Colorimetric Determination of Ammonia and Cyanate

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This report concerns a new sensitive method for the determination of small concentrations of ammonia by means of the purple color formed with a pyridine-pyrazolone reagent. The colored system is extracted into carbon tetrachloride for measurement. Cyanate, following hydrolysis to ammonia, may be determined in the same way. The effects of the following variables were determined: acidity, use of chloramine-T, composition of the reagent, nature of the extractant, and likely interferences. Studies of precision are reported for both ammonia and cyanate. Since cyanide interferes with the method for cyanate, results are included for the simultaneous determination of cyanide and cyanate.

THE determination of ammonia has long been an important part of water analysis. All types of water supplies, effluents, and sewage have to be tested frequently for this constituent. As the ammonia concentration in water is generally less than 10 p.p.m., a colorimetric method is essential. In addition to water analysis, the determination of ammonia formed as a by-product of chemical processes or by some biochemical reaction is frequently desirable.

The oldest and still most widely employed method for the colorimetric determination has been the use of Nessler's reagent (*4, 8, 11*), originally reported in 1856. Unfortunately, this procedure is beset by several major difficulties. The reagent is not easily prepared, and, although stable, may show some variation from day to day. Many common organic materials, such as ethyl alcohol, formaldehyde, or acetone, will form precipitates or colors with the reagent (*2, 5*) and thus prevent determination of ammonia. In addition, several inorganic ions—for example, magnesium, iron, manganese, and sulfide—interfere by forming precipitates or clouding the sample (*6, 10*). These interferences frequently necessitate the separation of the ammonia by distillation before the color can be developed. Also, in order to achieve adequate sensitivity in measurement, long cells or Nessler tubes are required (*9*).

In this paper a new method for the determination of ammonia is proposed. The process employs a pyridine-pyrazolone reagent to develop a purple color in aqueous solution, which is then extracted into carbon tetrachloride. The color in the extract is so sensitive that 1-cm. cells may be used with almost any photometer to measure as little as 0.05 p.p.m. of ammonia nitrogen. The color formed is stable, few ions interfere, and no turbidity is encountered.

A condensed step-by-step set of directions for applying this reagent to sewage analysis has been published (*7*). The object of this paper is to summarize the developmental work upon which the operating directions are based. Studies of the reagents, experimental conditions, and interferences are described, and a procedure for the determination of cyanate with the same reagent is included. Studies of the reproducibility of both of these determinations are reported.

GENERAL EXPERIMENTAL WORK

Apparatus and Reagents. The general transmittance curves were measured with a General Electric recording spectrophotometer, while absorbance measurements were made with a Beckman Model B spectrophotometer using 1-cm. cells. The pH measurements were made with a Beckman Model M pH meter. Ion exchange columns were constructed from a piece of 22-mm. tubing constricted at one end, and were packed to a height of about 20 cm. with the exchange resin.

Solutions containing 1 to 3% of chloramine-T are used for the reaction with ammonia and the hydrolysis of cyanate. The aqueous pyrazolone is prepared by dissolving 0.63 gram of recrystallized 3-methyl-1-phenyl-5-pyrazolone in 250 ml. of hot water (75° C.) and allowing the solution to cool to room temperature. Five parts of this solution are added to one part of pyridine containing 0.1% of bis-(3-methyl-1-phenyl-5-pyrazolone) to form the pyridine-pyrazolone reagent. The bispyrazolone is prepared by refluxing 17.4 grams of 3-methyl-1-phenyl-5-pyrazolone with 25 grams of phenylhydrazine in 100 ml. of 95% ethyl alcohol. The insoluble product, which is the bispyrazolone, is filtered off at intervals of a few hours and washed with hot ethyl alcohol. The reaction should be continued for at least 24 hours. The pyridine-pyrazolone reagent should be mixed only shortly before use, and the solution of bispyrazolone in pyridine should be freshly prepared. C.P. carbon tetrachloride is needed for the extraction, and a sodium acetate–acetic acid buffer of pH 3.7 for the pH adjustment.

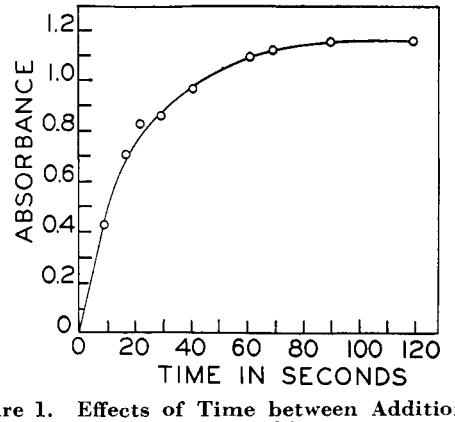

Figure 1. Effects of Time between Addition of Chloramine-T and Reagent

General Experimental Procedure. Preliminary experiments led to the adoption of the following procedure for subsequent checking of the effect of variable factors.

Adjust the pH of a 50-ml. sample to about 3.7 and then add 10 ml. of the buffer. Add 0.9 ml. of 3% chloramine-T and 90 seconds later add 30 ml. of the pyridine-pyrazolone reagent. After about 60 seconds for color development extract the solution with 25 or 50 ml. of carbon tetrachloride and measure the absorbance of this extract at 450 mμ. The preliminary treatment consists only of filtering off any suspended solids in the sample.

EFFECT OF VARIABLES

Sample Volume and Preliminary Treatment. As the color is measured in a carbon tetrachloride extract, the original sample volume is not too important as long as it is known exactly. In general, a 50-ml. sample was used, as this permitted the use of 125-ml. separatory funnels. If the sample volume was over 80 or under 30 ml., new calibration curves were needed.

The preliminary treatment consisted of filtering off any suspended solids from the sample and adjusting the pH to about 3.6 to 3.7. This pH adjustment was necessary with solutions which

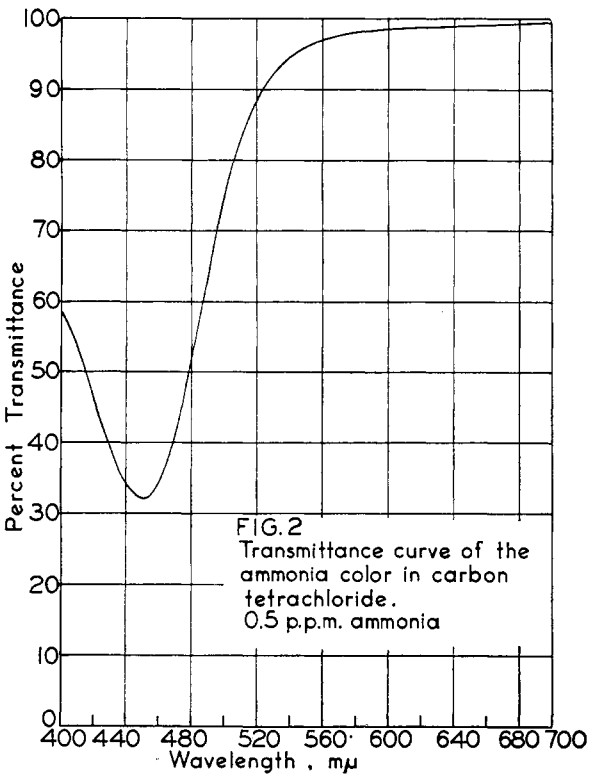

FIG. 2
Transmittance curve of the ammonia color in carbon tetrachloride.
0.5 p.p.m. ammonia

Table I. Dependence of Ammonia Color on pH

pH	A_s	pH	A_s
3.00	1.660	3.80	1.790
3.20	1.800	4.00	1.275
3.39	1.861	4.18	0.930
3.55	1.860	4.54	0.660
3.70	1.810		

All solutions contained 10 ml. of buffer and 3 p.p.m. of ammonia nitrogen. In every case 30 ml. of reagent was used.

were naturally buffered or whose pH differed considerably from the desired value of 3.7.

Effect of pH. The color development with the pyridine-pyrazolone reagent was found to be very dependent on the acidity of the sample. A study of the variation of absorbance with pH (Table I) showed that a pH between 3.2 and 3.8 was required for maximum color development. A pH of 3.7 was chosen for the procedure because adjustment to this value requires a minimum amount of acid. A sodium acetate–acetic acid buffer works very effectively at this pH.

Effect of Chloramine-T. An investigation of the effect of chloramine-T showed that the presence of this reagent was necessary, although its exact function is not understood. The chloramine-T does not oxidize ammonia to hydroxylamine, as the latter will not react with the pyridine-pyrazolone reagent. For a given ammonia concentration, the color intensity was found to increase with increasing amounts of chloramine-T up to a minimum of 23 mg. A further increase in the amount of chloramine-T did not affect the color intensity, although the addition of more than 35 mg. of this reagent resulted in some clouding of the sample. A fixed amount of 27 mg. has been advocated in the procedure, as this amount is well above minimum but does not cloud the sample. Since an excess over the minimum amount needed is used, some decrease in the reactivity of the reagent is not important. Consequently, a chloramine-T solution may be kept for 3 to 4 days.

Reagent Composition and Time of Addition. A study of reagent composition showed that the 1 to 5 ratio of pyridine to aqueous pyrazolone gave optimum color development, although variations in the ratio between 1 to 4 and 1 to 5 introduced no significant change. As the pyridine and the bispyrazolone are the most expensive reagents used in the procedure, the ratio using the least amount of these two constituents is advocated. Neither the concentration of the bispyrazolone in pyridine nor the concentration of the pyrazolone in water is very critical. The bispyrazolone concentration may vary between 0.06 and 0.1% in the pyridine. The upper value will result in the most stable solutions, but also it expresses the limit of solubility. The concentration of the aqueous pyrazolone may vary from about 0.2 to 0.3% with little effect on the color formation.

The time interval between the addition of the chloramine-T and the addition of the reagent was found to influence the final color (Figure 1). It is evident from this figure that a minimum interval of 75 seconds is necessary to obtain full color development. The chosen interval of 90 seconds represents an additional 15-second factor of safety.

Extraction. Liquid-liquid extraction is used to increase the sensitivity of the method for ammonia, to stabilize the color, to render the system independent of any color or turbidity in the sample, and to decrease any interferences by cyanide or thiocyanate. The range of ammonia concentrations that may be determined is increased by using different volumes of carbon tetrachloride. The extraction is so effective that a single 50-ml. portion of carbon tetrachloride is sufficient to remove all of the colored complex from the water layer except when more than 2 p.p.m. of ammonia are present. For more than 2 p.p.m. of ammonia, two 50-ml. extractions have to be used to withdraw all of the color and to bring the absorbance into a readable range. For very low concentrations of ammonia—i.e., below 0.1 p.p.m.—it is possible to double the sensitivity of the method by using a 25-ml. extract. All carbon tetrachloride extracts should be filtered through a cotton plug to remove water droplets.

A transmittance curve of the ammonia color in carbon tetrachloride (Figure 2) was recorded with a General Electric recording spectrophotometer, using a 1-cm. cell and a 10-mμ band width. The blank was carbon tetrachloride.

Beer's Law. Although the color in the extract does not follow Beer's law exactly, deviations are very small and a straight-line interpolation between any two adjacent points on the calibration curve is possible.

Interferences. There are essentially no serious interferences with the proposed procedure. Of the common anions, only cyanate and more than 0.2 p.p.m. of cyanide and thiocyanate interfere. These same ions also interfere with Nessler's method, but they can easily be removed with an anion exchange resin. Amberlite IRA-400 has been used with satisfactory results for this separation. Other anions such as chloride, sulfate, nitrate, and sulfide do not interfere.

A study of cation interferences is given in Table II. The only

Table II. Effect of Cations on Color of Ammonia with Pyridine-Pyrazolone Reagent

Cation	Effect of Cation	Permissible Concn. with 0.3 P.P.M. Ammonia[a], P.P.M.
Aluminum	Forms precipitate	100
Cadmium	None	1,000
Calcium	None	1,000
Chromium	None	1,000
Cobalt	Lowers absorbance	10
Copper	Lowers absorbance	1
Iron	Forms precipitate	10
Lead	None	1,000
Magnesium	None	1,000
Mercury(II)	Forms precipitate	500
Mercury(I)	None	Saturated Hg_2Cl_2
Nickel	Lowers absorbance	100
Potassium	None	10,000
Silver	Lowers absorbance	1
Sodium	Lowers absorbance slightly	1,000

[a] To give less than 2% error.

serious interferences are iron, zinc, silver, and copper. Because iron interferes by precipitating as the hydroxide filtration through the cotton plug alone will raise the permissible concentration to 25 p.p.m. For higher concentrations of iron, precipitation of the hydroxide and filtration before color development should be used. This same treatment will also remove the other cation interferences.

STUDY OF PRECISION

In order to gain an insight into the precision possible with the pyridine-pyrazolone method of determining ammonia, the procedure was tested with a series of sewage samples. In order to ensure the presence of some of the interferences, 0.1 p.p.m. of cyanide and 10 p.p.m. of iron were added to the sewage. The iron precipitated immediately and therefore was removed during the preliminary treatment along with other suspended matter. As the sewage originally contained about 2 p.p.m. of ammonia, this ammonia was removed with an ion exchange resin and known concentrations of ammonia were added as ammonium sulfate. A series of five determinations was made at each of ten different ammonia concentrations, and extraction with both 25 and 50 ml. of carbon tetrachloride was tested (Table III). All measurements were made at 450 mμ with a Beckman Model B spectrophotometer, using 1-cm. cells. The values in Table III are given in absorbance units as read from the instrument. In this way, no contribution due to plotting error is introduced into the standard deviation.

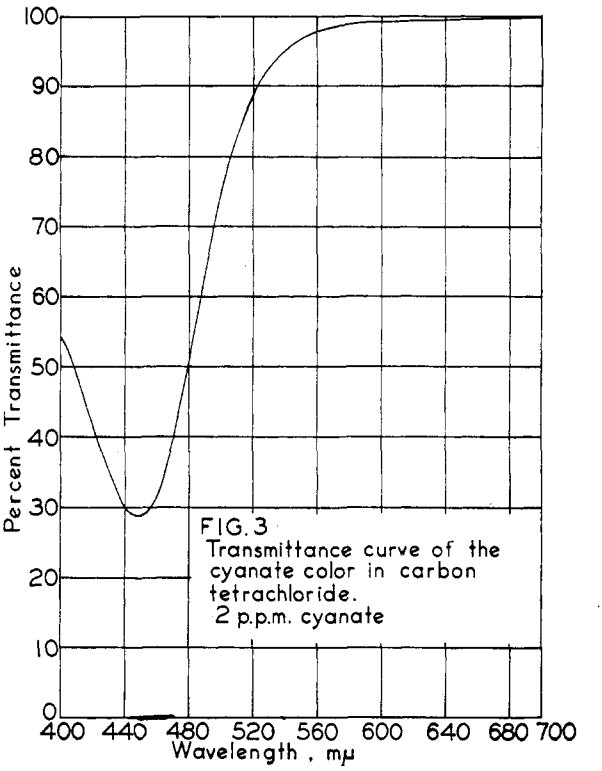

FIG. 3
Transmittance curve of the cyanate color in carbon tetrachloride.
2 p.p.m. cyanate

Table III. Determination of Ammonia with Pyridine-Pyrazolone Reagent

	NH₄, P.P.M.					
	0.025	0.05	0.10	0.25	0.50	1.00	2.50
Extraction with 50 ml. of carbon tetrachloride							
A_s calibration	..	0.023	0.046	0.138	0.276	0.622	1.471
	..	0.024	0.047	0.140	0.291	0.622	1.575
A_s samples	..	0.024	0.046	0.136	0.288	0.618	1.430
	..	0.023	0.046	0.135	0.281	0.615	1.404
	..	0.024	0.046	0.128	0.282	0.630	1.465
	..	0.022	0.045	0.133	0.292	0.608	1.500
	..	0.024	0.048	0.136	0.298	0.648	1.445
$\bar{X}$	..	0.0234	0.0462	0.1336	0.2888	0.6238	1.4488
R	..	0.002	0.003	0.008	0.017	0.040	0.096
σ^a	..	0.0008	0.0010	0.0062	0.0075	0.0141	0.0584
σ, %	..	3.42	2.20	4.45	2.65	2.28	3.88
Extraction with 25 ml. of carbon tetrachloride							
A_s calibration	0.023	0.043	0.088	0.244			
A_s samples	0.023	0.040	0.087	0.247			
	0.025	0.043	0.090	0.240			
	0.023	0.045	0.089	0.249			
	0.023	0.045	0.087	0.245			
	0.024	0.043	0.089	0.246			
$\bar{X}$	0.0236	0.0432	0.0884	0.2454			
R	0.002	0.005	0.013	0.009			
σ^a	0.0010	0.0018	0.0013	0.0033			
σ, %	4.35	4.28	1.45	1.35			

$^a\ \sigma = \sqrt{\frac{\Sigma(X - X\text{calib.})^2}{N}}$.

DETERMINATION OF CYANATE

Because cyanate is one of the main transformation products of cyanide, it frequently is desirable to determine the cyanate as a measure of this reaction. The determination of cyanate in plating wastes and other solutions is sometimes of importance in itself. Up to the present time any determination of cyanate has been limited by a lack of sensitivity. About the most sensitive method has been the use of a copper-pyridine reagent (*1, 12*), until Dodge and Zabban (*3*) recommended decomposition of cyanate to ammonia and determination of the latter with Nessler's reagent.

In order to apply the pyridine-pyrazolone reagent a new method was developed, which depends on the hydrolysis of cyanate to ammonia in acidic solution, and the subsequent determination of the ammonia. It is then possible to determine 0.1 to 10 p.p.m. of cyanate. The color development is identical to that described for ammonia, as cyanate is completely hydrolyzed to ammonia by the chloramine-T. This conversion is complete within the 90 seconds before the addition of the reagent, so that this period is still sufficient for full color development. The pH conditions also are identical with those described for the determination of ammonia. In fact, the only difference in factors is the use of a cation exchange resin prior to the color development to remove ammonia.

Stability of Cyanate. A study of the stability of cyanate in dilute solutions (0.1 to 10 p.p.m.) showed that this ion is stable only in alkaline media. In neutral solutions the ion decomposes slowly, while in acidic solution hydrolysis is rapid. Oxidizing agents, such as bromine, chlorine, or chloramine-T, catalyze this decomposition. Even in basic solutions the ion is not completely stable, so that a standard solution is usable for about 1 week.

Separation from Ammonia. The main problem in the determination of cyanate is the separation of this ion from ammonia. Because of the instability of cyanate, the sample cannot be treated by procedures such as boiling or aeration. The only procedure suitable for this separation is the use of an ion exchange resin to retain the ammonia and pass the cyanate.

Sulfonic acid resins could not be used directly for this purpose, as these strongly acidic resins decomposed cyanate. However, when these resins were converted to the sodium cycle with sodium chloride, no further decomposition was encountered. Some resins that were found satisfactory after treatment with sodium chloride are Amberlite IR 100 H, Amberlite IR 120, Nalcite HCR, and Dowex-50. It was found that the styrene resin Illco-211 could be used directly. The procedure for the determination of cyanate therefore includes the removal of am-

Table IV. Determination of Cyanate with Pyridine-Pyrazolone Reagent

	OCN, P.P.M.					
	0.20	0.40	1.00	2.00	5.00	10.0
Extraction with 50 ml. of carbon tetrachloride						
A_s calibration	0.034	0.085	0.162	0.380	1.010	1.958
A_s samples	0.040	0.090	0.160	0.381	1.046	1.971
	0.026	0.090	0.160	0.399	0.955	1.906
	0.046	0.082	0.180	0.378	0.988	1.963
	0.035	0.082	0.171	0.389	0.990	1.960
$\bar{X}$	0.0368	0.0860	0.1678	0.3868	0.9948	1.9500
R	0.020	0.008	0.020	0.021	0.091	0.065
σ^a	0.0078	0.0041	0.0102	0.0106	0.0361	0.0260
σ, %	23.0	4.85	6.26	2.80	3.57	1.33
Extraction with 25 ml. of carbon tetrachloride						
A_s calibration	0.041	0.103				
A_s samples	0.039	0.122				
	0.044	0.088				
	0.039	0.120				
	0.037	0.091				
$\bar{X}$	0.0398	0.1052				
R	0.007	0.034				
σ^a	0.0029	0.0159				
σ, %	7.00	15.4				

$^a\ \sigma = \sqrt{\frac{\Sigma(X - \bar{X})^2}{N}}$.

monia with one of these resins prior to color development. All cations except sodium are removed along with the ammonia, so that there are no cation interferences.

Interferences. The only anions interfering with the cyanate procedure are cyanide and thiocyanate, both of which form a very intense color with pyridine-pyrazolone reagent. Although the intensity of this color is greatly decreased by the use of the carbon tetrachloride extraction, no more than a total of 0.2 p.p.m. of either or both of these ions may be present.

Study of Precision. The precision obtainable in the determination of cyanate with the pyridine-pyrazolone reagent was studied (Table IV). Again, sets of five solutions were measured at different concentrations. The color was extracted with either 25 or 50 ml. of carbon tetrachloride. All measurements were made with a Beckman Model B spectrophotometer, using 1-cm. cells and a carbon tetrachloride blank.

DETERMINATION OF CYANIDE AND CYANATE

Because cyanide interferes with the determination of cyanate, the separation of these two ions was attempted. Unfortunately, no method of separation could be found, and consequently, these two ions had to be determined simultaneously. The use of carbon tetrachloride reduces the intensity of the cyanide color and enhances that of the cyanate color. This extraction was therefore used in the two-component analysis in order to make the determination of cyanate as accurate as possible.

Both the time of reagent addition and the time of extraction were found to influence the color development of solutions containing both cyanide and cyanate. From a study of the cyanate system, it was known that a 90-second interval between the addition of the chloramine-T and the reagent was satisfactory. This interval was also satisfactory for the simultaneous determination, as all cyanide is converted to cyanogen chloride in this time.

Effect of Time of Extraction. Although the interval between color development and the time of extraction is not important in the determination of cyanate alone, it is very important in the simultaneous determination of cyanate and cyanide. Even though cyanide forms a stable blue color after standing for 30 minutes, reproducible extraction of this color with carbon tetrachloride was found to be impossible. Further study showed that, as the time between the addition of the reagent and the extraction was increased, the relative absorbance of the color due to cyanide increased at wave lengths above 575 mμ, while the stability of the color in carbon tetrachloride and also the reproducibility decreased.

As the cyanate extract shows essentially no absorbance at wave lengths longer than 575 mμ (Figure 3), it is desirable to make the measurement of the cyanide component at a wave length greater than 575 mμ. Such a measurement would then give the cyanide concentration without any calculation and also greatly simplify the calculation of the amount of cyanate present. Some time interval which would be a compromise between reproducibility and intensity of the cyanide color was therefore necessary. As the cyanate color formed immediately, it did not have to be considered.

It was found that, as long as the interval between color development and extraction of the cyanide color was less than 2 minutes, the cyanide color was completely stable in carbon tetrachloride. At longer intervals of time, the reproducibility of the measurement became much poorer. A time of 150 seconds between color development and extraction was finally chosen because this was the maximum interval at which the color of the extract did not change and yet it was long enough to allow the cyanide component in the mixture to develop a noticeable absorbance at 575 to 600 mμ. As might be expected, the extraction should always be made within 150 ± 10 seconds after color development; greater variations in timing will result in considerable errors in the measurement of both cyanide and cyanate.

Effect of Cation Exchange Resin. Because ammonia is sometimes found along with cyanide and thiocyanate, the separation of ammonia from cyanide and cyanate prior to the determination of these two ions was necessary. As the separation of ammonia from cyanate had been found to be possible only with a cation exchange resin, the use of one of these resins was tried again. The same resins which had been used successfully for the separation of ammonia and cyanate decomposed cyanate when more than 0.3 p.p.m. of cyanide was present. All the resins previously mentioned were retested, but all decomposed cyanate. Nalcite HCR gave the least decomposition, but even this resin was useless for sewage analysis. Apparently, there is some interaction between cyanate, cyanide, and the resin which results in the decomposition of the cyanate. This study led to the conclusion that a simultaneous determination of cyanate and cyanide is impossible if ammonia is present. In the absence of ammonia, ion exchange resins need not be used and a simultaneous determination of cyanate and cyanide is possible.

Interaction between Cyanate and Cyanide. When solutions containing both cyanate and cyanide were tested with the pyridine-pyrazolone reagent, the cyanide component of the color in carbon tetrachloride showed major deviations from Beer's law. In fact, low and high concentrations of cyanide alone had different absorption curves in the extract. In the presence of cyanate this difference in curves for different cyanide concentrations is not very marked; for cyanide concentrations of less than 4 p.p.m., all curves very closely approximate the shape of the absorption curve of a 0.5 p.p.m. cyanide solution. A solution containing 0.5 p.p.m. of cyanide should therefore be used to determine the absorptivities of cyanide at 450 and 580 mμ (the two wave lengths used for measurement). The cyanate color is not affected by the presence of cyanide.

Proposed Procedure. The apparatus, reagents, and procedure used are identical with those described for the determination of ammonia, except that the following additional procedure had to be used:

After preparing the cyanate calibration curve at 450 mμ, prepare a calibration curve for 0.2 to 5 p.p.m. of cyanide by developing the color in 50 ml. of sample containing various cyanide concentrations. Then extract these samples with 50 ml. of carbon tetrachloride. Measure the absorbance of the extract at 580 mμ. From the extract of a sample containing 0.4 to 0.5 p.p.m. of cyanide, measure the ratio of absorptivities at 450 and 580 mμ. Then use this ratio for all concentrations of cyanide in mixtures

Table V. Simultaneous Determination of Cyanate and Cyanide

Present, P.P.M.		Found, P.P.M.			
		Trial 1		Trial 2	
OCN	CN	OCN	CN	OCN	CN
0.20	0.40	0.22	0.36	0.34	0.30
0.40	0.40	0.40	0.40	0.44	0.42
1.00	0.40	1.04	0.44	1.17	0.38
2.00	0.40	2.01	0.40	2.09	0.40
5.00	0.40	5.13	0.42	5.02	0.42
10.0	0.40	10.0	0.38	9.84	0.42
0.20	1.00	0.23	1.00	0.25	1.04
0.40	1.00	0.46	0.75	0.36	1.00
1.00	1.00	1.00	1.08	1.02	1.02
2.00	1.00	2.05	1.04	2.08	0.96
5.00	1.00	4.75	1.02	4.88	1.08
10.0	1.00	10.0	1.06	10.0	1.13
0.20	2.00	0.27	1.95	0.36	1.89
0.40	2.00	0.41	1.74	0.54	2.00
1.00	2.00	0.98	2.00	1.06	2.08
2.00	2.00	1.83	1.95	2.10	2.00
5.00	2.00	5.06	2.00	4.94	1.96
10.0	2.00	9.55	2.03	9.28	1.98
0.20	10.0	1.30	9.4	0.88	10.9
0.40	10.0	1.20	11.0	1.30	12.0
1.00	10.0	1.92	10.5	1.35	11.8
2.00	10.0	3.32	12.3	2.18	12.2
5.00	10.0	5.85	12.3	4.55	11.6
10.0	10.0	8.78	9.5	7.55	11.1

of cyanate and cyanide containing more than 0.2 p.p.m. of cyanide.

For the analysis of unknown samples, proceed as described for ammonia. Always make the extraction with 50 ml. of carbon tetrachloride 150 seconds after the addition of the reagent. Measure the absorbance of the extract at 450 and 580 mμ, and subtract the absorbance of the reagent blank. From the absorbance at 580 mμ, determine the concentration of cyanide and calculate the absorbance at 450 mμ due to cyanide by using the ratio of absorptivities found for the 0.5 p.p.m. cyanide solution. Subtract this absorbance from the total absorbance at 450 mμ; the residual absorbance is due to cyanate. From the cyanate calibration curve it is then possible to obtain the amount of cyanate present.

The above procedure and method of calculation were used for various mixtures of cyanate and cyanide (Table V). The method was found to be rapid, so that a set of nine solutions could be tested in 45 minutes.

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CALCIUM pantothenate is a member of the vitamin B complex vitamins and is used extensively as a constituent of multiple vitamin formulations and a variety of animal food supplements. Therefore, a rapid and accurate chemical method for its determination is highly desirable. Up to the present, control of this constituent in these mixtures has been based on microbiological assays (*2*, *7*). Chemical methods using 1,2-naphthaquinone-4-sodium sulfonate (*1*) and 3,4-dinitrophenylhydrazine (*5*) have been described for the determination of β-alanine, a hydrolytic cleavage product of pantothenic acid. A colorimetric method (*8*) has been reported based on Feigl's (*3*) reaction for esters and lactones after hydrolysis of pantothenic acid or panthenol to pantoyl lactone.

PRINCIPLE OF METHOD

Hydrolytic cleavage of pantothenic acid in acid medium results in the formation of β-alanine and α,γ-dihydroxy-β,β-dimethylbutyric acid. In an acid medium at a pH below 5, the dihydroxy acid undergoes lactonization to form α-hydroxy-β,β-dimethylbutyrolactone (*4*). Hydrolysis in alkaline solution will result in the formation of α,γ-dihydroxy-β,β-dimethylbutyric acid. The dihydroxy acid or the lactone produced by the hydrolysis reacts with 2,7-naphthalenediol in concentrated sulfuric acid to form a greenish yellow colored complex. The ratio between the amount of the colored complex formed and the hydrolyzed pantothenic acid is constant and can be estimated in dilute sulfuric acid spectrophotometrically at 465 mμ. Beer's law is followed over a suitable concentration range, with either acid or alkaline hydrolysis.

The specificity of this reaction with respect to other vitamins, organic and amino acids, and certain diluents usually encountered in vitamin mixtures has been studied. The presence of relatively large amounts of such compounds as thiamine, pyridoxine, choline, niacin, niacinamide, vitamins A and D, vitamin B_{12}, α-tocopherol, citric and tartaric acids, and β-alanine do not interfere with the pantothenate estimation. Such compounds as riboflavin, ascorbic acid, lactose, glucose, gluconic acid, glycolic acid, and furfural, however, do interfere with the reaction. Means of removing these interfering substances are given.

REAGENTS

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

Sulfuric acid.

Cupric sulfate, anhydrous.

Calcium hydroxide.

Diluted sulfuric acid. Mix 100 ml. of distilled water with 100 ml. of concentrated sulfuric acid. Cool in ice bath.

Naphthalenediol reagent. Dissolve 500 mg. of 2,7-naphthalenediol (Eastman) in 500 ml. of concentrated sulfuric acid. Allow this solution to stand until practically colorless (about 18 to 24 hours). Keep protected from light and prepare fresh weekly.

Acetic acid–pyridine solution. This is a mixture prepared with distilled water containing 20% of pyridine and 2% of acetic acid (v./v.).

Florisil. To a 500-ml. beaker add 100 to 200 grams of 60- to