

This equation indicates that titanium is somewhat more effective as a deoxidizer in steel than would be calculated from the equation of Chipman, which gives for the reaction

$$\Delta F^\circ = -151,000 + 57.3T$$

The deoxidation constants usually are expressed as the reciprocals of the constants of the deoxidation reactions; *i. e.*,  $K = [\%Ti] \times [\%FeO]^2$ . The values of these constants calculated from the authors' and from Chipman's equations are compared in Table II at several temperatures.

TABLE II  
DEOXIDATION CONSTANTS

Temp., °C.	1500	1600	1700
Chipman	$8.2 \times 10^{-7}$	$8.1 \times 10^{-6}$	$6.2 \times 10^{-5}$
Authors	$3.2 \times 10^{-9}$	$4.3 \times 10^{-8}$	$4.4 \times 10^{-7}$

The authors wish to thank Professor J. G. Aston

of Pennsylvania State College who made available the standard couple, S-6, for calibration of our calorimeter; Dr. C. S. Barrett of the Metals Research Laboratory of this Institute for the X-ray photographs of the oxide used in this investigation; and Mr. J. W. Stewart, of the same laboratory, for the spectroscopic analysis.

### Summary

1. The heat capacity of titanium dioxide (rutile) has been determined from 68–298°K.

2. The entropy of titanium dioxide has been computed from these measurements and found to be 12.45 e. u. at 298.1°K.

3. The thermodynamic properties of titanium dioxide have been evaluated, and applied to the problem of the deoxidation of steel by titanium.

PITTSBURGH, PENNA.

RECEIVED JUNE 28, 1939

[CONTRIBUTION FROM THE AMMONIA DEPARTMENT OF E. I. DU PONT DE NEMOURS & COMPANY, INC.]

## Analytical Procedures Employing Karl Fischer Reagent. I. Nature of the Reagent

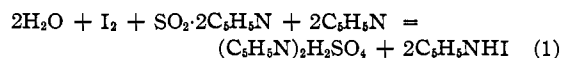
BY DONALD MILTON SMITH, W. M. D. BRYANT AND J. MITCHELL, JR.

The determination of water in mixtures by specific chemical methods has become an essential part of analytical chemistry. The senior authors<sup>1</sup> described such a procedure based on the hydrolysis of acetyl chloride in the presence of pyridine. Almost simultaneously another water method of unusual simplicity and range of application but based on a different chemical reaction was described by Karl Fischer.<sup>2</sup> The latter method involves a direct titration of the wet material with a solution of iodine, sulfur dioxide and pyridine in methanol, the reagent serving as its own indicator. The two methods have unique advantages in specific types of analysis, but the general analytical value of Fischer's method is sufficiently great to render desirable a detailed study of its underlying chemistry and a presentation of some of its significant applications.

Fischer has given a good practical discussion of the preparation and use of his reagent but has only briefly outlined the reactions involved. The latter, however, while partly correct as given, do not adequately explain the observed quantitative results. For example, his reaction

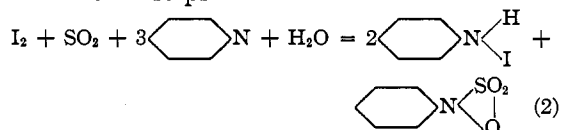
(1) Smith and Bryant, *THIS JOURNAL*, **57**, 841 (1935). This reference includes a brief review of earlier work.

(2) Karl Fischer, *Angew. Chem.*, **48**, 394 (1935).

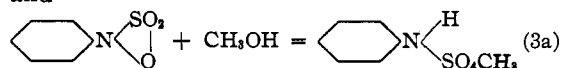


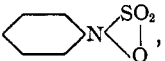
calls for the removal of two moles of water for each mole of iodine introduced, while the writers find that actually about one mole or less of water is removed by the unmodified reagent. In order to shed more light on the mechanism and stoichiometric relations upon which Fischer's method is based, the present paper includes a study of the fundamental molal ratios of the various constituents of the reagent and their relation to water removal. By working with concentrated solutions, crystalline intermediates and products were isolated and identified by optical crystallographic methods. Other bases were substituted for pyridine and other solvents for methanol, and the effect of the various modifications noted.

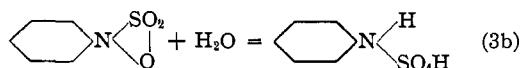
One of the most interesting results was the discovery that the fundamental reaction occurs in two distinct steps:



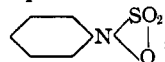
and



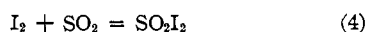
Only the first involves water absorption, and the stoichiometric amount of this substance is but half that required for Fischer's reaction 1. The intermediate compound, , can be isolated,<sup>3</sup> especially if methanol is omitted from the reagent. It was found possible to prepare a modified reagent (without methanol) by which the water absorbed approached the two moles required by equation 1



The general use of such a reagent, however, appears undesirable for the following reasons. First, reaction 3b is not specific for water as is reaction 2 but also takes place with alcohols (reaction 3a) and presumably with other compounds containing active hydrogen. Second, reaction 3b may be used only indirectly since it is not accompanied by a color change. (The indicator action of reaction 2 may be utilized in certain cases to follow the course of 3b.) Third, the water equivalence of the reagent varies greatly with minor differences of technique. Fourth, in the absence of methanol the reagent soon discolors.

By reducing the ratios of sulfur dioxide and pyridine to iodine, until in each case the iodine color no longer could be discharged on titration with a standard water solution, the stoichiometric ratios were found to be  $\text{I}_2:\text{SO}_2:3\text{C}_5\text{H}_5\text{N}$ , in support of equation 2. Further it was found that the presence of as much as one mole of methanol per mole of iodine would completely inhibit absorption of the second mole of water according to equation 3b, in support of equation 3a. In addition to the compound , all of the other products of reactions 2, 3a and 3b have been isolated and identified by comparison with the synthetic materials.

The literature gives little indication of the chemical nature of solutions of iodine and sulfur dioxide in pyridine. Since according to Mellor<sup>4</sup> iodine and sulfur dioxide do not combine appreciably to form sulfuryl iodide



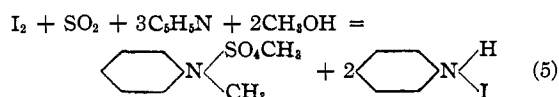
(3) This substance is really the "inner salt" of pyridinium hydroxide-N-sulfonic acid. It has been prepared previously by other methods. Baumgarten, *Ber.*, **59**, 1166 (1926); **64**, 1502 (1931); **64**, 1582 (1931).

(4) Mellor, "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Longmans, Green and Co., Ltd., London, 1930, Vol. X, p. 676.

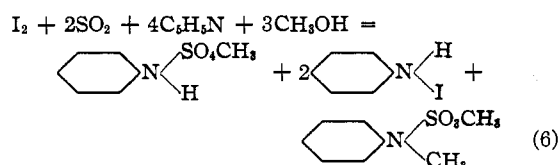
this iodide as such is not regarded as a likely constituent of the reagent although it may possibly be involved in the mechanism.

Binary combinations of pyridine with both iodine<sup>5,6</sup> and sulfur dioxide<sup>2,7</sup> are known, although accounts of their composition are conflicting. The existence of the binary compounds  $\text{C}_5\text{H}_5\text{NI}_2$  and  $\text{C}_5\text{H}_5\text{NSO}_2$  was verified in this Laboratory and some of their physical constants observed. The composition of the sulfur dioxide compound was established by melting point studies, and the formula  $2(\text{C}_5\text{H}_5\text{N})_2\text{SO}_2$  postulated by Karl Fischer shown to be incorrect. It is believed that the iodine and sulfur dioxide in Fischer reagent are present largely as the 1:1 compounds with pyridine. No ternary compounds have as yet been isolated.

While equations 2 and 3a fix the maximum absorption of water by the regular reagent at one mole per mole of iodine, this theoretical efficiency is rarely attained because of side reactions. Freshly prepared reagent is usually equivalent to 70% of the theoretical water but in the course of a month or two its strength falls to about 40%. The side reactions have been found to involve the reduction of iodine to the iodide ion and the formation of quaternary methylpyridinium salts, probably as follows



or



Contrary to earlier assumptions on this subject,<sup>8</sup> no evidence of the iodination of pyridine has been observed, since practically all of the iodine can be recovered from the spent reagent as iodide. The reagent is so readily standardized empirically that the lack of stability is only a minor consideration in connection with its analytical use.

Substitution of other bases for pyridine in the preparation of Fischer reagent proved feasible although not advantageous. For example, quino-

(5) Carlsohn, "Über eine neue Klasse von Verbindungen des positiv einwertigen Iods," S. Hirzel, Leipzig, 1932, p. 6.

(6) M. Chatelet, *Ann. chim.*, [2] **2**, 5 (1934).

(7) André, *Compt. rend.*, **130**, 1714 (1900).

(8) Ref. 2, p. 395; ref. 5, p. 45.

line, aniline, dimethylaniline, tri-*n*-butylamine and triethanolamine can be used although not with equal success. Close to four moles of tri-*n*-butylamine instead of three are necessary. The stability of the reagents prepared with aniline, dimethylaniline and triethanolamine was very poor. All factors considered, pyridine was the most satisfactory base employed.

The principal disadvantage of most organic solvents when incorporated in the reagent is their failure to dissolve the amine salts produced by the action of water. Where salts are thrown out of solution, the end-point is obscured to a serious degree. Only a few solvents were found free from this objection. Methanol, dimethylformamide and pyridine are satisfactory. Ethanol is fairly good but shows some tendency to deposit pyridine hydriodide crystals.

Fischer has discussed applications of his analytical technique in the determination of water in liquids and solids, both organic and inorganic, including benzene, liquid sulfur dioxide, methanol, acetone, petroleum fractions, fuller's earth, hydrated copper sulfate and calcium carbonate.<sup>9</sup> As a result of three years' laboratory experience with the method, the present writers recommend it for the determination of water in the following classes or organic compounds: alcohols, hydrocarbons, including saturated, ethylenic and aromatic types, carboxylic acids (except formic acid) and esters. Some weak tertiary amines can be titrated directly, but a better and more general procedure for amines involves the solution of the amine in an excess of glacial acetic acid of known water content, before titration. On titration with the regular reagent, the behavior of aldehydes and ketones is unsatisfactory due to the formation of acetals and ketals with the large excess of methanol in the reagent and the attendant liberation of water. Rapidly fading end-points and erroneously high apparent water contents are characteristic.<sup>10</sup> By greatly reducing the methanol content and increasing the pyridine in regular Fischer reagent, a modified reagent is obtained which reacts normally in the presence of ketones provided large amounts of the lower alcohols are absent. The writers have confirmed Fischer's surprising observation that the iodine in the reagent shows practically no tendency to react with ethylenic double bonds.

(9) Kaufmann and Funke [*Fette und Seifen*, **44**, 345 (1937)] describe the use of the reagent in determining water in fats.

(10) Fischer's apparent success with acetone is difficult to explain.

## Experimental

**Preparation of the Reagent.**—One-liter quantities of Karl Fischer reagent are prepared by dissolving 84.7 g. (0.33 mole) of Mallinckrodt resublimed U. S. P. iodine in a mixture of 269 ml. (3.3 moles) of pyridine and 667 ml. of du Pont synthetic methanol. The above solution is cooled in a slurry of chopped ice and 64 g. (1 mole) of liquid sulfur dioxide cautiously added. The final dark brown solution is about twice as strong as Fischer employed but is essentially the same in other respects. The water content of the solvents used should not exceed 0.1% since the presence of as much as 6 g. of water per liter will completely exhaust the reagent. (The color of the spent reagent is chromate yellow.) The solution deteriorates rapidly during the first week but more slowly thereafter.<sup>11</sup> The special reagent for use in the presence of ketones is prepared as above but using 860 ml. of pyridine and 90 ml. of methanol per liter instead of the quantities originally specified. A suction-filling reservoir buret<sup>12</sup> protected by calcium chloride towers is the most satisfactory dispensing device.

**Standardization of the Reagent.**—A supply of dry methanol (<0.1% water) is reserved for the preparation of a standard water solution. Several 10-ml. samples of it are titrated with the unstandardized reagent and the average titer recorded. About 18 ml. of distilled water is weighed carefully into a 1-liter volumetric flask, made up with the methanol and adjusted to volume after standing in a thermostat at  $25 \pm 1^\circ$ . Ten-ml. samples of this solution are titrated with the reagent until the brown color of iodine persists. The average titer is recorded. It can be assumed with sufficient precision that this titer corresponds to the aliquot of the water weighed in, plus 98% of the titer of the original methanol. Deduction of the titer due to water originally present with the methanol gives the titer due solely to the added water, which constitutes a standardization of the Fischer reagent. Reference to the uncorrected titer of the water solution now permits a calculation of the water in the final methanol solution. The latter solution is preserved as a standard for daily evaluation of the reagent.

**Establishment of the Stoichiometric Composition.**—Since the brown color of Fischer reagent evidently is due to the presence of free iodine, its disappearance on the addition of water affords a means of verifying the stoichiometric ratios of the individual constituents of the reagent. Starting with aliquots of a dimethylformamide solution each containing 8.5 g. of iodine (33 mmol.), the relative amounts of the other constituents, sulfur dioxide and pyridine were decreased individually until titration with a molar solution of water in dimethylformamide just failed to discharge the brown color of the solution. The minimum ratios were:  $I_2:SO_2:C_5H_5N = 1:1:3$ . Fischer's equation corresponds to the ratios,  $1:1:4$ <sup>13</sup> and is based on the assumption that pyridine forms a "neutral" sulfate,

(11) Little if any loss of strength occurs before the addition of sulfur dioxide, hence it is convenient to store the solution in this form and defer adding sulfur dioxide until a day or two before use. The reagent is conveniently prepared in 3-liter quantities.

(12) Item No. 750, supplied by Eck and Krebs, 131 West 24th St., New York, N. Y.

(13) The regular reagent contains a considerable excess of sulfur dioxide and pyridine, corresponding to ratios of 1:3:10.

TABLE I  
OPTICAL PROPERTIES AND MELTING POINTS OF CRYSTALLINE COMPOUNDS ISOLATED

Formula	M. p., °C. <sup>a</sup>	Optic axial angle <sup>b</sup>	Optical character	Dispersion	Miscellaneous observations
C <sub>5</sub> H <sub>5</sub> N·I <sub>2</sub>	62	2H <sub>a</sub> = 59 ± 1°; 2E = 96.5 ± 1°	+	r > v, moderate; distinct horizontal dispersion	Orange plates elongated    α
C <sub>5</sub> H <sub>5</sub> N·HI	231 (slow dec. at 195°)	Uniaxial	-		Compact colorless or pale yellow crystals
C <sub>5</sub> H <sub>5</sub> N·HI·I	193-195	Uniaxial	+		ω & ε > 1.75; pleochroism yellow    ω; red-brown to black    ε
C <sub>5</sub> H <sub>5</sub> N·H <sub>2</sub> SO <sub>4</sub>	101-105	2H <sub>a</sub> = 51 ± 1°	-	r > v, moderate	α = 1.430° ± 0.005; β = 1.615 ± .005
C <sub>5</sub> H <sub>5</sub> N·SO <sub>3</sub>		2H <sub>0</sub> = 135 ± 10°	-		α = 1.470 ± .003; β = 1.690 ± .003
C <sub>5</sub> H <sub>5</sub> N·HSO <sub>4</sub> ·CH <sub>3</sub>	112-117	2H <sub>a</sub> = 24 ± 1°; 2H <sub>0</sub> = 135 ± 5°		r > v, moderate	Needles and plates elongated    β

<sup>a</sup> Corrected melting points by Kofler-Hilbeck micro heating stage. <sup>b</sup> For details regarding this constant see Bryant, THIS JOURNAL, 60, 1394 (1938). <sup>c</sup> All refractive indices referred to 5461 Å. Hg light at 25 ± 3°.

(C<sub>5</sub>H<sub>5</sub>N)<sub>2</sub>H<sub>2</sub>SO<sub>4</sub>. Repeated attempts to prepare a "neutral" sulfate of pyridine were unsuccessful. Evaporation of sulfuric acid with large excesses of pyridine invariably gave the acid salt, C<sub>5</sub>H<sub>5</sub>NH<sub>2</sub>SO<sub>4</sub>, confirming the smaller ratios found in the above experiments. Although the absence of methanol did not prevent the removal of the brown iodine color by water, an abrupt change in the actual amount of water absorbed occurred as the molal ratio of methanol to iodine was reduced below one, while above this ratio only minor differences were observed. In the absence of methanol the water absorbed per mole of iodine at the characteristic end-point varied from 1.1 moles, when water was added to the reagent, to 1.9 moles in the reverse process. In the presence of one mole or more of methanol, about 0.7 mole of water was absorbed regardless of the order of mixing. This is preliminary evidence that, (a) the combination of the reagent with water occurs in two stages, and (b) that methanol can replace water in one stage. The nature of the intermediate and final products will be considered in the following section.

**Isolation and Identification of Crystalline Phases.**—A mixture of iodine, sulfur dioxide and pyridine in the stoichiometric ratios was not homogeneous at room temperatures but contained some C<sub>5</sub>H<sub>5</sub>NI<sub>2</sub> as a solid phase. Even after the addition of sufficient excess pyridine to produce a homogeneous solution, crystalline solid phases were obtained on the addition of water. When water was added directly to this mixture much heat was evolved and a slightly yellowish crystalline phase was obtained consisting of pyridine hydriodide and pyridine hydrogen sulfate. When, however, the reagent mixture was poured slowly onto chopped ice and stirred to prevent local heating, fine buff-colored plate-like crystals were formed. This material was identified as pyridine-sulfur trioxide, and is undoubtedly the intermediate compound predicted in the molal composition studies. In agreement with Baumgarten,<sup>3</sup> the compound was difficultly soluble and stable for an hour or two in ice water. It was converted rapidly to pyridine hydrogen sulfate by hot water and to pyridine methyl sulfate by methanol at room tempera-

ture. The action of methanol is evidently much less at 0°, since the "inner salt" was obtained by ice treatment of strong reagent containing up to ten moles of methanol. Vacuum evaporation of partly "spent" reagent also yielded some pyridine-sulfur trioxide along with brown crystals of pyridine hydroperiodide, C<sub>5</sub>H<sub>5</sub>NHI·I.

Identification of the above crystalline materials was made by comparing the optical crystallographic properties with those of the known salts. This did not involve a complete optical study but simply observation or measurement of a sufficient number of optical properties to ensure identification (Table I).

Analytical data for the compounds included in Table I are as follows

Compound	Calculated, %	Found, %
C <sub>5</sub> H <sub>5</sub> N·I <sub>2</sub>	I, 76.3	I, 75.4, 75.7
C <sub>5</sub> H <sub>5</sub> N·HI	HI, 61.8; C <sub>5</sub> H <sub>5</sub> N, 38.2	HI, 62.1; C <sub>5</sub> H <sub>5</sub> N, 37.4
C <sub>5</sub> H <sub>5</sub> N·HI·I	I, 38.0	I, 38.5
C <sub>5</sub> H <sub>5</sub> N·H <sub>2</sub> SO <sub>4</sub>	H <sub>2</sub> SO <sub>4</sub> , 55.4; C <sub>5</sub> H <sub>5</sub> N, 44.6	H <sub>2</sub> SO <sub>4</sub> , 53.8; C <sub>5</sub> H <sub>5</sub> N, 44.5; H <sub>2</sub> O, 0.6
C <sub>5</sub> H <sub>5</sub> N·SO <sub>3</sub>	SO <sub>3</sub> /C <sub>5</sub> H <sub>5</sub> N, 1.000	SO <sub>3</sub> /C <sub>5</sub> H <sub>5</sub> N, 1.002
C <sub>5</sub> H <sub>5</sub> N·HSO <sub>4</sub> ·CH <sub>3</sub>	HSO <sub>4</sub> ·CH <sub>3</sub> , 58.7; C <sub>5</sub> H <sub>5</sub> N, 41.3	HSO <sub>4</sub> ·CH <sub>3</sub> , 57.8; C <sub>5</sub> H <sub>5</sub> N, 41.1

Analyses of C<sub>5</sub>H<sub>5</sub>NSO<sub>3</sub> indicated the presence of a considerable amount of "adsorbed" water. Consequently the observed SO<sub>3</sub>/C<sub>5</sub>H<sub>5</sub>N ratio is given instead of the weight composition.

**Chemical Nature of the Reagent.**—It was first believed that the active constituents of Fischer's reagent were present as a complex compound of pyridine with sulfonyl iodide analogous to acetylpyridinium chloride formed from pyridine and acetyl chloride. However, all evidence to this effect has so far been negative and the reagent is now believed to be a mixture of the two binary compounds C<sub>5</sub>H<sub>5</sub>NI<sub>2</sub> and C<sub>5</sub>H<sub>5</sub>NSO<sub>2</sub>. In addition to the failure to isolate a crystalline ter-

nary compound, no appreciable thermal change was observed to accompany the mixing of the two binaries, although appreciable heat was evolved during the formation of the individual compounds. The two binary compounds deserve some attention because of the great confusion in the literature regarding their composition and physical properties. Pyridine diiodide was obtained from a 20% solution of iodine in pyridine both by spontaneous evaporation of the solution on a micro slide and also by precipitation by the gradual addition of several volumes of water or hexane. The compound was obtained in glistening orange plates or scales of probable monoclinic symmetry. The crystals decomposed rapidly in air to form a deep brown derivative of greater iodine content, probably  $C_5H_5N \cdot I_4$ . The compound was more stable under water, hexane or Nujol but gradually darkened. The structure is probably  $\left[ \begin{array}{c} C_5H_5N \\ I \\ I \end{array} \right]^6$ , involving an atom of positive monovalent iodine. The identity of the above preparations with a crystalline phase isolated from concentrated Fischer reagent was readily established by measurement of the optic axial angle and observation of the characteristic horizontal dispersion. The compound of pyridine with sulfur dioxide has been formulated both as  $C_5H_5NSO_2^7$  and  $(C_5H_5N)_2 \cdot SO_2$ .<sup>2</sup> Observations of the melting points in the vicinity of these compositions disclosed a 1:1 compound melting sharply at  $-4.4$  to  $-4.8^\circ$  while the 2:1 mixture melting over the range  $-11$  to  $-50^\circ$  was obviously not homogeneous. André's 1:1 compound<sup>7</sup> was described as a solid at room temperature, hence was probably polymeric. A small amount of a similar material of unknown composition was obtained on adding about 1% of water to the 1:1 compound.

**Nature of Parasitic Side Reactions.**—The deterioration of Fischer's reagent on standing was investigated in an effort to discover the reactions responsible and, if possible, prevent their occurrence. Considerable light was thrown upon the side reactions, although no method of inhibiting their progress was found. Chemical analyses of the strong reagent immediately after preparation and after standing for one month disclosed the following facts: (1) essentially complete recovery of iodine as iodide ion regardless of standing time; (2) a pronounced disappearance of both pyridine and total acidity after standing. In freshly prepared reagent, treatment with water and distilla-

tion from excess sodium hydroxide permitted a recovery of 97% of the initial pyridine. After standing for one month, similar treatment accounted for only 71% of the pyridine. After the distillation residue became more concentrated, a small amount of distillate with a very sharp amine odor somewhat like piperidine was obtained. This material was alkaline, and reduced potassium permanganate solution rapidly but did not form a crystalline picrate. A considerable amount of brown tarry material remained in the flask. A sample of methylpyridinium iodide distilled with aqueous sodium hydroxide apparently produced the same volatile base, hence it is assumed the methylpyridinium ion was present in both samples. According to Meyer and Jacobson<sup>14</sup> N-alkyldihydropyridines are formed when alkylpyridinium salts are distilled with strong alkali. These are reducing substances. It is not certain whether reaction 5 or 6 is responsible for the formation of the methylpyridinium ion. Reaction 6 is supported by the fact that pyridine and diethyl sulfite on heating reacted sufficiently to give an alkyldihydropyridine on subsequent distillation with alkali.

**Substitution of Other Nitrogen Bases for Pyridine.**—Separate solutions were prepared by dissolving 42.3 g. of iodine in 50 ml. of methanol and 125 ml. of one of a group of dry nitrogen bases, then cautiously adding 25 ml. of liquid sulfur dioxide to the mixture cooled in a bath of methanol and "dry ice." The solutions were finally made up to 250 ml. with methanol. Each solution was used at intervals to titrate known quantities of water in methanol. The relative stability of the different mixtures was found in this way. The bases tried were quinoline, aniline, dimethylaniline, tri-*n*-butylamine and triethanolamine, with pyridine as a control. All of the above bases had some value as substitutes for pyridine but the reagent employing aniline decomposed almost completely in twenty-four hours. Dimethylaniline was somewhat better. Decomposition of the reagent during preparation was difficult to avoid in the case of triethanolamine. Quinoline and tri-*n*-butylamine closely paralleled pyridine. Other experiments, however, showed that more of the aliphatic amine was required on a molal basis than in the case of pyridine. All of the above amines were analyzed carefully for water content.

**Substitution of Other Solvents for Methanol.**—Samples of iodine weighing 8.5 g. were each dissolved in 25 ml. of pyridine and 5 ml. of liquid sulfur dioxide carefully added. Each sample was diluted with 50 ml. of one of the following solvents: benzene, dioxane, glacial acetic acid, ethanol, acetonitrile, dimethylformamide, pyridine or methanol. On using these samples to titrate a standard solution of water in methanol, precipitates obscuring the end-point

(14) Meyer and Jacobson, "Lehrbuch der organischen Chemie," Walter de Gruyter and Co., Berlin, 1920, Vol. 11, Part 3, p. 799.

were obtained with benzene and dioxane. Ethanol, glacial acetic acid and acetonitrile were better and only occasionally showed a tendency to form a precipitate. Methanol, dimethylformamide and excess pyridine all were free from this objectionable feature.

**Analytical Methods Used.**—In the absence of sulfur dioxide, analyses for strong acidity as hydriodic or sulfuric acid were obtained by titration with standard 0.5 *N* sodium hydroxide to a phenolphthalein end-point. The samples were then titrated back to the brom phenol blue end-point with 0.5 *N* hydrochloric acid to obtain a measure of pyridine. This titrimetric procedure was found by control experiments to be reproducible within  $\pm 0.2\%$  and to account for at least 99% of the pyridine and inorganic acid added. Sulfate was determined by Raschig's benzidine method<sup>15</sup> and iodide argentimetrically using fluorescein as an adsorption indicator,<sup>16</sup> in both cases removing most of the pyridine by making alkaline to phenolphthalein with sodium hydroxide and boiling for fifteen to twenty minutes before proceeding with the analysis. Free and "periodide" iodine was obtained by thiosulfate titration after acidification to a *pH* of 4. In the presence of sulfur dioxide, the sample was boiled for ten to fifteen minutes with a known excess of standard

(15) Treadwell-Hall, "Analytical Chemistry," 8th ed., John Wiley and Sons, Inc., New York, N. Y., 1935, Vol. II, p. 660.

(16) Kolthoff and Sandell, "Textbook of Quantitative Inorganic Analysis," The Macmillan Co., New York, N. Y., 1936, p. 542.

hydrochloric or sulfuric acid, depending on whether sulfate or iodide was being determined. This modified analytical scheme was applicable to spent Fischer reagent. The alkali and acid titrations of mixtures containing free or "periodide" iodine were made successfully after destroying the iodine with sodium thiosulfate. The presence of this salt and its reaction product, sodium tetrathionate, did not interfere with the double titration procedure for strong acids and pyridine. When analyzing for sulfate and iodide ions, hydrazine hydrate in neutral or slightly alkaline solution was used instead of sodium thiosulfate to reduce iodine.

### Summary

1. The stoichiometric relations and mechanism of the reaction of water with Karl Fischer reagent have been investigated.

2. Crystalline intermediates and products of the reaction have been identified by optical crystallographic methods.

3. The nature of a parasitic reaction affecting the stability of the reagent has been clarified.

4. The effect of substituting other amines for pyridine and other solvents for methanol has been investigated.

WILMINGTON, DELAWARE

RECEIVED JULY 11, 1939

[CONTRIBUTION FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

## The Electro-optical Effect in Certain Viruses

BY MAX A. LAUFFER

### Introduction

Kerr<sup>1</sup> was the first to observe that many substances become doubly refracting when placed in a strong electrical field, behaving like uniaxial crystals with optic axes in the direction of the field. As a result of the studies of Raman and Sirkar<sup>2</sup> and of Kitchin and Mueller,<sup>3</sup> it is now known that this effect in homogeneous liquids is due to the orientation of molecules. Colloidal solutions containing rod-like or plate-like particles, such as those of vanadium pentoxide, benzopurpurin, ferric oxide, and bentonite, also show the Kerr effect. It is thought that this is due to the orientation of the anisometric colloidal particles. However, the studies of Errera, Overbeek, and Sack<sup>4</sup> on vanadium pentoxide and benzopurpurin sols and those of Mueller<sup>5</sup> and of

Norton<sup>6</sup> on bentonite sols show that the phenomenon in colloids is rather complex and not as yet encompassed by a single simple theory.

Bawden and associates<sup>7</sup> first reported that the particles of tobacco mosaic virus can be oriented by an electric field, *i. e.*, that the material exhibits electrical double refraction. This double refraction was reported recently by the author<sup>8</sup> to be positive with respect to the direction of the electric field. However, more detailed studies have substantiated this observation only in part, for it has been found that under some circumstances the electrical double refraction shown by the viruses may be negative. It is the purpose of this communication to describe further studies on the electrical double refraction of tobacco mosaic virus, the closely related aucuba mosaic of tomato virus, and latent mosaic of potato virus.

(1) Kerr, *Phil. Mag.*, [4] **50**, 337, 446 (1875).

(2) Raman and Sirkar, *Nature*, **121**, 794 (1928).

(3) Kitchin and Mueller, *Phys. Rev.*, **32**, 979 (1928).

(4) Errera, Overbeek, and Sack, *J. chim. phys.*, **32**, 681 (1935).

(5) Mueller, *Phys. Rev.*, **55**, 508, 792 (1939).

(6) Norton, *ibid.*, **55**, 668 (1939).

(7) Bawden, Pirie, Bernal and Fankuchen, *Nature*, **138**, 1051 (1936).

(8) Lauffer and Stanley, *Chem. Rev.*, **24**, 303 (1939).