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Automated Spectrophotometric Analyzer for Rapid Single-Point Titration of Seawater Total Alkalinity

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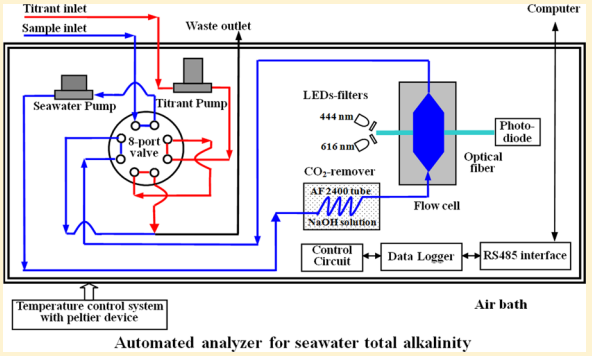
Supporting Information

ABSTRACT: An automated analyzer was developed to achieve fast, precise, and accurate measurements of seawater total alkalinity (A_T) based on single-point titration and spectrophotometric pH detection. The single-point titration was carried out in a circulating loop, which allowed the titrant (hydrochloric acid and bromocresol green solution) and a seawater sample to mix at a constant volume ratio. The dissolved CO_2 in the sample−titrant mixture was efficiently removed by an inline CO_2 remover, which consists of a gas-permeable tubing (Teflon AF2400) submerged in a sodium hydroxide (NaOH) solution. The pH of the mixture was then measured with a custom-made spectrophotometric detection system. The analyzer was calibrated against multiple certified reference materials (CRMs) with different A_T values. The analyzer features a sample throughput time of 6.5 min with high precision (±0.33−0.36 μmol kg^{-1}; n = 48) and accuracy (−0.33 ± 0.99 μmol kg^{-1}; n = 10). Intercomparison to a traditional open-cell A_T titrator showed overall good agreement of 0.88 ± 2.03 μmol kg^{-1} (n = 22). The analyzer achieved excellent stability without recalibration over 11 days, during which time 320 measurements were made with a total running time of over 40 h. Because of its small size, low power consumption requirements, and its ability to be automated, the new analyzer can be adapted for underway and *in situ* measurements.



Automated analyzer for seawater total alkalinity

INTRODUCTION

As a measure of seawater buffering capacity, total alkalinity (A_T) of a seawater sample is defined as "the number of moles of hydrogen ion equivalent to the excess of proton acceptors (bases formed from weak acids with a dissociation constant $K \leq 10^{-4.5}$ at 25 °C and zero ionic strength) over proton donors (acids with $K > 10^{-4.5}$) in 1 kg of sample".[1] Observations of A_T are extremely useful in identifying and assessing physical and biogeochemical processes in the ocean, such as inventory of anthropogenic CO_2, calcification by shell-building organisms, dissolution/precipitation of calcium carbonate minerals, aerobic versus anaerobic respiration, and water mixing.[2−6] As one of the four primary parameters of the marine CO_2 system, A_T can be used with one of the other three parameters, pH, pCO_2, and total dissolved inorganic carbon (DIC), to fully characterize the seawater−carbonic system through thermodynamic calculations.[7,8] In many ocean carbon studies, the required precision and accuracy of seawater A_T measurements are stringent (<0.2%) because of the high background values (mean seawater A_T ~ 2300 μmol kg^{-1}) compared to relatively small natural variations.

The traditional method for high-precision, high-accuracy measurements of seawater A_T involves a multi-point potentiometric titration in an open or a closed cell.[9,10] It uses a stepwise addition of a strong acid [hydrochloric acid (HCl)] to a known amount of seawater in a titration cell. The titration is monitored using a pH electrode, and the A_T is computed from the equivalence point located by a nonlinear least-squares or Gran function. This method is time-consuming (about 10−20 min for one sample) and requires precise weighing or precise delivery of the acid and sample. The pH electrode used to monitor the titration should have ideal Nernst response and be calibrated frequently.[11] These attributes make the traditional method challenging to be adopted for a rapid, automated operation with low maintenance.

To overcome these challenges, alternate methods have been developed in the past. Martz et al.[12] introduced a tracer-monitored titration for spectrophotometric measurements of seawater A_T. This method essentially shifts the burden of precise solution delivery or weighing to precise spectrophotometric measurements by monitoring titrant dilution during

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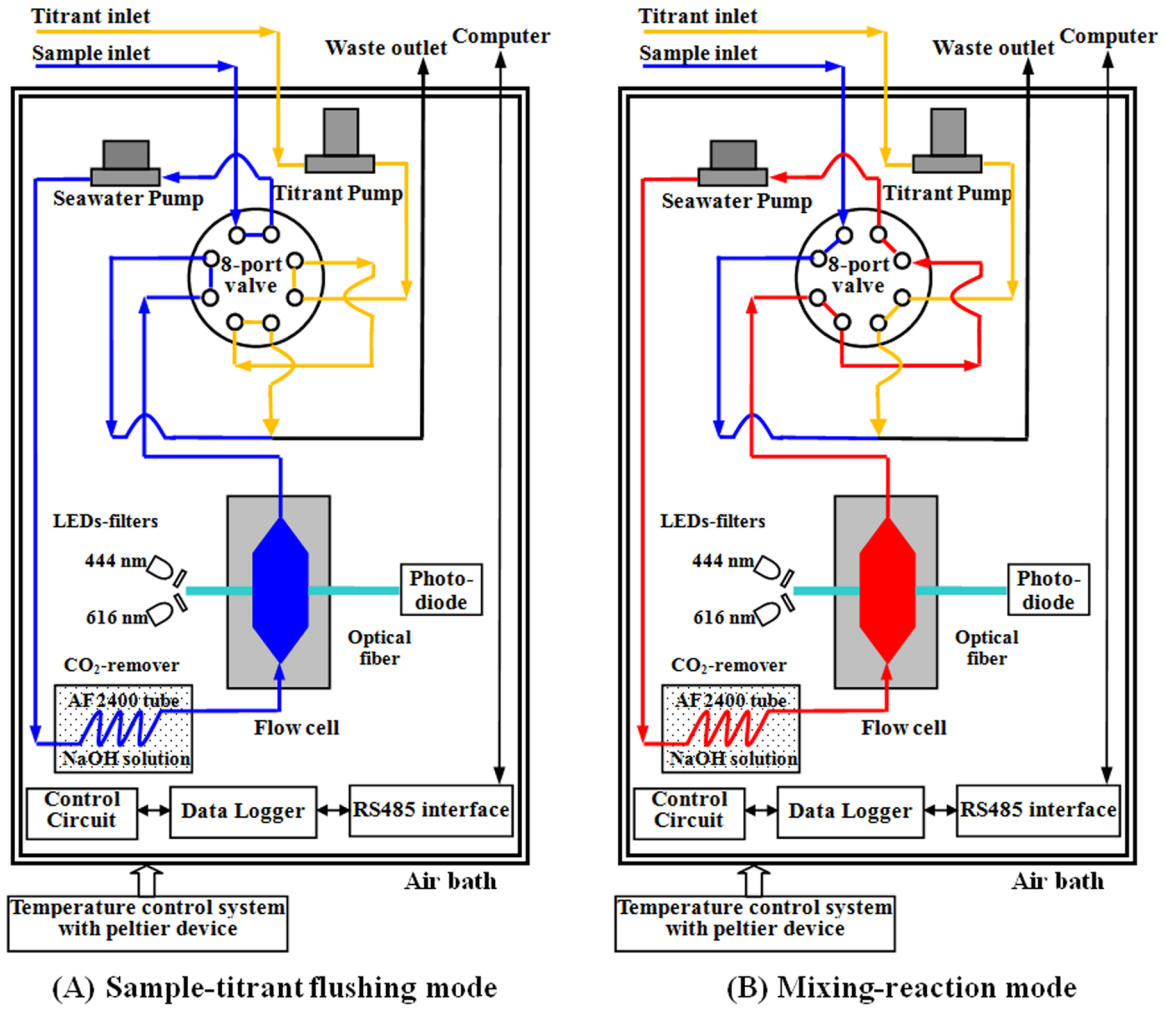

Figure 1. Schematic diagram of the A_T analyzer. During the sample−titrant flushing mode (A), the sample and titrant were introduced into the seawater flow path (blue) and the titrant flow path (yellow), respectively. During the mixing−reaction mode (B), a reaction loop (red), which consists of part of both the seawater and the titrant flow paths, was formed. The sample and titrant in the loop would mix and react rapidly when the seawater pump was turned on. In the mixing−reaction mode, the dissolved CO_2 would be removed by passing through the CO_2 remover. The pH of the mixture in the flow cell was spectrophotometrically measured.

stepwise additions of the titrant. The automated sequence has a sample throughput time of ∼16 min.

The other method for the determination of seawater A_T is the single-point, open-cell titration developed by Culberson et al.[13] It involves titration of a known amount of a seawater sample with an excess amount of a strong acid; after the sample−titrant mixture is purged of CO_2 with a stream of N_2 to reduce the effect of dissolved CO_2, the excess acid concentration (residual pH) is potentiometrically measured, allowing for calculation of alkalinity via the difference between added and excess amounts of acid. Byrne and co-workers modified this technique using spectrophotometric measurements of excess acid.[14,15]

The procedure for a single-point, open cell A_T titration is relatively simple and fast. A few automated flow-through systems have been developed on the basis of this technique using either spectrophotometric or potentiometric measurements of residual pH.[16,17] These systems can achieve excellent precision but require frequent calibration, precise solution delivery or weighing, or long throughput time (>10 min). The removal of CO_2 during these open-cell titrations requires purging gas and a stirring device, which make the systems bulky and complex.[16] As such, we believe that the single-point, open-cell titration of alkalinity has not been fully realized to its potential.

The ocean carbon and ocean acidification research communities have long been craving robust methodologies and *in situ* sensor technology for precise A_T measurements. *In situ* sensors for pH,[18−20] pCO_2,[21−23] and DIC[24] of seawater have been developed. *In situ* A_T sensors are lagging behind primarily because the existing methods are difficult to adapt for *in situ* applications. In this study, we describe the development of a fast, fully automated A_T analyzer based on single-point titration and spectrophotometric pH measurements. The analyzer features a throughput time of ∼6.5 min, high precision and accuracy (better than 2 μmol kg^{-1}), and requires less frequent calibration. The methodology applied to this analyzer is relatively simple and robust and has the potential to be further developed for underway instruments and *in situ* sensors. The major contribution of this study is the development of a circulating reaction loop, which allows for the titrant and seawater to mix at a constant volume ratio during the single-point titration. In addition, a novel inline CO_2 remover, which consists of a gas-permeable tube (Teflon AF2400) surrounded

Table 1. Standards and the Obtained Calibration Curves

	standards[a]			calibration curves[b]		
date		A_T (μmol kg^{-1})	salinity	R^2	slope (k)	intercept (C_t) (μmol kg^{-1})
July 5	1	2023.42	30.28			
	2	2124.91	31.80	0.99985	−0.94574	51704
	3	2229.75	33.37			
July 8	1	2032.77	30.42			
	2	2121.01	31.74	0.9999	−0.95007	51733
	3	2229.75	33.37			
July 9	1	2030.89	30.39			
	2	2129.66	31.87	0.99988	−0.94114	51686
	3	2229.75	33.37			
July 10	1	2034.04	30.44			
	2	2127.11	31.83	0.99984	−0.94196	51689
	3	2229.75	33.37			
July 16	1	2022.97	30.27			
	2	2129.68	31.87	0.99987	−0.94456	51710
	3	2229.75	33.37			
mean value				0.99986	−0.94469	51704
standard deviation				0.00002	0.0035	19

[a]Standard 3 is the CRM of batch 118, and standards 1 and 2 are the diluted CRMs of the same batch. [b]Calculated on the basis of eq 8.

by a NaOH solution, was used to efficiently remove the dissolved CO_2 in the sample−titrant mixture to achieve an "open-cell" condition without stirring or purging the titration solution. The performance of the analyzer was evaluated through measurements of certified reference materials (CRMs) and intercomparison samples measured with the traditional open-cell titration.

EXPERIMENTAL SECTION

Principle. Following Yao and Byrne's spectrophotometric A_T measurements,[15] we used a mixture of HCl and indicator [bromocresol green (BCG)] as the titrant. The addition of the titrant to the seawater sample is carried out by switching a two-position eight-port valve to construct a reaction loop (Figure 1). In the reaction loop, the titrant and sample mix at a constant volume ratio at a controlled temperature (25.0 ± 0.1 °C). The amount of HCl added is always more than enough to titrate either the sample or the CRM. The concentration of the excess acid in the sample−titrant mixture in the reaction loop can be expressed by

$$\frac{C_t V_t \rho_t - A_T V_{sw} \rho_{sw}}{V_t \rho_t + V_{sw} \rho_{sw}} = [H^+]_F + [HSO_4^-] + [HF] + [H_3PO_4] + [HI^-] - [HCO_3^-] - 2[CO_3^{2-}] - [B(OH)_4^-] - [OH^-] - [HPO_4^{2-}] - 2[PO_4^{3-}] - [SiO(OH)_3^-] - [NH_3] - [HS^-] \quad (1)$$

where C_t is the acid concentration of the titrant, including contributions from both HCl and the indicator, V_{sw} and V_t are the volumes of the seawater sample and titrant added into the reaction loop, respectively, ρ_{sw} and ρ_t are the densities of the seawater sample and titrant, respectively, $[HI^-]$ is the concentration of the protonated form of BCG, which is regarded as a proton donor, in the sample−titrant mixture, and $[H^+]_F$ is the free hydrogen ion concentration.

In this study, the pH of the sample−titrant mixture is kept in the range of 3.5−4.0 by adjusting C_t, such that the terms $[B(OH)_4^-]$, $[OH^-]$, $[H_3PO_4]$, $[HPO_4^{2-}]$, $[PO_4^{3-}]$, $[SiO(OH)_3^-]$, $[NH_3]$, and $[HS^-]$ on the right side of eq 1 can be omitted (see the Supporting Information). When the dissolved CO_2 in sample−titrant mixture is removed, $[HCO_3^-]$ and $[CO_3^{2-}]$ can also be omitted. As a result, eq 1 is reduced to

$$\frac{C_t V_t \rho_t - A_T V_{sw} \rho_{sw}}{V_t \rho_t + V_{sw} \rho_{sw}} = [H^+]_F + [HSO_4^-] + [HF] + [HI^-] \quad (2)$$

Using a volume mixing ratio γ_v ($\gamma_v = V_{sw}/V_t$), a density ratio γ_ρ ($\gamma_\rho = \rho_{sw}/\rho_t$), and a mass mixing ratio γ ($\gamma = \gamma_v \gamma_\rho$), eq 2 can be rearranged to obtain

$$\frac{C_t - A_T \gamma}{1 + \gamma} = [H^+]_F + [HSO_4^-] + [HF] + [HI^-] \quad (3)$$

Using dissociation equilibriums of the last three species, eq 3 can be rearranged as

$$A_T \gamma = C_t - (1 + \gamma)\left([H^+]_F + \frac{S_T \frac{\gamma}{1+\gamma} [H^+]_F}{[H^+]_F + K_S} + \frac{F_T \frac{\gamma}{1+\gamma} [H^+]_F}{[H^+]_F + K_F} + \frac{I_T \frac{1}{1+\gamma} [H^+]_F}{[H^+]_F + K_I} \right) \quad (4)$$

where S_T and F_T are the total sulfate and total fluoride in the seawater sample, respectively, I_T is the total concentration of BCG in the titrant, K_F and K_S are the dissociation constants of HF and HSO_4^-, respectively, K_I is the second dissociation constant of BCG, and $\gamma/(\gamma + 1)$ and $1/(\gamma + 1)$ are the dilution factors of the seawater sample and titrant, respectively. All dissociation constants are on free scale, and concentrations are expressed in moles per kilogram of solution.

$[H^+]_F$ or pH_F of the sample−titrant mixture can be measured spectrophotometrically and expressed as[14,15]

$$pH_F = -\log K_I + \log\left(\frac{R - e_1}{e_2 - R e_3} \right) \quad (5)$$

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where $e_1 = \lambda_2\varepsilon_{HI^-}/\lambda_1\varepsilon_{HI^-}$, $e_2 = \lambda_2\varepsilon_{I^{2-}}/\lambda_1\varepsilon_{HI^-}$, and $e_3 = \lambda_1\varepsilon_{I^{2-}}/\lambda_1\varepsilon_{HI^-}$, representing ratios of absorptivity coefficients, $\lambda\varepsilon_x$, for each indicator form, x, at wavelength 1 (λ_1) and wavelength 2 (λ_2), where the acid form (HI^-) and base form (I^{2-}) of the indicator have the maximum absorbance, respectively. R is the absorbance ratio ($R = \lambda_2A/\lambda_1A$) of λ_2 and λ_1. For BCG, λ_1 = 444 nm, λ_2 = 616 nm, and e_1, e_2, and e_3 at 25 °C, which are not salinity-dependent, are 0.0013, 2.3148, and 0.1299, respectively.[14,15] The salinity ($20 \le S \le 35$) dependence of K_I for BCG at 25 °C was reported by Breland et al.[14] as

$$-\log K_I = 4.4166 + 0.0005946(35 - S) \quad (6)$$

The pH of the sample−titrant mixture at 25 °C can then be calculated using

$$pH_F = 4.4166 + 0.0005946(35 - S) + \log\frac{R - 0.0013}{2.3148 - 0.1299R} \quad (7)$$

The sum of the four terms in the parentheses on the right side of eq 4 is the concentration of excess acid in the sample−titrant mixture. Because of inevitable measurement errors in parameters, such as the dissociation constants and $[H^+]_F$, there is a discrepancy between the actual and measured values of excess acid concentration. In practice, we may introduce an empirically calibrated coefficient, k, in eq 4 to describe this discrepancy

$$A_T\gamma = C_t - (1 + \gamma)Qk \quad (8)$$

where Q is the measured concentration of excess acid and given by

$$Q = [H^+]_F + \frac{S_T\frac{\gamma}{1+\gamma}[H^+]_F}{[H^+]_F + K_S} + \frac{F_T\frac{\gamma}{1+\gamma}[H^+]_F}{[H^+]_F + K_F} + \frac{I_T\frac{1}{1+\gamma}[H^+]_F}{[H^+]_F + K_I} \quad (9)$$

The k, which is experimentally shown to be a constant at a constant temperature and limited salinity range (see Table 1), can be accurately determined by calibrating the system with CRMs. γ in eqs 8 and 9 can be calculated with γ_v, which is determined experimentally, as shown in the Supporting Information, and with ρ_{sw} and ρ_t, which are known from the seawater salinity and the composition of the titrant. To have $A_T\gamma$ instead of A_T on the left side of eq 8 is a mathematical treatment to have a constant intercept, C_t, for the calibration equation. At least two standards with known A_T and salinity values are used to calibrate the analyzer. A linear calibration curve can be obtained by plotting $A_T\gamma$ against $(1 + \gamma)Q$; thus, C_t and k, the intercept and slope of the calibration curve, can be determined. Thereafter, the A_T of a measured seawater sample with a known salinity can be determined with eq 8 by spectrophotometrically measuring the pH of the sample−titrant mixture and calculating Q with eq 9.

Instrument. The schematic diagram of the A_T analyzer is shown in Figure 1. The main components include a two-position eight-port valve, two pumps, a custom-made flow cell, a custom-made spectrophotometric detection system with a light-emitting diode (LED) filter assembly and a photodiode, an inline CO_2 remover, control electronics, and a data logger. All components are thermostatted (25.0 ± 0.1 °C) inside an air bath using a Peltier device for heating and cooling. The two-position eight-port valve (C22Z-6188EH, Valco Instruments Co., Inc., Houston, TX) is used to switch between two modes of flow: a sample−titrant flushing mode and a mixing−reaction mode. In the former, the seawater and titrant pumps introduce the sample and titrant into the seawater and the titrant flow paths (Figure 1A), respectively, while the previous sample−titrant mixture is flushed out. During the mixing−reaction mode, the two-position eight-port valve is switched, so that a loop is created that contains both the seawater and titrant solutions (Figure 1B). The seawater pump drives circulation, and the two solutions mix. The volumes of the sample and titrant that mix in the loop depend only upon the internal volumes of the parts of the two flow paths, which form the loop. There are two advantages for this design: (1) Because the internal volumes of the two flow paths are constant under a constant temperature, a fixed sample-to-titrant volume mixing ratio, γ_v, is readily achieved for the single-point titration; If γ_v is experimentally determined, the mass mixing ratio, γ, can be calculated ($\gamma = \gamma_v\rho_{sw}/\rho_t$). (2) The use of a high-precision pump is not necessary.

The titrant pump is a solenoid pump (120SP1250-4EE, Bio-Chem Fluidics, Inc., Boonton, NJ) with a dispense volume of 50 μL per pulse, while the seawater pump is a peristaltic pump (WX10, Baoding Longer Precision Pump Co., Ltd., Baoding, China). To achieve a constant internal volume, the seawater pump was modified by positioning a magnet on the rotor and a hall sensor on the casing. The magnetic signal, which is produced when the magnet passes the sensor, is used to stop the magnet and the hall sensor at the same position every time. This way, the rollers of the pump will always stop at a fixed location relative to the rotor and, thus, achieve a constant internal volume. The seawater pump is equipped with a thick-wall pump tubing to reduce wear out, and the tubing is replaced regularly. The effect of the volume change caused by replacing the tubing upon measurement was discussed later.

Bubbles often present a challenge to optical detection in spectrophotometric pH measurements. To avoid bubbles and subsequent effects upon detection, a novel, cross-shaped flow cell with an internal volume of ∼1.3 mL and an optical path length of ∼1 cm was made of Plexiglas (Figure 1). The optical path and the flow path of the cell are perpendicular to each other ("cross-shaped"). When the cell is positioned with light path horizontally and flow path vertically, the bubbles in the cell float and leave the optical path free of bubbles. In addition, the relatively large internal volume of the cell allows the seawater and titrant to mix rapidly.

The inline CO_2 remover consists of Telfon AF2400 tubing (0.8 mm in outer diameter, 0.6 mm in inner diameter, and 120 cm in length, Biogeneral, San Diego, CA) located in a plastic box filled with 1 M NaOH solution. Teflon AF2400 is of high CO_2 permeability and has been used in the development of pCO_2 and DIC sensors.[5,22,23,25] When the acidified solution passes through the remover, the dissolved CO_2 would diffuse rapidly out of the tube and be absorbed by the NaOH solution.

A spectrophotometric detection system modified from our earlier development[22] was used to measure pH of the sample−titrant mixture. It is composed of two LEDs (Roithner Laser Technik Gmbh, Austria) with emission peak wavelengths at 444 and 616 nm, two optical filters (Shanghai Xinjing, Ltd., China) with central wavelengths of 443.8 and 615.8 nm and their respective half bandwidths of 6.7 and 7.4 nm, and a photodiode (S1226-44BQ, Hamamatsu Corp., Japan). A detection system with a narrow bandwidth of wavelength is

very important for accurate spectrophotometric pH measurement. The two LEDs, driven by pulsed current, are turned on for 500 ms one after the other within a detection cycle of 2.5 s. The outputs of the photodiode are recorded as the light intensities of 444 and 616 nm.

The spectrophotometric detection system, pumps, and valves are controlled by a control circuit based on a microcontroller unit (C8051F340, Silicon Laboratories, Inc., Austin, TX). A custom-made data logger is used for data acquisition and communication. The analyzer is interfaced with a personal computer via a RS485 interface. The software, written in C language, allows users to control all components of the system and run automated measurement cycles.

Before measurement, the titrant and sample to be analyzed are put in a thermostat water bath to bring the temperature to 25.0 ± 0.1 °C. The analyzer will then run in a series of steps to make A_T measurements: (1) The seawater and titrant pumps are turned on to flush their respective flow paths. The seawater pump is activated for 2.5 min, and about 20 mL of the sample is introduced into the seawater flow path. The acid pump then delivers 0.5 mL of titrant into the titrant flow path. These delivered volumes ensure that the previous sample−titrant mixture in both flow paths is flushed out completely. (2) The reference light intensity is taken. The spectrophotometric detection system is activated, and the light intensities of 444 and 616 nm are measured. The detection cycle is repeated 15 times to ensure a stable signal. The recorded signals in the last 10 detection cycles are averaged and used as the outputs. (3) The sample and titrant are mixed, and CO_2 is removed. The analyzer enters the mixing−reaction mode, and the seawater pump is activated for a period of time (mixing time), after which the solution has a pH range between 3.5 and 4.0. The sample−titrant mixture circulates through the remover to remove dissolved CO_2. A longer mixing time results in a lower CO_2 level in the solution. The mixing time is optimized experimentally as shown in the Experimental Section. (4) The light intensity for sample pH measurements is taken. These light intensities, with those taken in step 2, are used to calculate the absorbances for calculation of pH.

Solutions and Standards. The titrant was made of a mixture of ∼50 mmol kg^{-1} HCl and ∼0.5 mmol kg^{-1} BCG (Fisher Scientific) in 0.65 mol kg^{-1} NaCl solution and was stored in borosilicate glass bottles. The adding of NaCl to the titrant is to reduce the salinity difference between the sample and titrant. CRMs (batches 118, 119, and 120) were obtained from A. G. Dickson at Scripps Institution of Oceanography. Deionized (DI) water used throughout the experiment was obtained from a Barnstead Nanopure ultrapure water purification system (Thermo Scientific).

Calculation of γ and Q Value. The densities (ρ_t and ρ_{sw}) at 25 °C of the titrant, whose chemical composition was known, and the seawater sample, whose salinity should be determined before measurement, were calculated with the equations proposed by Dickson et al.[10] They were used together with γ_v, which was determined experimentally, as shown in the Supporting Information, to calculate γ. S_T and F_T in eq 9 were calculated from their conservative relationship with sample salinity. On the basis of the equations proposed by Dickson et al.,[10] K_F and K_S at 25 °C were calculated using the salinity of the sample−titrant mixture, which was estimated using γ and the salinity of the sample and titrant. After $[H^+]_F$ was measured spectrophotometrically, the concentration of excess acid in the sample−titrant mixture, Q, could be calculated with eq 9.

CO_2 Removal. In single-point, open-cell titration, dissolved CO_2 resulting from the A_T titration is usually removed from the sample−titrant mixture by purging the sample with gas.[13−15] Our analyzer uses an inline CO_2 remover to achieve this. The size (length, diameter and wall thickness) of CO_2 removal is related to the amount of Teflon AF2400 tubing used, the volume of the seawater in the reaction loop, and the mixing time. Once the analyzer is built, the mixing time is the only factor that can be easily adjusted to affect the efficiency of CO_2 removal. To investigate how much mixing time was required to effectively remove dissolved CO_2, the analyzer was used to measure a seawater sample with known DIC. Different mixing times were applied, and the solutions in the loop after CO_2 removal were analyzed with a DIC analyzer (AS-C3, Apollo SciTech, Bogart, GA), which was calibrated against CRM.

System Calibration. CRM batch 118 and two of its gravimetrically diluted solutions were used as standards to calibrate the analyzer. The diluted CRMs were prepared by adding a known amount of DI water into a known amount of CRM via weighing. The A_T and salinity values of the diluted CRMs were assigned according to the dilution factors.

Performance Evaluation. The precision of the analyzer was evaluated by repeatedly measuring two types of seawater samples, type I and type II. The type I sample was filtered coastal seawater from Environmental Ecosystem Lab of Woods Hole Oceanographic Institution (WHOI), and the type II sample was prepared by adding $NaHCO_3$ solution to the type I sample. Each of these samples was repeatedly measured with the analyzer 48 times in about 6 h.

The stability of the analyzer was investigated by observing the variations of the intercepts and slopes of the calibration curves obtained over 11 days. The accuracy of the analyzer was studied by comparing the measurements of the analyzer to the certified values or those made by a commercial open-cell alkalinity titrator (AS-ALK2, Apollo SciTech, Bogart, GA). The measured samples included CRMs, coastal water near WHOI, and open ocean water from the North Atlantic.

RESULTS AND DISCUSSION

Efficiency of CO_2 Removal. The Teflon AF2400-NaOH CO_2 remover (Figure 1) shows good CO_2 removal efficiency (Figure 2). For example, at a mixing time of 3 min, more than 92% of DIC in the sample−titrant mixture was removed. In this study, the pH of the mixture was kept in the range of 3.5−4.0. The DIC concentration in seawater usually ranges from 1800 to 2200 μmol kg^{-1}. When a sample with a DIC of 2200 μmol kg^{-1} is analyzed without removing the dissolved CO_2, $[HCO_3^-]$ resulting from the hydrolysis of carbonic acid would be in the range of 7−22 μmol kg^{-1} for a pH range of 3.5−4.0. If 92% of the dissolved CO_2 is removed, the result would be only 0.6 and 1.8 μmol kg^{-1}. Moreover, part of this small error can be incorporated into the calibration. As a result, it is reasonable to omit $[HCO_3^-]$ ($[CO_3^{2-}]$ is negligible in pH 3.5−4.0) as shown in eq 2. As a result, a mixing time of 3 min was adopted in this study and the total throughput time of the analyzer for measurement of one sample was about 6.5 min.

Volume Mixing Ratio (γ_v). The γ_v of the analyzer was determined to be 21.64 (see the Supporting Information), and it was used to calculate γ in eqs 8 and 9. For this analyzer, the accuracy of the A_T measurement is insensitive to the error of γ_v because the error can be incorporated into the calibration (i.e.,

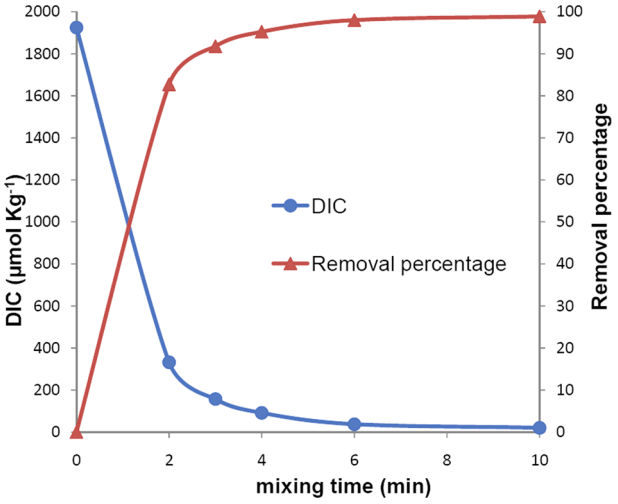

Figure 2. Concentrations of DIC of the sample−titrant mixture and CO_2 removal percentage in the reaction loop as a function of the sample−titrant mixing time.

k calculation). For example, if two values, 21.64 and 22.72 (5% higher than 21.64), were applied to the measurements, the difference between the two measured A_T values of the same sample was less than 0.05 μmol kg^{-1}. As such, when the seawater pump tube, which will wear out after long-term use, is replaced with a new tube of the same nominal dimension, the change of the γ_v value is less than 1%; therefore, it is not necessary to redetermine γ_v. With the determined γ_v value, the total BGC concentration in the sample−titrant mixture was about 25 μmol kg^{-1} and the absorbances at 444 and 616 nm were in the range of 0.1−0.4. When the tubing affecting the overall internal volume of the reaction loop is changed, the system requires a new calibration with CRMs.

Measurement Range. The pH of the sample−titrant mixture in the reaction loop depends upon the titrant acid concentration, C_t, and the sample A_T. The optimal pH indicating range of BCG is 3.4−4.6.[14] The sensitivity of pH measurements using BCG is not ideal when the pH is out of this range. At high pH, the effect of residual dissolved CO_2 in the sample−titrant mixture increases. As a result, the pH of the sample−titrant mixture in the reaction loop is maintained in the range of 3.5−4.0 by adjusting C_t. This pH range covers a Q value range of 145−429 μmol kg^{-1} when S is 35 (calculated from eq 9). For this analyzer, k and γ are 0.945 (Table 1) and 21.64 (S = 35 and $\gamma_\rho \approx 1$), respectively. The A_T measurement range is then from (C_t/21.64−424) to (C_t/21.64−143) μmol kg^{-1} based on eq 8. If C_t is 50 mmol kg^{-1}, the range would be about 1886−2167 μmol kg^{-1} with a concentration span of 280 μmol kg^{-1}. This range may be changed by adjusting C_t to match the A_T value range of the samples to be analyzed. The salinity range of the sample would be 20−35, because the salinity dependence of K_I in this range was used in eq 6. This range could be expanded if the salinity dependence of K_I in a wider salinity range was obtained.

Calibration, Stability, and Precision. Table 1 shows the three standards (one CRM and two diluted CRMs) and the corresponding calibration curves over 11 days. All of the R^2 values are more than 0.9998, indicating a tight linearity of the calibration curves described in eq 8.

Over the course of 11 days of repeated calibrations, 320 A_T measurements were made with a total running time of over 40 h. As shown in Table 1, the standard deviations (1σ) of k and C_t in this period are 0.0035 and 19 μmol kg^{-1}, resulting in A_T errors of ±0.5−1.6 μmol kg^{-1} (Q value ranges from approximately 145 to 429 μmol kg^{-1}) and ±0.88 μmol kg^{-1}, respectively. For further stability evaluation, the calibration curve obtained on July 5 was used to calculate A_T values and the standards used on the other 4 days were treated as samples. The agreement between the measured A_T values and the certified A_T values of those standards was −0.3 ± 1.1 μmol kg^{-1}. The above results show that the analyzer has achieved excellent stability over 11 days, and no recalibration was necessary during this period.

The precision of the analyzer was evaluated by measuring two seawater samples (type I and type II samples) repeatedly (Figure 3). The A_T values obtained from these measurements are 2091.69 ± 0.33 μmol kg^{-1} (n = 48) and 2203.22 ± 0.36 μmol kg^{-1} (n = 48), respectively. Such a level of precision is comparable to traditional A_T titration methods. Equations 8 and 9 indicate that the measurement precision of the analyzer depends upon the stabilities of C_t, I_T, γ, and the measurements of $[H^+]_F$. C_t and I_T are constant after the titrant is made and stored properly. Measurement stability of $[H^+]_F$ depends upon the spectrophotometric detection system. The stability of γ relies on γ_v, which is related to the variability of the internal volume of the two flow paths (Figure 1). The high precision that Figure 3 has shown clearly demonstrates that the analyzer has achieved excellent stability for all of these variables.

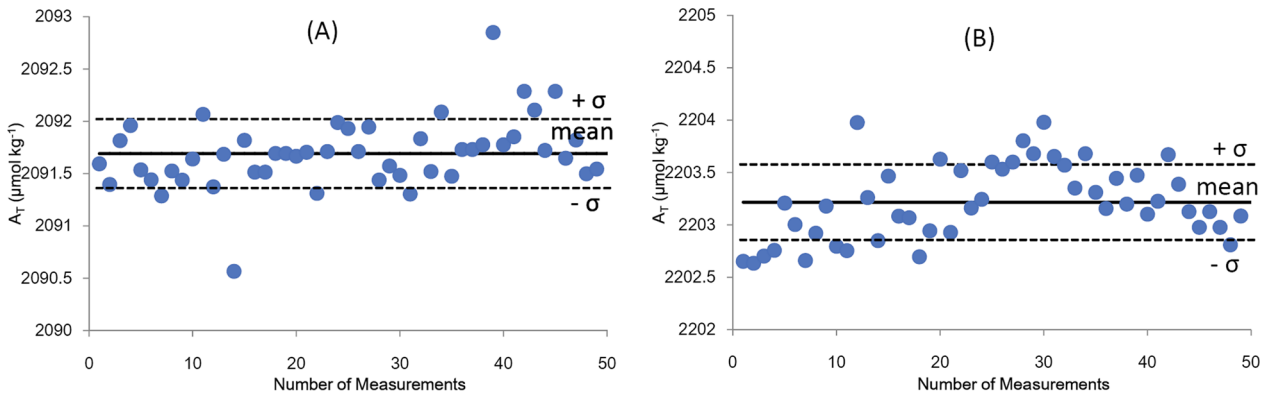

Figure 3. Residual plots of repeated measurements of a (A) type I sample with a mean A_T value of 2091.69 ± 0.33 μmol kg^{-1} (n = 48) and (B) type II sample with a mean A_T value of 2203.22 ± 0.36 μmol kg^{-1} (n = 48).

Accuracy. To evaluate the accuracy of the analyzer, two CRMs, batches 119 and 120, were measured as samples after the analyzer was calibrated with CRMs (batch 118) on different days and the results were compared to the certified A_T values (Table 2). The agreement between the measured and certified values is -0.33 ± 0.99 μmol kg^{-1} (n = 10). The analyzer thus has shown excellent measurement accuracy, which is comparable to traditional A_T titration.

Table 2. Comparison of A_T Measurements by the Analyzer and Certified Values of Two Batches of CRMs

date	CRMs[a]	measured A_T (n = 3; μmol kg^{-1})	ΔA_T[b] (μmol kg^{-1})
July 5	batch 119	2222.38 ± 0.30	1.14
	batch 120	2208.53 ± 0.70	0.10
July 8	batch 119	2220.02 ± 0.12	−1.22
	batch 120	2208.82 ± 0.14	0.39
July 9	batch 119	2219.52 ± 0.18	−1.72
	batch 120	2207.68 ± 0.62	−0.75
July 10	batch 119	2219.52 ± 0.63	−1.72
	batch 120	2208.30 ± 0.07	−0.13
July 16	batch 119	2221.06 ± 0.07	−0.14
	batch 120	2209.11 ± 0.14	0.71
mean ΔA_T ± standard deviation			−0.33 ± 0.99

[a]The certified A_T values for batches 119 and 120 are 2221.24 ± 0.61 and 2208.43 ± 0.79 μmol kg^{-1}, respectively. [b]ΔA_T = measured A_T − certified A_T.

We also assessed measurement agreement between the analyzer and a commercial open-cell titrator (AS-ALK2, Apollo SciTech, Bogart, GA). Both systems were calibrated with the same batch of CRMs before measurements. The measurement differences between the two analyzers for coastal samples were randomly distributed with a mean value of 0.79 ± 1.71 μmol kg^{-1} (n = 12; Figure 4A). At this time, the analyzer was calibrated with original and diluted CRMs; therefore, it could cover the A_T range up to the certified values of the CRM used. For samples with higher A_T than that of CRM, they can be diluted to lower the sample A_T value to the range of the calibration curve before measurement. In the future, we may use secondary standards with higher concentrations, which can be certified by traditional high-precision A_T titration. Figure 4B shows the agreement of A_T measurements between the analyzer and the Apollo titrator for open ocean samples, which were diluted with DI water by weighing before measurements. The two analyzers agreed within 0.99 ± 2.45 μmol kg^{-1} (n = 10). When all of the data in panels A and B of Figure 4 are combined, the overall agreement is 0.88 ± 2.03 μmol kg^{-1} (n = 22).

Effects of the Characteristics of BCG on A_T Measurements. The accuracy of the spectrophotometric pH measurement is related to the accuracies of the constants of BCG (eq 5), i.e., K_I, whose values vary in the literature,[14,26,27] and the ratios of the absorptivity coefficients (e_1, e_2, and e_3), whose values depend upon the temperature and the spectrophotometric detection system.[18] Yao et al. found that the impurities in the indicator led to an error up to 0.02 pH unit when seawater pH was spectrophotometrically measured.[28] The effects of these factors on the calibration curve or measurement accuracy were investigated, and insignificant influences on A_T measurement were found for the analyzer (see the Supporting Information).

There is great demand for precise, accurate, and robust A_T measurements of high spatial and temporal resolution in the oceanographic community. The presented A_T analyzer shows great potential to fulfill these requirements. Excluding the air bath, the weight and power consumption of the analyzer are about 3 kg and 8 W, respectively. Together with the nature of being automated, small sample size requirement, short sample throughput time, infrequent calibration need, and excellent precision and accuracy, the analyzer can be adapted for underway applications and *in situ* measurements. Future development will include making the system more robust, lowering the power consumption, and determining the temperature and pressure effects on A_T measurements.

■ ASSOCIATED CONTENT

Supporting Information

Determination of the volume mixing ratio (γ_v), calculation of the concentrations of the species in eq 1 with pH ranging from 3.5 to 4.0, and effects of the characteristics of BCG on A_T measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

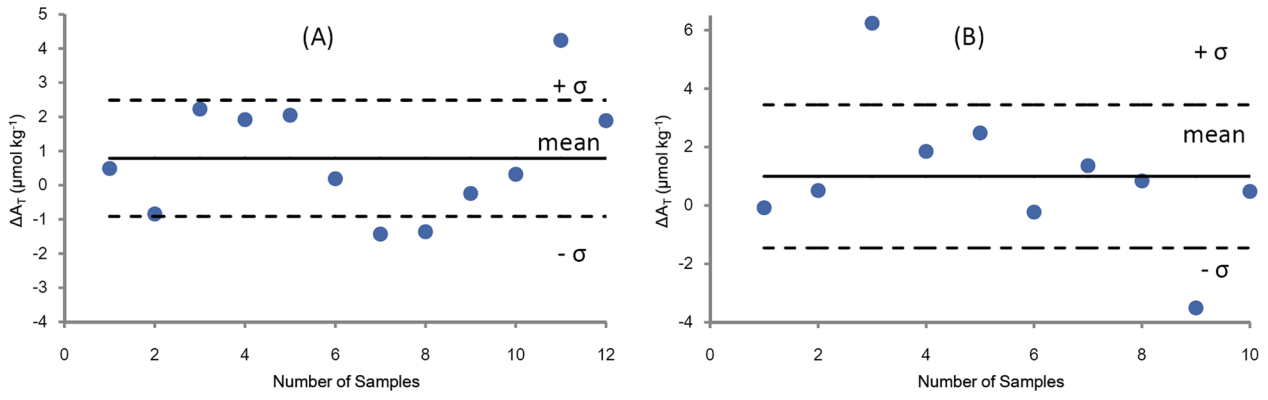

Figure 4. Residual plots for the intercomparison of A_T measurements between the analyzer and the commercial open-cell titrator for (A) coastal and (B) open ocean water samples.

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