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DETERMINATION OF TRACE LEVELS OF TOTAL CARBONATE-CARBON BY INDIRECT PHOTOMETRIC ION CHROMATOGRAPHY WITH NITROGEN PURGING

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SUMMARY

Purging with nitrogen substantially prevented atmospheric carbon dioxide from dissolving in the eluent used in indirect photometric ion chromatography. Total carbonate-carbon at trace levels could be determined as hydrogencarbonate by this method. By using an analytical column (250 × 4.6 mm I.D.) packed with MCI SCA-02 and $5.0 \cdot 10^{-4}$ *M* sodium hydrogenphthalate–$1.5 \cdot 10^{-4}$ *M* N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) (pH 6.5) as the eluent, hydrogencarbonate was detected as a negative peak at 250 nm. The detection limit (signal-to-noise ratio = 2) was $1.4 \cdot 10^{-11}$ mol ($7 \cdot 10^{-7}$ *M* with an injection volume of 20 μl) and the calibration graph was linear from $3.0 \cdot 10^{-11}$ to $3.8 \cdot 10^{-9}$ mol ($r = 0.994$). The coefficient of variation was less than 2% on injection of $4 \cdot 10^{-10}$ mol. Total carbonate-carbon at levels as low as 10^{-4}–10^{-6} *M* level could be determined accurately. The errors in the analysis of practical samples by the method without nitrogen purging are demonstrated.

INTRODUCTION

Carbon dioxide plays important roles as carbonic acid, hydrogencarbonate and carbonate in biological fluids, liquid foods and environmental water samples[1–3], and an accurate and sensitive method for the determination of total carbonate-carbon is required. Atmospheric carbon dioxide causes serious problems both in the use of ultra-pure water and in the sensitive determination of anions.

For the sensitive determination of aqueous total carbonate-carbon, flow-injection analyses based on an ultraviolet (UV)–visible absorption detector[4,5] and optical sensors with pH-sensitive fluorescent dyes[6,7] have been developed. However, these methods could not determine both total carbonate-carbon and other anions simultaneously. Ion exclusion chromatography with conductometric detection, which was effective for weak acids such as carboxylic acids, has also been used for the determination of total carbonate-carbon[8,9]. Recently, a modified ion exclusion chromatographic method with two enhancement columns after the analytical column

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has been reported[10]. Although the sensitivity of the method is high, the enhancement columns must be regenerated when exhausted.

Indirect photometric ion chromatography (IPIC) based on indirect photometric detection[11] has been used in the determination of anions and cations with a conventional high-performance liquid chromatographic system[12–14]. It was reported that IPIC could detect hydrogencarbonate when the eluent pH was maintained nearly neutral[15]. Although total carbonate-carbon at concentrations higher than 10^{-4} *M* could be determined in samples such as tap water and river water[16,17], lower concentrations could not be determined accurately. When a trace amount of sodium hydrogencarbonate was injected into an IPIC system using an eluent that had not been treated to remove atmospheric carbon dioxide, the negative peak (decrease in UV absorbance) observed was smaller than expected, whereas a small positive peak was observed at the same retention time on injection of deionized water. This peak at the retention time of hydrogencarbonate, which is termed the "carbonate-system peak" in this paper, disturbed the determination of hydrogencarbonate at low levels. Atmospheric carbon dioxide dissolved in the eluent was considered to be the cause.

It is necessary to remove this interference due to carbon dioxide in the sensitive and accurate determination of total carbonate-carbon. Purging with an inert gas[18] and gas headspace treatment[19] have been reported to remove gases such as oxygen from mobile phases. We found that gas purging also removed carbon dioxide from eluents for IPIC. This paper describes the effect of nitrogen purging on the determination of total carbonate-carbon by IPIC.

EXPERIMENTAL

Chemicals

Sodium hydrogenphthalate and N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) were purchased from Tokyo Kasei (Tokyo, Japan) and Wako (Osaka, Japan), respectively. All other chemicals were of analytical-reagent grade. Water was purified with a Millipore (Bedford, MA, U.S.A.) Milli-Q system. "Deionized water" in this paper means water just after repurification with this system.

Instrumentation

The HPLC system for IPIC consisted of a Jasco (Tokyo, Japan) 880-PU pump, a Rheodyne (Cotati, CA, U.S.A.) 7125 injector with a 20-μl loop, a Jasco 860-CO column oven, a Jasco 870 UV absorbance detector and a Jasco 805-GI graphic integrator. A Jasco 880-51 on-line degasser was also used. The nitrogen purging system consisted of a nitrogen gas bomb (purity over 99.99%), a gas washing bottle (glass, inner volume 200 ml) that contained 150 ml of water and an eluent reservoir (glass) with a gas purging filter. A schematic diagram of the system is shown in Fig. 1.

Operating conditions

An analytical column (250 × 4.6 mm I.D., stainless steel) and a guard column (50 × 4.6 mm I.D., stainless steel) were packed with MCI SCA-02 (styrene–divinylbenzene copolymer with an anion-exchange capacity of 0.01 mequiv./g and a particle size of 20 μm) (Mitsubishi Chemical, Tokyo, Japan). The eluent was $5.0 \cdot 10^{-4}$ *M* sodium hydrogenphthalate–$1.5 \cdot 10^{-4}$ *M* HEPES the pH of which was

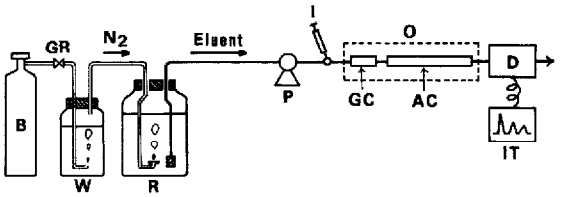

Fig. 1. Schematic diagram of IPIC with the nitrogen purging system. B = Nitrogen gas bomb; GR = gas regulator; W = gas washing bottle; R = eluent reservoir; P = pump; I = injector; O = column oven; GC = guard column; AC = analytical column; D = UV detector; IT = integrator.

adjusted to 6.5 ± 0.1 with sodium hydroxide. The eluent was treated with a 0.40-μm nylon membrane filter before use. Nitrogen was purged into the eluent continuously at a flow-rate of 50 ml/min during the experiment. The columns were equilibrated overnight with this nitrogen-purged eluent before use. The flow-rate of the eluent was 1.0 ml/min, the columns were kept at 40°C and the detection wavelength was 250 nm. Under these conditions, hydrogencarbonate, chloride and sulphate, which were standard anions, were separately determined as negative peaks at 6.0 min (capacity factor $k' = 0.8$), 7.7 min ($k' = 1.3$) and 12.9 min ($k' = 2.9$), respectively.

RESULTS AND DISCUSSION

According to Henry's law, the mass of a slightly soluble gas that dissolves in a definite mass of a liquid at a given temperature is very nearly directly proportional to the partial pressure of that gas, according to the following equation[20]:

$$CO_2(g) \overset{K_P}{\rightleftharpoons} CO_2(aq.) \tag{1}$$

where K_P is the Henry's law partition constant for carbon dioxide. In aqueous solution, carbon dioxide exists in three forms, carbonic acid (H_2CO_3), hydrogencarbonate (HCO_3^-) and carbonate (CO_3^{2-}), as indicated by the equation

$$H_2CO_3 \overset{K_1}{\rightleftharpoons} H^+ + HCO_3^- \overset{K_2}{\rightleftharpoons} 2H^+ + CO_3^{2-} \tag{2}$$

where pK_1 is 6.34 and pK_2 is 10.25[21]. In the eluent of pH 6.5 which was used in this work, the main species are HCO_3^- and H_2CO_3. H_2CO_3 in eqn. 2 is calculated as CO_2(aq.) in eqn. 1 and does not act as an anion in an anion-exchange column.

Let us consider here that the eluent contains hydrogencarbonate at the trace level and make the simplification that sample hydrogencarbonate elutes as a square pulse peak from an analytical column as shown in Fig. 2. If C_E and C_B are the concentration of UV-absorbing eluent anions and that of background hydrogencarbonate in the eluent, respectively, the total anion concentration in the effluent before the sample injection is $C_E + C_B$ and is constant. When the sample hydrogencarbonate peak is eluted at a concentration C_S, the increase in hydrogencarbonate concentration in the

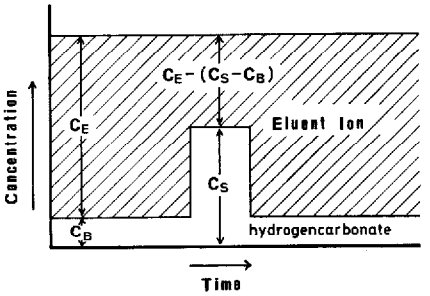

Fig. 2. Elution profile model for hydrogencarbonate.

effluent is given by $C_S - C_B$. Therefore, the signal (A_{S-E}) on the baseline is given by the equation

$$A_{S-E} = [C_E - (C_S - C_B)]\,A_E + C_S A_B - C_E A_E - C_B A_B$$

$$= (C_S - C_B)\,(A_B - A_E) \qquad (3)$$

where A_E and A_B are the absorbance of eluent species and that of hydrogencarbonate, respectively. Eqn. 3 is simplified into the following equation as A_B is zero at 250 nm:

$$A_{S-E} = -(C_S - C_B)A_E \qquad (4)$$

Eqn. 4 indicates that the signal is negative when C_S is higher than C_B, positive when C_S is lower than C_B and there is no signal when C_S is the same as C_B. If the eluent does not contain hydrogencarbonate ($C_B = 0$), the carbonate-system peak is not observed on injection of deionized water. Eqn. 1 suggests that carbon dioxide might be eliminated from an eluent with a decrease in its atmospheric partial pressure. The pressure can be decreased by displacing air to another gas. Nitrogen is readily available for purging an eluent and is advantageous from safety and economic points of view, although an inert gas such as argon or helium might be equally effective.

Fig. 3 shows chromatograms obtained by using $5.0 \cdot 10^{-4}$ M sodium

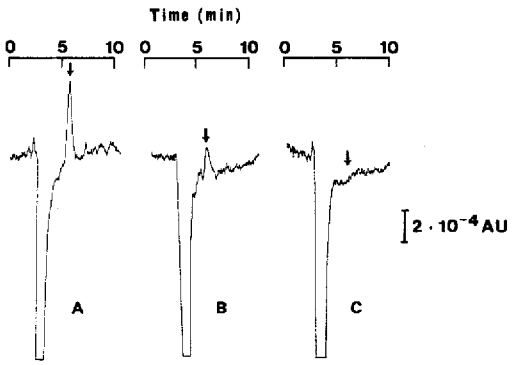

Fig. 3. Chromatograms of carbonate-system peak on injection of deionized water. The eluent was treated as follows: (A) none; (B) through an on-line degasser; (C) by the nitrogen purging system. The arrow indicates the position of the carbonate-system peak.

hydrogenphthalate–1.5 · 10^{-4} *M* HEPES (pH 6.5) as the eluent treated in the three different ways. Deionized water was injected as a sample. A positive peak (carbonate-system peak) was detected at 6.0 min without any treatment (Fig. 3A); the peak height decreased on passing through an on-line degasser as commonly used in HPLC (Fig. 3B); and the peak was not detected on using the nitrogen purging system (Fig. 3C). These results show that the nitrogen purging effectively prevents atmospheric carbon dioxide from dissolving in the eluent. Similar results were obtained when benzoate and benzenesulphonate eluents were tested. In the present system, a gas washing bottle was used to saturate the dry gas with water vapour and to wash out small amounts of contaminants from gas so as not to disturb the baseline stability.

Another interference from atmospheric carbon dioxide was observed in the preparation of hydrogencarbonate solutions. Dilute sodium hydrogencarbonate standard solutions were prepared by two different procedures. In the first series, a vial (inner volume *ca.* 26 ml) containing a small stirring bar was filled with deionized water under a nitrogen atmosphere and immediately stoppered with a silicone-rubber septum. After an aliquot of 0.1 *M* sodium hydrogencarbonate stock solution had been added to the vial by a microsyringe through the septum, the mixture was stirred for 2 min. In the second series, water was stirred overnight aerobically in a polyethylene bottle without a cap. Dilute sodium hydrogencarbonate solution was prepared with this water in a vial without a septum. Plots of peak area *vs.* amount injected for the two series are shown in Fig. 4. The former was a straight line ($r = 0.994$) from 3.0 · 10^{-11} to 3.8 · 10^{-9} mol (A), whereas the latter was curved (B). Line A could be used to represent dilute standard hyrogencarbonate solution. The difference between the two graphs might be due to the atmospheric carbon dioxide dissolved in the water. Therefore, the former procedure was used in subsequent experiments.

The coefficient of variation for the determination of hydrogencarbonate was less than 2% on injection of 4 · 10^{-10} mol. The determination error was as small as those of

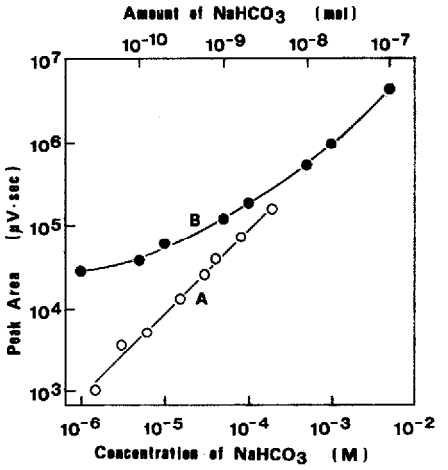

Fig. 4. Influence of atmospheric carbon dioxide on the calibration graphs for sodium hydrogencarbonate. Sodium hydrogencarbonate solutions were prepared in silicone rubber-stoppered vials with deionized water (A) and in open vials with air-equilibrated water (B).

TABLE I

DETERMINATION ERRORS OF THREE ANIONS

Anion	*Amount* (10^{-10} *mol*)[a]	*Coefficient of variation (%)* ($n = 5$)
Hydrogencarbonate	200	0.57
	20	1.4
	4	1.5
Chloride	200	0.50
	20	1.9
	2	1.5
Sulphate	200	0.20
	20	2.0
	2	3.4

[a] Injection volume, 20 μl.

chloride and sulphate, which were not affected by atmospheric carbon dioxide (Table I). The detection limit (signal-to-noise ratio = 2) of hydrogencarbonate was $1.4 \cdot 10^{-11}$ mol ($7 \cdot 10^{-7}$ *M* on injection of 20 μl). This value is more than ten times smaller than that obtained by the modified ion exclusion IC method, which gave the lowest limit in recent reports[10].

The concentration of total carbonate-carbon determined by IPIC without the nitrogen purging system is lower than the real concentration. The error is not negligible as the amount injected is small. Table II shows the results of the determination of total carbonate-carbon in aqueous samples by IPIC with and without the nitrogen purging system; the differences are given as relative values. Deionized water showed a negative peak with nitrogen purging but a positive peak without purging. The relative differences for air-bubbled water and for rain water were 37% and 13%, respectively. With an increase in concentration, the value becomes too small to be distinguished

TABLE II

ANALYTICAL RESULTS FOR TOTAL CARBONATE-CARBON BY INDIRECT PHOTOMETRIC ION CHROMATOGRAPHY (A) WITH AND (B) WITHOUT NITROGEN PURGING

Sample	*Concentration* (10^{-5} *M*)[a]		*Relative difference* [100 (*A* − *B*)/*A*] (%)
	A	*B*	
Deionized water	0	[b]	–
Air-bubbled water	3.0	1.9	37
Rain water	6.0	5.2	13
Tap water	43.3	42.4	2.1
River water	49.5	49.8	−0.6

[a] Mean values ($n = 3$).
[b] Positive peak.

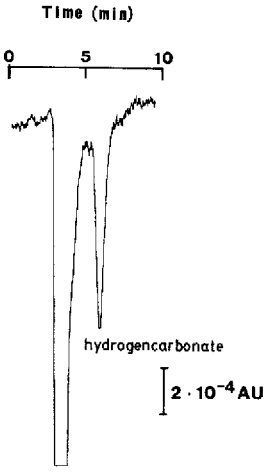

Fig. 5. Typical chromatogram of air-equilibrated water.

from the determination error. This was the reason why the value was positive (2.1%) for tap water but negative (−0.6%) for river water.

Total carbonate-carbon in air-equilibrated water could be determined by the present method, as shown in Fig. 5. The concentration was $1.2 \cdot 10^{-5}$ M when line A in Fig. 4 was used as a calibration graph. On the other hand, the concentration of carbon dioxide in air-equilibrated water can be calculated as

$$\begin{aligned} CO_2(aq.) &= 3.9 \cdot 10^{-2} \cdot 0.03 \cdot 10^{-2} \\ &= 1.17 \cdot 10^{-5}\ M \end{aligned} \tag{5}$$

as the Henry's law partition constant (K_p) of carbon dioxide is $3.9 \cdot 10^{-2}$ M per atom at 20°C and the concentration of carbon dioxide in air is 0.03% (v/v). This close agreement between the observed and calculated values indicates the reliability of the present method for the determination of low levels of carbonate-carbon.

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