A STUDY OF THE AUTOANALYSER II SYSTEM

AND

REGENERATION AND ASSIMILATION OF NITROGEN COMPOUNDS IN MARINE AND FRESHWATER ENVIRONMENTS USING THE N-15 ISOTOPE TECHNIQUE.

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INTRODUCTION.

This report is devided into two parts. The first part deals with nutrients (nitrate/nitrite, ammonia, phosphates and silicate) determinations using the Autoanalyser II system while the second part deals with the use of Nitrogen-15 isotope in studying ammonia diffusion and nitrogen assimilation and regeneration occuring in marine and freshwater environments. Since analysis of the different nutrient elements involves only the changing of the manifold part of the autoanalyser, only the manifold descriptions are presented in this report.

PART 1

1. THE AUTOANALYSER II SYSTEM.

1.1. General Principles.

The Autoanalyser II is basically composed of five distinctive parts, namely: the Sampler, the Proportioning pump, the Manifold, the Colorimeter and the Recorder. Below, is a short description of each part.

(I). The Sampler.

The sampler performs the following functions:

(a) it holds 40 sample cups that contain the sample and reference solutions.

(b) it has a sample probe through which the samples are aspirated into the analytical system.

(c) it controls the amount of time for aspirating each sample and for the wash cycle. This is usually done with the help of a timing device.

(d) it mixes, when equipped with optional rotary mixer assembly, the contents of sample cups to keep samples and reference solutions in a homogeneous suspension prior to and during sampling.

The sample probe on the sampler is connected to the sample pump tube on the manifold. The probe, on command from the sampler, automatically dips into the sample cup and aspirates the sample contained

therein. The volume of sample aspirated is determined by the flow rate of the sample pump tube on the manifold and by the timing cam on the sampler. Between each sample, a segment of air and a segment of wash solution are pumped through the sample probe to cleanse the probe and pump tubes and to segregate each sample as it is introduced into the analytical system. Fig. 1 shows a picture of the sampler.

(II). The Proportioning pump.

The Proportioning pump (Fig. 2) draws the liquid sample into the Manifold from the sampler. At the same time the Proportioning pump also draws the necessary liquid reagents from the reagent containers and propells them into the Manifold where they are combined and mixed with the sample. Movement of the analytical stream through all the hydraulically interconnected elements of the system is accomplished by means of the peristaltic pumping action of the Proportioning pump. An air bar assembly on the Proportioning pump releases air into the flowing liquid stream at two-second intervals. This air segmentation acts as a barrier to prevent sample interaction and cross contamination and to scrub the tube walls clean.

(III). The Manifold.

This is the section where the reactions take place. Mixing of the sample and reagents happens within the Manifold. As shown later, individual Manifolds are provided for each method. Each sample enters the Manifold as a segmented, continuously flowing stream and is combined with reagents, mixed, heated (if necessary) and otherwise prepared for measurement as specified by the method. The reagents are supplied to the

measurement as specified by the method. The reagents are supplied to the Manifold from reagent containers through the pump. After the sample has been processed it is passed into the colorimeter for measurement.

(IV) The Colorimeter.

The colorimeter (Fig. 3) is used to measure direct (colour development) or inverse (colour reduction) colorimetric methods. In the Autoanalyser II system, a single-channel colorimeter is used to measure the concentration level of a particular constituent within a sample. These colorimetric measurements are made as the analytical stream passes through a flowcell. The light that passes through the sample stream (as the stream is passing through the flowcell) strikes a phototube to produce an electrical signal output. This output is measured against the output of a reference (or blank) channel. The difference between the two outputs is the test result which is charted by the Recorder.

(V). The Recorder.

All test results that are derived from the measurements modules are graphically traced across a slowly moving strip of chart paper. Either a single-pen or a two-pen recorder can be used for the measurement. For our analysis, a single-pen recorder was used. Fig. 4 shows a picture of a single-pen recorder.

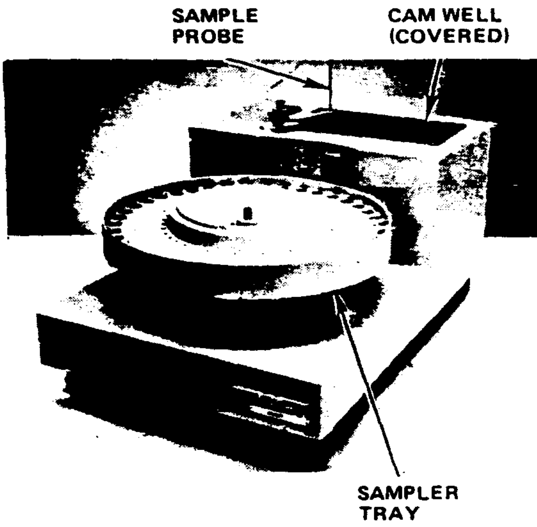


Fig. 1. The Sampler

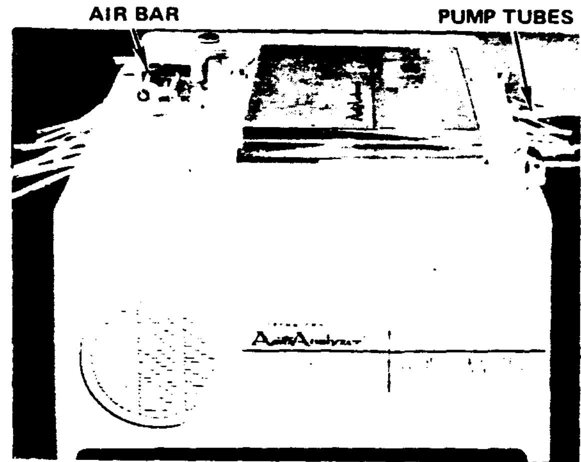


Fig. 2. Proportioning Pump

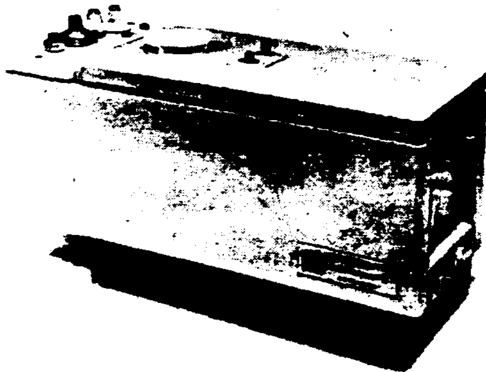


Fig. 3. Single - Channel Colorimeter

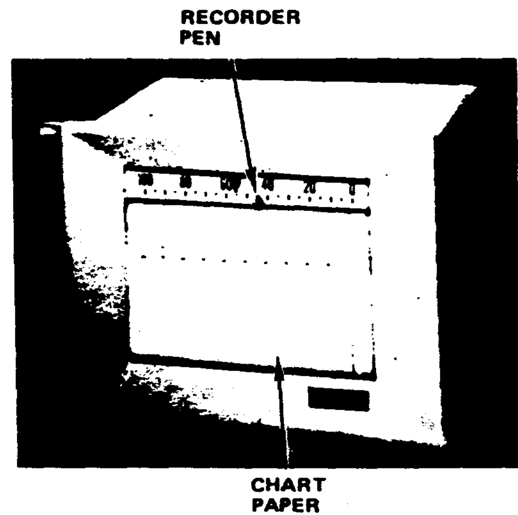


Fig. 4. Single - Pen Recorder

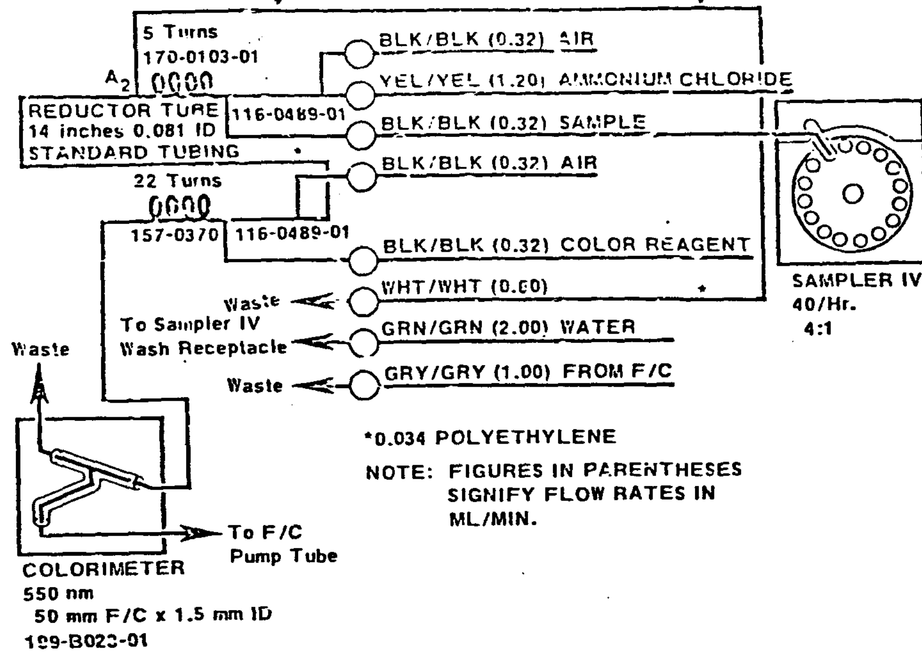


Fig. 5. Manifold for nitrate/nitrite determination.

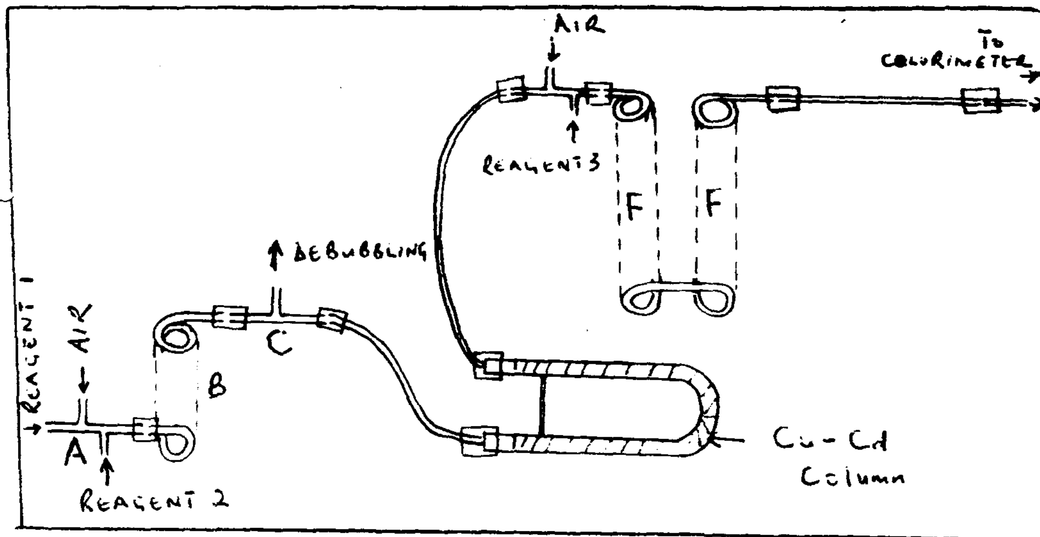


Fig. 6. Simplified drawing of the nitrate/nitrite manifold.

1.2. Determination of Nitrate/nitrite.

The automated method for the determination of nitrate and nitrite utilizes the procedure whereby nitrate is reduced to nitrite by a copper-cadmium reductor column. The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples wiht N-l-napthylethylenediamine dihydrochloride to form a reddish-purple azo-dye.

Since individual nutrients analysis depends on individual manifold used, only the manifold parts are discussed. Description of reagent preparation and tube connections will be found on annexes 1, 2, 3 and 4.

Fig. 5 shows the schematic drawing of the Manifold used for nitrate/nitrite determination. As seen in Fig. 6 (simplified drawing of the manifold), Reagent I (ammonium chloride solution) enters the Manifold through glass tube A. It is then broken into segments by the introduction of air bubbles. The sample is added through the Reagent 2 line. The ammonium chloride and sample are mixed thoroughly within coil B. Before the flow stream is passed through the Cu-Cd column for reduction to nitrite, the air bubble is debubbled out at glass tube C. From the Cu-Cd column, air bubbles are again re-introduced to segment the stream before the colour reagent is added through Reagent tube 3. Thorough mixing is done in coil F before the stream is passed into the colorimeter. In the colorimeter, all air bubbles are removed before the resultant stream passes through the flowcell for measurement.

General comments on nitrate/nitrite determination.

1) It is important to effectively debubble the air bubble before the sample enters the reduction column. Air bubbles should not be allowed to enter the column. Should this happen, the column should be reconditioned.

2) The reduction column should be filled with Cu-Cd carefully not to allow trapped air bubbles.

3) In case the flow in the manifold tubes is not uniform, pass ammonium chloride reagent (containing Brij-35 solution) for about 15 minutes while all the other tubes are in distilled water. The Brij-35 solution is a wetting agent.

4) At the end of the analysis, wash the system with ammonium chloride while the other tubes are in distilled water. Then, carefully disconnect the column. The column should always have ammonium chloride buffer in it and should be stored vertically.

1.3. Determination of Ammonia.

The automated procedure for ammonia determination utilizes the Berthelot reaction procedure. The Berthelot reaction is the name given to the reaction of ammonium ions and phenol, which under suitable oxidizing conditions, result in the formation of an indophenol dye. These dyes are highly conjugated and absorbed between 620 and 720 nm.

For reagent preparation, see annex 2.

Fig. 7 shows a schematic drawing of the ammonia manifold while Fig. 8 shows a simplified version of the same. Reagent 1 (complexing reagent) is introduced into the manifold through glass tubing A. Immediately after introduction, it is segmented into small segments by the introduction of air bubbles. Reagent 2 (sample) is then introduced and the two are allowed to mix in the first part of the mixing coil B, before the introduction of reagent 3. Sodium hypochlorite (Reagent 4) is then introduced and again mixing takes place in the first part of mixing coil D. After this mixing, reagent 5 (sodium nitroprusside) is introduced and the mixing repeated before the stream is passed through the heating bath (50°C). From the heating bath, the stream is mixed again in coil C and finally introduced into the colorimeter where its absorbance reading is passed onto the recorder which inturn plots the corresponding peak.

General comments on ammonia determination.

1) Avoid changing the tubings on the manifold unless it is absolutely necessary. For each specific analysis, it is necessary to have a particular "set of tubings" for the pump part. When changing from one analysis to another, just change this particular "set of tubing" and leave the manifold tubings intact.

2) Whenever a tubing is changed at the manifold, the system has to be conditioned before sample analysis is done. A common problem that arises when the system is not conditioned is getting uneven flow of the stream. Steady uniform flow is very essential before any analysis is made. The air introduction and separation must also be uniform.

3) When the flow is not uniform, wash the system with a solution containing Brij-35 (preferably 1 ml of Brij-35 in 100 ml of distilled water).

4) When a precipitate appears in the tubes, wash the system with 10% HCl.

5) Remember to set the temperature of the heating bath of 50°C.

6) The distilled water for washing the sampling probe should always be in a separate container. The GRN/GRN tube for washing should not be mixed with the other tubes when washing the system.

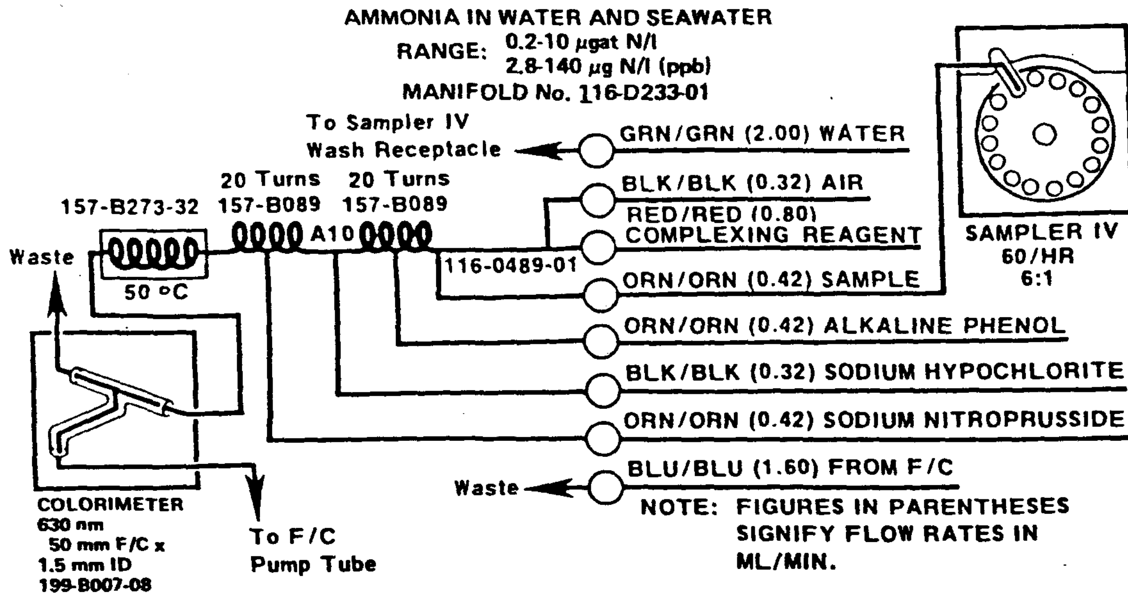


Fig. 7. Manifold for the Ammonia determination.

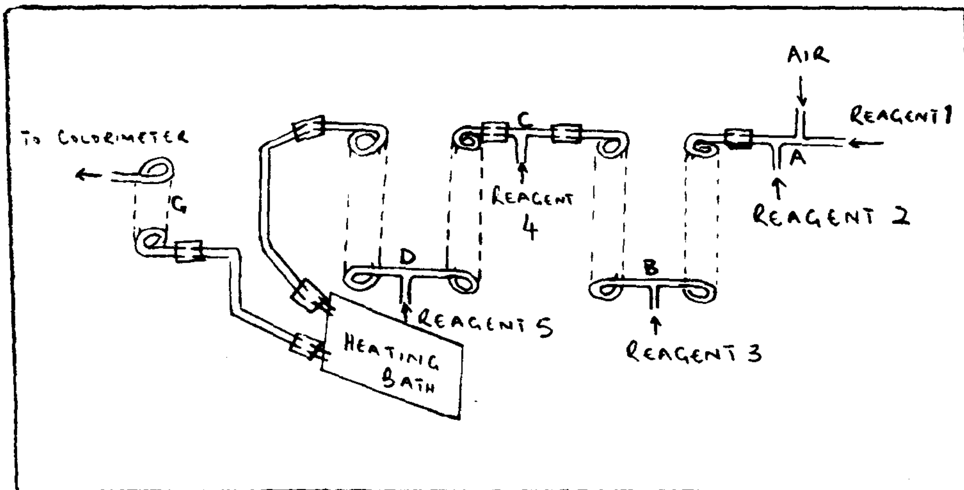


Fig. 8. Simplified drawing of the Ammonia Manifold.

1.4. Silicate determination.

This automated procedure is based on the reduction of silicomolybdate in acid solution to "molybdenum blue" by ascorbic acid. Oxalic acid is introduced to the sample stream before the addition of ascorbic acid to eliminate interference from phosphates.

For reagents preparation, see annex 3.

Manifold description.

Fig. 9 gives a complete silicate manifold flow chart while Fig. 8 can still be used as the simplified version. However, the reagent 5 tube should be closed. For silicate determination the reagent 1 tube is for ammonium molybdate reagent while reagent 2 tube is for the sample. Reagent 3 and 4 tubes are for oxalic acid and ascorbic acid respectively. Except for the different flow-rate tubes at the pump, mixing and transmition of the sample is exactly as described for the ammonia determination.

Comments on silicate determination.

1) When changing from ammonia to silicate or phosphate analysis, wash the system with 1.0% HCl, then distilled water, then 10% NaOH and finally with distilled water again.

2) Levor IV or Levor V is used as the wetting agent.

3) When the flow is not uniform, place all the tubes in a beaker containing

levor solution (1 ml/100 ml H_2O). Remember that the GRN/GRN tube should not be placed in the levor solution.

4) Though not shown on the manifold chart, it is necessary to set the temperature to 50°C as for ammonia determination.

5) Synthetic sea water may be prepared by just dissolving 34.5 g of NaCl in 1 liter distilled water (34.5 °/₀₀).

1.5 Phosphate determination.

The automated procedure for the determination of ortho-phosphate in sea water depends on the formation of a phosphomolybdenum blue complex which is read colorimetrically.

A single reagent solution is used consisting of an acidified solution of ammonium molybdate containing ascorbic acid and a small amount of antimony. The range covered by this determination is 0-4.0 µg at P/1.

For reagents preparation, see annex 4.

Manifold description.

While Fig. 10 gives a schematic drawing of the phosphate manifold flow chart, Fig. 8 can still be used as the simplified manifold chart. However, in this case, reagent 4 and 5 tubes are closed. Reagent 1 tube allows in water diluent which is essentially the levor solution used for wetting the tubes.

Reagent 2 tube is used to pass the sample while reagent 3 tube passes in the combined working reagent. Mixing and transmition takes place as described for the ammonia determination.

Comments on phosphate determination.

Same as for silicate except that for phosphate the temperature set should be 37.5°C.

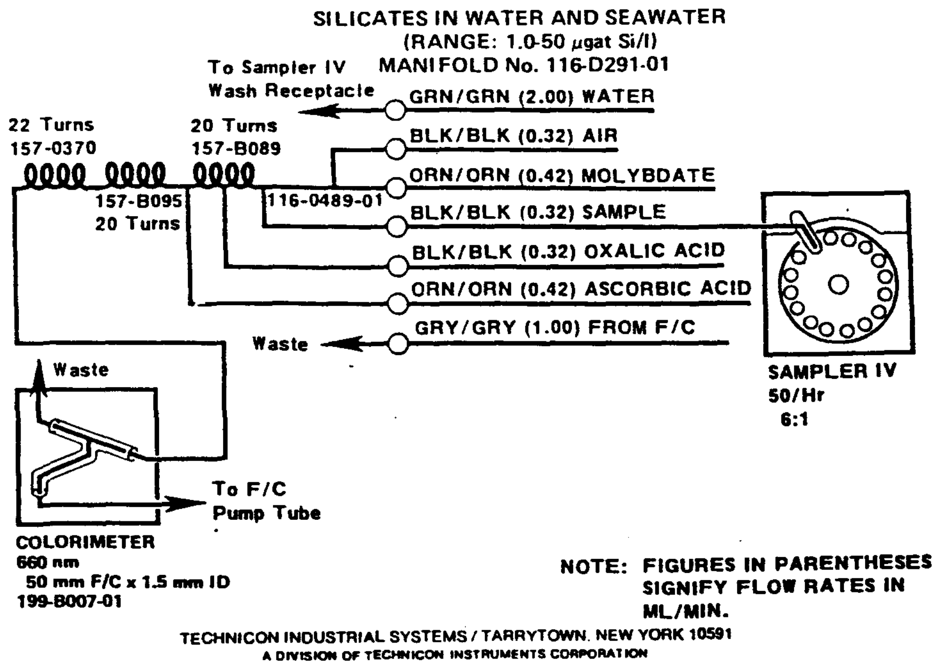


Fig. 9. Silicate Manifold flow-chart.

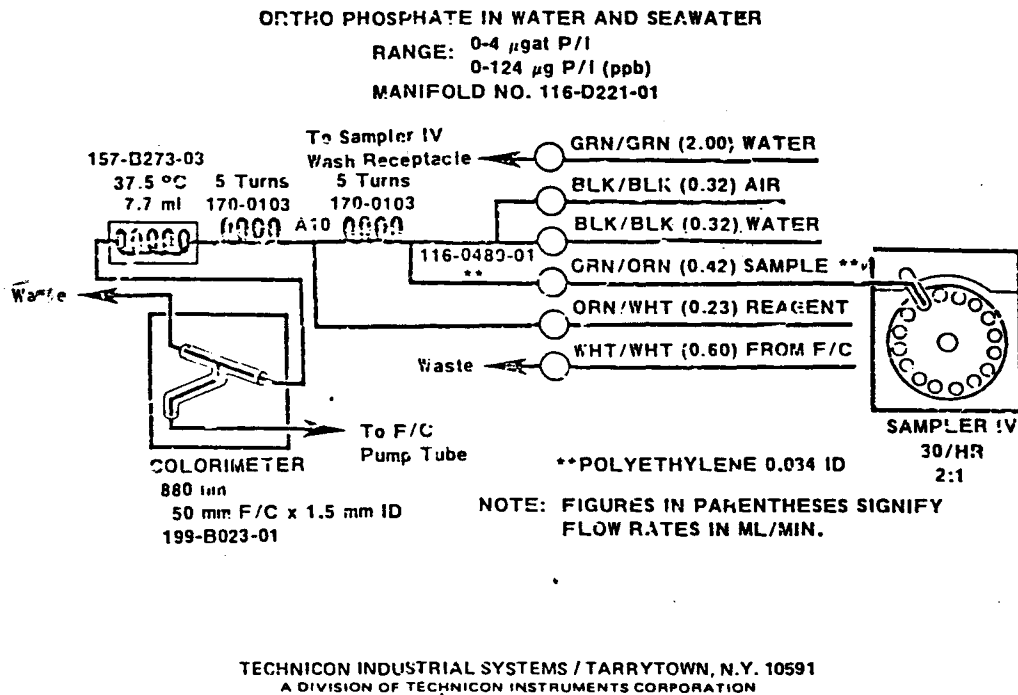


Fig. 10. Phosphate Manifold flow-chart

PART II

2. ASSIMILATION AND REGENERATION STUDIES USING NITROGEN-15 ISOTOPE TECHNIQUE.

2.1. General background.

Carbon, phosphorus and nitrogen have been shown to be the major inorganic nutrients required for primary production in both marine and freshwater environments. However, due to a surplus of carbon present as CO_2, the main limiting nutrients are phosphorus and nitrogen. Several sources, such as river runoff, sewage and anthropogenic means contribute to nutrients input in the marine environment. However, at the rapid rates of phytoplankton growth sometimes found, these relatively low concentrations of nutrients would be completely depleted if there were no regeneration.

Dugdale and Goering (1967), introduced the concept of new and regenerated production when they partitioned the primary production of oceanic waters according to the nitrogen source. New production is associated with inputs to the photic zone, mainly from upwelling nitrate, land runoff, nitrogen fixation and precipitation while regenerated production is associated with nitrogen input mainly from nitrogen nutrients remineralized within the photic zone. This is mainly ammonium regeneration. Traditionally, this ammonium regeneration has been regarded as a product of bacterial degradation of organic material and of macrozooplankton grazing and excretion (Selmer, 1988).

The present knowledge about assimilation and regeneration of nitrogen compounds is based largely on recent studies using isotope tracer

techniques. Though a number of radio isotopes (e.g. ^{13}N) have been used in nitrogen studies, stable N-15 isotope remains the most widely used tracer.

In any natural state, nitrogen occurs as ^{15}N and ^{14}N. Nitrogen-14 is usually the most abundant, being 99.63% while N-15 is only 0.37%. Most nitrogen studies in the marine environment have dealt with the utilization of dissolved nitrogen compounds by planktonic micro-organisms and the general experimental design has consisted of:

1) Confinement of a sea water sample - usually in a bottle
2) Inocculation with a labelled nitrogen (e.g. $^{15}NH_4^+$) compound
3) Incubation
4) Collection of the particulate organic material (plankton),
5) Measurement of isotopic tracer incorporation of the sample, usually by either mass or emission spectrometer.

After the sample isolation, its isotopic analysis generally follows two steps:

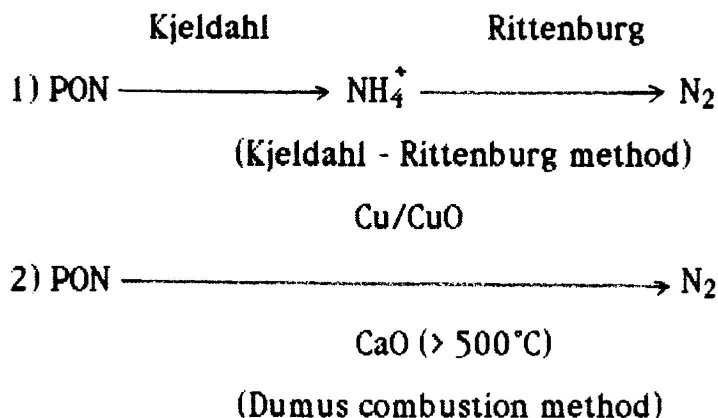
(1) Sample conversion;

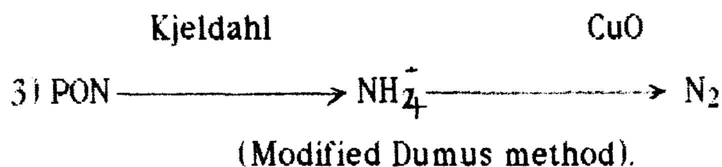
$$-\overset{|}{\underset{|}{N}}- \longrightarrow N_2 \text{ (gas)}$$

(2) Isotope detection, either by mass of emission spectrometer.

<u>Sample conversion</u>.

The most popular conversion methods are:



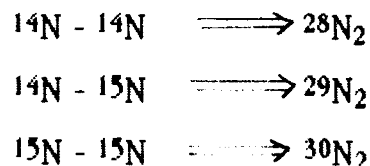


(Modified Dumus method).

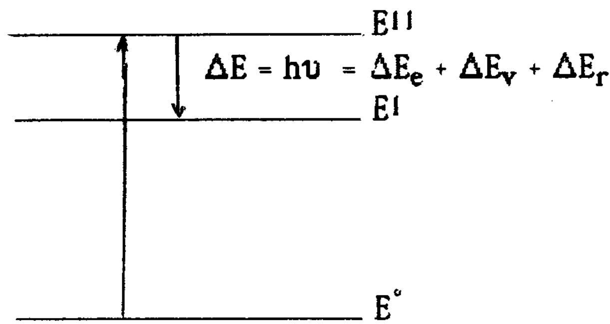
In earlier studies, Kjeldahl digestion was used to convert particulate organic nitrogen to NH_4^+ which was subsequently steam distilled and converted directly to N_2 by reaction with sodium hypobromite (Rittenburg method). The Dumus combustion method involves direct conversion of particulate organic nitrogen to N_2 by heating (> 500°C) in the presence of a copper (Cu + CuO) catalyst. The CuO is to oxidize the PON to N_2 while the Cu is to reduce exisiting NO_3^-, NO_2^{2-} to N_2. CaO acts as an absorbant. The third method (modified Dumus method) has become the most commonly used conversion method. It incorporates both the Kjeldahl and Dumus combustion methods.

Isotope detection.

Due to the two stable nitrogen isotopes (N-14 and N-15) nitrogen gas can exist as:



When the nitrogen gas is passed through a high frequency generator (EI), the electrons are excited to a higher energy level, E^{11}. When these electrons fall to a lower energy level E^{1}, ΔE energy is released.

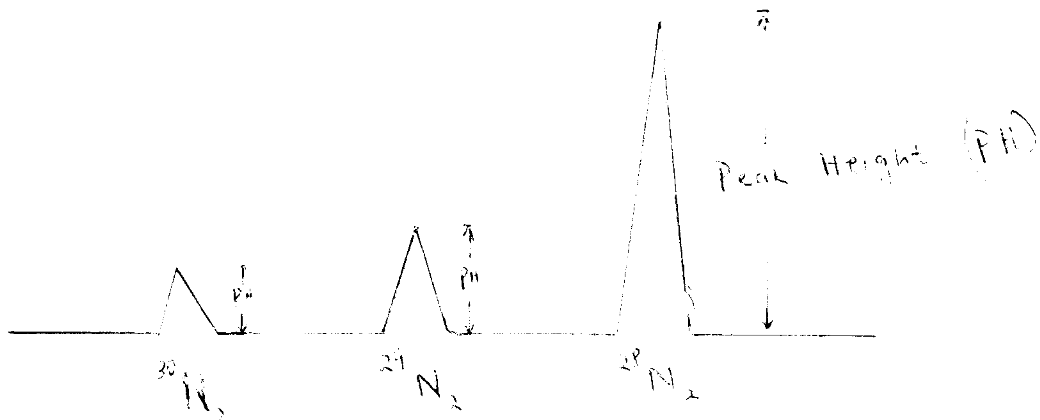


$$\therefore \quad h\upsilon = \Delta E_e + \Delta E_v + \Delta E_r$$

But $\upsilon \, \frac{c}{\lambda}$

$$\text{Hence} \quad h\frac{c}{\lambda} = \Delta E_e + \underbrace{\Delta E_v + \Delta E_r}_{f\,(\text{mass})}$$

Now since $\Delta E_v + \Delta E_r$ is a function of mass and h and c are constant, then any changes of $\Delta E_v + \Delta E_r$ will directly affect λ (wavelength). Since the three nitrogen forms would have different mass, they would then have different wavelengths (λ). So using a scanning system, the mass spectrometer or emission spectrometer would be able to register the difference.



By definition, % $^{15}N = \frac{100}{2R+1}$

Where $R = \frac{^{28}N_2}{^{29}N_2}$

But since the intensity is proportional to the peak height, then

$$R = \frac{I(^{28}N_2)}{I(^{29}N_2)} = \frac{PH\ (^{28}N_2)}{PH\ (^{29}N_2)}$$

Therefore, knowing the peak heights, % ^{15}N can easily be calculated.

2.2. Calculations of uptake and regeneration rates.

After sample collection and processing for N-15 analysis, uptake rates are calculated using the formulation given by Dugdale and Goering (1967);

$$\upsilon = [d\ (a_n)/dt]/\ (a_s - a_n) \qquad (1)$$

Where,

υ = specific uptake rates in units of time $^{-1}$

a_n = atom % ^{15}N in the particulate nitrogen (N)

a_s = atom % ^{15}N in the substrate.

For incubation of duration t, this equation becomes:

$$\upsilon = \frac{P}{N} = \alpha_t / R.t \qquad (2)$$

Where,

α_t - atom % excess (atom % ^{15}N minus 0.37, the natural abundance of ^{15}N) of PN at time t.

R = the atom % enrichment of the substrate $[(100.S_L/S_L + S_U)]$.

Where,

S_L = amount of labelled substrate added

S_L = amount of unlabelled substrate present

P = absolute uptake or transport in unit of mass (unit volume)$^{-1}$.time^{-1}.

For nitrogen regeneration studies, Harrison et al., 1977 used the equation of the form;

$$r = (S_t - S_0)/t + P$$

for calculating regeneration rates.

In this equation,

r = regeneration rate

S_0 = substrate concentration at time 0

S_t = substrate concentration at time t

P = substrate uptake rate, which is computed from equation 2.

However, a more generally applicable, linear differential-equation model that has the capability of estimating both regeneration and uptake (assimilation) from the change in concentration and specific activity of the substrate was introduced by Blackburn (1979) and Caperon, et al. (1979). In this ^{15}N isotope dilution technique, the rate of ammonium regeneration is determined by labelling the product, i.e. ammonium. The ^{15}N-labelled ammonium is initially added to the water sample and the dilution of the atom % ^{15}N with regenerated ammonium of only the natural background ^{15}N abundance (0.37%) is monitored with time. By determining the initial and final ammonium concentration and atom % ^{15}N of the ammonium pool, the rate of dilution of the ammonium pool (equal to the rate of ammonium regeneration) can be calculated. Once the regeneration rate is calculated, the uptake rate can also be calculated from the Blackburn's equations.

Blackburn's regeneration and uptake equations.

$$r = \left(\frac{P_f - P_i}{t} \right) \cdot \left(\frac{\ln \frac{A_i}{A_f}}{\ln \frac{P_f}{P_i}} \right) \qquad (4)$$

$$U = r. - \left(\frac{P_f - P_i}{t} \right) \qquad (5)$$

Where,

r = regeneration rate

U = uptake (assimilation) rate

P_i = initial ammonium concentration in the pool (immediately after adding

the spike).

P_f = final ammonium concentration in the pool (after incubation)

t = incubation time

A_i = excess % ^{15}N initially (immediately after adding the spike, t=0)

A_f = excess % ^{15}N finally (after incubation).

P_i and P_f are determined by the phenol-hypochlorite method (Solorzano, 1969) while A_i can be calculated (since we know the amount of labelled $^{15}NH_4^+$ added) and A_f is determined spectrometrically as described earlier.

2.3. Practical examples.

2.3.1. Ammonium isolation by diffusion process for nitrogen-15 isotope analysis.

Introduction.

The use of stable nitrogen-15 isotope has become very common in studies of assimilation and regeneration of nitrogen compounds in marine environment (Harrison, 1978; Axler et al., 1981; Selmer, 1988). Samples are incubated and inocculated with labelled nitrogen compounds, usually labelled $^{15}NH_4$ solution. The ^{15}N-labelled ammonium is initially added into the water sample (in a bottle) and the dilution of the natural background ^{15}N abundance (0.37%) is monitored with time (Selmer, 1988). One of the most common problems in these studies has been isolating the ammonium ion for isotope analysis. In the Kjeldahl-Rittenburg conversion method, the NH_4^+ is steam-distilled and converted to N_2 gas by reaction with sodium

hypobromite (Harrison, 1983; Caperon et al., 1979). While this remains the most common procedure, Blackburn (1979) used a diffusion process to isolate the NH_4^+ ion. With addition of a strong base in a sample (in a diffusion vial) the NH_4^+ ion was reduced to ammonia gas and diffused onto a glass capillary tube coated with a thin layer of sulphuric acid (Blackburn, 1979). Blackburn left the diffusion process to take place overnight before performing isotope analysis on the trapped ammonia.

In this short contribution, the time factor for the diffusion process and possibilities of isotope discrimination are examined.

Material and methods.

Using labelled ammonium sulphate, $(NH_4)_2SO_4$, of 97.5% ^{15}N and unlabelled ammonium sulphate, a solution of 4.0 mmol. $N\text{-}NH_4$/l with 10% ^{15}N was prepared. 5 ml of potassium hydroxide (50%) was then added and the vial stoppered immediately. The stopper had a long syringe needle passed through a plastic holder (Fig. 1). On the holder was a small tin cup with about 0.1 g Al_2O_3 and 10 µl sulphuric acid (0.25N). The OH reduces the NH_4 to NH_3 gas which diffuses into the sulphuric acid coated on the aluminium oxide.

Six diffusion vials were used. After 3, 6, 12, 30, 48 and 72 hours, the tin cups were removed, closed and folded, ready for the N-15 analysis.

For the N-15 isotope analysis, the Dumus combustion method was used (Harrison, 1983). The samples were put into thin glass tubes (Fig. 2) with copper oxide (CuO) and calcium oxide (CaO). The CaO was then heated and the air evacuated using a vacuum system before the tubes were sealed. The N-15 determinations were then obtained by getting the corresponding % N-15 values from a standard calibration graph obtained with certified N-15 standards.

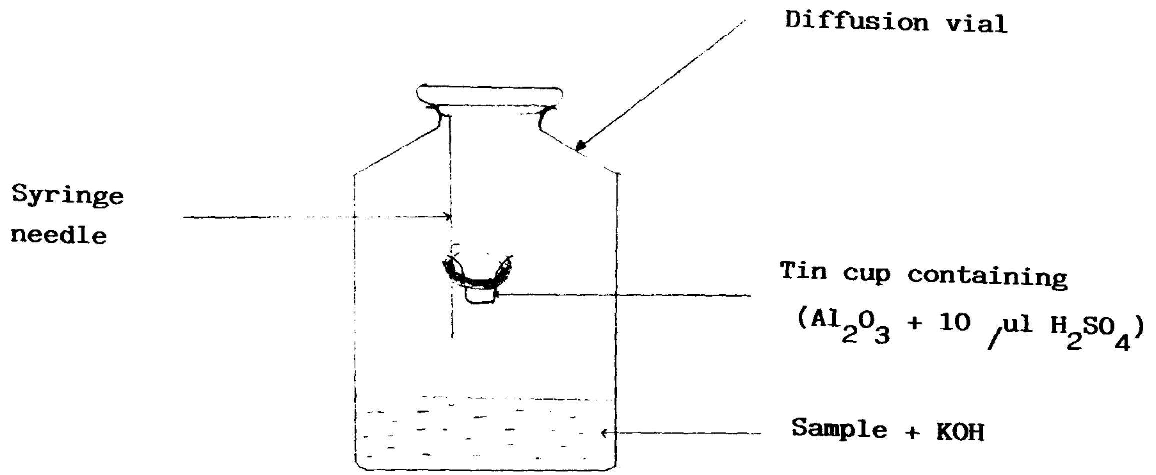


Fig. 1. Diffusion vial.

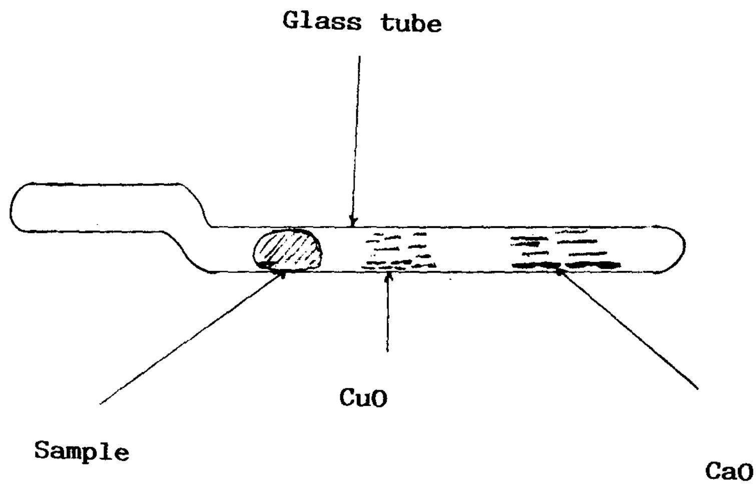


Fig. 2. Glass tube for N-15 isotope determination.

Results and Discussion.

Since we started with a labelled ammonium solution which had 10% ^{15}N, addition of a strong base (OH^-) would have generated ammonia whose N-15 percentage is 10. However from table 1, its seen that the maximum N-15 recovered, was 9.75% ^{15}N which is 97.5% of the original Nitrogen-15.

Fig. 3 shows the spread of % ^{15}N values with time. Apart from values obtained at 6 hrs., reproducibility appears very good. This is also reflected by the low standard deviation values obtained. A graph of mean corrected % ^{15}N values against time (Fig. 4) shows that the diffusion process is complete after 48 hrs.

These results indicate that isotope discrimination takes places during the diffusion process. Since % ^{15}N measurements are governed by monitoring $^{15}N/(^{15}N + ^{14}N)$ isotope ratio (Caperon et al., 1979), the relatively low % N-15 values obtained initially (i.e. at 3 hrs., 6 hrs., 12 hrs. and 30 hrs) means that more N-14 was being diffused. This can be attributed to mass difference between the two forms of nitrogen. Nitrogen-14 being lighter is released more easily. So in order to be sure complete diffusion has taken place, a time factor is very essential. From the results obtained, 48 hrs. is suggested to be the minimum time to be allowed for the diffusion process.

Table 1 Data obtained from the diffusion experiments.

Time (hrs)	Corr. % N-15(1)	Corr. % N-15 (2)	Corr. % N-15 (3)	Mean % N-15	S.D.	% Diffusion
3	8.90	8.82	9.00	8.91	0.07	89.10
6	9.40	9.60	9.12	9.37	0.20	93.70
12	9.50	9.60	9.21	9.44	0.17	94.40
30	9.50	9.40	9.75	9.55	0.15	95.50
48	9.60	9.80	9.82	9.74	0.10	97.40
72	9.83	9.70	9.71	9.75	0.06	97.50

Conclusion.

It is found that in the diffusion process for isolating ammonium for N-15 analysis, the maximum % ^{15}N recovered is 97.5%. Also, isotope discrimination takes place during the diffusion process due to the light nitrogen (N-14) being released relatively faster. Due to this, a time factor is very necessary to ensure complete diffusion. In this paper, 48 hrs. is shown to be the minimum time to be allowed to ensure complete diffusion before nitrogen-15 analysis is done.

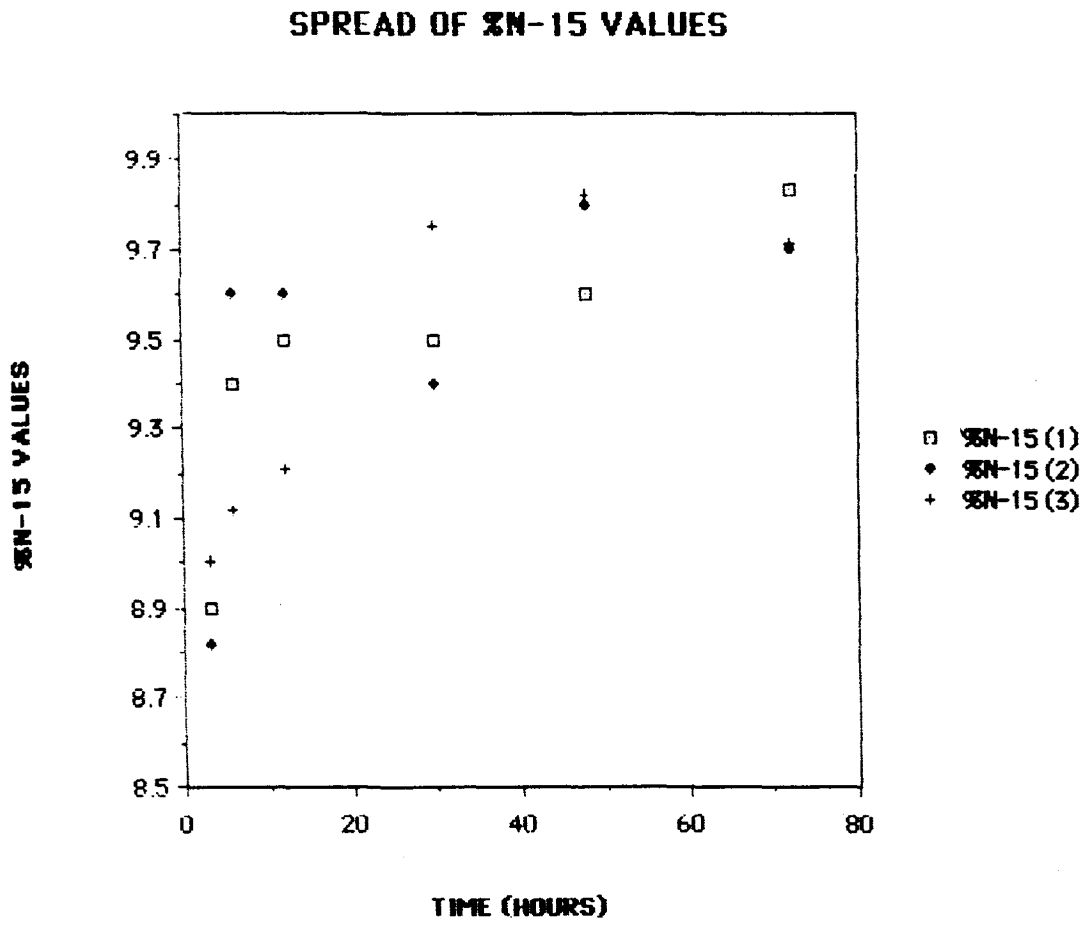


Fig. 3. Spread of % N-15 values with time.

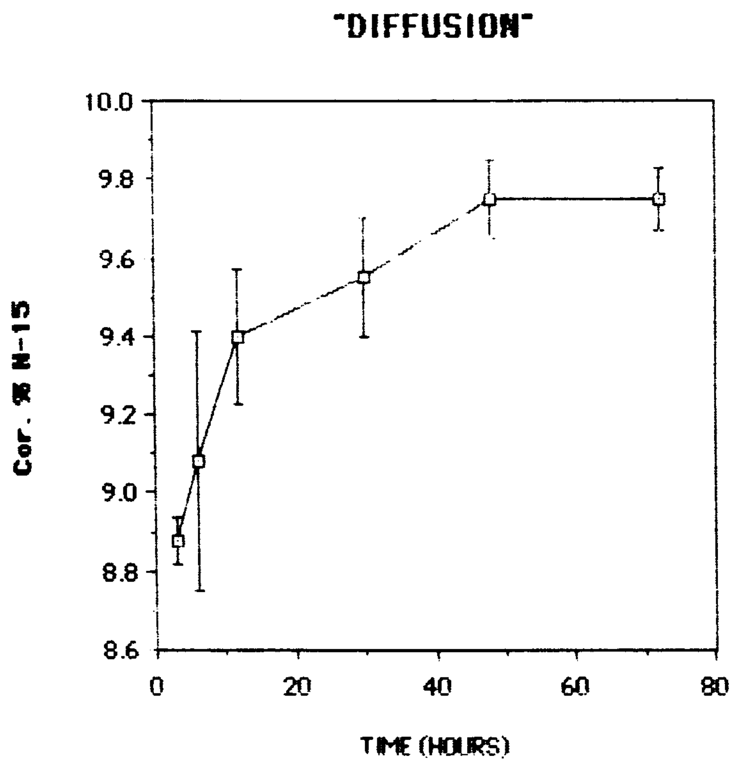


Fig. 4. Average corrected % N-15 values with time. The vertical bars denote standard deviation.

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1. Axler, R.P., and Goldman, C.R. (1981);
Isotope tracer methods for investigations of nitrogen deficiency in Castle Lake, California. Water Res. 15. 627-632.

2. Blackburn, T.H. (1979);
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3. Caperon, J., Schell, D., Hirota, J. and Laws, E. (1979)
Ammonium excretion rates in Kaneoke Bay, Hawai, measured by a ^{15}N isotope dilution technique. Mar. Biol. 54. 33-40.

4. Harrison, W.G. (1978)
Experimental measurement of nitrogen remineralization in coastal waters. Limnol. Oceanogr. 23. 684-694.

5. Harrison, W.G. (1983)
Use of isotopes. In "Nitrogen in the Marine Environment". (E.J. Carpenter and D.G. Capone eds.). pp 763-807. Academic Press, New York.

6. Selmer, J.S. (1988)
Ammonium regeneration in the marine environment.
Ph.D. thesis. Dept. of Microbiology Univ. of Goteborg. Sweden.

2.3.2. Uptake (assimilation) and regeneration measurements.

Introduction.

Using the presented discussion on uptake and regeneration calculations, a trial was made with water samples from Woluwe Lake park, Brussels. As pointed out earlier in equations 4 and 5, the most important parameters to be measured are; initial ammonium concentration (P_i, immediately after spiking), final ammonium concentration (P_f, at the end of incubation), initial % ^{15}N (A_i, usually calculated after adding the spike), final % ^{15}N value (A_f, usually obtained by mass or emission spectrometric analysis) and the incubation time, t.

Material and Methods.

Two 1-liter glass bottles were used for the incubation of the samples. Before the incubation process was started, the original ammonium concentration, P_0 was determined using the autoanalyser II system. This is a necessary prerequisite because the concentration of the labelled spike added is supposed to be about 10% of that of the sample concentration. The samples were then spiked with 1-ml of a 2.0 mmol. l^{-1} ammonium sulphate solution which had 97.5% ^{15}N. Immediately after spiking, the initial ammonium concentration, P_i was measured using the autoanalyser. After the incubation period (24 hrs.), the final ammonium concentration, P_f was also measured. Two 5 ml samples from each bottle were then taken and put in diffusion vial ready for the diffusion process as described earlier. However, from the P_f value obtained, a lot of ammonium seemed to have been used up. In this case we had to add a carrier NH_4^+ solution

(unlabelled) into our diffusion sample. 2 ml solution with 10 μg at N was added as the carrier. The main reason for adding the carrier is that the nitrogen isotope analyzer can only measure ^{14}N and ^{15}N only if they are within a certain concentration. So the carrier helps to dilute the N-15 if it is still too high and also raises the total nitrogen content if it is too low. So in our case, the total volume in the diffusion vial before the addition of potassium hydroxide was 7 ml. Corrections for the carrier added are done at the end of the analysis.

Results and Discussion.

Sample	Mean $[NH_4^+]_0$ (P_0) (μg-at N/1)	Mean $[NH_4^+]_i$ (P_i) (μg-at N/1)	Mean $[NH_4^+]_f$ (P_f) (μg-at N/1)	Initial excess % ^{15}N A_i.
1	14.00	16.90	9.35	16.66
2	14.10	16.90	9.45	16.09

Table 2: Shows mean original ammonium concentration, P_0, mean $[NH_4^+]_i$, P_i, mean $[NH_4^+]_f$, P_f, and mean calculated inital excess % ^{15}N, A_i.

As seen in table 2, a lot of NH_4^+ had been used after the incubation period (24 hrs.). Now since the P_f value appeared low, we had to add a carrier as discussed above.

Calculation of the initial % ^{15}N (A_i).

A_i = is usually calculated by using the formular.

$$A_m = \frac{(P_0 \times A_0) + (B \times A_B)}{P_0 + B}$$

Where,

A_m = % ^{15}N of the mixture immediately after adding the spike.

P_0 = original ammonium concentration.

A_0 = natural ^{15}N % abundance (0.37% ^{15}N) in the sample before the addition of the spike.

B = the difference in concentration between $[NH_4]_0$ and $[NH_4]_i$ which is P_i - P_0. This reflects the added ammonium concentration.

A_B = % ^{15}N of the added spike (97.5 % ^{15}N).

∴ For the first bottle we have:

$$A_m = \frac{(14.0 \times 0.37) + [(16.90 - 14.0) \times 97.5]}{14.0 + (16.90 - 14.0)} = 17.03 \% \ ^{15}N$$

But by definition A_i = excess % ^{15}N

∴ $A_i = A_m - 0.37$

$= 17.03 - 0.37$

$= 16.66 \% \ ^{15}N$

Similarly using the same calculation, 16.09 % ^{15}N is obtained as the excess % ^{15}N for the second bottle.

Calculation of the final % ^{15}N (A_f) after incubation.

Table 3 shows % N-15 obtained from the emission spectrometer after the diffusion process. Also shown are the corresponding standardized values obtained. The standardized values are obtained by getting the corresponding % N-15 values from a standard calibration graph prepared with certified N-15 standards. However, the final standardized % ^{15}N can't be used unless corrected for the carrier NH_4^+ added to the diffusion bottles.

For getting the corrected final % ^{15}N we again use the formular.

$$A_m = \frac{(P_f \times A_C) + (B \times A_B)}{P_f + B}$$

Where,

A_m = the obtained final standardized % ^{15}N value

P_f = final ammonium concentration after incubation

A_c = the % ^{15}N being calculated as final corrected % ^{15}N after the incubation

B = concentration of the added carrier (10 µg - at N)

A_B = natural abundance of the added carrier (0.37 %^{15}N).

Making Ac the subject we have

$$A_c = \frac{A_m (P_f + B) - (B \times A_B)}{P_f}$$

For the 1st. bottle we have

$$A_c = \frac{0.73\,(9.35 + 10.0) - (10 \times 0.37)}{9.35}$$

$= 1.12$ % ^{15}N

Using the same type of calculation, we get 0.84 % ^{15}N as the final corrected % ^{15}N after 24 hrs. of incubation for bottle 2.

Table 3 Shows the final obtained % ^{15}N, final standardized % ^{15}N, final corrected % ^{15}N and the final excess % ^{15}N (A_f).

Sample bottle	Final obtained % ^{15}N	Final standardized % ^{15}N	Final corrected % ^{15}N	Final excess % ^{15}N (A_f)
1	1.06	0.73	1.12	0.75
2	0.85	0.60	0.84	0.47

From definition

Final excess % ^{15}N = final corrected % ^{15}N - 0.37

For the first bottle

$$A_f = 1.12 - 0.37 = 0.75$$

and for the second bottle

$$A_f = 0.84 - 0.37 = 0.47$$

Calculation of uptake and regeneration.

As shown earlier, we now use equations 4 and 5 for uptake (assimilation) and regeneration rates for the Woluwe lake.

Equation 4

$$r = \left(\frac{P_f - P_i}{t} \right) \cdot \left(\frac{\ln \frac{A_i}{A_f}}{\ln \frac{P_f}{P_i}} \right)$$

and equation 5

$$U = r - \left(\frac{P_f - P_i}{t} \right)$$

Where,

r = regeneration rate

U = uptake rate

From out 1st. bottle

$$r = \left(\frac{9.35 - 16.90}{24}\right) \cdot \left(\frac{\ln \frac{16.66}{0.75}}{\ln \frac{9.35}{16.90}}\right)$$

$= (-0.315) \times (-5.24)$

$= 1.65$

$$\therefore \quad \text{uptake } U = 1.65 - \frac{9.35 - 16.90}{24}$$

$= 1.65 + 0.315$

$= 1.97$

Similarly for the 2nd. bottle

$$r = \left(\frac{9.45 - 16.90}{24}\right) \cdot \left(\frac{\ln \frac{16.09}{0.47}}{\ln \frac{9.45}{16.90}}\right)$$

$= (-0.31) \times (-6.075)$

$= 1.88$

$$\text{uptake } U = 1.88 - \left(\frac{9.45 - 16.90}{24} \right)$$

$$= 1.88 + 0.31$$

$$= 2.19$$

From the two bottles,

$$\text{Average regeneration rate, } r = \frac{1.65 + 1.88}{2}$$

$$= 1.77 \ \mu g \text{ at N } l^{-1} h^{-1}$$

$$\text{And average uptake rate, } r = \frac{1.97 + 2.19}{2}$$

$$= 2.08 \ \mu g \text{ at N } l^{-1} h^{-1}$$

Conclusion.

From the results uptake (assimilation) rate (2.08 µg at N $l^{-1}h^{-1}$) at lake Woluwe appears higher than regeneration rate (1.77 µg at N $l^{-1}h^{-1}$). This implies that regenertion process alone cannot sustain the ammonium-nitrogen required in lake Woluwe so external supply of ammonium-nitrogen is necessary.

ACKNOWLEDGEMENT.

I am very grateful to Dr. F. Dehairs for organizing a fellowship for me to study at the Free University of Brussels (V.U.B.), to Dr. L. Goeyens for introducing me to nitrogen isotope chemistry and to Prof. Ph. Polk and Mr. S. Allela for making it possible for me to get the A.B.O.S. fellowship.

I am equally grateful to Prof. I. Elskens for allowing me to conduct my study in his laboratory.

Technicon AutoAnalyzer II

INDUSTRIAL METHOD No. 158-71W/PRELIMINARY **DATE RELEASED: DEC. 1972**

NITRATE & NITRITE IN WATER AND SEAWATER – RANGE: 0-5.0 μgat N/l; 0-70 μg N/l (ppb)

GENERAL DESCRIPTION

This automated procedure for the determination of nitrate and nitrite utilizes the procedure whereby nitrate is reduced to nitrite by a copper-cadmium reductor column.[1,2] The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-napthylethylenediamine dihydrochloride to form a reddish-purple azo dye.

In surface waters normally encountered in surveillance studies, the concentration of oxidizing or reducing agents and potentially interfering metal ions are well below the limits causing interferences. When present in sufficient concentration, metal ions may produce a positive error, i.e., divalent mercury and divalent copper may form colored complex ions having absorption bands in the region of color measurement.[3]

PERFORMANCE AT 40 SAMPLES PER HOUR

USING AQUEOUS STANDARDS

Sensitivity at 5.0 μgat N/l (at 70 μg N/l)	0.17 absorbance units
Coefficient of Variation at 2.5 μgat N/l (35 μg N/l)	0.59%
Detection Limit	0.1 μgat N/l (1.4 μg N/l)

REAGENTS

AMMONIUM CHLORIDE REAGENT (Technicon No. T01-5064)

—Ammonium Chloride (NH_4Cl)	10 g
Alkaline Water, q.s.	1000 ml
Brij-35* (Technicon No. T21-0110)	0.5 ml

Preparation:

Dissolve 10 g of ammonium chloride in alkaline water and dilute to one liter. Add 0.5 ml of Brij-35.

NOTE: Alkaline water is prepared by adding just enough ammonium hydroxide to deionized distilled water to attain a pH of 8.5

COLOR REAGENT (Technicon No. T11-5065)

—Sulfanilamide ($C_6H_8N_2O_2S$)	20 g
—Concentrated Phosphoric Acid (H_3PO_4)	200 ml
—N-1-Naphthylethylenediamine Dihydrochloride ($C_{12}H_{14}N_2 \cdot 2HCl$)	1 g
Deionized, Distilled Water, q.s.	2000 ml
Brij-35 (Technicon No. T21-0110)	1.0 ml

Preparation:

To approximately 1500 ml of distilled water add 200 ml of concentrated phosphoric acid and 20 g of sulfanilamide. Dissolve completely. (Heat if necessary.) Add 1 g of N-1-naphthylethylenediamine dihydrochloride, and dissolve. Dilute to two liters. Add 1.0 ml of Brij-35. Store in a cold, dark place. STABILITY: one month.

CADMIUM POWDER** (Technicon No. T11-5063)

Use coarse cadmium powder (99% pure). Rinse the powder once or twice with a small quantity of clean diethyl ether or 1N HCl to remove grease and dirt. Follow with a distilled water rinse. Allow the metal to air-dry and store in a well-stoppered bottle.

PREPARATION OF REDUCTOR COLUMN

The reductor column tube is a fourteen inch length of 0.081 inch ID standard tubing. Before filling the column, prepare the cadmium in the following manner.

Wash 10 g of previously cleaned cadmium with 50 ml of 2% w/v copper sulfate ($CuSO_4 \cdot 5H_2O$) (Technicon No. T01-5068) until no blue color remains in solution and semi-colloidal copper particles begin to enter the supernatent liquid. Wash thoroughly with distilled water to remove all of the colloidal copper which is present. A minimum of ten washings is usually required.

[1] Armstrong, F.A.J., Sterns, C.R. and Strickland, J.D.H., 1967, Deep-Sea Res., *14*, pp. 381-389, "The Measurement of Upwelling and Subsequent Biological Processes by Means of the Technicon AutoAnalyzer and Associated Equipment".

[2] Grasshoff, K., Technicon International Congress, June, 1969.

[3] Federal Water Pollution Control Administration Methods for Chemical Analysis of Water and Wastes, November, 1969.

* Registered trademark of Atlas Chemical Industries, Inc.

** Use Pyrex Glass Wool, Corning No. 3950.

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Fill the reductor column tube with water to prevent entrapment of air bubbles during the filling operation. Transfer the prepared cadmium granules to the column using a Pasteur pipette. When the entire column is filled with granules, insert glass wool** in both ends of the tube. Insert an N5 nipple on one side of the tube.

Start pumping reagents. When the pump tubes are filled with reagents and all air is removed from transmission lines, attach the distal end of the tube to the 0.034 polyethylene resample line and connect the other end of the tube directly to the A_2 debubbler.

Preparing the column in this fashion keeps it effective for hundreds of samples.

To reactivate cadmium which has lost its reducing power, wash the cadmium with 1 *N* hydrochloric acid and rinse thoroughly with deionized distilled water. Re-treat the metal with copper sulfate.

STANDARDS

STOCK STANDARD A, 1000 μgat N/l (14,000 μg N/l)

Potassium Nitrate (KNO_3) (Technicon No. T13-5074)	0.101	g
Deionized, Distilled Water, q.s.	1000	ml
Chloroform ($CHCl_3$)	1	ml

Preparation:

Dissolve 0.101 g of potassium nitrate in deionized, distilled water and dilute to one liter. Store in a dark bottle. Add 1 ml of chloroform as a preservative.

STOCK STANDARD B, 50 μgat N/l (700 μg N/l)

Stock Standard A	5 ml
Synthetic SeaWater, q.s. or Deionized, Distilled Water, q.s.	100 ml

Preparation:

Dilute 5 ml of stock standard A in a volumetric flask to 100 ml with synthetic seawater or deionized, distilled water, Store in a dark bottle. Prepare fresh daily.

WORKING STANDARDS

ml Stock B	μgat N/l	μg N/l
0.20	0.1	1.4
2.0	1.0	14
4.0	2.0	28
6.0	3.0	42
8.0	4.0	56
10.0	5.0	70

Preparation:

Pipette stock B into a 100 ml volumetric flask. Dilute to 100 ml with synthetic seawater or deionized, distilled water. Store in a dark bottle. Prepare fresh daily.

OPERATING NOTES

1. Samples should be processed and analyzed as soon as possible. If this cannot be done immediately, they should be refrigerated at 5-10 °C or preserved with 1 drop of chloroform per 100 ml of sample.

2. Where particulate matter is present, the solution must be filtered prior to the determination. This can be accomplished by using the Technicon Continuous Filter as an integral part of the system if the sample is such that Whatman #4 or equivalent filter paper is satisfactory. (See Continuous Filter Manual No. CFO-1.)

3. It is of the utmost importance that the water used in preparing reagents and standards be completely free of contaminants.

4. In order to determine nitrate levels, the nitrite alone must be subtracted from the total (nitrate and nitrite). The nitrite value can be determined by eliminating the reductor column from the manifold, or by using the Technicon Method for Nitrite, Method No. 161-71W.

5. The reductor column must be clean and have good flow characteristics for the system to operate satisfactorily. Colloidal copper is the primary contaminant.

6. For initial activation of the reductor column, a midscale standard should be pumped through the system for about one hour.

7. The efficiency of the reductor column has been found to be 99%.

8. Before running the method, position the controls of the Modular Printer as follows:

CONTROL	POSITION
MODE Switch	Normal
SAMPLING RATE Switch	40
RANGE Switch	500
DECIMAL Switch	0.00

Details of Modular Printer Operation are provided in Technical Publication No. TA1-0278-10.

9. Alternate ranges may be obtained by utilization of the Std Cal control on the Colorimeter.

10. When analyzing seawater, blank reading for the particular seawater of interest should be determined by sampling the seawater while running distilled water only through the reagent lines. The blank reading obtained should then be subtracted from the readings of the unknowns.

11. Sample cups should be washed with 1 *N* hydrochloric acid and rinsed thoroughly with deionized, dis-

water in order to remove any traces of nitrate and nitrite.

12. The reagent baseline absorbance with reference to water should be approximately 0.06 absorbance units.

13. The Colorimeter should be operated in the Damp mode.

14. When analyzing seawater, standardization should be carried out with synthetic seawater standards. The Sampler IV wash receptacle should also contain synthetic seawater. The reagent baseline is adjusted to zero.

When running actual seawater samples, the Sampler IV wash receptacle should contain deionized, distilled water. The reagent baseline must be readjusted to zero.

Synthetic seawater is prepared in the following manner:

SYNTHETIC SEAWATER***

Sodium Chloride (NaCl)	31	g
Magnesium Sulfate Heptahydrate ($MgSO_4 \cdot 7H_2O$)	10	g
Sodium Bicarbonate ($NaHCO_3$)	0.041	g
Deionized, Distilled Water, q.s.	1000	ml

Preparation:

Dissolve 31 g of sodium chloride, 10 g of magnesium sulfate heptahydrate and 0.041 g of anhydrous sodium bicarbonate in deionized, distilled water and dilute to one liter.

15. When analyzing freshwater, use distilled water standards.

16. The use of multiple working standards is only to establish linearity. For day-to-day operation, the 3 μgat N/l standard is recommended for instrument calibration.

17. For the most accurate results only fresh Brij-35 should be used.

*** Strickland and Parsons, *A Practical Handbook of Seawater Analysis*.

NITRATE & NITRITE IN WATER AND SEAWATER

Range: 0-5 μgat N/l
0-70 μg N/l (ppb)

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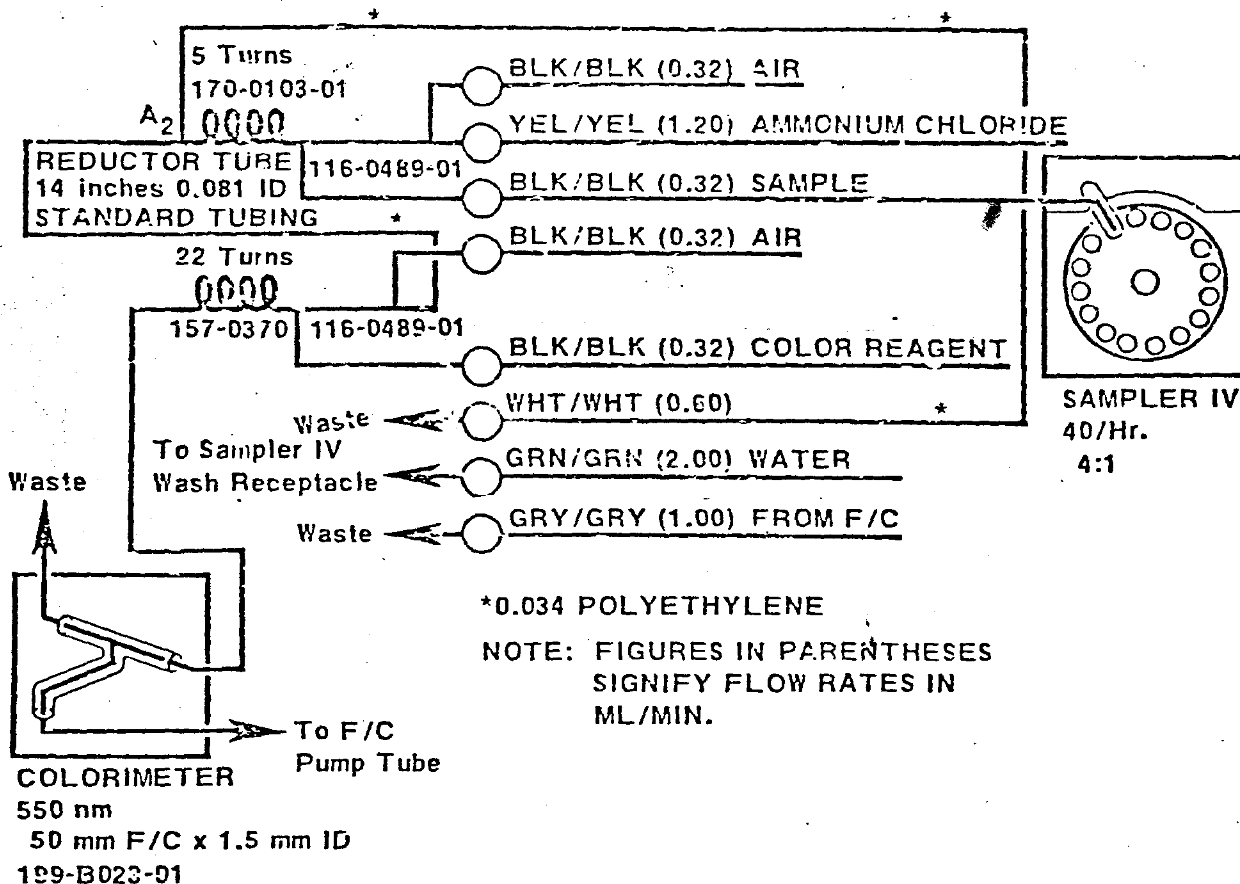

*0.034 POLYETHYLENE

NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.

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AutoAnalyzer™ II

INDUSTRIAL METHOD No. 154-71W†/B — **Released: FEBRUARY 1973/Revised: JANUARY 1978**

AMMONIA IN WATER AND SEAWATER

RANGE: 0.2-10 μgat N/l
2.8-140 μg N/l (ppb)

GENERAL DESCRIPTION

The automated procedure for the determination of ammonia utilizes the Berthelot Reaction, in which the formation of a blue colored compound believed to be closely related to indophenol occurs when the solution of an ammonium salt is added to sodium phenoxide, followed by the addition of sodium hypochlorite. A solution of potassium sodium tartrate and sodium citrate is added to the sample stream to eliminate the precipitation of the hydroxides of calcium and magnesium.[1,2,3,4,5,6]

PERFORMANCE AT 60 SAMPLES PER HOUR USING AQUEOUS STANDARDS

Sensitivity at 10 μgat N/l (140 μg N/l)	0.15 absorbance units
Coefficient of Variation at 8.0 μgat N/l (112 μg N/l)	0.31%
Detection Limit	0.2 μgat N/l (2.8 μg N/l)

REAGENTS

COMPLEXING REAGENT

Potassium Sodium Tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$)	33	g
Sodium Citrate [HOC (COONa) ($CH_2COONa)_2 \cdot 2H_2O$]	24	g
Distilled Water, q.s.	1000	ml
Brij-35* (Technicon No. T21-0110)	0.5	ml

Preparation:
Dissolve 33 g of potassium sodium tartrate and 24 g of sodium citrate in 950 ml of distilled water. Adjust the pH of this solution to 5.0 with concentrated sulfuric acid. Dilute to one liter with distilled water. Add 0.5 ml of Brij-35.

1Van Slyke, D.D. and Hillen, A.J., BioChem., *102*, p.499, 1933.
2Kallman, S., Presentation at Div. I Meeting of ASTM Committee E-3, April, 1967, San Diego, California.
3Bolleter, W.T., Bushman, C.J. and Tidwell, P.N., Anal. Chem., 33, p. 592, 1961.
4Tellow, J.A. and Wilson, A.L., Analyst, *89*, p. 453, 1964.
5Tarugi, A. and Lenci, F., Boll Chim. Farm., 50, p. 907, 1912.
6FWPCA Methods of Chem. Anal. of Water & Wastewater, November, 1969, p. 137

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ALKALINE PHENOL

Phenol (C_6H_5OH)	83	g
Sodium Hydroxide, 20% w/v (NaOH)	180	ml
Distilled Water, q.s.	1000	ml

Preparation:
Using a one liter Erlenmeyer flask, dissolve 83 g of phenol in 50 ml of distilled water. Cautiously add, while cooling under tap water, in small increments with agitation, 180 ml of 20% NaOH. Dilute to one liter with distilled water.

SODIUM HYPOCHLORITE (STOCK)

Any good commercially available household bleach having 5.25% available chlorine may be used.

SODIUM HYPOCHLORITE (WORKING)

Dilute 200 ml of stock sodium hypochlorite to one liter with water.

SODIUM NITROPRUSSIDE

Sodium Nitroprusside [$Na_2Fe(CN)_5NO \cdot 2H_2O$]	0.5	g
Distilled Water, q.s.	1000	ml

Preparation:
Dissolve 0.5 g of sodium nitroprusside in 900 ml of distilled water and dilute to one liter.

STANDARDS

STOCK STANDARD A, 5000 μgat N/l (70,000 μg N/l)

Ammonium Sulfate [$(NH_4)_2SO_4$]	0.3310	g
Distilled Water, q.s.	1000	ml
Chloroform	1	ml

Preparation:
In a one liter volumetric flask, dissolve 0.3310 g of ammonium sulfate in 900 ml of distilled water. Dilute to volume with distilled water. Add 1 ml of chloroform as a preservative.

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reparation:

Dilute 2 ml of stock standard A in a volumetric flask to 100 ml with distilled water. Prepare fresh laily.

WORKING STANDARDS

ml Stock B	μgat N/l	μg N/l
0.2	0.2	2.8
2.0	2.0	28
4.0	4.0	56
6.0	6.0	84
8.0	8.0	112
10.0	10.0	140

reparation:

Pipette stock B into a 100 ml volumetric flask. Dilute to 100 ml with distilled water. Prepare fresh aily.

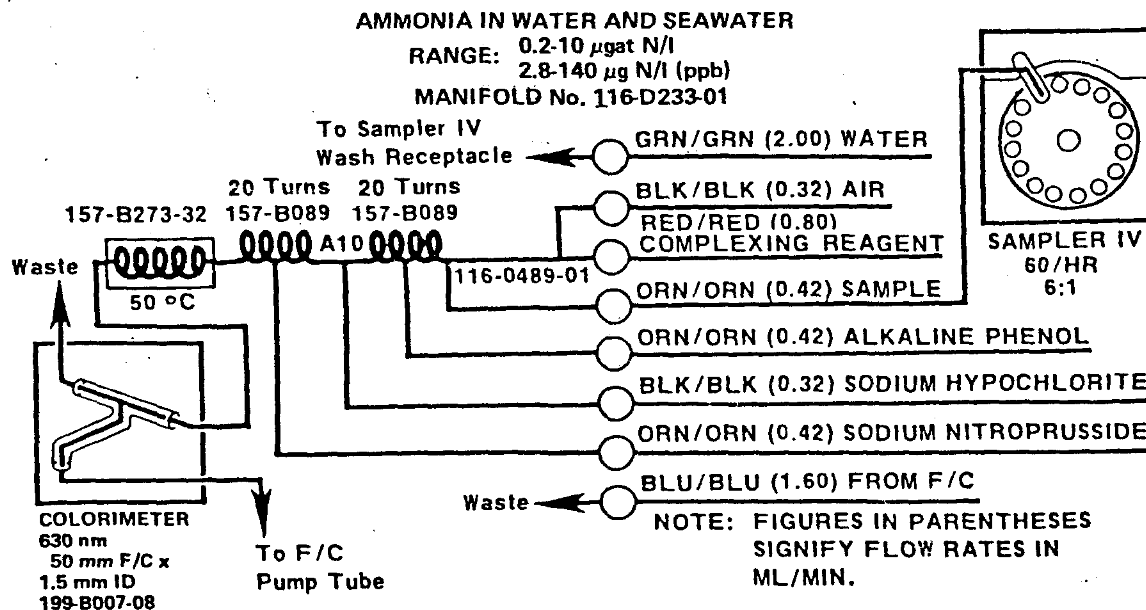
OPERATING NOTES

1. All water used in the preparation of reagents should be deionized acid distilled or distilled water which has been passed through a hydrogen form resin.
2. The alkaline phenol reagent should be filtered through a glass filter prior to use.
3. Where particulate matter is present, the solution must be filtered prior to the determination. This can be accomplished by having the Technicon Continuous Filter as an integral part of the system if the sample is such that Whatman #4 or equivalent filter paper is satisfactory.
4. If the system is being run in an ammonia contaminated environment, the air for seg-
5. Before running the method, position the controls of the Modular Printer as follows:

CONTROL	POSITION
MODE Switch	Normal
SAMPLING RATE Switch	60
RANGE Switch	100
DECIMAL Switch	00.0

Details of Modular Printer Operation are provided in Technical Publication No. TA1-0278-10.

6. Alternate ranges may be obtained by utilization of the Std Cal control on the Colorimeter.
7. The Colorimeter should be operated in the Damp 1 mode.
8. The reagent baseline absorbance with reference to water should be approximately 0.180 absorbance units.
9. Glassware for the preparation of reagents and standards should be washed with one normal hydrochloric acid and rinsed thoroughly with distilled water in order to remove any traces of ammonia. Sample cups should be treated in a similar manner and then rinsed with the solution to be measured.
10. When analyzing seawater, a blank reading for the particular seawater of interest should be determined by sampling the seawater while running distilled water only through the system. The blank reading obtained should then be subtracted from the readings of the unknowns.
11. The use of multiple working standards is only to establish linearity. For day-to-day operation, the 6 μgat N/l standard is recommended for instrument calibration.



Annex 3

SILICATES IN WATER AND SEAWATER
(RANGE: 1.0-50 μgat Si/l)

GENERAL DESCRIPTION

This automated procedure for the determination of soluble silicates is based on the reduction of a silicomolybdate in acidic solution to "molybdenum blue" by ascorbic acid. Oxalic acid is introduced to the sample stream before the addition of ascorbic acid to eliminate interference from phosphates.

Tannin, large amounts of iron, color, turbidity and sulfide interfere.

PERFORMANCE AT 50 SAMPLES PER HOUR

USING AQUEOUS STANDARDS

Sensitivity at 50 μgat Si/l	0.38 absorbance units
Coefficient of Variation (at 25 μgat Si/l)	± 0.95%
Detection Limit	1.0 μgat Si/l

REAGENTS

AMMONIUM MOLYBDATE REAGENT

Ammonium Molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	10 g
Sulfuric Acid, 0.1 *N*	1000 ml

Preparation:
Dissolve 10 g of ammonium molybdate in one liter of 0.1 *N* sulfuric acid (2.8 ml concentrated sulfuric acid, sp. gr. – 1.84/liter). Filter and store in an amber plastic container.

OXALIC ACID

Oxalic Acid $(H_2C_2O_4) \cdot 2H_2O$	50 g
Distilled Water, q.s.	1000 ml

Preparation:
Dissolve 50 g of oxalic acid in 900 ml of distilled water and dilute to one liter.

ASCORBIC ACID REAGENT

Ascorbic Acid, U.S.P. $(C_6H_8O_6)$	17.6 g
Acetone (CH_3COCH_3)	50 ml
Distilled Water, q.s.	1000 ml
Levor V	0.5 ml

Preparation:
Dissolve 17.6 g of U.S.P. quality ascorbic acid in 500 ml of distilled water containing 50 ml of acetone. Mix and dilute to one liter with distilled water. Add 0.5 ml of Levor V* per liter of reagent.

SYNTHETIC SEAWATER**

Sodium Chloride (NaCl)	31 g
Magnesium Sulfate Heptahydrate $(MgSO_4 \cdot 7H_29)$	10 g
Sodium Bicarbonate $(NaHCO_3)$	0.041 g
Deionized, Distilled Water, q.s.	1000 ml

Preparation:
Dissolve 31 g of sodium chloride, 10 g of magnesium sulfate heptahydrate and 0.041 g of anhydrous sodium bicarbonate in deionized, distilled water and dilute to one liter.

STANDARDS

STOCK STANDARD A, 10,000 μgat Si/l

Sodium Fluosilicate (Na_2SiF_6)	1.88 g
Distilled Water, q.s.	1000 ml

Preparation:
Dissolve 1.88 g of sodium fluosilicate in one liter of recently boiled and cooled distilled water. Store this stock solution in a tightly stoppered plastic bottle.

STOCK STANDARD B, 1000 μgat Si/l

Stock Standard A	10 ml
Synthetic Seawater, q.s.	100 ml

Preparation:
Dilute 10 ml of stock standard A in a volumetric flask to 100 ml with synthetic seawater. Store in a dark bottle. **Prepare fresh daily.**

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** Strickland and Parsons, A Practical Handbook of Seawater Analysis.

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ml Stock	µgat Si/l
0.10	1.0
1.0	10
2.0	20
3.0	30
4.0	40
5.0	50

Preparation:

Pipette stock into a 100 ml volumetric flask. Dilute to 100 ml with synthetic seawater. Store in tightly stoppered plastic bottles.

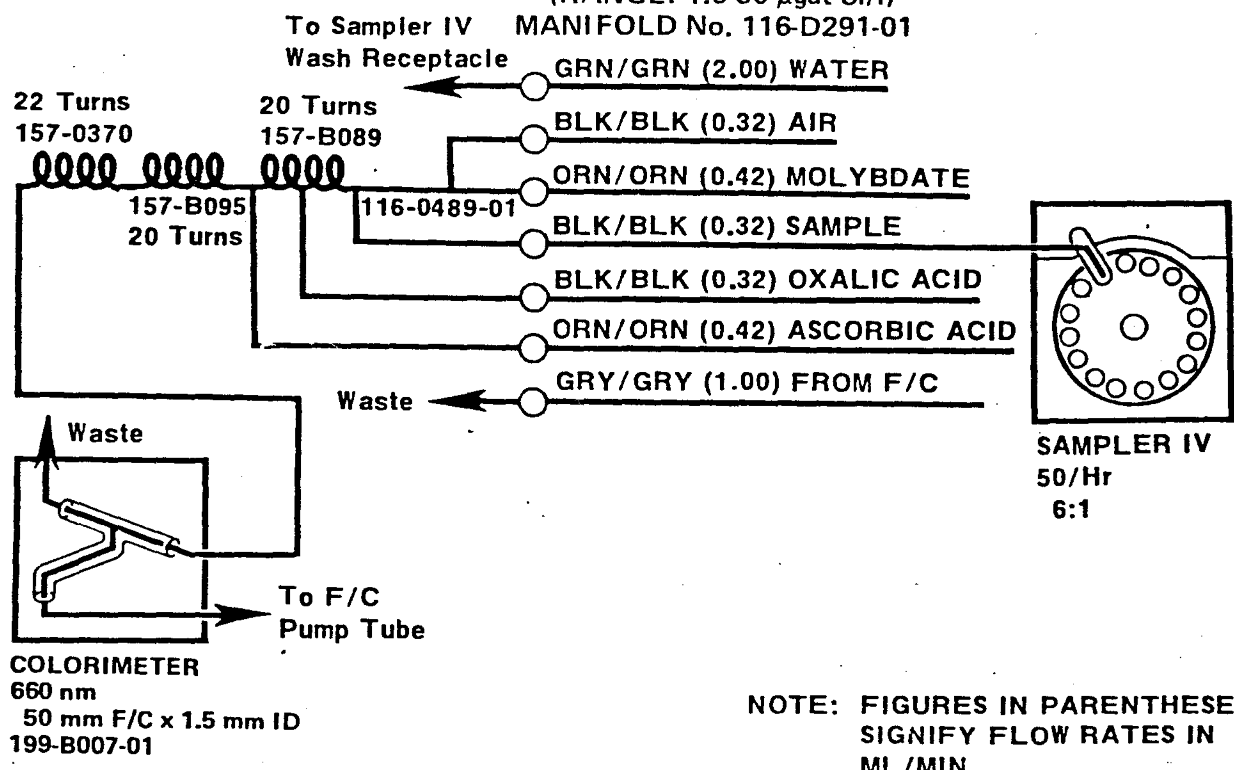
OPERATING NOTES

1. The use of glassware should be avoided as much as possible since it may contribute silica.
2. The chemicals used for reagents and distilled water should be low in silica.
3. Alternate ranges may be obtained by utilization of the Std Cal control on the Colorimeter.
4. A blank reading for the particular seawater of interest should be determined by sampling the seawater while running distilled water only through the reagent lines. The blank reading obtained should be subtracted from the sample recording.
5. Where particulate matter is present, the solution must be filtered prior to the determination. This may be accomplished by having the Technicon Continuous Filter as an integral part of the system if the sample is such that Whatman #4 or equivalent filter paper is satisfactory. (See Continuous Filter Manual #CFO-1.)
6. Before running the method, position the controls of the Modular Printer as follows:

CONTROL	POSITION
MODE Switch	Normal
SAMPLING RATE Switch	50
RANGE Switch	500
DECIMAL Switch	00.0

Details of Modular Printer Operation are provided in Technical Publications No. TA1-0278-10.

7. Since there is a salt effect with this procedure, standardization should be carried out with synthetic seawater standards. The Sampler IV wash receptacle should also contain synthetic seawater. The reagent baseline is adjusted to zero.
8. When running actual seawater samples, the Sampler IV wash receptacle should contain distilled water. The reagent baseline must be readjusted to zero.
8. The reagent baseline absorbance with reference to water should be approximately 0.015 absorbance units.



NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.

Annex 4

ORTHO PHOSPHATE IN WATER AND SEAWATER

RANGE: 0-4 µgat P/l
0-124 µg P/l (ppb)

GENERAL DESCRIPTION

The automated procedure for the determination of ortho phosphate in seawater depends on the formation of a phosphomolybdenum blue complex which is read colorimetrically at 880 nm.[1]

A single reagent solution is used consisting of an acidified solution of ammonium molybdate containing ascorbic acid and a small amount of antimony.

Interference from copper and iron is insignificant. Silicon at a level of 100 µgat Si/l causes an interference equivalent to approximately 0.04 µgat P/l.

Although arsenate produces a similar color to phosphate, sea water rarely contains arsenate in concentrations high enough to interfere. The salt error has been found to be less than 1%.

PERFORMANCE AT 30 SAMPLES PER HOUR USING AQUEOUS STANDARDS

Sensitivity at 4.0 µgat P/l (124 µg P/l)	0.15 absorbance units
Coefficient of Variation at 2 µgat P/l (62 µg P/l)	1.98%
Detection Limit	0.08 µgat P/l (24 µg P/l)

REAGENTS

SULFURIC ACID, 4.9N

— Sulfuric Acid, concentrated (sp. gr. 1.84) (H_2SO_4)	136 ml
Deionized, Distilled Water, q.s.	1000 ml

Preparation:

Add 136 ml of concentrated sulfuric acid to 800 ml of deionized, distilled water while cooling. After this solution has cooled, dilute to one liter with deionized, distilled water.

AMMONIUM MOLYBDATE

— Ammonium Molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$)	40 g
Deionized, Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 40 g of ammonium molybdate in 800 ml of deionized, distilled water. Dilute to one liter with deionized, distilled water.

ASCORBIC ACID

— Ascorbic Acid, U.S.P. (Technicon No. T11-5070) ($C_6H_8O_6$)	18 g
Deionized, Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 18 g of U.S.P. quality ascorbic acid in 800 ml of deionized, distilled water. Dilute to one liter with deionized, distilled water.

ANTIMONY POTASSIUM TARTRATE ?

— Antimony Potassium Tartrate [$K(SbO)C_4H_4O_6 \cdot 1/2H_2O$]	3.0 g
Deionized, Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 3.0 g of antimony potassium tartrate in 800 ml of deionized, distilled water. Dilute to one liter with deionized, distilled water.

COMBINED WORKING REAGENT

Sulfuric Acid, 4.9N	50 ml
Ammonium Molybdate	15 ml
Ascorbic Acid	30 ml
Antimony Potassium Tartrate	5 ml

Preparation:

Combine reagents together in the order listed above: 50 ml of sulfuric acid, 15 ml of ammonium molybdate, 30 ml of ascorbic acid and 5 ml of antimony potassium tartrate. This reagent is stable for about eight hours.

[1] Murphy, J., and Riley, J.P., A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. Anal. Chim. Acta, 27, p. 30, 1962.

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er liter.

STANDARDS

STOCK STANDARD A, 1000 µgat P/l (31,000 µg P/l)

Anhydrous Potassium Dihydrogen Phosphate (Technicon No. T13-5069) (KH_2PO_4)	0.136	g
Deionized, Distilled Water, q.s.	1000	ml
Chloroform	1	ml

Preparation:

Dissolve the potassium dihydrogen phosphate in 500 ml of deionized, distilled water in a volumetric flask. Dilute to one liter with deionized, distilled water. Add 1 ml of chloroform as a preservative.

STOCK STANDARD B, 40 µgat P/l (1240 µg P/l)

Stock Standard A	4 ml
Deionized, Distilled Water, q.s.	100 ml

Preparation:

Dilute 4 ml of stock standard A in a volumetric flask to 100 ml with deionized, distilled water. Prepare fresh daily.

WORKING STANDARDS

ml Stock B	µgat P/l	µg P/l
0.20	0.08	9.9
2.0	0.8	24.8
4.0	1.6	49.6
6.0	2.4	74.4
8.0	3.2	99.2
10.0	4.0	124

Preparation:

Pipette stock B into a 100 ml volumetric flask. Dilute to 100 ml with deionized, distilled water. Prepare fresh daily.

OPERATING NOTES

1. When analyzing seawater, a blank reading for only through the reagent lines. The blank reading obtained should then be subtracted from the readings of the unknowns.

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2. Glassware for the preparation of reagents and standards should be washed with one normal hydrochloric acid and rinsed thoroughly with deionized, distilled water in order to remove any traces of phosphate. Sample cups should be treated in a similar manner and then rinsed with the solution to be measured.

3. The ascorbic acid solution is stable for about two months if kept in a freezer or refrigerator. It is stable for about two weeks if not refrigerated. However, the container must be kept well stoppered.

4. Samples which are not run immediately should be preserved with 1 ml/l of chloroform.

5. The reagent baseline absorbance with reference to water should be approximately 0.015 absorbance units.

6. Alternate ranges may be obtained by utilization of the Std Cal control on the Colorimeter.

7. Before running the method, position the controls of the Modular Printer

CONTROL	POSITION
MODE Switch	Normal
SAMPLING RATE Switch	30
RANGE Switch	400
DECIMAL Switch	0.00

Details of Modular Printer Operation are provided in Technical Publication No. TA1-0278-10.

8. The Colorimeter should be operated in the Damp 1 mode.

9. The use of multiple working standards is only to establish linearity. For day-to-day operation, the 2.4 µgat P/l standard is recommended for instrument calibration.

ORTHO PHOSPHATE IN WATER AND SEAWATER

RANGE: 0-4 μgat P/l
0-124 μg P/l (ppb)

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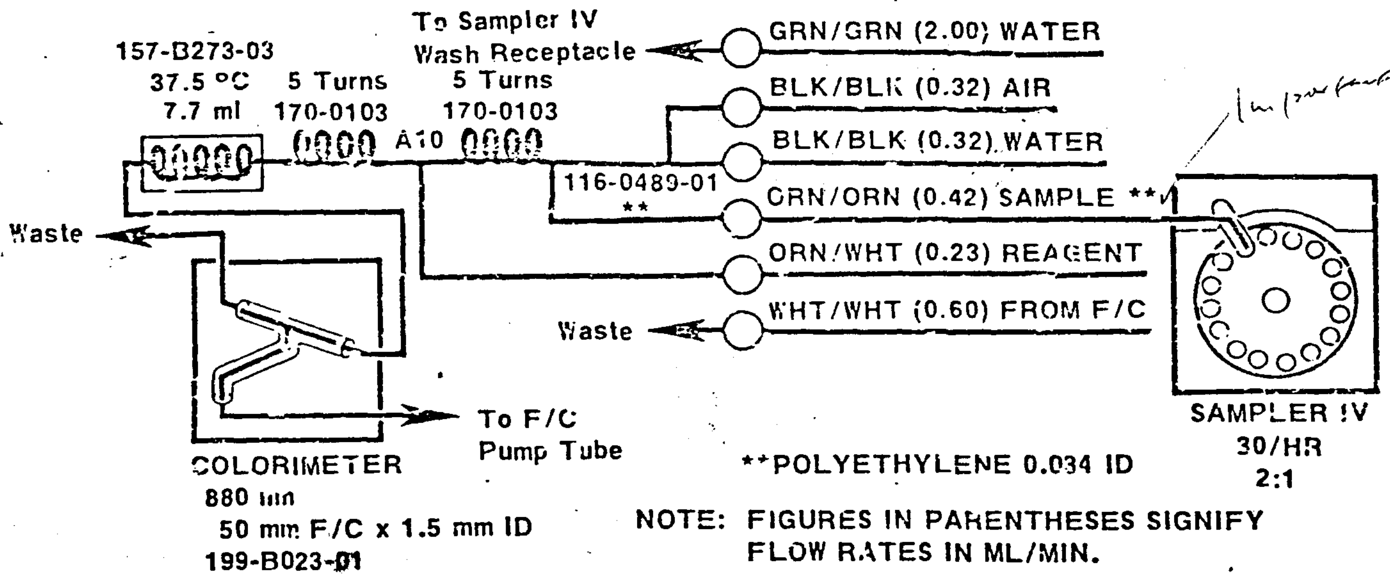

**POLYETHYLENE 0.034 ID

NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.

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