La mer 47 : 119–127, 2010
Société franco-japonaise d'océanographie, Tokyo

Short communication

Automated colorimetric determination of trace silicic acid in seawater by gas-segmented continuous flow analysis with a liquid waveguide capillary cell

Fuminori HASHIHAMA* and Jota KANDA

Abstract: We have developed a highly sensitive automated colorimetric system in which molybdenum blue reaction is used to determine nanomolar concentrations of silicic acid in seawater. The analytical system consists of a gas-segmented continuous flow analyzer equipped with a 1-m long-path liquid waveguide capillary cell. A detection limit of 11nM and a linear dynamic range of >2000nM were achieved. The reproducibility at 100-nM standards had a coefficient of variation of 2.2% ($n=6$). A sample time of 150s was sufficient to reach the maximum absorbance output for a 100-nM solution. Field observations using this system revealed that the concentration of silicic acid in the surface waters of the southwest Indian Ocean varied from 1460 to 1790nM. This analytical method has a detection limit that is considerably lower than that of conventional analytical method. The fairly large dynamic range, high reproducibility, and relatively rapid sample processing are suitable for the high-resolution measurement of temporal and spatial variations in trace concentrations of silicic acid.

Keywords: *silicic acid, high sensitivity, automated colorimetry, liquid waveguide capillary cell*

1. Introduction

Marine silicon cycling through the processes of biogenic silica production, dissolution, and preservation has received considerable attention in recent biogeochemical studies (RAGUENEAU *et al.*, 2000). Biogenic silica production generally takes place through the uptake of silicic acid by planktonic organisms in the surface waters of the ocean. Silicic acid is an important nutrient for the growth of diatoms, and its availability has a major impact on carbon cycling because diatoms are currently responsible for 40% of the primary production in the ocean (NELSON *et al.*, 1995; FALKOWSKI *et al.*, 2004).

Water masses with low concentrations of silicic acid are frequently found in many regions of the world's oceans. There are chronically low silicic acid concentrations of $<1\,\mu$M in the surface waters of the subtropical Atlantic throughout the annual cycle (BRZEZINSKI and NELSON, 1995, 1996). Such low concentrations were also observed in the surface waters of the equatorial Pacific and the Southern Ocean; these areas are termed as high-nutrient, low-silicic acid, low-chlorophyll (or HNLSLC) areas (DUGDALE *et al.*, 1995; DUGDALE and WILKERSON, 1998). In addition to these oceanic regions, low concentrations have also been detected in neritic waters during diatom blooms (e.g., OFFICER and RYTHER, 1980; TSUNOGAI and WATANABE, 1983; EGGE and AKSNES, 1992). Silicic acid concentrations in these waters occasionally fall to levels below the detection limit of the conventional analytical method that uses the molybdenum blue reaction (ca. 0.1 μM;

Department of Ocean Sciences, Tokyo University of Marine Science and Technology, Konan, Minato, Tokyo, 108–8477 Japan
*Corresponding author: Tel./fax: 03–5463–0731;
Email: f-hashi@kaiyodai.ac.jp

STRICKLAND and PARSONS, 1972), and they are frequently less than 0.5–1.5 μM; this concentration range has often been suggested as a threshold concentration for net silicic acid uptake by silicon-starved diatoms (PAASCHE, 1973).

An accurate determination of low silicic acid concentration is critical to a thorough understanding of silicon cycling and its relationship with diatom dynamics, especially in silicic acid-depleted environments. However, highly sensitive methods for measuring nanomolar concentrations of silicic acid in seawater are poorly established. Although a solvent extraction method (BRZEZINSKI and NELSON, 1986) and magnesium-induced coprecipitation (MAGIC) method (RIMMELIN-MAURY *et al.*, 2007) have been developed to measure nanomolar silicic acid, these methods are not automated and are unsuitable for rapid analysis. Furthermore, highly sensitive automated colorimetry using a gas-segmented continuous flow analyzer with a liquid waveguide capillary cell (LWCC) has been employed for the nanomolar determination of nitrate and nitrite (ZHANG, 2000), phosphate (ZHANG and CHI, 2002), and ammonium (LI *et al.*, 2005) ; but, such as method has not yet been applied for silicic acid determination. In the present study, we develop a highly sensitive automated colorimetric system for the nanomolar determination of silicic acid by incorporating an LWCC into a gas-segmented continuous flow analyzer.

2. Experiment

Automated analytical system

A gas-segmented continuous flow colorimetric method was employed for the automated analysis of trace silicic acid in seawater. The manifold configuration and a flow diagram are shown in Fig. 1. We installed a 1-m long-path LWCC (LWCC–2100, World Precision Instruments), a fiber optic light source (FO–6000, World Precision Instruments), and a miniature fiber optic spectrometer (USB2000, Ocean Optics) in the detector position. The 1-m long-path LWCC is composed of quartz tubing with a 550-μm inner diameter and 250-μL internal sample volume, and its outer surface is coated with a low refractive index cladding material, Teflon-AF. The LWCC was connected via fiber optic cables to a light source and spectrometer. The spectrometer was connected to a computer via a universal serial bus, and it was operated using SpectraSuite software (Ocean Optics).

The colorimetric analysis involved a conventional molybdenum blue reaction. The reaction path in the manifold (Fig. 1) was similar to that described by HANSEN and KOROLEFF (1999). First, silicic acid in a seawater sample reacts with molybdate in an acidic solution to form a yellow silicomolybdate complex. The complex is then reduced by ascorbic acid into silicomolybdenum blue to increase its spectrophotometric sensitivity. Oxalic acid is added to prevent the reduction of the excess molybdate and to diminish the influence of the phosphate present in the sample. The absorbance of the blue silicomolybdate complex was measured at 660nm, and the silicic acid concentration was quantified from the peak height calibrated against that of the standard solution. The measurement at 810nm generally yields the maximum absorbance (HANSEN and KOROLEFF, 1999), but we selected the wavelength of 660nm because of the effective detection of the 1-m long-path LWCC in the range from 230 to 730nm. Flow rates were controlled by Tygon pumping tubes and the peristaltic pump of a Technicon AutoAnalyzer II. The tubes, mixing coils, and fittings of the AutoAnalyzer II were used to construct the manifold. Polyethylene filters with pore size of 20 μm were fitted to the suction lines of the sample and the analytical reagent to remove large particles, which could potentially clog the quartz tubing of the LWCC.

Analytical reagents and standards

All reagents used were of Wako analytical reagent grade. The pure water used in preparing the analytical reagents and stock standard was purified by a reverse osmosis and deionization system (Auto Pure WEX3 and WR600A, Millipore) that produces water with a resistance of 18.2MΩ. The preparation of the analytical reagents was based on those used for a flow analysis described by HANSEN and

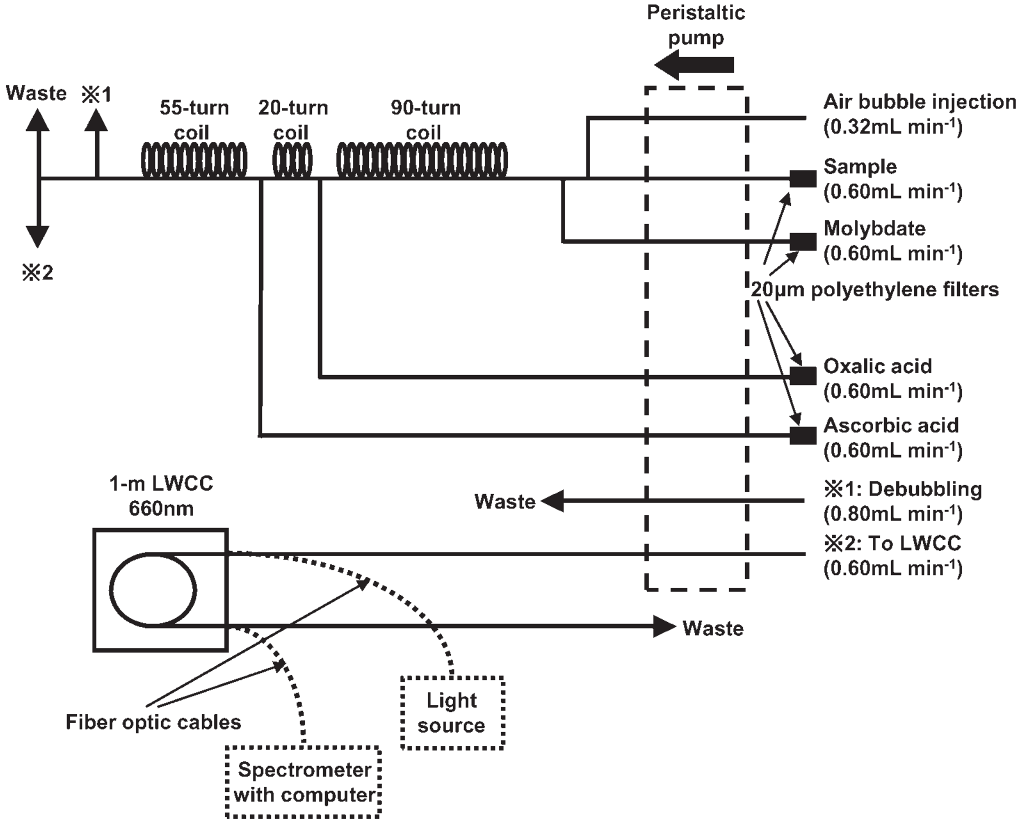

Fig. 1. Manifold configuration and flow diagram of the gas-segmented continuous flow analysis of silicic acid with an LWCC.

Koroleff (1999), except for an ascorbic acid solution. The molybdate regent was prepared by dissolving 7g of sodium molybdate dehydrate ($Na_2MoO_4 \cdot 2H_2O$) and 21mL of 4.5-M H_2SO_4 in 1L of pure water. The oxalic acid solution was prepared by dissolving 6g of oxalic acid dehydrate ($(COOH)_2 \cdot 2H_2O$) in 1L of pure water. The ascorbic acid solution was prepared by dissolving 16g of ascorbic acid ($C_6H_8O_6$) in 1L of pure water, and then adding 10mL of 15% (w/v) sodium dodecyl sulfate ($CH_3(CH_2)_{11}OSO_3Na$) solution as a surfactant. The prepared reagents were stored in polyethylene bottles, which were first rinsed with 0.3-M HCl and pure water.

Disodium hexafluorosilicate (Na_2SiF_6) dried at 110℃ was used to prepare a 10-mM stock standard solution of silicic acid (Hansen and Koroleff, 1999). The dried salt was dissolved with pure water in a plastic measuring flask using ultrasonication (Oku, 2002). The stock solution was stored in an acid-cleaned polyethylene bottle and placed in a refrigerator. Working standard solutions were prepared by the serial dilution of the stock solution with silicic acid-free seawater. The silicic acid-free seawater was also used as blanks and wash solutions. The silicic acid-free seawater was prepared by removing silicic acid from seawater collected from the surface of the western North Pacific (30° N, 138° E). The addition of 1-M NaOH to the seawater at a 1:40 ratio (v/v) produced a Mg $(OH)_2$ precipitate that effectively scavenged the silicic acid (Rimmelin-Maury *et al.*, 2007). The supernatant was then used as silicic acid-free seawater.

Cleaning

The reaction path in the manifold was cleaned by the sequential injection of 10% (v/v)

Merck Extran MA 03 and 1-M HCl with injections of pure water before and after each cleaning solution. As for the LWCC, the sequential injection of 2-M NaOH, 2-M HCl, methanol, and acetone, along with the pure water injection, proved effective. A 0.2-μm DISMIC–25cs syringe filter (Advantec) and 0.2-μm Anotop 25 syringe filter (Whatman) were used for the injections of 2-M NaOH and 2-M HCl, respectively. Pure water, methanol, and acetone were injected through a 0.2-μm Millex syringe filter (Millipore).

Shipboard observation

The automated analytical system was applied to shipboard measurements in the southwest Indian Ocean during the RT/V *Umitaka-maru* 2008/2009 cruise. Silicic acid concentrations in the ship's intake water, which was pumped from a depth of ca. 5m were determined continuously from 34.76° S, 46.17° E to 35.72° S, 45.43° E on 19 December 2008, along with temperature and salinity measurements. Data for the silicic acid concentration, temperature, and salinity were taken at 1-min intervals.

3. Results and discussion

A typical output signal of trace silicic acid was derived from analysis using the gas-segmented continuous flow analyzer equipped with the LWCC (Fig. 2). The absorbance of pure water was not significantly different from that of silicic acid-free seawater ($P > 0.05$, n=10; Fig. 2a), suggesting that the silicic acid in the surface water of the western North Pacific was completely removed by the alkaline treatment. However, a ghost peak was observed in the transition from pure water to silicic acid-free seawater. Such a ghost peak was not observed in the transition from western North Pacific water to silicic acid-free seawater. This peak may be attributed to an increase in the refractive index caused by the mixing between the pure water and silicic acid-free seawater inside the quartz tubing of the LWCC. Since this peak hampers accurate measurement, the consistent usage of silicic acid-free seawater as blanks and wash solutions, and in the preparation of working standards, was essential for completely eliminating this peak.

The molybdenum blue reaction in the silicic acid determination is vulnerable to interference by high concentrations of phosphate, hydrogen sulphide, fluoride, and transition metals such as Fe, Cu, Co, and Ni; however, such interference is generally negligible for the concentrations commonly present in the oligotrophic open ocean (HANSEN and KOROLEFF, 1999). In the present study, the effect due to the interference by phosphate was examined by using our analytical system. A sample of 5000-nM phosphate dissolved in silicic acid-free seawater was injected into the analytical system; its measured absorbance was not significantly different from that of silicic acid-free seawater ($P >$ 0.05, n=4; Fig. 2b). Since the phosphate concentration in this sample was much higher than that in oligotrophic subtropical surface waters (<300nM; WU *et al.*, 2000; KARL *et al.*, 2001; MOUTIN *et al.*, 2008; HASHIHAMA *et al.*, 2009), the phosphate interference in the determination of low silicic acid concentration was almost completely negligible. Furthermore, we measured the absorbance of 500-nM silicic acid in the silicic acid-free seawater and in natural seawater, because the alkaline treatment to produce the silicic acid-free seawater removed not just silicic acid, but also magnesium hydroxide and other compounds, which could potentially interfere in the determination of silicic acid. However, no significant difference was observed between them ($P > 0.05$, n=3; Fig. 2b), indicating that the compounds removed by the alkaline treatment did not affect the molybdenum blue reaction in the present analytical method.

A calibration curve was established from a duplicate measurement of six different concentrations of working standards (Fig. 3). A linear absorbance response to silicic acid concentrations above 2000nM was obtained with a significant correlation (r^2=0.9989). The linear dynamic range was greater than the ranges afforded by the solvent extraction method (<750nM; BRZEZINSKI and NELSON, 1986) and the MAGIC method for silicic acid (<500nM; RIMMELIN-MAURY *et al.*, 2007). BRZEZINSKI *et al.* (1998) reported that surface silicic acid concentrations in the subtropical

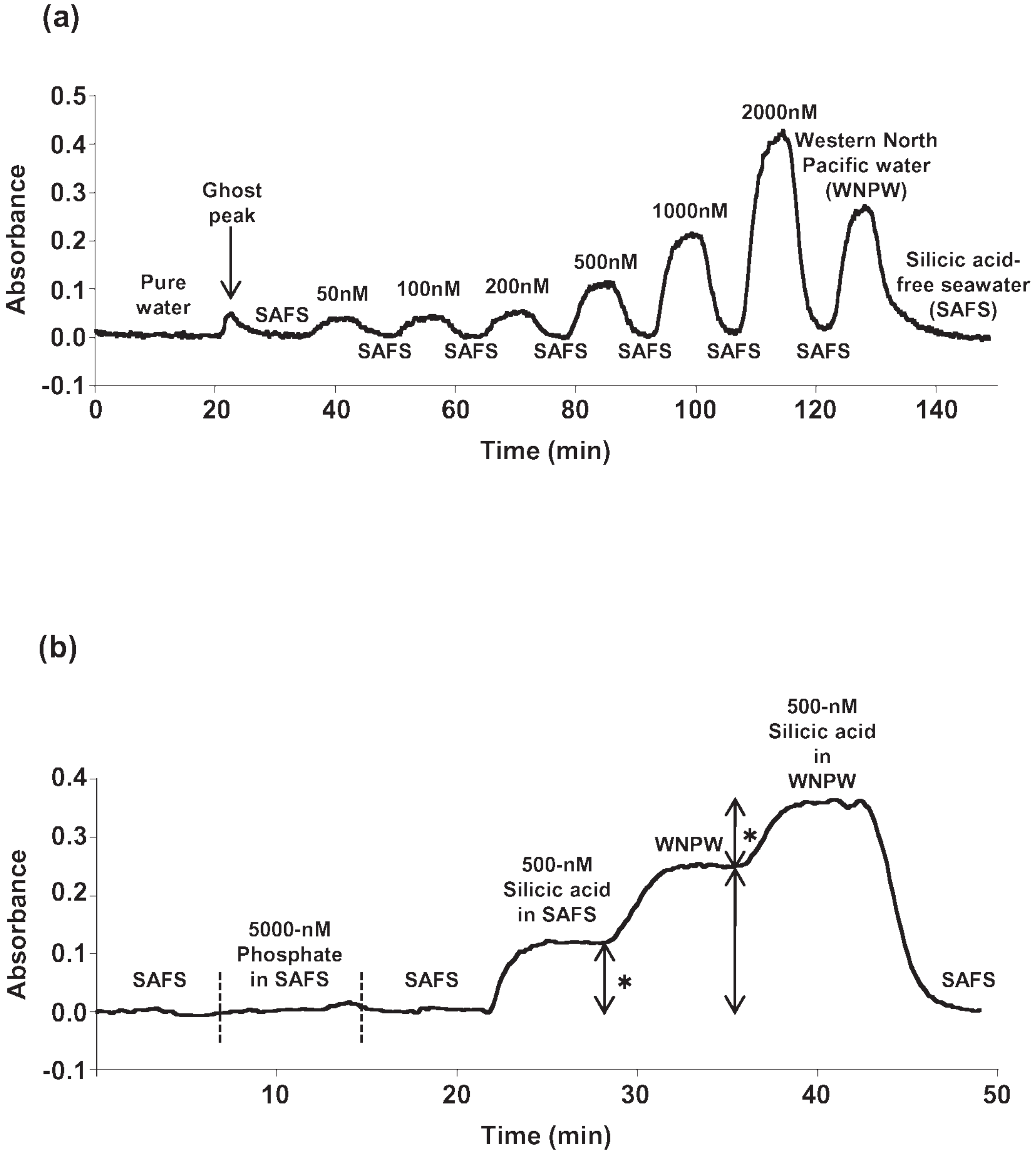

Fig. 2. Output signal of silicic acid derived from the gas-segmented continuous flow analyzer equipped with an LWCC. (a) Pure water was injected for the first 20min, then various concentrations of silicic acid standards (50–2000nM) and western North Pacific water (WNPW) were sequentially measured every 7min with injections of silicic acid-free seawater (SAFS) between them. The arrow indicates a ghost peak between pure water and SAFS. (b) A sample of 5000-nM phosphate dissolved in SAFS was measured at 7min between the SAFS injections, and then sequential samples of 500-nM silicic acid in SAFS, WNPW, WNPW with 500-nM silicic acid, and SAFS were measured every 7min. Dashed lines indicate the boundaries between the 5000-nM phosphate solution and SAFS. Asterisks denote the absorbance of 500-nM silicic acid in SAFS and in natural water.

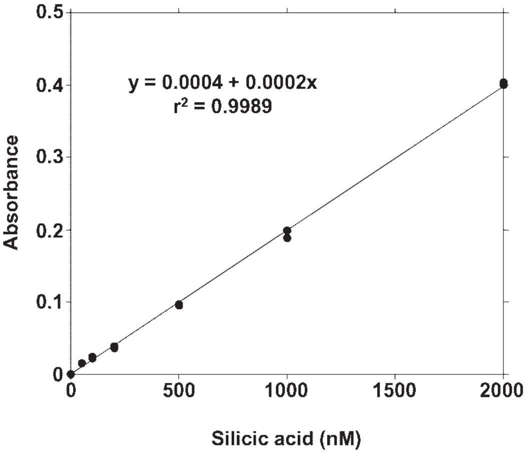

Fig. 3. Linearity of the determination of silicic acid concentration by gas-segmented continuous flow analysis with an LWCC.

North Pacific varied horizontally from 900 to 3000nM. Thus, the relatively wide dynamic range of our analytical method is appropriate for shipboard observations in oligotrophic subtropical surface waters.

A detection limit of 11nM was estimated as being three times the standard deviation of the measurement blanks ($n=6$), and the reproducibility at 100-nM standards had a coefficient of variation of 2.2% ($n=6$). The detection limit is clearly lower than that of the conventional analytical method (ca. 0.1 μM; STRICKLAND and PARSONS, 1972), and is similar to the detection limits afforded by the solvent extraction method (ca. 3nM; BRZEZINSKI and NELSON, 1986) and the MAGIC method for silicic acid (3nM; RIMMELIN-MAURY *et al.*, 2007).

Air bubble injections significantly reduce carryover and sample dispersion in the gas-segmented continuous flow analysis. In an experiment, where a sample line was switched from blank to 100-nM standards, a sample suction of 150s was sufficient for reaching maximum absorbance (Fig. 4). Since the peak shapes were almost symmetric (Fig. 2a), sample or wash time of >150s is considered to be effective for eliminating the influence of carryover and sample dispersion.

The shipboard observation using this analytical method detected a spatial variation of silicic acid concentration in the surface waters of the southwest Indian Ocean (Fig. 5). The time period of 150s mentioned above corresponds to a traversed distance of 1.16km, assuming a cruising speed of 15 knots (27.8km h^{-1}). Hence, the horizontal spatial resolution of this method was approximately 1km. Silicic acid concentrations on a ship transect varied from 1460 to 1790nM with an increase or decrease in the small horizontal scale of several tens of kilometers. This spatial variation showed no clear correspondence with the variations in temperature and salinity over the transect except for the region from 34.8 to 35.0° S where a regional inverse relationship between the silicic acid concentration and temperature was observed. A sporadic occurrence of diatom *Rhizosolenia* was recently found in the surface waters of the southwest Indian Ocean (POULTON *et al.*, 2009). Thus, the small-scale variations in the low silicic acid concentration could be ascribed to the uptake by diatoms.

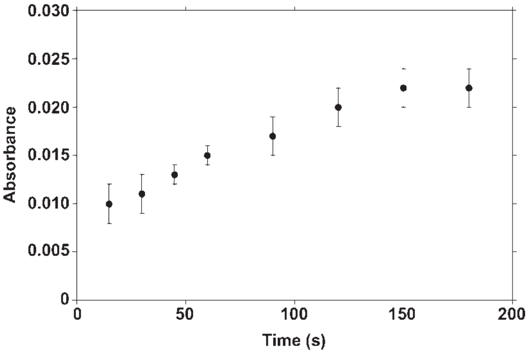

Fig. 4. Output signal of 100-nM silicic acid standards as a function of the duration of sample suction. Error bars indicate standard deviations ($n=3$).

As described above, a highly sensitive automated colorimetric system for the detection of silicic acid was developed by incorporating an LWCC into a gas-segmented continuous flow analyzer. This system offers the advantages of having a wide dynamic range, low detection limit, and high reproducibility. Furthermore, since this method is automated, it can be used for rapid analysis; it is also appropriate for shipboard measurements. This method permits high-resolution measurement of temporal and spatial variations in low concentrations of silicic acid. Recently, nanomolar concentra-

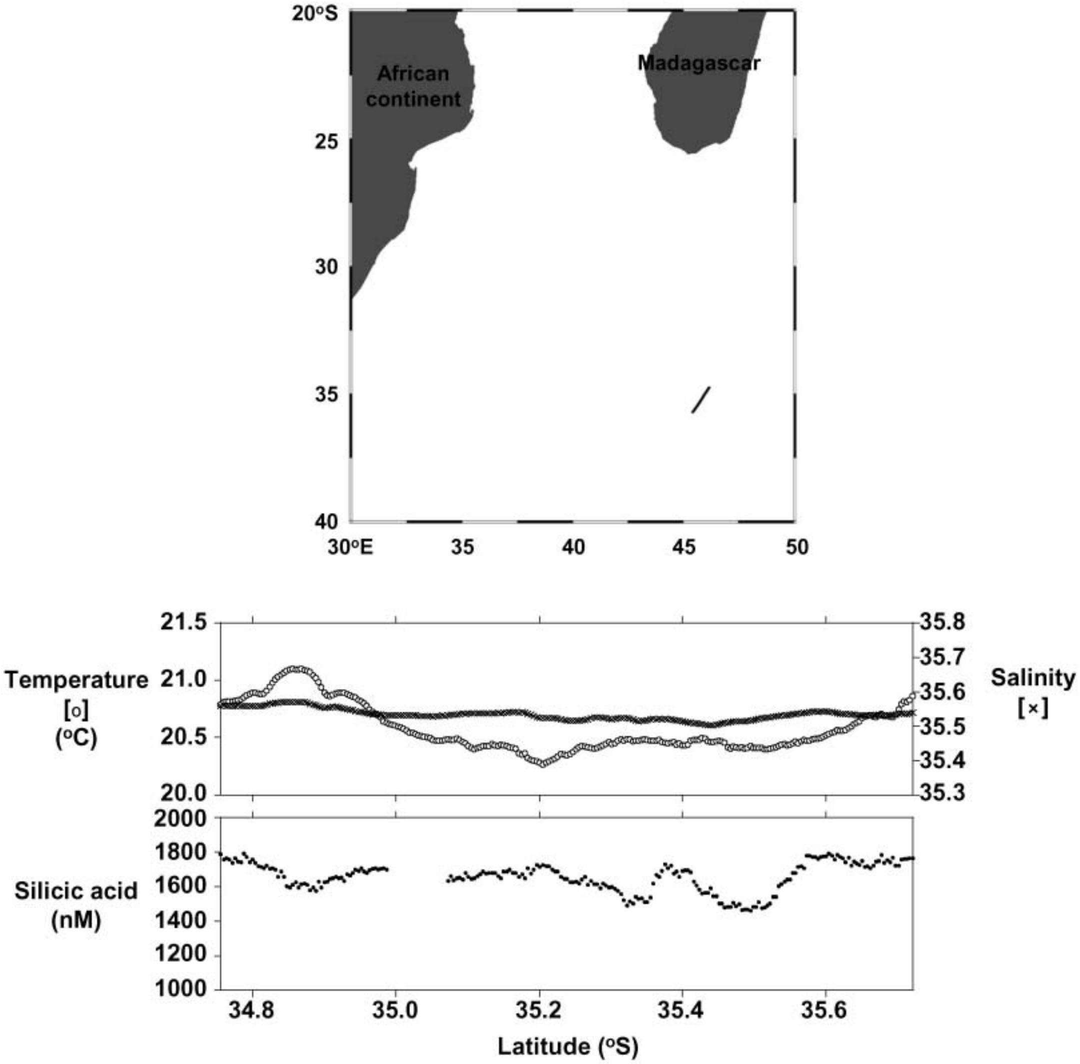

Fig. 5. Temperature, salinity, and silicic acid concentration as measured on a transect in the surface waters of the southwest Indian Ocean. The transect is denoted on the map by a black line. The silicic acid concentration on the transect between 34.99 and 35.07° S was not available.

tions of nitrate, nitrite, and phosphate in oligotrophic waters have been intensively measured to understand nutrient dynamics at low concentration levels (e.g., GARSIDE, 1985; EPPLEY and RENGER, 1988; WU *et al.*, 2000; KARL *et al.*, 2001; KANDA *et al.*, 2007; MOUTIN *et al.*, 2008; HASHIHAMA *et al.*, 2009). However, accurate silicic acid measurements have not been conducted. HASHIHAMA *et al.* (2009, 2010) revealed basin-scale and meso-scale distributions of nitrate + nitrite and phosphate in the tropical and subtropical Pacific using highly sensitive automated colorimetric system using the LWCC. By combining the new silicic acid analytical system with these nitrate + nitrite and phosphate systems, we can perform simultaneous measurements of three nutrients, and gain a better understanding of nutrient dynamics in oligotrophic waters.

Acknowledgments

We thank the officers, crew members, and members of the scientific party of the RT/V *Umitaka-maru* cruise for their cooperation at sea. We are grateful to Mr. T. Kodama for his assistance in our laboratory work. This work was financially supported by a Sasakawa Scientific Research Grant from the Japan Science Society, Grant-in-Aid No. 17510005 for Scientific Research from the Japan Society for Promotion of Science (JSPS), and Grant-in-Aid No. 18067007 for Scientific Research in Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

Brzezinski, M.A. and D.M. Nelson (1986): A solvent extraction method for the colorimetric determination of nanomolar concentrations of silicic acid in seawater. Mar. Chem., **19**, 139–151.

Brzezinski, M.A. and D.M. Nelson (1995): The annual silica cycle in the Sargasso Sea near Bermuda. Deep-Sea Res. I, **42**, 1215–1237.

Brzezinski, M.A. and D.M. Nelson (1996): Chronic substrate limitation of silicic acid uptake rates in the western Sargasso Sea. Deep-Sea Res. II, **43**, 437–453.

Brzezinski, M.A., T.A. Villareal and F. Lipschltz (1998): Silica production and the contribution of diatoms to new and primary production in the central North Pacific. Mar. Ecol. Prog. Ser., **167**, 89–104.

Dugdale, R.C., F.P. Wilkerson and H.J. Minas (1995): The role of a silicate pump in driving new production. Deep-Sea Res. I, **42**, 697–719.

Dugdale, R.C. and F.P. Wilkerson (1998): Silicate regulation of new production in the Equatorial Pacific upwelling. Nature, **391**, 270–273.

Egge, J.K. and D.L. Aksnes (1992): Silicate as regulating nutrient in phytoplankton competition. Mar. Ecol. Prog. Ser., **83**, 281–289.

Eppley, R.W. and E.H. Renger (1988): Nanomolar increase in surface layer nitrate concentration following a small wind event. Deep-Sea Res., **35**, 1119–1125.

Falkowski, P.G., M.E. Katz, A.H. Knoll, A. Quigg, J.A. Raven, O. Schofield and F.J.R. Taylor (2004): The evolution of modern eukaryotic phytoplankton. Science, **305**, 354–360.

Garside, C. (1985): The vertical distribution of nitrate in open ocean surface water. Deep-Sea Res., **32**, 723–732.

Hansen, H.P. and F. Koroleff (1999): Determination of nutrients. *In* Methods of Seawater Analysis. Grasshoff, K., K. Kremling and M. Ehrhardt (eds.), Wiley, Weinheim, p.159–228.

Hashihama, F., K. Furuya, S. Kitajima, S. Takeda, T. Takemura and J. Kanda (2009): Macro-scale exhaustion of surface phosphate by dinitrogen fixation in the western North Pacific. Geophys. Res. Lett., **36**, L03610, doi:10.1029/2008GL036866.

Hashihama, F., M. Sato, S. Takeda, J. Kanda and K. Furuya (2010): Mesoscale decrease of surface phosphate and associated phytoplankton dynamics in the vicinity of the subtropical South Pacific islands. Deep-Sea Res. I, **57**, 338–350.

Kanda, J. T. Itoh and M. Nomura (2007): Vertical profiles of trace nitrate in surface oceanic waters of the North Pacific Ocean and East China Sea. La mer, **45**, 69–80.

Karl, D. M., K.M. Björkman, J.E. Dore, L. Fujieki, D.V. Hebel, T. Houlihan, R.M. Letelier and L.M. Tupas (2001): Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA. Deep-Sea Res. II, **48**, 1529–1566.

Li, Q.P., J.Z. Zhang, F.J. Millero and D.A. Hansell (2005): Continuous colorimetric determination of trace ammonium in seawater with a long-path liquid waveguide capillary cell. Mar. Chem., **96**, 73–85.

Moutin, T., D.M. Karl, S. Duhamel, P. Rimmelin, P. Ranimbault, B.A.S.V. Mooy and H. Claustre (2008): Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean. Biogeosciences, **5**, 95–109.

Nelson, D.M., P. Tréguer, M.A. Brzezinski, A. Leynaert and B. Quéguiner (1995): Production and dissolution of biogenic silica in the ocean: Revised global estimates, comparison with regional data and relationship to biogenic sedimentation. Glob. Biogeochem. Cycles, **9**, 359–372.

Officer, C.B. and J.H. Ryther (1980): The possible importance of silicon in marine eutrophication. Mar. Ecol. Prog. Ser., **3**, 83–91.

Oku, O. (2002): Know-how of Spectrophotometric Method: Quantitative Analyses of Silicic Acid, Phosphate, Nitrate. Gihodo Shuppan, Tokyo, 127 pp (in Japanese).

Paasche, E. (1973): Silicon and the ecology of marine plankton diatoms. II. silicate-uptake kinetics in five diatom species. Mar. Biol., **19**, 262–269.

Poulton, A.J., M.C. Stinchcombe and G.D. Quartly (2009): High number of *Trichodesmium* and diazotrophic diatoms in the southwest Indian Ocean. Geophys. Res. Lett., **36**, L15610, doi:10.1029/2009GL039719.

Ragueneau, O., P. Tréguer, A. Leynaert, R.F. Anderson, M.A. Brzezinski, D.J. DeMaster, R.C. Dugdale, J. Dymond, G. Fischer, R. Françoise, C. Heinze, E. Maier-Reimer, V. Martin-Jézéquel, D.M. Nelson and B. Quéguiner (2000): A review of the Si cycle in the modern ocean: recent progress and missing gaps in the application of biogenic opal as a paleoproductivity proxy. Glob. Planet. Change, **26**, 317–

365.

RIMMELIN-MAURY, P., T. MOUTIN and B. QUÉGUINER (2007): A new method for nanomolar determination of silicic acid in seawater. Anal. Chim. Acta, **587**, 281–286.

STRICKLAND, J.D.H. and T.R. PARSONS (1972): A practical handbook of seawater analysis. Bull. Fish. Res. Bd. Can., **167**, 310pp.

TSUNOGAI, S. and Y. WATANABE (1983): Role of dissolved silicate in the occurrence of a phytoplankton bloom. J. Oceanogr. Soc. Japan, **39**, 231–239.

WU, J., W. SUNDA, E.A. BOYLE and D.M. KARL (2000): Phosphate depletion in the western North Atlantic Ocean. Science, **289**, 759–761.

ZHANGE, J.Z. (2000): Shipboard automated determination of trace concentrations of nitrite and nitrate in oligotrophic water by gas-segmented continuous flow analysis with a liquid waveguide capillary flow cell. Deep-Sea Res. I, **47**, 1157–1171.

ZHANGE, J.Z. and J. CHI (2002): Automated analysis of nanomolar concentrations of phosphate in natural waters with liquid waveguide. Environ. Sci. Technol., **36**, 1048–1053.

Received: April 28, 2009
Accepted: September 8, 2009