Title	An Efficient Method for Determining the Total Amount of Dissolved Free Amino Acids in Natural Waters by Fluorometric Analysis
Author(s)	YOSHIDA, Hidemi; 吉田, 秀見
Citation	北海道大學水産學部研究彙報, 36(4), 210-215
Issue Date	1985-12
Doc URL	https://hdl.handle.net/2115/23892
Type	departmental bulletin paper
File Information	36(4)_P210-215.pdf

Bull. Fac. Fish. Hokkaido Univ.
36(4), 210-215. 1985.

An Efficient Method for Determining the Total Amount of Dissolved Free Amino Acids in Natural Waters by Fluorometric Analysis*

Hidemi YOSHIDA**

Abstract

The total amount of dissolved free amino acids (DFAA) in natural waters was determined fluorometrically by the use of the smaller amount of the reagent including *o*-phtalaldehyde (6.44 mg l^{-1} as the final concentration). By this method, a low level of DFAA (0.016 μmol l^{-1} Leu units) could be determined without the desalting and preconcentration of the seawater sample. Variation coefficient was 12% at 0.1 μmol l^{-1} level, 0.64% at 1 μmol l^{-1} level and 1.9% at 10 μmol l^{-1} level, in leucine units. The method can be applied to combined and particulate amino acids by means of an adequate pretreatment and hydrolysis.

Introduction

Starting in the 1970's, fluorescence reactions have been applied to the analysis of amino acids (Roth, 1971; Roth and Hampai, 1973). The ninhydrin method, previously most in use, is excellent for its precision of measurements and for its constancy of the relative molar absorptivity of individual amino acids. The fluorometric method, however, is suited for the determination of trace amounts of amino acids in seawater, because this method is several orders of magnitude more sensitive than the ninhydrin method. And also, since the fluorometric method can determine amino acids without the desalting and preconcentraion of seawater samples, it has been used as an automated analysis of total amino acids in seawater (Josefsson et al., 1977), and has been recommended (Dawson and Liebezeit, 1981) and adopted (Parsons et al., 1984) as a routine method for ecological studies.

In this study, the author critically examined the method of Josefsson et al. (1977), determining total *o*-phtalaldehyde reactive substances (ORS), to analyze by hand for a rapid, simple and sensitive method to determine the total amount of dissolved free amino acids in natural waters.

---

* *Presented in part at the Fall Meeting of the Oceanographical Society of Japan, Hakodate, Japan, October 1983, Abstracts, p. 208-209.*
(この研究の一部は1983年10月に函館で開かれた日本海洋学会秋季大会において講演発表された。講演要旨集, 208-209.)

* *Taken in part from the Ph. D. Thesis of H. Yoshida, Hokkaido University, 1985.*
(この研究は吉田秀見が北海道大学審査学位論文 (1985) の一部として行ったものである。)

** *Division of Marine Biochemical Science, Research Institute of North Pacific Fisheries, Faculty of Fisheries, Hokkaido University*
(北海道大学水産学部北洋水産研究施設海洋生産学部門)

Procedures

Apparatus

The fluorophotometer and pH meter used were Hitachi 650-10S and Hitachi F-5, respectively.

Reagents

Analytical quality reagents used in this experiment were supplied from Wako Pure Chemicals, Co. Ltd. 2-Mercaptoethanol and *o*-phtalaldehyde were stored in vessels replaced with N_2 to avoid contamination by ammonia.

o-Phtalaldehyde solution. *o*-Phtalaldehyde, 100 mg, was dissolved in 10 ml of 99.5% ethanol.

Borate buffer. Boric acid, 13 g, was dissolved in 500 ml of redistilled water. This solution was adjusted pH to 10.4±0.02 with 1 N NaOH (ca. 200 ml).

Buffered reagent solution. One ml of 2-mercaptoethanol and 5 ml of *o*-phtalaldehyde solution were added to 700 ml of the borate buffer. The reagent was used after 1 h and stored in a refrigerator until about a week afterwards.

Sample solutions

Stock solution. Leucine was dissolved in redistilled water to give 1 m mol l^{-1} solution. About 1 ml of toluene was added to this solution and stored in a refrigerator. The working solutions of the stock solution were prepared for calibration freshly every day.

Water samples. Water samples were filtered through precombusted glass fiber filters (Whatman GF/C, 450℃ for 12 h) and stored in polyethylene bottles at 4℃ until analyses or at below −20℃ if not for immediate use.

Procedure

A water sample, 3 ml, was pipetted into a alkali-cleaned test tube (0.1 N NaOH) and kept at room temperature. The buffered reagent solution, 0.3 ml, was added to the sample, and transfered immediately to a 1 cm×1 cm quartz cell. After just 2 min, the fluorescence intensity was measured at 340 nm for excitation and 455 nm for emission.

Results and discussion

Mixing ratio in the volume of the reagent solution to water sample

The variations of fluorescence intensity with the change of the mixing ratio in the volume of the reagent solution to water samples are shown in Fig. 1. The findings in the leucine and the glycine indicate that maximum fluorescence intensity varies slightly depending on the species of amino acid. The result of the natural seawater shows that the highest fluorescence intensity can be obtained in the range of the mixing ratios from ca. 0.1 to ca. 1. In this study, the mixing ratio of 0.1 was adopted both because of the lower fluorescence intensity of the reagent itself and because it was economical.

Fig. 2 shows the variation of pH with the change of the mixing ratio. It is known that ORS solutions pH value between 9.0 and 11.5 will yield effective

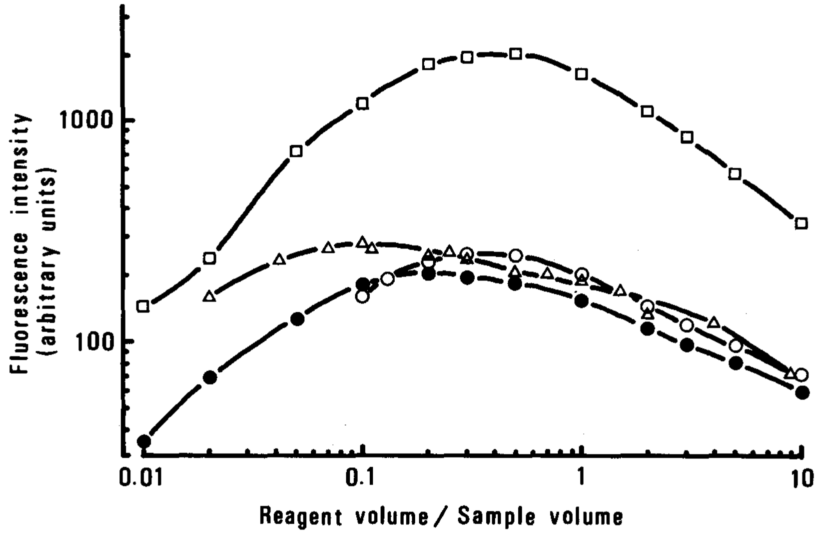

Fig. 1. Variations of the fluorescence intensity with the change of the mixing ratio in the volume of the reagent to water samples (□, 10 μmol l^{-1} Leu ; ○, 1 μmol l^{-1} Leu ; △, 1 μmol l^{-1} Gly ; ●, the seawater collected from 5 m depth at station 2 (35° 31.0′N, 139°52.8′E) of Tokyo Bay).

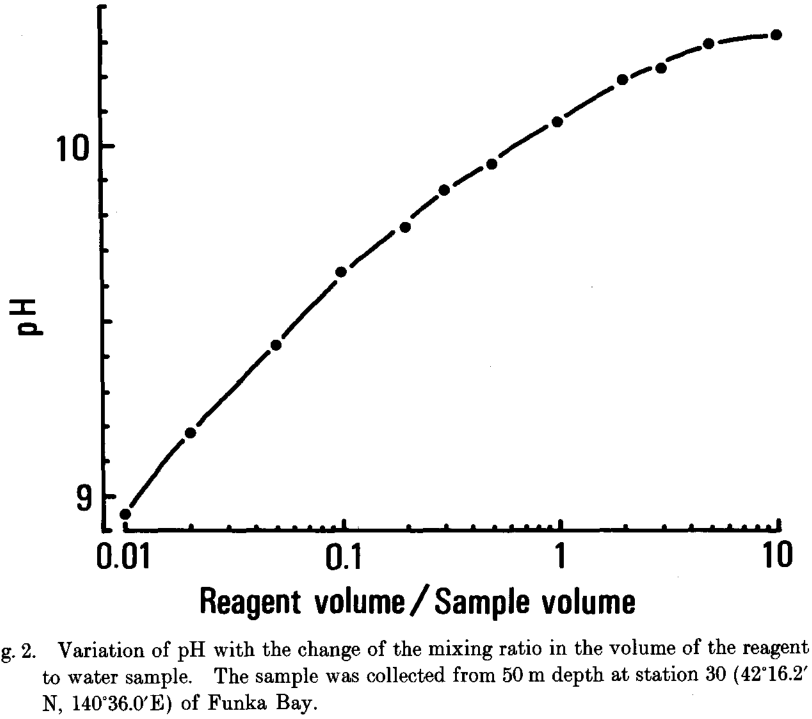

Fig. 2. Variation of pH with the change of the mixing ratio in the volume of the reagent to water sample. The sample was collected from 50 m depth at station 30 (42°16.2′ N, 140°36.0′E) of Funka Bay.

Table 1. Relative fluorescence intensities of 10 μmol l^{-1} amino acid solutions.

Amino acid	Fluorescence intensity (arbitrary units)				(M)/Gly	(M)/Leu
	1st	2nd	3rd	Mean (M)		
Gly	66	69	70	68	1.0	2.5
Leu	27	26	28	27	0.40	1.0
Ile	9.0	9.6	9.4	9.3	0.14	0.34
Nle	25	26	26	26	0.38	0.96
Ser	29	29	31	30	0.44	1.1
Arg	22	22	23	22	0.32	0.81
Glu	17	17	17	17	0.25	0.63
Val	12	12	12	12	0.18	0.44
Asp	13	12	12	12	0.18	0.44

fluorescence. At the adopted mixing ratio 0.1, pH indicates 9.6. Therefore, the above condition suffices for practical use.

The final concentration of *o*-phtalaldehyde at the measurement of the fluorescence intensity of samples is 6.44 mg l^{-1} by this method. This value is below 10% of the values of Josefsson et al. (1977): 66.5 mg l^{-1} for the range of amino acids concentration 0.05 to 2.5 μmol l^{-1} and 177 mg l^{-1} for the range of amino acids concentration 2.0 to 15 μmol l^{-1}. In addition, this value is below 3% of the value of Parsons et al. (1984): 250 mg l^{-1}.

Relative responses of individual amino acids

The fluorescence intensities of 10 μmol l^{-1} of the several amino acids using this method are shown in Table 1. The method of Josefsson et al. (1977) used glycine units to express total amino acids. The method used here, however, adopted leucine units for total amino acids because of the projection of the fluorescence intensity of glycine.

Calibration curve

A calibration curve for leucine solution is shown in Fig. 3. The molar concentration of amino acids in the water samples was calculated with the following equation:

$$\mu\text{mol } l^{-1} \text{ Leu units} = F(F_S - F_B)$$

where F_S is the fluorescence intensity of the water sample, F_B is the fluorescence intensity of Blank using redistilled water instead of the water sample and F is the slope of the calibration curve expressed as μmol l^{-1} (fluorecence unit)$^{-1}$. In this study, the F value was 2.94×10^{-3} for the range of leucine concentration 0.016 to 0.1 μ mol l^{-1} and 7.59×10^{-3} for the range of leucine concentration 0.1 to 10 μ mol l^{-1}.

Molar concentrations were converted to the concentrations by carbon and the concentrations by weight when necessary by the following equations:

$$\mu\text{g C } l^{-1} \text{ Leu units} = \mu \text{ mol } l^{-1} \text{ Leu units} \times 72$$

and

$$\mu\text{g } l^{-1} \text{ Leu units} = \mu \text{ mol } l^{-1} \text{ Leu units} \times 131,$$

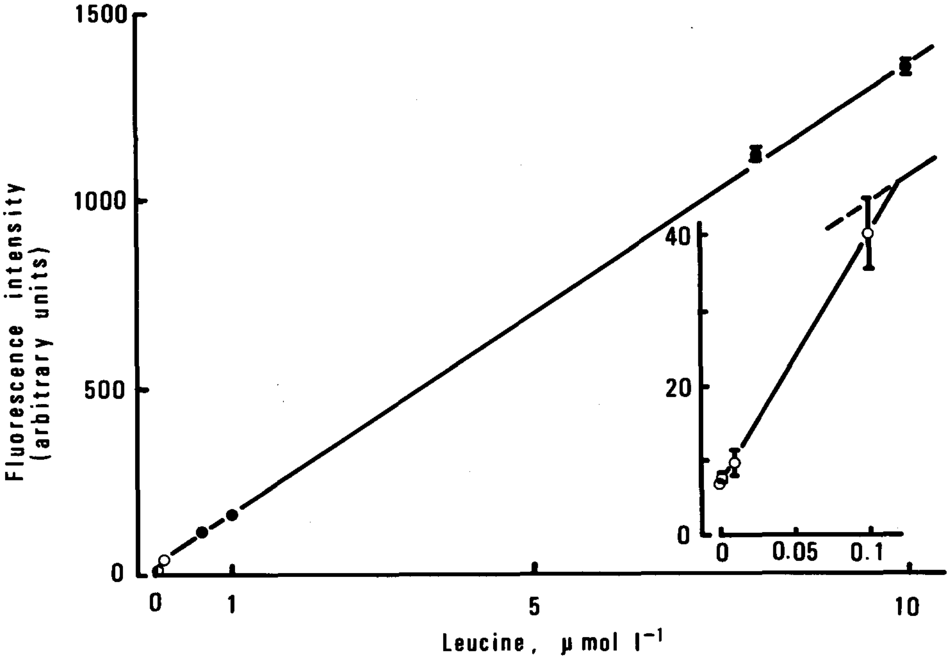

Fig. 3. Calibration curve of amino acid, leucine. The fluorescence intensity was expressed as the value under the sensitivity condition of Hitachi 650−10S : Range=3 and Fine=5.

Table 2. Reproducibility of analytical results in 0.01, 0.1, 1 and 10 μ mol l^{-1} concentrations of amino acid, leucine. The fluorescence intensity was expressed as the value under the sensitivity condition of Hitachi 650-10S : Range=3 and Fine=5.

No.	Fluorescence intensity (arbitrary units)				
	Blank	0.01 μM	0.1 μM	1 μM	10 μM
1	5.76	11.73	35.4	163.2	1383
2	6.82	9.42	44.9	163.2	1344
3	7.50	8.22	40.3	161.4	1335
4	7.89				1317
5	6.14				1380
6	6.62				1356
7					1380
8					1341
9					1317
10					1335
Mean	6.79	9.79	40.2	162.6	1351
σ_{n-1}	0.80	1.78	4.7_5	1.0_4	25
% σ_{n-1}	12	18	12	0.64	1.9

0.01 μmol l^{-1} level, $3\sigma_{n-1}=5.34$ (correspond to 0.016 μmol l^{-1})

respectively.

Precision and sensitivity

As shown in Table 2, the variation coefficient for the stock solution was 18% at 0.01 μ mol l^{-1} level, 12% at 0.1 μmol l^{-1} level, 0.64% at 1 μ mol l^{-1} level and 1.9% at 10 μ mol l^{-1} level. The detection limit calculated from the 3 σ defined by Strickland and Parsons (1968) is 0.016 μ mol l^{-1}.

Acknowledgements

The author is grateful to Professor Y. Maita for critical reviews of the manuscript and his comments on this report, and to Dr. S. Noriki for valuable criticism. The author also thanks Dr. S. Fukase, Dr.K. Matsunaga and Dr.M. Yanada for their help and encouragement, and the officers and crew of R.V. Tansei Maru and R.V. Ushio Maru for the assistance in the collections of seawater samples.

References

Dawson, R. and Liebezeit, G. (1981). The analytical methods for the characterisation of organics in seawater. p. 445-496. In Duursma, E.K. and Dawson, R. (eds.), *Marine Organic Chemistry*. 521 p. Elsevier, Amsterdam.

Josefsson, B., Lindroth, P. and Östling, G. (1977). An automated fluorescence method for the determination of total amino acids in natural waters. *Anal. Chim. Acta*, **89**, 21-28.

Parsons, T.R., Maita, Y. and Lalli, G.M. (1984). *A Manual of Chemical and Biological Methods for Seawater Analysis*. 173 p. Pergamon Press, Oxford.

Roth, M. (1971). Fluorescence reaction for amino acids. *Anal. Chem.*, **43**, 880-882.

Roth, M. and Hampai, A. (1973). Column chromatography of amino acids with fluorescent detection. *J. Chromatogr.*, **83**, 353-356.

Strickland, J.D.H. and Parsons, T.R. (1968). *A Practical Handbook of Seawater Analysis*. Fish. Res. Bd. Canada, Bull., **167**, 311 p. Ottawa.