Simultaneous Determination of Nitrite and Nitrate Ions by Air-Segmented Amplitude-Modulated Multiplexed Flow Analysis

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The concept of amplitude-modulated multiplexed flow analysis has been extended to the simultaneous determination of multiple analytes in a sample. A sample solution containing nitrite and nitrate ions is delivered from two channels, but the flow rates are varied at different frequencies. One of the channels has a reduction column for converting nitrate ions to nitrite ions. Downstream, the absorbance of the diazo-coupling product is monitored after the merging of both solutions with a Griess reagent. The signal is analyzed by a fast Fourier transform (FFT) in real time. From the thus-obtained amplitude, a $\mu\text{mol dm}^{-3}$ level of the ions can be determined. The introduction of air bubbles is effective to reduce any axial dispersion, and hence to improve the sensitivity.

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Introduction

Amplitude-modulated multiplexed flow analysis (AMMFA) is a new concept of continuous flow analysis.¹ The flow rates of sample solutions to be merged are periodically varied at different frequencies. Downstream, the analytical signal, V_d , is monitored and analyzed by a fast Fourier transform, FFT. Multiple samples can be analyzed simultaneously from the amplitudes of the wave components of interest. We have applied AMMFA to the determinations of food dyes,¹ ferrous ion,¹ chloride ion² and nitrite ion.³ The segmentation of a liquid stream with air bubbles is effective to increase the sensitivity, because the bubbles act as barriers against the axial dispersion of analytes.³ The applications of AMMFA remained, however, a single analyte per sample. The purpose of the present study is, therefore, to extend the concept of AMMFA to the simultaneous determination of multiple analytes in a sample. We selected nitrite and nitrate ions as analytes, because their quantifications are of importance in the field of environmental water analysis.⁴⁻⁶ A sample solution containing the ions is fed through two channels, one of which has an inline reduction process. The proposed AMMFA with air-segmentation has made it possible to determine the $\mu\text{mol dm}^{-3}$ level of the analytes from a single continuous analytical signal.

Experimental

Flow system

Figure 1 shows a schematic diagram of the flow system. Two channels (Channels 1 and 2) were used for delivering a sample solution, and one channel for a coloring reagent solution, unless otherwise stated. A Cd-Cu column (C; 2.2 mm i.d. $\times$ 15 cm

long, Ogawa & Co., Japan) was set in Channel 1 in order to reduce nitrate ions to nitrite ions. Three peristaltic pumps ($P_1 - P_3$; Rainin Dynamax RP-1, USA) were used for delivering solutions, and one pump (P_4 ; the same model as $P_1 - P_3$) for air. The pump tubes used were Pharmed® tubings (0.80 mm i.d. for P_3 and 0.51 mm i.d. for others). The flow rates with P_1 and P_2 were varied in the range from 0 to $0.4 \text{ cm}^3 \text{ min}^{-1}$ in response to sinusoidal control voltages (V_{c1} and V_{c2}), each having a different frequency (typically 0.03333 and 0.01666 Hz for P_1 and P_2 , respectively). The voltages were generated from a signal generator (SG; NF Corp. WF1974, Japan). The total flow rate was kept constant at $1.0 \text{ cm}^3 \text{ min}^{-1}$ with P_3 . Sample solutions were merged with a coloring reagent that was aspirated from the third channel. Air bubbles were introduced from the forth

Fig. 1 Schematic diagram of the flow system. A three-channel system was used for the simultaneous determination of nitrite and nitrate ions. S, Sample solution; R, reagent solution; C, Cd-Cu reduction column; w, waste; $P_1 - P_4$, peristaltic pumps; MC, mixing coil; T, thermostat bath; DT, deaeration tubing; D, spectrophotometer; SG, signal generator; PC, laptop computer with card type A/D-D/A converter; V_{c1} and V_{c2} , controller output voltages; V_d , detector output voltage.

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channel with P₄ at a flow rate of 0.2 cm³ min⁻¹ in order to segment the merged solution. Both fluids were propelled to a mixing coil (MC; 0.8 mm i.d. × 0.5 m) immersed in a thermostat bath (T; 50°C). The temperature of the bath was controlled with an AS ONE CA061 heater, a Shinko PT100 resistance thermometer and a Shikoku-Riken ACS-13A PID controller. After the removal of air bubbles using a porous PTFE tubing (DT; Sumitomo Electric Fine Polymer TB-0201), the deaerated solution was introduced into a hand-made flow cell (quartz tubing; 1 mm optical path length),⁷ which was placed in a spectrophotometer (D; Shimadzu SPD-6AV, Japan). The detector output voltage (V_d , 0.8 V/AU) as well as V_{c1} and V_{c2} were quantitized by an A/D-D/A converter (Measurement Computing, PC-CARD-DAS16/12-AO, USA) and acquired in a computer (PC; IBM ThinkPad R51e 1843-BLJ, USA) as a Microsoft Excel format. An in-house program written in Excel VBA was used for the acquisition, analysis and graphical display of the data. The previous program³ was improved so that it could remove noise from air bubbles that were accidentally introduced to the flow cell.

Determination of analyte

Nitrite ion was determined by a Griess method:⁸ a spectrophotometry at 538 nm based on the coupling of diazotized sulfanilamide and *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED).^{9,10} An aqueous solution containing 0.24 mmol dm⁻³ sulfanilamide, 4 mmol dm⁻³ NED and 0.6 mol dm⁻³ hydrochloric acid was used as a coloring reagent. Nitrate ion needs to be reduced to nitrite ion before a measurement by the spectrophotometry.

Reagents of analytical reagent grade purchased from Kanto Chemicals (Tokyo) or Nacalai Tesque (Kyoto) were used without further purification. Zartorius Arium 611DI grade deionized water was used throughout.

FFT analysis

The principle of FFT for AMMFA was described in detail elsewhere.¹ Briefly, if V_{c1} and V_{c2} for controlling sample flow rates are varied periodically, the analytical signal, V_d , shows a periodic profile, the period of which equals to the least common multiple of the V_{c1} and V_{c2} periods. Figure 2A shows an example of real data for an aqueous solution containing nitrite and nitrate ions. The V_d period (*i.e.*, 60 s) is used as the length of the window for FFT analysis. Eight data obtained during 60 s are used for FFT analysis, as expressed by

$$X_k = \frac{1}{N} \sum_{n=0}^{N-1} x_n \exp\left(-j \frac{2\pi nk}{N}\right) \quad N = 8, \quad (1)$$

where j is an imaginary number. The total concentration of nitrate and nitrite ions can be determined from the amplitude of the second-harmonic wave component, $A_2 (= 2|X_2|)$, because the flow rate of Channel 1, which has the Cd-Cu column, is controlled by V_{c1} with a 30-s period. The concentration of nitrite ion is determined from the amplitude of the fundamental wave component, $A_1 (= 2|X_1|)$, because the flow rate of Channel 2, which has no pretreatment column, is controlled by V_{c2} with a 60 s period. A temporal profile of the amplitudes are obtained in real time by moving the FFT window with time, as shown in Fig. 2B, where the obtained A_1 and A_2 values (mean $\pm$ s.d., $n = 60$) are 0.3162 ± 0.0078 and 0.5331 ± 0.0059 V, respectively.

Fig. 2 (A) Typical flow signals of amplitude-modulated multiplexed flow analysis. V_{c1} and V_{c2} , controller output voltages with periods of 30 and 60 s, respectively; V_d , detector output voltage; sample, aqueous solution containing 40 $\mu\text{mol dm}^{-3}$ nitrite ion and 80 $\mu\text{mol dm}^{-3}$ nitrate ion. (B) Temporal profile of FFT analysis for the data shown in Fig. 2A. DC, direct current component. A_1 – A_4 , amplitudes of fundamental, second-harmonic, third-harmonic and fourth-harmonic wave components, respectively. Amplitudes for the third- and fourth-harmonic waves components ($A_3 (= 2|X_3|)$ and $A_4 (= |X_4|)$, respectively) obtained should theoretically be zero because there are no control signals with a period of 20 or 15 s.

Results and Discussion

Optimization of analytical conditions for a coloring reaction

The mixing coil length (0–3 m) and the reaction temperature (30–60°C) were optimized. For this purpose, the Cd-Cu reduction column was removed from Channel 1. A sample solution containing nitrite ion, and the coloring reagent solution were delivered from Channels 1 and 2, respectively. The flow rate of the former channel was varied from 0 to 0.375 cm³ min⁻¹ with a period of 30 s; that of the latter was held constant at 0.25 cm³ min⁻¹. From the third line (R), water was aspirated instead of the coloring reagent. The coil length was optimized to be 0.5 m, because the highest amplitude was obtained under this condition. Shorter and longer coils were not appropriate because of insufficient coloration and amplitude damping through the dispersion, respectively. As for the coloration temperature, 50°C gave the highest amplitude; this temperature was selected as the optimum condition.

The performance of the Cd-Cu column for the reduction of nitrate ion to nitrite ion was evaluated. It was confirmed that almost a 100% reduction efficiency was obtained for 10–40 $\mu\text{mol dm}^{-3}$ nitrate ions for at least 6 h.

Analytical performance

Calibration curves were constructed from five standard solutions, each containing the same concentrations of nitrite and nitrate ions (0, 10, 20, 30, and 40 $\mu\text{mol dm}^{-3}$). The flow rates of P₁, P₂, P₃ and P₄ were 0–0.75 (period: 30 s), 0–0.75 (period: 20 s), 2.00 and 0.67 (or closed) cm³ min⁻¹, respectively. In this

Table 1 Analytical performance of air-segmented amplitude-modulated flow analysis

Mode	Analyte	Linear regression equation	r^2	LOD/ $\mu\text{mol dm}^{-3}$
Air-segmented AMMFA (proposed method)	$\text{NO}_2^- + \text{NO}_3^-$	$A_2 = 0.00391C_T + 0.0033$	0.9985	5.90
	NO_2^-	$A_3 = 0.00368C_{\text{NO}_2^-} - 0.0010$	0.9992	2.16
AMMFA (previous method)	$\text{NO}_2^- + \text{NO}_3^-$	$A_2 = 0.00322C_T + 0.0001$	0.9974	7.80
	NO_2^-	$A_3 = 0.00255C_{\text{NO}_2^-} - 0.0015$	0.9950	5.39

AMMFA, amplitude modulated multiplexed flow analysis; $C_{\text{NO}_2^-}$, concentration of nitrite ion in $\mu\text{mol dm}^{-3}$; C_T , total concentration of nitrite and nitrate ions in $\mu\text{mol dm}^{-3}$; A_2 and A_3 , amplitudes of the second- and third-harmonic wave components, respectively, in the analytical signal.

Table 2 Determination of nitrite and nitrate ions in river-water samples

Sample	Nitrite ion			Nitrate ion		
	$C_{\text{NO}_2^-, \text{add}} / \mu\text{mol dm}^{-3}$	$C_{\text{NO}_2^-, \text{find}} / \mu\text{mol dm}^{-3}$	Rec., %	$C_{\text{NO}_3^-, \text{add}} / \mu\text{mol dm}^{-3}$	$C_{\text{NO}_3^-, \text{find}} / \mu\text{mol dm}^{-3}$	Rec., %
Akui River	0	ND ^a	—	0	42.0	—
	30	30.3	97.5	0	41.3	—
	0	(2.7)	—	30	72.5	101.8
Yoshino River	30	30.7	98.8	30	70.9	96.4
	0	(2.5)	—	0	28.8	—
	30	32.5	100.0	0	28.5	—
	0	(2.3)	—	30	58.6	99.3
	30	32.4	99.5	30	57.3	95.1

Nitrite and nitrate ions were added as sodium potassium salts, respectively. The data in parentheses are below the limit of quantification.

a. The obtained value ($1.0 \mu\text{mol dm}^{-3}$) was below the LOD ($2.2 \mu\text{mol dm}^{-3}$).

case, the amplitude of the second-harmonic wave component corresponds, therefore, to the total concentration of nitrite and nitrate ions ($0 - 80 \mu\text{mol dm}^{-3}$) supplied from Channel 1, which has the reducing column. On the other hand, that of the third-harmonic wave component corresponds to the concentration of nitrite ions alone ($0 - 40 \mu\text{mol dm}^{-3}$) supplied from Channel 2, which has no reducing column. The results are summarized in Table 1 together with those obtained through the previous method (AMMFA without air-segmentation).^{1,2} The linearity of the obtained calibration curves was good ($r^2 > 0.997$). By introducing air-segmentation, sensitivity was increased by factors of 1.22 and 1.45 for the total and nitrite concentrations, respectively. The difference between the factors is attributed to that in the control period. That is, the effect of air-segmentation is more pronounced when the control period becomes shorter.³ The difference in the slopes for the total and nitrite concentration can also be attributed to that in the control period; amplitude damping is more significant when the control period becomes shorter.² Table 2 gives the results of recovery tests for river-water samples. The concentration of nitrate ions was estimated from the difference between the total concentration (Channel 1) and the nitrite concentration (Channel 2). Good recoveries of around 100% were obtained for analytes spiked in the samples.

A number of methods have still been reported on the simultaneous determination of nitrite and nitrate ions in the flow mode, even since an excellent review by Moorcroft *et al.*¹¹ These include, for example, flow injection analysis,^{12,13} sequential injection analysis^{14,15} and their analogous technique.¹⁶ Their basic configuration has two channels for a sample. That is, one has a reduction process and the other has no pretreatment process. Analytical signals for the total and nitrite concentrations are obtained sequentially, based on the difference in the

residence time between the channels, or by switching the channels. The present study, where the amplitudes of the components in a single continuous analytical signal were used for quantifications, suggests an alternative approach to the conventional methods mentioned above.

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