotometric data were obtained with a Beckman Model DU quartz spectrophotometer, using 1-cm. silica cells.

**Reagents.** Distilled water was employed throughout this work.

CUPFERRON. C.P. grade cupferron (Coleman and Bell Co.) was recrystallized as follows: Hot boiling ethyl alcohol was saturated with cupferron and suction-filtered while still hot. The solution was chilled in an ice bath. The resulting crystals were filtered and repurified by a second recrystallization from alcohol. After the second recrystallization the crystals were suction-filtered and washed with ethyl ether and air dried. With cupferron, which is especially impure, it is sometimes more desirable to add Norit to the hot alcohol solution during the first recrystallization, and then filter the Norit from the solution while it is hot.

CHLOROFORM, Merck reagent grade.

HYDROCHLORIC ACID, Baker & Adamson reagent grade, specific gravity 1.89.

HYDRAZINE DIHYDROCHLORIDE, Eastman Kodak No. 1117.

CHLOROFORM SOLUTION OF HYDROGEN CUPFERRATE. Approximately 0.5 gram of cupferron was dissolved in 50 ml. of chloroform. (The cupferron was dissolved in water, sufficient dilute sulfuric acid was added to convert the ammonium salt to the acid, and the solution and precipitate were extracted to obtain the reagent solution.) Chloroform solutions of acidified cupferron are rather unstable. For this reason, these solutions should be prepared immediately before use.

2*N* SODIUM HYDROXIDE SOLUTION. Approximately 80 grams of sodium hydroxide were dissolved to make 1 liter of solution.

2*N* SULFURIC ACID SOLUTION. Approximately 56 ml. of concentrated sulfuric acid were diluted to 1 liter.

POTASSIUM PERRHENATE, University of Tennessee 99.8% grade.

**Development of Method.** All four of the hexahalide complexes of rhenium(IV) exhibit weak visible and strong ultraviolet absorptions. The hexafluoride is difficult to prepare. The hexabromide and hexaiodide salts hydrolyze rather readily and must be supported in rather concentrated solutions of the corresponding hydrohalide acids; such solutions introduce a strong background absorption. Accordingly, rhenium(IV) hexachloride was selected as the most suitable basis for a spectrophotometric determination.

A pure standard of potassium chlororhenate was first prepared by the conventional iodide reduction synthesis (*1*), but by using two extra recrystallizations from concentrated hydrochloric acid. The absorbance spectrum of this compound in 4*N* acid

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is shown in Figure 1. The maximum absorbance occurs at 281.5 mμ, and a molar-absorbance index of 11,800 was found at 25° C.

The validity of Beer's law was tested at the wave length of optimum absorbance by readings on carefully diluted aliquots of a standard hexachlororhenate solution. A Beer's law relation was found to be rigorously valid for 0.01 to 0.10 millimolar solutions, and the straight-line relationship extrapolates through 0.0 if $4N$ hydrochloric acid is used in "zero-setting" the instrument.

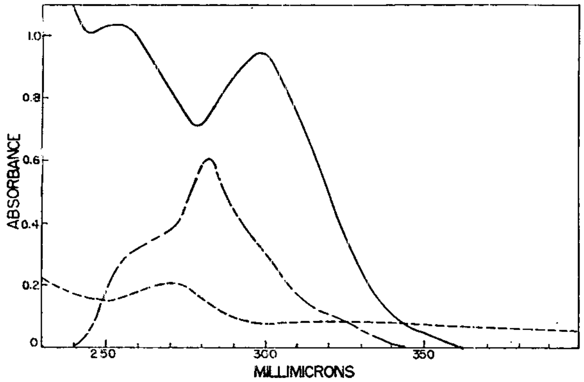

**Figure 1. Absorption spectra of rhenium, molybdenum, and hydrazine in $4N$ hydrochloric acid**

—— Pentavalent molybdenum 0.5m$M$
— – — Hexachlororhenate(IV) 0.05m$M$
- - - - Hydrazine dihydrochloride 0.05$M$

The synthesis of hexachlororhenate(IV) ion by the iodide reduction of perrhenate in hydrochloric acid is not a convenient technique. Neither is it a complete quantitative conversion at the desired level of concentrations. Moreover, it is difficult to remove the last traces of iodine which is a strong absorber in the ultraviolet region. Formaldehyde, sulfur dioxide, and hydroxylamine hydrochloride gave an appreciable conversion, but each of these was subject to some limitations. Hydrazine dihydrochloride proved to be the most reliable reducing agent for the conversion of 0.05- to 10.0-mg. quantities of rhenium as perrhenate to hexachlororhenate(IV) in hydrochloric acid. Test reductions were conducted in which the time of reaction at boiling temperature, the quantity of excess hydrazine employed, and the acid concentration were varied over wide ranges to define the optimum conditions.

Fortunately, hydrazine has only a weak ultraviolet absorption. It can be seen from Figure 1 that a 1000-fold excess of hydrazine over rhenium results in only one fourth as much absorbance at 281.5 mμ. A correction for the hydrazine absorbance may be applied by taking absorbance readings at two different wave lengths. Using the data at 265 mμ and 281.5 mμ, gives for the molarity of rhenium,

$$C_{\mathrm{Re}} = \frac{A_{281.5} + 1.92A_{265}}{1.61 \times 10^4}$$

But best practice would dictate that independent workers using this method should determine their own values of the constants for their particular instrument.

Figure 1 also shows the spectrum of a molybdate solution after treatment by the hydrazine reduction procedure. The resulting quinquevalent (?) molybdenum absorbs less strongly than the rhenium, and shows a minimum near 280 mμ. Therefore, one could readily determine rhenium in the presence of at least equal quantities of molybdenum. For this purpose, readings at wave lengths of 281.5 mμ and about 298 mμ would be suitable. If the (small) hydrazine correction was also to be made, the system could be treated as a three-component mixture taking readings at 265 (258 to 260 would actually be preferable for this case), 281.5, and 298 mμ. With disproportionate quantities of molybdenum, a preliminary separation is essential.

From the existing methods for the separation of rhenium and molybdenum the following conclusions were drawn. The separation methods using the distillation of perrhenic acid from sulfuric acid solution, the reduction and ether extraction of molybdenum as the thiocyanate, and the extraction of the molybdenum after the formation of the ethyl xanthomolybdate complex seemed rather cumbersome. The extraction of tetraphenyl-arsonium perrhenate into chloroform seemed to be the most promising of the methods previously developed, but the necessity of recovering the rhenium from the chloroform layer presented a problem.

The possibility of re-extracting the perrhenate into concentrated hydrochloric acid was investigated. It was found that some of the rhenium was recovered in the aqueous phase in all cases, but the recovery was never quantitative; however, tetraphenylarsonium chloride in chloroform extracted rhenium from solutions of potassium hexachlororhenate(IV). Attempts were made to obtain absorption spectra of the compound in chloroform, but the background absorption of chloroform and tetraphenylarsonium chloride in the region of the rhenium absorption peak interfered.

It seemed desirable, in view of the facts mentioned, to develop another means of separating rhenium and molybdenum. According to a recent article (*3*) it is possible to extract molybdenum quantitatively from dilute sulfuric acid solution by means of a chloroform solution of cupferron. Since perrhenates have never been reported as being extractable with cupferron, this method seemed to offer a possibility for separation of these two elements. However, the substitution of hydrochloric for sulfuric acid was first tried as being a more desirable preliminary to the hexachlororhenate step.

The results on a series of samples were in fair agreement with each other but were not in agreement with the amount of rhenium which was known to be present. Evidently, some of the rhenium is extracted by the cupferron in hydrochloric acid medium.

This same procedure was tried again with the substitution of sulfuric acid for the hydrochloric acid. In this case the results not only agreed with each other but also agreed with the value of rhenium known to be present. When sulfuric acid is employed, it is necessary to neutralize the solution before evaporating down to a small volume in order to prevent the loss of rhenium as volatile perrhenic acid.

The following complete procedure for the determination of rhenium in the presence of molybdenum evolved from these studies. The cupferron extraction steps can be omitted, of course, whenever molybdenum is absent or present in small amount. The entire procedure, including the necessary extractions (about three) for the removal of 0.4 gram of molybdenum, is easily conducted in less than 2 hours.

## PROCEDURE

The perrhenate solution ("ores" may be converted to perrhenate by means of a sodium peroxide fusion) is made approximately $2N$ in sulfuric acid and the volume adjusted to approximately 25 ml. This solution is transferred to a separatory funnel to which are added 50 ml. of the chloroform solution of cupferron. The funnel is shaken for about 3 minutes; the chloroform layer is allowed to separate, and is drained off. If the aqueous layer is still colored, another extraction is necessary. After sufficient reagent has been added to remove all of the molybdenum the aqueous layer is extracted once more with pure chloroform to remove the last traces of cupferron.

The aqueous layer is quantitatively transferred to a beaker and neutralized with sodium hydroxide solution. The beaker is covered with a cover glass and the solution evaporated to approximately 5 ml. Twenty-five milliliters of concentrated hydrochloric acid is added to the solution followed by approximately 1 gram of hydrazine dihydrochloride. The solution is allowed to

**Table I. Results of Spectrophotometric Determinations of Rhenium**

(Molybdenum absent)

| Molarity of Rhenium × $10^5$ | | |
|---|---|---|
| Actual | Calculated | % Error |
| 1.00 | 1.04 | 4.0 |
| 2.00 | 1.97 | 1.5 |
| 3.00 | 2.94 | 2.0 |
| 5.00 | 5.01 | 0.2 |
| 7.00 | 7.01 | 0.1 |
| 100.0[a] | 99.6 | <1.0 |

[a] This sample was diluted to 500 ml. rather than 50 ml.

**Table II. Results of Spectrophotometric Determinations of Rhenium after Separation from 0.4 Gram of Molybdenum**

| | Molarity of Rhenium × $10^5$ | | |
|---|---|---|---|
| Sample No. | Actual | Calculated | % Error |
| 1 | 5.00 | 5.04 | 0.8 |
| 1a | 5.00 | 4.98 | 0.4 |
| 2 | 5.00 | 4.92 | 1.6 |
| 3 | 5.00 | 5.10 | 2.0 |
| 3a | 5.00 | 5.07 | 1.4 |
| 4 | 1.00 | 1.07 | 7.0 |
| 5 | 1.00 | 0.99 | 1.0 |

boil for 45 to 60 minutes. After cooling the solution is transferred to a 50-ml. volumetric flask and diluted to the mark with water. The solution is placed in a quartz cell and the absorbances are recorded at 265 and 281.5 mμ. The blank solution used in the spectrophotometer may be either one which has been carried through the same boiling procedure without the rhenium and the hydrazine, or it may be a water blank. In the latter case, however, it is necessary to record the difference in absorbance between a water blank and a hydrochloric acid blank and to use this as a correction factor in all subsequent determinations.

### RESULTS AND DISCUSSION

When tested with known amounts of perrhenate, the hydrazine reduction to hexachlororhenate gave results such as those shown in Table I. With the complete procedure, including the cupferron removal of 0.4 gram of molybdenum, the results shown in Table II were obtained. All of these figures were corrected for the small absorption due to hydrazine. But since no correction was needed for possible molybdenum present in series II, it is evident that the cupferron separation is adequate.

Including a cupferron separation where necessary, an average precision and accuracy of about ±2% may be expected to apply for the determination of 0.1 mg. and more of rhenium. Appreciably lower quantities are readable in 50-ml. volumes, but concentration into 5- to 10-ml. volumes would be preferable at lower levels.

The complete procedure should be free from interference of the alkali metals, the alkaline earths, most anions and nonmetals, titanium, vanadium, iron, copper, gallium, zirconium, columbium, molybdenum, indium, tin, tungsten, and small amounts of mercury and bismuth. If one incorporates a precipitation from 9*N* sulfuric acid solution with hydrogen sulfide into this procedure, one can remove all interferences except ruthenium, rhodium, palladium, antimony, platinum, and gold. Small amounts of all of these metals may be removed in the reduction with hydrazine; if large amounts are present, it may be necessary to reduce these metals to the metallic state immediately after solution of the sample.

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# Differential Spectrophotometric Determination of Zirconium

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**This investigation was undertaken to ascertain the feasibility of differential spectrophotometry for the determination of zirconium. The reaction between zirconium and sodium alizarin sulfonate in perchloric acid solution to produce the intensely red-colored complex was chosen for this application. The reference standard solution contained 1.0 mg. of zirconium per 25 ml. of 1*M* perchloric acid. Beer's law was conformed to cover the range of 1.0 to 1.6 mg. of zirconium per 25 ml. of 1*M* perchloric acid. The coefficient of variation of the method is less than 1% and compares favorably with the best precision obtainable by the gravimetric mandelic acid method. Chief advantage over gravimetric method is that method is extremely rapid; it is particularly suited for samples when zirconium concentration is known generally. Fluoride, sulfate, and phosphate are primary interferences.**

THE ever-increasing applicability of zirconium and its compounds has led to the recent development of a number of methods for its determination. Most of the investigations have been concerned with organic precipitants such as mandelic acid and its halo-derivatives (*6, 11, 12*). These reagents are decidedly superior to phosphate (*7*) and cupferron (*8*) as quantitative precipitants. Volumetric modifications have been devised. Kolthoff and Johnson (*10*) reported an amperometric method based on the use of *m*-nitrophenylarsonic acid. Recently Fritz and Fulda (*4*) demonstrated that zirconium can be titrated with ethylenediaminetetraacetic acid (EDTA) [(ethylenedinitrilo)-tetraacetic acid].

This investigation was undertaken to apply the advantages of differential spectrophotometry to the determination of zirconium. Hiskey (*9*) and Bastian (*1*) have emphasized that the accuracy and precision which can be attained by differential spectrophotometry are equivalent to volumetric and gravimetric techniques. In the differential method the absorbance scale is set at zero with a solution of a highly colored or light-absorbing substance in the place of a reagent blank. Higher concentrations of the given component are then measured against this zero point in the usual way. In order to obtain the increased light needed to adjust the zero scale by this procedure, the spectrophotometer slit is set at wider apertures than normal. For systems obeying Beer's law at wider slit openings, the resultant absorbance readings are directly proportional to the difference in concentration between the zero point standard and the solution in question.

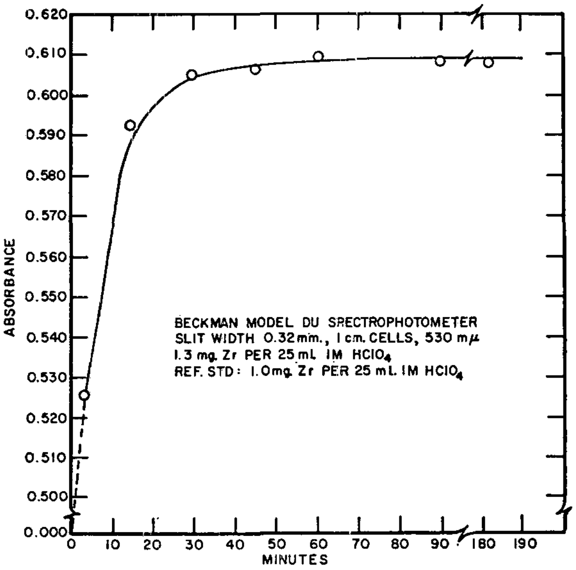

**Figure 1. Effect of time on color development of zirconium-Alizarin Red S complex**

The reaction between zirconium and sodium alizarin sulfonate is an excellent color reaction for the determination of zirconium and was used in this study.

## EXPERIMENTAL

**Application of Differential Spectrophotometry to Zirconium-Alizarin Red S Reaction.** Green (*5*) applied the reaction between zirconium and Alizarin Red S to the determination of low concentrations of zirconium. The "lake" is formed in either hydrochloric or perchloric acid media and its absorbance is measured at 530 mμ. Since zirconium forms a very weak complex with perchloric acid, this medium was used. The concentration of hydrogen ion is not critical over the range of acidity from pH 0 to 2. The acidity generally used is in the pH range of 1 to 2. Higher acid concentrations decrease the intensity of the complex, whereas hydrolysis readily occurs in less acidic solutions. In this particular application, sensitivity is of minor importance so that some sensitivity was sacrificed to permit the use of 1*M* perchloric acid. The color formed in this medium reaches its maximum intensity within 0.5 hour (Figure 1) and is stable for at least 3 hours.

For systems which obey Beer's law, the accuracy possible by differential colorimetry increases as the absorbance of the zero point standard (reference standard) increases. Eventually, however, because of the combined effects of broad band widths of light emitted by the widened slit apertures, and excessive concentrations, an optimum concentration is reached beyond which decreased accuracy results. A thorough discussion of these effects and a detailed procedure for the determination of the optimum reference standard is presented by Bastian (*2*). The optimum concentration of zirconium in the reference standard as determined by Bastian's procedure was 1.0 mg. in a final volume of 25 ml. of 1*M* perchloric acid. With this solution as a reference, a standard zirconium curve was prepared.

**Reagents.** STANDARD ZIRCONIUM STOCK SOLUTION, 2.5 mg. per ml. Dissolve 9.3 grams of zirconium nitrate in 10 ml. of concentrated nitric acid and 200 ml. of concentrated perchloric acid by heating to fumes for 1 hour. Cool and dilute with approximately 200 ml. of water. Cool and filter through Whatman No. 2 paper, or equivalent, and dilute to 500 ml. Standardize by determining the zirconium gravimetrically in a 25-ml. aliquot of the solution.

ZIRCONIUM STANDARD SOLUTIONS. Zirconium, 1 mg. per ml. Dilute an appropriate aliquot of the stock solution with water. Zirconium, 0.10 mg. per ml. Dilute 10 ml. of the solution containing 1 mg. of zirconium per ml. to 100 ml. with 1*M* perchloric acid. Zirconium, 0.05 mg. per ml. Dilute 5 ml. of the solution, containing 1 mg. of zirconium per ml., to 100 ml. with 1*M* perchloric acid.

SODIUM HYDROXIDE, 0.25*M* SOLUTION. Dissolve 10 grams of sodium hydroxide in 1 liter of water.

PERCHLORIC ACID, 1*M*. Dilute 86 ml. of perchloric acid (70–72%) to 1 liter with water.

SODIUM ALIZARIN SULFONATE (ALIZARIN RED S). Prepare by dissolving 0.5 gram of sodium alizarin sulfonate in approximately 80 ml. of water. Filter and adjust the acidity of the solution to pH 1 with perchloric acid and dilute to 100 ml. with 0.1*M* perchloric acid.

**Procedure.** Transfer the appropriate volumes of standard solutions of zirconium into 25-ml. volumetric flasks so that they contain 1.00, 1.05, 1.10, 1.20, 1.30, 1.40, and 1.50 mg. of zirconium, respectively. Dilute to about 15 ml. with 1*M* perchloric acid and add 3 ml. of Alizarin Red S reagent. Swirl, then add 5 ml. of acetone. Dilute to volume with 1*M* perchloric acid and shake. Allow the color to develop for 30 minutes and measure the absorbance of each of the solutions on the Beckman Model DU spectrophotometer at 530 mμ in 1-cm. cells against the reference standard, which is the solution containing 1 mg. of zirconium. To obtain the proper slit width, adjust the galvanometer to zero by setting the slit width constant and make the necessary adjustments with the sensitivity control. Plot the absorbance as the abcissa *vs.* the concentration of zirconium on the ordinate. Since the reference standard must remain in the cell for some time it is necessary to place a cap on this cell. Acetone was added to the solution following formation of the complex to prevent its precipitation in the aqueous medium. The solubility product in aqueous solution is exceeded above 1.2 mg. of zirconium in 25 ml. of 1*M* perchloric acid.

**Table I. Determination of Zirconium in Standard Solutions by Differential Spectrophotometric Method**

(Reference solution—1 mg. of Zr per 25 ml. Slit width—1.7 mm., Beckman Model DU spectrophotometer)

| | Standard Solution | |
|---|---|---|
| | Pure | Mixed |
| Zirconium, mg. per ml. | | |
| Taken | 1.27 | 3.80 |
| Found | 1.27 | 3.79 |
| Difference | 0.00 | 0.01 |
| Number of determinations | 6 | 4 |
| Standard deviation, mg. per ml. | 0.02 | 0.03 |

## RESULTS

With the establishment of a standard curve, (Figure 2) the method was tested by determining zirconium in a solution containing 0.38 mg. per ml. in 1*M* perchloric acid. Aliquots of 3 ml. were taken and the described procedure was followed. Additional tests were made on solutions with known concentrations of zirconium, in the presence of such cations as sodium, potassium, iron, nickel, chromium, and uranium. The results of these tests are shown in Table I.

The method was also applied to a number of solutions of mixtures of sodium and zirconium fluorides. These samples had been analyzed by the mandelic acid method (*11*). The procedure consisted of the following steps.

Dissolve 0.1-gram samples in 10 ml. of concentrated nitric acid and 15 ml. of 72% perchloric acid. Evaporate to fumes of perchloric acid. Avoid evaporation of the solution to near dryness because dehydrated zirconium salts do not dissolve readily upon dilution. Add 10 ml. of 1*M* perchloric acid, cool, transfer to a 150-ml. beaker, and dilute with 25 ml. of water. Adjust the pH to 0 with sodium hydroxide. Transfer to a 100-ml. volumetric flask and dilute to the mark with 1*M* perchloric acid. Transfer an aliquot containing between 1 and 1.5 mg. of zirconium to a 25-ml. volumetric flask and follow the outlined procedure. The results are shown in Table II.

## DISCUSSION

Samples 1 through 14 were supposedly identical in composition. Therefore, the statistical variance ratio, or *F* test can be applied to determine if there is significant difference in the precision of the two sets of data. The experimental *F* was less than the theoretical *F* value at the 95% confidence level. This test reveals that the precisions of the two methods are essentially the same and that no significant difference exists.

Since the method of differential colorimetry involves the measurement of absorbance of an unknown solution *vs.* a reference standard, extreme care must be exercised in the preparation of zirconium standards for establishing the reference curve, and for the reference standards.

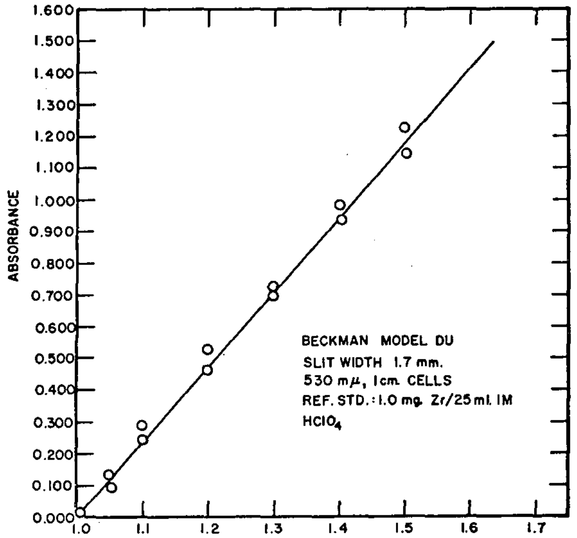

**Figure 2. Standard curve for determination of zirconium with Alizarin Red S by differential spectrophotometry**

Zirconium metal and zirconium hydride initially were used to prepare standard solutions, because of the high purity of these materials (in excess of 99%). These materials were dissolved in dilute hydrofluoric acid and taken to fumes of perchloric acid several times. The standard curves, which were prepared from solutions of these reagents, were not reproducible. This difficulty was considered to be due to the presence of traces of fluoride ion. Fluoride (*3, 13, 14*) is the most serious interference in this method, because it forms a strong complex with zirconium which effectively bleaches the alizarin complex. Zirconium oxide is available in a pure state, but must be dissolved in either fluoride or sulfate media. Sulfate, like the fluoride, forms a strong complex with zirconium and also diminishes the intensity of the complex. Zirconium nitrate, however, is relatively impure and, therefore requires standardization. The reproducibility of the standard curve, which was prepared from a solution of zirconium nitrate, was determined by repeating the procedure on five different occasions. The results are shown in Table III.

**Table II. Comparison of Differential Spectrophotometric Method for Determination of Zirconium with Gravimetric Mandelic Acid Method**

| | Zirconium, % | | |
|---|---|---|---|
| Sample | Spectrophotometric A | Gravimetric B | Difference B-A |
| 1 | 37.1 | 37.9 | 0.8 |
| 2 | 38.2 | 38.3 | 0.1 |
| 3 | 37.8 | 38.1 | 0.3 |
| 4 | 38.9 | 38.2 | −0.7 |
| 5 | 37.9 | 38.3 | 0.4 |
| 6 | 37.4 | 38.3 | 0.9 |
| 7 | 39.0 | 38.3 | −0.7 |
| 8 | 39.1 | 38.4 | −0.7 |
| 9 | 38.7 | 39.6 | −0.9 |
| 10 | 37.3 | 37.9 | 0.6 |
| 11 | 38.7 | 38.3 | −0.4 |
| 12 | 39.2 | 38.9 | −0.3 |
| 13 | 39.4 | 39.0 | −0.4 |
| 14 | 38.1 | 38.3 | 0.2 |
| 15 | 43.0 | 43.2 | 0.2 |
| 16 | 35.3 | 34.9 | −0.4 |
| 17 | 43.7 | 43.1 | −0.6 |
| 18 | 31.7 | 31.5 | −0.2 |
| 19 | 30.0 | 30.2 | 0.2 |
| 20 | 39.2 | 38.5 | −0.7 |
| | | Average difference | 0.5 |

**Table III. Reproducibility of Standard Zirconium Curve**

Reference standard—1.0 mg. of Zr
Acidity—1*M* $HClO_4$
Final volume—25 ml.
Slit width—1.7 mm., Beckman Model DU spectrophotometer, 1-cm. cells

| Zirconium, Mg. | Average Absorbance | Coefficient of Variation, % |
|---|---|---|
| 1.05 | 0.122 | 3.1 |
| 1.10 | 0.262 | 2.0 |
| 1.20 | 0.504 | 2.8 |
| 1.30 | 0.708 | 1.8 |
| 1.40 | 0.946 | 2.9 |
| 1.50 | 1.153 | 1.1 |
| | Average | 2.3 |

An evaluation of the data reveals that the curve can be reproduced to within 2.3%. The importance of the standard reference curve is illustrated by this data. To obtain an order of precision comparable to that obtainable by gravimetric methods, the standard reference curve should be re-established with each set of determinations. The precision of the method can be considered to be less than 1% under these conditions. If the curve is not established with the set of determinations, the maximum precision obtainable is represented by the reproducibility of the standard curve, approximately 2.5%.

## CONCLUSION

Over-all evaluation of the data obtained by this method leads to the conclusion that the differential spectrophotometric method, under ideal conditions, is satisfactory for the determination of zirconium with a coefficient of variation of less than 1%. The method has the major advantage of being considerably less time-consuming than precipitation methods. It is particularly suited for samples in which the concentration of zirconium is generally known. The interferences are relatively few. Cationic interferences (*5*), with the exception of aluminum, are eliminated by the use of 1*M* perchloric acid. Such anions as fluoride and sulfate, which form strong complexes with zirconium, interfere seriously. Phosphate forms an insoluble zirconium salt and must be absent also.

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# Spectrophotometric Determination of Vanadium(V) with Benzohydroxamic Acid and 1-Hexanol

## Application to Steels and Crude and Residual Oils

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**A spectrophotometric method for quantitatively determining minute amounts of vanadium is based on the extraction with 1-hexanol of the colored product, which is formed when vanadium(V) reacts with benzohydroxamic acid, and the subsequent measurement of the absorbance of the extract. The effects of the following variables have been investigated: the amount of complex extracted with respect to the kind of alcohol used, amount of reagent, pH, volume of the aqueous phase, ionic strength, and diverse ions. The system obeys Beer's law between the limits of $5.00 \times 10^{-7}$ and $1.00 \times 10^{-5}$ mole of vanadium(V). The method is accurate and precise. A satisfactory procedure for determining vanadium in steels was developed. The interfering effect caused by large amounts of iron(III) was eliminated by removing the iron. An electrolysis using a mercury pool as a cathode was employed. The method was advantageously applied to crude and residual oils.**

The results of the studies conducted by Bhaduri and Ray (*1*) show that in aqueous solutions minute amounts of vanadium(V) will react with salicylohydroxamic acid to give a colored product. Das Gupta (*4*) used benzohydroxamic acid to develop a colorimetric method for vanadium. It was also observed that certain oxygenated organic solvents such as 1-pentanol, amyl acetate, and isobutyl acetate have the ability to extract one or more of these colored products (*1*). Because versatile analytical procedures for determining minute amounts of vanadium are not abundant, the system was investigated in order to determine whether an extraction process could be employed to develop a more sensitive spectrophotometric method.

## APPARATUS

Beckman Model B spectrophotometer equipped with two matched 1.00-cm. cells.

General Electric recording spectrophotometer equipped with two matched 1.00-cm. cells.

Leeds & Northrup pH meter (line-operated).

## REAGENTS

Ammonium metavanadate, $1.00 \times 10^{-3}$ *M*.
Potassium hydroxide.
Sodium hydroxide, 2*M*.
Sulfuric acid, 6*M*.

All of the alcohols which were employed as extractants were distilled prior to use. Solutions of diverse ions were prepared from analytical grade compounds and distilled water.

The potassium benzohydroxamate was prepared according to published techniques (*2*). The product was recrystallized from a solution containing 20% distilled water and 80% methanol. Benzohydroxamic acid is very difficult to dissolve in water, and since potassium benzohydroxamate decomposes rapidly upon standing in basic solutions, the reagent solution was prepared in the following manner: The required amount of purified potassium benzohydroxamate was dissolved in distilled water, the pH adjusted to 5.0, and the solution diluted to volume. A 0.200 *M* reagent solution prepared in this manner is stable for at least 1 month.

## DEVELOPMENT OF METHOD

**Experimental.** The required amount of reagent was added to the solution containing the vanadium in the +5 oxidation state. (When the effects of diverse ions were investigated, they were added before the reagent.) The ionic strength, pH, and volume were adjusted. The extraction was performed with a single, pipetted 20.0-ml. portion of the alcohol, and the extract was centrifuged to remove the droplets of water. The absorbance of the extract was measured at 450 mμ. In each case the alcohol which was employed for the extraction was used as the blank for the absorbance measurements.

**Results and Discussion.** Preliminary Studies. Vanadium(V) in aqueous solution will react with benzohydroxamic acid, yielding a colored product which can be extracted with some oxygenated organic solvents. It was found that all of the vanadium must be in the +5 oxidation state in order to obtain immediate maximum color formation in the nonaqueous phase. Curve *A* in Figure 1 exhibits the spectrophotometric properties of a system containing $5.00 \times 10^{-6}$ mole of ammonium metavanadate and $2.00 \times 10^{-4}$ mole of benzohydroxamic acid in 20.0 ml. of water at pH 2.0. Curve *B* shows the increase in sensitivity that can be obtained when the system is extracted with 20.0 ml. of 1-hexanol. Thus, the molar absorptivity of the complex at 450 mμ is increased from $1.0 \times 10^3$ to $3.5 \times 10^3$ liter per mole-cm. by the nonaqueous solvent.

Effects of Using Various Alcohols for Extraction of Complex. An investigation showed that hydrocarbons and nonoxygenated solvents will not extract the colored complex, while aliphatic alcohols appear to be the best and most inexpensive reagents for this purpose. A study was conducted at pH 2.0 in order to find a satisfactory alcohol for the extraction of the complex. All the extracts were found to have absorbance maxima at 450 ± 5 m$\mu$. The data given in Table I show the effects of using different alcohols. Since with one extraction 1-hexanol removed 97% of the complex under the conditions used and was readily available, it was chosen for the purpose. The complex has an absorbance maximum at 450 m$\mu$ in this solvent, and this wave length was selected for all absorbance measurements.

**Table I. Effects of Using Different Alcohols for Extraction of the Complex**[a]

| Alcohol | $A_{450\ m\mu}$ |
|---|---|
| 2-Methyl-4-butanol | 0.885 |
| Benzyl alcohol | 0.885 |
| 1-Heptanol | 0.885 |
| 1-Octanol | 0.880 |
| 2-Ethyl-1-hexanol | 0.870 |
| 1-Hexanol | 0.865 |
| 1-Decanol | 0.865 |
| 1-Pentanol | 0.860 |
| 2-Hexanol | 0.850 |
| 2-Ethylbutanol | 0.230 |
| Cyclohexanol | 0.020 |

[a] Vanadium(V) = $5.00 \times 10^{-6}$ mole; reagent = $2.00 \times 10^{-4}$ mole.

EFFECT OF AMOUNT OF REAGENT. The effect of the amount of reagent used was examined at pH 2.0. The results listed in Table II exhibit the fact that at least a 10 to 1 ratio of reagent to vanadium(V) is required for $1.00 \times 10^{-5}$ mole of vanadium(V). This amount of vanadium(V) appears to be the upper limit of the method when 20.0 ml. of 1-hexanol are used. In all of the following work $2.00 \times 10^{-4}$ mole of reagent was employed for each extraction.

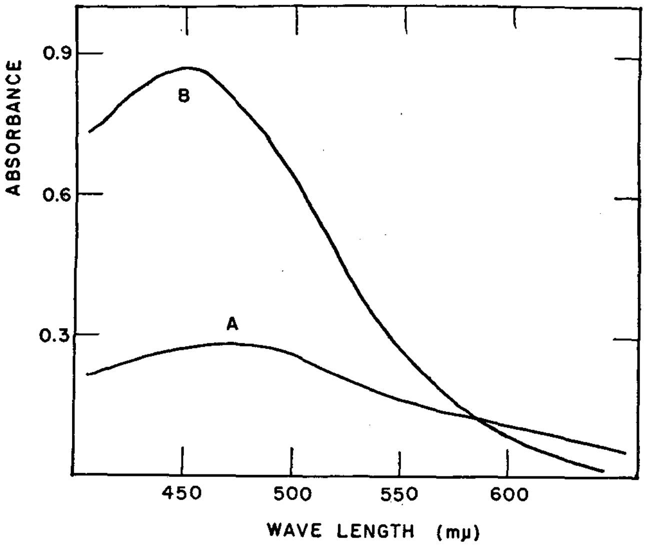

Figure 1. Vanadium(V)–benzohydroxamic acid complex

A. In water
B. In hexanol

It has been noted that the 1-hexanol used in the extraction procedure must be redistilled at intervals of about 2 weeks. Apparently some decomposition product is formed upon storage which causes low results.

EFFECTS OF pH, VOLUME OF AQUEOUS PHASE, AND IONIC STRENGTH. The system was studied with respect to the effect of the pH of the aqueous phase on the amount of complex extracted. The average of 10 absorbance values at pH 2.0 was obtained, and the standard deviation was calculated. If the permissible limits are established as this average $\pm 2\sigma$, constant results are obtained within the pH range 1.2 to 5.5. The system changes below pH 1.2, until at pH 0.2 only 84% of the amount extracted at pH 2.0 is removed. Above pH 5.5 a rapid change takes place, until at pH 8.5 no color is present in the extractant. On the basis of these data, pH 2.0 was chosen for all subsequent work.

It was found that the volume of the aqueous phase can vary between 30 and 90 ml. without effect. For simplicity the volume of the aqueous phase in all subsequent work was adjusted to approximately 50 ml. before extractions.

When potassium chloride is used to adjust the ionic strength, the effect is negligible up to 2.0. Above 2.0 the absorbance decreases slowly, until at $\mu = 4.0$ it is 78% of what it is at $\mu = 0.04$. This variable was neglected since none of the investigations were conducted at higher ionic strengths.

**Table II. Effect of Amount of Reagent**

| Molar Ratio of Reagent to Vanadium(V) | $A_{450\ m\mu}$ |
|---|---|
| 1:1 | 0.400 |
| 2:1 | 0.910 |
| 4:1 | 1.480 |
| 8:1 | 1.720 |
| 10:1 | 1.760 |
| 20:1 | 1.780 |
| 40:1 | 1.750 |

**Table III. Calibration Curve Data and Results of Precision Studies**

| Moles of Vanadium(V) $\times 10^7$ | $A_{450\ m\mu}$[a] | Standard Deviation, $\sigma$[b] |
|---|---|---|
| 5.00 | 0.085 | 0.0038 |
| 20.0 | 0.357 | 0.0070 |
| 50.0 | 0.878 | 0.0080 |
| 100 | 1.754 | 0.0074 |

[a] Average of 10 values.

[b] $\sigma = \sqrt{\frac{(x - \bar{x})^2}{n - 1}}$.

BEER'S LAW. The data given in Table III show that the system obeys Beer's law between the limits of $5.00 \times 10^{-7}$ and $1.00 \times 10^{-5}$ mole of vanadium(V). The standard deviation for each amount of vanadium(V) given in Table III was calculated using 10 absorbance values. The results show that good precision can be obtained within the chosen concentration range.

EFFECTS OF DIVERSE IONS. The effects of diverse ions were investigated. The desired compound was added to the system before the reagent was introduced. The experimental procedure was not altered in those cases in which incomplete dissolution or precipitate formation resulted. An interference was considered to be caused if the absorbance obtained for $5.00 \times 10^{-6}$ mole of vanadium(V) fell $\pm 2\sigma$ from the average value given in Table III.

The following compounds do not interfere when the molar ratio of each to vanadium(V) is 100 to 1: ammonium sulfate, magnesium chloride, calcium nitrate, strontium nitrate, barium chloride, tantalum oxide, chromic nitrate, copper sulfate, zinc chloride, cadmium chloride, potassium dihydrogen phosphate, sodium fluoride, potassium bromide, sodium carbonate, potassium thiocyanate, sodium citrate, sodium-potassium tartrate, potassium perchlorate, boric acid, disodium hydrogen arsenate, ammonium peroxydisulfate, and uranyl acetate.

The data given in Table IV show the compounds which interfere with the method. They can be grouped into one of three main classifications. The powerful oxidizing agents attack the reagent. Strong reducing agents convert the vanadium(V) to vanadium(IV). Removal of these groups should be accomplished without difficulty. Ions which form complexes with the reagent deprive the vanadium(V) of a sufficient amount of reagent to

**Table IV. Interfering Ions**

| Substance Added | Molar Ratio to Vanadium(V) 100:1 | 10:1 |
|---|---|---|
| $K_2TiO(C_2O_4)_2.2H_2O$ | −[a] | − |
| $ZrO(NO_3)_2$ | − | − |
| $K_8Nb_6O_{19}.16H_2O$ | +[b] | 0[c] |
| $MnSO_4$ | − | 0 |
| $FeCl_3$ | − | + |
| $CoCl_2$ | + | 0 |
| $NiSO_4$ | + | 0 |
| $Hg(NO_3)_2$ | + | + |
| $Th(NO_3)_4$ | − | − |
| $Al_2(SO_4)_3$ | − | − |
| $SnCl_4$ | − | − |
| KI | − | 0 |
| $Na_2C_2O_4$ | − | − |
| $K_2Cr_2O_7$ | − | 0 |
| $Na_2MoO_4$ | − | 0 |
| $Na_2WO_4$ | − | − |
| $NaBiO_3$ | − | − |
| $K_2Sb_2O_7.4H_2O$ | − | − |
| $KIO_4$ | − | − |
| NaCN | − | − |
| $Na_2S.9H_2O$ | − | − |
| $NaNO_2$ | − | − |
| $Na_2SO_3$ | − | 0 |

[a] − denotes low results.
[b] + denotes high results.
[c] 0 denotes no interference.

effect maximum color formation in the alcohol layer. The latter appear to be the following: titanium(IV), zirconium(IV), manganese(II), iron(III), thorium(IV), aluminum(III), tin(IV), molybdenum(VI), and tungsten(VI). The addition of excess reagent should remove the interferences of those which give no color: zirconium(IV), manganese(II), thorium(IV), aluminum(III), and tungsten(VI).

**Suggested Procedure for Determining Vanadium(V) in Absence of Interfering Amounts of Diverse Ions.** Add 10.0 ml. of 0.0200*M* benzohydroxamic acid to the solution containing not more than $1.00 \times 10^{-5}$ mole of vanadium(V). Adjust the pH to 2.0 with 6*M* sulfuric acid, and dilute to between 30 to 90 ml. with distilled water. Extract the solution with a pipetted 20.0-ml. portion of 1-hexanol, and centrifuge the extract. Read the absorbance of the extract at 450 mμ against a 1-hexanol blank. Convert the absorbance to amount to vanadium(V) with the aid of a calibration curve. By using larger amounts of reagent and 1-hexanol, greater quantities of vanadium(V) can be determined.

## APPLICATION OF METHOD TO STEELS

As the method is capable of determining minute amounts of vanadium(V), an attempt was made to apply it to the determination of vanadium in steels and alloys. The chief difficulty that was encountered in this investigation was the elimination of the interfering effect caused by iron(III) when its molar ratio to vanadium(V) was 100 to 1 or greater.

**Experimental.** SUMMARY OF ATTEMPTS TO ELIMINATE IRON (III) INTERFERENCE. Qualitative tests showed that the extractable iron(III)-benzohydroxamic acid complex is not readily formed at a low pH. Experiments were conducted at pH 1.3 in the presence of iron(III), but the iron(III) always interfered with the determination of the vanadium(V). Attempts were made to mask the iron(III) with acetylacetone, salicylic acid, fluoride, citrate, tartrate, Versene, and phosphate, but these agents did not prove to be satisfactory. A cation exchange resin was employed to separate vanadium(V) and iron(III) without success. Complexing agents such as fluoride and thiocyanate were used to convert the iron(III) present to negatively charged complexes so that it could be removed with an anion exchange resin. However, some iron(III) always passed through the column.

Hydrochloric acid and isopropyl ether were used to extract iron(III). However, enough vanadium(IV) also was extracted to cause appreciably low results after the vanadium(IV) was oxidized to the +5 state and determined in the usual manner. Attempts were made to remove the iron(III) by complexing it with salicylic acid, thiocyanate, and benzohydroxamic acid and extracting the complexes with aliphatic alcohols, but it was observed that too many extractions are required, and some of the vanadium(V) is also extracted. An attempted distillation of the vanadium(V) from aqua regia was unsuccessful. The hydrous iron(III) oxide was precipitated with ammonium hydroxide and by hydrolyzing urea, but some of the vanadium(V) was always coprecipitated. The previously mentioned methods which were tried in order to eliminate the iron(III) interference failed chiefly because of the large ratio of iron(III) to vanadium(V).

A satisfactory procedure for quantitatively removing large amounts of iron(III) from acidic solutions of vanadium(V) and iron(III) was found. An electrolysis using a mercury pool for a cathode as described by Cain (*3*) is very satisfactory for this purpose.

**Results and Discussion.** The method as given can be used to determine quantitatively amounts of vanadium in steels ranging from 0.03 to 0.5%. The data listed in Table V show the results obtained using several different steels. The low values received for NBS No. 153 are probably due to the presence of some interfering constituent. However, the values obtained for the other samples agree with the accepted ones.

It was found that as the concentration of the sulfuric acid is increased the time of electrolysis also is increased. The presence of nitrate and chloride ions in the electrolyte is undesirable. The former is reduced to nitrogen dioxide at the cathode prolonging the electrolysis, and the latter is oxidized to chlorine which attacks the anode. The treatment of the electrolyzed solution with an oxidant was found to be necessary. If the step is omitted, low results are obtained. Potassium dichromate solution is unsatisfactory; however, potassium peroxydisulfate in the presence of a trace of silver ion was found to be adequate. The treatment with peroxidisulfate and silver ion also converts the small amount of manganese(II) left after the electrolysis to permanganate. This serves to indicate that all of the vanadium is in the +5 state. The permanganate is subsequently destroyed when the solid potassium hydroxide is added.

**Table V. Analyses of Some Steels**

| Sample[a] | Description | Vanadium, % Found | Average value | Accepted value |
|---|---|---|---|---|
| NBS 30a | Cr-V steel | 0.23, 0.20, 0.18, 0.18 | 0.20 | 0.21 |
| NBS 30c | Cr-V steel | 0.25, 0.26, 0.22, 0.26 | 0.25 | 0.24 |
| NBS 153 | Cr-V-Co-Mo-W steel | 1.63, 1.81, 1.86, 1.86 | 1.79 | 2.04 |
| NBS 160 | Cr-Mo-Ni steel | 0.036, 0.033, 0.034, 0.038 | 0.035 | 0.038 |
| Commercial sample | Cr-V-W steel | 0.26, 0.24, 0.22, 0.23 | 0.24 | 0.25 |

[a] NBS, National Bureau of Standards.

In the following procedure an excess of reagent is required in order to give the correct result for the amount of vanadium(V) present. At least $4.00 \times 10^{-4}$ mole must be used. This is probably due to a reaction between the excess peroxydisulfate and the reagent.

**Suggested Procedure for Determining Vanadium in Steels and Alloys.** Dissolve the sample, 0.02 to 0.1 gram, with gentle heating in an acid or suitable mixture of acids. After the sample has dissolved, add 1.5 ml. of sulfuric acid if none was present initially. Evaporate to fumes of sulfur trioxide, cool, and dilute to 40 ml. with distilled water. Heat the solution to boiling, and stir to dissolve any solids. Add 500 grams of mercury, and make the cathode contact with a platinum wire lead sealed in a piece of glass tubing filled with mercury. For an anode immerse a strip of platinum 0.5 inch wide to a depth of about 1 inch in the electrolyte. Stir and electrolyze with 6 or 7 volts and 4 or 5 amperes until the solution gives no pink color when a drop is placed on a few crystals of 1,10-phenanthroline. From 20 to 40 minutes are required for complete removal of the iron.

Isolate the electrolyte with a separatory funnel, and transfer it to a 400-ml. beaker. Add 1 gram of potassium peroxydisulfate, 2 drops of 0.3*M* silver nitrate, a few glass beads, and boil until the volume is about 50 ml. Cool to room temperature, and slowly add solid potassium hydroxide with stirring until the pH is about 1.4. Cool the solution, and add 20.0 ml. of 0.0200*M* reagent. Adjust the pH to 2.0 with 2*M* sodium hydroxide, and extract with 20.0 ml. of 1-hexanol. (The volume of the aqueous layer should be between 70 and 90 ml. at this point.) Wash the beaker and the glass beads with a portion of the aqueous phase, and return the washings to the separatory funnel. Shake the mixture again. Centrifuge the alcoholic extract for a few minutes, and read the absorbance at 450 mμ against a 1-hexanol blank. Convert the absorbance to per cent vanadium with the aid of a calibration curve.

## APPLICATION OF METHOD TO CRUDE AND RESIDUAL OILS

Current methods (*5, 6, 8*) for determining vanadium in crude and residual oils are frequently time-consuming or yield unsatisfactory results. An attempt was made to utilize the sensitivity of the method in its application to samples of crude and residual oils.

Table VI. Results of Analyses of Some Crude and Residual Oils

| Sample | % V (Found) (Wet-Ashing) | | | Average Value | % V (Reported) (Dry-Ashing) |
|---|---|---|---|---|---|
| Crude oil | 0.021 | 0.022 | 0.022 | 0.022 | 0.020 |
| Residual oil A | 0.028 | 0.030 | 0.028 | 0.029 | 0.034 |
| Residual oil B | 0.0015 | 0.0014 | | 0.0014 | 0.0010 |
| Residual oil C | 0.0044 | 0.0038 | | 0.0041 | 0.0032 |
| Residual oil D | 0.013 | 0.015 | | 0.014 | 0.013 |
| Asphalt | 0.031 | 0.030 | | 0.030 | 0.051 |

**Experimental.** The sample of oil was wet-ashed, using sulfuric, nitric, and perchloric acids in that order. In order to maintain a high reaction temperature, the water formed was driven off before each addition of acid. The perchloric acid was not added until all of the easily oxidizable material was eliminated. After the wet-ashing and the volatilization of the excess acids, the procedure for determining vanadium in steel was used.

**Results and Discussion.** The data given in Table VI show the results of the analyses of some crude and residual oils. Considering the fact that dry-ashing procedures were used for the comparative values, it is apparent that the results are in good agreement except in the case of sample 6. This discrepancy has not been explained.

The sensitivity of the vanadium determination permits the use of relatively small samples, thus aiding the speed of decomposition. If iron(III) is known to be absent or present only in small amounts, the electrolysis and subsequent oxidation with peroxydisulfate may be omitted. The treatment with nitric and perchloric acids leaves the vanadium in the quinquivalent state.

The wet-ashing procedure (*7*) is comparatively rapid, requires little attention, and does not involve the larger sample and possible physical losses that dry-ashing does. Thus the combination of the wet-ashing and the benzohydroxamic acid method provides a reliable and convenient approach to the determination of a wide range of concentrations of vanadium in petroleum products.

**Recommended Procedure.** Place the sample (1 to 2 grams) in a round-bottomed flask and attach a condenser by means of a ground-glass connection. Add 5 ml. each of concentrated sulfuric acid and concentrated nitric acid and a few glass beads. Heat at reflux, adding additional 5-ml. portions of nitric acid as the reaction subsides. Before each addition of nitric acid remove the water from the flask by stopping the flow of water through the condenser. Continue the nitric acid treatment (two to 3 additions are usually sufficient) until all particles of carbon are absent, and cool the solution. Add cautiously 3 to 5 ml. of 60 or 72% perchloric acid, slowly heat, reflux until the solution is a light yellow or orange color (5 to 15 minutes), and cool. Remove the condenser and fume off the remaining acids until the volume is about 1 ml. Cool, transfer to an electrolytic beaker, and dilute to 40 ml. Complete the determination as for steel.

## CONCLUSIONS

The method is a satisfactory and versatile one for determining microgram quantities of vanadium. It is sensitive and precise. The system is stable for prolonged periods. The procedure does not require an exceedingly large excess of reagent, and the common variables such as pH, volume of the aqueous layer, and ionic strength can vary within wide limits without affecting the results. Because almost all of the complex is extracted with the first 20.0-ml. portion of 1-hexanol, only one extraction is required. Therefore, the amount of extractant used is the only exact measurement of volume that must be made during the entire procedure. The method as given can be satisfactorily applied to steels ranging from 0.03 to 0.5% vanadium. However, by altering the procedure it can easily be extended outside of these limits. The authors feel that with the proper modifications this method can be used for determining minute amounts of vanadium in carnotite ores, petroleum products, and biological materials.

## ACKNOWLEDGMENT

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# Quantitative Determination of Amino Acids by Circular Paper Chromatography

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**A procedure for the separation of 16 amino acids from a mixture, on a series of circular paper chromatograms by using different solvent mixtures is described. The solvent mixtures used are phenol–1-butanol–acetic acid saturated with water, benzyl alcohol–*tert*-amyl alcohol saturated with water, 1-butanol–acetic acid saturated with water, *m*-cresol saturated with pH 8.4 buffer, and phenol saturated with pH 12 buffer. The chromatograms of the unknown amino acid mixture and known mixture of standard amino acids are developed side by side on the same paper. For the quantitative determination of the amino acids the chromatograms are treated with ninhydrin and the colored bands due to each amino acid in the standard and unknown chromatograms are cut. The color is extracted with 5 ml. of 75% alcohol and estimated in a Klett-Summerson photoelectric colorimeter using 560 mμ filter. The method yields highly reproducible results in the determination of the amino acid content of casein, in agreement with those reported by other workers.**

SINCE the appearance of the now classical publication of Consden, Gordon, and Martin (*3*) on partition paper chromatography, several variations of the technique have been described by many workers (*1*). One of these is due to Rutter (*19*), who employed circular filter paper on which the components separated out in concentric rings instead of the usual spots obtained in the ascending or descending technique with filter paper sheets (*1, 3*). The circular paper chromatographic technique has been applied by different workers to the separation of amino acids (*6–11, 18, 20*). It has been reported to possess certain advantages over the ascending or descending one-dimensional technique, the most important of these being: speed, sharpness of separations, and simplicity and compactness of apparatus; but it suffers from the same disadvantages as the one-dimensional technique in that it has not been found possible so far to separate and identify the different amino acids using any one solvent mixture (*8, 20*). A method for the separation of the overlapping amino acids by circular paper chromatographic technique has recently been described by Giri and Rao (*8*), but this involves a complicated procedure of cutting out, eluting and spotting the overlapping amino acids, and again running a chromatogram with suitable solvents. Recently McFarren (*15*) published a new modification of one-dimensional technique for the separation and identification of the different amino acids from a mixture, by employing filter paper and solvents buffered at selected pH values. McFarren and Mills (*16*) have successfully applied the above procedure for the quantitative determination of amino acids in lactoglobulin.

The present investigation was undertaken with the object of developing a circular paper chromatographic procedure, similar in principle to the buffered paper technique of McFarren (*15*), in view of the numerous advantages possessed by the former method over the ascending or descending techniques on filter paper sheets (*20*). As a result of this investigation, procedures have been developed for the separation of 16 commonly occurring amino acids by circular paper chromatographic technique using different solvent mixtures (see Table I). The separated amino acids have been quantitatively determined by reaction with ninhydrin according to the method described by Thompson *et al.* (*21*) and Giri *et al.* (*10*). Preliminary accounts of the present investigation have already appeared (*12–14*).

## EXPERIMENTAL

**Apparatus.** The apparatus used for the development of chromatograms is shown in Figure 1 and is briefly described below.

A circular glass trough 30 cm. in diameter and 15 cm. in height inverted over a glass plate served as a chromatographic chamber. A circular glass stand (26 cm. in diameter) with four legs (each 5 cm. in height) served as the support for the filter circle. The developing solvent was kept in a small Petri dish (10 ml. in volume) at the center over a raised platform so that the edge of the Petri dish was on the same plane as the circular glass ring. Three Petri dishes containing the components of the solvent mixture were placed at the bottom of the chamber to keep the atmosphere saturated with respect to the solvents and water.

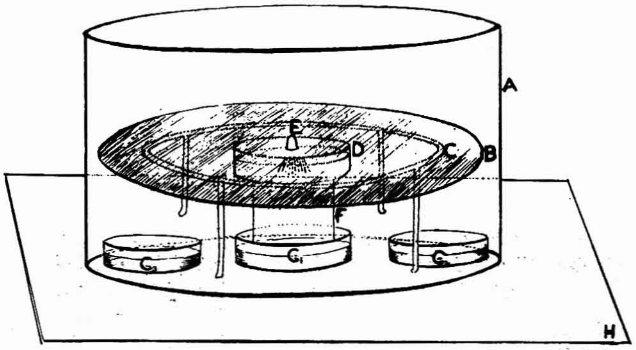

**Figure 1. Apparatus for development of circular paper chromatograms**

**A.** Circular glass trough
**B.** Filter circle
**C.** Circular glass stand
**D.** Petri dish to hold developing solvent
**E.** Wick
**F.** Inverted beaker
**$G_1$, $G_2$, and $G_3$.** Petri dishes to hold components of solvent mixture
**H.** Glass plate

**Table I. Summary of Quantitative Paper Chromatographic Procedure**

| Solvent | pH of Buffer: For saturating solvent | pH of Buffer: For buffering filter circle | Amino Acids Separated |
|---|---|---|---|
| 1-Butanol–acetic acid–water (40:10:50)[a] | .. | .. | Tyrosine, proline, alanine, arginine, and cystine |
| Phenol–1-butanol–acetic acid–water (20:20:8:40)[a] | .. | 2.0 | Tyrosine, alanine, glutamic acid, threonine, lysine, cystine, and histidine |
| Benzyl alcohol–*tert*-amyl alcohol (1:1)[a] saturated with water | .. | No buffer or 8.4 | Phenylalanine, leucine, isoleucine, and methionine |
| Phenol | 12.0 | 12.0 | Threonine, alanine, glycine, serine, aspartic acid, and glutamic acid |
| *m*-Cresol | 8.4 | 8.4 | Phenylalanine, methionine, valine, histidine, tyrosine, arginine, and alanine |

[a] Ratio by volume.

**Table II. Relative Distances Traveled by Amino Acids in Different Solvents**

| | Amino Acids | Phenol–1-Butanol–Acetic Acid–Water (20:20:8:40)[a] | Benzyl Alcohol–*tert*-Amyl Alcohol–water (1:1:2)[a] | Phenol Saturated with pH 12.0 Buffer | *m*-Cresol Saturated with pH 8.4 Buffer | 1-Butanol–Acetic Acid–Water (40:10:50)[a] |
|---|---|---|---|---|---|---|
| 1. | Aspartic | 0.51 | 0.00 | 0.25[b] | 0.00 | 0.46 |
| 2. | Glutamic | 0.64[b] | 0.00 | 0.35[b] | 0.06 | 0.51 |
| 3. | Arginine | 0.51 | 0.14 | 1.00 | 0.42[b] | 0.41[b] |
| 4. | Histidine | 0.48[b] | 0.24 | 0.85 | 0.58[b] | 0.36 |
| 5. | Cystine | 0.38[b] | 0.14 | 0.50 | 0.12 | 0.30[b] |
| 6. | Lysine | 0.44[b] | 0.11 | 0.99 | 0.22 | 0.34 |
| 7. | Serine | 0.52 | 0.12 | 0.47[b] | 0.15 | 0.47 |
| 8. | Glycine | 0.53 | 0.14 | 0.54[b] | 0.21 | 0.47 |
| 9. | Threonine | 0.69[b] | 0.24 | 0.65[b] | 0.27 | 0.54 |
| 10. | Alanine | 0.76[b] | 0.25 | 0.74[b] | 0.36[b] | 0.61[b] |
| 11. | Proline | 0.96 | 0.50 | 1.00 | 0.94 | 0.64[b] |
| 12. | Tyrosine | 0.83[b] | 0.57 | 0.82 | 0.50[b] | 0.70[b] |
| 13. | Tryptophan | 0.97 | ... | 0.96 | 0.87 | 0.88 |
| 14. | Methionine | 0.96 | 0.70[b] | 0.96 | 0.85[b] | 0.84 |
| 15. | Valine | 0.96 | 0.57 | 0.91 | 0.71[b] | 0.82 |
| 16. | Phenylalanine | 0.98 | 1.00[b] | 1.00 | 1.00[b] | 0.94 |
| 17. | Leucine | 1.00 | 0.87[b] | 0.99 | 0.90 | 0.98 |
| 18. | Isoleucine | 1.00 | 0.91[b] | 0.99 | 0.91 | 1.00 |

[a] Ratio by volume.

[b] Amino acids separated from all others sufficiently for identification and quantitative determination.

**Hydrolysis of Proteins.** The hydrolysis of protein was accomplished with 6*N* hydrochloric acid (25 ml. per gram of the sample) by autoclaving at 15-pound pressure for 8 hours. The acid was removed by repeated evaporation at reduced pressure. The residue was taken up in hot water and made up to a known volume, so that the solution contained nearly 2 mg. of amino nitrogen per ml. Aliquots of the extracts were neutralized, before used for the separation of amino acids, with *m*-cresol (at pH 8.4).

**Buffers.** The buffer solutions used in these investigations were prepared according to McFarren (*15*).

**Filter Paper.** Filter circles (diameter 28 cm.) cut from Whatman No. 1 filter paper sheets were used throughout this investigation.

**Solvent Mixtures.** All the solvents used for preparing the solvent mixtures were of analytical reagent quality and were distilled before use.

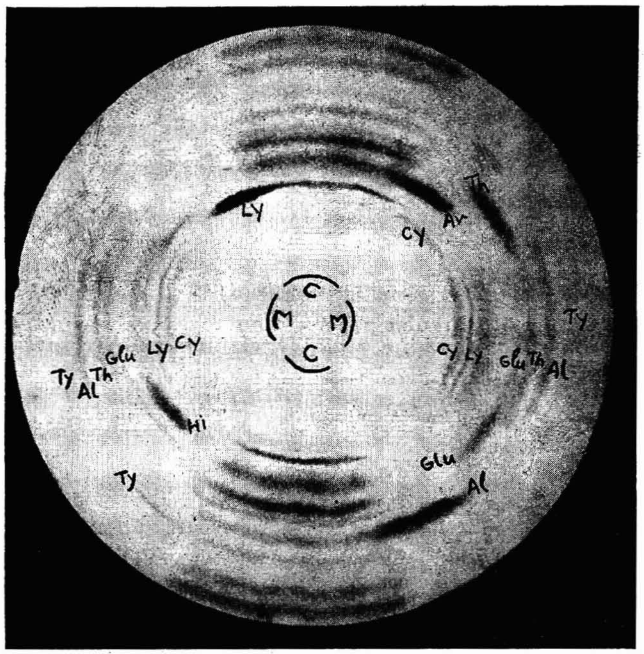

**Figure 2. Circular paper chromatogram showing separation of cystine, lysine, glutamic acid, threonine, alanine, and tyrosine**

**Solvent. Phenol-1-butanol-acetic acid-water (20:20:8:40). Filter circle buffered at pH 2.0.**

| | | | |
|---|---|---|---|
| ***M.*** | **Mixture of known amino acids** | **Glu.** | **Glutamic acid** |
| ***C.*** | **Acid hydrolyzate of casein** | **Th.** | **Threonine** |
| **Cy.** | **Cystine** | **Al.** | **Alanine** |
| **Ly.** | **Lysine** | **Gly.** | **Glycine** |
| **Hi.** | **Histidine** | **Ty.** | **Tyrosine** |
| **Ar** | **Arginine** | | |

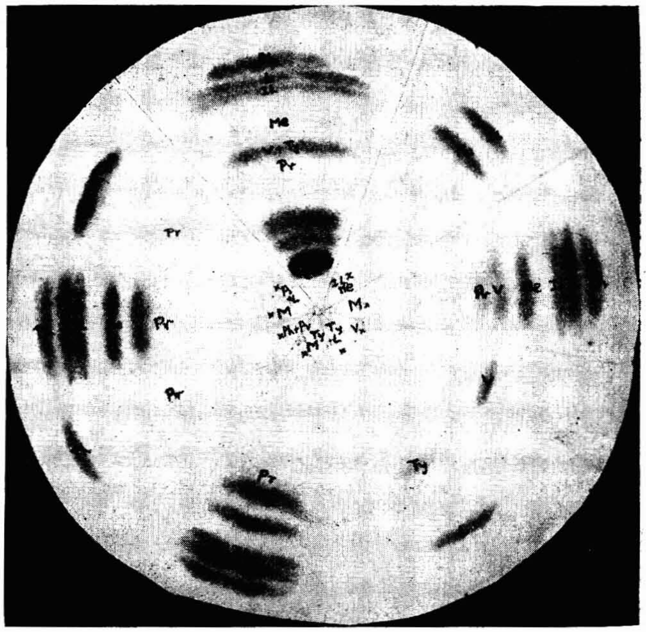

**Figure 3. Circular paper chromatogram showing separation of phenylalanine, leucine, isoleucine, and methionine**

**Solvent. Benzyl alcohol, *tert*-amyl alcohol (1:1) saturated with water.**

| | | | |
|---|---|---|---|
| ***M.*** | **Mixture of known amino acids** | **Ph.** | **Phenylalanine** |
| **Ca.** | **Acid hydrolyzate of casein** | **V.** | **Valine** |
| **Il.** | **Isoleucine** | **Ty.** | **Tyrosine** |
| **L.** | **Leucine** | **Pr.** | **Proline** |
| **Me.** | **Methionine** | | |

Phenol–1-butanol–acetic acid–water. Twenty volumes of phenol were mixed with 20 volumes of 1-butanol, 8 volumes of acetic acid, and 40 volumes of water. The mixture was shaken for 10 minutes and allowed to separate. The top layer consisting of a mixture of solvents was separated and used in conjunction with filter papers buffered at pH 2.0.

Benzyl alcohol–*tert*-amyl alcohol–water. A mixture of 50 volumes of benzyl alcohol and 50 volumes of *tert*-amyl alcohol was saturated with water, by shaking with 100 volumes of water for 10 minutes, and the two layers were then allowed to separate. The top layer was used as solvent in conjunction with either unbuffered filter paper or filter paper buffered at pH 8.4.

Phenol saturated with pH 12.0 buffer was prepared according to McFarren (*15*). This solvent was used in conjunction with filter circles buffered at pH 12.0.

*m*-Cresol saturated with pH 8.4 buffer was prepared according to McFarren (*15*). This solvent was used in conjunction with filter papers buffered at pH 8.4.

1-Butanol–acetic acid–water. This solvent mixture was prepared according to Partridge (*17*) by shaking 40 volumes of 1-butanol with 10 volumes of acetic acid and 50 volumes of water. After settling, the top layer was used as the solvent.

**Procedure for Separation and Determination of Amino Acids.** On the circumference of a circle (about 4 cm. in diameter) drawn from the center of a circular filter paper (Whatman No. 1) of 28 cm. in diameter, six equidistant points were marked. The test sample and known quantities of standard amino acid solutions were spotted at alternate points. Usually in the case of the test solution, a sample containing 20 $\gamma$ of amino nitrogen was spotted to get good chromatograms. In the case of known amino acids, 4 to 8 $\gamma$ of each amino acid was used. Spotting of the solutions was done with a calibrated micropipet in a thin streak (1 cm. in length) along the line of the circumference of the circle on either side of the marked points. In the case of dilute standard amino acid or unknown solutions, it is necessary to build up the required concentration on paper by repeated spotting in 5-$\mu$l. quantities with drying in between in a current of warm air.

A wick rolled from a strip of filter paper (2 × 3 cm.) and cut at one end in the form of a brush, similar to that described by Giri *et al.* (*9*), was fixed to a hole at the center of the filter paper. The filter paper was placed on the glass stand with the brushlike end of the wick dipping in the solvent in the Petri dish inside the chromatographic chamber. The chromatogram was irrigated for 8 to 12 hours until the solvent front reached the edge of the paper, the time taken being controlled by the size of the wick. The filter paper was taken out, dried in a current of warm air, and irrigated again in the above manner. After the chromatogram had been irrigated two or three times, the filter paper was dried and sprayed with 0.5% ninhydrin in acetone containing 5% acetic acid. The chromatogram was heated at 60° C. for 15 minutes to develop the color of the amino acids.

For quantitative determination of the amino acids, the ninhydrin stained bands corresponding to the standard and unknown of each amino acid were cut, rolled, and placed in separate test tubes containing 5 ml. of 75% alcohol. The color was eluted by gently agitating the contents of the test tube for 5 minutes. The color of the extract was measured in a Klett-Summerson colorimeter using a 560 mμ filter. The quantity of the amino acid in the test sample was estimated from the calibration curve drawn for each of the amino acids. It is desirable to draw the calibration curve for the amino acids by running the mixture of different amino acids of known concentrations on the same paper, side by side, with the test samples.

### Table III. Determination of Certain Amino Acids in Casein Using Different Solvent Mixtures

| Amino Acids | Solvent | Amino Acid Values (Calculated to 16 Grams of Nitrogen) |
|---|---|---|
| Cystine | (1) Phenol-1-butanol-acetic acid-water | 0.62 |
| | (2) 1-Butanol-acetic acid-water | 0.60 |
| Arginine | (1) 1-Butanol-acetic acid-water | 4.9 |
| | (2) *m*-Cresol saturated with pH 8.4 buffer | 5.0 |
| Threonine | (1) Phenol saturated with pH 12.0 buffer | 5.0 |
| | (2) Phenol-1-butanol-acetic acid-water | 4.8 |
| Alanine | (1) 1-Butanol-acetic acid-water | 3.1 |
| | (2) *m*-Cresol saturated with pH 8.4 buffer | 3.0 |
| | (3) Phenol saturated with pH 12.0 buffer | 3.2 |
| | (4) Phenol-1-butanol-acetic acid-water | 3.0 |
| Tyrosine | (1) 1-Butanol-acetic acid-water | 6.1 |
| | (2) Phenol-1-butanol-acetic acid-water | 6.0 |
| Methionine | (1) *m*-Cresol saturated with pH 8.4 buffer | 3.3 |
| | (2) Benzyl alcohol-*tert* amyl alcohol-water | 3.4 |
| Phenylalanine | (1) *m*-Cresol saturated with pH 8.4 buffer | 6.2 |
| | (2) Benzyl alcohol-*tert* amyl alcohol-water | 6.0 |

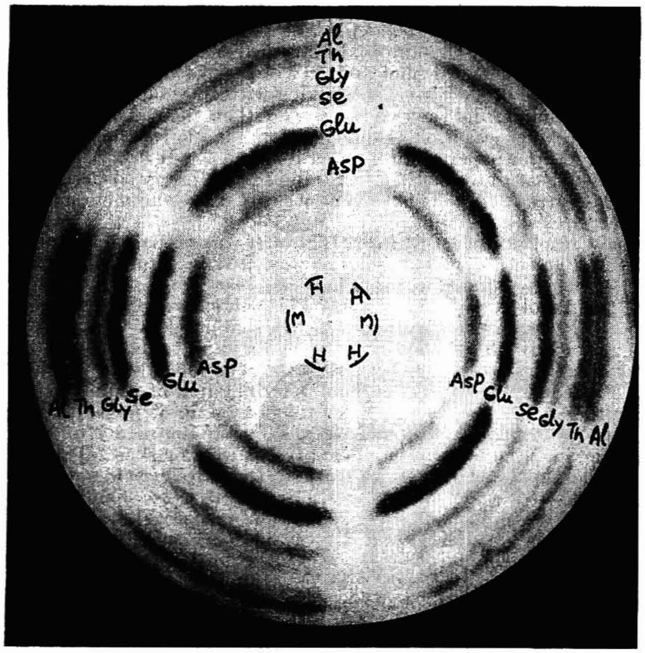

**Figure 4. Circular paper chromatogram showing separation of aspartic acid, glutamic acid, serine, glycine, threonine, and alanine**

**Solvent. Phenol-saturated with pH 12.0 buffer. Filter circle buffered at pH 12.0.**

| | | | |
|---|---|---|---|
| ***M.*** | **Mixture of known amino acids** | **Se.** | **Serine** |
| ***H.*** | **Acid hydrolyzate of protein** | **Gly.** | **Glycine** |
| **Asp.** | **Aspartic acid** | **Th.** | **Threonine** |
| **Glu.** | **Glutamic acid** | **Al.** | **Alanine** |

### Table IV. Amino Acid Composition of Casein

Calculated to 16 grams of nitrogen

| Amino Acid | Present Results | Block (*2*) |
|---|---|---|
| Aspartic | 6.4 | 7.0 |
| Glutamic | 25.1 | 23.0 |
| Arginine | 4.9 | 4.2 |
| Lysine | 8.6 | 8.5 |
| Histidine | 3.7 | 3.2 |
| Cystine | 0.6 | 0.4 |
| Serine | 5.5 | 6.8 |
| Glycine | 2.4 | 2.1 |
| Threonine | 4.9 | 4.5 |
| Tyrosine | 6.1 | 6.4 |
| Alanine | 3.1 | 3.3 |
| Methionine | 3.4 | 3.5 |
| Valine | 6.8 | 7.7 |
| Phenylalanine | 6.1 | 6.3 |
| Leucine | 10.7 | 10.0 |
| Isoleucine | 7.0 | 7.5 |

## RESULTS

The composition of the different solvent mixtures used for the separation of various amino acids is presented in Table I.

Table II gives the relative distance traveled by different amino acids with the various solvent mixtures.

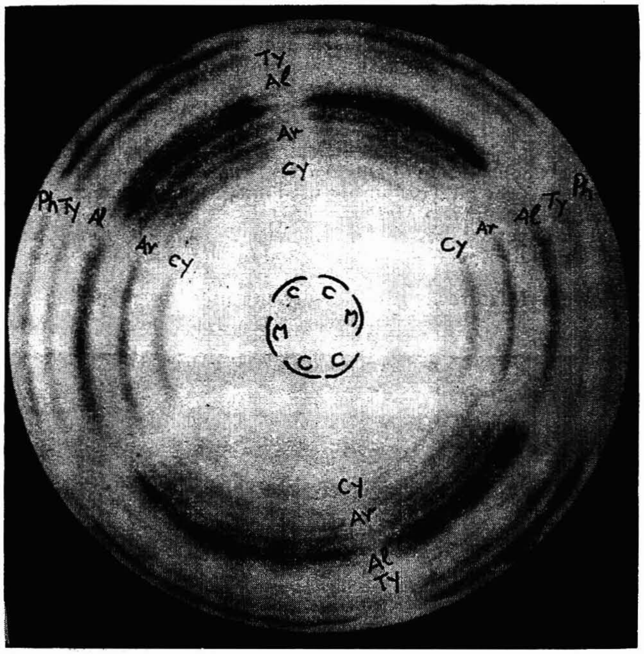

**Figure 5. Circular paper chromatogram showing separation of cystine, arginine, alanine, and tyrosine**

**Solvent. 1-Butanol-acetic acid-water (40:10:50)**

| | | | |
|---|---|---|---|
| ***C.*** | **Acid hydrolyzate of protein** | **Al.** | **Alanine** |
| ***M.*** | **Mixture of known amino acids** | **Ty.** | **Tyrosine** |
| **Cy.** | **Cystine** | **Ph.** | **Phenylalanine** |
| **Ar.** | **Arginine** | | |

Typical chromatograms showing the separation of different groups of amino acids by the different solvent mixtures are shown in Figures 2 to 6.

Table III gives the content of certain amino acids in casein determined after separating them on the circular paper chromatograms by different solvent systems. There is good agreement between the values obtained. The figures obtained for the content of 16 amino acids in casein by the present procedure together with the values reported in the literature (*2*) are presented in Table IV.

## DISCUSSION

The results obtained in the present investigation show that 16 amino acids could be quantitatively determined by the procedure outlined in this paper. The advantages of the present

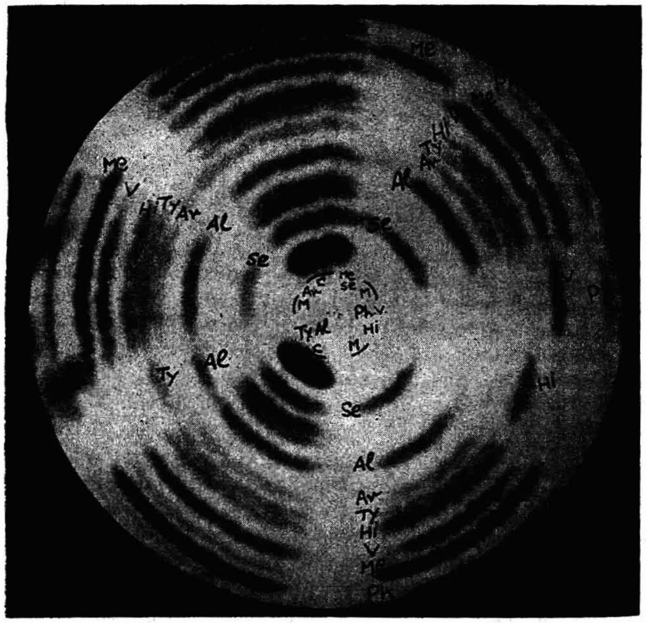

**Figure 6. Circular paper chromatogram showing separation of phenylalanine, methionine, valine, histidine, tyrosine, arginine, and alanine**

**Solvent.** *m*-Cresol saturated with pH 8.4 buffer. Filter circle buffered at pH 8.4.

| | | | |
|---|---|---|---|
| *C.* | Acid hydrolyzate of casein | V. | Valine |
| *M.* | Mixture of known amino acids | Hi. | Histidine |
| Me. | Methionine | Al. | Alanine |
| Se. | Serine | Ty. | Tyrosine |
| Ph. | Phenylalanine | Ar. | Arginine |

method over the techniques described by other workers are speed of separation, clarity and compactness of amino acid zones, and the ready availability, at low cost, of the solvents required. Table III shows that some of the amino acids can be separated and estimated by more than one solvent mixture. This has served as a check both on the values obtained for these amino acids and also on the accuracy of the method.

The separation of lysine from the other basic amino acids has engaged the attention of many workers in the past. Consden, Gordon, and Martin (*3*) and Dent (*4*) succeeded in separating the above amino acids from a mixture, by two-dimensional chromatography using phenol and collidine as the developing solvents. McFarren (*15*) recommended the use of lutidine saturated with pH 6.2 buffer for the same purpose by the undimensional technique. In the present method the separation of lysine from other amino acids has been achieved by a cheap solvent system consisting of phenol–1-butanol–acetic acid–water. This solvent mixture is easy to handle, unlike collidine and lutidine which possess an unpleasant odor.

Very few solvents have so far been suggested in the literature for the separation of leucine and isoleucine from each other and from other amino acids. Consden, Gordon, and Martin (*3*) used benzyl alcohol–water or 1-butanol–water or a mixture of the two in equal proportions in unidimensional chromatograms, for this purpose. Edman (*5*) and Wretlind (*23*) suggested the use of pyridine–amyl alcohol–water mixture. Work (*22*) reported that *tert*-amyl alcohol saturated with water was satisfactory for the separation of leucine, isoleucine, and phenylalanine, when the paper strip was irrigated for 3 days. McFarren (*15*) recommended the use of benzyl alcohol–1-butanol saturated with pH 8.4 buffer in conjunction with a filter paper buffered at the same pH. None of the above solvent systems proved quite satisfactory in the authors' hands for this purpose. The separation of leucine, isoleucine, and phenylalanine was finally achieved by using as solvent a mixture of *tert*-amyl alcohol and benzyl alcohol saturated with water.

Work is now in progress on the analysis of some food proteins using the procedure described in this paper.

## ACKNOWLEDGMENT

The authors wish to thank V. Subrahmanyan for his keen interest and helpful suggestions.

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# CORRECTIONS

## Precision in X-Ray Emission Spectrography

In the article on "Precision in X-Ray Emission Spectrography" [Liebhafsky, H. A., Pfeiffer, H. G., and Zemany, P. D., Anal. Chem., 27, 1257 (1955)], in the third paragraph from the end the last sentence should read: Further, when $s_A \gg s_C$, an increase in the number of the counts will not necessarily reduce $s_A$.

## Determination of Nordihydroguaiaretic Acid in Creosote Bush

In the article on "Determination of Nordihydroguaiaretic Acid in Creosote Bush" [Page, J. O., Anal. Chem., 27, 1266 (1955)], the first line of data on unrecrystallized nordihydroguaiaretic acid tetraacetate should read:

| | | | | | | |
|---|---|---|---|---|---|---|
| NDGA, twice recryst. m.p. 186–7° C. | 0.9792 | 1.5214 | 1.5237 | −1.5 | 66.52 | 6.65 |

# Paper Chromatography of Sugar Alcohols and Their Glycosides

J. CERBULIS[1]

*Stephen F. Whitman & Son, Inc., Philadelphia 5, Pa.*

**$p$-Anisidine is found to be a very valuable reagent for the detection of sugar alcohols and their glycosides on paper chromatography. The filter paper is sprayed with the reagent, and after being heated at 100° C. for 10 to 15 minutes, turns light brown. Sugar alcohols and their glycosides leave white spots. These white spots can be seen after several months and can be distinguished from yellow or brown sugar spots. The heat and reagent do not affect the consistency of filter paper which can be handled and stored without any difficulties. $p$-Anisidine is a useful reagent of greater sensitivity than ammoniacal silver nitrate for detection of sugar alcohols and their glycosides.**

MANY reagents have been used for the detection of sugars by paper chromatography (*2*), but only a few reagents have been used for the detection of sugar alcohols. Ammoniacal silver nitrate in different variations has been the most widely used reagent for polyols (*14, 16*).

During the last several years boric acid–phenolphthalein, boric acid–phenol red (*6*), boric acid–methyl red (*13*), vanillin–perchloric acid (*5*), lead tetraacetate (*4*), and alkaline periodate–permanganate (*7*) have also been recommended as reagents for the detection of polyols.

The author has found $p$-anisidine phosphoric acid (*12*) to be a very valuable reagent for the detection of polyols on paper strips. This reagent, used for 2 years in research on carbohydrates in the cacao bean, has been proved to be somewhat superior to ammoniacal silver nitrate (*17*) and the procedure is much simpler. With $p$-anisidine detection is possible for sugar alcohols, glycosides of sugar alcohols, and reducing and nonreducing sugars in the same run.

## EXPERIMENTAL

**Apparatus and Materials.** Chromatographic apparatus consisted of a cylindrical glass jar, 25 cm. in diameter and 45 cm. in height, with a tight-fitting ground-glass cover, which served as the chamber.

Filter paper, Whatman No. 1, H. Reeve Angel & Co., Inc., New York.

**Reagent.** $p$-Anisidine reagent was prepared by dissolving 0.5 gram of $p$-anisidine in 3 ml. of phosphoric acid (sirupy, 85 to 88%). Small amounts of precipitate, if any, were not filtered off. The solution was diluted with 100 ml. of 80 volume % aqueous methanol.

**Procedure.** The solutions of polyols (about 8% concentration) were applied in triplicate as spots (5 mm. in diameter) about 2 cm. apart on a starting line 4 cm. above the edge of the paper sheet. The chromatograms were developed by the ascending method for 24 hours. After the solvent had advanced to about 32 cm., the sheet was dried in air for 1 hour, immersed in a shallow pan containing the $p$-anisidine reagent, removed, and gently blotted to remove excess reagent. Then the sheet was heated at 100° C. for approximately 10 to 15 minutes until the paper turned light brown. The sugar alcohols appeared as white spots on a light brown background. Floridoside and umbilicin gave brown spots (Table I). A marked contrast in the color was noted between the spots and the background.

Replicate $R_f$ determinations were found to agree within ±2%. As a check, sugar alcohols were also determined by ammoniacal silver nitrate (*17*) and vanillin–perchloric acid (*5*).

## RESULTS AND DISCUSSION

The $p$-anisidine reagent is recommended for the detection of sugar alcohols and their glycosides on paper chromatography. The analytical results are shown in Table I. The $R_f$ values were determined using Whatman No. 1 filter paper and a mixture of 1-propanol–ethyl acetate–water as the irrigant. The spots of almost all polyols were clearly distinguishable. D-Volemitol, 1-D-mannitol-monoacetate, and dulcitol gave somewhat weaker spots, which were recognizable. Only D-perseitol gave a very weak spot test, or none at all, with the 8% concentration used. Paper strips on which $p$-anisidine reagent was used could be stored very easily and the spots could be distinguished after several months.

The sensitivity of ammoniacal silver nitrate reagent was similar to the $p$-anisidine reagent. Umbilicin and 1-D-mannitol monoacetate treated with ammoniacal silver nitrate gave very weak spots. Chromatograms were handled without any difficulties.

Vanillin–perchloric acid reagent proved to be as satisfactory as the other reagents, but the procedure was more complex. According to the recommendations of Godin (*5*), the strips were heated for a longer period at 85° C. The disadvantages of this reagent were: The spots faded rapidly and required marking immediately upon removing the strips from heat; and on heating the paper strips became very brittle.

**Table I. $R_f$ Values of Polyols**

| Polyols | Lit. Cited | Average $R_f$[a] | Color of Spot with $p$-Anisidine Reagent |
|---|---|---|---|
| 1-D-Mannitol monoacetate | (*8*) | 0.63 | White |
| Glycerol | | 0.58 | White |
| Erythritol | | 0.49 | White |
| L-Arabitol | | 0.43 | White |
| Adonitol (ribitol) | | 0.40 | White |
| Pinitol | | 0.36 | White |
| Floridoside (α-D-galactopyranosyl-2-glycerol) | (*15*) | 0.36 | Brown |
| Umbilicin (3-D-arabitol-β-D-galactofuranoside) | (*11*) | 0.35 | Brown |
| D-Mannitol | | 0.34 | White |
| D-Sorbitol (D-glucitol) | | 0.31 | White |
| Dulcitol (galactitol) | | 0.31 | White |
| D-Volemitol | | 0.28 | White |
| D-Perseitol | | 0.25 | No spot |
| 3-Floridoside-α-D-mannoside | (*9*) | 0.18 | White |
| 1-D-Mannitol-β-D-glucopyranoside | (*8*) | 0.15 | White |
| Inositol | | 0.11 | White |
| 1- and 7-D-Volemitol-β-D-glucopyranoside[b] | (*10*) | 0.11 | White |
| 1,6-D-Mannitol-di-(β-D-glucopyranoside) | (*8*) | 0.05 | White |
| Galactinol(*O*-α-galactopyranosyl-*myo*-inositol) | (*3*) | 0.03 | White |

[a] Values represent average of triplicate determinations on Whatman No. 1 paper by ascending method run in solvent system of 1-propanol–ethyl acetate–water (7:1:2, vol. %) (*1*) at 20–21° C.

[b] Mixture of both substances.

## ACKNOWLEDGMENT

The author wishes to express his appreciation to C. Clay for stimulating comments and suggestions and to T. H. Sharp for his interest in this work. He also wishes to thank the following for generous supply of various compounds:

| | |
|---|---|
| Pinitol | A. B. Anderson, Forest Products Laboratory, University of California, Berkeley, Calif. |
| D-Perseitol | A. A. Benson, Radiation Laboratory, University of California, Berkeley 4, Calif. |
| Galactinol | R. J. Brown and R. F. Serro, The Great Western Sugar Co., Denver, Colo. |
| Floridoside<br>Umbilicin<br>D-Volemitol<br>1-D-Mannitol monoacetate<br>3-Floridoside-α-D-mannoside<br>1-D-Mannitol-β-D-glucopyranoside<br>1- and 7-D-Volemitol-β-D-glycopyranoside<br>1,6-D-Mannitol-di-(β-D-glucopyranoside) | B. Lindberg, The Royal Institute of Technology, Division of Organic Chemistry, Stockholm 70, Sweden |

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# Colorimetric Determination of Low Concentrations of Dissolved Oxygen in Water

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**There is a need for a less complex and more accurate method for determining dissolved oxygen concentrations in the 0 to 50 parts per billion range. A colorimetric method has been developed employing reduced indigo carmine. The oxygen is estimated either by comparing by eye with artificial color standards, or with a spectrophotometer. Oxygen in concentrations of 0 to 25 p.p.b. can be determined by eye with an average deviation of 2 p.p.b. The procedure adopted is free of the difficulties of air entrapment, reagent blank, and complicated sampling. It can be used by inexperienced boiler plant personnel to determine dissolved oxygen accurately in boiler feed water, or in precise laboratory determination.**

FEED water for high-pressure boilers must be free essentially of dissolved oxygen, which causes pitting of the steel surfaces. Mechanical deaeration can reduce the dissolved-oxygen content of feed water to the range of approximately 0.01 p.p.m. (10 p.p.b.). Chemical reducing agents, notably sodium sulfite, are employed for scavenging the last traces of dissolved oxygen. As insurance that these mechanical and chemical processes are effective, boiler operators need a method for accurate determination of dissolved-oxygen concentration of 20 p.p.b. or less.

Methods currently employed for the determination of dissolved oxygen are based upon that of Winkler. The so-called "straight Winkler" is insensitive to dissolved oxygen in concentrations below 20 p.p.b., and is subject to interference by either oxidizing or reducing materials which may be present in the sample. Modifications developed by Schwartz and Gurney (*6*), and by Adams, Barnett, and Keller (*1*), offer precision and accuracy of the order of 2 p.p.b. Both have been used widely for referee work, that of Adams *et al.* having been adopted as the referee method of the American Society for Testing Materials. However, both of these methods are cumbersome and demanding. They require duplex sampling in special glassware, skilled manipulation in sampling and titration, and the use of a comparatively expensive electrometric titrator. Neither is ideal for frequent control analysis by power-plant operators.

The existing need for simplicity and sensitivity to low concentration suggested a colorimetric method. This paper recounts a successful search for, and development of, such a method.

A search of the literature showed that several previous investigators have explored this field. Procedures based on color developed with the iodine or chlorine released in a modified Winkler procedure were tried but abandoned, because of the manipulations involved (*5*). Records of several reagents which react directly with dissolved oxygen to yield colors were found, and four of these colors were surveyed. Amidole (*2*), adurol (*9*), pyrogallol (*8*), and indigo carmine (*4*) produced colors which are related to dissolved-oxygen concentration. Trail confirmed the findings of previous investigators that all of these reagents were unstable, the developed colors were inconsistent, or the test was insensitive. Despite this negative information, further investigation was devoted to indigo carmine, because of the wide range of colors which it develops. Upon oxidation the bright yellow-green color of reduced indigo carmine changes to orange, to red, to purple, to blue, and finally to blue-green in the completely oxidized form. Three problems needed to be solved to provide a usable indigo carmine method: development of an air-stable, reduced, indigo carmine reagent; development of a procedure by which a measured amount of reagent could be added to a representative, segregated water sample; and calibration of developed colors and provision of color standards for comparator determinations.

## DEVELOPMENT OF INDIGO CARMINE REAGENT

Efimoff (*4*) employed a 0.1% solution of indigo carmine in water to which was added 1% of glucose as reducing agent and 1% of potassium carbonate as pH controller. This was stored under mineral oil to prevent atmospheric oxidation. This barrier is not completely effective, and pipetting the reagent with a protective slug of oil above and below it in the pipet is too complicated for boiler-plant operators. Potassium hydroxide was substituted for potassium carbonate in the reducing solution. In an attempt to sharpen the colors, a one to one mixture of glycerol and water was substituted for water as solvent for the reagent. This not only brightened the colors, but provided also for more rapid initial reduction of indigo carmine and a more air-stable reagent solution.

## STABILITY OF SOLUTIONS

The air stability of the stock indigo carmine solution was evaluated by storage tests. Typical stock solutions were prepared

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and stored in bottles in a refrigerator, in a dark cabinet at ambient temperature, and on a reagent shelf in the laboratory. The stock solution is usable for at least 90 days if stored in the refrigerator and for at least half that long if stored in the dark at ambient temperature. It deteriorates rapidly if permitted to stand at ambient temperature in an ordinary reagent bottle.

## SAMPLING AND ADDITION OF REAGENT

Having provided a suitable colorimetric reagent, successful use required devising a means for sampling the water and adding the reagent to it. The water sample must be collected directly in the container in which the final color comparison is to be made. First, attempts were made using square bottles and underwater sampling as suggested by Dougherty (*3*). These bottles were not sufficiently uniform to be ideal comparator tubes, but more serious was the difficulty with reagent addition. The sampling tube, which was finally devised and proved satisfactory, is illustrated in Figure 1.

## REAGENTS

**Indigo Carmine,** stock solution. Dissolve 0.018 gram of reagent grade indigo carmine and 0.20 gram of reagent grade glucose in enough water to make 5 ml. of solution. Add 75 ml. of reagent grade glycerol and mix thoroughly. Application has been made for letters patent covering the indigo carmine reagent.

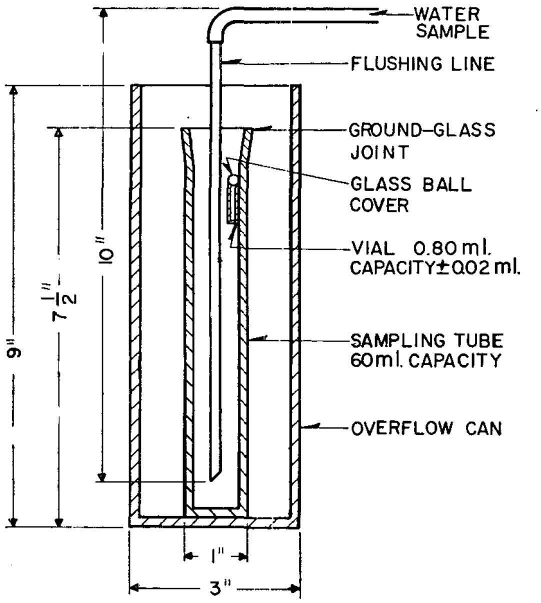

**Figure 1. Sampling assembly**

**Potassium Hydroxide Solution.** Dissolve 37.5 grams of reagent grade potassium hydroxide in enough water to give 100 grams of final solution.

**Leuco Reagent.** In a small bottle mix 8 ml. of stock indigo carmine solution and 2 ml. of 37.5% potassium hydroxide. Cap and invert several times, until the solutions are well mixed. Allow the reagent to stand undisturbed until the initial dark red color changes to lemon yellow in approximately 10 minutes.

## ARTIFICIAL COLOR STANDARDS

**Master Solutions** (*7*). RED SOLUTION. Dissolve 59.29 grams of cobaltous chloride, $CoCl_2.6H_2O$, in sufficient 1% hydrochloric acid to produce 1 liter of solution.

YELLOW SOLUTION. Dissolve 45.05 grams of ferric chloride, $FeCl_3.6H_2O$, in sufficient 1% hydrochloric acid to produce 1 liter of solution.

BLUE SOLUTION. Dissolve 62.45 grams of cupric sulfate, $CuSO_4.5H_2O$, in sufficient 1% hydrochloric acid solution to produce 1 liter.

WORKING SOLUTIONS. In 60-ml. glass-stoppered Nessler tubes place the amounts of stock color solutions shown in Table I, and 0.5 ml. of concentrated hydrochloric acid. Add distilled water within 1/4 inch of the top of the tubes and cap them. The diluted color standards are stable for 24 hours, but are reliable only when viewed under a daylight lamp.

**Table I. Artificial Color Standards**

| Equivalent P.P.B. Dissolved Oxygen | Ml. of Master Solutions | | | |
|---|---|---|---|---|
| | Yellow | Red | Blue | Color of Mixture |
| 0 | 7.0 | 0.15 | ... | Yellow-green |
| 5 | 4.00 | 1.00 | ... | Greenish yellow |
| 10 | 2.50 | 1.25 | ... | Yellow |
| 15 | 2.0 | 1.88 | ... | Orange |
| 22 | 1.00 | 2.88 | ... | Pink |
| 28 | 0.70 | 2.90 | ... | Red |
| 44 | 0.40 | 3.20 | 0.55 | Purplish red |
| 62 | 0.20 | 4.60 | 3.63 | Reddish purple |
| 79 | 0.05 | 5.80 | 5.35 | Purple |
| 94 | 0.15 | 6.10 | 8.45 | Purple |
| 114 | .. | 5.70 | 10.40 | Purplish blue |
| 122 | .. | 3.70 | 11.10 | Blue |
| 126 | .. | 3.90 | 11.50 | Blue |
| 142 | 0.05 | 2.30 | 13.90 | Blue-green |
| 170 | 0.10 | 0.35 | 9.05 | Blue-green |

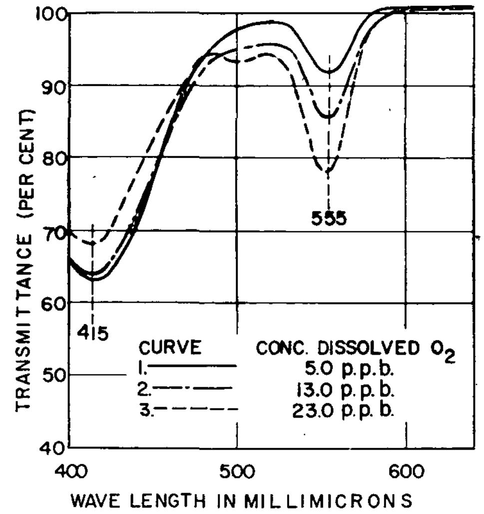

**Figure 2. Spectral curves of sample solutions**

## APPARATUS

**Sampling Tube.** [The sampling tube—without vial—used in the development work was supplied by the Will Corp., Baltimore, Md. (Figure 1).] Attach a glass-stoppered Nessler-type tube of 60-ml. capacity with a vial of exactly 0.8-ml. capacity to the inner wall with open top up, 1 inch below the bottom of the glass stopper; a glass ball approximately equal to the outside diameter of the vial serves as a cover for it. Application has been made for letters patent covering this sampling tube.

**Container for Submerged Sampling.** Use a can or metal cylinder 3 inches in diameter and 9 inches in height.

**Sampling Line.** Use an 8-mm. glass tube 10 inches long with a bevel end.

**Guide Tube.** Use a 4-inch length of glass tubing with an inside diameter slightly larger than the glass ball used to seal the vial.

## PROCEDURE

**Sampling.** Start the sample water flowing in the sampling container at a temperature below ambient and not above 70° F. Allow it to fill the container and overflow for at least 10 minutes.

With a dropping pipet fill the sample tube's inner vial with the leuco reagent. If air bubbles are trapped in the dense reagent allow them to rise and disperse so that the vial is completely full and free of air. Put a glass ball on the top of the vial by dropping it through the guide tube. Submerge the sample tube in the sampling container, being careful not to dislodge the ball. Transfer the sampling line from the sampling container to the sampling tube, and allow the water to flow for at least 10 minutes at a rate of 200 to 1500 ml. per minute. Transfer the line from the sampling tube back to the sampling container without breaking the water surface above the can. Wet the stopper by immersing it in the sampling container and put it in the sample tube without trapping air.

**Determination.** Remove the tube from the jacket, invert it so that the glass ball falls off the vial, and mix the contents by repeated inversions. A color indicative of the dissolved-oxygen concentration will develop. Match this color under a daylight or fluorescent lamp by placing the lamp above the tube stopper, and view the colors in an oblique mirror held below the tubes. Use standards representing various increments of dissolved oxygen in the expected range (see Table I).

**Table II. Variation of Transmittance with Oxygen Content**

| Dissolved Oxygen, P.P.B. | Per Cent Transmittance[a] |
|---|---|
| 5 | 91.5 |
| 12 | 85.0 |
| 23 | 77.5 |

[a] All readings were taken on a General Electric recording spectrophotometer at 555 mμ.

## SPECTROPHOTOMETRIC STUDIES

Preliminary studies were made on the possible use of spectrophotometer in determining concentrations of dissolved oxygen. After developing the color in the described fashion, the transmittance curves were taken on the unopened sample with a General Electric recording spectrophotometer. Transmittance curves of color developed at various dissolved oxygen concentrations are given in Figure 2. Similar runs were carried out on the Bechman Model B spectrophotometer. Transmittance minima appear at 415, 555, and 620 mμ. The best wave length for low oxygen determinations seem to be 555 mμ.

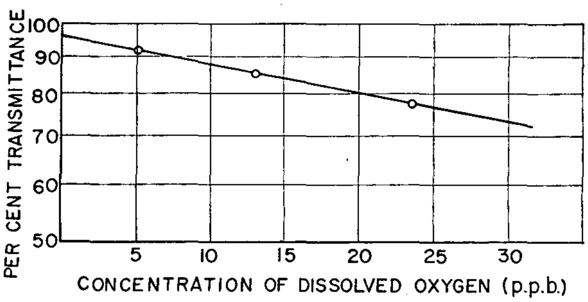

**Figure 3. Transmittance curve of sample solutions at 555 mμ**

Table II shows per cent transmittance on the General Electric recording spectrophotometer at 555 mμ for varying concentrations. These points follow Beer's law, as shown in Figure 3. Either of these instruments might be the basis for accurate laboratory or industrial method for the determination of dissolved oxygen.

## EXPERIMENTAL DATA

In order to determine the reproducibility of the method, samples of deaerated water were analyzed simultaneously by it and ASTM referee method D 888 (*1*). One series of runs was made on water from a laboratory deaerator. Another series was made on a large power plant deaerator at the Consolidated Gas Electric Light and Power Co. at Baltimore, Riverside Station, Baltimore, Md. In some of the tests, oxygen saturated water was bled into the system to give various dissolved-oxygen concentrations. In others, water containing sodium sulfite was used to test the effect of this reducing agent. The results of these tests are given in Tables III and IV.

**Table III. Experimental Data for Laboratory Deaerator**

| | Dissolved Oxygen Found, P.P.B. | | |
|---|---|---|---|
| Time[a] and Date | ASTM method D 888 | Indigo carmine method | Difference |
| Aug. 22, 1952 | | | |
| 1015 | 25 | 25 | 0.0 |
| 1045 | 4[b] | 21 | 0.0 |
| 1115 | 13 | 17 | 4.0 |
| 1245 | 13 | 15 | 2.0 |
| 1345 | 12 | 10 | 2.0 |
| 1430 | 6 | 8 | 2.0 |
| 1530 | 9 | 6 | 3.0 |
| Aug. 25, 1952 | | | |
| 1230 | 17 | 16 | 1.0 |
| 1345 | 12 | 12 | 0.0 |
| 1450 | 11 | 10 | 1.0 |
| 1545 | 9 | 10–11 | 1.5 |
| Aug. 26, 1952 | | | |
| 1030 | 16 | 17–18 | 1.5 |
| 1215 | 13 | 12.5 | 0.5 |
| 1300 | 12 | 9.5 | 2.5 |
| 1345 | 9 | 8 | 1.0 |
| Aug. 29, 1952 | | | |
| 1215 | 12 | 14 | 2.0 |
| 1330 | 10 | 10 | 0.0 |
| 1500 | 21 | 23 | 2.0 |

[a] 24-hour clock.
[b] Incomplete reaction with oxygen.

No interference was found from nickel, cupric, or zinc ions in concentrations of 1000 p.p.b., nor ferric ion at 3000 p.p.b. Sulfite ions showed no interference in concentrations of 8 to 12 p.p.b. as shown in Table IV. Of the metals tested only ferrous ion was found to interfere. This ion, or intolerable concentrations of other ions, can be removed by a mono-bed ion exchange demineralizer in series with the sample container. Care must be taken to remove all air from the exchanger by up-flow flushing.

The data in Tables III and IV show very satisfactory agreement between the new method and ASTM method D 888, in both laboratory tests and the power plant tests. Of the 34 comparisons made, 33 agreed within 4 p.p.b., 26 agreed within 2 p.p.b., and 15 agreed within 1 p.p.b. The data in Table III show that

**Table IV. Experimental Data for Power Plant Deaerator**

| | Dissolved Oxygen Found, P.P.B. | | |
|---|---|---|---|
| Time and Date | ASTM method D 888 | Indigo carmine method | Difference |
| Sept. 6, 1952 | | | |
| 1115 | 7 | 10 | 3.0 |
| 1415 | 6.8 | 4.5 | 2.3 |
| 1530 | 6 | 6 | 0.0 |
| 1600 | 6 | 6 | 0.0 |
| Sept. 8, 1952 | | | |
| 1040 | 15 | 5 | 10.0 |
| 1100 | 4.1 | 5 | 0.5 |
| 1320[a] | 8.4 | 8.5 | 0.1 |
| 1410[a] | 13 | 16.5 | 3.5 |
| 1505[a] | 10 | 11 | 1.0 |
| 1600 | 7.6 | 8 | 0.4 |
| Sept. 9, 1952 | | | |
| 1515 | 5 | 7.5 | 2.5 |
| 1545 | 5 | 5.5 | 0.5 |
| Sept. 10, 1952 | | | |
| 1430 | 4.4 | 4.5 | 0.1 |
| Sept. 10, 1952. Sodium sulfite bled into line to make final solution 8 to 12 p.p.b. | | | |
| 1000 | 8 | 15[b] | .. |
| 1030 | 6 | 26[b] | .. |
| 1105 | 12 | 13.5 | 1.5 |
| 1245 | 7 | 8 | 1.0 |
| 1325 | 7 | 8 | 1.0 |
| 1400 | 7 | 7.5 | 0.5 |

[a] Saturated water bled into line.
[b] Stopper on sampling tubes leaked; changed in subsequent runs.

the dissolved-oxygen content of the laboratory-deaerated water declined in a uniform manner from the beginning to the end of each day as indicated by indigo carmine, whereas it was somewhat erratic as determined by ASTM method D 888. Since the laboratory deaerator was started up each morning, it is logical to assume that the dissolved-oxygen content would decrease as the deaerator approached steady operation. Therefore, the large differences between the two methods may not be due to deficiencies on the part of the colorimetric procedure.

Colors developed in distilled water with a dissolved-oxygen concentration of less than 50 p.p.m. are stable for at least 45 minutes. Colors developed in high dissolved-oxygen concentration tend to drift toward lower oxygen colors on standing. This can be remedied partially by using less glucose in the reagent. In deaerated tap water there was, occasionally, rapid changes toward the high oxygen colors although the initial colors appeared to correspond to the "true values."

### APPLICABILITY OF METHOD

This procedure is suitable for oxygen concentrations up to 100 p.p.b. The range can be extended by using more indigo carmine in the stock solution. It may be necessary to increase the glucose if over five times the quantity of indigo carmine recommended is used. The tubes must be viewed diametrically for high concentrations, since the colors will be too intense for axial comparison.

### CONCLUSIONS

The improved analytical method described in this paper is applicable to the determination of dissolved oxygen in most low oxygen water. The advantages of the test lie in its speed, sensitivity, and simplicity. It can be applied satisfactorily by persons of very limited experience. The reagent, when properly stored, has excellent stability. If implemented by slide-comparator equipment, this colorimetric method could be further simplified to the extent of eliminating the artificial liquid standards.

### ACKNOWLEDGMENT

Acknowledgment is made to R. C. Adams and F. E. Clarke for their advice and editing of this article, and to Natalie Scholtz for her work on the early development of the reagent.

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# Colorimetric Determination of 2-Hydroxy-4,6-dimethylpyrimidine

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**In the manufacture of nicarbazin the purity of 2-hydroxy-4,6-dimethylpyrimidine must be controlled. Existing methods for the pyrimidines were found to be unsatisfactory. A method has been devised based on the reaction of 2-hydroxy-4,6-dimethylpyrimidine with sulfanilic acid and sodium nitrite in an alcoholic sulfuric acid solution. A red-colored complex is formed having absorption peaks at 450 and 540 mμ. The quantitative characteristics and simplicity of the reaction make it useful for routine analysis. The assay is specific, the only other substances that form a similar color are the 2 amino- and 2 mercapto-analogs.**

NICARBAZINE is a complex of 4,4′-dinitrocarbanilide and 4,6-dimethyl-2-pyrimidinol. It is a prophylactic drug, effective in preventing outbreaks of coccidiosis in chickens. A need arose in these laboratories for an accurate method of analysis to control the purity of the 2-hydroxy-4,6-dimethylpyrimidine used in the manufacturing of the nicarbazine complex. Several methods that have been reported in the literature for the determination of pyrimidines were tried, but none had been found satisfactory for 2-hydroxy-4,6-dimethylpyrimidine in substance and in complex.

Several of these methods are based on the reaction of the pyrimidine with diazotized sulfanilic acid in alkaline solution (*1–3*). These methods suffer from lack of specificity. Shepherd (*4*) reported that the color-forming reaction with 2-thiobarbituric acid was found to be specific for the pyrimidine ring among the heterocyclic nuclei and related groupings. This reaction of the pyrimidine ring was prevented by one or more substituents in the 4-, 5-, or 6-position unless these substituents were removed during the test. This test was negative on 2-hydroxy-4,6-dimethylpyrimidine.

In an alcoholic sulfuric acid medium 2-hydroxy-4,6-dimethylpyrimidine formed a red color when treated with sulfanilic acid and sodium nitrite, whereas other pyrimidines and related compounds did not form the red color. In order to apply the reaction of 2-hydroxy-4,6-dimethylpyrimidine with sulfanilic acid and sodium nitrite to the development of a photometric method, it was necessary to determine the exact conditions under which reproducible results were obtained. This resulted in the development of the highly specific quantitative method reported.

### REAGENTS

Unless otherwise indicated, all reagents are C.P. or reagent grade.

**Absolute Ethyl Alcohol.**

**Sulfuric acid,** 0.4*N* standard solution.

**Sulfanilic acid reagent,** 1.60 grams of sulfanilic acid dissolved in 100.0 ml. of 0.4*N* sulfuric acid.

**Sodium Nitrite.** Sodium nitrite, 1.00 gram, is dissolved in sufficient distilled water to make 100 ml. of solution.

**Reference Standard 2-Hydroxy-4,6-dimethylpyrimidine.** Pure 2-hydroxy-4,6-dimethylpyrimidine was recrystallized three times from absolute ethyl alcohol after treatment with Norit.

**Standard 2-Hydroxy-4,6-dimethylpyrimidine Solution.** The reference standard of 2-hydroxy-4,6-dimethylpyrimidine, 100.0 mg., is dissolved in sufficient absolute alcohol to make 100 ml. of solution. Working standards are prepared by further dilution with absolute alcohol.

**Table I. Reproducibility of Absorbance at Four Concentrations at 540 mμ**

| | 15 γ | 30 γ | 45 γ | 60 γ |
|---|---|---|---|---|
| | 0.190 | 0.385 | 0.565 | 0.770 |
| | 0.193 | 0.375 | 0.560 | 0.765 |
| | 0.185 | 0.380 | 0.575 | 0.760 |
| | 0.187 | 0.373 | 0.560 | 0.775 |
| | 0.195 | 0.388 | 0.570 | 0.780 |
| Av. | 0.190 | 0.380 | 0.566 | 0.770 |
| Av. dev. | ±0.003 | 0.005 | ±0.005 | ±0.006 |

## APPARATUS

Absorbance measurements were made in a Cary recording spectrophotometer and a Beckman Model B spectrophotometer. A constant temperature water bath maintained at 65° C. was used.

## RECOMMENDED PROCEDURE

Prepare an alcohol solution of the substance to be tested containing 2 to 3 γ of 2-hydroxy-4,6-dimethylpyrimidine per ml.

Pipet 15.0 ml. of the sample solution into a 50-ml. glass-stoppered cylinder or tube. Add 5.0 ml. of the sulfanilic acid reagent and mix. Then add 5.0 ml. of sodium nitrite solution and mix. Place in a water bath at 65° C. for 15 minutes. Remove from bath and let stand at room temperature for 1 hour. Read the color formed in a suitable instrument set at 540 mμ with a blank prepared at the same time, from 15.0 ml. of alcohol, 5.0 ml. of sulfanilic acid reagent, and 5.0 ml. of sodium nitrite solution.

**Preparation of Standards.** Prepare standards containing 15, 30, 45, and 60 γ of 2-hydroxy-4,6-dimethylpyrimidine reference standard per 15.0 ml. of alcohol and run simultaneously with the unknown.

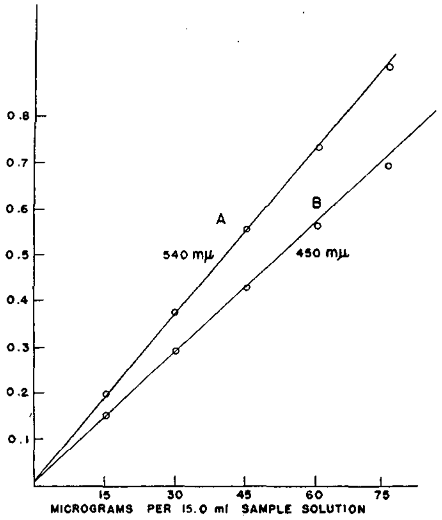

Figure 1. Calibration curves

**Measurement of Color.** Since the red color produced by 2-hydroxy-4,6-dimethylpyrimidine has two absorption peaks, it may be measured at either 450 or 540 mμ. In Figure 1, *A* represents the plot of absorbances *vs.* concentration measured at 540 mμ, and Figure 1, *B*, that measured at 450 mμ. Since the red color at 540 mμ is more sensitive and more specific, this absorption maximum is preferred. Table I gives the absorbance obtained at 540 mμ at four levels of 2-hydroxy-4,6-dimethylpyrimidine. Because of the many variables, the exact directions of the procedure must be followed, or exact proportioned aliquots of all volumes used.

## EXPERIMENTAL

**Spectral Characteristics.** The spectral characteristics of the color produced by the reaction were determined by means of a Cary recording spectrophotometer in the range 400 to 600 mμ. Figure 2 shows the absorption curves obtained when 150 γ of 2-hydroxy-4,6-dimethylpyrimidine (HDP) dissolved in 15.0 ml. of the solvent specified were treated with 5.0 ml. of sulfanilic acid reagent and 5.0 ml. of 1% sodium nitrite solution under the conditions of the recommended procedure. Ethyl, methyl, and isopropyl alcohol solutions show absorption peaks at 450 and 540 mμ. Dimethyl formamide solution of 2-hydroxy-4,6-dimethylpyrimidine produced a red color with maximum absorption at 475 mμ. Wholly aqueous solutions produce an orange yellow with maximum absorption at 430 mμ. Ethyl alcohol solution showed the greatest absorption at 540 mμ, and, therefore, was chosen as the solvent.

**Effect of Alcohol.** The red color reaction will not take place in a wholly aqueous solution under the recommended conditions of the procedure. Figure 3 illustrates the effect alcohol has on

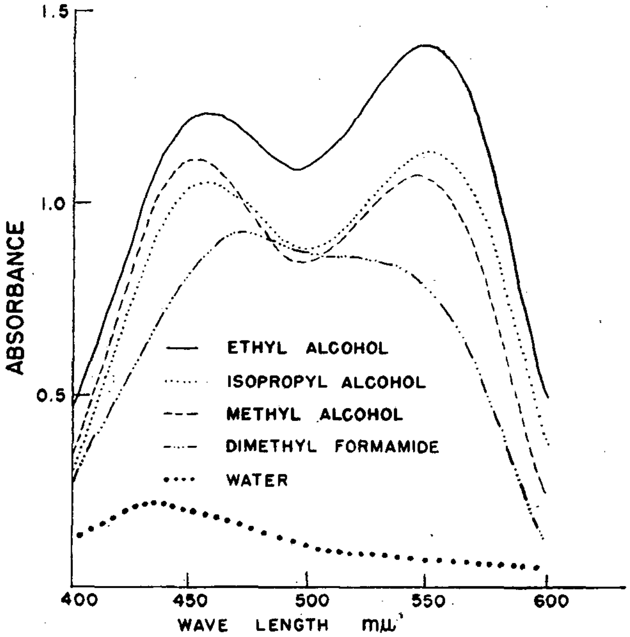

Figure 2. Absorption curves

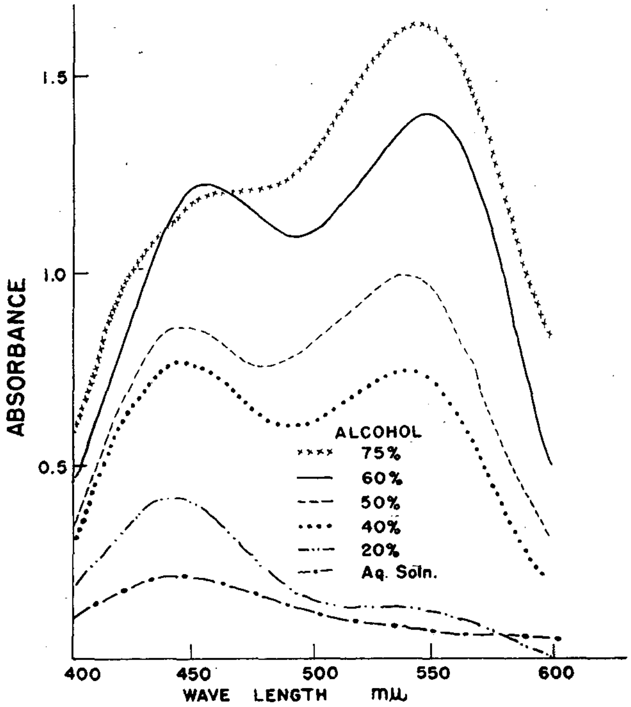

Figure 3. Effect of alcohol

the color formation. In these experiments, 5.0 ml. (150 γ) of an alcohol stock solution of 2-hydroxy-4,6-dimethylpyrimidine were diluted with alcohol or water, so that the total volume after addition of 5.0 ml. of the sulfanilic acid reagent and 1.0 ml. of 5.0% sodium nitrite solution was 25.0 ml. and the desired alcohol content, 0 to 75 volume %, was obtained. The color was then developed under the conditions of the procedure. Precipitation of reagents occurs when the alcohol content of the solution exceeds 75 volume %.

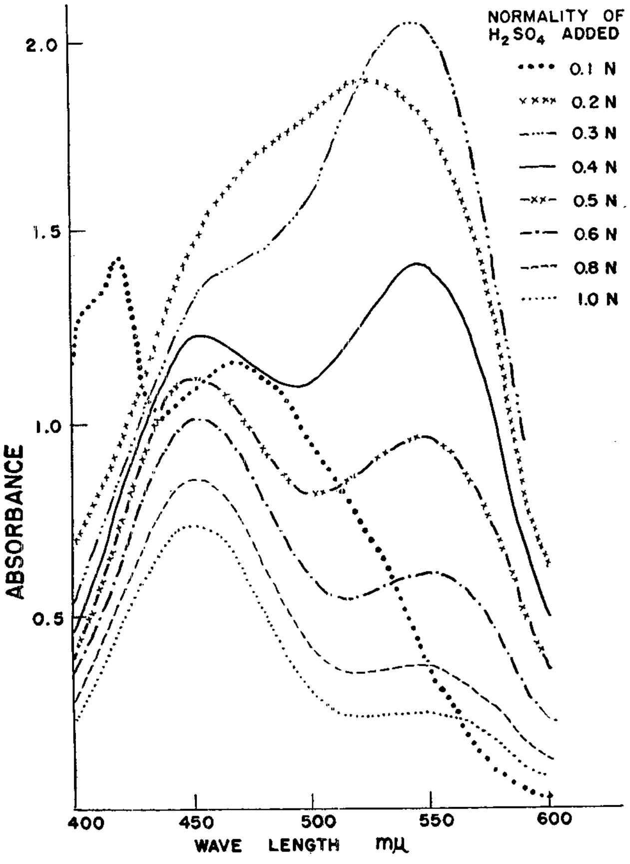

Figure 4. Effect of sulfuric acid

**Effect of Sulfuric Acid.** Figure 4 illustrates the absorption characteristics of the color complex depending upon the normality of the sulfuric acid added. In this series of experiments, 15.0 ml. (150 γ of 2-hydroxy-4,6-dimethylpyrimidine) of alcohol stock solution were treated with 5.0 ml. of sulfuric acid containing 16 mg. sulfanilic acid per ml. and 5.0 ml. of 1.0% sodium nitrite solution under the conditions specified. Two absorption peaks were obtained when the normality of the sulfuric acid added was kept between 0.3 and 1.0*N*. The absorption at 540 mμ increased as the normality of the sulfuric acid decreased. The ratio of the absorbance at 540 mμ to the absorbance at 450 mμ increased as the normality of the sulfuric acid decreased.

A wide absorption peak at 510 to 530 mμ was obtained when 0.2*N* sulfuric acid was added. Peaks at 415 and 470 mμ were obtained with 0.1*N* sulfuric acid.

**Effect of Sodium Nitrite.** Figure 5 illustrates the effect of sodium nitrite on color formation. Again 15.0 ml. (150 γ of 2-hydroxy-4,6-dimethylpyrimidine) of the alcohol stock solution were treated with 5.0 ml. of sulfanilic acid reagent and 5.0 ml. of sodium nitrite solution containing 4 to 20 mg. of sodium nitrite per ml. under the conditions of the procedure. An orange color, maximum absorption 450 mμ, was obtained with 20 mg. of sodium nitrite. As the amount of sodium nitrite was increased from 20 to 80 mg. the color changed to red, maximum absorption at 450 to 460 and 540 mμ. The color changed to orange, maximum absorption at 475 mμ, as the amount of sodium nitrite exceeded 80 mg.

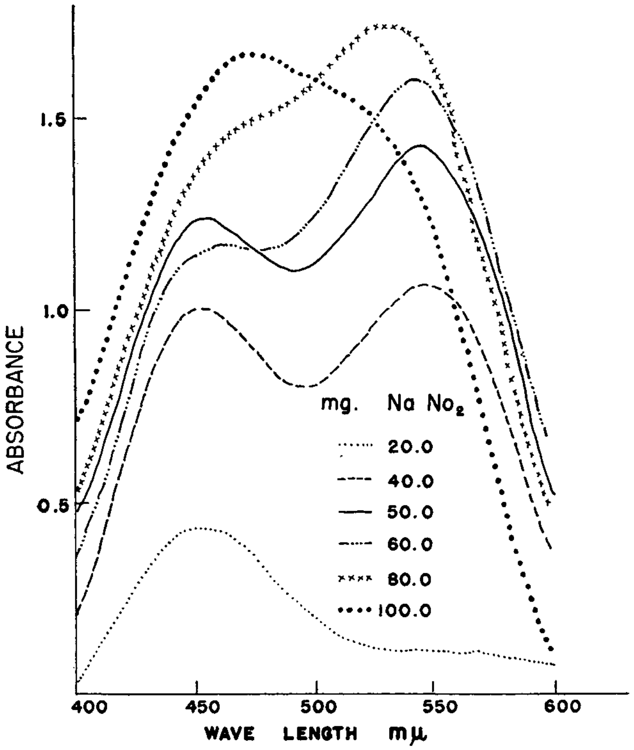

Figure 5. Effect of sodium nitrite

**Effect of Sulfanilic Acid.** Figure 6 illustrates the effect of the amount of sulfanilic acid added on the color formation. In these tests 15.0 ml. (150 γ 2-hydroxy-4,6-dimethylpyrimidine) of alcohol stock solution were treated with 5.0 ml. of 0.4*N* sulfuric acid containing from 4 to 20 mg. of sulfanilic acid per ml. and 5.0 ml. of 1% sodium nitrite solution. With amounts of 40 mg. or more of sulfanilic acid, red color was formed, maximum absorption at 450 and 540 mμ. With amounts of 30 mg. or less of sulfanilic acid, an orange to yellow color, maximum absorption at 450 mμ, was formed. More than 90 mg. of sulfanilic acid produced turbid solutions which required filtration or centrifuging in order to make spectrophotometer readings.

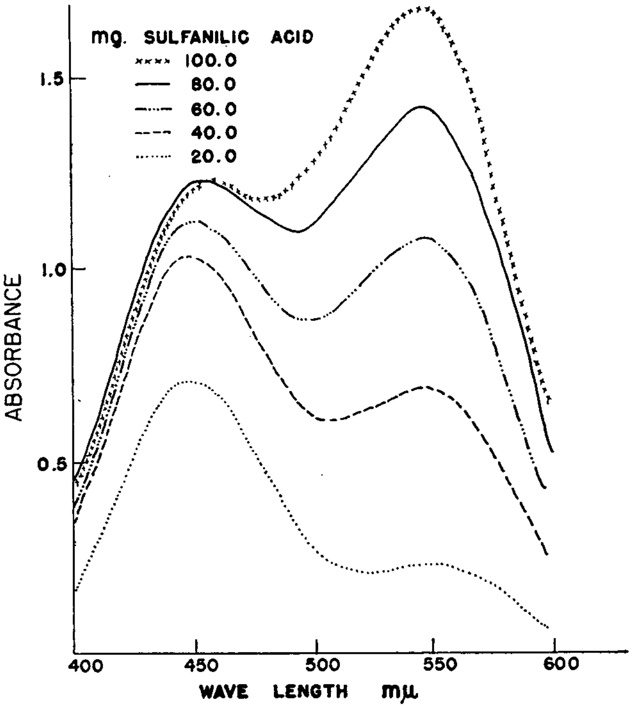

Figure 6. Effect of sulfanilic acid

**Effect of Time and Temperature.** This effect was studied by treating 15.0-ml. (45 $\gamma$ of 2-hydroxy-4,6-dimethylpyrimidine) aliquots of an alcoholic solution of 2-hydroxy-4,6-dimethylpyrimidine with 5.0 ml. of sulfanilic acid reagent and 5.0 ml. of 1.0% sodium nitrite solution for various periods of time at different temperatures. The spectral characteristics and intensities of the colored solutions were determined with a Cary recording spectrophotometer. At room temperature a minimum of 20 hours was required for maximum color development. Maximum color development was reached after heating for 1 hour at 40° C. and standing for 30 minutes at room temperature. In an 80° C. bath, maximum color intensity was reached in 5 minutes and the red color rapidly faded to a brown.

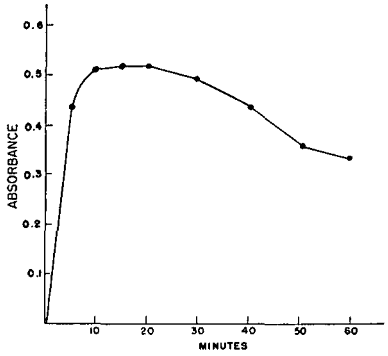

Figure 7. Rate of reaction

Table II. Specificity of Reaction

Substances Developing No Color

2-Amino-5-chloropyrimidine
2-Amino-5-methylpyrimidine
2-Amino-5-nitropyrimidine
2,4-Dihydroxy-5-methylpyrimidine
2-Hydroxypyrimidine
2-Methyl-4-amino-5-ethoxymethylpyrimidine
2-Methyl-4-amino-5-bromomethylpyrimidine
2-Methyl-4-amino-5-nitratomethylpyrimidine
2-Methyl-4-amino-5-chloromethylpyrimidine
2-Methyl-4-hydroxy-6-ethoxymethylpyrimidine
Pyrimidine
Allantoin
Alloxan
Arsanilic acid
Biuret
Barbital
Caffeine
Cyanuric acid
Hexamine
Histamine
Hydroxyproline
2-Hydroxypyridine
4,4'-Dinitrocarbanilide
Niacin (nicotinic acid)
Niacinamide
Nitrofurazone
Pyridine
Pyridoxine
Phenobarbital
Proline
Theobromine
Thiamine
Uric acid
Guanine

Substances Forming a Yellow Color

2-Amino-4-methylpyrimidine
2-Hydroxy-4-methylpyrimidine
Aminopyrine
Aniline
Dinitrodiphenyl disulfide
*p*-Aminosalicylic acid
*p*-Nitroaniline
*p*-Aminobenzoic acid
Sulfanilamide
Sulfathiazole
Sulfamerazine
Sulfadiazine
Sulfapyridine
Sulfamethazine
4-Hydroxy-3-nitrophenylarsonic acid

Figure 7 shows that at least 10 minutes in the bath at 65° C. and standing for 1 hour at room temperature are required for maximum color development at 540 m$\mu$. Increasing the heating time in the bath to 20 minutes produces no greater intensity in color; while heating for 30 minutes or longer decreased the intensity and stability of the color. The color required standing for 1 hour at room temperature to become stable for reproducible readings. An increase of 1 to 2% in absorbance was observed over a 2-hour period of standing. Gradual fading of the color was observed after 8 hours.

**Specificity of Reaction.** The specificity of the reaction with respect to other pyrimidines and similar compounds was investigated. The color reaction was found to be specific for 2-hydroxy-4,6-dimethylpyrimidine with the exception of 2-amino, and 2-mercapto-4,6-dimethylpyrimidine. Figure 8 shows the absorption curves obtained when 15.0 ml. (150 $\gamma$) of an alcohol solution of the substance indicated were treated according to the conditions of the recommended procedure. Table II lists the sub-

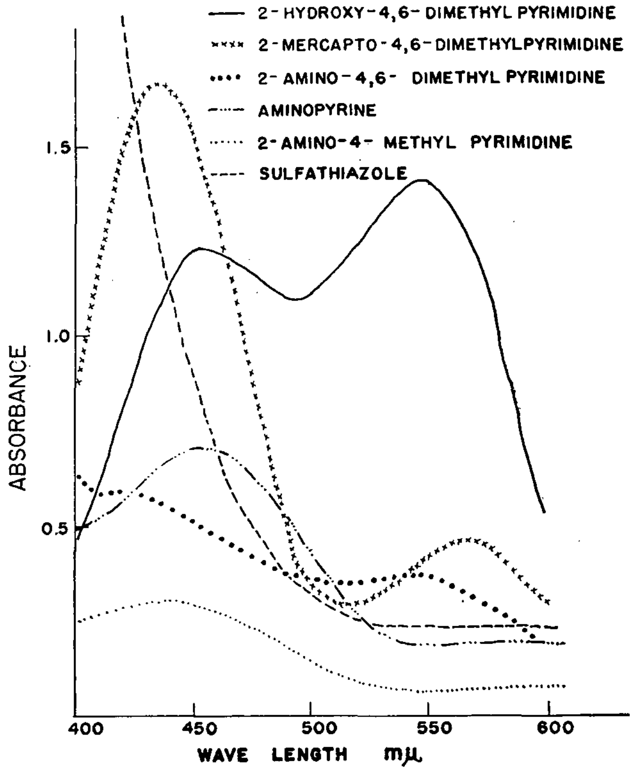

Figure 8. Absorption curves

Table III. Assay of 2-Hydroxy-4,6-dimethylpyrimidine

| Sample | % HDP | Average | Av. Dev., % |
|---|---|---|---|
| HDP, pure | 97.0 | 97.3 | ±0.2 |
| | 97.2 | | |
| | 97.3 | | |
| | 97.5 | | |
| HDP, pure | 99.0 | 99.6 | ±0.3 |
| | 100.0 | | |
| | 99.6 | | |
| | 99.0 | | |
| HDP, crude | 77.7 | 77.6 | ±0.3 |
| | 77.3 | | |
| | 78.0 | | |
| | 77.5 | | |
| HDP, crude hydrate | 56.0 | 55.7 | ±0.7 |
| | 55.0 | | |
| | 55.6 | | |
| | 56.2 | | |
| HDP, crude hydrate | 56.2 | 56.0 | ±0.4 |
| | 56.0 | | |
| | 55.6 | | |
| | 56.2 | | |
| Nicarbazin | | | |
| No. 1 | 27.9 | 27.9 | ±0.2 |
| | 27.8 | | |
| | 28.3 | | |
| | 27.6 | | |
| No. 2 | 29.6 | 29.5 | +0.1 |
| | 29.4 | | |
| | 29.6 | | |
| | 29.3 | | |
| No. 3 | 29.2 | 29.15 | +0.08 |
| | 29.0 | | |
| | 29.2 | | |
| | 29.2 | | |
| No. 4 | 26.6 | 26.95 | ±0.18 |
| | 27.0 | | |
| | 27.0 | | |
| | 27.2 | | |

stances which did not give the color reaction, and those which formed a yellow color. Under the conditions of the test, the colors formed by these substances faded rapidly and showed no definite absorption maximum.

**Reproducibility of Method.** Satisfactory reproducibility of the method is achieved by carefully observing the procedure outlined. Table III shows the reproducibility of the method when applied to different samples of 2-hydroxy-4,6-dimethylpyrimidine and nicarbazin.

## CONCLUSION

A study of the effects of reagent variations indicates the conditions optimal for 540 mμ absorption: 75% alcohol (by volume), 0.3$N$ sulfuric acid, 100 mg. of sulfanilic acid, and 60 mg. of sodium nitrite in a 25-ml. volume for 15-minute heating period. To satisfy these conditions, the following procedure was set up. Fifteen milliliters of an alcohol solution of the sample containing 2 and 4 $\gamma$ of 2-hydroxy-4,6-dimethylpyrimidine per ml. were added to 5.0 ml. of 0.3$N$ sulfuric acid containing 20 mg. of sulfanilic acid per ml. in a 25-ml. volumetric flask and mixed. Then 1.0 ml. of 6% sodium nitrite was added and the volume made up to 25 ml. with alcohol. After heating for 15 minutes in a bath and standing for 1 hour at room temperature, a red, turbid solution was obtained. This required an additional step, centrifuging, to obtain a clear solution for spectrophotometric reading.

The clear solution showed absorption peaks at 450 and 540 mμ. Four separate runs on 30 $\gamma$ gave absorbances of 0.245, 0.260, 0.280, and 0.300; for 60 $\gamma$, the values were 0.500, 0.550, 0.610, and 0.580. These variations, ±7%, were too large; therefore, another set of conditions had to be established. As a result the conditions specified in the recommended procedure were tested and proved to be most satisfactory.

The 540 mμ absorption was chosen over the 450 mμ because less interference of background is encountered at this point.

Best results are obtained when the color reaction is carried out under the strict conditions of the recommended procedure. The method provides a rapid and accurate procedure for routine analyses of 2-hydroxy-4,6-dimethylpyrimidine, nicarbazin, nicarbazin premixes, and feeds.

## ACKNOWLEDGMENT

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# Colorimetric Determination of Niobium in the Presence of Titanium

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**A critical examination of the acetone-intensified thiocyanate procedure was made with the aim of developing a colorimetric procedure for the determination of niobium without the prior time-consuming, tedious, and uncertain separation of titanium. Absorption studies made between wave lengths of 320 and 800 mμ showed that a marked shift occurred in the titanium absorption maximum with change in thiocyanate concentration, whereas the niobium maximum remained constant. The shift in maximum brought about a condition in which total absorption of a mixture of titanium and niobium at 400 mμ was due mainly to niobium while at 360 mμ the absorption was due mainly to titanium. This condition enabled determination of both niobium and titanium from measurements made at two different wave lengths. The niobium color was not proportional to concentration, but a ratio procedure permitted the determination of the fraction of total absorption contributed by either component at a given wave length. The actual amount was then determined by use of a calibration curve prepared from pure solutions. The absorption spectra of impurities and their effect on the niobium results were also investigated.**

THE determination of niobium in the presence of titanium has long been recognized as a difficult problem, and no satisfactory, simple method exists for the separation of these two elements (*1*, *4*, *8*). In addition to the similarity of niobium and titanium reactions, the separations are further complicated by the marked solvent action that titanium exerts on forms of niobium that are normally insoluble. This solvent effect is exemplified by the inhibition of niobium hydrolysis from dilute solutions of sulfuric or hydrochloric acid. Even in the presence of tannin the solvent action is pronounced and complete precipitation of the niobium is assured only when the acidity is adjusted so that substantial titanium precipitates along with the niobium.

The studies described relate to the development of a colorimetric procedure for the determination of niobium as a supplement to spectrographic methods for materials containing titanium as the major component and which eliminate the time-consuming, tedious, and uncertain separation of titanium necessary in the conventional chemical procedures. Of the various methods proposed for the colorimetric determination of niobium (*2*, *3*, *6–10*) the thiocyanate procedure appeared to offer the most promise of eliminating interference due to titanium. The yellow color produced by niobium in the thiocyanate procedure may be extracted with ether (*6*), or intensified in aqueous solutions by addition of acetone (*2*). The low interference of titanium reported for these methods was not fully realized, and since preliminary test indicated no appreciable difference in titanium interference between these two procedures, the more convenient acetone-intensified procedure of Freund and Levitt (*2*) was selected for further study to determine the factors influencing titanium interference.

## EXPERIMENTAL PROCEDURE

Standard solutions of niobium and titanium were prepared and used in determining the absorption spectra of each element to find the region in which titanium interference was at a minimum and to establish the effect of the various reagents on the relative absorptivities.

**Standard Niobium Solution.** A 0.1000-gram sample of niobium pentoxide was fused with 2.5 grams of potassium pyrosulfate, dissolved in a solution containing 75 grams of tartaric acid, and diluted to 1 liter.

**Standard Titanium Solution.** A 0.5000-gram sample of C.P. titanium dioxide was dissolved in a boiling mixture of 15 ml. of concentrated sulfuric acid and 5 grams of ammonium sulfate, precipitated with excess ammonia, heated 5 minutes, washed, redissolved in 25 ml. of hydrochloric acid, again precipitated with

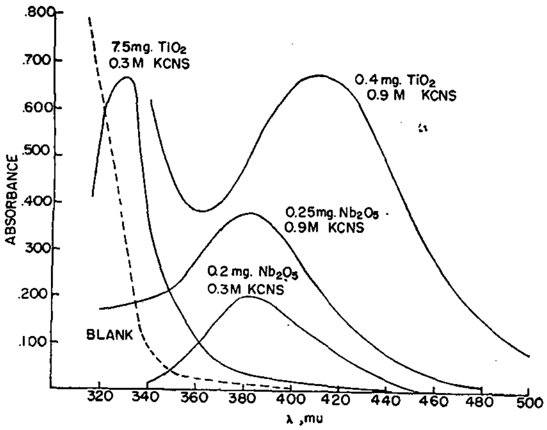

Figure 1. Titanium and niobium absorption curves

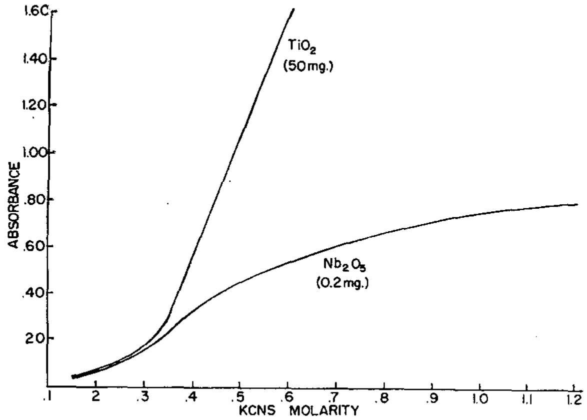

Figure 2. Effect of potassium thiocyanate concentration

excess ammonia, boiled 5 minutes, filtered, and washed. Excess ammonia was present after each boiling to obtain a form of hydrate readily soluble in acids. The final precipitate was transferred with the aid of about 50 ml. of water, to a beaker containing 7.5 grams of solid tartaric acid and heated for 2 hours or more at 50° to 60° C. to produce a crystal clear solution. The tartrate solution was transferred to a 100-ml. volumetric flask and diluted to volume.

The C.P. titanium dioxide was prepared from specially purified titanium ammonium sulfate by calcining the double salt at 1000° C.

**Color Development.** Aliquots of the standard niobium solution and standard titanium solution were separately transferred to 50-ml. volumetric flasks. A sufficient amount of a stock solution of tartaric acid containing 75 grams per liter was added so that the standard solutions plus tartaric acid were equal to 10 ml. Twenty milliliters of 1 to 1 hydrochloric acid, 10 ml. of acetone, and 2 ml. of 1*M* stannous chloride in 1 to 1 hydrochloric acid were then added. The solutions were cooled to room temperature, 5 ml. of 3*M* potassium thiocyanate added, immediately diluted to 50 ml., and mixed. The solutions were permitted to stand for one-half hour to effect complete color development, after which absorbances were measured in a Beckman Model B spectrophotometer at maximum sensitivity with matched 1-cm. Corex cells. Flasks and cells were kept sealed at all times to prevent loss of acetone. All reagents were accurately measured from burets or pipets.

## EFFECT OF REAGENT CONCENTRATION

Freund and Levitt showed that the intensity of the niobium thiocyanate color was markedly influenced by the concentration of each reagent used. Similar tests were made in this laboratory to determine the effect of reagents on the brownish yellow titanium color that forms with thiocyanate.

**Thiocyanate.** A study of the thiocyanate effect at different concentrations and wave lengths showed that a marked shift occurred in the absorption curve of titanium with change in thiocyanate concentration, whereas no shift occurred in the niobium curve. At low concentrations of 0.3*M* thiocyanate, titanium produced a single absorption peak at 320 mμ which was followed by a rapid drop in absorbance at higher wave lengths. At increased concentrations the slope of the absorbance curve from the peak at 320 mμ flattened out, and eventually a new absorption peak developed at 410 mμ. At a concentration of 0.9*M* thiocyanate two peaks were present, one at 320 mμ and the other at 410 mμ. This effect is shown in Figure 1.

The absorptivity of both niobium and titanium changed with thiocyanate concentration. At 400 mμ and a concentration below 0.3*M* the absorptivity of niobium increased at a somewhat greater rate than the titanium with increasing concentrations of thiocyanate, but above 0.3*M* thiocyanate the absorptivity of titanium increased at a markedly greater rate than the niobium color. At a concentration of 0.9*M* thiocyanate the absorptivity ratio of titanium to niobium at 400 mμ was 35 times higher than at 0.3*M* and at 1.2*M*, the relative absorptivity increased by a factor of over 100. These effects are illustrated in Figure 2. Further data within narrower limits are given in Table IA.

The thiocyanate study revealed that optimum conditions for reduction of titanium interference were realized when measurements were made at 400 mμ with a thiocyanate concentration of 0.3*M*. Further considerations led to the use of absorption values at both 360 mμ and 400 mμ for correction of titanium present.

**Acetone.** The effect of acetone was similar to that of thiocyanate. The 400 mμ absorptivity of niobium increased at a greater rate than that of titanium up to a given point, after which the rate of increase in titanium absorptivity exceeded that of niobium to a very pronounced extent. This effect resulted in an initial decrease in ratio of titanium to niobium absorptivity followed by an increase at the higher acetone concentrations. The absorptivity at 360 mμ likewise increased with increasing

Table I. Effect of Reagent Concentration

| KCNS (Ml. 3*M*) | $Nb_2O_5$ (0.5 Mg.) | | | $TiO_2$ (50 Mg.) | | | $TiO_2/Nb_2O_5$ Absorbance Ratio at 400 mμ |
|---|---|---|---|---|---|---|---|
| | Absorbance | | Ratio, 360 mμ/ 400 mμ | Absorbance | | Ratio, 360 mμ/ 400 mμ | |
| | 360 mμ | 400 mμ | | 360 mμ | 400 mμ | | |
| | | | A. Effect of Potassium Thiocyanate | | | | |
| 2.5 | 0.059 | 0.056 | 1.05 | 0.307 | 0.051 | 6.02 | 0.77 |
| 4.0 | 0.232 | 0.270 | 0.859 | 0.555 | 0.088 | 6.31 | 0.32 |
| 5.0 | 0.395 | 0.476 | 0.829 | 0.825 | 0.156 | 5.37 | 0.33 |
| 6.0 | 0.581 | 0.757 | 0.770 | 1.164 | 0.278 | 4.19 | 0.37 |
| 10.0 | 0.976 | 1.410 | 0.692 | 3.090 | 2.290 | 1.35 | 1.6 |
| | | | B. Effect of Acetone | | | | |
| Acetone, Ml. | | | | | | | |
| 5 | 0.099 | 0.087 | 1.138 | 0.753 | 0.103 | 7.31 | 1.18 |
| 9 | 0.312 | 0.364 | 0.857 | 0.777 | 0.119 | 6.53 | 0.33 |
| 10 | 0.408 | 0.493 | 0.828 | 0.805 | 0.151 | 5.33 | 0.31 |
| 11 | 0.496 | 0.627 | 0.791 | 0.886 | 0.192 | 4.51 | 0.31 |
| 20 | 1.130 | 1.540 | 0.734 | 2.440 | 3.140 | 0.777 | 2.04 |
| | | | C. Effect of Hydrochloric Acid | | | | |
| HCl (Equiv. Ml.) 1 to 1 | | | | | | | |
| 10 | 0.037 | 0.033 | 1.12 | 0.447 | 0.057 | 7.84 | 1.73 |
| 18 | 0.336 | 0.381 | 0.882 | 0.740 | 0.133 | 5.56 | 0.35 |
| 20 | 0.417 | 0.498 | 0.828 | 0.834 | 0.155 | 5.38 | 0.31 |
| 22 | 0.515 | 0.623 | 0.826 | 0.905 | 0.201 | 4.50 | 0.32 |
| 40 | 0.667 | 0.832 | 0.802 | 1.102 | 0.412 | 2.68 | 0.50 |
| | | | D. Effect of Tartaric Acid | | | | |
| Molarity of Tartaric Acid | | | | | | | |
| 0.05 | 0.479 | 0.601 | 0.797 | 0.660 | 0.123 | 5.37 | 0.20 |
| 0.10 | 0.407 | 0.492 | 0.827 | 0.804 | 0.155 | 5.19 | 0.32 |
| 0.20 | 0.309 | 0.371 | 0.833 | 0.845 | 0.181 | 4.67 | 0.49 |
| 0.50 | 0.222 | 0.248 | 0.895 | 1.290 | 0.352 | 3.67 | 1.42 |

acetone concentration, but at a different rate than at 400 mμ, to produce a 360 to 400 mμ ratio for both niobium and titanium, which decreased with increasing reagent concentration. Data are given in Table IB.

**Hydrochloric Acid.** The data in Table 1C show that the same general trends were found for variations in hydrochloric acid concentration as existed with thiocyanate and acetone, but to a lesser degree. No sharp rise in absorptivity of titanium was apparent at the highest concentration studied.

**Tartaric Acid.** The effect of tartaric acid on the absorptivity of niobium was different from the above reagents. Increasing concentration produced a decided decrease in the absorptivity at both 400 mμ and 360 mμ and produced a relatively slight increase in the 360 mμ to 400 mμ absorbance ratio instead of the marked decrease found with other reagents. The titanium absorptivity followed the usual trend of increasing with increasing concentration of reagent which resulted in a continual rise in the titanium dioxide–niobium pentoxide absorbance ratio at 400 mμ. Data are given in Table ID.

**Stannous Chloride.** Stannous chloride is not necessary for color development, but is essential for elimination of iron interference and stabilization of reagent blank values. In the absence of stannous chloride the blank values were high and continued to increase rapidly with time. Increasing amounts of stannous chloride appeared to have no effect on the absorbance of either niobium or titanium when the total amount of hydrochloric acid was held constant. Data given in Table IIA appear to indicate slightly higher absorbances in the absence of stannous chloride, but the high and unstable blank values made these values questionable.

### Table II. Effect of Reagent Concentration

| $SnCl_2$ Ml. 1*M* | $Nb_2O_5$ (0.5 Mg.) Absorbance 360 mμ | $Nb_2O_5$ (0.5 Mg.) Absorbance 400 mμ | $Nb_2O_5$ (0.5 Mg.) Ratio 360 mμ/400 mμ | $TiO_2$ (50 Mg.) Absorbance 360 mμ | $TiO_2$ (50 Mg.) Absorbance 360 mμ | $TiO_2$ (50 Mg.) Ratio 360 mμ/400 mμ | Reagent Blank 360 mμ | Reagent Blank 400 mμ |
|---|---|---|---|---|---|---|---|---|
| | | | | A. Effect of Stannous Chloride[a] | | | | |
| 0 | 0.410 | 0.507 | 0.809 | 0.780 | 0.158 | 5.00 | 0.070 | 0.030 |
| 2 | 0.403 | 0.483 | 0.836 | 0.799 | 0.152 | 5.26 | 0.018 | 0.004 |
| 4 | 0.414 | 0.482 | 0.827 | 0.812 | 0.148 | 5.27 | 0.020 | 0.004 |
| 2[b] | 0.315 | 0.370 | 0.851 | 0.706 | 0.153 | 4.61 | 0.124 | 0.010 |
| | | | | B. Effect of Sulfate | | | | |
| $K_2SO_4$ Molarity | | | | | | | | |
| 0.00 | 0.410 | 0.491 | 0.835 | 0.798 | 0.153 | 5.21 | | |
| 0.01 | 0.394 | 0.475 | 0.829 | 0.800 | 0.155 | 5.16 | | |
| 0.05 | 0.278 | 0.335 | 0.830 | 0.763 | 0.151 | 5.05 | | |
| 0.10 | 0.220 | 0.266 | 0.827 | 0.775 | 0.156 | 4.97 | | |

[a] Amount of 1 to 1 HCl reagent adjusted to maintain constant total HCl concentration.
[b] $SnCl_2$ over 6 months old.

### Table III. Effect of Organic Solvents

| Organic Solvent | Absorbance[a] Ti-CNS | Absorbance[a] Nb-CNS[b] |
|---|---|---|
| Acetone | 0.158 | 0.344 |
| Dioxane | 0.134 | 0.230 |
| Methyl Cellosolve | 0.381 | 0.141 |
| Methanol | 0.634 | [c] |
| Ethyl alcohol | 0.938 | [c] |

[a] Constant amount of Ti and Nb for all solvents.
[b] Data given by Freund and Levitt.
[c] Absorbance less than for acetone.

### Table IV. Absorptivity and Absorbance Ratio

| $Nb_2O_5$ Mg./50 ml. | $Nb_2O_5$ Molar absorptivity × $10^{-3}$ at 400 mμ | $Nb_2O_5$ Absorbance ratio 360 mμ/400 mμ | $TiO_2$ Mg./50 ml. | $TiO_2$ Molar absorptivity at 400 mμ | $TiO_2$ Absorbance ratio 360 mμ/400 mμ |
|---|---|---|---|---|---|
| 0.1 | 14.2 | 0.81 | 12.5 | 11.8 | 5.24 |
| 0.2 | 13.8 | 0.83 | 25.0 | 12.0 | 5.38 |
| 0.4 | 13.7 | 0.82 | 37.5 | 11.9 | 5.33 |
| 0.6 | 12.4 | 0.83 | 50.0 | 12.1 | 5.30 |
| 0.8 | 11.7 | 0.82 | | | |

Although essentially the same absorbance values were obtained in the presence and absence of stannous chloride, a solution of stannous chloride which was over 6 months old produced greatly decreased absorbance values for niobium. The high blank values given for this run in Table IIA indicated nearly complete oxidation of the stannous chloride. Apparently stannic tin in large amounts interferes with color development whereas stannous tin does not.

**Sulfate.** Sulfates had little or no effect on the absorbance of titanium but inhibited development of the niobium thiocyanate color. Data in Table IIB show that the presence of 0.1*M* potassium sulfate decreased the niobium absorbance about 50% whereas 0.01*M* potassium sulfate brought about a decrease of about 3%.

**Solvents.** A limited amount of work was done to determine the effect of different water-soluble organic solvents on the relative intensification of the niobium and titanium thiocyanate colors. Tests were confined to titanium and the results compared with the effect of the same solvents on niobium which were given in the literature (*2*). Acetone, dioxane, methyl Cellosolve, methanol, and ethyl alcohol were examined. Acetone produced the lowest relative intensity for titanium. Data are given in Table III.

## CORRECTION FOR PRESENCE OF TITANIUM

The marked difference in relative absorbance of niobium and titanium thiocyanate colors at different wave lengths was used as a means to correct for the presence of titanium. The normal procedure of taking measurements in a two component system at the absorption peaks for the two components could not be conveniently applied, because of high blank values at the low wavelength titanium peak and increased interference of titanium at the longer wave-length niobium peak. However, the absorbances measured at 360 and 400 mμ were sufficiently different to permit the use of these wave lengths for the determination of each element in a mixture.

The absorbance of the niobium at 400 mμ was not proportional to the concentration, and therefore, the usual procedure of simultaneous calculations could not be applied. However, it was found that the ratio of absorbances at 360 and 400 mμ for niobium, and also for titanium, were constant (Table IV). Calculations based upon these two wave lengths enable determination of the fraction of the total absorbance at 400 mμ contributed by each of these elements. The concentration of niobium present in solution was then found from a standard calibration curve consisting of a plot of concentration *vs.* absorbance.

The equation used to determine the fraction of the total absorbance at 400 mμ due to niobium is derived as follows:

$$R_T = \frac{\mathrm{Nb}_{360} + \mathrm{Ti}_{360}}{\mathrm{Nb}_{400} + \mathrm{Ti}_{400}} \qquad (1)$$

$R_T$ = measured $\frac{360\ \mathrm{m}\mu}{400\ \mathrm{m}\mu}$ absorbance ratio
$\mathrm{Nb}_{360}$ = absorbance due to niobium at 360 mμ
$\mathrm{Ti}_{360}$ = absorbance due to titanium at 360 mμ
$\mathrm{Nb}_{400}$ = absorbance due to niobium at 400 mμ
$\mathrm{Ti}_{400}$ = absorbance due to titanium at 400 mμ

Equation 1 can be rearranged as follows:

$$R_T = \frac{\mathrm{Nb}_{360}}{\mathrm{Nb}_{400} + \mathrm{Ti}_{400}} + \frac{\mathrm{Ti}_{360}}{\mathrm{Nb}_{400} + \mathrm{Ti}_{400}} \qquad (2)$$

and numerator and denominator of the first right hand member of Equation 2 can be multiplied by $\mathrm{Nb}_{400}$, without changing its value, to produce:

$$\frac{\mathrm{Nb}_{360}}{\mathrm{Nb}_{400} + \mathrm{Ti}_{400}} = \frac{\mathrm{Nb}_{400}}{\mathrm{Nb}_{400} + \mathrm{Ti}_{400}} \times \frac{\mathrm{Nb}_{360}}{\mathrm{Nb}_{400}} = F_{\mathrm{Nb}400} \times R_{\mathrm{Nb}} \qquad (3)$$

where $F_{\mathrm{Nb}400}$ is a fraction of total absorbance at 400 due to niobium

$R_{\mathrm{Nb}}$ is $\frac{360\ \mathrm{m}\mu}{400\ \mathrm{m}\mu}$ absorbance ratio for pure niobium

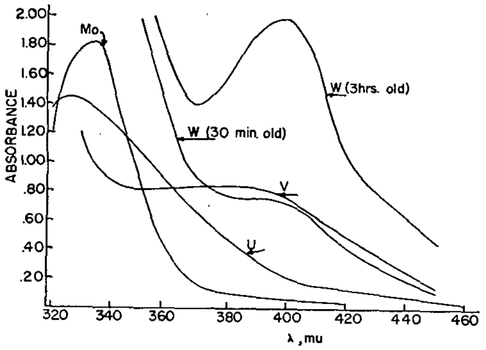

Figure 3. Absorption curves of impurities

likewise the numerator and denominator of the second right hand member of Equation 2 can be multiplied by $Ti_{400}$ without changing its value:

$$\frac{Ti_{360}}{Nb_{400} + Ti_{400}} = \frac{Ti_{400}}{Nb_{400} + Ti_{400}} \times \frac{Ti_{360}}{Ti_{400}} = F_{Ti400} \times R_{Ti} \qquad (4)$$

where, $F_{Ti400}$ is a fraction of total absorbance at 400 due to titanium

$R_{Ti}$ is $\frac{360\ m\mu}{400\ m\mu}$ absorbance ratio for pure titanium

substituting Equation 3 and 4 in Equation 2

$$R_T = F_{Nb400} \times R_{Nb} + F_{Ti400} \times R_{Ti} \qquad (5)$$

since the fraction of each element present can be expressed in terms of the other

$$F_{Ti400} = 1 - F_{Nb400} \qquad (6A)$$

$$F_{Nb400} = 1 - F_{Ti400} \qquad (6B)$$

substitution of first Equation 6A, then 6B into Equation 5 and solving gives

$$F_{Nb400} = \frac{R_T - R_{Ti}}{R_{Nb} - R_{Ti}} \text{ and } F_{Ti400} = \frac{R_T - R_{Nb}}{R_{Ti} - R_{Nb}}$$

**Recovery of Niobium Values in Mixtures of Niobium and Titanium.** Synthetic mixtures of titanium and niobium were prepared with niobium contents ranging from the equivalent of 0.5 to 4.0% niobium pentoxide based on the titanium dioxide value. In one series of tests, standard tartaric acid solutions were used and the color was developed directly by the addition of color developing reagents. In another series the niobium and titanium contained in hydrochloric acid solutions were precipitated with ammonia and the precipitate dissolved in tartaric acid prior to developing the color. Data given in Table V show that the accuracy of recovery in all cases was at least 95%.

**Color Development and Stability.** In the initial studies the niobium color was measured shortly after addition of the thiocyanate and over various periods of time following the first measurement. During the period between measurements the solutions were left in the cell compartment of the spectrophotometer. Under these circumstances the solutions either faded or failed to produce full color development. This difficulty was eventually traced to poorly fitting cell covers which permitted loss of acetone. Removal of cell covers after complete color development produced a decrease in absorbance values equivalent to 10% of the original value after 10 minutes of exposure, while a well-covered duplicate showed no change over the same period of time. After taking proper precautions, color development was complete in 20 minutes and stable for about 1 hour. Beyond 1 hour, the absorbance gradually decreased after an initial equal rise in blank and sample absorbance which did not change the absolute absorbance of the sample.

Table V. Recovery of Niobium Values from Synthetic Mixtures

| $TiO_2$ Present, Mg. | $Nb_2O_5$ Present, Mg. | % $Nb_2O_5$ Based on $TiO_2$ | $Nb_2O_5$ Found, Mg. | |
|---|---|---|---|---|
| | | | Using theoretical $TiO_2$ correction | Using ratio correction for $TiO_2$ |
| 25 | 0.125 | 0.5 | 0.123 | 0.125 |
| 25 | 0.250 | 1.0 | 0.254 | 0.255 |
| 25 | 0.500 | 2.0 | 0.493 | 0.495 |
| 12.5 | 0.500 | 4.0 | 0.509 | 0.515 |
| 25[a] | 0.500 | 4.0 | 0.525 | 0.520 |
| 25[a] | 0.500 | 4.0 | 0.525 | 0.520 |

[a] Chloride solution precipitated with $NH_4OH$ and dissolved in tartaric acid.

Table VI. Effect of Impurities on $Nb_2O_5$ Values

| Elements Present in Final Solution of 50 Ml., Mg. | | | | Error Introduced Based on Equal Amount of $Nb_2O_5$ |
|---|---|---|---|---|
| $TiO_2$ | $Fe_2O_3$ | $Nb_2O_5$ | Impurity | Impurity, % |
| | | | $Co_3O_4$ | |
| 0.0 | 0.0 | 0:0 | 24.2 | 0.0[a] |
| | | | $MnO_2$ | |
| 0.0 | 0.0 | 0.0 | 87.8 | 0.0[a] |
| | | | $MoO_3$ | |
| 0.0 | 0.0 | 0.0 | 8.1 | 4.4 |
| 22.5 | 2.5 | 0.25 | 2.5 | 0.0[b] |
| | | | $U_3O_8$ | |
| 0.0 | 0.0 | 0.0 | 8.27 | 2.8 |
| 22.5 | 2.5 | 0.25 | 2.5 | − 5.0[b] |
| | | | $WO_3$ | |
| 0.0 | 0.0 | 0.0 | 14.1 | 4.0 |
| 25.0 | 5.0 | 0.25 | 2.5 | +68 |
| 22.5 | 2.5 | 0.25 | 2.5 | +68[b] |
| 0.0 | 0.0 | 0.0 | 2.5 | 5.6 |
| 0.0 | 5.0 | 0.0 | 2.5 | 17.2 |
| 25.0 | 0.0 | 0.0 | 2.5 | 18.8 |
| 25.0 | 2.5 | 0.25 | 0.25 | 58.0[c] |
| | | | $V_2O_5$ | |
| 0.0 | 0.0 | 0.0 | 5.0 | 15.2 |
| 0.0 | 0.0 | 0.0 | 0.4 | 15.8 |
| 50.0 | 10.0 | 0.0 | 0.4 | 72.8 |
| 22.5 | 2.5 | 0.25 | 0.25 | 51.6[b] |
| 50.0 | 10.0 | 0.0 | 0.4 | 67.6 |
| 0.0 | 10.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 10.0 | 0.0 | 0.4 | 33.6 |
| 50.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 50.0 | 0.0 | 0.0 | 0.4 | 21.6 |
| | | | $WO_3$ $V_2O_5$ | |
| 25.0 | 0.0 | 5.0 | 25 25 | 28.0[b] |
| 25.0 | 250 | 5.0 | 25 25 | 0.27[d] |
| 0 | 250 | 5.0 | 25 25 | 0.19[d] |

[a] Impurity can be 100 times $Nb_2O_5$ without producing error in $Nb_2O_5$ values.

[b] Precipitated with ammonia prior to developing color.

[c] Aliquots of solutions containing impurities and niobium evaporated to dryness; $TiO_2$ and $KHSO_4$ added; fused and carried through precipitation steps prior to developing color.

[d] Precipitated with NaOH at pH 12.0 to 12.5 after oxidizing with $H_2O_2$.

**Interferences.** Molybdenum, tantalum, tungsten, uranium, and vanadium, in addition to titanium, were known to interfere in the determination of niobium according to the method of Freund and Levitt. As the present method differs from that of Freund and Levitt by the use of a lower concentration of thiocyanate and longer wave length, the effect of these and other elements on the modified procedure was investigated.

The effect of the impurities was tested under several different conditions. In some cases the impurity element in the form of a soluble salt was examined for its ability to produce a color in the absence of other elements. In other cases the impurity was added to solutions of titanium, iron, and niobium, either separately or in combination, and the color developed by direct addition of the color producing reagents as well as after carrying out an ammonia precipitation. Data are given in Table VI. Absorption curves for impurities producing colors are given in Figure 3.

**Cobalt.** Cobalt produced an intense blue color but did not absorb at wave lengths used for the determination of niobium. No interference was produced when the cobalt to niobium ratio was 100 to 1.

**Manganese.** Manganese did not produce interference when present in the ratio of 100 to 1 manganese to niobium.

**Molybdenum.** In the absence of other elements, molybdenum produced a color with an intensity which was 4% as intense as an equal amount of niobium. This interference was eliminated when solutions containing titanium, iron, niobium, and molybdenum were precipitated with ammonia prior to color development.

**Tantalum.** Tantalum did not produce a color with thiocyanate, but a turbidity occurred when the color developing reagents were added to solutions containing high concentrations of tantalum. The maximum permissible before development of a turbidity was about 5 mg. of tantalum pentoxide per 100 ml.

**Uranium.** In the absence of other elements, uranium produced a weak color which was about 2 to 3% as intense as an equal amount of niobium. In the presence of titanium, iron, and niobium, uranium appeared to inhibit development of the niobium color since equal amounts of niobium and uranium produced a color that was about 5% low.

**Tungsten.** Solutions containing only tungsten produced a color which was 5% as intense as that produced by an equal amount of niobium. In the presence of titanium the color due to tungsten was found to be about 20% as intense as the niobium color after making a correction for titanium. The interference of tungsten was also intensified in the presence of iron. With a combination of titanium, iron, and niobium, the interference produced by tungsten was well in excess of the sum of individual absorbances. Under these circumstances the color produced by tungsten was 80% as intense as an equal amount of niobium.

The marked increase in interference, due to tungsten in the presence of mixtures, was attributed to the acceleration of the gradual increase of absorbance which occurred with pure solutions of tungsten over extended periods of time.

**Vanadium.** Vanadium acted in a manner similar to tungsten. In the absence of other elements it produced an intensity which was 15% as intense as an equal amount of niobium. This value increased to 22% in the presence of titanium and 34% in the presence of iron. In presence of titanium, iron, and niobium the color produced by vanadium was 68% as intense as an equal amount of niobium.

Vanadium and tungsten in oxidized solutions can be separated from niobium and titanium by precipitating the latter two elements from carbonate solutions with the aid of magnesia (*5*). A less involved procedure found satisfactory in the presence of relatively large amounts of either titanium or iron was precipitation from an oxidized 20% hydrochloric acid solution with sodium hydroxide at a pH of 12.0 to 12.5, boiling for 15 minutes, filtering, and washing with hot 15% sodium chloride and a final water wash. The precipitate was washed back into the beaker containing a predetermined weight of tartaric, heated to 50° to 60° C. to dissolve the precipitate, and diluted to a volume such that no more than a 10-ml. aliquot was required for color development. The weight of tartaric acid used was that amount which produced a 0.5$M$ solution.

## APPLICATION

The procedure should be applicable to a wide variety of minerals and other products containing niobium and titanium. No acids other than tartaric should be present in the solutions which are used for color development. Large amounts of sulfates and other extraneous salts should also be absent. Such conditions are best established by dissolving the sample in a suitable acid or fusion mixture, precipitating the niobium and titanium with ammonium hydroxide, and dissolving the hydrates in tartaric acid. In the presence of interfering elements, such as vanadium and tungsten, a similar precipitation may be carried out with sodium hydroxide. The melts of samples that are solubilized by fusion with potassium pyrosulfate, and which contain no interfering elements, may be dissolved directly in tartaric acid. This may be used as an alternative procedure in dissolving the precipitated hydrates of niobium and titanium. Since sulfates reduce the absorbance of the niobium color, the amount of pyrosulfate should be kept at an insignificant final concentration of about 25 mg. to 50 ml. or sufficient sulfate added to solutions used in establishing the standard calibration curve to compensate for its effect (*2*).

Certain precautions must be rigidly observed in carrying out ammonia precipitation of titanium so that the precipitate may be dissolved in tartaric acid. Precipitation must be carried out with excess ammonia and the boiling limited to 5 minutes so as not to drive off the excess ammonia. If insufficient ammonia is used or the boiling extended too long, the hydrate will not dissolve in solutions containing as much as 15% tartaric acid. In spite of these precautions, the temperature during solution in tartaric acid must not be above 60° C. The time required to produce a crystal clear solution may take as long as 2 hours with pure titanium dioxide. The presence of iron in the precipitate reduced the time requirement considerably.

The method has been applied mainly to the determination of niobium in titanium products with a titanium dioxide–niobium pentoxide ratio of about 100 to 1. Under these circumstances the niobium accuracy is at least 95%. At a titanium dioxide–niobium pentoxide ratio of 1000 to 1 the titanium absorbance is 75% of the total absorbance at 400 m$\mu$, and the accuracy is still about 95%. At higher ratios the accuracy drops off rapidly because of the greater contribution of the titanium to the total absorbance.

The following procedure was used for the determination of niobium in products containing principally 70 to 90% titanium dioxide, 5 to 15% ferric oxide, and 1 to 20% niobium pentoxide. Samples ranging between 0.500 and 0.100 gram were fused with 8 grams of potassium pyrosulfate, and the melts dissolved in 100 ml. of water containing 25 ml. of concentrated hydrochloric acid. Small amounts of residue remaining were filtered off, ignited, treated with hydrofluoric and sulfuric acids, fused in pyrosulfate, and returned to the main solution. Excess ammonia was added and the solutions were boiled for 5 minutes to precipitate the hydrates. The precipitates were thoroughly washed to remove sulfates, transferred to beakers containing 15 grams of tartaric acid, diluted to 100 ml., and heated for about 1 hour at 50° to 60° C. to dissolve the hydrates. After cooling, the solutions were transferred to a 200-ml. volumetric flask and diluted to volume.

Aliquots of 2 to 5 ml. were transferred to 50-ml. flasks and sufficient amounts of 0.5$M$ tartaric acid added so that sample volumes plus tartaric acid reagent were equal to 10 ml. Twenty milliliters of 1 to 1 hydrochloric acid, 10 ml. of acetone, and 2 ml. of 1$M$ stannous chloride in 1 to 1 hydrochloric acid were added, and the solutions cooled to room temperature. The flasks were stoppered between addition of reagents and during cooling. After attaining room temperature, 5 ml. of 3$M$ potassium thiocyanate was added and the solutions were immediately diluted to volume, mixed, stoppered, and allowed to stand 30 minutes to permit complete color development. Measurements were then made at 360 and 400 m$\mu$ in a Beckman Model B spectrophotometer with maximum sensitivity setting. The fraction of total absorbance at 400 m$\mu$ due to niobium was calculated by use of the ratio equation, and the niobium content found by use of a calibration curve of absorbance *vs.* niobium concentration.

## ACKNOWLEDGMENT

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# Determination of Trace Kjeldahl Nitrogen in Petroleum Stocks

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**The only previously reported method for determining very low levels of total nitrogen in petroleum stocks is unsuited to routine analysis. In this work, a reproducible low blank has been achieved by purification of the Kjeldahl reagents, permitting detection of as little as 1 p.p.m. total nitrogen by Kjeldahl digestion of a 5-gram sample. A systematic study was made of the phenol–sodium hypochlorite colorimetric method of ammonia assay, resulting in improved reliability of this hitherto unpopular method. The procedure lends itself well to multiple analyses.**

THE nitrogen content of petroleum stocks is of interest because even small quantities of nitrogen poison the catalyst in reformer units and promote the formation of gum in stored products. Traces of basic nitrogen may be determined readily down to 1 p.p.m. by perchloric acid titration (*5*), but basic nitrogen may be only a minor fraction of the total nitrogen to be found in a petroleum stock. A recent publication (*11*) reviews the limitations of the Kjeldahl (*3*) and ter Muelen (*2*) procedures and solves the problem by catalytic hydrogenation of a very large (1000-ml.) sample. The method is not suited to routine analysis.

This investigation covers the necessary modifications of the basic Kjeldahl procedure to permit its use for the detection of as little as 1 p.p.m. total nitrogen with the digestion of only a 5-gram sample. The investigation consisted of three parts: the establishment of a colorimetric procedure for routine determination of low concentrations of ammonia, the purification of the Kjeldahl reagents to give a reproducible low blank value, and a study of the ability of the Kjeldahl procedure to convert, without loss, trace amounts of nitrogen in organic media to ammonia. The trace nitrogen so determined is termed Kjeldahl nitrogen because it is consistent with macro Kjeldahl nitrogen on the crude oils on which the method has been tested. No claim is made for it being an absolute total nitrogen method.

## COLORIMETRIC DETERMINATION OF AMMONIA

The ammonia detection procedure had to be selected and its reliability established before reagent blanks could be determined. The phenol and sodium hypochlorite reagent, which gives a clear blue color with ammonia, was chosen because the blue color would be more suitable for spectrophotometric evaluation than the colloidal Nessler color. It was also thought the reaction would be less sensitive to reagent formulation, pH, and temperature than the Nessler reaction. The phenol–sodium hypochlorite method is included in laboratory manuals (*8*) and has been studied by many investigators (*1, 7, 9, 10*). It is recommended for its high sensitivity but warnings usually appear concerning poor reproducibility and color instability. A systematic study was made of the variables in this reaction, and a sensitive and reproducible procedure was developed as follows:

### APPARATUS

Cenco Photelometer with 1- and 5-cm. cells and 610-mμ filter.
Water bath maintained at 100° ± 0.5° C.
Usual laboratory glassware.

### REAGENTS

Freshly distilled water, ammonia-free.

Sodium hypochlorite solution, commercial Clorox, labeled as 5.25% by weight NaOCl.

Phenol solution, made by dissolving 8 grams of C.P. phenol in 100 ml. of freshly distilled water. If the solution becomes cloudy, warm it slightly prior to use.

Standard nitrogen solution for calibration, made by dissolving 0.382 grams of dry C.P. ammonium chloride in 1 liter of fresh distilled water. Pipet 100 ml. of this solution into a 1-liter volumetric flask and make up to mark. This standard contains 0.01 mg. of nitrogen per ml.

### COLORIMETRIC PROCEDURE

The Kjeldahl distillate, in which the ammonia is fixed with two drops of 0.5*N* sulfuric acid, is concentrated by evaporation in the presence of boiling beads to 20-ml. volume in the receiving flask. Two standard samples are prepared in similar flasks by adding 0.0 and 25.0 ml. of the nitrogen standard to 175 ml. of fresh distilled water containing two drops of 0.5*N* sulfuric acid and then concentrating by boiling to 20-ml. volume. The flask contents are transferred to 50-ml. borosilicate mixing cylinders, and the volume is increased to 40 ml. with washings from the flask. At this point it is convenient to carry six to eight cylinders through the remaining steps together. Pipet 5.0 ml. of the 8% phenol solution into each of the series of cylinders. Mix the contents by upending several times. Pipet 5.0 ml. of the sodium hypochlorite solution into each cylinder and mix again. Plunge the cylinders into boiling water to a depth sufficient to cover liquid contents. Loosen the ground-glass stoppers. After 6 but before 8-minute exposure to the boiling water bath, remove the cylinders and cool them quickly in tap-water bath. Measure the absorbance of the solution in 1-cm. cells using a 610-mμ filter, and estimate the milligrams of nitrogen in the samples from the linear curve established by the two reference samples. If samples contain under 0.1 mg. of nitrogen, it is advantageous to use 5-cm. absorption cells and a standard curve based on 0 and 10 ml. of standard nitrogen solution.

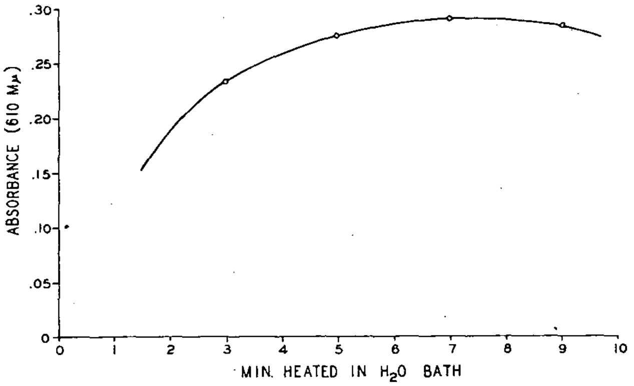

Figure 1. Time *vs.* color intensity

0.25 mg. N as $NH_4^+$

Table I. Effect of Sequence of Addition of Reagents on Color Intensity

| Treatment[a] | Absorbance with 610-mμ Filter |
|---|---|
| Add phenol, add NaOCl, stand 5 minutes, heat | 0.276 |
| Add phenol, add NaOCl, heat | 0.276 |
| Add phenol, stand 5 minutes, add NaOCl, heat | 0.282 |
| Add NaOCl, add phenol, stand 5 minutes, heat | 0.187 |
| Add NaOCl, add phenol, heat | 0.200 |
| Add NaOCl, stand 5 minutes, add phenol, heat | 0.032 |

[a] Each treatment was performed on 0.25 mg. N as $NH_4Cl$ and one drop 0.5*N* $H_2SO_4$ made up to 40 ml. with distilled water. Additions were made as listed under treatment. Each sample was inverted twice after each addition of reagent to mix and heated 7 minutes in boiling water bath.

**Table II. Effect of Volume to Which Reagents Are Added on Ultimate Color Intensity**

| Treatment[a] | Absorbance with 610-mμ Filter |
|---|---|
| Add phenol, add NaOCl, add 20 ml. water, heat | 0.274 |
| Add phenol, add NaOCl, add 20 ml. water, heat | 0.268 |
| Add phenol, add 20 ml. water, add NaOCl, heat | 0.218 |
| Add phenol, add 20 ml. water, add NaOCl, heat | 0.212 |
| Add phenol, add 10 ml. water, add NaOCl, add 10 ml. water, heat | 0.238 |
| Add phenol, add 10 ml. water, add NaOCl, add 10 ml. water, heat | 0.235 |

[a] Each treatment performed on 0.20 mg. N as $NH_4Cl$ in 20 ml. of distilled water.

## EXPERIMENTAL

The effect of one to five drops excess of 0.5*N* sulfuric acid was studied over the range of 0 to 0.25 mg. of nitrogen and found to have no effect. The effect of heating time was studied over the range of 3 to 9 minutes, and 7 minutes in boiling water was found to be optimum. This is shown in Figure 1. The stability of the color formed was observed for several hours and found to be stable even in the presence of air.

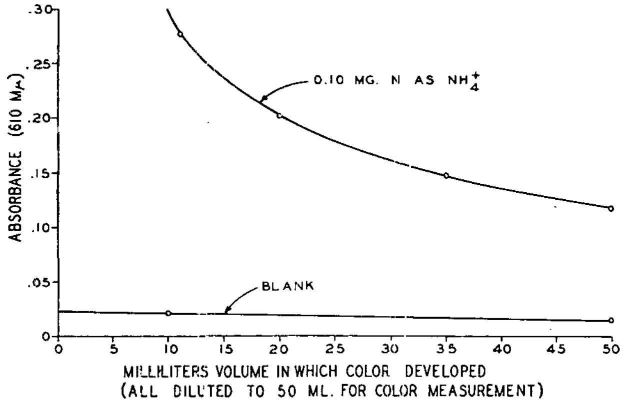

Figure 2. Effect of volume in which color developed *vs.* color intensity

The sequence of the addition of the phenol and sodium hypochlorite solution was found to be important. The phenol solution must be added first and mixed in well. The time between steps is of little significance, provided this is done. The data are shown in Table I.

The volume of solution in which the color is developed affects the color intensity. If the color is formed in a volume of less than 50 ml. and then diluted to 50 ml. when cool, a more intense color results. This effect is shown in Figure 2. Also if the volume of solution is less than 40 ml. when the 5 ml. of phenol and 5 ml. of sodium hypochlorite are added, and then the volume is increased to a total of 50 ml. before heating, a more intense color results. The smaller the initial volume, the greater the color intensity as shown in Table II. If the volume is 45 ml. before adding the sodium hypochlorite, however, it appears to make no difference at what dilution the phenol is added.

The effect of phenol concentration was studied and is shown in Figure 3. The optimum amount is near 5 ml. but is not critical. The effect of sodium hypochlorite concentration is shown in Figure 4. Here, too, the optimum is at about 5 ml. and is not too critical. In Figure 5, the spectral absorption curve of the blue solution between 540- and 800-mμ wave length shows a peak near 610 mμ, which is the reason for choice of the designated filter. In Figure 6, typical concentration curves versus absorbance show the linear relationship with either the 1- or 5-cm. cell.

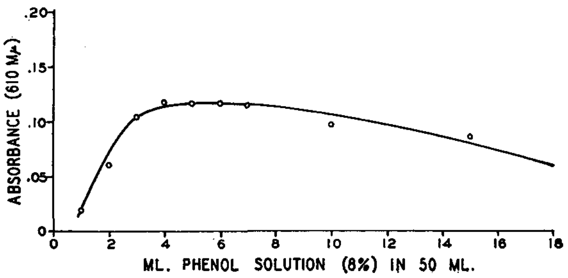

Figure 3. Effect of phenol concentration on color intensity

0.10 mg. N as $NH_4^+$

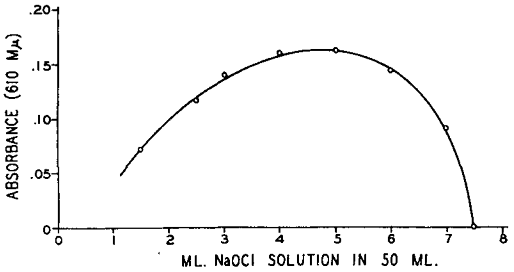

Figure 4. Effect of sodium hypochlorite concentration on color intensity

**Table III. Nitrogen Found in Kjeldahl Reagents**

| | | Nitrogen Content | |
|---|---|---|---|
| Reagent | Quantity Tested | In quantity tested, mg. | P.P.M. nitrogen blank if 5-g. sample digested |
| $H_2SO_4$, C.P. | 50 ml. | 0.03–0.12 | 7–25 |
| $H_2SO_4$, distillation heart cut | 50 ml. | 0.01 | 2 |
| $K_2SO_4$, C.P. | 10 g. | 0.03–0.06 | 6–12 |
| $K_2SO_4$, recrystallized | 10 g. | 0.01 | 2 |
| Hg, C.P. triple distilled | 1.0 g. | 0.00 | 0 |
| HgO, C.P. | 0.7 | 0.01 | 2 |
| $CuSO_4$, C.P. | 1.0 g. | | |

## DISCUSSION

It is evident from Figure 2 and Table II that sensitivity beyond the normal effect of concentration can be obtained by developing and measuring the color in a minimum volume. It was not necessary to take advantage of this greater sensitivity for this use of the method. Since this work was done, Riley (*6*) has reported a systematic study of development of the indophenol color. His procedure differs considerably from the one developed here in that it uses more complex reagents and a longer color development period.

## REDUCTION OF KJELDAHL BLANK

Chemically pure sulfuric acid gave a high blank value which differed widely from lot to lot. A simple distillation in all glass equipment yielded a heart cut (10 to 80%) of uniformly low nitrogen content. Chemically pure potassium sulfate also gave a high and variable blank value. A simple recrystallization reduced the blank to a uniform low value. Catalytic materials were also tested for their nitrogen content. The nitrogen content of various reagents is summarized in Table III.

If the Kjeldahl procedure is run on a 5-gram sample with 50 ml. of distilled sulfuric acid, 10 grams of recrystallized potassium sulfate, and mercury metal as a catalyst, the blank corresponds to 4 p.p.m. of nitrogen in the result, 2 p.p.m. coming from the acid, and 2 p.p.m. coming from the sulfate.

In this range a difference of 1% transmittance, which is the accuracy of the detecting instrument, is equivalent to just under

1 p.p.m. of nitrogen using 1-cm. cells and about 0.3 p.p.m. using the 5-cm. cells. Consequently, since the blanks vary less than 1%, they are considered to be sufficiently reproducible to determine ±2 p.p.m. of Kjeldahl nitrogen in a sample up to 50 p.p.m. and to detect as little as 1 p.p.m. with certainty.

### FURTHER MODIFICATIONS AND RESULTS

**Reagents.** POTASSIUM SULFATE. Recrystallize C.P. grade potassium sulfate by saturating 2000 ml. of boiling distilled water and then allowing it to cool overnight. Filter off the crystals, rinse lightly with distilled water, and store wet for future use.

COPPER SULFATE, C.P. anhydrous powder.

MERCURIC OXIDE, C.P. powder.

MERCURY, C.P. triple distilled.

SULFURIC ACID, 10 to 80% heart cut from C.P. concentrated acid of 1.84 gravity prepared in an all-glass still. Distill the acid batchwise at atmospheric pressure from a 2-liter round-bottomed flask with a ground-glass standard-tapered neck, connected to an air condenser by a 75° connector with standard tapered-ground glass fittings at both ends. It is not necessary to lubricate the joints, to record the temperatures, or to protect the overhead from air. Approximately 4 hours are required to obtain the 10 to 80% cut from a 1700-ml. charge.

FIFTY PER CENT SODIUM HYDROXIDE SOLUTION. Dissolve 2 kg. of C.P. sodium hydroxide pellets in 2 liters of water. Let the solution stand 24 hours to cool and permit the carbonate to settle out. Filter through glass wool into a gallon bottle.

**Kjeldahl Digestion Procedure.** Weigh into a clean, dry 300-ml. Kjeldahl flask 10 grams of purified potassium sulfate, 1 gram of copper sulfate, and 0.7 gram of mercuric oxide. (One gram of mercury may be used instead of the copper sulfate and mercuric oxide.) Add three or four glass beads and 50 ml. of the distilled sulfuric acid. Weigh into the flask 5 grams of sample to the nearest 0.01 gram. If more than 50 p.p.m. of nitrogen are anticipated, take a smaller weight of sample, or complete analysis by distilling into boric acid and titrating with hydrochloric acid.

Several blanks should be run to establish the amount of nitrogen contributed by the sulfuric acid and catalysts. In the place of the sample, use 5 ml. of a pure hydrocarbon such as isooctane. Once the blank value is known, it need not be redetermined until different reagents are used.

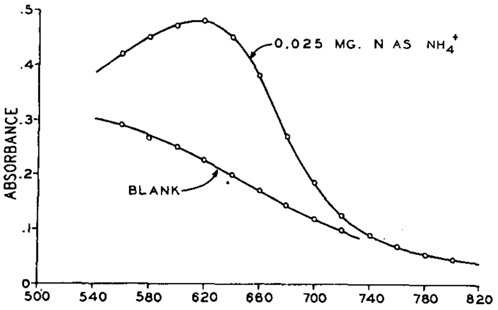

**Figure 5. Spectral absorption curve**

**Sodium phenate reagent**
**10-cm. cell**

Swirl the flasks vigorously until the sample and acid are well mixed. (If the material being analyzed, or any fraction thereof, has a boiling point below about 100° C., digest for 1 hour on a steam plate with periodic vigorous swirling.) Place the flasks on the Kjeldahl digestion rack and proceed with the digestion, using as much heat as necessary. Excessive foaming can be largely controlled by periodically removing the flasks and swirling them for a few seconds, or small additions of sulfuric acid may be used. However, the additional sulfuric acid is not desirable and consequently should be avoided if possible.

After the acid has become clear and light-colored, and the neck of the flask has been cleaned down by increasing the heat and letting the sulfuric acid reflux, reduce the total volume about 25 ml. with a little additional heat. Reduce the flame and boil another 3 hours to ensure complete reduction of the nitrogen.

Cool the contents of the flasks, slowly add 175 ml. of distilled water, and cool again. Incline the Kjeldahl flask at 45° and add 55 ml. of 50% caustic solution slowly, so that it settles to the bottom without mixing. Add a pinch of 20-mesh metallic zinc. Place the flask in position on the distillation apparatus. (If the apparatus has been used for other work, the inside of the still should be cleaned by drawing distilled water through it by vacuum, or by distilling off a flask full of water.) Add 20 ml. of distilled water and two drops of 0.5*N* sulfuric acid to a 250-ml. Erlenmeyer flask and mount on the distillation apparatus to receive the distillate. Swirl the Kjeldahl flask to mix the contents thoroughly, and then distill until serious bumping commences.

The Kjeldahl portion of the procedure required relatively little investigation or modification. Steps in the procedure, which differed from the conventional Kjeldahl procedure, were checked to make certain that no ammonia or nitrogen was lost.

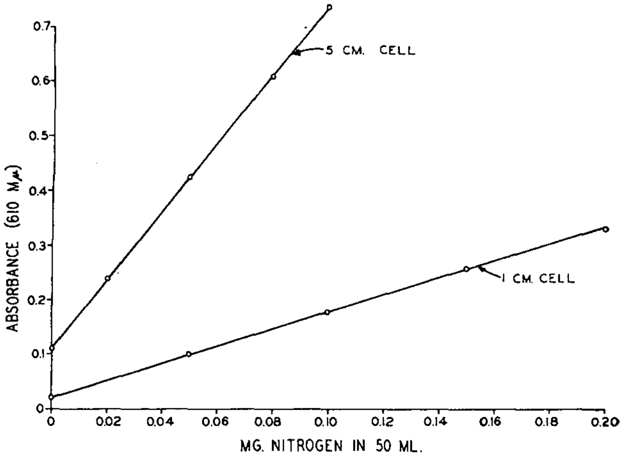

**Figure 6. Standard curves**

**Cenco photelometer with 610-mμ filter**

When a 5- to 6-gram sample was digested in 50 ml. of concentrated sulfuric acid plus the catalyst, a large portion of the lower boiling paraffinic and naphthenic hydrocarbons distilled off and was lost. However, digesting at a lower temperature for longer periods, so that no sample distilled off, did not increase the apparent nitrogen recovery. Because of the rapid distillation of hydrocarbon, a large quantity of sulfuric acid remained after the sample had cleared. To obtain a temperature high enough to ensure complete reduction of the nitrogen (*4*), the amount of sulfuric acid for the 10 grams of potassium sulfate present must be reduced. Raising the temperature of the burner after the solution had cleared to distill off the excess sulfuric acid resulted in no discernible loss of nitrogen from standard samples.

**Table IV. Results of Analysis of Diluted Crude Oils**

| Crude Oil Diluted | | Known Total N by Dilution, P.P.M. | Basic Nitrogen Found, P.P.M. | Total Nitrogen Found, P.P.M. | |
|---|---|---|---|---|---|
| Name | Nitrogen content, % | | | Catalyst 1[a] | Catalyst 2[a] |
| Midway | 0.50 | 5 | 1.6 | 6 | 5 |
| Midway | 0.50 | 10 | 2.5 | 10 | 8 |
| Santa Maria | 0.81 | 5 | 0.8 | 5 | 4 |
| Santa Maria | 0.81 | 10 | 2.2 | 11 | 12 |

[a] Catalyst 1, $CuSO_4$ and HgO.
Catalyst 2, Hg.

Two California crudes, one Santa Maria and the other Midway, which were high in sulfur and nonbasic nitrogen, were analyzed and then diluted to 5 and 10 p.p.m. of total nitrogen with nitrogen-free kerosine. The analyses of these stocks are shown in Table IV. This shows good recovery of the total Kjeldahl nitrogen at very low levels and is the basis for extending the method to naphtha analysis. The procedure has been in routine use for about a year and a half and has been of value in distinguishing basic from total nitrogen at low levels. The method has been cross checked with the ter Muelen and basic nitrogen procedures where applicable, and the analyses have been thoroughly consistent.

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# Precipitation of Barium Carbonate

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**Critical studies of the several factors affecting the solubility of barium carbonate in analytical procedures have not been reported. It was found that a dense, easily filtered, and easily washed product was obtained by bubbling carbon dioxide into an ammoniacal solution containing barium. Using radiochemical tracer techniques, the solubility loss of barium carbonate was determined with respect to final pH, alcohol concentration of the wash solution, addition of alcohol to the mixture after precipitation of the barium, and the presence of excess ammonium salts. The recommended procedure results in a solubility loss of 0.00015 gram of barium in 375 ml. of solution.**

THE quantitative precipitation of barium carbonate is recommended in various texts (*8, 14*) of qualitative analysis. A quantitative barium carbonate precipitation has been recommended by Fresenius and Hintz (*3*), although the presence of ammonium salts, present in the procedure, have been reported as preventing complete precipitation (*9*).

A survey of the literature (*11*) indicated that no critical study of the factors affecting the solubility of barium carbonate under analytical conditions has been reported. Towley, Whitney, and Felsing (*13*) have reported the solubility of barium carbonate in pure water and in the presence of alkali chlorides. The solubility in water of barium carbonate at various pressures of carbon dioxide was investigated by Haehnel (*5*). Semiquantitative estimations of the solubility of barium carbonate in the presence of varying concentrations of ammonia, ammonium carbonate, and ethyl alcohol have been published by Bray (*2*). Sidgwick (*12*) has accepted the solubility in water at 18° C. as being 8.6 mg. per liter.

This paper describes tests made to determine the solubility loss (solubility in mother liquor plus wash solution) of barium carbonate formed by the introduction of carbon dioxide into an ammoniacal solution containing barium followed by the addition of alcohol. The precipitate is then washed with aqueous alcohol. The solubility loss was investigated with respect to the final pH, the addition of alcohol to the reaction mixture after precipitation, the presence of excess ammonium salts, and the composition of the wash solution.

## APPARATUS AND MATERIALS

**Apparatus.** The reaction vessel is illustrated in Figure 1. During precipitation carbon dioxide was introduced through the medium-porosity glass frit. Filtration was performed by connecting the exit tube to a bell jar which could be evacuated by gentle suction. A beaker placed in the bell jar served to collect the filtrate and washings.

**Reagents.** Barium nitrate. General Chemical Co. reagent grade barium nitrate was recrystallized twice from water and dried overnight at 110° C.

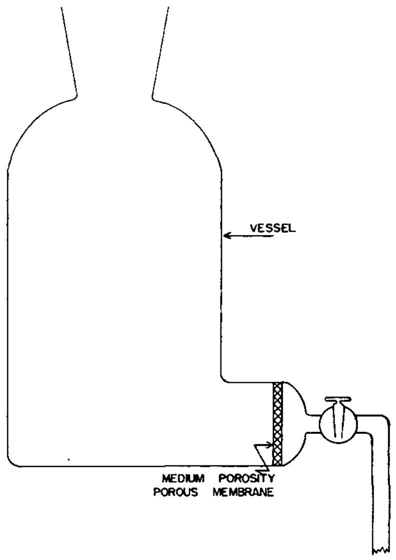

**Figure 1. Reaction vessel**

Barium-140. This material, carrier-free, was obtained from Oak Ridge National Laboratory in dilute nitric acid and with a radiochemical purity greater than 98%. Two solutions were prepared from this material: One solution contained approximately 0.1 mc. per 0.3 ml. and the second solution, which was used in the preparation of counting standards, contained approximately 25,000 counts per 0.1 ml.

**Table I. Effect of Precipitation Time on Total Solubility Loss**

| Alcohol Added, Ml. | Alcohol Concentration, % | Barium Lost, % | |
|---|---|---|---|
| | | Time, 30 min. | Time, 40 min. |
| 0 | 0.0 | 0.037 | 0.036 |
| 10 | 4.4 | 0.025 | 0.089 |
| 20 | 8.5 | 0.051 | 0.051 |
| 30 | 12.2 | 0.036 | 0.040 |
| 40 | 15.6 | 0.029 | 0.050 |

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Carbon Dioxide. The carbon dioxide was obtained from the Pure Carbonic Corp., New York, N. Y. The gas was used directly from the cylinder without further purification.

Ethyl alcohol. Absolute alcohol was used, although no special precautions were taken to maintain this grade.

Shelf Reagents. Nitric acid, concentrated, and ammonium hydroxide, concentrated, were reagent grade chemicals supplied by the General Chemical Co.

pH Paper. The pH measurements were made with Precision pH control paper supplied by the Precision Laboratories, Rossmoyne, Ohio. The accuracy was ±0.1 to 0.2 unit.

## EXPERIMENTAL

**Initial Work.** Exploratory tests using the standard procedures for precipitating barium carbonate produced a voluminous and gelatinous precipitate which clogged all filters used. This was caused by the relatively large amount of barium used, about 3 grams, and the high supersaturation condition produced by the ammonium carbonate–ethyl alcohol procedure.

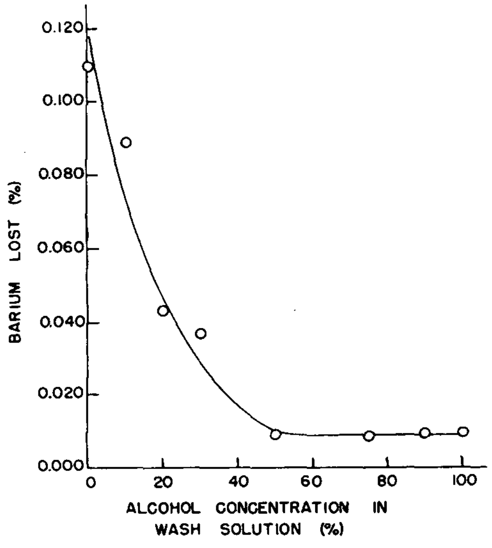

**Figure 2. Effect of composition of wash solution on solubility loss**

Initial efforts to improve the physical nature of the precipitate were directed to eliminating or minimizing the supersaturation. Precipitation from homogenous solution was tried by hydrolyzing urea (*4*, *15*) or potassium cyanate (*10*) in slightly acidic solutions of barium nitrate. The pH was raised slowly and carbonate ion was generated uniformly throughout the solution. The reaction with cyanate proved more satisfactory, producing large, easily filtered, and readily washed crystals. However, precipitation was not complete in 2 hours at elevated temperatures.

When gaseous carbon dioxide was bubbled into an ammoniacal solution, the precipitate obtained was intermediate between the product of the standard procedure and that obtained from homogeneous solution. This was indicated by the relative volumes and the settling and filtering characteristics of the precipitate. More undesirable filtering characteristics were observed with the carbon dioxide procedure when the initial pH was too high, about 11, and when ethyl alcohol was present during the initial stages of precipitation. Addition of ethyl alcohol after precipitation was essentially complete, however, had no noticeable effect upon the physical characteristics of the precipitate, but did make precipitation complete.

## PROCEDURE

A solution was prepared containing 5.76 grams of barium nitrate in 150 ml. of water, 2.0 ml. of concentrated nitric acid was added, and the solution was transferred to the reaction vessel. The volume was adjusted to 200 ml. and approximately 0.1 mc. of an equilibrium mixture of barium-140 and lanthanum-140 was added. The pH was adjusted to 9.5 by the addition of 10 ml. of concentrated ammonium hydroxide, and carbon dioxide was introduced for the appropriate time. No effort was made quantitatively to control the rate of flow of carbon dioxide other than to avoid spattering. During precipitation, 3-ml. portions of ammonium hydroxide were added at 10-minute intervals to maintain an alkaline pH. The final pH was varied by adding varying volumes of ammonium hydroxide. Varying quantities of ethyl alcohol were then added. The mixture was thoroughly mixed and allowed to stand 10 minutes before filtration. The mother liquor was filtered by gentle suction and the precipitate was washed.

The combined filtrate and wash solution was acidified with nitric acid and then evaporated to a volume of approximately 50 ml. After cooling, the solution was transferred to a 100-ml. volumetric flask. Duplicate 25-ml. aliquots were transferred to a centrifuge tube, 10 mg. of carrier barium were added, and barium sulfate was precipitated by the dropwise addition of sulfuric acid to the vigorously stirred solution. Stirring was continued for 15 minutes and the precipitate was centrifuged, washed, and mounted on slides for counting by proportional counting. The mounting procedure has been described by Ames and coworkers (*1*). Activity standards were prepared at the same time and by the same procedure. Duplicate slides usually agreed within 1 to 2% even at rates as low as several hundred counts per minute. The barium-140 activity was differentiated from its shorter-lived daughter, lanthanum-140, by application of Kirby's (*6*) differential decay equation.

## RESULTS

**Effect of Precipitation Time.** Table I shows the results obtained when precipitation was allowed to proceed for 30 and 40 minutes, to a final pH of 9.1, whereupon varying quantities of alcohol were added, and the precipitate was washed with 100 ml. of water in 25-ml. portions.

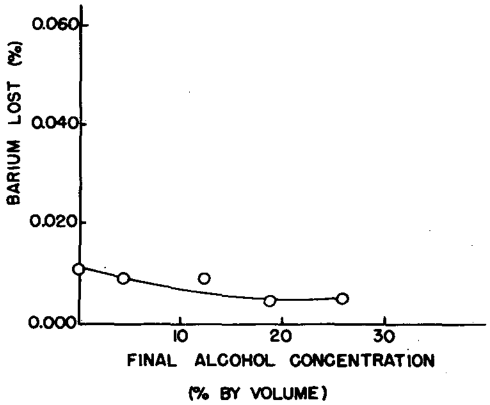

**Figure 3. Effect of addition of alcohol following precipitation**

The data appear to indicate that for a given alcohol concentration and a precipitation time of 30 minutes, some factor other than time affects the solubility loss. As shown below, the critical factor appeared to be the composition of the wash solution.

**Effect of Composition of Wash Solution.** The effect of alcohol concentration of the wash solution on the solubility loss of barium carbonate is illustrated in Figure 2. The final pH, following a 30-minute precipitation time, was adjusted to 9.1, and 10 ml. of ethyl alcohol was added. It was observed that four 25-ml. portions of wash solution were required to remove the ammonium salts completely. It is apparent that the combined solubility loss is reduced by a factor of 10 by use of a 50% (by volume) aqueous–ethyl alcohol wash solution. This corresponds to a recovery of 99.991% of the barium. These results indicated the need for further study of the effect of alcohol addition to the mixture after precipitation.

**Effect of Addition of Alcohol After Precipitation.** Figure 3 summarizes the observations made when varying amounts of ethyl alcohol were added to the reaction mixture after 30 minutes of precipitation time, the final pH being 9.1, and the precipitate being washed with four 25-ml. portions of 90% alcohol. The solubility loss becomes essentially constant when the ethyl alcohol concentration is about 20% by volume, and the recovery is 99.995%.

**Effect of Final pH.** Since the physical characteristics of the barium carbonate precipitate obtained depended to some extent on the pH of the solution, a study of the effect of final pH on the solubility loss was necessary in order to determine the most satisfactory final pH. The observations at varying ethyl alcohol concentrations are illustrated in Figure 4. Precipitation proceeded for 10 minutes, and the product was washed with four 25-ml. portions of 90% ethyl alcohol.

It is apparent that a sharp rise in the solubility occurs below pH 7, and the depressing effect of alcohol has its greatest relative effect in this region. Above pH 10 the solubility loss at 0 to about 20% alcohol becomes essentially the same.

Table II. Effect of Excess Ammonium Nitrate

| Ammonium Nitrate Added, Grams | Barium Lost, % |
|---|---|
| 0 | 0.0046 |
| 15 | 0.015 |
| 30 | 0.037 |

**Effect of Excess Ammonium Salts.** Several experiments were performed to determine the effect of excess ammonium salts on the solubility loss. Precipitation was performed as previously described, except that varying amounts of ammonium nitrate were added to the mixture before precipitation. In all cases the final pH was adjusted to 9.1, more ammonia being required than was necessary in the absence of the salts. The data are summarized in Table II.

The results show that the presence of excess ammonium nitrate exerts a solubilizing effect on barium carbonate at a given pH and alcohol concentration.

## RECOMMENDED PROCEDURE

Using the vessel described, or some modification thereof, the barium nitrate should be present in 200 ml. of solution. Add 10 ml. of concentrated ammonium hydroxide, stir thoroughly, introduce carbon dioxide for 30 minutes at a rate such as to avoid spattering, and add 3 ml. of ammonium hydroxide to the solution after 10 and 20 minutes have elapsed. After the 30 minutes, add 10 ml. of ammonia, turn off the carbon dioxide, add 50 ml. of ethyl alcohol, mix, and allow to stand for 10 minutes. Filter the mother liquor and wash the precipitate with four 25-ml. portions of 90% ethyl alcohol.

## DISCUSSION AND CONCLUSIONS

The results have indicated the relative effects of final pH, alcohol concentration in the wash solution, the addition of alcohol after precipitation, and the presence of excess ammonium nitrate on the solubility loss of barium carbonate. Precipitation from solutions at a high initial pH or containing alcohol, both of which decrease the solubility, produces a product with unfavorable filtering and washing characteristics. These observations are in accord with Von Weimarn's (*16*) precipitation rules concerning the relationship between the physical nature of a precipitate and initial supersaturation, the latter depending upon the solubility.

The solubility loss by the procedure recommended results in a loss of 0.5 mg. of barium carbonate per liter at 22° C., compared with Sidgwick's (*12*) value of 8.6 mg. at 18° C. in water. These results indicate the need for more careful evaluation of solubility data under analytical conditions.

Complete washing of ammonium salts required 100 ml. of 50% alcohol when 3 grams of barium were precipitated as the carbonate. The procedure of Fresenius and Hintz (*3*) recommends 50 ml. and not only is a more voluminous precipitate produced, but a greater concentration of ammonium salts is present. The combined filtration and washing of the barium carbonate required 15 minutes in the apparatus described.

The data also confirm previous reports on the solubilizing effect of ammonium salts on barium carbonate (*9*). This may account for Kolthoff's (*7*) report that sodium carbonate was a more sensitive reagent for detecting barium ion than ammonium carbonate.

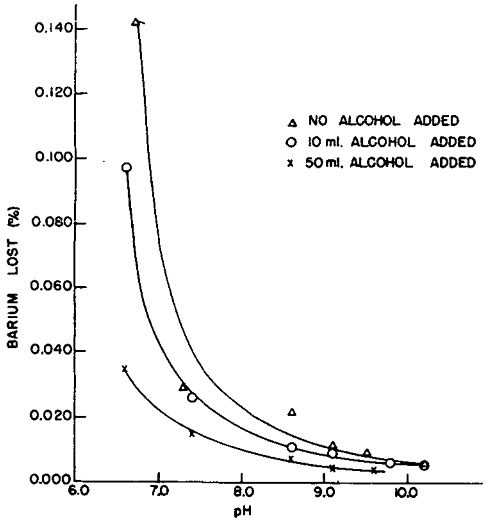

Figure 4. Effect of final pH

The production of crystalline barium carbonate by precipitation from homogeneous solution offers further evidence for the superiority of this technique in improving the physical characteristics of the precipitate. Further study of this procedure for barium should yield interesting results.

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# Cobalt(III) Oxidimetry

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**The extremely high reduction potential of the cobalt (III)–cobalt(II) system (1.8 volts *vs.* the standard hydrogen electrode) is well known but because of the rapid oxidation of water by cobalt(III) ions, the use of this oxidant as a titrimetric reagent has not been proposed. Efforts to find a stable cobalt(III) complex ion which would decompose in acid solution or to prepare a reproducible suspension of cobalt(III) oxide were unsuccessful. However, it was found that a cobalt(III) sulfate solution which was prepared by electrolytic oxidation from cobalt(II) sulfate could be stabilized if kept cold and prepared in the proper acid concentration. Cobalt(III) sulfate solutions have been kept for more than 3 months without any detectable change in titer. Variations in stability of these solutions with changes in acid concentration, cobalt(III) concentration, and temperature were investigated. A study was made of the use of cobalt(III) sulfate solutions for the titrimetric determination of iron(II), ferrocyanide, and cerium (III), with preliminary studies on the titration of arsenite, oxalate, and peroxide. An accuracy within about 1% was achieved in all quantitative determinations. End points of the titrations were detected by spectrophotometric methods.**

THE extremely high oxidation potential of cobalt(III) when it undergoes reduction to cobalt(II) is well known (1.8 volt *vs.* the standard hydrogen electrode) (*6*, *12*). Cobalt(III) has been used only in a few instances as an analytical oxidant (*1*, *11*) and, because of its oxidizing action on water, no record of the use of this substance as a titrimetric reagent was found. Since the potentialities of a standard cobalt(III) solution in titrimetric determinations would appear to be extensive, the feasibility of preparing a stable solution of this reagent was investigated.

Uncomplexed cobalt(III) salts can be prepared by only the strongest oxidizing agents of which fluorine (*5*), ozone (*4*), sodium persulfate or sodium bismuthate (*7*), and electrolytic methods (*2*, *11*) have been suggested. Regardless of the method of preparation, the resulting cobalt(III) solution has been considered too unstable previously to serve as a standard solution. Studies have been made to determine the stoichiometric relation in the reaction between cobalt(III) and iodide (*9*), oxalate (*11*), and iron(II) (*8*). The oxidation of chromium(III) to dichromate and manganese(II) to permanganate (*8*) as well as the products and kinetics of the reaction of cobalt(III) with a variety of alcohols, aldehydes, and carboxylic acids has also been investigated (*1*, *11*). In this study it has been shown that some, at least, of these reactions can be made the basis of titrimetric methods and that the equivalence point in these titrations can be detected conveniently by spectrophotometric measurements.

## EXPERIMENTAL

**Preparation and Stability of Cobalt(III) Sulfate.** The cobalt(III) sulfate was prepared by electrolytic oxidation of cobalt(II) sulfate solution according to the method of Swann and Xanthakos (*11*). A schematic diagram of the apparatus used in this investigation for the oxidation is shown in Figure 1.

A cobalt(II) sulfate solution was prepared by saturating 10*N* sulfuric acid with reagent grade cobalt sulfate heptahydrate ($CoSO_4.7H_2O$) at room temperature. This solution of cobalt(II) sulfate was cooled to approximately 0° C. by means of an ice-salt bath and allowed to stand for 2 hours at this temperature. The saturated solution was then decanted from the crystallized solid into a separate container. This saturation procedure must be followed, using a temperature below that to be used in the electrolysis, in order to prevent contamination of the subsequent product with large amounts of solid cobalt(II) sulfate.

Approximately 210 ml. of the saturated cobalt(II) sulfate were added to the porous cup for the anolyte and 550 ml. to the outer container for the catholyte. The electrodes were immersed, the solution was cooled to approximately 3° C., and the electrolysis at a current of 1 ampere (anodic current density was about 0.032 ampere per square centimeter) was continued for about 4 hours. After the first few minutes of the electrolysis, the anolyte became noticeably blue. The color gradually became more intense and the final solution in the anode compartment contained a large amount of dark blue-green crystals, presumably $Co_2(SO_4)_3.18H_2O$. Upon completion of the electrolysis the solution and crystals in the anode compartment were transferred to a 250-ml. glass-stoppered flask and placed in a freezer at a temperature of −7° C. for storage. Under these conditions, the dark green solution and crystals did not undergo any apparent change over a period of at least 3 months.

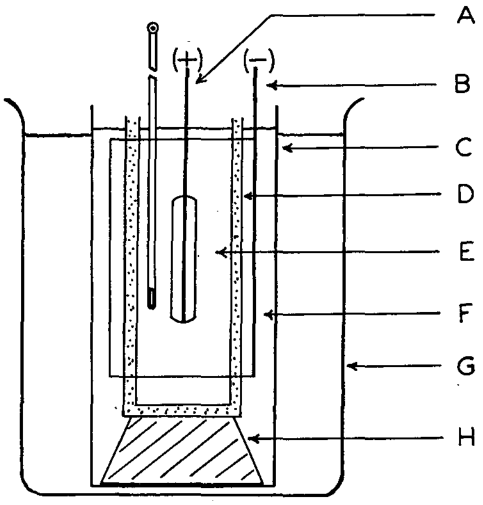

**Figure 1. Diagram of electrolysis cell**

| | | | |
|---|---|---|---|
| *A.* | Platinum anode | *E.* | Anode compartment |
| *B.* | Copper foil cathode | *F.* | Cathode compartment |
| *C.* | Glass container | *G.* | Beaker, 1500-ml. |
| *D.* | Porous cup | *H.* | Rubber stopper |

If, on the other hand, the oxidized anolyte was allowed to stand at room temperature instead of being stored in a freezer, evolution of oxygen and the disappearance of the dark green cobalt(III) sulfate crystals were observed. Although this decomposition was initially very rapid, it appeared to slow down as the cobalt(III) concentration decreased. Even after 2 weeks of standing at room temperature, some oxidizing power remained as suggested by the faint blue color of the solution and its ability to oxidize ferroin indicator.

Quantitative data on the stability of cobalt(III) sulfate solutions were obtained by preparing several dilutions of the concentrated material and then either by measuring the absorbance of the solution at 610 mμ as a function of time or by titrating against a standard iron(II) sulfate solution. Even though absolute concentrations of cobalt(III) were not determined by the optical measurements, this method was very convenient to follow

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the decomposition of these solutions, since cobalt(III) absorbs in the 610- to 620-mμ region whereas cobalt(II) absorbs at 510 mμ and only very slightly at 610 mμ. Absorption curves for cobalt(III) and cobalt(II) sulfate solutions are shown in Figure 2.

**Effect of Acid Concentration on Stability.** Several solutions, ranging in sulfuric acid concentration from 1 to 18*N*, were prepared by diluting 6-ml. aliquots of the anolyte slurry to 100 ml. with the proper strength acid to yield final solutions of the desired acid concentration. Care was taken so that the solutions were at all times very close to room temperature, and not noticeably warmed by the mixing of acid and water. Absorbance measurements at 610 mμ were made immediately and at intervals over a period of approximately 3 days. The per cent decomposition of the solution at a given time was calculated from the ratio of the measured absorbance at this time and the original absorbance of the solution. Values for three different acid concentrations are shown in Figure 3. Solutions less acid than 10*N* in sulfuric acid decomposed more rapidly than those shown in Figure 3 and fell into the expected positions between the next lowest and next highest acid concentration on the graph. Even though the rates of decomposition of a cobalt(III) solution appear to be practically the same in 14 and 18*N* sulfuric acid, 18*N* sulfuric acid was used in preparing all subsequent cobalt(III) sulfate solutions.

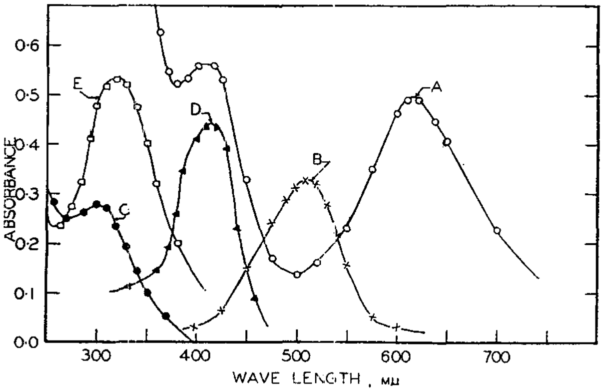

Figure 2. Absorption curves of various substances

*A.* $1 \times 10^{-2} M$ cobalt(III) sulfate in 18*N* sulfuric acid
*B.* $5 \times 10^{-2} M$ cobalt(II) sulfate in 2*N* sulfuric acid
*C.* $1.2 \times 10^{-4} M$ iron(III) sulfate in 2*N* sulfuric acid
*D.* $4.4 \times 10^{-4} M$ $K_3Fe(CN)_6$ in 2*N* sulfuric acid
*E.* $9.4 \times 10^{-5} M$ cerium(IV) sulfate in 2*N* sulfuric acid

**Effect of Temperature on Stability.** Three solutions were prepared from the anolyte slurry so that the concentration of cobalt(III) was about 0.01*M* and the sulfuric acid was 18*N*. After the absorbance of each of these solutions was measured, they were stored at 23°, 7°, and −7° C., respectively. The decrease in cobalt(III) concentration with time was again determined from the change in absorbance at 610 mμ and the results of this study are shown in Figure 4. It is apparent that complete or nearly complete stabilization of approximately 0.01*M* cobalt(III) sulfate solutions in 18*N* sulfuric acid can be affected at 7° C. and possibly slightly above this temperature. The effect of temperature on stability is extremely interesting, as somewhere between +7° and −7° C. decomposition apparently stops almost completely.

**Effect of Cobalt(III) Sulfate Concentration on Stability.** Properly sized aliquots of the anolyte slurry were diluted to 100 ml. with cold sulfuric acid of such a concentration to yield a final concentration approximately 18*N* in acid. The solutions were shaken until all of the crystals of cobalt(III) sulfate had dissolved, after which the flasks were placed in a freezer which maintained a temperature of −13° C. Absorbance readings on these solutions were taken periodically at a wave length of 610 mμ and the results are tabulated in Table I. These results show very little about the variation in stability of cobalt(III) sulfate solutions with changes in cobalt(III) concentration, since, at the temperature used, all solutions were apparently stable. The results indicate, however, that at this temperature and in 18*N* sulfuric acid, crystallization of cobalt(III) sulfate at concentrations between 0.02 and 0.03*M* occurs within a few days. Solutions as dilute as 0.015*M* in cobalt(III) appear to precipitate on long standing. These observations, while very encouraging from the standpoint of stability, definitely suggest an upper limit for the concentration of a cobalt(III) solution for titrimetric determinations.

**Table I. Stability of Different Concentrations of Cobalt(III) Sulfate in 18*N* Sulfuric Acid**

| Cobalt(III) Concentration, *M* | Time in Days 0 | 9 | 17 | 46 | 64 | 87 | 267[a] |
|---|---|---|---|---|---|---|---|
| | Absorbance at 610 mμ | | | | | | |
| 0.0017 | 0.107 | 0.106 | 0.108 | 0.105 | 0.100 | 0.105 | 0.086 |
| 0.0034 | 0.202 | 0.195 | 0.199 | 0.195 | 0.194 | 0.195 | 0.174 |
| 0.0068 | 0.389 | 0.382 | 0.387 | 0.387 | 0.379 | 0.380 | 0.330 |
| 0.010 | 0.529 | 0.525 | 0.525 | 0.520 | 0.522 | 0.515 | 0.453 |
| 0.015 | 0.887 | 0.885 | 0.882 | 0.880 | 0.878 | 0.805[b] | ... |
| 0.020 | 1.162 | 1.155 | 1.153 | 1.155 | 1.148 | [b] | ... |
| 0.030 | 1.625 | [b] | ... | ... | ... | ... | ... |
| 0.040 | 2.126 | [b] | ... | ... | ... | ... | ... |

[a] Between 87 and 267 days, freezer was turned off for 3 days and temperature rose to room temperature for a time.
[b] Precipitate of solid cobalt(III) sulfate was present.

## PREPARATION, STANDARDIZATION, AND APPLICATIONS OF 0.01*M* COBALT(III) SULFATE SOLUTION

**Solutions.** The approximately 0.01*M* cobalt(III) sulfate solution was prepared by adding 60 ml. of the anolyte slurry to sufficient 18*N* sulfuric acid to make 1 liter. This solution was stored at −7° C. and stood 1 day before the initial standardization.

A standard iron(II) solution was prepared by dissolving 2.8 grams of ferrous ammonium sulfate hexahydrate in 1 liter of 1*M* sulfuric acid which had been deaerated with a stream of nitrogen. This solution was transferred to a bottle fitted with an automatic microburet and was standardized at frequent intervals against a standard cerium(IV) sulfate solution using a photometric method for detecting the end point (*3*).

**Standardization of Cobaltic Solution.** All titrations were carried out in a titration cell which has been described previously (*12*) and all end points were determined from photometric measurements. A Beckman Model B spectrophotometer was used for all the photometric measurements described in this paper.

A measured quantity (4.90 ml.) of the standard ferrous ammonium sulfate was run into the titration cell and 85 ml. of 6*N* sulfuric acid were added. A fresh portion of the cold cobalt(III) sulfate solution was transferred to a dry microburet and 5 minutes

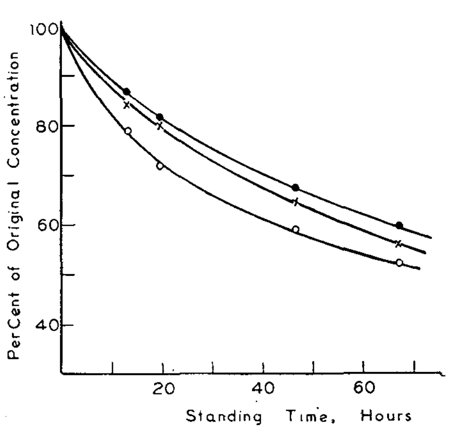

Figure 3. Stability of cobalt(III) sulfate at room temperature in various concentrations of sulfuric acid

Open circles, 10*N*; closed circles, 14*N*; crosses, 18*N*.

**Table II. Molarity of a Cobalt(III) Sulfate Solution as a Function of Time**

| Days | Absorbance at 610 mμ | Days | Molarity by Titration |
|---|---|---|---|
| 0 | 0.546 | 0 | 0.01055 |
| | | | 0.01048 |
| 1 | 0.547 | | |
| | | 2 | 0.01048 |
| | | | 0.01051 |
| | | 4 | 0.01055 |
| | | | 0.01051 |
| | | | 0.01055 |
| 5 | 0.546 | | |
| 7 | 0.545 | 7 | 0.01048 |
| | | | 0.01045 |
| 10 | 0.544 | | |
| 14 | 0.544 | | |
| | | 15 | 0.01045 |
| | | | 0.01045 |
| 16 | 0.544 | | |
| | | 22 | 0.01045 |
| 23 | 0.544 | | |
| | | 30 | 0.01048 |
| 31 | 0.545 | | |
| | | 36 | 0.01051 |
| 37 | 0.542 | | |
| | | 40 | 0.01048 |
| 41 | 0.542 | | |
| | | 48 | 0.01045 |
| 49 | 0.543 | | |
| | | 59 | 0.01048 |
| 60 | 0.542 | | |
| | | 105[a] | 0.01004 |
| | | Average[b] | 0.1048 |
| | | Av. dev. | ±0.0003 |

[a] Electrical power was off 1 day prior to this last titration.
[b] Last value not used in computing average.

were allowed for this solution to establish temperature equilibrium. A wave length of 360 mμ was employed to follow the formation of iron(III) and the absorbance was set at some convenient value with the slit adjustment approximately 1 ml. before the end point. Readings were then taken every 0.20 ml. and the end point was deduced by extrapolating a plot of milliliters of cobalt(III) solution added *vs.* absorbance as shown in Figure 5, curve *B*. Each titration was completed in 10 or 15 minutes after the titrant reached room temperature.

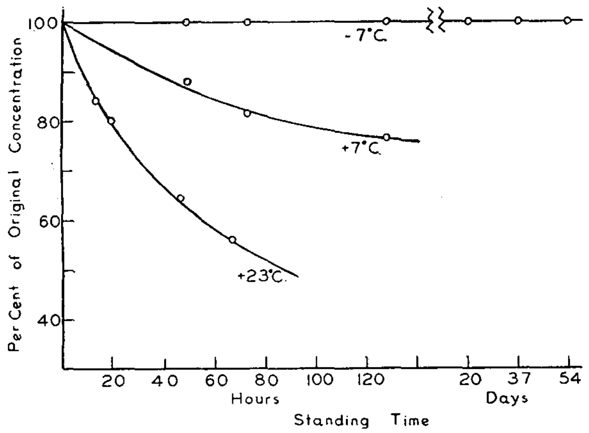

**Figure 4. Stability of cobalt(III) sulfate in 18*N* sulfuric acid at various temperatures**

The cobalt(III) sulfate solution was standardized every few days for about 9 weeks and again about 6 weeks later. In addition, the absorbance of this solution was measured periodically at 610 mμ. A tabulation of the molarities found by titration and the absorbance measurements is given in Table II. The absorbance measurements suggest that the concentration of the cobalt(III) may have decreased as much as 8 parts per thousand in the 60-day interval. The titrations, on the other hand, show no consistent trend and the stability of the cobalt(III) solution would appear to be as good as the reproducibility of the titration which is 3 parts per thousand.

**Determination of Iron.** Examination of the absorption curves of iron(III) sulfate, cobalt(II) sulfate, and cobalt(III) sulfate shown in Figure 2 suggests that spectrophotometric detection of the equivalence point in the titration of iron(II) with cobalt(III) solution should be possible by following the formation of the iron(III) up to the equivalence point at a wave length of 300 to 400 mμ, or by detecting the excess cobalt(III) at a wave length of 610 mμ. End points detected by following the course of the titration at 610 mμ (Figure 5, curve *C*) were uncertain for two reasons. First, the molar absorbance index of cobalt(III) is rather low and, therefore, the excess titrant did not produce a large change in the observed absorbance. Secondly, since cobalt(II) absorbs slightly at 610 mμ, the absorbance prior to the end point was not zero, but increased gradually. The error in determining this small slope accurately caused appreciable uncertainty in determining the end point.

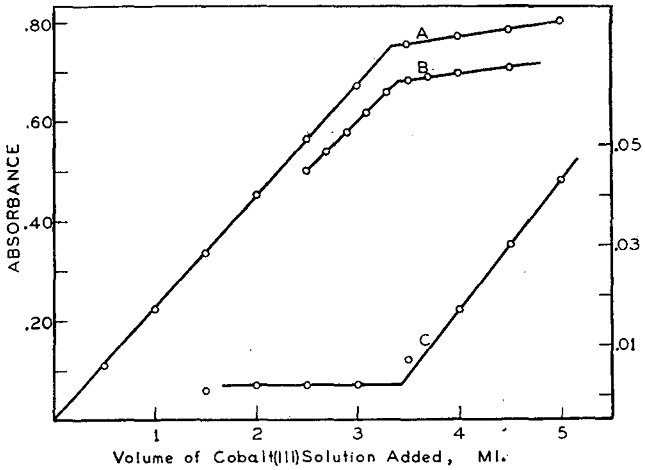

**Figure 5. Titration curves for iron(II) with cobalt(III)**

*A*, *B*. Titration of iron(II) with cobalt(III) using 360 mμ
*C*. Titration of iron(II) with cobalt(III) using 610 mμ
Absorbance values on left apply to *A* and *B*; values on right to *C*

Titrations of iron(II) with cobalt(III) sulfate solutions which were followed at a wave length of 360 mμ (Figure 5, curve *A*) were subject to an error caused by the temperature change during the titration. During the course of this titration, the addition of the 18*N* sulfuric acid to the less concentrated acid solution in the titration cell caused a rise in temperature. When the titration required the addition of only 3 or 4 ml. of titrant, the stoichiometry appeared to be good. However, with samples which required more than this amount of titrant, end points became increasingly low. This was shown to be due to the increase in absorbance of iron(III) with temperature which amounts to about 0.9% of its absorbance at 25° C. per degree rise of temperature. Consequently, points near the end points in titrations which required larger amounts of titrant (5 to 10 ml.) were "overcorrected" when volume corrections were applied to the absorbance reading—that is, the slope of the line prior to the end point was too great and low end points resulted. This error was minimized by starting to take the absorbance reading only 1 ml. or less before the end point so that the temperature change in the interval measured was small. Furthermore, this procedure required such a short extrapolation to the end point as shown in Figure 5, *B*, that volume corrections are not necessary.

The procedure used for determining iron in an iron(II) salt is exactly the same as that described previously for the standardization of the cobalt(III) solution, except that a weighed sample of the unknown or an aliquot of an unknown iron(II) sulfate solution is dissolved in 6*N* sulfuric acid. The resulting solution is then titrated with a standardized cobalt(III) sulfate

Table III. Determination of Iron(II) with 0.01042*M* Cobalt(III) Sulfate

| Sample Weight Diluted to 100 Ml., Gram | Aliquot Taken, Ml. | Cobalt(III) Sulfate Used, Ml. | Iron Found, % | Iron Theoretical, % |
|---|---|---|---|---|
| 0.1610 | 10.00 | 3.94 | 14.24 | 14.24 |
| | 5.00 | 1.98 | 14.31 | |
| | 20.00 | 7.96 | 14.39 | |
| | 15.00 | 5.94 | 14.31 | |
| | | Av. | 14.31 | |
| 0.1694 | 10.00 | 2.91 | 10.00 | 10.23 |
| | 20.00 | 6.05 | 10.39 | |
| | 5.00 | 1.51 | 10.39 | |
| | 15.00 | 4.64 | 10.63 | |
| | | Av. | 10.35 | |
| 0.1911 | 10.00 | 3.21 | 9.78 | 9.86 |
| | 5.00 | 1.62 | 9.87 | |
| | 20.00 | 6.56 | 9.99 | |
| | 15.00 | 4.92 | 9.99 | |
| | | Av. | 9.91 | |

solution and the end point is determined from photometric measurements at 360 mμ.

The results of some iron determinations in iron(II) salts are given in Table III. The four values on a given sample were obtained in the order shown in the table without emptying and filling the microburet with fresh cobalt(III) sulfate solution between determinations. Instead fresh solution was merely added to the buret as needed. Some of the inaccuracy and nonreproducibility of the values given in Table III can be explained undoubtedly by the slight decomposition of the titrant in the buret.

**Determination of Cerium.** The standard potentials of the cerium(IV)–cerium(III) and the cobalt(III)–cobalt(II) systems are sufficiently separated to suggest that cerium(III) could be titrated directly with cobalt(III). Furthermore, preliminary qualitative experiments showed the immediate production of the yellow cerium(IV) color upon the addition of a drop of cobalt(III) sulfate to an acid solution of cerium(III) sulfate. Inspection of the adsorption curves shown in Figure 2 indicates that this titration could be followed photometrically either at 400 mμ where the formation of cerium(IV) is observed or at 610 mμ where the excess cobalt(III) is detected. However, when these titrations were tried, it was found that the reaction between cobalt(III) and cerium(III) was very slow in the vicinity of the equivalence point. Since Noyes and Deahl (*8*) used silver ion to catalyze the reaction of cobalt(III) with manganese(II) and with chromium(III), a solution of silver nitrate was tried as a catalyst for the cerium(III) reaction and was found to be very effective.

A cerium(III) sulfate solution in 1*M* sulfuric acid was prepared and then standardized by the sodium bismuthate method (*10*). Various sized aliquots of this solution were taken for the determinations with cobalt(III) sulfate.

A known aliquot of the cerium(III) solution was added to the titration cell and the volume was adjusted to 90 ml. with 1*M* sulfuric acid. Ten drops of 0.1*M* silver nitrate were then added. A microburet was emptied and filled with standardized cobalt(III) sulfate and 5 minutes were allowed for the titrant to reach equilibrium before each titration. The reaction was followed at 400 mμ, but absorbance readings were not taken until about 1 ml. before the end point. Readings of the volume of titrant added and absorbance were then taken after each addition of 0.1 to 0.2 ml. of titrant until about 1 ml. was added beyond the end point. The end point was determined after plotting the data similar to that shown in Figure 5, *B*. Each titration required about 10 minutes for completion.

The results of several titrations of cerium(III) sulfate with cobalt(III) sulfate are given in Table IV. The reproducibility of these titrations is about 5 parts per thousand, whereas the disagreement between the values obtained by the sodium bismuthate method and the cobalt(III) titrations is 1.5%. No effort was made to determine the reason for this discrepancy or which of the two values was correct.

**Determination of Ferrocyanide.** Aliquots of a potassium ferrocyanide solution were pipetted into a titration cell, diluted to 90 ml. with 6*N* sulfuric acid, and then titrated with a standardized cobalt(III) sulfate solution in the same manner as described for the iron or cerium determinations. A wave length of 370 mμ was used to determine the end point of these titrations.

This determination of ferrocyanide is limited to small amounts, since cobalt(II) ferricyanide precipitates and interferes with the photometric detection of the end point when larger quantities are titrated. However, the results of titrations of small amounts of ferrocyanide with cobalt(III) sulfate and with cerium(IV) sulfate are given in Table V and show very good agreement.

## DISCUSSION

Several attempts were made to titrate solutions of sodium oxalate, arsenious acid, and hydrogen peroxide with cobalt(III) sulfate. Experiments were performed in both 2 and 6*N* sulfuric acid and in all cases the titrations were followed at a wave length of 610 mμ, since none of these reducing agents or their oxidation products have characteristic absorption bands. All titrations proceeded too slowly to be practical. Attempts to catalyze these reactions with osmium tetroxide, phosphoric acid, or chloride ion were fruitless but no study was made of the effect of silver ion on these reactions.

Table IV. Determination of Cerium(III) with 0.01033*M* Cobalt(III) Sulfate

| Cerium(III) Sulfate Taken, Ml. | Cobalt(III) Sulfate Added, Ml. | Molarity of Cerium(III) Sulfate |
|---|---|---|
| 5.00 | 1.15 | 0.00238 |
| 10.00 | 2.31 | 0.00239 |
| 15.00 | 3.52 | 0.00242 |
| 15.00 | 3.46 | 0.00238 |
| 20.00 | 4.63 | 0.00239 |
| | Av. | 0.00239 |
| Molarity of cerium(III) sulfate from sodium bismuthate method | | 0.00236 |
| | | 0.00235 |
| | Av. | $0.00235_5$ |

Table V. Determination of Ferrocyanide with 0.01165*M* Cobalt(III) Sulfate

| $K_4Fe(CN)_6$ Solution Taken, Ml. | Cobalt(III) Sulfate Added, Ml. | Molarity $K_4Fe(CN)_6$ Solution |
|---|---|---|
| 5.00 | 1.32 | 0.00307 |
| 10.00 | 2.62 | 0.00305 |
| 15.00 | Precipitation of $Co_3[Fe(CN)_6]_2$ occurred | |
| | Av. | 0.00306 |
| Molarity of $K_4Fe(CN)_6$ from cerium(IV) sulfate titration | | $0.00305_5$ |

Although no thorough or quantitative study was made to find what foreign substances would interfere in the titrations described, the reaction of cobalt(III) on several substances was noted. Chloride ion as well as bromide and iodide ions is rapidly oxidized by cobalt(III) and therefore the presence of any of these ions in a titration would have a very deleterious effect. Acetic acid, acetic anhydride, ethylene glycol, and glycerol are oxidized rapidly by cobalt(III). A concentrated solution of disodium ethylenediaminetetraacetic acid appeared to reduce cobalt(III) immediately. Although the reaction of cobalt(III) on ethylenediamine was not studied, it is likely that this compound is also oxidized by cobalt(III). If so, some of the error in the iron analyses shown in Table III may be explained by this interference, since the iron(II) salts used were ferrous ethylenediamine sulfate–potassium sulfate mixtures.

All titrations with cobalt(III) were followed photometrically because it was found that in a solution containing iron(II) sulfate and ferroin, the indicator was oxidized more rapidly than the iron(II). That is, the blue oxidized form of the indicator appeared after each addition of cobalt(III) sulfate and then reverted back slowly to the red-colored reduced form. Although the indicator did not appear to be destroyed, it was evident that the detection of end points by this visual indicator would be extremely laborious. No further study of other indicators was made.

It is obvious that the inherent difficulties involved in the stabilization and use of cobalt(III) sulfate make it a much less practical titrant than cerium(IV) or permanganate. However, as it is almost certain that the accuracy of cobalt(III) titrations can be improved by using ice-jacketed burets or refrigerated automatic pipets, this reagent should be very useful in simple and direct titrations of substances such as cerium(III), chromium(III), and manganese(II) where very high oxidation potentials are needed. Furthermore, although all organic compounds studied were oxidized by cobalt(III), it is possible that this reagent may be selective and stoichiometric in its attack. Further work is being done along these lines.

## ACKNOWLEDGMENT

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# Constant Current Sources Based on Transistors

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**This paper describes the characteristics of some electrical circuits based on the use of a germanium transistor of the p-n-p junction type. After a preliminary warm-up of 15 minutes the circuits are used to give currents constant to within 0.02% for half-hour intervals, at any setting in the range from 100 μa to 5 ma. The transistor is protected from ambient temperature changes by a simple thermostat. The current output varies only 0.01 to 0.1% when the resistance in this circuit is varied by 100 ohms.**

THERE is a need for circuits of simple nature that will deliver efficiently a small constant current. At present the majority of such circuits have been either the simple but rather inefficient ones that employ a battery of high voltage and a high resistance in series, or the vacuum tube circuits that make use of electrical or electromechanical feed-back loops. For the latter systems the efficiency is rather low, but they are capable of high current outputs.

Recently fairly reproducible germanium transistors have been produced. Since transistor action may be obtained with bias batteries of a few volts and at high efficiency, their use in certain types of instruments offers much promise.

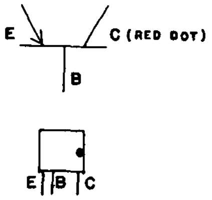

**Figure 1. Schematic diagram of transistor**

*E.* Emitter
*C.* Collector
*B.* Base

## GENERAL DISCUSSION

In Figure 1 a diagram of a transistor and the connections, for the junction type is given. The familiar cathode, grid, and plate of the vacuum tube have their counterparts in the emitter, base, and collector of the transistor. When used in the grounded base assembly the characteristic curves indicated in Figure 2 are obtained with the junction-type transistor. The family of curves indicate the variation of collector current with collector voltage at the designated constant emitter currents. These curves show that the current output, $I_c$, is essentially independent of the collector voltage, which strongly suggests its use as a constant current generator. Its vacuum tube counterpart would be the pentode. The point contact transistor does not have the same characteristic curves as the junction unit, but has curves that resemble those of a vacuum tube triode; therefore, only junction type and not point contact transistors must be used here.

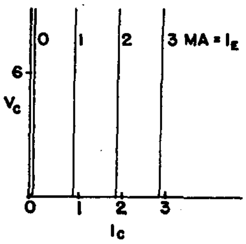

**Figure 2. Insensitivity of current output with collector voltage at various operating points**

$V_c$. Collector voltage
$I_c$. Collector current
$I_e$. Emitter current

The fundamentals of transistors, are covered in a standard treatise (*2*). Two types of junction transistor exist—namely, the p-n-p (positive-negative-positive) and the n-p-n. It is therefore essential that the proper polarity be observed—that is, for a p-n-p, the emitter is biased positively with respect to the base and the collector negatively relative to the base. For the n-p-n type the polarity is reversed. A transistor may be permanently damaged by failure to observe the right polarity, or by overstepping the maximum ratings. Because transistors are semiconductors, they are sensitive to temperature changes, an important disadvantage. As there is no filament to supply the carriers in a transistor, the problem of a stable filament supply does not exist. These temperature changes affect the transistor by essentially changing its operating point. Thus, it is fortunate that the grounded base network is less sensitive to temperature variations than the grounded emitter and gounded collector networks. If one plots the log of $I_{c0}$ (the output for zero-emitter current input) against the temperature, a straight line is obtained. In order to make a transistor as insensitive percentagewise to temperature as possible, a transistor with low $I_{c_0}$ should be used. This is apparent since $I_c = I_{c_0} + aI_e$, where $a$ is a constant.

Because of the changes due to temperature fluctuations the low current range of the transistor circuit is limited, as is the upper current. The output resistance falls at the higher current levels, because the increased power dissipation at the base-collector junction increases the temperature of this junction and also $I_{c_0}$. Hence from the previous equation $I_c$ will increase, which effectively decreases the output resistance. Preliminary experiments with a silicon-junction transistor gave inconclusive results, since the reproducibility was poor. As the silicon transistors have a much smaller $I_{c_0}$ value than the germanium transistors, they should be much less sensitive to temperature variations percentagewise. This would tend to increase the upper range and decrease the lower range of operation.

## APPARATUS

The transistor was covered with a urethan plastic and maintained in a Dewar vessel filled with water. When changes in output current were measured *vs.* time, it was found that the unit was feasible as a source of constant currents.

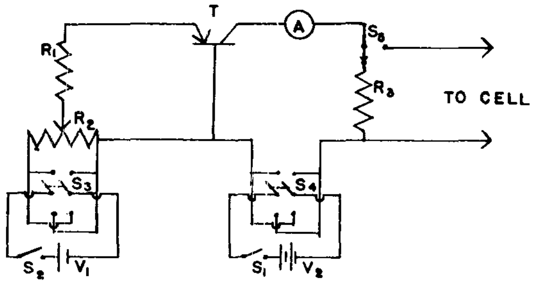

**Figure 3. Circuit for constant current**

| | |
|---|---|
| $V_1$. | 1.5-volt dry cell |
| $V_2$. | 6-volt dry cell |
| $S_1$, $S_2$. | S.P.S.T. toggle switches |
| $S_3$, $S_4$. | D.P.D.T. toggle switches |
| $S_5$. | S.P.D.T. toggle switch |
| $R_1$. | 100-ohm wire wound resistor, 1/4 watt |
| $R_2$. | 50,000-ohm wire wound potentiometer, 1/2 watt, 1 to 3 turns |
| $R_3$. | Dummy resistor to match resistance of the electrolytic cell |
| $T$. | R.C.A. 2N34 p-n-p junction transistor, or equivalent |
| $A$. | Ammeter, 0 to 50 $\mu$a., with suitable shunts to extend range to 5 ma. |

The circuit and its components are shown diagrammatically in Figure 3. The transistor socket was connected to a two-wire shielded cable, and the transistor connections were made to the socket (Figure 4). This overcame the danger of overheating and of ruining the transistor by soldering. The unit with the transistor in the socket was dipped into carbon tetrachloride, allowed to dry, and then coated with a plastic of the following composition:

1. Castor oil Grade D.B. (Baker Co.), 29.8 grams.
2. Dioctyl sebacate (Rohm & Haas Co., Resinous Products Division) 5.76 grams.
3. Dipropylene glycol (Carbide and Carbon Chemicals Co.), 5.96 grams.
4. *m*-Tolylene 2,4-diisocyanate (Monsanto Chemical Co.), 16.10 grams.

The components were mixed as follows: Nos. 1, 2, and 3 were mixed together and then No. 4 was added. The mixture was poured into a 22 × 135 mm. test tube, to which the transistor unit was lowered and properly positioned so that it was completely covered. After the mixture had gelled a day or so at room temperature, drying was completed at about 35° C. in an oven for 4 days. If an oven is not available, drying may be completed at room temperature over a 6-day period. The glass tube was then cracked, and the fragments were removed. As shown in Figure 4 the plastic was cut half-way down the transistor with a razor blade. Next a radiation fin, *R*, was attached as indicated in Figure 4. This fin is a sheet of copper foil, 4 × 1 1/2 inches, and is held in place by an alligator clip. The plastic coating, which has extremely low water absorption, was ideal for insulating the unit from the water. When immersed in water in a Dewar vessel of 1-quart capacity the transistor was stable over half-hour periods.

**Constant Current Generator.** The resistors and switches were housed in an aluminum box, 4 × 5 × 6 inches. The insulated transistor was connected to the other components by a four-pronged jack. Switches $S_4$ and $S_3$ (Figure 3) are reversing switches, so that the unit may be used with either p-n-p or n-p-n transistors. With $S_1$ and $S_2$ open when the transistor was put in circuit, the right polarity was set with $S_3$ and $S_4$. It was extremely important that the right polarity be used when $S_1$ and $S_2$ were closed. $S_1$ was closed with $S_5$ in the dummy position and $R_3$ in the circuit. Potentiometer $R_2$ was turned to the limit that makes $V_e = 0$, then $S_2$ was closed. It is important to have $V_e$ initially zero, since the full battery voltage might pass more than the maximum current, 8 ma., and ruin the transistor. Resistor $R_1$ was inserted in series as an extra protection against mistakes. It limited the input current to about 7 ma. for any setting of $R_2$ assuming the input resistance $R_e$ was about zero. Actually it was of the order of 100 ohms, so that this would protect it from any mistake in making the connection. With $S_2$ closed, $R_2$ was adjusted to give the current output that was required. For this particular circuit the minimum current is of the order of 100 $\mu$a. and the maximum about 5 ma. The upper limit depends on the power dissipation, which in the case of the R.C.A. 2N34 transistor is about 50 mw. It is obvious that the potential drop across the cell should never exceed $V_2$; otherwise there will be no current regulation.

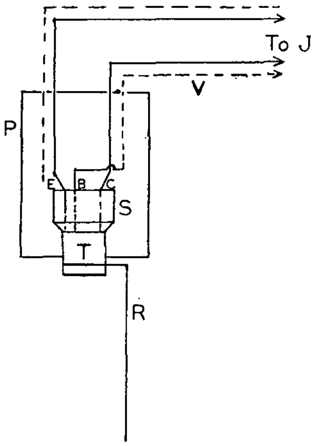

**Figure 4. Transistor and accessories**

| | |
|---|---|
| *J*. | Four-prong jack |
| *V*. | Shield of two-wire cable |
| *P*. | Plastic coating |
| *S*. | Socket |
| *T*. | Transistor |
| *E*, *B*, *C*.. | Emitter, base, and collector, respectively |
| *R*. | Radiation fin |

## EXPERIMENTAL AND RESULTS

With the unit set at various operating points, readings were taken with a Type K potentiometer, Leeds & Northrup Co., measuring the voltage drop across a standard resistor. A GM Laboratories Co., Chicago, Ill., galvanometer of 0.02 $\mu$a. per mm. sensitivity was used for balancing. The data are based on the procedure of allowing a 15-minute stabilization period for each operating current (Table I). The results are summarized in Table I. The effect of a change of 100 ohms in resistance is included in the table. The need for a 15-minute stabilization period can be seen from Figure 5, which shows the rapid initial rise in current as the transistor, immersed in water at room temperature, was warmed by the current. The steady state was reached in about 5 minutes if the transistor unit was placed in ice water. If the initial reading was taken after 15 minutes and the final one at the end of 45 minutes, the reading at 30 minutes was the average of the two.

**Table I. Operation of Water-Cooled Transistor, Radiation Fin, and Dewar Vessel Used**

| Mean Current, 3 Readings | Average Current, 1st and 3rd Readings | Average Deviation, % | Maximum Deviation, % | Effect of 100 Ohm Change (Output), % |
|---|---|---|---|---|
| 104.48 $\mu$a. | 104.48 $\mu$a. | 0.02 | 0.03 | 0.01 |
| 521.30 $\mu$a. | 521.29 $\mu$a. | 0.02 | 0.03 | 0.01 |
| 1.0493 ma. | 1.0492 ma. | 0.01 | 0.01 | 0.015 |
| 3.0880 ma. | 3.0879 ma. | 0.01 | 0.01 | 0.055 |
| 5.1662 ma. | 5.1661 ma. | 0.01 | 0.01 | 0.12 |

Water was used because of its high heat capacity, and because it served to thermostat the transistor against rapid changes in the temperature of the air of the room. Ice water was the best simple stabilizing medium. When run in air the current was unsteady and not reproducible from one experiment to another, because of ambient temperature changes.

In other experiments it was found that a change of 1000 ohms in the output circuit caused only a 0.03% change in output in the 100-μa. range and a 0.07% change in the 500-μa. range.

**Use of Transistor for Variable Controlled Currents.** Since the output of transistor circuit (Figure 3) may be varied continuously, the unit can be used to take polarograms in which the current is the controlled variable (*1*). For this application it is unnecessary to coat the transistor with plastic and to thermostat it as the transistor need not be stable over a 30-minute period. The current is set, its value is read, and the corresponding voltage is noted. The transistor unit offers decided advantages because the current held constant regardless of changes in resistance such as those at the dropping mercury electrode. The ideal type of transistor would again be one with low $I_{c_0}$ since this would require a smaller B battery in the circuit that is used to buck out this $I_{c_0}$.

The circuit for this application is shown in Figure 6. The operation is the same as current-scan polarography. After connecting the dropping mercury electrode and reference half cell, $R_2$ (Figure 6) was set so that the microammeter read about 5 μa.; at this point $I_e$ was approximately zero. Then $S_5$ was closed and $R_4$ was adjusted to give zero current. From then on it was only necessary to adjust $R_2$ to give currents up to approximately 100 μa., which is ample for the dropping mercury electrode. $R_1$ was made large to reduce $I_{c_0}$ and thus require less biasing current. $R_3$ was large in order to obtain a biasing current of the order of $I_{c_0}$ without effectively decreasing the output resistance of the unit. This unit has an exceptionally high output resistance; therefore the current will fluctuate little, if any, with drop size. The constancy of current during drop formation was demonstrated for a mixture of 25 ml. of $0.1M$ cadmium chloride plus 15 ml. of saturated potassium chloride. Only at the lowest scale of the recorder (2 μa.) were there visible fluctuations on the record of the order of 0.02 microamp. or less. Fluctuations of the same size were found with a fixed resistor in place of the dropping electrode assembly.

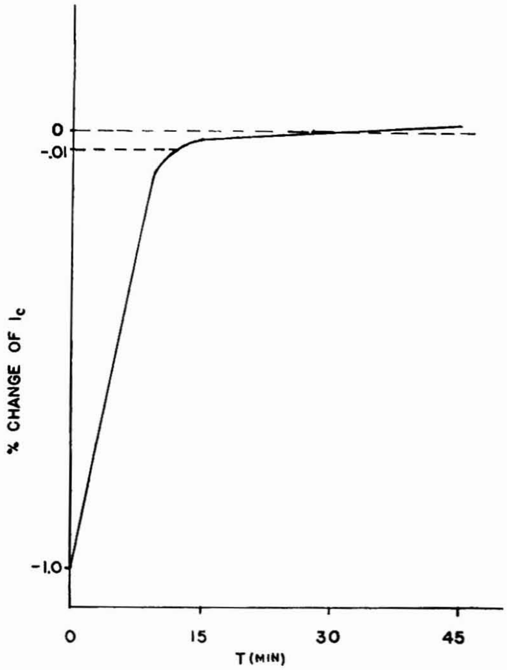

**Figure 5. Change of current with time**

## CONCLUSION

With proper attention to temperature control, there are numerous possibilities for taking advantage of the high output-resistance characteristic of the junction transistor in the grounded-base configuration. In this paper two possible applications have been explored—namely, the use of the transistor in a constant current circuit for coulometry or other applications, and the development of a convenient source of steady and continuously variable current for current scanning polarography. In the case of the germanium transistor of the junction type (R.C.A. p-n-p), the stability of the unit after a 15-minute warm-up period is from 0.02% to better than 0.01% for the range from 100 μa. to 5 ma., respectively. Changes in load resistance or cell resistance from 0 to 100 ohms produced a change of 0.01 to 0.1% in current output. The chief advantage of the transistor is its long life and stability toward shock. The power requirements are much less than for vacuum tube units, and the circuits are simplified.

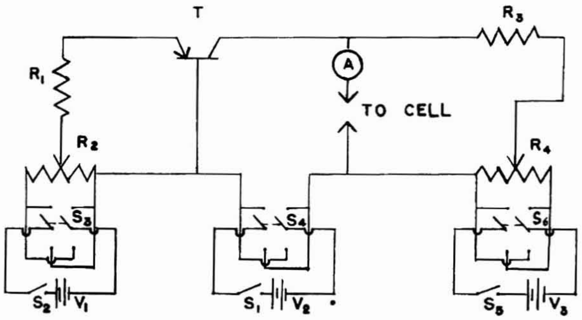

**Figure 6. Circuit for current scan applications**

| | |
|---|---|
| $V_1$. | 45-volt dry cell |
| $V_2$. | 6-volt dry cell |
| $V_3$. | 90-volt dry cell, or two 45-volt cells in series |
| $R_1$. | 270,000-ohm carbon resistor, 1/2 watt |
| $R_2$. | 50,000-ohm wire-wound potentiometer, 1/4 watt, 1 to 3 turns |
| $R_3$. | 10- to 15-megohm carbon resistor, 1/4 watt |
| $R_4$. | 5-megohm carbon potentiometer, 1/4 watt |
| *A*. | Ammeter, 0 to 50 microamp., with shunts to extend range |
| $S_1$, $S_2$, $S_5$. | S.P.S.T. toggle switches |
| $S_3$, $S_4$, $S_6$. | D.P.D.T. toggle switches |
| *T*. | R.C.A. 2N34 p-n-p junction-type transistor or equivalent |

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# Analysis of Lacquers Containing Nitrocellulose, Alkyd Resins, and Phthalate-Type Plasticizers

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**Lacquers containing nitrocellulose, alkyd resins, and plasticizers such as esters of phthalic or phosphoric acid are used as protective coatings for automotive equipment and other ordnance applications. New methods have been developed for solvent separation of nitrocellulose, direct spectrophotometric determination of total phthalate, separation and analysis of phthalate ester plasticizers by charcoal adsorption, and nonvolatile and phosphate ester determinations.**

IN THE analysis of lacquer compositions containing nitrocellulose, phthalate-type alkyd resins, and phthalate-type or phosphate-type monomeric plasticizers, difficulty is encountered in determining each component. All methods of determining phthalic anhydride in phthalate-type alkyd resins involve saponification in alcohol to precipitate dipotassium phthalate which can be isolated and weighed or measured by other methods (*2*, *3*). Phthalate-type plasticizers also yield dipotassium phthalate on saponification, so that all known methods would give a measure of total phthalate. Nitrocellulose decomposes during saponification and interferes with the determination of total phthalate.

Physical separation of the nitrocellulose from such formulations would greatly simplify the analysis for either component, and a procedure for effecting this separation quantitatively is described in this paper. The usual technique of precipitating nitrocellulose from lacquers, by adding benzene or chloroform, is not satisfactory in the presence of alkyd resins due to excessive coprecipitation of the latter. Shaefer and Becker (*1*) developed excellent procedures for determining nitrocellulose and total phthalate in lacquers using separate samples for each determination; however, solvent separation of the nitrocellulose from the alkyd resins permits a simpler technique for the determination of either component. Saponification of the filtrate from the separation will precipitate dipotassium phthalate which can also be weighed to measure total phthalate.

Efforts have been made to obtain the direct analysis of lacquers for their phthalic anhydride content without first separating the nitrocellulose. This has always been prevented by the decomposition of the nitrocellulose during saponification. This decomposition product is water-soluble, but when dried becomes insoluble in glacial acetic acid or methanol so that the method (*3*) of determining phthalic acid with lead acetate cannot be used. It was thought likely that the specific method of analysis for phthalic anhydride as originally presented by Shreve and Heether (*2*), now ASTM Method D 1307–54T, would be applicable; but it was found that the decomposition products of the nitrocellulose show some absorption in the ultraviolet range at a wave length of 276 mμ at which phthalic acid absorbs and is measured (Figure 1). By determining the nature of the absorption of the interfering materials, it has been found possible to measure the phthalic anhydride, correcting for the interference by determining the absorption of each sample at two distinct wave lengths.

In order to measure accurately the phthalic anhydride in an alkyd resin when phthalate plasticizers are present, it is necessary to effect a separation of the two. This has been accomplished by adsorptive filtration. A variety of solvents and adsorbents have been tested for this purpose, the adsorption of known alkyd resins and plasticizers being tested separately before being tested in lacquers. Best results were obtained with a specially prepared mixture of animal charcoal and short-fiber asbestos. The lacquer is dissolved in a small amount of acetone and diluted with absolute ethyl alcohol. When the acetone is removed by distillation, the plasticizers remain in solution while the bulk of the alkyd resin and nitrocellulose are precipitated. When this is filtered and the filtrate passed through the adsorbent, the residual alkyd resin and nitrocellulose are retained in the column. Phthalic anhydride can then be determined in the separated plasticizer and correction made by deducting from the total phthalic anhydride, determined by one of the other methods described.

The usual procedures for determining nonvolatile content of resin solutions were tested with a variety of lacquers and none were found satisfactory for lacquers containing diethyl phthalate; even dibutyl phthalate is lost to some extent in most methods. Table I gives some of the results obtained and shows the loss of plasticizers due to volatility to be appreciable in some methods. The new and excellent method (ASTM D 1259–53T) for determining nonvolatile content of heat-stable resin solutions by spreading small samples over a large area as a very thin film gives lowest results and greatest loss of plasticizer and is least suitable for use with lacquers in general. However, if this method is modified so that the sample is allowed to air-dry for 12 to 18 hours and the oven-drying is omitted, excellent results are obtained that agree with those obtained by the recommended procedure. ASTM method 330–40 for determining the nonvolatile in lacquers is the most suitable of the existing methods but is not satisfactory for

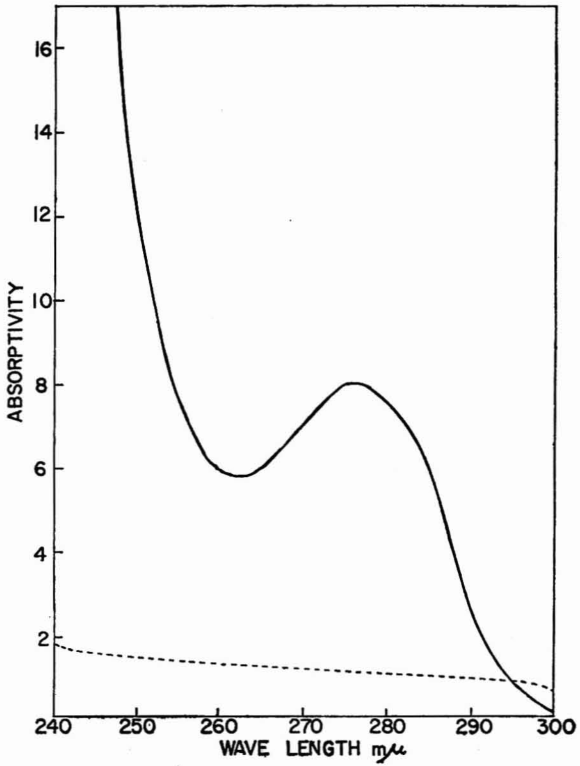

**Figure 1. Relationship of absorptivity and wave length**

**—— 0.1*N* hydrochloric acid solutions of phthalic acid**
**----- Nitrocellulose saponification product**

**Table I. Nonvolatile Determinations on Lacquers and Plasticizers**

| Sample | Nonvolatile (Theoretical), % | Nonvolatile (Determined), % | Deviation, % |
|---|---|---|---|
| Method 404.1 of Fed. Spec. TT-P-141 (3 hours' drying in 105° C. oven) | | | |
| Diethyl phthalate | 100.0 | 64.2 | −35.8 |
| Dibutyl phthalate | 100.0 | 72.9 | −27.1 |
| Tricresyl phosphate | 100.0 | 99.9 | − 0.1 |
| Lacquer with diethyl phthalate | 43.9 | 39.5 | − 4.4 |
| Lacquer with dibutyl phthalate | 42.5 | 39.0 | − 3.5 |
| Lacquer with tricresyl phosphate | 42.8 | 41.1 | − 1.7 |
| ASTM Method D 1259-53T for heat-stable resin solutions (dried 30 minutes in 105° C. oven after pressing between two sheets of aluminum foil) | | | |
| Dibutyl phthalate | 100.0 | 38.4 | −61.6 |
| Lacquer with diethyl phthalate | 42.9 | 36.0 | − 6.9 |
| Lacquer with dibutyl phthalate | 45.3 | 40.3 | − 5.0 |
| Lacquer with tricresyl phosphate | 42.0 | 40.5 | − 1.5 |
| ASTM Method 330-40 for determining nonvolatile on lacquer solutions (acetone and water added, evaporated on steam bath, dried 1 hour at 105° C.) | | | |
| Diethyl phthalate | 100.0 | 40.7 | −59.3 |
| Dibutyl phthalate | 100.0 | 87.7 | −12.3 |
| Lacquer with diethyl phthalate | 39.8 | 38.3 | − 1.5 |
| Lacquer with dibutyl phthalate | 38.5 | 38.5 | 0 |
| Lacquer with tricresyl phosphate | 38.9 | 38.9 | 0 |
| Recommended method (air-dry 18 hours, oven-dry 2 hours at 60° C.) | | | |
| Diethyl phthalate | 100.0 | 99.4 | − 0.6 |
| Dibutyl phthalate | 100.0 | 99.4 | − 0.6 |
| Tricresyl phosphate | 100.0 | 100.0 | 0 |
| Lacquer with diethyl phthalate | 39.7 | 39.4 | − 0.3 |
| Lacquer with dibutyl phthalate | 34.9 | 34.7 | − 0.2 |
| Lacquer with tricresyl phosphate | 40.4 | 40.2 | − 0.2 |

diethyl phthalate and is inconvenient to use, since it requires considerable attention and the borosilicate glass dishes must be cleaned for re-use. A method was developed which is satisfactory for all lacquers including those containing diethyl phthalate, which involves air-drying of the sample followed by oven-drying at 60° C. Disposable pans can be used and very little attention is required. Solvents present were according to Federal Specification TT-T-266a.

Phosphate-type plasticizers can be estimated in lacquer compositions by applying the usual molybdate precipitation to the water soluble product obtained by fusing a dried lacquer sample with potassium hydroxide as described in the method (*4*) for determining chlorine in vinyl alkyds. If the specific phosphate ester in the sample is known or can be identified, the determination can be made accurately by applying the appropriate calculation factor. A qualitative test for tricresyl and triphenyl phosphate is included which does not distinguish between these two plasticizers but makes possible a semiquantitative determination because of their similar molecular weights.

## PROCEDURES

**Determination of Nitrocellulose.** A 50-ml. Erlenmeyer flask is provided with a cork stopper and weighed. A sample of the clear lacquer vehicle representing 0.6 gram of nonvolatile matter is poured into the flask which is reweighed. Two milliliters of acetone are added, and, when the sample has dissolved, the weight of the mixture is recorded. A wide-mouthed 500-ml. flask containing 200 ml. of boiling water is placed on a hot plate just inside the window of a hood in such manner that the draft causes the water vapors to be drawn away sharply. The diluted lacquer sample is poured very slowly into the boiling water with care to avoid condensation of moisture on the sample flask. Immediately after pouring the sample flask is restoppered and weighed. From the four weighings the actual amount of lacquer sample involved in the analysis can be calculated. The sides of the large flask are washed down with water and the contents swirled several times to collect the precipitate into one portion as much as possible. Boiling is continued for a total of 5 minutes. The sample is filtered while hot through rapid, hardened filter paper such as Whatman No. 54. Water is used for washing and transferring the precipitate to the paper. Without delay the paper is opened up on blotting paper. The sample is picked up and the paper blotted with the bulk of it so as to pick up any stray portions. It is then pressed repeatedly between folds of dry, hardened filter paper until the paper shows no dampness. It is immediately transferred to a 250-ml. flask, exactly 83 ml. of dry benzene and a bumping stone are added, an air condenser is attached, and the benzene is refluxed for 30 minutes. Excessive delay at any point between the first pouring of the sample and the addition of the benzene should be avoided. The water-precipitated sample will normally be soft and pliable; if the lacquer precipitates as a hardened mass in water, it will be necessary to start the analysis again, adding 2 drops of tricresyl phosphate to the lacquer sample before diluting it with acetone. At the end of the 30-minute reflux period exactly 17 ml. of absolute ethyl alcohol are added slowly (about 2 drops per second) from a separatory funnel through the condenser to the boiling benzene portion. Some agitation is advisable during the addition of the ethyl alcohol. Refluxing is continued for 3 hours. During this time the resin and plasticizer are completely dissolved so that the nitrocellulose can be separated by filtration. When cool, the sample is filtered through a fritted-glass crucible of medium porosity which was previously prepared with a thick mat of asbestos, dried, and weighed. Benzene is used to transfer and wash the nitrocellulose. The filtrate is collected in a clean, dry filtering flask and held for analysis of the alkyd resin. The crucibles are dried at 105° C., cooled over desiccant, and weighed, and the percentage of nitrocellulose is calculated.

**Determination of Total Phthalic Anhydride (Gravimetric).** The filtrate, which contains alkyd resin and plasticizers, should be refiltered through retentive paper into a flask of suitable size and the paper washed with benzene. Sixty milliliters of 1.0*N* alcoholic potassium hydroxide are added and the sample refluxed for 1.5 hours. The phthalic anhydride, calculated in the usual manner from the weight of dipotassium phthalate formed, will represent total phthalic anhydride in the lacquer sample. If phthalate-type plasticizers are present, correction is made after completing the analysis of a separate sample by the procedure which follows. Some analytical results are shown in Table II.

**Separation of Lacquer Plasticizer and Determination of Phthalic Anhydride Content (Adsorptive Filtration).** Twenty grams of short-fiber filtering asbestos are thoroughly mixed with 20 grams of powdered bone black (Fisher Scientific Co. Catalog No. C-263) and added in 10-gram portions to a column 20 mm. in diameter and 400 mm. long which contains a plug of glass wool covered with a 1-inch layer of filtering asbestos. Each 10-gram portion is saturated with alcohol and packed lightly with a stirring rod. A cotton or glass-wool plug is used at the top. If properly prepared, a column of adsorbent mixture 20 mm. in diameter will be approximately 220 mm. long.

**Table II. Analysis of Lacquers of Solvent Separation**

Composition
30% nitrocellulose
10% tricresyl phosphate (plasticizer)
60% alkyd resin (35% phthalic anhydride), equivalent to 21% phthalic anhydride in lacquer

| Nitrocellulose (by Analysis), % | Phthalic Anhydride (by Analysis), % |
|---|---|
| 30.4 | 22.0 |
| 29.4 | 21.9 |
| 29.0 | 21.5 |
| 31.0 | 21.6 |
| 31.1 | 21.4 |

A sample of the clear lacquer vehicle, representing 1 gram of nonvolatile material, is weighed into a 250-ml. flask. It is dissolved in 5 ml. of acetone, and 75 ml. of absolute ethyl alcohol are added slowly with agitation. A bumping stone is added and 25 ml. of solvent are removed by distillation. The sample is cooled to room temperature and approximately 0.3 gram of filter paper pulp added and mixed. The sample is then placed in an ice bath for 20 minutes and filtered while cold through a fritted-glass crucible of coarse porosity which contains a thick mat of filtering asbestos. Sometimes the filtrate will be cloudy, but this does not alter the procedure; most of the alkyd resin and nitrocellulose have been removed.

The filtrate is poured onto the adsorption column and the elution rate is adjusted to 40 to 60 drops per minute, using vacuum. When the upper level of the sample liquid reaches the top of the adsorbent, 10 ml. of ethyl alcohol are added. When this level again reaches the adsorbent, another 10 ml. of ethyl alcohol wash are used, followed by the addition of 130 ml. of ethyl alcohol in any convenient portions. It is important that the alcohol level does not drop below the top of the adsorbent and that new portions of alcohol are withheld until the previous portion has been drawn down to the adsorbent surface. The eluate is transferred to a suitable flask, 75 ml. of 1.0*N* alcoholic potassium hydroxide added, and the mixture refluxed for 1.5 hours. It is then filtered through a weighed crucible. The phthalic anhydride is calculated in the usual manner and deducted from the total phthalic anhydride, determined by a separate procedure, to determine the phthalic content of the alkyd resin portion of the lacquer.

A variety of phthalate-type alkyds were tested to determine their adsorbability on the above column. The resins were dispersed in 50 ml. of absolute ethyl alcohol, filtered, and allowed to flow through the column followed by 150 ml. of alcohol. The percolates were tested for the presence of alkyd resins by adding alcoholic potassium hydroxide and refluxing. The absence of precipitate formation is indication of complete adsorption of the resin by the column. The composition of the alkyds tested varied from 22 to 43% phthalic anhydride and were modified with soybean, linseed, castor, and coconut oils of both drying and nondrying types. All plasticizers tested by the same procedure were recovered completely in the eluate.

To test the effectiveness of adsorption for lacquer analysis, a group of lacquers was prepared having the approximate composition: 10% plasticizer, 30% nitrocellulose, and 60% alkyd resin. The results of analysis with different plasticizers are shown in Table III.

**Direct Determination of Total Phthalic Anhydride in Lacquer Vehicles (Two-Component, Ultraviolet Spectrophotometric Method).** Figure 1 shows the absorptivities of 0.1*N* hydrochloric acid solutions of phthalic acid and the decomposition product of nitrocellulose on saponification, plotted against the wave length. Tenth normal hydrochloric acid was used in the blank cell. Several wave-length combinations were tried in the calculation of the amount of phthalic anhydride in known samples. It was found that the use of 270 and 276 mμ gave best results.

CALIBRATIONS. The absorptivities of both phthalic acid and the acidified saponification product of nitrocellulose must be determined at 270 and 276 mμ. Details for determining absorptivity of the phthalic acid are found in the original literature (*2*). The following procedure is used to obtain the absorptivity of the saponified nitrocellulose.

**Table III. Analysis of Lacquer Plasticizers Separated from Lacquers by Adsorptive Filtration**

| Plasticizer | Plasticizer (Present), % | Phthalic Anhydride (Present), % | Phthalic Anhydride (by Analysis), % |
|---|---|---|---|
| Separated from castor oil–modified alkyd resin; phthalic anhydride 35% | | | |
| Diethyl phthalate | 10.5 | 6.9 | 6.7; 6.6 |
| Dibutyl phthalate | 11.8 | 6.3 | 5.9 |
| Dioctyl phthalate | 12.0 | 4.5 | 4.5; 4.8 |
| Diphenyl phthalate | 10.0 | 4.7 | 4.6 |
| Butyl phthalyl butyl glycollate | 10.2 | 4.5 | 4.3 |
| Separated from soybean oil–modified alkyd resin; phthalic anhydride 22% | | | |
| Diethyl phthalate | 10.0 | 6.7 | 6.5 |
| Dibutyl phthalate | 10.0 | 5.2 | 4.9 |
| Dioctyl phthalate | 10.0 | 3.7 | 3.3 |
| Butyl phthalyl butyl glycollate | 10.0 | 4.3 | 3.9 |
| Separated from coconut oil–modified alkyd resin; phthalic anhydride 43% | | | |
| Diethyl phthalate | 10.0 | 6.7 | 7.0 |
| Dibutyl phthalate | 10.0 | 5.2 | 5.1 |
| Dioctyl phthalate | 10.0 | 3.7 | 3.5 |
| Butyl phthalyl butyl glycollate | 10.0 | 4.3 | 4.0 |

A sample of nitrocellulose solution estimated to contain 0.3 gram of lacquer-grade nitrocellulose is weighed accurately into a 500-ml. Erlenmeyer flask. It is dissolved in 50 ml. of methyl isobutyl ketone and 200 ml. of 0.125*N* alcoholic potassium hydroxide (made with absolute ethyl alcohol) are added. The mixture is refluxed 3 hours and filtered while warm through a weighed glass crucible of coarse porosity which contains a mat of filtering asbestos. After washing with absolute ethyl alcohol the crucible is dried at 105° C., cooled, and weighed. The residue is dissolved in distilled water, 10 ml. of concentrated hydrochloric acid are added, and the mixture is diluted to 1 liter with water. This solution is allowed to stand 18 hours or longer; then a portion is filtered through the most retentive filter paper, the first 25 to 30 ml. being discarded. The absorbance of this filtered solution is determined at 270 and 276 mμ by following the same procedure used for phthalic acid. The absorptivity of the nitrocellulose saponification product is calculated from the following equation:

$$a_n = \frac{A}{C_n}$$

where $a_n$ is the absorptivity of this material at the particular wave length measured, $A$ is the average absorbance at the same wave length, and $C_n$ is the concentration in grams per liter of the precipitate resulting from saponification of the nitrocellulose.

**Table IV. Analysis of Some Known Lacquers for Phthalic Anhydride, Using Two-Component, Ultraviolet Spectrophotometric Method**

| Phthalic Anhydride (Present), % | Method 702.1 of Spec. TT-P-141b | Original Spectrophotometric Method (ASTM D 1307–54T) | Two-Component Spectrophotometric Method |
|---|---|---|---|
| 20.6 | 35.4, 26.1 | 25.6, 25.5 | 19.6, 20.7 |
| 22.7 | 37.0, 36.7 | 26.3, 26.5 | 21.8, 21.4 |
| 22.0 | 29.7, 29.4 | 23.2, 23.1 | 21.0, 21.0 |
| 21.0 | 31.7, 31.9 | 23.8, 24.1 | 21.3, 20.8 |

ANALYSIS OF UNKNOWN LACQUERS. A sample of the clear lacquer vehicle estimated to contain 0.1 gram of phthalic acid is weighed into a 500-ml. Erlenmeyer flask. The solvents, alkali, and saponification are the same as outlined in the previous paragraph. The dried precipitate is dissolved in water, acidified with 10 ml. concentrated hydrochloric acid, and diluted to 1 liter. A portion of this solution is filtered, the first 25 to 30 ml. being discarded. A 50-ml. aliquot of the filtrate is diluted to 100 ml. with 0.1*N* hydrochloric acid and the absorbance determined at 270 and 276 mμ as in calibration. The absorbance should fall within the range of 0.25 to 0.50 for best results. If the readings are outside this range, the dilution factor should be adjusted, but anything greater than a fourfold final dilution should be avoided.

For this two-component system the following equations apply:

$$A_{270} = C_p a_{270,p} - C_n a_{270,n} \qquad (1)$$

$$A_{276} = C_p a_{276,p} - C_n a_{276,n} \qquad (2)$$

where $A_{270}$ and $A_{276}$ are the average absorbances at 270 and 276 mμ. respectively; $C_p$ is the concentration in grams per liter of phthalic acid in the final diluted solution; $C_n$ is the concentration in grams per liter of the nitrocellulose saponification product in the final diluted solution; $a_{270,p}$ and $a_{276,p}$ are the absorptivities of phthalic acid at 270 and 276 mμ; and $a_{270,n}$ and $a_{276,n}$ are the absorptivities of the nitrocellulose saponification product at 270 and 276 mμ as determined in the calibration procedure. The following equation is obtained by solving Equations 1 and 2 simultaneously:

$$C_p = \frac{a_{270,n}\,A_{276} - a_{276,n}\,A_{270}}{a_{270,n}\,a_{276,p} - a_{276,n}\,a_{270,p}}$$

The per cent phthalic anhydride in the lacquer vehicle is then calculated by the equation

$$\%\ \text{P.A.} = \frac{C_p \times 0.8916 \times f \times 100}{W \times S}$$

where $f$ is the aliquot factor, $W$ is the original sample weight, and $S$ is the nonvolatile vehicle fraction.

Some analytical results are shown in Table IV. The results by the usual saponification, Method 702.1 of Federal Specification TT-P-141b, show the extent of interference from nitrocellulose. This interference is greatly reduced but still excessive in the application of the usual spectrophotometric method (ASTM D 1307–54T).

**Determination of Nonvolatile.** A lacquer sample representing 0.5 gram of nonvolatile is weighed into a small, disposable aluminum dish of approximately 2-inch diameter. Two milliliters of acetone are added and after 12 to 24 hours of exposure to the air at room temperature, the sample is placed in an oven at 60° C. for 2 hours, cooled, and weighed, and the per cent nonvolatile calculated.

A suitable alternative method is ASTM D 1259–53T modified so that oven-drying is omitted and the lacquer films are air-dried 12 to 24 hours.

## DISCUSSION

Pigmented lacquers must be centrifuged until a clear vehicle is obtained, thinning if necessary prior to centrifuging. In the separation of nitrocellulose care must be exercised to prepare a filtering crucible sufficiently retentive to hold the nitrocellulose. A small part of it is finely divided and may pass through most crucibles; this is the reason for refiltering the filtrate before saponifying the alkyd resin. Unless asbestos is used, glass crucibles will clog and filtration is difficult.

It is apparent that the separation by adsorption of alkyd resins of low molecular weight from plasticizers of high molecular weight will be increasingly difficult as these weights approach each other. It is possible that the procedure presented here may be unsuited for some separations; the technique could undoubtedly be modified for such special purposes. The eluates in these plasticizer separations also contain some oil and for this reason cannot be evaporated and weighed to measure the plasticizer separated. It has been noted that the oil separated varies with the oil length of the alkyd resin used and there are indications that a slight modification of this technique will permit measurement of uncombined oils in oil-modified alkyds.

All lacquers tested which contained diphenyl phthalate, as plasticizer, developed a bright red color when alcoholic alkali was added, probably due to the presence of some phenolphthalein. The phosphate plasticizers, tricresyl and triphenyl phosphate, give positive qualitative tests for phenol when a dried sample of lacquer is tested according to Method 514.1 of Federal Specification TT-P-141b, paragraph 2.1.3.

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# Determination of Fluorine in Catalysts Containing Alumina and Silica

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**Alumina and silica interfere with determination of fluorine. A one-precipitation method for removing aluminate and silicate has been developed using low temperature sodium peroxide fusion for decomposition of sample. Sodium peroxide fusion gives thorough decomposition of sample and yields a readily water-soluble melt. Aluminate and silicate are precipitated with zinc sulfate. One precipitation and filtration remove more than 99% of the silica present. Fluorine is isolated by distillation as fluosilicic acid and titrated with thorium nitrate. The method is accurate to within ±2.9% with 95% confidence limit for samples containing as little as 0.1% of fluorine, and has a standard deviation of 1.2%.**

IN RECENT years the presence of fluorine in catalysts containing alumina and silica has become a matter of interest in the petroleum industry, and this in turn has stimulated interest in methods of determining the fluorine content of these catalysts. A simple and rapid method for determination of fluorine in presence of alumina and silica has been developed in this laboratory.

Both alumina and silica interfere with determination of fluorine. Berzelius' gravimetric method (*3*) is tedious and not suitable for samples containing less than 10 mg. of fluorine. Steiger's peroxidized titanium procedure (*10*), as modified by Parrish and others (*6*), is rapid, but is not accurate for fluorine contents higher than 2%. Willard and Winter's method (*11*) of volatilizing fluorine as hydrofluosilicic acid, followed by titrating with thorium nitrate, covers a wider concentration range than either Berzelius' or Steiger's method, but requires triple filtration for removing large amount of silica prior to distillation.

A simple procedure for removal of alumina and silica has been developed in which one precipitation with zinc sulfate at pH 11.5 is sufficient to remove interfering amounts of alumina and silica; residual alumina and silica are too low to interfere with recovery of fluorine in the subsequent distillation step. Sodium peroxide is used rather than sodium carbonate in fusing the sample, since sodium peroxide is a more effective fusion agent. The fusion is performed at 500° to 540° C., permitting use of platinum crucibles (*7*). The constant-temperature steam-distillation still designed by Huckabay and others (*4*) is used with slight modification. Hydrofluosilicic acid is distilled from sulfuric acid at 145° C. Fluorine in the distillate is determined by the thorium nitrate titration method of Rowley and Churchill (*8*). A half-neutralized monochloroacetic acid buffer, prepared according to Kimball and Tufts (*5*), is used to maintain pH 3.0 during titration. An artificial color standard of cobaltous nitrate and potassium chromate, proposed by Eberz and others (*1*), is used for matching the end point. A suitable amount of thorium fluoride in the color standard helps in matching to a precise end point (*2*).

This method apparently was developed concurrently with that of Shell and Craig (*9*), the two methods being similar in broad outline but differing in a number of details. The present method is believed to be more suitable for accurate determination of low concentrations of fluorine and has several other advantages.

Use of sodium peroxide fusion not only gives thorough decomposition of sample but also yields a melt that is entirely and readily soluble in water. Furthermore, the completeness of dissolution provides an indication of the completeness of decomposition. In Shell and Craig's sodium carbonate–zinc oxide fusion method, the melt contains insoluble zinc salts which hinder the dissolution of soluble salts and the completeness of decomposition is assured only after a repetition of fusion and distillation. In the titration step, use of a buffer is much simpler than monitoring with a pH meter as required by Shell and Craig. Two milliliters of buffer was found to maintain the pH at 3.0 to 3.2 for titrating fluorine concentrations up to 10 mg. of fluorine in 100 ml. of distillate, the largest amount of fluorine normally encountered. Use of a color standard is desirable, as it enables an inexperienced worker to detect the end point.

## REAGENTS

Sodium peroxide, reagent grade.

Zinc sulfate, 36 grams of zinc sulfate heptahydrate per 100 ml. of solution.

Sodium sulfate, reagent grade, 1% aqueous solution.

Sulfuric acid, concentrated, reagent grade, tested by distillation to be fluorine-free.

Sodium hydroxide, reagent grade, 20% and 1% aqueous solution.

Mixed indicator, 0.4 gram of 1,3,5-trinitrobenzene and 0.4 gram of alkaline blue per 100 ml. of 95% ethyl alcohol.

Soda-lime glass, finely ground, washed with sulfuric acid and water.

Sodium alizarin sulfonate, 0.05% aqueous solution.

Hydrochloric acid, reagent grade, diluted 1 to 250 with water.

Buffer solution, pH 3.0, prepared according to Kimball and Tufts (*5*) by dissolving 9.4 grams of monochloroacetic acid in 30 ml. of water, diluting to 60 ml., neutralizing 38 ml. with 20% sodium hydroxide solution and mixing with remainder, and diluting to 100 ml. This solution decomposes within 2 weeks on standing at room temperature and then gives appreciable turbidity on addition of dilute silver nitrate solution and nitric acid. The buffer solution is stable for a few months if stored in a refrigerator.

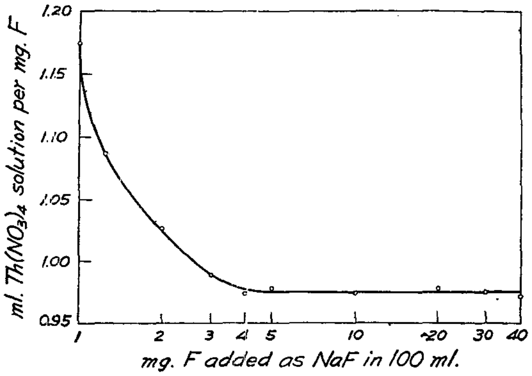

**Figure 1. Effect of fluorine concentration on titration equivalent of thorium nitrate solution**

Sodium fluoride in 0.05*N* solution, containing about 2.2 grams of dry, pure, accurately weighed sodium fluoride per liter of solution.

Thorium nitrate in 0.05*N* solution, prepared by dissolving 7.3 grams of thorium nitrate tetrahydrate per liter of water. This solution is standardized against standard sodium fluoride solution that has been carried through the distillation step of the method. This is necessary because only 98.5% of fluorine is recovered.

Color standard, containing 1.236 grams of cobaltous nitrate hexahydrate and 0.0093 gram of potassium chromate per liter of water.

Silver sulfate, reagent grade.

1,1,2,2-Tetrachloroethane, redistilled, for constant-temperature steam-distillation still.

## ANALYTICAL PROCEDURE

**Fusion of Sample.** A finely pulverized sample of not more than 2 grams, containing preferably 5 to 25 mg. of fluorine, is weighed into a platinum or nickel crucible. Eight to 10 times the sample weight of sodium peroxide is added and thoroughly mixed with the sample. The mixture is heated in a muffle furnace for 1 hour at 500° to 540° C., usually resulting in complete fusion. After cooling to room temperature, the melt is dissolved in 100 ml. of water with heating, and the solution simmered for about 1 hour to destroy any remaining peroxide. Water should be added as required to keep volume up to 75 ml.

**Removal of Alumina and Silica.** About 20 ml. of zinc sulfate solution per gram of sample is added slowly, with stirring, to the solution prepared in the previous step. One milliliter of mixed indicator solution is added and pH is adjusted to 11.5 by dropwise addition of sulfuric acid (1 to 3) until color of suspension changes from orange red to muddy violet. An extra drop of acid gives the suspension a definitely blue color; the pH may be readjusted by dropwise addition of 20% sodium hydroxide solution. The curdy precipitate of zinc aluminate and zinc silicate is digested on a steam bath until it becomes crystalline, usually requiring 1 hour. The hot solution is decanted through a medium-porosity sintered-glass filter funnel. The precipitate is then transferred into the filter funnel and washed thoroughly with hot 1% sodium sulfate solution. Filtrate and washings are combined and concentrated on a steam bath. This one-precipitation procedure removes more than 99% of the silica originally present in the sample.

**Distillation of Fluorine as Hydrofluosilicic Acid.** The concentrated filtrates, together with 0.5 gram of soda-lime glass powder and a suitable amount of silver sulfate for binding any chloride present, are charged to the constant-temperature steam-distillation still and followed by addition of 20 ml. of concentrated sulfuric acid. Distillate is collected in volumetric flasks at a distillation rate of 2.5 to 5 ml. per minute. Distillation is continued until 100 ml. of distillate consumes no more than 0.04 ml. of 0.05*N* thorium nitrate solution, an amount required in a typical distillation blank. If distillation is carried out at a faster rate, appreciable amount of sulfuric acid is carried over and results in a higher distillation blank. The volume of distillate to be collected varies with amount of fluorine present. Usually, collection of 200 ml. is sufficient for 1 to 5 mg. of fluorine, 500 ml. for 15 to 25 mg. of fluorine.

**Titration.** The distillate is thoroughly mixed, and to a 100-ml. aliquot taken for titration, 0.80 ml. of 0.05% sodium alizarin sulfonate solution is added. Enough 1% sodium hydroxide solution is added to give a red color, then dilute hydrochloric acid (1 to 250) is added dropwise until the red color fades. Two milliliters of buffer solution is added and the solution is titrated with thorium nitrate solution using a 5-ml. microburet. The end point is reached when color of the solution matches that of 100 ml. of color standard containing thorium fluoride, precipitated by adding 4 ml. of each of the standard solutions of thorium nitrate and sodium fluoride.

**Table I. Analytical Results on Synthetic Samples**

| Compn., Grams | | Peroxide Fusion and Separation | Fluorine | | |
|---|---|---|---|---|---|
| Alumina | Silica | | Present, mg. | Found, mg. | % Recovery |
| 1.0 | 0.1 | No | 27.18 | 27.22 | 100.1 |
| 0.2 | 0.02 | No | 21.74 | 21.62 | 99.4 |
| 1.0[a] | 0.1 | No | 54.35 | 54.32 | 99.9 |
| 1.0[a] | 0.1 | No | 10.87 | 10.92 | 100.3 |
| 0.13 | 0.87 | Yes | 18.70 | 18.31 | 98.0 |
| 0.13 | 0.87 | Yes | 17.04 | 16.64 | 97.6 |
| 0.13 | 0.87 | Yes | 4.925 | 4.938 | 100.2 |
| 0.13 | 0.87 | Yes | 1.970 | 1.999 | 101.5 |

[a] Added as $AlK(SO_4)_2.12H_2O$.

**Table II. Analytical Results on Catalysts and Minerals**

| Sample | Peroxide Fusion and Separation | Sample Size, Grams | % Fluorine |
|---|---|---|---|
| Alumina-rich catalyst A | No | 0.23 | 7.14 |
| | No | 0.21 | 7.22 |
| Silica-rich catalyst B | No | 1.07 | 1.42 |
| | Yes | 1.02 | 1.60 |
| | Yes | 1.03 | 1.60 |
| Lepidolite, Calif. | Yes | 0.22 | 7.56 |
| | Yes | 0.21 | 7.50 |
| Idocrase, Maine | Yes | 0.21 | 2.10 |
| | Yes | 1.09 | 2.06 |
| Zunyite, Utah | Yes | 0.42 | 4.73 |
| | Yes | 0.32 | 4.79 |

Optimum concentration of fluorine for titration is 3.5 to 10 mg. per 100 ml. As shown in Figure 1, the amount of thorium nitrate needed per milligram of fluorine required for titrating undistilled sodium fluoride solution remains constant for concentrations of fluorine ranging from 3.5 to 40 mg. per 100 ml. Below 3.5 mg. per 100 ml., thorium nitrate consumption increases steadily as fluorine concentration decreases. Therefore, for distillates containing less than 3.5 mg. of fluorine per 100 ml., a suitable amount of standard sodium fluoride solution is added to bring total fluorine up to 4 mg.

## DISCUSSION

Synthetics were prepared by adding sodium fluoride solution to mixture of alumina and silica gels, and evaporating to dryness; the alumina dissolved in sulfuric acid and the silica became gelatinous in sulfuric acid. Analytical results on these synthetics are summarized in Table I. Results on the first four synthetics show that quantities up to 1 gram of alumina gel and 0.1 gram of silica gel can be tolerated in distillation. Therefore, for catalysts rich in alumina and containing less than 10% of silica, the pulverized sample can be subjected to distillation without removal of alumina and silica, provided that sample size is 1 gram or less. For silica-rich catalysts, separation is necessary before distillation; without separation, only about 90% of the fluorine is recovered. In Table II, good agreement of duplicate fluorine determinations on various mineral samples shows that this method may prove useful in mineral analysis.

Accuracy of this method has been tested with synthetics and found to be ±2.9% with 95% confidence limit for samples containing as little as 0.1% of fluorine. Duplicate determinations of catalysts and minerals showed a standard deviation of 1.2%.

## ACKNOWLEDGMENT

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# Determination of Oxygen in Calcium Metal

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**A rapid procedure is presented for the determination of oxygen in calcium metal. The method consists of the reaction of calcium with anhydrous methanol and dissolution of the reaction products by addition of an anhydrous salicylic acid–pyridine solution. The water formed by the reaction of the oxide with the salicylic acid is easily determined by titration with Karl Fischer reagent. A simple apparatus for the reaction and titration operations is described. Data are presented to show that pyridine effectively inhibits esterification between the salicylic acid and the methanol. Results are given on the determination of anhydrous calcium oxide equivalent to 2.5 to 13 mg. of oxygen with a precision of about 0.1 mg. Calcium metal containing 0.4 to 2% of oxygen was analyzed readily.**

LITERATURE reviews have disclosed no recorded method for the determination of oxygen in calcium metal. Recently, White, Ross, and Rowan (*7*) have determined oxygen in sodium by the reaction between sodium and excess *n*-butyl bromide in hexane solution. The sodium monoxide which does not react with the reagent was determined by titration, after addition of water. Pepkowitz and Judd (*6*) developed a method for oxygen in sodium which essentially depends upon the extraction of sodium with mercury in an inert atmosphere and the separation of the residual sodium monoxide, which is insoluble in mercury. Williams and Miller (*9*) have reported a modification of the method of Pepkowitz and Judd. None of these methods was found to be adaptable to the determination of oxygen in calcium.

The authors have developed a new method which is based upon the fact that methanol reacts with calcium to form calcium methylate, but does not react with calcium oxide. Following this reaction, dissolution of the calcium methylate and calcium oxide by addition of a salicylic acid–pyridine solution yields soluble calcium salicylate, methanol, and water. The water which is liberated stoichiometrically from the oxide present is determined by the Karl Fischer (*5*) method using the dead-stop end point technique of Foulk and Bowden (*1*, *4*).

## APPARATUS

The apparatus used for the reaction of calcium with methanol is shown in Figure 1.

The borosilicate glass reaction and titrating flask of 250-ml. capacity has a ₮ 24/40 center neck and a ₮ 14/20 side neck which is fitted with a ground-glass stopper. The refluxing condenser is 300 mm. in length and connected to the center neck of the flask through a bushing type adapter of ₮ 24/40 outer ground zone and ₮ 14/40 inner ground zone. A straight stopcock is sealed to the top of the condenser and is then connected through a ball-and-socket joint to a Nesbitt absorption bulb containing a layer of Drierite covered with anhydrous magnesium perchlorate. Solutions are stirred with the aid of a magnetic stirrer. The glass covered stirring bar is 35 mm. in length and 5 mm. in diameter.

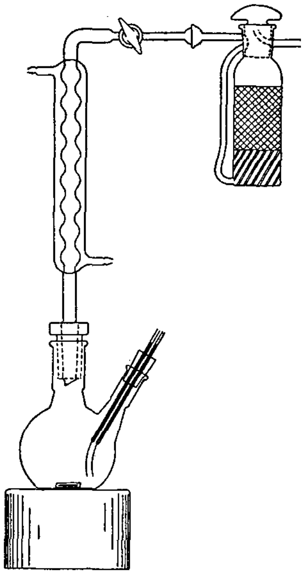

Figure 1. Reaction and titration apparatus

A Beckman Model K-F Aquameter is employed for carrying out the Karl Fischer titration. The standard 125-ml. titrating flask and duel platinum electrodes supplied with the Aquameter are used in determining the water (blank) content of an aliquot of the methanol drawn from the reaction flask prior to addition of the calcium. The smaller flask is also used for titrating the salicylic acid–pyridine solution before addition of the acid to the reaction flask. In this procedure no correction for water in the salicylic acid–pyridine solution is necessary since the acid is pretitrated. A special set of dual platinum electrodes is constructed for direct use in the 250-ml. reaction flask.

## REAGENTS

**Absolute Methanol.** Reduce the water content of the alcohol to less than 0.005% by reaction of the alcohol with magnesium ribbon (*8*) followed by distillation.

**Salicylic Acid–Pyridine Solution.** Dissolve 100 grams of salicylic acid in 100 ml. of pyridine. Pyridine can be dried to less than 0.01% water by adding a calculated 10% excess of benzene over that required to remove the water initially present and fractionating out the benzene-water azeotrope and excess benzene (*3*).

**Karl Fischer Reagent.** Prepare in the usual manner with 269 ml. of pyridine, 84.7 grams of iodine, 667 ml. of absolute methanol, and 45 ml. of liquid sulfur dioxide.

**Table I. Reproducibility of Blanks for Oxygen Content**

| Oxygen, Mg. | | |
|---|---|---|
| Blank[a] | Found | Difference |
| 4.08 | 3.78 | −0.30 |
| 4.98 | 4.56 | −0.42 |
| 5.61 | 5.28 | −0.33 |
| 32.70 | 32.31 | −0.39 |
| 52.35 | 51.99 | −0.36 |
| | Average | −0.36 |

[a] Calculated from water found in an aliquot of alcohol.

## PROCEDURE

Dry the reaction flask, adapter, stopper, stirring bar, and condenser in the oven at 120° C. and then assemble with the glass stopper in the side arm. With the stopcock on the condenser closed, rinse the apparatus with two 50-ml. portions of absolute methanol.

Place 100 ml. of absolute methanol in the reaction flask through the side arm and rapidly replace the stopper. Rinse the flask and condenser, but do not discard the alcohol. Attach the condenser through the ball-and-socket joint to the Nesbitt absorption bulb and open the stopcock.

Rinse a 25-ml. pipet twice with absolute methanol and then quickly withdraw through the side arm a 25-ml. aliquot of the alcohol from the reaction flask. Transfer the aliquot rapidly to the 125-ml. titrating flask, which contains 25 ml. of "pretitrated" methanol. Titrate the aliquot with Karl Fischer reagent to a dead-stop end point and record the titration. Multiply by a factor of 3 to give the blank titration. Discard the solution.

The weight of the sample should not exceed 5 grams. Although a 2-gram sample reacts completely with the methanol within 30 minutes with stirring, the dissolution of a 5-gram sample may take an hour. Place the sample of calcium metal chips in the reaction flask through the side arm and tightly replace the stopper. Water-cool the condenser and heat the flask gently with a small flame until the calcium reacts rapidly with the alcohol. Discontinue the heating. The reaction is very rapid at the start and goes nearly to completion within 15 minutes. Stir constantly and occasionally warm the alcohol to dissolve the residual small particles of calcium metal which are coated with calcium methylate. Allow one hour for complete reaction. As would be expected, large pieces of metal dissolve very slowly. The samples which were analyzed passed through a 6 mesh per inch sieve.

Titrate 50 ml. of salicylic acid–pyridine solution in the previously used 125-ml. flask with the Karl Fischer reagent. Transfer the acid rapidly to the reaction flask and stir the contents with the magnetic stirrer for 10 minutes. Close the stopcock and disconnect the condenser from the drying bulb. Rinse the flask and condenser with the solution. Allow the condenser to drain for a few minutes and then remove the condenser and stopper from the flask. Insert the dual platinum electrodes made for the reaction flask. Connect the reaction flask to the buret of the Karl Fischer apparatus and titrate the solution with Karl Fischer reagent. Record the titration and subtract the blank titration.

With a Kirk ultramicro transfer pipet add 25 mg. of water to the titrated solution. Titrate with Karl Fischer reagent, and determine the oxygen equivalent in milligrams per milliliter of reagent. Calculate the per cent oxygen in the calcium metal.

## EXPERIMENTAL RESULTS AND DISCUSSION

Experiments were conducted on the reproducibility of blanks to find if any error could be attributed to atmospheric moisture pick-up and to the possible esterification of the acid with the methanol. Some of these tests were carried out on days of high humidity. Each test was carried through the procedure without the calcium. The alcohol was refluxed for 30 minutes. The salicylic acid–pyridine solution was added while the alcohol was still warm and a period of 10 minutes was allowed for stirring the solution before titrating with the Karl Fischer reagent. The results of these tests are given in Table I.

No error was found to be introduced from atmospheric moisture and the results showed that the pyridine effectively inhibited any esterification. In all tests, less oxygen was found than calculated to be present in the alcohol, but the difference was small and fairly constant. The cause of this constant difference has not been established, but it may possibly be attributed to the employment of two different sets of platinum electrodes in carrying out the Fischer titrations. The results also showed that a high blank could be tolerated.

In Table II are listed the results on the recovery when known amounts of anhydrous calcium oxide were added to the methanol. The alcohol, with the added calcium oxide, was refluxed for 30 minutes before the addition of the salicylic acid–pyridine solution. The results for the recovery of total oxygen were nearly quantitative.

Typical results on the analysis of samples of calcium metal are shown in Table III. All the metals passed a sieve of 6 meshes to the inch. The surface texture of individual pieces of the metal, especially sample NBL B-8123, varied from light gray, to dark gray and to black. Guntz and Röderer (*2*) found that when the clean bright metal was exposed to air the surface immediately became dull, and later gray, owing to the formation of oxide. This observation was verified by the analyses made on sample NBL B-8123. The results showed that selected dark pieces of the metal contained much more oxygen than selected bright pieces. The results on the analyses of five portions of this sample, drawn at random, showed a considerable scattering. This scattering of results was not due to the method of analysis, but to the heterogeneity of the metal. Sample NBL B-8525 was by observation a much more uniform light gray and the individual pieces of the metal were much smaller than those of sample NBL B-8123. The results on eight determinations showed some scattering, but not nearly so great as for sample NBL B-8123.

**Table II. Recovery of Known Amounts of Oxygen in Methanol**

| Oxygen, Mg. | | | | | |
|---|---|---|---|---|---|
| Added[a] | Blank[b] | Total (blank + added) | Found | Difference | Recovery, % |
| 13.44 | 4.35 | 17.79 | 17.81 | +0.02 | 100 |
| 2.57 | 4.95 | 7.52 | 7.61 | +0.09 | 101 |
| 2.60 | 45.72 | 48.32 | 48.45 | +0.13 | 100 |

[a] Added as anhydrous calcium oxide.

[b] Calculated from water found in aliquot of alcohol and corrected for average blank difference of −0.36 mg. as found in Table I.

**Table III. Oxygen Content of Calcium Metal**

| Sample No. | Manner Drawn | Calcium, Grams | Oxygen Found Mg. | Oxygen Found % |
|---|---|---|---|---|
| NBL B-8525 | At random | 2.002 | 15.9 | 0.79 |
| | | 2.004 | 17.0 | 0.85 |
| | | 2.003 | 17.8 | 0.89 |
| | | 2.005 | 15.1 | 0.76 |
| | | 2.001 | 16.0 | 0.80 |
| | | 4.005 | 29.0 | 0.72 |
| | | 5.000 | 37.4 | 0.75 |
| | | 5.002 | 43.4 | 0.87 |
| | | | Mean | 0.80 |
| | | | Standard deviation | ±0.06 |
| NBL B-8123 | At random | 2.00 | 21.0 | 1.05 |
| | | 2.00 | 15.4 | 0.77 |
| | | 2.00 | 12.2 | 0.61 |
| | | 2.00 | 20.0 | 1.00 |
| | | 2.00 | 12.0 | 0.60 |
| | | | Mean | 0.81 |
| | | | Standard deviation | ±0.19 |
| | Selected bright pieces | 2.00 | 8.4 | 0.42 |
| | Selected dark pieces | 2.00 | 39.2 | 1.96 |

To test the precision of the method was difficult because of the heterogeneous nature of the calcium metals. The most uniform metal available analyzed 0.80 ± 0.06% oxygen and was used for the preparation of standards. The standards were prepared by adding known amounts of calcium oxide to portions of the metal. The results of determinations of these standards are given in Table IV. All results fell within the limits of the natural oxygen variation in the metal.

**Table IV. Recovery of Known Amounts of Oxygen in Calcium Metal**

| | Oxygen, Mg. | | | | | |
|---|---|---|---|---|---|---|
| Calcium, Grams | Added[a] (A) | In alcohol[b] (B) | In metal[c] (C) | Total (A + B + C) | Found | Difference |
| 2.004 | 28.5 | 7.5 | 16.0 ± 1.1 | 52.0 | 53.0 | +1.0 |
| 2.001 | 14.6 | 38.4 | 16.0 ± 1.1 | 69.0 | 69.3 | +0.3 |
| 4.000 | 7.1 | 11.3 | 32.0 ± 2.3 | 50.4 | 52.3 | +1.9 |
| 5.002 | 7.1 | 19.0 | 40.0 ± 2.8 | 66.1 | 63.9 | −2.2 |

[a] Added as calcium oxide.
[b] Calculated from water found in an aliquot of alcohol and corrected for average blank difference of −0.36 mg. as found in Table I.
[c] Average of eight determinations. Metal was heterogeneous.

In Table II it can be seen that 2.6 mg. of oxygen, added as calcium oxide, can be determined with an error of less than 5%. For a 5-gram sample, this is equivalent to the determination of 0.05% oxygen in calcium metal.

Because of the relatively slow reaction of calcium with atmospheric water and oxygen as compared to sodium, no elaborate precautions must be taken in weighing and transferring the calcium to the reaction flask. These operations, however, should be carried out as quickly as possible. However, in production sampling at the manufacturing plant, the metal taken for analysis should be sealed immediately in a dry inert atmosphere since days may elapse before an analysis is made. Moreover, samples in containers once opened show a continuous increase in oxygen content unless this precaution is taken.

For the currently available calcium metal, as a result of sample heterogeneity, several determinations are required.

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# Determination of Wolframite in Scheelite

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**Both the natural and artificial forms of scheelite (a calcium tungstate with the formula $CaWO_4$) must be low in iron and manganese to meet government specifications. These elements indicate the presence of the iron and/or manganese tungstate mineral wolframite $Fe(Mn)WO_4$ which is only slightly soluble in dilute acids, thus obviating total extraction of the tungsten in industrial processes. The method described here suggests solution of the sample in hydrochloric acid in the absence of oxidizing agents and determination of the iron(II), the form in which iron exists in wolframite. Other hydrochloric acid–soluble minerals found in scheelite either are present in the trivalent form or are oxidized by a heating at 400° C. for 4 hours. Experiments are described which indicate that virtually all of the manganese found in scheelite is present as the tungstate. The proposed method is expected to aid in avoiding controversies in the tungsten industry concerning the actual amount of wolframite found in scheelite.**

WOLFRAMITE, an iron and/or manganese mineral of the formula $Fe(Mn)WO_4$, is less soluble in dilute acids than the calcium tungstate mineral scheelite ($CaWO_4$). Therefore, Material Purchase Specifications of the General Services Administration covering the purchase of Class I, Natural and Synthetic Scheelite, under the National Stockpiling Program (*2*) strictly limit the amount of wolframite permissible in scheelite. These specifications of the General Services Administration are based on its assumption that all the manganese in a scheelite is present as hübnerite and all the iron as ferberite. Hübnerite is defined principally as manganese tungstate with no more than 20% of the tungsten present as ferrous tungstate, while ferberite is described as $FeWO_4$ with no more than 20% of the tungsten as manganese tungstate. Combinations of hübnerite and ferberite in varying proportions thereby form wolframite.

Based on a 23.41% manganese oxide (MnO) and a 76.59% tungstic oxide ($WO_3$) content, 1% of manganese found in scheelite is calculated to be due to 5.52% of manganese tungstate, resulting in a penalty for 4.22% of tungstic oxide. Fortunately, the amount of manganese found in scheelite rarely exceeds 0.20% and therefore offers less of a problem than does iron, which frequently is present in excess of 1%. In the case of iron, based on a 23.64% ferrous oxide and 76.36% tungstic oxide content, 1% of iron found in scheelite is calculated to be due to 5.44% of ferrous tungstate resulting in a deduction of 4.15% of tungstic oxide from the total amount of tungstic acid found.

## FERBERITE

The assumption that all the iron in a scheelite is present as ferberite appears to be unwarranted. On many occasions, the yellow color of ferric chloride was noted when dissolving scheelite in concentrated hydrochloric acid in absence of air and oxidizing agents. Because iron is present in ferberite in the iron(II) form (*1*), any trivalent iron obviously denotes the presence of iron minerals other than ferberite.

Ten wolframite concentrates originating from various locations throughout the world were recently analyzed in this laboratory for the three major components, using standard methods of analysis (*5*, *6*). The results presented in Table I positively demonstrate that wolframite concentrates frequently contain an excess of iron and/or manganese to satisfy the formula $Fe(Mn)WO_4$.

In addition to the iron introduced into scheelite from wolframite, either as part of the mineral or as contaminant, the scheelite concentrate may contain various other iron minerals which could not be removed during the concentration process. No chemical methods found in the literature differentiate between the iron of wolframite and that of other iron minerals. In the past, the Bureau of Mines has provided a very limited service by submitting the most controversial samples to microscopic tests including grain count. Since the need for a routine procedure has become apparent, an attempt was made to develop a rapid chemical method.

**Table I. Comparison of $WO_3$ by Analysis and by Calculation**

| Sample No. | Fe Found, % | Fe × 4.15 = % $WO_3$, % | Mn Found, % | Mn × 4.22 = % $WO_3$, % | Total $WO_3$, % Calculated | Total $WO_3$, % By analysis |
|---|---|---|---|---|---|---|
| 1 | 15.45 | 64.12 | 1.65 | 6.96 | 71.08 | 66.15 |
| 2 | 8.00 | 33.20 | 10.67 | 45.03 | 78.23 | 68.61 |
| 3 | 11.70 | 48.56 | 7.63 | 32.20 | 80.76 | 70.98 |
| 4 | 15.70 | 65.16 | 3.03 | 12.79 | 77.95 | 74.31 |
| 5 | 17.42 | 72.29 | 2.15 | 9.07 | 81.36 | 68.40 |
| 6 | 16.10 | 66.82 | 2.37 | 10.00 | 76.82 | 68.21 |
| 7 | 12.25 | 50.84 | 7.44 | 31.40 | 82.24 | 69.33 |
| 8 | 6.59 | 27.35 | 7.82 | 33.00 | 60.35 | 46.97 |
| 9 | 10.45 | 43.37 | 7.76 | 32.75 | 76.12 | 72.79 |
| 10 | 7.11 | 29.51 | 8.57 | 36.17 | 65.68 | 65.66 |

**Table II. Accuracy of Proposed Method**

| $Fe^{++}$ Added to 1 Gram of Scheelite, Mg. | $Fe^{++}$ Found, Mg. | $Fe^{++}$ Added to 1 Gram of Scheelite, Mg. | $Fe^{++}$ Found, Mg. |
|---|---|---|---|
| 1.6 | 1.8 | 4.6 | 4.8 |
| 1.6 | 1.4 | 4.6 | 4.8 |
| 1.6 | 1.5 | 7.6 | 7.2 |
| 3.1 | 3.2 | 7.6 | 7.2 |
| 3.1 | 2.9 | 7.6 | 7.6 |
| 4.6 | 4.2 | 15.2 | 14.7 |
| 4.6 | 4.6 | 15.2 | 15.3 |

**Principle of Proposed Method.** The proposed method is based on the fact that iron exists in wolframite in the iron(II) state (*1*) which forms, in the absence of oxidizing agents, ferrous chloride upon treatment with concentrated hydrochloric acid. Other hydrochloric acid–soluble iron minerals—such as pyrite, siderite, and garnets—associated with wolframite or contaminating scheelite, either are present in scheelite in the trivalent form or can, upon heating for 4 hours at 400° C., be converted to the trivalent state.

The method does not anticipate the presence of metallic iron (from crushing or grinding equipment) or magnetite, both of which should be removed, if present, with the help of a magnet.

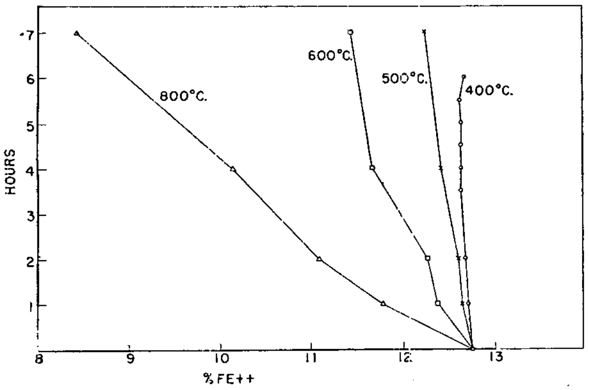

Figure 1. Oxidation rate of iron(II) in ferberite at various temperatures

**Apparatus and Reagents.** Burrell high-temperature electric furnace, thermostatically controlled or equivalent.

Standard Potassium Dichromate Solution, 0.1*N* (1 ml. = 5.584 mg. Fe). Dissolve 4.9035 grams of NBS No. 136 in water, transfer to 1-liter volumetric flask, and dilute to the mark with water.

Standard Potassium Dichromate Solution, 0.01*N*. Transfer 100.0 ml. of 0.1*N* standard potassium dichromate solution to a 1-liter volumetric flask and dilute to mark with water.

Sodium Diphenylamine Sulfonate Indicator. Dissolve 100 mg. of the reagent in 100 ml. of cold water.

**Procedure.** Transfer approximately 5 grams of the finely pulverized sample (−200*M*) to a 20- to 30-ml. round-bottomed porcelain crucible. Heat in a thermostatically controlled furnace, with free access of air, for 4 hours at 400° C., removing the crucible once every hour from the furnace and mixing the sample. Since the 400° C. roasting procedure provides for the oxidation of ferrous-iron minerals soluble in hydrochloric acid, other than wolframite, particularly pyrite and siderite, this step may be omitted in the known absence of such compounds.

Cool in a desiccator, weigh 1-gram portions into 500-ml. Erlenmeyer flasks, add 100 ml. of concentrated hydrochloric acid, and immediately close the flasks with rubber stoppers containing a glass tube extending on the outside to the bottom of the flask. Seal the end of the tube with a solution of hot water containing a little sodium bicarbonate, heat the flasks on a hot plate to boiling, and allow to simmer gently for 45 minutes. Replace the beaker with the hot water (now saturated with hydrochloric acid) with a fresh beaker containing 10 grams of sodium bicarbonate dissolved in 250 ml. of hot water. Remove the flasks from the hot plate and cool to below 15° C., always keeping the end of the glass tube sealed with the solution of bicarbonate.

Remove the stopper from the flask, add 100 ml. of cold water, previously boiled and cooled, add 1 ml. of the sodium diphenylamine indicator, and immediately titrate the ferrous iron, depending on the iron content, with the 0.1 or 0.01*N* potassium dichromate solution to a faint purple end point which can be easily noticed in the presence of the precipitated tungstic oxide. One milliliter of the 0.01*N* potassium dichromate solution is equivalent to 0.0558% of iron in the ferrous state or 0.30% of ferberite.

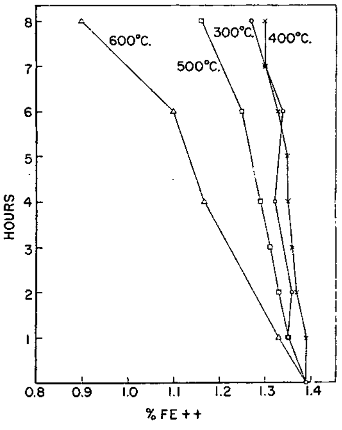

Figure 2. Oxidation rate of iron(II) in 10% wolframite-scheelite mixtures at various temperatures

**Accuracy of Proposed Method.** Various quantities of a sulfur-free wolframite, containing 7.60% iron(II) (determined by solution of the sample in hydrochloric acid in absence of air, and titration with standard potassium dichromate solution) were mixed with 5-gram portions of a scheelite with an iron content of 0.01%. The material was heated at 400° C.; 1-gram portions were dissolved in hydrochloric acid and titrated with standard potassium dichromate solution. The results are presented in Table II.

**Experimental.** Figure 1 shows the rate of oxidation of a sulfur-free sample of wolframite at various temperatures and time intervals. Figure 2 indicates the effect of heat on a sample of scheelite which was intimately mixed with 10% by weight of wolframite. Figure 3 demonstrates the rate of oxidation of the two ferrous-iron minerals, pyrite and siderite, at various temperatures. Figure 1 shows that the iron(II) of ferberite is not significantly oxidized by a 4-hour heating at 400° C.; Figure 2, that ferberite, when mixed with an excess of scheelite, is not affected to a large extent; Figure 3, that the ferrous-iron minerals, pyrite and siderite are oxidized sufficiently and do not cause serious positive errors when present in small quantities. Iron sulfide minerals, other than pyrite, are expected to react similarly.

**Table III. Solubility of Wolframite-Manganese in 2% Acetic Acid Containing Oxalic Acid**

| Sample No. | Total Mn Present Volhard Method (*6*), Mg. | Manganese Found, Mg. 1st extraction | Manganese Found, Mg. 2nd extraction | Manganese Found, Mg. 3rd extraction |
|---|---|---|---|---|
| 2 | 106.7 | 1.4 | 0.5 | 0.4 |
| 3 | 76.3 | 2.1 | 0.7 | 0.4 |
| 4 | 30.3 | 0.4 | 0.4 | 0.5 |
| 5 | 21.5 | 0.3 | 0.5 | 0.3 |
| 7 | 74.4 | 1.0 | 0.7 | 0.4 |
| 8 | 78.2 | 1.8 | 0.2 | 0.3 |
| 9 | 77.6 | 1.3 | 0.4 | 0.4 |
| 10 | 77.6 + 10 mg. Mn[a] | 11.6 | 0.8 | 0.4 |
| 11 | 77.6 + 20 mg. Mn[a] | 21.8 | 0.7 | 0.5 |

[a] Manganese added in form of pyrolusite ore.

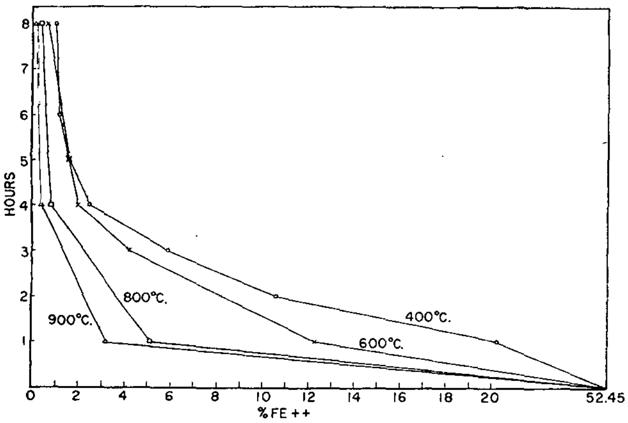

**Figure 3. Oxidation rate of iron(II) in 50 to 50 mixture of pyrite and siderite at various temperatures**

Oxidized iron minerals—such as hematite or limonite—do not interfere with the proposed method in the absence of reducing agents.

Occasionally, ferrous-iron minerals other than pyrite or siderite, occur in small quantities in scheelite as gangue material. They are usually silicates, mainly garnets, with either part of the soluble iron in the oxidized form or easily oxidizable by the roasting procedure at 400° C. Such a garnet suspected to contain 2% of wolframite was obtained through the cooperation of the Wah Chang Smelting and Refining Co., Glen Cove, N. Y., from the weakly magnetic fraction of a high-grade scheelite concentrate (73% tungstic oxide) which was passed through a laboratory magnetic separator. Part of the iron [1.42% iron(II)] was found to be in the ferrous state using the hydrofluoric-sulfuric acid–permanganate titration method (*4*). However, after roasting, the iron(II) content dropped to 0.38% using the proposed method, indicating the presence of 0.38 × 5.44 = 2.07% of ferberite. Additional tests indicated no errors when small amounts of ilmenite and columbite were added to scheelite.

### HÜBNERITE

No methods are described in the chemical literature to distinguish manganese of hübnerite from that of other manganese minerals. Based on experiments indicating that hübnerite is only slightly soluble in dilute acetic acid containing a small amount of oxalic acid, manganese from most manganese minerals was found to be soluble in the above medium.

**Experimental.** One-gram samples of wolframite concentrates were heated for three 1-hour periods (with intermittent filtration) with 50-ml. portions of 2% acetic acid containing 100 mg. of oxalic acid. The filtrates, after addition of sulfuric acid, were evaporated separately by fuming and the manganese was determined by using the silver nitrate–ammonium persulfate method (*2*). The data (see Table III) indicate that the solubility of manganese of wolframite in the medium chosen amounts to about 0.4 mg. per 50 ml., and, that none of the wolframites tested contained more than 3% of the manganese in a nonhübnerite form. Since scheelite rarely contains more than 0.20% of manganese, the assumption is that all the manganese found exists in this mineral as hübnerite.

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# Use of γ-(4-Nitrobenzyl)pyridine as Analytical Reagent for Ethylenimines and Alkylating Agents

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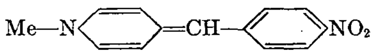
**Procedures are described for colorimetrically estimating very low concentrations of ethylenimine and substituted ethylenimines in water and various alkylating materials in a nonaqueous solvent by the reaction of these compounds with γ-(4-nitrobenzyl)pyridine followed by alkalinization. The procedure for ethylenimine and substituted ethylenimines is adaptable to routine analysis. Concentrations of ethylamine and ethanolamine (the hydrolysis product of ethylenimine) as much as 1000 times that of the ethylenimine do not interfere in the determination. Concentrations of the imines from 0 to 5 p.p.m. produce intensities in color in this reaction which adhere to the Beer-Bouguer law. In nonaqueous medium, the procedure has been applied to α-halogenated esters, diethyl sulfate, alkyl iodides, bromides, and chlorides; and various organic arsenic, phosphorus, silicon, and nitrogen chlorides. The procedure in nonaqueous solvents is recommended for estimation of reactive alkylating materials in the presence of nonreactive alkylating material, but not vice versa. Development of optimum reaction conditions for individual compounds is recommended.**

KOENIGS and others (*8*), reported that γ-(4-nitrobenzyl) pyridine, as well as 2- and 4-benzylpyridine, reacted with methyl iodide to form a salt which yielded a blue dye when treated with potassium hydroxide solution. The dye was assigned the following structure:



The application of this reaction to the detection of mustard gas was first discovered by Brown (*3*). Gehauf (*5*) and Braun (*1*) utilized the general reaction to detect a number of organic alkylating compounds. The vapors of alkyl halides were adsorbed on silica gel impregnated with γ-(4-nitrobenzyl)pyridine. Addition of alkali produced colors varying from blue to violet to brown. The group of compounds detected in this manner included a variety of examples such as diethyl sulfate, butyl thiocyanate, benzene sulfonyl chloride, diphenylchloroarsine, and diethyl phosphorofluoridate. No attempt was made to develop a procedure for quantitative estimation of the reacting compounds, or to determine the limits of sensitivity of the test. Swift and others (*11*) expanded the qualitative studies of Gehauf and Braun

Organic molecules containing $—COX$, $—CH_2X$, and $—OCH_2X$ groupings were found to react similarly. By qualitatively comparing the intensities of colors, the activity of functional groups as summarized as follows: $RCH_2CH_2X > RCH_2CHX_2 > RCH_2CX_3$; $ArCH_2X > ArX$; $ROCH_2X > ROCH_2CH_2X$; and $COCH_2X > COX$. No attempt was made to correlate the concentration of the alkyl halide with the absorbance of the solution formed following reaction. Holzman (*7*) utilized this reaction for the estimation of 2,2′-dichlorodiethyl sulfide and 2,2′,2″-trichlorotriethylamine.

Since a variety of alkylating agents had been shown to form a dye precursor when reacted with $\gamma$-(4-nitrobenzyl)pyridine, it was desirable to investigate these reactions further in order to develop procedures suitable for quantitative estimation. Two procedures are given in this publication. One is carried out in essentially nonaqueous medium and is generally applicable to a large number of organic solvent soluble alkylating materials; the other is carried out in essentially aqueous solution and is specifically for the estimation of ethylenimine and nitrogen or carbon substituted ethylenimines in water. The procedure for estimation of concentrations of the alkylating material in nonaqueous medium is based upon studies with only one alkyl halide —viz., *n*-butyl bromide—and may not be the optimum one for other alkylating materials, especially with respect to the time of heating. For specific analyses, the analyst should determine the optimum conditions using the procedure given herein as a guide.

## SYMBOLS AND DEFINITIONS

The terms transmittance, *T*, absorbance, *A*, and molecular extinction coefficient, *E*, are used in this report as defined by Miller (*6*). The M301 for any compound is defined as the number of moles of that compound which will react with $\gamma$-(4-nitrobenzyl)pyridine (NBP) under the conditions of the relevant procedure to form a colored solution having 0.301 absorbance (50% transmittance) at the wave length of maximum absorption in a cell of 1.3-cm. length.

## APPARATUS

Coleman Universal spectrophotometer (Model 11A).
Matched square couvettes, 1.3-cm. light path length.
PC-4 filter.

## REAGENTS

$\gamma$-(4-Nitrobenzyl)pyridine Reagent. Five per cent of $\gamma$-(4-nitrobenzyl)pyridine, recrystallized from cyclohexane, melting point 70° to 71° C., in methyl ethyl ketone or C.P. acetone.

Potassium Carbonate Solution, 1*M* aqueous.

Triethylamine Reagent, 50% C.P. triethylamine in acetone.

Alkylating Agents. All alkylating agents were either purchased as C.P. chemicals or synthesized by standard methods and distilled prior to use.

The ethylenimines were obtained from Monomer-Polymer Inc., Chicago, Ill. The materials were distilled prior to use. The boiling points and refractive indices of the distilled compounds were as follows:

| Compound | Boiling Point, ° C./ca. 760 mm. | Refractive Index at 25° C. |
|---|---|---|
| Ethylenimine | 55.2–56.0 | 1.4088 |
| 2-Ethylethylenimine | 88.0–88.3 | 1.4165 |
| 2,2-Dimethylethylenimine | 68.0–69.0 | 1.4088 |
| *N*-Ethylethylenimine | 51.3–52.3 | 1.3938[a] |

[a] Manufacturer's value.

Solutions of the ethylenimine compounds were made up by diluting accurately weighed quantities of the distilled materials to the mark in a volumetric flask. Aliquots of the solutions were further diluted to give stock solutions containing from 0 to 5 p.p.m. The stock solutions were stable for at least 24 hours.

Buffer Solution, pH 4.0, Clark and Lubs (*4*).

## ESTIMATION OF ETHYLENIMINES

**Preparation of Standard Curve.** To each of a series of calibrated 10-ml. tubes are added 3 ml. of the stock solutions, 1 ml. of the buffer solution, and 1 ml. of the $\gamma$-(4-nitrobenzyl)pyridine reagent. The solutions are mixed well, heated in a boiling water bath for 20 minutes, and then cooled in an ice-water bath. The reaction mixture at this point is stable for about 30 minutes and the remaining steps in the procedure (including the measurement of color intensity) should be carried out on each tube, one at a time. Four milliliters of acetone, 1 ml. of potassium carbonate solution, and sufficient distilled water to make up to a volume of 10 ml. are added in rapid succession. The solutions are again mixed well and read immediately at 600 m$\mu$ against distilled water. The absorbance of a reagent blank (0 p.p.m.) is determined as above and subtracted from that of the other samples. The standard curve is constructed by plotting the net absorbances against the concentration of ethylenimine on arithmetic graph paper.

**Table I. Effect of pH of Initial Solution on Absorbance**

| Initial pH | Absorbance |
|---|---|
| 2.4 | 0.199 |
| 2.8 | 0.211 |
| 3.2 | 0.215 |
| 4.0 | 0.226 |
| 4.4 | 0.224 |
| 5.0 | 0.220 |
| 5.6 | 0.172 |
| 6.0 | 0.152 |

**Procedural Notes and Results.** The importance of initial pH of the solution containing the ethylenimine was evaluated by tests performed on solutions of ethylenimine in Clark and Lubs buffers ranging in pH from 2.4 to 6.0. The absorbances found after reaction are shown in Table I.

**Table II. Absorbance of Solutions Heated for Different Periods**

| Heating Period, Min. | Absorbance |
|---|---|
| 5 | 0.262 |
| 10 | 0.300 |
| 15 | 0.306 |
| 20 | 0.311 |
| 25 | 0.307 |
| 30 | 0.315 |

The absorbances are, within experimental error, the same and at a maximum in solutions of initial pH between 4.0 and 5.0. A maximum and constant absorbance was obtained by heating the ethylenimine with $\gamma$-(4-nitrobenzyl)pyridine agent in a boiling water bath for approximately 15 minutes, but the heating period could be extended to 30 minutes without any definite effect upon the absorbance of the final colored solution (Table II).

**Table III. Stability of Reaction Product of Ethylenimine and $\gamma$-(4-Nitrobenzyl)pyridine**

| No. of Detn. | Absorbances | |
|---|---|---|
| | a[a] | b[b] |
| 5 | 0.214 ± 0.002 | 0.210 ± 0.004 |
| 5 | 0.424 ± 0.009 | ±0.413 ± 0.004 |
| 5 | 0.575 ± 0.02 | 0.558 ± 0.02 |

[a] Reaction product between ethylenimine and NBP made alkaline immediately after heated product had been cooled to room temperature.

[b] Reaction product between ethylenimine and NBP cooled to room temperature, allowed to sit for 30 minutes, and then made alkaline.

A heating period of 20 minutes (see procedure) is therefore not critical. It may be inferred from these observations that the product of the reaction between ethylenimine and $\gamma$-(4-nitrobenzyl)pyridine is stable. By comparing the absorbances of solutions obtained in tests in which the intermediate had been allowed to sit at room temperature for zero and 30 minutes prior to alkalinization, the intermediate was found to be stable for at least that length of time at room temperature (Table III).

The drop in absorbance over the 30-minute period indicates a decomposition of 2 to 3%.

Measurement of the absorbance immediately after color development is imperative, as the final color fades appreciably with time. However, if the procedure is followed, good reproducibility of absorbances is obtained, as seen by the average deviations from the mean shown in Table III.

Tests performed with the other ethylenimines were limited to the preparation of standard curves using the recommended procedure. In all cases, the curves adhered to the Beer-Bouguer law.

Dilute solutions of diethylamine and ethanolamine (the hydrolysis product of ethylenimine) did not interfere with the determination of the ethylenimine in concentrations up to 1000 times that of the ethylenimine. Absorbances obtained in the presence of diethylamine and ethanolamine did not vary from the expected values by amounts greater than those appraised in the absence of these substances.

## ESTIMATION OF ALKYLATING AGENTS IN NONAQUEOUS SOLUTION

**Preparation of Standard Curve.** To 6 ml. of a methyl ethyl ketone solution containing different quantities of alkylating agent in dry round-bottomed flasks is added 5 ml. of γ-(4-nitrobenzyl)pyridine reagent. (A significant amount of water—e.g., 1 ml., was found to have an effect on the results, and whereas it was not necessary to keep reagents and equipment absolutely water-free, some care was taken to keep water out of the reaction.) Two glass beads are added to the flask, and the flask is connected to a condenser, refluxed for 45 minutes in a boiling water bath, and then cooled for 1 minute in an ice-water bath. Five milliliters of acetone is added through the condenser and allowed to drain for 1 minute into the flask. The condenser is separated from the flask, 1 ml. of triethylamine reagent is added, and the solutions are mixed well. The purple color, which appears immediately, is read within 2 minutes after addition of the triethylamine reagent at 565 mμ. All concentrations of the samples, including blanks (no alkylating agent) are measured against distilled water.

**Procedural Notes and Results.** The variation in reactivity of alkylating agents to nitrogen compounds (*2, 9, 10*) is well known. This procedure was developed using a comparatively unreactive alkyl halide—viz., *n*-butyl bromide—and hence may not be the optimum procedure for others. Attempts to cause the reaction between *n*-butyl bromide in aqueous solution and γ-(4-nitrobenzyl)pyridine were unsuccessful presumably because of the insolubility of *n*-butyl bromide in water in concentrations necessary to give a test. Other solvents, which overcame the solubility factor, were unsatisfactory for various reasons: Ether, cyclohexane, and methyl and ethyl Cellosolves gave colored solutions which faded too quickly; nitrobenzene and nitromethane gave colored solutions when heated with γ-(4-nitrobenzyl)pyridine in the absence of alkyl halides. Methyl ethyl ketone and acetone were satisfactory and the former was chosen because of its higher boiling point.

**Table IV. Moles of Alkyl Halide Required to Produce Colored Solution Having 0.301 Absorbance**

| Halide | Series | Alkyl Group | M301 |
|---|---|---|---|
| Iodide | Normal | Ethyl | $1.6 \times 10^{-6}$ |
| | | Butyl | $7.0 \times 10^{-7}$ |
| | | Amyl | $3.7 \times 10^{-7}$ |
| Bromide | Normal | Propyl | $8.2 \times 10^{-6}$ |
| | | Butyl | $5.3 \times 10^{-6}$ |
| | | Amyl | $3.3 \times 10^{-6}$ |
| | | Hexyl | $1.3 \times 10^{-6}$ |
| | | Heptyl | $7.4 \times 10^{-7}$ |
| | | Decyl | $2.8 \times 10^{-7}$ |
| | | Allyl | $3.0 \times 10^{-7}$ |
| | | Benzyl-(α-bromo-toluene) | $1.5 \times 10^{-7}$ |
| | | 2-Methylbenzyl | $1.5 \times 10^{-7}$ |
| | Iso | Butyl | $8.1 \times 10^{-5}$ |
| | | Amyl | $7.6 \times 10^{-6}$ |
| | Secondary | Isopropyl | $1.3 \times 10^{-4}$ |
| | | Butyl | $5.5 \times 10^{-5}$ |
| | | Octyl | $4.6 \times 10^{-6}$ |
| | Tertiary | Butyl | $2.9 \times 10^{-4}$ |
| Chloride | Normal | Butyl | $6.6 \times 10^{-4}$ |
| | | Amyl | $4.4 \times 10^{-4}$ |
| | | Benzyl | $4.9 \times 10^{-7}$ |
| | | *p*-Methylbenzyl | $2.2 \times 10^{-7}$ |
| | | *p*-Nitrobenzyl | $2.5 \times 10^{-7}$ |
| Fluoride | Normal | Amyl | $>10^{-2}$ |
| Cyanide | Normal | Amyl | $>10^{-3}$ |
| Bromobenzene | | | $>10^{-3}$ |

Addition of varying quantities of acid quickly revealed that acid promoted the decomposition of γ-(4-nitrobenzyl)pyridine without assisting the reaction between it and the alkyl halide. The color intensity of both the sample and the blank increased with increasing concentration of acid.

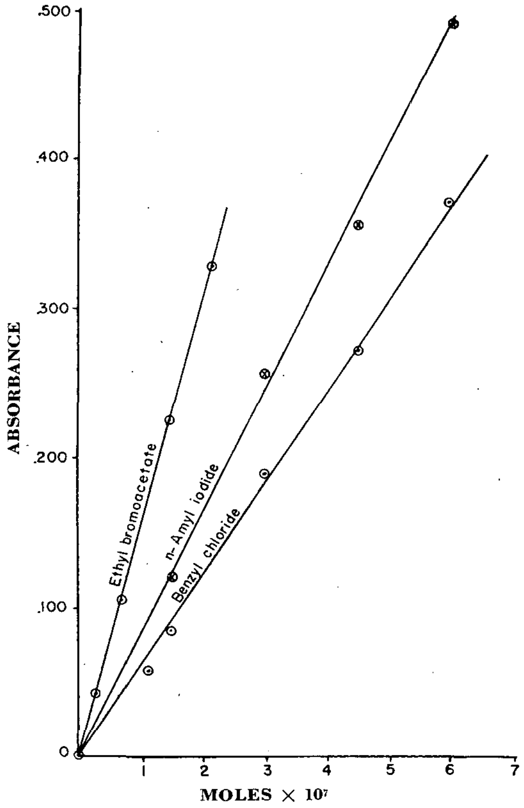


**Figure 1. Calibration curves of some organic halides**

The concentration of γ-(4-nitrobenzyl)pyridine recommended in the procedure was chosen because under the conditions of the experiment—i.e., a 45-minute reflux—blank determinations using this concentration of γ-(4-nitrobenzyl) pyridine gave transmittances of 95% or greater, and this concentration produced a maximum reaction with *n*-butyl bromide in 45 minutes. A linear absorbance *vs.* concentration of alkyl halide was obtained even if 2 ml. of water was added to the reaction mixture prior to refluxing. A slight increase in sensitivity was observed by addition of water, but was unnecessary, except in the case of the 2,2′,2″-trichlorotriethylamine and arsenic halides, where omission of water produced nonreproducible results (see Table VII). As alkalizing agents, potassium carbonate, piperidine, and sodium bicarbonate gave colored blanks, while pyridine failed to produce a color with the samples. Ammonia solution formed colorless blanks and highly colored solutions with the samples, but gave inconsistent results, probably due to the difficulty in reproduction of ammonia concentrations. In contrast, triethylamine, which is less volatile, gave intensities of color which were reproducible and stable for at least 5 minutes.

Figure 1 shows typical graphs of absorbances of solutions *vs.* concentrations of various halides. The sensitivities of the various halides in this procedure are given in the last column of Table IV.

From the data in Table IV it can be seen that in an homologous series the sensitivity of the test increases with increase in molecular weight, in a series of halides having the same halogen and number of carbon atoms, the sensitivity follows the order normal > iso and secondary > tertiary, and in a series of halides having the same alkyl groups, sensitivity follows the order iodide > bromide > chloride. *n*-Amyl fluoride, *n*-amyl cyanide, and bromobenzene in concentrations as high as $10^{-2}$, $10^{-3}$, and $10^{-3}$ mole, respectively, failed to react under the test conditions.

**Table V. Observed Absorbances from Mixtures of *n*-Amyl Bromide and *n*-Amyl Chloride Reacted with γ-(4-Nitrobenzyl)pyridine**

| Moles of *n*-Amyl Bromide Present | Moles of *n*-Amyl Chloride Present | Observed Absorbance | Absorbance[a] Increase Due to *n*-Amyl Chloride |
|---|---|---|---|
| $1.4 \times 10^{-6}$ | .... | 0.163 | ... |
| $1.4 \times 10^{-6}$ | $0.8 \times 10^{-5}$ | 0.164 | 0.001 |
| $1.4 \times 10^{-6}$ | $1.6 \times 10^{-5}$ | 0.192 | 0.029 |
| $1.4 \times 10^{-6}$ | $3.2 \times 10^{-5}$ | 0.211 | 0.048 |
| $1.4 \times 10^{-6}$ | $6.4 \times 10^{-5}$ | 0.232 | 0.069 |

[a] Figures should be directly proportional to concentration of *n*-amyl chloride. Errors inherent in analysis are believed to be responsible for deviations of observed from theoretical values.

The differences observed in M301 values of two halides having identical alkyl groups suggests the possibility of estimating the concentrations of one halide in the presence of another. As an example, the absorbances of mixtures of *n*-amyl bromide and *n*-amyl chloride, after reaction with γ-(4-nitrobenzyl)pyridine and subsequent color development, are shown in Table V.

Several experiments with mixtures showed that the calculated sum of the absorbances due to the individual components and the observed absorbance were within experimental error—i.e., the absorbances were additive. If in the analysis for *n*-amyl bromide an increase of 0.008 (5% of 0.163) in the absorbance is allowable, then between 0.8 and $1.6 \times 10^{-5}$ mole of *n*-amyl chloride can be present without seriously interfering with the accuracy of the analysis. Thus, it is possible to estimate the concentration of *n*-amyl bromide in the presence of at least as much as five times the concentration of *n*-amyl chloride.

The reactivities of several alpha-halogenated esters in this reaction may be compared by reference to Table VI.

**Table VI. Moles of Ester of α-Halogenated Acid Required to Produce a Colored Solution Having 0.301 Absorbance**

| Compound | M301 |
|---|---|
| Methyl fluoroacetate | $2.6 \times 10^{-4}$ |
| Ethyl chloroacetate | $1.9 \times 10^{-6}$ |
| Ethyl bromoacetate | $2.1 \times 10^{-7}$ |
| Ethyl trifluoroacetate | $1.0 \times 10^{-3}$ |

Although the number of compounds in the series tested is far too small to allow any general conclusions, it appears that the carboalkoxyl group activates the halogen-containing compound, as shown by a comparison of the M301 of methyl fluoroacetate (Table VI) with that of *n*-amyl fluoride (Table IV). It also appears that, just as was found in the alkyl halide series, the bromo compound has a lower M301 than the chloro compound in this reaction. The presence of more than one fluorine atom on the same carbon seemed to increase the M301 of the compound, as is roughly shown by a comparison in the M301 of the methyl fluoroacetate and ethyl trifluoroacetate.

In Table VII are given the M301 results of diethyl sulfate, benzoyl chloride, and organic compounds of nitrogen, phosphorus, arsenic, and silicon. The reaction with benzoyl chloride was almost instantaneous; a yellow solution, with an absorption maximum at 457 mμ, was formed without the addition of base. The 45-minute heating period did not increase the color. This reaction could be used for spot test detection as well as quantitative estimation.

With triethoxychlorosilane, diethoxydichlorosilane, trimethylchlorosilane, phenyltrichlorosilane, and methyltrichlorosilane, a plot of the concentration of the silicon compound against the absorbance of the solution was not linear and hence the procedure cannot be recommended for quantitative work. In the cases of diphenylchloroarsine, phenyldichloroarsine, dimethylchloroarsine, and methyldichloroarsine, the addition of a small quantity of water to the reaction mixture and use of a 20-minute heating period increased the sensitivity of the test.

Chloramine T was tested and gave no color, whereas 20% dichloramine T in glyceryl triacetate gave a color which did not come from the solvent. *p*-Dibromo- and *p*-diiodoarsinobenzenesulfonamide gave light colors, but were not studied because of their insolubility in methyl ethyl ketone. 2,6-Dichloroquinonechlorimide gave no color in the reaction other than a red color caused by its own reaction with base.

## NATURE AND EXTENT OF REACTION

Holzman (*7*) isolated and identified the intermediates of the reaction between 2,2′-dichlorodiethyl sulfide and γ-(4-nitrobenzyl)pyridine. He reported that the sequence of reactions are as given in the following equations:

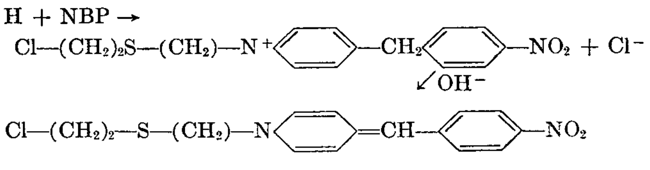


**Table VII. Moles of Nitrogen, Phosphorus, and Miscellaneous Compounds Required to Produce Colored Solution Having 0.301 Absorbance**

| Compound | M301 |
|---|---|
| Nitrogen compounds | |
| 2,2′,2″-Trichlorotriethylamine[a] | $1.3 \times 10^{-7}$ |
| *n*-Bromosuccinimide | $4.0 \times 10^{-7}$ |
| Phosphorus compounds | |
| Butoxydichlorophosphite (butylphosphorodichloridite)[b] | $8.0 \times 10^{-6}$ |
| Cyclohexanephosphodichloride (cyclohexylphosphonic dichloride)[b] | $1.5 \times 10^{-5}$ |
| Phenyldichlorophosphine (phenylphosphonous dichloride)[b] | $5.9 \times 10^{-6}$ |
| Arsenic compounds | |
| Lewisite [dichloro(2-chlorovinyl)arsine] | $3.0 \times 10^{-6}$ |
| Diphenylchloroarsine[c] | $6.3 \times 10^{-5}$ |
| Phenyldichloroarsine[c] | $2.3 \times 10^{-4}$ |
| Methyldichloroarsine[c] | $2.7 \times 10^{-5}$ |
| Dimethylchloroarsine[c] | $6.2 \times 10^{-5}$ |
| Silicon compounds[d] | |
| Triethoxychlorosilane | $7.4 \times 10^{-5}$ |
| Diethoxydichlorosilane | $8.5 \times 10^{-7}$ |
| Trimethylchlorosilane | $3.3 \times 10^{-4}$ |
| Phenyltrichlorosilane | $9.3 \times 10^{-4}$ |
| Methyltrichlorosilane | $1.5 \times 10^{-4}$ |
| Miscellaneous compounds | |
| Diethyl sulfate | $1.4 \times 10^{-7}$ |
| Benzoyl chloride[e] | $2.1 \times 10^{-7}$ |

[a] 2 ml. of water added to reaction mixture before heating period.

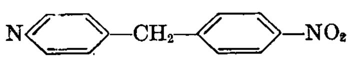
[b] A.C.S. nomenclature.

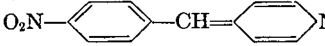
[c] 1 ml. of water was added to reaction mixture and 20-minute heating period was used.

[d] Approximate values only as plot of absorbance against concentration was not a straight line.

[e] Measured at 457 mμ.

where H = $Cl—(CH_2)_2—S—(CH_2)_2—Cl$

NBP = 

The colored materials formed in the reaction between butyl, heptyl, and decyl bromides with γ-(4-nitrobenzyl)pyridine were isolated by the present authors. Elemental analyses (by Analytical Branch, Chemical Corps Chemical and Radiological Laboratories, Army Chemical Center, Md.) of the dyes for carbon and hydrogen agree fairly well with those calculated for the structure  (R being butyl, heptyl, or decyl) (Table VIII).

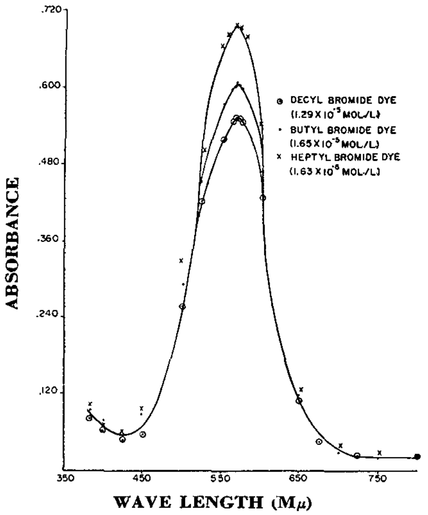


**Figure 2. Absorption spectrum of alkyl halide–γ-(4-nitrobenzyl)pyridine**

Dye dissolved in solution containing 6 ml. of methyl ethyl ketone, 5 ml. of acetone, 5 ml. of 5% NBP in acetone, and 1 ml. of 50% triethylamine in acetone

The molecular extinction coefficients were calculated from absorbance measurements of dye solutions in solvents of the same composition and ratio as those used in the experimental procedure for alkylating agents in nonaqueous medium. The spectral curves of the isolated dyes (Figure 2) show the wave length of maximum absorption to be 565 mμ. By visual inspection, all alkylating agents except benzoyl chloride gave the same color. The color produced by the reaction of benzoyl chloride with γ-(4-nitrobenzyl)pyridine has a maximum absorbance at 457 mμ. Under the reaction conditions described for the ethylenimines, the wave length of maximum absorbance is 600 mμ.

If it is assumed that the molecular extinction coefficients of the dyes are not dependent upon the alkyl adduct (Table VIII), then under the test conditions the theoretical M301 for any alkyl halide can be shown to be $1.3 \times 10^{-7}$. For the most reactive halides, the M301 was $1.5 \times 10^{-7}$. Therefore, for these halides the reaction was, for all practical purposes, complete. The less reactive compounds had M301's of $10^{-3}$ to $10^{-5}$. It is likely that the reaction of γ-(4-nitrobenzyl)pyridine with ethylenimines is also complete, inasmuch as similar calculations from absorbance-concentration data show that their M301's were approximately $1.5 \times 10^{-7}$.

The order of reactivity toward γ-(4-nitrobenzyl)pyridine observed in a homologous series of alkyl halides is opposite of that found by Noller and Dinsmore (*10*) in the study of the reaction of alkyl bromides with pyridine; Brown and Cahn (*2*) in the study of the reaction of alkyl iodides with 4-methyl pyridine; and Larsen and Kraus (*9*) in the study of the reaction of alkyl halides with tertiary amines. This discrepancy may be due to different temperature sensitivities of the reaction products with γ-(4-nitrobenzyl)pyridine. It is well known, for example, that prolonged heating tends to destroy the dye precursor, resulting in less than maximal color yields.

**Table VIII. Dyes Formed from Reaction of γ-(4-Nitrobenzyl)pyridine with Alkyl Bromides**

| Alkyl Group | Analysis %C Calcd. | Analysis %C Obsd. | Analysis %H Calcd. | Analysis %H Obsd. | Molecular Extinction Coefficient, Cm. Mole/Liter |
|---|---|---|---|---|---|
| Butyl | 71.0 | 69.2 | 6.7 | 6.3 | $2.82 \times 10^4$ |
| Heptyl | 73.3 | 69.7 | 7.4 | 7.15 | $3.30 \times 10^4$ |
| Decyl | 74.6 | 74.2 | 8.5 | 8.4 | $3.04 \times 10^4$ |

## GENERAL APPLICABILITY AND LIMITATIONS

The results of this work offer limited but interesting possibilities for the use of γ-(4-nitrobenzyl)pyridine. It is limited by the fact that a trace of an impurity of high reactivity in an alkyl halide of comparatively low activity would introduce large errors in the analysis of low activity halides. The method should be applied to estimation of relatively reactive alkyl halides that are pure and of known identity, or to estimation of reactive halides in mixtures containing unreactive halides. Its use to analyze for a relatively unreactive alkyl halide cannot be recommended unless it is definitely known that no relatively reactive alkyl halides are present as impurities. The analytical procedure may be used to follow the kinetics of a reaction of a particular alkyl halide.

For analysis of concentrations of ethylenimine compounds in air, the gas might be collected in bubblers containing solutions of 0.01*M* potassium acid phthalate. The γ-(4-nitrobenzyl)pyridine reagent is then added directly to 4 ml. of the collected solution, followed by heat, cooling, etc. The ethylenimine compounds hydrolyze slowly in buffered media, but the extent of hydrolysis is such that changes are not appreciable for at least 24 hours.

## ACKNOWLEDGMENT

The technical assistance of Mary M. Demek, William Spall, Jack Florence, and William Duff is acknowledged.

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# Automatic Instrumental Methods for Determination of Critical Solution Temperatures

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**Several instrumental means for the determination of critical solution temperatures using photoelectric detection and control are described. Heater control is effected either through a Schmitt trigger circuit or with limit switches mounted on standard recorders. Temperature is recorded on standard recording potentiometers. Precision of measurement to ±0.01° C. is obtainable although limitations in volume measurement and reagent purity may easily limit useful results to about ±0.05° C.**

APPARENTLY, Brown (*2*) was the first to use photoelectric methods for the automatic determination of critical solution temperatures—specifically, the aniline point of petroleum products. In his application a simple photoelectric relay was used to control the heating or cooling of the test sample and cause it to attain temperature equilibrium automatically. The present work was undertaken for two reasons. First, the authors believe that Brown's technique can find analytically useful extensions to many other chemical systems and, second, so many commercially recording techniques and instruments are now available that several new and original approaches are possible. This brief report affords the analyst several choices for the automatic recording of critical solution temperatures and for analytical information which such systems can provide. Each method reveals its own advantages as well as limitations and can best be adapted to the particular instrument which is at hand.

When a binary liquid–liquid system which exhibits the critical solution phenomenon is heated to a certain critical temperature, it passes from a state of miscibility to immiscibility, or vice versa, depending upon the particular system under study. This critical temperature depends not only on the components of the system, but also on their relative concentrations. Within certain concentration limits, depending on the system in question, the determination of the critical solution temperature constitutes a sensitive quantitative analysis of the system. Conversely, when one component and the concentrations are given, a qualitative analysis may be made for the other component. This analysis is the basis for the ASTM (*1*) aniline point determination. Both methods require a previous calibration in which components and concentrations are known, but the literature contains many examples and even empirical equations for some series of compounds.

If the system is stirred while in its immiscible state, a turbid mixture results; when the miscible state is reached, this turbidity disappears.

Simple photoelectric methods can be used to detect this transition and to initiate a variety of control functions.

## EXPERIMENTAL

**Glass Sample Container.** A cylindrical shape was selected in order to use ordinary standard-taper glassware. The dimensions were adjusted so that a vessel using a 55/50 standard-taper top had a total volume of about 300 ml., and a 200-ml. sample was used each time. The upper portion of the vessel was fitted with a reflux condenser, two wells for heating coil outlets, and a hole for inserting a thermocouple. Magnetic stirring was used.

**Heating System.** The heating system was divided into two parts: a main heater consisting of a 200-watt glass-insulated tape wrapped around the outside of the glass container, and a 10-watt control heater which was a coil of Nichrome wire immersed in the liquid. Both heaters were energized initially to bring the system up to the critical temperature rapidly. When the system reached the critical temperature for the first time, the main heater was permanently removed from the heating circuit by a lock-in relay.

**Temperature Determination.** A copper-constantan thermocouple with reference junction at 0° C. and a Type B Rubicon potentiometer were used for most of the temperature determinations.

**Control and Measurement.** The transmission of light through the liquid system was measured with a Type 921 phototube with a projection type lamp, 75 watts at 110 volts, as a light source. This lamp, however, was operated at only 40 volts to provide a phototube signal of desired magnitude. The phototube was shielded from stray light with a metal box which had a window and collimating tube facing the liquid mixture.

Several methods were used for the control and measurement of the critical solution temperatures.

METHOD I. The phototube signal controlled a Schmitt trigger (*6*) circuit, which in turn controlled the heater. The mixture was heated to its critical temperature, around which it then hovered, alternately heating and cooling with a variation in temperature of about ±0.05° C. The average was taken as the critical solution temperature. The temperature was measured either with a mercury thermometer or various combinations of thermocouple, Rubicon potentiometer, galvanometer, amplifier, and recorder. The potentiometer provided a wider range and higher sensitivity than obtainable with direct recording of

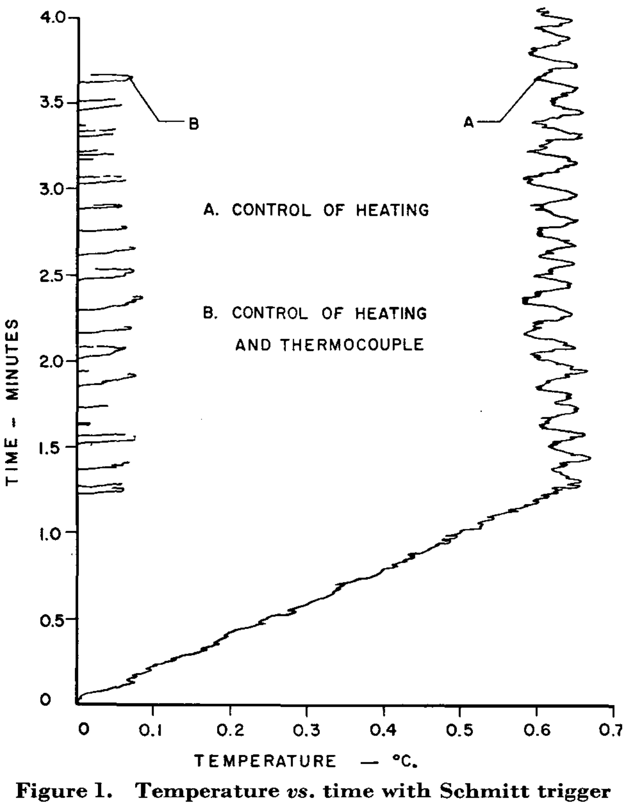

**Figure 1. Temperature *vs.* time with Schmitt trigger**

*A.* Control of heating
*B.* Control of heating and thermocouple

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thermocouple electromotive force on standard strip chart recorders.

1. The first method of recording temperature involved the use of a Beckman Photopen recorder. This recorder follows the beam of light reflected from the potentiometer galvanometer and traces a record of the deflections on standard recorder paper. Sufficient amplification from the length of the light beam was obtained so that there was about 1.3 microvolts per division on the chart corresponding to about 0.03° per division, and the temperature could be read to about ±0.006° C. The temperature is read as a potentiometer setting plus a value read from the chart paper.

2. The second variation in measuring the temperature was to feed the unbalance voltage from the galvanometer binding posts of the Rubicon potentiometer to a Leeds & Northrup direct current microvolt amplifier. This amplified unbalance voltage then was recorded on a 2.5-mv. full-scale Brown recorder. Temperatures could be obtained from the potentiometer setting plus chart reading again to about ±0.006° C. Figure 1, *A*, is an example of the record obtained. Again, the temperature scale is to be added to the base temperature obtained from the potentiometer setting.

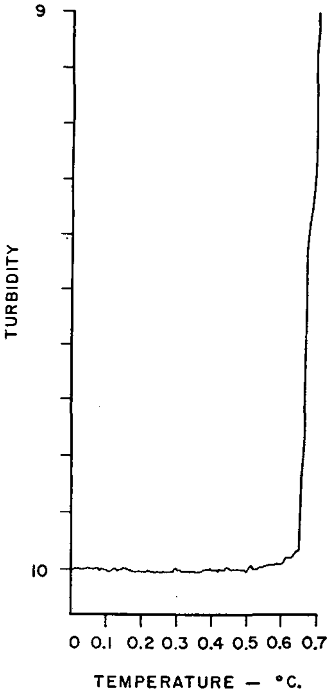

**Figure 2. Temperature *vs.* turbidity in arbitrary units**

3. A third variation to improve the readability of the temperature from the chart was to connect another relay to the Schmitt trigger so that when it turned off the heater, it also shorted the thermocouple leads, and the recorder pen moved to zero. When the heater was turned on again, the recorder pen returned to a position corresponding to the critical temperature. Thus a series of cusps was obtained from which the critical temperature could be read with no necessity for calculating or estimating the average value of a saw-tooth line. Figure 1, *B*, is an example of this method.

METHOD II. A disadvantage of using a Schmitt trigger circuit for control in a turbidity measurement is that the circuit can be set to trigger at practically any degree of turbidity. Thus the critical temperature obtained is varied somewhat by the rather arbitrary determination of triggering level. A calibration could be made with known mixtures, but this calibration would be affected if later determinations were made with dark-colored substances. A more fundamental approach is indicated by an investigation of the actual changes in turbidity near the critical temperature.

1. A Speedomax X-Y recorder, 10-mv. full scale, was available for this portion of the investigation. The thermocouple electromotive force was recorded on the $X$ axis with the aid of the Rubicon potentiometer and the Leeds & Northrup direct current amplifier. The phototube signal was passed through a simple cathode follower to the $Y$ axis of the recorder. As shown in Figure 2, a very sudden change occurs in the turbidity value at a certain temperature, after which the turbidity changes more gradually until a clear solution is reached, as shown in Figure 3 recorded at lower sensitivity. The slowness of the total turbidity change is probably due to lack of thermal equilibrium, and the temperature at the sharp break should be taken as the critical solution temperature. This sharp break in the turbidity curve occurs before any change is apparent to the eye.

2. While the X-Y recorder affords a precise method of critical temperature determination, manual control of heating is required, and the recorder chart must be moved for every heating and cooling cycle to avoid overlapping the curves. This latter disadvantage can be avoided by the use of a multipoint recorder to record both temperature and turbidity against time. A Speedomax 10-mv. 10-point recorder with odd points wired to record turbidity and the even ones temperature was used to produce Figure 4. The critical temperature is found by observing the time at which the sudden change in turbidity took place, and reading the temperature at this time from the same chart. A continuous record of the same type may be made with a two-pen recorder. Figure 5 is a record obtained with a Brown two-pen recorder, 10-mv. full scale. The pens are offset on the time scale so one may pass under the other. The turbidity curve was

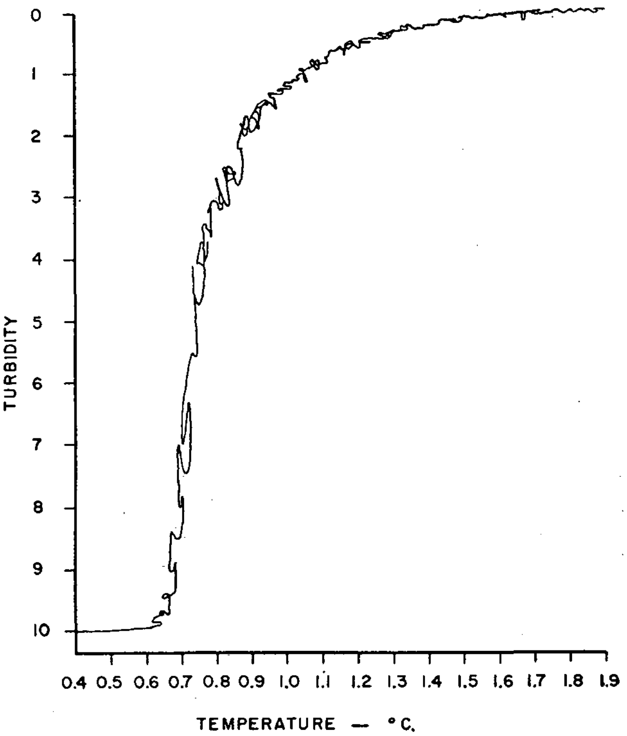

**Figure 3. Temperature *vs.* turbidity in same arbitrary units**

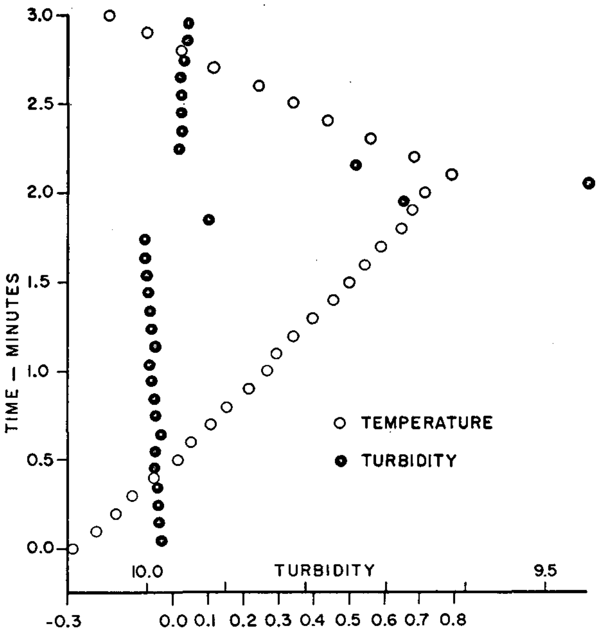

**Figure 4. Temperature and turbidity *vs.* time with manual control of heating, using 10-point recorder**

arbitrarily cut off at point *A* because the important part of the curve is the initial sharp change, and the time of return depends merely on the manual control of the heating current.

3. The next step was to use the sharp change in turbidity to control the heater. For this purpose, the two-pen recorder was fitted with a standard Brown mercury switch controller on one pen. This mercury switch activated a high-current mercury plunger-type relay which controlled the heater. When the output of the cathode follower, a measure of turbidity, was connected to the controlling pen, and the temperature recorded with the other pen, the record reproduced in Figure 6 was obtained. The control point was set slightly after the sharp break in the turbidity curve to ensure positive control of the heater. Slight drifts in the turbidity record are apparent, but they are so small compared to the total turbidity change (many times full scale) that they have negligible effect on the control point. The temperature can be read as an average of the extreme values, or the time at the sudden change in turbidity may be observed, and the temperature at this time determined on the same chart, allowing for the time offset of the pens. This latter method affords the most precise of all those mentioned for the determination of critical solution temperatures. The successive values agree within ±0.01°, and even the total excursions are only ±0.025°.

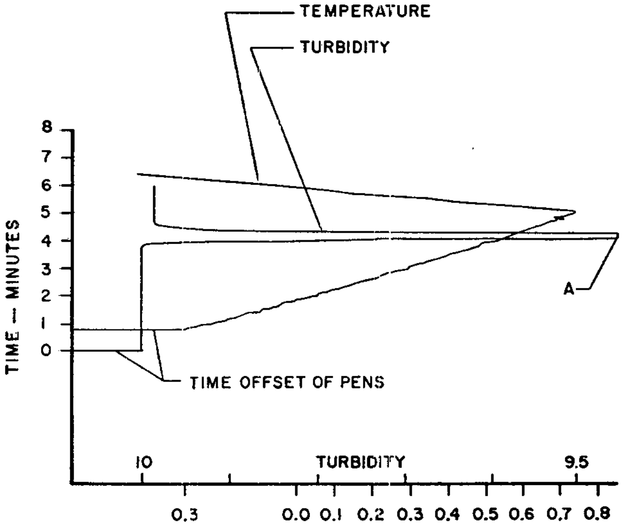

**Figure 5. Temperature and turbidity *vs.* time with manual control of heating**

**Table I. Summary of Results for Hexane-Aniline System**

| % Hexane | Experiment Result, ° C. at 590 Mm. Hg | Literature Value, ° C. (4) at 760 Mm. Hg |
|---|---|---|
| 10.40 | 38.70 | 30.0 |
| 10.40 | 38.70 | 30.0 |
| 10.40 | 38.68 | 30.0 |
| 13.20 | 44.88 | 40.0 |
| 13.20 | 44.88 | 40.0 |
| 19.40 | 56.92 | 50.0 |
| 19.40 | 56.98 | 50.0 |

## RESULTS

Apparently very few studies have been made of critical solution temperatures at pressures below atmospheric. The effect of low atmospheric pressure (about 585 mm. of mercury at Los Alamos) immediately became apparent with the investigation of the aniline-hexane system, results for which are shown in Table I. The system of *n*-butyl alcohol–water was found to be not completely miscible at any temperature below atmospheric pressure, and so, no longer exhibits the critical solution temperature phenomenon. The *sec*-butyl alcohol–water system was known (*3*) to behave in a similar manner. However, at the aniline point of heptane (equal volumes of aniline and heptane) the pressure effect is evidently less, since a critical temperature of 67.98° C. was obtained, compared with literature values of 68° (*7*), 70.6° (*5*), 68.9° (*2*), and 69.2° (*1*).

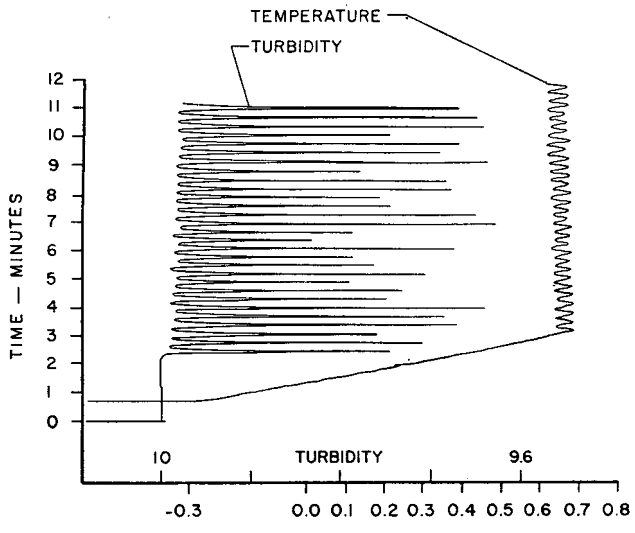

**Figure 6. Temperature and turbidity *vs.* time with recorder control of heating**

As is shown in Table I, measurements of critical solution temperatures may be repeated with new mixtures with variations usually less than ±0.06°. A practical limit probably has been reached in the measurement of those temperatures in that errors arising from the volume measurements and impurities in the reagents are apt to be greater than the observed precision in the actual temperature measurements.

A mercury thermometer may give misleading results as to the degree of control owing to its large lag. In one instance where a thermometer was checked against the thermocouple, the thermocouple showed a variation of ±0.05° C. with Schmitt trigger control while a thermometer with 0.1° C. divisions showed a variation of less than 0.01° C.—i.e., no movement of the mercury column. However, where high precision is not desired, a thermometer might be a simpler method of obtaining an average value for the temperature at the control point.

Another point to be observed is that there is some preferential distillation of the more volatile component, even with the reflux condenser, and the mixture constantly changes in composition. This effect was observed both with the aniline-hexane system, where the critical solution temperature decreased with time, and with water-triethylamine at low triethylamine concentrations where the critical temperature increased with time. Therefore, to obtain the true critical temperature for the initial mixture, the temperature reading must be taken as early as possible. If the time when temperature control begins is taken as zero time, the temperature may be read at this time, or a series of temperature readings may be plotted against time and extrapolated back to a temperature at zero time. Either method yields substantially the same result. Because of this distillation effect, overshooting the critical temperature is to be avoided the first time it is approached. A sufficiently low heating rate may be used to avoid this overshooting, or if the approximate critical temperature is known, additional switches on the recorder may be used to turn off the main heater 5° to 10° C. before critical temperature is reached, and additional heating done only with the small control heater. In any case, the distillation effect is small, amounting to only about 0.5° per hour in this case for the aniline-hexane system.

ACKNOWLEDGMENT

The authors wish to express their indebtedness to D. M. Olson for mechanical assistance and for the preparation of the drawings.

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# Sampling for In-Line Instrumentation

## A Degasser for Obtaining Air-Free Samples

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**The degassing device described was designed for use on sample streams containing up to 98% air, as obtained with an 8-cubic feet per minute air jet and air lift, and delivers a sample stream of at least 99.95% liquid content, comprising 50 to 80% of the liquid content of the input stream. For the optimum orifice size, which yields this wide-range operation, a minimum liquid input flow of 0.05 gallon per minute (~200 ml. per minute) is required for positive sample flow (at 8- to 12-inch lift above the degasser input) when an air flow of less than 0.5 gallon per minute is involved. At air flow rates of 0.5 to 1 gallon per minute, the degasser functions on as little as 0.015-gallon-per-minute liquid input. For 0.05- to 1-gallon-per-minute liquid content in the input stream, air contents up to at least 2 gallons per minute can be tolerated. Higher flow rates were not obtainable under test conditions.**

IN OBTAINING samples from process lines and vessels, particularly where corrosive or dangerous conditions are involved, one common engineering practice is to employ jet aspirators to lift the sample stream to the sampling position. In many instances, introduction of air or an inert gas into the input riser is necessary to ensure adequate lift; and frequently air is admitted inadvertently through leaks into the below-atmospheric-pressure system. In such cases, the gas content of the sample stream may amount to 90% or more of the total flow.

In many instrumental applications it is necessary or desirable that the measured sample be air-free, or that the sample cup be completely filled at all times. To this end, the degasser described herein was developed. This device was designed to operate in the flow range obtainable with an air jet of 8 cubic feet per minute (capacity, 3 gallons per minute). The data given here are for water inputs only, but similar operation has been obtained for both organic and high-concentration aqueous streams. Special designs and modifications for specific unusual applications are discussed.

### PRINCIPLE OF OPERATION

Degassing of an air-liquid mixture is effected by the disengagement of phases in the degasser chamber through gravity separation during low velocity flow of the liquid phase (Figure 1). The liquid thus passes out from the bottom of the chamber and through the sample cell, while the air is bled off through the orifice, the two paths converging into the jet stream. The orifice diameter must be large enough that the pressure drop for air flow alone is not sufficient to depress the air-liquid interface to the liquid output port, yet small enough that when a small amount of liquid is included in the air stream, the pressure difference is sufficient to ensure flow through the sample leg. With the proper orifice, the liquid level maintains itself just below the orifice position over a large range of air and liquid flow rates. A purpose of this study was to determine the orifice dimensions necessary to attain this condition and still cause an adequate fraction of the liquid phase to pass through the sample cell.

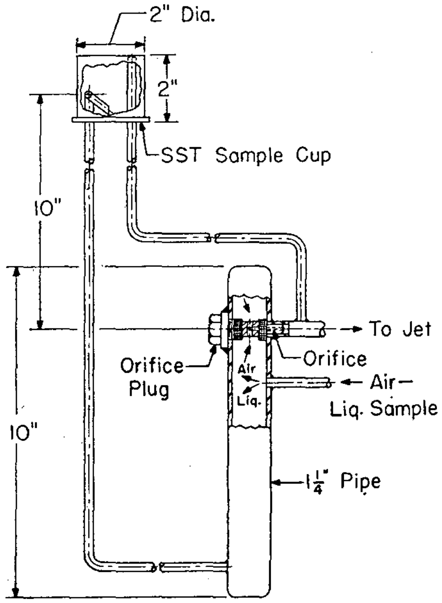

**Figure 1. Degasser, basic design**

### EXPERIMENTAL

A full-scale model was constructed of Lucite to permit visual observation of the phase conditions within the degasser during operation. Interchangeable orifice plugs were made up having orifice dimensions of $^{15}/_{16}$ inch long by $^{1}/_{64}$, $^{1}/_{32}$, $^{1}/_{16}$, and $^{3}/_{32}$ inch in diameters—sizes calculated to bracket the usable range. Similarly, a suitable sample cell was constructed of Lucite, and these components were connected by means of Tygon tubing of $^1/_8$-inch inside diameter and 24 inches long. The sample cell was located about 10 inches above the degasser input level. A laboratory aspirator was used to simulate the sampler jet. The air and water inputs were separately controlled and were separately measured by means of rotameters. In this manner any desired sampling condition could be simulated, within the flow limitations of the test equipment. Because of the lesser capacity of the laboratory aspirator as compared to a 3-gallons-per-minute air jet, positive pressures were applied to the air and/or water streams whenever necessary, in order to obtain higher flow rates.

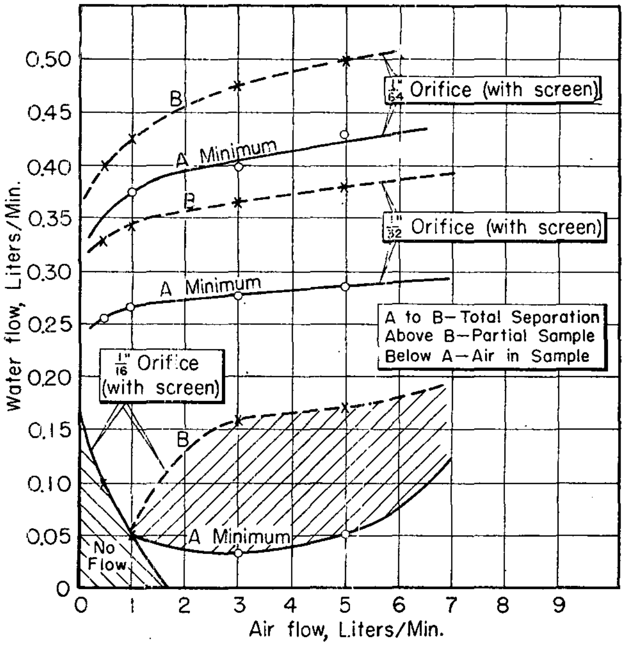

**Figure 2. Phase separation conditions**

The initial test was to determine, for the different orifices, the minimum water input rates necessary to maintain all-liquid flow through the sample cell, for various rates of air input (Figure 2, *A*). Also determined were the maximum water input rates which could be handled with complete separation of both phases—i.e., the rates at which the water level in the degasser rose to just below the orifice level (Figure 2, *B*). These two limits were determined both with and without a 30-mesh screen protecting the orifice, and the effect of the screen was found to be slight. At higher liquid input rates (above Figure 2, *B*), a fraction of the liquid passes with the air through the bypass orifice, and this constitutes the condition during normal operation.

For the orifice design used, an orifice $^1/_{16}$ inch in diameter yields optimum performance. Smaller orifice diameter requires greater liquid input to ensure an all-liquid sample (see Figure 2), while at greater than $^1/_{16}$-inch diameter, greater liquid input is required to ensure flow through the sample cell.

A further series of measurements was made to determine the fraction of total liquid flowing through the sample cell as a function of liquid and air flow rates for the different orifice sizes. The sample fraction was found to be substantially independent of liquid flow rate and to vary only moderately with air flow variation. The per cent of sample, average of four water input rates, and expressed as a function of air-flow rate and orifice diameter, is shown in Figure 3. These data were obtained at 0, 1, and 3 liters per minute of air input flow. Additional data were obtained at air flows of 2 liters per minute and 5 liters per minute for the $^1/_{16}$-inch orifice (Figure 4) and for each liquid input flow rate the sample flow obtained at about 1 liter per minute of air flow is unexpectedly high, as compared with other air-flow rates. This is beieved to be a result of a resonance, or "whistle" effect, at the oriice at this rate. This effect, while not particularly significant, is favorable to rapid sample change in the normal operating range of a 3-gallon-per-minute air jet installation. The rate of sample change for a typical flow condition, 3 liters per minute of air and 0.3 liters per minute of water, is satisfactory. When a dye was injected into the input stream, observations indicated that a complete sample change is effected in about 1.5 minutes.

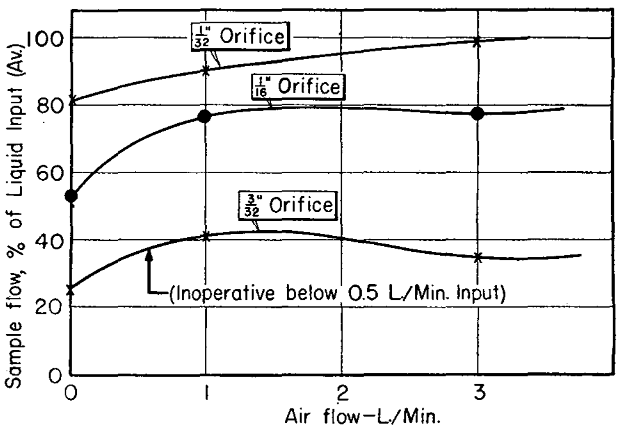

**Figure 3. Sample yield *vs.* orifice size**

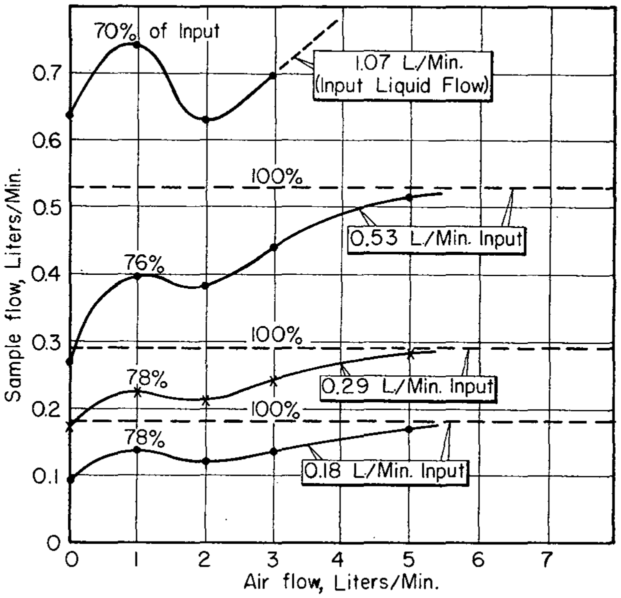

**Figure 4. Sample flow for $^1/_{16}$-inch orifice**

**Design Modifications.** Some slight modifications to the original design are believed desirable. Tests were made on the degasser with an input stream containing both flocculent and heavy particles. Paper pulp fibers and sand were introduced into the liquid feed stream. The light fibers tended to plug the unprotected orifice, while turbulence in the chamber at high flow rates swept some sand into the sample cell. Protecting the orifice with a screen sleeve was found to prevent difficulty at this point, and a design is given for a modified orifice plug with such a screen (Figure 5, upper). Replacement of the screen is easily performed if plugging should occur. Almost any type of baffle was found to be sufficient to reduce turbulence in the lower chamber to the point where heavy particles were not swept into the outlet; but at very high air-to-liquid ratios, especially at high flow rates, the impact of water droplets against the chamber wall opposite the input had been observed to produce a fine dispersion of air in the liquid phase flowing downward in the degasser. Convection currents produced by these impacts caused this dispersed air to circu-

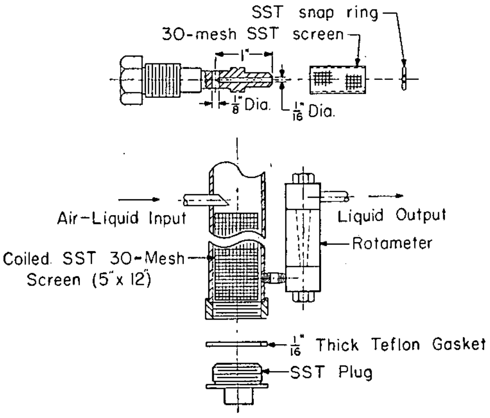

**Figure 5. Screened orifice plug (upper) and baffle screen and rotameter (lower)**

late downward to the output port. Some of these bubbles would be swept into the sample cell and there would collect and coalesce into bubbles of appreciable size before being swept out of the sample cell. After baffles were installed in the chamber, this effect was almost entirely eliminated, increasing the sample from ~99.5% to ~99.95% liquid. A coil of wire screen, made by rolling a 5 by 12 inch, 30-mesh stainless steel screen on the shorter axis, was found to produce a simple and effective baffle. It offered the further advantage of a strainer on the sample outlet. In order to insert or replace the baffle screen, a screw plug is specified at the bottom of the degasser chamber (Figure 5, lower). The input pipe is extended into the chamber to act as a stop for positioning the screen, and is terminated with a diagonal cut to direct turbulent flow away from the sample outlet port.

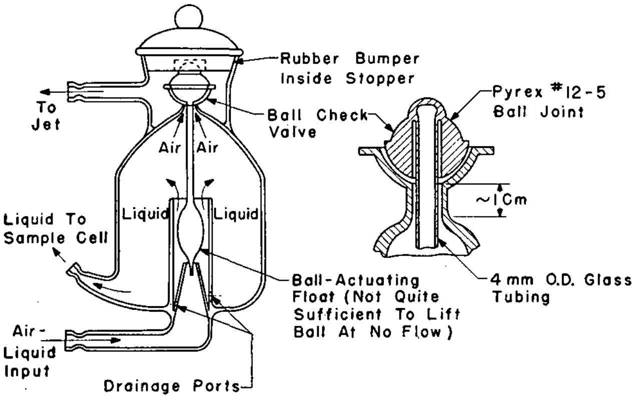

**Figure 6. Check-valve degasser**

Since the operation of sampler jets is often uncertain, and sample flow cannot be assumed on the basis of apparently satisfactory jet operation, and since knowledge of sample flow often is essential to the interpretation of instrumental data, some indication of this flow may be required. For the author's purpose, a rotameter was installed at the degasser liquid output, mounted directly on the degasser body. A tube and float combination having a sensitive range of 0.02 to 0.38 gallon per minute, or approximately 0.075 to 1.4 liters per minute, was used.

## SPECIAL DEGASSER DESIGNS

In some installations it may be desirable to obtain an empty cell reading by turning off the jet air and allowing the sample to siphon from the cell. The inclusion of a normal degasser in this system would provide an air bypass, and thus might prevent the sample cell from being siphoned empty. A special borosilicate glass degasser containing a check valve was constructed for such an installation. To ensure opening of the valve with minimum impedance to flow and yet to assure a positive checking action, an activating piston and float was imposed on the degasser input stream. The annular orifice surrounding the connecting arm was proportioned to give the desired pressure difference. The design of this degasser is shown in Figure 6. Although it does not possess the wide range of the basic degasser, inputs of over 95% air were found to be successfully degassed by this device.

In other installations, such as for colorimetric devices, in which the presence of even small amounts of air cannot be tolerated, two-stage degassing may be desirable. A simple second-stage degasser was designed to operate on a high-liquid input and to yield excellent separation of any residual dispersed air. This cell was placed in parallel with the basic degasser, in place of the sample cell, and the sample cell was then connected to the output from the second stage (Figure 7). No gas formation on the sample cell walls was observed when this system was used on a constant-temperature liquid stream. No study was made of the effects of changing temperature upon dissolved air, but increasing the temperature of an air-saturated stream might be expected to result in bubble formation in the sample cell. The mixing obtained in the two degasser stages results in an averaging effect to transient sample variations, equivalent to about a 3-minute time constant at input flow rates of 1 to 3 liters per minute of air and 0.5 to 1.5 liters per minute of liquid.

The present design was intended for applications where a holdup of 200 ml. in the degasser will not present a problem. For installations where the holdup volume must be reduced, a similar design of smaller dimensions may be used, with corresponding loss in flow capacity. The degasser cross section should be chosen to give similar linear liquid flow rates, and the degasser height may be reduced for lower air-to-liquid ratios.

While no one degasser can be expected to yield optimum phase separation under all conditions, an extremely wide range of flow conditions can be adequately handled by a single design as that described. Any special conditions for which this degasser might not adequately perform can be met by simple modifications of the basic design.

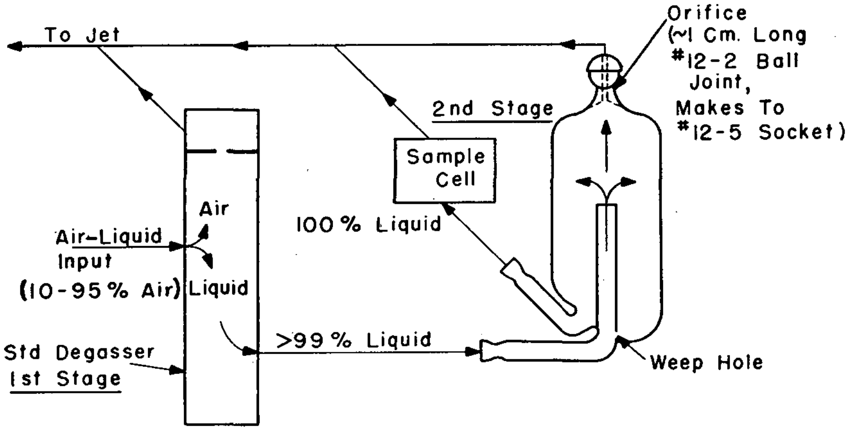

**Figure 7. Two-stage degasser**

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# High Temperature Recording Polarimeter

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**A recording photoelectric polarimeter has been developed to meet the need for a means of continuously monitoring the optical rotation of a plant stream of molten resin at 180° C. The instrument contains an arrangement of two photovoltaic cells in a balanced electrical network. A change in the optical rotation of the sample stream creates an unbalance signal in the network which, after amplification, actuates the balance motor of a recorder. This motor simultaneously moves the recorder pen and rotates a polarizer in the instrument until balance is restored. The polarimeter has been in service more than 2 years. It is rugged and stable, and has an over-all accuracy within about 1%.**

IN CHEMICAL processes involving optically active materials, the optical rotation of the product is often used as a primary measurement for quality control. However, the usual periodic optical rotation measurements by a control laboratory may introduce serious time lags between the recognition and correction of unusual operating conditions. An instrument which would continuously indicate and record the optical rotation of the product would obviously provide a more reliable guide for close control of the process.

Heller gives an excellent survey of the field of polarimetry (*4*). Other polarimeters have been described (*2*, *3*, *6*, *7*); however, none of these could be easily adapted to meet the requirement of reliable continuous plant service at elevated temperatures. Hence, a recording photoelectric polarimeter suitable for plant use was developed in this laboratory. It has been in operation on a plant stream of molten resin for more than 2 years.

## PRINCIPLE OF OPERATION

The instrument is similar in operation to an ordinary laboratory polarimeter, except that an electromechanical feedback loop replaces the human operator. A schematic diagram of the polarimeter is shown in Figure 1.

Light from a tungsten lamp is collimated by a lens combination, monochromatized by a multilayer interference filter, and plane-polarized by a rotatable Polaroid polarizer. The beam then passes through the sample cell and is split by a half-aluminized mirror. Each of the resulting two beams passes through a Polaroid disk used as an analyzer and finally impinges on a photovoltaic cell. The polarizing axes of the two analyzers are 90° apart.

The beam reflected from the mirror is actually elliptically polarized. However, by mounting the mirror so that the angle of incidence is small, the beam may be considered as plane-polarized for all practical purposes.

The photocells are connected in a current-balancing network. The condition of balance is attained when the plane of polarization of the light beam from the sample cell is in a unique position, approximately midway between the axes of the analyzers. At balance, there is no output from the photocell network. If the intensities of the two beams were equal, and if the two photocells were matched, the angle between the plane of polarization of the light beam from the sample cell and the axis of each analyzer would be 45° at balance.

Rotation of the plane of polarization of the light beam from the balance position, caused by a change in optical rotation of the liquid flowing through the sample cell, results in an off-balance signal in the photocell circuit which is fed into the recorder amplifier. The output from the amplifier actuates a balance motor which simultaneously moves the recorder pen and rotates the balancing polarizer until balance is restored.

At balance, the rotation of the balancing polarizer from its zero position is always equal and opposite to the optical rotation of the sample.

## THEORETICAL CONSIDERATIONS

From the law of Malus:

$$I = I_0 \cos^2 \theta \tag{1}$$

where $I_0$ = intensity of the plane-polarized beam entering the analyzer, $I$ = intensity of the beam leaving the analyzer, and $\theta$ = angle between the plane of polarization of the beam and the polarizing axis of the analyzer.

Then
$$\frac{dI}{d\theta} = -2I_0 \cos \theta \sin \theta \tag{2}$$

The angle at which $\frac{dI}{d\theta}$ is a maximum is found by equating $\frac{d^2I}{d\theta^2} = -2I_0 (\cos^2\theta - \sin^2\theta)$ to zero and solving for $\theta$. It is thus seen that with the polarizing axis of each analyzer at 45° to the plane of polarization of the light beam, the response of the detecting circuit to a small change in rotation of the plane of polarization of the light beam is a maximum.

The photocells are connected in the current-balancing circuit shown in Figure 2. By considering the characteristics of this circuit (*8*) and with the aid of the law of Malus and Lambert's law of absorption, an optimum cell length may be derived for a sample having a given absorption coefficient.

For simplicity, assume that the intensities of the beams striking

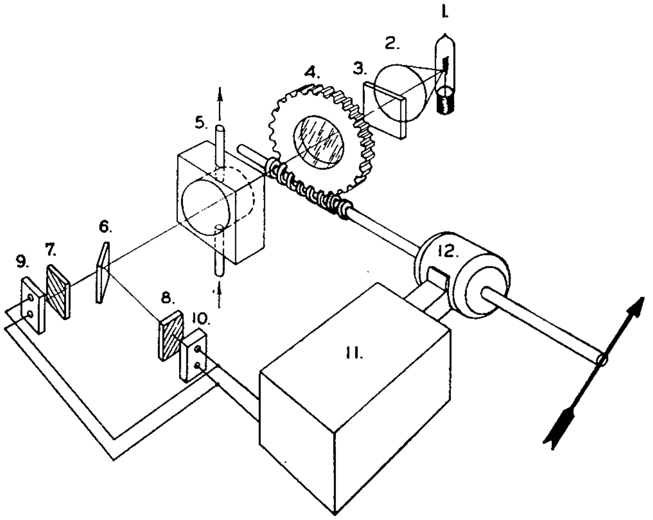

**Figure 1. Schematic diagram of recording polarimeter**

1. **Lamp**
2. **Lens**
3. **Filter**
4. **Polarizer and worm gear**
5. **Sample cell**
6. **Half-aluminized mirror**

**7, 8. Analyzer**
**9, 10. Photocells**
**11. Amplifier**
**12. Balance motor**

the two photocells are equal at balance, and that the current-illumination characteristics of the two photocells are equal. For a small change ($\Delta\alpha$) in the specific rotation of the sample:

$$i = c(I_1 - I_2) \tag{3}$$

where

$i$ = off-balance current
$c$ = constant
$I_1$ = intensity of light beam striking photocell 1
$I_2$ = intensity of light beam striking photocell 2

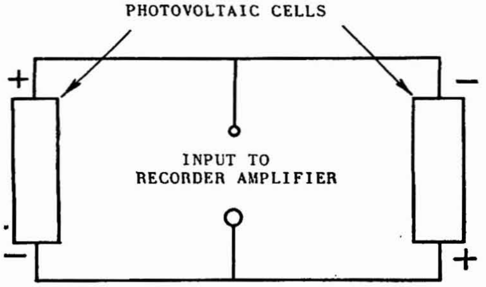

Figure 2. Photocell current-balancing circuit

From the law of Malus:

$$I_1 = 0.5\, I_3 \cos^2 (45° - \Delta\alpha L) \tag{4}$$

$$I_2 = 0.5\, I_3 \cos^2 (45° + \Delta\alpha L) \tag{5}$$

where $I_3$ = intensity of light leaving sample cell, $\alpha$ = specific rotation of sample, and $L$ = length of sample cell.

From Lambert's law:

$$I_3 = I_0 e^{-kL} \tag{6}$$

where $I_0$ = intensity of light entering sample cell, and $k$ = absorption coefficient of the sample at 589 m$\mu$.

Combining Equations 3, 4, 5, and 6

$$i = 0.5\, cI_0e^{-kL}[\cos^2 (45° - \Delta\alpha L) - \cos^2 (45° + \Delta\alpha L)] \tag{7}$$

Simplifying

$$i = 0.5\, cI_0e^{-kL} \sin (2\Delta\alpha L) \tag{8}$$

Since $\Delta\alpha L$ is very small, $\therefore \sin 2\Delta\alpha L \cong 2\Delta\alpha L$

Therefore:

$$\frac{i}{\Delta\alpha} = LKe^{-kL} \tag{9}$$

where $K = cI_0$

The value of $L$ to make $\frac{i}{\Delta\alpha}$ a maximum is desired; therefore:

$$\frac{d}{dL}\left(\frac{i}{\Delta\alpha}\right) = Ke^{-kL}(1 - kL) = 0 \tag{10}$$

Solving for $L$:

$$L = \frac{1}{k} \tag{11}$$

From measurements of the spectral transmittance of the resin at 589 m$\mu$, the absorption coefficient $k$ was found to be 0.67 per inch. Therefore, the optimum value of $L$ becomes 1.5 inches.

### OPTICAL UNIT

The optics of the recording polarimeter are entirely enclosed under a positive pressure of dry, clean air in a housing which is mounted on the back of a Minneapolis-Honeywell circular chart recorder as shown in Figure 3.

**Light Source.** The light source is a General Electric 200-watt, 120-volt projection lamp with a Type 2CC8 filament and medium prefocused base. The lamp housing (*1*) is both water-cooled and air-ventilated.

**Lenses.** Light from the lamp is collimated by two achromatic lenses, 54 mm. in diameter and 140 mm. in focal length.

**Filter.** A 2 × 2 inch Baird multilayer interference filter having a transmittance of 73% at the peak wave length of 589 m$\mu$ and a half-band width of only 5 m$\mu$ monochromatizes the light beam.

**Polarizers.** Type H, high-transmittance Polaroid polarizers are used in this instrument.

The rotatable balancing polarizer is a 2.5-inch-diameter disk mounted in a housing which is press-fitted into the inner race of a New Departure No. N5L16-A double-row angular contact ball bearing.

The two analyzers are 1.75 × $^7/_8$ inch rectangles with polarizing axes 45° to the edges. One is mounted in front of each photocell so that the polarizing axes are 90° apart.

**Sample Cell.** The sample cell is designed to handle molten resin at 180° C.—the average temperature of the resin in the plant stream. The stainless steel body of the cell is channelled for the flow of the high pressure steam used to heat the cell to this temperature. The sample flows through a cylindrical cavity, 2.5 inches in diameter and 1.5 inches long, in the cell body. The cell compartment is sealed with 2.75-inch optically polished borosilicate glass windows pressed against Teflon gaskets.

To prevent excessive heat transfer from the sample cell to other parts of the polarimeter, the cell is encased in a housing with the space between the cell and the housing filled with expanded vermiculite. In addition, the cell is fitted with double windows, separated by a dead air space, and the cell assembly is mounted within a compartment, the walls of which are covered by a layer of magnesia.

**Beam Splitter.** The beam splitter is a 2 × 2 inch, half-aluminized mirror which reflects approximately 40% of the incident light to one analyzer and transmits 40% to the other. A piece of optical glass is cemented over the aluminized surface in order to protect it from corrosion and mechanical injury.

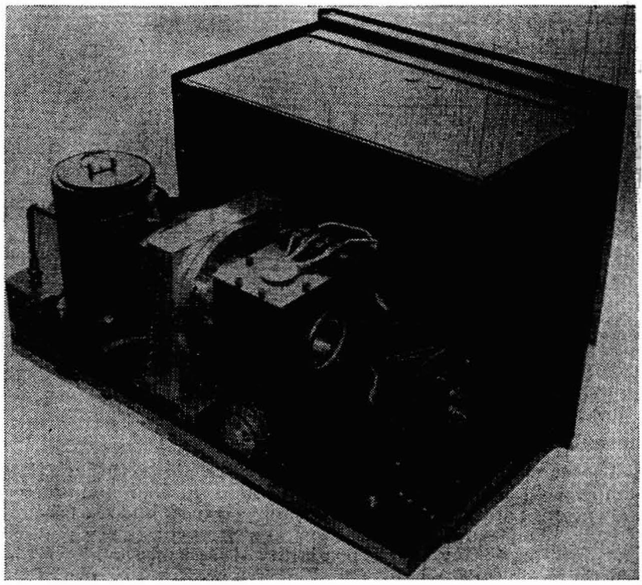

Figure 3. Recording polarimeter

**Photocells.** Two matched, General Electric Type 8PV1AAB hermetically sealed photovoltaic cells are used as detectors. They are mounted in Dural holders which are wrapped with copper tubing through which cooling water flows. Since photovoltaic cells must not be operated continuously above 50° C., cooling is necessary.

### ELECTRICAL, BALANCING, AND RECORDING SYSTEMS

**Power Supply.** A Sola, 115-volt, 250-watt, constant voltage transformer regulates the power for the projection lamp. A parallel combination of a resistor and an indicator lamp in series with the projection lamp drops the voltage across it to approximately 100 volts. The life of the lamp varies with the applied voltage as follows:

$$\frac{L_V}{L_v} = \left(\frac{v}{V}\right)^{1.31}$$

where $L_V$ is the life of the lamp with the applied voltage, $V$, and $L_v$ is the life of the lamp with the applied voltage, $v$ (*5*). Hence, this 20-volt drop from the rated voltage increases the nominal life of the bulb from 50 to 550 hours. The indicator lamp is mounted in the front of the recorder and acts as a warning signal to the operator if the projection lamp burns out.

**Photocell Circuit.** The photocells are connected in the current-balancing circuit shown in Figure 2. Photocell fatigue is at a minimum as a result of effective zero external resistance at balance (*8*). The output of the photocell circuit is connected directly to the input of a high gain amplifier associated with a Minneapolis-Honeywell recorder.

**Recording and Balancing System.** The amplifier converts the direct current signal from the photocell circuit to an alternating current, which is then amplified. The output of the amplifier drives a balancing motor, which is coupled not only to the indicator pointer and recorder pen, but also, through a universal joint and long shaft, to a worm and pinion gear combination on the balancing polarizer assembly.

The worm gear is mounted on a tube press-fitted into the inner race of the ball bearing. The worm is mounted between two small ball bearings located on the base of the large bearing assembly.

An unbalance signal detected by the amplifier causes the motor to rotate the polarizer in such a direction as to restore balance. The angle through which the polarizer rotates from its zero position is equal and opposite to the optical rotation of the sample. Since the polarizer is mechanically linked to the recorder pen and pointer, the optical rotation can be read directly from the recorder scale.

## SAMPLE HANDLING SYSTEM AND TEMPERATURE CONTROL

The sample handling system is designed to deliver a flow of molten resin to the polarimeter at a relatively constant temperature of 180° C. The flow diagram in Figure 4 shows the details of the system.

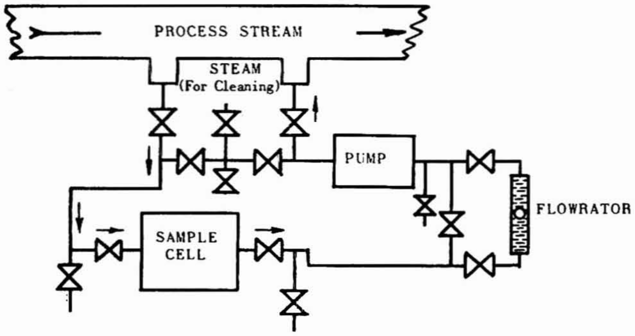

**Figure 4. Sample handling system**

The sample is tapped from the process, flows through the sample cell, then through a steam-traced Fischer and Porter Flowrator to a Marco pump which pumps it back into the process stream. The sample cell and Flowrator are kept on the suction side of the pump to avoid the possibility of exposing the Flowrator and the sample cell windows to excessive pressures in case of an obstruction on the discharge side.

Several points in the sampling system are tapped to provide for blowing out sections of the sample lines with steam.

Primary temperature control is attained through pressure control of the steam used to heat the sample lines and the sample cell. In this application, a K-Master regulating valve reduces 250 pounds per square inch steam to a controlled pressure of about 140 pounds per square inch. The sample lines consist of $^3/_8$-inch tubes mounted inside 0.75-inch tubes. The steam flows in the annular space between the two tubes. The lines are lagged with magnesia insulation. Swadgelok heat exchanger tees are used for the connections. With this arrangement, the temperature of the resin within the sample cell is controlled well within the required limits of ±5° C.

## CALIBRATION

Previous plant experience has been based on the measurement of the resin at 25° C. As the resin is an amorphous solid at 25° C., however, any continuous measurement must be made on the molten plant stream at 180° C. The calibration of the recording polarimeter was made by carefully measuring the optical rotations of samples of resin with a laboratory polarimeter at 25° C., and comparing these measurements with the recording polarimeter readings of the same samples at 180° C. A linear relationship between the instrument readings and the optical rotations was found. The instrument scale was finally calibrated to read specific rotation at 25° C. directly.

## ADJUSTMENTS

The range of the polarimeter is fixed by the chosen gear ratios and sample cell length. Inside the recorder case a zero adjustment is provided. This consists of a knurled knob and set-screw arrangement at the universal joint by means of which the coupling shaft may be disconnected from the balance motor and rotated to a new position. With a liquid of known rotation in the sample cell and the coupling shaft disconnected from the balance motor, the worm shaft is turned until the recorder indicates the correct rotation. The shaft is then reconnected to the balance motor by means of the set-screw.

## SAFETY PRECAUTIONS

The optical unit, the recorder, and the constant voltage transformer are air-purged for safety as well as for cooling purposes. A pressure relay is connected in the air line and will disconnect the electrical power to the instrument should the air pressure fail.

A pressure relay is also installed in the steam line. Should the steam pressure in the jackets fail, the relay will disconnect the sample pump to prevent possible damage to the system.

## PERFORMANCE

The recording polarimeter is a rugged and relatively trouble-free instrument designed for a high temperature application where a high precision is not necessary. It has been in plant operation for over two years and has shown excellent stability and more than adequate sensitivity for a required accuracy of ±0.5° specific rotation over a range of 40°.

The precision of the instrument is limited primarily because of lack of close temperature control and because of fluctuations in readings due to vapor bubbles in the sample stream. However, under the more ideal conditions of the laboratory, the polarimeter is capable of considerably higher precision. With sugar solutions at 25° C., the recording polarimeter is precise to ±0.2° specific rotation, being limited by the precision of the recorder. Somewhat higher precisions could be obtained by using a longer sample cell with a decreased range of rotations. However, for very high precision (better than ±0.02° specific rotation), very careful optical alignment and elimination of fluctuating strains, although minute, in the borosilicate glass windows would be necessary.

The excellent stability of the recording polarimeter is indicated by the fact that, although the zero setting is checked daily, zero adjustments have not been required for month-long periods of continuous operation.

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# Rotating Blade Stirrer and Small Sample Technique in the Determination of Freezing Points

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**Two new stirrers have been developed which markedly increase the usefulness and scope of the established cryoscopic technique for the determination of purity. A stirrer with a flat blade revolving coaxially about the thermometer has been designed for melting point determinations on organic sulfur compounds. The uniformity and shape of the time-temperature curves obtained using this stirrer indicate that thermal equilibrium is established with compounds which give very unsatisfactory results with the conventional reciprocating wire coil type. A second stirrer in the form of an internally threaded aluminum tube of 10-ml. capacity differs in principle from the first in that the tube containing the sample rotates and the stirring action is provided by the combined effect of the stationary thermometer and the rotating thread. This arrangement enables freezing points to be determined on samples as small as 8 ml. with an accuracy of ±0.01° C., and the corresponding freezing points for zero impurity to be calculated with an accuracy of ±0.1° C. These values are within the accepted limits of accuracy for freezing point determinations. The purity of a compound may be assessed satisfactorily with this form of freezing point apparatus on a much smaller sample, providing that the cryoscopic constant is known.**

The estimation of purity by cryoscopic methods requires a knowledge of the cryoscopic constant and an accurate determination of the time-temperature curve from which the freezing points of the sample and of the pure compound can be obtained. The procedure developed for the determination of freezing points by Rossini and coworkers (*12, 16, 17*) in connection with the American Petroleum Institute Research Project 6 and now generally accepted as a standard procedure, while satisfactory with hydrocarbons, is frequently unsatisfactory with compounds other than hydrocarbons. Organic sulfur compounds in particular, because of their rapid increase in viscosity at or near their freezing points and their tendency to melt unevenly, give very irregular time-temperature curves with the reciprocating wire helix stirrer recommended by Rossini. By replacing the latter with a blade on a rotating shaft, these difficulties can be overcome, while at the same time this type of stirrer not only gives more efficient stirring but also less frictional heat input to the sample.

This is shown with a somewhat similar stirrer which has been described by Kaye (*13*), who encountered difficulties of the same nature with certain hydrocarbons and some of the intermediates involved in the preparation of high purity fuels. The advantages of the rotating blade stirrer described in the present work, compared with Kaye's, are its much simpler construction, general applicability, and resistance to corrosion by sulfur compounds. Further, the screw stirrer designed by Kaye requires several sets of vanes of different pitch to deal with various crystal sizes. This has not been found necessary with the blade stirrer.

Both helix and blade stirrers suffer from the disadvantage that a relatively large sample is required to enable an accurate determination to be made. Even though the introduction of the rotating blade stirrer made possible a reduction in sample size from 50 to 35 ml., this amount of purified material is frequently not available, and a further appreciable reduction in volume was desirable. This has been accomplished by constructing a small aluminum tube to hold the sample. The tube can be screwed on to the rotating shaft in place of the blade and is threaded internally throughout its length so that when rotated the thread acts as a spiral fin causing mixing by vertical displacement of the sample. With this type of stirring it has been possible to reduce the volume of sample required to 6 to 8 ml. without appreciably affecting the accuracy of the method.

## APPARATUS

**Rotating Blade Stirrer.** This is used in conjunction with the freezing tube assembly described by Rossini and coworkers (*12, 16, 17*). Diagrams of the stirrer blade and shaft and their dimensions are given in Figures 1 and 2. The blade was constructed from a solid aluminum rod through which a central hole was drilled to take the platinum resistance thermometer. Slots were cut along the length of the rod to allow free access of the sample to the thermometer. Below the head (which was threaded internally for attachment to the shaft) the rod was cut away as shown to leave only two fins at opposite ends of one diameter.

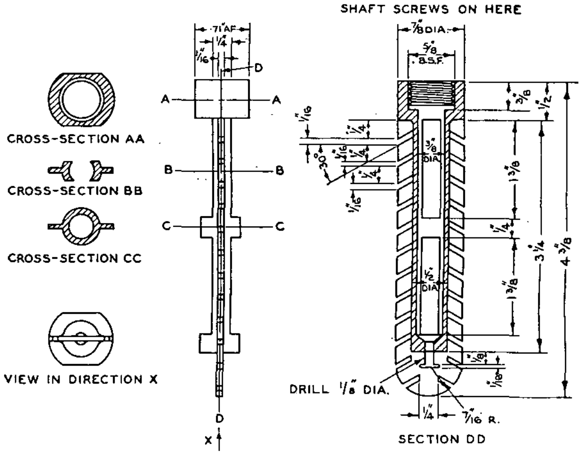

**Figure 1. Blade stirrer constructed of aluminum**

**For clarity all hidden lines have been omitted**

The stirrer shaft consists of a hollow Tufnol tube threaded at its lower end to secure the blade, which can be removed easily for cleaning. Tufnol, a laminated synthetic resinoid material, is used rather than metal to prevent heat being conducted to the sample down the stirrer shaft. The Tufnol tube is supported by a journal bearing and rotated by a belt drive from an electric motor at a speed of 300 r.p.m. The belt drive must be slack, or some other similar clutch device provided to prevent damage to the thermometer should the sample freeze completely. The platinum resistance thermometer is inserted through the hollow Tufnol shaft and clamped in position with the coil of the thermometer centrally placed inside the stirrer. The liquid level should be below the lower end of the Tufnol shaft but such that the thermometer coil is completely immersed.

**Tube Stirrer for Small Samples.** This consists of a small aluminum tube (Figure 3) machined from 7/8-inch aluminum rod, interchangeable with the blade of the rotary stirrer described above and rotated in the same manner. The tube is placed inside the normal glass freezing tube which provides the means of con-

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[1] Present address, Socony-Vacuum Oil Co., Ltd., London, England.

trolling the cooling rate. The internal thread functions as an Archimedean screw and the direction of rotation is such that the material in contact with the wall of the tube is carried downward providing an efficient stirring action. The thread form is B.S.W. with a pitch of 10 turns per inch; any similar coarse thread may be used. [B.S.W. and B.S.F. refer to British Standard Whitworth and British Standard Fine thread scales, respectively (*15*)].

The tube is covered on the outside with a layer of sheet cork which reduces the annular space between the aluminum tube and the inner wall of the glass freezing tube to give the minimum running clearance. The cork is primarily to protect the glass tube from damage, but at the same time it serves to reduce the air space which would otherwise give rise to convection currents and erratic heat transfer.

## PROCEDURE

**Rotating Blade Stirrer.** The experimental procedure is essentially the same as that described by Rossini and coworkers (*12, 16, 17*). In assembling the apparatus, care must be taken to ensure that the thermometer, stirrer, and freezing tube are correctly aligned to minimize the frictional heat and prevent damage to the platinum resistance thermometer.

**Tube Stirrer for Small Samples.** Using this stirrer, there is a greater time lag in the heat transfer from the sample to the cooling bath and the normal technique has therefore been altered. The sample (8 ml.) is pipetted into the aluminum tube which is then screwed on to the Tufnol shaft. The thermometer is inserted down the shaft and clamped in position. The glass freezing tube is fitted over the aluminum tube and connected to the vacuum system by a universal glass joint. By slowly rotating the stirrer assembly the alignment can be suitably adjusted, whereupon the glass tube is isolated by a tap from the rest of the system and the system evacuated. An external cooling bath is placed around the freezing tube and the sample cooled rapidly to approximately 25° C. above its freezing point. The glass freezing tube is then evacuated to a pressure of approximately 0.001 mm. of mercury. Readings are taken on the resistance bridge at 1-minute intervals until a constant rate of cooling is obtained. This can then be adjusted to the optimum value of 0.3° to 0.5° C. per minute, by either further evacuation of the jacket or raising the pressure. Owing to the small amount of sample employed, the time of freezing or melting is much shorter than with a 50-ml. sample using the accepted method. This necessitates a reduction in the time interval between readings in order that the curve may be as well defined as possible. Thus, when the temperature of the sample is approximately 6° C. above its freezing point, resistance readings are commenced at 0.5-minute intervals and continued until the completion of the determination.

If the sample tends to supercool, crystallization may be induced by switching off the motor and inserting down the gap between the thermometer and the inner wall of the Tufnol shaft, a length of thin Nichrome wire which has been cooled in liquid nitrogen.

## RESULTS

**Rotating Blade Stirrer.** About 1000 determinations have been carried out using the rotating blade stirrer. Although the majority of these were on sulfur compounds, the stirrer has also been used successfully with ketones, alcohols, and paraffinic, naphthenic, and aromatic hydrocarbons. Some of the data on sulfur compounds compiled as a result of these determinations have already been reported (*8–11, 14, 18*).

Table I illustrates the agreement between values obtained using the rotary and reciprocating stirrers. On account of the poor performance of the latter with sulfur compounds, the examples are confined to hydrocarbons. API values (*1–7*) are included for comparison.

Melting curves for 3-methyl-2-thiabutane (Figure 4) show the difference in performance between the rotating blade stirrer and the conventional type. Although the variation is very marked in this instance, such behavior has been noted to the same or lesser degree in a large number of melting point determinations on sulfur compounds, many of which gave such irregular curves with a reciprocating stirrer that not even an approximation to the melting point could be obtained.

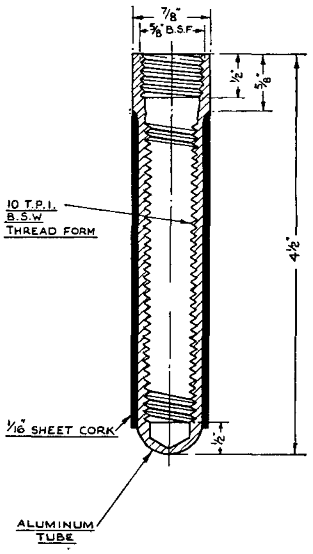

Figure 3. Tube stirrer for small samples

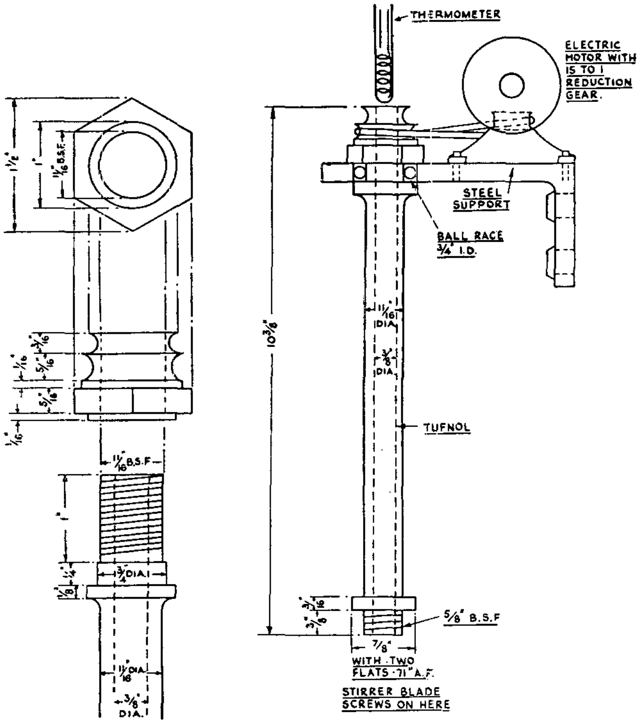

Figure 2. Shaft assembly

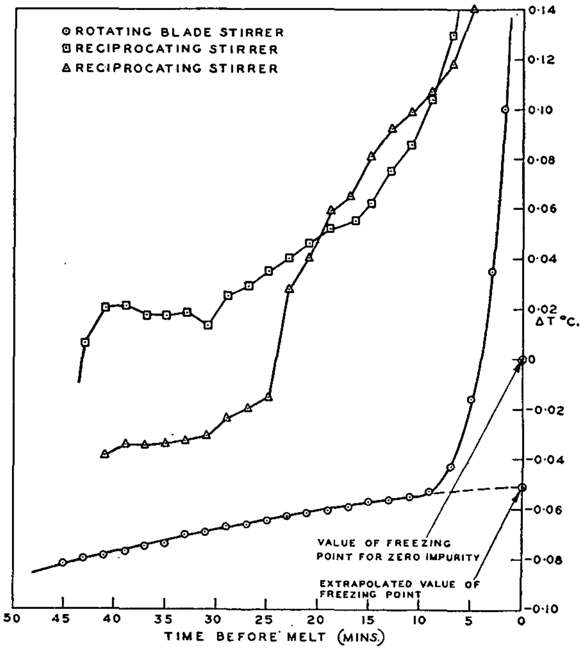

Figure 4. Comparison of rotating blade and reciprocating stirrers on 3-methyl-2-thiabutane

$\Delta T$ = ° C. above or below freezing point for zero impurity

### Table I. Comparison of Rotating Blade and Reciprocating Stirrers

| | M.P./F.P., ° C. | | | Calculated M.P./F.P. for Zero Impurity, ° C. | | | |
|---|---|---|---|---|---|---|---|
| Compound | Rotating blade stirrer | Reciprocating stirrer | M.P. or F.P. | Rotating blade stirrer | Reciprocating stirrer | API value | Ref. |
| *n*-Octane | −56.800 | −56.801 | F.p. | −56.798 | −56.800 | −56.795 | (*4*) |
| | −56.798 | | | −56.796 | | | |
| *n*-Dodecane | −9.712 | −9.710 | F.p. | −9.615 | −9.70 | −9.587 | (*4*) |
| *n*-Tetradecane | 5.831 | 5.835 | F.p. | 5.84 | 5.85 | 5.863 | (*4*) |
| 2,2,4-Trimethylpentane | | | | | | | |
| First sample | −107.414 | −107.417 | F.p. | −107.411 | −107.413 | | |
| | −107.416 | | | −107.415 | | | |
| Second sample | −107.376 | | | −107.375 | | −107.380 | (*1*) |
| 1,2-Dimethylbenzene | −25.200 | −25.200 | F.p. | −25.182 | ... | −25.182 | (*2*) |
| | −25.198 | | | | | | |
| 1,4-Diethylbenzene | −43.133 | −43.136 | M.p. | −42.84 | −43.09 | −42.850 | (*3*) |
| Methylcyclopentane | −142.449 | −142.450 | F.p. | −142.449 | −142.450 | −142.455 | (*5*) |
| Methylcyclohexane | −126.596 | −126.594 | | −126.584 | −126.55 | −126.593 | (*6*) |
| | | −126.594 | M.p. | | −126.54 | | |
| | | −126.598 | | | −126.585 | | |

### Table II. Test Determinations—Tube Stirrer for Small Samples

| | M.P./F.P., ° C. | | | Calculated M.P./F.P. for Zero Impurity, ° C. | | | |
|---|---|---|---|---|---|---|---|
| Compound | Tube stirrer | Blade stirrer | M.P. or F.P. | Tube stirrer | Blade stirrer | API value | Ref. |
| *n*-Dodecane | −9.687 | −9.687 | F.p. | −9.616 | −9.615 | −9.587 | (*4*) |
| 1-Thiatetralin | −15.572 | −15.565 | M.p. | −14.90 | −14.91 | ... | |
| 3,4-Dithiahexane | −101.558 | −101.555 | M.p. | ... | −101.46 | ... | |
| Isobutylbenzene | −51.562 | −51.569 | M.p. | ... | −51.452 | −51.48 | (*3*) |
| Ethylcyclopentane | −139.232 | −139.234 | M.p. | ... | ... | −138.446 | (*5*) |
| Thiophene | −38.309 | −38.307 | F.p. | −38.193 | −38.237 | −38.21 | (*7*) |
| | −38.294 | −38.305 | | −38.215 | | | |

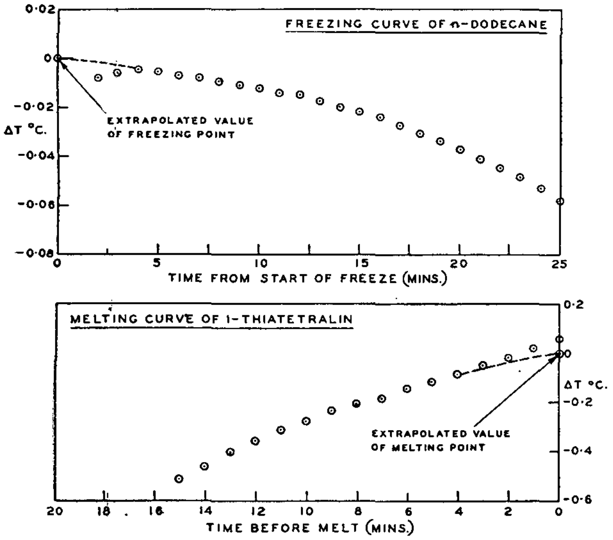

**Figure 5. Specimen curves obtained with tube stirrer**

**$\Delta T$ = ° C. above or below extrapolated value of freezing point or melting point**

**Tube Stirrer for Small Samples.** To assess the accuracy and characteristics of the rotating tube stirrer, a number of compounds were examined typical of those being investigated in these laboratories. In each instance the freezing point or melting point was checked on a 35-ml. sample, using the rotating blade stirrer, immediately prior to the determination using the rotating tube. The results are given in Table II together with the API values of the freezing points for zero impurity where available. As with the blade stirrer, the stirring efficiency was judged from the nature of the curves obtained and examples of these are given in Figure 5.

The combination of the rotating thread and the stationary thermometer appears to give a satisfactory equilibrium state, the rate of attainment of which is assisted by the high thermal conductivity of the aluminum tube. The accuracy of the method has not suffered appreciably as a result of the reduction in sample volume and a freezing point or melting point accurate to 0.01° C. is obtained which is within the limits of accuracy previously reported for the conventional method (*12*, *16*, *17*).

In freezing point determinations, thermodynamic equilibrium appeared to be maintained until slightly more than half of the material had crystallized. When this stage was reached, a sharp increase in the gradient of the curve took place, indicating loss of equilibrium, although the tube continued to rotate until the sample had almost completely frozen. This effect is not shown in Figure 5, which illustrates only those portions of the curves suitable for extrapolation. It was found that an estimation of the freezing point for zero impurity correct to 0.1° C. could be obtained using standard geometrical or analytical methods (*12*, *17*). In melting point experiments however, it seemed that equilibrium was only attained for the last few points of the curve following a sharp rise in temperature from the nonequilibrium state. Sufficient points were usually obtained to enable the melting point for zero impurity to be calculated, but for a number of the compounds examined only the melting point of the sample could be measured with any degree of accuracy.

In conclusion, the rotating blade stirrer is recommended for use with those samples which do not respond well to the reciprocating stirrer. The small sample tube allows satisfactory determinations to be made on samples too small for conventional apparatus.

## ACKNOWLEDGMENT

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# Mutual Interferences and Elimination of Calcium Interference in Flame Photometry

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**The use of flame photometric methods has made possible the rapid estimation of potassium, sodium, and calcium where large numbers of analyses are required. Interference effects, however, constitute a serious disadvantage to the method, especially in soil and plant extracts where the solutions are frequently complex in nature. This paper discusses the effects of mutual cationic interference with respect to potassium, sodium, and calcium in the Lange flame photometer. A method is given for the elimination of calcium interference. Aluminum is added to the solution under test. This has but little effect on the potassium or sodium readings but suppresses the calcium emission considerably, avoiding the necessity for chemical separation.**

IN RECENT years, the flame photometer has been increasingly used in laboratories where the determinations of small amounts of sodium, potassium, and calcium are required.

It has been shown by many workers that the presence of alien cations frequently causes erroneous results, due to interference. Domingo (*6*) shows that potassium and sodium mutually increase emission, but that calcium does not interfere with potassium in his instrument. Barnes and others (*2*) report that sodium gives a positive interference with calcium. West and others (*17*) show that the presence of calcium increased the apparent potassium content. Halstead and Chaiken (*10*) state that calcium interferes with sodium and potassium. Gilbert (*3*), Brown, Lilleland, and Jackson (*3*), and Toth (*16*) report the interference of calcium with other elements. Seay, Attoe, and Truog (*14*) using glass filters in the Perkin-Elmer instrument show that the 603.5 m$\mu$ calcium line penetrates the sodium filter to give a relative transmittance of 44%. In the interpretation of findings in the literature, it is important to consider the type of instrument used, as the results for a filter instrument may not necessarily hold for those from a prism instrument.

Eggertson, Wyld, and Lykken (*7*) state that negative errors are produced in the estimation of sodium and potassium owing to the presence of acids and salts within the spray chamber. It has been shown by Caton and Bremner (*5*) that because of their effect upon the nature of the spray, "inert" substances such as glucose or gelatin may cause a serious depression in flame intensity. Other outstanding effects are generally due to the spectral emission of an undesired element passing the filter, absorption of energy within the flame, or a general increase in background.

Improved filters of the interference type have been shown to have a high selectivity but none is yet truly monochromatic. It is possible to overcome certain interference effects by making up standards of the same composition as the solution under test, although this is not always practicable in the case of soil extracts, which may be variable in composition.

West and coworkers (*17*) have used the "radiation buffer" technique successfully by the addition of high concentrations of interfering ions to the standards and samples. This served to minimize the effects of small concentration variations of the diverse ions within the samples under investigation. An alternative method is the reduction of interference by high dilution, but this is frequently impractical where the desired element is present only in small quantity. The use of an internal standard serves to reduce interference effects, provided that the interferences affect the required element and standard equally.

The instrument described below, the Lange Photometer Model 2, (manufactured by B. Lange, 14–18 Hermannstrasse, Berlin, Germany) has been in use for some time at the Imperial College of Tropical Agriculture for the determination of potassium, sodium, and calcium in soils and biological materials. This paper discusses the effects of mutual interference and describes a method for the elimination of calcium interference by the addition of aluminum to the solution under test.

The effects of "inert" interferences in this instrument are generally the same as those reported in the literature (*5*, *7*) and where necessary have been obviated by compounding the standards in the same fashion as the samples.

## DESCRIPTION OF INSTRUMENT

Air at a controlled pressure of 0.4 atmospheres is supplied from a rotating piston pump (Figure 1) to the atomizer, *A*. The solution under test contained in a 10-ml. beaker is drawn into the chamber via the capillary, *B*, and atomized at the nozzle, *C*.

The finer droplets of the spray enter the burner, *D*, the larger droplets running to waste, *E*. The gas burns at the removable quartz nozzle, *G*, entering the burner at *F*.

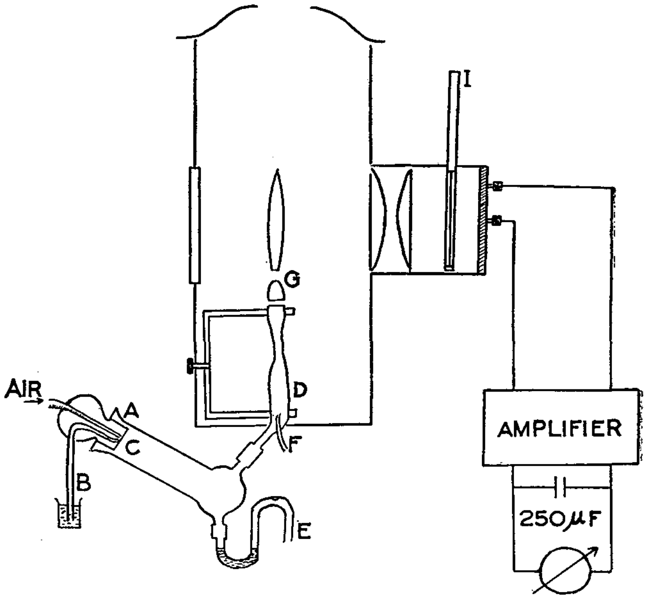

**Figure 1. Flame photometer**

Acetylene, propane, or coal gas may be used. Acetylene was chosen because it burns at a much higher temperature than coal gas and thus increases the intensity of flame coloration. This increases the over-all sensitivity of the instrument, although mutual interference is increased at high temperatures. The flame emission spectrum of the given element passes via the condenser lens system to the appropriate interference filter. This filter isolates the brightest line, allowing it to fall on a selenium barrier layer photocell. The filter consists of a dielectric spacer sandwiched between two semitransparent silver films. This type of filter has been termed a Fabry-Perot filter by Greenland (*9*), because its behavior is essentially the same as a low-order Fabry-Perot etalon. The thickness of the dielectric film determines the

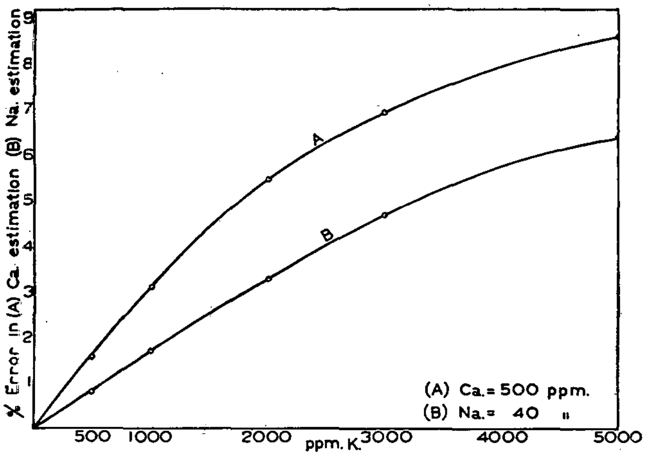

Figure 2. Effect of potassium on calcium and sodium

wave length transmitted and the selectivity is further increased by including a suitable colored filter which serves to eliminate undesired transmittance bands. The filter is assembled into a mount and protected by a cover glass. It is highly selective, but the light absorbed by the silvered surfaces reduces its efficiency. Knickman (*11*) points out that a special measuring instrument is required to determine low values of potassium when similar filters are used. In this case it is necessary to include an amplifier between the photocell and galvanometer in order to obtain a full scale deflection for a concentration of 10 p.p.m. of potassium. This is a unique feature of the instrument, as difficulty is generally experienced when attempting to amplify photoelectric currents from selenium barrier layer cells because of the high input resistance of most amplifiers (*4*, *15*).

The photo current is fed to a vibrator (which also acts as a synchronous rectifier). This converts it to "chopped" d.c. which is then amplified using two E.F.12 (Telefunken) tubes. The output then passes to a sensitive light beam galvanometer (sensitivity $2 \times 10^{-9}$ A. per mm.), the over-all amplification being approximately ten times.

The interference filters or a blank are easily introduced by rotating the holder, *1* (Figure 1).

The gas is controlled by a sensitive flowmeter, and the air by a hand valve, the pressure of the latter being read on a mercury manometer.

The galvanometer is equipped with a multirange switch and two rheostats for electrical zero. When interference filters are used, it is essential to ensure that the beam incident on the filter is truly collimated, a point not always made clear by the manufacturers.

Increased sensitivity was obtained by the insertion of a small mirror behind the flame.

In order to stabilize the amplifier it is necessary to modify the circuit by placing a 250-microfarad condenser in parallel with the output stage. This reduces the oscillating drift considerably. No increase in sensitivity is gained by electrically heating the atomizing chamber, contrary to the reports of some workers.

## REAGENTS

Double distilled water was obtained from an all borosilicate glass still.

Stock Solutions. Spectroscopically pure chlorides of sodium and potassium were dried to constant weight at 110° C. A stock solution of 2000 p.p.m. of sodium was prepared by dissolving 5.0833 grams of sodium chloride in distilled water and making up to 1 liter. Similarly a stock solution of 10,000 p.p.m. of potassium was made by dissolving 19.069 grams of potassium chloride and making up to 1 liter. In the case of calcium, 24.970 grams of calcium carbonate were dissolved in a minimum of dilute hydrochloric acid, allowed to cool, and then brought up to 1 liter, giving a solution of 10,000 p.p.m. of calcium. A molar solution of aluminum was prepared by dissolving 26.97 grams of the metal in hydrochloric acid and then diluting to 1 liter.

Standard Solutions. These were prepared by diluting suitable aliquots of the appropriate stock solutions. All volumes were measured by Grade A National Physical Laboratory calibrated glassware.

In the case of samples containing inert materials, such as 0.5*N* acetic acid extracts of soils, a separate set of stock solutions and standards was prepared such that the concentration of the acid in the standards was the same as that in the samples.

## OPERATIONAL PROCEDURE

With the blank in position and gas turned on the flame is lighted. The initial air pressure is approximately 0.15 atmosphere, being gradually increased to 0.4 atmosphere. The instrument is then left for 45 minutes to warm up. The selenium cell has a high temperature coefficient and if readings are taken before the body of the instrument is warm erroneous results are likely to occur due to the ambient temperature. The ASTM Committee E-2 on Emission Spectroscopy (*1*) recommend a warm-up time of approximately 30 minutes after the gas and electrical circuits are turned on. In agreement with their suggestion it is also found advisable to switch on the amplifier at the same time as the gas. This is an important consideration when working under the prevailing humid conditions in the wet tropics, as the slight increase in temperature of the system is sufficient to prevent condensation of moisture.

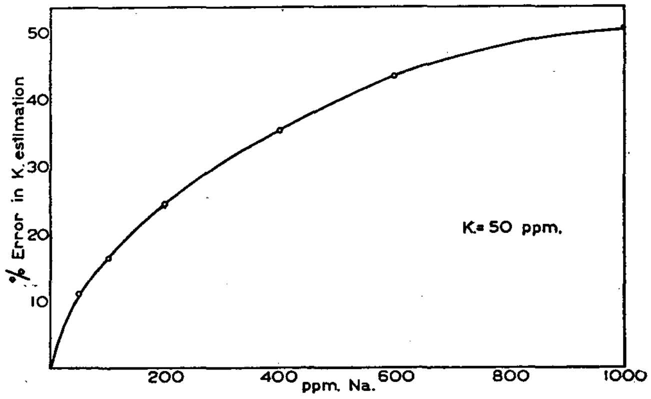

Figure 3. Effect of sodium on potassium

When the initial warm-up period has elapsed, the required filter is switched in and distilled water is sprayed into the flame. The galvanometer is set to zero mechanically by adjusting the torsion of the suspension.

The strongest standard is next atomized and the galvanometer is adjusted to its maximum reading electrically. The atomizer is then rinsed thoroughly and the standards are sprayed into the flame in ascending order of concentration, the deflection being noted and a graph plotted.

In routine use the standards are sprayed in prior to each run of determinations and the initial graph is replotted if the readings have altered by more than ±1% of the true value. Generally, however, the graphs remain within these limits for 12 months or more. The working curves are linear over small concentrations—e.g., 0 to 10 p.p.m. of potassium—in accordance with the findings of other workers.

## MUTUAL INTERFERENCE

The spectrum of an element is produced when it is sprayed into a flame, and it may be simple or complex. However, only one line is normally selected by the filter, usually the brightest. If several elements are present, there will be a general increase in flame background spread over most of the spectrum, and if any lines fall within the region of the desired line interference may ensue depending on the selectivity of the filter. Gilbert (*8*) showed that sodium interference is due in part to this phenomenon by the interposition of a didymium filter in the path of the beam. The bright line 5895 A. was practically eliminated, the background increase at other wave lengths remaining unaltered.

In the investigation of mutual cationic interference potassium, calcium, and sodium were chosen as being the most commonly determined by flame photometric methods.

Standards were made up from the stock solutions, the values being within the ranges encountered in the analysis of soil and plant materials. The results are illustrated in Figures 2 to 6; all the errors are positive. For instance, 1000 p.p.m. of potassium give an error of 3% in the estimation of 500 p.p.m. of calcium—that is, when estimated in the flame a true content of 500 p.p.m. of calcium appears to be 515 p.p.m. Similarly, a 1.6% error is obtained in the determination of 40 p.p.m. of sodium. In soil extracts the potassium level seldom exceeds 10 p.p.m. and thus the interference due to potassium may be considered negligible.

Fifty parts per million of sodium give an error of 11% in potassium readings for a value of 50 p.p.m. of potassium, and 200 p.p.m. of sodium give an error of 2.2% where the calcium concentration is 500 p.p.m. Apart from soils of coastal regions, sodium seldom gives trouble.

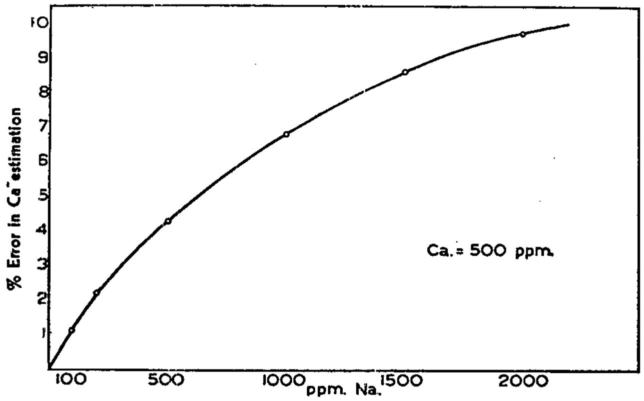

**Figure 4. Effect of sodium on calcium**

As may be observed from Figures 2 to 4, the effects of mutual interference tend to reach a maximum—that is, they are asymptotic to a given value. For example, in Figure 2 with a value of 5000 p.p.m. for potassium, the error at a calcium content of 500 p.p.m. is 8.6%. A considerable increase in potassium content above this value would give rise to only a very small additional percentage error in the calcium value—e.g., 10,000 p.p.m. of potassium give an error of 10.8% in the calcium reading.

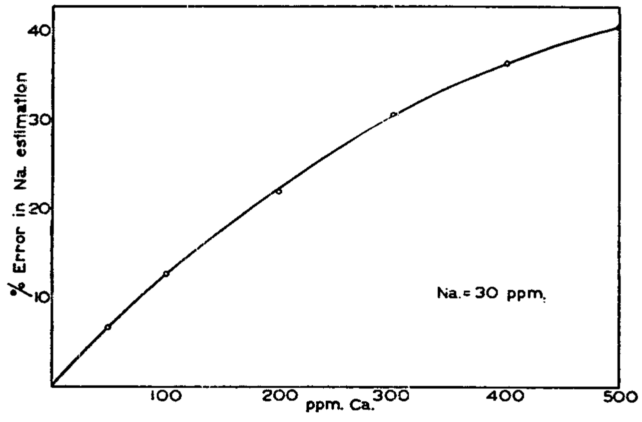

**Figure 5. Effect of calcium on sodium**

Thus, in this case, an excess of potassium may be added to the solution under test (and the standards) especially in the case of soil extracts, where the potassium content is considerably less than 10,000 p.p.m. This excess would then act as a "radiation buffer" (*17*) and the appropriate correction could be applied by the addition of an excess of potassium to the standards. The use of the radiation buffer method may also be applied for the determination of calcium or potassium where sodium is present. When Morgan's reagent (10% sodium acetate in 0.5*N* acetic acid) has been used for the extraction of plant material, the sodium content is generally in excess of the asymptotic value required for buffering and thus a constant difference may be observed, allowing a simple correction.

### METHOD FOR ELIMINATION OF CALCIUM INTERFERENCE

One of the methods for the determination of "soluble" or "available" potassium in soils consists of shaking 25 grams of soil with 200 ml. of 0.5*N* acetic acid. After standing overnight the solution is filtered into a 1-liter graduated flask and made up to the mark by leaching the soil remaining in the filter paper with additional acetic acid of the same concentration. The potassium content of such an extract does not normally exceed 10 p.p.m. A content of 10% free calcium carbonate in a soil would give rise to a large error in the potash estimation. Owing to the frequent occurrence of calcium in soil extracts, this interference effect is serious.

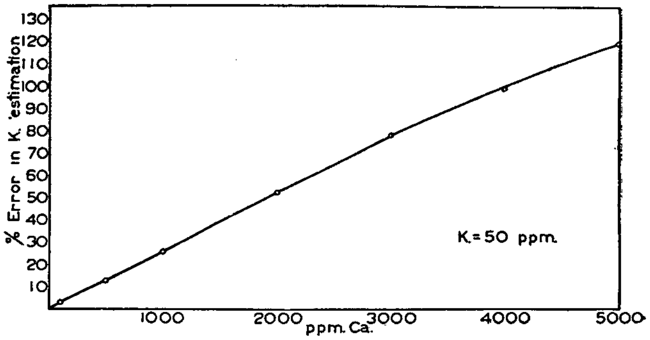

**Figure 6. Effect of calcium on potassium**

Figure 6 shows that 5000 p.p.m. of calcium give an error of 119% in the determination of 50 p.p.m. of potassium. In the estimation of values up to 10 p.p.m. of potassium this error would be increased considerably. West and coworkers (*17*) using the Beckman instrument report that 500 p.p.m. of calcium give a 40% error in the determination of 10 p.p.m. of potassium.

The addition of a large excess of calcium to the solution under test would not be suitable, owing to the low content of potassium in the soil extract—that is, the galvanometer deflection for calcium plus potassium would hardly differ from that of calcium alone and might even be of the order of the amplifier drift. In addition, Figure 6 illustrates that even at a concentration of 5000 p.p.m. of calcium the curve is still linear and not yet asymptotic to a given error.

An alternative procedure would be the precipitation of the calcium as oxalate. This is indicated by Lundegårdh and Boratynski (*12*) who, working with filters capable of transmitting the dark red potassium line, state that a concentration of more than 0.002 mg.-atom per liter of calcium interferes and must be removed. Halstead and Chaiken (*10*) give a correction curve for calcium content. Seay, Attoe, and Truog (*14*) give a correction based on pounds of calcium per acre, but prefer the addition of solid ammonium oxalate to the actual sample prior to extraction.

Apart from the additional tedium due to precipitation, etc., the possibility of interference due to the molecular spectrum of the oxalate ion is introduced. This constitutes a depression of line intensity, and thus the oxalate in turn must be removed.

Mitchell and Robertson (*13*) show that aluminum seriously interferes in the determination of calcium in the Lundegårdh method. This effect was investigated in the Lange flame-photometer. Solutions were prepared from the standards to contain concentrations ranging from 1$M$ to 0.00001$M$ aluminum and 1000 p.p.m. of calcium.

It was found that the apparent calcium content of a given solution in the flame decreased rapidly with increasing aluminum content. Solutions of varying concentrations of potassium and aluminum were next prepared and it was found that even a concentration of 0.3$M$ aluminum had no effect on the potassium reading (Table I). Finally, solutions containing 50 p.p.m. of potassium, 1000 p.p.m. of calcium, and varying concentrations of aluminum were prepared. The percentage error in the potassium reading due to calcium decreased markedly with increasing aluminum content, until at a concentration of 0.2$M$ aluminum there was no error in the potassium determination (Figure 7). The effect was found to be similar in the determination of sodium (Figure 8).

**Table I. Effect of Aluminum on Potassium**

| Aluminum Concn., $M$ | Potassium Added, P.P.M. | Potassium Found, P.P.M. |
|---|---|---|
| 0.01 | 50 | 50.0 |
| | 10 | 10.1 |
| 0.1 | 50 | 49.5 |
| | 10 | 10.0 |
| 0.3 | 50 | 50.0 |
| | 10 | 9.9 |

The calcium extracted in most soils is less than 1000 p.p.m. (in the extract), and thus it was considered that in the determination of potassium the addition of aluminum to a final concentration of 0.1$M$ should be adequate to annul calcium interference.

A set of Jamaican soils, rich in calcium carbonate, was chosen and extracted by the normal procedure as outlined above. In each case an aliquot of 10 ml. of the extract was taken and 10 ml. of 0.2$M$ aluminum solution were added.

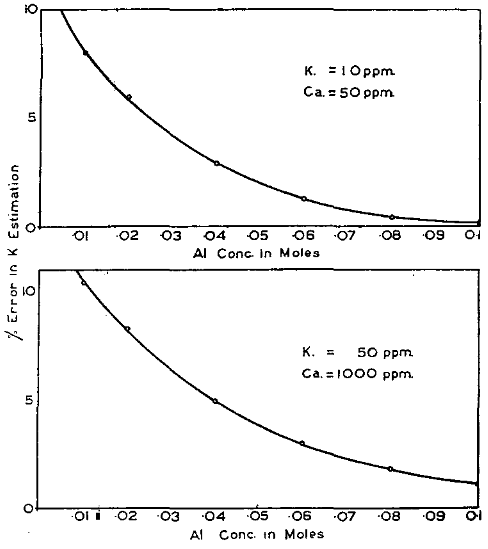

**Figure 7. Effect of aluminum on interference of calcium with potassium**

Determinations of potassium were made by the flame photometer on the extract alone and on the extract plus aluminum. The results are compared with those obtained by the chemical methods in Table II.

In the cases of low calcium content the results are in good agreement, whereas in the case of high calcium content only the treated extracts give comparable results. In the determination of calcium in soils by flame photometric methods caution must be observed because of the depressing effect of aluminum, which is usually present in the extracts. Mitchell and Robertson (*13*) using a prism instrument indicate that this interference may be overcome by the addition of strontium. This is not applicable in the case of filter instruments, however, as the strontium emission itself penetrates the calcium filter.

**Table II. Analysis of Soil Extracts**

| Sample No. | % Free $CaCo_3$ in Soil | Apparent P.P.M. of K in Untreated Extract | P.P.M. of K in Extract Plus 0.2$M$ Al | P.P.M. of K, Wet Chemical Method |
|---|---|---|---|---|
| J.C.A. | | | | |
| 77 | 6.1 | 10.55 | 5.85 | 5.38 |
| 78 | 7.0 | 9.05 | 3.20 | 3.15 |
| 87 | 13.47 | Too high for reading | 8.20 | 7.97 |
| 107[a] | ... | 5.35 | 4.15 | 4.30 |
| 142[a] | ... | 4.90 | 3.53 | 3.49 |

[a] Calcium is present in samples 107 and 142 but not determined as free $CaCO_3$.

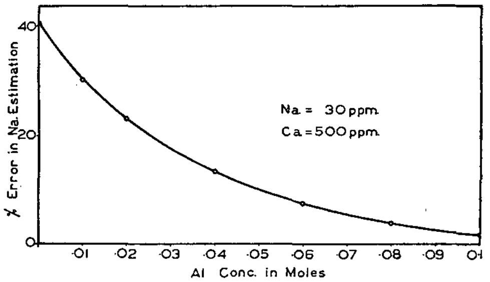

**Figure 8. Effect of aluminum on interference of calcium with sodium**

The interference of aluminum with calcium may be used to determine aluminum. If a known amount of calcium be added to a solution containing aluminum, the deviation of the apparent calcium content from the true, as determined in the flame, will give an estimate of the aluminum present. This presupposes that other calcium depressants—e.g., phosphate ion—are absent

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# Determination of Trace Metals in Petroleum

## Wet Ash–Spectrographic Method

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**Previous investigators have established that the quantitative determination of trace metals in petroleum and its products is of considerable significance. Data are presented in this paper which confirm that trace metals in some petroleum distillates are more completely recovered by a wet ashing or partial sulfuric acid decomposition method than by the conventional dry ashing procedure. A wet ashing–spectrographic procedure utilizing magnesium nitrate as an ashing and spectrographic aid has been developed. Nickel, vanadium, and manganese can be accurately determined at the 0.1- to 2.0-p.p.m. level using 10- to 50-gram samples. This procedure should also be applicable to trace quantities of iron, sodium, and copper, when magnesium nitrate free of these elements becomes available.**

VARIOUS investigators have reported that small amounts of metallic elements in crude petroleum and its products are of considerable significance in the petroleum industry. The presence of vanadium in fuel oils is undesirable because of its corrosive effect on turbine blades and its destructive effect on fire clay brick (*4*, *7*, *10*). Trace quantities of nickel, vanadium, copper, and iron in catalytic cracking charge stocks contaminate the catalyst and cause greatly increased gas and coke yields and reduced gasoline production (*2*, *9*).

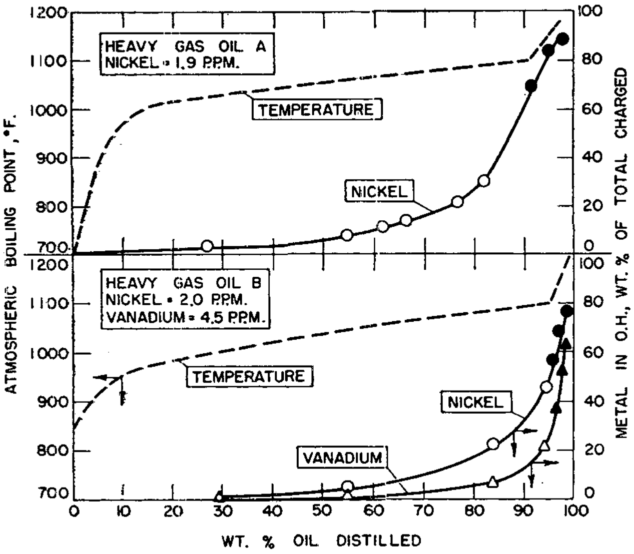

Figure 1. Redistillation of heavy gas oils

○. Laboratory vacuum distillation
●. Molecular distillation on bottoms from vacuum still

For years the usual method for determining the mineral content of petroleum oils has consisted of burning the oil to a char and igniting the residue in a muffle furnace to remove the carbonaceous material. A brief resume of the analytical methods used in determining ash and its metallic components has been reported by Milner and others (*10*) and Karchmer and Gunn (*8*).

Milner and coworkers presented a wet oxidation method for determining metals in crudes, residua, and overhead stocks. In this procedure the sample is partially decomposed (coked) with sulfuric acid and the carbonaceous residue burned in a muffle furnace with added oxygen at 885° to 975° F. The metals are determined colorimetrically and polarographically. These authors concluded that this novel sulfation or wet ashing method should be used on distillate fractions to prevent loss of metals by volatilization.

Milner has further pointed out that few data are available to show that metals are lost during the conventional ignition procedure. However, most of the investigators reporting no loss of metals by dry ashing have dealt with crudes, residual stocks, or synthetics prepared from nonvolatile metal organic compounds.

It is reasonable to postulate that the loss of metals, percentagewise, during dry ashing would be greater on distillate stocks which contain volatile metal compounds. Woodle and Chandler (*15*) concluded that crude oils contain iron, nickel, and vanadium compounds which volatilize during vacuum distillation. Wrightson (*16*) has shown that in the preparation of catalytic cracking charge stocks by vacuum distillation of reduced crudes, the metals content increases with depth of cut. Data (Figure 1) obtained in the authors' laboratory on the distribution of nickel and vanadium in the redistillation of heavy gas oils are supporting evidence for metal volatility.

### STUDY OF ASHING PROCEDURES

Considerable work has been done in this laboratory on the determination of metals in catalytic cracking feed stocks. Both the dry ashing (*8*) and the wet ashing (*10*) techniques have been studied, using colorimetric and spectrographic methods for determining the various metals.

The wet ashing technique of Milner has been modified so that the operation is relatively simple and a considerable saving of time is effected. Using a ratio of 1 ml. of concentrated sulfuric acid to 1 gram of oil, samples up to about 100 grams can be quickly coked in Vycor beakers by rapidly heating the mixture on a high temperature hot plate (800° to 1000° F.). The coking reaction for most samples takes place in a fairly vigorous manner, and foaming or frothing over is prevented by skillful stirring and handling during the critical period which usually lasts only a few minutes. Heating of the carbonaceous residue on the hot plate is continued with occasional stirring until oxides of sulfur are no longer

**Table I. Effect of Burning Rate on Metal Recovery by Dry Ashing 700-Gram Samples**

| | | | | |
|---|---|---|---|---|
| Dish size, ml. | 100 | 750 | 100 | 750 |
| Burning rate, grams/hour | 30 | 175 | 30 | 175 |
| | Total Ash, P.P.M. | | Nickel (as Ni), P.P.M. | |
| | (Average Values) | | | |
| Heavy Gas Oil | | | | |
| M | 26.4 | 17.4 | 1.3 | 0.9 |
| K | 9.4 | 4.0 | 1.1 | 0.4 |
| D | 8.8 | 4.0 | 0.9 | 0.3 |
| C | 8.1 | 3.3 | 1.0 | 0.5 |
| A | 8.6 | 3.6 | .. | .. |

**Table II. Comparison of Wet and Dry Ashing by Nickel Analyses on Heavy Gas Oils**

| Heavy Gas Oil | Nickel in Oil, Parts per Million | | | |
|---|---|---|---|---|
| | Wet Ashing | | Dry Ashing | |
| | Individual determinations | Average | Individual determinations | Average |
| A | 0.7, 0.7, 1.0, 0.7, 0.9, 0.7 | 0.8 | 0.5, 0.5, 0.6 | 0.5 |
| B | 1.1, 1.1, 1.4 | 1.2 | 0.7, 0.6, 0.9 | 0.7 |
| C | 1.7, 1.9, 2.0, 1.7 | 1.8 | 0.8, 1.2, 1.0 | 1.0 |
| D | 1.9, 2.0 | 2.0 | 1.0, 1.3, 0.9, 0.6 | 0.9 |
| E | 0.6, 0.7, 0.8, 0.6 | 0.7 | 0.2, 0.2, 0.3 | 0.2 |
| F | 1.7, 1.5 | 1.6 | 0.8, 1.0 | 0.9 |
| G | 2.3, 2.3, 2.3 | 2.3 | 1.3, 1.3, 1.5 | 1.4 |
| I | 0.8, 0.7, 0.6, 0.6, 0.8, 0.6, 0.6 | 0.7 | 0.3, 0.3 | 0.3 |
| J | 2.4, 1.7, 1.5 | 1.8 | 1.0, 1.0, 1.0, 0.9 | 1.0 |
| K | 2.5, 2.5, 2.7, 2.7 | 2.6 | 1.0, 1.1 | 1.1 |

evolved. The dry coke is finally burned to an inorganic residue in a muffle furnace at 1000° F. The ash is dissolved in concentrated hydrochloric acid for subsequent metals analyses.

The colorimetric methods for iron, nickel, and vanadium as described by Milner (*10*), as well as the colorimetric method for vanadium as recommended by Wrightson (*16*), have been used. The spectrographic method of Gamble and Kling (*5*) has been applied to both wet and dry ash residue from samples of 700 grams or more.

In applying the dry ashing procedure to heavy gas oils, the recovery of metals varies with the burning rate. The data in Table I show lower recoveries of total ash and of nickel when 700-gram samples were allowed to burn freely in 4 hours using 750-ml. dishes as compared to 24 hours employing 100-ml. dishes. Thus, some loss of ash material might be expected from burning a sample of any size.

A comparison of dry ashing and wet ashing has been made on several gas oils. The results in Table II show that the nickel content is, on the average, 75% higher by the wet ashing procedure.

While the wet ashing method gives consistently higher metal recovery than dry ashing, there is no absolute proof of complete recovery. Obviously, the preparation of known synthetics which simulate natural samples in volatility characteristics of metals present is a difficult problem at the present time. However, in the investigation of the wet ashing procedure an increase in the weight ratio of sulfuric acid to oil from 1 to 16 and also an increase in time of contacting at lower temperatures gave no greater nickel recovery. Thus, the wet ashing technique probably gives essentially complete metal recovery and only this type of ashing is reliable for distillate fractions.

## WET ASH–SPECTROGRAPHIC METHOD

In analyzing oils for metals at the 0.1-p.p.m. level, it is usually necessary to wet ash separately 100-gram or larger samples for each element in order to obtain reliable colorimetric determinations. The requirement of large samples is objectionable from the standpoint of time, size of beakers, muffle space, and quantity of sulfuric acid used. Also, in many cases the amount of sample available may be limited.

A wet ash–spectrographic method has been developed for the simultaneous determination of several metals. Using samples as small as 10 to 20 grams, nickel, vanadium, chromium, and manganese can be determined with reasonable accuracy at the 0.1-p.p.m. level. Lower concentrations can be determined, of course, by using larger samples. Briefly, the method consists of wet ashing the oil sample to which magnesium nitrate and cobalt naphthenate, both dissolved in alcohol, have been added. The magnesium nitrate acts as a carrier (mechanically and spectrographically), buffer, and intensifier, while the cobalt serves as the internal standard.

Studies show that this procedure should also be applicable to trace quantities of iron, sodium, and copper when magnesium nitrate free of these elements is made available.

**Apparatus and Materials.** SPECTROGRAPH. The instrument ARL Model 2060, 1.5-meter grating spectrograph, is equipped with a 24,000-line per inch concave grating with 5 cm. (2 inches) of ruled surface. The linear dispersion is 7 A. per mm. in first order. A cylindrical quartz lens of 5-inch focal length was used to form an image of the arc at the grating. An intensity control stand employing metallized quartz filters was used for adjusting light intensity.

EXCITATION SOURCE. ARL Model 4700, high precision multi-source unit.

COMPARATOR-DENSITOMETER. ARL Model 2050, nonrecording type with voltage regulator.

DEVELOPING UNIT. ARL, temperature controlled rocking machine.

FILM. Eastman Kodak No. SA-1.

ELECTRODES. Special high purity graphite obtained from National Carbon Co., United Carbon Co. (see Figure 2).

MAGNESIUM NITRATE. Merck's C.P.; 0.20 gram per ml. of ethyl alcohol.

COBALT NAPHTHENATE. Nuodex Concentrate; 0.015 mg. of cobalt per ml. of ethyl alcohol.

**Analytical Procedure.** PREPARATION OF STANDARDS. Synthetic samples for calibration were prepared by diluting chemically analyzed metal concentrates with iso-octyl alcohol. Analyses of the metal concentrates are as follows:

| Compound | Wt. % Metal in Concentrates |
|---|---|
| Nickel oleate | 5.42 ± 0.09 |
| Iron oleate | 2.84 ± 0.05 |
| Vanadium oleate | 1.36 ± 0.01 |
| Copper naphthenate | 1.93 ± 0.05 |
| Chromium oleate | 0.61 ± 0.02 |
| Manganese oleate | 0.95 ± 0.03 |

Using these compounds, individual synthetic stock solutions containing 100 p.p.m. of metal were prepared. A composite stock solution containing 10 p.p.m. of each metal was then prepared. By further dilution of this blend, synthetics used in the spectrographic calibration were obtained.

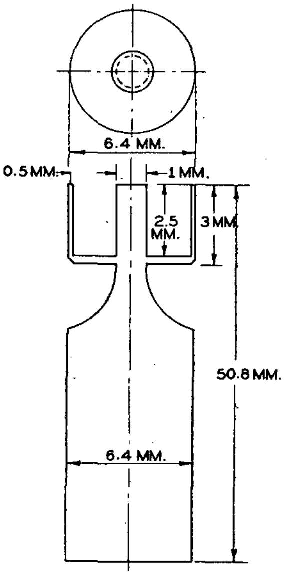

**Figure 2. Modified platform electrode**

FILM EMULSION CALIBRATION CURVE. Using an iron arc, selected homogeneous lines between 3200 and 3300 A. suggested by Dieke (*1*) were employed in constructing the curve.

ANALYTICAL WORKING CURVES. The synthetic standard samples were ashed and arced under the same conditions as outlined in the procedure. The curves were obtained by plotting micrograms of metal versus intensity ratio. Typical calibration curves are shown in Figures 3, 4, and 5.

SULFURIC ACID DECOMPOSITION AND ASHING. The oil sample, warmed if necessary, is weighed into a Vycor beaker. The sample size varies depending on the metal concentration level. Ten-gram samples give ideal photographic densities for metal concentrations ranging from 0.3 to 2.0 p.p.m. Below the 0.3-p.p.m. level, larger samples of the order of 20 to 50 grams are recommended. For samples up to about 25 grams, a 250-ml. Vycor beaker is adequate.

Two milliliters of the alcoholic magnesium nitrate solution and exactly 1 ml. of the cobalt naphthenate solution are added to the oil and thoroughly mixed. For each gram of oil, 1 to 2 ml. of metal-free 95% sulfuric acid is added. This mixture is then heated rapidly on a hot plate. Considerable frothing occurs when the sulfuric acid begins to react, and cautious stirring and heating are necessary at this point. When the foaming subsides, the reaction is essentially complete and the oil has been reduced in only a few minutes to a char. Some refractory samples, such as refined paraffinic mineral oil, require longer times of heating. The charred mass is heated on the hot plate for 1 to 3 hours until sulfur trioxide($\alpha$) fumes are no longer evolved.

The resulting char is then ashed in the muffle furnace at 1000° F. The time required for complete ashing varies from 4 to 16 hours, depending on the type and quantity of oil sample. In routine work the sample is normally left in the muffle overnight. The ash is then cooled and transferred with the aid of a camel's hair brush to a small agate mortar for mixing.

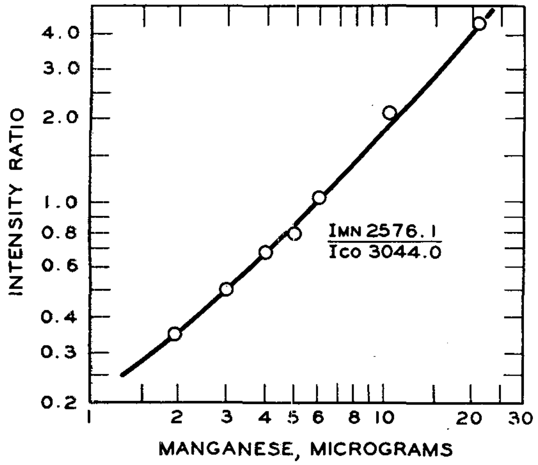

**Figure 3. Analytical working curve for manganese**

Spectrographic Excitation and Densitometry. The ash is packed in the modified platform electrodes and arced against a $^1/_8$-inch upper electrode. Details for excitation and film development are outlined in Table III. Normally, three electrodes are arced for one analysis. Thus, by using a split filter, six intensity ratios per sample are available.

The transmittance of selected analytical lines (Table IV) is determined on a nonrecording densitometer. The densitometer is adjusted to 100% transmittance for any background adjacent to the line. The intensity ratios, analytical line to cobalt line, are determined by applying the transmittance values to the film calibration curve. By referring the intensity ratio to the appropriate analytical working curve the metal content is determined.

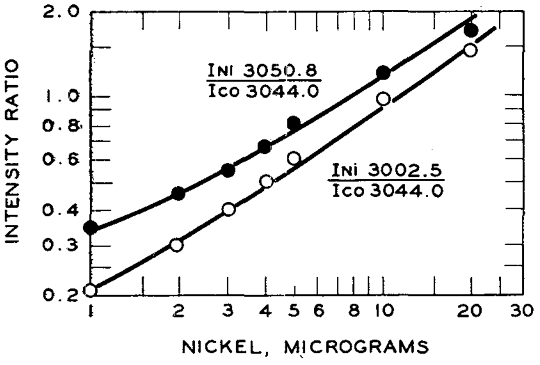

**Figure 4. Analytical working curves for nickel**

**Choice of Buffer.** Murray and Plagge (*11*) described a method which employs silica as an ash-aid in the metal analysis of petroleum oils. Others (*3, 5, 6, 14*) have used lithium carbonate as a matrix for determining the composition of ash obtained by the dry ashing procedure. O'Conner and Heinzelman (*12, 13*) recommended the use of magnesium nitrate for trace metals in vegetable oils and fats. In their work an alcoholic solution of magnesium nitrate was added prior to dry ashing.

In this laboratory the use of alcoholic solutions of lithium nitrate and magnesium nitrate was investigated. Although both compounds were found to be satisfactory as an ashing aid, carrier, and buffer, magnesium nitrate was chosen because of the increased sensitivity that it provided.

**Table III. Spectrographic Conditions for Excitation and Film Development**

| | |
|---|---|
| **Spectrograph** | |
| Grating aperture | Open |
| Slit, microns | 40 |
| Filters in intensity control stand | 2-6-9 |
| % transmittance | 32 |
| **Electrodes, Special High Purity** | |
| Upper | $^1/_8$ inch in $^1/_4$ inch |
| Lower | Modified platform |
| Electrode gap, mm. | 3 |
| **Power** | |
| Type unit | ARL high precision |
| Capacitance, mfd. | 60 |
| Inductance, mh. | 360 |
| Resistance, ohms | 25 |
| Volts | 300 |
| Amps | 10 |
| Rotary gap pointer | 90 |
| **Exposure Time, Sec.** | |
| Preburn | None |
| Exposure | 30 |
| **Film** | |
| Type | SA-1 |
| Development | |
| Developer | D-19 |
| Time, min. | 3 |
| Fixing time, min. | 2 |
| Drying time, min. in infrared dryer | 2 |

**Table IV. Analytical Lines**

| Element | Line, A. | Excitation Potential, E.V. |
|---|---|---|
| Cobalt (internal standard) | 3044.0 | 4.05 |
| Nickel | 3002.5<br>3050.8 | 4.14<br>4.07 |
| Vanadium | 3183.4 | 3.89 |
| Manganese | 2576.1 | ... |

**Acid Blanks.** The sulfuric acid is composited in 3-gallon batches and both chemical and spectrographic metal analyses are obtained on 300- to 400-gram samples prior to use in coking. Typical analyses are illustrated in Table V.

**Accuracy and Precision.** The calibration data on seven synthetic samples ranging from 0.1 to 2.0 p.p.m. were obtained over a 4-week period. A total of about 45 to 50 samples were ashed and analyzed. Thus, the analytical working curves prepared from these data include errors involved in both the ashing and spectrographic parts of the procedure. The precision of the method for nickel was further studied by making replicate analyses of several gas oils in which the nickel content varied from 0.1 to 2 p.p.m. (Table VI).

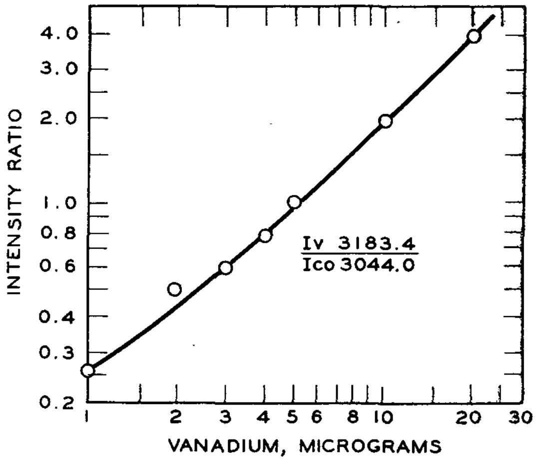

**Figure 5. Analytical working curve for vanadium**

### Table V. Sulfuric Acid Blanks

| Batch | Method | Analyses, P.P.M. in $H_2SO_4$ Iron | Nickel | Vanadium |
|---|---|---|---|---|
| A | Chem. | 0.04 | 0.004 | |
| | Spectro. | <0.05 | 0.003 | Nil |
| B | Chem. | 0.02 | 0.004 | |
| | Spectro. | <0.05 | 0.002 | Nil |

### Table VI. Reproducibility of Nickel Analyses by Wet Ash-Spectrographic Method

(Standard deviation equals ±0.06 p.p.m.)

| Heavy Gas Oil | Sample Size, Grams | Parts Per Million in Oil Individual determinations | Average |
|---|---|---|---|
| U | 10 | 0.16 | 0.12 |
| | 20 | 0.17, 0.18, 0.12, 0.09, 0.08 0.12 | |
| | 30 | 0.08, 0.10 | |
| | 40 | 0.13, 0.10, 0.13 | |
| | 50 | 0.07, 0.09 | |
| Q | 10 | 0.38, 0.49, 0.49, 0.44, 0.47, 0.40, 0.47, 0.46, 0.46 | 0.45 |
| R | 10 | 0.45, 0.46, 0.45, 0.47, 0.47, 0.52, 0.41, 0.45, 0.50 | 0.46 |
| I | 10 | 0.68, 0.80, 0.73, 0.72, 0.68, 0.57 | 0.70 |
| S | 10 | 0.9, 1.0, 0,9, 0.9, 1.0, 1.2, 0.9, 1.0 | 0.96 |
| T | 10 | 1.9, 2.2, 2.2, 2.2 | 2.1 |

From these data, the standard deviation in parts per million of the method in the 0.1- to 2.0-p.p.m. range have been calculated as follows:

| | Nickel | Vanadium | Manganese |
|---|---|---|---|
| From calibration data | ±0.05 | ±0.02 | ±0.06 |
| From analysis of gas oils | ±0.06 | .. | .. |

Several gas oils have been analyzed for nickel and vanadium by both the spectrographic and colorimetric methods. The results (Table VII) are in generally good agreement.

## SUMMARY

Data have been presented which confirm that trace metals in petroleum distillates are more completely recovered by a wet ashing or partial sulfuric acid decomposition method than by the conventional dry ashing procedure. A wet ashing-spectrographic procedure utilizing magnesium nitrate as an ashing and spectrographic aid has been developed wherein several metals at the 0.1- to 2.0-p.p.m. level can be accurately determined using 10- to 50-gram samples.

## ACKNOWLEDGMENT

The authors wish to acknowledge the aid of W. E. Price, E. W. Seefield, and J. L. Jones who made the chemical analyses reported in this paper.

### Table VII. Comparison of Chemical and Spectrographic Methods for Nickel and Vanadium on Wet Ash Residues

| Heavy Gas Oil | Spectrographic No. of detns. | Spectrographic Average analysis | Colorimetric No. of detns. | Colorimetric Average analysis |
|---|---|---|---|---|
| | | Nickel, P.P.M. in Oil | | |
| C | 4 | 1.85 | 15 | 1.93 |
| I | 6 | 0.70 | 7 | 0.70 |
| N | 5 | 0.45 | 11 | 0.35 |
| K | 9 | 1.90 | 4 | 2.6 |
| O | 4 | 0.51 | 2 | 0.50 |
| P | 5 | 0.22 | 2 | 0.15 |
| Q | 6 | 0.32 | 2 | 0.30 |
| | | Vanadium, P.P.M. in Oil | | |
| C | 4 | 0.12 | 3 | 0.10 |
| I | 3 | 0.13 | 3 | 0.08 |
| N | 6 | <0.10 | 6 | 0.06 |
| K | 9 | 0.16 | 3 | 0.20 |
| O | 4 | 0.76 | 2 | 0.75 |
| P | 4 | 0.13 | 2 | 0.25 |
| Q | 6 | 0.21 | 2 | 0.40 |

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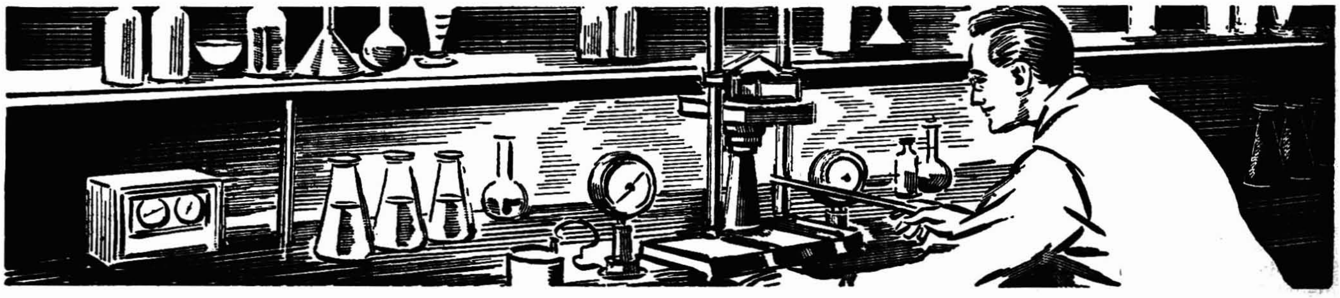

# A New Spot Test for Formaldehyde

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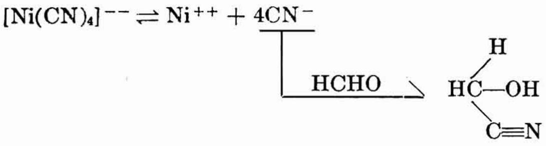
**A new method for detecting formaldehyde by means of a spot test on reagent paper has been developed. The reagent papers are prepared by impregnating filter paper in an equilibrium mixture of potassium tetracyanonickelate and dimethylglyoxime. As little as 0.5 $\gamma$ of formaldehyde can be detected. The test can be used as a semiquantitative method and is essentially free from interference.**

METHODS for the detection of formaldehyde are numerous and can be classified into four groups: methods depending upon Schiff's type reagents (*17*); methods using phenolic-type compounds (*2–4*, *15*, *16*, *20*); methods based on reactions with miscellaneous aromatic compounds; and methods based on complicated reactions with inorganic reagents (*1*, *5*, *10*, *11*, *21*). Besides these, a number of other detection methods have been reported which cannot be classified into any of these groups. The various methods of detection have been reviewed in a number of communications (*12–14*). The present authors also investigated many of the proposed procedures, and the results of their investigation will be published shortly in connection with studies of the quantitative determination of small amounts of formaldehyde.

A new approach for the detection of formaldehyde is proposed. In principle, the test depends on a demasking reaction. The reagent solution from which the test papers are prepared is an equilibrium mixture of potassium tetracyanonickelate(II) and dimethylglyoxime. The nickelcyanide complex dissociates as follows:

$$[Ni(CN)_4]^{--} \rightleftharpoons Ni^{++} + 4CN^-$$

In the equilibrium mixture, the amount of nickel ion is very low [The stability constant (*19*) of the complex is $5.6 \times 10^{13}$. The concentration of nickel ion in a normal solution of the complex is only $10^{-3}N$.] and the formation of nickeldimethylglyoximate cannot take place. When formaldehyde is added to the above equilibrium mixture, cyanide is removed through the formation of cyanhydrin



The equilibrium is thus shifted toward the right with the liberation of nickel ions and consequent development of red color, the intensity of which is a function of the amount of formaldehyde.

## EXPERIMENTAL

**Reagents.** All reagents used were of analytical reagent grade. Test papers were prepared from Whatman No. 1 filter paper.

Nickeldimethylglyoximate was prepared according to standard procedures of quantitative analysis.

A 0.1% stock solution of formaldehyde was prepared and its strength established according to standard procedure (*18*). Dilute solutions were prepared by the quantitative dilution of the stock solution.

**Preparation of Reagent Solution.** The following solution was found to have satisfactory sensitivity for the preparation of the reagent paper. Nickeldimethylglyoximate (0.5 gram) was suspended in 100 ml. of water and potassium cyanide (0.4 gram) added. The suspension was shaken and then allowed to stand for at least 24 hours. After this period it was filtered, and the filtrate was stored in a borosilicate glass bottle and kept away from light.

Test papers prepared from this solution were almost white. The color of the reagent solution was light yellow. Test papers prepared from reagent solutions obtained by using lesser amounts of nickeldimethylglyoximate and cyanide were less sensitive, whereas those prepared from reagent solutions obtained by using larger amounts of nickeldimethylglyoximate and cyanide were too highly colored.

**Method of Preparing Test Paper.** Whatman No. 1 filter paper circles (11 cm.) were dipped in the reagent solution and were quickly dried under an infrared lamp and stored in a stoppered bottle. The test papers were stable when properly kept.

**Procedure for Detecting Formaldehyde.** The test paper was treated with a drop of test solution, and the spot was quickly dried under an infrared lamp or in an oven. In the presence of formaldehyde, a distinct red spot was developed. When amounts less than 2 $\gamma$ of formaldehyde were detected, the spot due to the test solution was always compared with the slightly reddish blank spot due to water. In actual procedure, the test paper was cut into two semicircles, one was spotted with the test solution and the other with a drop of water, and the two were dried as usual. This was done to avoid the possibility of isothermal distillation of formaldehyde from the test spot on to the blank spot and thus indicating a spurious positive test.

Formaldehyde can also be detected with the reagent solution. About 2 ml. of the reagent was taken in a test tube to which was added a drop of aldehyde solution (2.5 $\gamma$) and the mixture continuously shaken. A distinct turbidity and a pink coloration appeared within 5 minutes. It was observed that the time of ap-

**Table I. Interferences**[a]

| Name of Compound | Positive Interference | Masking Interference | Comments |
|---|---|---|---|
| Acetaldehyde | + | + | |
| Acrolein | − | − | |
| Butyraldehyde | − | − | |
| Benzaldehyde | − | − | |
| Crotonaldehyde | − | − | |
| Hexaldehyde | − | − | |
| Heptaldehyde | − | − | |
| Acetone | − | − | |
| Acetophenone | − | − | |
| Acetylacetone | − | − | |
| Methanol | − | − | |
| Ethyl alcohol | − | − | |
| Propyl alcohol | − | − | |
| Amyl alcohol | − | − | |
| Acetic acid | − | + | Interference eliminated by addition of ammonia or exposure to ammonia fumes |
| Butyric acid | − | + | |
| Formic acid | − | + | |
| Trichloroacetic acid | − | + | |
| Acetic anhydride | − | + | |
| Phenol | − | + | |
| Methylamine | − | − | |
| Ethylamine | − | − | |
| Butylamine | − | − | |
| Dithylamine | − | − | |
| Ethylenediamine | − | + | |
| Aniline | − | − | |
| Pyridine | − | − | |
| Quinoline | − | − | |
| Ether | − | − | |
| Carbon tetrachloride | − | − | |
| Carbon disulfide | − | − | |
| Benzene | − | − | |
| Bromobenzene | − | − | |
| Chlorobenzene | − | − | |
| Fluorobenzene | − | − | |
| Nitrobenzene | − | − | |
| Toluene | − | − | |
| Ethyl acetate | − | − | |
| Ammonia | − | − | |
| Hydrazine | − | − | |
| Phenylhydrazine | − | − | |
| Hydroxylamine | − | − | |
| Ammonium acetate | − | − | |
| Sodium acetate | − | − | |
| Mineral acids | − | + | Acid neutralized with a drop of ammonia or sodium acetate |

[a] + indicates positive interference or masking effects by compound.
− indicates absence of interference.

pearance of turbidity and pink coloration was dependent upon the amount of formaldehyde. Because of its greater sensitivity the procedure based on the use of reagent paper is recommended.

**Sensitivity.** As little as 0.5 γ of formaldehyde can be easily detected by the proposed test; and spots containing 0.5, 5, 25, and 50 γ of formaldehyde are distinguishable from each other. A semiquantitative determination is possible by matching the test colors with standard spots.

**Interferences.** Neither sulfite nor sulfide interferes with the test. A large number of organic compounds were tested for interference effects and the results of the investigations are shown in Table I.

### DISCUSSION

It is evident that the method is very simple, rapid, and sensitive. Very few of the present detection methods are as sensitive as the proposed test, and most of the known methods are much more complicated and require rigid control (*6–8*). The methods for the detection of formaldehyde by spot tests are very few, and that due to Frehden and Furst (*9*) is not very sensitive and failed to produce reliable results in the hands of the present investigators.

### ACKNOWLEDGMENT

The financial assistance of the Mine Safety Appliances Co. is gratefully acknowledged.

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# Rapid Microtitration of Sulfate

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**Small concentrations of sulfate can be determined by a direct titration with 0.005*M* barium perchlorate using Thorin [2(2-hydroxy-3,6-disulfo-1-naphthylazo)benzenearsonic acid] as the indicator. The titration is carried out in 80% alcohol, in the apparent pH range 2.5 to 4.0. The end point is sharp and equilibrium is rapidly attained, so that the titration can be rapidly carried out. The method is more versatile when cations are removed by passage of the sample through an ion exchange column prior to the titration. Phosphate makes the method inaccurate, but can be removed by a rapid precipitation with magnesium carbonate. The method has been applied to the determination of sulfate in raw water, treated city water, and boiler water. Concentrations of sulfate as low as 10 p.p.m. can be determined which are accurate to ±1 p.p.m. Higher concentrations can be determined with an error of 1% or less. In the absence of phosphate the time required for a complete single determination is only 3 to 5 minutes.**

NO REALLY satisfactory method is available for the determination of small amounts of sulfate. Gravimetric methods are time-consuming and unsuited for the determination of very low concentrations of sulfate. Turbidimetric procedures are rapid, but are often unreliable and difficult to reproduce. The direct titration of sulfate with barium using tetrahydroxyquinone (THQ) or rhodizonic acid indicator has been widely used by some but rejected by others, largely because of the poor end point (*1*, *3*, *5*).

A rapid titrimetric method for macro amounts of sulfate has recently been reported (*2*). This involves direct titration of sulfate in 40% alcohol with barium chloride using Alizarin Red S as the indicator. The end point is sharp and easily detectable. This method can only be applied for the determination of macro quantities of sulfate, however, because a certain minimum of precipitate is required to provide enough surface for the end point reaction.

The present method is applicable to very low concentrations of sulfate. The determination is carried out by first removing cations by means of a small ion exchange column in the hydrogen form. Alcohol is added to bring the alcohol concentration to 80% by volume and the resulting solution is titrated with 0.005*M* barium perchlorate using Thorin [2(2-hydroxy-3,6-disulfo-1-naphthylazo)benzenearsonic acid] as the indicator. The end point is marked by a sharp change from yellow to pink.

### CONDITIONS FOR TITRATION

The titration is successful only if a high concentration of some nonaqueous solvent is present. Using the lower alcohols the concentration of nonaqueous solvent should be between 70 and 90% by volume. Ethyl alcohol, 2-propanol, or methanol serves equally well. Barium sulfate precipitated from solutions containing a high concentration of alcohol appears to be gelatinous and not at all like the familiar fine crystalline precipitate which is obtained from aqueous solution. Titration of very low concentrations of sulfate is rapid in alcohol-water, indicating that the induction period observed in water for precipitation of small concentrations of sulfate is not encountered here.

The titration can be successfully carried out anywhere in the "apparent" pH range 2.5 to 4.0. Passage of the sample through the cation exchange column automatically brings the apparent pH within this range after the addition of the alcohol unless the foreign salt concentration is too high. The maximum foreign salt concentration permissible is approximately five to ten times the molar concentration of sulfate. The pH is controlled by adjusting the quantity of sample taken so that the total salt concentration will not be great enough to lower the pH excessively after passage through the hydrogen form ion exchange column.

The titrant used in barium perchlorate in 80% alcohol. The perchlorate ion introduced with the barium causes less coprecipitation error than chloride; also barium perchlorate is much more soluble in alcohol than is barium chloride. Preparing the titrant in alcohol-water solution keeps the alcohol concentration constant throughout the titration.

The optimum concentration of the titrant and of sulfate depends on the size of the increments of titrant added near the end point and on the total volume of sulfate solution taken for titration. Adding approximately 0.01-ml. increments of barium perchlorate near the end point and taking 10 ml. of sulfate solution (plus 40 ml. of alcohol) for titration, it is best to use 0.005*M* barium perchlorate. Using these conditions, the range of sulfate titrated is 0.05 to 5.0 mg. or approximately 5 to 500 p.p.m. (before addition of alcohol).

The rate of titration is not critical. Equilibrium is rapidly attained, so that the titration can be quickly carried out. It is best to allow 2 or 3 seconds between addition of the last few increments of titrant before the end point. Efficient magnetic stirring improves the speed and convenience of the titration.

## EFFECT OF FOREIGN IONS

The effect of foreign cations was studied by titrating sulfate in the presence of various ions added as the perchlorate or chloride. The results given in Table I show that several metal ions cause errors of 2 to 3% due to coprecipitation. Numerous other metal ions completely interfere with the titration by forming a colored complex with the Thorin indicator. Many metal ions which do not form colored complexes with Thorin in aqueous solution do so in solutions containing a high percentage of alcohol.

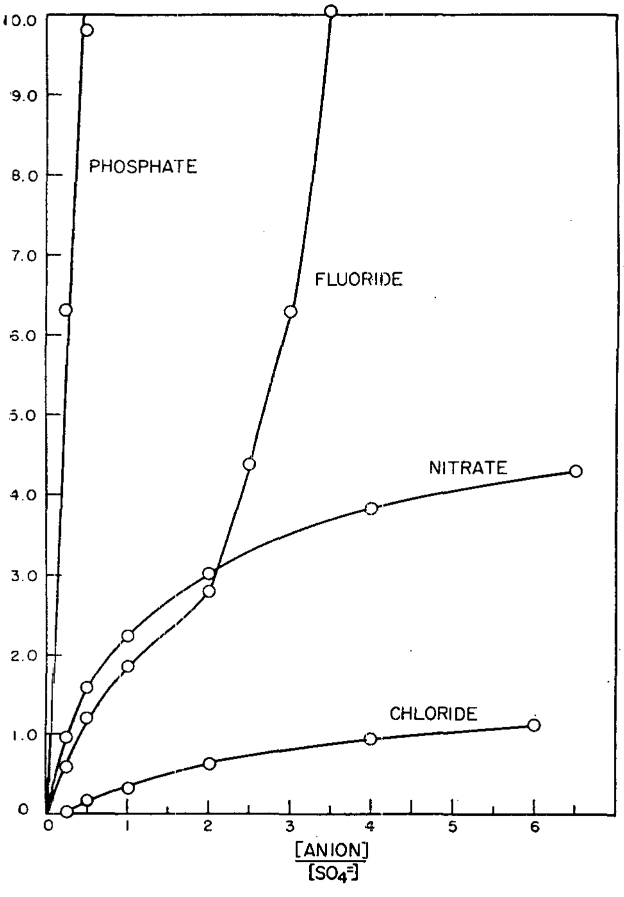

**Figure 1. Results of titration of sulfate in presence of different concentrations of common anions**

**Table I. Effect of Cations**

| Salt Added, (= 2[$SO_4^{--}$]) | $Ba^{++}$ Theory, Ml. | $Ba^{++}$ Actual, Ml. | Difference, % |
|---|---|---|---|
| $NaClO_4$ | 4.83 | 4.69 | −2.9 |
| KCl | 6.76 | 6.32 | −6.6 |
| $NH_4ClO_4$ | 6.76 | 6.62 | −2.0 |
| $Zn(ClO_4)_2$ | 4.83 | 4.74 | −1.9 |
| $Mg(ClO_4)_2$ | 2.95 | 2.90 | −1.7 |

**Table II. Sulfate Titration after Ion Exchange Removal of Cations**

| Added | $Ba^{++}$ Theory, Ml. | $Ba^{++}$ Actual, Ml. | Difference, % |
|---|---|---|---|
| $NaClO_4$ | 2.42 | 2.42 | ±0.0 |
| KCl | $2.63_7$ | 2.64 | +0.1 |
| $LiClO_4$ | 2.42 | 2.42 | ±0.0 |
| $NH_4ClO_4$ | $2.63_7$ | $2.63_5$ | −0.1 |
| $Cu(ClO_4)_2$ | $2.63_7$ | 2.64 | +0.1 |
| $Ca(ClO_4)_2$ | $2.63_7$ | 2.64 | +0.1 |
| $Zn(ClO_4)_2$ | $2.63_7$ | $2.63_5$ | −0.1 |
| $LaCl_3$ + HCl | $2.63_7$ | $2.68_5$ | +1.8 |
| $Al(ClO_4)_3$ | $2.63_7$ | $2.63_5$ | −0.1 |

The preliminary removal of cations by means of an ion exchange column is recommended in almost every case. This operation can be quickly accomplished and permits excellent results in the sulfate titration. Table II gives data for the titration of small amounts of sulfate following the ion exchange removal of various cations. Virtually all cations can be successfully removed by this process; only metals such as chromium (*4*) and zirconium, which form anion complexes with sulfate, cause difficulty.

Sulfate was titrated in the presence of common anions added as the appropriate acid. The error caused by different concentrations is plotted in Figure 1. Of the anions commonly associated with sulfate, the error due to phosphate is by far the most serious. It was found, however, that phosphate can be removed by precipitation with magnesium carbonate. Apparently the excess magnesium carbonate serves as a carrier to ensure complete precipitation of the phosphate. Table III gives results for titration of sulfate following the removal of phosphate by this method. Phosphate removal is essentially complete if the precipitate is filtered cold, but a small amount of phosphate apparently remains if the filtration is carried out at room temperature.

**Table III. Titration of Sulfate Following Removal of Phosphate**

| Method of $PO_4^{---}$ Removal | $Ba^{++}$ Theory, Ml. | $Ba^{++}$ Actual, Ml. | Difference, % |
|---|---|---|---|
| $MgCO_3$ cooled to 10° C. | 0.74 | 0.74 | ± 0.0 |
| | 0.74 | 0.74 | ± 0.0 |
| | 0.74 | $0.74_3$ | + 0.4 |
| $MgCO_3$ cooled to 25° C. | 0.74 | $0.75_7$ | + 2.3 |
| | 0.74 | $0.74_7$ | + 1.0 |
| Phosphate not removed | 0.74 | 0.85 | +15.0 |

Sulfate interferes seriously. Attempts to remove the sulfite by acidifying and boiling were unsuccessful. Interference can be avoided, however, by titrating the sulfite to sulfate with a standard iodine solution, and determining the total sulfate by the general procedure. The original sulfate can then be calculated by subtracting the amount of sulfate equivalent to the amount of iodine used.

## APPLICATION TO WATER ANALYSIS

The titrimetric microprocedure has been adapted to the determination of sulfate in raw water, treated city water, and in boiler water. As in the general method, cations are first removed by passage of the sample through an ion exchange column. In the conventional ion exchange method, the sample is diluted on rinsing through the column. To avoid this a continuous flow technique has been adopted. The apparatus employed is shown in Figure 2. The use of a rather coarse resin, and of a water sample large enough to give a head of pressure, permits a very rapid rate of flow through the column. After the first 30 to 50 ml. of effluent are discarded, a portion of the next effluent (which has the same sulfate concentration of the original sample) is taken for the sulfate titration. Using this method, a complete single determination of sulfate in water requires only 3 to 5 minutes.

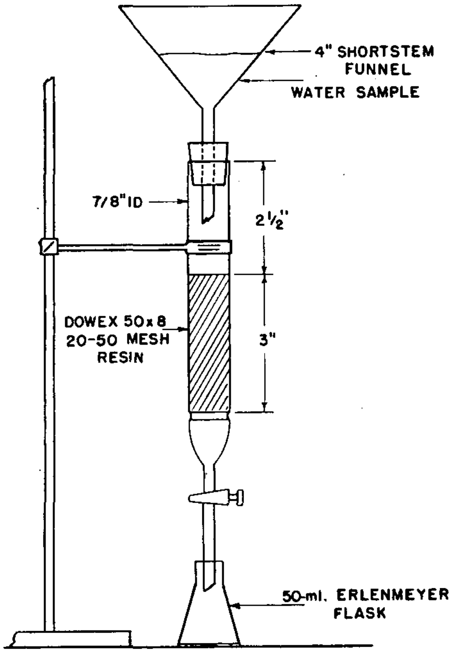

**Figure 2. Apparatus used for determination of sulfate in water**

Tables IV and V compare the results obtained in the analysis of several water samples by gravimetric and titrimetric methods. In the gravimetric method a 500-ml. sample was first passed through an ion exchange column, then concentrated by evaporation to about 250 ml. before precipitation. The samples of boiler water had been titrated with sodium phosphate and contained a slight excess over that required to treat the hardness.

## APPARATUS

The apparatus used in the general procedure for sulfate is shown in Figure 3 and that for the determination of sulfate in water is described in Figure 2. The capacity of the small column is approximately 30 meq. The columns should be regenerated when about two thirds of the capacity has been exhausted, as indicated by the progress of the lighter colored upper band.

**Table IV. Determination of Sulfate in Water**

| Sample Source | $SO_4$ Gravimetric, P.P.M. | $SO_4$ Titrimetric, P.P.M. | Difference, P.P.M. |
|---|---|---|---|
| ISC water | 118.8 | 120.2 | 1.4 |
| ISC water | 109.2 | 109.4 | 0.2 |
| Diluted ISC | 43.8 | 44.1 | 0.3 |
| Diluted ISC | 10.9 | 11.2 | 0.3 |
| Boiler water | 918 | 926 | 8 |
| Boiler water | 1203 | 1210 | 7 |
|  | 1203 | 1240[a] | 37 |

[a] Phosphate not removed.

Regeneration is carried out by passing about 100 ml. of 2 to 3*N* hydrochloric acid through the column. After thorough washing with distilled water, the column is ready for use.

## REAGENTS AND SOLUTIONS

Alcohol. Absolute ethyl alcohol, 2-propanol, methanol, or mixed ethyl alcohol and methanol as purchased commercially.

Barium perchlorate, 0.005*M*. Dissolve 2.0 grams of barium perchlorate trihydrate in 200 ml. of water and add 800 ml. of 2-propanol or ethyl alcohol. Adjust the apparent pH to about 3.5 with perchloric acid. Standardize against 0.005*M* sulfuric acid according to the general procedure.

**Table V. Titration of Diluted Samples of Raw Water**

| Dilution |  | $Ba^{++}$, Ml. | $SO_4^{-2}$, P.P.M. | $SO_4^{-2}$ Gravimetric, P.P.M. | Difference, P.P.M. |
|---|---|---|---|---|---|
| None |  | 2.46 |  |  |  |
|  |  | 2.47 |  |  |  |
|  |  | 2.47 | 115.8 | 114.9 | +0.9 |
|  | Av. | 2.47 |  |  |  |
| 10:100 |  | 0.25 |  |  |  |
|  |  | 0.25 | 11.7 | 11.5 | +0.2 |
|  | Av. | 0.25 |  |  |  |
| 20:100 |  | 0.49 |  |  |  |
|  |  | 0.50 |  |  |  |
|  |  | 0.50 | 23.4 | 23.0 | +0.4 |
|  | Av. | 0.50 |  |  |  |
| 40:100 |  | 1.00 |  |  |  |
|  |  | 1.00 | 46.9 | 46.0 | +0.9 |
|  | Av. | 1.00 |  |  |  |
| 70:100 |  | 1.73 |  |  |  |
|  |  | 1.72 |  |  |  |
|  |  | 1.73 | 81.1 | 80.4 | +0.7 |
|  | Av. | 1.73 |  |  |  |

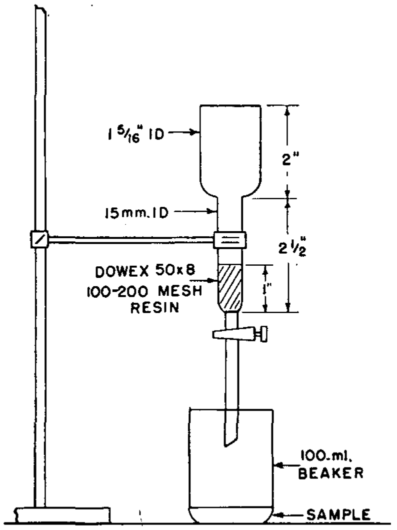

**Figure 3. Apparatus used in the general procedure for sulfate**

Sulfuric acid, 0.005*M*. Prepare a 0.005*M* solution and standardize by titration with 0.02*N* sodium hydroxide.

Thorin. Prepare a 0.2% solution in water. The Thorin used was purchased from the Hach Chemical Co., Ames, Iowa.

Starch. To 1 gram of starch add sufficient water to make a paste. To this add 100 ml. of boiling 50% glycerol.

Iodine, 0.01*M*. Dissolve 1.27 grams of recrystallized iodine and 10 grams of potassium iodide in 100 ml. of water. Dilute to 500 ml.

## PROCEDURES

**General Procedure.** Take a sample for analysis which is 10 ml. or less in volume and which contains 0.12 to 12.0 mg. of sulfate. Pass the sample through the small ion exchange column (Figure 3) and wash through with 12 to 15 ml. of wash water added in three or four portions (the liquid level should be allowed to recede to the top of the resin before each portion of wash water is added). Receive the effluent from the column in a 25-ml. volumetric flask and dilute exactly to volume. Pipet a 10-ml. aliquot of the effluent into a 100-ml. beaker, and add 40 ml. of alcohol and 1 drop of Thorin indicator. Titrate with $0.005M$ barium perchlorate, taking the first permanent change from yellow or yellow-orange to pink as the end point. Add the bulk of the titrant rapidly, but allow 2 or 3 seconds between addition of the last few increments.

The total salt content of the sample (including sulfate and foreign salts) should not exceed about 0.6 meq.; otherwise the pH of the solution after passage through the ion exchange column will be too low. If the salt concentration is not known, determine the pH of a small portion of the 25-ml. effluent; a pH lower than 1.5 indicates that additional dilution is required. Discard this portion of the effluent, as it will be contaminated with potassium chloride from the calomel electrode.

**Procedure for Sulfate in Water.** Fill the reservoir of the larger ion exchange column (Figure 2) with the water sample and pass through the column at a rapid rate. Discard the first 40- or 50-ml. portion of the effluent. Pipet 10 ml. of the next portion into a small beaker or flask, add 40 ml. of alcohol and 1 drop of Thorin indicator, and titrate with $0.005M$ barium perchlorate as described in the general procedure.

In the presence of sulfite and phosphate (as in boiler water), pipet 25 ml. of the water into a 100-ml. beaker. Titrate the sulfite with $0.01M$ iodine solution using 1 ml. of starch solution as the indicator. Add 10 drops of $0.1M$ perchloric acid and 0.3 gram of magnesium carbonate. Boil gently for 5 minutes, cool to 10° C., and filter (using Schleicher & Schuell No. 589 filter paper or equivalent) directly into the small ion exchange column (Figure 3). Titrate a 10-ml. aliquot with $0.005M$ barium perchlorate according to the general procedure. This gives the total sulfate. The original sulfate is the total sulfate less the amount of sulfite determined iodometrically.

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# Determination of Carboxylic Acid Anhydrides by Reaction with Morpholine

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**A titrimetric method employing an indicator was sought for the direct determination of carboxylic acid anhydrides in the presence of and to the exclusion of the corresponding acids. A satisfactory method was developed based on the reaction of the anhydride with morpholine and titration of the excess reagent with methanolic hydrochloric acid. Data are presented on the determination of the purity of eight anhydrides and the determination of low concentrations of acetic anhydride in glacial acetic acid. The procedure is rapid, generally applicable to a variety of acid anhydrides, and accurate and precise over a wide range of concentrations.**

EARLIER methods for the determination of carboxylic acid anhydrides were based on the simultaneous measurement of the acid and the anhydride. Either the aniline reaction of Radcliffe and Medofski (*6*) or the sodium methylate titration of Smith and Bryant (*9*) was used in conjunction with a total hydrolysis with sodium hydroxide to arrive at the anhydride and acid content of a sample.

Two direct methods for the determination of anhydrides, which do not involve the measurement of the acid originally present in the sample, have been reported. Smith, Bryant, and Mitchell developed a procedure employing the use of Karl Fischer reagent (*10, 11*). Malm and Nadeau (*4*) determined acetic anhydride in cellulose esterification baths by reaction with aniline and titration of the excess aniline by mineral acid in nonaqueous solution. A general procedure employing this principle was developed by Siggia and Hanna (*8*), in which the excess aniline is titrated potentiometrically in the glycol–2-propanol medium employed by Palit (*5*).

The method described in this paper also measures the anhydride independent of the acid content of the sample. It possesses the speed and convenience of an indicator titration and excellent precision for both high and low concentrations of anhydride.

The principle was originally described at the April 1951 Round Table Discussions on Titrations in Nonaqueous Solutions (*7*), and later its application to the determination of chrysanthemum acid anhydride in commercial allethrin was reported (*3*).

Morpholine reacts with carboxylic acid anhydrides to produce equimolar quantities of amide and acid. In a methanolic medium using mixed methyl yellow–methylene blue indicator, all the components of the reaction are neutral except morpholine. If a measured excess of morpholine reacts with a sample containing anhydride, the anhydride reacts preferentially with morpholine, and the excess can be titrated with standard methanolic hydrochloric acid. The morpholine consumed, represented by the difference in titration between a blank and sample, is a measure of anhydride.

**Table I. Purity of Anhydrides by Morpholine Method**[a]

| Anhydride | Minimum Reaction Time at Room Temperature, Min. | Purity, Wt. %[b] | Acid Content, Wt. %[c] | Total, Wt. % |
|---|---|---|---|---|
| Acetic | 5 | 99.7 ± 0.1 (5) | 0.2 (2) | 99.9 |
| Butyric | 5 | 98.0 ± 0.2 (4) | 1.9 (2) | 99.9 |
| Chrysanthemum | 5 | 98.6 ± 0.1 (2) | 0.5 (2) | 99.1 |
| 2-Ethylhexanoic | 30 | 98.9 ± 0.2 (6) | 0.1 (2) | 99.0 |
| Glutaric | 5 | 97.1 ± 0.1 (3) | 3.4 (2) | 100.5 |
| Phthalic | 5 | 99.6 ± 0.1 (3) | 0.3 (2) | 99.9 |
| Propionic | 5 | 99.5 ± 0.1 (3) | 0.5 (2) | 100.0 |
| Succinic | 5 | 96.9 ± 0.1 (5) | 2.4 (2) | 99.3 |

[a] $0.5N$ reagents.
[b] Figures in parentheses indicate number of analyses.
[c] Calculated from difference between Radcliffe-Medofski (*6*) and morpholine reactions.

**Table II. Determination of Acetic Anhydride in Glacial Acetic Acid by the Morpholine Method**

| | Wt. %[a] |
|---|---|
| Sample 1 | |
| Anhydride added | 0.065 |
| Anhydride found | 0.066 ± 0.002 (5) |
| Sample 2 | |
| Anhydride added | 0.011 |
| Anhydride found | 0.016 ± 0.007 (4) |

[a] Figures in parentheses indicate number of analyses.

When used in conjunction with other procedures which measure total acid plus anhydride (*6*, *9*), the amount of acid originally present in the sample also may be determined.

## REAGENTS

**Hydrochloric Acid, 0.5*N* Methanolic Solution.** Transfer 84 ml. of 6*N* hydrochloric acid to a 1000-ml. volumetric flask and dilute to volume with methanol. Standardize daily against standard 0.5*N* sodium hydroxide using phenolphthalein indicator. The reagent is best handled in an automatic buret assembly.

**Morpholine Solution, 0.5*N* Methanolic Solution.** Transfer 44 ml. of redistilled morpholine to a 1-liter reagent bottle and dilute to 1 liter with methanol. Fit the bottle with a two-hole rubber stopper and through one hole insert a 50-ml. pipet so that the tip extends below the surface of the liquid; through the other hole insert a short piece of glass tubing to which is attached a rubber atomizer bulb.

**Methyl Yellow–Methylene Blue Mixed Indicator.** Dissolve 1.0 gram of methyl yellow (*p*-dimethylaminoazobenzene) and 0.1 gram of methylene blue in 125 ml. of methanol.

## PROCEDURE

Carefully pipet 50 ml. of the morpholine solution into each of two 250-ml. glass-stoppered flasks. Fill the pipet by exerting pressure in the reagent bottle with the atomizer bulb.

Reserve one of the flasks as a blank and introduce not more than 20 meq. of anhydride into the second flask. Swirl the flask to effect solution. Allow both the sample and blank to stand at room temperature for the time indicated for each specific anhydride (Table I).

Add 4 or 5 drops of indicator to each flask and titrate with the 0.5*N* acid to the disappearance of the green color. At this point the color is best described as amber.

The difference in titration between blank and sample is a measure of anhydride.

## RESULTS

The data obtained on eight anhydrides are shown in Table I. All samples used were commercial grade materials containing some of the corresponding acid and, in some cases, small but significant amounts of other impurities. In order to show the recoveries to be expected from the morpholine procedure, the amount of acid in each sample is also shown. This was determined by measuring the acid after reaction with aniline according to the procedure of Radcliffe and Medofski (*6*) and correcting for the anhydride present in the sample.

Table II shows the accuracy and precision obtained in the determination of low concentrations of acetic anhydride in acetic acid.

## DISCUSSION

**Reaction Rates.** Rate studies of the reaction of acetic anhydride with 0.02, 0.1, and 0.5*N* morpholine indicate that the reaction is practically instantaneous, being quantitative in less than 30 seconds. With the exception of 2-ethylhexanoic anhydride, which requires a 30-minute reaction, all the anhydrides in this work react quantitatively within 5 minutes at room temperature, and in most cases the reaction is complete in less than 1 minute.

**Potentiometric Titration.** Potentiometric titration studies showed that the indicator end point occurs on the steep break of the curve. The true equivalence point does not exactly coincide with the indicator end point described, but the error is not significant, particularly in view of the fact that the blank titration is in error to nearly the same degree. A number of indicators have been tried, but none has been found superior to a methyl yellow–methylene blue mixture.

The method is not applicable to anhydrides if the acids have ionization constants in water greater than $2 \times 10^{-2}$, since such acids are somewhat acidic to the indicator in a methanol medium. Thus, maleic and citraconic anhydrides cannot be determined by this method. Potentiometric titrations could possibly be substituted satisfactorily in these cases.

**Effect of Solvents.** Siggia and Hanna (*8*) state that they were unable to obtain satisfactory indicator end points in acetic acid medium when aniline was used as the reactant. As a result they resorted to potentiometric titrations in an ethylene glycol–2-propanol, medium. The poor end points in the acetic acid medium were undoubtedly due to the enhanced basicity of the resulting amide. Amides are appreciably more basic in acetic acid than in methanol solution.

This adverse effect of amides on the titration of free amino groups in acetic acid medium is also apparent in the determination of tertiary amines by the acetylation procedure of Blumrick and Bandel (*1*) and Wagner, Brown, and Peters (*12*). In these laboratories, tertiary amine content is also determined in a methanol medium after acetylating primary and secondary amines with acetic anhydride (*2*). A report of this work possibly will be published at a later date.

**Reaction with Other Amines.** Morpholine has been selected for use in the method because of its high reactivity with anhydrides and because it is a secondary amine which should be subject to less interferences than compounds having primary amino groups. Obviously, in certain cases other amines, primary or secondary, could be substituted for morpholine as the reagent.

**Low Concentrations.** For the determination of low concentrations of anhydride, it is convenient to use 0.1*N* reagents as previously described for chrysanthemum anhydride in allethrin (*3*). For the determination of acetic anhydride in glacial acetic acid, a 10-ml. sample was made to react with 50 ml. of 0.02*N* morpholine. Excess morpholine was titrated with 0.1*N* methanolic hydrochloric acid. Samples in excess of 10 ml. are not recommended, inasmuch as the resultant amides tend to buffer the end points if more than this amount of acid is present (see above).

**Interferences.** The only known interferences are compounds which can react with morpholine to destroy its basic behavior. These include ketene and diketene, which react quantitatively under certain conditions, and acid chlorides, which can react quantitatively only in a dilute solution, as they react with both morpholine and methanol. If a dilute solution of an acid chloride is added slowly to the reagent while the latter is being swirled, the morpholine reacts preferentially with the acid chloride. Under these conditions the equivalent weight of the acid chloride is one half the molecular weight as a mole of amide and a mole of mineral acid are formed. Mineral acids, of course, interfere. In certain cases the interference is quantitative and appropriate corrections may be applied.

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# Group-Type Nitrogen-Hydrogen Analysis of Pyrrole-Indole-Carbazole Type Compounds

## An Infrared Absorption Method

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**The absorptivities for the free nitrogen-hydrogen stretching frequency in the 3480-cm.$^{-1}$ region for six compounds of the pyrrole-indole-carbazole type of molecules were found to be reasonably constant. This makes it possible to determine total pyrrole-indole-carbazole type nitrogen-hydrogen by the usual group-type method. Errors resulting from the use of a group-type calibration curve and the nonconstancy of band position have been evaluated. The method can be applied to Diesel-type fuel oils by the use of a base-line technique to correct for hydrocarbon interference in the 3480-cm.$^{-1}$ region.**

A QUICK and simple method is presented for determining the approximate concentration of nitrogen-hydrogen type compounds of the pyrrole-indole-carbazole classes in materials such as Diesel oils by infrared absorption. It is a group-type method and as such it provides the total concentration of these types of materials under the limitations of accuracy imposed by the use of an average absorptivity.

Thompson and coworkers (*8*) demonstrated the presence of pyrrolic nitrogen in certain distillate fuel oils and the possible effect of these compounds on the stability of the oils. The nitrogen content of these oils generally is less than 0.05%, sulfur less than 1%, and ash less than 0.01%. The greater relative increase in nitrogen content as compared to sulfur content in going from fuel oil to sludge suggests that certain nitrogen compounds may play a more active part than the sulfur components in the reactions leading to sludging, and this has been the basis for studies of nitrogen compounds in fuel oils (*1*, *2*, *5–7*, *9*, *10*).

Nitrogen compounds of the pyrrole type, which are invariably accompanied by indole- and carbazole-type compounds, are included in the group of suspected sludge-forming compounds. An infrared absorption peak at about 3480 cm.$^{-1}$ does indeed appear in the spectra of dilute solutions of many Diesel-type fuel oils and is ascribed to the stretching frequency of the N-H group present in the type compounds shown in Table I (*3*).

**Table I. Position of N-H Absorption Band in Dilute Carbon Tetrachloride Solution (16-Mm. Cell)**

| Compound | Frequency, Cm.$^{-1}$ |
|---|---|
| Pyrrole | 3492 |
| 2,5-Dimethylpyrrole | 3475 |
| Indole | 3489 |
| 3-Methylindole | 3489 |
| 2-Phenylindole | 3479 |
| Carbazole | 3479 |

### EXPERIMENTAL

All the data were obtained with dilute solutions in carbon tetrachloride with a Perkin-Elmer Model 12B spectrometer equipped with a lithium fluoride prism. The use of a cell having sodium chloride faces shellacked onto a glass body and a path length of approximately 16 mm. permitted the material to be examined in sufficiently dilute solutions so that hydrogen bonding effects are practically eliminated. It also allows the determination of absorptivity values completely corrected for cell and solvent absorption effects. In calibrations or analyses the nitrogen-hydrogen transmittance minimum was located by manual scanning in the 3480-cm.$^{-1}$ region and the data then were obtained at this point.

The $I_0$ values used in calculating the corrected absorbances were obtained with the cell filled only with solvent. A fixed spectrometer slit width of 0.230 mm. was employed. The method of utilizing the transmittance minimum and an average absorptivity was chosen rather than a method utilizing integrated absorptivities in the interest of rapidity of analyses. The compounds shown in Table I were used for calibration; the concentration range is shown in Figure 1. Indole, carbazole, and 2-phenyl indole (Eastman Kodak white label) and 3-methylindole (Bios Laboratories) are white crystalline solids; both pyrroles are liquid and were freshly distilled prior to their use in obtaining calibration data.

The band positions vary over a range of 17 cm.$^{-1}$ and this imposes a limitation on the expected absolute accuracy of the method. The calibration curves for the individual compounds dissolved in carbon tetrachloride are shown in superposition in Figure 1 as absorbance (cell plus solvent corrected) versus concentration in millimoles per liter. These curves were determined at the transmittance minimum specific to each compound shown in Table I. It is apparent that these curves do not obey Beer's law under the experimental conditions used in the analysis. However, Linnell (*4*) has reported that pyrrole itself does indeed obey Beer's law when data are obtained under conditions of resolution which apparently are higher than used in this study.

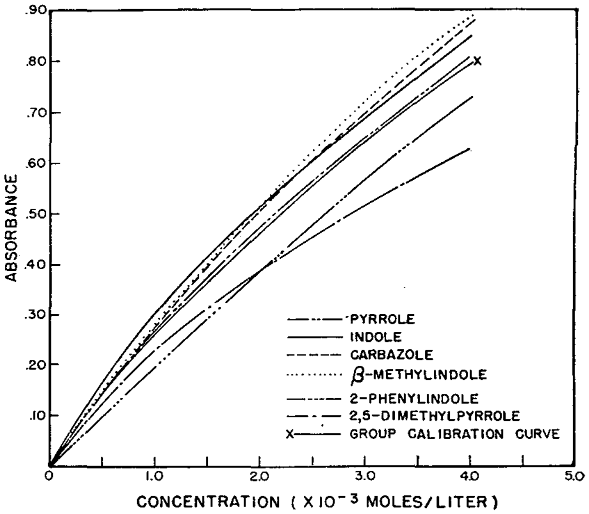

**Figure 1. Calibration curves for N-H compounds in 3480$^{-1}$ region**

Dilute $CCl_4$ solutions with 16-mm. cell

The absorbances at fixed concentrations were averaged from the six calibration curves, and these averages then were used to plot the group calibration curve, also shown in Figure 1. (The group curve is very close to the calibration curve of pyrrole itself.)

### ACCURACY LIMITATIONS DUE TO SPECTRAL VARIATIONS

Errors in analyzing mixtures of these materials for the total concentration of the class obviously can arise from two sources: nonconstancy of band positions, and variations in individual

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absorptivities. In applying this method these two effects are intermingled and cannot be evaluated independently. The total errors from both sources were determined by blending mixtures of known concentrations and random compositions and analyzing them for total class concentration. Prior to this it was of interest to evaluate the approximate range of error that would result when a mixture of two of the class was analyzed using data specific to one of them. It was felt that this would give some indication of the errors that could result from the nonconstancy in band position. In determining the total nitrogen-hydrogen content for each mixture, it was assumed that the total material was one or the other of the two components, and the appropriate calibration curve (Figure 1) specific to that component was used. The results are shown in Table II. From Table I it can be seen that for mixtures of pyrrole and carbazole one would expect a large error due to the separation of absorption peaks (13 cm.$^{-1}$). The smaller separation between the absorption peaks of indole and pyrrole (3 cm.$^{-1}$) would lead one to expect a smaller error in the analysis. As seen in the table, the results for the carbazole-pyrrole mixture are the poorest, as expected.

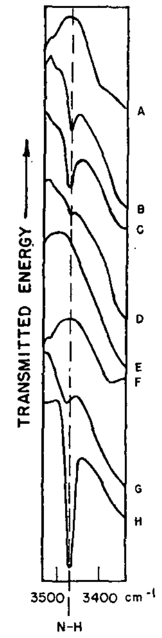

**Figure 2. Qualitative spectra of typical fuel oils in N-H absorption region**

Spectra have been displaced vertically

To evaluate the errors for mixtures by the use of an average absorptivity curve, synthetic mixtures containing various compositions of the different compounds were prepared and analyzed. Some typical results are shown in Table III. An analysis of eighteen synthetics of the types that appear in this table gave a standard deviation of 0.34 × 10$^{-3}$ mole per liter. Such a large standard deviation is a natural consequence of the inherent limitations due to variations in wave length of minimum transmission and in absorptivities. Errors of this magnitude make the method unsuitable for analyses requiring higher precision and accuracy. However, they can be tolerated in many cases wherein an approximate value is required by a simple analysis.

## MODIFICATION OF METHOD FOR USE ON DIESEL-TYPE OILS

A major purpose of these investigations was to determine total nitrogen-hydrogen content of the pyrrole-indole-carbazole types in Diesel-type oils. It is anticipated that such information would aid in the evaluation of the role such compounds play in sludge formation and discoloration. Hence, it was necessary to determine the background interference in applying the method to typical distillate fuel oils. The synthetic samples discussed above evaluated the limitations on accuracy for sample types where there was no optical interference. However, in Diesel-type oils the total nitrogen-hydrogen content is very low, and the background absorbance due to the other components in the oil at the analytical point can be appreciable. This background absorbance might be expected to be appreciable because of the proximity of the heavy absorption arising from the carbon-hydrogen stretching vibrations in the hydrocarbons. Consequently, in order to determine the nitrogen-hydrogen absorbance of these oils, it was necessary to evaluate the interference and make appropriate corrections for it. Several typical fuel oils were examined qualitatively for nitrogen-hydrogen content by inspection of the nitrogen-hydrogen absorption region in the recorded spectra. These spectra are shown in Figure 2. Oils A (37.4° American Petroleum Institute gravity, from Kuwait and Eastern Venezuelan crudes) and F (42.3° American Petroleum Institute gravity, from Mid-Continent crudes) show no qualitative evidence for the presence of detectable amounts of nitrogen-hydrogen components in the 3480-cm.$^{-1}$ region. These two oils, then, were chosen as the base oils to which known concentrations of nitrogen-hydrogen compounds were added and analyzed.

**Table II. Analysis of Synthetics to Evaluate Effect of Nonconstancy of Transmittance Minima on Accuracy**

| | Total N-H Content, Moles/Liter | | | |
|---|---|---|---|---|
| Components | Synthetic | Analyzed as indole | Analyzed as carbazole | Analyzed as pyrrole |
| Carbazole-indole | 2.23 × 10$^{-3}$ | 2.15 × 10$^{-3}$ | 2.18 × 10$^{-3}$ | ..... |
| Carbazole-pyrrole | 2.31 × 10$^{-3}$ | ..... | 1.78 × 10$^{-3}$ | 1.92 × 10$^{-3}$ |
| Indole-pyrrole | 2.41 × 10$^{-3}$ | 2.38 × 10$^{-3}$ | ..... | 2.57 × 10$^{-3}$ |

**Table III. Group-Type Analyses for Total N-H Content for Synthetic Samples**

| Sample No. | No. of Components | Total N-H Content, Mole/Liter | |
|---|---|---|---|
| | | Synthetic | Observed |
| 1 | 1 | 2.12 × 10$^{-3}$ | 2.32 × 10$^{-3}$ |
| 2 | 2 | 2.23 | 2.43 |
| 3 | 3 | 2.36 | 2.27 |
| 4 | 6 | 2.36 | 2.03 |
| 5 | 6 | 2.11 | 2.00 |
| 6 | 6 | 2.93 | 2.53 |

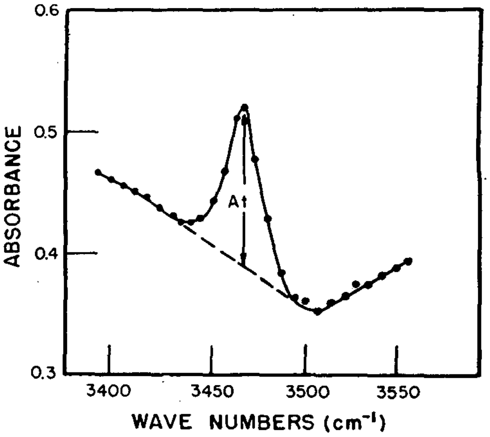

**Figure 3. Base-line method for determining total N-H absorbance in fuel oils**

— — — — Extrapolated base line
$A_t$ = Absorbance of N-H compounds
2 ml. (1.7256 grams) of oil diluted with 20 ml. of carbon tetrachloride. Each of the absorbance points has been corrected for cell plus solvent

The analysis was carried out by manually scanning the solution containing a known amount of nitrogen-hydrogen compound (indole or 3-methylindole) in a fixed volume of oil dissolved in carbon tetrachloride. Point-by-point absorbance values in the region of 3400–3570 cm.$^{-1}$ were obtained for this solution contained in the 16-mm. cell, against a suitable dummy. Corresponding point-by-point measurements then were obtained with the cell filled only with carbon tetrachloride. From the two sets of data, the absorbance of the oil plus nitrogen-hydrogen compound was calculated for each point. This corrected absorbance was plotted against the wave numbers (cm.$^{-1}$). A straight line then was drawn through the points of minimum absorbance on either side of the absorption maximum to give the "base line." The true absorbance, $A_t$, of the nitrogen-hydrogen compound was taken as the length of the ordinate between the spectral curve (at its maximum) and the extrapolated base line. Figure 3 shows the base-line method as applied to an actual Diesel fuel.

In order to evaluate the accuracy of this base-line procedure, only one nitrogen-hydrogen compound at a time was added to the oil as a synthetic and the specific calibration curve of this compound (from Figure 1) was used in the calculations. This procedure was employed solely to evaluate the errors due to the fuel oil interference without the complications associated with the use of a group absorptivity. The data for these tests are given in Table IV, showing the amounts of nitrogen-hydrogen component added to the fuel oils and the amounts determined. A straightforward application of the previous data was employed, except that the concentrations have been converted into weight per cent nitrogen.

It is clear from above results that the base-line technique overcorrects so that the observed concentrations are about 11% low, on the average, when calculated directly. Although there is considerable scattering in the degree to which the observed results are low, it is recommended that the average be used as an empirical correction factor when analyzing actual distillate oils by this group-type method.

**Table IV. Synthetic Samples of N-H Compounds in Fuel Oils**

(Analyzed by base-line method for specific component)

| Synthetic | Oil | N-H Component | N-H Content Calculated as Wt., % N in Oil: Synthetic | Observed | Difference |
|---|---|---|---|---|---|
| 1 | A | Indole | 0.022 | 0.018 | −0.004 |
| 2 | A | 3-Methylindole | 0.025 | 0.022 | −0.003 |
| 3 | F | Indole | 0.022 | 0.020 | −0.002 |
| 4 | F | 3-Methylindole | 0.026 | 0.023 | −0.003 |
| 5 | F | Indole | 0.044 | 0.040 | −0.004 |
| 6 | F | 3-Methylindole | 0.052 | 0.049 | −0.003 |
| 7 | F | Indole | 0.0069 | 0.0059 | −0.001 |

The spectra in Figure 2 show that not all oils are sufficiently high in nitrogen-hydrogen components to permit one to apply this spectral method, except to set a limit (approximately 0.002% nitrogen by weight) to the minimal nitrogen-hydrogen concentration necessary before it can be determined by this method. In special cases, less than 0.002% nitrogen in the form of nitrogen-hydrogen compounds probably could be determined by slightly modified techniques. The low content of nitrogen-hydrogen components in certain fuel oils may actually prove to be of value in predicting fuel oil stability. For the examples recorded in Figure 2, good storage stability was found for A and E, poor stability for B and C, as well as fuel oil blends which contained G and H. For comparison purposes as to order of magnitude, the catalytic cracked fuel oil shown as *H* in Figure 2 was found to contain 0.016% nitrogen in the form of nitrogen-hydrogen compounds.

These nitrogen-hydrogen components have been concentrated by chromatographic procedures here and elsewhere (*7*, *10*). These studies have shown carbazole-type compounds to be relatively more abundant in distillate fuels than pyrrole- and indole-type compounds—i.e., compounds containing at least one hydrogen atom on a carbon atom in the pyrrole nucleus as determined colorimetrically by the method of Thompson, Symon, and Wankat (*9*). This greater abundance in fuel oils of carbazoles relative to pyrroles is also shown in Table V, which is a typical analysis of nitrogen compounds for a 50-50 mixture of virgin distillate and catalytically cracked fuel oil (*E* and *H* of Figure 2). The base-line technique for measuring the absorbance, $A_t$, due to the nitrogen-hydrogen compounds in this mixture is illustrated in Figure 3.

**Table V. Nitrogen Content of a 50-50 Volume Mixture of Virgin Distillate[a] and Catalytically Cracked[b] Fuel Oil**

| Nitrogen Compounds As | Nitrogen, Wt. % |
|---|---|
| Total nitrogen (Kjeldahl) | 0.02 |
| Basic nitrogen (perchloric acid method)[c] | 0.004 |
| Nitrogen as pyrrole and indole (*9*)[d] | 0.0015 |
| Nitrogen as pyrrole, indole, and carbazole | 0.008[e] |

[a] Curve E of Figure 2.

[b] Curve H of Figure 2.

[c] A colorimetric procedure with methyl violet as the indicator and 1:1 benzene–acetic acid as the solvent was substituted for the potentiometric method of Deal (*2*).

[d] Pyrroles having at least one hydrogen atom on a carbon atom in the pyrrole nucleus.

[e] The concentration value (millimoles/liter from Figure 1) is multiplied by $\frac{14.008 \times V \times 10^{-4}}{w}$, where $V$ is the total volume in milliliters to which the oil was diluted with carbon tetrachloride, and $w$ is the weight of oil sample in grams.

In a typical analysis for nitrogen-hydrogen content, the procedure is to dilute a weighed quantity of oil to 25 ml. with spectral grade carbon tetrachloride. The sample amount will vary, of course, with different oils but in order to get absorbance of about 0.5 at the nitrogen-hydrogen absorption maximum, 1.5 to 4 grams of oil will be required. The same procedure is followed as described previously for obtaining the nitrogen-hydrogen absorption maximum where a known nitrogen-hydrogen compound had been added to an oil.

There is the possibility that interferences in this analysis could arise from the presence of certain functional groups which are not due to the nitrogen-hydrogen of the pyrrole-indole-carbazole types. The carboxylic acid hydroxyl group stretching frequency occurs at approximately 3520 cm.$^{-1}$ Unsubstituted aromatic amines have two bands due to nitrogen-hydrogen stretching at about 3400 and 3480 cm.$^{-1}$, while nitrogen-substituted amines have a single band at approximately 3430 cm.$^{-1}$ Certain amides exhibit a free nitrogen-hydrogen stretching frequency at about 3450 cm.$^{-1}$ Interferences from the above types of materials can generally be recognized by studying the complete spectrum of the sample, or by simple chemical tests. Such compounds have not been detected spectrally in any of the fuel oils studied to date. Aliphatic amines should give no interference because of their very low absorptivity in the fundamental N-H stretching region.

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# Amperometric Determination of Ethylenediaminetetraacetic Acid with Zinc Ions

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**A method for the determination of total and available ethylenediaminetetraacetic acid in solutions containing the calcium or magnesium chelates is described. The solution is titrated amperometrically with zinc sulfate at a low pH for total ethylenediaminetetraacetic acid. The available ethylenediaminetetraacetic acid is determined at a higher pH when calcium is not displaced from its chelate. If magnesium is present, glycine is added to prevent the zinc displacing the magnesium from its chelate with ethylenediaminetetraacetic acid.**

THE sodium salts of ethylenediaminetetraacetic acid (EDTA) recently have become prominent owing to their ability to form soluble chelate compounds with many metal ions. The chelate formed with calcium ions is of special interest, and because the formation of this particular chelate ethylenediaminetetraacetic acid is applicable in many industrial applications. In investigating the use of ethylenediaminetetraacetic acid for dissolving scale from factory evaporator tubes, determination of both the free ethylenediaminetetraacetic acid and the calcium chelate in the aqueous solutions used was required. These solutions acquired a deep reddish-brown color during their use. The available methods (*1–4*) for the determination of ethylenediaminetetraacetic acid and its chelates were found to be in this case unsatisfactory.

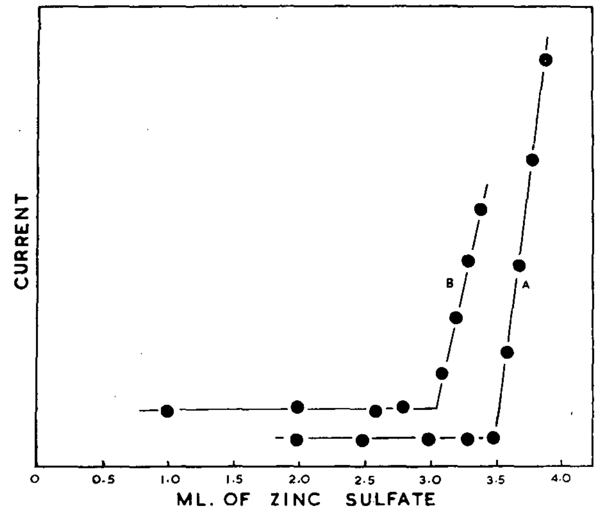

**Figure 1. Amperometric titration curves**

*A.* 10 ml. of 0.0171*M* EDTA in 25 ml. of acetate buffer, titrated with 0.0493*M* $ZnSO_4$

*B.* 15 ml. of 0.0171*M* EDTA, 5 ml. of 0.0199*M* $CaCl_2$, in 25 ml. of 0.1*N* KOH, titrated with 0.0515*M* $ZnSO_4$

Many workers have studied the polarography of the metal chelates of ethylenediaminetetraacetic acid, and, therefore, it should be possible to titrate amperometrically the ethylenediaminetetraacetic acid with a metal ion, the chelate of which was not reduced at the dropping mercury electrode. The zinc chelate was found to give no reduction wave, at least in the buffers considered, while reduction of free zinc or zincate ions indicated well defined polarographic waves. The addition of zinc ions to a solution containing ethylenediaminetetraacetic acid results in no polarographic wave until free zinc ions are in excess of those required to chelate all of the ethylenediaminetetraacetic acid.

## DETERMINATION OF TOTAL ETHYLENEDIAMINETETRAACETIC ACID

**Experimental.** A sample of pure ethylenediaminetetraacetic acid was prepared by precipitation from a solution of analytical grade disodium salt of ethylenediaminetetraacetic acid with hydrochloric acid. The acid was recrystallized from water and dried. A standard solution of ethylenediaminetetraacetic acid was prepared by dissolving a weighed amount of this acid in sufficient potassium hydroxide to form the dipotassium salt. A standard zinc solution was prepared by dissolving (analytical reagent grade) zinc in dilute sulfuric acid and partly neutralizing with sodium hydroxide.

A known amount of the ethylenediaminetetraacetic acid solution was pipetted into a polarographic cell containing 25 ml. of a buffer, 0.5*N* in both acetic acid and sodium acetate. The dissolved oxygen was then removed by gassing out with nitrogen; the applied potential was kept constant at −1.3 volts (*vs.* saturated calomel electrode) and the solution was titrated with 0.0515*M* zinc sulfate solution added from a microburet.

**Results.** A typical titration curve shown in Figure 1, *A*, is the expected form. Such curves were obtained in solutions containing the ethylenediaminetetraacetic acid chelates of calcium or magnesium and the current readings became constant almost immediately, indicating that the displacement of calcium and magnesium ions by zinc ions was very rapid under these conditions. Some of the results obtained by this method are given in Table I.

**Table I. Determination of Total Ethylenediaminetetraacetic Acid by Titration in Acetate Buffer**

| Substance Added | Moles of Added Substance × 10⁵ | Titration, Ml. of 0.0515*M* Zinc Sulfate | Total EDTA, Moles × 10⁵ | |
|---|---|---|---|---|
| | | | Added | Found |
| ... | 0 | 0.00 | 0 | 0 |
| ... | 0 | 0.35 | 1.8 | 1.8 |
| ... | 0 | 1.17 | 6.1 | 6.0 |
| ... | 0 | 2.37 | 12.2 | 12.2 |
| ... | 0 | 1.72 | 9.0 | 8.9 |
| $CaCl_2$ | 80 | 1.73 | 9.0 | 9.0 |
| $MgSO_4$ | 72 | 1.74 | 9.0 | 9.0 |
| $Na_2SO_4$ | 2100 | 1.74 | 9.0 | 9.0 |
| $Na_2HPO_4$ | 100 | 1.73 | 9.0 | 9.0 |

All determinations were carried out in acetate buffer of pH 4.6, but further experiments showed that the method is valid at least over the range from pH 3.5 to 5.5.

The accuracy of the determination depends upon the accuracy of reading the end point of the titration curve (Figure 1). This accuracy was found to be within 0.02 ml., or within ±1% when approximately 2 ml. of the zinc solution are required for the titration. The experiments were completely reproducible within the accuracy of the determination.

Among other uses of the method, are the determination of ethylenediaminetetraacetic acid content of commercial samples (giving very satisfactory results), the standardizing of solutions made from analytical grade sodium salts of ethylenediaminetetraacetic acid, and a convenient way of standardizing zinc solutions by a volumetric method. The use of an amperometric titration to determine zinc has been published previously (*6, 7*).

### DETERMINATION OF AVAILABLE ETHYLENEDIAMINETETRAACETIC ACID

Although zinc is able to displace calcium from its ethylenediaminetetraacetic acid chelate under acid or neutral conditions, it seemed possible that conditions could be found where zinc is not able to displace calcium. If this were so, the available ethylenediaminetetraacetic acid—i.e., that not chelated by calcium—could be determined in solutions containing both ethylenediaminetetraacetic acid and its calcium chelate. If the amperometric titration was carried out in an alkaline medium then zinc could not displace calcium from its chelate, but calcium could displace zinc from the zinc chelate with ethylenediaminetetraacetic acid. The same effect was described by Laitinen and Sympson (*5*) in a method of titrating calcium amperometrically.

The results obtained for the determination of free ethylenediaminetetraacetic acid in solutions containing calcium are given in Table II. In this method $0.1N$ potassium hydroxide solution was substituted for the acetate buffer and the applied potential was $-1.7$ volts (*vs.* S.C.E.). A typical titration curve is shown in Figure 1, *B*. The potassium hydroxide concentration must range from 0.05 to $0.5N$ in order to obtain satisfactory titration.

**Table II. Determination of Available Ethylenediaminetetraacetic Acid in Solutions**

| Total EDTA, Moles × $10^5$ | Calcium, Moles × $10^5$ | Titration, Ml. $0.0515M$ Zinc Sulfate | Available EDTA, Moles × $10^5$ | |
|---|---|---|---|---|
| | | | Theoretical | Found |
| 3.4 | 0 | 0.66 | 3.4 | 3.4 |
| 8.6 | 0 | 1.64 | 8.6 | 8.5 |
| 17.1 | 0 | 3.33 | 17.1 | 17.2 |
| 25.7 | 9.9 | 3.05 | 15.8 | 15.7 |
| 17.1 | 9.9 | 1.37 | 7.2 | 7.1 |
| 8.6 | 4.0 | 0.87 | 4.6 | 4.5 |
| 8.6 | 8.0 | 0.10 | 0.6 | 0.5 |

The results of Table II show that in solutions containing both free ethylenediaminetetraacetic acid and ethylenediaminetetraacetic acid combined with calcium, the free ethylenediaminetetraacetic acid can be determined by amperometric titration in alkaline solution with zinc sulfate.

However, when the same method was applied to solutions containing magnesium, the zinc could displace magnesium from its chelate with ethylenediaminetetraacetic acid when the titration was carried out in a potassium hydroxide solution. A titration curve similar to Figure 2, *D*, was obtained. Unlike the previous titration curves obtained, no definite end point was observed; but, when a large amount of zinc was added the titration curve tended toward a straight line which extrapolated to the point corresponding to the total ethylenediaminetetraacetic acid in solution. This behavior shows that in the presence of a large hydroxyl ion concentration zinc is just able to displace magnesium from its chelate and does so completely only when in excess.

In order, therefore, to prevent zinc from displacing magnesium a substance must be added to the solution which sets up another equilibrium with the zinc ions present and further reduces the amount of free zinc ions available for ethylenediaminetetraacetic acid chelation.

Alkaline tartrate was first tried, but this chelate was so strong that the zinc could not chelate with ethylenediaminetetraacetic acid to any extent. Therefore, no titration curve was obtained (Figure 2, *A*). In an ammonia–ammonium chloride buffer, zinc was still able to displace magnesium. However, glycine was found to possess the desired characteristics. Titration curves obtained with two concentrations of glycine are shown in Figure 2, *B* and *C*. With a buffer containing small concentrations of glycine the magnesium was displaced from its chelate, but as the glycine concentration was increased an end point was observed at the titration corresponding to the amount of free ethylenediaminetetraacetic acid (Figure 2, *B*).

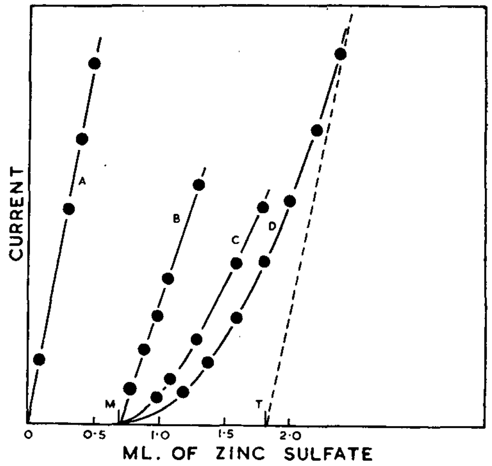

**Figure 2. Amperometric titration curves of EDTA solutions containing magnesium**

*A*. 5 ml. of $0.0171M$ EDTA, 2 ml. of $0.0204M$ $MgSO_4$ in 20 ml. of tartrate buffer (10% potassium hydrogen tartrate in $2N$ NaOH)

*B, C, D*. 5 ml. of $0.0171M$ EDTA, 2 ml. of $0.0204M$ $MgSO_4$, titrated with $0.0493M$ $ZnSO_4$, in presence of:

- *B*. 20 ml. of glycine buffer ($0.5M$ in KOH and $0.5M$ in glycine)
- *C*. 5 ml. of glycine buffer and 15 ml. of water
- *D*. 2 ml. of glycine and 18 ml. of water

*M*. Theoretical end point for available EDTA

*T*. Theoretical end point for total EDTA

When the experimental end points were compared with the theoretical end points there was a small constant error. In using an ethylenediaminetetraacetic acid solution without added magnesium, the error was proportional to the glycine concentration, and an impurity in the glycine apparently was responsible for the error. This was confirmed by the fact that the ash from glycine was found to chelate ethylenediaminetetraacetic acid. A correction for the impurity in the glycine was determined by titrating a known volume of a standard ethylenediaminetetraacetic acid solution in the glycine buffer.

**Table III. Determination of Available Ethylenediaminetetraacetic Acid in Solutions Containing Magnesium Using Glycine Buffer**

| Total EDTA, Moles × $10^5$ | Magnesium, Moles × $10^5$ | Titration | | Available EDTA, Moles × $10^5$ | |
|---|---|---|---|---|---|
| | | Ml. of $0.0493M$ zinc sulfate | Corrected | Theoretical | Found |
| 5.2 | 2.0 | 0.50 | 0.65 | 3.2 | 3.2 |
| 8.6 | 2.0 | 1.17 | 1.32 | 6.6 | 6.5 |
| 12.1 | 2.0 | 1.86 | 2.01 | 10.1 | 9.9 |
| 20.6 | 8.1 | 2.39 | 2.54 | 12.5 | 12.5 |
| 12.1 | 4.1 | 1.46 | 1.61 | 8.0 | 7.9 |

As a result of this work the following procedure is established for the determination of available ethylenediaminetetraacetic acid in solutions which may contain magnesium. A known amount of the solution to be analyzed is added to 20 ml. of a buffer, $0.5M$ for glycine and $0.5N$ for potassium hydroxide, giving pH 11.1. The solution is gassed out with nitrogen and titrated amperometrically with zinc sulfate solution at a potential of $-1.7$ volts (*vs.* S.C.E.). Purification of the glycine is not necessary before use, provided the amount of impurity is determined and the appropriate correction applied.

The results obtained using this procedure are given in Table III. Allowing for the error due to the impurity in the glycine, good results were obtained. Since this method can be used when calcium is present, the use of the glycine buffer is preferable for routine determinations of available ethylenediaminetetraacetic acid; if a sample contains both magnesium and calcium, erratic results are obtained only when potassium hydroxide is used.

ACKNOWLEDGMENT

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# Paper Chromatography of Alkali and Alkaline Earth Cations

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**This method separates and specifically detects 0.1 to 10 $\gamma$ of the common cations potassium, sodium, calcium, and magnesium on one-dimensional paper chromatograms run for 2 hours. Complete separation of barium, strontium, calcium, magnesium, and beryllium is also possible. Many other inorganic and organic cations and anions can be separated and identified. Polyvalent anions may interfere with cation analysis, but can usually be removed by precipitation as insoluble lead salts on the paper strip. The method is usually applicable to 1-$\mu$l. volumes of blood and other biological fluids without preliminary ashing.**

PAPER partition chromatography makes possible the rapid separation and detection of microgram quantities of inorganic ions such as potassium, sodium, calcium, magnesium, and chloride, which are widely distributed in minerals, soil, water, and living organisms. Flame photometry has been perfected to measure less than 0.1 $\gamma$ of sodium, potassium, and calcium (*7, 14*), but paper chromatography may prove to be equally useful. It is a semiquantitative technique in its own right, and, because it does not destroy the analytical sample, it can be used for separations preliminary to final analysis by colorimetry, fluorimetry, or flame photometry.

The objective of the research reported in this paper was to obtain complete separation of potassium, sodium, calcium, and magnesium on chromatograms spotted with 1 $\mu$l. or less of blood drawn from small organisms such as insects, without preliminary ashing to destroy organic matter. While many solvent systems have been used for inorganic ion separations on paper (*4, 9*), a new four-component system was devised for this work, especially for ascending one-dimensional chromatography of the alkali and alkaline earth cations. The chromatographic technique has also been slightly modified to improve reproducibility of $R_f$ values.

## REAGENTS

**Paper-Washing Solvent.** Mix distilled water, pyridine (spectrochemical grade), and acetic acid (glacial, conforming to ACS qualifications) in the volume ratio 80 to 15 to 5.

**Developing Solvent.** Mix isopropyl alcohol (98 to 99%, such as Eastman 212), pyridine (spectrochemical grade), acetic acid (glacial, reagent), and distilled water in the volume ratio 8 to 8 to 1 to 4.

**Lead Acetate Solution.** Prepare 0.1*M* basic lead acetate in dilute acetic acid, by dissolving 580 mg. of basic lead acetate in 9.5 ml. of water plus 0.5 ml. of glacial acetic acid.

**Solution ID-1-A** consists of 0.1% of reagent 8-quinolinol in c.p. or spectrochemical grade acetone.

**Solution ID-1-B.** Prepare by dissolving 145 mg. of 8-quinolinol and 360 mg. of 2-aminoethanol (Eastman 1597) in 50 ml. of c.p. acetone.

**Solution ID-2.** Dissolve 100 mg. of bromocresol purple indicator (Eastman 745) in 10 ml. of absolute ethyl alcohol and dilute to 100 ml. with c.p. acetone. Add one or two drops of 28% ammonia in water to give the solution a red-yellow color.

**Solution ID-3** consists of 1% uranyl zinc acetate in absolute ethyl alcohol containing 5% by volume of acetic acid (glacial, reagent). Heating on a water bath is necessary to obtain complete solution. Uranyl zinc acetate is obtainable from Fisher Scientific (Catalog No. U-11).

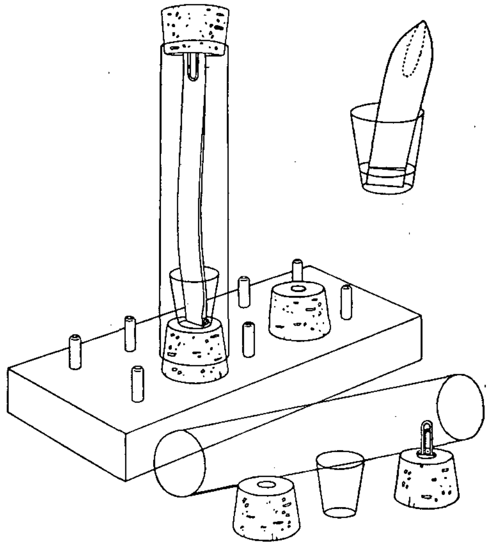

**Figure 1. Simplified apparatus for ascending one-dimensional paper chromatography**

**Inset, upper right-hand corner, illustrates use of concentrating strip for solutions more dilute than 0.001*M***

Traces of inorganic ions are common contaminants in chemicals, and it is desirable to use the purest obtainable reagents in this work. Samples of pyridine and bromocresol purple from some sources have given very poor results.

## APPARATUS

The chromatographic apparatus consists of borosilicate glass tubes, 51 mm. in diameter and 380 mm. long, open at both ends (Figure 1). The ends are fitted with No. 26 corks through which center holes 12 mm. in diameter have been bored. One of these corks is inserted on a wood peg on a heavy wood base (a piece of 2 × 6 lumber 16 inches long into which ten 3-inch lengths of 0.5-inch doweling are driven in a pegboard arrangement), and the tube mounted on the cork to support it in a vertical position. The upper cork is fitted with a paper clip; the wide end of a No. 3 cork is slitted with a razor blade and the single-wire end of a paper clip inserted in the slit. When the No. 3 cork is pressed into the hole in the upper No. 26 cork, the clip is held firmly (but easily replaceable when the small cork is pushed out). The upper end of a paper strip is supported within the tube by this clip. The lower end of the paper strip dips in solvent contained in a special glass which rests on the lower cork. The solvent container is a Glasco medical prescription glass, which has been reduced in size by cutting with a glass saw at the 30-ml. mark. This type of glass is used because it is molded to uniform size, has a volumetric calibration, and has sides that slope outward (this ensures uniform ascent of the solvent, as the edges of the paper strip do not touch the sides of the glass). It is important that all components be of nearly identical dimensions, so that chromatograms will be reproducible. A ten-tube unit of this kind is stable and easily handled, and any one of the tubes can be removed and dismantled in a few seconds.

The use of corks makes this apparatus unsuitable for use with volatile solvents (such as methyl ethyl ketone or ethyl ether) because of vapor leaks through the cork. Chromatographic developments requiring more than 2 or 3 hours, even with relatively nonvolatile solvents, may also be somewhat erratic because of leakage. This apparatus gives very reproducible chromatograms with developments of 2 hours or less and solvents boiling above 80° C.

## PROCEDURE

**Preparation of Paper Strips.** Whatman No. 4 paper is obtainable in rolls 1 inch wide and 600 feet long. It is cut into 660-mm. lengths, which are folded in the center to form "double strips" 330 mm. long, and a pencil line is drawn 38 mm. from the fold on each side. This is the "starting line," to which all measurements are referred. The double strips are washed by ascending chromatography in a mixture of water, pyridine, and acetic acid (80 to 15 to 5); within 2 hours the "washing solvent" ascends nearly to the top of the strips (a bundle of four double strips can be washed in one tube, using 15 to 20 ml. of solvent). Cations present as impurities in the paper move with the front and form a brown band at the top of the strips. The washed strips are thoroughly dried in air for 2 hours in a fume hood. These strips show very low background color or fluorescence when treated with ion-detector solutions. Washed strips must be handled carefully by the upper end or edges to avoid contamination by fingerprints. They can be stored in tightly corked tubes for months without change.

Whatman No. 4 strips 0.5 inch wide can also be used. $R_f$ values are the same, but smaller spots (0.5-µl., 3 to 4 mm. in diameter) and smaller quantities of ions must be used to obtain resolution equal to that on 1-inch-wide strips.

**Spotting of Paper Strips.** One-microliter aliquots of the solutions to be chromatographed are pipetted with Misco No. 282-B micropipets and applied to the center of the penciled starting lines to form spots about 3.5 mm. in diameter and dried. Even viscous and quick-clotting fluids can be directly pipetted with special short uniform-capillary Misco pipets, which are self-filling in less than 1 second; these have been used for human blood and insect hemolymph taken directly from a wound without anticoagulants. Several 1-µl. aliquots can be applied to the same spot without enlarging its area if the paper is dried after each application. This micro-volume application requires ion concentrations of 0.001 to 1 micromole per µl. (0.001 to 1$M$).

Larger volumes of dilute solutions can be concentrated by the following modification of the method of Decker (*3*): A 100-mm. length of washed 1-inch paper strip is held by stainless steel forceps and one end is cut with scissors to form a tapering point. The solution to be concentrated is applied in aliquots of 20 µl. in a band extending backward about 25 mm. from the tip (shown as a dotted line in the inset at upper right of Figure 1); the band must be narrow to ensure concentration into the tip. The paper is quickly dried (a current of hot air from an electric hair dryer is very effective) and a second application made. This operation is continued until 100 to 1000 µl. have been applied. The square end of the strip is placed in a glass containing 5 ml. of the water-pyridine-acetic acid washing solvent. Capillary ascent drives the ions with the solvent front up to the tip of the concentrating strip. The strip is removed, dried, and replaced in the solvent; the second ascent concentrates the ions closer to the tip. The tip is then touched to the center of the starting line of a washed chromatographic strip, so that the solvent flows to form a spot 3 to 4 mm. in diameter. The chromatographic strip is removed and the spot dried. The transfer operation from the tip of the concentrating strip to the starting spot may be repeated to increase the percentage transfer of ions, but some loss of material on the edges of the concentrating strip is unavoidable.

**Development of Chromatograms.** The chromatographic strips are developed in the 8-8-4-1 isopropyl alcohol-pyridine-water-acetic acid solvent for exactly 2 hours at 30° C. Double strips, spotted on each side, are normally used; a short length of glass rod is placed in the loop to weight the strip and act as a separator. The solvent glass is filled to the 10-ml. mark, and the strip suspended so that it dips in the solvent to a depth of 10 mm. The solvent ascends 190 mm. in the 2-hour period, and the solvent front is 165 to 170 mm. above the starting line. Careful control of solvent volume, temperature, and position of the strip in the solvent makes chromatograms exceptionally reproducible. It is not necessary to run known reference spots simultaneously with unknowns, since the latter can be identified by measuring $R_f$. A double strip can be cut at the fold into two single strips, which can be developed independently, but it is better to spot both sides of a double strip, either with duplicate 1-µl. spots or with one 1-µl. and one multiple-spotted 5-µl. spot.

It is better not to re-use solvent. Solvent used for one development gives slightly lower $R_f$ values if re-used. The paper strips apparently alter the solvent composition, by preferential absorption of water and, if highly reproducible results are desired, it is best to use a large volume of solvent, so that the volume and composition of the solvent flowing into the paper will be relatively constant throughout the development period.

Developed strips are air-dried in a fume hood for 15 to 30 minutes. The solvent front should be marked by a pencil line.

**Precipitation of Interfering Anions by Lead Acetate.** Polyvalent anions such as sulfate, phosphate, citrate, and many proteins interfere with the normal movement of cations on paper chromatograms. In many analytical procedures for divalent cations preliminary removal of such interfering anions is necessary. Sulfate and phosphate can be selectively adsorbed by an alumina column (*8*), and all anions can be replaced by acetate if the solution is passed through an anion exchange column in the acetate form (*10*). These purification methods require relatively large volumes of solution, however. The authors have devised a special method for removal of interfering anions from 1-µl. volumes by precipitation as lead salts directly on the chromatographic strip.

A single strip of 1-inch-wide Whatman 4 paper is cut 370 mm. long and washed by ascending chromatography with the paper-washing solvent. Two 2-µl. spots of 0.1$M$ lead acetate solution are applied at points 10 and 15 mm. from the lower end of the strip to form two overlapping circles 6 mm. in diameter each containing 0.4 micromole of lead. A 1-µl. spot of the ion solution from which as much as 0.1 micromole of sulfate, phosphate, oxalate, etc., is to be removed is then applied to the region of overlap of the lead spots about 12 mm. from the lower end. A visible white precipitate may form. The edge of the lower end of the strip is then dipped in a beaker of distilled water. In a few minutes the water rises by capillarity to a height of 60 to 70 mm. The strip is removed and the lower end blotted to remove excess water, but the water front will rise 10 mm. farther. A pencil line is drawn at the water front; it will be the starting line. The strip is then cut 38 mm. below and 292 mm. above the starting line, to convert it into a standard 330-mm. single strip. This is dried and chromatographed in the usual way. The chromatogram has a large lead spot at R 70, but the pattern of cations is normal, whereas the original ion solution might give only an unidentifiable streak.

**Detection of Ion Spots on Chromatograms.** Many ion-detector solutions can be used to develop color or fluorescence in the ion spots on a chromatogram. In this work, some reagent solutions devised by other workers (*1, 9*) have been modified to eliminate spraying. The upper end of a paper strip is held in one hand, and the tip of a 1-ml. bacteriological pipet filled with the ion-detector solution is applied to the strid at the solvent front line.

The 1 ml. of solution is allowed to flow down the length of the strip for a few seconds, until the paper is saturated down to the starting line. This "pouring" technique is quicker and cleaner than spraying or dipping, wastes none of the detector solution, and gives very uniform impregnation and color development.

Each ion-detector solution is numbered—e.g., ID-2 means "ion-detector solution number 2"—and this identifying symbol is written on every chromatogram treated with the solution.

Solution ID-1-A gives fluorescent spots (in 360-mμ ultraviolet light) with beryllium, magnesium, calcium, strontium, barium, zinc, cadmium, aluminum, yttrium, indium, zirconium, gallium, silver, and lithium, while manganese, iron, cobalt, nickel, copper, and lead show up as dark spots on the faintly fluorescent background (*5*, *9*). At levels of 0.1 micromole or more some of these cations show up as light yellow or brown spots, but fluorescence detection is 10 to 100 times more sensitive. Fluorescence is intensified by passing the paper strip slowly over an open bottle of 28% ammonia in water, and the perimeter of each spot is outlined in pencil; the strip is first observed without ammonia, to reveal the central areas of large intense spots, which are also outlined in pencil. Since fluorescent oxinates form even in the presence of strong acid anions, calcium can be detected even when a large chloride spot overlaps a calcium spot. High levels (0.2 micromole) of potassium or sodium give faintly fluorescent spots.

Solution ID-1-B gives fluorescent spots similar to those developed by ID-1-A, but does not require intensification by ammonia vapor because it contains the nonvolatile base 2-aminoethanol. Sensitivity of detection is nearly equal to ID-1-A. Qualitative differences between cation fluorescence colors are enhanced by the 2-aminoethanol: magnesium is greenish yellow, calcium is green, strontium is greenish white, and barium is bluish white. Solution ID-1-A is preferable to ID-1-B, however, since solution ID-2 can be applied to strips previously treated with ID-1-A.

**Table I. Mobility of Inorganic Ions and of Various Organic Biochemicals in Isopropyl Alcohol–Pyridine–Water–Acetic Acid (8-8-4-1)**

(2 hours, 30° C.)

| Ion | Micromoles Spotted | Detection[a] by ID- | | | *R* Values of Spot Center and Limits[b] | Organic Compounds | ID-2 Color[a] | R |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | | |
| $SO_4^-$ | 0.2 | | + | | 3 (9-0) | | | |
| $Ba^{++}$ | 0.01 | + | ± | | 9 (12-6) | Aspartic acid | Y | 11 |
| $Cs^+$ | 0.2 | − | ++ | − | 16 (18-14) | Cysteic acid | Y | 12 |
| $Rb^+$ | 0.03 | | + | ± | 15 (17-13) | Arginine | P | 15 |
| $K^+$ | 0.01 | | + | ± | 13 (15-11) | Glycerophosphoric acid | Y | 20 |
| | 0.05 | − | + | ++ | 15 (16-11) | Glycine | − | 20 |
| | 0.4 | ± | ++ | | 15 (19-10) | Glutamic acid | Y | 23 |
| | 2.0 | + | | | 20 (22-18-11) | | | |
| $Sr^{++}$ | 0.02 | + | ± | | 19 (21-13) | Riboflavin-5-phosphoric acid[c] | Y | 24 |
| $Na^+$ | 0.01 | | ± | + | 24 (26-22) | 3-Adenylic acid | Y | 28 |
| | 0.05 | − | + | ++ | 26 (28-24) | Oxalic acid | Y | 32 |
| | 0.3 | ± | ++ | | 30 (31-29-27) | Alanine | − | 34 |
| | 2.0 | + | | | 32 (36-28-22) | Proline | − | 36 |
| $Ca^{++}$ | 0.002 | ± | | | 35 (36-34) | Gluconic acid | Y | 38 |
| | 0.02 | + | ± | | 35 (38-32) | Tartaric acid | Y | 43 |
| | 0.1 | ++ | + | − | 41 (44-39-34) | Xanthopterin[c] | − | 43 |
| | 0.4[d] | | ++ | | 39 (44-35-31) | Tetramethylammonium on | P | 47 |
| | 0.6[d] | | | | (47-18) | | | |
| $H_2PO_4^-$ | 0.1 | | + | | 42 (45-38) | Hydantoic acid | Y | 49 |
| | | | | | | Valine | − | 51 |
| | 0.2[d] | | ++ | | (48-28) | Phenylalanine | − | 51 |
| | | | | | | *l*-Quinic acid | Y | 51 |
| | | | | | | Choline | P | 52 |
| $H_2AsO_4^-$ | 0.1 | | + | | 47 (50-43) | Orotic acid | Y | 55 |
| $Cl^-$ | 0.04 | | ± | | 47 (50-44) | Guanidine | P | 56 |
| | 0.4 | | ++ | | 51 (54-48) | | | |
| | 1.6[d] | | | | 48 (53-43-30) | | | |
| $Mg^{++}$ | 0.001 | ± | | | 53 (55-51) | Thiamine | P | 59 |
| | | | | | | Glyceric acid | Y | 60 |
| | 0.01 | ++ | ± | | 57 (61-47) | Citric acid | Y | 62 |
| | 0.1 | | + | − | 60 (63-57-54) | Leucine | − | 62 |
| | | | | | | Isoleucine | − | 62 |
| | 1.6 | | ++ | | 61 (67-50-47) | Acetylcholine | P | 62 |
| $Li^+$ | 0.02 | ± | − | − | 58 (61-55) | β-Dimethylaminoethano | P | 65 |
| | 0.1 | + | + | + | 61 (63-59) | Riboflavin[c] | − | 65 |
| | | | | | | Glucose | − | 65 |
| $Br^-$ | 0.1 | | + | | 66 (69-63) | Tetraethylammonium ion | P | 68 |
| $Al^{+++}$ | 0.05 | ++ | | | 69 (77-57-8) | Malic acid | Y | 68 |
| $Pb^{++}$ | 0.2 | ++ | ++ | | 71 (82-60) | α-Ketoglutaric acid | Y | 68 |
| | | | | | | Glycolic acid | Y | 71 |
| $NO_3^-$ | 0.1 | | + | | 75 (78-71) | Pyruvic acid | Y | 72 |
| | | | | | | *trans*-Aconitic acid | Y | 75 |
| $Mn^{++}$ | 0.05 | ++ | ± | | 84 (89-80) | *p*-Hydroxyphenylpyruvic acid | Y | 76 |
| $Cu^{++}$ | 0.05 | + | | | 88 (98-78) | Shikimic acid | Y | 78 |
| | | | | | | *cis*-Aconitic acid | Y | 78 |
| | | | | | | Fumaric acid | Y | 80 |
| $Be^{++}$ | 0.02 | ++ | − | | 91 (95-87-82) | Nicotinic acid | Y | 83 |
| | | | | | | Ascorbic acid | Y | 83 |
| | | | | | | Lactic acid | Y | 83 |
| $Zn^{++}$ | 0.2 | ++ | | | 91 (97-82) | Hippuric acid | Y | 88 |
| | | | | | | Maleic acid | Y | 89 |
| $Ni^{++}$ | 0.05 | + | | | 92 (98-85) | Aesculin[c] | − | 89 |
| $Co^{++}$ | 0.1 | + | | | 92 (99-84) | Gentisic acid[c] | Y | 89 |
| $Fe^{+++}$ | 0.05 | + | | | 96 (99-90) | α-Ketobutyric acid | Y | 90 |
| $Cd^{++}$ | 0.1 | ++ | | | 97 (99-92) | Tetra-*n*-propylammonium ion | P | 93 |
| | | | | | | Succinic acid | Y | 93 |
| | | | | | | *p*-Aminobenzoic acid | − | 98 |
| | | | | | | *p*-Hydroxybenzoic acid | Y | 98 |

[a] Ion-detector solutions 1, 2, and 3 are described in text. ID-1 is 0.1% 8-quinolinol in acetone. ID-2 is 0.1% bromocresol purple in alcohol-acetone. ID-3 is 1% uranyl zinc acetate in alcohol.

− Spot not detectable.
± Faint spot, near limit of detection.
+ Distinct spot of less than maximum intensity.
++ Spot with center area of maximum intensity.
Y = yellow spot, P = purple spot.

[b] $R = 100\ R_f$.

[c] Fluoresces strongly in ultraviolet light.

[d] This quantity is an overload that causes streaking and/or significant displacement of some other ion spots from their normal *R* value.

Solution ID-2 detects "acid" and "alkaline" spots. When this solution is poured on strips freshly developed and dried, only the central areas of strong cation spots are detectable as purple spots on a bright yellow background. Fresh strips retain much adsorbed pyridine and acetic acid; these gradually evaporate and the ion spots become larger and more deeply colored if the strips stand overnight. It is preferable, however, to intensify the spots immediately by holding the strips over a beaker of boiling distilled water (or some other generator of pure steam); the "steaming" drives out pyridine and acetic acid, and the background color becomes a dark greenish yellow. The faintly alkaline spots of the acetates of strong-base, nonvolatile cations show up as bright purple, the limit of sensitivity being about 0.02 micromole of acetate in a spot 5 mm. in diameter. The faintly acidic spots of the pyridinium salts of anions show up as bright yellow, the limit of sensitivity being about 0.04 micromole. All spots should be outlined in pencil when steaming for several minutes has developed them to maximum intensity, since they slowly fade on standing. When an anion spot overlaps a cation spot there is mutual masking by formation of a neutral salt; chloride commonly masks calcium spots in this way. It is better to treat a chromatogram first with ID-1 and ammonia, and mark the fluorescent spots on one side of the strip; and then treat the same strip with ID-2 and steam, and mark the ID-2 spots in pencil on the other side of the strip. Divalent cations are masked by the oxine and do not give purple spots with the ID-2 indicator; anions or monovalent cations are therefore independently revealed by the ID-2 even when they overlap divalent cation spots; thus, both calcium and chloride can be independently detected on the same chromatogram. The presence of oxine, however, makes the ID-2 color reaction less sensitive. Because ID-2 is not specific but detects many organic acids and bases (cf. Table I), a third ion-detector solution is necessary for positive identification of sodium and potassium.

Solution ID-3 is poured on a strip, air-dried for a few minutes, and heated in an oven at 120° C. for 2 to 5 minutes. The ion spots do not fluoresce blue in ultraviolet light, as reported by other workers using zinc uranyl acetate reagents (*1, 9*), but are yellow on a dark blue background. The probable reason for the difference is that ion spots in the present work are slightly alkaline spots of the acetate salts, not neutral or acidic spots. Spots of potassium and rubidium fluoresce bright yellow, spots of sodium and lithium pale yellow; heating for 10 minutes brightens the yellow fluorescence. Cesium is not detected by ID-3 even at 0.2 micromole. Although heating above 100° for 5 to 15 minutes will develop fluorescence in spots of many organic compounds (*6*), this does not seem to occur on strips treated with ID-3. Such possible interference can be checked by heating a control strip, not treated with ID-3, together with the treated strip, or by heating the strip and observing in ultraviolet light before treating with ID-3. Because ID-3 is incompatible with ID-1 and ID-2, it must be used on a separate chromatogram, as a specific test for lithium, sodium, potassium, and rubidium.

Any intense and well-filtered source of ultraviolet radiation in the 360-mμ region will reveal fluorescent spots with approximately the sensitivity indicated in Table I. A Black Raymaster TF8 lamp B, from Geo. W. Gates & Co., Franklin Square, L. I., N. Y., was used in this work; it gives a dark blue background and illuminates an area 28 × 10 cm., so that several chromatograms can be inspected simultaneously for intensity comparisons.

## QUANTITATIVE FLUORIMETRY ON PAPER STRIPS

Measurement of the fluoresced radiation from spots on paper chromatograms is possible with simple apparatus (*11*). The reproducibility of direct fluorimetry has been tested in this laboratory using a more complex automatic scanning-recording apparatus constructed by Craig (*2*). Four strips, to each of which an initial spot of 0.5 μl. of a solution containing 0.1 micromole of barium, strontium, calcium, magnesium, and beryllium acetates had been applied, were developed, dried, and treated with solution ID-1-B. The strips were scanned and the areas under the peaks traced by the recorder were measured. The values were: barium, 18 ± 4; strontium, 16 ± 4; calcium, 19 ± 2; magnesium, 50 ± 10; beryllium, 29 ± 4. When observed by eye under ultraviolet light the four strips seemed to be very uniform except for minor variations in size and shape of the spots. The $R_f$ values were identical. The Craig apparatus gives very reproducible tracings for any one chromatogram; the variation, of the order of 10%, found in this experiment, may be due to nonuniformity of the Whatman No. 4 paper.

## RESULTS AND DISCUSSION

Table I summarizes the data on the movement of ions in the isopropyl alcohol-pyridine-water-acetic acid solvent. Mobilities are given in $R$ units ($R = 100\ R_f$) measured from the starting line to the estimated center of the ion spot. The $R$ value tends to be higher the larger the quantity of the ion in the spot; therefore Table I includes values over a 100- or 1000-fold range of concentration, together with a more detailed description of the spot. The numbering 20 (22-18-11) indicates that the approximate mass center of the spot is at R 20, its main mass lies between R 22 and R 18, and a faint "tail" extends down to R 11. The numerical description given is usually obtained with the most sensitive detector solution; a less sensitive detector reveals a smaller spot, because the concentration of ion is not uniform within a spot but higher in the center region. Spot area increases with the quantity of ion in the spot, but not linearly (*10*); however, for very large intense spots detected with ID-2, quantity can be roughly estimated by multiplying the dark purple area (not including the fainter "tail") in square millimeters by the factor 0.001 micromole per square millimeter, which seems to be the maximum capacity of Whatman No. 4 paper.

The common cations (potassium, sodium, calcium, and magnesium) are well resolved, even when one is present in a mole ratio of 100 to 1 over the others. Potassium, rubidium, and cesium are not resolved; they can be separated by a phenol-hydrochloric acid solvent (*12*). Ammonium ions are not detected by any of the ion-detector solutions used in this work.

There is a limit to the quantity of any one ion, above which the spot streaks badly and ruins the chromatogram; this "overload limit" is noted for several ions in Table I. "Overloading" is a breakdown of the paper partition system and causes streaking or displacement of other ion spots also. It is a quite different phenomenon from simple "overlapping" of two spots caused by increasing spot area at heavy loads. Polyvalent ions tend to have relatively low overload limits. Anions such as sulfate or phosphate distort the normal movement of divalent cations even at levels of 0.1 micromole, and affect even monovalent cations at levels of 0.3 micromole. Trivalent cations such as aluminum or gallium streak instead of moving as spots.

These results agree with those of Tewari (*13*), who studied the separation of heavy metal ions by a different solvent. The $R_f$ of an ion spot increases as the quantity of the ion is increased, and decreases as the solvent front travels a greater distance. Rigid control of temperature, solvent volume and composition, development time, and geometry of the paper strip system are essential to minimize variation in $R_f$. Tewari believes that the cellulose fibers selectively remove water from the solvent as it ascends the paper strip. A continuous gradient will result from this process, the lower end of the strip containing a solution somewhat richer in water than the original developing solvent, while the water content progressively falls to a minimum at the solvent front. If this gradient can be exactly reproduced in all chromatograms, $R_f$ values will also be reproducible. Large quantities of an ion, however, spread out over a considerable area and modify the water gradient within this area. Presumably the cellulose is less able to remove water from a strong salt solution, and the water concentration at any point will be higher than it would be if there were less salt; therefore, $R_f$ is higher. Overloading will occur when the solubility of the salt in the moving liquid is relatively low; removal of water then causes precipitation of salt along a streak. The fact that calcium has a much lower overload limit than magnesium correlates well with the relatively low solubility of calcium acetate in alcohol.

Table I also lists $R$ values for many organic compounds, some of which may occur in biological fluids or extracts and be detected by ID-2, or interfere with the movement or detection of cations, or be fluorescent or fluorescence-quenching. Enough different types of organic compounds are included to make possible some generalizations about the effect of structure on $R_f$ in this solvent, and the probable position of many related compounds can therefore be estimated.

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# Application of Coulometric Titrations to Microvolumes of Solution

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**The coulometric generation of titrants was found to be applicable to microvolumes of solution. Ten microliters of hydrochloric acid, sodium hydroxide, and arsenite were titrated with coulometrically generated reagents with an accuracy comparable to that obtained using conventional methods. The technique is not limited to these particular examples, but is general and can be applied to a variety of titrations using small volumes of solution.**

IN MANY instances, the problems facing the analytical chemist are rendered more difficult by the fact that only a small amount of material is available for analysis. In volumetric procedures, the amount of solution may be as small as 0.01 ml. and many ingenious devices have been devised for the handling of such titrations (*3*). Microburets have been developed which are capable of delivering volumes of titrant small enough to be useful in such work. It would seem, however, that the coulometric generation of titrants, when applicable, would have some advantages over conventional methods. The apparatus is simple, a wide variety of titrants can be generated (*4*), and the reagent can be added in extremely small increments with high accuracy.

It was found that thin platinum probes could be used as generating electrodes, and the experimental arrangement was both compact and versatile. The technique is illustrated by typical redox and acidimetric titrations but is by no means limited to these particular examples. Undoubtedly the method is general in nature and can be applied to a variety of titrations involving small volumes of solution.

## EXPERIMENTAL

The arrangement of the titration vessel and electrodes was varied to compensate for experimental difficulties associated with each type of titration explored. In titrations involving visual detection of the end point, the sample was pipetted onto a glass or porcelain slide which was treated with an organosilicon compound (Desicote, Beckman Instruments, Inc.) to prevent the drop from spreading over the surface. When the end point was detected potentiometrically, a platinum foil was used as the titrating surface. The foil then served as the generating electrode as well as a container for the solution. The latter arrangement was difficult to use with visual indicators because of the reflection of light from the metallic surface. Two arrangements which were used in this work are shown in Figure 1. The solutions were stirred by means of a fine glass rod attached to a vibrator (*5*).

The electrical apparatus necessary for a source of constant current was greatly simplified because the amount of material was small and hence currents of only 100 $\mu$a. or less were required. A simple circuit consisting of two 45-volt B batteries and the appropriate series resistances (0.5 to 10 megohms) was adequate (*1*). The current was found by measuring the voltage drop across a known resistance by means of potentiometer, but a microammeter of the required accuracy could be substituted for the resistance-potentiometer combination if desired. The time of generation was measured by means of a synchronous motor clock ganged into main circuit in the usual fashion.

**Iodine Titrations of Arsenite.** The apparatus for the visual titration of arsenite with electrically generated iodine is shown in Figure 1, A.

Iodide ion was oxidized at the platinum wire anode, while hydrogen was evolved at the cathode. Thirty microliters of potassium iodide solution, buffered at pH 6.4 and containing the starch indicator, was used as the background electrolyte. Ten-microliter volumes containing known amounts of arsenic were pipetted into the drop and the titration carried out. Results are shown in Table I. To extend the range of this procedure to more dilute solutions, the same titration was performed using a modified potentiometric end point (*2*). In this procedure the reagents are titrated to an arbitrary end point, the unknown is added, and the solution is then titrated back to the original potential. The apparatus described above was slightly modified for use with this method of end-point detection. A piece of platinum foil, indented and treated with Desicote, served as the generator anode in place of the glass slide. A capillary calomel electrode and platinum wire were used as the indicator system, and the potentials were measured by means of a pH meter.

**Titrations of Acids.** Small volumes of dilute solutions of hydrochloric acid were titrated with electrically generated hydroxyl ion using methyl red as an indicator. The generator cathode consisted of a 0.007-inch platinum wire and an attackable anode of 0.07-inch silver wire completed the circuit. (After each titration the silver chloride was removed from the electrode surface with potassium cyanide solution to decrease the electrical resistance of the film.) A measured sample of hydrochloric acid solution was pipetted into 30 $\mu$l. of 1% potassium chloride solution containing 1 $\mu$l. of 0.1% methyl red indicator solution. The acid was then coulometrically titrated to a yellow end point. To eliminate the indicator blank, the methyl red was added to the supporting electrolyte and brought to the end point color before addition of the sample. This procedure was felt to be more accurate than subtraction of a blank titration because of the relatively large value of the latter (10% of the total titration) with more dilute solutions.

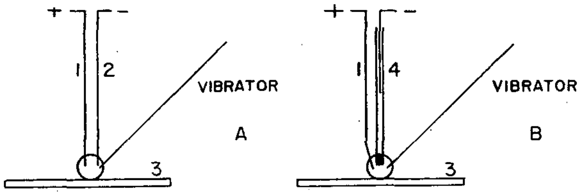

**Figure 1. Titration assemblies**

1. **Generating platinum anode**
2. **Platinum cathode**
3. **Glass slide treated with Desicote**
4. **Cathodic compartment**

**Titration of Bases.** The titration of sodium hydroxide was similar to that of hydrochloric acid, except that the apparatus was somewhat modified. Since an attackable cathode was not available, it was necessary to isolate this electrode as shown in Figure 1, *B*. The cathode compartment contained sodium sulfate solution and a plug of filter paper was used to isolate the hydroxyl ion formed at this electrode.

## DISCUSSION

It has been previously thought that coulometric generation of acid is not feasible in the presence of chloride ion because of prefer-

**Table I. Summary of Results**

| Titration | Sample | Present, $\gamma$ | Approximate Generating Current, $\mu$a. | No. of Titrations | Found, $\gamma$ | Average Deviation % |
|---|---|---|---|---|---|---|
| Arsenite (visual) | Arsenic | 7.49 | 100 | 8 | 7.39 | 1.8 |
| Arsenite (potent) | | 7.49 | 100 | 3 | 7.49 | 1.5 |
| | | 0.749 | 100 | 6 | 7.44 | 0.6 |
| | | 0.374 | 20 | 5 | 0.366 | 2.9 |
| | | 0.149 | 10 | 5 | 0.155 | 5.8 |
| Acid | Hydrochloric acid | 3.63 | 100 | 6 | 3.60 | 2.0 |
| | | 1.82 | 100 | 5 | 1.78 | 1.8 |
| | | 0.726 | 10 | 4 | 0.722 | 2.6 |
| | | 0.403 | 10 | 2 | 0.343 | 14.0 |
| Base | Sodium hydroxide | 4.13 | 100 | 6 | 3.93 | 5.3 |
| | | 4.13 + 300 potassium chloride | 100 | 7 | 3.92 | 4.2 |

ential formation of chlorine (*5*). It was found, however, that at least under the conditions described, the presence of chloride had no effect on the stoichiometry of the coulometric process. Even in solutions that were 2*M* with respect to chloride ion, small amounts of base could be titrated. It was obvious in such titrations that some free chlorine was being evolved at the generating anode, but the electrolytic stoichiometry of the process is probably preserved by the reaction

$$Cl_2 + 2OH^- \rightarrow OCl^- + Cl^- + H_2O$$

To check this hypothesis, macrovolumes of sodium hydroxide were titrated in the presence of chloride ion using a glass-calomel system for the detection of the end point. No significant errors were involved, and the presence of hypochlorite ion was indicated by testing the titrated solution with iodide ion in the presence of starch. The error in the base titration was lowered by sweeping the solution with nitrogen.

The above examples of typical titrations indicate that coulometric methods can be applied to the titration of small volumes of dilute solutions. The volume of sample was usually 10 $\mu$l., and the concentration of the arsenite solution ranged from 0.01 to 0.0002*M* while in the acidimetric titrations the range of concentration was 0.01 to 0.001*M*. The accuracy attained is comparable to that of conventional methods.

### ACKNOWLEDGMENT

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# Colorimetric Determination of Platinum with Stannous Chloride

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**In 0.3*N* acid solution, sometimes recommended in the literature for the reaction between stannous chloride and chloroplatinic acid, platinum probably forms a "hybrid" complex or mixture of complexes containing both stannous and stannic tin. The reaction product is sensitive to slight changes in acidity, time of standing, and concentration of reacting ions, so that rigorous control of conditions is necessary to ensure reproducible results. At higher acidities (1.5 to 2.5*N*), a true stannous-platinum chloride complex is formed. This reaction is unaffected by the presence of stannic tin, and is generally more suitable as a basis for quantitative photometry.**

ONE of the most suitable methods for routine determination of platinum is the spectrophotometric determination based on the reaction of platinum with stannous chloride in hydrochloric acid solution. In the course of adapting this method to the analysis of platinum-bearing materials, some interesting observations were made regarding the nature of the reaction.

## REACTION OF PLATINUM AND STANNOUS CHLORIDE IN 0.3*N* ACID

**Effect of Acidity and Time.** Sandell (*4*) suggests that the reaction between chloroplatinate and stannous chloride involves a simple reduction to chloroplatinous acid, and he reports that the intensity of the color varies with the acidity. Although the authors subsequently found the reaction to be different, a study of the effect of the acid concentration on the intensity and stability of the color confirmed Sandell's statement in regard to acidity. This effect is shown in Table I.

In general, the intensity of the color increases with time at acidities below 0.3*N*, and decreases with time at acidities above 0.3*N*. At an acidity of 0.3*N* the color develops immediately and is stable for at least 2 hours. Consequently, careful control of the acid concentration is necessary to ensure reproducible results. It was also found that a 400 to 1 mole ratio of stannous chloride to platinum was necessary to yield complete color development. These effects cast some doubt on the validity of the theory of simple reduction to chloroplatinous ion. Nonetheless, by careful control of conditions, the authors were able to obtain reliable results by the method recommended by Sandell.

**Variation between Different Grades of Stannous Chloride.** Some time after the adoption of the method, it was observed that the intensity of the color depended on the grade of stannous chloride used. Technical grade stannous chloride, as used in the original adaptation of the method, yielded consistently reproducible results; however, with reagent grade material, the colors were not reproducible and were less intense. The color reaction was therefore re-examined to determine the cause of this anomaly.

The difference between the two grades of stannous chloride was first investigated by reacting various amounts of each with 5 p.p.m. of platinum, the acidity of the final solution being kept constant at 0.3*N*. The technical grade reagent gave constant absorbance with different quantities, but the reagent grade stannous chloride did not. Although the absorbance increased with increasing amounts of the reagent solution, results were lower than those with the technical grade material (Table II).

**Table I. Variation of Absorbance with Acidity and Time**

(Pt = 3 p.p.m.)

| Acidity, *N* | Absorbance at 350 m$\mu$<br>Immediate | 10 min. | 30 min. | 60 min. | 120 min. |
|---|---|---|---|---|---|
| 0.20 | 0.279 | 0.297 | 0.299 | 0.300 | 0.290 |
| 0.26 | 0.270 | 0.274 | 0.286 | 0.288 | 0.294 |
| 0.30 | 0.298 | 0.290 | 0.293 | 0.290 | 0.293 |
| 0.35 | 0.241 | 0.229 | 0.221 | 0.220 | 0.218 |
| 0.40 | 0.212 | 0.211 | 0.211 | 0.210 | 0.201 |

**Table II. Comparison of Different Grades of Stannous Chloride**

| Quantity of $SnCl_2$, (Ml. of 10% Soln.) | Absorbance<br>Technical grade | Reagent grade |
|---|---|---|
| 4.0 | 0.479 | 0.357 |
| 5.0 | 0.471 | 0.390 |
| 8.0 | ... | 0.431 |

**Table III. Effect of Addition of Stannic Chloride**

| $SnCl_4$, Ml. | Absorbance, $SnCl_2$ Reagent grade | Technical grade |
|---|---|---|
| 0.0 | 0.340 | 0.461 |
| 1.0 | 0.395 | 0.467 |
| 10.0 | 0.467 | 0.467 |

**Table IV. Effect of Stannic Chloride on Reaction of Stannous Chloride with Bivalent Platinum**

| Reagent Added, Ml. | | Absorbance | |
|---|---|---|---|
| $SnCl_4$ (1% soln.) | $SnCl_2$ (10% soln.) | Observed | Theory |
| 4.0 | 0.0 | 0.000 | ... |
| 4.0 | 4.0 | 0.403 | 0.418 |
| 0.0 | 4.0 | 0.348 | 0.418 |

**Effect of Stannic Tin.** It was believed that the most likely contaminant of the technical grade material was stannic tin. To test the effect of stannic tin, 5-p.p.m. amounts of platinum were made to react with both grades of stannous chloride, and various amounts of a 1% solution of stannic chloride pentahydrate were added. The reagent grade material initially gave a much lower color intensity than the technical grade, but the intensity increased with the amount of stannic tin present. With 10 ml. of added stannic tin solution, the intensity was identical to that yielded by the technical grade material, which showed no change with the addition of stannic chloride (Table III).

The following experiment confirms that stannic tin was involved in the reaction: A solution of reagent grade stannous chloride was prepared fresh and divided into two portions. Air was bubbled through one portion for 2 hours, after which both were made to react with 5 p.p.m of platinum. The platinum solution that contained the nonoxidized reagent gave an absorbance of 0.391, whereas the solution that contained the oxidized portion gave an absorbance of 0.461.

In view of the above findings, it was thought possible that the stannous chloride acted only as a reductant, and that the color measured was actually a platinous-stannic reaction product, with the technical grade material containing sufficient stannic tin to carry the reaction to completion. (If 1% of the tin in the stannous chloride were present as stannic tin, the amount added would correspond to a tin-platinum mole ratio of about 7 to 1.) To test this possibility, solutions containing 4.4 p.p.m. of bivalent platinum as potassium chloroplatinite, prepared according to the method of Fernelius (*2*), were made to react with various combinations of reagent grade stannous and stannic chloride (Table IV).

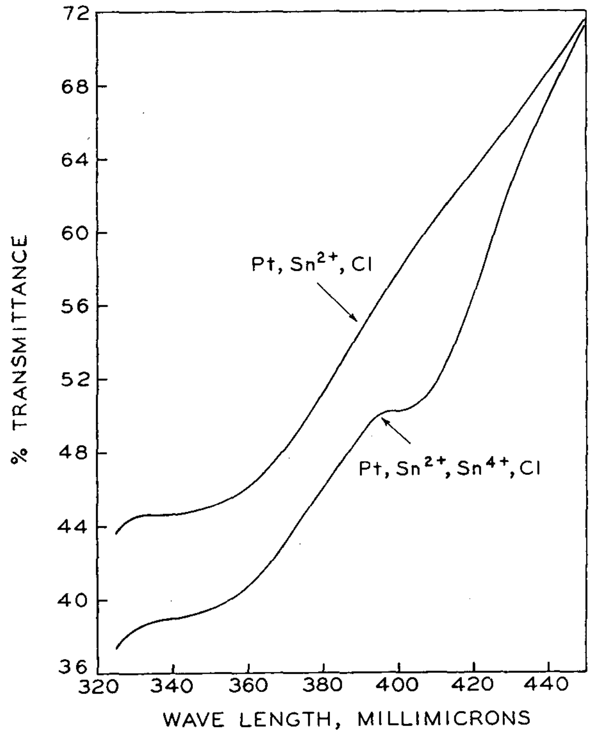

**Figure 1. Transmittance of platinum-tin chloride complexes in 0.3*N* hydrochloric acid**

Stannic chloride alone did not react at all; a spectrogram of the solution showed no absorbance between 350 and 450 mμ. When both stannous and stannic chlorides were present, the color intensity was reasonably close to the calculated value for 4.4 p.p.m. of platinum. With only stannous chloride present, the color developed, but showed a 17% deficiency, indicating that both stannic and stannous ions were necessary for full color development at 0.3*N* acidity.

Spectrograms of the two colored solutions were similar (Figure 1), but the presence of an absorption peak (at about 400 mμ) in the curve of the solution containing both stannic and stannous tin, indicates that a second complex is involved in the reaction. Since stannic tin itself gives no reaction, the inference is that this complex probably contains both stannous and stannic tin.

From the above, it is apparent that platinum cannot be determined by reaction with stannous chloride in 0.3*N* acid, unless the stannic tin content of the reagent is carefully controlled. As this is extremely difficult, in view of the ease with which tin is oxidized by air, a variation of the method which might overcome this problem was sought.

## REACTION OF PLATINUM AND STANNOUS CHLORIDE IN 2*N* ACID

Meyer and Ayres (*3*) showed that if the reaction is carried out in 2*N* acid, the absorbing species is a complex of the form $(PtSn_4Cl_4)^{++++}$. It was also found that the final color is independent of acid concentration, provided the concentration exceeds 0.7*N*, and that stannous chloride concentration has no effect (*1*). The authors' data confirm many of the findings of these investigators, and show the advantages of the higher acidity.

**Spectral Characteristics.** A solution of chloroplatinic acid 2*N* in hydrochloric acid, was made to react with reagent grade stannous chloride. A spectrogram of the reaction product showed it to have a true minimum transmittancy at 405 mμ, in agreement with the work of Ayres.

Although the color intensity of the complex at this wave length was only approximately half that of the complex formed in 0.3*N* acid, the same sensitivity was maintained in practice by developing the color in half the volume used at the lower acidity.

**Table V. Variation of Absorbance with Acidity and Time at 2*N* Acid Level**

| Acidity, *N* | Absorbance at 405 mμ Immediate | 10 min. | 20 min. | 30[a] min. |
|---|---|---|---|---|
| 1.5 | 0.204 | 0.202 | 0.205 | 0.204 |
| 2.0 | 0.207 | 0.204 | 0.207 | 0.205 |
| 2.5 | 0.208 | 0.205 | 0.208 | 0.208 |

[a] No change during additional 3 days.

**Variation of Absorbance with Acidity.** The effect of varying the acidity at the 2*N* level was checked on a solution containing 5 p.p.m. of platinum. In contrast to the results at the lower acidities, the absorbance of the complex at the 2*N* level shows no significant change with time or acid concentration (Table V). For convenience, a concentration of 2*N* was selected. Although the values refer to the final acidity of the solution, the stannous chloride must not be added until the acidity has been adjusted to the approximate required strength. If the reagent is added

**Table VI. Variation of Absorbance with Stannous Chloride Concentration**

(Pt = 5 p.p.m.)

| $SnCl_2$, Ml. of 20% Soln. | Absorbance |
|---|---|
| 1.0 | 0.200 |
| 2.0 | 0.202 |
| 5.0 | 0.202 |
| 10.0 | 0.203 |
| 15.0 | 0.206 |
| 20.0 | 0.207 |

**Table VII. Effect of Stannic Chloride on Reaction in 2*N* Hydrochloric Acid**

(Pt = 5 p.p.m.)

| $SnCl_4$, Ml. of 1% Soln. | Absorbance |
|---|---|
| 0.0 | 0.201 |
| 0.5 | 0.201 |
| 1.0 | 0.201 |
| 5.0 | 0.200 |
| 10.0 | 0.200 |

**Table VIII. Variation of Absorbance with Platinum Concentration**

(Beckman Model B spectrophotometer; 1-cm. light path; $\lambda$ = 405 m$\mu$)

| Pt, P.P.M. | Absorbance | Absorption Coefficient (Absorbance/P.P.M.) |
|---|---|---|
| 2.0 | 0.078 | 0.0390 |
| 4.0 | 0.162 | 0.0405 |
| 6.0 | 0.239 | 0.0398 |
| 8.0 | 0.319 | 0.0399 |
| 10.0 | 0.400 | 0.0400 |
| 12.0 | 0.473 | 0.0394 |
| 16.0 | 0.639 | 0.0399 |
| 20.0 | 0.800 | 0.0400 |

to a solution that is only weakly acidic, the reaction appears to proceed irreversibly along the lines already discussed; subsequent addition of acid does not change the absorbance.

**Variation of Absorbance with Stannous Chloride Concentration.** In 2*N* acid, 1.0 ml. of 20% stannous chloride, or a stannous chloride-platinum mole ratio of 300 to 1, was sufficient to yield essentially full color development; the absorbance of the solution showed a negligibly slight change with increasing amounts of stannous chloride (Table VI). A 5-ml. portion of the reagent per 100 ml. of solution was selected to ensure an adequate excess.

**Effect of Stannic Tin.** Since stannic tin had a pronounced effect on the reaction with stannous chloride in 0.3*N* acid, its effect on the complex at the higher acidity was studied by adding stannic chloride in varying amounts to the reaction mixture. In contrast to the effect at the lower acidity, there was no change in the intensity of the color (Table VII).

**Linearity.** The color intensity is directly proportional to the platinum concentration (Table VIII). The average absorption coefficient (absorbance units per p.p.m.) is 0.0398 with a standard deviation of ±0.00044.

**Application of Method.** The method has proved accurate and precise when applied to a variety of platinum-bearing materials and in the presence of numerous other elements. Details of this work cannot be revealed at present. However, aside from gold and some of the other platinum metals, or high concentrations of ions which are themselves colored, few interferences have been encountered.

Rhodium, which is a particularly troublesome interference in the determination of platinum, interferes less in 2*N* acid than in 0.3*N* acid. At the lower acidity, the rhodium absorbance increases rapidly with time until it is about double that of an equal concentration of platinum; in 2*N* acid, the absorbance gradually decreases to about one third that of platinum. By allowing the solution to stand for approximately 3 hours before measuring the absorbance, the rhodium interference can be held to a minimum.

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# Sensitive Determination of Low Boiling Organic Sulfur Compounds

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**Improvements in the reduction of disulfide in organic solution permit determinations of 99% accuracy. Direct determination of hydrogen sulfide in the presence of thiol is effected by separating precipitated sulfide from mercaptides by adjustment of acidity. Procedures, suitable especially where homologs of low molecular weight may be present, form a convenient scheme for sensitive determination of hydrogen sulfide and low boiling thiols and disulfides in gaseous organic mixtures.**

The analysis of sulfur compounds in organic mixtures has been widely studied, particularly by the petroleum industry (*1, 5*), and a number of methods of determining the various compound types have been developed. However, methods of improved accuracy or sensitivity are often desired, as well as methods applicable to anomalous cases, and a group of procedures may be of interest which have been in use in this laboratory in kinetic studies of gas phase reactions of organic sulfur (*6*). Significant improvements in the reduction of disulfide in organic solutions, and a method of determining hydrogen sulfide directly in the presence of thiol(mercaptan) are described. Procedures designed for the case where homologs of low molecular weight may be present are presented as a convenient scheme for the sensitive determination of hydrogen sulfide and low boiling thiols and disulfides in gaseous samples which may contain double bonds.

In kinetic studies by static methods gaseous samples are normally obtained at low pressure in pipets of several hundred milliliters; procedures are in a form suitable for such samples. Under typical conditions, 0.5 mm. of gas in a 250-ml. reaction vessel at 600° K. will correspond to about 0.000003 mole of sample, or when dissolved in 100 grams of solvent to the order of 0.0001% sulfur. The procedures have a sensitivity of this order.

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**Table I. Estimation of Gaseous Hydrogen Sulfide**[a]

| S in Basic Solution of 100 Ml., % | | | Error, % |
|---|---|---|---|
| | $H_2S$ | | |
| $CH_3SH$ | Present | Found | |
| 0 | 0.00037 | 0.00034[b] | .. |
| 0 | 0.00499 | 0.00482[b] | −3 |
| 0 | 0.00499 | 0.00476 | −5 |
| 0 | 0.01225 | 0.01218[b, c] | −1 |
| 0.0083 | 0.00258 | 0.00266[d] | +3 |
| 0.0067 | 0.00418 | 0.00414 | −1 |

[a] Uncertainty in samples = 0.00003% sulfur; 1% sulfur = 0.31 molar.
[b] Filtered at pH 4.
[c] Warmed before filtering.
[d] Duplicated at pH 2.1, 2.4, and 2.8.

Thiols of one or two carbon atoms show anomalous behavior due to relatively high volatility and solubility in water. The well-known procedures, such as the Borgstrom-Reid method (*4*), for determining thiol directly are impractical because of large losses in the preliminary partition separation from hydrogen sulfide. Ball (*1*) reports 12% loss of ethanethiol and the authors have obtained losses of methanethiol greater than 20%. The losses are enhanced when hydrogen sulfide is present, presumably because thiol which passes into the aqueous phase adsorbs on precipitated sulfide (*7*), and thus are not reproducible. Therefore, thiol is determined indirectly.

The direct method for hydrogen sulfide is based on the well-known iodimetric determination of cadmium sulfide. The gas is absorbed in weak base, and precipitated sulfide is separated from mercaptides by careful adjustment of acidity. In concentrations of about 0.005% sulfur, cadmium ethyl mercaptide dissolves exactly at the methyl orange end point (pH 4); the methyl mercaptide requires a solution slightly more acid. At pH 1, however, loss of hydrogen sulfide is appreciable. The allowable range is about pH 2 to 3. In the procedure, pH 2.4 has been used, but results have been duplicated with thiol present with pH of 2.1, 2.4, and 2.8. An asbestos filter is quantitative, although the sulfide precipitated from basic solution is very finely divided, and does not interfere with the oxidation. The procedure is given for samples totaling less than 0.00005 mole of hydrogen sulfide; for larger samples the filtration is difficult and smaller aliquots must be taken.

With hydrogen sulfide known, thiol is readily determined from the total sulfhydryl. For small gaseous samples a simple and sensitive procedure is to oxidize the gases directly with standard iodine.

In the presence of double bonds, the only feasible chemical method of determining disulfide appears to be by reduction to the corresponding thiols. Gaseous samples must first be dissolved in an organic solvent to allow removal of hydrogen sulfide, thiols, and elemental sulfur, which interfere. However, the quantitative reduction of disulfide in organic solution has proved difficult. Ball (*1*) surveyed and tested many methods and concluded that refluxing with zinc and glacial acetic acid gives the best results. These are, however, far from precise. A marked improvement has been obtained by using a stronger acid, reduced standard temperature, and a time of heating sufficiently long to complete the reduction and direct all volatile thiol to the trap. Acetic acid containing about 6% water is used and heating is on a water bath at 100° C. Apart from these modifications and the lower concentrations, the procedure is similar to that of Bell and Agruss (*2*), but the small error is reproducible to ±1% and can be corrected. The procedure is convenient for samples less than about 0.0003 mole.

## MATERIALS

**Hydrogen Sulfide Gas,** prepared by the method of Bickford and Wilkinson (*3*).

**Other Sulfur Compounds,** Eastman Kodak products of 98 to 100% purity fractionated under vacuum before use. Before fractionation dimethyl disulfide had refractive index $n_D^{20}$ = 1.5261 ± 0.0002 in agreement with the value of Vogel and Cowan (*9*).

**Petroleum Ether, Reagent Grade.** The organic solvent used was a completely saturated sulfur-free material with boiling range 120° to 160° C. Thiol solutions are stable.

**Preparation of Samples.** Gaseous samples were admitted to suitable evacuated pipets from a glass reservoir maintained at a uniform temperature close to 600° K. and fitted with a glass Bourdon gage for measuring the pressure; their quantity was calculated by the ideal gas law from the pressure change in the reservoir during sampling. This technique assumes ideal behavior only at the high temperature, and it avoids errors arising from adsorption of the sulfur compound on the pipet surface. It was checked with pure hydrogen sulfide. The standard solutions of dimethyl disulfide were prepared by weighing the pure disulfide in sealed capillary tubes and breaking them under the solvent.

## PROCEDURES

**Hydrogen Sulfide.** Admit 50 ml. of 2% (0.5*N*) aqueous sodium hydroxide to the sampling pipet, but no air, and shake 15 minutes. Transfer to a 100-ml. volumetric flask and dilute to volume with water. Treat 25-ml. aliquots in glass-stoppered flasks with 25 ml. of neutral 5% cadmium chloride. Add a drop of methyl orange, followed by 1*N* hydrochloric acid dropwise with alternate vigorous shaking until both cadmium hydroxide and the cadmium mercaptides have dissolved and the pink acid color is just permanent. Neutralize exactly with 0.5*N* sodium hydroxide (total now 60 ml.), and add 0.25 ml. of the acid in excess. Shake vigorously, filter through a Gooch crucible fitted with a thick asbestos mat, and wash the precipitate thoroughly with distilled water to remove any adsorbed thiol (for thiols higher than ethanethiol, alcohol washing may be necessary). Transfer precipitate and mat to a 125-ml. stoppered flask, add 0.01*N* standard iodine solution, at least 5 ml. in excess, and add 6*N* hydrochloric acid to an acidity 0.5 to 1*N*. Stopper, shake to disperse precipitate and mat, and let stand 20 minutes. Titrate the excess iodine with 0.01*N* standard sodium thiosulfate to a starch iodide end point. The hydrogen sulfide equals the moles of iodine used. A blank should use less than 0.1 ml. of iodine.

**Thiol.** Admit a measured excess of neutral 0.01*N* standard iodine to the sampling pipet with a minimum of air, and shake 20 to 30 minutes, depending on the excess. Titrate the remaining iodine with 0.01*N* sodium thiosulfate, and calculate thiol from the equations:

$$2RSH + I_2 \longrightarrow RSSR + 2HI \tag{1}$$

$$H_2S + I_2 \longrightarrow S + 2HI \tag{2}$$

**Disulfide.** Shake sample with 50 ml. of reagent grade petroleum ether (or similar solvent) 15 minutes, transfer to a 100-ml. volumetric flask, and dilute to volume. For duplicate analyses treat about 60 ml. with 10 ml. of 0.1*N* silver nitrate in a separatory funnel to remove hydrogen sulfide and thiols, decant, repeat the treatment, and wash with distilled water. Shake remaining solution vigorously with metallic mercury in a stoppered flask several hours to remove free sulfur; filter and repeat treatment until no more sulfide is formed.

Place aliquots of 20 ml. in 250-ml. Erlenmeyer flasks with 15 grams of 30-mesh zinc and 50 ml. of prepared acetic acid, 94% by weight. Through a ground-glass joint attach to an efficient reflux condenser connected at the top through a stopcock to a trap containing 25 ml. of standard 0.01*N* silver nitrate to collect volatile thiol. With rich samples attach a second trap. Immerse flask in boiling water 3 hours. Titrate silver nitrate remaining in the trap with 0.01*N* ammonium thiocyanate to an iron(III) alum end point. From residue in the flask separate the oil phase by diluting with water, wash it with water, and determine any thiol by the method of Borgstrom and Reid (*4*). Calculate disulfide as half the mercaptan formed, and correct by the factor previously determined for the acid used.

## RESULTS AND DISCUSSION

**Hydrogen Sulfide.** The oxidation procedure was tested with weighed samples of pure reprecipitated cadmium sulfide and is quantitative. Table I shows the results of analyzing gaseous samples of hydrogen sulfide with and without added thiol. The concentrations refer to the solutions prepared from these samples according to the procedure. When no thiol is present the results are in general a few per cent low, whereas they are more accurate when thiol is present. This phenomenon might be connected with the grain size of the sulfide, with adsorption, or with air oxidation in the alkali solution. In any case the method is accurate to within a few per cent in the range 0.002 to 0.01% sulfur. It is sensitive to 0.0000002 mole of hydrogen sulfide per aliquot (0.05 ml. of 0.01*N* iodine) or 0.00003% sulfur. In application to complex mixtures, duplicate determinations have checked to within twice these figures, corresponding to 0.5 mm. of gas in the typical reaction vessel. There appears no likelihood of other sulfur compounds interfering; even if they dissolved in the alkali solvent and survived the filtration they would not interfere with the oxidation.

**Thiol.** The accuracy of the thiol determination is limited more by that of the hydrogen sulfide determination than by that of the sulfhydryl. Equation 2 was checked directly, analysis of 0.000259 ± 0.000002 mole of hydrogen sulfide giving 0.000258 mole. Equation 1 was checked indirectly for methanethiol by showing the stability of dimethyl disulfide, its oxidation product, to iodine plus iodide. The method is not affected by free sulfur monosulfides, and disulfides. It can be used in the presence of most unsaturated hydrocarbons, though not those particular double bonds which react with iodine. Where "reactive" double bonds are present, the less convenient procedure of precipitating the gases directly is more satisfactory.

**Disulfide.** Table II shows the results of a series of reductions of dimethyl disulfide in petroleum ether under various conditions. Refluxing with glacial acetic acid gives results strongly dependent on the time of heating; results of refluxing with 94% acid are too high and poorly reproducible, and the presence of side reactions is evidenced by darkening of the precipitate in the trap. With 94% acid and a water bath the results are 10 to 15% too high, but the error is reproducible and independent of disulfide concentration, so that a correction factor can be determined on a standard solution for any batch of acid.

**Table II. Reduction of Dimethyl Disulfide in Petroleum Ether**[a]

| Conditions | Acid Strength, % | Acid Volume, Ml. | Heating Time, Hr. | Wt. of Zn, Grams | RSSR Present, % S | RSH Found, % S | Reduction, % |
|---|---|---|---|---|---|---|---|
| Reflux, 120° C. | Glacial | 30 | 3 | 10 | 0.0310 | 0.0200 | 65 |
| | | 35 | 4.75 | 10 | 0.0280 | 0.0230 | 82 |
| | | 35 | 6 | 10 | 0.0295 | 0.0271 | 92 |
| | 94 | 35 | 6 | 25 | 0.0306 | 0.0345 | 113 |
| | 94 | 50 | 4 | 15 | 0.0276 | 0.0343 | 124 |
| Water bath, 100° C. | 94 | 50 | 4 | 15 | 0.0392 | 0.0436 | 111 |
| | Glacial | 50 | 3 | 15 | 0.0254 | 0.0002 | 1 |

[a] Volume of solution is 20 ml.

The extent of reduction depends markedly on the strength of the acid used, and a new correction factor must be determined for each batch of acid prepared. Table III shows the results of a series of analyses for disulfide using two different batches of roughly 94% acid. For either acid the error is constant, but the two correction factors differ by 4%. Glacial acid gives only 1% reduction in 3 hours.

Most of the results in Table III refer to analyses of pure solutions in which the preliminary purifications were omitted. It was verified that disulfide is not removed by the silver nitrate wash. The final result refers to a gaseous sample analyzed by the complete procedure; precipitated silver sulfide was added to the silver nitrate wash to simulate actual conditions.

The results are not affected by monosulfide. They are independent of the particular reduction apparatus. Amounts of zinc and acid and the time of heating are not critical. With dimethyl disulfide the reaction is complete in about 2.5 hours, and longer heating has no effect on the results. In practice, duplicate analyses generally check within 0.000001 mole of disulfide per aliquot or 0.0005% sulfur. With heating at a constant temperature instead of reflux, the reduction should be the same with many solvents, a point of interest for the analysis of petroleum products.

The method was developed and tested specifically for dimethyl disulfide. With this homolog the thiol is found entirely in the trap, and it is unnecessary to analyze the residue in the flask. The reduction time may be slightly different for higher homologs. If several homologs are present at once, there is the possible difficulty of small differences in correction factors. In such a case all the factors could be made closer to unity by using a weaker acid—e.g., 97%—but the longer heating needed is a disadvantage.

**Table III. Determination of Dimethyl Disulfide in Petroleum Ether**[a]

| Time of Heating, Hr. | S Present, % | S Found, % | S Corrected, % | Error, % |
|---|---|---|---|---|
| Reductions with Acid A = 94%. Correction Factor 100/110.5 | | | | |
| 4 | 0.0392 | 0.0436 | 0.0394 | 0.6 |
| 3 | 0.0290 | 0.0320 | 0.0289 | 0.3 |
| 3 | 0.0290 | 0.0318 | 0.0288 | 0.7 |
| 3 | 0.0290 | 0.0320 | 0.0289 | 0.3 |
| 3 | 0.0290[b] | 0.0322 | 0.0291 | 0.3 |
| Reductions with Acid B = 94%. Correction Factor 100/115 | | | | |
| 3 | 0.0073 | 0.0088 | 0.0076 | 4 |
| 3 | 0.0292 | 0.0335 | 0.0291 | 0.3 |
| 3 | 0.0802[c] | 0.0963 | 0.0805 | 0.4 |

[a] 1% sulfur = 0.12 molar.
[b] Contained 0.03% sulfur of methyl sulfide.
[c] Complete procedure on gaseous sample from 70 mm. in 250 ml. at 460° C.

The more than quantitative reductions may be due to formation of hydrogen sulfide. Darkening of the precipitate in the trap when refluxing suggests silver sulfide, and the result of Moses and Reid (*8*) is noteworthy that reduction of dibenzyl disulfide by sodium amalgam in alcohol produces bibenzyl and hydrogen sulfide. The analogous reaction

$$CH_3SSCH_3 \xrightarrow{Zn/HAc} CH_3CH_3 + 2\ H_2S$$

would make 1 mole of disulfide appear to be 2 moles.

The disulfide method cannot be used if polysulfides of the type $R - S_{x+2} - R$ are present, as these compounds reduce to hydrogen sulfide and thiol. The serious error introduced is particularly unfortunate for applications to the study of pyrolysis, as polysulfides form rather easily from disulfides under pyrolysis conditions. The development of a method independent of polysulfides would be of considerable interest.

## ACKNOWLEDGMENT

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# Morphology of Barium Sulfate as Seen through Electron Microscopy

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**This investigation was undertaken to determine the morphology of barium sulfate, which is precipitated from barium chloride and sulfuric acid at various concentrations and temperatures. The morphology of barium sulfate is affected by the reaction temperature and the concentration of both reagents. Barium sulfate has been studied by the use of an electron microscope, employing the replica method, which is of value in studying powdery substances. The results are shown in photographs. Generally speaking, the smaller the concentration of each reagent, the simpler is the form of the precipitate, and the higher the reaction temperature, the greater is the rate of growth of the precipitate. The precipitate from a definite concentration and temperature has a definite characteristic shape.**

THE study of the precipitate of barium sulfate has a considerable history, going back nearly half a century. More recently electron microscopical studies have been published (*1, 5*), and the authors have reported on the mechanism of precipitation of barium sulfate (*2, 3*). In this paper they describe the morphology of barium sulfate, which is precipitated from various concentrations of barium chloride and sulfuric acid at room and near-boiling temperatures.

## EXPERIMENT

Barium chloride and sulfuric acid were mixed directly to cause precipitation. Concentrations of both reagents were 0.1, 0.05, 0.01, 0.005, and 0.001*M*. The chemicals used in this experiment were C.P. barium chloride from Merck in Germany and C.P. sulfuric acid for battery use. The barium chloride solution was filtered after a few days' aging. Two milliliters of barium chloride of a definite concentration were poured drop by drop into 2.5 ml. of sulfuric acid of the same concentration. Each time the sulfuric acid was in excess it prevented the crystallization of barium chloride. After 24-hour aging, the precipitates were washed several times with distilled water. To examine the precipitate, the authors employed the replica method of electron microscopy. As a study on the replica of a powdery substance has been published (*4*), only a brief description is given here.

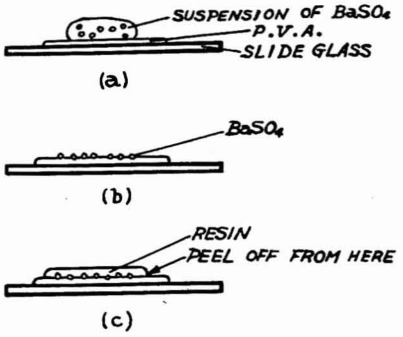

Figure 1. Technique used in replica method

A drop of a suspension of barium sulfate was placed on a poly(vinyl alcohol) sheet which had been dried on a slide glass (see Figure 1, *a*). When allowed to dry, the precipitates settled on the poly(vinyl alcohol) sheet (Figure 1, *b*). Then a benzene solution of poly(methyl methacrylate) was poured over it (Figure 1, *c*). After drying, the poly(methyl methacrylate) was peeled off the poly(vinyl alcohol) sheet. The arrow in Figure 1, *c*, indicates how it was peeled off. As some of the precipitates adhered to the resin, to obtain a good replica the authors were obliged to peel the resin many times. Then the resin, placed in a vacuum evaporator, was coated with aluminum and shadowed with chromium in the usual manner.

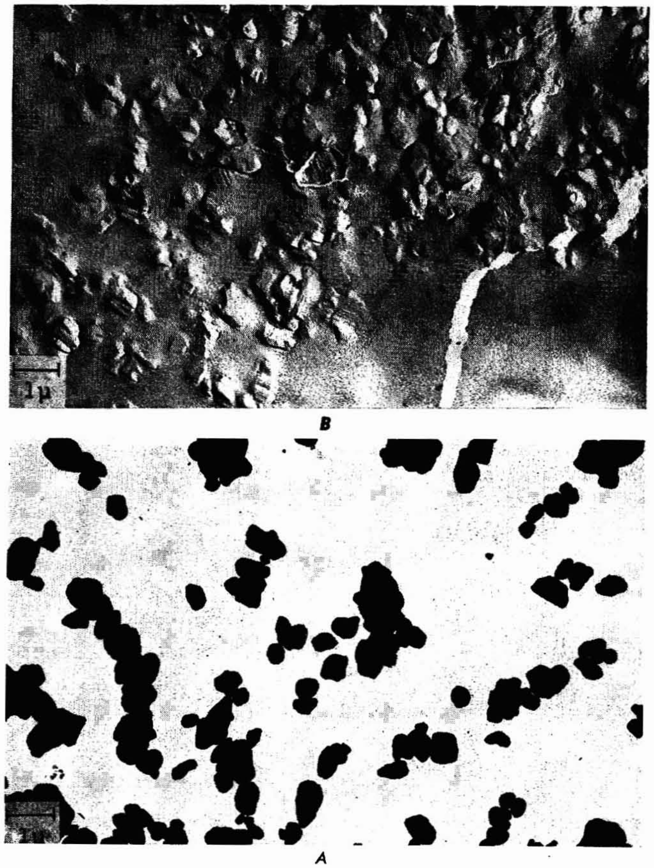

Figure 2. Electronmicrographs of barium sulfate

From 2 ml. of 0.1*M* barium(II) plus 2.5 ml. of 0.1*M* sulfate at room temperature
*A*. Transmission image
*B*. Replica

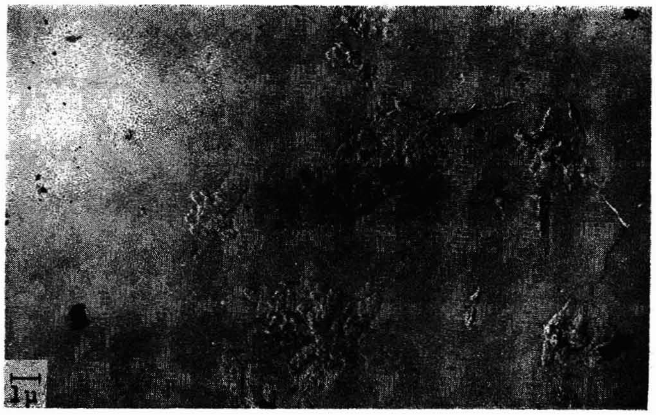

Figure 3. Replica of barium sulfate

From 2 ml. of 0.05*M* barium(II) plus 2.5 ml. of 0.05 *M* sulfate at room temperature

**Figure 4. Replica of barium sulfate**

From 2 ml. of $0.01M$ barium(II) plus 2.5 ml. of $0.01M$ sulfate at room temperature

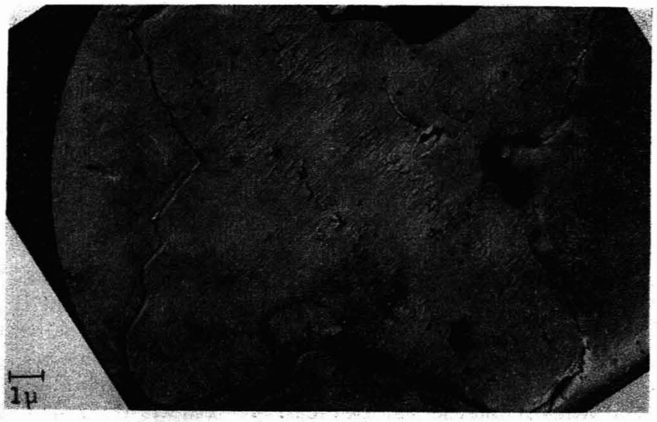

**Figure 5. Replica of barium sulfate**

From 2 ml. of $0.005M$ barium(II) plus 2.5 ml. of 0.005 $M$ sulfate at room temperature

**Figure 6. Replica of barium sulfate**

From 2 ml. of $0.001M$ barium(II) plus 2.5 ml. of $0.001M$ sulfate at room temperature

Photographs from the electron microscope show almost perfect precipitates.

**Precipitation at Room Temperature.** The replica method is compared with the direct transmission method of electron microscopy in Figure 2. Figure 2, *A*, shows an image, obtained by the direct method, of barium sulfate precipitated from $0.1M$ solution while Figure 2, *B*, shows the surface of the barium sulfate. (In all this work 2 ml. of barium chloride solution were poured dropwise into 2.5 ml. of sulfuric acid.) Figure 2, *A*, does not show definitely that each precipitate has a crystalline structure composed of flaky crystals not highly aggregated, but this is confirmed by Figure 2, *B*. In a previous paper (*3*) the authors expressed the opinion that a large, irregularly shaped particle of barium sulfate is an aggregate of $\alpha$-aggregates, and designated it as $\beta$-aggregate, but Figure 2, *B*, shows that an irregularly shaped particle is not always an aggregate of $\alpha$-aggregates but may be an $\alpha$-aggregate in a growing state. This fact is recognized everywhere in this experiment.

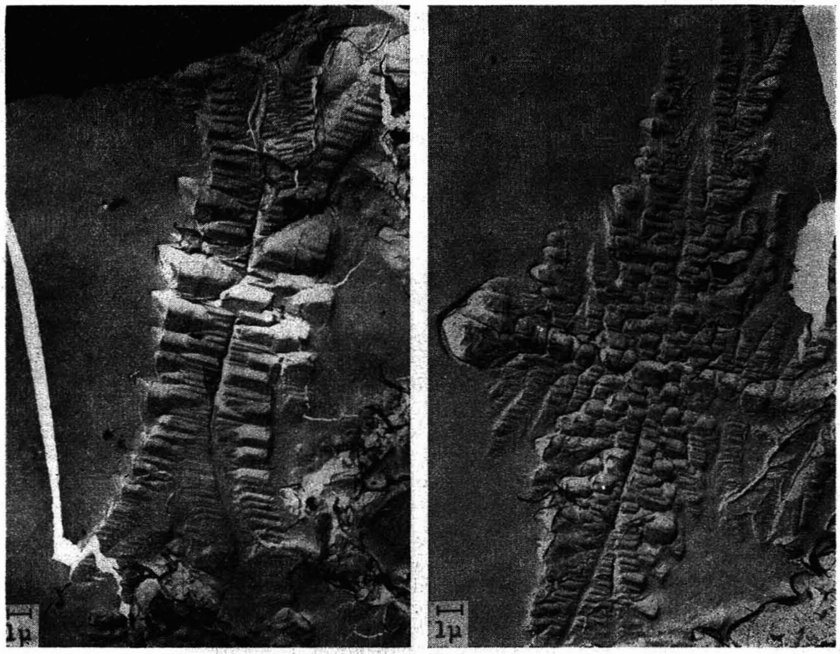

**Figure 7. Replicas of barium sulfate**

From 2 ml. of $0.1M$ barium(II) plus 2.5 ml. of $0.1M$ sulfate at boiling

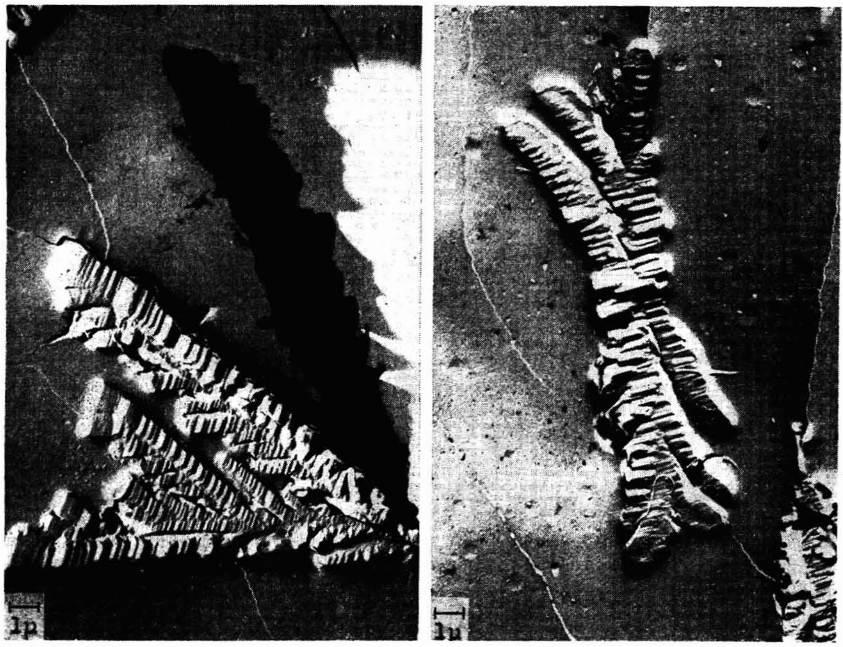

**Figure 8. Replicas of barium sulfate**

From 2 ml. of $0.05M$ barium(II) plus 2.5 ml. of $0.05M$ sulfate at boiling point

Figure 3 shows precipitates of barium sulfate from $0.05M$ solution. In this case the precipitate has no fixed form. In the authors' opinion, it is an imperfect form of a dendrite-shaped precipitate, because precipitates from $0.01M$ solution, as seen in Figure 4, have a typically dendrite form. The authors also believe as a result of experiments in electron microdiffraction, that it is a single crystal. These dendrite-shaped precipitates are very thin and measure about 2 microns at their thickest point. (Thickness means a length perpendicular to the photograph.) They contain many stripes, which are parallel to the short axis and are about 30 mμ wide. From Figure 4, *B*, the authors conclude that the growth velocity of a short axis is less than that of a long axis, so that the branches of a short axis grow parallel to the long axis, and are longer than the branches of the long axis. The precipitate seen in Figure 5 has a similar cross-shaped crystal, and is simpler than from those of Figure 4. Figure 6 shows precipitates from $0.001M$ solution.

These precipitates have a very simple shape and look like a cylindrical lens, but their sizes are not uniform.

**Precipitation at Boiling Point.** In the experiments illustrated in Figures 2 to 6, the precipitation occurred at room temperature. To study the effects of temperature on the morphology of barium sulfate, the authors repeated the experiment at near-boiling temperature.

Equal concentrations of barium chloride and sulfuric acid were heated separately to near-boiling temperature, and then the barium chloride (2 ml.) was poured dropwise into the sulfuric acid (2.5 ml.). The other conditions were the same as in the previous experiment.

**Figure 9. Replica of barium sulfate**

From 2 ml. of $0.01M$ barium(II) plus 2.5 ml. of $0.01M$ sulfate at boiling point

**Figure 10. Replica of barium sulfate**

From 2 ml. of $0.005M$ barium(II) plus 2.5 ml. of $0.005M$ sulfate at boiling point

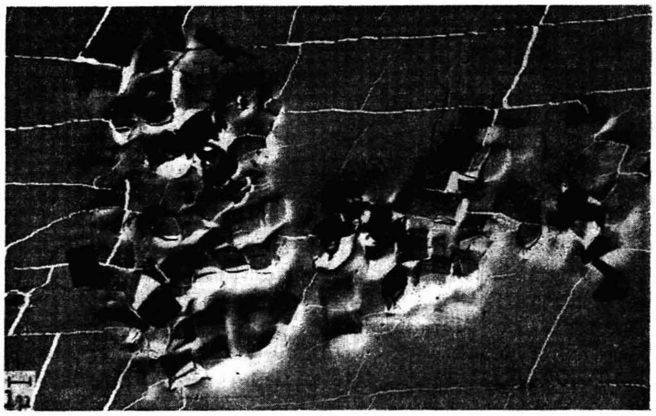

**Figure 11. Replica of barium sulfate**

From 2 ml. of $0.001M$ barium(II) plus 2.5 ml. of $0.001M$ sulfate at boiling point

Precipitates from $0.1M$ solution have two shapes, as seen in Figure 7. The precipitates consist of a regular combination of mosaic blocks. These precipitates are also derived from $0.05M$ solution. Figure 8, *B*, shows the same shape as in Figure 7, *A*, but the shape in Figure 8, *A*, is not derived from $0.1M$ solution. In the experiment shown in Figure 8, *A*, a very interesting development occurred—the authors were able to see the surface of the barium sulfate powder on the bottom, the shadow of the powder generated by the chromium in the center, and the replicated powder on the right side. In the same experiment they were also able to compare the replica image with the direct transmission image. It is clear that without the replica image it would scarcely be possible to deduce the regular structure of the precipitate from the direct image alone. The shape in Figure 8, *A*, has some branches stemming from the *V*-axis. The branches have an almost uniform width of about 1 micron, or rather slightly wider at the farther ends of the *V*-axis. This *V*-shaped precipitate is, in the authors' opinion, one side of an *X*-shaped axis, the branches appearing only within the acute angle of the *V*-axis, so that it also seems to belong to a cross-shaped type. Figure 9 shows precipitates from 0.01*M* solution. They are similar to those in Figure 8, *A*, but not so large in size, and the mosaic blocks are not so clear. Figure 10 shows precipitates from 0.005*M* solution. They have a butterfly shape, and the authors believe that they grow to an *X*-axis, with branches both inside and outside of the acute angle.

Figure 11 shows the simplest type of precipitates obtained in this experiment. They are also similar to a cylindrical lens as are those in Figure 6, but they are not so large, and they are uniform in size. Were it not for the replica method, one might suppose that these precipitates have rectangular and spindle shapes. But the work reported here confirmed that they have only one shape, and that the convex surface of the precipitate is not round like a lens, but is composed of four planes.

## DISCUSSION

In the case of the direct mixing of both reagents at room temperature, the size of the precipitates is smallest at the highest concentration (0.1*M*), and is largest at medium concentration (0.01*M*). Below 0.01*M*, the size of the precipitate is proportional to the concentration and the precipitate has a definite shape, depending upon the concentration.

Mixed at the boiling point, the size of the precipitates is proportional to the concentration throughout this experiment, and the velocity of growth is increased, so perfectly shaped precipitates can be seen even at 0.1*M*.

Independent of the mixing temperature, precipitates from higher concentrations have a complex crystal shape, and precipitates from 0.001*M* have the simplest shape and are small. Generally speaking, the large precipitate is easy to filter and the simply shaped precipitate is free from contamination. In the case of barium sulfate, the larger precipitates have a complex shape and a large surface area. The size of the precipitate from 0.001*M* solution at the boiling point is small (about 2 microns) and its shape is the simplest, so the authors feel that these precipitates are the most free from contamination.

The predominant shape of precipitates at room temperature is a perpendicular cross shape (Figure 4), and that at the boiling point is an oblique cross shape. From this experiment it is clear that precipitates from a definite concentration have a definite characteristic shape, so one can deduce the conditions of the precipitation from the shape of the precipitates.

It is obvious that the replica method is of value in the study of powdery substances, because the surface of the powder is shown by the use of the replication, and no attention need be given to specimen change through electron bombardment.

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# Flame Photometric Determination of Manganese

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**A rapid method for the flame photometric determination of manganese in a variety of materials is described. The intensity of the emission of the manganese line at 403.3 mμ is measured for this determination. Any enhancement or inhibition of this intensity by other ions present can be corrected by a standard addition technique. General background radiation in the vicinity of 403.3 mμ can be detected from the intensity of the emission at 400 and 406 mμ, respectively. Correction for such interference can be applied without any adverse effect to the standard addition procedure.**

ALTHOUGH several authors (*2, 3, 7–9*) have reported that manganese can be determined by means of flame spectroscopy, the details of the procedures, in most cases, have not been reported, and the instruments used have been homemade adaptations of Lundegårdh's apparatus employing photographic recording of intensities. All of the previously reported methods have been concerned with the determination of manganese in organic matter or in minerals or rocks.

After preliminary studies confirmed that several parts per million of manganese could be determined easily by flame photometry, an investigation was undertaken in order to establish rapid procedures for the determination of this element in complex materials such as rocks and alloys. As Kuemmel and Karl (*5*) have pointed out, the application of flame photometry in the metallurgical field has not been very widespread. In addition to their paper on the determination of alkali and alkaline earth metals in cast iron, the only other metallurgical analyses employing flame photometry previously described are the determination of sodium and potassium in lithium metal (*4*), the analysis of lithium in magnesium-lithium alloys (*10*), the determination of traces of sodium in aluminum (*1*), and the determination of indium in aluminum (*6*).

This paper describes rapid procedures for the determination of manganese and presents simple techniques for reducing the errors in this determination caused by interference phenomena encountered with complex solutions containing a number of different chemical components present in the original samples or introduced by the dissolution procedure.

## APPARATUS

Measurements of emission intensities were made with a Beckman Model DU spectrophotometer equipped with a Model 4030 atomizer-burner employing an oxygen-acetylene mixture. The regular blue-sensitive phototube in the spectrophotometer was replaced by the Model 4300 photomultiplier accessory furnished by the manufacturer. The manganese emission intensities were measured at a wave length of 403.3 mμ employing a slit width of 0.06 mm.

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**Table I. Results of Manganese Determinations**

| Sample | Manganese, % Present | Manganese, % Found | Relative Error, % |
|---|---|---|---|
| NBS burned magnesite No. 104 | 0.43 | 0.42[a] | −2.3 |
| NBS manganese bronze, 626 | 1.29 | 1.26<br>1.38 | +2.3 |
| | Av. | 1.32 | |
| NBS nickel-copper alloy, No. 162 | 2.34 | 2.28<br>2.45 | +0.86 |
| | Av. | 2.36 | |
| Stainless steel F | 1.71 | 1.68<br>1.67 | −1.7 |
| | Av. | 1.68 | |
| Stainless steel No. 8 | 0.76 | 0.76[b] | 0.0 |
| Manganese ore No. 134 | 42.3 | 46.1<br>40.4 | +2.1 |
| | Av. | 43.2 | |
| Aluminum manganese No. 1 | 15.6 | 16.3[b] | +4.5 |
| Aluminum manganese No. 2 | 1.82 | 1.83[b] | +0.5 |

[a] Reported as manganese oxide.
[b] Values corrected for very large inhibition on emission of manganese.

## REAGENTS AND STANDARDS

All chemicals used were of analytical reagent or C.P. grade. A standard manganese solution containing 1000 p.p.m. of manganese was prepared by dissolving 0.3957 gram of previously dried Baker's analyzed reagent grade manganese dioxide (less than 0.3% impurities) in concentrated hydrochloric acid. This solution was evaporated to dryness, and the residue was diluted with water to 250 ml.

Suitable portions of the standard manganese solution were diluted with water so that solutions containing 50, 100, 150, and 200 p.p.m., respectively, were obtained. When the intensity of the emission at 403.3 m$\mu$ from each of these solutions was plotted against the concentration of manganese, a linear calibration curve was obtained. That is, if the sensitivity of the instrument was adjusted so that the solution containing 200 p.p.m. of manganese gave a reading of 100 on the per cent transmittance scale, the solution with only 50 p.p.m. of manganese showed an emission of 25 on this same scale. Thus, the calibration curve for manganese is linear up to at least 200 p.p.m. of manganese, as contrasted to a considerable number of metallic ions which do not give linear calibration curves over this same concentration range. The reproducibility of the individual readings for the calibration curve was usually within ±0.5 division on the per cent transmittance scale of the spectrophotometer.

## PROCEDURES

**Elimination of Interferences.** In connection with a broad program of study on flame photometric interferences, it was found that the emission from an $0.0018M$ manganese solution which contained either $0.1M$ phosphate or sulfate was reduced 25 and 17%, respectively. On the other hand, the same concentration of chloride and perchlorate ions increased the emission intensity of $0.0018M$ manganese 4 and 10%, respectively. Copper and zinc ions when present in amounts equivalent to the manganese concentration had no effect on the intensity of the emission at 403.3 m$\mu$, but when these ions were present in larger amounts, as in a brass sample, a 5 to 10% decrease in the intensity of the manganese emission was observed.

In addition to the interferences just mentioned, the effect of unresolved radiation has to be considered when determining the intensity of the manganese emission. This type of interference is simply a consequence of the inability of the monochromator to distinguish between the manganese line at 403.3 m$\mu$ and the energy emitted by some other element present in the unknown solution. This unresolvable background emission commonly occurs as broad bands which result in a general increase in the background intensity.

A simple but effective standard addition procedure was used to overcome the first type of interference. In this method, intensity measurements are obtained on two solutions, one (solution A) containing an aliquot of the unknown solution and the other (solution B) containing the same quantity of unknown solution plus a measured volume of the standard manganese solution. The quantity of manganese in each of these solutions is then determined from their measured emission intensities and the standard calibration curve. Subtracting the quantity of manganese found in solution A from that found in solution B yields an amount of manganese equal to that added when there is no inhibition or enhancement. When one of these effects is present, however, the quantity of manganese found by subtraction is greater or less than that added. In such cases, the true manganese content of solution A is found by multiplying the observed manganese content by a factor which corrects for the interference. This factor is found by dividing the quantity of manganese added to solution B by the amount of manganese found by subtracting the observed manganese content of solution B from that of solution A.

The type of interference resulting from the presence of broad band emission can be eliminated by subtracting this background radiation from the intensity measured at the wave length of the analysis line. In the determination of manganese employing the spectral line at 403.3 m$\mu$, this was done by measuring the background intensity at both 400 and 406 m$\mu$, where the sharp manganese line did not show any measurable emission. Measurements are taken on both sides of the manganese line in order to establish the fact that there is no fine structure in the background in this region of the spectrum. If the background is found to be of equal intensity on either side of 403.3 m$\mu$, it is assumed that the intensity level is constant within this narrow wave length interval, and that correction for this type of interference can be made by merely subtracting this background intensity from the intensity measured at 403.3 m$\mu$. If, however, the intensities measured at 400 and 406 m$\mu$ are not equal, the average of these two readings is taken as the background correction. This procedure assumes that the change in background intensity within this narrow wave length interval is linear. After correction has been made for the background in this manner, the standard addition procedure can be used to correct for enhancement or inhibition.

**Dissolution Procedures.** MINERALS. Approximately 0.5-gram samples of the dried powdered material were weighed into platinum crucibles. In the case of carbonate rocks, in order to prevent loss of sample by the evolution of carbon dioxide, the samples were first covered with distilled water before perchloric acid was cautiously added. After the evolution of carbon dioxide was complete, 5 ml. of perchloric acid and 5 ml. of hydrofluoric acid were added and the solution was evaporated to dryness. In order to prevent bumping during this evaporation, the crucible was heated with a medium hot plate and an infrared lamp. The white residue obtained by this treatment was dissolved in distilled water and diluted to a final volume of 100 ml. For the determination of manganese, aliquots of this solution were taken and diluted to contain less than 100 p.p.m. of manganese.

ALLOYS. Brasses, bronzes, and alloys of nickel and copper were easily dissolved in concentrated nitric acid. In each case, accurately weighed samples containing 0.2 to 1 gram of fine turnings were dissolved in 10 ml. of concentrated nitric acid. After dissolution of the samples was complete, the solutions were evaporated to dryness and the residues dissolved in dilute hydrochloric acid. The solutions were then diluted to 100 ml. Aliquots were taken for analysis and diluted to contain less than 100 p.p.m. of manganese.

A stainless steel sample was treated with dilute sulfuric acid (1 to 4) and then warmed until solution was complete. After the addition of 5 ml. of nitric acid, the solution was evaporated to a small volume. A heavy residue was formed which was difficult to redissolve. Consequently, prolonged heating and excessive evaporation were avoided in subsequent samples, and the solution containing a large excess of acid was diluted to 100 ml. with distilled water. Aliquots of this solution, diluted to contain less than 100 p.p.m., of manganese, were taken for analysis. The standard addition technique was successful in these cases even in the presence of a very high concentration of sulfate ion which normally behaves as a strong inhibitor.

## RESULTS AND DISCUSSION

The standard addition technique revealed no examples of enhancement of the manganese line in the analyses reported here. On the contrary, inhibition was found in every case except for the magnesite and manganese ore samples, with correction factors varying from 1.25 to 2.38. Corrections for unresolved background emission were necessary with the magnesite, the stainless steels, and the mixture of aluminum and manganese containing a small amount of manganese. The background emission in the magnesite sample was attributed to the magnesium oxide band which has its maximum emission at 383 mμ. The quantity of magnesium in this material was over 200 times the manganese content. The interfering background emission in the stainless steels was probably caused by iron which has a series of emission lines in the vicinity of 400 mμ or by iron oxide which produces a band spectrum in this region. In the case of aluminum-manganese mixtures, the background emission is detectable only when the sample contains over 85% aluminum and is presumably due to a weak band spectrum from aluminum oxide.

The results of several determinations for manganese on six widely differing materials are given in Table I. The accuracy found in these determinations is consistent with that reported by other workers who have employed the flame photometric technique and is superior to that obtained by other emission spectrographic methods. Although this accuracy may be surpassed in absorptiometric or titrimetric methods for manganese, the flame photometric method should be especially valuable where a simple, reasonably accurate, and rapid determination is required. After dissolution of the samples the time necessary to complete a duplicate analysis for manganese by the method suggested was never greater than 20 minutes.

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# Infrared Spectra of Adrenocortical Hormones in Potassium Bromide Disks

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**The infrared spectra of six adrenocortical hormones dispersed in potassium bromide disks are presented. The spectra are reproducible and can be used for identification purposes. The corticosteroids possessing a 17-hydroxyl group and those corticosteroids in which this group is absent exhibit significant differences in the 1100 to 1000 cm.$^{-1}$ region.**

THE need for a method of identifying small amounts of adrenocortical steroids by infrared spectroscopy has arisen in the course of work in progress in this laboratory on the assay of individual steroids in biological extracts. As the more highly hydroxylated adrenocortical steroids are difficultly soluble in the usable solvents, attention was focused on the study of these compounds in the solid state.

Earlier work on the infrared spectra of adrenocortical hormones in the solid state involved the preparation of melted or deposited films (*5, 8*) and suspensions in Nujol (*3, 6, 8*). Some difficulties which may be encountered with these methods are reflection losses, inconsistent optical reproducibility, and interference of bands of the mulling agent. In general, these methods require milligram quantities of material; however, it has been reported (*8*) that a complete spectrum of a crystalline film can be obtained on 0.5 to 2.0 mg. of substance.

In an effort to avoid the above-mentioned difficulties, the method of dispersing a compound, under external pressure, in solid potassium bromide (*10*) was applied to the adrenocortical steroids. In this way as little as 300 γ of the free alcohols can be analyzed rapidly and conveniently. The resulting spectra are well resolved, of optimum intensity, reproducible, and qualitatively useful for identification purposes. The disks may be stored under anhydrous conditions for future reference. With micro infrared equipment (*1, 2*) and smaller die sizes (*2, 9*), smaller amounts of sample should be sufficient.

## EXPERIMENTAL

The following adrenocortical steroids were recrystallized from alcohol: 11-deoxycorticosterone (Q), 11-dehydrocorticosterone (A), corticosterone (B), 17-hydroxy-11-deoxycorticosterone (S), 17-hydroxy-11-dehydrocorticosterone (E), and 17-hydroxycorticosterone (F). Each of the crystalline compounds was dissolved in sufficient absolute alcohol to give a concentration of 10 γ per ml. Thirty milliliters (300 γ) of each of the solutions was concentrated in vacuo at 50° C. to approximately 0.5 ml.

The potassium bromide was obtained by pulverizing absorption cell plates. The powder (200 mesh) was dried in a vacuum oven at 105° C. for 16 hours. The steroid concentrate was dried with 65 mg. of potassium bromide in a porcelain spot plate at room temperature under reduced pressure for 16 hours. Afterward the mixture was ground and mixed with a mullite pestle on the spot plate.

An additional 135 mg. of potassium bromide was arranged in the form of a ring, about 2.8 mm. wide, inside a die similar to the one used by Stimson and O'Donnell (*10*). The mixture of potassium bromide and sample was placed in the center of this ring. The die was assembled and placed in the larger cylinder. The system was evacuated to 0.15 mm. of mercury and was subjected to approximately 9 tons of force by a Carver hydraulic press for 10 minutes. At the end of this time air was admitted to the system and the pressure was released. Under these conditions a disk of 12.6-mm. diameter and 0.56-mm. thickness, with the sample occupying a circular area of about 7-mm. diameter, was obtained.

The disk was placed in a holder which fitted into the microcell adapter of a Perkin-Elmer Model 21 double-beam infrared spectrophotometer. The spectra were taken from 5000 to 660 cm.$^{-1}$ without compensation for the potassium bromide. Atmospheric water vapor and carbon dioxide in addition to ammonia vapor were used for calibration. Band positions were accurate within ±5 cm.$^{-1}$ with somewhat less accuracy for broad bands.

## RESULTS

In Figure 1 is shown the spectrum of a disk of the potassium bromide used in this study. The presence of a small amount of water is indicated by the weak band at 3400 cm.$^{-1}$

All of the spectra in Figures 1 to 7 exhibit absorption bands characteristic of associated hydroxyl(s) (3401 to 3344 cm.$^{-1}$), 20-

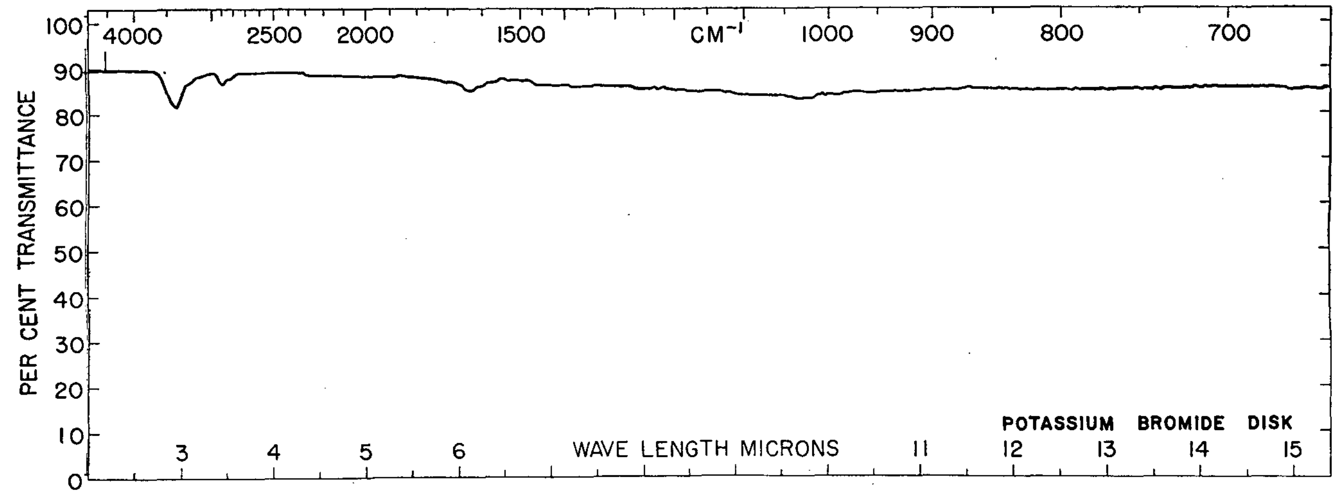

Figure 1. Infrared spectrum of potassium bromide disk

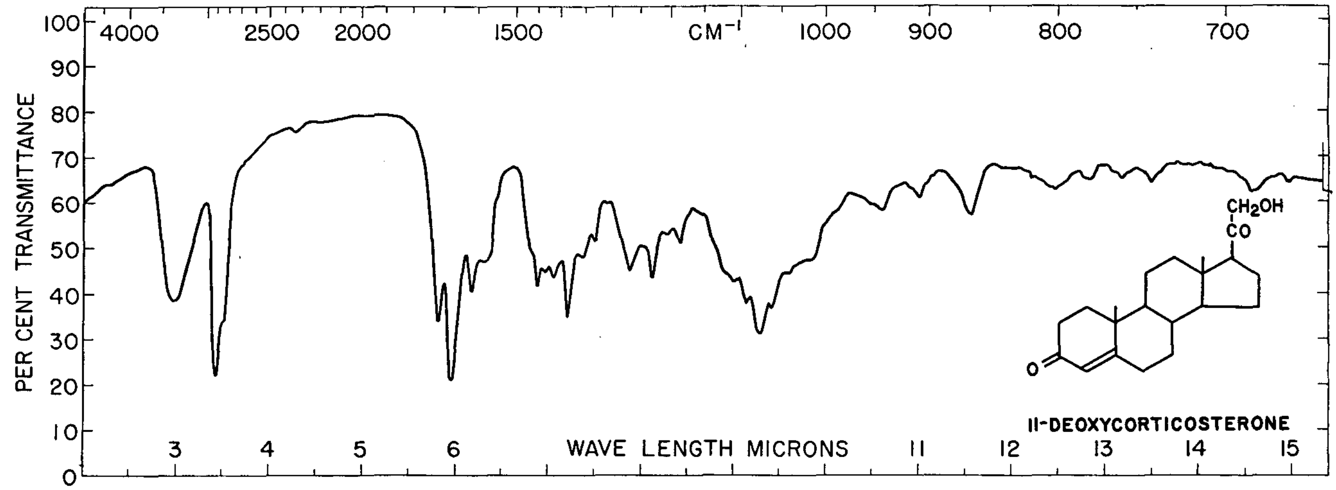

Figure 2. Infrared spectrum of 11-deoxycorticosterone (Compound Q)

ketone (1706 cm.$^{-1}$), $\Delta^4$-3-ketone (1667 to 1661 cm.$^{-1}$), and the $\Delta^4$-double bond (1618 to 1608 cm.$^{-1}$). Compound A, which has an additional carbonyl at $C_{11}$, exhibits a band at 1721 cm.$^{-1}$ The origin of the shoulder near 1575 cm.$^{-1}$ is not known.

All spectra given show bands at 1276 to 1259 cm.$^{-1}$ and 1236 to 1230 cm.$^{-1}$ Compounds A, S, and E show additional absorption near 1218 cm.$^{-1}$

In the C–OH stretching region (1100 to 1000 cm.$^{-1}$) there are significant differences between the spectra of the 17-hydroxyl corticosteroids and those corticosteroids in which this group is absent. In the spectra of compounds S, E, and F, the most intense bands occur at 1096 cm.$^{-1}$ and 1048 to 1034 cm.$^{-1}$. Compounds B and A possess corresponding maxima at 1079 to 1072 cm.$^{-1}$ and 1040 to 1034 cm.$^{-1}$ Compound Q exhibits the band at 1076 cm.$^{-1}$; however, the maximum at 1040 to 1034 cm.$^{-1}$ is not detected.

The spectra of all compounds except E exhibit a band of moderate intensity between 880 and 860 cm.$^{-1}$ which is attributable to some structural vibration involving the $\Delta^4$-3-ketone (*4*, *7*). The spectrum of compound E displays two weak bands in this region.

These spectra can be reproduced by analyzing replicate disks prepared under the given experimental conditions. Although these disks may vary slightly in transparency, the resulting spectra are qualitatively useful for identification purposes.

## DISCUSSION

In general, the spectra of the adrenocortical steroids (Figures 2 to 7) are similar to those published by other workers. In the 1200 to 800 cm.$^{-1}$ region they are nearly identical with the spectra of solutions published by Dobriner and others (*4*). However, they are somewhat different from the spectra reported previously in which solid films, melts, and suspensions in Nujol were employed.

Furchgott and others (*5*) reported the presence of a band near 1727 cm.$^{-1}$ in the spectra of compounds A and E which was assigned to the 11-ketone.

Dobriner and others (*4*) reported that some six-membered ring ketones possess two or more bands of moderate intensity between 1300 and 1100 cm.$^{-1}$ Jones and Herling (*7*) reported that there are characteristic bands in the spectra of $\Delta^4$-3-ketones between 1332 and 1187 cm.$^{-1}$ in addition to bands in other regions. The two bands at 1276 to 1259 cm.$^{-1}$ and 1236 to 1230 cm.$^{-1}$ are in agreement with these earlier observations.

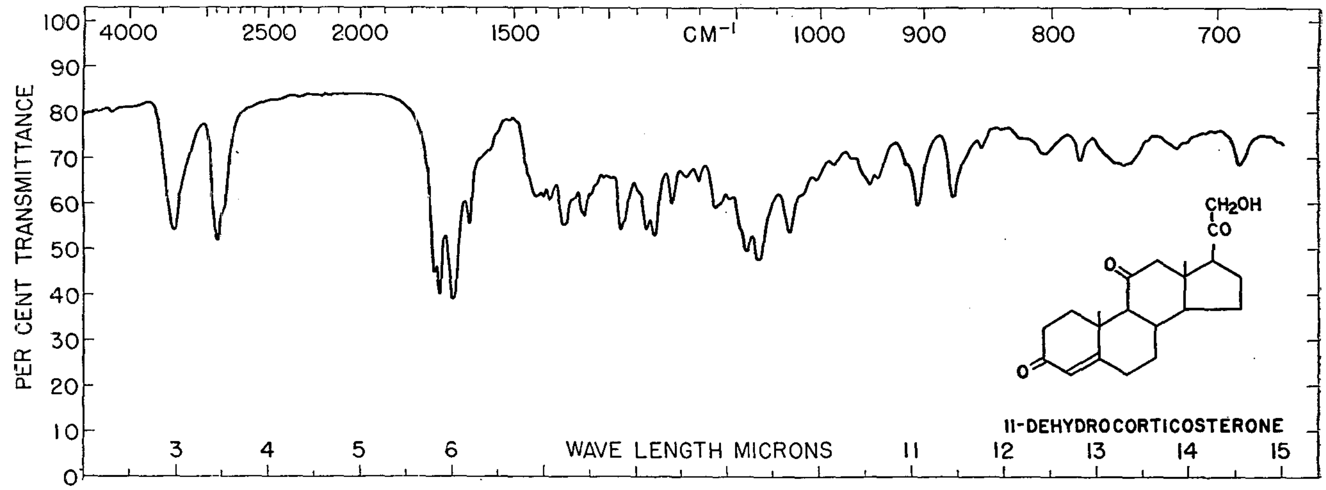

Figure 3. Infrared spectrum of 11-dehydrocorticosterone (Compound A)

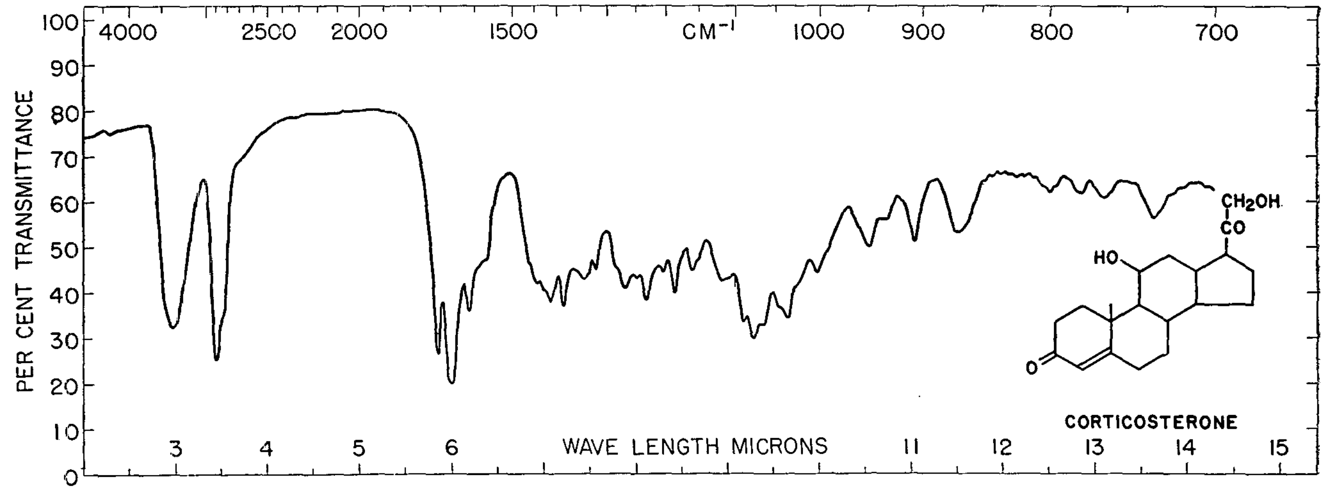

Figure 4. Infrared spectrum of corticosterone (Compound B)

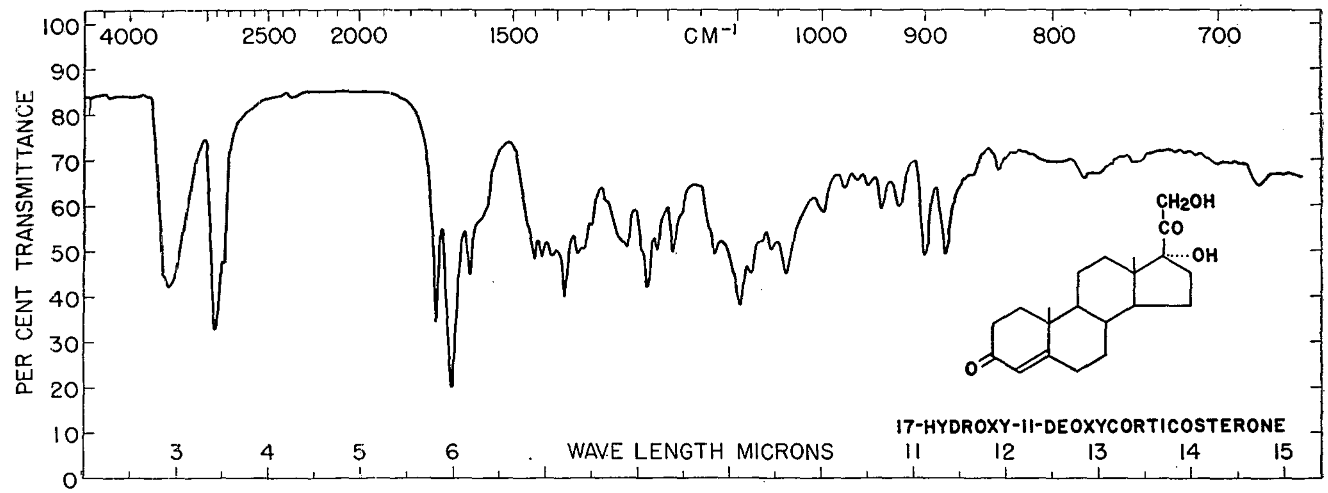

Figure 5. Infrared spectrum of 11-deoxy-17-hydroxycorticosterone (Compound S)

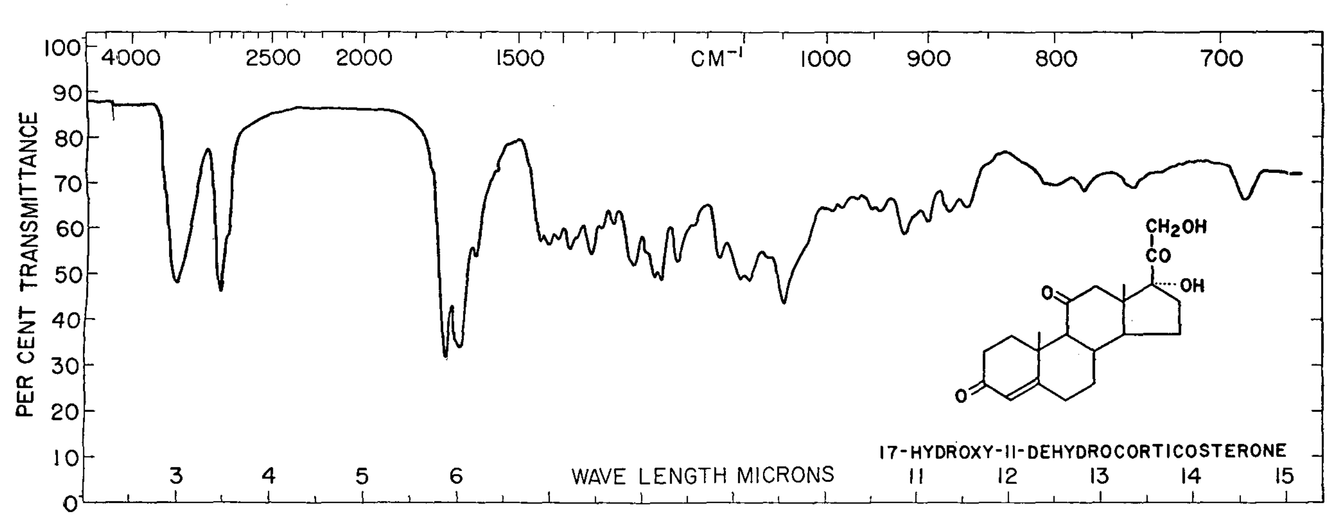

Figure 6. Infrared spectrum of 11-dehydro-17-hydroxycorticosterone (Compound E)

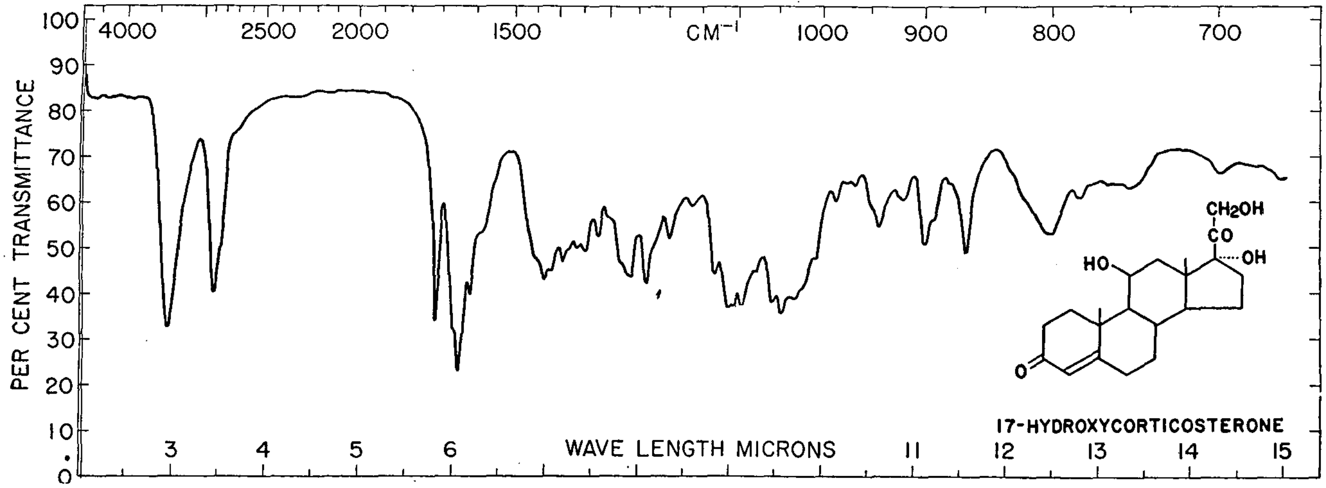

Figure 7. Infrared spectrum of 17-hydroxycorticosterone (Compound F)

The possible usefulness of the differences in band positions in the 1100 to 1000 cm.$^{-1}$ region to distinguish between the three 17-hydroxyl corticosteroids and the three corticosteroids in which this group is absent must await further investigation. Conceivably, hydroxylic or ketonic substituents could alter the appearance of bands in this region.

Although the method, as reported here, is not quantitative, its usefulness for the qualitative identification of microgram quantities of adrenocortical hormones is established.

## ACKNOWLEDGMENT

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The infrared spectra were taken by Harold K. Miller and W. Madison Jones of this laboratory.

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# Modification of Ferrous Thiocyanate Colorimetric Method for Determination of Some Atmospheric Oxidants

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**No satisfactory method for measuring the oxidants produced by the reaction of ozone with hydrocarbons in the gas phase has been available. The ferrous thiocyanate method for the determination of peroxides was modified and used for the determination of gaseous oxidants. The reagent responds to very low concentrations of the reaction products of ozone and hexene or gasoline while being fairly insensitive to ozone alone. It is somewhat sensitive to nitrogen dioxide but not to nitrous oxide, formic acid, formaldehyde, valeric acid, or isovaleraldehyde. It appears to be useful in sampling naturally polluted atmospheres as well as atmospheres artificially polluted with various ozonated hydrocarbons.**

ATMOSPHERIC pollution (or smog) in the Los Angeles basin has become a problem of much interest in the past few years. Certain constituents in this pollution produce a type of damage to agricultural crops that appears to be unique (*9*). It has been postulated that the plant damaging substances are organic peroxides produced by the reaction of ozone and volatile unsaturated hydrocarbons found in the atmosphere (*6*). Haagen-Smit has demonstrated the presence of short-chain organic peroxides in the Pasadena atmosphere during a period of aggravated pollution, using the enzyme peroxidase (*4*). However, the enzyme is unable to utilize the longer chain peroxides as a substrate. These latter peroxides have not been identified as such in the atmosphere.

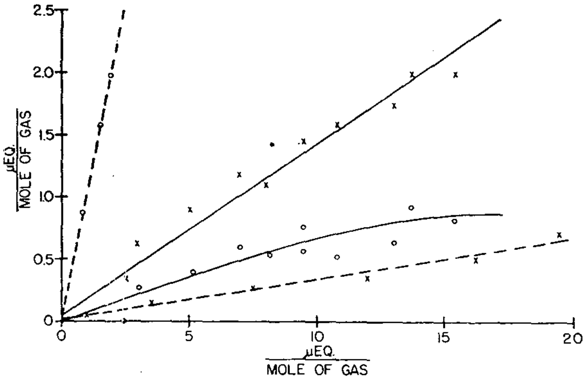

**Figure 1. Response of potassium iodide and thiocyanate reagents to ozone alone and ozone plus hexene**

X. Ferrous thiocyanate
- - - Ozone only
—— Ozone plus hexene
O. Potassium iodide
- - - Ozone only
—— Ozone plus hexene

A common technique for measurement of the level of pollution is to determine the oxidant concentration with a 20% buffered potassium iodide solution, which, under the conditions used, measures atmospheric ozone (*10*), nitrogen dioxide (*3*), and some peroxides (*12*). Since ozone damage to plants has been observed in the area only rarely, a measure of other oxidants present might appear to give a better indication of the amount of plant damaging substances present. Another reagent that is used for measuring atmospheric oxidants is phenolphthalin (*5, 8*). This reagent measures some peroxides as well as ozone, but its greatest sensitivity seems to be to ozone.

Most other reagents used for the detection of organic peroxides were found to be extremely sensitive to ozone. The ferrous thiocyanate reagent previously used to determine organic peroxides in the liquid phase (*11*) is not nearly so sensitive to ozone as the other reagents tested and, therefore, is useful in measuring other atmospheric oxidants which are present in only minute amounts.

## MATERIALS AND METHODS

The ferrous thiocyanate reagent for peroxides, which is a modification of that given by Wagner and others (*11*), was prepared in the following manner:

One-half gram of ammonium thiocyanate and 1.0 ml. of 6$N$ sulfuric acid were added to 100 ml. of water. To this 0.1 gram of ferrous ammonium sulfate was added and shaken. The reagent was then diluted with 100 ml. of water to give a low blank colorimetric density. The solution, which darkens upon standing, should be prepared fresh daily. The pigment formed can be removed with decolorizing carbon; thus the reagent probably could be used with an automatic recorder of the type described by Littman and others (*7*). The amount of color developed was read with a Fisher Electrophotometer using a 425-mμ filter (the primary absorption band lies at about 460 mμ). A standard curve was prepared by adding known amounts of ferric iron to the reagent. A standard curve may also be prepared using known amounts of hydrogen peroxide (1 mole releases 2 equivalents of ferric iron). The results are expressed as the number of microequivalents (μ eq.) of ferric iron produced by 22.4 liters (1 mole) of the gas.

**Table I. Response of Potassium Iodide and Thiocyanate Reagents to Ozone, Hydrocarbons Alone, and Reaction Products from Ozonated Hydrocarbons**

| | μ eq. per 22.4 Liters | | |
|---|---|---|---|
| Sample | Peroxides determined with ferrous thiocyanate | Ozone determined with potassium iodide | Rubber Cracking |
| Hexene only | 0.006 | 0.0 | No |
| Ozone only | 0.031 | 0.382 | Yes |
| Ozone plus hexene | 0.117 | 0.104 | No |
| Gasoline only | 0.237 | 1.01 | ... |
| Ozone only | 0.498 | 6.34 | ... |
| Ozone plus gasoline | 1.187 | 1.22 | ... |

Ozone was determined by passing the gas through a 20% potassium iodide solution buffered at pH 7.0 with 0.1$M$ phosphate. The total iodine released after acidification with sulfuric acid was titrated with 0.005$N$ sodium thiosulfate using a starch end point. The results are expressed as the number of microequivalents of iodine released by 22.4 liters (1 mole) of the gas.

In the experiments reported in Figure 1, the ozone was produced by passing cylinder oxygen through a corona discharge tube (manufactured by the Mueller Neon Co., 1231 Sunset Boulevard, Los Angeles 26, Calif.). The vapor of 1-hexene (obtained from Phillips Petroleum Co.; technical grade 95 mole % minimum) was introduced into the system by passing a stream of cylinder nitrogen through the liquid. The rates of flow of the various components were measured by passing them through small calibrated capillaries. The rate of oxygen flow (containing the ozone) was 35 liters per hour. The stream of nitrogen was regulated to a flow of 2 liters per hour and was bubbled through the liquid 1-hexene at 20° C. The ozone and hexene were in contact for about 45 seconds (calculated mean residence time). The mixture was then diluted with carbon-filtered air at the rate of 800 liters per hour. This gave a final hexene concentration of about 570 $\mu$ moles per mole of gas assuming no reaction with the ozone. The final ozone concentration is presented with the data assuming no reaction with the hydrocarbons (hexene). Where gasoline was substituted for 1-hexene the amount used was much higher because a high proportion of nonreactive materials is present (only the olefinic compounds are easily ozonated).

In one experiment (reported in Table I) where ozone and hexene were used, they were mixed in a small tube and further placed into a large chamber (187 cubic feet) which gave a mean residence of about 1 minute. The final hexene concentration was 17 $\mu$ moles per mole and the ozone concentration was 0.2 $\mu$ mole per mole. One micromole of a substance per 22.4 liters of a gas equals one part per million by volume. The final concentrations were calculated as though none of the reactants were used up.

The cracking of rubber as used in one experiment is a very sensitive indicator of the presence of ozone (*2*). The strips of rubber remained in the air stream for 20 minutes (a flow of 2.8 liters per minute) and were subsequently examined for cracking. When the ozone concentration exceeds about 0.16 $\mu$ eq. per mole, cracking is observed in this length of time.

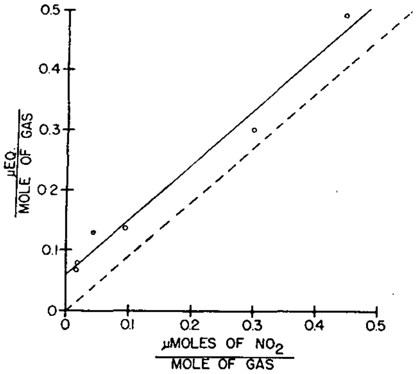

**Figure 2. Response of ferrous thiocyanate reagent to low concentrations of nitrogen dioxide gas in air**

- - -. Translation line through origin

The nitrous oxide (98%) and nitrogen dioxide (98%) were obtained in cylinders from the Matheson Chemical Co.

### RESULTS AND DISCUSSION

Preliminary studies indicated that the response of the thiocyanate reagent was definitely marked to ozonated hexene while the response to ozone alone was low. Table I shows such an experiment where the ozone (0.4 $\mu$ eq. per mole of gas) and 1-hexene (17 $\mu$ moles per mole of gas) were mixed in a very large chamber (187 cubic feet) and the mean residence time was 1 minute. When ozone is present alone the amount measured by the thiocyanate reagent is less than one tenth the value of the determination of ozone with potassium iodide. The rubber-cracking test indicated that the ozone concentration was less than 0.16 $\mu$ eq. per mole after the addition of hexene. The thiocyanate reagent value was much higher after hexene was added indicating that ozonated intermediates were present, presumably peroxides. The results of a similar experiment where gasoline was used as the hydrocarbon, together with a shorter reaction time, is also given in Table I. Again the thiocyanate reagent indicates the presence of ozonated intermediates when the gasoline and ozone are mixed. The high value obtained with gasoline alone with both reagents is probably due to oxidizing agents present in the gasoline.

A more complete investigation was made of the sensitivity of both potassium iodide and ferrous thiocyanate to ozone alone and to ozone and hexene. Figure 1 shows that the ferrous thiocyanate reagent is not very responsive to ozone but is much more sensitive to the reaction products of ozone plus hexene. The potassium iodide reagent releases 1 equivalent of iodine for each equivalent of ozone present, whereas the thiocyanate reagent releases only 1 equivalent of ferric iron for each 30 equivalents of ozone present. After the addition of hexene to the ozone, the response of the thiocyanate reagent increased to more than four times the response to ozone alone. The potassium iodide reagent gave a fairly low response to the reaction products of ozone plus hexene. There may have been some unreacted ozone remaining at the higher concentrations accounting for the somewhat erratic experimental points with the potassium iodide reagent which is extremely sensitive to ozone.

Since this reagent was modified for use in atmospheric sampling it was desirable to know what other substances might occur in the air which would interfere with the test. Ozone does show a slight interference. The oxides of nitrogen are also present in the Los Angeles atmosphere and occur to the extent of 0.4 p.p.m. (0.4 $\mu$ mole per mole of gas during a period of extended pollution) (*1*). The results of a test of the response of ferrous thiocyanate to nitrogen dioxide in air are given in Figure 2. The high blank represents substances that color the thiocyanate present in the air used. If the curve is translated so that it passes through the origin, then 0.5 $\mu$ mole of ferric iron is released for each micro mole of nitrogen dioxide present in the sample. However, according to Effenberger (*3*), neutral buffered potassium iodide releases 1 mole of iodine for each mole of nitrogen dioxide present. Thus, when nitrogen dioxide occurs in the atmosphere it will cause color development of these reagents, although to a greater extent with the potassium iodide than with the thiocyanate.

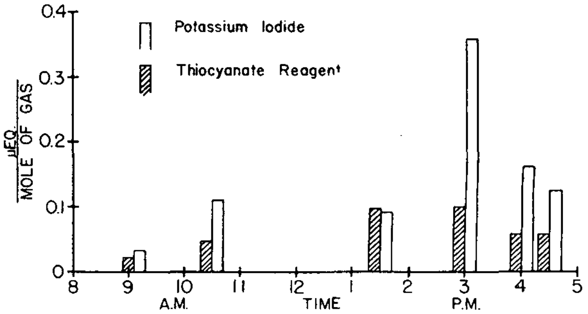

**Figure 3. Comparison of response of potassium iodide and thiocyanate reagents to contaminants present in naturally polluted air**

Several other substances that occur in the Los Angeles atmosphere during periods of smog, or might be expected as end products in the reaction of ozone and hexene, caused no color development of the thiocyanate reagent. These were: 98% nitrous oxide, 5000 p.p.m. of formaldehyde, formic acid, valeric acid, or isovaleraldehyde.

The usefulness of the thiocyanate reagent for sampling naturally occurring pollution was determined on outside Riverside air (Figure 3). Five cubic feet of outside air were passed through 20 ml. of the thiocyanate reagent. It is compared to the amount of iodine released from buffered potassium iodide reagent. When the visibility decreased and the amount of iodine released from the potassium iodide solution increased (as measured by a continuous oxidant recorder) (*7*), a parallel increase was also shown by the ferrous thiocyanate reagent. This indicates that when the amount of oxidant present in the atmosphere increases, the amounts of substances (possibly peroxides) giving a reaction with the thiocyanate reagent also show an increase, probably being dependent on the amount of hydrocarbons present.

### ACKNOWLEDGMENT

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# Analysis of Certain Alkylated Phenol Mixtures by Bromination

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**By taking advantage of the different number of ortho or para positions available for bromination in nonalkylated and alkylated phenols, it has been possible to apply the acid bromate-bromide method to the analysis of certain phenol mixtures. Attention to the percentage of excess bromine and the reaction time is necessary, the best conditions being determined by a study of known mixtures before applying the method to unknowns.**

THE quantitative bromination of phenols by acid bromate-bromide solution has been reviewed and examined thoroughly by Ruderman (*4*). The effect of acid concentration, bromination time, temperature, and substituents was investigated. In particular, Ruderman showed that an appreciable excess of bromine and long reaction times very often resulted in overbromination—i.e., bromination in excess of the number of unsubstituted ortho and para positions. But in certain cases—for example, *m*-cresol and phenols with *tert*-alkyl substituents—excellent results were obtained regardless of the percentage of excess bromine. Others—for example, *p*-cresol—gave good results when a reasonable (say 10 to 30%) excess of bromine was used. The most critical variable with most phenols appeared to be the percentage of excess bromine.

To study the kinetics of the alkylation of several phenols with α-phenylethyl chloride (*2*), it became necessary to analyze mixtures of phenols and their alkylated homologs. One possible method would take advantage of the different number of ortho or para positions available for bromination in the nonalkylated and alkylated phenols. Despite the limitations cited above, it has been found that the bromate-bromide technique can give satisfactory results, provided one uses a limited excess of bromine and short bromination times. The conditions must be carefully established with known mixtures. This paper describes the application of the bromate-bromide method to mixtures of certain alkylated phenols.

**Table I. Analysis by Bromination of Phenol, *o*-(α-Phenylethyl)phenol, *p*-(α-Phenylethyl)phenol, and Mixtures of These**

| Millimole | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Phenol | *o*-(α-Phenylethyl)-phenol | *p*-(α-Phenylethyl)-phenol | Reaction Time, Sec. | Meq. $BrO_3^-$-$Br^-$ | Meq. $S_2O_3^{--}$ | Net Meq. $Br_2$ Absorbed | R[a] | Phenol, % Found | Phenol, % Calcd. |
| 0.402 | | | 120 | 2.7160 | 0.3726 | 2.3434 | 2.92 | | |
| 0.402 | | | 120 | 2.7160 | 0.3575 | 2.3585 | 2.93 | | |
| 0.402 | | | 240 | 2.7160 | 0.3575 | 2.3585 | 2.93 | | |
| 0.402 | | | 20 | 2.7160 | 0.3520 | 2.3640 | 2.95 | | |
| 0.402 | | | 25–30 | 2.7160 | 0.3320 | 2.3840 | 2.97 | | |
| 0.402 | | | 25–30 | 2.7160 | 0.3370 | 2.3790 | 2.96 | | |
| | 0.384 | | 20 | 1.6490 | 0.1913 | 1.4577 | 1.92 | | |
| | 0.384 | | 20 | 1.6490 | 0.1913 | 1.4577 | 1.92 | | |
| | 0.384 | | 30 | 1.6390 | 0.1720 | 1.4770 | 1.95 | | |
| | | 0.1656 | 60 | 0.7760 | 0.0504 | 0.7256 | 2.20 | | |
| | | 0.1656 | 10–15 | 0.7760 | 0.0957 | 0.6803 | 2.06 | | |
| | | 0.1656 | 10–15 | 0.7760 | 0.0856 | 0.6904 | 2.09 | | |
| | | 0.1656 | 5 | 0.7760 | 0.1007 | 0.6753 | 2.05 | | |
| | | 0.3312 | 25–30 | 1.4550 | 0.0705 | 1.3845 | 2.09 | | |
| 0.396 | 0.0212 | 0.0229 | 20 | 2.7160 | 0.206 | 2.510 | | 85.3 | 90.0 |
| 0.396 | 0.0212 | 0.0229 | 25–30 | 2.7160 | 0.156 | 2.550 | | 89.8 | 90.0 |
| 0.396 | 0.0212 | 0.0229 | 25–30 | 2.7160 | 0.156 | 2.550 | | 89.8 | 90.0 |
| 0.396 | 0.0772 | 0.0557 | 30–35 | 3.100 | 0.216 | 2.884 | | 72.6 | 74.9 |
| 0.396 | 0.0772 | 0.0557 | 25 | 3.100 | 0.201 | 2.899 | | 73.9 | 74.9 |
| 0.396 | 0.0772 | 0.0557 | 25 | 3.100 | 0.212 | 2.888 | | 73.0 | 74.9 |
| 0.396 | 0.193 | 0.209 | 20 | 4.171 | 0.236 | 3.935 | | 46.4 | 49.7 |
| 0.396 | 0.193 | 0.209 | 25–30 | 4.171 | 0.201 | 3.970 | | 48.7 | 49.7 |
| 0.396 | 0.193 | 0.209 | 25–30 | 4.171 | 0.201 | 3.970 | | 48.7 | 49.7 |
| 0.396 | 0.193 | 0.209 | 25–30 | 4.171 | 0.201 | 3.970 | | 48.7 | 49.7 |
| 0.158 | 0.278 | 0.201 | 20–25 | 3.100 | 0.246 | 2.854 | | 24.0 | 24.8 |

[a] Number of reactive positions per molecule—i.e., for phenol, $R = 3$.

### SOLUTIONS AND REAGENTS

**Sodium Thiosulfate,** 0.1*N*, standardized against a weighed sample of potassium iodate using standard analytical procedure.

**Bromide-Bromate Solution,** 0.1*N*, prepared following the directions of Ruderman (*4*).

**Hydrochloric Acid,** concentrated acid, specific gravity 1.19.

**Potassium Iodide,** 10% aqueous solution.

**Methanol.** Merck & Co. absolute methanol, c.p., acetone-free, was used without further purification.

**Phenol.** c.p. grade phenol was triply distilled, the final distillation being carried out in a nitrogen atmosphere, boiling point 179° to 180° C.

***o*-Cresol.** Water-white, c.p. grade *o*-cresol (Fisher Scientific Co.) was used without further purification.

***p*-Cresol.** Practical grade *p*-cresol was triply distilled to give

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a water-white product, boiling point 199–200° C.; recrystallized from petroleum ether gave a melting point 36–37° C.

**2-α-Phenylethylphenol.** A sample obtained from Chemical Division, Koppers Co., Pittsburgh, Pa., was distilled through a Podbielniak column; boiling point 127–127.5° C. at 0.9 mm. of mercury, $n_D^{20} = 1.5923$.

**4-α-Phenylethylphenol.** A sample obtained from Koppers, was recrystallized from petroleum ether, melting point 56.0–56.3° C.

**4-α-Phenylethyl-2-methylphenol.** This compound was prepared by the alkylation of *o*-cresol with α-phenyl ethyl chloride (*3*). The crude 4-α-phenethyl-2-methylphenol was doubly distilled and collected as a water-white viscous liquid, boiling point 158–159° C. at 6 mm. of mercury. The reported boiling point is 179° C. at 13 mm. (*1*).

**2-α-Phenylethyl-4-methylphenol.** This was prepared in a manner identical to that described for 4-α-phenylethyl-2-methylphenol using *p*-cresol instead of *o*-cresol. It was obtained after double distillation as a water-white, viscous liquid, boiling point 165° to 170° C. at 9 mm. of mercury. The reported boiling point is 175° to 177° C. at 13 mm. (*1*).

## PROCEDURE

The procedure was essentially a modification of the method of Ruderman (*4*). Stock solutions of phenol, *p*-cresol, and *o*-cresol and their respective α-phenylethyl substituted derivatives were prepared by dissolving a weighed sample of the phenol in methanol and diluting the solution to volume with 60% aqueous methanol. These solutions were usually about 0.04*M*. It was shown that 60% aqueous methanol solutions could be used safely without bromine consumption by the methanol, since dilution with acetone-free methanol instead of water in standardization of the bromate-bromide solution did not alter the titer of sodium thiosulfate required.

An appropriate aliquot of the stock solution (or solutions in the case of mixtures) was pipetted into a 250-ml. iodine flask. The calculated excess of standard bromate-bromide solution was added together with sufficient distilled water or methanol to make the combined volume about 40 to 50 ml. Five milliliters of concentrated hydrochloric acid was then added, and the flask was quickly stoppered, and swirled for the desired reaction time, as measured by a stop watch. The excess bromine was then destroyed with 10 ml. of a 10% potassium iodide solution. The iodine liberated was titrated within 30 seconds with standard sodium thiosulfate solution to the disappearance of the iodine color, or to a starch end point. The results are tabulated in Tables I, II, and III.

The amount of bromine consumed is a measure of the relative amount of the phenol and its α-phenylethyl derivative present in a mixture, while the total amount of both phenols is known from the composition of the original mixture. Thus, the percentage of phenol or alkylated phenol found by the bromination technique may be calculated from a pair of simultaneous equations. For example, in the case of mixtures of phenol and *o*- or *p*-α-phenylethylphenol, the following equations were used:

$$x + y = \text{total moles of phenols present}$$
$$6x + 4y = \text{equivalents of bromine consumed}$$

where $x$ = moles of phenol present and $y$ = moles of α-phenylethylphenol present.

Evaluation of $x$ and $y$ in the above equation permitted the determination of the percentages of each phenol as given in Tables I, II, and III. Similar equations were used for mixtures of *o*- and *p*-cresol and their alkylated derivatives.

The number of reactive positions, *R*, for the cases in which only one phenol was present, was found by dividing the milliequivalents of bromine consumed by twice the millimoles of phenol present (as 2 moles of bromine were required per position brominated).

**Table II. Analysis by Bromination of *p*-Cresol,2-(α-phenylethyl)-4-methylphenol, and Mixtures of These**

| Millimole | | Reaction Time, Sec. | Meq. $BrO_3^-$–$Br^-$ | Meq. $S_2O_3^{--}$ | Net Meq. $Br_2$ Absorbed | *R* | *p*-Cresol, % | |
|---|---|---|---|---|---|---|---|---|
| *p*-Cresol | 2-(α-Phenylethyl)-4-methylphenol | | | | | | Found | Calcd. |
| 0.1930 | | 15 | 1.183 | 0.470 | 0.713 | 1.85 | | |
| 0.1930 | | 25 | 1.183 | 0.415 | 0.768 | 1.99 | | |
| 0.1930 | | 25 | 1.183 | 0.405 | 0.778 | 2.02 | | |
| 0.1930 | | 25 | 1.183 | 0.408 | 0.775 | 2.01 | | |
| | 0.2165 | 25 | 0.592 | 0.179 | 0.413 | 0.96 | | |
| | 0.2165 | 25 | 0.592 | 0.170 | 0.422 | 0.98 | | |
| | 0.2165 | 25 | 0.592 | 0.159 | 0.433 | 1.00 | | |
| | 0.2165 | 25 | 0.592 | 0.144 | 0.448 | 1.03 | | |
| 0.1544 | 0.0217 | 25 | 0.986 | 0.328 | 0.658 | | 86.9 | 87.4 |
| 0.1544 | 0.0217 | 25 | 0.986 | 0.326 | 0.660 | | 87.4 | 87.4 |
| 0.1544 | 0.0433 | 25 | 1.085 | 0.382 | 0.703 | | 77.8 | 77.8 |
| 0.1544 | 0.0433 | 20 | 1.085 | 0.392 | 0.693 | | 75.3 | 77.8 |
| 0.1544 | 0.0433 | 25 | 1.085 | 0.385 | 0.700 | | 77.3 | 77.8 |
| 0.1544 | 0.0433 | 25 | 1.085 | 0.387 | 0.698 | | 76.8 | 77.8 |
| 0.1544 | 0.0433 | 25 | 1.085 | 0.378 | 0.707 | | 78.8 | 77.8 |
| 0.1351 | 0.0650 | 25 | 0.986 | 0.322 | 0.664 | | 66.0 | 67.5 |
| 0.1351 | 0.0650 | 25 | 0.986 | 0.324 | 0.662 | | 65.5 | 67.5 |
| 0.1351 | 0.0650 | 35 | 0.986 | 0.310 | 0.676 | | 69.0 | 67.5 |
| 0.1351 | 0.0650 | 30 | 0.986 | 0.316 | 0.670 | | 67.5 | 67.5 |
| 0.1351 | 0.0650 | 30 | 0.986 | 0.314 | 0.672 | | 68.0 | 67.5 |
| 0.1158 | 0.0866 | 30 | 0.986 | 0.352 | 0.634 | | 56.9 | 57.4 |
| 0.1158 | 0.0866 | 30 | 0.986 | 0.347 | 0.639 | | 57.9 | 57.4 |
| 0.1158 | 0.0866 | 35 | 0.986 | 0.342 | 0.644 | | 59.4 | 57.4 |

**Table III. Analysis by Bromination of *o*-Cresol,4-α-phenylethyl-2-methylphenol, and Mixtures of These**

| Millimole | | Reaction Time, Sec. | Meq. $BrO_3^-$–$Br^-$ | Meq. $S_2O_3^{--}$ | Net Meq. $Br_2$ Absorbed | *R* | *o*-Cresol, % | |
|---|---|---|---|---|---|---|---|---|
| *o*-Cresol | 4-α-Phenylethyl-*o*-cresol | | | | | | Found | Calcd. |
| 0.200 | | 15 | 1.183 | 0.372 | 0.811 | 2.03 | | |
| 0.186 | | 15 | 1.085 | 0.338 | 0.746 | 2.01 | | |
| 0.186 | | 15 | 1.085 | 0.344 | 0.741 | 2.00 | | |
| 0.186 | | 25 | 1.183 | 0.405 | 0.778 | 2.10 | | |
| | 0.204 | 15–20 | 0.6902 | 0.2867 | 0.404 | 0.99 | | |
| | 0.204 | 25 | 0.6902 | 0.2847 | 0.406 | 1.00 | | |
| | 0.204 | 27 | 0.6902 | 0.2796 | 0.411 | 1.01 | | |
| | 0.204 | 15 | 0.5916 | 0.1925 | 0.399 | 0.98 | | |
| | 0.204 | 15 | 0.5916 | 0.2026 | 0.389 | 0.96 | | |
| 0.180 | 0.0200 | 15 | 1.183 | 0.415 | 0.768 | | 91.7 | 90.0 |
| 0.360 | 0.0400 | 15 | 2.268 | 0.729 | 1.538 | | 91.8 | 90.0 |
| 0.334 | 0.081 | 15 | 2.169 | 0.672 | 1.497 | | 80.3 | 80.5 |
| 0.334 | 0.081 | 15 | 2.169 | 0.669 | 1.500 | | 80.8 | 80.5 |
| 0.278 | 0.122 | 15 | 1.972 | 0.598 | 1.374 | | 71.6 | 69.5 |
| 0.278 | 0.122 | 15 | 1.972 | 0.612 | 1.360 | | 70.0 | 69.5 |
| 0.278 | 0.122 | 15 | 1.972 | 0.598 | 1.374 | | 71.6 | 69.5 |
| 0.241 | 0.163 | 15 | 1.873 | 0.580 | 1.293 | | 60.2 | 59.8 |
| 0.241 | 0.163 | 15 | 1.873 | 0.573 | 1.300 | | 60.8 | 59.8 |

## DISCUSSION

In order to develop a reproducible method for the analysis of phenol mixtures by a bromination technique, the variables effecting the bromination must be carefully controlled. Analysis of known mixtures must be carried out until reproducibility is obtained before the method can be used with confidence on mixtures of unknown composition. Thus, it becomes necessary to carry out the bromination on known mixtures covering the entire composition range that may be encountered in unknown mixtures.

Probably the two primary factors contributing to overbromination are the bromine excess used and the time of reaction with bromine. In this procedure, the percentage of excess bromine was kept constant for the entire composition range of each mixture—e.g., for all compositions containing phenol, *o*-α-phenylethylphenol and *p*-α-phenylethylphenol (Table I) the excess bromine was kept at 6 ± 2%. The reaction time was then varied until reproducible results within 1 to 2 absolute % of the calculated values were obtained.

As shown in Table I, the reaction time, while not critical for phenol itself, must be maintained at 25 to 30 seconds for the *o*- and *p*-α-phenylethylphenol and for mixtures of these with phenol. If this is done, results in satisfactory agreement with the calculated values are obtained.

For *p*-cresol, 2-(α-phenylethyl)-*p*-cresol and mixtures of these, conditions essentially identical to those employed for phenol and its alkylated derivatives gave very satisfactory results (Table II). However, when *o*-cresol, 4-(α-phenylethyl-)-*o*-cresol and mixtures of these were analyzed, it was found that a shorter reaction time was necessary in order to obtain reproducible and accurate results. This rapid overbromination of *o*-cresol has also been observed by other investigators (*4*, *5*), but may be successfully overcome by careful control of the reaction time. Lower temperatures may also be helpful (*4*).

This analytical procedure has been successfully applied to analysis of mixtures arising from the alkylation of phenol, *o*-, and *p*-cresol by α-phenyl ethyl chloride, permitting a kinetic study of that reaction (*2*). However, in using the bromination technique for the analysis of phenol mixtures, determinations on mixtures of known compositions must first be made before applying this method to unknown mixtures. It is very likely that this technique cannot be adapted to all phenol mixtures successfully, and experimentation must be undertaken to determine its suitability for each specific case.

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# Displacement of the Nitro Group during Determination of Nitrophenols and Nitroanilines by the Koppeschaar Method

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**During a study of the determination of 2,4-dinitrophenol and picric acid in mixtures by the Koppeschaar method, it was found that picric acid consumes bromine under the conditions of the procedure and a displacement of the nitro group may occur, rendering the final titration inaccurate. A procedure has been developed in which both phenols are titrated initially and the Koppeschaar method is applied to the titrated liquid to determine 2,4-dinitrophenol; picric acid is calculated by difference.**

The Koppeschaar procedure (*10*), though capable of excellent results with compounds that brominate normally (*5*), is by no means general for the classes named and is subject to errors from several recognized causes. These errors may be due to incomplete bromination because of conflicting directive influences or to the low solubility of the sample or of the partially brominated sample [the latter, in the case of nitrophenols, is the hypobromite compound (*11*)]; oxidation of the sample by bromine or hypobromous acid, as with aniline (*5*) and the nitroresorcinols examined in the present study; bromination of side chains (*15*); displacement of certain nuclear substituents by bromine—e.g., conversion of anthranilic acid and of sulfanilic acid to tribromoaniline (*5*, *8*); and replacement of alcoholic—e.g., hydroxymethyl—groups (*13*). In the course of work on the analysis of mixtures of picric acid and 2,4-dinitrophenol (*16*) it was observed that picric acid, presumably immune to bromination, consumed measurable amounts of bromine if the period of contact exceeded a few minutes, this result being accompanied by a fugitive end point in the final titration with thiosulfate. A similar displacement under preparative conditions—the conversion of picric acid to 6-bromo-2,4-dinitrophenol—was reported by Armstrong (*1*) and by Dhar (*6*). This paper presents the findings of a study of the displacement of the nitro group under the relatively mild conditions of the Koppeschaar analysis.

Of the 18 compounds examined (Table I) all except *o*-nitroaniline, *m*-nitroaniline, the nitroanisoles, and dinitrophenetole were found to consume bromine in excess of that attributable to normal bromination. The nitrophenolic ethers yielded no significant results because of their insolubility in the Koppeschaar liquid. The nitroanilines formed bulky amorphous precipitates that obstructed action of bromine (*5*). The nitroresorcinols consumed abnormally large amounts of bromine owing to oxidation.

## DETECTION OF NITROUS ACID FOLLOWING DISPLACEMENT OF NITRO GROUP BY BROMINE

Preliminary tests showed that the consumption of more than the calculated bromine is associated with the presence of nitrous acid in the liquid; this is detectable by application of the Griess-Ilosvay test, following alkalization of the mixture and acidification with sufficient formic acid to reduce excess bromine and to leave the liquid at an acidity suitable for the diazotization and subsequent coupling.

Attempts to estimate colorimetrically the amount of nitrous acid formed and to correlate this with the excess bromine consumed revealed that the amount of nitrous acid in some cases increased to a maximum and then decreased slightly.

It appears that the momentary concentration of nitrous acid is not a dependable index of the extent of the displacement reaction. Analysis of the reaction liquid for nitrogen (other than nitro nitrogen) by use of Devarda's alloy (*4*) proved to be unfeasible, as this yielded ammonia from picric acid and reduced the nitro group to some extent. To exclude the effect of nitrous acid on the end point of the titration several reagents capable of destroying nitrous acid (urea, sulfamic acid, hydrazine sulfate) were tested, but none was completely effective and all consumed bromine. Analogy with the behaviors of anthranilic acid and sulfanilic acid suggests that displacement of the nitro group is followed by introduction of bromine in its place. Thus far, a number of attempts to isolate and identify such a product (bromodinitrophenol from picric acid or tribromophenol from 2,6-dibromo-4-nitrophenol or from 4,6-dibromo-2-nitrophenol) following prolonged action under the conditions of the Koppeschaar analysis have been unsuccessful. The scantiness of the recovered material and its indefinite character (no increase in bromine and small loss of nitrogen) suggest that action was destructive.

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**Table I. Effect of Time on Consumption of Bromine by Nitrophenols, Nitroanilines, and Other Compounds**[a]

| Compound | Atoms of Bromine Consumed per Molecule | | | | Test for $HNO_2$ | |
|---|---|---|---|---|---|---|
| | 5 min. | 20 hr. | 167 hr. | Calcd. | 20 hr. | 167 hr. |
| *o*-Nitrophenol, m.p. 44–45° C. | 3.990 | 4.091 | 4.209 | 4 | – | + |
| *m*-Nitrophenol, m.p. 95–96° C. | 5.987 | 6.027 | 5.915 | 6 | – | + |
| *p*-Nitrophenol, m.p. 112–113° C. | 3.941 | 3.985 | 4.332 | 4 | Bulky ppt. | + |
| 2,4-Dinitrophenol, m.p. 113–114° C. | 2.003 | 2.124 | 2.723 | 2 | | + |
| Picric acid, m.p. 120–121° C. | 0.0309 | (1.504 22 hr.) | 3.937 | 0 | + | + |
| *o*-Nitroaniline, m.p. 70–71° C. | 3.978 (3.974 30 min.) | 3.981 | 3.606 | 4 | Bulky ppt. | |
| *m*-Nitroaniline, m.p. 110–111° C. | 5.977 | 5.977 | 5.477 | 6 | Bulky ppt. | |
| *p*-Nitrodimethylaniline, m.p. 161–162° C. | 4.020 | 6.741 | 9.325 | 4 | + | + |
| 2,6-Dibromo-4-nitrophenol, m.p. 143–144° C. | 0.0443 | 1.489 | 8.097 | 0 | + | + |
| 4,6-Dinitro-*o*-cresol, m.p. 85–86° C. | 0.011<br>0.055 | 4.785 | ... | 0 | + | |
| 2,4-Dinitro-1-naphthol, m.p. 81–82° C. | 0.292 | 1.374 | 3.491 | 0 | + | + |
| 2-Nitroresorcinol, m.p. 81–82° C. | 10.008 | 12.143 | 14.042 | 4 | + (+ in 5 min.) | + |
| 2,4-Dinitroresorcinol, m.p. 160° C. | 0.647 (0.704 10 min.) | 11.680 (11.222 11 hr.) | ... | | + | |
| 2,4,6-Trinitroresorcinol, m.p. 177–178° C. | 0.522 (1.922 30 min.) | (6.559, 11 hr.) (8.43 22 hr.) | 9.642 | | + (+ in 30 min.) | + |
| 2,4-Dinitrothymol, m.p. 53–54° C. | 0 | ... | 5.203 | | | |
| 2,4-Dinitroanisole, m.p. 94–95° C. | Insoluble, bromination slight | | | | | |
| 2,4,6-Trinitroanisole, m.p. 67–68° C. | Insoluble, bromination slight | | | | | |
| 2,4-Dinitrophenetole, m.p. 85–86° C. | Insoluble, bromination slight | | | | | |

[a] Temperature 20–25° C.; samples 0.09–0.15 gram.

**Table II. Effects of Temperature and Time on Bromination of Nitrophenols**

| Compound | Temp., ° C. | Atoms Bromine Consumed per Molecule | | | |
|---|---|---|---|---|---|
| | | 5 min. | 10 hr. | 20 hr. | Calcd. |
| *o*-Nitrophenol | 0–5 | ... | ... | 3.921 | 4 |
| | 5–10 | 3.935 | 3.981 | ... | |
| | 20–25 | 3.990 | ... | 4.091 | |
| | 25–30 | 4.004 | 4.035 | 4.209 | |
| *m*-Nitrophenol | 0–5 | ... | ... | 5.905 | 6 |
| | 5–10 | 5.914 | 5.959 | ... | |
| | 20–25 | 5.987 | ... | ... | |
| | 25–30 | 5.987 | 5.990 | ... | |
| *p*-Nitrophenol | 0–5 | ... | ... | 3.964 | 4 |
| | 5–10 | 3.965 | 3.968 | ... | |
| | 20–25 | 3.941 | ... | ... | |
| | 25–30 | 4.004 | 4.002 | ... | |
| 2,4-Dinitropheno | 0–5 | ... | ... | 2.006 | 2 |
| | 5–10 | ... | 1.989 | ... | |
| | 20–25 | 2.003 | ... | 2.124 | |
| | 25–30 | 2.003 | 2.137 | 2.121 | |
| Picric acid | 0–5 | ... | ... | 0.1617 | 0 |
| | 5–10 | 0.0236 | 0.1338 | ... | |
| | 25–30 | 0.0309 | 0.6989 | 1.465 | |
| 2-Nitroresorcinol | 5–10 | ... | 8.463 | 8.611 | 4 |
| | 20–22 | ... | 8.658 | ... | |
| 2,4-Dinitroresorcinol | 5–10 | ... | 9.262 | ... | 2 |
| | 20–22 | ... | 11.222 | ... | |
| 2,4,6-Trinitroresorcinol | 5–10 | ... | 4.035 | 6.490 | 0 |
| | 20–22 | ... | 6.559 | | |

## INFLUENCE OF EXPERIMENTAL CONDITIONS ON KOPPESCHAAR BROMINATION

**Effect of Time.** Normal bromination is rapid, and because the effects of the displacement reaction may not become apparent in the usually brief bromination periods, it is clear that this reaction is relatively slow. Table I presents the results of experiments to test the effect of time upon the two reactions.

Except for *o*- and *m*-nitroanilines, the amount of bromine consumed increases with time and, even with some compounds which can be accurately determined by brief bromination, at some point exceeds the theoretical [especially *o*- and *p*-nitrophenols, 2,4-dinitrophenol, and *p*-nitrodimethylaniline (*8*)]. Several compounds (picric acid, 2,6-dibromo-4-nitrophenol, 2,4-dinitronaphthol, and 4,6-dinitro-*o*-cresol) not capable of normal bromination consume bromine in amounts that increase markedly with time, though the amounts consumed in 5 minutes are small. Prolonging the bromination period does not improve results by the Koppeschaar method when applied to nitro compounds and may vitiate them. The rapid and excessive consumption of bromine by dinitro-*o*-cresol, 2,4-dinitrothymol, and especially the several nitroresorcinols, is attributable to oxidation.

**Effect of Temperature.** Table II records the results for eight nitrophenols, showing the combined effects of temperature and time.

The effect of increasing time and temperature upon the bromination of nitrophenols is toward consumption of more than the theoretical amount of bromine. In Table II the separate effects of the two variables can be seen by comparing the results horizontally or vertically. Bromination of the isomeric nitrophenols and of 2,4-dinitrophenol is substantially normal up to room temperature, if the time of contact is brief. Of the four compounds referred to, *o*-nitrophenol and 2,4-dinitrophenol, show abnormality for periods of 10 hours or more at 25° to 30° C. The results for picric acid indicate that to restrain attack by bromine so that it is negligible contact should be brief and below room temperature. The results for the nitroresorcinols show, for both time and temperature, pronounced effects that suggest concurrent bromination, displacement of the nitro group, and oxidation; these results have no analytical significance.

**Effect of Acidity.** Table III presents the results of experiments in which the quantity of hydrochloric acid was varied from somewhat less than the usual amount to four times the usual amount.

The results indicate that for brief periods of bromination the amount of acid used in the standard procedure (5 ml. of concentrated acid in a total volume of 80 ml.) is satisfactory, and that increasing acidity retards somewhat both normal bromination and displacement of the nitro group. The two effects are of

**Table III. Effect of Acid Concentration on Bromination of Nitrophenols**

| Compound | Concd. HCl, Ml. | Atoms of Bromine Consumed per Molecule | | |
|---|---|---|---|---|
| | | 5 min. | 20 hr. | Calcd. |
| *o*-Nitrophenol | 3 | 3.957 | 5.009 | 4 |
| | 5 | 4.004 | 4.691 | |
| | 10 | 3.964 | 4.265 | |
| | 20 | 3.909 | 4.095 | |
| *m*-Nitrophenol | 3 | 5.951 | 5.972 | 6 |
| | 5 | 5.987 | 5.859 | |
| | 10 | 5.864 | 5.941 | |
| | 20 | 5.854 | ... | |
| *p*-Nitrophenol | 3 | 3.987 | 3.970 | 4 |
| | 5 | 4.004 | 3.980 | |
| | 10 | 3.940 | 3.900 | |
| | 20 | 3.840 | 3.779 | |
| 2,4-Dinitrophenol | 3 | 1.968 | 2.295 | 2 |
| | 5 | 2.003 | 2.121 | |
| | 10 | 1.971 | 1.995 | |
| | 20 | 1.930 | 1.929 | |
| Picric acid | 3 | 0.1003 | 1.973 | 0 |
| | 5 | 0.0309 | 1.465 | 0 |
| | 10 | ... | 0.638 | |
| | 20 | ... | 0.059 | |

**Table IV. Effect of Amount of Bromine upon Bromination of Nitrophenols**[a]

| Compound | Bromine Taken (1 = Usual Amt.)[b] | Atoms of Bromine Consumed per Molecule | | |
|---|---|---|---|---|
| | | 5 min. | 20 hr. | Calcd. |
| *o*-Nitrophenol | 1.0 | 4.004 | ... | 4 |
| | 1.4 | 3.998 | 4.687 | |
| *m*-Nitrophenol | 1.0 | 5.987 | ... | 6 |
| | 1.4 | 5.959 | 5.961 | |
| *p*-Nitrophenol | 1.0 | 4.004 | ... | 4 |
| | 1.4 | 3.992 | 4.015 | |
| 2,4-Dinitrophenol | 1.0 | 2.005 | ... | 2 |
| | 1.4 | 1.994 | 2.149 | |
| Picric acid | 1.0 | 0.0309 | ... | |
| | 1.4 | 0.0309 | 1.404 | |

[a] Temperature 25–30° C.
[b] Bromine from 25 ml. of $0.1N$ Koppeschaar solution.

about the same magnitude, so that one cannot expect control of acidity to exclude the displacement reaction while permitting normal bromination. The influence of acidity is most strikingly shown by the 20-hour results for picric acid: A sevenfold increase in acid decreases the displacement reaction about 97%.

**Effect of Amount of Bromine.** The results of experiments in which the amounts of Koppeschaar solution were varied from the usual amount (about twice the theoretical) to 40% more appear in Table IV.

The effect of change in the amount of bromine is slight, especially for the 20-hour trials; the influence of time exceeds that of amount of bromine. These results, together with visual observation, suggest that the amount of bromine ordinarily used maintains virtual saturation with respect to bromine, and that increase in its amount does not much increase its effective concentration.

**Chemistry of Displacement Reaction.** The displacement of the nitro group by aqueous bromine may be attributed to free bromine or to hypobromous acid or both:

$$\begin{aligned} &ArNO_2 + Br_2 \rightarrow ArBr + BrNO_2 \\ &\qquad\qquad \text{[hypothetical nitroxyl bromide (4)]} \\ &BrNO_2 + H_2O \rightarrow HBr + HNO_3 \end{aligned} \tag{1}$$

$$\begin{aligned} &Br_2 + H_2O \rightarrow HOBr + HBr \\ &ArNO_2 + HOBr \rightarrow ArBr + HNO_3 \end{aligned} \tag{2}$$

The ability of free bromine to effect this replacement appears to be established (*1, 3, 6, 17*); that of hypobromous acid to do so was ascertained experimentally. It was found that, following contact of aqueous hypobromous acid with the isomeric nitrophenols or with picric acid, the liquids contained nitrous acid. In several cases precipitates appeared; analysis of one of these showed it contained mercury, indicating mercuration by the mercuric bromide present in the reagent as prepared by the Balard method (*2*). Separate experiments showed that mercuric bromide alone is not able to displace the nitro group from the same nitrophenols.

The primary displacement product is probably nitric acid, and its conversion to nitrous acid appears to be inevitable because of interaction with hydrobromic acid which is present in abundance.

$$2HNO_3 + 6HBr \rightarrow 3Br_2 + 2NO + 4H_2O \tag{3}$$

The nitric oxide thus produced may react with bromine to form nitrosyl bromide and then nitrous acid and hydrobromic acid:

$$\begin{aligned} &2NO + Br_2 \rightarrow 2NOBr \\ &2NOBr + H_2O \rightarrow HNO_2 + HBr \end{aligned} \tag{4}$$

or with air (oxygen) to yield nitrogen dioxide, which with water yields nitrous and nitric acids:

$$\begin{aligned} &2NO_2 + O_2 \rightarrow 2NO_2 \\ &2NO_2 + H_2O \rightarrow HNO_3 + HNO_2 \end{aligned} \tag{5}$$

or with nitric acid to yield nitrous acid:

$$2NO + HNO_3 + H_2O \rightarrow 3HNO_2 \tag{6}$$

Nitrous acid formed by Reactions 4 and 6 may be oxidized to nitric acid by hypobromous acid, or by bromine water, or by air:

$$HNO_2 + HOBr \rightarrow HNO_3 + HBr \tag{7}$$

$$2HNO_2 + Br_2 + 2H_2O \rightarrow 2HNO_3 + 2HBr \tag{8}$$

$$2HNO_2 + O_2 \rightarrow 2HNO_3 \tag{9}$$

To test the extent to which oxygen may be involved (Equations 5 and 9) experiments were performed with air displaced from the liquid and the space above it by nitrogen or carbon dioxide. These showed that picric acid consumed less bromine in absence of air than in its presence (in 22 hours 0.994 atom *vs.* 1.504 atoms, respectively). It may be assumed that little or no oxygen is formed by the reaction $2HOBr \rightarrow 2HBr + O_2$, as light is excluded during the Koppeschaar analysis and the amount of hypobromous acid momentarily present is probably small. Further, the final result of action of oxygen is the formation of nitrous and nitric acids and not an increase in the bromine consumed. Reactions 9 and 8 (which consume, respectively, oxygen and bromine to oxidize nitrous acid) are in competition, with an enormous statistical advantage for Reaction 8. The nitric acid formed is thereafter involved with hydrobromic acid (Reaction 3) to yield ultimately both nitrous and nitric acids and to regenerate bromine.

**Table V. Effects of Temperature, Time, and Acidity on Determination of 2,4-Dinitrophenol in Presence of Picric Acid**

| Sample Mixture | Temp., ° C. | Time, Min. | Concd. HCl, Ml. | Atoms Br Consumed | Indicated Purity of DNP, % |
|---|---|---|---|---|---|
| 2,4-Dinitrophenol,<br>0.08030 gram<br>Picric acid,<br>0.03024 gram | 20–25 | 5 | 5 | 2.050 | 102.5 |
| | | 30 | 5 | 2.023 | 101.2 |
| | | 60 | 5 | 2.022 | 101.1 |
| | | 5 | 10 | 2.043 | 102.2 |
| | | 30 | 10 | 2.023 | 101.2 |
| | | 60 | 10 | 1.964 | 98.2 |
| | | 5 | 20 | 2.012 | 100.6 |
| | | 30 | 20 | 1.992 | 99.6 |
| | | 60 | 20 | 1.960 | 98.0 |
| 2,4-Dinitrophenol,<br>0.09034 gram<br>Picric acid,<br>0.03564 gram | 0–10 | 5 | 5 | 1.987 | 99.4 |
| | | 30 | 5 | 1.988 | 99.4 |
| | | 60 | 5 | 1.995 | 99.8 |
| | | 5 | 10 | 1.983 | 99.2 |
| | | 30 | 10 | 1.988 | 99.4 |
| | | 60 | 10 | 1.988 | 99.4 |
| | | 5 | 20 | 1.964 | 98.2 |
| | | 30 | 20 | 1.983 | 99.2 |
| | | 60 | 20 | 1.983 | 99.2 |

The system contains, or by primary or secondary reactions produces, a group of incompatible substances, and after a lapse of sufficient time it may reach an apparently steady state that represents a balance among a number of interdependent reactions, with the reactants either in a condition of interlocking equilibria or at concentrations below the threshhold values for reaction. At any moment there could then be present bromine, hypobromous acid, hydrobromic acid, nitric acid, nitrous acid, and nitric oxide, and this conclusion may account for the apparently paradoxical facts: nitrous acid is present after prolonged periods of time during which free bromine has been continuously present, and in some brominations—e.g., of *m*-nitrophenol and *m*-nitroaniline—the total apparent consumption of bromine may, beyond a certain point, show a decrease with time. The possibility that adding formic acid before the test for nitrous acid may cause reduction of nitric to nitrous acid was excluded by the inability of formic acid to effect this reduction at low concentration. It is possible that no nitrous acid persists so long as an excess of bromine is present, but that following its reduction by formic acid some nitrous acid is formed and survives.

When displacement of the nitro group occurs it is doubtful that results by the Koppeschaar method are trustworthy, owing to the secondary reactions mentioned. Reaction 3 produces free bromine while Reactions 1, 2, 4, and 8 consume bromine. The bromine liberated in Reaction 3 may exceed the bromine consumed to effect displacement of the nitro group. All that can be determined in the Koppeschaar analysis is the over-all total amount of bromine consumed, and there is no measurable and unique criterion of the extent of the displacement reaction. Following the displacement reaction there exists a situation that cannot be delt with using only the analytical data; it is aggravated by the fugitive nature of the end point, which is presumably already delayed on its first appearance.

**Table VI. Analyses of Mixtures of 2,4-Dinitrophenol and Picric Acid**[a]

| Mixtures Analyzed | Wt., Gram | % | Found Gram | Found % |
|---|---|---|---|---|
| DNP | 0.3326 | 100.0 | 0.3332 | 100.18 |
| PA | 0.0000 | 0.0 | 0.00 | 0.00 |
| DNP | 0.3346 | 98.35 | 0.3336 | 98.71 |
| PA | 0.0056 | 1.65 | 0.0044 | 1.29 |
| DNP | 0.3108 | 86.81 | 0.3114 | 86.91 |
| PA | 0.0472 | 13.19 | 0.0469 | 13.09 |
| DNP | 0.2608 | 85.34 | 0.2609 | 85.23 |
| PA | 0.0448 | 14.66 | 0.0452 | 14.77 |
| DNP | 0.2358 | 76.85 | 0.2358 | 76.83 |
| PA | 0.0710 | 23.15 | 0.0711 | 23.17 |
| DNP | 0.1566 | 50.16 | 0.1572 | 50.14 |
| PA | 0.1556 | 49.84 | 0.1563 | 49.86 |
| DNP | 0.1244 | 31.12 | 0.1255 | 31.87 |
| PA | 0.2754 | 68.88 | 0.2683 | 68.13 |
| DNP | 0.0711 | 24.09 | 0.0713 | 24.31 |
| PA | 0.2240 | 75.91 | 0.2220 | 75.69 |
| DNP | 0.0590 | 15.73 | 0.0557 | 15.86 |
| PA | 0.3162 | 84.27 | 0.2957 | 84.14 |
| DNP | 0.0264 | 8.32 | 0.0265 | 8.27 |
| PA | 0.2912 | 91.68 | 0.2942 | 91.73 |
| DNP[b] | 0.0142 | 3.60 | 0.0195 | 2.50 |
| PA | 0.3808 | 96.40 | 0.3606 | 97.50 |
| DNP | 0.0000 | 0.00 | 0.0000 | 0.00 |
| PA | 0.1908 | 100.00 | 0.1911 | 100.15 |

[a] Temperature 20–25°; time 30 minutes; concentrated hydrochloric acid, 5 ml.

[b] Three other analyses of mixtures in this range gave poor results, indicating interference by picric acid when present to the extent of about 95% or more.

## ANALYTICAL APPLICATION OF FINDINGS

**Analysis of Mixtures of 2,4-Dinitrophenol and Picric Acid.** In the light of the disclosures outlined above, the method for analysis of mixtures of dinitrophenol and picric acid was improved to a state of usefulness. The need for a method to estimate 2,4-dinitrophenol and picric acid when present in mixtures arose during work on the oxynitration process for preparation of picric acid from benzene by a research team at the University of Pennsylvania during World War II. The product consists largely of dinitrophenol, with some picric acid, and the amount of the former must be known in order both to judge the effectiveness of the oxynitration and to calculate the amount of 98% nitric acid required for nitration of dinitrophenol to picric acid. This preliminary work disclosed the fact that picric acid consumes bromine under the conditions of the Koppeschaar analysis at a rate affected by both time and temperature, and led to the further study reported in this paper.

The effects of time, temperature, and acidity upon the bromination of mixtures of the two compounds were determined, and the results (Table V) were used to assist in the selection of conditions that would permit quantitative determination of dinitrophenol by the Koppeschaar method with minimal, or at most tolerable, interference by picric acid.

The effects of temperature and time are seen to exceed that of acidity; at 0° to 10° C. interference by picric acid is negligible and results for dinitrophenol are reasonably good, with only slight decrease in dinitrophenol values at higher acidities. As a compromise in the interest of convenience it was decided to operate at room temperature (20° to 25° C.) and at the usual acidity and to omit replacement of air by inert gas, with the bromination period reduced to 30 minutes or less. The entire analytical procedure involves an initial acidimetric titration of both phenols and application to the titrated liquid of the Koppeschaar method to determine dinitrophenol; picric acid is calculated by difference. Test results for the procedure appear in Table VI.

## EXPERIMENTAL

The compounds examined are listed in Table I. All were purified to constant melting points, *o*-nitrophenol by steam distillation and the others by recrystallization: *o*-nitroaniline from water, picric acid and 2,4,6-trinitroresorcinol from water containing hydrochloric acid, and the others from aqueous alcohol. The melting points appear in Table I. Each value listed in Tables I to V represents the average of two determinations. Each value listed in Table VI represents the average of three determinations.

The Koppeschaar solutions were $0.1N$ with respect to available bromine: potassium bromide, 50.0 grams; potassium bromate, 2.8 grams per liter. The $0.1N$ thiosulfate solutions were standardized weekly against potassium iodate (*12*). Extended brominations were effected in specially made, thin-walled glass ampoules of about 100-ml. capacity, each with two concavities pushed into the wall (to facilitate crushing the ampoule under water); an 8-mm. tubular side arm permitted introduction of liquids through an improvised funnel tube with semicapillary stem, drawn from a thistle tube. After charging, the ampoule was closed by sealing off the neck while the ampoule was being cooled in an ice bath. For briefer brominations there were used 500-ml. iodine flasks with stoppers of tested tightness.

**General Procedure for Bromination.** Samples were taken as 25-ml. aliquots of solutions of the compounds in water or dilute sodium hydroxide. To the sample, in a 500-ml. iodine flask or in the glass ampoule, were added 25 ml. of water, 25 ml. (from a pipet) of $0.1N$ Koppeschaar solution, and finally, without agitation, 5 ml. of concentrated hydrochloric acid. The flask was stoppered, or the ampoule was sealed, at once. The amount of sample in each case was such that the Koppeschaar solution taken would provide approximately a 100% excess of bromine over that calculated for normal bromination. The flask or ampoule was allowed to stand in the dark at the temperature and for the period specified.

Analyses in iodine flasks were completed in the usual manner, using 5 ml. of 40% potassium iodide solution for combining the free bromine, after which the equivalent iodine was titrated with $0.1N$ thiosulfate using 5 ml. of 1% soluble starch solution added near the end point. Analyses in sealed ampoules were completed by immersing the ampoule in 150 ml. of 10% potassium iodide solution and by breaking it by pressure with a heavy glass rod applied at one of the thin-walled concavities. The operations following introduction of potassium iodide were completed without interruption, and the titration was completed rapidly, as displacement of the nitro group led to delayed liberation of iodine and to reappearance of the starch-iodine color after the first end point was reached. With each series a blank analysis (25 ml. of Koppeschaar solution, 50 ml. of water, and 5 ml. of concentrated hydrochloric acid) was run, to determine the value of the Koppeschaar solution in terms of equivalent standard thiosulfate.

Results stated as "atoms of bromine consumed" in the tables are calculated thus:

$$\text{Atoms of bromine per molecule of sample} = \frac{(V - V_1) \times \text{mol. wt.} \times 0.001N}{S}$$

in which $V$ is milliliters of thiosulfate used in the blank titration, $V_1$ is milliliters of thiosulfate used in the main titration, $N$ is the normality of the thiosulfate, and $S$ is the weight of sample in grams. Results stated in terms of per cent purity are calculated as 100 times the quotient of the number of atoms of bromine consumed per molecule of sample divided by the theoretical number of atoms of bromine.

**Identification of Nitrous Acid Following Displacement of Nitro Group.** The analysis liquid, at the end of the bromination period, was neutralized by addition of 25% sodium hydroxide solution, and was then reacidified by addition of 3% formic acid until pH was about 3. The formic acid first liberated the bromine combined by the alkali and then reduced it. To the prepared solution were added 1 ml. of 0.5% 1-naphthylamine in 30% acetic acid and, after 2 minutes, 1 ml. of 1% sulfanilic acid solution prepared as directed by Snell and Biffen (*14*). For each series a blank analysis was similarly treated. In presence of nitrous acid a violet-pink color appeared within a few minutes, the intensity increasing for about an hour. This test gave negative results following normal brominations of nitrophenols, but positive results when excessive bromine was consumed and the end points were impermanent.

**Influence of Experimental Conditions and Structure.** In all experiments the standard procedure was changed only to the extent required to ascertain the effects of changes in the variable(s) under study. Brominations continued for 167 hours were effected in sealed glass ampoules. Temperatures above or below room temperature were maintained by keeping the iodine flasks or ampoules in a constant-temperature oven or in a refrigerator, with periodic inspections. Variations in acidity were made at constant volume by reciprocal adjustments of the amounts of water and hydrochloric acid added. Variations in the amounts of bromine were made at constant volume by compensating adjustments of the amounts of Koppeschaar solution and water. The results of the tests appear in Tables I, II, III, and IV.

**Action of Hypobromous Acid on Nitrophenols.** An aqueous solution of hypobromous acid was prepared by the method of Balard (*2*); the brown-red solution was filtered from the solid residue and was extracted several times with carbon tetrachloride and finally with ether to remove free bromine. This reagent contains hypobromous acid and mercury(II) bromide:

$$HgO + 2Br_2 \rightarrow 2HOBr + HgBr_2$$

To test the action of hypobromous acid upon nitrophenols, 25 ml. of the colorless or straw-colored reagent was added to a 25-ml. aliquot of a solution of *o*- or *p*-nitrophenol or of picric acid for periods from 1 to 20 hours. In all cases, positive tests for nitrous acid were obtained by the test procedure described. Both mononitrophenols yielded precipitates during contact with the reagent; one of these precipitates was removed and washed, and analysis proved it contained mercury. The possibility that the nitro group might be replaced by mercuration (rather than by action of hypobromous acid) was excluded by tests in which the nitrophenols (25-ml. aliquots) were treated with saturated aqueous mercuric bromide (25 ml.) and concentrated hydrobromic acid (2 ml.) for 1 to 20 hours, after which the liquids were tested for nitrous acid with negative results.

In separate tests it was found that the normal Koppeschaar bromination is not noticeably accelerated or retarded by presence of mercuric bromide. Samples of *o*- and *p*-nitrophenol, brominated for 5 to 30 minutes at 20° to 25° C. in presence of 25 ml. of saturated aqueous mercuric bromide, yielded 100.05 and 99.92%, respectively, comparing well with the results by the usual procedure (*5*).

Experiments that resulted indecisively and are not described here (*9*) include the colorimetric determination of nitrous acid formed in the displacement reaction, the attempted determination of nitrogen other than nitro nitrogen in the analysis liquid by use of Devarda's alloy (*4*), attempts to destroy nitrous acid prior to the titration (using urea, hydrazine sulfate, or sulfamic acid), and attempts to prove the replacement of nitrogen dioxide by bromine by isolation and identification of the brominated product.

**Determination of 2,4-Dinitrophenol and Picric Acid in Admixture.** The procedure consists of two operations performed on the same sample: determination of total acidity by titration with standard alkali and Koppeschaar analysis of the resulting solution under conditions such as to minimize involvement of picric acid. The bromine consumed is calculated to dinitrophenol, and picric acid is calculated by difference. The samples, in 500-ml. iodine flasks, were first dissolved by addition (from a buret) of a slight excess of 0.1*N* sodium hydroxide (standardized against 99.95% picric acid, using phenolphthalein indicator), after which a small excess of 0.1*N* acid was added (from a buret) and the titration was completed with 0.1*N* alkali using phenolphthalein indicator. For reproducibility and certainty the titrations were continued to a rather deep color, which was compensated in the standardizations in which like volumes and procedure were used. To the titrated and nearly neutral solution was added 25 ml. of 0.1*N* Koppeschaar solution followed by 5 ml. of concentrated hydrochloric acid, and analyses were completed by the general procedure.

The results of preliminary trials to determine favorable conditions with respect to temperature, time, and acidity appear in Table V. In consideration of the findings and as a compromise between optimum conditions and convenience, it was decided to operate at 20° to 25° C., to use 5 ml. of concentrated hydrochloric acid, and to continue bromination for 30 minutes. This procedure, applied to mixtures of dinitrophenol and picric acid over a wide range of compositions, yielded the results collected into Table VI, which indicate that satisfactory analysis is possible with mixtures containing at least 25% dinitrophenol.

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# Zinc Mercurithiocyanate Method and Its Application to Yellow-Metal Alloys

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**The results of over 80 student analyses for zinc on 26 different alloys, containing from 0.3 to 23% of zinc, are reported. A comparison of these results by the mercurithiocyanate method with others obtained by the classical pyrophosphate procedure shows a definite superiority for the former method from the standpoints of precision and accuracy. The selectivity is also improved—iron, for example, need not be removed. The time for analysis is approximately one half of that for the phosphate method. Some misconceptions in the literature are mentioned.**

THE mercurithiocyanate method for the determination of zinc was first investigated by Lundell and Bee (*6*). They advocated solutions of 5% acidity. Large amounts of salts (potassium sulfate, ammonium acetate, ammonium thiocyanate) were shown to give high results. Their factor of 0.1266 for converting the weight of precipitate to a weight of zinc was based upon the assumption that the monohydrate prevailed on drying at 102° to 108° C. Later, Jamieson (*5*) showed that the precipitate was anhydrous and that the gravimetric factor must therefore be 0.1312. The interferences of arsenic(III) and small amounts of nickel were shown to be negligible. Jamieson (*5*),

**Table I. Determination of Zinc in Yellow-Metal Alloys by Mercurithiocyanate and Phosphate Methods**

| | Mercurithiocyanate Method, % | | | Phosphate Method, % | | |
|---|---|---|---|---|---|---|
| Standard value[a], % | Av. analysis | Av. deviation, relative | Av. error, relative | Av. analysis | Av. deviation, relative | Av. error, relative |
| 0.29 | 0.27 | .. | .. | 0.29 | .. | .. |
| 1.61 | 1.68 | 2.82 | 4.20 | 1.46 | 4.56 | 9.32 |
| 1.84 | 1.81 | 4.24 | 4.71 | 1.76 | 6.15 | 6.10 |
| 2.18 | 2.29 | 3.78 | 5.66 | 2.25 | 7.11 | 6.28 |
| 2.53 | 2.60 | 3.97 | 5.01 | 2.44 | 6.42 | 6.46 |
| 2.63 | 2.66 | 3.38 | 3.55 | 2.59 | 3.92 | 3.87 |
| 2.84 | 2.91 | 1.84 | 2.46 | 2.95 | 4.46 | 4.53 |
| 3.07 | 3.18 | 1.65 | 3.66 | 3.04 | 3.18 | 9.32 |
| 4.23 | 4.35 | 2.14 | 2.83 | 4.33 | 6.81 | 6.57 |
| 4.81 | 4.93 | 4.04 | 2.49 | 4.80 | 1.04 | 0.97 |
| 5.70 | 5.68 | 0.24 | 0.35 | 5.70 | 1.26 | 1.26 |
| 5.96 | 6.06 | 0.78 | 1.64 | 5.88 | 1.50 | 1.65 |
| 6.22 | 6.20 | 0.36 | 0.44 | 6.14 | 0.53 | 1.58 |
| 6.75 | 6.87 | 1.56 | 1.73 | 6.66 | 2.38 | 2.64 |
| 7.16 | 7.14 | 2.38 | 2.46 | 7.02 | 3.68 | 3.60 |
| 7.25 | 7.40 | 1.38 | 2.03 | 7.21 | 0.74 | 0.83 |
| 7.27 | 7.19 | 1.62 | 1.97 | 7.13 | 1.24 | 2.08 |
| 7.32 | 7.36 | 2.49 | 2.32 | 7.28 | 1.08 | 1.12 |
| 7.73 | 7.61 | 1.88 | 1.68 | 7.53 | 0.84 | 2.58 |
| 7.94 | 7.89 | 0.21 | 0.67 | 7.84 | 0.60 | 1.26 |
| 8.41 | 8.48 | 0.94 | 1.23 | 8.38 | 0.86 | 0.91 |
| 8.70 | 8.85 | 0.60 | 1.72 | 8.64 | 0.94 | 1.17 |
| 11.70 | 11.76 | 0.82 | 0.99 | 11.88 | 1.60 | 2.12 |
| 12.66 | 12.66 | 1.42 | 1.42 | 12.59 | 0.61 | 0.92 |
| 12.93 | 12.88 | 0.65 | 0.52 | 12.89 | 1.46 | 1.36 |
| 23.66 | 23.34 | 0.98 | 1.39 | 23.47 | 0.52 | 0.45 |

[a] Based on Standard Sample Co.'s assays by phosphate procedure. These student "brasses" also contain major amounts of copper, moderate amounts of tin and lead, and up to about 0.5% of iron and nickel in some samples.

and later Vosburgh and others (*10*), used ammonium thiocyanate with no adverse effects on the results. Vosburgh's work definitely confirmed the value of the method. The amount of salts in solution should be kept low, but up to 5 grams of sulfuric acid and 2.5 grams of nitric acid in a total volume of 150 ml. are permissible. Results with 0.02 to 0.09 gram of zinc were reported. The method has been used satisfactorily in several phase studies (*3, 7*).

The ASTM (*2*), Alcoa (*1*), and Snell (*9*) factor of 0.1289 is an average of the correct value and that for the monohydrate. Duval's pyrolysis curve for this substance (*4*) indicates only a stable horizontal (corresponding to the anhydrous compound) up to 270° C., with subsequent decomposition.

A recent Spanish paper (*8*) advocates the addition of glycerol to facilitate the precipitation and minimize adsorption. This variation has not been tested, but its use does not seem to be necessary.

Despite proof of the utility of this method, it has not received the credit it deserves. Student results in this laboratory indicate that the method is more acceptable than the phosphate method for the determination of zinc in brass and similar alloys. The feasibility of wider industrial use for the determination of zinc in yellow-metal alloys is apparent.

The mercurithiocyanate precipitation requires only a medium of approximately 5% acidity; pH control in the phosphate procedure is extremely critical. Ferric ion does not interfere and may be left in solution; indeed, the large quantities of salts accumulated during its removal are best avoided. Small amounts of iron, nickel, manganese, etc., cause a very highly colored precipitate, but this gives no detectable error in the results. When the amount of cobalt, nickel, or copper exceeds about 0.2, 0.7, or 0.1%, respectively, a preliminary acid-sulfide separation is indicated.

## PROCEDURE

The procedure used by students follows the preliminary separations in the brass analysis as given by Willard and Furman (*11*). One-gram samples of brass are weighed and dissolved in concentrated nitric acid. Tin is separated as metastannic acid by concentrating the solution to about 5 ml., diluting with 30 ml. of warm water to dissolve soluble salts, and filtering. Two to three milliliters of sulfuric acid are added to the filtrate from the tin separation, and the solution is evaporated almost to dryness (cover glass dry). Twenty milliliters of water are added to the cool solid mass; then the solution is warmed, diluted to 80 to 100 ml., and warmed further to dissolve all soluble salts, and the lead sulfate is filtered. After concentrating to 75 ml., 1 ml. of nitric acid is added to the filtrate from the lead separation. The copper is electrolytically deposited at a current of 1.5 to 2.0 amperes.

The spent electrolyte from the electrodeposition of copper should contain 2 to 3 ml. of sulfuric acid, and 1 ml. of nitric acid in 100 to 150 ml. of solution. The electrolyte solution is transferred quantitatively to a 250-ml. volumetric flask and diluted to the mark with distilled water. A 50-ml. aliquot of this solution is transferred to a 250-ml. beaker and diluted with 25 to 50 ml. of distilled water, and the mercurithiocyanate reagent is added, with stirring, to slight excess. After standing 1 hour at room temperature, the solution is filtered on a Gooch crucible, washed with 50 to 75 ml. of cold (5° to 10°) $0.001M$ reagent, dried 1 hour at 105° to 110° C., and weighed. The factor for converting zinc tetrathiocyanatomercurate(II) to zinc is 0.1312.

Students should be instructed not to wear jewelry when working with mercury, to avoid ingestion of mercury salts, and to guard against igniting crucibles to high temperature (as in the phosphate method), because of the toxicity of mercury vapor.

## REAGENTS

Mercurithiocyanate Reagent. Dissolve 31.5 grams of ammonium thiocyanate (Mallinckrodt analytical and reagent grade, or equivalent) in 600 ml. of water. Dissolve 27.1 grams of mercuric chloride (Merck reagent, or equivalent) in the solution, filter, and dilute to 1 liter.

Wash Solution. Dilute 10 ml. of the above solution to 1 liter.

## RESULTS

Table I shows a comparison of the student results obtained on a representative group of brasses by the mercurithiocyanate and phosphate methods for zinc. The first column lists the values submitted by the Standard Sample Co., Ames, Iowa; for the purpose of calculating errors, etc., these values have been chosen (rather arbitrarily) as the correct analyses for the samples. The per cent relative deviation (*vs.* average analysis) and per cent relative error (*vs.* standard value) are tabulated for the two methods. Six analyses were averaged for the phosphate method, and three to five values were available for the mercurithiocyanate method. On comparing the per cent deviations and per cent errors for the two methods, it is apparent that the mercurithiocyanate procedure is preferable from the standpoints of precision and accuracy. In addition, the time requirements are much less than those of the phosphate procedure.

Work in this laboratory also indicates that the mercurithiocyanate procedure is equally applicable on a semimicro scale.

From 2 to 10 mg. of zinc in 6 to 8 ml. of solution containing 1 drop of $3M$ hydrochloric acid are precipitated with 4 ml. of $0.1M$ reagent. After standing 1 hour at room temperature, the precipitate is filtered through a Pregl tube, washed with 6 to 8 ml. of $0.001M$ reagent, and dried 30 minutes at 105° to 110° C. Copper, cadmium, cobalt, nickel, manganese, bismuth, and mercury(I) interfere; chromium(III and VI), iron(III), arsenic, aluminum, magnesium, cadmium, and alkali metals do not interfere. As in the macro technique, a large excess of salts is undesirable. From 2 to 10 mg. of zinc were determined with an average deviation of −1.1 parts per thousand. All weighings were made on an Ainsworth-type TCX micro balance, accurate to ±0.01 mg.

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# Use of Capillary Trap in Microdetermination of Carbon

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**A rapid and precise manometric method is described for the measurement of carbon dioxide produced by combustion in microdetermination of carbon. In the very low range 1 $\gamma$ of carbon corresponds to a manometer reading of 4 mm. Excluding the effects of sample loading, the blank is negligible. With the sacrifice of some precision the measuring unit may be revised to possess a dual range, accommodating both micro and macro amounts of carbon.**

A SINGLE unit for measuring carbon dioxide has been developed by Smiley (*4*) which he has used in methods for determining oxygen and carbon. In view of considerable interest shown recently in methods for determining low carbon in metals (*1–3*, *5*), the following brief description is given of a very satisfactory adaptation of Smiley's capillary trap to the standard combustion method. The result of the adaptation is a simple, rapid method, giving good precision in the microgram range.

Figure 1 shows a schematic diagram of the all-glass apparatus. The indicated components were made large enough to accommodate an oxygen flow rate of 150 ml. per minute, and were connected together by semiball joints. The manometer used was constructed with dimensions approximating those described by Smiley (*4*), except that the manometer tube was made of 1.0-mm. precision-bore capillary and was made longer because of lower altitude. Another minor change was the substitution of 1-mm. oblique-bore stopcocks for the brass valves. The stopcocks were sealed on to become permanently incorporated in the calibrated unit. The outer stems were equipped with 18/9 semiball joints to facilitate easy dismantling from the line for cleaning. The inlet stopcock had a grooved plug to give precise control of gas flow. For this manometer 1 $\gamma$ of carbon (or 0.0001% for a 1-gram sample) was equivalent to about 4 mm. at the upper portion of the scale and to about 2 mm. at the lower. The maximum amount of carbon measurable with this manometer was about 200 $\gamma$. The various purification tubes indicated in the diagram adequately protected the cold trap of the measuring unit from contaminants such as moisture and oxides of sulfur and selenium. A liquid nitrogen trap, not shown, was used between the measuring unit and the Duo-Seal pump to protect the unit from pump oils.

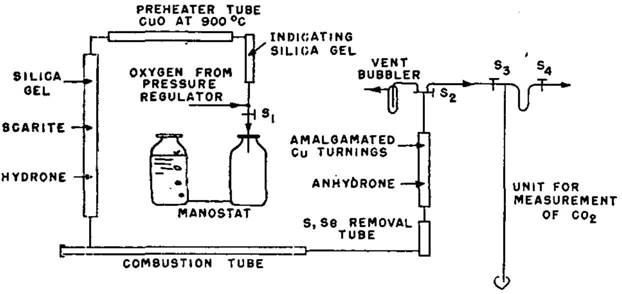

**Figure 1. Schematic diagram of carbon train**

The time required for measurement of the carbon dioxide after completion of sample combustion was 2 minutes or less. This constituted a considerable saving of time over the previous method of weighing Ascarite tubes on a microbalance. Another outstanding advantage over the gravimetric method was the absence of a blank attributable to the carbon dioxide measurement. When only the sample loading operation was omitted from the procedure the manometer gave zero readings.

Results obtained on two National Bureau of Standards samples containing low carbon are shown in Table I.

## EXTENDED RANGE

The range of measurement was extended to accommodate macro as well as micro quantities of carbon by constructing a unit with two additional modifications. A hollow stopcock reservoir (Figure 2) was sealed on between the manometer tube and the U-bend; and the lower portion of the U-bend was made of a 5-cm. section of 7-mm. tubing containing a plug of glass wool.

This arrangement offered a choice of two calibration curves. For low carbons the reservoir was not used, whereas for higher percentages it was opened to increase the volume of the unit, so that the scale divisions represented correspondingly larger amounts of carbon. The unit used had a low scale range of 0 to 400 $\gamma$ and a 7.5-ml. reservoir which afforded a high scale range of 0 to 2500 $\gamma$. The carbon in a 0.1-gram sample running as high as 2.5% could be measured on the high scale, while that in a 1-gram sample of a material containing as low as 0.001% could be measured on the low scale. The low scale of such a revised unit is not as highly sensitive as the unit described above because of the increased volume of the glass-wool section. The ratio of scale reading to weight of carbon was about 1 mm. per $\gamma$.

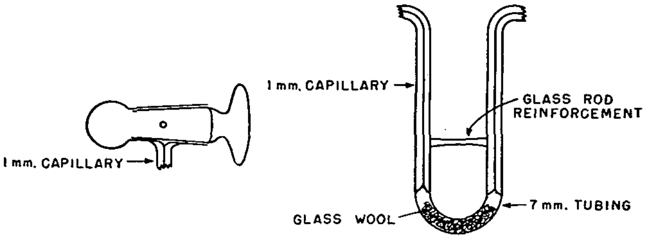

**Figure 2. Hollow stopcock reservoir (*left*) and glass wool U-tube (*right*)**

The glass-wool section was found to be necessary to condense out completely and to retain all the carbon dioxide coming from a sample exceeding about 200 $\gamma$ of carbon. The plain capillary U-bend adequately retained all the carbon dioxide below this amount; but as additional amounts entered the cold zone of the trap carbon dioxide crystals apparently grew to the extent that some of them broke loose from the walls of the capillary into the oxygen stream, which carried them out of the measuring unit, thus giving slightly low results. This explanation of the low results was verified by operating two measuring units in series. The combined amounts in the two units gave complete recoveries. After the glass-wool section was added to the primary unit it

**Table I. Analyses of National Bureau of Standards Standard Samples 166 and 59**

| Sample | Carbon Recommended, % | | Carbon Found, % |
|---|---|---|---|
| NBS steel 166 | 0.0274 | | 0.0275<br>0.0275<br>0.0273<br>0.0281<br>0.0281<br>0.0272 |
| | | Average | 0.0276 ± 0.0003 |
| NBS ferrosilicon 59 | 0.015 | | 0.0158<br>0.0148<br>0.0155<br>0.0151 |
| | | Average | 0.0153 ± 0.0004 |

**Table II. Analyses for Carbon in Macro Range Using Revised Measuring Unit**

| Sample | Carbon Recommended % | | Carbon Found, % |
|---|---|---|---|
| $CaCO_3$ | 12.00 | | 11.97 |
| | | | 12.08 |
| | | | 11.99 |
| | | | 12.04 |
| | | | 12.02 |
| | | | 12.05 |
| | | | 12.14 |
| | | | 11.87 |
| | | | 11.98 |
| | | | 12.00 |
| | | Average | 12.01 ± 0.05 |
| NBS steel 82, 8 samples | 2.78 | | |
| | | Average | 2.79 ± 0.09 |
| NBS steel 132, 6 samples | 0.803 | | |
| | | Average | 0.793 ± 0.005 |

gave complete recoveries and no carbon dioxide was found to escape into the secondary unit. Other designs tried for the cold trap before adopting the U-bend containing glass wool were a capillary helix coil, a multiple loop coil, and a U-bend containing a fritted-glass filter. These were all improvements over the plain capillary U-bend when large amounts (over 200 $\gamma$) of carbon dioxide were involved, but all permitted varying degrees of loss. The glass wool not only served as a filter to retain the crystals but it also substantially increased the cold surface on which the condensation could occur.

Table II shows the results obtained on materials containing carbon in the macro range, using the modified measuring unit.

## ACKNOWLEDGMENT

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# Detection of Amino Acids on Paper Chromatograms

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**Amino acids and other amino compounds after being oxidized by periodate may be detected on paper chromatograms by benzidine and by starch-iodide reagents. These methods have enabled certain of the amino acids to be distinguished. Mixtures of *tert*-amyl alcohol–1-propanol–water have been found useful as irrigating solvents for separating amino acids.**

NINHYDRIN is a satisfactory reagent for detecting amino acids on paper chromatograms (*3*) and the more recent method involving chlorination followed by a starch-iodide spray makes it possible to detect proteins as well as amino acids (*2, 8*). However, the identification of the amino acids in a mixture still presents considerable difficulty. Since no single solvent mixture permits the separation and identification of all the amino acids on a single one-dimensional chromatogram, recourse has been had to the use of two dimensional chromatography (*3*), and of specific tests. Thus diazo reagents have been employed for detecting tyrosine and histidine (*4, 5, 9*); *p*-aminobenzaldehyde for glucosamine (*7*); and sodium azide (*1*), iodoplatinate (*11*), and nitroprusside (*10*) for locating sulfur-containing amino acids.

In an attempt to simplify the identification of amino acids on paper chromatograms the authors have found that certain periodate sprays with or without a subsequent benzidine spray are useful for detecting certain amino acids and groups of amino acids. These new reagents, which have already proved valuable in the paper chromatographic analysis of carbohydrates (*2*) are also valuable for the identification of the difficultly separable amino acids such as glycine and serine, threonine and alanine, and valine and methionine on one-dimensional chromatograms.

## EXPERIMENTAL

**Solvents for Irrigating the Chromatograms.** Mixtures containing *tert*-amyl alcohol and 1-propanol were found to be useful as a second solvent following phenol in two-dimensional chromatographic separation of amino acids. Such solvent mixtures can also be used for one-dimensional chromatograms (see Table II).

## REAGENTS

Benzidine Reagent A. Mix equal volumes of $0.1M$ benzidine in ethyl alcohol and $0.8N$ hydrochloric acid.

Benzidine Reagent B. Mix 10 volumes of $0.1M$ benzidine in 50% ethyl alcohol with 1 volume of $0.2N$ hydrochloric acid and add 2 to 3 volumes of acetone to dissolve the benzidine hydrochloride.

Neutral Periodate. Saturated aqueous solution of potassium metaperiodate.

Alkaline Sodium Iodide. A 4% aqueous sodium iodide solution saturated with sodium bicarbonate.

Starch, 0.1% aqueous solution.

Sodium Iodide, 5% aqueous solution.

Glycine, 5% aqueous solution.

## PROCEDURE AND RESULTS

**Treatment of Chromatograms with Periodate.** The chromatograms were freed from volatile solvents by allowing them to stand in air. When phenol was used it was removed by extraction with either ether or acetone-ether. The papers were then sprayed with the saturated aqueous potassium metaperiodate solution and allowed to stand for 1 to 2 minutes.

**Procedure 1. Detection of Amino Acids with Benzidine Reagent A.** When the periodate treated paper is sprayed with Reagent A it gives within 1 minute a blue color with glycine and a yellow color with methionine on an almost colorless background. With insufficient hydrochloric acid in the reagent, the background will turn blue, while too much acid reduces the sensitivity of the reagent.

Maximum sensitivity for all reactive amino acids is obtained by spraying the chromatogram lightly with Reagent A and, after the blue spots appear, respraying with Reagent A; this intensifies the spots due to the sulfur-containing amino acids and tryptophan. The blue spots generally fade under this treatment, but they can usually be made to reappear by respraying the chromatogram with potassium metaperiodate.

The more basic amino acids appear as blue spots, while the sulfur-containing amino acids and tryptophan appear yellow or yellow-brown (see Table I group IA).

**Table I. Detection of Amino Compounds on Paper Chromatograms**[a]

| Compound | Group I | | Group II Procedure 3 | | Group III Procedure 4 |
|---|---|---|---|---|---|
| | Group IA by Procedure 1 | Group IB by Procedure 2 | Before starch | After starch | |
| Alanine | Blue | | | | |
| Arginine[b] | Blue | | | | |
| Cysteine[b] | Yellow[c] | Colorless[c] | Light brown | Blue | Colorless |
| Cysteic acid | | | Dark brown | Blue | Colorless |
| Cystine | Yellow | Colorless | Dark brown | Blue | Colorless |
| Glucosamine[b] | Blue | Colorless | Dark brown | Blue | Colorless |
| Glycine | Blue | | | | |
| Histidine[b] | Blue | | Dark brown | Blue | Colorless |
| Hydroxyproline | Blue | | | | |
| Lysine | Blue | | | | |
| Methionine | Yellow[d] | Colorless | | | Colorless |
| Serine | | Colorless | | | Colorless |
| Threonine | | Colorless | | | Colorless |
| Tryptophan[e] | Yellow-brown | Colorless | | | Colorless |
| Valine | Blue | | | | |

[a] Blank spaces in table indicate compound not detectable.
[b] Applied as hydrochloride.
[c] Colorless spot detected by Procedure 2 corresponds in $R_f$ value to colorless area within yellow trailing spot produced by Procedure 1.
[d] Colorless center can generally be detected within yellow ring.
[e] Dissolved in 0.1*N* HCl.

Cysteine hydrochloride gives a long, trailing, rapidly darkening yellow spot usually containing a colorless area when the chromatogram is developed with such solvents as phenol saturated with water, *tert*-amyl alcohol–acetic acid–water, *tert*-amyl alcohol–1-propanol–water or methyl Cellosolve–water. The colorless area is in the center of the yellow trailing spot when *tert*-amyl alcohol–1-propanol–water is used as the irrigating solvent, but when phenol saturated with water is used the colorless area is at the lower edge of the trailing spot. The colorless area is best seen if the chromatogram is given an additional spraying with glycine solution, since this produces a blue background (see later).

It is possible to detect the amino acids of groups IA and IB on the same chromatogram by spraying the periodate treated chromatogram first with the benzidine Reagent A, to detect the amino acids in group IA, and then, within 5 minutes, with the 5% glycine solution whereupon colorless spots due to the amino acids of groups IB soon appear on a blue background.

In this manner, pairs of overlapping spots due to amino acids of group IA and IB such as glycine-serine, alanine-threonine, and valine-methionine can be differentiated. Concentrations of 10 $\gamma$ of amino acids were detected in this way on one-dimensional chromatograms that had been irrigated with methyl Cellosolve–water (9 to 1).

**Procedure 2. Detection of Amino Acids with Benzidine Reagent B.** The periodate treated paper is sprayed lightly with reagent B. Colorless spots on a blue background appear at the location of $\alpha$-hydroxyamino acids, glucosamine, tryptophan, cysteine, and methionine (see Table I, group IB).

**Procedure 3. Detection of Amino Acids with Neutral Periodate and Sodium Iodide.** The chromatogram, after being lightly sprayed with a saturated solution of potassium metaperiodate, is warmed for 20 to 30 minutes at 50° to 60° C. Upon spraying with 5% aqueous sodium iodide the amino acids are located by the formation of brown spots which turn blue when the paper is sprayed with starch solution (see Group II, Table I) (*6*).

The warming of the chromatogram prior to spraying with sodium iodide appears to be necessary to produce maximum color stability of the spots.

Cysteic acid may be distinguished from other amino acids by the formation of a blue color upon spraying the periodate treated chromatograms with the starch solution.

**Procedure 4. Detection of Amino Acids with Alkaline Periodate and Sodium Iodide.** After the irrigating solvent has been removed, the chromatogram is sprayed with the sodium iodide–sodium bicarbonate solution and then with starch. After drying in the air, the paper is lightly sprayed with potassium metaperiodate. Cystine, histidine, and cysteic acid are revealed by the immediate formation of brown spots (the color disappears with excessive spraying). After a few minutes a number of the other amino compounds listed in Table I appear as colorless spots on a blue background.

With this reagent reducing sugars will also form colorless spots (*2*), but they may be distinguished from the colorless spots due to amino compounds by the fact that they turn brown on being heated for 15 to 20 minutes at 110° C. whereas the colorless spots due to the amino acids remain unchanged. In this test tryptophan develops a slight color (Table I, group III).

**Table II. $R_f$ Values of Amino Compounds Using Mixtures of *tert*-Amyl Alcohol (A), 1-Propanol (B), and Water (C)**[a]

| Compound | $R_f$ Values Using Solvent Mixture | |
|---|---|---|
| | A, 4; B, 1; C, 1 vol. | A, 4; B, 0.8; C, 1.8 vol. |
| Alanine | 0.24 | 0.26 |
| Arginine[b] | 0.08 | 0.13 |
| Aspartic acid | 0.04 | 0.11 |
| Cysteic acid | 0.10 | 0.12 |
| Cysteine[b] | 0.27[c] | .. |
| Cystine | 0.04 | 0.08 |
| Glucosamine[b] | 0.23 | 0.27 |
| Glycine | 0.16 | 0.20 |
| Histidine[b] | 0.10 | 0.14 |
| Hydroxyproline | 0.24 | 0.24 |
| Leucine | 0.62 | 0.57 |
| Lysine | 0.06 | 0.10 |
| Methionine | 0.46 | 0.43 |
| Proline | 0.27 | 0.31 |
| Serine | 0.16 | 0.20 |
| Threonine | 0.21 | 0.24 |
| Tryptophan[d] | 0.44 | 0.42 |
| Valine | 0.43 | 0.38 |

[a] Ascending technique used; compounds detected by ninhydrin unless stated otherwise.
[b] Applied as hydrochloride.
[c] Cysteine hydrochloride not easily detected by ninhydrin. $R_f$ value refers to colorless spot detected by Procedure 1 followed by treatment with 5% glycine.
[d] 1% solution in 0.1*N* HCl.

Since this work was completed, a similar procedure using periodate followed by a starch-iodide buffered spray reagent has been published for detecting periodate oxidizable compounds including amino acids (*6*).

The above procedures have enabled the authors to show that in the hydrolyzate of a purified preparation of yeast invertase the following amino acids are present: alanine, aspartic acid, cysteic acid, glycine, glutamic acid, leucine, lysine, serine, threonine, tyrosine, valine, and one as yet unidentified.

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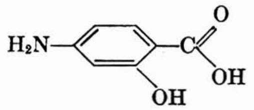
Received for review October 18, 1954. Accepted April 19, 1955. This paper (No. 3230, Scientific Journal Series, Minnesota Agricultural Experiment Station) forms part of a thesis submitted by J. A. Cifonelli to the graduate school of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree, 1952.

# No. 98. 4-Aminosalicylic Acid

**Contributed by JOHN KRC, JR., and W. C. McCRONE, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.**



**Structural Formula for 4-Aminosalicylic Acid**

Excellent crystals of 4-aminosalicylic acid can be obtained from 1 to 1 acetone-water solution. Sublimation at ordinary pressure apparently gives a decomposition product; hence this procedure cannot be used. There was no evidence of polymorphism.

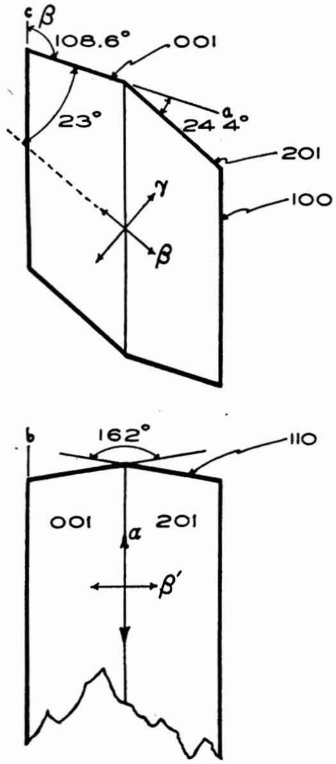


**Figure 1. Orthographic projection for typical crystal of 4-aminosalicylic acid**

CRYSTAL MORPHOLOGY
Crystal System. Monoclinic.
Form and Habit. Needles, rods, and plates elongated parallel to *b*, lying on negative hemiorthodome, {201}, or orthopinacoid, {100}, showing basal pinacoid, {001}, and prism, {110}.
Axial Ratio. $a:b:c = 6.487:1:1.908$.
Interfacial Angles (Polar). 001 Λ $\bar{1}00$ = 108.6°; 001 Λ $\bar{2}01$ = 24.4°; 201 Λ $\bar{1}00$ = 47°; 201 Λ $\bar{1}00$ = 133°; 110 Λ $\bar{1}10$ = 18°.
Beta Angle. 108.6°.

X-RAY DIFFRACTION DATA. (See table, page 1504.)
Cell Dimensions. $a = 24.62$ A.; $b = 3.795$ A.; $c = 7.239$ A.
Formula Weights per Cell. 4 (3.91 calculated from x-ray data).
Formula Weight. 153.14.

Density. 1.545 (flotation in benzene–carbon tetrachloride, pycnometer); 1.582 (x-ray).

OPTICAL PROPERTIES
Refractive Indices (5893 A.; 25° C.). $\alpha = 1.536 \pm 0.001$. $\beta = 1.695 \pm 0.005$. $\gamma = 1.99 \pm 0.02$ (calculated from $\alpha$, $\beta$, and $2V$).
Optic Axial Angles (5893 A.; 25° C.). $2H = 100°$. $2V = 87$ (calculated from $\beta$ and $2H$).
Dispersion. Strong, $r > v$.
Optic Axial Plane. 010.
Sign of Double Refraction. Positive.
Acute Bisectrix. $\gamma$.
Extinction. $\gamma \Lambda a = 26°$ in acute $\beta$.

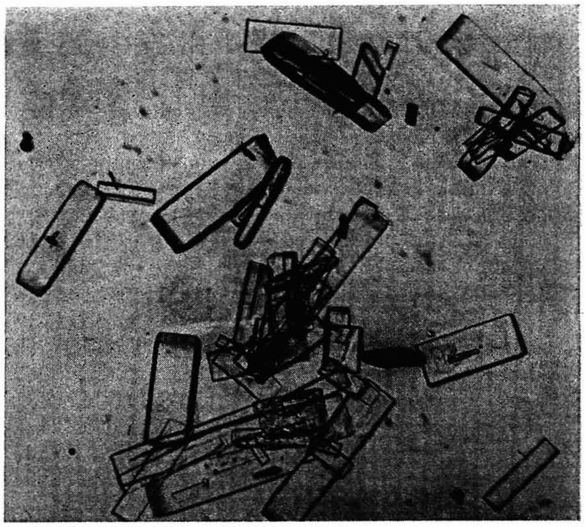


**Figure 2. Crystals of 4-aminosalicylic acid recrystallized from benzyl alcohol on microscope slide**

**Figure 3. Crystals of decomposition product obtained on melting or subliming 4-aminosalicylic acid**

**Principal Lines**

| d | $I/I_1$ | d | $I/I_1$ | d | $I/I_1$ |  | $I/I_1$ |
|---|---|---|---|---|---|---|---|
| 12.1 | 5 | 2.40 | 4 | 3.45 | 9 | 1.802 | 2 |
| 6.96 | 7 | 2.35 | 2 | 3.35 | 10 | 1.770 | 3 |
| 6.15 | 6 | 2.27 | 3 | 3.24 | 7 | 1.688 | 2 |
| 5.64 | Weak | 2.14 | 2 | 3.04 | 4 | 1.662 | 1 |
| 5.27 | 9 | 2.09 | 4 | 2.99 | 3 | 1.633 | 1 |
| 5.03 | Weak | 2.05 | 1 | 2.79 | 1 | 1.606 | 1 |
| 4.09 | 2 | 1.992 | 1 | 2.74 | 1 | 1.581 | Weak |
| 3.75 | 2 | 1.955 | 2 | 2.60 | 4 | 1.540 | 2 |
| 3.63 | 5 | 1.920 | Weak | 2.50 | 3 | 1.506 | 1 |
| 3.53 | Weak | 1.840 | 2 | 2.46 | 3 | 1.440 | Weak |

Molecular Refraction ($R$) (5893 A.; 25° C). $\sqrt[3]{\alpha\beta\gamma} = 1.735$. $R$ (calcd.) = 33.1; $R$ (obsd.) = 38.8.

Fusion Data. 4-Aminosalicylic acid decomposes on melting (220° C.) to give a pure decomposition product melting, in turn, at 122° C. The latter crystallizes spontaneously from the melt after considerable supercooling. The melt is fairly viscous, the crystallization velocity is very slow, and the crystals are small. On warming, the crystal size and growth rate both increase. Bisectrix figures are possible with $2V$ about 90°.

## ACKNOWLEDGMENT

Irene Corvin is responsible for powder x-ray diffraction data.

Contributions of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Research, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

# CORRESPONDENCE

# Conductometric Titrations with Dimethylglyoxime

Sir: This note reports the conditions under which conductometric methods, including high-frequency methods, may be used to determine the end point for the titration of solutions of nickel(II) ion and dimethylglyoxime and shows that for some recently reported titrations of dimethylglyoxime with cobalt, nickel, lead, and manganese (*3*), no changes that occur under the conditions specified permit detection of the claimed end points by two recognized types of high-frequency apparatus or by a conventional conductance method.

Nakano, Hara, and Yashiro (*3*) report high-frequency titration curves having very abrupt changes of slope at molar ratios 2 to 1 and 1 to 1 for the addition of 0.005$M$ nickel sulfate, cobalt nitrate, lead nitrate, or manganese chloride to 0.001$M$ aqueous dimethylglyoxime in the absence of any buffer. It is the opinion of the present authors that their results must be related to factors other than the reported reactions and would not be substantiated over any considerable range of concentration and volume.

The high-frequency titrations, using concentrations and conditions specified by Nakano, Hara, and Yashiro, have been repeated in this laboratory using the apparatus based on the General Radio Twin-T impedance measuring circuit (*1*) at a number of frequencies from 2 to 10 Mc., and using a crystal oscillator apparatus (*2*) at 5 Mc. A thorough study has also been made of these reactions, at the same and other concentrations, with and without added ammonia and buffers. These titrations were followed with the highly precise 1000-cycle conductance bridge built around the Leeds and Northrup Co. No. 1553 ratio box, as well as with the radio-frequency apparatus just listed.

## DIMETHYLGLYOXIME-NICKEL

For all unbuffered solutions, a plot of instrument response against volume of nickel sulfate added to the aqueous dimethylglyoxime gave a smooth curve, indicating a continuous increase in conductance with no abrupt changes. For the addition of 0.005$M$ nickel sulfate to 100 ml. of 0.001$M$ aqueous dimethylglyoxime (for which the 2 to 1 and 1 to 1 ratios would occur at 10 and 20 ml. of nickel sulfate, respectively), the first 1 or 2 ml. of salt solution resulted in a faint yellow color. More nickel sulfate gradually changed the color to a faint pink, with a slight turbidity increasing to a just visible precipitate at 15 to 25 ml. of added solution. Greatly increased precipitation may be produced at any point during the titration by addition of a few drops of ammonia. Similar conductance curves and color changes were produced over concentrations ranging from 0.001$M$ to 0.005$M$. Similar color changes and converse conductance changes were produced by addition of dimethylglyoxime to nickel sulfate solution. More concentrated solutions of dimethylglyoxime were made by using a small amount of ethyl alcohol in the water, but this did not affect the nature of the results.

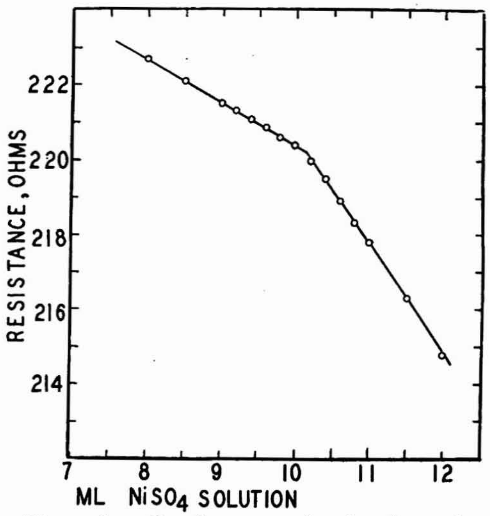


**Figure 1. Conductometric titration of dimethyl glyoxime with nickel ion in presence of ammonia**

These observations suggest that in these unbuffered solutions a small amount of the usual 2 to 1 complex is formed and precipitated but that no reaction is completed. This conclusion was further supported by pH measurement as dimethylglyoxime was added to nickel sulfate solution. Upon addition to a 0.001$M$ salt solution the pH falls continuously to well past the 2 to 1 ratio, hydrogen ion being released as the 2 to 1 complex is formed.

Prior addition of ammonia to the dimethylglyoxime solution resulted in immediate precipitation as each increment of nickel sulfate was added, and a distinct break occurred in the conductance or high-frequency plot at the 2 to 1 ratio. No break was detected at the 1 to 1 ratio under any of the conditions tried. One or two drops of $3N$ ammonia for each 100 ml. of $0.001M$ dimethylglyoxime produced distinct 2 to 1 end-point breaks with considerable curvature of the lines leading to them. As the ammonia concentration was increased, the linearity of the plots on the two sides of the end point improved. Addition of ammonia beyond 6 ml. or addition of acetate buffer decreased the change of slope at the end point.

The conditions selected as best for the conductometric titration of dimethylglyoxime with nickel were as follows.

An $0.005M$ stock solution was made by dissolving a weighed amount of Eastman Kodak dimethylglyoxime in the minimum amount of alcohol and diluting to volume with water. An $0.005M$ stock solution was made by dissolving a weighed amount of Baker & Adamson nickel sulfate hexahydrate reagent and diluting to volume. This solution was found to be $0.00496M$ by gravimetric dimethylglyoxime determination. Twenty milliliters of the dimethylglyoxime solution and 5 to 6 ml. of approximately $3N$ ammonia were mixed and diluted to approximately 100 ml. The resistance was measured with the Leeds and Northrup Co. bridge following each addition of nickel sulfate solution. The end points, as determined from graphical plots, agreed within 0.1 ml.

A sample curve is shown in Figure 1. The cell constant for the cell used in this titration was 0.0962. An average of three determinations gave the value 10.14 ml. Using the gravimetric value for the concentration of the nickel, the concentration of the dimethylglyoxime was calculated to be 0.00503, in good agreement with the value from the weight used. This method should permit accurate standardization of dimethylglyoxime solutions.

### OTHER IONS

The high-frequency and conventional conductance procedures described above for nickel were repeated for cobalt(II), nitrate, lead(II), nitrate, and manganese(II) chloride. As for nickel, the conditions of Nakano, Hara, and Yashiro were first duplicated as nearly as possible, then other conditions were tried. In no case, with or without the addition of ammonia, was there any indication of end points at any ratio. The cobalt solutions gave a yellow to amber color and whenever ammonia was present the lead solution yielded a small amount of white precipitate. Addition of the first 5 ml. of dimethylglyoxime to the $0.001M$ cobalt solution produced no change of pH from the initial pH of 4.8. A gradual drop to pH 4.6 occurred between 5 and 25 ml., possibly indicating some slight displacement of hydrogen ion from the dimethylglyoxime by coordination. For both the lead and manganese salts the pH rises continuously on addition of dimethylglyoxime, indicating no coordination involving displacement of hydrogen ion.

### ACKNOWLEDGMENT

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# Effects of Impurities upon Rate of Precipitation and Particle Size

SIR: It has been observed (*1*, *2*) that crystals of barium sulfate, precipitated with a freshly prepared barium chloride solution, are distinctly smaller than those obtained with a reagent solution which has been standing for some time. If "barium chloride" is understood to mean pure barium chloride, the explanation of the "aging" of its solution invites speculations involving equilibria between hydrated and complex ions and considerations of kinetics including that of the growth of an anhydrous lattice of barium sulfate. With such possibilities in mind, a quick experimental survey was carried out, which soon led to the suspicion that an impurity of the reagent might be responsible for the phenomena observed.

In the final series of experiments, about 15-ml. portions of $0.02M$ barium chloride solution and of a solution $0.01M$ with sulfuric acid and $0.1M$ in hydrochloric acid were briefly heated in test tubes by inserting them into a steam bath. The hot solutions were simultaneously poured into an empty beaker which was being heated upon the steam bath. The mixture was kept hot for 5 minutes with frequent mixing by swirling. It was then allowed to cool to room temperature during 10 minutes, after which the average diameter of the crystals of barium sulfate was determined under the microscope.

**Batch A.** Barium chloride dihydrate, maximum impurities listed "heavy metals 0.0." When a freshly prepared solution of the salt was used within 10 minutes from the time of dissolution, the crystals of the precipitated barium sulfate had a diameter of approximately 3 microns. This diameter increased to 5 microns when the freshly prepared solution of the barium chloride was filtered through S. & S. Blue Ribbon, ash-free filter paper and used within 10 minutes from the time of dissolution; average particle diameters of 6 microns were obtained with solution of the salt, filtered or not filtered, which had been standing in glass containers for one to several days. The results were not affected if the solution was $0.2M$ during the aging period and was diluted with water to $0.02M$ just previous to use in precipitating barium sulfate.

**Batch B.** Some of batch A was recrystallized from hot water. The hot saturated solution of about 50 grams of batch A assumed, for a moment, a dark gray color and this was immediately followed by flocculation of a very small amount of partly tawny and partly brownish black precipitate which was removed by filtration and gave a positive test for iron.

A freshly prepared solution of batch B used within 10 minutes from the dissolution of the salt gave barium sulfate with average grain diameters of 6 microns. Aging or filtration of the solution had no effect on the particle size of the barium sulfate.

**Batch C.** Barium chloride dihydrate meeting ACS specifications and furnished by a different firm. Solutions of this pure salt behaved exactly like solutions of batch B—i.e., the aging of solutions had no effect on the particle size of precipitated barium sulfate.

The conclusion seems unavoidable that solutions of pure barium chloride do not age and that in the instance of my brand A, impure barium chloride, the increase of the particle size of precipitated barium sulfate is caused by a removal of the impurity. Adsorption on the walls of storage bottles or on the surface of filter mats could bring this about, since the amount of impurity is very small.

Freshly prepared solutions of batch A gave a turbidity of barium sulfate immediately upon addition of sulfate solutions.

With aged solutions of batch A and with pure barium chloride, the turbidity is perceived after a slight delay. The time lapse between addition of the reagent and perception of the first turbidity is much more pronounced with strontium sulfate and was studied with $0.01M$ solutions of strontium salts and sulfuric acid. The turbidity became visible 20 to 30 minutes after combining the reagents if a solution of our strontium chloride hexahydrate was used. The time lapse increased to 1 hour if our strontium chloride hexahydrate was first rendered anhydrous by drying in an oven and then dissolved in water. Our strontium nitrate gave the visible turbidity within 2 to 5 minutes, but the time lapse was extended to 2 hours if the solution was acidified with hydrochloric acid and allowed to stand 2 hours before adding the sulfuric acid. These strontium salts retained the described behavior also after attempted purification by recrystallization from water.

After the experience with the barium chloride, it does not seem proper to attach any importance to the odd behavior of our strontium salts unless it were proved that they are entirely free from impurities. Traces of barium could be responsible for the observed phenomena, and they would not be removed by recrystallization from water.

All these observations show that the rate of precipitation and the particle size of precipitates may be strongly affected by trace impurities in solutions. In the instance of the barium sulfate, the particle size decreased by more than 50% without any noticeable change in the habit of the crystals of barium sulfate. Considering this remarkable sensitivity of the particle size, the analyst will do well to consider the origin and history of his solutions when trying to obtain easily filterable precipitates. In addition, fine-grained precipitates might be contaminated by the trace constituents which are responsible for the small particle size.

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A. A. Benedetti-Pichler

Sir: Benedetti-Pichler's work on the "Effects of Impurities upon Rate of Precipitation and Particle Size" is interesting. His conclusions on the aging and filtering of a solution of recrystallized barium chloride dihydrate do not agree with mine. The differences could well be explained by the differences in the precipitation. His conditions are: $0.02M$ barium chloride added to a solution $0.12N$ in hydrochloric and sulfuric acids after a 5-minute heating period compared to mine: 5% barium chloride added to sodium sulfate in $0.004N$ hydrochloric acid at once at room temperature. Our filtering of the solutions was also different, his being filtered through S. & S. Blue Ribbon while mine was filtered through Pyrex F filters with suction.

In my work (*1*), a sedimentation method was used to compare particle sizes. Barium chloride dihydrate, without previous treatment, was used to make a 5% solution. After precipitation with this solution, 66 minutes were required for 28 mg. of barium sulfate to settle. This same barium chloride dihydrate was dissolved in hot water, filtered through a Pyrex F filter with suction, and recrystallized. This salt was used to make a 5% solution. The barium sulfate obtained required 48 minutes for 28 mg. to settle. This same 5% solution of recrystallized salt was filtered through a Pyrex F filter with suction. It was used within 5 minutes of the solution above and the barium sulfate took only 5.2 minutes for 28 mg. to settle. This last was not included in my thesis, for I felt that recrystallization had little to do with the effects on particle size.

Also not included in my thesis was an experiment on recrystallization where the hot concentrated solution was filtered through paper. The recrystallized salt, used in a 5% solution, required 102 minutes for 28 mg. of barium sulfate to settle. When this same solution was used 48 hours later, the barium sulfate took 49 minutes for 28 mg. to settle. These sedimentation results cannot be compared with those above, for they were carried out at a lower temperature.

It was suggested during the examination over my thesis that air was serving as nuclei. This was checked with a 5% solution: Standing for 1 hour at 757.5-mm. pressure gave barium sulfate that required 47.4 minutes for 30 mg. to settle, standing over the same period of time but under 27.5-mm. pressure, with vigorous agitation every 5 minutes, gave barium sulfate that required 26.6 minutes for 30 mg. to settle.

I have no doubt that impure barium chloride will cause fine precipitates of barium sulfate. I believe, too, that no one single item will explain all the interesting facts about this precipitation

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# Separation of Orthophosphates from Organic Phosphates

Sir: In attempting to follow the procedure of Martin and Doty [Martin, J. B., and Doty, J. H., Anal. Chem., 21, 965 (1949)] for the separation of orthophosphates from organic phosphates, we found that the aqueous phase developed a blue color of greater intensity than the isobutyl alcohol-benzene phase. In an effort to eliminate this trouble, we prepared a new set of reagents, using only redistilled water and chemicals of the highest purity.

The persistence of a deep blue color in the aqueous phase suggested that a contaminant in the ethyl alcohol used to rinse the pipet was responsible. In fact, addition of 1 ml. of absolute ethyl alcohol to a mixture of 2 ml. of 4% ammonium molybdate in $4N$ sulfuric acid and 0.50 ml. of stannous chloride caused a blue color to develop within a few seconds. The addition of 1 ml. of acetone gave a very intense blue color (absorbance $>2$ at 730 m$\mu$ in a Beckman Model DU spectrophotometer with 1-cm. cuvettes), while 0.3 ml. of acetone gave a pale blue (absorbance $\sim 0.05$). One milliliter of acetone-free methanol (as checked by a mass spectrometer) had an absorbance of about 0.05 at 200 seconds and about 0.15 at 800 seconds.

Methyl ethyl ketone, isopropyl alcohol, and isobutyl alcohol also caused some blue color to develop. Formaldehyde and ether did not cause the formation of any color. Heptyl and hexyl alcohol caused the formation of a bright yellow color in the aqueous phase, while cyclohexanone caused a yellow color in the cyclohexanone phase.

In addition to suggesting a rather sensitive method for acetone and possibly for some alcohols, these results explain the residual blue color in the aqueous phase even after extraction of all the orthophosphate.

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## Reduction of Rapid Random Fluctuations of the Beckman Flame Spectrophotometer

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In order to reduce the random, rapid fluctuations present when a Beckman DU spectrophotometer with direct-atomizing flame attachment and photomultiplier is used at near maximum photomultiplier sensitivity, capacitors have been installed across the null-meter. Johnson and Wischmeier (Beckman Application Bull. **DU-24-A**) mention the use of a 4000-microfarad capacitor. However, to have only one large value of dampening and to have it permanently installed would be inconvenient, because of the sluggish response of the meter, especially when the wavelength control is to be set for a spectral line at narrow slit widths. By the nature of capacitor dampening, slow changes are not affected and thus the sensitivity and accuracy of the instrument are not impaired. A marked increase in precision of setting the luminosity dial as well as greatly reduced eye strain has resulted from using these capacitors.

### CONSTRUCTIONAL DETAILS

Excellent fluctuation suppression has been obtained by using the circuit shown in Figure 1.

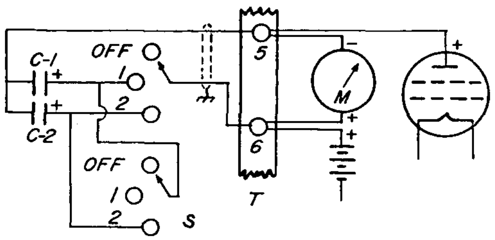

**Figure 1. Circuit diagram of fluctuation suppressor**

*S.* Two-gang, three-position switch with following positions:
- Off.
- 1. 500-μfd. dampening for routine determinations
- 2. 3500-μfd. dampening for extreme conditions

*C*-1. 500-μfd., 15-volt Mallory type WP dry electrolytic capacitor
*C*-2. 3000-μfd., Mallory Type WP dry electrolytic capacitor
*T.* Terminal strip of photomultiplier battery box
*M.* Null-meter on monochromator

The capacitors are readily available at radio supply houses for less than $5 each. It was found necessary to place 15 volts (direct current) across the capacitors for a few seconds to restore the electrolytic film lost after long shelf life and thus to increase the internal resistance of the capacitors to above 10,000 ohms. After this treatment, switching the capacitors out of the circuit gives no detectable deflection of the meter. Proper polarity is essential. Connections are easily made at the terminal strip of the photomultiplier battery box. Two-wire shielded cable should be used. The capacitors are mounted in a metal box which is fastened, by means of a clamp, to one leg of the fuel regulation panel to provide a convenient and firm mounting for the switch.

A further increase in precision and decrease of eye strain may be obtained by placing a small hand lens of about three power in a wire frame over the null-meter, so that it is at a convenient focus.

Another addition to the DU, which aids materially in flame spectrophotometric exploration and routine determinations, is the replacement of the sensitivity and dark current control knobs by turns-counting Duodials, Model RB (made by the Helipot Division, Beckman Instruments). These dials, giving the number of turns and fractions thereof, permit rapid and accurate resetting of the controls for various determinations. When fully "warmed up" and in equilibrium, so that drift is negligible for long periods, the dark current setting with the shutter off and the instrument nulled is highly reproducible. The sensitivity Duodial gives an accurate indication of the degree of sensitivity and the ratio of luminosity dial movement to null-meter deflection.

### ACKNOWLEDGMENT

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## Partial Acetylation of Paper for Chromatography

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There appears to be need for a paper that is less hydrophilic than ordinary filter paper for use in specialized applications of the paper chromatographic method (*10*). Materials that are difficultly soluble or insoluble in water would tend not to be held by the stationary hydrophilic phase; if the hydrophobic phase is stationary and the hydrophilic phase mobile, the problem can be solved. Kostir and Slavik (*6*) have used acetylated filter paper as the carrier for the stationary phase with two organic solvents for the separation of dinitrophenylhydrazones of the carbonyl fission products of sugar. Micheel and Schweppe (*7*) have partially acetylated Schleicher & Schuell No. 602 ED filter paper for use in separation of sugar acetates.

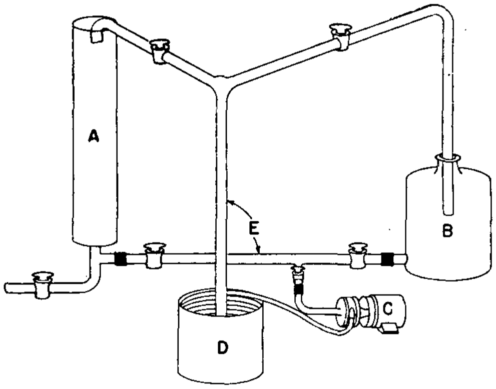

**Figure 1. Apparatus used for partial acetylation of paper**

*A.* Reaction tube, containing paper to be acetylated
*B.* Mixing bottle, 4 liters
*C.* Small centrifugal pump, stainless steel
*D.* Cooling coil, 15 feet of 1/2 inch-stainless steel tubing, in beaker of ice-water mixture
*E.* Glass tubing and stopcocks for controlling direction and rate of flow

For the laboratory preparation of acetylated paper from ordinary filter paper, Burton (*3*) suggested the use of an acetic anhydride–benzene mixture with perchloric acid as catalyst in a two-step procedure, but some difficulties were encountered in retaining paper structure. More recently Scott and Goldberg (*9*) have tried the method of Hess and Ljubitsch (*5*), but the acetylation reaction took 9 days to produce 25% acetyl content in the paper. The writers have adapted for the treatment of filter paper the method used on cotton cloth by Cooper and others (*4*).

The use of a nonswelling diluent, amyl acetate, in the acetylation solution helped to retain original density and texture in the acetylated paper product. The washing procedure was also changed by the addition of two methanol and two hot water rinses in order to remove the reagents more effectively. These modifications have been used successfully for the partial acetylation of both Schleicher & Schuell No. 602 ED and Whatman No. 1 papers. Best uniformity with satisfactory acetyl content was obtained by the following procedure.

Three 22 × 18 inch sheets of the selected filter paper are interleaved with glass cloth, rolled into a cylinder, and placed in a glass tube of suitable dimensions to allow proper circulation of acetylating solutions. A convenient apparatus, based on that of Cooper and others (*4*), is shown in Figure 1. The activation reagent of glacial acetic acid, containing 5% acetic anhydride by volume, is added and allowed to stand overnight at room temperature. This solution is then replaced by the acetylation solution made of 4 parts of 22.5% acetic anhydride by volume in technical grade amyl acetate and 0.5 part of 1.8% perchloric acid by volume in glacial acetic acid, the latter being slowly added to the former while it is circulating through the cooling coil to maintain a temperature of 28–29° C.

The acetylation solution maintained at 28–29° C. is circulated through the paper by the pump for 2.5 hours. Following this treatment, the product is washed several times with cold water until the odor of amyl acetate can no longer be readily detected, then with methanol to remove the last traces of amyl acetate, and finally with hot water. The paper is suspended on a cord and dried at room temperature. The acetyl contents of the products obtained have varied over a narrow range around 26% by weight (dry basis).

The properties of filter paper before and after typical treatment are shown in Table I. Physical properties were determined under standard conditions for testing textiles—namely, 65% relative humidity at 21° C. The Draves test was modified by using distilled water, a 1 × 6 inch test specimen, and no hook.

**Table I. Properties of Filter Paper Before and After Acetylation**

| Property | Before | After | Method |
|---|---|---|---|
| Thickness, inch | 0.0065 | 0.0081 | ASTM D 76–53 |
| Density, lb./cu. inch | 0.0187 | 0.0181 | Mass/area × thickness |
| Tear strength, lb. | | | |
| Machine direction | 0.19 | 0.08 | Elmendorf |
| Cross direction | 0.16 | 0.07 | Elmendorf |
| Breaking strength, lb. | | | ASTM D 39–49 |
| Machine direction | 17.0 | 8.7 | (1-inch cut strip) |
| Cross direction | 10.1 | 5.7 | ASTM D 39–49 (1-inch cut strip) |
| Wet breaking strength, lb., machine direction | 1.8 | 2.5 | ASTM D 39–49 (1-inch cut strip) |
| Moisture content, % | 7.16 | 3.67 | Fed. Spec. CCC-T-191b, Method 2601 |
| Water absorption, sec. | Ca. 1 | 3000 | (*1*) |
| Wettability, sec. | Ca. 1 | 6000 | (*2*) |
| Water-repellency index | 72 | 72 | (*8*) |

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The mention of trade products is for convenience only and is not a recommendation by the U. S. Department of Agriculture over equivalent products of other companies.

## Silver Electrode

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In the titration of halides (*2*), the silver electrode must be protected from contact with air (*3*), in order to avoid irregular measurements. A silver electrode that has been used for a halide titration must be cleaned (*1*), or polished with crocus cloth, and treated with nitric acid before re-use. Electrodes having a small nonreplacable silver disk are unsatisfactory, in that they become unserviceable in a short period of use. A sturdy electrode, which can be easily resurfaced, and is useful for some other purposes where a known small area is needed, can be prepared as illustrated in the figure.

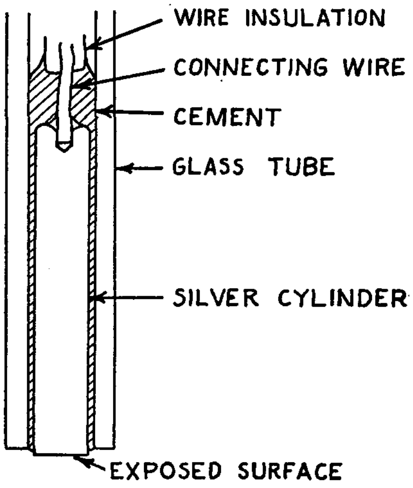

Figure 1. Silver electrode

The silver cylinder is conveniently made by drawing molten silver, by suction, into a selected piece of glass tubing and, after cooling, removing the glass from it. For the electrode tube another piece of tubing can be found, such that the cylinder will fit snugly into it. After the end of the flexible lead-wire has been soldered to the end of the sliver cylinder, the connection and the cylinder are coated with a laboratory cement such as Cementyte or Picein, and inserted into the tube. The cement chosen should be inert in the system studied and should completely fill the space between the cylinder and the tube. Excess cement can be wiped off while soft, or, in the case of the cements mentioned, wiped off with a cloth moistened with acetone.

The end of the electrode constructed as above is nearly flat. The glass tube can be tapered by hand lapping against a plate using an emery paste or in a rotating cone, again using an emery paste. When the exposed silver surface is dissolved by repeated cleaning, so that it no longer protrudes slightly from the glass and cannot be polished with the crocus cloth, the end may be renewed by warming the cement portion and pushing the cylinder out sufficiently to restore the original shape of the electrode. For this method a sufficiently stiff connecting wire through the electrode tube is helpful. Alternatively, the end may be restored by grinding away some of the glass, to produce a tapered end on the glass tube.

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