Simultaneous Measurement of N₂, O₂, Ar, CO₂, CH₄, H₂ and CO in Aqueous and Gaseous Samples : a Detection of N₂/Ar Shifts in Environments

Muneoki YOH, Makoto TAKEUCHI and Hideshige TODA

ABSTRACT

A gas chromatographic system which accurately analyzes major gas constituents in environments is described. The system, which consists of three separation columns and two gas chromatographs equipped with a thermal conductivity detector, is capable of determining N₂, O₂, Ar, CO₂, CH₄, H₂ and CO simultaneously in both aqueous and gaseous samples. For gaseous samples, a special injection port was devised in the analytical line to eliminate air contamination during sample handling. A test measurement of air-equilibrated water confirmed that the measured concentrations of dissolved gases agreed well with their theoretical values calculated from solubility and atmospheric partial pressures. The preliminary results of groundwater and wetlands samples demonstrate that the entire gas composition in environments could be fully accounted for by the detected gas species. The N₂/Ar ratio given by this analysis could be an effective parameter to evaluate both N₂ production (denitrification) and N₂ consumption (nitrogen fixation) in nature.

Key words : gas analysis, gas chromatograph, gas composition, N₂/Ar ratio

INTRODUCTION

The measurement of gases has attracted considerable interest in a variety of fields such as soils, oceans and lakes. Gas chromatography is employed as a useful technique for gas analysis because it has the merit of permitting the simultaneous determination of several species using small sample sizes.

A number of procedures are proposed according to the gas components to be studied (SMITH and ARAH, 1991). Among gases measured with gas chromatography, the determination of Ar is relatively difficult. This is because it necessitates cooling the separation column down to the subambient temperature requisite for the separation of O₂ and Ar by either porous polymer columns (OBERMILLER and CHARLIER, 1968 ; BLACKMER and BREMNER, 1977) or molecular sieve 5A (LARD and HORN, 1960).

For this reason, researches which have actually determined the composition of major gases including Ar in natural environments are fairly limited.

GAMO and HORIBE (1980) developed a set of gas chromatographs for simultaneous analysis of four dissolved gases in seawater; N_2 , O_2 , Ar and ΣCO_2 . After extraction from seawater by an on-line gas stripping technique (SWINNERTON *et al.*, 1962), the gases in this system are sequentially separated by three columns and determined by a thermal conductivity detector and an ultrasonic detector.

All natural gas species except inert ones are produced or consumed by biological activities. Beside the four gases measured by GAMO and HORIBE (1980), methane or other species may be present as dominant gas constituents in certain environments. It is desirable to measure the full gaseous compositions in a variety of environments to extend the understanding of gas metabolisms. The same column assemblage as given by GAMO and HORIBE (1980) was modified in the present study to enable a simultaneous determination of N_2 , O_2 , Ar, CO_2 , CH_4 , H_2 and CO, taking advantage of the separation capability of molecular sieve 5A (KRYACOS and BOORD, 1957).

Special interest is focused on the applicability of Ar as a conservative tracer in both aqueous and gaseous phases. Since atmospheric contamination from sampling to measurement is a great concern in the gas analysis of major gas species, the overall accuracy was verified by examination with an air-equilibrated water. The results of some preliminary measurements of field samples are presented which demonstrate that the present gas analysis actually determines almost all the gas components in natural environments. In addition, data are presented showing that the present method can be effective in detecting not only N_2 production (denitrification) but N_2 consumption (nitrogen fixation) in terms of the shifts in the N_2 /Ar ratio.

MATERIALS AND METHODS

Apparatus

The schematic diagram of the gas chromatographic system is shown in Figure 1. Two gas chromatographs equipped with a thermal conductivity detector are used under the conditions shown in Table 1 (a). Helium was used as the carrier gas at a flow rate of 50 ml min^{-1} with a head pressure of 160 k Pa. Computer integrators (Shimadzu, CR 6A; System Instrument, Chromatocorder 11; or subsequently, Dynamax[®] MacIntegratorTMI, Rainin Instrument Co., Inc.) recorded the output from the detectors.

The separation of gases was carried out by three columns connected in series. The detailed descriptions of separation columns are given in Table 1 (b). Column 3 was kept in a dry ice-ethanol bath (-72°C) for the separation of O_2 and Ar. This column was warmed up to ambient temperature every twelve measurements to release N_2 and CH_4 , which were retained under that temperature (TOLK *et al.*, 1969; GAMO and HORIBE, 1980). CO_2 was removed by a Soda Talc (10-24 mesh) column before entering column 2, because molecular sieve 5A irreversibly absorbs it (TOLK *et al.*, 1969).

The all-glass sample chamber for dissolved gas extraction has a round bottom and a sintered glass ball filter having a pore size of $5\text{--}10 \mu\text{m}$. This

Fig. 1. Flow chart of the gas analytical system for both aqueous and gaseous samples.

Table 1. Analytical conditions : gas chromatography (a) and columns (b).

(a)	GC 1	GC 2
Instrument	Model 373 (Gasukuro-Kogyo Co., Ltd.)	GC 8A (Shimadzu Co., Ltd.)
Detector	TCD	TCD
Current	150 mA	200 mA
Detector temp.	60 °C	50 °C
Injection temp.	120 °C	80 °C

(b)	Column 1	Column 2	Column 3
Adsorbent	Silica Gel	Molecular Sieve 5A	Molecular Sieve 5A
Mesh	30/60	30/60	80/100
Size	3 mm ID ; 40 cm long	3 mm ID ; 200 cm long	3 mm ID ; 150 cm long
Temperature	25 °C	25 °C	-72 °C

chamber is designed so as to have a minimal dead volume to produce sharp peak. The outlet arm of the chamber is connected to a drying tube containing Drierite and Mg(ClO₄)₂. A buffer column is inserted between the sample chamber and the detector cell to reduce recording noises arising from bubbling vibration.

Procedure

Aqueous samples : Water samples were gently filled into a sample vessel having a fixed volume of around 2.5 ml. This vessel is made of a glass tube with two ground glass stoppers, as illustrated in Figure 1. After attachment to the line with Casion Ultra-Torr® Fittings, the air within the arms was forced out by repeated evacuation and He filling using a vacuum manifold. Three repetitions of this procedure completely removed the contaminated air.

By turning a 6-way valve, the inner water was introduced to a stripping chamber where 1 ml of 1 : 10 phosphoric acid had been injected to extract ΣCO_2 in water. Dissolved gases were quickly extracted from the solution by the carrier gas in a stream of fine bubbles at a flow rate of 50 ml l⁻¹. The sample volume of 2.5 ml in the present work was optimal for both gas-stripping efficiency and analytical sensitivity. An injection of the entire volume rather than an aliquot of a collected sample permits quantitative analysis even when some bubbles may be degassed from an aqueous solution in a sample container. The gas mixture in the carrier gas stream passes first through the drying column to eliminate water vapor, then into separation columns.

The degassed water was drained out by turning an 8-way valve by which the flow direction in the chamber was reversed. During this time the carrier is short-circuited, passing through a needle valve installed on the 8-way valve to balance the flow rate.

Gaseous samples: Although it is convenient to use syringes for the injection of gaseous samples, small quantities of air entering the bore of the syringe needles during sample transfer could cause considerable error, especially during a close analysis of major air constituents such as N₂ or O₂. Therefore, gaseous samples were introduced via a gas injection port which was devised to minimize such air contamination. This injection port is made of a 6-way valve and a 2×1 mm stainless tube equipped with a silicone septum at the end and having a small gas sampling loop (0.2613±0.005 ml in volume).

Gaseous samples were collected into evacuated vials. The vials were prepared a few days before sampling using a sealing-chamber (Tokyo Rikakikai Co., Ltd.), which created a vacuum without making a puncture. A test indicated that the decrease in vacuum was less than 1 % during a few days. Two ml of sample gas was injected into this port using a gas-tight syringe having a lock valve. During this procedure, the air contaminated by the needle is flushed out by a sample gas. The excess gas flows out from a vent placed just under the water surface (Fig. 1), and the gas sample collected in the loop is sealed against the atmosphere under ambient pressure. Turning the 6-way valve starts the analysis.

Calibration

The gas for calibration was prepared by mixing known volumes of desired

gases (CO₂, CH₄, H₂ and CO) with fresh air in a gas-tight glass bottle. The atmospheric concentrations of N₂, O₂ and Ar (78.084 %, 20.946 % and 0.934 % in volume, respectively) corrected for the above dilution were employed as their concentrations. Trace gases in air were negligible for the concentrations of mixed gas.

The calibration was performed every 7 or 8 samples by measuring the mixed gas in the same way as the gaseous samples described above.

RESULTS AND DISCUSSION

Analytical examinations

Typical chromatograms of both detectors are shown in Figure 2. Quick extraction of dissolved gases by the carrier gas stream produced peaks sharp enough for their precise determination. The gas mixture was sequentially separated by the three columns into a single gas species and detected by two detectors. First, CO₂ was separated from the mixed components by column 1 and detected by GC 2. The remaining gas mixture was separated into H₂, Ar+O₂, N₂, CH₄ and CO by column 2 and passed through GC 1. Finally, Ar+O₂ was separated into Ar and O₂ by column 3 for detection by GC 2. The CO₂ peak was negative, because the opposite cell of GC 2 was used for the CO₂ detection (it can be calculated as a positive peak when another integrator is connected to the GC with the polarity reversed).

Fig. 2. The chromatograms on GC 1 and GC 2. The retention times are denoted in the figure. CO is omitted because of its much later appearance. The amounts of measured gases were 6835 n mole for N₂, 1835 n mole for O₂, 669 n mole for CH₄, 401 n mole for CO₂, 267 n mole for H₂ and 81.7 n mole for Ar.

Typical retention times were 1.6, 2.2, 8.9, 10.8, 13.1, 13.6 and 46.9 min for H_2 , CO_2 , N_2 , Ar, CH_4 , O_2 and CO, respectively. The operation was repeated every 16 min for natural samples, because practically no CO is present in them.

The analytical sensitivity in the present system was maximized by raising the detector current and by constructing the analytical line free of leaks, which improved the baseline qualities. In addition, it was desirable for increased precision to keep the room temperature and column temperature as stable as possible. (For this reason, the columns were kept in water in Dewar bottles).

The estimated detection limits are 10, 1, 1, 5, 10, 200 and 100 n mole for N_2 , O_2 , Ar, CO_2 , CH_4 , H_2 and CO, respectively. GAMO and HORIBE (1980) used an ultrasonic detector for the detection of Ar, O_2 and N_2 because of its high sensitivity. The sensitivity of the present system by thermal conductivity detector was high enough to determine Ar concentrations in water with satisfactory precision, as shown subsequently by the analysis of natural samples.

A thermal conductivity detector has the advantage of providing a linear response. The relationship between peak area and the amount of gas is shown in Figure 3. CH_4 was used as a standard gas because of its low background level. The data indicates that the outputs are quite proportional, up to two orders of magnitude at least. Such linearity also held good for other gas species. The sensitivity of an ultrasonic detector is not proportional to the amount of gas. Excellent linearity as shown in the figure is of great merit for precise determination; especially for gases having wide concentration ranges, such as CH_4 .

The present gas chromatographic system is insensitive to H_2 and CO because the thermal conductivity is similar between H_2 and the carrier gas of He, and CO has a long retention time. Though they were under the detection limits in all of the natural samples we measured, the measurement of H_2 and CO by the present system is of practical use for experimental studies in which the culture conditions are artificially controlled.

The overall accuracy of the determination for dissolved gases is examined in Table 2. In this test, pure water equilibrated with air at a known temperature and pressure was poured into the sample vessel and analyzed for dissolved concentrations of N_2 , O_2 and Ar in the same way as the samples. As shown in the Table, the determined concentrations agreed closely with their theoretical values calculated from solubility (WEISS, 1970) and the partial pressures in the atmosphere. The results show that dissolved gas constituents were measured with satisfactory accuracy and precision by the present procedure.

Gas composition differs greatly with environments: under anaerobic conditions, oxygen is depleted, and CH_4 could be present as a dominant gas species. A test was thus carried out to make sure that coexisting gases had no effect on the analytical results. The ratio of N_2 to Ar was measured in the presence of different concentrations of CH_4 . The result shown in Figure 4

Fig. 3. Output versus the amount of gas. Varying amounts of CH₄ diluted with air were used. The lower figure is shown in log scale.

Table 2. The determination of N₂, O₂ and Ar concentrations in air-equilibrated water by the present analytical system and their theoretical values. Pure water equilibrated with air at 25.0 °C and under the atmospheric pressure of 99.5 k Pa was analyzed.

	Theoretical	Determined (n=3)
N ₂	10.71	10.67 ± 0.01
O ₂	5.58	5.39 ± 0.09
Ar	0.276	0.282 ± 0.011

unit : ml STP·l⁻¹

confirmed that the N₂/Ar ratio was invariable irrespective of concentrations of coexisting CH₄, suggesting no mutual interference in the present gas analysis.

Fig. 4. The effect of varying amounts of CH₄ on the N₂/Ar ratio.

Example 1 : Groundwater samples

The present gas analytical method was applied to samples collected from natural environments. The results for groundwater are shown in Table 3. Samples were collected from a spring at the bottom of a river terrace. A large part of the catchment area is occupied by upland fields. The results of bubbles concurrently emitted from the spring and the atmosphere at the site, which were sampled into vials, are also shown in this table.

Table 3. The gas composition of the waters of a spring, concurrent bubbles and the atmosphere *in situ*.

	N ₂	O ₂	Ar	CO ₂	Σ	N ₂ /Ar	R. N ₂ /Ar [#]
	ml STP l ⁻¹	ml STP l ⁻¹	ml STP l ⁻¹	ml STP l ⁻¹	ml STP l ⁻¹		%
Spring water (n=4)	13.8 ±0.1	5.0 ±0.1	0.338 ±0.005	24.6 ±0.7	43.7 ±0.7	40.7 ±0.8	107.3
	10 ³ ppmv	10 ³ ppmv	10 ³ ppmv	10 ³ ppmv	10 ³ ppmv		%
Bubbles (n=3)	819.1 ±1.1	153.6 ±0.1	9.53 ±0.07	15.2 ±0.2	997.4 ±1.1	85.9 ±0.6	104.1
Atmosphere (n=2)	779.3 ±0.6	207.8 ±0.4	9.45 ±0.01	—	996.5 ±0.1	82.5 ±0.0	(100.0)

R. N₂/Ar means the relative ratio of observed N₂/Ar to the theoretical values.

The gas species detected in the groundwater samples were N₂, O₂, CO₂ and Ar. The measured gas compositions accounted for 99.74 and 99.65 % of the contents in the bubbles and in the atmosphere, respectively, implying that the bubbles consisted mostly of these gas species.

The ratio of N₂ to Ar was 40.7 for the spring water. This was 7.3 % higher than the equilibrium value calculated from the temperature of spring water, as shown in the last column of the table. No temperature difference at equilibrium could explain such an anomaly in the N₂/Ar ratio. The N₂

content ($819.1 \pm 1.1 \cdot 10^3$ ppmv) and the N₂/Ar ratio (85.9 ± 0.6) of bubbles were also substantially higher than those in the atmosphere, with an N₂ enrichment of 4.1 %. Even though spring water and bubbles are well oxygenated, the present gas analysis applied to a groundwater ecosystem provides evidence of a process causing an N₂ excess, probably due to denitrification.

Example 2 : Wetland samples

Table 4 shows another example of the determination: gases trapped in peat ('peat bubbles') collected from the Kushiro Wetlands, Japan (43°10' N, 144°40' E). The analysis demonstrated that the peat bubbles consisted almost entirely of detected species, N₂, O₂, Ar, CO₂ and CH₄, since these components represented 98.9 ± 1.0 % of the total volume. The remainder was attributable to water vapor in the vials. Dominant components were N₂, CH₄ and CO₂, though their proportions varied somewhat among the sites from which trapped gases were collected. Ar and O₂ were only in trace amounts, and no H₂ was detected by the present method. The fact that the O₂ content was quite low, usually less than the Ar content, indicates that the sample vials were well protected from atmospheric contamination not only during sampling but during the period from vial evacuation to analysis.

Table 4. The gas composition in peat bubbles trapped at several sites in the Kushiro Wetlands and that in the atmosphere *in situ*.

	N ₂	O ₂	Ar	CO ₂	CH ₄	Σ	N ₂ /Ar	R. N ₂ /Ar
Site 1	663.5	3.49	8.42	22.7	288.7	986.9	78.8	0.940
Site 2	743.5	4.96	9.08	24.2	203.9	985.6	81.9	0.977
Site 3	783.5	7.96	9.41	17.4	169.3	987.3	83.3	0.994
Site 4	672.9	5.62	8.56	30.8	264.0	981.9	78.6	0.938
Site 5	597.4	3.90	8.17	48.7	336.4	994.6	73.1	0.872
Atmosphere	771.4	206.6	9.21	—	—	987.2	83.8	(1.000)

Units are 10³ ppmv (except for N₂/Ar). R. N₂/Ar means the relative ratio of the N₂/Ar observed to that in the atmosphere. CO₂ and CH₄ in the atmosphere are under the detection limits.

The N₂/Ar ratios in the peat bubbles, ranging from 73.1 to 83.3, were found to be significantly lower than the atmospheric ratio of 83.6. Since the air collected into vials at wetland site had an N₂/Ar ratio of 83.8, the observed decrease in the N₂/Ar ratio did not arise from the sampling or analytical procedures. Apparently, there is no physical mechanism which could discriminate N₂ from Ar. The contribution from radiogenic Ar from the earth's crust is also negligible for the gaseous composition of bubbles. Thus, it is highly likely that the decreased N₂/Ar ratios in bubbles were due to N₂ removal by biological nitrogen fixation. The presence of nitrogen fixation activity is ubiquitously shown in wetland ecosystems by the acety-

lene reduction technique (WAUGHMAN and BELLAMY, 1980). Our results provide a novel piece of evidence for this process taking place in the Kushiro Wetlands from a gas compositional aspect.

CONCLUSIONS

The present gas analytical system provides a full and accurate composition of natural major gases, and will be of versatile use in clarifying the entire gas metabolism in a variety of environments. Atmospheric contamination during the analytical procedures was insignificant allowing inert Ar to be a natural tracer of practical availability. The capability to detect both N₂ excess (denitrification) and N₂ deficiency (nitrogen fixation) as suggested here seems especially valuable. The procedure described has been used extensively in our laboratory for studies on both processes including experimental approaches. More details on denitrification in groundwater and nitrogen fixation in wetlands will be given in subsequent papers.

ACKNOWLEDGEMENTS

The authors wish to thank Drs. K. Ae, T. OTANI and I. NOUCHI for providing facilities for the present work, Drs. K. MINAMI, H. TSURUTA and K. YAGI for their helpful discussion, and M. KOHMARU (the Environment Agency), Prof. M. SAKAMOTO and Prof. H. TERAII for making the wetland research feasible. This work was financially supported by a research fellowship from the Science and Technology Agency, Japan.

REFERENCES

- BLACKMER, A. M. and J. M. BREMNER (1977) : Gas chromatographic analysis of soil atmosphere. *Soil Sci. Soc. Am. J.*, 41 : 908-912.
- GAMO, T. and Y. HORIBE (1980) : Precise determination of dissolved gases in sea water by shipboard gas chromatography. *Bull. Chem. Soc. Jpn.*, 53 : 2839-2842.
- KYRYACOS, G. and C. E. BOORD (1957) : Separation of hydrogen, oxygen, nitrogen, methane, and carbon monoxide by gas absorption chromatography. *Anal. Chem.*, 29 : 787-788.
- LARD, E. W. and R. C. HORN (1960) : Separation and determination of argon, oxygen, and nitrogen by gas chromatography. *Anal. Chem.*, 32 : 878-879.
- OBERMILLER, E. L. and G. O. CHARLIER (1968) : Gas chromatographic separation of nitrogen, oxygen, argon, carbon monoxide, carbon dioxide, hydrogen sulfide and sulfur dioxide. *J. Gas Chromatogr.*, 6 : 446-447.
- SMITH, K. A. and J. R. M. ARAH (1991) : Gas chromatographic analysis of the soil atmosphere. In *Soil Analysis - Modern Instrumental Techniques, Second Edition*, K. A. SMITH (ed.) : 505-546. Marcel Dekker, Inc., New York·Basel·Hong Kong.
- SWINNERTON, J. W., V. J. LINNENBOM and C. H. CHEEK (1962) : Determination of dissolved gases in aqueous solutions by gas chromatography. *Anal. Chem.*,

34 : 483-485.

TOLK, A., W. A. LINGERAK, A. KOUT and D. BORGER (1969) : Determination of traces of hydrogen, nitrogen and oxygen in aqueous solutions by gas chromatography. *Anal. Chim. Acta*, 45 : 137-142.

WAUGHMAN, G. J. and D. J. BELLAMY (1980) : Nitrogen fixation and the nitrogen balance in peatland ecosystems. *Ecology*, 61 : 1185-1198.

WEISS, R. F. (1970) : The solubility of nitrogen, oxygen and argon in water and seawater. *Deep-Sea Res.*, 17 : 721-735.

Muneoki YOH : Faculty of Agriculture, Tokyo University of Agriculture and Technology, Hachioji 192-0355 (楊 宗興 : 〒192-0355 八王子市堀ノ内 1528, 東京農工大学農学部波丘地実験施設)

Makoto TAKEUCHI : National Institute of Agro-Environmental Sciences, Tsukuba 305-0856 (竹内 誠 : 〒305-0856 つくば市観音台 3-1-1, 農業環境技術研究所)

Hideshige TODA : Faculty of Science, Shinshu University, Matsumoto 390-0802 (戸田任重 : 〒390-0802 長野県松本市旭 3-1-1, 信州大学理学部)

(Received 11 September 1997 ; Accepted : 20 January 1998)

水および気体試料中の N₂, O₂, Ar, CO₂, CH₄, CO, H₂ の 同時分析：環境中の N₂/Ar 比変動の検出

楊 宗興・竹内 誠・戸田任重

摘 要

環境中の主要気体を精密に測定する分析システムを製作した。このシステムは、3つの分離カラムと2つの TCD (熱伝導度検出器) ガスクロマトグラフを直列に連結したもので、水および気体試料中に存在する N₂, O₂, Ar, CO₂, CH₄, CO および H₂ を同時測定できる。分析ラインには、シリンジ採取時の混入空気を除去して気体サンプルを正確に測定する特別な注入口を取り付けてある。大気と平衡にさせた水を用いたテストの結果から、本システムで測定される溶存ガス濃度が溶解度と大気中分圧から計算される理論値と一致することを確認した。環境中のガス組成が本法の検出気体でほぼ完全に説明されること、測定の結果えられる N₂/Ar 比が、自然界で行われる N₂ の生成(脱窒)あるいは消費(窒素固定)を評価する有効なパラメーターとなることを、地下水および湿原試料の測定データにより例示する。