METHOD #: 353.1   Approved for NPDES and SDWA (Reissued w/
                                     Rev. 1978)

TITLE: Nitrogen, Nitrate-Nitrite (Colorimetric,
                                     Automated, Hydrazine Reduction)

ANALYTE: CAS #   N Nitrogen 7727-37-9
                                     NO_3 Nitrate
                                     NO_2 Nitrite

INSTRUMENTATION: Spectrophotometer

STORET No.   Total 00630

1.0   Scope and Application

        1.1   This method is applicable to drinking and surface water, and domestic and
                industrial wastes. The applicable range of this method is 0.01-10 mg/L
                nitrate-nitrite nitrogen. Approximately 20 samples per hour can be analyzed.

2.0   Summary of Method

        2.1   Nitrate is reduced to nitrite with hydrazine sulfate and the nitrite (that
                originally present plus reduced nitrate) is determined by diazotizing with
                sulfanilamide and coupling with N-(naphthyl)-ethylenediamine
                dihydrochloride to form a highly colored azo dye which is measured
                colorimetrically.

3.0   Sample Handling and Preservation

        3.1   Analysis should be made as soon as possible. If analysis can be made within
                24 hours, samples should be preserved by refrigeration at 4°C. When samples
                must be stored for more than 24 hours, they should be preserved with 2 mL of
                sulfuric acid (H_2SO_4) per liter and refrigerated.

4.0   Interferences

        4.1   Sample color that absorbs in the photometric range used for analysis will
                interfere.
        4.2   The apparent NO_3 and NO_2 concentrations varied ± 10 percent with
                concentrations of sulfide ion up to 10 mg/L.

5.0   Apparatus

        5.1   Sampler
        5.2   Manifold AAI or AAII
        5.3   Proportioning Pump
        5.4   Heating bath 32°C AAI or 37°C AAII

5.5 Continuous filter
5.6 Colorimeter equipped with an 8 mm, 15 mm or 50 mm flow cell and 529 nm filters.

6.0 Reagents

6.1 Color developing reagent: To approximately 500 mL of distilled water add 200 mL concentrated phosphoric acid (sp. gr. 1.834), 10 g sulfanilamide ($H_2NC_6H_4SO_2NH_2$) followed by 0.8 g N (1-Naphthyl) ethylenediamine-dihydrochloride. Dilute the solution to 1 liter with distilled water and store in a dark bottle in the refrigerator. This solution is stable for approximately 1 month.

6.2 Copper sulfate stock solution: Dissolve 2.5 g of copper sulfate ($CuSO_4 \cdot 5H_2O$) in distilled water and dilute to 1 liter.

6.3 Copper sulfate dilute solution: Dilute 20 mL of stock solution (6.2) to 2 liters with distilled water.

6.4 Sodium hydroxide stock solution, (ION): Dissolve 400 g NaOH in 750 mL distilled water, cool and dilute to 1 liter.

6.5 Sodium hydroxide (1.0 N): Dilute 100 mL of stock NaOH solution (6.4) to 1 liter.

6.6 Hydrazine sulfate stock solution: Dissolve 27.5 g of hydrazine sulfate ($N_2H_4 \cdot H_2SO_4$) in 900 mL of distilled water and dilute to 1 liter. This solution is stable for approximately 6 months.
CAUTION: Toxic if ingested. Mark container with appropriate warning.

6.7 Hydrazine sulfate dilute solution.
6.7.1   AAI: Dilute 55 mL of stock solution (6.6) to 1 liter.
6.7.2   AAII: Dilute 22 mL of stock solution (6.6) to 1 liter.

6.8 Stock nitrate solution (100 mg/L NO_3-N): Dissolve 0.7218 g of KNO_3, oven dried at 100-105°C for 2 hours, in distilled water and dilute to 1 liter. Add 1 mL chloroform as a preservative. Stable for 6 months. 1 mL = 0.1 mg N.

6.9 Stock nitrite solution (100 mg/L NO_2-N): Dissolve 0.6072 g KNO_2 in 500 mL of distilled water and dilute to 1 liter. Preserve with 2 mL of chloroform and keep under refrigeration. 1 mL = 0.1 mg N.

6.10 Standard nitrate solution: Dilute 100 mL of stock nitrate solution (6.8) to 1 liter. 1 mL = 0.01 mg N.

6.11 Using the stock nitrate solution (6.8), prepare the following standards in 100 mL volumetric flasks. At least one nitrite standard should be compared to a nitrate standard at the concentration to verify the efficiency of the reduction.

Conc., mg NO_3-N/L	mL of stock solution/100 mL
0.5	0.5
1.0	1.0
2.0	2.0
3.0	3.0
4.0	4.0
5.0	5.0
8.0	8.0
10.0	10.0

For standards in the range of 0.01 mg/1 use the standard nitrate solution.

7.0   Procedure

7.1   Set up the manifold as shown in Figure 1 (AAI) or Figure 2 (AAII). The continuous filter must be used to remove the precipitate.

7.2   Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a stable baseline with all reagents, feeding distilled water through the sample line.

7.3   Run a 2.0 mg/L NO_3-N and a 2.0 mg/L NO_2 -N standard through the system to check for 100% reduction of nitrate to nitrite. The two peaks should be of equal height. If they are not, the concentration of the hydrazine sulfate solution must be adjusted as follows. If the NO_3 peak is lower than that of the NO_2 peak the concentration of hydrazine sulfate should be increased until they are equal. If the NO_3 peak is higher than the nitrite, the concentration of the hydrazine sulfate should be reduced. When the correct concentration of hydrazine sulfate has been determined, no further adjustment should be necessary.

7.4   Place appropriate nitrate standards in the sampler in order of decreasing concentration of nitrogen. Compete loading tray with unknown samples.

7.5   For both the AAI and the AAII use a 2 minute sampling rate.

8.0   Calculation

8.1   Prepare a standard curve by plotting peak heights of processed standards against known concentrations. Compute concentrations of samples by comparing sample peak heights with the standard curve.

9.0   Precision and Accuracy

9.1   In a single laboratory using drinking water, surface water and industrial waste at concentrations of 0.39, 1.15, 1.76 and 4.75 *u*g NO_3-N/L, the standard deviations were± 0.02, ± 0.01, ± 0.02 and± 0.03, respectively. In a single laboratory using drinking water at concentrations of 0.75 and 2.97 the recoveries were 99% and 101%.

Bibliography

1 .   Kamphake, L., Hannah, S., and Cohen, J., "Automated Analysis For Nitrate by Hydrazine Reduction", Water Research 1, 205 (1967).
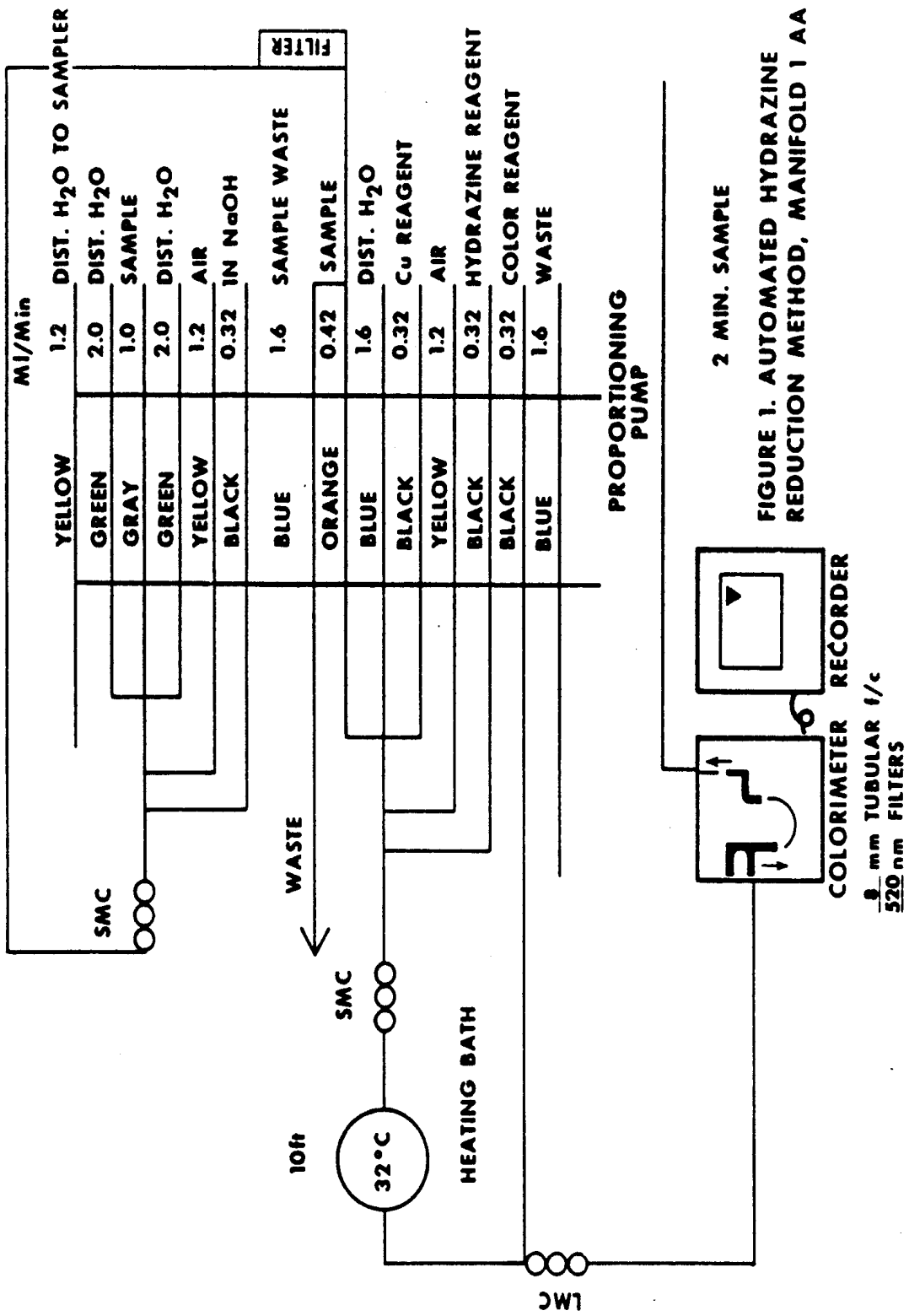
FIGURE 1. AUTOMATED HYDRAZINE REDUCTION METHOD, MANIFOLD 1 AA 1

Ml/Min

1.0	GRAY	DIST. H$_2$O TO SAMPLER
0.32	BLACK	AIR
0.80	RED	1N NaOH
0.23	ORANGE WHITE	SAMPLE
1.0	GRAY	DIST. H$_2$O
1.2	YELLOW	SAMPLE WASTE
0.32	BLACK	AIR
0.32	BLACK	Cu REAGENT
0.32	BLACK	SAMPLE
0.32	BLACK	HYDRAZINE REAGENT
0.32	ORANGE	DIST. H$_2$O
0.32	ORANGE WHITE	COLOR REAGENT
1.0	GRAY	WASTE

116—0489

20 TURNS
157—B089

A—10

116—0489

20 TURNS
157—B089

HEATING BATH

37°C

A—10

20 TURNS

PROPORTIONING PUMP

FILTER

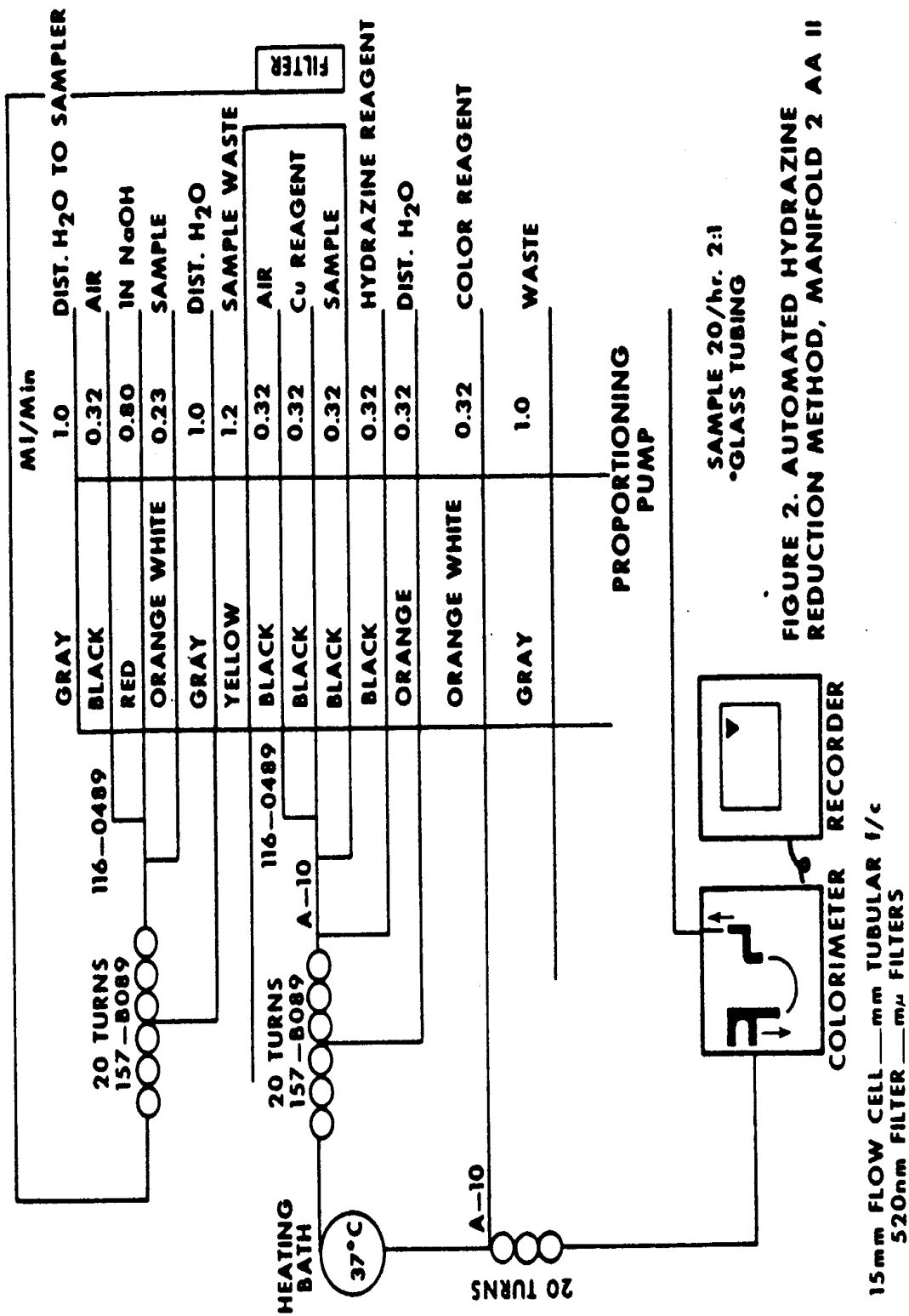COLORIMETER   RECORDER

SAMPLE 20/hr. 2:1
*GLASS TUBING

FIGURE 2. AUTOMATED HYDRAZINE
REDUCTION METHOD, MANIFOLD 2 AA II

15mm FLOW CELL ——mm TUBULAR f/c
520nm FILTER —— mµ FILTERS