Technical Report No. 97

THE SPECTROPHOTOMETRIC DETERMINATION OF AMMONIA AND LABILE AMINO COMPOUNDS IN FRESH AND SEAWATER BY OXIDATION TO NITRITE

by

Francis A. Richards and Richard A. Kletsch

Office of Naval Research
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SEATTLE, WASHINGTON 98105
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ERRATA

Page 4, line 8 from bottom should read:

"... A concentration of 0.66 N NaOH was chosen ..."

Page 5, last line should read:

"to 40 mg/liter."

Page 7, paragraph 4 should read:

"Sodium Arsenite: Dissolve 9 g of analytical reagent grade \( \text{As}_2\text{O}_3 \) in 111 ml of 1 N NaOH. Neutralize the solution with 1 N HCl, and adjust the pH to 7.0 with HCl or NaOH. Dilute to 1 liter."

Page 7, first sentence of paragraph on \( \text{N-(1-naphthyl) ethylenediamine 2HCl} \) should read:

"Dissolve 0.6 g of \( \text{C}_{10}\text{H}_7\text{NHCH}_2\text{CH}_2\text{NH}_2 \cdot 2\text{HCl} \) in 500 ml of ..."

Page 7, first sentence of paragraph on \text{Sulfanilamide} should read:

"Dissolve 0.6 g of \( \text{NH}_2\text{SO}_2\text{C}_6\text{H}_4\text{NH}_2 \) in 500 ml of ..."

Page 7, footnote 1 should read:

"... the titration of the iodine formed requires 3.19 ml of 0.1 N sodium thiosulfate solution."

Page 9. To the paragraph on Procedure for Seawater, add the sentence:

"If the salinity of the sample is less than 19%, follow the procedure for distilled and freshwater."
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RICHARD H. FLEMING
Chairman

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ABSTRACT

A new method has been developed for the determination of small concentrations of ammonia and labile amino compounds in distilled, fresh, and seawater. It employs the oxidation of these compounds to nitrite by sodium hypochlorite in strongly basic solution. The excess sodium hypochlorite is reduced with sodium arsenite solution, and the nitrite is then determined by a colorimetric method. Bromide and iodide ions catalyze the oxidation. The precipitation of calcium and magnesium hydroxide from seawater samples by the base does not interfere with the determination. Concentrations as low as 1 μg NH₄⁺-N/liter can be detected. The average standard deviation is ± 3 μg NH₄⁺-N/liter at the level of 100 μg NH₄⁺-N/liter in seawater. The comparable average standard deviation is ± 1.1 μg in distilled water. On the average, 73% of the ammonia is converted to nitrite. Of six amino acids investigated, 12 to 67% of their amino nitrogen was converted to nitrite. The present method is compared with the vacuum distillation method of Riley (1953) and Riley and Sinhaseni (1957).
INTRODUCTION

Several methods have been used for the determination of small concentrations of ammonia in natural waters. Perhaps the best known is the Nessler method, but it is generally insufficiently sensitive for the concentrations of ammonia occurring in seawater, and the precipitate formed by the highly basic reagent with seawater requires special treatment by either the Witting and Buch (Witting 1914; Buch 1915) or Wattenberg (1929; Föyn 1950) method.

Vacuum distillation has been used to concentrate ammonia and to separate it from interfering substances in methods described by Krogh (1934), Riley (1953), and Riley and Sinhaseni (1957), and the latter methods now are in reasonably wide use. Although these distillation methods appear to successfully remove the inorganic ammonia from other amino compounds, they are time consuming, the apparatus is fragile, and the procedure has not gained widespread acceptance for use at sea. Riley (1953) reviewed the concentration and removal of ammonia from interfering substances by diffusion. These methods are all designed to accomplish the separation under the mildest conditions of basicity and temperature with the aim of removing the inorganic ammonia fraction without the concurrent degradation of labile amines and the consequent return of apparent ammonia from them. The method of Riley (1953) involves the development of an indol blue color, proportional to the ammonia concentration, in the distillate.
The colorimetric method proposed by Kruse and Mellon (1953) and first applied to seawater by Atkins (1957) requires a solvent extraction and has been found to be difficult to reproduce.

Zitomer and Lambert (1962) described the conversion of ammonia to NCl₃ by hypochlorite in acid solution. They destroyed the excess hypochlorite with nitrite, and the NCl₃ was reduced by iodide, yielding an equivalent amount of iodine. The starch-iodine color was measured spectrophotometrically. This method is not applicable to seawater, because the bromide ions react with hypochlorite in acid solution to give bromine, which in turn oxidizes ammonia to free nitrogen.

The present method employs the oxidation of ammonia to nitrite by hypochlorite in strongly basic solution. The excess hypochlorite is destroyed with sodium arsenite, and the nitrite is then determined by an adaptation of the method of Bendschneider and Robinson (1952). Their sulfanilamide and N-(1-naphthyl) ethylenediamine reagents (Shinn 1941) give about 15% more color and somewhat more consistent results than sulfanilic acid and o-naphthylamine (Ilosvay 1889; Robinson and Thompson 1948).

In preliminary work, the excess hypochlorite ions were destroyed by reduction with iodide ions (added with the sulfanilic acid reagent) followed by reduction of the liberated iodine with Na₂S₂O₃. To avoid the decomposition of nitrite ion in acid solution, the sulfanilic acid reagent containing potassium iodide was added to the sample while still basic. The solution was then acidified, releasing iodine. However, the diazotization was only about 90% complete under these conditions, and the procedure was abandoned. It was found that Na₂S₂O₃ would destroy the excess NaOCl in basic solution, but little dye was formed during the subsequent diazotization. Sodium arsenite reduces the excess NaOCl in basic solution, and does
not interfere with the diazotization reaction in acid solution.

The sodium hypochlorite oxidizes approximately 73% of the ammonia to nitrite, but the mechanism of the reaction is not known. According to Riley et al. (1954) the oxidation of ammonia to N₂ and N₂O by OCl⁻ and OBr⁻ (at much higher concentrations than are involved in this procedure) does not involve hydrazine or hydroxylamine as intermediates. During our work, it was found that the oxidation by OCl⁻ is catalyzed by iodide and bromide ions, suggesting iodate or bromate ions as intermediates, but their addition had no effect on the fraction of the ammonia oxidized to nitrite, nor did iodate or bromate ions alone oxidize ammonia to nitrite.

F. L. Margolis of the Scripps Institution of Oceanography, in an unpublished report dated 1957, proposed using the phenol-hypochlorite reaction for the direct determination of ammonia in seawater. He stated that at pH values under 9.8 the products of the reaction between NaOCl and NH₃ are NH₄Cl and NaOH. However, at the higher pH values we have employed, it is apparent that about 75% of the ammonia nitrogen is converted to nitrite.

EXPERIMENTAL

Effect of Concentration of Sodium Hypochlorite

The effect of the strength of the oxidizing agent was investigated by carrying out the reaction in 50 ml samples containing 100 μg NH₄⁺-N/liter. Ten ml of 1 N NaOH containing between 0.084 and 0.220 of full strength Clorox were about equally effective in distilled water samples, converting approximately 73% (Fig. 1A) of the ammonia to nitrite, but markedly smaller fractions were converted with weaker or stronger solutions.

In seawater samples containing 56 μg NH₄⁺-N/liter (Fig. 1B), the
Fig. 1 Effect of strength of oxidizing reagent on the conversion of ammonia to nitrite.
Fig. 2 Effect of the NaOH concentration on the conversion of ammonia to nitrite.
conversion was poorer with 10 ml of 4 N NaOH containing less than 0.048 ml and more than 0.180 ml of Clorox. A reagent containing 0.7 ml of Clorox/100 ml of 4 N NaOH was adopted for use with seawater samples.

**Effect of the Concentration of Sodium Hydroxide**

The effect of the concentration of NaOH on the oxidation was determined by carrying out the reaction in 50 ml samples containing 100 μg NH₄⁺-N/liter and 0.12 ml 100% Clorox (Fig. 2A). In distilled water, about the same fraction of ammonia was converted to nitrite in the presence of 0.26 to 1.10 N NaOH, but poorer conversions were obtained at lower concentrations.

In seawater samples (Fig. 2B), the fraction of ammonia oxidized to nitrite decreased in the presence of less than 0.32 N NaOH, but in the range 0.32-1.10 N, about the same fraction of ammonia was oxidized to nitrite. Although the recovery decreased at concentrations of less than 0.26 N in distilled water and at concentrations of less than 0.32 N in seawater, the precipitation of calcium and magnesium hydroxide in 50 ml of seawater used approximately 5.0 milliequivalents of the base, so that seawater actually requires a smaller final concentration of base than does distilled water. A concentration of 0.8 N NaOH was chosen from this range. Waters with a salinity as high as 55 °/oo can be analyzed when this concentration of NaOH is used.

**Effects of Time and Temperature**

To determine the optimal time for the maximum conversion of ammonia to nitrite, the reaction was carried out in distilled water solutions containing 100 μg NH₄⁺-N/liter. At 25 °C (Fig. 3A), the fraction of ammonia converted to nitrite was independent of time between 1 and 24 hr, but
Fig. 3 Relationship between time, temperature, and the conversion of ammonia to nitrite in distilled water.
markedly less conversion was obtained in less than 1 hr. At 32 °C (Fig. 3B), the maximum conversion was attained more slowly than at 25 °C (1.5 hr compared to 1 hr at 25 °C). At 6.6 °C (Fig. 3C), maximum conversion was about 5% less than at higher temperatures.

The oxidation is slower in seawater than in distilled water. At 21-25 °C, maximum conversion was realized only after 3 hr, compared with 1 hr in freshwater. At 32 °C, maximum conversion was realized in one-half hour less time than at 21-24 °C, and at 11.6 °C, maximum conversion was achieved only after 6 hr.

**Effect of Iodide and Bromide Ions**

Bromide and iodide ions catalyze the oxidation. In distilled water solutions containing between 30 and 130 mg bromide ion/liter and 100 μg NH₄⁺-N/liter, about the same fraction of ammonia was converted to nitrite (Fig. 4), but smaller fractions were converted at concentrations lower than 30 mg bromide ion/liter. In distilled water samples containing between 2 and 10 mg iodide ion and 100 μg NH₄⁺-N/liter, about the same fraction of ammonia was converted to nitrite, but appreciably less was converted at iodide ion concentrations of less than 2 mg/liter. The effectiveness of the iodide ion varied with the concentrations of both NaOH and NaOCl.

Since seawater of salinity of 34.3 °/oo contains approximately 65 mg of bromide ion/liter, it is unnecessary to add either bromide or iodide ion to it. Additions of 20 mg of bromide ion/liter to seawater samples had no effect on the fraction of ammonia oxidized to nitrite. There was no decrease in the fraction of ammonia converted to nitrite, even when dilution by the addition of NaOH solutions decreased the bromide ion concentration to 30 mg/liter.
Fig. 4 Relationship between bromide ion concentration and the conversion of ammonia to nitrite.
Salt Effect

The effect of salinity on the conversion of ammonia to nitrite was investigated by carrying out the reaction in distilled water and seawater (salinity 32.5 °/oo) to which 100 µg NH₄⁺-N had been added. After subtraction of the blanks, the optical density values were essentially the same, indicating that there is no salt effect.

Effect on Nitrite Initially Present

The effect of the oxidation on nitrite initially present was investigated by carrying out the determination in distilled water samples containing 100 µg NH₄⁺-N/liter, and in similar samples containing the same amount of ammonia plus 20 µg NO₂⁻-N/liter. After the subtraction of the absorbance values which were obtained for samples containing only 20 µg NO₂⁻-N/liter, and not subjected to the oxidation procedure, the optical density values were the same. Thus, the nitrite initially present is unchanged by the analytical procedure, and must be determined separately to correct the apparent ammonia values.

Effect of Insufficient Sodium Arsenite

If insufficient sodium arsenite to destroy all the NaOCl is used, a yellow color is formed on the addition of the sulfanilamide, or a slight brown color is formed on adding the N-(1-naphthyl) ethylenediamine. Up to 3 ml excess sodium arsenite in some samples had no effect on the determination.

Interfering Substances

Solutions of six amino acids and of urea were subjected to the procedure (Table 1). There was a 6.4% return of apparent ammonia from urea, and the return from the amino acids varied between 15.9 and 91.3%.
In general, we have detected no difference in the apparent ammonia content of unfiltered offshore surface seawater samples and duplicates filtered through AA Millipore filters. However, samples with high amounts of particulate matter should be filtered.

The Analytical Method

Apparatus

A model DU Beckman spectrophotometer and 5-cm Pyrex glass cells were used for optical density measurements. A slit width of 0.04 mm was used. Distilled water was used in the reference cell.

Reagents

Sodium Hypochlorite: A solution of commercial NaOCl (Clorox) containing approximately 5.25% NaOCl is used. The solution decomposes slowly and should be discarded after two months. 1

Sodium Arsenite: Dissolve 9 g of analytical reagent grade As₂O₃ in 111 ml of 1 N NaOH. Neutralize the solution with 1 N HCl, and adjust the pH to 7.0 with HCl or NaOH.

N-(1-naphthyl) ethylenediamine 2HCl: Dissolve 0.6 g of C₁₀H₇CH₂NH₂·2HCl in 500 ml of nitrite-free distilled water. Store in an amber glass-stoppered bottle, protect from direct sunlight. Replace the solution when it becomes discolored.

Sulfanilamide: Dissolve 0.55 g of NH₂SO₂C₆H₄NH₂ in 500 ml of 4 N HCl. Store in an amber glass-stoppered bottle.

1 When an acid solution of KI (2 g/300 ml) is added to 50 ml of distilled water containing 0.40 ml of 50% (V/V) Clorox, the titration of the iodine formed requires 4.76 ml of 0.1 N sodium thiosulfate solution.
1 N Sodium Hydroxide; made up in ammonia-free distilled water.

1 N Hydrochloric Acid; made up in nitrite-free distilled water. The strength of the HCl should be adjusted so that when 10.7 ml of the sulfanilamide reagent is added to a 50-ml sample containing the OCl⁻ and As₂O₃ solutions, there will be an excess of 2.8 milliequivalents HCl.

Oxidizing Solutions: (a) For distilled and freshwater: add 1.5 ml of full strength sodium hypochlorite solution (Clorox, approximately 5.25% NaOCl) to 100 ml of 1 N NaOH, just before using. (b) For seawater: add 0.7 ml of Clorox to 100 ml of 1 N NaOH.

Potassium Bromide: Dissolve 5.8 g of analytical grade KBr in 1 liter of ammonia-free distilled water. A new solution should be made each day.

Ammonia-Free Distilled Water: Pass the distilled water through a Deeminac ion exchange column, or a similar column packed with Dowex 50 cation-exchange resin, at a rate no faster than 1 ml/second.

Standard Ammonia Solution (Primary): Dissolve 0.1717 g of dry analytical reagent grade (NH₄)₂SO₄ and make up to 1 liter with ammonia-free distilled water. Add 0.5 ml of chloroform to prevent bacterial growth.

Standard Ammonia Solution (Secondary): Dilute 2.5 ml of the primary solution to 100 ml with ammonia-free distilled water. Two ml of this solution added to 48 ml of ammonia-free distilled water gives a concentration of 100 µg NH₄⁻N/liter.

---

2 Smaller fractions of ammonia were oxidized to nitrite when a solution two days old was used.
Procedure for Distilled and Freshwater

To 50 ml of the sample in a soft glass bottle, add 1 ml of 0.58% (w/v) KBr solution. Add 10 ml of the oxidizing reagent specified for distilled and freshwater, shake well, and let stand for at least 1.5 hr at room temperature. After the oxidation period, add 2 ml of sodium arsenite solution from a buret and again shake well. Follow this by 10.7 ml of the sulfanilamide reagent. After 2-8 minutes, add 1 ml of the N(1-naphthyl) ethylenediamine reagent. The color is fully developed after 10 minutes, and is stable for 2 hr. Determine the absorbance at 540 nm in a 5-cm cell.

Procedure for Seawater

To 50 ml of the sample in a soft glass bottle, add 10 ml of the oxidizing reagent specified for seawater, shake well, and let stand for at least 3.5 hr at room temperature. After this period, add 1 ml of sodium arsenite, and proceed as for distilled and freshwater samples.

Value of Blank

Significant color develops when the procedure is carried out in "ammonia-free" distilled water. On the average, absorbance of the blanks is 0.130 in a 5-cm cell. Part of the color is caused by the nitrite contained in the HCl (11.9%), sodium arsenite (1.3%), NaOH (7.9%), NaOCl (0.4%), and the color-forming reagents in distilled water alone (2.7%). Boiling the NaOH does not decrease the blank value.

Standardization in Distilled Water

The standard curve using the sulfanilamide and N-(1-naphthyl) ethylenediamine reagents is linear, i.e., the color is proportional to the nitrite (ammonia converted to nitrite) concentration in conformity with Beer's law (Fig. 5) at least to the 80 μg/liter level. The slope of the
Fig. 5 Standard curves run in seawater, distilled water, and lake water.
curve is fairly constant from run to run (and has a value of \( \Delta \) absorbance: \( \Delta N\it{H}_4^+\text{-N (\mu g/liter)} \) of approximately 0.0098, but standards should be run with each set of determinations.

**Standardization in Seawater**

Since there is no salt effect, standard curves can be run in either seawater or distilled water. For the latter procedure, 48 ml standards are made up in ammonia-free distilled water and the procedure given for distilled water is followed. The final volume is then equal to that of seawater samples. Deviation from Beer's law was a little greater in seawater at the 100 \( \mu g \) \( N\it{H}_4^+\text{-N/liter} \) level than for distilled water (Fig. 5), and the standard deviation is a little greater at all concentrations (see discussion of errors below).

**Standardization in a Lake Water**

A standard curve was run in surface water from Lake Washington using the distilled water procedure (Fig. 5). The standard deviation was a little greater at all concentrations than in distilled water, but the high blank value accounts for part or perhaps all of the greater scatter.

**Sensitivity and Errors**

Concentrations as low as 1 \( \mu g \) \( N\it{H}_4^+\text{-N/liter} \) can be detected by the method, but the magnitude of the blank prevents the detection of smaller concentrations.

The determinations in seawater had an average standard deviation of about \( \pm 3 \mu g \) \( N\it{H}_4^+\text{-N/liter} \) at the 100 \( \mu g \) \( N\it{H}_4^+\text{-N/liter} \) level, \( \pm 1.4 \) at the 60 \( \mu g/liter \) level, and \( \pm 1.3 \mu g \) \( N\it{H}_4^+\text{-N/liter} \) at the 20 \( \mu g \) \( N\it{H}_4^+\text{-N/liter} \) level. In distilled water samples, the standard deviation was \( \pm 1.1 \mu g \) \( N\it{H}_4^+\text{-N/liter} \) at the 100 \( \mu g/liter \) level.
Fraction of Ammonia Converted to Nitrite

It was found, by comparing the optical density values obtained after oxidation of 100 µg NH$_4^+$-N/liter with those obtained for 100 µg NO$_2^-$-N/liter (not subjected to oxidation), that on the average 73% of the ammonia was converted to nitrite. Without the bromide catalyst, the conversion was about 50%.

Tests of the Method

Analyses of samples prepared by adding varying known amounts of \((\text{NH}_4)_2\text{SO}_4\) to seawater and analyses of duplicate samples of natural seawater by the hypochlorite method and by a modification of the method of Riley (1953) and Riley and Sinhaseni (1957) have been compared.

The recovery of inorganic ammonia added as \((\text{NH}_4)_2\text{SO}_4\) was nearly identical by both methods. In a series of 81 analyses of duplicates in which from 0 to 125 µg NH$_4^+$-N/liter were added to the same seawater, the relationship between the determined return of ammonia and the ammonia added was, for the hypochlorite method

\[
\text{NH}_4^+\text{-N (µg/liter)} = 0.914 \times \text{(NH}_4^+\text{-N added)} + 1.96.
\]

The standard deviation in the ammonia returned was 3.81 µg/liter, and the standard error of the coefficient was 0.014. For the samples run by the Riley method

\[
\text{NH}_4^+\text{-N (µg/liter)} = 0.905 \times \text{(NH}_4^+\text{-N added)} + 2.80.
\]

The standard error of the ammonia returned was 4.24 µg/liter, and the standard error of the coefficient was 0.015.

A series of 128 natural seawater samples was analyzed by both methods. At concentrations below 14 µg/liter (1 µg-at./liter), there was little
agreement between the two sets -- the correlation coefficient was only 0.465. At the higher concentrations, there was a more nearly one-for-one relationship between the two methods, but the hypochlorite method generally returned higher values than the Riley method. The data have been analyzed for linear correlations by the method of least squares, first as a whole, second for those samples for which either method returned less than 14 µg/liter (86 samples), and third, for the samples for which either method returned more than 14 µg/liter (75 samples) (Fig. 6).

For all samples,

\[ \text{NH}_4^+ - \text{N (hypochlorite, µg/liter)} = 0.896 \times (\text{NH}_4^+ - \text{N, Riley}) + 9.24. \]

The correlation coefficient was 0.911, the standard error of the coefficient was ± 0.036, and the standard error of the ammonia returned by the hypochlorite was ± 10.93 µg/liter, assuming the Riley method to give the correct value.

For the 75 higher concentration samples,

\[ \text{NH}_4^+ - \text{N (hypochlorite, µg/liter)} = 0.790 \times (\text{NH}_4^+ - \text{N, Riley}) + 16.31. \]

The correlation coefficient was 0.894, the standard error of the coefficient was ± 0.046, and the standard error in the ammonia returned by the hypochlorite method was ± 12.15 µg/liter, again assuming the Riley method to give the correct value.

It should be pointed out that several of these samples were collected in anoxic parts of Saanich Inlet, a fjord on the coast of Vancouver Island, British Columbia, Canada, and thus contain considerably higher concentrations of ammonia than can be expected in most natural seawater.
Fig. 6 Analysis of determinations run in natural seawater samples by the hypochlorite and by Riley's Method.
DISCUSSION

It is clear from the investigation of solutions of amino acids that a high proportion of these materials will be returned as if they were ammonia. The method thus differs from both the Krogh (1934) and Riley (1953) methods, in which pains are taken to distill the ammonia from the amino acids and other interfering substances under gentle conditions (vacuum distillation at low temperature, mildly basic solutions) designed not to degrade labile amines. Our method can thus be expected to return a different fraction of the amino compounds present in natural waters. The modification of the Riley method used in this work employs distillation at a higher pH (approximately 10.3) than Riley recommends, so our distilled values may include a greater fraction of amino nitrogen than does the originally described procedure. We found this modification necessary for the complete return of ammonia added as \((\text{NH}_4\text{)}_2\text{SO}_4\). Our investigations are still inadequate to judge the significance of the difference between the hypochlorite method and Riley’s method in the analysis of natural waters, but the differences might be used to estimate the presence of labile organic amines. On the other hand, the differences may well partly owe to incomplete recovery of the ammonia during the vacuum distillation, particularly if the concentration is low.

The method has the distinct advantage over other methods in its simplicity both in operation and apparatus. A skilled analyst, working alone, can make up to 100 determinations in a day.
ACKNOWLEDGMENT

The career of Professor Ken Sugawara has provided outstanding contributions to the study of chemical oceanography. His work and his personality have been an inspiration to us, and we take pleasure in acknowledging this help.

The reaction used in the determination was, to the best of our knowledge, discovered by Dr. Ernst Føyn, Universitetets Biologiske Laboratorium, Adv. A, of the University of Oslo, while one of us (R) was working with Doctor Føyn on the problem of estimating ammonia in the waters of Oslofjord. We want to give full credit to Doctor Føyn and to express our gratitude for his permitting us to develop the described method.

This paper, in part, is from a M.S. thesis prepared by one of us (K) under the sponsorship of Professor Rex J. Robinson, to whom we want to express our thanks. The technical assistance and encouragement of Joel Cline, Dr. Harry Christoffers, Peter Paine, W. W. Broenkow, C. Paul Falls, E. V. Grill, Meredythe Meller, Judith Witkin, Ralph Riley, and Fred Palmer is gratefully acknowledged.

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REFERENCES


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