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# COULOMETRIC TOTAL CARBON DIOXIDE ANALYSIS FOR MARINE STUDIES: AUTOMATION AND CALIBRATION

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## ABSTRACT

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An automated instrument for the coulometric detection of total carbon dioxide ($TCO_2$) was developed from the manual prototype of Johnson et al. Pure $CO_2$ was used to calibrate the detector, the whole analytical procedure was controlled by a microcomputer, and the unit was compact enough for field and shipboard use. Some 67 samples from the Askö field station on the Baltic Sea (salinity = 6; $TCO_2$ ranging from 1224.3 to 1276.1 $\mu$mol l$^{-1}$ with a mean of 1245.6 $\mu$mol l$^{-1}$) and 31 samples from Swedish lakes ($TCO_2$ ranging from 19.5 to 252.3 $\mu$mol l$^{-1}$ with a mean of 72.9 $\mu$mol l$^{-1}$) were analyzed. The pooled standard deviation of these analyses was ± 1.0 $\mu$mol l$^{-1}$ (% CV of 0.08 and 1.3%, respectively). Precisions of $< 0.1\%$ were routine for waters exceeding 1000 $\mu$mol l$^{-1}$ $TCO_2$. At Askö on the Baltic Sea, the mean absolute difference between coulometric and pH-alkalinity determinations of $TCO_2$ on 22 parallel samples was 6.7 $\mu$mol kg$^{-1}$ or 0.5% of the mean. Community metabolism studies gave maximum rates of net production ($TCO_2$ uptake) and respiration ($TCO_2$ production) of 0.9–1.2 and 0.3–0.5 $\mu$mol l$^{-1}$ h$^{-1}$, respectively. The results of this study provide solutions to the problems observed with the earlier manual prototype.

## INTRODUCTION

The coulometric determination of $CO_2$ is a standard method (see ASTM Publication D 513-82) adapted for the determination of total carbon dioxide ($TCO_2$) in marine waters by Johnson et al. (1985). With a commercial coulometer and custom glassware for $CO_2$ extraction, successful comparisons of $TCO_2$ uptake with $^{14}C$ uptake have been made in estuarine waters (Bender et al., 1987). The original analytical procedure was laborious, but its potential for measurements of marine metabolism encouraged us to automate the analytical and calibration procedures. For calibration, a stainless steel loop is filled with pure $CO_2$ at ambient temperature and pressure, its contents analyzed, and the result compared with the loop content calculated from the gas law to ensure that the coulometer and cell are performing to theory. This procedure replaces the use of weighed samples of $CaCO_3$. For automation, the original glassware is simplified, and in-line electric solenoid valves under the direction of a microcomputer or programmable controller replace manual valves and stopcocks.

During this work two automated systems were built. A prototype in Narragansett, RI, (hereafter called URI-I), and a fully developed system in Gothenburg, Sweden, (called UG-I). For system URI-I, the coulometer was purchased in October 1982, the solenoid valves controlled by a stand-alone programmable controller, and a small microcomputer detected end points and printed the results. For system UG-I, the coulometer was purchased in December 1984, the solenoid valves are controlled by a custom built 16 channel relay controller coupled to and controlled by a desk-top computer. This computer also controls all other aspects of the analysis. The URI-I system was used to develop the $CO_2$ calibration, while all of the marine and fresh water analyses, including samples from lakes of high acidity and a field location on the Baltic Sea, were run on the UG-I system.

For the Baltic samples, coulometrically determined $TCO_2$ variation was compared with $O_2$ variation and with total carbonate concentrations, calculated from pH and alkalinity. The absolute difference between parallel analyses of duplicate samples for total carbonate ranged between 0.1 and 1.0%. The automated and improved coulometric technique was precise enough to enable estimates of productivity and respiration in waters showing only 4–6 $\mu$molar changes day$^{-1}$. In our experience this system may offer an alternative to $^{14}C$ measurements of productivity in some situations, it, of course, also provides respiratory measurements. Many of the problems encountered in the original work were resolved by the improved calibration procedure which also was of help in the interpretation of data. At the level of precision (% coefficient of variation $\leqslant 0.1\%$) and accuracy provided by automated coulometry, sample handling and storage becomes critical, and further work may be needed before a completely unbiased sampling procedure for $TCO_2$ is assured.

## MATERIALS AND METHODS

### *Modification of the analytical procedure for automation*

The original scheme of $CO_2$ extraction of Johnson et al. (1985) has been retained. Figure 1 shows the modifications for the automated UG-I system. These include the $CO_2$ stripper, substitution of 115 V normally closed two or three way pinch solenoid valves (100 P2WNC 115-05B or 02B and 100 P3WDV 115–02B, Bio-Chem Valve Corp., East Hanover, NJ 07936) for glass stopcocks, and the installation of three way teflon (4-47-901) or stainless steel (9-317-900) solenoid valves from General Valve Corporation (Fairfield, NJ 07006) in the

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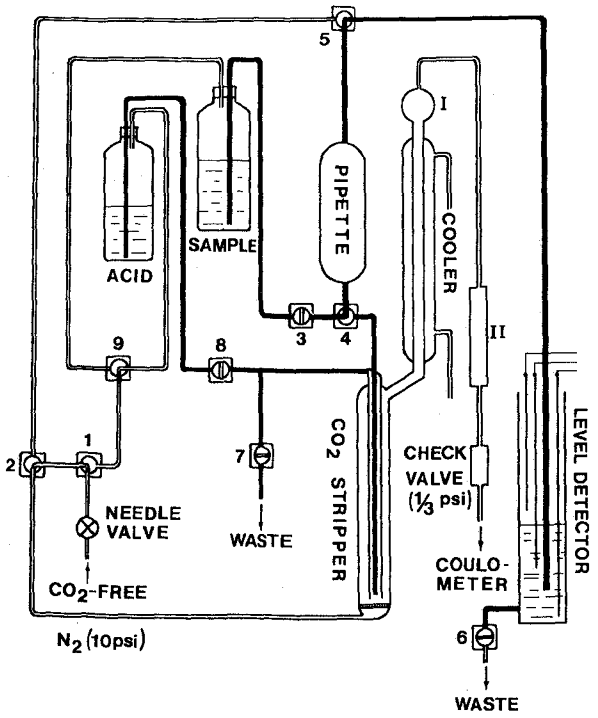

Fig. 1. The sample dispensing, extraction and gas scrubbing components after Johnson et al. (1985) as modified in the UG-I system for the automated coulometric determination of $TCO_2$. Lines for liquids are closed and pass through pinch valves 3,4,5,6,7, and 8, while those conducting gases are open lines controlled by three-way solenoid valves 1,2, and 9. All valves are shown de-energized. Note: the connection between the 1/16 in. od teflon tube from the pipette (valve 4) into the stripper was made via a 1/16 to 1/8 in. Swagelok reducing union (SS-200-6-1-BT, Crawford Fitting Co., Solon, OH 44139) which was bored out so that the teflon tube could pass through the union. This union was coupled to a male pipe thread 1/4 in. connector (SS-4-UT-1-2, Ultra-Torr, Cajon Co., Macedonia, OH 44056) mounted on the distal end of the central stripper glass tube with a Swagelok 1/8 in. port connector (SS-201-PC) and a female pipe thread to tube adapter (SS-200-7-2).

carrier gas lines. Carrier gas pressure is used for fluid transfer in lieu of pumps.

Three way valves (1, 2 and 9 in Fig. 1) are configured to connect the carrier gas ($N_2$) at 10 psl to the stripper until power is applied to them which reroutes the carrier gas supply to and pressurizes either the acid (8.5% $H_3PO_4$, V/V) reservoir or the 250 ml sample bottle. After pressurization, opening two-way valve 8 forces acid into the stripper, while opening valve 3 fills the pipette with sample. The volume of acid added (~2 ml) is a function of the time that pinch valve 8 is held open. The microcomputer, which is coupled to a model 346-609 electronic liquid level sensor (RS components, Corby, UK), senses that the pipette is full when the overflow from the pipette empties into a 100 ml graduated cylinder which immerses the sensor electrodes. Then pinch valve 3 is closed, and power is applied to valves 4, 5 and 2 so that carrier gas pressure forces the contents of the pipette past valve 4 into the stripper. Draining of the pipette requires ~30 s after which the pipette is flushed with carrier gas for an additional 30 s. Power is then removed from all valves to restore carrier gas flow to the stripper. The $CO_2$ stripped from solution is passed successively through a water-cooled condenser, a glass adapter (I, Fig. 1) containing magnesium perchlorate (dehydrite, A.H. Thomas, C260-M61) and glasswool, and a 100 mm × 6 mm od glass tube (II, Fig. 1) packed with silica gel (ORBO-53 active adsorbent tube, Supelco, Inc., Bellefonte, PA 16823) which removes traces of water vapor, acidic ions, and acidic gases ($H_2S$, etc.) (W. Betz, Supelco Inc., personal communication, 1984, 1986). When the titration of $CO_2$ is completed, pinch valve 7 is opened so that back pressure due to the check valve (B-2C-1/3, Nupro Company Willoughby, OH 44094) downstream from the stripper causes the spent sample to exit the stripper. The system is made ready for the next replicate with a fresh aliquot of acid, and the liquid level in the graduated cylinder reservoir is lowered by opening valve 6. The carrier gas flow is ~200 ml $\text{min}^{-1}$, and stripping is continued until the software determines that the coulometer is titrating no more $CO_2$. For every new sample a rinse is run as above except that the sample is drained from the stripper after 75–90 s, but the first replicate is delayed until the software senses that the system is clear of $CO_2$.

The three separate inputs or outputs on the original $CO_2$ stripper were replaced with a single central glass tube (6 mm od) originating ~2 mm above the frit and protruding 2 cm from the top of the stripper. Through this tube, acid is added to the stripper and sample is exhausted from the stripper, while a 1/16 in. (1.6 mm) od teflon tube running through the tube connects the stripper with the sample pipette. The diameter of the stripper is 25 mm and its overall height is ~250 mm. The pipette outlets are 15 mm long 1/8 in. (3 mm) od glass tubing, and flexible pinch valve tubing (1.6 mm id) connects valves 4 and 5 (Fig. 1) to them. The components are mounted in a rectangular enclosure (40 cm wide, 75 cm high, and 17 cm deep) of clear PVC (10 mm) or polycarbonate (12 mm) with a detachable cover so the unit can be packed and shipped intact to the field or ship.

### *Instruments and apparatus*

For the URI-I system, the solenoid valves were activated by a MicroMaster programmable controller (WP6200) with input/output expander (WP6300) manufactured by Minarik Electric Company (Los Angeles, CA 90013). These units are cabinet mounted and wired to binding posts on the cabinet front. The latter are connected to the valves via banana jacks on the valve leads. Analytical sequences, initiated from the controller panel, use programmable software built into the controller. The coulometer is coupled to an AIM 65 micro computer (Rockwell International, Richardson, TX 95080) via a parallel data interface card (PN 110–013) from Coulometrics Inc. (Wheat Ridge, CO). The UG-I system consists of a Hewlett-Packard (HP) 86 microcomputer (Sunnyvale, CA 94086), disk drive (HP 9130A), monitor (HP 82913A), and printer (HP

Thinkjet), and a custom built 16 channel relay controller (Woodmill Electronics, Woodmill Lane, Southampton, UK) connected through a HP GPI0 interface. The coulometer interface card is slightly modified according to directions from Coulometrics for use with the HP 86. A program in BASIC activates the valves in sequence, displays the valve status, reads the coulometer, logs the samples, determines the end points, makes the calculations, and undertakes the statistics.

A refrigerated bath (M 20C, Julabo, West Germany or Model RTE-8 from Neslab Portsmouth, NH 03801) provided coolant as close to 0°C as possible without freezing the water vapor in the condenser (Fig. 1). All tubing used in the automated systems was soaked for 1 h in 6N $HNO_3$, rinsed with distilled water, and dried before installation.

### *Determination of accuracy and gas calibration*

A hastalloy, electrically actuated, six port gas chromatographic (GC) valve (E66P) with 15 cm standoff, and a 0.5 or 1.0 ml 1/8 in. (3 mm) od 304 stainless steel sample loop (SL-0.5 K6P and SL-1.0 K6P, respectively, Valco Instruments Co. Inc., Houston, TX 77255) were used for calibration. This unit is mounted so that the valve is immersed in a small plastic bucket packed with insulation to minimize temperature fluctuation. With the GC valve in the 'inject' position the sample loop is connected to the carrier gas, while in the 'load' position it is connected to a 1 l cyclinder of research grade $CO_2$ (99.995–99.998%) from Matheson (Gloucester, MA 01930) or Aga Gas (Lidingö, Sweden). The regulator (Matheson 19-320 or Aga 1181-500-24) diaphragm, and the $CO_2$ lines (1/8 in. od) are of stainless steel to prevent air infiltration. A stainless steel input solenoid valve located between the regulator and the GC valve, and a vent solenoid valve downstream from the valve (loop), are controlled as described above. To fill the loop, we moved the GC valve to 'load', the $CO_2$ circuit alternately pressurized and depressurized three times by opening the input and alternately closing the vent solenoid, then 5–10 circuit volumes (25–50 ml) of $CO_2$ at 150 ml $\min^{-1}$ were passed through the loop. The flow of $CO_2$ was then terminated, and after an equilibration period of 4 s, the GC valve was moved to 'inject' so carrier gas could sweep out the $CO_2$ for titration. The $N_2$ and $CO_2$ flows were adjusted with stainless steel fine metering valves.

For an absolute calibration using mercury, the volume of the loop ($V_c$) in ml is the difference in weight ($W_d$) between the empty and mercury filled loop divided by the density (g $ml^{-1}$) of mercury ($d_{Hg}$) at its weighing or calibration temperature ($T_c$). Multiplying this result by the density of moist air at $T_c$ from Weast and Astle (1983), adding the product to $W_d$, and redividing by $d_{Hg}$ gives $V_c$ corrected for the buoyancy of air. The term $V_c$ is unaffected by pressure changes, but for temperatures different from $T_c$ multiplying the coefficient of linear expansion ($a'$) for 304 stainless steel by 3 according to Marks (1967) gives the coefficient of cubical expansion ($a'''$) so that the volume of the loop ($V_a$) at any temperature ($T_a$) after correction for thermal expansion is

$$V_a = V_c(1 + (T_a - T_c)a''') \quad (1)$$

where $a'''$ was $5.184 \times 10^{-5}$ ml $ml^{-1}$ $°C^{-1}$ (Eshbach, 1975). The total absolute volume of the $CO_2$ sampling system ($V_t$) in ml is found by adding the internal volume of the GC valve ($V_v$ = 0.01282 ml) to $V_a$

$$V_t = V_a + V_v \quad (2)$$

The mass of carbon ($m_c$) in the metering system for volume ($V_t$), temperature ($T_a$), and pressure ($P$) in $\mu$gC may be calculated from the gas law as

$$m_c = V_t\,(12.01/22.263) \times P(\text{atm}) \times (1/T_a\ °\text{K}) \times 1000 \times 0.99998 \quad (3)$$

Because $CO_2$ is not an ideal gas, its molar volume at STP is 22.2631 and 0.99998 is the purity of $CO_2$. If the absolute difference ($A_{diff}$) between $m_c$ and coulometrically determined mass ($m_d$) was $< 1.1$ $\mu$gC or $< 0.1\%$ of $m_c$, the coulometer and cell are considered to be performing to theory. Temperature was measured with a primary reference thermometer (ASTM 63, Fisher Scientific, Medford, MA 02155) and pressure was measured with a model IDW-1990 digital barometer from Heathkit (Benton Harbor, MI 49022) calibrated against a mercury barometer (Henry J. Green, Brooklyn, NY 90096) at the Rhode Island Nuclear Science Center, Narragansett, RI. The sample loop (SL-0.5 K6P) was filled with mercury and emptied until six consecutive weighings of the empty loop gave the same result to ensure that no mercury remained after emptying. The average of six pairs of weighings ($T_c$ = 22.9°C = $T_a$), gave a $V_a$ of 0.5191 ml and $V_t$ was 0.5319 ± 0.00021 ml.

For a relative calibration using $CO_2$, the coulometrically determined quantity $m_d$ is substituted for $m_c$ to solve for $V_t$ (eq. 3). Next $V_v$ is subtracted from $V_t$ to give $V_a$ at $T_a$. The volume of the loop at 25°C ($V_{25}$) correcting for thermal expansion is

$$V_{25} = V_a\,(1 + (25 - T_a)a''') \quad (4)$$

The value of $V_t$ for the nominally 1.0 ml loop (SL-1.0 K6P) was determined 12 times on the UG-I system. The highest and the lowest results were discarded, and the remaining results were averaged to give a $V_{25}$ of 0.9164 ± 0.00094 ml. For all subsequent calibrations, $V_a$ is calculated with 0.9164 ml substituted for $V_c$ and 25°C for $T_c$ in eq. 1. After solving for $V_a$, eqs. 2 and 3 are used as for the absolute calibration procedure. If $A_{diff}$ is $< 1.1$ $\mu$gC or $< 0.1\%$ of $m_c$, the system response is considered unchanged and ready for analysis. For this work $P$ was read to ± 0.1 mbar with an aneroid barometer manufactured by Negretti and Sambra (Croydon, UK) obtained from and calibrated by the Swedish Meterological and Hydrological Institute (SMHI) in Norrköping, Sweden.

### *Calibration of the sample pipette and correction for temperature and salinity*

The volume of the sample pipette in system UG-I (Fig. 1) was determined with a sodium carbonate solution. For diluent, fresh distilled water was adjusted to a pH of between 7.5 and 8.0 with a few crystals of NaOH, and its $CO_2$ content determined. For the standard solution, 190–200 mg of primary standard grade $Na_2CO_3$ (heated 1–2 h at 270°C, cooled in a desiccator) were weighed out on a Cahn Electrobalance (Cahn Instruments Inc., Cerritos, CA) and dissolved in 1000 ml of diluent. The standard solution was immediately analyzed, corrected for the blank, and used to determine the volume of the pipette ($V_{pc}$) in ml at $T_c$. Using $W_T$ and $W_{25}$ from the Volumetric Glassware Table (Weast and Astle, 1983), we can calculate the volume of the pipette at 25°C ($V_{p25}$) using

$$V_{p25} = W_{25}\ (V_{pc}/W_T) \tag{5}$$

For the pipette shown in Fig. 1, $V_{p25}$ ($n = 9$) is $31.273 \pm 0.006$ ml (1 s.e.; %CV = 0.06). From $V_{p25}$ the true volume of the pipette ($V_{pt}$) at the analytical temperature ($T_a$), salinity (S), and density ($d^0$) of the sample is

$$V_{pt} = (d_{25}/d_{T_a}\ (1 + 1 \times 10^{-5})(T_a - 25))\,V_{p25} \tag{6}$$

where $d_{25}$ and $d_{T_a}$ are the densities of the sample at 25 and $T_a$ °C, respectively, calculated from the expression given by Chen and Millero (1976), and $1 \times 10^{-5}$ $deg^{-1}$ is the volume coefficient of expansion for borosilicate glass (Weast and Astle, 1983). As in the manual system (Johnson et al., 1985), the temperature of the pipette is read with a digital thermometer for each replicate analysis, and $\mu mol\ l^{-1}$ $TCO_2$ was calculated from

$$TCO_2 = (1000\ ml\ l^{-1}/V_{pt}\ ml) \times \mu gC/12.01\ \mu gC\ \mu mol^{-1} \tag{7}$$

Dividing $TCO_2$ by $d_{T_a}$ gives $\mu mol\ kg^{-1}$. For system URI-I, the reproducibility of sample delivery was also checked gravimetrically after draining the pipette contents into preweighed serum bottles at $T_a$.

### *Sampling*

Samples (salinity = 33) were collected from 40 m at the Kristineberg Marine Station (Lysekil, Sweden). The samples were kept in the dark at 0–5°C, and before analysis they were allowed 24 h to equiliberate to room temperature. Seawater samples from the Baltic with a salinity of 6 were taken over a depth range of 1–6 m from a 150 m³ mesocosm deployed during field studies at the Askö field station, Sweden. These samples were analyzed within 2 h of collection. S. Larsson of the Zoological Institute, University of Gothenburg, Gothenburg, Sweden, provided the fresh-water samples from Gårdsjön, Norra Hästevatten, and Södra Hästevatten (Västergötland, Sweden). The latter two lakes exhibited pH values below 6.0. The fresh-water samples were kept in the dark for 12–48 h at 1–5°C until analyzed.

## RESULTS

Table I summarizes the results for absolute calibration using the URI-I system over a 5 month period. A difference of +2.0 μgC (0.71%) between the determined ($m_d$) and calculated ($m_c$) contents of the 0.5 ml steel sample loop was constant over this period which indicates either a miscalibration of the coulometer at the factory or an error in our calibration procedure. For the measurement of temporal or spatial $TCO_2$ variation in the environment, this is of little concern. Consequently, absolute calibration of the UG-I system was omitted.

TABLE I

Determination of the absolute accuracy in the URI-I system by analysis of the mass of $CO_2$ contained in a stainless steel sample loop whose volume (0.5319 ml) was determined with mercury

| Date (1984) | No. of analyses ($n$) | Means | | |
|---|---|---|---|---|
| | | $m_c$(μgC)[a] | Error (μgC)[b] | Error (%) |
| 27 July | 10 | 267.5 | +1.9 | +0.69 |
| 28 July–13 Dec | 10 | 267.5 | +1.9 | +0.69 |
| 14 Dec | 10 | 271.6 | +2.1 | +0.75 |
| Mean | | | 2.0 | +0.71% |

[a] The mean mass of $CO_2$ calculated ($m_c$) from the gas law (eq. 3) for $n$ analyses of the sample loop contents.

[b] The mean difference between the mass of $CO_2$ determined by coulometric titration ($m_d$) and $m_c$($m_d - m_c$) for $n$ analyses of the sample loop contents.

TABLE II

Precision for $TCO_2$ analyses with the automated UG-I coulometric system (Fig. 1)

| Source/period (1985) | Conc. range (μmol l⁻¹) | Samples[a] analyzed ($k$) | Mean | Precision[b] (μmol l⁻¹) | %CV |
|---|---|---|---|---|---|
| $Na_2CO_3$[c] | | | | | |
| 12 June–1 July | 1900–5735 | 5 | 3023.8 | ± 2.2 | 0.07 |
| KBSW[d] | | | | | |
| 11 June–2 July | 1700–2190 | 12 | 2099.7 | ± 1.9 | 0.09 |
| Askö[e] | | | | | |
| 7 July–18 July | 1220–1275 | 67 | 1245.6 | ± 1.0 | 0.08 |
| Lake[f] | | | | | |
| 12 June–24 Sept | 19–255 | 31 | 72.9 | ± 1.0 | 1.37 |

[a] $k$ is the number of samples for which there were at least three replicates ($n$) run.

[b] Pooled standard deviation ($s$) = $\sqrt{\dfrac{\sum_{i=1}^{K}\left(\sum_{j=1}^{n_i} x_{ij} - \sum\left[\left(\sum_{j=1}^{n_i} x_{ij}\right)^2 \Big/ n_i\right]\right)}{\sum_{i=1}^{K} n_i - K}}$

[c] $Na_2CO_3$ solutions.

[d] Aged Kristineberg seawater (salinity = 33).

[e] Nearshore Baltic seawater (salinity = 6).

[f] Fresh waters from Gårdsjön, Norrahästevatten, and Södrahästevatten, Sweden.

TABLE III

Daily precision for the coulometric determination of $TCO_2$ with system UG-I on samples of Baltic seawater (salinity = 6) during the Askö mesocosm experiment of July 1985

| Day | Samples analyzed ($k$)[a] | Mean ($\mu$mol l$^{-1}$) | $S^b$ | % CV |
|---|---|---|---|---|
| 8 | 9 | 1243.4 | 1.16 | 0.09 |
| 9 | 3 | 1226.4 | 1.27 | 0.10 |
| 10 | 5 | 1234.0 | 1.30 | 0.11 |
| 11 | 2 | 1232.5 | 0.72 | 0.06 |
| 12 | 5 | 1234.3 | 1.00 | 0.08 |
| 13 | 4 | 1239.0 | 1.24 | 0.10 |
| 14 | 1 | 1243.5 | 0.76 | 0.06 |
| 15 | 16 | 1252.2 | 0.83 | 0.07 |
| 16 | 4 | 1258.1 | 0.93 | 0.07 |
| 17 | 9 | 1264.8 | 1.43 | 0.11 |
| 18 | 9 | 1273.0 | 0.66 | 0.05 |

[a] As in Table II.
[b] Precision calculated as in Table II.

The precision of the $TCO_2$ analyses with the UG-I system are given in Table II. For samples of differing $TCO_2$ concentrations and salinities, the best precision was obtained at Askö (salinity ≈ 6) where 67 ($n = 3$) samples having a mean $TCO_2$ of 1245.6 $\mu$mol l$^{-1}$ were analyzed with a pooled standard deviation of ± 1.0 $\mu$mol l$^{-1}$ (%CV = 0.08%), and a range of individual standard deviations from ± 0.14 to ± 2.66 $\mu$mol l$^{-1}$ (%CV of 0.01–0.21%, respectively). The same precision was obtained with non-saline waters. Table III shows the day by day variation in the pooled standard deviations at Askö between 8 and 18 July

TABLE IV

A comparison of $\Sigma CO_2$ calculated from total alkalinity and pH($TCO_2^*$) and $\Sigma CO_2$ determined coulometrically ($TCO_2$) on parallel samples from the Askö mesocosm in July 1985

| Day | $k^a$ | Mean $TCO_2^{*b}$ ($\mu$mol kg$^{-1}$) | Mean $TCO_2^c$ ($\mu$mol kg$^{-1}$) | Diff. ($TCO_2 - TCO_2^*$) ($\mu$mol kg$^{-1}$) | Difference (%) |
|---|---|---|---|---|---|
| 9 | 4 | 1211.8 | 1224.6 | + 12.8 | + 1.05 |
| 10 | 3 | 1230.3 | 1232.5 | + 2.2 | + 0.18 |
| 11 | 2 | 1224.5 | 1229.0 | + 4.5 | + 0.36 |
| 12 | 4 | 1229.3 | 1231.1 | + 1.8 | + 0.15 |
| 17 | 9 | 1248.7 | 1260.8 | + 12.1 | + 0.97 |
| Summary | 22 | 1228.9 | 1235.6 | + 6.7 | + 0.54% |

[a] $k$ = no. of comparisons (two replicates for each $TCO_2^*$ sample and three for each parallel $TCO_2$ sample).
[b] Total alkalinity by the method of Anderson and Wedborg (1983), and pH by the method of Almgren et al. (1975).
[c] System UG-I.

TABLE V

A comparison of net $\mu$molar changes calculated from the mean $\Sigma CO_2$ and $O_2$ concentrations in the Askö mesocosm on 9 July (start), 12 July (midpoint), and 17 July (end) derived from three automated high precision phototitration techniques

| Date (1985) | $TCO_2^*$ ($\mu$mol kg$^{-1}$) | Net change ($\mu$molar) | $TCO_2$ ($\mu$mol kg$^{-1}$) | Net change ($\mu$molar) | $O_2^a$ ($\mu$mol kg$^{-1}$) | Net change ($\mu$molar) |
|---|---|---|---|---|---|---|
| 9/7 | 1211.8 | – | 1224.6 | – | 303.0 | |
| 12/7 | 1229.3 | + 17.5 | 1231.1 | + 6.5 | 298.0 | − 5.0 |
| 17/7 | 1248.7 | + 19.4 | 1260.8 | + 29.7 | 272.9 | − 25.1 |
| | | + 36.9 | | + 36.2 | | − 30.1 |

Abbreviations and explanations as in Table IV.
[a] Method of Williams and Jenkinson (1982).

TABLE VI

The relative accuracy, sensitivity, and stability of the coulometric detector shown by the difference between the mass of pure $CO_2$ in a steel sample loop (volume = 0.9164 ml) determined coulometrically ($m_d$) and calculated from the gas law ($m_c$)

| Date/Time | $\Sigma T$ (h) | $\Sigma C_p$ (mgC) | $T$ (°C) | $P$ (mbar) | $m_c$ ($\mu$gC) | $m_d$ ($\mu$gC) | Diff. |
|---|---|---|---|---|---|---|---|
| 8VII85 | | | | | | | |
| 09:53 | 0.0 | 0.0 | 25.8 | 1011.2 | 457.0 | 457.4 | + 0.4 |
| 18:15 | 8.4 | 14.4 | 26.4 | 1011.2 | 456.2 | 456.4 | + 0.2 |
| $\Delta m$ | | | | | − 0.9 | − 1.0 | |
| 15VII85 | | | | | | | |
| 07:40 | 0.0 | 0.5 | 25.2 | 1009.7 | 457.3 | 458.0 | + 0.7 |
| 18:23 | 10.7 | 25.4 | 27.2 | 1004.6 | 452.0 | 451.7 | − 0.3 |
| $\Delta m$ | | | | | − 5.3 | − 6.3 | |
| 17VII85 | | | | | | | |
| 08:34 | 0.0 | 0.0 | 24.0 | 1013.6 | 460.9 | 460.7 | − 0.2 |
| 23:34 | 15.0 | 23.1 | 25.0 | 1014.0 | 459.6 | 460.0 | + 0.4 |
| $\Delta m$ | | | | | − 1.3 | − 0.7 | |
| 18VII85 | | | | | | | |
| 09:08 | 0.0 | 0.0 | 24.7 | 1015.3 | 460.6 | 462.2 | + 1.6 |
| 18:44 | 9.6 | 20.0 | 26.2 | 1014.3 | 457.9 | 459.6 | + 1.7 |
| $\Delta m$ | | | | | − 2.7 | − 2.6 | |

Abbreviations; length of use (h) of the coulometer cell reagents, $\Sigma T$; amount of carbon (mg) titrated before analysis, $\Sigma C_p$; temperature of the loop, $T$; ambient pressure, $P$.

1985. They ranged from ± 1.43 μmol $l^{-1}$ (individual standard deviations from ± 0.26 to 2.27 μmol $l^{-1}$) on 17 July to ± 0.66 μmol $l^{-1}$ (individual standard deviations from ± 0.18 to 0.89 μmol $l^{-1}$) on 18 July.

In Table IV the coulometric $\Sigma CO_2$ determinations ($TCO_2$) are compared with total carbonate concentrations ($TCO_2$*) calculated from pH and total alkalinity determined on parallel samples. Total alkalinity was determined by photometric titration (Anderson and Wedborg, 1983), and pH determined according to Almgren et al. (1975). For the 22 comparisons at Askö, the mean difference was + 6.7 μmol $kg^{-1}$ ($TCO_2$ − $TCO_2$*) or 0.5%. The mean difference of + 2.5 μmol $kg^{-1}$ (0.2%) for nine analyses between 10 and 12 July is within the analytical precision shown in Table III, while the mean difference of 12.5 μmol $kg^{-1}$ or 1% of $TCO_2$* on 9 and 17 July probably indicates a systematic error or a real difference in these samples. Table V compares the apparent net μmolar changes in $\Sigma CO_2$ with that of $O_2$. Coulometrically determined $TCO_2$ behaves very much as $O_2$ with a relatively small net production and net consumption, respectively, between 9 and 12 July followed by large net changes between 12 and 17 July. Some 83.4% of the total apparent $O_2$ consumption (30.1 μmolar) and 82.0% of the total apparent $TCO_2$ production (36.2 μmolar) occurred between 12 and 17 July. For $TCO_2$* (36.9 μmolar) the comparable result was 52.6%, but $TCO_2$* and $TCO_2$ apparent net productions were equivalent for the period 9–17 July. These data were not corrected for diffusion, invasion to the atmosphere, or for $O_2$ consumption by purported photo-oxidizing processes (Laane et al., 1985).

Table VI shows the accuracy, sensitivity, and stability of the 'relative calibration' procedure as it functioned in the field. The response ($m_d$ − $m_c$) of the coulometer was unchanged until at least 25 mgC had been titrated in the cell, and the changes in the quantity of $CO_2$ due to variations in $P$ and $T_a$ appear to be properly corrected for. Differences between $m_d$ and $m_c$ were always $< 1.0$ μgC between 8 and 17 July, but on 18 July a constant difference of + 1.6 μgC was observed.

In-situ $TCO_2$ profiles at four depths in the Askö mesocosm (15 and 16 July) are shown in Fig. 2. Rates of net production from 0.93 to 1.1 μmol $l^{-1}$ $h^{-1}$ and respiration rates from 0.3 to 0.5 μmol $l^{-1}$ $h^{-1}$ were observed in the enclosure. On 15 July between 07:54 and 23:33 (cell solutions were changed at 18:40) 16 samples each of four replicates along with appropriate blanks and calibration analyses were run with a pooled standard deviation of ± 0.8 μmol $l^{-1}$ on the UG-I system.

## DISCUSSION

Coulometer current and stripping efficiency were originally (Johnson et al., 1985) checked by introducing precisely weighed amounts of 99.9% pure $CaCO_3$ into a stripper filled with acidified $CO_2$-free distilled water or seawater. Recoveries of $CO_2$ stripped from solution and analyzed coulometrically ranged from 99.7 to 100.5%, but the majority of recoveries slightly exceeded 100.0%. These data were interpreted to mean that stripping was complete and accurate to ± 1.0 μgC from a few hundred to over 2000 μg $CaCO_3$–C, and provided the basis for proceeding with automation. Calibration with $CaCO_3$ is laborious, and cannot be automated. This led us to determine the volume of a stainless steel loop with mercury, and compare its analyzed $CO_2$ content ($m_d$) with its content calculated from the gas law ($m_c$, eq.3). Table I shows that the mean difference between $m_c$ and $m_d$ for system URI-I did not change with time, although $m_d$ was consistently larger than $m_c$. This result may be due to a failure to correctly determine $V_t$, $P$, or $T_a$ during the initial calibration. The determination of $V_t$ with Hg may be affected by changes in its purity during storage, and accurate pressure readings require a stable sensitive barometer plus the opportunity to calibrate it with a certified barometer. D. Chipman (personal communication, 1986) also found a positive error of + 0.7 to 0.8% with a coulometer purchased in 1983, but he attributed this to miscalibration of the coulometers' calibration resistor by the manufacturer. The small positive errors for analyses of $CaCO_3$–$CO_2$ in Johnson et al. (1985), and for $CO_2$ gas in this work (Table I) may or may not be due to the same causes. These data indicate that neither technique can be considered absolutely accurate, and therefore no absolute calibration of the coulometer in system UG-I was attempted.

'Relative calibration' obtained by determining $V_c$ coulometrically on system UG-I is, however, compatible with the primary focus of this method, namely the measurement of $TCO_2$ variation. A steel sample loop is an ideal reference because barring damage and a small correction for temperature, its volume is

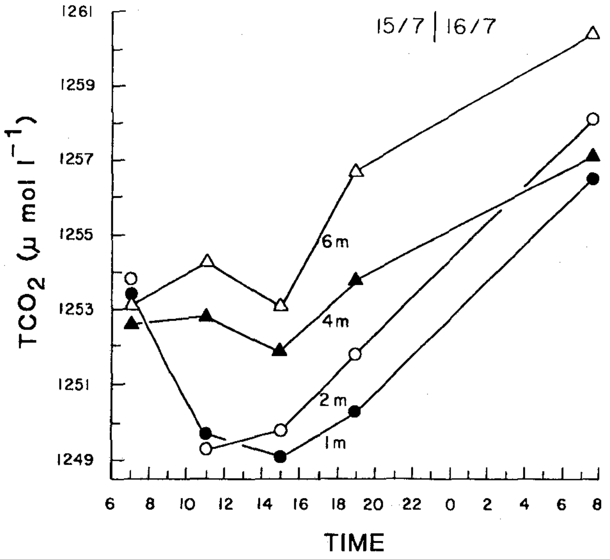

Fig. 2. The apparent diel variation in $TCO_2$ determined coulometrically for four depths in a 150 $m^3$ mesocosm at Askö on the Baltic Sea on 15 and 16 July 1985.

constant at ordinary pressures. Having once determined its volume ($V_c$) with the coulometer at 25°C to $\pm 1\,\mu l$ (see Results), we find that each subsequent successful calibration ($m_d - m_c \leqslant 1.1\,\mu gC$) means that $V_c \pm 2\,\mu l$ ($\pm 2$ standard deviations) still satisfies eq. 1. Because $2\,\mu l$ of pure $CO_2$ at ambient temperatures and pressures is $\sim 1\,\mu g$ $CO_2$–C, the criterion for $m_d - m_c$ is $1.0\,\mu gC$. Actually, the difference $m_d - m_c$ includes not only titration errors, but also those arising from the measurement of $T_a$ and $P$. If $m_d - m_c$ remains constant during an experiment, then the system response (coulometer calibration, cell, and measurement of $T_a$ and $P$) is unchanged with reference to $V_c$, and any significant $TCO_2$ variation observed is probably real. The value $V_c$, determined in May 1985 with system UG-I, held for calibration analyses through October 1985 using the same barometer and thermometer.

Table VI shows that the coulometer can detect the $\Delta m_c$ (eq.3) due to diurnal variation in ambient pressure and temperature, while Fig. 2 shows that small temporal and spatial $TCO_2$ changes in the water column can be detected. Table VI also shows that at least 25 mgC can be titrated in the cell without error which is a significant improvement over the original system (Johnson et al., 1985) when errors developed after 10 mgC. This increased life of the cell solutions is probably due to the removal of acid mists, ions, and traces of acid gases through the addition of the condenser, dehydrite, and the replacement of the wet $Ag_2SO_4$ scrubber with the dry Orbo-53 silica-gel trap, so that the coulometer cell receives only pure dry $CO_2$ in $N_2$. We did observe a positive error ($m_d - m_c = +2.5\,\mu gC$) on 19 June, but only after 35 mgC had been titrated. Errors normally develop if a cell is used, left overnight, and then reused. These are probably the type IV errors of Johnson et al. (1985), because sufficient time elapses for contaminants to diffuse from the anode through the cell frit to the cathode where they are electrolyzed. Therefore, on present information, we recommend changing the cell solutions daily or after 25 mgC have been titrated. On 18 July 1985, $m_d - m_c$ did exceed $1.1\,\mu gC$ even with fresh cell solutions (Table VI), however, this difference remained constant throughout the experiment. Subsequent calibrations over the next 2 months were normal so we have no explanation for this one aberrant result.

Coulometrically determined ($TCO_2$) and total carbonate concentrations calculated from pH and alkalinity ($TCO_2^*$) at Askö were on average, within 0.5% as shown in Table IV. The 12 $\mu$molar difference (1%) observed on 9 and 17 July may be real, but because electrical interference prevented the alkalinity titrations on 15 and 16 July (L. Anderson, personal communication, 1985) a systematic error in $TCO_2^*$ cannot be ruled out. Even so, Table V shows that $TCO_2$ and $TCO_2^*$ based estimates of net metabolism between 9 and 17 July at Askö are equivalent. Questions may remain concerning absolutes, but Tables IV and V clearly show that the functional accuracy of the system is high, and large errors in either coulometer calibration, the estimation of pipette volume ($V_{pt}$) with $Na_2CO_3$ solutions, or $V_{pt}$ corrected for temperature and salinity seem unlikely for system UG-I. The $TCO_2^* - TCO_2$ differences at Askö, in the MERL mesocosms (Johnson et al., 1985), and the open sea (Bainbridge, 1981; Bradshaw and Brewer, 1987) suggest that these discrepancies are widespread. Additional comparative analyses of $\Sigma CO_2$ should be made, because they may not measure the same thing, and this may help us to understand the short term departures from theoretical PQ and RQ so often observed in natural waters (Johnson et al., 1981; Oviatt et al., 1986).

Precision (Tables II, III) for samples exceeding 1000 $\mu$mols $l^{-1}$ $TCO_2$ was routinely < 0.1%. Nevertheless better precision with the Kristineberg (KB) samples was expected during the testing of the UG-I hardware and software. For the 12 KB samples shown in Table II, Cochran's Test (Guenther, 1964) for homogeneous variance ($n = 3$) indicated that a hypothesis of equal variance could be rejected ($\alpha = 0.05$). At Askö high precision was obtained, but Table III does show an unexplained day to day variation in the pooled standard deviation. Evidence for interactions between sample handling and chemistry comes from 12 Askö samples stored in the dark at 0–5°C. After a few hours storage, these samples always gave higher results when compared with the fresh material, and they often showed poorly defined end points and reduced precision. We also attempted to analyze deep ground waters for $TCO_2$ without success. If these samples were kept in cold storage before analysis, end point detection was often impossible. Some of this difficulty must involve carbonate fines which slowly dissolve at lower temperatures. Filtering these samples through glass wool did not correct the problem, and vacuum filtration through fine porosity filters would of course result in loss of $CO_2$. Cold storage did not appear to affect the fresh-water analyses. Previous work in the nearshore waters of Bermuda (unpublished data) also suggested that the poisoning of samples with $HgCl_2$ as a means of preservation was unsatisfactory because some samples showed biological activity for more than 1 h after treatment. The data in this report are therefore confined to samples collected as $O_2$ and analyzed as pH samples (within 2 h) after recommendations in Grasshof et al. (1983). For biological work, we believe further study is needed to improve methods for $TCO_2$ sampling and storage.

Sample delivery precision was checked with the URI-I system using a pipette having a known volume ($V_{P25}$) of 26.3387 ml at 25°C. Configured as in Fig. 1, its output was collected in preweighed serum bottles so that $V_{pt}$ could be gravimetrically determined. On January 14 1985, nine weighings of distilled water ($T_c = 22.7°C$) gave a mean of 27.5603 ± 0.0099 ml (%CV = 0.036), and on 15 January the five weighings from the same pipette ($T_c = 23.0°C$) gave 27.5603 ± 0.0063 ml (%CV = 0.023%). The difference (1.222 ml) between $V_{P25}$ and $V_{pt}$ is due primarily to the liquid contained in the tubing between the pipette and solenoids at positions 4 and 5 (Fig. 1), and the small volumetric correction for temperature. In the UG-I system this difference is < 0.1 ml because the total tubing length has been reduced to ~ 2 cm, but for clarity this is not shown to scale in Fig. 1. Nevertheless, $V_{pt}$ remained constant in the test above, and volumetric errors of 0.02% can probably not be reduced further and represent the upper limit for precision. A new coulometer (Model 5011), capable of titrating in increments of 0.01 $\mu$gC is available, and this could lead to a small improvement in precision as end point detection is more precise. The lowest pooled standard errors of 0.38 and 0.36 $\mu$mol l$^{-1}$ were found for nine samples at Askö (mean pH = 8.33; mean $TCO_2$ = 1273.0 $\mu$mol l$^{-1}$) on 18 July and for seven samples from Gårdsjön (mean pH = 7.46; mean $TCO_2$ = 173.6 $\mu$mol l$^{-1}$; individual standard errors 0.20 to ± 0.55 $\mu$mol l$^{-1}$) on September 19, 1985, respectively. It may not be possible to improve on this precision, but it should be achievable on a routine basis.

The sidearm cell design (Johnson et al., 1985) has been retained because during a titration its large cross-sectional frit area minimizes resistance to current flow, and therefore minimizes the resistive heating of the cell solution which could affect the equilibrium constants of the indicator (thymolphthalein) reaction (D. Chipman, personal communication, 1985). Whenever blanks exceeding 0.15 $\mu$gC min$^{-1}$ occur, the cells are cleaned by soaking them overnight in a 10% (w/v) solution of NaOH and 3% (w/v) $KMnO_4$, rinsing with hot tap water until the purple disappears, and then soaking in a 3% (w/v) solution of $Na_2SO_3$ containing 1.5% HCl until all traces of brown $MnO_2$ disappear. After rinses with hot tap water, distilled water, and acetone drawn through the cell frit under vacuum, the cell is thoroughly dried.

The new pipette and stripper design with less dead volume and sample addition through a hydrophobic teflon insert, probably improves the precision of sample delivery. This new design is also easier and cheaper to manufacture. A serum stoppered sampling port may be mounted on the front of the stripper to allow gas samples (e.g. headspace analysis) or small water samples to be injected, scrubbed, and titrated. The calibration system can check scrubber performance by titrating the sample loop contents before and after passage through the scrubber. Using this procedure, we determined that the silica-gel trap was not adsorbing or desorbing $CO_2$.

In summary, the automated coulometric method described can be used to measure temporal and spatial $TCO_2$ variation, test the validity of $^{14}C$ bottle assays, provide an alternative procedure for estimating productivity, estimate respiration, and with further work it should provide an absolute measure of $TCO_2$. It fulfils the requirement of Herczeg et al. (1985) for a sensitive and precise measurement of temporal trends in $TCO_2$ in lakes impacted by acid precipitation without risking the inter-laboratory variation common to empirical analyses because the primary coulometric standard is the electron. This method joins a new family of computer assisted field techniques for measuring $CO_2$ and $O_2$ (Williams and Jenkinson, 1982; Anderson and Wedborg, 1983; Langdon, 1984; Bradshaw and Brewer, 1987; Bender et al., 1987). Although the total capability now exists at only two laboratories, Table V indicates that the high precision tools needed to 'balance' the books and elucidate the major pathways of aquatic metabolism are now available.

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