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EVALUATION OF THE TECHNICON BLOCK DIGESTOR SYSTEM FOR TOTAL KJELDAHL NITROGEN AND TOTAL PHOSPHORUS

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.

- Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water. Conduct studies to determine the responses of aquatic organisms to water quality.

- Conduct an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

There is an ever-increasing interest in the use of automated methods to analyze water and waste samples, whether the resulting data are to be used for research, surveillance, compliance monitoring, or enforcement purposes. Accordingly, the Environmental Monitoring and Support Laboratory has an on-going methods research effort in the development, evaluation, and modification of automated colorimetric procedures. This particular report pertains to the simultaneous and semi-automated determination of two key nutrient parameters: total phosphorus and total Kjeldahl nitrogen. The method has potential routine application for the analysis of these constituents in drinking waters, surface water, and domestic and industrial wastes.

Dwight G. Ballinger
Director
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ABSTRACT

Technicon's block digestor method for the simultaneous determination of total phosphorus and total Kjeldahl nitrogen has been evaluated and modified for the semi-automated determination of these constituents in surface water and domestic and industrial wastes. The Technicon digestion mixture was replaced with the Kjeldahl nitrogen digestion solution. The applicable range is 0.1 to 20 mg per liter for phosphorus and nitrogen.

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SECTION I

INTRODUCTION

Organic nitrogen and phosphorus have been considered important water quality parameters because of their association with human and industrial waste. Before these parameters can be analyzed, samples have to be digested. For analysis of phosphorus the sample is digested using sulfuric acid and persulfate (1) to convert all the phosphorus to orthophosphate. The Kjeldahl nitrogen method (1) is used for the determination of organic nitrogen. This method converts nitrogen components of biological origin to ammonia and may or may not be applicable for industrial wastes. An automated vanadium method (2) was developed by the Environmental Monitoring and Support Laboratory (EMSL) for simultaneous determination of organic nitrogen and phosphorus in surface water and industrial wastes; however, the use of this method was discontinued because of the hazard involved with using perchloric acid as the oxidant. The National Pollution Discharge Elimination System (NPDES) has increased the need for a method to replace this automated method. Technicon has developed another system, the Block Digestor , that can be used to determine organic nitrogen and phosphorus for the same sample.

The objective of this study was to evaluate the Technicon Block Digestor (BD) system (3) and determine its applicability to water and waste analyses. This system consists of a block digestor that holds 40 digestion tubes and an AutoAnalyzer for subsequent colorimetric analysis. Technicon's method can be used to determine organic nitrogen and phosphorus over a range of 0.1 to 20 mg per liter in water and wastewaters at a rate of 20 to 30 samples per hour.

SECTION II

CONCLUSION

The digestion system employed is similar to one used by two other EPA laboratories where less sample is taken; however, the same ratio of sample to digestion solution is maintained. The data obtained by the three laboratories have shown that this system can be used to analyze surface water, drinking water, and domestic and industrial wastes. The recovery of 73% nitrogen from nicotinic acid shows that this system has the potential of giving higher results than the conventional manual TKN method.

SECTION III

EXPERIMENTAL PROCEDURES

APPARATUS

Technicon Block Digestor* - BD-40
1 x 10" or 1 x 8" Pyrex digestion tubes**
Chemware TFE (Teflon) boiling stones (Markson Science Inc., Box 767, Delmar, CA 92014)
Technicon Manifold No. 325-74W for Ammonia
Technicon Manifold No. 327-74W for Phosphorus

REAGENTS

For Digestion

Mercuric sulfate solution: Dissolve 8 g red, mercuric oxide (H_gO) in 50 ml of 1:5 sulfuric acid (10.0 ml conc. H_2SO_4: 40 ml distilled water) and dilute to 100 ml with distilled water.

Digestion solution (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of K_2SO_4 in 700 ml of distilled water and 200 ml of conc. H_2SO_4. Add 25 ml of mercuric sulfate solution and dilute to 1 liter.

For Ammonia

Sulfuric acid solution, 4%: Add 40 ml of conc. sulfuric acid to 800 ml of ammonia-free distilled water; cool and dilute to 1 liter.

Stock sodium hydroxide, 20%: Dissolve 200 g sodium hydroxide in about 800 ml of ammonia-free water and dilute to 1 liter.

Stock sodium potassium tartrate solution, 20%: Dissolve 200 g sodium potassium tartrate in about 800 ml of ammonia-free distilled water and dilute to 1 liter.

Stock buffer solution: Dissolve 134 g of sodium phosphate, dibasic (Na_2HPO_4), in about 300 ml of ammonia-free water. Add 20 g of sodium hydroxide and dilute to 1 liter.

* The Environmental Research Center, Corvallis, OR has also developed a block digestion system that can be used on a hot plate.
**If the 8" tubes are used, two inches have to be cut off of the bottom of the tube rack.

Working buffer solution: Combine the reagents in the stated order; add 250 ml of stock sodium potassium tartrate solution to 200 ml of stock buffer solution and mix. Add ml amounts *** sodium hydroxide solution and dilute to 1 liter. The pH of this solution should be above 12.9.

Sodium salicylate/sodium nitroprusside solution: Dissolve 150 g of sodium salicylate and 0.3 g of sodium nitroprusside in about 600 ml of ammonia free water and dilute to 1 liter.

Sodium hypochlorite solution: Dilute 6.0 ml of sodium hypochlorite solution to 100 ml with ammonia-free distilled water.

Stock standard solution: In distilled water, dissolve 3.819 g of anhydrous ammonium chloride (NH_4Cl) dried at 105°C, and dilute to 1000 ml. 1.0 mg = 1.0 mg NH_3-N.

For Phosphorus

Sulfuric acid solution, 0.72 N: Add 10 ml of conc. sulfuric acid to 300 ml of distilled water, mix, and dilute to 500 ml.

Molybdate/Antimony Solution: Dissolve 8 g of ammonium molybdate and 0.2 g of antimony potassium tartrate in about 800 ml of distilled water and dilute to 1 liter.

Ascorbic Acid Solution: Dissolve 60 g of ascorbic acid in about 600 ml of distilled water. Add 2 ml of acetone and dilute to 1 liter.

Sodium chloride solution: Dissolve 40 g of NaCl in about 600 ml of distilled water and dilute to 1 liter.

Stock standard phosphorus solution: Dissolve 0.4393 g of pre-dried (105°C for 1 hour) KH_2PO_4 in distilled water and dilute to 100 ml. 1.0 mg = .1 mg P.

PROCEDURE

Digestion

a. To 20 or 25 ml of sample, add 5 ml of digestion solution and mix by use of vortex mixer.

b. Add boiling chips (4 to 8 Teflon boiling stones). NOTE: Too many boiling chips will cause the sample to boil over.

c. Set the low temperature setting at 200°C and the high temperature setting at 200°C and preheat to 200°C. Place tubes in the digestion and set the timer for the low temperature for 1 hour and the timer for the high temperature for 2 1/2 hours. Set the high temperature setting at 380°C.

***See concentration range, Table 1 of appendices for ml amounts of sodium hydroxide solution.

NOTE: If eight inch tubes are used, the high temperature timer should be set for 30 minutes after the temperature has reached 380°C. Longer time and higher temperature may result in complete loss of the acid.

d. After the digestion has been completed, cool the samples and add 25 ml of ammonia free water and mix.

Colorimetric Analysis

a. Check the level of all reagent containers to ensure an adequate supply.

b. Excluding the salicylate and molybdate/antimony lines, place all reagent lines in their respective containers, connect the sample probe to the Sampler IV, and start the proportioning pump.

c. Flush the Sample IV wash receptacle with about 25 ml of 4% sulfuric acid.

d. When the reagents have been pumping for at least 15 minutes, place the salicylate and molybdate/antimony lines in their respective containers and allow the system to equilibrate.

NOTE 1: If a precipiate forms after the addition of salicylate, the pH is too low. Immediately stop the proportioning pump and flush the coils with water using a syringe. Before restarting the system, check the concentration of the sulfuric acid solutions and/or the working buffer solution.

NOTE 2: To prevent precipitation of sodium salicylate in the waste tray (which can clog the tray outlet), keep the nitrogen flowcell pump tube and the nitrogen colorimetric "To Waste" tube separate from all other lines, or keep tap water flowing in the waste tray.

e. After a stable baseline has been obtained, start the Sampler.

SECTION IV

DATA

TECHNICON SYSTEM

To 20 ml of sample 0.01 g of mercuric oxide, 10 ml of conc. sulfuric acid and 6 g potassium sulfate are added. The samples and standards are digested for 3 hours. After digestion, the samples are cooled, diluted to 75 ml, and placed on the AutoAnalyzer for ammonia and phosphate determination.

The reliability of this system was determined by analyzing a variety of organic compounds for nitrogen and phosphorus. Table 2* shows the recovery of nitrogen from cysteine and nicotinic acid. The recovery of nitrogen from cysteine was equal to the recovery obtained using the total Kjeldahl nitrogen method (1). The recovery of nitrogen from nicotinic acid using the total Kjeldahl method was less than 1%; however, with the block digestor system, 52% recovery was obtained from 10 mg per liter. The recovery of phosphorus and nitrogen from adenosine-5 phosphate was equal to the recovery obtained using the automated vanadium TKN method (2).

The precision of this method was determined by analyzing 7 replicates of a sewage sample. The organic nitrogen plus ammonia nitrogen concentration was 11.4 mg N per liter and the standard deviation was $\pm$ 0.5. The phosphorus concentration was 1.1 mg P per liter and the standard deviation was $\pm$0.2. Because of this relatively large standard deviation for phosphorus, 20%, it was felt that improvement could be made. The fact that 10 ml of conc. sulfuric are added makes it very difficult to mix the sample after digestion. The 6 grams of potassium sulfate required for each sample is a considerable quantity.

MODIFIED METHOD

As an alternate possibility, the digestion solution used for the total Kjeldahl nitrogen method was tried. With this method only 0.65 g of potassium sulfate and 1 ml of conc. sulfuric acid are required for each sample. Because only 5 ml of the digestion solution is used, an increase in the sample size from 20 to 25 ml is possible. Comparison of the Technicon system and the modified method is shown in Table 4.

In the Technicon method the samples were digested for a total of 3 hours: 1 hour at low temperature, 200°C, and 1 hour at 370°C With this

*For all tables and figures, see the Appendices.

time and temperature about 1.5 hours was required to remove the water from the samples. With the modified method, the water was evaporated in 1 hour; therefore, the total elapsed time was reduced to 2.5 hours. It was noted that all the holes in this block did not have the same temperature. Because of this variation in temperature, poor precision was obtained for some compounds. It was also noted that better precision and higher recoveries of highly refractory compounds, such as nicotinic acid and adenosine-5-phosphate, were obtained when the high temperature was set at 380°C and the total time was set at 3 hours (retaining the 1 hour at low temperature).

Jirka and Carter (4) recommended the use of 1 x 8" digestion tubes in place of 1 x 10" tubes. Table 5 shows the comparison of results obtained for three concentrations of nicotinic acid with the two types of tubes. All samples and standards were digested at the same time and analyzed with the same reagents and colorimeter settings. The digestor was set for 1 hour at 200°C and total time was 2.5 hours. Higher values were obtained for samples digested in the 8-inch tubes. The higher values indicated that the water was removed from the 8-inch tubes sooner than from the 10-inch tubes. Therefore, these samples were digested longer. Eight-inch tubes may be used in place of 10-inch tubes; the digestion must not exceed 30 minutes at 380°C. Except for the data in Table 5, the other results in this study were obtained using the 10-inch tubes.

In both the original method and the modified method, the samples were diluted to 75 ml after digestion. Jirka and Carter (4) found that greater sensitivity could be obtained by diluting the samples to 10 ml. This was an improvement in the method when samples with low concentrations of TKN were analyzed, but does not provide sufficient sample if a rerun is necessary. Therefore, for this study the samples were diluted to 25 ml. The 25 ml sample is sufficient to fill two sample tubes. Table 1 shows the dilution loops used for various concentration ranges of ammonia. Best results are obtained when dilution loops 3,4,5 and 6 are used. Figures 1 and 2 show the manifolds used for the determination of ammonia and phosphate. For this study, the sampling rate was decreased from 40 per hour (9:1) to 30 per hour (4:1). The chart speed was 3/16 inch per minute. The salicylate-hypochlorite reaction with ammonia was found to be as sensitive as the phenate-hypochlorite reaction.

Basically, only minor changes in Technicon's method for determining ammonia and phosphate following digestion were made. The sulfuric acid wash solution was reduced from 13% to 4% and the sulfuric acid in the phosphorus method was reduced from 2.0 N to 0.72 N because of the smaller amount of acid used for digestion. The ammonium molybdate was reduced from 30 g per liter to 8.0 g per liter because 30 g per liter was found to be an excessive amount. Two milliliters of acetone were added to preserve the ascorbic acid and prevent precipitation in the system.

The reliability of this modified procedure was determined for a solution of ammonium chloride, a reference sample from EMSL, Quality Assurance Laboratory, and several sewage samples. The ammonium chloride was included to determine if ammonia would be lost during the digestion step. The reference sample was analyzed to determine the recovery of organic nitrogen. As shown in Table 6, the coefficients of variation for the two compounds are about

equal. This reference sample was also added as a spike to sewage samples. As shown in Table 7, the recovery of phosphate was approximately 93% and the recovery of organic nitrogen was 92%.

Accuracy was also determined by analyzing several organic compounds for both nitrogen and phosphorus. Sewage samples were also analyzed for organic nitrogen plus ammonia and the results compared to those obtained using the ammonia probe. Table 8 lists the recovery of nitrogen and phosphorus from two organic compounds (nicotinic acid and adenosine-5-phosphate) added to distilled water. These samples were digested with the block digestor and analyzed using the colorimetric methods. For nicotinic acid the recovery of nitrogen was 76% for 0.5 mg N per liter and 71% for 10 mg N per liter. The average recovery of nitrogen from adenosine-5-phosphate was 85% for the range of 1.1 to 6.7 mg N per liter. The recovery of phosphorus for the range 0.5 to 3.0 mg per liter was 94%. Table 9 lists the results obtained using the colorimetric method versus the ammonia probe. The same digested sample was used for both methods. The probe was used in this set to determine whether the ammonia mercury complex was broken. Because about the same results were obtained for the two methods, it was concluded that the complex was broken.

The precision of this method was determined at three separate concentration levels. The samples used for this study were sewage, two river waters, and an industrial waste. They included a low and two intermediate concentrations. The nitrogen concentrations were 5.7, 0.98, and 1.92 mg per liter. The precision was ±0.11, ±0.03, and ±0.05 mg per liter, respectively.

Jirka and Carter (4) also made a study of the interferences using the automated phenate and single reagent methods. They found that high concentrations of chromium, zinc, copper, vanadium, and manganese can cause low recovery of nitrogen. High concentrations of chromium will also cause low recovery of phosphorus. Arsenic is a positive interference for phosphorus. The same interferences were noted for the salicylate and the two reagent phosphorus methods. Nitrate also interferes with the TKN when it is present in concentrations ten times or greater than the TKN levels present(5).

SECTION V

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TABLE 1

CONCENTRATION RANGES
(NITROGEN)

	Dilution loops				Approx.	Range	ml stock NaOH
	Initial sample		Resample		std.cal.	PPM N	per liter
No.	Sample line	Diluent line	Resample line	Diluent line	setting	(+10%)	working buffer solution
1	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0-0.5	250
2	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0-1.5	250
3	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0-1	120
4	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0-5	120
5	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	700	0-2	80
6	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	100	0-10	80

TABLE 2

RECOVERY OF NITROGEN FROM CYSTEINE AND NICOTINIC ACID WITH THE BLOCK DIGESTOR SYSTEM

Sample	Amounts added mg N/l	mg/l found	%recovery
Cysteine	1.0	0.96	96
	10.0	9.8	98
Nicotinic	1.0	0.53	53
	10.0	5.20	52
Sewage	0	0.79	--
Sewage & Cysteine	4.0	4.73	99
	8.0	8.74	99
Sewage & Nicotinic	4.0	2.95	54
	8.0	4.17	42

TABLE 3

RECOVERY OF PHOSPHORUS AND NITROGEN FROM ADENOSINE-5-PHOSPHATE WITH THE BLOCK DIGESTOR SYSTEM

Sample	Phosphorus added mg/l	Phosphorus found mg/l	%recovery
Distilled H_2O	1.0	0.9	90
	2.0	1.71	85
	5.0	3.94	79
Sewage	0	6.90	--
	2.0	8.67	89
	Nitrogen added mg/l	**Nitrogen found mg/l**	**%recovery**
Distilled H_2O	0.45	0.45	100
	0.90	0.91	101
	2.26	2.10	93
	4.52	3.86	85
	11.30	9.2	81
Sewage	0	6.90	--
	4.5	9.98	68

TABLE 4

COMPARISON OF TECHNICON'S SYSTEM AND THE MODIFIED SYSTEM

Item	Technicon	Modified
Sample	20 ml	20 or 25
Digestion	10 ml H_2SO_4	1 ml H_2SO_4
	6.3 g K_2SO_4	0.66 g K_2SO_4
	.1 g HgO	.01 g HgO
Time of digestion	3 hours	2.5 hours
Manifold	Technicon	Technicon

TABLE 5

COMPARISON OF TKN DATA OBTAINED FOR NICOTINIC ACID USING THE 1 X 8" AND 1 X 10" DIGESTION TUBES

mg N/l added	8-inch tubes mg N/l found	%recovery	10-inch tubes mg N/l found	%recovery
1.0	0.99	99	.80	80
3.0	2.70	90	2.48	83
10.0	8.20	82	6.30	63

TABLE 6

RECOVERY OF AMMONIA FROM AMMONIUM CHLORIDE AND QAL REFERENCE SAMPLE

Ammonium chloride - Actual value: 2.0 mg N/l

Mean	2.1
Number of samples	32
Standard deviation	$\pm$0.08
Coefficience of var.	3.9%

Sample from QAL - Actual value: 5.8 mg N/l

Mean	5.6
Number of samples	32
Standard deviation	$\pm$0.22
Coefficience of var.	4.0%

TABLE 7

RECOVERY OF NITROGEN AND PHOSPHORUS FROM SEWAGE SAMPLES

Total Phosphorus

Sample	Found mg/l	Added mg/l	Recovered mg/l	%recovery
1	1.14	0.71	1.86	101
2	1.29	0.71	1.89	85
3	1.17	0.71	1.84	94

Organic Nitrogen

Sample	Found mg/l	Added mg/l	Recovered mg/l	%recovery
1	3.66	5.25	8.27	89
2	1.54	5.25	6.45	93
3	1.33	5.25	6.30	95

TABLE 8

RECOVERY OF NITROGEN AND PHOSPHORUS FROM ORGANIC COMPOUNDS ADDED TO OHIO RIVER WATER

Compound added	N in sample	Conc. added	N found	%recovery
Nicotinic acid mg/l	0.85	0.5	1.23	76
	0.85	1.0	1.53	68
	0.85	5.0	4.27	68
	0.85	10.0	7.90	71
Adenosine - 5- phosphate mg/l	0.85	1.13	1.80	84
	0.85	2.26	2.66	80
	0.85	4.50	4.77	87
	0.85	6.70	6.79	89
	P in sample	**Conc. added**	**P found**	**%recovery**
Adenosine - 5-phosphate mg/l	0.06	0.5	0.55	98
	0.06	1.0	1.00	94
	0.06	2.0	1.94	94
	0.06	3.0	2.78	91

TABLE 9

COMPARISON OF NITROGEN RESULTS,
COLORIMETRIC METHOD VS. THE AMMONIA PROBE, MG N/1

Sample	Colorimetric Method	Ammonia Probe
1	10	11.2
2	1.2	1.3
3	2.6	2.2
4	1.7	1.6

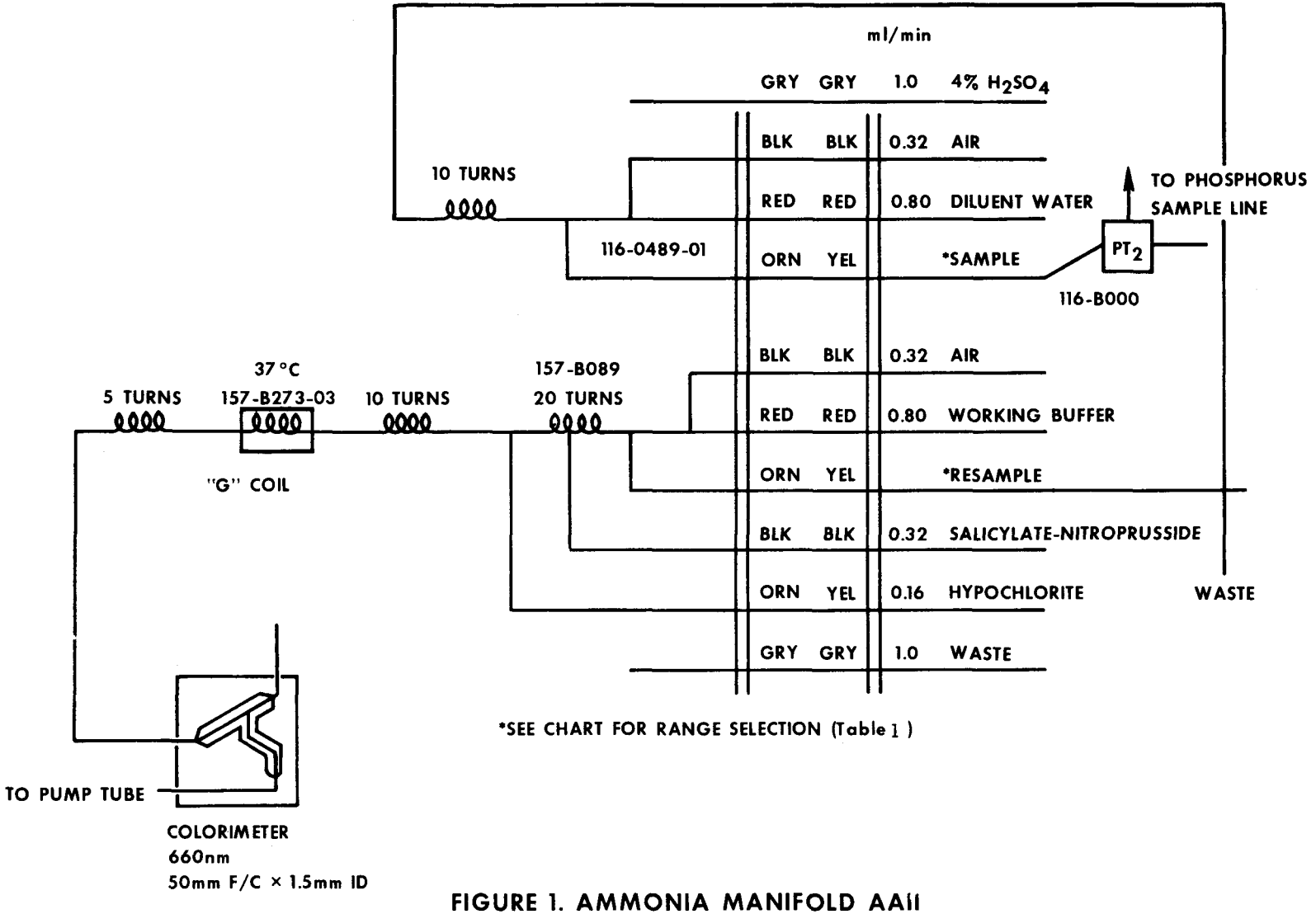

FIGURE 1. AMMONIA MANIFOLD AAII

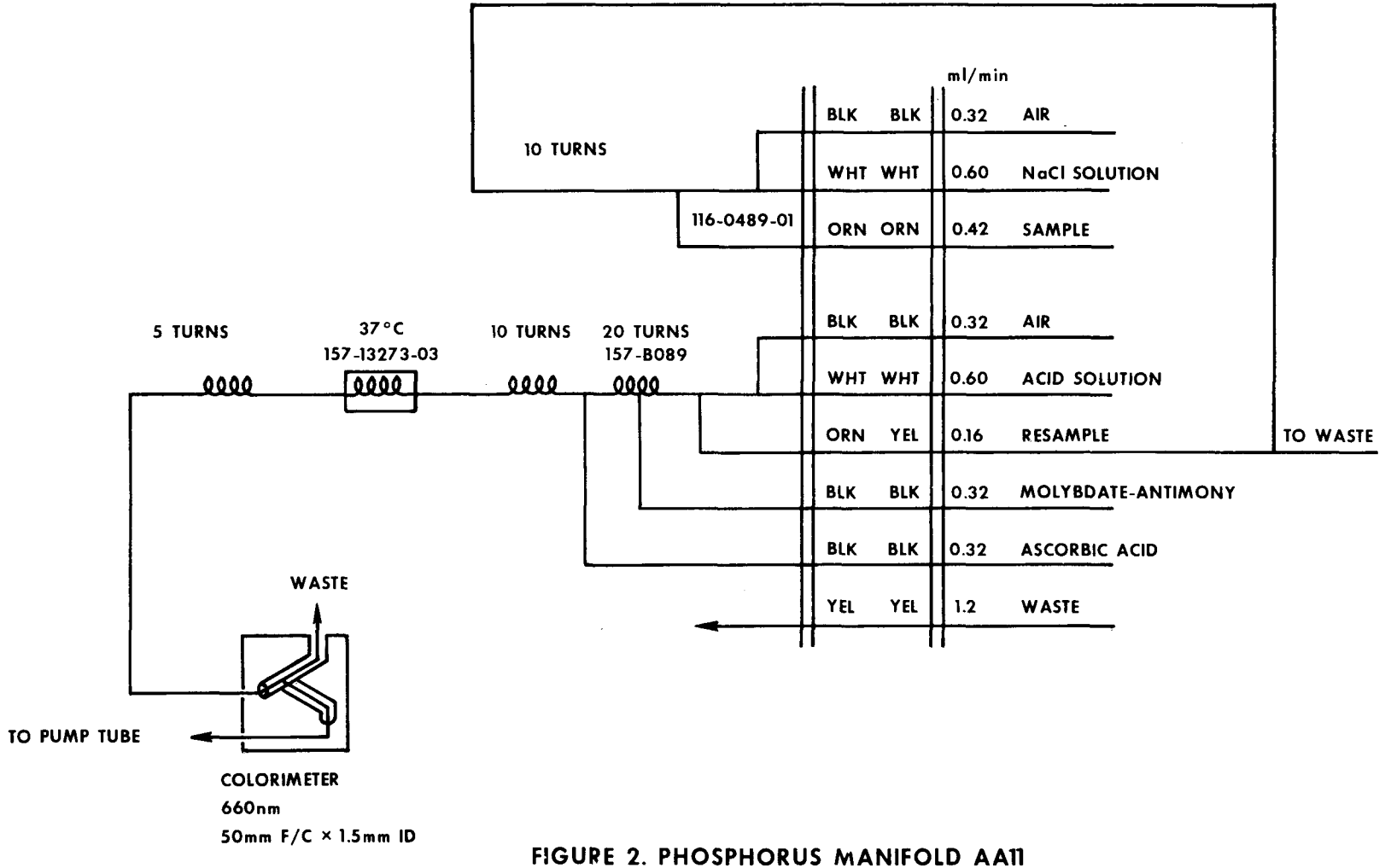

FIGURE 2. PHOSPHORUS MANIFOLD AA11

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16. ABSTRACT

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