

## Continuous In-Situ Analysis of Total Carbonate in Water by a Reversed-Flow Injection Method

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### Abstract

By coupling a gas-diffusion (GD) unit with a portable FIA system, a reversed-flow injection analysis (rFIA) system was assembled for the continuous in-situ determination of total carbonate in natural waters. In this system, only 0.02 ml injection of a reaction solution (diluted sulfuric acid) was sufficient for one measurement. The flow system was incorporated two six-way injection valves in the sample stream before the gas diffusion unit. One valve was used for injecting the reaction solution (dil. H<sub>2</sub>SO<sub>4</sub>), the other being used for injecting standard carbonate solutions for the preparation of a calibration graph. The standard deviation of 8 replicate measurements of a water sample was  $3 \times 10^{-4}$  absorbance unit, and the relative standard deviation was 0.7%. The detection limit of the method was  $1.2 \times 10^{-5}$  M. The consumption of the reaction solution (dil. H<sub>2</sub>SO<sub>4</sub>) can be reduced to less than one hundredth, compared with a conventional FIA (cFIA).

**Keywords:** spectrophotometry; reversed FIA; gas diffusion unit; total carbonate; river water

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### 1. Introduction

Earth warming (green-house effect), abnormal weather and climate observed in recent years are caused partly by the enrichment of carbon dioxide in the atmosphere, the concentration of which is now more than 0.03%. Carbon dioxide content in the atmosphere has been increasing gradually year by year; therefore, the amount of its generation has been restricted. Carbon dioxide can dissolve in environmental waters, and is kept at the equilibrium between the atmosphere and the aquatic environment. The oceans are a huge reservoir of CO<sub>2</sub>, and can act as both a source and a sink of CO<sub>2</sub>, depending upon the region, season, and the occurrence of episodic events. Widespread mapping of the partial pressure of CO<sub>2</sub> of surface seawater is essentially vital for determining the present and future global CO<sub>2</sub> air-sea flux. Long-term ship-based measurements are a serious endeavor because of their high cost and rare chance, and it is, in general, preferable to move instruments easily at strategic ocean locations when

they are used. That is why the instruments must be simple, inexpensive, and can work for a long-term monitoring.

There are several methods for the determination of total carbonate (TC) in aqueous solutions. Sensors for CO<sub>2</sub> have been extensively studied [1,2]. However, their sensitivity and precision are not enough for their application to seawater. The single operator multiparameter metabolic analyzer (SOMMA) proposed by Johnson *et al.* [3] has been widely used in practical determination of total carbonate in seawater. The system, however, is very big, complicated and difficult to operate [4].

Gas permeation methods coupled with FIA have been proved to be simple, sensitive, rapid and easy to operate. In the methods, the changes of physicochemical property of the CO<sub>2</sub> receptor, which are caused by permeated carbon dioxide, can be detected by potentiometry [5], spectrophotometry [6-10] and conductometry [10,11]. Kuban and Dasgupta [10] compared the conductometry with spectrophotometry; they decided that the conductometric procedure with a weak alkaline receptor solution provided better day-to-day reproducibility, sensitivity and detection limits. Aoki *et al.* improved a conductometric method based on a

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continuous flow reported by Carlson [12]; the limit of detection corresponding to the signal-to-noise ratio of 3 was  $1.0 \times 10^{-5}$  M [11]. Motomizu *et al.* reported a gas-diffusion/FIA (GD/FIA) for carbonate [6-8] and ammonium [8,9], and recently Higuchi *et al.* developed a stable GD apparatus for ammonia determination [13].

Reversed-FIA (rFIA) was firstly proposed by Johnson and Petty [14] for the determination of phosphate in seawater. Since then, rFIA has been applied to the determination of nitrite, nitrate [15], and ammonia [15,16] in natural water, and total dissolved iron and total dissolved manganese in underground water [17]. In the case that a lot of samples must be analyzed successively, continuous monitoring is necessary, and/or saving reagents in measuring processes is requested, rFIA is one of the best choices.

There are few instruments currently available, which can fit the strict criteria for continuous in-situ determination of total carbonate in natural waters. In this work, a rFIA system was developed by coupling a GD unit with a portable FIA system, and was used for long-term automatic and continuous in-situ monitoring of  $\text{CO}_2$  in natural waters, such as river water and seawater.

## 2. Experimental

### 2.1. Apparatus

The schematic diagram of a rFIA system for the continuous determination of total carbonate in water samples was shown in Fig. 1. A double plunger micropump, installed in a portable FIA system (PFA,

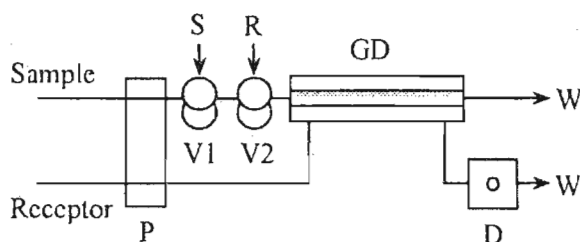


Fig. 1 Schematic diagram of a rFIA system. Receptor:  $2.5 \times 10^{-5}$  M cresol red solution (pH = 9.0); P: pump; S: six-way valve (V1) with a loop (sample size of 1.0 ml) for standard carbonate samples; R: six-way valve (V2) with a loop of 0.02 ml for reaction solution (dil.  $\text{H}_2\text{SO}_4$ ); GD: gas diffusion unit; D: detector and signal recorder; W: waste.

F·I·A Instruments, Tokyo), was used for propelling a sample and a receptor solution: each flow rate was  $0.3 \text{ ml min}^{-1}$ . Two six-way valves (V1 and V2) were installed in the flow line of the sample. V1 was used for injecting the standard carbonate solutions (loop size 1.0 ml) into the flow line of the sample stream to change the flow stream from the sample to the standard carbonate solutions when the calibration of the system is necessary. V2 was used for injecting the reaction solution (diluted sulfuric acid; injection volume 0.02 ml) into the sample stream. The GD unit was constructed with a PTFE membrane tube (Gore-Tex TA001: 1.0 mm inner diameter and 1.8 mm outer diameter, pore size  $2 \mu\text{m}$ , porosity 50%) as an inner tube, and the outer tube was made of glass (2.0 mm inner diameter and 5.0 mm outer diameter)[13]. Absorbance was measured at 450 nm by a visible detector installed in the PFA, which consists of a light emitting diode (LED) as a light source with an interference filter (450 nm) and a micro flow cell (light path 10 mm, volume  $8 \mu\text{l}$ ). All of these components were packed in a small box (16 cm width x 16 cm height x 32 cm depth) in order to be moved easily. The system was equipped with a controller for controlling flow rate, temperature of the reaction compartment and a dynamic range of the detector. Signals were recorded with a strip chart recorder (Toa Dempa FBR-251A) or stored in a note-type personal computer. The main system was 7.5 kg, worked with 12 V battery, and could be used outdoors.

### 2.2. Reagents

All chemicals used in this work were of analytical reagent grade (Kanto Chemical Co., Inc.). Distilled water was purified with a Milli-Q Labo (Elix 3 / Milli-Q Element, Millipore Co., Japan), and then  $\text{CO}_2$  in the purified water was removed with  $\text{N}_2$  bubbling for 1.5 h in a water bath at  $50^\circ\text{C}$  under ultrasonic radiation. This carbonate-free water was used for preparing the reaction, the receptor, and the standard carbonate solutions.

The receptor solution contained  $2.5 \times 10^{-5}$  M cresol red and  $3 \times 10^{-3}$  M sodium hydrogen carbonate; the pH of the solution was adjusted to 9.0 with sodium hydroxide.

A solution,  $3.6 \times 10^{-3}$  M  $\text{H}_2\text{SO}_4$ , was prepared by diluting a 1.8 M  $\text{H}_2\text{SO}_4$  stock solution with the carbonate-free water, and was used as a reaction solution.

The stock solution of standard carbonate solution was prepared by dissolving 0.420 g of sodium hydrogen carbonate in 100 ml of water to give a  $5.0 \times 10^{-2}$  M solution. This solution was diluted

accurately with the carbonate-free water.

### 3. Results and Discussion

#### 3.1. Experimental variables in rFIA

Effect of flow streams in the GD unit on signals was examined. To increase sample throughput or to

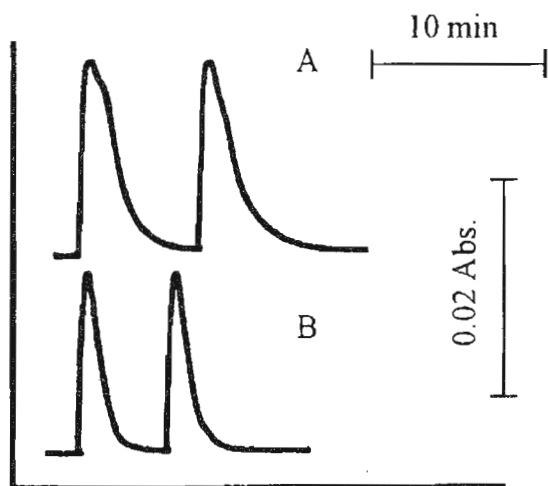


Fig. 2 Effect of flow stream in GD unit on signals

Sample solution:  $2 \times 10^{-4}$  M  $\text{NaHCO}_3$  solution; A: sample solution flows in the outer tubing; B: sample solution flows in the inner tubing; each flow rate:  $0.3 \text{ ml min}^{-1}$ ; injection volume of the reaction solution (dil.  $\text{H}_2\text{SO}_4$ ):  $0.02 \text{ ml}$ .

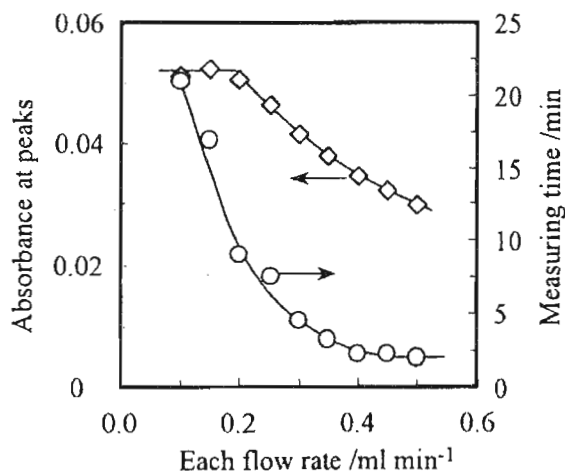


Fig. 3 Effect of flow rate on the absorbance at peaks ( $\diamond$ ) and measuring time ( $\circ$ )

Sample solution:  $0.2 \text{ M}$   $\text{NaHCO}_3$ ; injection volume of the reaction solution:  $0.02 \text{ ml}$ ; reaction solution:  $3.6 \times 10^{-3} \text{ M}$   $\text{H}_2\text{SO}_4$ .

reduce a measuring time (from injection of the reaction solution to the signals return to the baseline), which affects the time corresponding to the peak width at the baseline, the dispersion of  $\text{CO}_2$  in the receptor stream must be lowered. Previously, when the proposed GD unit was used, usually the carrier stream with a sample zone flows through the outer tubing<sup>13</sup>. However, as shown in Fig. 2, when the sample stream flowed through the inner tubing, the measuring time could be decreased significantly from about 7 min to 3.5 min. For shortening the measuring time, the sample stream was flowed through the inner tubing.

The effect of each flow rate on the absorbance at peaks and the measuring time was examined; the result is shown in Fig. 3. When the flow rate was less than  $0.2 \text{ ml min}^{-1}$ , there was no influence on the absorbance at peaks. However, when the flow rate was larger than  $0.2 \text{ ml min}^{-1}$ , the absorbance at peaks decreased gradually with increasing the flow rate. This is because the residence time of the sample zone in the GD unit became short. When the flow rate was greater than  $0.4 \text{ ml min}^{-1}$ , the measuring time became almost constant. Considering the measuring time and the sensitivity, the flow rate of  $0.3 \text{ ml min}^{-1}$  was adopted as a compromise in this work, though the sensitivity was low.

The effect of the injection volume of the reaction solution (dil.  $\text{H}_2\text{SO}_4$ ) on the absorbance at peaks and width was studied by varying the volume from  $0.02$

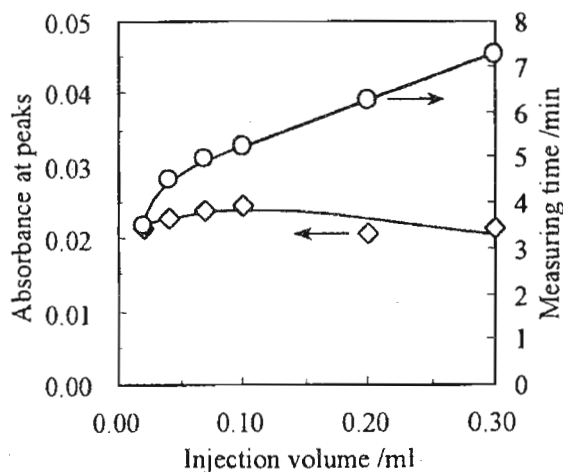


Fig. 4 Effect of injection volume of the reaction solution on the absorbance at peaks ( $\diamond$ ) and measuring time ( $\circ$ )

Sample solution:  $2 \times 10^{-4} \text{ M}$   $\text{NaHCO}_3$ ; reaction solution:  $3.6 \times 10^{-3} \text{ M}$   $\text{H}_2\text{SO}_4$ ; each flow rate:  $0.3 \text{ ml min}^{-1}$ .

to 0.3 ml; the results obtained are shown in Fig. 4. At the region of the injection volume of less than 0.1 ml, the absorbance at peaks increased gradually with an increase in the injection volume, and at the region of the injection volume larger than 0.1 ml, the absorbance at peaks decreased gradually with an increase in the injection volume. Furthermore, with an increase in the injection volume, the measuring time became longer, and double peaks for each injection occurred. In this work, 0.02 ml of the injection volume was adopted in practical use.

The concentration of a sulfuric acid solution as a reaction solution was examined. The sulfuric acid solution was used to convert carbonate and hydrogen carbonate ion in sample solutions into  $\text{CO}_2$  gas. The effect of the concentration of the sulfuric acid on the absorbance at peaks and peak width was investigated: the results are shown in Fig. 5. As expected, the absorbance at peaks and the peak width were both

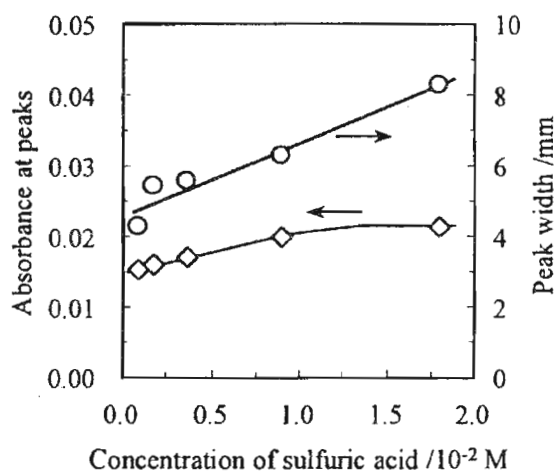


Fig. 5 Effect of the concentration of sulfuric acid on the absorbance at peaks (◇) and peak width at the baseline (○)  
Sample solution:  $2 \times 10^{-4}$  M  $\text{NaHCO}_3$ ; injection volume of sulfuric acid: 0.02 ml; each flow rate:  $0.3 \text{ ml min}^{-1}$ .

increased with an increase in the concentration of sulfuric acid solution. However, when the concentration of the sulfuric acid solution was equal to or less than  $1.8 \times 10^{-4}$  M, the peaks were too small to detect them, because the acidification is not sufficient to generate  $\text{CO}_2$ . In the range from  $9 \times 10^{-4}$  M to  $9 \times 10^{-3}$  M, the absorbance at peaks was increased linearly. When the sulfuric acid concentration exceeded  $9 \times 10^{-3}$  M, the absorbance at peaks was almost identical with each other, though the peak width became wider. Appropriate sulfuric

acid concentration is related to carbonate concentrations of sample solutions. The total carbonate concentration in natural water is generally  $10^{-4}$  M level, and therefore  $3 \times 10^{-3}$  M of sulfuric acid solution is sufficient. For convenience,  $3.6 \times 10^{-3}$  M of  $\text{H}_2\text{SO}_4$  solution was adopted in this work.

### 3.2. Calibration procedure

Calibration of the rFIA system was done by using a series of standard solutions of sodium hydrogen carbonate. In general, it was inconvenient and time-consuming to change the flow stream from practical sample solutions to standard sample solutions for making a calibration graph. In this work, it was realized by installing a six-way injection valve ( $V_1$  in Fig.1) in the sample stream just before the injection valve of the reaction solution. Each standard carbonate solution was injected into the sample stream by  $V_1$ , and then the reaction solution (dil.  $\text{H}_2\text{SO}_4$ ) was injected by using the other six-way injection valve ( $V_2$ ), which was installed just after the valve  $V_1$ , into less dispersed zone of the standard sample solutions flowing into the sample stream.

To obtain an appropriate loop size of the standard sample solutions, the effect of loop size (0, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) on the absorbance at peaks was examined by using  $1.2 \times 10^{-3}$  M carbonate solution as a standard, Zasu River as a sample solution and  $3.6 \times 10^{-3}$  M  $\text{H}_2\text{SO}_4$  as a reaction solution. After one half of the standard solutions in the loop were flowed

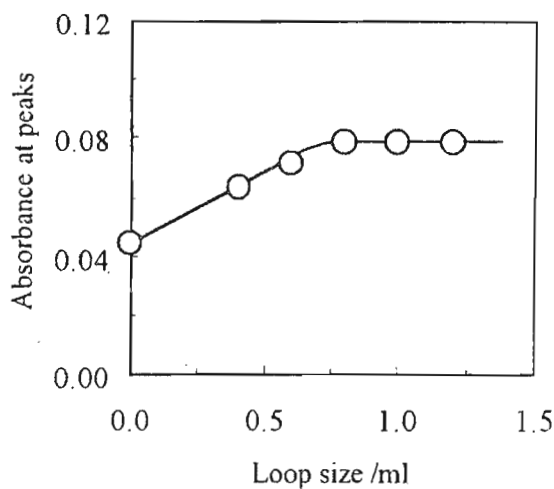


Fig. 6 Effect of loop size for injected standard carbonate solutions on the absorbance at peaks  
Sample: Zasu River ( $6.7 \times 10^{-4}$  M carbonate); standard carbonate solution:  $1.2 \times 10^{-3}$  M; reaction solution: 0.02 ml of  $3.6 \times 10^{-3}$  M  $\text{H}_2\text{SO}_4$ .

out of the outlet of V1, the reaction solution was injected into the less dispersed zone of the standard carbonate solutions by turning the six-way valve V2 to the injection position. The injection timing of the reaction solution, after the V1 was turned, was changed corresponding to the loop size of V1: when the loop size of V1 were 0, 0.4, 0.6, 0.8, 1.0 and 1.2 ml, the V2 valve was turned 0, 40, 60, 80, 100 and 120 s, respectively. As shown in Fig. 6, the absorbance at peaks was increased with an increase in a loop size till the loop size reached 0.8 ml. These increases indicate that in the region below 0.8 ml of the loop size, the standard carbonate solutions were diluted with the river water, whose concentration of carbonate is lower than that of the standard solution. When the loop size become larger than 0.8 ml, the absorbance at peaks become constant, which corresponds to the absorbance at peaks of  $1.2 \times 10^{-3}$  M standard carbonate solution. In this work, a 1.0 ml loop for standard samples was adopted.

The effect of injection time of the reaction solution ( $3.6 \times 10^{-3}$  M  $\text{H}_2\text{SO}_4$ ) after the standard sample solutions ( $1.2 \times 10^{-3}$  M carbonate solution) was injected into the sample stream (Zasu River,  $6.7 \times 10^{-4}$  M carbonate) by using a standard sample loop of 1.0 ml was examined in detail. In the front and the rear of the standard sample zone, where a dispersion must be occurred, the standard sample solutions would be mixed with the sample stream; as a result the standard sample solution was diluted with the sample stream and the concentration of the carbonate was decreased. Therefore, optimum timing for the injection of reaction solution into the standard sample zone is very important to get a real absorbance at peaks corresponding to the standard sample solution. As shown in Fig. 7, in the proposed system, the absorbance at peaks was constant when the injection time of reaction solution was varied from 80 s to 100 s, where the absorbance at peaks obtained was corresponded to the real ones of  $1.2 \times 10^{-3}$  M standard carbonate solution. In this work, turning V2 to the injection position was carried out 90 s (1.5 min) after the standard sample solution was injected into the sample stream by turning V1 to the injection position.

### 3.3. Application to the successive determination of total carbonate in water

The differences in absorbance between the standard carbonate solutions and the blank solution (non-carbonate solution: purified water) were used for the preparation of a calibration graph. The calibration graph was linear over the concentrations

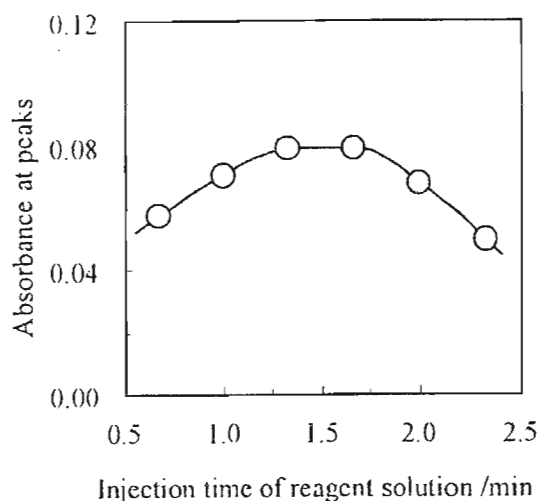


Fig. 7 Effect of injection time of reaction solution on the absorbance at peaks  
Sample: Zasu River ( $6.7 \times 10^{-4}$  M carbonate); standard carbonate solution:  $1.2 \times 10^{-3}$  M; reaction solution: 0.02 ml of  $3.6 \times 10^{-3}$  M  $\text{H}_2\text{SO}_4$ .

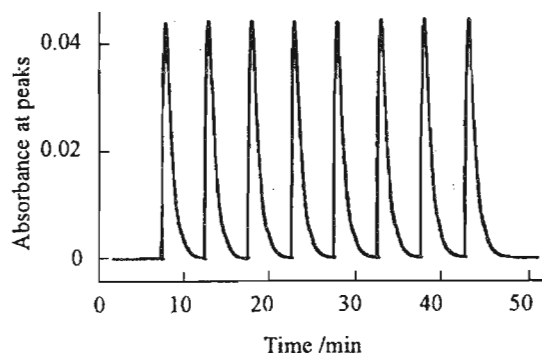


Fig. 8 Signals for the continuous determination of total carbonate in river water  
Sample: Zasu River ( $6.7 \times 10^{-4}$  M); a mean value ( $\bar{x}$ ), a standard deviation (SD) and a relative standard deviation (RSD) of the absorbance at peaks:  $\bar{x} = 4.40 \times 10^{-2}$  Abs., SD =  $3 \times 10^{-4}$  Abs. and RSD = 0.7%.

of  $10^{-5}$  -  $10^{-3}$  M of carbonate. The regression equation of the calibration graph is as follows:  $\Delta A = 6.55 \times 10^{-3} \cdot C$  and  $r = 0.99$ , where  $\Delta A$  (Abs.) is the difference in absorbance between the standard carbonate solution and the blank,  $C$  ( $10^{-4}$  M) is the concentrations of the standard solutions of sodium hydrogen carbonate.

The application of the proposed system to the

continuous determination of carbonate in natural waters was examined. The total carbonate concentration in tap water, a river water and a seawater determined by the proposed system were  $5.4 \times 10^{-4}$  M,  $6.7 \times 10^{-4}$  M and  $1.8 \times 10^{-3}$  M, respectively. The level was as the same as those determined by similar method or ion chromatography. [18] As an example, the signals for the determination of total carbonate in the river water are shown in Fig. 8. The standard deviation (SD) of 8 replicate measurements was  $3 \times 10^{-4}$  Abs., and the relative standard deviation (RSD) was 0.7%. The detection limit, corresponding to three times of the standard deviation of the  $2 \times 10^{-4}$  M standard carbonate solution, was  $1.2 \times 10^{-5}$  M.

#### 4. Conclusion

In this work, the total carbonate in water samples can be continuously measured by using a rFIA system with 0.02 ml injection volume of the reaction solution (dil.  $\text{H}_2\text{SO}_4$ ). If the measurement is carried out continuously for one month, and a sampling frequency is 12 samples/h, about 170 ml of the reaction solution ( $3.6 \times 10^{-3}$  M sulfuric acid) is necessary in the proposed rFIA, whereas in a conventional FIA, about 26 l of the diluted  $\text{H}_2\text{SO}_4$  is necessary. This is advantageous to the continuous in-situ analysis of total carbonate in natural waters. The proposed method will be very useful for continuous determination of total carbonate in natural water samples.

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