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Low Level Kjeldahl Nitrogen Determination on the Technicon AutoAnalyzer

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■ The existing procedures for the determination of Kjeldahl nitrogen on the AutoAnalyzer have been improved to provide greater sensitivity and reliability. Digestion temperatures of 400° C. provided improved stability. Sample flow rates of 2.5 ml. per minute and colorimeter flow rate of 3.6 ml. per minute, in combination with 0.01*M* Na nitroprusside as a color catalyst and 5-cm. colorimeter light path, provided a detection limit of 0.03 mg. per liter of nitrogen. Fifteen Kjeldahl analyses were performed per hour.

Difficulty was experienced in obtaining the sensitivity and stability required for the analysis of natural water samples using previously published procedures (Catanzaro, Goldgraben *et al.*, 1966; Ferrari, 1960; Ferrari, Catanzaro *et al.*, 1965) for the determination of low level total Kjeldahl nitrogen on the Technicon AutoAnalyzer. The following procedure is a modification of the previous procedures that enable the water analyst to perform large numbers of routine Kjeldahl nitrogen determinations with greater sensitivity and reliability.

The basic procedure involves digestion of the sample (natural water, in this case) with a sulfuric acid solution containing

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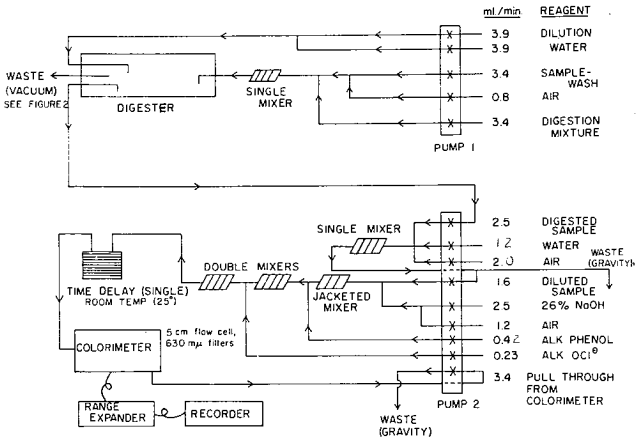

Figure 1. AutoAnalyzer flow diagram for low-Level Kjeldahl nitrogen

perchloric acid and selenium as a catalyst to convert amine- and amide-type organic nitrogen to ammonium. The digested sample is then made alkaline with a sodium hydroxide solution and treated with an alkaline phenol solution followed by an alkaline hypochlorite solution. This treatment results in the formation of an intense blue color described as an indophenol (Bolleter, Bushman, *et al.*, 1961; Russell, 1944) that obeys Beer's law and is measured colorimetrically.

Experimental Procedure

Apparatus. The Technicon AutoAnalyzer system consists of: one sampler; two proportioning pumps with the manifolds shown in Figure 1; one colorimeter with 630-mμ filters, 5-cm. flow cell, range expander, and recorder; one continuous digester; one vacuum pump; two 5-gallon carboys; three 1-liter suction flasks for use as traps; and one single, unheated time delay coil.

Reagents. ACS grade chemicals were used unless otherwise noted.

DIGESTION SOLUTION (1 liter). Add 3.0 ml. of perchloric acid (60%) to 1 liter of 50% v./v. sulfuric acid solution, stir, then add 4.8 ml. of selenium oxychloride (technical grade, Baker Chemical Co.) to the solution and stir until the selenium oxychloride is completely dissolved.

SODIUM HYDROXIDE SOLUTION, 26% (about 1 liter). Dissolve 260 grams of NaOH pellets in 740 ml. of distilled water; cool the solution and transfer it to an alkaline-resistant bottle for storage.

ALKALINE PHENOL SOLUTION (1 liter). Place 250 grams of crystalline phenol in a 2-liter Erlenmeyer flask. Place the flask in a large vessel containing water and ice, or cool it with cold running water. Add 500 ml. of 20% NaOH solution to the flask slowly with constant mixing while keeping the flask cool. When the phenol is dissolved, transfer the contents of the Erlenmeyer to a 1-liter volumetric flask and dilute to volume with 20% NaOH solution. Store the solution in a polyethylene or amber glass bottle. Proper cooling and stirring during the slow addition of the NaOH will give a light-colored solution with a correspondingly low reagent blank.

ALKALINE HYPOCHLORITE SOLUTION (0.5 liter). Dissolve 35.7 grams of calcium hypochlorite (Matheson HTH, 70%) in 300 ml. of hot water (70° to 80° C.). Solution will probably be incomplete. Dissolve 40 grams of anhydrous K_2CO_3 in 200 ml. of distilled water; add 135 ml. of this solution to the calcium hypochlorite solution and stir. Heat the combined solution to 90° C. and cool rapidly. Dilute the solution to 500 ml. with distilled water. Filter a small portion of the solution and test for the presence of calcium ion as follows: To 1m l. of filtered solution, add 2 to 3 ml. of the K_2CO_3 solution prepared previously and heat the resulting mixture in a boiling water bath for a few minutes. If Ca is absent, the solution will remain clear. If the solution becomes cloudy, indicating the presence of Ca, add more K_2CO_3 solution to the hypochlorite solution and repeat the Ca test. When the test is negative, filter the entire solution and store it in an amber glass bottle. Commercial Clorox may be substituted for the alkaline hypochlorite solution.

AMMONIUM SULFATE STANDARD SOLUTION. Dilute 0.472 gram of ammonium sulfate to 1 liter with distilled water. The resulting solution is equivalent to 100 mg. per liter of ammonia nitrogen. Dilute this stock solution to give standards in the range needed.

The two pumping manifolds, shown in Figure 1, are constructed and arranged with the other basic AutoAnalyzer components as shown. The vacuum trap system used in conjunction with the digester is shown in Figure 2. The AutoAnalyzer instruction manual should be consulted for the details of putting the system in operation.

The following basic instrument settings were utilized:

Sampler	Digester
Sample time, 1 minute	Stage I, 4 amperes (350° C.)
Total cycle, 4 minutes	Stage II, 6.7 amperes (400° C.)

Range Expander

10× for levels 0 to 5.0 mg. per liter of nitrogen

Discussion

Most of the work that has been done on automated Kjeldahl nitrogen procedures has been directed toward clinical analyses (Ferrari, 1960; Ferrari, Catanzaro, *et al.*, 1965) and industrial monitoring and quality control operations (Hofstader, 1966). These methods cover a concentration range of 50 to 5000 mg. per liter of nitrogen. Hofstader (1966) used the AutoAnalyzer to determine nitrogen in fertilizer and petroleum samples that had been digested manually. The ammonium ion formed was measured colorimetrically as the indophenol blue complex. Apparently, no range expansion was applied; standards ranged from 10 to 90 mg. per liter of nitrogen. For work with relatively unpolluted natural waters, 0.1 mg. per liter of nitrogen is a convenient maximum lower limit of detection.

Catanzaro *et al.* (1966) reported a procedure for low level nitrogen determinations that is a modification of an earlier procedure presented by Ferrari (1960). Increased sensitivity was attained by increasing the sample volume used, applying range expansion, and increasing the length of the colorimeter flow cell. Analyses were reported using this procedure for

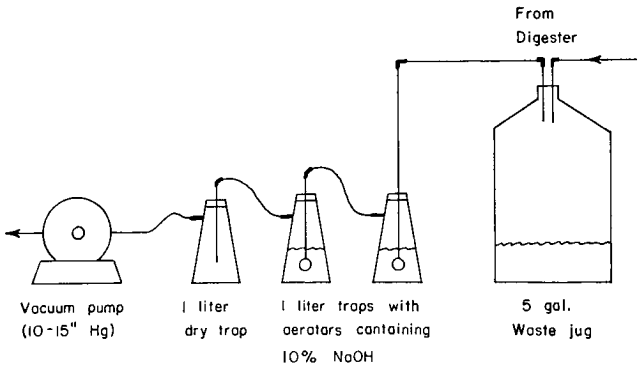

Figure 2. Vacuum trap system used with Kjeldahl digester

samples containing as little as 0.3 mg. per liter of ammonia nitrogen.

The procedure reported here is a modification of the Catanzaro procedure, which gives greater sensitivity and increased stability.

Sensitivity was increased by increasing the amount of digested sample withdrawn from the digester for analysis from 2.0 to 2.5 ml. per minute.

Stability was another problem. When the second stage of the digester was operated at temperatures above 400° C. for several hours, drying occurred in parts of the helix during rotation. This apparently caused sample material to be fused to the glass, causing a gradual decrease in sensitivity accompanied by a rising, noisy base line. A digestion temperature of 400° C. was adequate for complete digestion and recovery of organic standards tested (quinoline, urea, glycine) and did not affect the values obtained on previously analyzed water samples. The flow through the colorimeter cell was increased from 2.9 to 3.6 ml. per minute in an effort to prevent particulate matter from settling out in the flow cell.

The optimum pH for color formation is considered to be 11.4 to 11.6 (Mann, 1963). With the system at this pH, color formation is said to be enhanced by heating (Catanzaro, Goldgraben, *et al.*, 1966; Ferrari, 1960; Russell, 1944). This study showed that passing the sample stream containing the color-forming reagents through a heated 5-minute time delay coil decreased the intensity of the color formed. This decrease could be due to the length of the heating time or to the presence of excess sodium hydroxide. The presence of excess sodium hydroxide alone greatly decreases the sensitivity of the method.

The use of sodium nitroprusside as a catalyst for the color formation reaction has been reported (Mann, 1963). In the present work, a 0.01*M* solution of sodium nitroprusside was added to the system in place of the dilution water used to dilute the sample drawn from the digester. Roughly a fivefold increase in sensitivity was observed. The addition of more concentrated nitroprusside solutions (up to 0.03*M*) increased the sensitivity slightly, but also raised the base line by 10% transmission. No effort was made to determine the optimum nitroprusside concentration since the sensitivity available, without the use of a color catalyst, was adequate for the samples encountered.

The lower limit of detectability, using the system described without a color catalyst, was 0.1 mg. per liter of nitrogen, using a range expansion of 10×. The estimated error for concentrations read on the 10× scale is ±0.1 mg. per liter for routine analysis. A range of 0.1 to 5.0 mg. per liter of nitrogen may be covered on the 10× scale; for larger concentrations, the range expansion may be decreased.

The lower limit of detectability, using the system with a 0.01*M* sodium nitroprusside color catalyst, appears to be about 0.03 mg. per liter of nitrogen. The precision has not been estimated for values obtained using a nitroprusside color catalyst.

Recovery of Nicotinamide. Added quinoline, urea, and glycine were completely recovered with this procedure; nicotinamide was only partially recovered. Solutions of nicotinamide in distilled water were analyzed, and the following recoveries were noted:

Mg. per Liter of Nitrogen		
Calcd.	Found	Recovery, %
2.0	1.4	70
2.0	1.3	65
0.5	0.4	80

Interference of NO_2^- and NO_3^-. Although NO_2^- and NO_3^- should not be measured by the procedure used in this investigation, a check was made to be certain this was the case. Separate samples of untreated Lake Mendota water were dosed with concentrated solutions of sodium nitrite and sodium nitrate to give final concentrations of 5 mg. per liter of nitrite nitrogen, 10 mg. per liter of nitrite nitrogen, 5 mg. per liter of nitrate nitrogen, and 10 mg. per liter of nitrate nitrogen. These samples were analyzed along with a control that had been dosed with a volume of distilled water equal to the volume of nitrate or nitrite solution used (1 ml. per 100-ml. sample). The analyses for all samples were identical within the range of experimental error (±0.1 mg. per liter of nitrogen) and equal to 0.6 mg. per liter of total Kjeldahl nitrogen.

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