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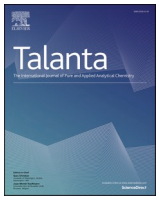

Autocalibration based on dilution of a single concentrated standard is used for the determination of silicate in sea water by the modified molybdenum blue method

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ABSTRACT

The silicate (Si) molybdenum blue method was modified by combining oxalate and ascorbic acid into a single reagent and was used for determining Si in sea water samples. The first step of this automated assay protocol was designed to perform either a calibration by a single Si standard prepared in deionized (DI) water, or to dilute samples in the range of 0–160 μM Si to fit into 0–20 μM Si calibration range using a 20 cm flow cell. By designing the assay protocol to function in batch mode, the influence of salinity on calibration was eliminated, thus making the method suitable for analysis of samples collected in the open ocean, coastal areas, or rivers. Reproducibility and accuracy of this method were evaluated by analysis of certified sea water reference materials. Phosphate (P) does not interfere significantly if the Si:P ratio is 4:1 or larger. The limit of detection was 514 nM Si, r.s.d. 2.1 %, sampling frequency 40 s/h, reagent consumption 700 μL/sample, and using deionized water as the carrier solution.

1. Introduction

There are three goals of this communication: To present a modified and optimized method for the determination of dissolved silicate in natural waters (in the form of H_4SiO_4, silicic acid, hereinafter referred to simply as Si or silicate) based on the formation of silica molybdenum blue (SiMoB) using programmable flow injection (pFI). To use autodilution, based on flow programming, to bring environmental samples to within an optimized narrow calibration range. To verify that a single concentrated standard solution autodiluted in deionized water (DI) water provides results consistent with sea-water based certified reference materials.

1.1. Silicate determinations in oceanography

Dissolved Si in sea water is one of the most frequently determined nutrients by analysts around the world using the classic molybdenum blue method [1]. The interest in making this measurement arises from the fact that Si is the 2nd most abundant element in the Earth's crust as a result of being a major component of the aluminosilicate rocks that make up the continents. The weathering of that rock by dissolved atmospheric carbon dioxide in rain water brings dissolved Si to the oceans, where surface dwelling organisms that construct Si skeletons remove it from solution reducing values to $\leq$ 1 μM in surface waters [2]. The remnants of these surface-dwelling organisms are remineralized as they sink into deep waters leading to a large vertical concentration gradient of Si between the surface and the deep waters of the ocean where values can reach 160 μM or more. Assessing the temporal and spatial distributions of dissolved Si through shipboard measurements is thus important in the field of Oceanography as it underpins our understanding of marine food webs, the biogeochemical cycles they support, as well as the weathering rates of continental material which is directly related to atmospheric carbon dioxide levels [2].

However, this wide range of concentrations, while providing a rich source of information, poses a problem for shipboard determinations, as in order to obtain high sensitivity at the lowest concentrations, as well as

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accurately constraining the values at the highest concentrations, requires the development of separate calibrations in order to cover the entire concentration range.

Here we adopt a new approach to solving this problem by constructing a narrow concentration range calibration line and using the autodilution technique to bring samples so that they fall within this single range.

1.2. Calibration issues of sea water analysis

Changes in salinity interfere in the spectrophotometric determination of nutrients in seawater by two mechanisms: First, by affecting the rate of reaction of reagent-based methods and second, by the schlieren effect, which causes irreproducible variations of absorbance due to changes of refractive index in a moving stream as it passes through a detector [3,4]. Increased salinity can decrease the rate of reaction resulting in a nonlinear decrease in the slope of the calibration line from 12 % [5, Fig. 7] up to 20 % [6, Fig. 6] as the sample salinity increases from zero to 3.5 % (w/w)-roughly equivalent to a salinity of 35 practical salinity units, a typical value in the open ocean. Also, changes in the calibration slope of up to 54 %, due to an increase in salinity from 1.4 % to 3.4 % (w/w), were reported for the reverse flow injection analysis (FIA) method [7, Table 1]. To mitigate this interference, a number of approaches have been suggested including standard addition of the target analyte to individual samples, collecting a series of calibration lines covering the salinity gradient of collected samples [6, Fig. 6], and salinity matching of calibration standards with the samples to be analyzed – the latter method being widely used for analysis of open ocean water, where salinity is relatively constant. Mitigating the schlieren effect is as equally challenging as explained in a comprehensive review on the use of Flow Injection for analysis of nutrients in sea water [3, Section 4, Table 1 Fig. 3]. Therefore, while spectrophotometry at dual wavelengths led to improvement [5, Fig. 6] and modifications of flow cell geometry, it failed to eliminate the schlieren interference [3, Table 1].

From these options, matrix matching is most widely used to alleviate salinity effects. It is a feasible approach to analysis of open ocean water, where salinities are relatively constant, but impractical for analysis of the high variability of salinity in estuarine samples. Yet there is, unfortunately, also a drawback to matrix matching of calibration standards with open ocean sea water samples. To make a reliable calibration, standards must be prepared in sea water with low nutrient concentrations, usually collected in the open ocean far from shore. Unavoidably, there is no uniform "Low Nutrient Sea Water", because different research groups collect SW at different locations and store it in different ways [4, Section 6.5 and 6.6].

However, there is a simple and obvious solution to this problem: the use of DI water for preparation of calibration standards and an automated analyzer and an assay protocol designed to yield identical calibration lines for DI water and open ocean sea water (3.5 % w/w salinity). This will not only position data obtained at different laboratories on the same calibration basis (DI water) but will also allow use of the same calibration for samples collected in the open ocean, coastal areas and estuaries.

Such a method must reproduce the conditions of batch techniques used to analyze nutrients in seawater manually. In that case, each sample was assigned an individual container into which sample and reagents were sequentially metered, homogenously mixed, and when reactions neared completion, the absorbance was measured on a solution held statically in a cuvette. Therefore, absorbance measurements that were performed on stationary, homogenous solutions at equilibrium, were not influenced by variations of salinity or the schlieren effect. An example of such an approach is a manual method for determination of silica in brackish waters [8].

The Achilles Heel of all continuous flow methods, air segmented flow analysis, and classical Flow Injection analysis, is that they yield calibration data obtained and while the concentration gradient of the analyte is moving through the flow cell. Therefore, to eliminate the salinity effect the assay protocol must be designed in such a way that absorbance is measured on a homogenous stationary solution which is at equilibrium conditions (Section 1.4).

1.3. Determination of silicate by molybdenum blue spectrophotometry

The molybdenum blue (MoB) method has been used to determine the two most frequently analyzed nutrients in sea water (P and Si). Discovered by Scheele in 1783, the MoB method has been described in several thousands of papers; its underlying chemistry is so complex that authors who published a comprehensive review [9], entitled it "Molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box".

The determination of silicate by the MoB method is comprised of two main steps and is affected greatly by acidity. First, in the presence of soluble phosphate (PO_4) and silicate in an acidic solution, yellow phosphomolybdate (PMo) and silicomolybdate (SiMo) are formed. Addition of a reducing agent (ascorbic acid) yields three species: phosphomolybdenum blue (PMoB, absorbance maxima at 880 nm and 700 nm), silicomolybdenum blue (SiMoB, absorbance maximum at 810 nm), and molybdenum blue (MoB, broad absorbance range 400–900 nm). In a strongly acidic solution (pH 0–1), the formation of PMoB becomes selective, even in the presence of 1000-fold excess of silicate. A less acidic solution (pH 1–3) allows the formation of SiMoB, but does not prevent the formation of PMoB, causing a false positive readout for Si. The widely accepted way of removing the PO_4 interference is to use oxalate reagent (OX), which is said to decompose PMo and also binds to the molybdate reagent (MO), preventing production of interfering colloidal MoB produced by ascorbic acid reduction of the molybdate reagent [3–19].

Therefore, methods for the determination of silicate are based on three steps: formation of SiMo + PMo, decomposition of PMo and binding excess MO, followed by reduction of the remaining SiMo into SiMoB to be measured at 810 nm. In manual (batch) format, sample, molybdate reagent, oxalate reagent, and reducing agent are sequentially metered into a container, mixed and allowed to react long enough to reach equilibrium [8]. When automated in flow mode [3,6,11–19] the three reagents are added in sequence. Thus, the sample is injected into a DI water carrier, merged with the molybdate reagent forming SiMo + PMo. Next the sample stream merges with oxalate to decompose any PMo and to complex the remaining molybdate reagent. Finally, SiMoB is produced by reduction of SiMo with ascorbic acid. There are several reported modifications of the SiMoB-FIA configuration [11–14] of which [11] was meticulously optimized. While reversed FIA [7], sequential injection [15–19] and lab-in-syringe configuration [6], all use the same sequence of reagents, none of them applied the simplified protocol of mixing the oxalate with the reducing agent. The main advantage of using this two, rather than three, reagent protocol is higher sensitivity, because the sample is less diluted on the way to the flow cell. Another advantage is that since the pFI PO_4 [20] method uses the same flow scheme and software protocol as Si, this facilitates switching between these two most frequently performed oceanographic determinations.

1.4. Programmable flow injection

Programmable flow injection (pFI) [21] offers two unique features that are exploited in this work.

- Flow programming that simulates manual batch-type assay protocol [20].
- Autodilution of a standard solution for automated calibration [22], and
- Use of the autodilution technique to bring samples of a wide range of concentrations within a single calibration range.

The pFI is performed in a lab-on-valve (LOV) manifold (Fig. 1) run by two milliGAT pumps (P1 and P2) via two holding coils (HC1 and HC2). A long light path flow cell, mounted on port 2 within the LOV is connected by optical fibers to a light source and a spectrophotometer [20–22]. The remaining ports are used to introduce the sample (port 4), MO reagent (port 3) and the masking/reducing mixture reagent of oxalate (OX) and ascorbic acid (AA) (port 5).

The two-reagent flow protocol (Fig. 2), designed to function in batch-flow mode is comprised of three parts: sample metering, sample derivatization, and monitoring of the reaction products. This is accomplished by means of the following sequence.

1/Silicate containing sample is aspirated upstream into a holding coil (HC1).
2/Flow reversal carries the sample through a confluence point within the LOV, where the MO reagent is added, thus transporting the reaction mixture into a second holding coil (HC2).
3/Flow reversal carries the reaction mixture of SiMo + MO back through the confluence point, where a mixture of OX and AA is added on the way back into HC1.
4/In the last part of the protocol, 600 μL of the SiMo + MO + OX + AA reaction mixture is transferred from HC1 into the flow cell, where it is arrested until chemical equilibrium is reached.

In this way, the absorbance is measured on a stationary homogenous mixture of sample after chemical equilibrium has been reached since:

- The sample was homogenously mixed by local turbulence when passing through the confluence point multiple times.
- The volume of the sample and reagent zone (600 μL) is much larger than the flow cell volume (100 μL).
- The centroid of the homogenous sample and reagent zone is, during the stop flow period, in the middle of the flow cell light path having been transported into the flow cell by 150 μL of forward flow, thus leaving the front and tail sections, which have been diluted by the carrier of the zone, outside the flow cell.
- The absorbance is measured when the solution is stationary after a 20 s stop flow period when the signal has reached steady state chemical equilibrium.

Auto calibration by a single standard solution [22] (Section 3.1) and autodilution of SW samples to be analyzed (Section 3.2), is accomplished in the first step of the flow protocol (Fig. 2) by varying the flowrate and volume of the carrier solution.

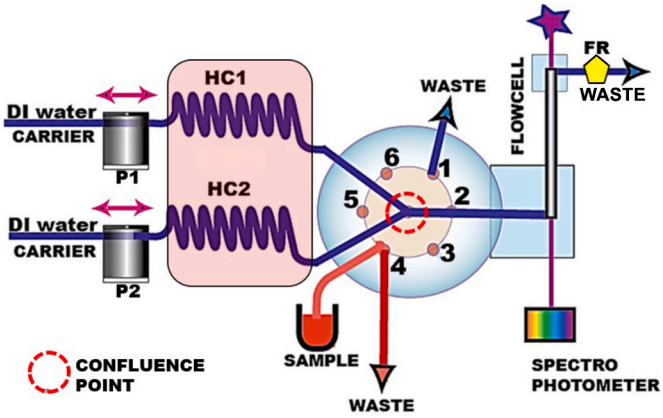


Fig. 1. Flow diagram of apparatus used in this work. Holding coils (HC 1, HC 2) were thermostated at 40 °C. The confluence point is situated at the center of the 6-position valve, milliGAT pumps (P1, P2), a flow restrictor (FR). A 20 cm long flow cell, internal volume 100 μL. Port 3 MO reagent; port 4 sample; port 5 OX + AA reagent.

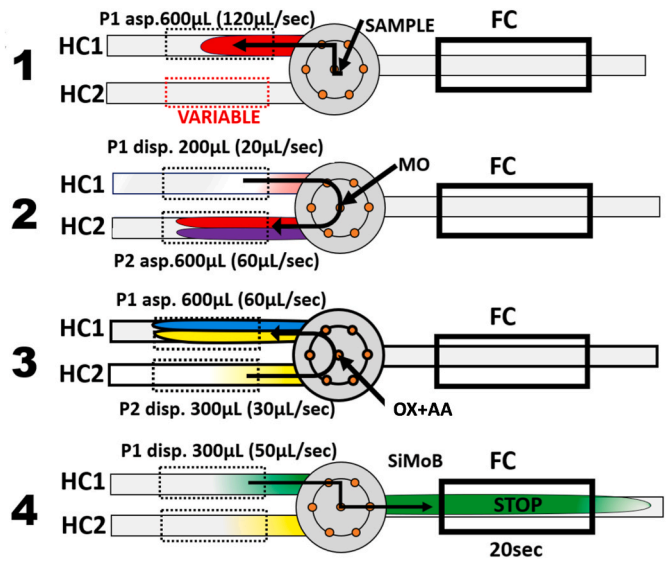


Fig. 2. Flow scheme for two reagent assay for determination of silicate by molybdenum blue spectrophotometry. P- milliGAT pump, HC- holding coil, FC- Flow cell, MO- molybdenum reagent, OX + AA oxalate/ascorbic acid reagent. SiMoB- silicomolybdenum blue. Asp refers to aspiration, disp refers to dispensing.

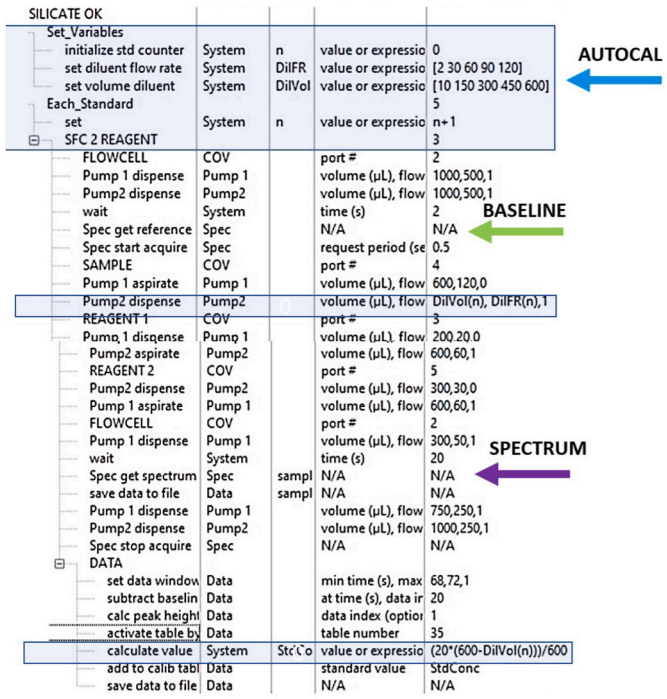


Fig. 3. Screenshots of the software protocol for the AUTOCAL calibration of the two-reagent silicate determination. AUTOCAL steps are in blue, BASELINE is determined at the green arrow, SPECTRUM is where the data is recorded.

2. Experimental

2.1. Instrumentation

The instrument (Fig. 1), miniSIA-2 (Global FIA, Fox Island, WA, USA), comprises two high precision, synchronously refilling milliGAT pumps, two thermostated holding coils, a 6-port lab-on-valve (model COV-MANI-6) furnished with a module for an external flow cell. All tubing connections, downstream from the milliGAT pumps including the

holding coils (internal volume 1000 µL), were made with 0.8 mm I.D. polytetrafluoroethylene (PTFE). The tubing between the carrier stream reservoirs and the milliGAT pump was made from 1.6 mm I.D. PTFE tubing in order to minimize degassing under reduced pressure at higher aspiration flowrates. A spectrophotometer (USB 4000) and a light source HL-2000-LL Fiber Optic Tungsten Halogen Source (Ocean Optics, Dunedin, FL) were connected to a 20 cm Long Light Path flow cell (LLP, internal volume 100 µL) by optical fibers with 500 µm silica cores encased in 0.8 mm I.D. PEEK tubing. The outlet of the LLP flow cell, mounted in close proximity to port 2 of the LOV module was fitted with a 40-psi flow restrictor (GlobalFIA, Fox Island, WA, USA), that, by elevating the pressure within the flow cell, efficiently prevented the formation of microbubbles from spontaneous outgassing (Fig. 1).

2.2. Reagents and materials

Si stock solution, containing 1000 µM Si was prepared weekly by diluting a commercial 1000 ppm Si standard (15747-100 ML, Fluka) with 0.5 N hydrochloric acid (HCl). This stock solution was further diluted daily by DI water, to obtain a single standard containing 20 µM Si. The Si solution had to be neutralized by HCl, because the Si standard is prepared in sodium hydroxide solution.

The mixed OX and AA reagent was prepared by dissolving 2 g of oxalic acid anhydrous, 2 g of ascorbic acid, and 2 g of sodium dodecyl sulfate (SDS) surfactant (CAS151-21-3 Thermo Scientific), in 100 mL of DI water. The solution, prepared ½ hour before use to stabilize its reducing strength, was stable for a week when stored in darkness at room temperature.

The MO reagent was prepared by dissolving 1.0 g of ammonium molybdate tetrahydrate crystalline (A674-500, CAS12054-85-2, certified ACS, Fisher Scientific) in 50 mL of DI water. Next, 0.5 mL of conc. sulfuric acid (A300-500, CAS7664-93-9, certified ACS, Fisher Scientific) was added and then DI water was added to make a final volume of 100 mL. This solution of 8.09 mM MO in 0.09 M sulfuric acid is stable for one month.

The 20 µM phosphate solution was prepared by diluting a commercial PO_4 standard (LC185701, LabChem) in DI water.

3. Results and discussion

3.1. Optimizing the silicomolybdate method by autocalibration

The goal of optimizing this assay is to maximize the sensitivity and the selectivity of SiMoB. In this work we are using the slope of the calibration line to optimize the assay protocols, an approach that is seldom used. The reason is that manual calibration procedures are time and labor consuming. However, the single standard calibration method offers easy automation of optimizing the assay conditions by comparison of the slopes of calibration lines. In the following sections, auto calibration is used to optimize the SiMoB method performed with OX reagent (Section 3.1.1), selectivity in the presence of PO_4 (Section 3.1.2), and to evaluate the salinity effect (Section 3.2.1)

3.1.1. Elimination of molybdenum blue interference by oxalate

It is well established that OX in a three-reagent protocol efficiently eliminates the formation of MoB by forming a complex with the MO reagent prior to its reduction with AA. However, a two-reagent protocol will be feasible only, if the kinetics of the MO-OX complex formation are much faster than the kinetics of MO reduction by AA.

Using the two-reagent flow protocol (Fig. 2) in the first step, a single concentrated standard solution is diluted by DI water carrier into five known standards used to create the calibration line (AUTOCAL, Fig. 3).

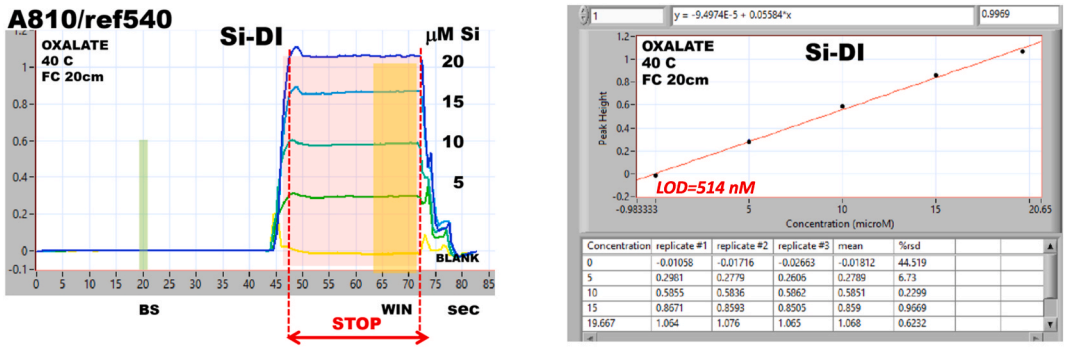


Fig. 4. Calibration by single standard solution (20 µM Si) prepared in DI water in the presence of oxalate. Left Response curves from an autocalibration (20 µM Si) prepared in DI. Right. Calibration obtained by plotting absorbance, collected during WIN period versus analyzed concentration, while the instrument baseline (BS) was set to zero.

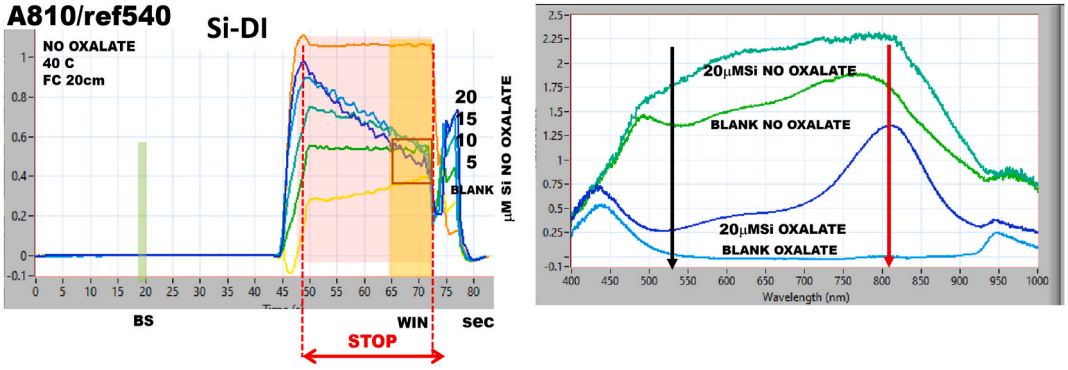


Fig. 5. Left. Response curves from an autocalibration by a single standard solution (20 µM Si) prepared in DI water in the absence of oxalate. Overlaid is a 20 µM Si standard with oxalate shown as a red line at the top. Right. Absorbance spectra recorded in the red box at the end of stopped flow period (WIN, left panel). Red and black arrows denote the target wavelength and the reference wavelength respectively.

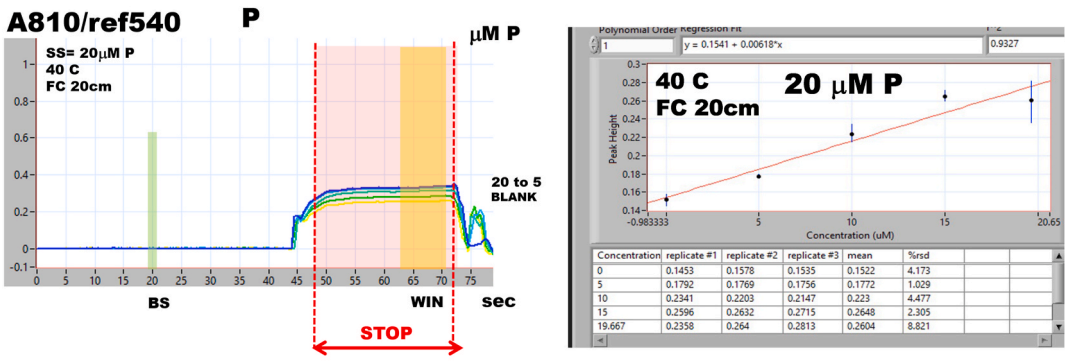


Fig. 6. Phosphate interference of the silicate determination. Calibration by single standard solution (20 µM PO_4) prepared in DI water in the presence of oxalate. Left. Response curves obtained in the BLANK to 20 µM PO_4 range. Right. Calibration obtained by plotting absorbance, collected during WIN period versus analyzed concentration, while instrument baseline (BS) was set to zero. Values under the figure compare the slopes of the calibration lines for Si (Fig. 4) and P (this figure).

This is accomplished by adjusting the dispensing volumes and flow rates of P2, while keeping P1 aspiration volumes and flow rates constant [22].

The calibration run obtained with the two-reagent protocol in the presence of OX (Fig. 4, left) confirms that equilibrium has been reached at all concentration levels and the absorbance values obtained towards the end of stop flow period (WIN) yield a strictly linear calibration response within the range 0–20 µM Si (Fig. 4 right).

In contrast, the calibration run obtained in the absence of OX (Fig. 5 left) yields a series of peaked responses, where the peak height is proportional to the concentration of Si. However, instead of a plateau (as shown in Fig. 4), the absorbance rapidly decreases. To compare the reaction kinetics in the presence of the complexing OX, a red response curve obtained with 20 µM Si with OX is overlaid, shown at the top of Fig. 5 (Left).

To explore this further, four spectra obtained during the WIN period were overlayed: blank with and without OX and a 20 µM Si standard with and without OX (Fig. 5 right). By comparing the blank runs in the presence and absence of OX, it is seen that the formation of colloidal MoB results in a broad absorption spectrum that obscures the formation of SiMoB at 810 nm and dramatically increases the absorbance at the 540 nm reference wavelength. Therefore, as MoB is formed, the corrected target absorbance peak for SiMoB is decreased due to the formation of the MoB colloid (Fig. 5 left). Therefore, the use of the mixed OX/AA prevents the formation of the interfering MoB complex.

3.1.2. *Elimination of phosphate interference by oxalate*

It has been widely accepted [3–9] that oxalate destroys PMo thus preventing the formation of PMoB in the final reduction step. However, the product of the destruction was never identified and a tolerable P:Si ratio was not quantified. To investigate the magnitude of the interference from PO_4 in this two-reagent silicate method, a standard solution containing 20 µM PO_4 was analyzed by using the Si autocalibration protocol (Fig. 3).

The calibration run (Fig. 6 left) shows that absorbance values under Si assay protocols and in the presence of OX, increase proportionally with increasing PO_4 concentrations as shown in (Fig. 6 right). This indicates that under these conditions the presence of OX does not completely eliminate the PO_4 interference.

Interference of PO_4 on the determination of silicate can be evaluated by comparison of the calibration slopes of PO_4 and silicate (Fig. 6 bottom) where P:Si = 0.11. Therefore, for samples containing [P] = [Si] the concentration of Si will be overestimated by 11 %. However, the PO_4 interference will be only be approximately 3 %, (similar to our analytical error), if the silicate concentration is more than 4 times higher than the PO_4 concentration. Fortunately, phosphate concentrations in the open ocean are much less than those of silicate (i.e. in shallow depths P:Si = 1:10, deep water P:Si = 1:50), thus this method can be used in most open ocean samples. However, this might, not be true for samples collected in coastal regions, in estuaries or in sedimentary pore-water.

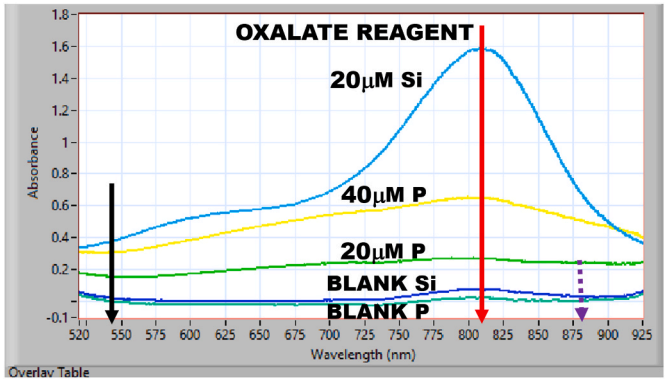


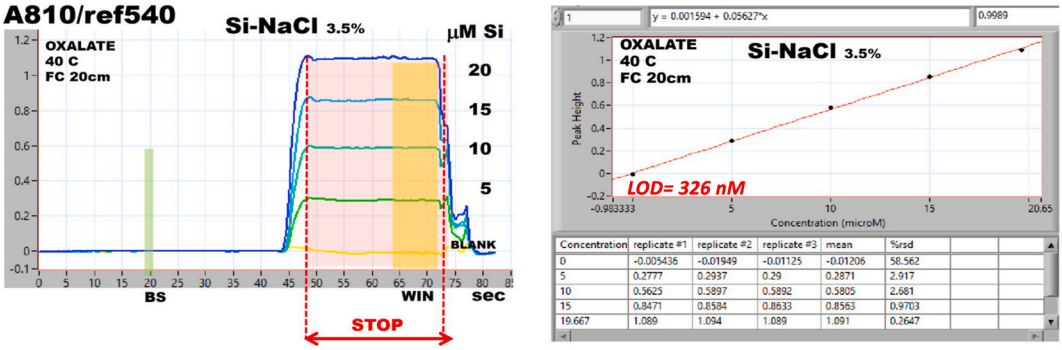
Fig. 7. Absorbance spectra recorded at the end of the stop flow period (WIN Fig.6) Red arrow marks SiMoB absorbance maximum. Black arrow is the reference wavelength. Dashed arrow shows the location of the PMoB absorbance maximum. For details see text.

It is beyond the scope of this work to speculate on the mechanism of PMoB "destruction". However, Fig. 7 shows in the presence of OX using Si assay conditions, that P does not show the typical PMoB spectrum, but instead is similar to the broad spectrum of MoB seen in Fig. 5 (Right). In contrast the spectrum of SiMoB is well defined.

3.2. *Analysis of sea water*

3.2.1. *Elimination of salinity and schlieren effect*

To evaluate the effect of salinity on the calibration data of the Si method, an autocalibration (Figs. 2 and 3) was performed with standard solutions being prepared either in DI water or in 3.5 % (w/w) sodium



SLOPE DI/NaCl = 0.0558/0.0563 = 0.99 INTERCEPT DI – NaCl = 0.001 – 0.0016 = -0.0006

Fig. 8. Calibration by single standard solution (20 µM Si) prepared in NaCl solution in the presence of oxalate. Left Response curves from an autocalibration (20 µM Si) prepared in NaCl solution. Right. Calibration obtained by plotting absorbance, collected during WIN period versus analyzed concentration, while the instrument baseline (BS) was set to zero. The text compares the slopes of DI (Fig. 4) and NaCl (this figure) AUTOCAL Si standard curves. Also shown is the difference between the DI and NaCl intercepts.

chloride (NaCl) solution, the latter solution serving as simulated open ocean sea water. Two calibration runs were performed.

- DI calibration with Si standard prepared in DI water, while both carrier streams were made of DI water.
- Simulated sea water calibration with Si standard prepared in NaCl solution, while the carrier stream (P1) was made of DI water, and carrier stream (P2) was NaCl solution.

In this way, the DI calibration (Fig. 4), is based on deionized water since the 20 µM Si single standard was prepared in DI water and was stepwise diluted by DI water carrier, while Fig. 8 shows a Si standard prepared in NaCl which is stepwise diluted by NaCl.

Since the ratio of the calibration slopes obtained from Figs. 4 and 8 is $>$ 0.99, and the difference in intercept offsets is 0.0006 AU (equivalent to 10.6 nM Si) (Fig. 8), we conclude that DI and NaCl calibrations are, within our methodological errors, identical and the DI calibration is suitable for analysis of seawater, estuarine and fresh water samples.

3.2.2. *Accuracy and reproducibility of sample autodilution*

In the open ocean, the concentration of Si increases with increasing depth. To accommodate the 0–160 µM Si concentration range, sea water samples must be diluted because it is practical to use only one calibration range for any given assay. Therefore, for this work, the most sensitive, 0–20 µM Si calibration range was selected. In order to be able to do so, this was accomplished for sea water samples within the first step of the assay protocol (Fig. 9), using the autodilution technique by merging the sample solution with a specific volume of DI water dispensed by P2. In this way samples within the 0–20 µM Si range are analyzed undiluted Fig. 9 (A), samples in 20–80 µM Si range are 4 times diluted (B) and samples in 80–160 µM Si range are 8 times diluted (C). The corresponding script is in Fig. 10. Since the increase of Si values with increasing depth takes place gradually and in a predictable concentration gradient, it is easy to implement this dilution scheme.

The accuracy and reproducibility of the sample dilution was evaluated by comparison of results obtained by analyzing 3 manually diluted standards which were measured using the two-reagent protocol, followed by their automated dilution into the 0–20 µM Si calibration range

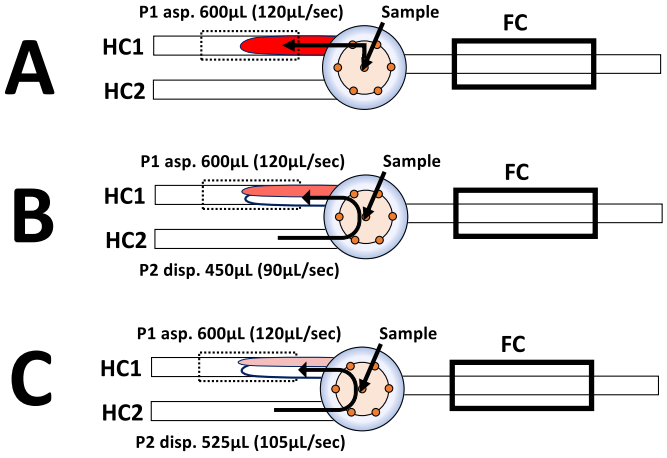


Fig. 9. Schematic showing flow and flowrates for sample autodilution. Panel A, undiluted sample, Panel B 4x dilution, Panel C 8x dilution. HC/FC/P components as in Fig. 2.

(Table 1). This data which was developed to evaluate the limits of this dilution process shows that 4-fold automated dilution of concentrated samples results in recoveries that are within the methodological errors. However, an 8-fold and 10-fold dilution of the lowest concentration is distorted by the error inherent the measurement of low concentrations. The 8-fold dilution recovery however, is within our analytical error at the higher concentration standards. The 10-fold dilutions show larger errors at all concentrations. It follows that automated dilution can be reliably applied to cover the entire Si concentration range by judiciously selecting the degree of dilution up to 8 times.

3.2.3. *Analysis of reference materials*

The accuracy and reproducibility of the method (Table 2) was established by determining the concentration of certified reference materials [23]. We see clearly in these values that as the dilution factor increases beyond 4x, the precision of the values decreases and the recovery of the method starts to be outside our expected analytical error.

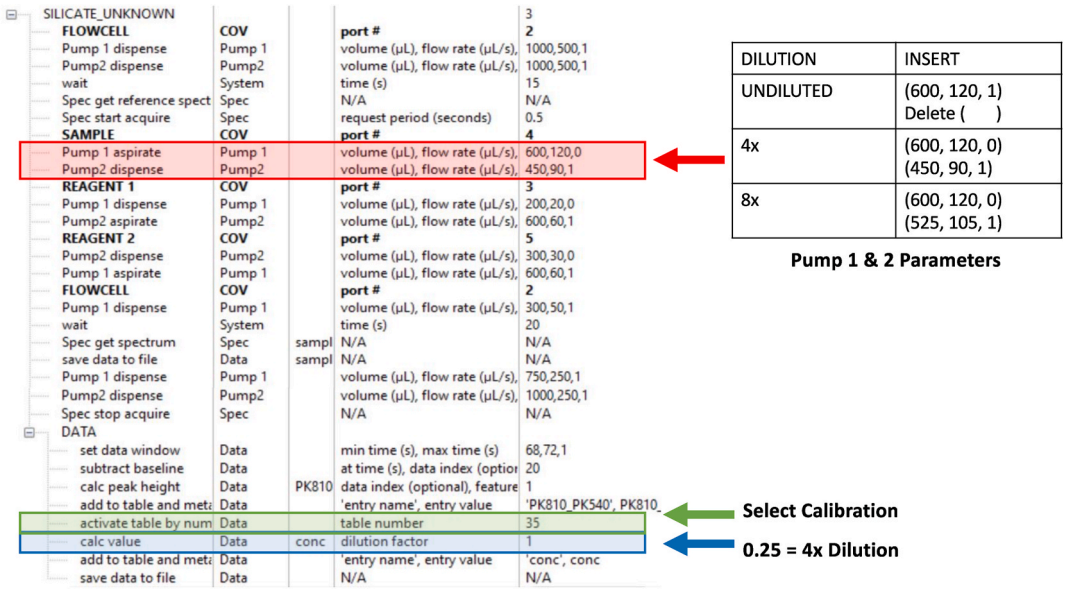


Fig. 10. Screenshots of the software protocol for four times sample autodilution. To change the dilution factor use the values in the table to the right to change the values within the red box. Green box selects the calibration table number (produced in Fig. 3 as 35.) to be used for data calculation. Blue box contains the inverse of the dilution factor to be used for data calculation. For details see text.

Table 1
Precision and accuracy of sample autodilution.

Manual Standard Value (µM)	Found (µM)	Recovery (%)	RSD (%)	Dilution Factor (DF)
39.92	38.96	98	2.3	2x
39.92	39.74	100	4.8	4x
79.43	77.06	97	3.9	4x
39.92	42.71	107	8.9	8x
79.43	77.82	98	5.7	8x
159.95	158.58	99	1.8	8x
39.92	35.36	89	23	10x
79.43	67.39	85	43	10x
159.95	147.83	92	17	10x

The increase in error seems to be primarily a result of the degree of dilution rather than the resulting smaller concentrations, as the 4x dilution of the CL material (originally 13.8 µM) yields recovery at 102 % and a r.s.d. of 3.7 %, still close to our expected analytical error despite the low concentration this dilution would produce.

It is important to note that our certified reference materials and our artificial sea water samples were particle free. We are seeing some issues with natural seawater samples containing particles that may result in problems with autodilution of these samples. This is an area which requires further investigation.

Table 2
Analysis of reference materials.

CRM	Reported value (µM)[a] [17]	Found Recovery (µM)	Recovery (%)	RSD (%)	Dilution Factor (DF)
CL	13.8	13.47	98	2.4	1x
CL	13.8	13.44	97	2.4	2x
CO	34.72	34.43	100	2.3	2x
CL	13.8	14.05	102	3.7	4x
CO	34.72	35.88	103	3.6	4x
CL	13.8	13.22	96	21	8x
CO	34.72	38.12	110	5.1	8x
CM	100.5	112.03	111	14	8x

[a] Represented the reported value for the CRMs [23].

4. Conclusion

Programmable flow injection enables operations that cannot be done in any other way, such as calibration with a single standard solution, autodilution of samples, or elimination of interferences, caused by salinity and the schlieren effect. In this work, in the first step of the assay protocol, the flow programming facilitates automated calibration and sample dilution, while the last step of the assay protocol is designed to operate in a batch mode, when chemical reactions reach equilibrium and the absorbance measurement is performed on a stationary solution arrested in the flow cell. In this way the same calibration protocol, and resulting DI water-based calibration can be used for analysis of open ocean waters as well as of estuarine and fresh waters – a task not possible using continuous flow techniques [24].

Our results confirmed that oxalate prevents formation of interfering MoB and PMoB (Fig. 5), but it also revealed that phosphate still interferes in the determination of silicate (Fig. 7) by promoting the formation of a colloid of unknown composition.

Determination of silicate in sea water is a widely used method: thus, in a 2-year period (2010-11) some 51,938 new silicate measurements from 5317 vertical oceanographic profiles were reported to the NOAA global data base (A. Barna pers. comm.). The use of an autocalibration system for standardization simplifies shipboard use and reduces the possibility of human error. These results show the ability to adapt traditional continuous flow techniques to newer discontinuous flow systems that use smaller reagent volumes and produce less chemical waste.

CRediT authorship contribution statement

M. Hatta: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Funding acquisition, Formal analysis, Conceptualization. **J. Ruzicka:** Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Conceptualization. **C. Measures:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **M. Davis:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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