An automated technique for total dissolved free amino acids in seawater[1]

Abstract—An automated method for total dissolved free amino acid in seawater, using an air-stable ninhydrin solution, is most suitable as a field method providing real time information. Data taken in two estuaries are presented to show the distribution of total amino acid.

The increased interest in amino acids as a nutrient source for phytoplankton (Bruce 1969; Schell 1971) has made it desirable to have a rapid, sea-going technique for measuring dissolved, free amino acids in seawater. Technicon makes a complete amino acid analyzer system for the separation and analysis of amino acids with ninhydrin (Technicon Instr. Corp. 1970); we have adapted this method to our AutoAnalyzer system. Our method is intended as a field technique that can be used to detect the presence (>0.5 μM) of total free amino acids in seawater samples, to allow more adequate assessment of nitrogen nutrient budgets, and to permit the biological oceanographer to decide whether additional samples should be taken and returned to the laboratory for more complete analysis.

A seawater sample, the ninhydrin reagent, and the hydrazine sulfate solution (*see Table 1 for rates*) are introduced into the system and mixed in a long coil. The segmenting gas must be nitrogen rather than air as the ninhydrin-hydrazine complex is rapidly oxidized by air. The stream then passes through a 95°C heating bath with a delay time of about 17 min; this requires a long (12 m) heating bath coil. The sample is then debubbled and read at 570 nm in a 50-mm flow cell. One advantage of this method over other ninhydrin methods is that the stock ninhydrin reagent is air-stable before mixing with the hydrazine.

Ammonia interferes, producing additional color, but the reaction is not always stoichiometric (Yemm and Cocking 1954) depending on the condition of the ninhydrin reagent (i.e. age, pH). Ammonia must be measured at the same time in the samples (Head 1971) and a fraction of the measured value subtracted from the ninhydrin estimate. The fraction is determined by running an ammonia standard through the amino acid system.

There is a salt effect associated with this method and we use artificial seawater as the blank solution. For seawater of 20–35‰ the salt effect is not significant. However, if a wide salinity range is expected a salt correction may be necessary.

The calculation of total free amino acid concentration is

$$\mu\text{M total amino acid} = (A \times 2/B) - K[(D \times 2/B) \div 6],$$

where A is the absorbance of seawater sample through amino acid analysis, B the absorbance of 2 μM mixed amino acid standard, C the absorbance of seawater sample through ammonia analysis, D the absorbance of 6 μM ammonia standard through the amino acid analysis, H the absorbance of 6 μM ammonia standard through ammonia analysis, and K $(C \times 6/H)$ is the absorbance of ammonia in the seawater sample. In the above it is assumed that all blank corrections have been made.

Reagents—1. 4 N sodium acetate buffer (pH 5.5): Dissolve 656 g of anhydrous sodium acetate in 1,500 ml of distilled water with continuous stirring. Add 200 ml of glacial acetic acid and dilute to 2 liters with distilled water. Adjust pH to 5.51 ± 0.03 if necessary and store at 4°C.

2. Stock ninhydrin (pH 5.45–5.50): Dissolve 13.34 g of ninhydrin in 1,000 ml of methyl cellosolve (2-methoxyethanol). Add 56 ml of glacial acetic acid and 200 ml of acetate buffer. Dilute to 2,000 ml with distilled water. Prepare at least 3 hr before use and store in amber glass bottle open to air.

3. 0.002 M hydrazine sulfate: Dissolve 0.26 g of hydrazine sulfate in 1,000 ml of distilled water. Add two or three drops of

[1] Supported by Sea Grant project 061 through NOAA contract 2-35187.

Table 1. Reagents and pumping rates for amino acid method.

Reagent	Pumping rate (ml/min)
Stock ninhydrin*	0.80
Hydrazine sulfate	0.32
Sample	0.60
N_2	0.23
Flow cell draw	0.60

*Solvaflex pump tubing.

concentrated sulfuric acid and 10 drops BRIJ-35 (Technicon Instr. Corp.) per liter. Store in borosilicate container.

4. Mixed amino acid standard (amino acid standard H, Pierce Chem. Co., Rockford, Ill.): 1 ml standard H + 44 ml of artificial seawater, 1 ml of STD = 1 μM amino acid, artificial seawater = 118 g of NaCl + 19 g of $MgSO_4$ + 0.2 g of $NaHCO_3$ + 4 liters of distilled water. Standard H does contain some ammonia but as diluted above only 0.06 $\mu M\ ml^{-1}$.

Some amino acids are underestimated and some overestimated by this technique (Table 2). Proline is underestimated because its reaction with ninhydrin produces a color maximum at a different wavelength and can be measured more accurately if a second colorimeter is used, or if the sample is run a second time and measured at 440 nm. Proline can support growth of some marine diatoms (Bruce 1969) and may be present in seawater up to 1 μg-atom $liter^{-1}$.

Cystine, arginine, and histidine all are overestimated. Each contains more than one amino group and under the conditions of this technique more than the alpha-amino group may react with the ninhydrin. Cystine is not generally abundant in seawater (Bruce 1969; Siegel and Degens 1966) and its overestimation is not critical. Arginine is an excellent source of nitrogen for *Melosira nummuloides* (Hellebust and Guillard 1967; Bruce 1969) and most other neritic diatoms, but histidine did not support growth of *Melosira*.

Our results with the mixed standards (Table 2) are the best evidence that the technique measures an average of the total free amino acids. Analysis of standards made up in artificial seawater obeyed Beer's law for concentrations in the 1–13 μM range. The standard deviation for 38 samples containing 2.0 μM amino acids was 0.5.

This technique has been used on two cruises in large estuaries: Auke Bay, Alaska, and Hood Canal, Washington. Table 3 provides data from three stations from cruise

Table 2. Color yields from amino acids and related compounds on a molar basis relative to leucine.

Compound	Color yield	Compound	Color yield
Glutamic acid	1.07	Cystine	1.24
Leucine	1.00	Arginine	1.92
Serine	1.07	Phenylalanine	0.97
Alanine	1.07	Threonine	1.03
Histidine	1.50	Ammonia	0.52
Proline	0.14	Adenine	0.05
Glycine	1.10	Urea	0.03
Methionine	1.07	Mixed 1*	0.97
Tryptophan	0.90	Mixed 2†	0.93
Tyrosine	1.18	Mixed 3‡	1.01
Valine	1.10	Mixed 4§	0.97
Aspartic acid	0.97		

*Glycine, valine, isoleucine, adenine, tyrosine 1:1:1:1:1 molar basis.
†Serine, glutamic acid, proline 2:2:1 molar basis.
‡Leucine, aspartic acid, phenylalanine 2:2:1 molar basis.
§Commercially prepared mixture of 17 amino acids and ammonia (amino acid standard H, Pierce Chem. Co.).

Table 3. Total free amino acid data from Auke Bay, Alaska, compared to values in the literature. Concentration in μM. A plus is <0.5 μM.

Depth (m)	Sta 27*	Sta 29*	Sta 25*	S†	CR‡	SD§
0	0.6	+	+	0.9	0.7	0.67
5	0.6	0.7	0.5	0.6		
10	0.6	+	+	+		
15	+	+	+	+		
20	+	+	+	+		
25	+	+	+	+		
30	+	+	+	+		
40	2.0+	+	0.7	0.1		
50	0.5	+	1.0	+		
60	0.8	+	+	+		
70	1.2	+	+	+		

*From cruise C7306C, June 1973, Auke Bay.
†Schell 1971, Auke Bay (totaled from estimates of individual amino acids).
‡Chan and Riley 1966, Irish Sea (totaled from estimates of individual amino acids).
§Siegel and Degens 1966, Buzzards Bay.

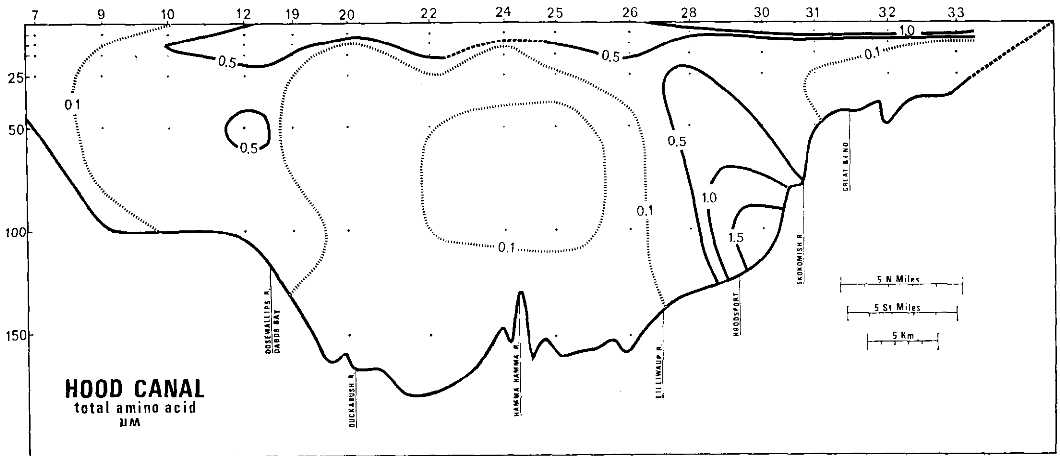

Fig. 1. Total free amino acid data from Hood Canal, C7309D. Unexpectedly low values occur below 10 m at stations 32 and 33, possibly due to overcorrection of ammonia values when H_2S is present.

C7306C to Auke Bay as well as from three other sources: Auke Bay, the Irish Sea, and Buzzards Bay. The comparative data are close to ours. Literature values are scarce. Total amino acid is seldom reported (presumably due to lack of a method) and analyses of discrete amino acids are laborious. Data are usually from a single station.

Amino acids measured in a total of 260 samples in Auke Bay in June 1973 were 2.0 μM or less, with minimum values at middepths. Concentrations in other parts of the Inside Passage showed a similar distribution. Surface values are frequently irregularly distributed. The highest values near the surface appear to be positively correlated with feeding by crustacea and cetaceans; bottom maxima appear to be a result of release from seston and sediments (Curl and Coughenower in prep.). Because our method measures a mixture of amino acids (proportions unknown) and because it depends on another measurement for corrections (ammonia), variability can be large. It cannot be used to detect small concentrations (<0.5 μM) of total free amino acid accurately. Our normal procedure is to list values lower than 0.5 μM as zero.

We urge caution in interpreting values below 0.5 μM because small negative amino acid values sometimes occur after the ammonia correction is made. We have, however, contoured data calling all negative values zero and plotting all positive values (even those <0.5 μM). These contours (Fig. 1) are not inconsistent with other nonconservative and conservative properties and show patterns that are intuitively reasonable.

One puzzling feature of the data shown in Fig. 1 are the zero amino acid values below 10 m at stations 32 and 33. The ammonia values at these stations were some of the highest observed on the cruise and we expected correspondingly high amino acids. Hydrogen sulfide (H_2S) was noted in many samples from these two stations. Reducing sediments were also found at station 30 but ammonia values were much lower. Subsequent testing has shown that H_2S causes overestimation in both the ammonia and amino acid methods, apparently more so for ammonia. Overestimation of ammonia will in turn result in low, even negative, amino acid values and this may account for the difference in calculated amino acid values between station 30 and stations 31–33. The effect of anoxic conditions on the ammonia and amino acid analysis was also evaluated; no effect was found.

D. Douglas Coughenower[2]
Herbert C. Curl, Jr.

School of Oceanography
Oregon State University
Corvallis 97331

References

Bruce, H. E. 1969. The role of dissolved amino acids as a nitrogen source for marine phytoplankton in an estuarine environment in southeastern Alaska. Ph.D. thesis, Oregon State Univ. 124 p.

Chan, Y. K., and J. P. Riley. 1966. The determination of amino acids in sea-water. Deep-Sea Res. **13:** 1115–1125.

Head, P. C. 1971. An automated phenolhypochlorite method for the determination of ammonia in sea-water. Deep-Sea Res. **18:** 531–532.

Hellebust, J. A., and R. R. L. Guillard. 1967. Uptake specificity for organic substrates by the marine diatom *Melosira nummuloides.* J. Phycol. **3:** 132–136.

Schell, D. M. 1971. Uptake and regeneration of dissolved nitrogen in southeastern Alaskan marine waters. Ph.D. thesis, Univ. Alaska. 142 p.

Siegel, A., and E. T. Degens. 1966. Concentration of dissolved amino acids from saline waters by ligand-exchange chromatography. Science **151:** 1098–1101.

Technicon Instruments Corporation. 1970. Revised operation manual for the Technicon amino acid analyzer system model No. NC-1. Tech. Publ. TAO-0155-00. Terrytown, N.Y.

Yemm, E. W., and E. C. Cocking. 1954. The determination of amino acids with ninhydrin. Analyst **80:** 209–213.

Submitted: 6 March 1973
Accepted: 26 August 1974

[2] Present address: Florida Cooperative Extension Service, P.O. Box 338, Palmetto 33561.

The determination of ammonia in seawater

Abstract—A simple phenolhypochlorite method for the estimation of ammonia in seawater obeys Beer's Law over the concentration range 0–20 μg-atoms NH_3-N liter^{-1}; the standard deviation on a set of samples containing 4 μg-atoms NH_3-N liter^{-1} is 0.04.

The estimation of ammonia in seawater has always proved difficult and several methods have been tried in the Plymouth Laboratory with varying degrees of success. Solórzano has published a phenolhypochlorite method (Solórzano 1969) which has since been used by many workers, some of whom have reported difficulties (McCarthy and Kamykowski 1972). We also obtained anomalous results and after a series of experiments have modified the method. This modified procedure has been in use for several months and has given us consistent results.

Initially we found that the blank absorbancies were high and erratic. By substituting potassium ferrocyanide in place of sodium nitroprusside as the catalyst, we got stable reproducible blanks of about 0.050 absorbance, the same level as the one given by Solórzano, in a 10-cm cuvette. To make certain that the blank could be applied to seawater in our method, we checked it against aged irradiated seawater samples. Commercial hypochlorite solutions too were a source of variation because of their instability, making necessary a daily check on the concentration of available chlorine. Sodium dichloroisocyanurate has been used as an alternative hypochlorite donator by Grasshoff and Johannsen (1972) and also by Benesch and Mangelsdorf (1972) in their ammonia procedures. This salt has the advantage of being a stable solid, and the yield of hypochlorite on hydrolysis is both rapid and quantitative.

Even with these modifications the intensity of the blue indophenol color still varied from day to day, being almost twice as strong on a bright sunny day as on a dull overcast one. Various sources of artificial light were therefore investigated to find the optimum conditions for color development. The most efficient source was an ultraviolet lamp. We used a Hytek mer-