A RAPID AUTOMATED SYSTEM FOR THE ANALYSIS OF DISSOLVED TOTAL ORGANIC NITROGEN IN AQUEOUS SOLUTIONS

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Abstract—An automated system for the analysis of dissolved organic nitrogen has been developed and applied to natural waters. It is based on subjecting sample to u.v. irradiation followed by a heterogeneous reduction of the nitrogen containing irradiation products to ammonia which is detected by an ammonia gas sensing probe. Quantitative recoveries of different organic nitrogen compounds common to natural waters were obtained after 17 min of irradiation. Determination with and without irradiation make possible the separate determination of total and inorganic nitrogen, respectively.

INTRODUCTION

It came to our attention during our participation in a water monitoring program that there exists a need for the development of a convenient automated analytical system for dissolved organic nitrogen that has an appropriate sensitivity for natural waters. In such a system, the digestion of organic nitrogen and the measurement of combustion products are the two most important steps. One promising digestion procedure requiring little attention is u.v. irradiation, in spite of its limited application in the field of water analysis. Armstrong, Williams & Strictland (1966) proposed a manual u.v. light combustion procedure as an alternate method to the classical Kjeldahl digestion (Kjeldahl, 1883) and its semi-automated (Stevens, 1975) and automated (Tenny, 1966) versions for the analysis of organic nitrogen in seawater. Manny, Miller & Wetzel (1971) demonstrated u.v. irradiation as a practical quantitative manual technique for organic nitrogen in lake waters. An automated u.v. irradiation system for organic nitrogenous compounds was studied by Goulden, Afghan & Ryan (1971). The automated procedure involved two steps: (1) oxidation of organic compounds to a mixture of nitrate and nitrite; and (2) colorimetric measurement of nitrite following nitrate reduction on a cadmium column. The oxidation was carried out both in acidic and alkaline media to allow for the quantitative recovery of different compounds having varying pH dependences. This system, with some improvisions, became the starting point of our study.

The time of irradiation in such an automated system has to be sufficiently long to allow for the decomposition of organic nitrogen to an inorganic form, and to oxidize any ammonium or ammonia produced to nitrite or nitrate, which can be easily measured. Recently, Mertens, VandenWinkel & Massert (1975) described an automated procedure for the

simultaneous analysis of ammonium, nitrite and nitrate based on the analysis of ammonia after a heterogeneous reduction utilizing a Devarda's alloy column. The use of this unique reduction column in our automated system, together with the ammonia gas sensing electrode, eliminates the irradiation time necessary for the oxidation of ammonium or ammonia, since quantitation is based on all three forms of inorganic nitrogen. Quantitative recoveries of organic nitrogenous compounds in aqueous media are obtained with considerably shorter times of irradiation (17 min as compared to 1 to 2 h).

EXPERIMENTAL

Apparatus

The autoanalysis system for total nitrogen is shown diagrammatically in Fig. 1. Basically, the system consisted of an automatic sampler, a proportionating pump, a water bath, a series of quartz coils irradiated by an u.v. lamp, a reduction column, an electrode assembly, a high impedance pH/mV meter and a strip chart recorder. The system was driven by a Technicon II proportionating pump. A Gilson automatic sampler utilizing a sample time of 2.5 min and a rinse time of 3.5 min facilitated sampling. The constant temperature bath was fashioned from an aquarium heater, a glass container, and a delay coil. The u.v. light assembly consisted of quartz delay coils centered around a 0.75 × 12 in 1200 W high pressure quartz mercury vapor arc lamp housed in a sheet metal reflector. The lamp and voltage stabilizing transformer were obtained from Conrad-Hanovia, Inc., Newark, New Jersey. The mercury lines as a function radiated energy are given in Table 1. The quartz delay coils were in two sections, each 2.4 mm i.d., with a coil diameter of 5.5 in and each section approximately 15 coils. A high speed fan placed at one end of the housing maintained the temperature at the coils at about 32° C. An Orion 95-10 ammonia selective electrode fitted with an Orion 94-00-25 flow through cap was the sensing assembly. The potentiometric output of the electrode assembly was continuously recorded using an Orion model 801 digital pH/mV meter and a Health/Schlumberger strip chart recorder.
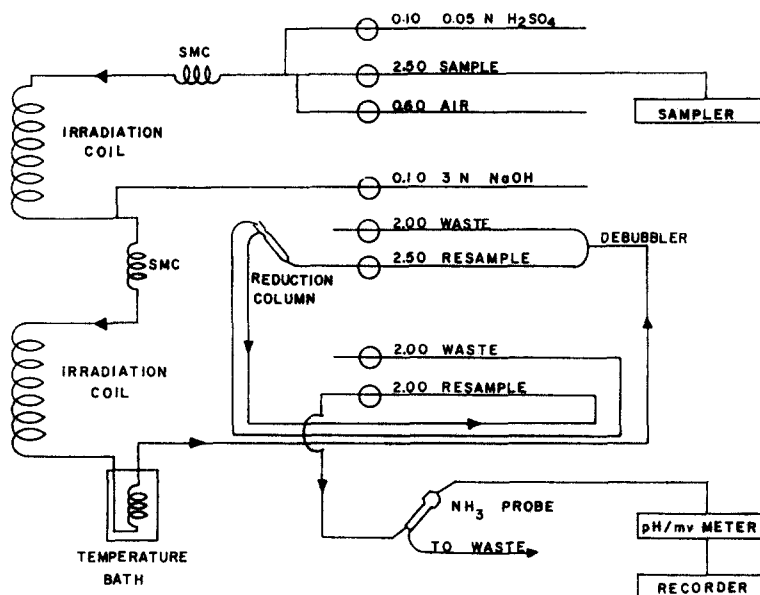
Fig. 1. Flow diagram of the automated system.

Table 1. Spectral energy distribution of the mercury vapor lamp

Mercury lines (Å)	Radiated energy (W)
13,673–10,140 (Infrared)	48.68
5780–4045 (Invisible)	187.07
3660–3341 (Near u.v.)	104.03
3130–2804 (Med. u.v.)	117.01
2753–2224 (Far u.v.)	116.15

Column

The reduction column, usually 5.5 mm i.d. × 30 mm formed from disposable Pasteur pipets, was packed with Devarda's alloy that had been sized to 100 to 140 mesh and washed 10 to 15 times with distilled water to eliminate fines. To prevent channeling, quartz wool was packed every 10 mm. Conventional slurry packing techniques utilizing a vibrator allowed maximum packing of the alloy. The degassing system, necessary due to the large H_2 gas production, consisted simply of having the column open to the air and drawing off the sample below a set volume (0.1 ml).

Reagents

All the reagents were reagent grade. Stock solutions of 1000 ppm N of all compounds examined were prepared and stored at 4° C in amber glass bottles. Dilutions were made with ammonium free water made by double acid distillation of deionized water in a silica apparatus. The Devarda's alloy was obtained from Fischer Scientific.

Procedure

Aliquots from a discrete automatic sampler were aspirated into a stream of air-segmented solutions flowing at a constant rate. The air was bubbled through a 1 M H_2SO_4 solution to prevent ammonia uptake. Sample irradiation was done in acidic medium (pH 2.0) in one of the coils of the irradiation assembly. After leaving the first coil, the

solution's pH was adjusted to above 13.0 with alkali and passed through the second coil. After irradiation, the solution was debubbled and passed through the reduction column. Finally, the sample stream was resampled and pumped to the electrode cell.

The inorganic nitrogen analysis was accomplished by bypassing the irradiation assembly. The ammonium content of the irradiation products was determined by simply bypassing the reduction column. A 300 ppb N as NO_3^- blank usually was utilized to facilitate a better sample to wash ratio over the concentration range 300 to 3000 ppb N. A sampling rate of 10 h^{-1} was employed. The time to reach the steady state response of the electrode and the time to rinse back to the background level as function of concentration, is given in Table 2. Organic nitrogen in a mixed sample was obtained by the difference in total nitrogen analysis and the total inorganic analysis.

Table 2. Analysis time as a function of concentration

Standard ppb $N-NO_3$	Response time (s)	Rinse time (s)
500	180	60
1000	150	120
2000	120	150
3000	100	180

RESULTS AND DISCUSSIONS

Choice of irradiation conditions

Manny *et al.* (1971) observed that irradiation of a number of organic nitrogenous compounds resulted in a distribution of nitrogen among ammonium, nitrite and nitrate. The distribution was dependent on pH, oxygen concentration, and the compound being irradiated. Goulden, Afghan & Ryan (1971) optimized conditions such that a quantitative analysis

Table 3. Per cent distribution of the irradiation products of urea and glycine as a function of time and pH

| Time of irradiation (min) | pH | Product distribution for | | | |
| | | Urea | | Glycine | |
		% $NH_3 + NH_4^+$	% $NO_3^- + NO_2^-$	% $NH_3 + NH_4^+$	% $NO_3^- + NO_2^-$
8.5	2	40	60	69	—0—
17.0	2	87	13	74	21
8.5	13.2	5	5	100	—0—
17.0	13.2	5	5	100	—0—

could be obtained by observing the nitrite and nitrate concentrations after 1 to 2 h of irradiation. In our system, the use of a higher intensity u.v. lamp (1200 W as compared to 550 W) and the advantage offered by the unique reduction column were effective in reducing the total time of irradiation.

A number of experiments were conducted to define the distribution of nitrogen combustion products of glycine and urea, after irradiation in acidic and caustic media for varying times of exposure. Table 3 contains the results of these experiments. As previously reported (Goulden et al., 1971), irradiation in an acidic and alkaline media was necessary for the decomposition of organic compounds. A quantitative recovery of urea was obtained in an 8.5 min acidic irradiation, but not so for glycine. Glycine, however, could be quantitatively recovered in an 8.5 min caustic digestion, which resulted in little decomposition of urea. It can also be observed that the distribution of nitrogen would require the simultaneous analysis of ammonium, nitrite and nitrate offered by the Devarda's alloy reduction colums to observe a quantitative recovery of total nitrogen in a single analysis.

Recovery of organic nitrogen

The decomposition of different organic compounds was confirmed by irradiation of these compounds in distilled water and by spiking same amounts of these compounds in actual natural waters. Table 4 reveals

Table 4. The recovery of nitrogen from various organic compounds following u.v. irradiation

Name of compound	Known concentration of nitrogen (ppb)	Actual concentration found (ppb)
Glycine	500	485
Urea	500	505
dl-Alanine	500	500
Leucine	500	500
EDTA	1000	950
NTA	500	420
Pyridine	1000	900
s'-Diphenylcarbazone	1000	140
Semicarbazone	1000	300
Dinitrophenylhydrazine	1000	200
3-Methyl-1-phenyl-2 pyrazolin-5-one	1000	310

that the carbon–nitrogen bonds were being quantitatively cleaved, even in a ring such as pyridine. The more stable nitrogen–nitrogen bonds seemed to resist the conditions employed, and poor recoveries for similar compounds were previously reported (Goulden et al., 1971).

The inorganic nitrogen irradiation decomposition products of some of the organic compounds were examined. Bypassing the reduction column allowed for the analysis of ammonium and not nitrate or nitrite. Table 5 indicates that cleavage of the amino acid type compounds readily occurred. Urea was the only compound found to consist mostly of either

Table 5. Ammonia produced by u.v. irradiation of various organic compounds

Name of compound	Known concentration of nitrogen (ppb)	Concentration of nitrogen found as ammonia (ppb)
Glycine	500	520
EDTA	1000	930
NTA	500	440
dl-Alanine	500	540
Leucine	500	490
Urea	500	75

nitrate and/or nitrite. To insure that no contamination was in the test compounds, the u.v. lamp was shut off. No nitrogen was recovered for any of the organic test compounds. Samples of natural waters were obtained from Saginaw Bay in Michigan. This water was filtered through 0.45 μm membrane filters, reaerated with oxygen, analyzed for total nitrogen, and spiked separately with 1 ppm N as urea and glycine. Under these conditions, recoveries of 93 and 98% were observed for both urea and glycine.

Performance of reduction column

Previous investigators (Mertens et al., 1975) found that it was necessary to form plastic bonded Devarda alloy beads since the available powder was not conducive to the high flow rates utilized with the automated electrode detector system. We had no problem in obtaining the commercial alloys in the 50 mesh or less variety. In this study, the columns were prepared

from the pure alloy, thus eliminating the task of forming the plastic beads.

Columns of varying size from 5.5 × 20 mm to 5.5 × 50 mm were utilized throughout our study. A column (5.5 × 30 mm) packed with 200–270 mesh metal performed will with 100% reduction after 16 h of continuous use. Back pressure problems were sometimes encountered. Columns of the same size, but packed with 100–150 mesh metal, performed with 100% efficiency of reduction for 20 h of use with a 12 h storage period. The life of the columns was not limited to 20 h, but that was the maximum time that most were utilized. The life of the column can be extended by washing and storing the column in distilled water.

Sensitivity, precision and accuracy

Calibration curves obtained for both nitrate and ammonia concentration ranges of 300 to 3000 ppb N fell within 0.5 mV for each comparable concentration. The ammonia and nitrate curves had slopes of 53 mV/decode.

The limit of detection was found to be dependent on the concentration of the blanking solution and the sampling rate. Blanking with a 300 ppb N as NO_3^- solution and a sampling rate of $10\,h^{-1}$, deviation from linearity appears for concentration below 300 ppb N and results in a detection limit of about 75 ppb N. Blanking with 50 ppb N and a sampling rate of $6\,h^{-1}$ has a detection limit of about 20 ppb N. The lowest concentration detected while blanking with acid distilled water was 10 ppb N with a sampling rate of $3\,h^{-1}$.

The precision of the method can be divided into that for the sensor response, the reduction column, and the entire system for organic nitrogen. The per cent relative standard deviation determined for seven replicate samples containing 359 ppb N as NH_3 was 1.7%. Seven replicate measurements for a sample 270 ppb N as NO_3^- resulted in a per cent relative standard deviation of 2.6%. The precision of the entire organic nitrogen system, based on seven replicate measurements of the concentrations of each of the organic compounds presented in Table 3 and expressed as per cent relative standard deviations was between 3.1 and 4.5%.

Assessment of accuracy can be divided into that for the reduction column and for the entire system. The reduction column was changed when the efficiency fell below 98%, determined in continuous comparison to ammonia standards. This was accomplished by measuring a nitrate and an ammonia standard every eight samples. The accuracy of the nitrate determination is also shown by standard addition. The recovery of a 200 ppb N as NO_3^-, an addition to a Saginaw Bay sample of 279 ppb N, was 97%. The accuracy of the entire organic nitrogen system based on the average recovery of nitrogen from organic compounds (Table 4) was 97% for carbon–nitrogen bound compounds and 24% for the nitrogen–nitro-

Table 6. Recovery of nitrogen as urea from natural water after u.v. irradiation

Sample nitrogen concentration (ppb)	Known nitrogen spike (ppb)	Concentration found (ppb)	% Recovery
760	1000	1710	95
550	2000	2510	98
840	1000	1750	92
550	1500	2000	97

gen bound compounds. Spiking of natural water samples with urea gave further insight into the accuracy of the analysis. Table 6 illustrates typical recoveries of urea.

CONCLUSIONS

An improved sensitive automated procedure for the analysis of dissolved organic nitrogen, common to natural waters, was developed. Although compounds containing nitrogen–nitrogen bonds are not quantitatively recovered, the organic-nitrogen content of natural waters is usually attributed to products of biological processes (amino acids, polypeptides, and proteins) which consist of carbon–nitrogen bonds. It should be pointed out that a Kjeldahl nitrogen determination fails to account for the nitrogen in some of the compounds found to give poor recoveries by our system. Since carbon–nitrogen bonds were quantitatively recovered by the automated system, this technique should prove quite satisfactory for the analysis of dissolved organic nitrogen in natural waters. This statement is further substantiated by a comment made in the article by Armstrong *et al.* (1966). Since pyridine is quantitatively recovered, in view of its refractory nature, leaves little doubt that most likely nitrogenous compounds dissolved in water would also be quantitatively recovered.

A principal shortcoming of this system is the relatively long time of analysis required at low concentration levels, e.g. less than 50 ppb N. This is due to the slow diffusion of ammonia gas across the membrane of the electrode system. A dichotomy exists in that a main advantage of the system is the high selectivity of the sensor electrode which renders the system applicable to a wide variety of aqueous solutions. This reduces interferences more effectively than in colorimetric techniques. Furthermore, this system has the advantage of a short irradiation time, facilitated by a unique reduction system, which allows this system to be used for both total inorganic and organic nitrogen dissolved in natural waters.

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