COLORIMETRIC DETERMINATION OF COPPER WITH 1-(2-PYRIDYLAZO)-2-NAPHTHOL, PAN

by

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A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1958

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Date thesis is presented Aug 9, 1957

Typed by Dolores F. Kowcun

ACKNOWLEDGMENT

The author would like to express his gratitude to Dr. Max B. Williams for his helpful suggestions and aid in the research and organization of this thesis. Also to Dr. J. P. Mehlig for his cooperation and advice in the application of the colorimetric method developed.

The author also wishes to express his thanks to the graduate students of this Department for their constructive criticism during the preparation of this thesis.

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INTRODUCTION

The analysis of trace amounts of copper is performed on a variety of substances such as plant tissue, oils, blood, milk, paper pulp, and many other materials of biological nature. An extensive survey was recently made by Bochard and Butler (4, pp. 414-419) of the methods available for the determination of trace amounts of copper in biological materials. The survey compares a number of colorimetric methods found in the literature as applied to the analysis of paper pulp samples. The methods which worked best for this type of sample within a concentration range of 0.1-40 ppm copper, were methods which required the extraction of a copper complex into a suitable organic solvent in order to obtain the specificity and sensitivity of the coloring agent necessary for this concentration range. A study by Cheng and Bray (6, pp. 782-785) and a more recent study by Pease (13) of the dye 1-(2-pyridylazo)-2-naphthol, PAN, as a possible analytical reagent indicated the possibility of using the dye as a sensitive coloring agent for a colorimetric copper method. The latter study suggests the use of the dye in an aqueous miscible organic solvent system. A sensitive dye which can be used in an aqueous system eliminates a tedious and often complicated extraction procedure.

PREVIOUS WORK ON PAN

The synthesis of the dye 1-(2-pyridylazo)-2-naphthol, PAN, was first reported in 1915 and 1918 by Tschitschibabin (14, pp. 513-516 and 15, p. 1582). Lui (10) found that many heavy metals formed chelates with the dye. Cheng and Bray (6, pp. 782-785) investigated the use of PAN as an analytical reagent. They applied it as an indicator in the complexometric titration of zinc, copper, and cadmium with ethylenediaminetetraacetic acid. The latter study included an investigation of the reaction of a number of metal ions with PAN and the color stability relative to pH for a number of the chelates. They found that the colored PAN chelates of copper II, zinc II, cobalt III, and nickel II, when extracted into isoamyl alcohol, followed Beer's law. A study of the effect of various masking agents on 16 different PAN chelates (6, p. 784) was also included. The complexometric titration procedure was extended to the determination of indium by Cheng (5, pp. 1582-1583) and for scandium by Cheng and Williams (7, p. 96). The latter investigation included a procedure for the back titration of excess EDTA with a standard copper solution (10, pp. 96-97). The back titration technique, using PAN as the indicator, was applied by Flaschka and Abdine for the determination of iron, copper, nickel, cobalt, zinc, cadmium, lead, gallium, calcium, magnesium, manganese, mercury, and vanadium (8, pp. 2-3 and 9, pp. 58-61). The properties of PAN and its copper, nickel, and palladium chelates were recently investigated by Pease (13). Cheng (5, p. 1583) reported the dye 1-(2-pyridylazo-

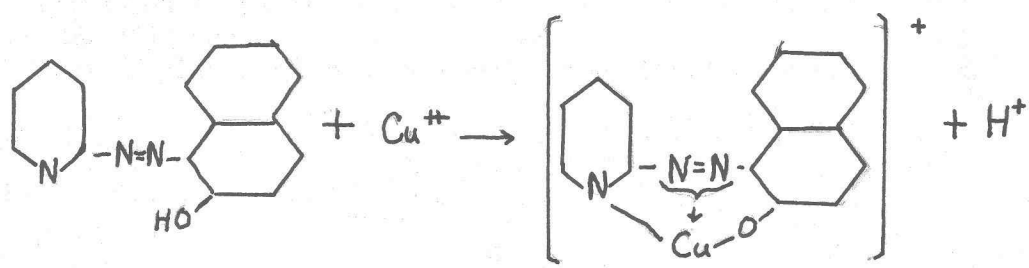
2-naphthol, to be an acid base indicator in aqueous media, being yellow in neutral solution and red in the strongly basic region. A study of the absorption spectrum of PAN (Figure 1) by Pease shows that actually three forms of the dye exist; a yellow green acid form, a yellow neutral form, and a red basic form. The equilibrium reactions of the three forms are expressed by the following equations (13, p. 34):

$$\underset{H_2KE^+}{\text{yellow green}} \rightleftharpoons \underset{HKE + H^+}{\text{yellow}}$$

$$\underset{HKE}{\text{yellow}} \rightleftharpoons \underset{KE^- + H^+}{\text{red}}$$

The dissociation constants for the above reactions were reported to be 1.26×10^{-2} and 6.3×10^{-13} respectively (13, p. 50). These constants were determined in 20% dioxane solutions due to the insolubility of the dye in water above a pH of 2. It may be noted that the value of Ka_2 is only approximate because of the instability of the basic form of the dye in 20% dioxane solutions.

The reaction of copper II with PAN is postulated as follows (6, p. 782 and 10, p. 18):



$$HKE + Cu^{++} \rightleftharpoons CuKE^+ + H^+$$

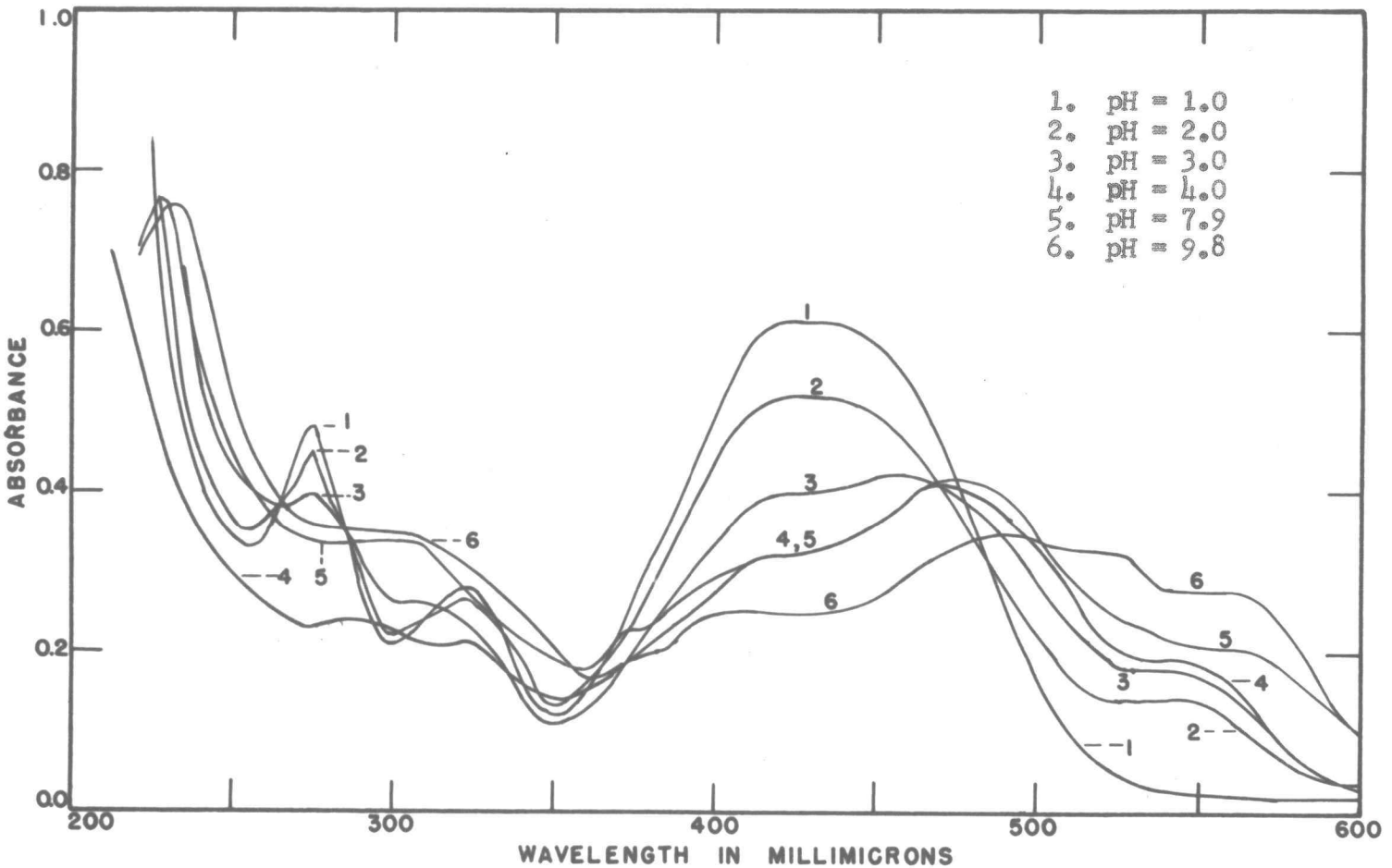


Figure 1. (13, p. 27) Absorption Spectra of PAN in Water

The equilibrium constant for the reaction was found to be 6.4×10^3 (13, p. 75). Pease verified the formation of a 1 to 1 Cu - PAN chelate in aqueous and 20% dioxane solutions at all pH values by Job's method of continuous variation and by spectrophotometric titrations (13, pp. 52-72). Lui (10, p. 19) has also found that copper II forms a 1 to 1 chelate with the dye in 95% alcohol. The copper chelate has a strong absorbance at 550 mμ while that of the dye alone is very small as shown in Figure 2 (13, p. 52). The molar absorbancy index of the dye was found to be 2.4×10^4. It was also found that the color formed by the copper was stable for five days. No maximum time limit was determined. Because of the high molar absorbancy index and the stability of the copper chelate, Pease suggested the possibility of an excellent colorimetric copper determination using PAN as the coloring agent.

The purpose of this investigation is to develop a simple and sensitive colorimetric method for the determination of trace amounts of Cu, using the dye 1-(2-pyridylazo)-2-naphthol as a coloring agent. An investigation will be made of the optimum conditions for an analytical procedure such as concentration range, effect of pH, minimum dye concentration, and the effects of interferences. The method will be applied to some hay samples which have been analyzed by a proven colorimetric method. The two systems will also be compared on an artificial sample of a known copper concentration.

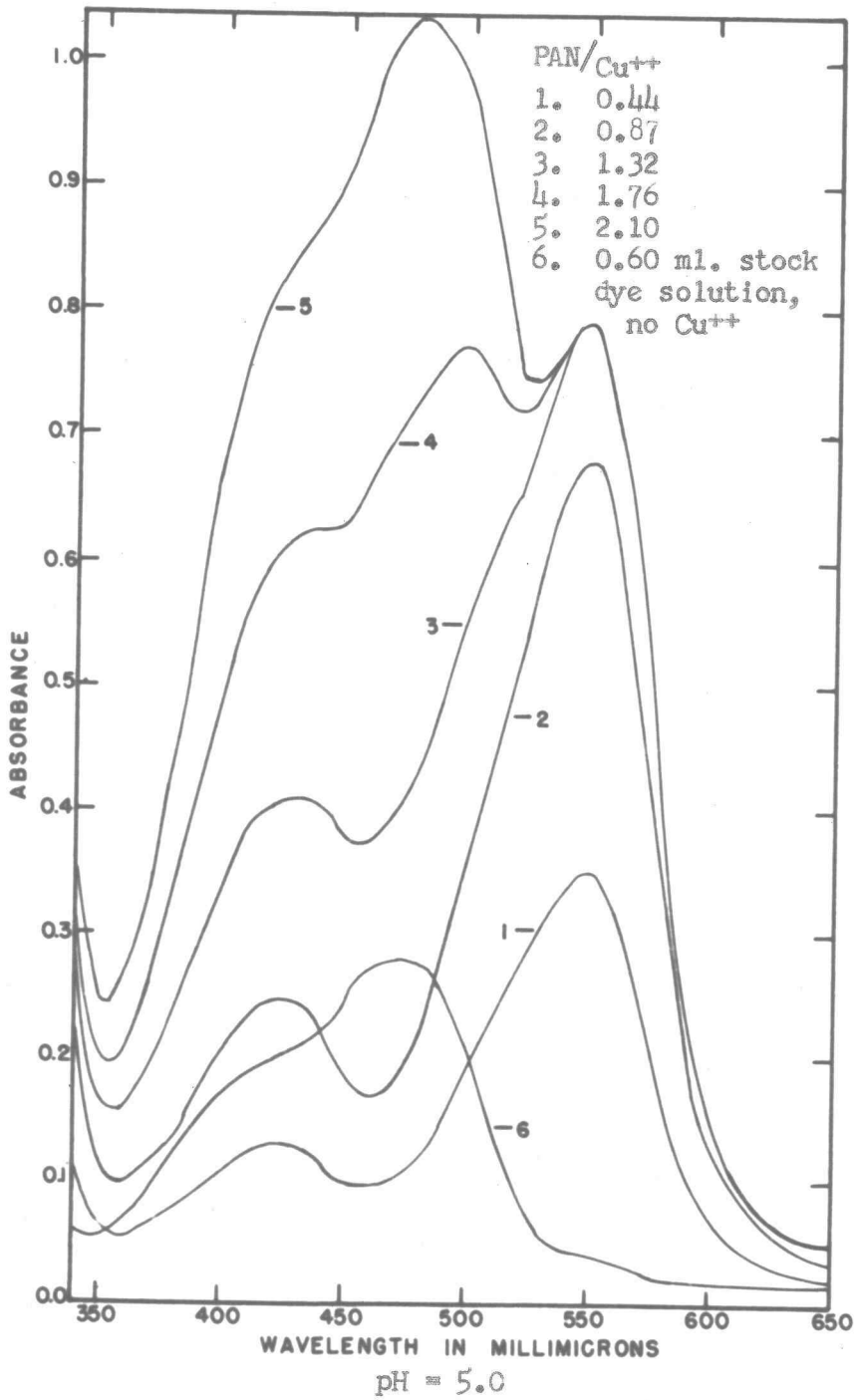


Figure 2. Absorption Spectra of Copper Chelate in 20% Dioxane (13, p. 53)

SPECTROPHOTOMETRIC PROCEDURE

Spectrophotometric methods of analysis are based upon the absorbance of light by solutions according to the equation $It = Io\ e^{-kd}$ or $\ln \frac{Io}{It} = kd$. This is known as Lambert-Bouques Law.

Io = the intensity of the transmitted light beam.
It = the intensity of the light beam after passing through an absorbing material for a distance d.
k = the proportionality constant characteristic of the absorbing solution.

The term absorbance refers to the $\ln \frac{Io}{It}$. The absorbance of a solution depends on the concentration of the absorbing material present. If the distance d is kept constant, it can be said that the absorbance is directly proportional to the concentration. This is known as Beer's Law. In order for an absorbing material to follow Beer's Law, monochromatic light must be used, and, in addition, the nature of the absorbing material must remain constant at different concentrations. In setting up an analytical procedure for a metal or some other component, a set of standard solutions of varying known amounts of the component is prepared. The absorbance is measured for each of the solutions at a characteristic wave length with a suitable instrument. A standardization curve is then prepared by plotting absorbance vs total concentration of the desired material. If the absorbance follows Beer's Law, a straight line will be obtained. Deviation from Beer's Law simply results in a curve which still can be used in much the same way as a

straight line plot. The concentration of an unknown is determined by measuring its absorbance and using the standard curve to find the concentration which corresponds to that absorbance. The concentration range of a standard curve is governed by the relative absorbance of the constituent in solution. If no interferences are present, the absorbance of the constituent will correspond to its concentration.

APPARATUS AND REAGENTS

Apparatus.

All absorbance measurements were made with a Beckman Model DU quartz spectrophotometer using a set of matched 1 cm pyrex cells. A Beckman Model H-2 pH meter with glass electrode and calomel cell was used to obtain all pH values. Ordinary good grade burets, pipets, and volumetric flasks were employed.

Standard metal solutions.

A standard copper solution was prepared by dissolving 1.0026 grams of chemically pure electrolytic copper in 5 ml of 1-3 nitric acid. The solution was boiled for 15 minutes to drive off the oxides of nitrogen, transferred to a one-liter volumetric flask and diluted to volume with distilled water. Other standard copper solutions of desired concentration were prepared from this solution.

Standard solutions of metals for the purpose of studying their interference effects were prepared by dissolving the following salts

in distilled water: $FeCl_3.6H_2O$, $Ni(NO_3)_2.6H_2O$, $Co(NO_3)_2.6H_2O$, $SnCl_4.5H_2O$, $PbNO_3$, $Zn(NO_3)_2.6H_2O$, $Cu(NO_3)_2.4H_2O$, $HgCl_2$, and $UO_2(NO_3)_2.6H_2O$. Bismuth nitrate ($Bi(NO_3)_3.5H_2O$) and stannous chloride ($SnCl_2.2H_2O$) are insoluble in water and were therefore dissolved in 1 M nitric acid and 12 M hydrochloric acid respectively. All salts used were reagent grade chemicals and were made up in 100 ml volumetric flasks at a concentration of 5×10^{-1} gram of metal ion per 100 ml of solution. Aliquots of these solutions were used for the preparation of desired concentrations of the metal ions.

Stock PAN solutions.

Stock PAN solutions were prepared for two procedures, one for a nitric acid dye system and one for a phosphoric acid dye system. The first dye solution was prepared by dissolving 0.1 gram of PAN, (92-95% pure) (13, p. 64) obtained from Baker Chemical Company lot number 4312, in 100 ml of redistilled methyl alcohol. This was added to a solution of 1.2 ml of concentrated (70%) nitric acid diluted to 200 ml with distilled water. The second stock dye solution was prepared by adding 100 ml of the same PAN-methyl alcohol solution to a solution containing 30 ml of 85% phosphoric acid and 68 ml of 5% sodium hydroxide, diluted to a total volume of 200 ml with distilled water. The sodium hydroxide was added to the second dye solution to adjust the pH.

Methyl alcohol.

The reagent grade methyl alcohol which is stored in metal drums was found to contain traces of impurities which show some absorbtion at a wave length of 550 mμ (13, p. 18). To remove impurities all methyl alcohol used was redistilled. The redistilled alcohol has no absorption peak at 550 mμ.

PRELIMINARY EXPERIMENTS

In order to establish an aqueous organic solvent system for PAN, a number of preliminary experiments were performed. Previous work by Pease (13, p. 22) concludes that redistilled methyl alcohol is a suitable solvent for PAN. This study also shows that the dye is not soluble in an aqueous system above a pH of 2 (13, p. 31). The author decided to try an aqueous methyl alcohol system adding enough nitric acid so that a 20 ml aliquot of the stock dye solution when diluted to 50 ml in volumetric flask would give a final pH of 1.60. Nitric acid was chosen because copper is usually dissolved in nitric acid and also the nitrate ion is not noted for complexing strongly with metal ions. A stock dye solution was then made up according to the procedure previously described with the exception that a less concentrated methyl alcohol dye solution was used. Later experiments showed that more dye was needed to insure an excess for the formation of the copper chelate at higher concentrations.

Another experiment was to determine whether or not the absorp-

tion spectrum for this system was the same as the one previously determined by Pease (13, p. 53) shown in Figure 2, for a 20% dioxane system. A Beckman Model DK-2 spectrophotometer was employed in this experiment. The following procedure was used. To a 50 ml volumetric flask, 20 ml of stock dye solution and an aliquot of copper solution were added. When diluted to volume, the solution contained 2 ppm copper with a ten fold excess of dye at a pH of 1.60. The absorption spectrum of the solution was found to be very similar to that shown in Figure 2 and will not be included in this manuscript.

A study of the effect of methyl alcohol on pH was considered to be important. Bates (3, p. 244) states that little difficulty is encountered in the use of the glass electrode for pH measurements in ethanol-water mixtures containing less than 30 weight percent of ethanol. The solutions to be measured in the proceeding experiments contain a maximum of 14% by volume of methyl alcohol. A series of three solutions were prepared by adding a constant amount of nitric acid and varying the concentration of methanol. The results are tabulated in Table 1. Methanol concentrations up to 40% by volume showed no change in pH. This indicates that no difficulty should be encountered in measuring the true pH of the 14% methanol solutions.

The effect of pH on absorbance over a pH range of 1-2 was determined. Two runs were made in this procedure, one using the stock dye solutinn made up as previously described and one using

TABLE 1

THE EFFECT OF METHYL ALCOHOL ON pH

ml HNO_3	ml Methanol	Total volume	% Methanol	pH
1.58	0	50 ml	0	1.52
1.58	10	50 ml	20%	1.52
1.58	20	50 ml	40%	1.52

a modified stock dye solution in order to study the effect on absorbance above a pH of 1.60. The modified dye solution was prepared by using 0.95 ml of concentrated nitric acid instead of the 1.2 ml in the usual 300 ml volume of stock dye. When a 20 ml aliquot of the modified dye was added to a 50 ml volumetric flask and diluted to volume, the pH was 1.78. Absorbance measurements were not taken at this pH because the dye slowly precipitated out. The same procedure was followed for both runs. To a series of 50 ml volumetric flasks an aliquot of standard copper solution, containing 4×10^{-5} gram copper, and 20 ml of dye solution were added. To each volumetric flask an increasing amount of 1 M nitric acid was added and the solutions diluted to volume. When absorbance measurements were taken within one-half hour after dilution, erratic results were obtained. After letting the solutions stand for several hours, more measurements were taken. The new readings were uniform. This is probably due to the copper dye reaction slowly shifting equilibrium upon addition of the nitric acid. The data for this experiment is tabulated in Table 2, and the combined results of the two runs are shown in Figure 3. Since the dye precipitated at a pH of 1.78 and a relatively small change in absorbance over the pH range of 1.5 to 1.7 was noted, the author decided that the best pH for the acid dye system would be 1.60.

The final preliminary step was to determine the rate at which equilibrium of the acid, dye, and copper system was reached. A 20 ml aliquot of stock dye was added to a 50 ml volumetric flask

TABLE 2

THE EFFECT OF pH ON ABSORBANCE

(Part 1 Modified Stock Dye Used)

ml of 1 M HNO_3	pH	Absorbance
0	1.78	ppt
0.25	1.72	0.255
0.50	1.63	0.247
0.75	1.57	0.244
1.00	1.50	0.233

(Part 2 Stock Dye Used)

ml of 1M HNO_3	pH	Absorbance
0	1.60	0.250
1	1.42	0.228
2	1.30	0.198
3	1.12	0.171
4	1.05	0.145

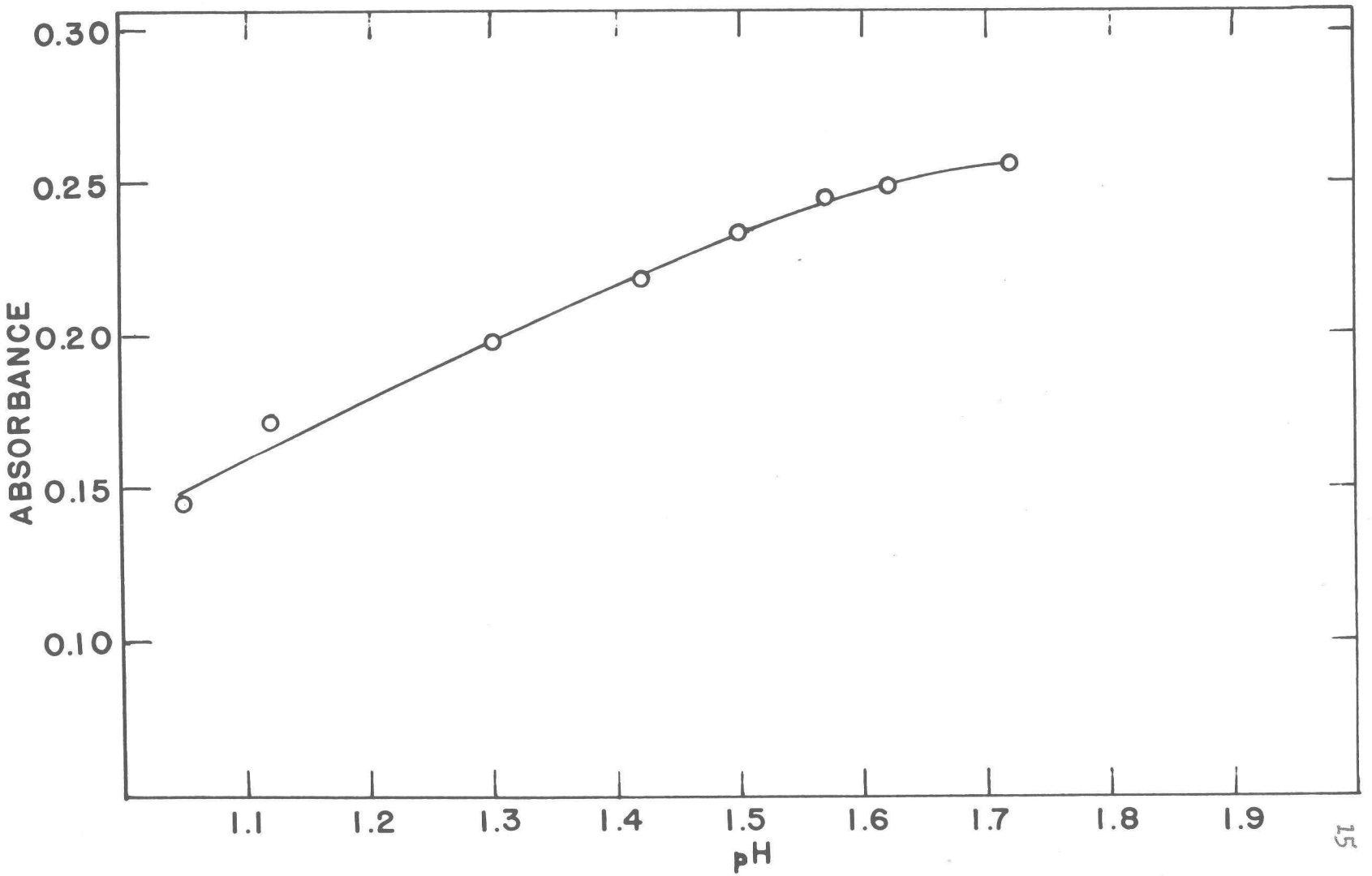


FIGURE 3. COPPER—PAN IN METHYL ALCOHOL AND NITRIC ACID

containing a solution of 4×10^{-5} gram copper, and the solution was diluted to volume. The absorbance was measured at three five-minute intervals and at a one-hour interval. The results tabulated in Table 3 show that equilibrium is established almost instantaneously. The stability of the copper-PAN chelate over long periods of time was determined by Pease (13, p. 59). Pease also made a study of the nickel-PAN equ librium (13, p. 80). In this case at least one half hour was necessary for equilibrium conditions.

Preparation of standard copper curve.

A standard curve was prepared as follows: suitable aliquot portions of a standard copper solution were added to a series of 50 ml volumetric flasks in which, when diluted to volume, the copper concentration ranged from 0.5 to 3 γ/ml. Then 20 ml aliquots of stock nitric acid dye solution were added to each flask and all were diluted to volume with distilled water. No adjustment of pH was necessary because the aliquots of standard copper solution were not acid enough to effect the pH. Absorbance measurements were made at 550 mμ. A typical standard curve is shown in Figure 4.

Determination of optimum concentration range.

A study of the optimum concentration range relative to absorbance to obtain the best accuracy was made by Ayers (1, pp. 652-657). Ayres pointed out that straight line plots such as

TABLE 3

VARIATION OF ABSORBANCE OF COPPER-PAN CHELATE WITH TIME

Time in minutes	Absorbance
5	0.245
10	0.245
15	0.245
60	0.248

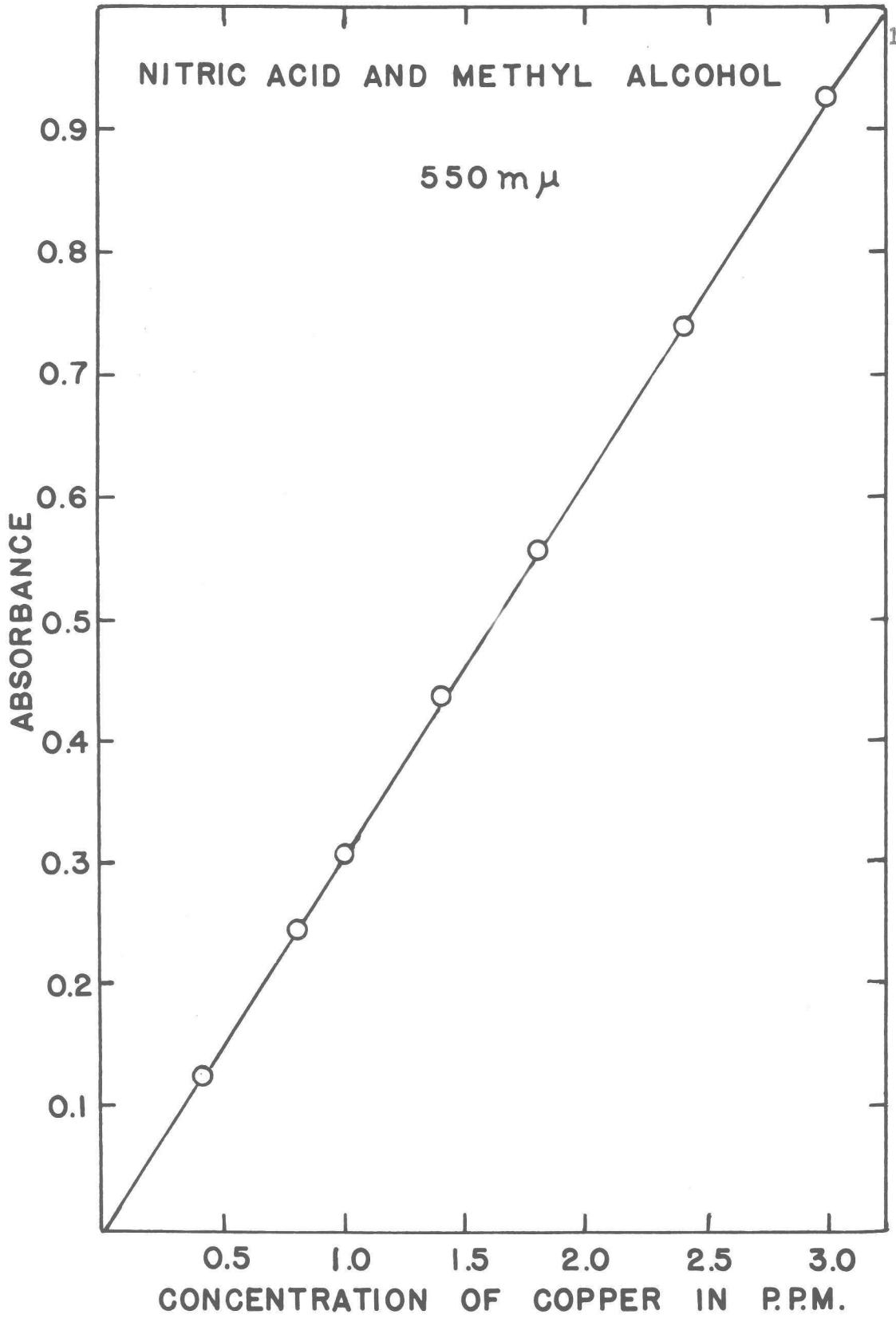


FIGURE 4. STANDARDIZATION OF COPPER–PAN

Figure 4 only show conformity to Beer's Law and do not show directly the concentration range for best accuracy. The proposed method for determining the optimum concentration range is to plot percent absorptancy (100 minus percent transmittancy) vs log concentration. An s curve is obtained and the linear portion corresponds to the optimum rangefor best accuracy. The absorbance readings obtained in preparation of the standard curve were converted to percent transmittancy and these values were subtracted from 100 to get percent absorptancy. The data is summarized in Table 4. A plot was then made of percent absorptancy vs log concentration. The plot, Figure 5, shows the optimum concentration to be from 0.6 to 2.5 ppm copper.

Interfering ions.

The effect on the absorbance of the copper-PAN chelate of a number of the common metal ions which were reported by Cheng and Bray (6, p. 782) as forming colored chelate with the dye PAN were investigated. Test tube checks were also made on several of the cations and anions reported as not reacting with the dye. No reactions occured for ions tested. The following ions were reported as not reacting with the dye: (6, p. 782) aluminum, calcium, magnesium, strontium, barium, antimony, chromium (VI), potassium, sodium, lithium, titanium, zirconium, mercury (I), germanium, tellurium, ruthenium, rhodium, iridium, cesium, beryllium, osmium, arsenate, arsenite, nitrite, nitrate, chloride,

TABLE 4

DATA OBTAINED USING AYRES METHOD FOR FINDING THE OPTIMUM CONCENTRATION RANGE IN A HNO_3-DYE SYSTEM

Copper concentration	A	% T	% Absorptancy
0.3 ppm	0.092	81.0	19
0.5 ppm	0.155	70.0	30
1.0 ppm	0.308	49.2	50.8
1.5 ppm	0.462	34.5	65.5
2.0 ppm	0.617	24.2	75.8
2.5 ppm	0.772	16.9	83.1
3.0 ppm	0.926	12.0	88.0

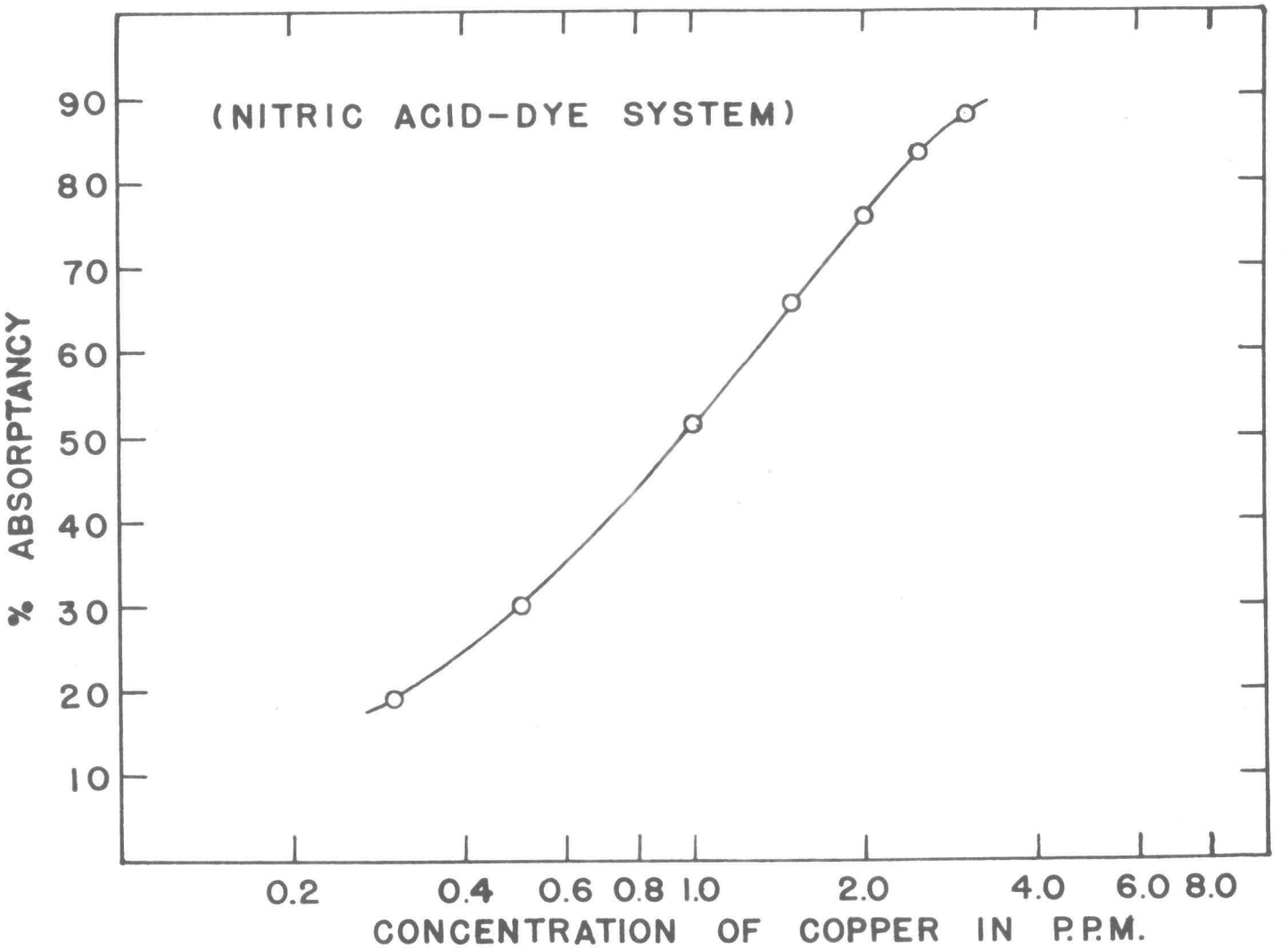


FIGURE 5. **OPTIMUM** CONCENTRATION RANGE

bromide, iodide, fluoride, sulfate, sulfite, perchlorate, phosphate, tungstate, molybdate, and bromate. The investigation was carried out as follows: a series of successive dilutions were made with the previously described metal ion solutions by adding 10 ml aliquots to 100 ml volumetric flasks, diluting to volume and repeating this step with the diluted solution until four dilutions were made. When 10 ml of each of these solutions were added to 50 ml volumetric flasks along with a suitable aliquot of standard copper solution and 20 ml of stock dye solution, the series contained 1000, 100, 10, 1 and 0.1 ppm interfering ion plus 1 ppm copper. The absorbances of these solutions were measured at 550 mμ. The results for Hg II, Pb II, Zn II, UO_2 II, Cd II, Co II, Ni II, Fe III, Bi II, Sn II, and Sn IV are summarized in Table 5. The most seriously interfering ions were found to be Ni II, Co II, and Fe III. Although all of the ions studied form chelates with the dye most of them do not interfere because the chelates are not stable at a pH of 1.6. Minute amounts of Fe III, Ni II, and Co II can be tolerated but higher concentrations precipitate with the dye. A modification of the system will be described for the determination of copper in the presence of higher concentrations of Fe III in the section on application of the colorimetric copper method. A number of attempts were made to mask the effect of Ni II and Co II but none of these were successful. A preliminary separation would be required to determine copper in the presence of 1 ppm or more of these ions using the copper-PAN method.

TABLE 5

EFFECT OF INTERFERING IONS

ppm Interfering ions -		0.1	1	10	100	1000
Interfering ion	Cu taken		PPm Cu found			
Hg^{++}	1.0	-	-	-	1.0	1.1
Pb^{++}	1.0	-	-	-	-	1.0
Zn^{++}	1.0	-	-	-	1.0	1.1
UO_2^{++}	1.0	-	1.0	1.1	1.2	ppt
Cd^{++}	1.0	-	-	-	-	1.0
Co^{++}	1.0	*1.1	ppt	∞	∞	∞
Ni^{++}	1.0	1.0	1.1	ppt	∞	∞
Fe^{+++}	1.0	1.1	1.3	ppt	∞	∞
Bi^{+++}	1.0	1.1	1.1	1.2	2	∞
Sn^{++}	1.0	-	-	1.0	.8	Bleached
Sn^{++++}	1.0	-	-	-	1.0	ppt

∞ extreme interference
\- no interference
* after standing for several hours, precipitation occured

Cheng and Bray (6, p. 784) postulated that in an acid water solution, the Co II ion is rapidly oxidized to the Co III ion, since the red Co II chelate formed in water solution turned green when acid was added. A Beer's Law curve prepared by Cheng and Bray (6, p. 784) for the Co III chelate in an aqueous solution at 640 mμ, suggests the possibility that a colorimetric method might be developed for cobalt in samples containing both copper and cobalt, provided the cobalt concentration is low enough to permit the determination of copper without interference.

APPLICATION

The author decided to try the colorimetric copper method on some plant tissue samples because of the availability of such samples at the Agricultural Experiment Station, Oregon State College, Corvallis, Oregon. Several hay samples which had been analyzed for traces of Fe, Cu, and Co were obtained from the Experiment Station. The procedure which they used to ash the samples and analyze for traces of the above elements was a modification of the procedure followed by Parks, R. Q., et al. (12, pp. 527-533) The procedure was modified by J. P. Mehlig of the Agricultral Experiment Station. The method used for the colorimetric determination of copper involved a dithizone extraction into carbon tetrachloride and the use of diethyldithiocarbamate as a coloring agent. (13, p. 529). A range of 100 - 500 ppm Fe, 0.03 - 0.3 ppm Co and 3 - 10 ppm Cu was found in the analysis of the hay samples determined at the

Experiment Station. Previous study of the interfering ions, Table 5, shows that the Co concentration is low enough to be tolerated, but the Fe concentration is too high and a modification of the colorimetric procedure must be made in order to analyze for copper in the hay samples. In a study of the use of masking agents (6, p. 784) Cheng and Bray reported the citrate and fluoride ions as effectively masking the Fe III-PAN chelate. Fluoride was ruled out because of the effect of acid fluoride solutions on glass. Test tube checks showed that 100 ppm Fe are not successfully masked by the presence of an excess of citrate although it worked for lower concentrations. At 100 ppm Fe, precipitation still occurred with the dye. Tartrate was also tried but the results were the same as those for the citrate ion. When an excess of phosphoric acid was added to a solution of 100 ppm Fe before adding the dye, no precipitation or color reaction occurred with the dye. An interference study similar to the one previously discussed was made on the effect of Fe III on the absorbance of the copper-PAN chelate in the presence of 10, 25, 50, and 100 ppm iron using phosphoric acid as the masking agent. The addition of phosphoric acid to the system lowered the pH of the solution, therefore, a pH adjustment was necessary. Aliquot portions of standard copper and iron solutions, plus 1 ml of 85% phosphoric acid were added to a 100 ml beaker. After a 20 ml aliquot of stock dye had been added, the pH was adjusted with a pH meter by adding 5% sodium

hydroxide solution slowly until the pH reached 1.60. The solutions were then carefully transfered to 50 ml volumetric flasks and diluted to volume. The pH was rechecked after dilution and no change was observed. The results of the masking effect of 1 and 2 ml of 85% phosphoric acid are tabulated in Table 6. The decrease in the absorbance of 1 ppm copper in the presence of the phosphoric acid is probably due to the copper ions weakly complexing with the phosphate ions present. The addition of more than 2 ml of phosphoric acid would decrease the effect of the iron to a greater extent, but it would also decrease the sensitivity of the copper PAN reaction. The error due to 100 ppm iron is relatively minute in the presence of 2 ml of phosphoric acid, therefore, the author decided this would be the best concentration of acid to use. A new stock dye solution was prepared as previously described, containing enough phosphoric acid so that a 20 ml aliquot of the dye contained 2 ml of acid and also enough 5% sodium hydroxide to bring the pH up to 1.60 when the aliquot is added to a 50 ml volumetric flask and diluted to volume. A new standard curve was prepared because of the reduced sensitivity of the phosphoric acid dye system. The same procedure was followed as that used in the preparation of the standard curve for the nitric acid dye system. The new standard curve is shown in Figure 6. The molar absorbancy index dropped from 19,650 in the nitric acid dye system to 17,440 for the phosphoric acid dye system.

TABLE 6

THE INTERFERENCE OF IRON WITH A

PHOSPHORIC ACID MASKING AGENT

Run #1 (1 ml 85% H_3PO_4 per 50 ml solution)

ppm Fe Added	Absorbance of 1 ppm Cu	Absorbance of 1 ppm Cu + Fe	Change in ppm Cu found
10	0.290	0.302	+ 0.05
25	0.290	0.309	+ 0.08
50	0.290	0.334	+ 0.18
100	0.290	0.358	+ 0.25
Run #2	(2 ml 85% H_3PO_4 per 50 ml solution)		
10	0.275	0.276	0.00
25	0.275	0.282	+ 0.03
50	0.275	0.293	+ 0.07
100	0.275	0.303	+ 0.10

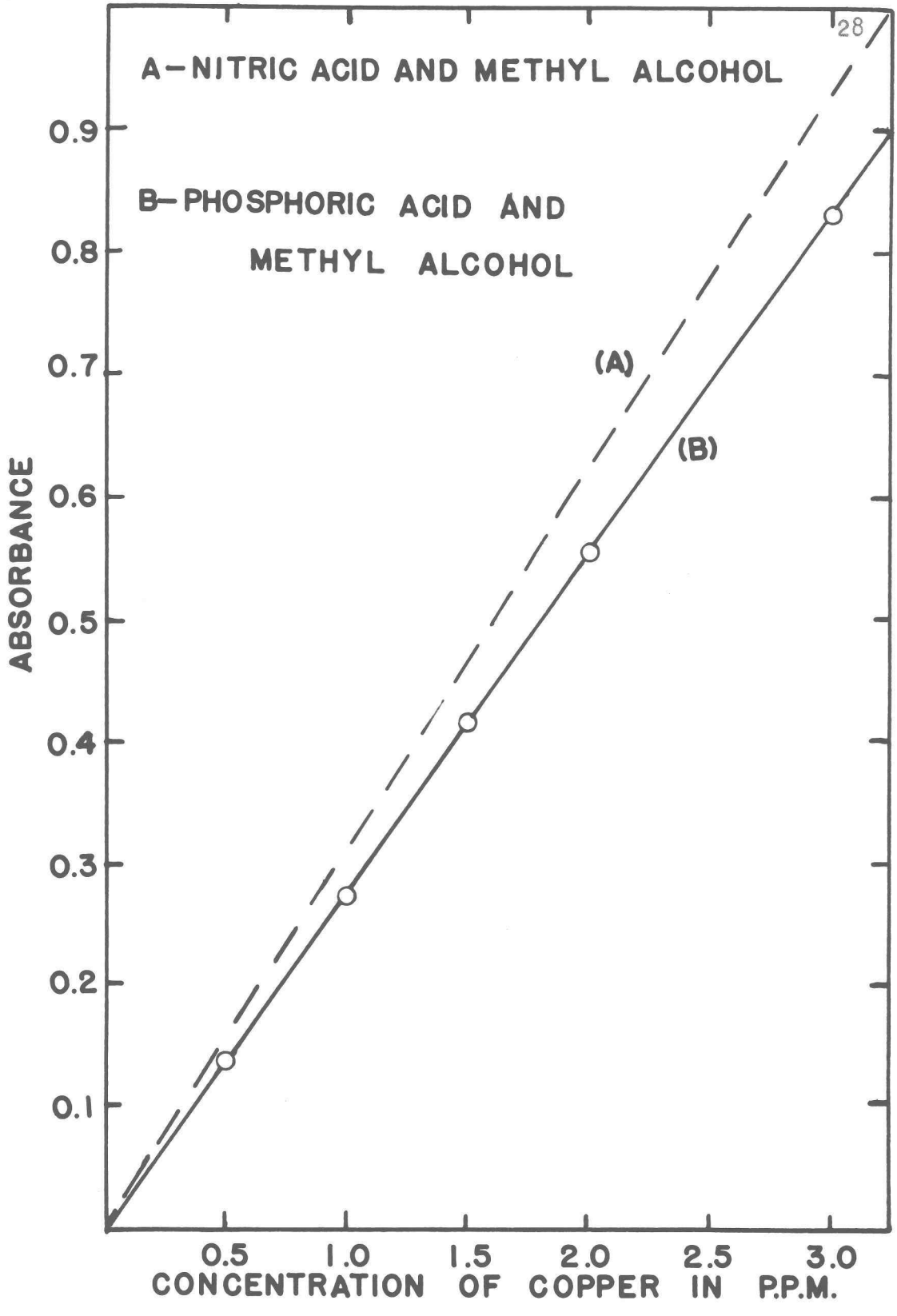


FIGURE 6. STANDARDIZATION OF COPPER–PAN

The modified colorimetric copper-PAN method was applied to two hay samples which the author ashed and to nine more samples, which were analyzed using aliquots from solutions of hay samples which had been ashed at the experiment station. The ashing procedure employed was the same as the one previously referred to. In the analysis of the two samples ashed by the author, 20 ml aliquots of the resulting solutions and 20 ml aliquots of stock phosphoric acid dye solution were added to 100 ml beakers. The pH was adjusted to 1.60 as in the former procedure. The solutions were then carefully transferred to 50 ml volumetric flasks, diluted to volume and absorbance readings taken at 550 mμ. A similar procedure was followed on the solutions of hay samples obtained from the experiment station. A 25 ml aliquot of sample was added to a 50 ml beaker and the volume reduced to 5 ml by evaporation on a hot plate. Only 10 ml of stock dye was added and after the pH had been adjusted, the solutions were transferred to 25 ml volumetric flasks. These changes were necessary because of the lower concentration of copper in the solutions. The samples which were reported as having concentrations of Co as high as 0.1 to .2 ppm Co showed precipitation with the dye upon standing for 2 hours. All absorbance measurements were made before precipitation occured. The results of the analysis are summerized in Table 7. In general the results of the author were slightly higher than those of the experiment station. No correlation was found between the difference in the values of the two methods of analysis and the concen-

TABLE 7

RESULTS OF ANALYSIS OF HAY SAMPLES

Sample #	ppm Cu Reported	ppm Cu by PAN method	Deviation in ppm Cu
* 1	8.2	8.3	+0.1
* 2	3.6	4.4	+0.8
3	10.5	12.2	+1.7
4	5.2	8.3	+3.1
5	4.9	7.9	+3.0
- 6	7.8	8.5	+0.7
* 7	5.6	6.3	+0.7
* 8	5.4	6.2	+0.8
‡ 9	4.8	5.7	+0.9
‡10	4.6	5.7	+0.9
11	4.3	5.5	+1.2

* samples ashed by author
- rerun on sample #1
‡ numbers 7 and 8, 9 and 10 are duplicate samples

tration of iron and cobalt present.

The two-color systems were compared on a synthetic sample by the author and Mehlig. A solution was prepared containing 50 ppm iron, 0.025 ppm cobalt and a concentration of copper unknown to the analysts. The same procedures were followed as those used on the hay samples. A comparison of the results revealed that both analyses were slightly high. The unknown solution was prepared to contain 0.50 ppm copper, the author's value was 0.73 ppm, and Mehlig's value 0.62 ppm. A correction for the iron present, from the previous study of iron interference in the system, would lower the author's value to 0.66 ppm. The positive deviation of both analyses indicates the possibility that some copper had been picked up in the preparation of the unknown. One likely source may be the distilled water used in the solution. The two values followed the same pattern as the results of the hay samples in that the value obtained by the author was slightly higher than that of the extraction procedure.

SUMMARY AND CONCLUSION

A colorimetric copper method has been developed in which the dye 1-(2-pyridylazo)-2-naphthol is used. An aqueous solvent system using nitric acid and methyl alcohol at an optimum pH of 1.60 was investigated. A standard curve was prepared and the concentration range for best accuracy was found to be 0.6 to 2.5 ppm. The effect of eleven ions was studied of which only Fe III, Ni II, and Co II were found to seriously interfere with the method.

The method was applied to a number of hay samples previously analyzed at the Agricultural Experiment Station, Oregon State College. A modification of the solvent system was developed using phosphoric instead of nitric acid in order to mask the effect of high concentrations of iron in the samples. A standard curve was prepared for the phosphoric acid system. Eleven samples were analyzed and the results compared to the previous analysis. The color systems were also compared on a synthetic sample. The values obtained compared favorably with those of the experiment station, showing an average positive deviation of 1.2 ppm. A positive deviation was also found in the analysis of the synthetic sample but no conclusions could be made as to the validity of the two results because both values were high. The new method is a more rapid and simpler procedure than the method employed at the experiment station in that it eliminates the extraction step from the analysis. The method appears to be well adapted to materials of biological origin.

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