Advanced Flow Analysis Systems for Sensitive Automated Chemical Analysis

ALEJANDRO AYALA QUEZADA

The Graduate School of Engineering

Aichi Institute of Technology

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Contents

Chapter 1 Introduction

Flow analysis techniques have been gaining interest for their versatility and sensitivity in the analytical chemistry field. Since flow injection analysis (FIA) was introduced by Ruzicka and Hansen[1] in 1975, this flow technique has become the most widely employed. The typical system is shown in Fig. 1-1. The basic components of a FIA system include a peristaltic pump, an injection valve, a reaction coil and a detector. The pump is continuously propelling carrier and reagent solutions to establish a baseline. A constant volume of sample is inserted into the carrier stream through the injection valve to merge with the reagents in the reaction coil and to obtain the detectable form by the analytical method applied. Finally, the desired response or responses of the reaction product is measured by using a detector such as a spectrophotometer, a fluorescence spectrophotometer and potentiometer, etc. The major advantages of FIA over conventional batch wise methods are its simplicity, ease of implementation, sample and reagent economy and cost-effectiveness.

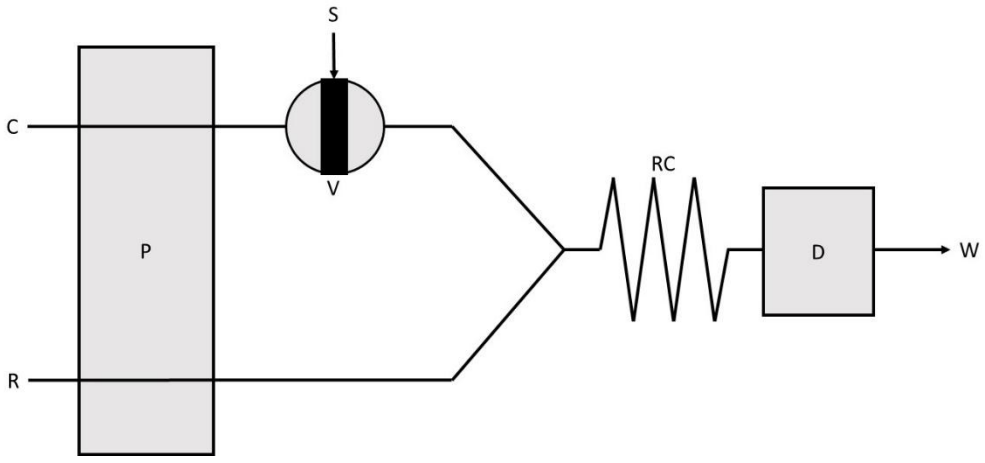

Fig. 1-1. The schematic diagram of a basic flow injection analysis system.

C, carrier; S, sample; R, reagent; P, pump; V, injection valve; RC, reaction coil; D, detector; W, waste.

Sequential injection analysis (SIA) was developed as an alternative to FIA by Ruzicka and Marshall[2] in 1990. Fig. 1-2 shows the basic configuration of a SIA system. The manifold of a typical SIA system is composed of a syringe pump, a holding coil connected to the central port of a multiport selection valve and a detector. The preset volumes of sample and reagents are aspirated sequentially into the holding coil, then the reaction products are dispensed through the detector. In SIA, the syringe pump only works during the time needed to aspirate the amount of sample and reagents required for a given determination. Therefore, the consumption of sample and reagents is dramatically reduced in comparison with the conventional FIA system. Furthermore, using a selection valve with an appropriate number of ports to hold different reagents, SIA can easily be turned into a multiparameter analysis system.

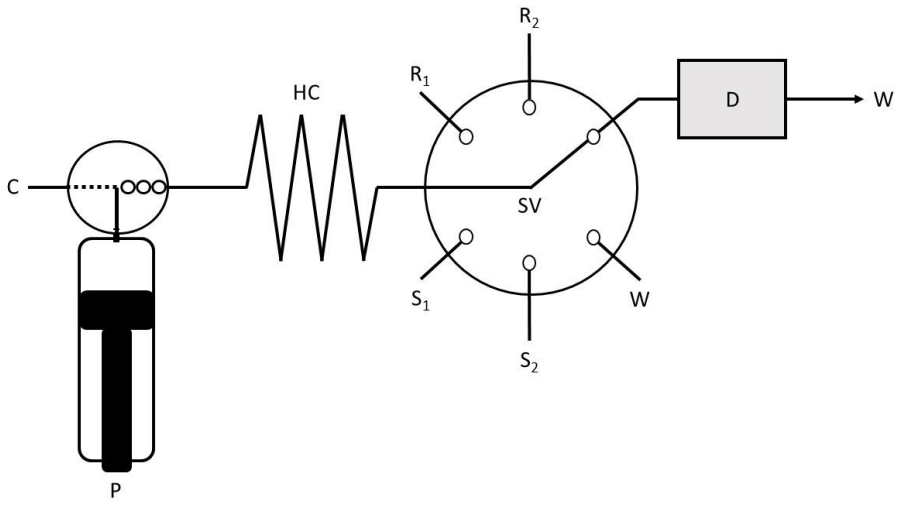

Fig. 1-2. The schematic diagram of a typical SIA system.

C, carrier; S_1 and S_2, samples; R_1 and R_2, reagents; P, pump; HC, holding coil; SV, six-port selection valve; D, detector; W, waste.

FIA and SIA have many merits as is mentioned above. However, they also present some disadvantages. For instance, FIA requires continuous aspiration of carrier and reagent solution in order to establish a baseline, leading to a large amount of waste. In the other hand, SIA aspirates just

the needed volumes of reagent and sample solutions into a holding coil, the mixing efficiency is lower than in FIA. New concepts of flow based techniques have been proposed to improve their function. In 2007, Teshima *et al.*[3] introduced a novel concept called stopped-in-loop flow analysis (SIL-FA). In SIL-FA, the sample and reagent solutions are well-mixed before being loaded into the loop. The mixed solution is separated by a six-way valve from the main flow stream line and all the pumps are stopped during the stationary step of the method for color development. After that, the colored product by the reaction is dispensed to the detector. Fig. 1-3 shows the schematic flow diagram of a SIL-FA system. The SIL-FA system does not require a baseline continuously leading to a reduction in reagent consumption and waste generation.

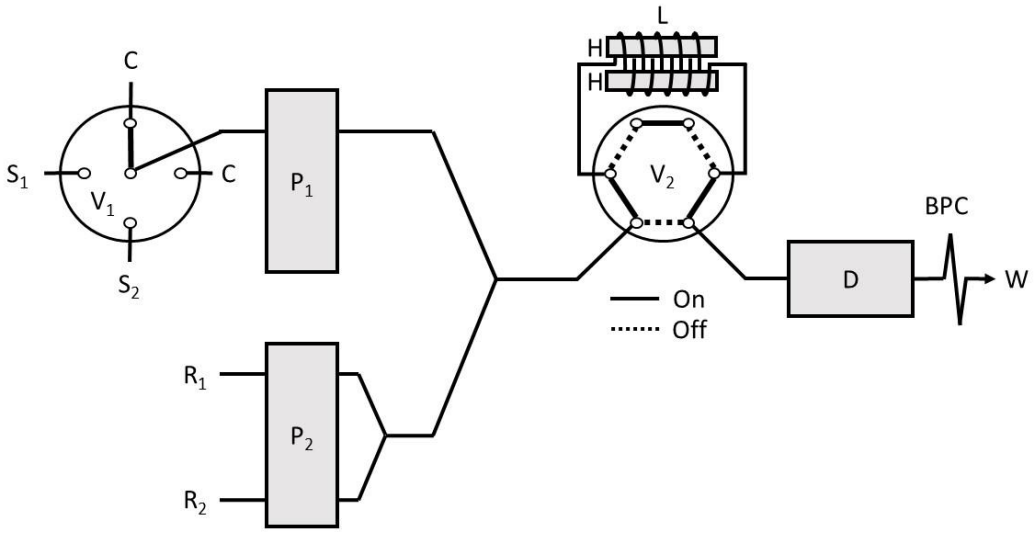

Fig. 1-3. The schematic diagram of a SIL-FA system.

C, carrier; S_1 and S_2, samples; R_1 and R_2, reagents; P_1 and P_2, pumps; V_1, four-ports selection valve; V_2, six-ports two-positions valve; L, loop; H, heated bars; D, detector; BPC, back pressure coil; W, waste.

For improving the mixing efficiency, the employment within micro-liter range of reagent volume and robustness of the system. The simultaneous injection effective mixing analysis (SIEMA) was introduced by Teshima *et al.*,[4] in 2010. The SIEMA system is based on the simultaneous aspiration of

small amounts of sample and reagent and effectively mixed at a confluence multiple port connector. The basic SIEMA system is shown in Fig. 1-4. It consists of a syringe pump, three three-way solenoid valves and a two-way solenoid valve all connected via PTFE tubing and two four-way cross connectors. Defined volumes of sample and reagent solutions are aspirated into separate holding coils through individual solenoid valves before dispensing simultaneously towards the detector. The SIEMA system performs a rapid analysis because all the solutions are simultaneously managed by the syringe pump achieving low reagent consumption as only micro-liters of reagents are employed.

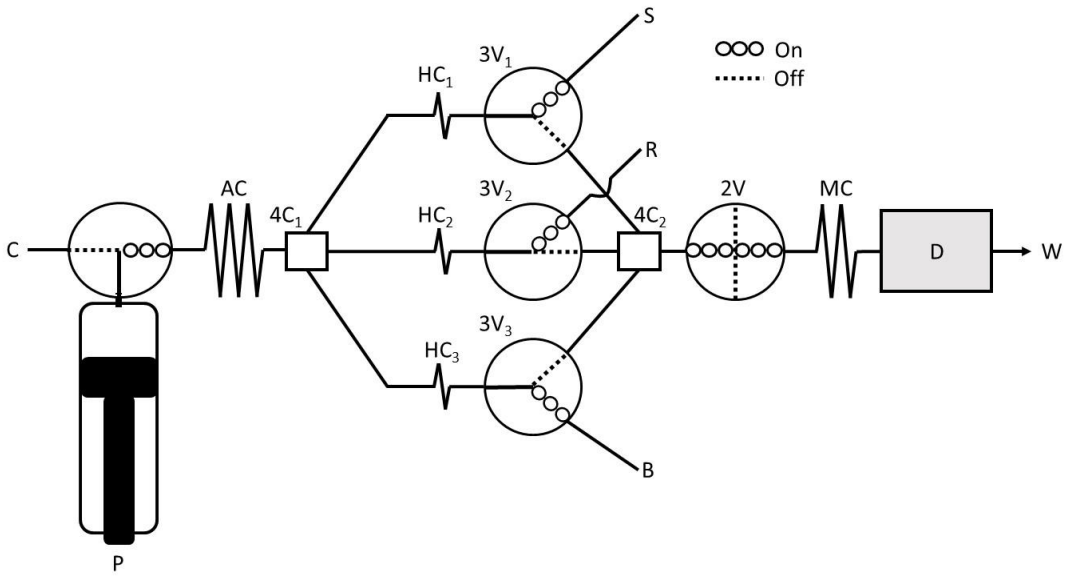

Fig. 1-4. The schematic diagram of a SIEMA system.

C, carrier; P, pump; AC, auxiliary coil; $4C_1$ and $4C_2$, four-way cross connectors; HC_1, HC_2 and HC_3, holding coils; $3V_1$, $3V_2$ and $3V_3$, three-way solenoid valves; S, sample; R, reagent; B, buffer; 2V, two-way solenoid valve; MC, mixing coil; D, detector; W, waste.

A combined use of flow techniques for the development of a specific application is plausible in order to maximize the advantages of each individual choice. One potentially effective combination can be that of SIL-FA and SIEMA. In fact, the latter allows a fast and precise management of the reagent and sample solutions (simultaneous aspiration in micro-liter range), meanwhile, SIL-FA can contribute improving the accuracy of the analysis by performing the stopped-flow technique.

Sensitivity can be improved by using pre-concentration with, for example, a column packed with an ion exchanger and/or chelating agent. This additionally allows the removal of potential interferences. Solid phase extraction (SPE) is widely used for pre-concentration techniques. Although, batch-wise SPE methods are complicated and time-consuming due to the need of operations such as conditioning, sampling, elution, and so on. FIA and SIA possess high capabilities on automated solution management systems such as chemical derivatization, dilution and on-line SPE. Several works have been developed for sensitive and selective analysis with on-line pretreatment flow-based systems coupled with spectrophotometry,[5-7] inductively coupled plasma atomic emission spectrometry (ICP-AES),[8-14] inductively coupled plasma mass spectrometry (ICP-MS),[15-19] flame atomic absorption spectrometry (FAAS),[20-26] and electrothermal atomic absorption spectrometry (ETAAS).[27-33]

In this dissertation, automated flow analysis methods for biological and environmental samples are proposed and their usefulness novel techniques are discussed. In the chapter 2, the SIA method for sulfate, nitrite and nitrate in water samples was investigated. The sulfate formed a precipitate after its reaction with barium chloride was observed. Nitrate was reduced to nitrite by the action of a cadmium column allowing its determination by difference of concentrations after comparison with the result of nitrite determination with a modified Griess-Illosvay reaction. These reactions were introduced into a SIA system and it was applied to the determination of sulfate, nitrite and nitrate in drinking water and wastewater samples. The proposed method is available to the automated monitoring of drinking water and wastewater. In the chapter 3, SIL-FA was proposed for vanadium and iron determination in water samples. The developed automated technique gave a high sensitivity for both analytes with low reagent consumption. In the chapter 4, the SIEMA concept was proposed for palladium determination

in alloys. It was found that 2-(5-bromo-2-pyridylazo)-5-[*N*-*n*-propyl-*N*-(3-sulfopropyl)amino]aniline (5-Br-PSAA) reacts with palladium to form a red colored complex. This chemistry was introduced into a proposed SIEMA system providing a highly efficiency mixing and low reagent consumption. The developed automated technique was applied to the determination of palladium in dental alloys and palladium carbon catalysts. In the chapter 5, an advanced SIL-FA system was developed for vanadium and iron in water samples. The same chemistry introduced to the SIL-FA system in chapter 4 was employed in this study. The proposed advanced SIL-FA provided a selective method for the determination of vanadium and iron employing specific required small amounts of reagents reducing the waste generation. The advanced SIL-FA was applied to the determination of vanadium and iron in drinking waters. In the chapter 6, the automated pre-concentration system combined with a graphite furnace atomic absorption spectrometer was developed for the determination of trace vanadium, cadmium and lead. The SPE procedure was automated by the sequential injection technique. Vanadium, cadmium and lead at ppt level could be determined using the proposed system and the sensitivity obtained was comparable to that by ICP-MS. The proposed system was applied to the determination of vanadium, cadmium and lead in human urine samples.

The technologies proposed here are novel and useful in the environmental, clinical and quality control fields.

Chapter 2 Multiparametric Automated System for Sulfate, Nitrite and Nitrate Monitoring in Drinking Water and Wastewater Based on Sequential Injection Analysis[34]

2.1 Introduction

The determination of sulfate, nitrite and nitrate is of general relevance because they are important indicators of water pollution. These species at high levels can cause adverse effects in aquatic environments such as acidification, indicated by the presence of sulfate, or eutrophication, caused by nitrogenous compounds including nitrite and nitrate of water bodies as result of their role in the biogeochemistry of those processes.[35,36] The presence of sulfate in drinking water does not pose immediate threat to human health. However, nitrate can be reduced to nitrite, which in turn can react with secondary or tertiary amines present in the body resulting in the formation of nitrosamine.

They are known to be carcinogenic and at high concentrations in blood can react with iron(II) of the hemoglobin forming methemoglobin, which has no ability for carrying oxygen. This condition is known as methemoglobinemia.[35,37] Thus, the monitoring of these parameters in drinking water is significant, since high concentrations of them can lead to a potential risk to human health.[38]

Hence, World Health Organization (WHO)[39] and the United States Environmental Protection Agency (EPA)[40] have established the maximum concentration of sulfate, nitrite and nitrate in drinking water as 500, 3 and 50 mg L^{-1} (WHO), and 250, 1 and 10 mg L^{-1} (EPA), respectively.

The most common method for determination of nitrite in water is the colorimetric method. Meanwhile, for nitrate methods such as the cadmium reduction method, selective UV spectrometry and ion-selective electrode method can be used. Furthermore, for sulfate the turbidimetric method, the method gravimetric, and ion-selective electrode can be applied. Moreover, ionic chromatography can be used for determination of three parameters.[41] Those techniques mentioned previously have been established as standard methods.[41]

Flow analysis represents a good alternative over the traditional methods used in the environmental field due to its characteristics, such as low volumes of sample and reagents consumption, high sample throughput and mainly the possibility to develop automated monitoring systems adapting different techniques and instruments.

Several methodologies have been adapted to flow analysis for nitrite and nitrate determination, but the most used has been the colorimetric method in combination with an on-line reduction of nitrate to nitrite, measuring each one or both anions simultaneously;[36,38,42-52] meanwhile, for sulfate determination the turbidimetric method has been employed.[53-57] Also, other flow analysis methods have been developed adapting different techniques to determine nitrite and nitrate such as detection by sensors,[58,59] catalytic methods,[60] techniques based on the oxidation capacity of different compounds,[37] chemiluminiscent methods,[61,62] an indirect determination through of atomic absorption spectrometry[63] and gas phase molecular absorption.[64] For sulfate, different arrangements have been proposed like the use of a sensor,[35] a reactor column[65] and an indirect determination by atomic fluorescence spectrometry.[66]

The sequential injection analysis (SIA) allows the integration of different techniques in a single manifold for monitoring of multiple environmental parameters with a low consumption of sample and reagents and a minimal waste generation. Also, due to its control through software, the potential application of this technique in situ is highly possible.

However, the well-known limitations of the conventional methods and the drawbacks of some flow analysis systems, have been limited its application to determine nitrite, nitrate and sulfate in drinking water and wastewater with the same system; mainly, due to the difficulty to keep the manifold free of precipitate after the sulfate determination.

In this work, the coating of $BaSO_4$ on tubing walls was avoided with the use of a colloidal emulsifier such as gum arabic (gum acacia), which is added during sulfate analysis. A cleaning cycle was programmed after this analytical determination.

Thus, the main objective of this work is the development of a multiparametric system based on sequential injection analysis (SIA) for sulfate, nitrite and nitrate monitoring in drinking water and wastewater in a single procedure. Turbidimetric method for sulfate determination and direct nitrite determination using the Griess-Ilosvay reaction (Shinn method) in combination with on-line cadmium reduction column for nitrate following by nitrite determination, were used in the proposed system.

2.2 Experimental

2.2.1. Chemicals and reagents

All reagents employed were of analytical grade and deionized water was used throughout. Stock standard solutions of 1000 mg NO_2^--N L^{-1}, 1000 mg NO_3^--N L^{-1} and 1000 mg SO_4^{2-} L^{-1} were prepared by dissolving sodium nitrite, sodium nitrate and sodium sulfate in water, respectively. Working standard solutions were obtained by dilution.

Chromogenic Shinn reagent: 39.6 g of sulphanilamide, 0.99 g of *N*-(1-naphthyl) ethylene diamine and 99 mL of concentrated HCl (37%) were dissolved in 1000 mL of water.

Buffer solution A: 80 g L^{-1} NH_4Cl, 15 g L^{-1} $Na_2B_4O_7 \cdot 10\ H_2O$ and 0.5 g L^{-1} EDTA (disodium salt).

Regeneration solution: 38 g L^{-1} EDTA (disodium salt) and 12.5 g L^{-1} $CuSO_4$, adjusted at 7 the pH by adding NaOH and were dissolved in 1000 mL of water.

Buffer solution B: 30 g L^{-1} $MgCl_2 \cdot 6H_2O$, 5 g L^{-1} $CH_3COONa \cdot 3H_2O$, 1 g L^{-1} KNO_3, 20mL CH_3COOH (99%) and 5% (m/v) gum arabic were dissolved in 1000 mL of water.

Cleaning solution: 0.3% (m/v) EDTA (disodium salt) in 0.2 mol L^{-1} NaOH.

Precipitating agent: 4% (m/v) $BaCl_2$ solution.

2.2.2. Apparatus and software

A schematic diagram of the SIA system used in this work is shown in Fig. 2-1. The system included a piston pump type, a selection valve and a spectrophotometer, all of them connected to a personal computer. The piston pump type consisted on an autoburette (BU4S; Crison Instruments, Barcelona, Spain) equipped with a 10 mL syringe (Hamilton, Switzerland) which is used as liquid driver.

Spectrophotometric measurements were made using an Ocean Optics USB2000 UV‑VIS (Florida, USA) detector equipped with a flow-through cell (18 mL inner volume, 1 cm optical path). Absorbance was measured at 540 nm. An eight-port selection valve was used as a liquid distributor (VA 1+1, Crison Instruments, Barcelona, Spain). The central port of the selection valve is connected on one side to the syringe via a holding coil, and on the other to the peripheral ports of the unit (1‑8), for sequential aspiration of the various constituents for reduction/reaction/precipitation processes of the analytical protocol.

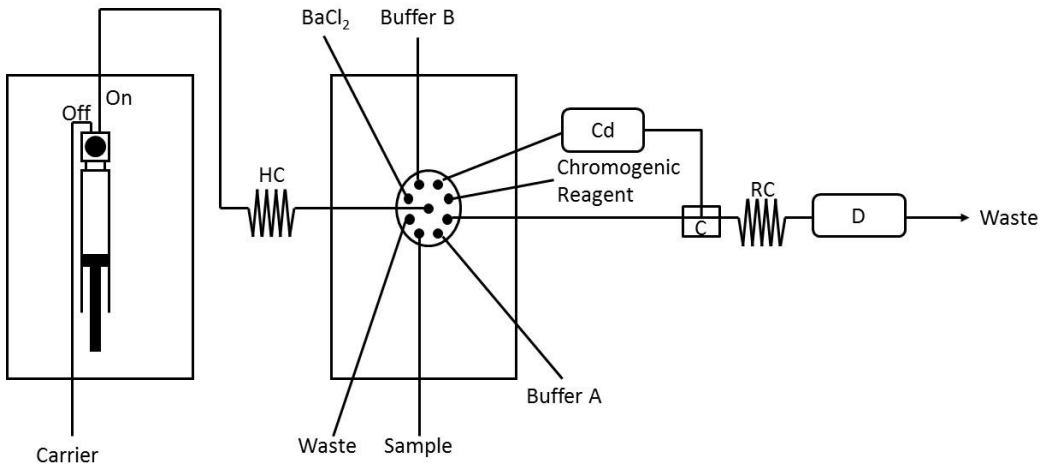

Fig. 2-1. SIA monitoring system proposed for SO_4^{2-}, NO_2^- and NO_3^-.

HC: Holding coil; RC: Reaction coil; C: Three-way connector; Cd: Cadmium column; D: UV-VIS Detector; Buffer A: Ammonium Chloride-EDTA solution; Buffer B: Magnesium Chloride-Acetic Acid- Arabic Gum solution.

The manifold is constructed with 0.8 mm i.d. poly(tetrafluoroethylene) (PTFE) tubing, except for the loading coil in which 1.5 mm i.d. tubes were employed. The reduction column was inserted in the detector line between the eight-port selection valve and reaction coil, using a three-way connector.

Instrument control is performed using the software package Auto-Analysis 5.0 (Sciware, Palma de Mallorca, Spain). The distinctive feature of developed software based on dynamic link libraries (DLLs) at 32 bits is the possibility of using a single and versatile application without further modification for whatever instrumentation and detection system needed. It involves a basic protocol which allows the implementation of specific and individual DLLs, addressing the configuration of the assembled flow analyzer.

2.2.3. Preparation of the reduction column

The column was prepared using cadmium granules as described by Cerdà *et al.*[44] The activation of the reduction column was as follows: the granules were first washed with 0.1 mol L^{-1} HCl and subsequently coated with copper by dipping them into a 1% CuSO4 solution for ca. 1 min. The copperized cadmium granules were washed with deionized water, and packed into a methacrylate tube (55 mm length and 4 mm i.d.), plugging it with glass wool.

2.2.4. Analytical procedure

To carry out the proposed SIA system, one syringe with its corresponding solenoid commutation valve was employed. The position “off” (solenoid disabled) of the valve connects syringe to the right channel and “on” (solenoid enabled) to the left one. The position “on” of the valve connects to the system through the central port of the selection valve, and the position “off” to the carrier bottle.

The management of the sample and reagents through the selection valve were as follows: Port 2 is used for sample aspiration, port 4 for the chromogenic solution, buffer solution A and buffer solution

B flow through port 5 and port 7, respectively, and the $BaCl_2$ solution is aspirated through port 8. The port 1 was employed as a waste and for loading cleaning solution. The reduction column was connected to the port 6.

Table 2-1. SIA program used for sulfate, nitrite and nitrate determination.

Analyte	Step	Vol. (mL)	Flow rate (mL min^{-1})	Operation	Solenoid valve position	Selection valve position	Description
SO_4^{2-}	1	1.7	1	Pick up	On	2, 7, 8	Reagents and sample loading using sandwich technique
	2	10	10	Dispense	On	3	Reagents and sample injection to the detector
	3	2	5	Pick up	On	1	Washing solution loading
	4	3	4	Dispense	On	3	Precipitate removal from the system
NO_2^-	5	2.5	1.8	Pick up	On	2, 4	Shinn reagent and sample loading using sandwich technique
	6	10	15	Dispense	On	3	Shinn reagent and sample injection to the detector
NO_3^-	7	2.58	1.8	Pick up	On	2, 4, 5	Reagents and sample loading using sandwich technique
	8	10	15	Dispense	On	6	Reagents and sample injection through the column to the detector

Table 2-1 depicts a general scheme of the method with the corresponding flow rates and volumes used. The steps of the process are the following: the initial step was the filling of the reagents tubes with the corresponding solutions. Then, the instrumental blank was set and the measuring method was started. For NO_2^- determination, 2 ml of sample and 0.5 ml of chromogenic solution were loaded and sent to the reaction coil and then to the detector by the port 3. For NO_3^-, 2 ml of sample, 0.5 ml of chromogenic reagent and 0.08 ml of buffer solution A were loaded from their containers and then impelled through the reduction column located in the port 6; after the reduction of NO_3^- to NO_2^-, they pass through the reaction coil and the measurement was carried out by the detector. For SO_4^{2-}

determination, 1 ml of sample, 0.5 ml of $BaCl_2$ solution and 0.2 ml of buffer solution B were loaded through their respective ports and then sent to the reaction coil and after that to the detector. After each SO_4^{2-} determination, a washing process was performed to remove the precipitate added to the tubing. For this purpose, 2 ml of the cleaning solution were loaded through port 1 and then impelled by port 3 to the reaction coil and the detector, leaving the system ready for further analysis.

2.2.5 Optimization of experimental conditions

Previous assays were carried out in order to establish the best type of injection in order to improve the analytical signal. Besides, due to the maintenance of formed precipitate as homogeneous as possible is mandatory in a turbidimetric determination to improve the precision of the measurements, stabilizing solution concentrations were also tested.

The optimal value of each variable (sample and reagent volumes, reagents concentrations, flow rate and reaction coil length) was determined by performing a Box-Behnken response surface experimental design with the model 2k. The computer statistics package Minitab 15 (Minitab Inc.) was used to build a response surface experimental design, achieving a total of 53 experimental runs. Effects of individual factors and their second order interactions were thus investigated.

2.3. Results and discussion

2.3.1. System dimensioning

The measuring cycle was carried out with a sandwich technique. It consists on inserting sample plugs between two reagents zones, improving the mixture of them.[44] The selected sandwich arrangement (0.35 of reagent / 1.00 ml of sample / 0.35 ml of reagent for sulfate; 0.25 ml of reagent / 2.00 ml of sample / 0.25 ml of reagent for nitrite; 0.29 ml of reagent / 2.00 ml of sample / 0.29 ml of

reagent for nitrate) led to the highest readout per injected sample. The optimal values of each studied variable are shown in Table 2-2.

Table 2-2. Optimal operation conditions.

Variable	Optimal value
Loading coil	200 cm
Reaction coil	400 cm
Carrier	H_2O
SO_4^{2-}	
Sample volume	1 mL
$BaCl_2$ volume	0.5 mL
Buffer B volume	0.2 mL
$BaCl_2$ concentration	4 % (w/v)
Flow rate of sample and reagents	10 mL min^{-1}
NO_2^- and NO_3^-	
Sample volume	2 mL
Buffer A volume	0.08 mL
Chromogenic reagent volume	0.5 mL
Chromogenic reagent concentration	0.23 mol L^{-1}
Flow rate of sample and reagents	15 mL min^{-1}

The dimensions of the reduction column were 55 mm length and 4 mm i.d. They were chosen to allow a better packed of cadmium granules, avoiding bubbles formation, since they reduce the area for conversion of nitrate to nitrite. The use of a miniaturized reduction column diminishes consumption of cadmium in comparison with those employed in several works[38,45-47,49,50] and subsequently, decreases operation costs and waste generation.

In the turbidimetric determination, 5% (m/v) gum arabic was added to buffer solution B, as stabilizer of the suspension (protecting colloid).

2.3.2 Analytical figures of merit

Under optimized conditions, the analytical performance of the system was evaluated in terms of detection and quantification limits, sensibility, regression coefficient (R^2), linear range, repeatability, reproducibility and injection frequency. Table 2-3 summarizes the figures of merit presented by each parameter studied in the present work.

Table 2-3. Figures of merit for SIA system.

	Sulfate	Nitrate	Nitrite
Detection limit (mg L^{-1})	3	0.0207	0.0022
Quantification limit (mg L^{-1})	10	0.0692	0.0073
Sensibility (AU L mg^{-1})	0.0024	0.5531	2.0887
Regression coefficient (n = 7)	0.9952	0.9951	0.9994
Lineal range (mg L^{-1})	75-300	0.14-1.82	0.01-0.42
Repeatability (% RSD) (n = 10)	1.34	1.96	0.46
Reproducibility (% RSD) (n = 5)	2.13	4.76	3.39
Injection frequency (h^{-1})	12	12	15

Detection and quantification limits were calculated from the standard deviation of the blank (3σ and 10σ, respectively; n=10) divided by the slope of the calibration curve (Fig. 2-2). The detection limits of the proposed system were below the maximum contaminant levels regulated by United States Environmental Protection Agency (US EPA) of 10 mg L^{-1} for nitrate, 1 mg L^{-1} for nitrite, 250 mg L^{-1} for sulfate;[40] and the recommended values given by the World Health Organization (WHO) of 50 mg L^{-1} for nitrate, 3 mg L^{-1} for nitrite and 500 mg L^{-1} for sulfate.[39]

The detection limits of 0.18 mg N L^{-1}, 0.1 mg N L^{-1} and 1 mg SO_4^{2-} L^{-1}, established in the standard methods[41] were almost completely surpassed by the ones achieved in this work, with the exception of the sulfate detection limit, which was very similar.

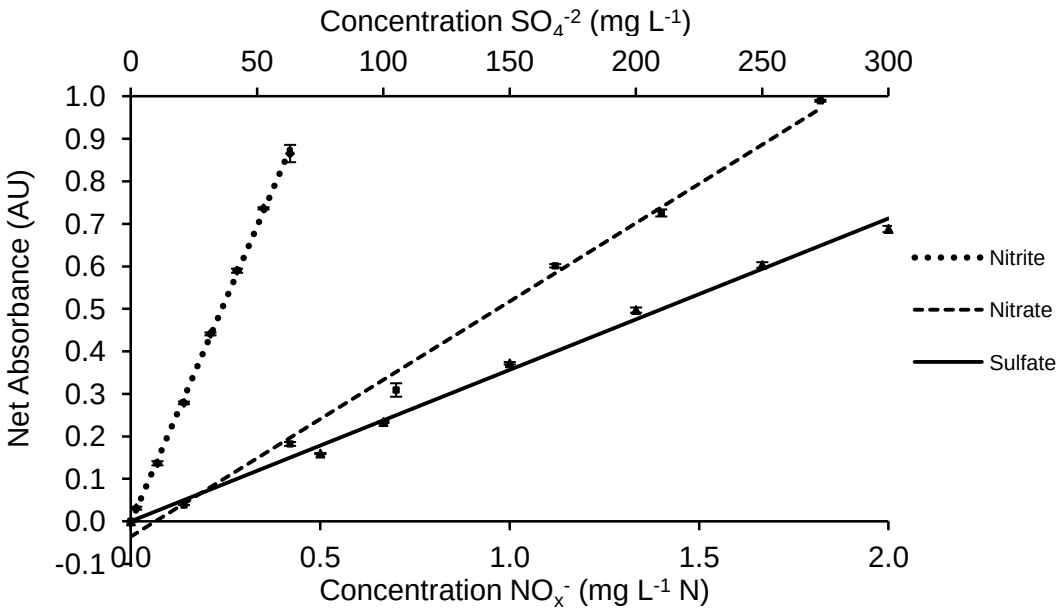

Fig. 2-2. Calibration graphs for sulfate, nitrite and nitrate.

The repeatability and reproducibility were expressed as relative standard deviation (RSD). The repeatability was evaluated from 10 successive injections of 1.4 mg L^{-1} N, 0.28 mg L^{-1} N and 200 mg L^{-1} SO_4^{-2} reference solutions for nitrate, nitrite and sulfate, respectively. The repeatability values accomplished of 1.34% for sulfate and 0.46% for nitrite , were lower than those acquired in several works which varies in a range of 2.40-3.81% and 0.50-2.80% for sulfate and nitrite, respectively.[36,38,42,44-50,54-57] Reproducibility was determined from results obtained on different working days (n=5), changing the column packing and using the same standard solutions mentioned above. As can be seen in Table 2-3, the results obtained with the proposed system have a good level of accuracy, being below the statistical reference of acceptance of 5%.

The injection frequencies were also calculated for the proposed system. Values of 12 and 15 injections h^{-1} were obtained for the system as a consequence of the two-step procedure (sampling into a holding coil and delivering towards the reaction coil) and the washing steps required in this flow methodology.

2.3.3 Efficiency of the reduction column

The effectiveness of the reduction column was tested running three calibration curves (Fig. 2-3), one for nitrate under reductive conditions and two for nitrite, one under non-reductive and the other under reductive conditions.

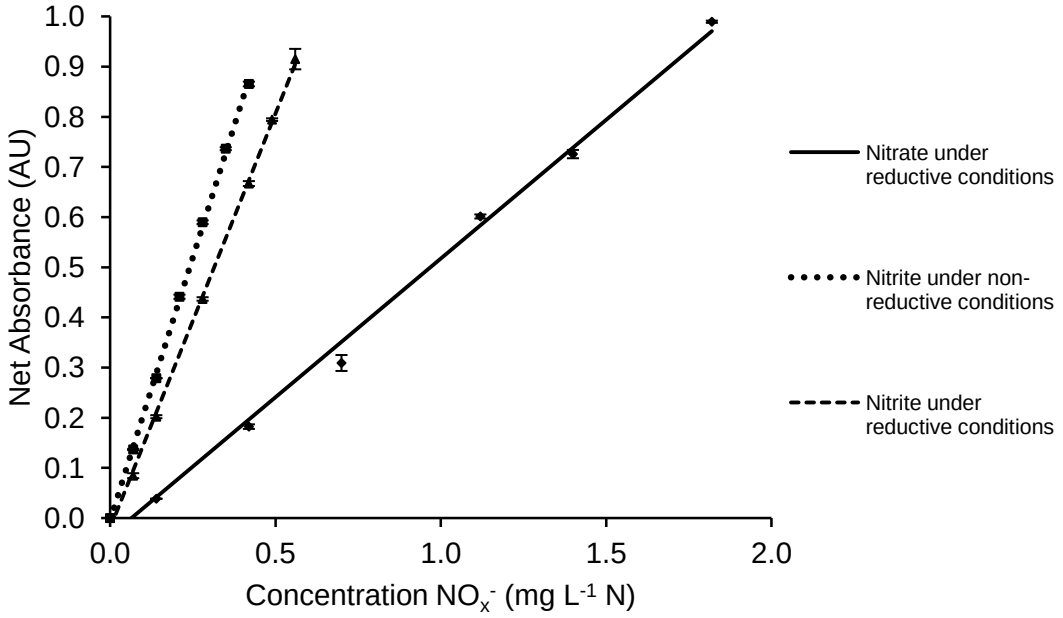

Fig. 2-3. Calibration graphs for nitrate under reductive conditions and nitrite under reductive and non-reductive conditions.

When the reduction activity of the column falls below 85%, a regeneration of the column was required. For this purpose, the on-line method proposed by Cerdà *et al.*[44] was modified, consisting on aspiration of 2 ml of regeneration solution through port 1 of the selection valve and subsequent dispensed through port 6. The regeneration solution passed through the column, providing new copper to regenerate the external coating of the cadmium granules and EDTA to avoid the precipitation of $Cd(OH)_2$ or other hydroxides on the reduction column. The column was finally flushed with water, leaving the system ready for further analysis.

Thus, the repeatability value accomplished of 1.96% for nitrate, was lower than those acquired in different works which varies in a range of 2.30-3.70%.[45,46,49,64]

The repetitive running of the regeneration solution not only decreased the sampling rate but also shortened the lifetime of the column due to the breakdown of the cadmium granules into smaller particles.

2.3.4 Interferences

The selectivity of the proposed technique has been evaluated by studying the effect of adding foreign ions in the determination of 1.12 mg N L^{-1} of nitrate and 200 mg SO_4^{2-} L^{-1} of sulfate. A given ion was considered to interfere when the signal variation is greater than 10%.

The effect of PO_4^{3-} on the reduction column for the analytical determination of nitrate was studied, since it was found as the most important interference by Cerdà *et al.*[44] (up to 1 mg PO_4^{3-} L^{-1}). In this work, the presence of PO_4^{3-} does not interfere due to higher flow rate used, causing a short contact time between the ion and the column, despite the high concentration of the phosphate ion. A concentration range from 2 up to 300 mg PO_4^{3-} L^{-1} was studied without signal variations greater than 10%. The interfering effect of three cations on the determination of sulfate was also studied: Ca^{2+}, Mg^{2+} and K^+, which are commonly found in water samples.[35] A concentration of 500 mg L^{-1} Ca^{2+} and 40 mg L^{-1} K^+ had an interfering effect in the precipitation of Ba_2SO_4. On the other hand, it was found higher tolerance level of Mg^{2+}, up to 800 mg L^{-1} Mg^{2+} did not interfere. The addition of EDTA keeps a constant low value of pH during the precipitation of Ba_2SO_4, decreasing interferences of Ca^{2+} and K^+. Also, Fung *et al.*[35] studied the effects of Ca^{2+} (50 and 100 mg L^{-1}), K^+ (5 and 10 mg L^{-1}) and Mg^{2+} (10 and 20 mg L^{-1}) in a solution of 20 mg SO_4^{2-} L^{-1} of sulfate, finding that Mg^{2+} and Ca^{2+} had an interfering effect at the concentrations used. While, the cation K^+ did not show an interfering effect at the chosen concentrations.

2.3.5 Validation of the methodology

The proposed technique was validated by the analysis of a certified river water sample (ION-96.3, LGC Standards). Three replicates of the certified sample were analyzed. The results obtained were compared with the given reference values. The *t*-test for comparison of means revealed there were no significant differences at the 95% confidence level between the certified value and the results obtained with the proposed method. As can be seen from Table 2-4, satisfactory recoveries were obtained with the proposed methodology.

Table 2-4. Analytical results and recovery tests obtained for the CRM River water ION-96.3.

Analyte	CRM River water ION-96.3	SIA	Recovery (%)
Sulfate (mg L^{-1})	110.00 ± 8.50	112.60 ± 1.31	102
Nitrite + Nitrate (mg N L^{-1})	4.30 ± 0.35	4.45 ± 0.04	103

The results are expressed as three replicates average ± standard deviation.

2.3.6 Application of the proposed method to water samples

In order to investigate the usefulness of the proposed method in monitoring sulfate, nitrite and nitrate, three types of water samples were analyzed: tap water, groundwater and wastewater (from inlet and outlet of a treatment plant). Before injecting the samples into the system, the wastewater samples were filtered. Tables 2-5, 2-6 and 2-7 show the comparison of the analytical results and recovery tests obtained with the proposed method and the conventional standard method.[41] The analytical results of the samples analyzed were found below the different international legislations for nitrate, nitrite and sulfate.[39,40] In the recovery tests performed, the results were obtained without a variation larger than 10% in the absorbance signal. Furthermore, the *t*-test at 95% confidence level was applied to compare the results obtained by both methods and no significant differences were found.

Table 2-5. Analytical results and recovery tests obtained for sulfate with the proposed SIA system and the standard methodology.

Sample	Addition (mg SO_4^{-2} L^{-1})	SIA		Standard Method [4 1]	
		Sulfate (mg SO_4^{2-} L^{-1})	Recovery (%)	Sulfate (mg SO_4^{2-} L^{-1})	Recovery (%)
Groundwater	0	80.30 ± 5.04		63.58 ± 1.79	
	80	153.52 ± 2.92	92	150.46 ± 1.07	109
Tap water	0	32.59 ± 0.11		25.08 ± 0.26	
	80	113.05 ± 3.18	101	110.30 ± 0.84	107
Wastewater inlet*	0	28.85 ± 0.71		30.48 ± 0.20	
	80	106.37 ± 0.54	97	106.62 ± 0.45	95
Wastewater outlet*	0	47.39 ± 1.51		43.58 ± 1.18	
	80	132.93 ± 4.87	107	130.34 ± 0.28	108

The results are expressed as three replicates average ± standard deviation.
Sample was diluted 1:3 (*) before the analysis.

Table 2-6. Analytical results and recovery tests obtained for nitrite with the proposed SIA system and the standard methodology.

Sample	Addition (mg N L^{-1})	SIA		Standard Method [4 1]	
		Nitrite (mg N L^{-1})	Recovery (%)	Nitrite (mg N L^{-1})	Recovery (%)
Groundwater	0	N.D.		N.D.	
	0.2	0.1962 ± 0.0041	94	0.2048 ± 0.0041	104
Tap water	0	0.0145 ± 0.0007		N.D.	
	0.2	0.2053 ± 0.0033	95	0.2077 ± 0.0018	105
Wastewater inlet*	0	N.D.		N.D.	
	0.2	0.1983 ± 0.0044	97	0.2026 ± 0.0020	102
Wastewater outlet*	0	0.0309 ± 0.0009		N.D.	
	0.2	0.2274 ± 0.0050	98	0.2152 ± 0.0057	109

The results are expressed as three replicates average ± standard deviation.
N.D.: No detectable.
Sample was diluted with water 1:1 (*) before the analysis.

Table 2-7. Analytical results and recovery tests obtained for nitrate with the proposed SIA system and the standard methodology.

Sample	Addition (mg N L^{-1})	SIA		Standard Method [41]	
		Nitrate (mg N L^{-1})	Recovery (%)	Nitrate (mg N L^{-1})	Recovery (%)
Groundwater*	0	0.9962 ± 0.0154		0.9527 ± 0.0132	
	1	1.9211 ± 0.0423	93	1.9105 ± 0.0120	96
Tap water	0	0.5626 ± 0.0199		0.5742 ± 0.0098	
	1	1.5256 ± 0.0173	96	1.5187 ± 0.0121	95
Wastewater inlet^{+}	0	0.7269 ± 0.0610		0.6352 ± 0.0002	
	1	1.6757 ± 0.0331	95	1.6607 ± 0.0128	103
Wastewater outlet^{+}	0	0.8573 ± 0.0129		0.8032 ± 0.0130	
	1	1.8293 ± 0.0629	97	1.8200 ± 0.0133	102

The results are expressed as three replicates average ± standard deviation.
Samples were diluted 1:10(*) and 1:25 ($^{+}$) before the analysis.

2.4. Conclusions

The proposed SIA system allows the automated and multiparametric monitoring of nitrate, nitrite and sulfate in water, offering some advantages such as low consumption of sample and reagents, a minimal waste generation, optimum sensitivity, good reproducibility and repeatability and a higher sample throughput compared with the standard methods. Besides, the developed system does not suffer the typical interferences of phosphate ion in the determination of sulfate reported at the bibliography. The proposed technique shows a good accuracy in its working range for monitoring of the three ions, making it suitable for drinking water and wastewater samples.

Although regeneration process for the column is mandatory in order to avoid the decreasing efficiency of nitrate reduction to nitrite and washing steps are required to prevent the adhesion of Ba_2SO_4 to the tubes walls, these minor drawbacks do not affect the system performance. Moreover, the cleaning cycles are carried out in a fully automated approach.

Due to the quite simplicity, portability and rapid response of the proposed method, it could be used to monitor nitrate, nitrite and sulfate in certain fields where the continuous assessment of these ions is required (e.g. wastewater treatment plants, drinking water treatment plant or industrial processes).

Chapter 3 Stopped-in-loop Flow Analysis System for Successive Determination of Trace Vanadium and Iron in Drinking Water Using Their Catalytic Reactions[67]

3.1 Introduction

Vanadium has attracted much attention[68] since Tolman *et al.*[69] reported its insulin mimetic effect in in vitro experiments. Heyliger *et al.*[70] reported *in vivo* assessments of the insulin-like effect of vanadate using diabetic rats in 1985. In recent years, supplements and bottled mineral water containing vanadium are sold, advertising its insulin-like effect. Furthermore, clinical trials in humans revealed that the consecutive administration of the Mt. Fuji underground water, which contained natural vanadium (probably vanadate), resulted in the significant reduction of blood glucose level.[71] However, the physiological effect of vanadium on humans has not yet been perfectly revealed.

On the other hand, iron is an essential element for hemoglobin and myoglobin generation, cell proliferation and differentiation and is a substance necessary to cellular respiration in living organisms. However, the presence of free radicals of iron ion brings a detrimental in active oxygen and DNA damage occurs.[72] In recent years, it was reported that we need to avoid the excessive intake of iron because its accumulation may increase the risks of type 2 diabetes[73] and cancer development.[74] Therefore, the strict control of the concentrations of both elements contained in drinking water and in food must be performed by reliable quality control tests.

Catalytic reactions have been utilized as indicator reactions for kinetic-catalytic methods of trace analysis.[75,76] Since the measurement includes time as an experimental variable, care is needed to ensure that mixing of reagents takes place at regular time intervals to obtain highly accurate results. Such precise control of the reaction conditions can be easily accomplished by employing flow injection analysis (FIA) techniques.[77] Highly sensitive flow injection (FI) catalytic spectrophotometric methods are a very useful and versatile technique for trace metal determinations in natural and/or drinking water samples.[78] Many reports on FI catalytic spectrophotometric methods for the determination of vanadium[79-81] and iron[82-84] have been published so far.

However, among FI catalytic spectrophotometric methods of analysis, there are few reports on the determinations of two or more analytes in a mixed solution. The authors have developed FI catalytic spectrophotometric methods for the successive determinations of copper and iron based on their catalytic effects on the oxidative coupling reaction of *p*-anisidine with *N*,*N*-dimethylaniline.[85,86] Fortes *et al.*[87,88] reported a novel flow-based strategy for implementing simultaneous catalytic spectrophotometric determinations of vanadium and iron in Fe-V alloys.

Recently some concepts of flow-based analytical systems have been proposed: e.g. air-carrier continuous analysis system,[89] sequential injection analysis,[2] multi-commutation in flow analysis,[90] multisyringe flow injection analysis,[91] all injection analysis,[92] multi-pumping in flow analysis,[93] sequential injection chromatography[94] and sequential injection lab at valve.[95] Our laboratory also has conceived new flow-based analysis methods, such as stopped-in-loop flow analysis (SILFA)[3] and simultaneous injection effective mixing flow analysis (SIEMA).[96,97] These techniques are capable of lower reagent(s) consumption and lower waste generation compared with a conventional FIA technique.

The SILFA method is an alternative stopped flow technique in which the reaction proceeds in a coiled loop on a six-way injection valve; such feature is able to avoid a stain on the flow cell window caused by a colored reaction product. In a previous paper, we proposed a SILFA system with two loops (called stopped-in-dual loop flow analysis, SIDLFA) for the catalytic determination of vanadium based on its catalytic effect on the oxidation reaction of *p*-anisidine with bromate.[98] We found that the *p*-anidine oxidation with hydrogen peroxide was catalyzed by iron.[99] In the present paper, we introduce these two catalytic reactions into a SILFA system in order to develop a spectrophotometric method for the successive determination of trace vanadium and iron in drinking water.

3.2. Experimental

3.2.1. Reagents

All the reagents were of analytical grade. Deionized water used to prepare solutions was obtained from an Aquarius GSH-210 apparatus (Advantec, Tokyo).

3.2.1.1. Common reagents

Commercially available 1000 mg L^{-1} vanadium(V) and 1000 mg L^{-1} iron(III) standard solutions (Wako, Osaka) were used as stock solutions. Each working solutions were prepared by serial dilution of the standard solutions with 0.01 mol L^{-1} nitric acid (Sigma-Aldrich Japan, Tokyo).

A 0.25 mol L^{-1} *p*-anisidine stock solution was prepared by dissolving 6.16 g of *p*-anisidine (Alfa Aesar, US) in 200 mL of 3 mol L^{-1} hydrochloric acid (Sigma-Aldrich Japan).

A 4 mol L^{-1} acetate buffer stock solution was made from solutions of acetic acid (Sigma-Aldrich Japan) and sodium acetate trihydrate (Nacalai Tesque, Kyoto).

3.2.1.2. Vanadium determination

A 0.2 mol L^{-1} Tiron stock solution was prepared daily by dissolving 1.57 g of 1,2-dihydroxy-3,5-benzenedisulfonic acid disodium salt monohydrate (Tokyo Chemical Industry, Tokyo) in 25 mL of water.

A mixed reagent solution of 0.06 mol L^{-1} *p*-anisidine, 1 mol L^{-1} acetate buffer and 0.05 mol L^{-1} Tiron was prepared by mixing daily these three stock solutions and adjusting the pH to 2.9 with 10 mol L^{-1} sodium hydroxide. When the initial optimization studies (described in the sections 3.3.1.1.–3.3.1.7.) were carried out, the mixed solution constituted RS_1 referred to in the section 3.2.3.

A 0.1 mol L^{-1} sodium diphosphate stock solution was made dissolving 4.46 g of sodium diphosphate decahydrate (Wako) in 100 mL of water.

In the section 3.3.1.8. and later sections, another mixed solution of 0.06 mol L^{-1} panisidine, 1 mol L^{-1} acetate buffer, 0.05 mol L^{-1} Tiron and $5x10^{-3}$ mol L^{-1} sodium diphosphate (as a masking agent for iron) was delivered from RS_1 shown in Fig. 3-1.

A bromate solution of 0.08 mol L^{-1} delivered from RS_2 was daily prepared by dissolving 0.668 g of potassium bromate (Wako) in 50 mL of water.

3.2.1.3. Iron determination

A 0.01 mol L^{-1} 1,10-phenanthroline (phen) solution was prepared by dissolving 0.099 g of 1,10-phenanthroline monohydrate in 50 mL of 0.1 mol L^{-1} HCl.

In order to prepare a mixed reagent solution of 0.04 mol L^{-1} *p*-anisidine, 1 mol L^{-1} acetate buffer, $5x10^{-4}$ mol L^{-1} phen (delivered from RS_3) was prepared daily, and the pH of the solution was adjusted to 4.1 with 10 mol L^{-1} sodium hydroxide.

A 0.5 mol L^{-1} hydrogen peroxide solution was prepared from a 30% hydrogen peroxide solution (Sigma-Aldrich Japan) and was delivered from RS_4.

3.2.2. Apparatus

A schematic diagram of the proposed SILFA system is shown in Fig. 3-1. The system included an Ogawa & Co. (Kobe) touchscreen automatic controller device (Mode1 OG-V3-P2) composed by two peristaltic pumps, two six-port switching valves and one four-port selection valve.[98] All of these components are able to be operated in either manual or automatic mode by a built-in programmable logic controller. A Shimaden (Tokyo) SR91 proportional-integral-derivative (PID) digital controller

was used to control two cartridge heaters (C3JX4A, Watlow Japan, Tokyo) and a thermocouple (type K chromel-alumel, Sakaguchi E.H VOC, Tokyo). In order to assemble a heater device, each cartridge heater was installed into a tubular brass on which Teflon tubing was coiled to make a heated loop. Spectrophotometric measurements were made using a Soma (Tokyo) visible detector (S-3250) equipped with a flow-through cell (8 μL volume, 10 mm path length). Absorbance was measured at 510 nm for both analytes. The detector output signal was recorded with a FIA monitor (Ogawa & Co.) controlled by a PC. The flow line was built using 0.5 mm inner diameter Teflon tube, and only the back pressure coil was of 0.25 mm inner diameter Teflon tube (3 m long).

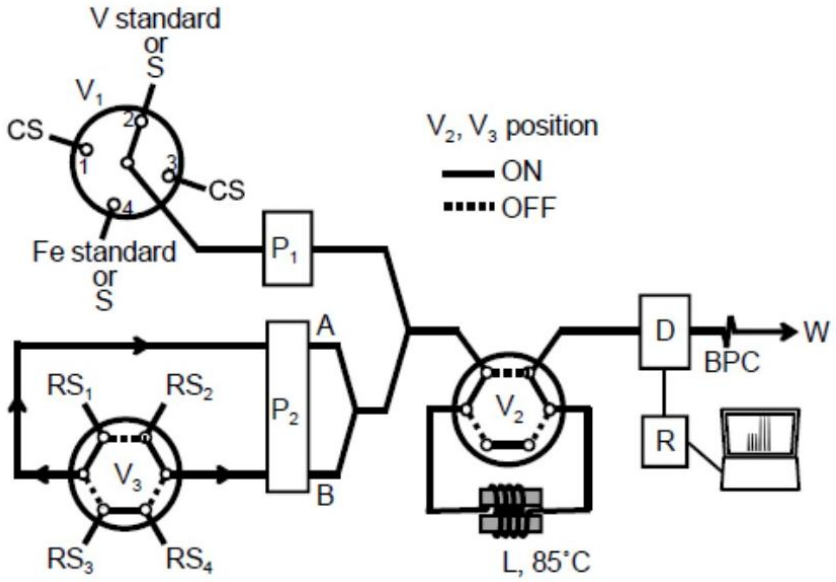

Fig. 3-1. Schematic diagram of the proposed SILFA system. CS: 0.01 mol L^{-1} HNO_3 carrier solution; RS_1: reagent solution 1 containing 0.06 mol L^{-1} *p*-anisidine, 1 mol L^{-1} acetate buffer (pH 2.9), 0.05 mol L^{-1} Tiron (in case higher iron was present, $5x10^{-3}$ mol L^{-1} diphosphate was added); RS_2: reagent solution 2 containing 0.08 mol L^{-1} $KBrO_3$; RS_3: reagent solution 3 containing 0.04 mol L^{-1} *p*-anisidine, 1 mol L^{-1} acetate buffer (pH 4.1), $5x10^{-4}$ mol L^{-1} 1,10-phenanthroline; RS_4: reagent solution 4 containing 0.5 mol L^{-1} H_2O_2; V_1: four-port selection valve; V_2, V_3: six port switching valve; P_1 (3.9 mL min^{-1}), P_2 (line A, 0.56 mL min^{-1}; line B, 0.38 mL min^{-1}): peristaltic pump; D: detector (510 nm); R: recorder; BPC: back pressure coil; W: waste.

3.2.3. Analytical procedure

As shown in Fig. 3-1, pump 1 (P_1) was connected to the central port of the four-port selection valve (V_1) which managed a carrier solution (CS, 10 mM HNO_3) and standards/samples as follows; port 1 and port 3 were used for the carrier solution aspiration, the vanadium standard/sample was aspirated through port 2 and the iron standard/sample was aspirated through port 4. Pump 2 (P_2) connected to a switching valve (V_3) was used to switch the mixed reagent solutions (RS_1 and RS_3) and the oxidants (RS_2 and RS_4); *i.e.* a couple of solutions in reservoirs, RS_1 and RS_2, for vanadium determination and another couple of solutions in reservoirs, RS_3 and RS_4, for iron determination were delivered alternately. Meanwhile, another switching valve (V_2) with a heated loop was used to perform the stopped-in-loop step.

The temporal operation protocol of the SILFA system shown in Table 3-1 for the automated performance of the analytical operations was set through the touchscreen controller. The procedures for vanadium determination are described as follows. First, in mode 1, a vanadium standard or sample solution through the port 2 (in the case of blank measurement, a 0.01 mol L^{-1} nitric acid through the port 1) was aspirated by the P_1, and the mixed solution of *p*-anisidine, acetate buffer and Tiron (in case higher iron was present, diphosphate was added) in the RS_1 and bromate in the RS_2 were aspirated by the P_2. These solutions were well mixed and loaded into the heated loop on the V_2. In mode 2, the loop on the V_2 was disconnected from the flow line to facilitate the catalytic reaction at 85° C, and the P_2 was stopped. Meanwhile, the P_1 was aspirating a 0.01 mol L^{-1} nitric acid solution through the port 1 to clean the flow line. The next step is mode 3, the P_1 was turned off, so that the net waste can be equal to 0. In mode 4, only the P_1 was turned on to send a 0.01 mol L^{-1} nitric acid solution through the port 1, so that a baseline can be established. After that (mode 5), the recorder was activated to perform the baseline measurement for five seconds. In mode 6, the reaction mixture was finally dispensed by switching the V_2 (ON) towards the detector obtaining a peak for vanadium at 510 nm.

In the case of iron determination, the same procedures (modes 1 to 6) were carried out except the aspiration ports on the V_1 and the position of V_3.

Table 3-1. Operation protocol of the SILFA system.

Mode	V_1 position V/Fe	V_2 position	V_3 position V/Fe	P_1	P_2	R	Working time (s)	Description
1	2/4[a]	ON	ON/OFF	ON	ON	OFF	125	Loading reagents and blank or sample into loop
2	1/3	OFF	ON/OFF	ON	OFF	OFF	80	Reaction in loop and washing line
3	1/3	OFF	ON/OFF	OFF	OFF	OFF	85	Reaction in loop (net waste = 0)
4	1/3	OFF	ON/OFF	ON	OFF	OFF	10	Reaction in loop and establishing baseline
5	1/3	OFF	ON/OFF	ON	OFF	ON	5	Reaction in loop and signal monitoring for baseline
6	1/3	ON	ON/OFF	ON	OFF	ON	40	Signal monitoring for peak

[a] When a blank signal for each analyte, the position of V_1 was 1 for vanadium or 3 for iron.

3.3. Results and discussion

3.3.1. Optimization

The optimal conditions for *p*-anisidine, bromate, hydrogen peroxide, Tiron, phen, pH, reaction temperature, stopping time and flow rate were studied for the determination of 1 μg L^{-1} vanadium and 10 μg L^{-1} of iron, respectively. The optimal experimental conditions for iron determination will be established by these studies described in the sections 3.3.1.1.–3.3.1.7.

For vanadium determination, the above mentioned optimization studies were performed in the absence of diphosphate as a masking agent for iron. Eventually, the optimal experimental conditions for vanadium determination will be established in the presence of diphosphate described in section 3.3.1.8.

3.3.1.1. Effects of *p*-anisidine concentration

The effects of *p*-anisidine concentration on the peak heights for vanadium and iron are shown in Fig. 3-2 (a) and (b), respectively. For both analytes, uncatalyzed (○) and catalyzed (□) reactions were promoted at the higher *p*-anisidine concentration. Taking into accounts the net absorbance values (▲) and lower blank peak heights, *p*-anisidine concentrations of 0.06 and 0.04 mol L^{-1} were chosen for vanadium and iron determination, respectively.

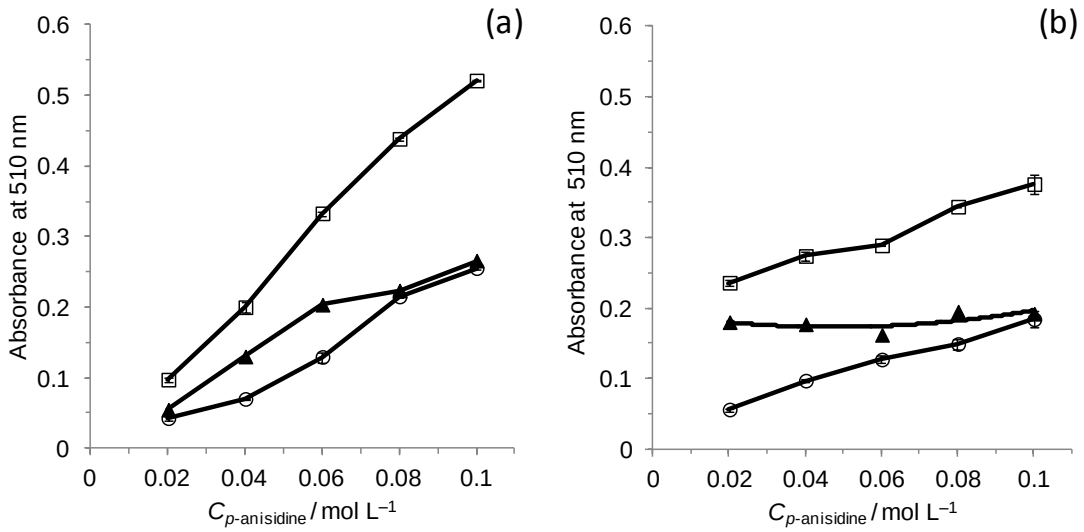

Fig. 3-2. Effects of *p*-anisidine concentration.

3.3.1.2. Effects of oxidants

The effects of bromate and hydrogen peroxide on the responses were studied in ranges of 0.01 to 0.1 mol L^{-1} and 0.1 to 1.5 mol L^{-1}, respectively. The results are shown in Fig. 3-3. In the case of bromate (Fig. 3-3 (a)), the response of the uncatalyzed reaction (○) increased drastically compared with the increase in the response of the vanadium-catalyzed reaction (□) over a concentration of 0.08 mol L^{-1}, diminishing the net absorbance value. Therefore, a bromate concentration of 0.08 mol L^{-1} was chosen. As shown in Fig. 3-3 (b), the responses for iron with poor repeatability were obtained

with hydrogen peroxide concentrations greater than 0.5 mol L^{-1} for the iron determination. We chose therefore a hydrogen peroxide concentration of 0.5 mol L^{-1}.

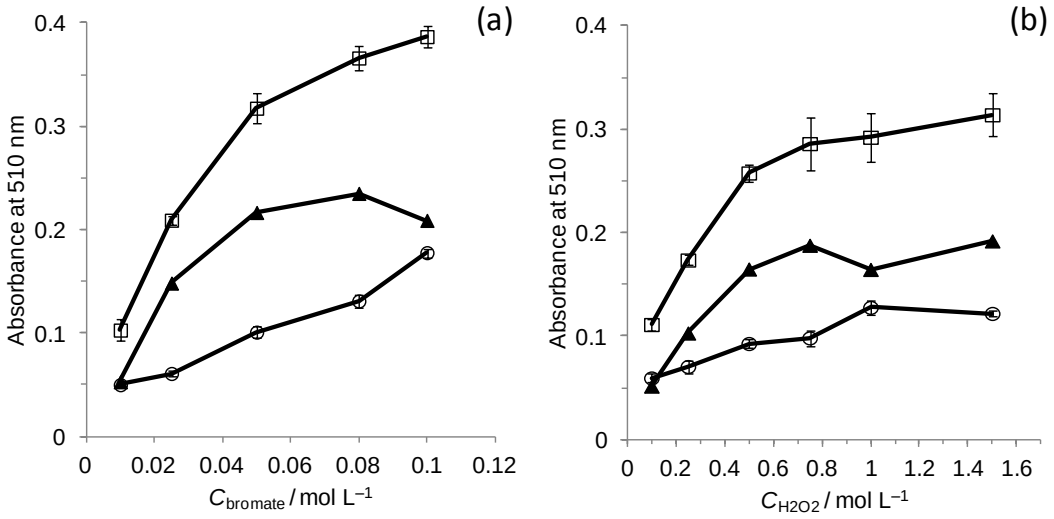

Fig. 3-3. Effects of oxidants.

3.3.1.3. Effects of activators

The addition of an activator increases the catalytic action of a particular metal ion.[78,99] In the present study, Tiron and phen were employed as activators for vanadium and iron determination, respectively. The absorbance was measured in the concentration ranges of 0.01 to 0.3 mol L^{-1} for Tiron and $4x10^{-5}$ to $1x10^{-3}$ mol L^{-1} for phen.

As shown in Fig. 3-4 (a), the net absorbance for vanadium increased with increasing the concentration of Tiron. Although a higher Tiron concentration was therefore suitable for the sensitivity of vanadium, too high Tiron concentration resulted in lowered selectivity of vanadium; *i.e.* the serious positive interference from iron was observed at a Tiron concentration of 0.2 mol L^{-1} Tiron, probably because of the absorbance of iron-Tiron complex (560 nm). However, when a Tiron concentration of 0.05 mol L^{-1} was chosen, the presence of 50 μg L^{-1} iron was tolerable for 1 μg L^{-1} vanadium determination. The tolerance limit will further be improved by the addition of diphosphate

as a masking agent (see section 3.3.1.8.). Meanwhile, at a phen concentration of $5x10^{-4}$ mol L^{-1}, the highest net absorbance value was obtained, and the signal responses decreased as higher concentrations were employed (Fig. 3-4 (b)). Hence, a phen concentration of $5x10^{-4}$ mol L^{-1} was chosen.

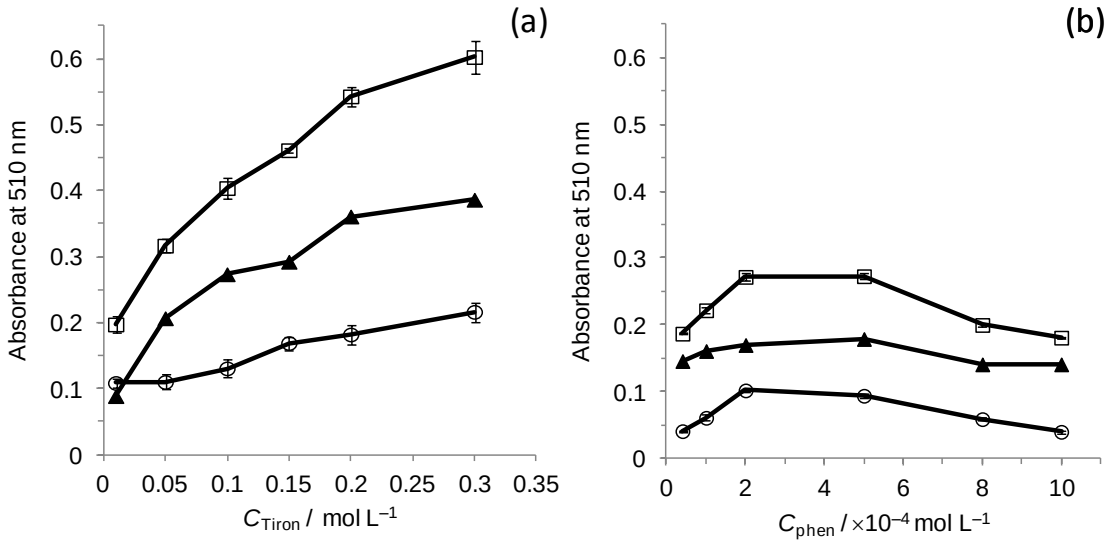

Fig. 3-4. Effects of activators.

3.3.1.4. Effects of pH

The pH values were varied from 2.3 to 3.5 for vanadium and from 2.6 to 4.5 for iron determination, respectively. The results obtained showed maximum absorbance at pH values of 2.9 for vanadium determination and 4.1 for iron determination. Thus, the further studies were carried out at the pH values mentioned above.

3.3.1.5. Effects of reaction temperature

The temperature setting of the heater device was changed in the range of 70–95° C. The absorbance values of the uncatalyzed and catalyzed reactions for both analytes began to increase as

the temperature was raised. However, the peak signal showed poor repeatability for temperatures above 85° C. Thus, the heater device was set at the temperature of 85° C.

3.3.1.6. Effects of stopping time in loop

The stopping time in the heated loop was varied from 2 to 5 min. The longer stopping time in the loop resulted in the increase in the responses of the uncatalyzed and catalyzed reactions of both analytes. A stopping time of 3 min in loop was selected for both analytes in consideration of the sample throughput rates and the sensitivities.

3.3.1.7. Effects of flow rates

The effects of flow rates on the responses were studied by varying the rotation speeds of the peristaltic pumps, P_1 and P_2. The flow rate of the P_1 was changed in the range of 1.1–3.9 mL min^{-1} (Fig. 3-5 (a) and (b)). On the other hand, the flow rates of the lines A and B in the P_2 shown in Fig. 3-1 were simultaneously varied in the ranges of 0.14–0.56 mL min^{-1} for the line A and 0.10–0.38 mL min^{-1} for the line B (Fig. 3-5 (a') and (b')). The flow rates in the lines A and B were different because the inner diameters of PharMed tubing were different between the lines A (2.0 mm i.d.) and B (1.0 mm i.d.). A P_1 flow rate of 3.9 mL min^{-1} was chosen in order to give priority to the sensitivity of iron. Fig. 3-5 (a') shows that the signal responses for the uncatalyzed and vanadium-catalyzed reactions increased drastically with the P_2 flow rate. Referring to Fig. 3-5 (b'), the maximum net absorbance for iron was obtained at flow rates of 0.42 mL min^{-1} for the line A and 0.29 mL min^{-1} for the line B. For the final system, we chose flow rates of 0.56 mL min^{-1} and 0.38 mL min^{-1} were selected for the lines A and B, respectively, taking into account the sensitivity of vanadium.

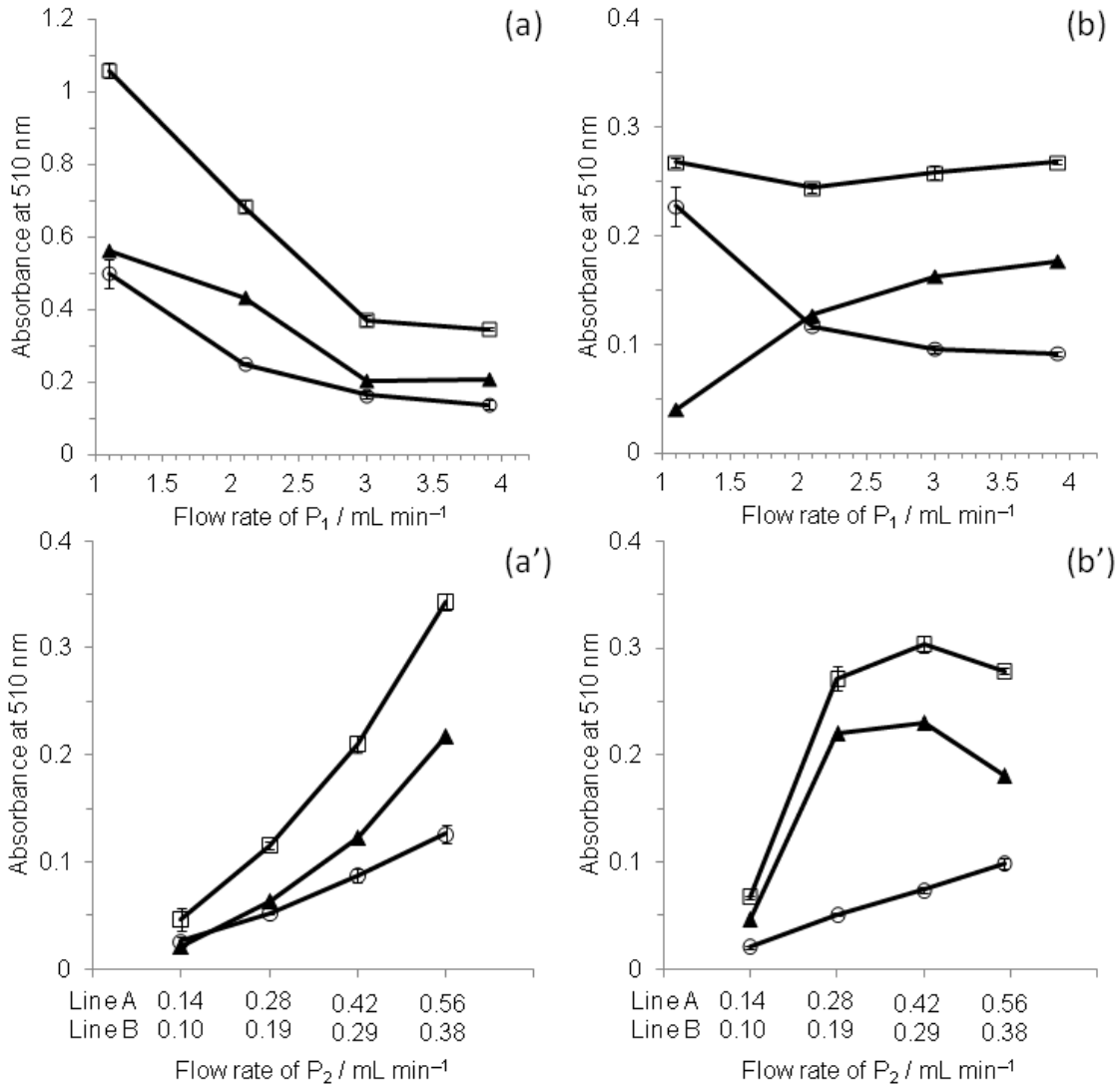

Fig. 3-5. Effects of flow rates.

3.3.1.8. Effect of diphosphate for vanadium determination

In order to improve the selectivity of the vanadium determination, the effect of diphosphate in the reservoir RS_1 on the signal responses was examined. The result is shown in Fig. 3-6. Diphosphate had no effect on the blank signals (– ○ –), while the response for 1 μg L^{-1} vanadium (– □ –) gradually decreased with diphosphate concentration. However, at a diphosphate concentration of $5x10^{-3}$ mol L^{-1}, the peak height for 1 μg L^{-1} vanadium including 400 μg L^{-1} iron (– Δ –) was almost same as that for a

standard solution containing only 1 μg L^{-1} vanadium. For the following studies, we therefore added $5x10^{-3}$ mol L^{-1} diphosphate into the RS_1 including *p*-anisidine, acetate buffer and Tiron.

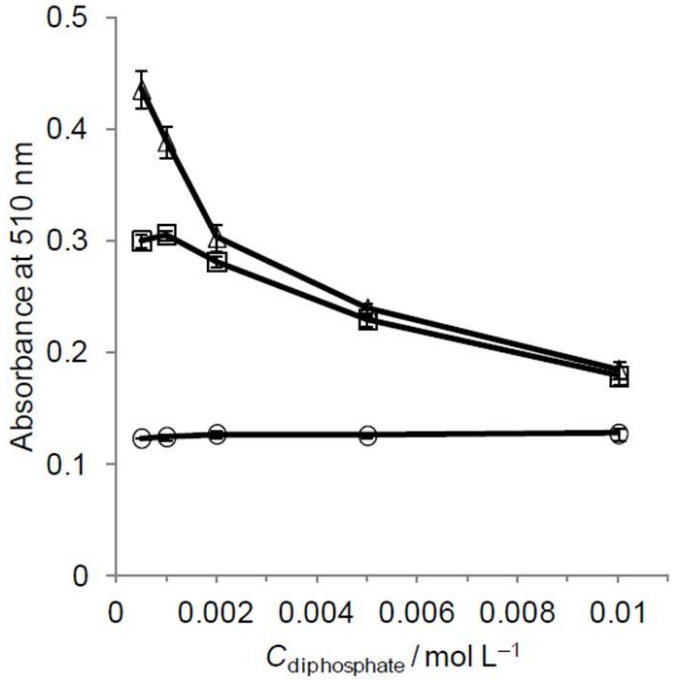

Fig. 3-6. Effect of diphosphate for vanadium determination

3.3.2. Analytical characteristics

Fig. 3-7 shows typical calibration sequences for vanadium and iron standards under the optimized conditions. Linear calibration curves for vanadium and iron were obtained from the signal responses in the ranges of 0–2.0 and 0–20 μg L^{-1}, respectively.

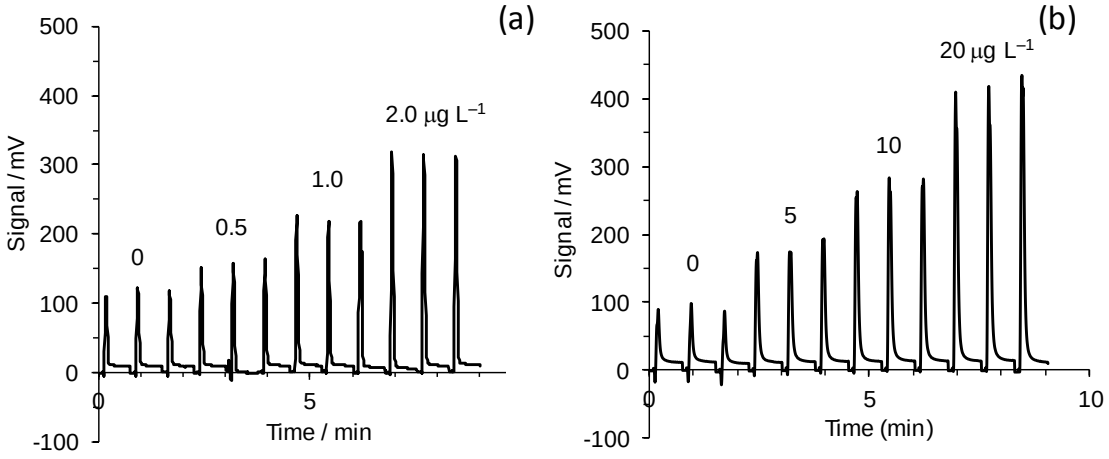

Fig. 3-7. Calibration sequences for vanadium and iron standards under the optimized conditions.

Table 3-2 summarizes the analytical performances of the proposed system in terms of parameters of the calibration curves, limits of detections, limits of quantitation, repeatabilities and injection frequencies. The limits of detection of the proposed system were unfortunately higher than those obtained by similar catalytic FIA methodologies which achieved limits of detection ranged 0.008–0.01μg L^{-1} for vanadium[79-81] and 0.02–0.05 μg L^{-1} iron,[82-84] respectively.

However, the sensitivities for vanadium and iron of the proposed method were better than a catalytic spectrophotometric method for the simultaneous determination of mg L^{-1} levels of vanadium and iron in the same alloy samples.[87,88]

Table 3-2. Analytical characteristics for SILFA system.

	Vanadium	Iron
Slope[a]	0.101	0.0164
Intercept[a]	0.002	0.006
Regression coefficient (r^2)	0.996	0.996
Limit of detection / μg L^{-1}	0.052	0.55
Limit of quantitation / μg L^{-1}	0.17	1.8
%RSD (n = 5)	2.2[b]	2.3[c]
Injection frequency / h^{-1}	10	10

[a] Net absorbance = slope[vanadium or iron, μg L^{-1}] + intercept.
[b] 1 μg L^{-1} vanadium.
[c] 10 μg L^{-1} iron.

3.3.3. Interferences

The selectivities of the proposed method were evaluated by studying the effects of foreign ions in the determination of 1 μg L^{-1} of vanadium and 10 μg L^{-1} of iron. The results are summarized in Table 3-3. The tolerance limit for a given ion was defined as the interference that yielded a relative error less of ±5% or less. As described in the section 3.3.1.8., 400 μg L^{-1} iron was tolerable for the determination of 1 μg L^{-1} vanadium, while 500 μg L^{-1} vanadium was tolerable for the determination of 10 μg L^{-1} iron. Among the tested foreign ions, Mo(VI) showed the largest interference in the determination of vanadium. For iron determination, Al(III), Cr(VI), Cu(II) and Ni(II) had remarkable interferences. However, we believe the proposed method is capable of successive determination of both analytes in drinking water, because the concentrations of the ions mentioned above are not so large in drinking water samples.

Table 3-3. Interference of foreign ions in the determination of 1 μg L^{-1} of vanadium and 10 μg L^{-1} of iron.

Tolerance limit (μg L^{-1})	Ion added[a]	
	Vanadium	Iron
50000	Ba(II), BO_3^{3-}, Ca(II), Cd(II), Cl^-, Co(II), K(I), Mg(II), Na(I), NO_3^-, SO_4^{2-}	Ca(II), Cl^-, K(I), Na(I), NO_3^-, SO_4^{2-}
20000	Mn(II), Ni(II), Pb(II), Zn(II)	
10000	Si(IV)	Ba(II), Se(IV)
5000	Al(III), Se(IV)	BO_3^{3-}, Cd(II), Mo(VI), Si(IV)
2000		Mg(II)
1000	Ti(IV)	Zn(II)
500	Cr(VI)	Pb(II), V(V)
400	Cu(II), Fe(III)	
200	Mo(VI)	Co(II), Ti(IV)
100		Mn(II)
50		Al(III), Cr(VI), Cu(II), Ni(II)

[a] An error of ±5% or less is considered to be tolerable.

3.3.4. Application

The proposed SILFA method was applied to the analysis of vanadium and iron in tap water and commercial mineral water samples. Table 3-4 shows the comparison of the analytical results between

the proposed method and other methods (GFAAS and ICP-MS). In the analytical values of both analytes in tap water samples, there were no significant difference between the proposed method and GFAAS. Although the iron concentrations in bottled mineral water samples were less than the LOQ of the proposed method, the analytical values of vanadium were in fair agreement with those obtained by GFAAS and ICP-MS and with the labeled values.

Table 3-4. Determination of vanadium and iron in mineral water and tap water samples.

Sample	SILFA [a] (µg L^{-1})		Other methods (µg L^{-1})		Labeled[d] (µg L^{-1})
	Vanadium	Iron	Vanadium	Iron	Vanadium
Tap water 1	0.54±0.05	23.2±0.16	0.57±0.00[b]	23.1±0.08[b]	–
Tap water 2	0.65±0.11	19.4±0.28	0.61±0.09[b]	19.5±0.04[b]	–
Mineral water 1	55.8±0.04	<LOQ	54.4±0.89[b]	0.81±0.29[b]	55
Mineral water 2	61.4±0.03	N.D.	61.3±0.46[b]	<LOQ[b]	62
Mineral water 3	160.6±0.05	<LOQ	160.7±0.07[c]	1.58±0.58[c]	160

[a] Average value (n=3)
[b] Average value (n=3) obtained by GFASS
[c] Average value (n=3) obtained by ICP-MS
[d] Value displayed in the label of the bottle

3.4. Conclusions

We have described here an automated SILFA system for the catalytic determination of vanadium and iron in drinking water. The developed method offered also typical characteristics of the multicommutated systems, as low reagents consumption and the subsequently minimization of waste generation compared with batchwise and conventional FIA methodologies. Such affordable SILFA system can be expanded into any kinetic-catalytic methods of analysis.

Chapter 4 Spectrophotometric Determination of Palladium using 5-Br-PSAA by Simultaneous Injection Effective Mixing Flow Analysis[100]

4.1 Introduction

Flow injection analysis (FIA), conceived by Ruzicka and Hansen,[1] is one of the promising techniques used to obtain a highly sensitive method, provided that a suitable chemical reaction is introduced into FIA, and that a highly stable baseline is obtained. Since FIA appeared in 1975, other sophisticated flow-based analytical systems have been proposed so far. However, basically FIA needs to establish a baseline continuously, which leads to continuous reagent consumption and a large amount of waste generation.

In 1989, Petersen and Dasgupta[89] developed an air-carrier continuous analysis system (ACCAS). A peristaltic pump placed downstream in an ACCAS is used for aspiration of the sample and reagent(s). Each solution is systematically aspirated by using several 3-way solenoid valves. Finally, air is aspirated to transport the resulting solution to a detector. Such ACCAS is capable of low reagent consumption and low waste generation. This concept, except for the air carrier, was modified by Reis et al., and they call it multicommutation in flow analysis (MCFA).[90] Recent developments concerning MCFA have been reviewed by Feres *et al.*[101]

Sequential injection analysis (SIA), which appeared in 1990, has played important roles in the automation and miniaturization of analytical methods.[2] In the SIA format, mutual penetration of sample and reagent(s) zones is essential for a successful chemical reaction. In SIA, however it is usually difficult to obtain a well-mixed condition of these zones aspirated into a common holding coil. This lower mixing efficiency plagues the utilization of SIA in some cases.

Recently, we have developed an alternative stopped-flow technique, called stopped-in-loop flow analysis, SILFA.[3,98] This technique permits lower reagent consumption and waste generation

compared with conventional FIA. The SILFA technique should be attractive for a relative slow chemical reaction.

We propose here a hybrid flow analysis, called simultaneous injection-effective mixing analysis (SIEMA), which has advantages of FIA, SIA and MCFA. In 2006, the initial study on SIEMA was reported by Motomizu.[102] In the present work, we demonstrated a spectrophotometric determination of palladium by using a SIEMA system, and obtained experimental evidence that proved the reliability of the SIEMA technique.

4.2. Experimental

4.2.1. Reagents

All reagents used were of analytical grade, and de-ionized water purified by an Aquarius GSH-210 (Advantec, Tokyo) was used throughout.

Stock standard solution (100 mg L^{-1}) of palladium was prepared by suitable dilutions of commercially available 1000 mg L^{-1} palladium standard solution for atomic absorption spectrometry (Wako, Osaka) with water. Working solutions were prepared daily by diluting the solutions with 0.01 mol L^{-1} hydrochloric acid.

A 3.0×10^{-3} mol L^{-1} 5-Br-PSAA stock solution was prepared dissolving 0.1435 g of 2-(5-Bromo-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)amino]aniline, sodium salt (Dojindo, Kumamoto) in 100 mL of water, stored in a dark container and accordingly diluted for its use.

Acetate buffer with a concentration of 0.2 mol L^{-1}, was prepared from dissolving 3 mL of acetic acid (Sigma-Aldrich Japan, Tokyo) in 205 mL of water and 6.8 g of sodium acetate trihydrate (Nacalai Tesque, Tokyo) in 250 mL of water. The acetate buffer adjusted a pH of 4.5 was prepared per each use.

4.2.2. Apparatus

In a previous study, Sakai and Ohno[103] found that 2-(5-bromo-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)amino]aniline (5-Br-PSAA) reacts with palladium to form a complex that has an absorption maximum at 612 nm, and an FIA system was assembled for the determination of palladium. However, the above mentioned FIA system was not fully automated and not compact; especially a double plunger pump was relatively large and expensive.

The same chemistry was introduced into a newly proposed SIEMA system. The SIEMA system shown in Fig. 4-1 basically consisted of a bidirectional syringe pump (P), three 3-way solenoid valves (3V) and a 2-way solenoid valve (2V) that were controlled by homemade software in a laptop computer. The manifold was built using Teflon tube inner diameter of 0.8 mm, but the auxiliary coil (AC) having inner diameter of 2 mm.

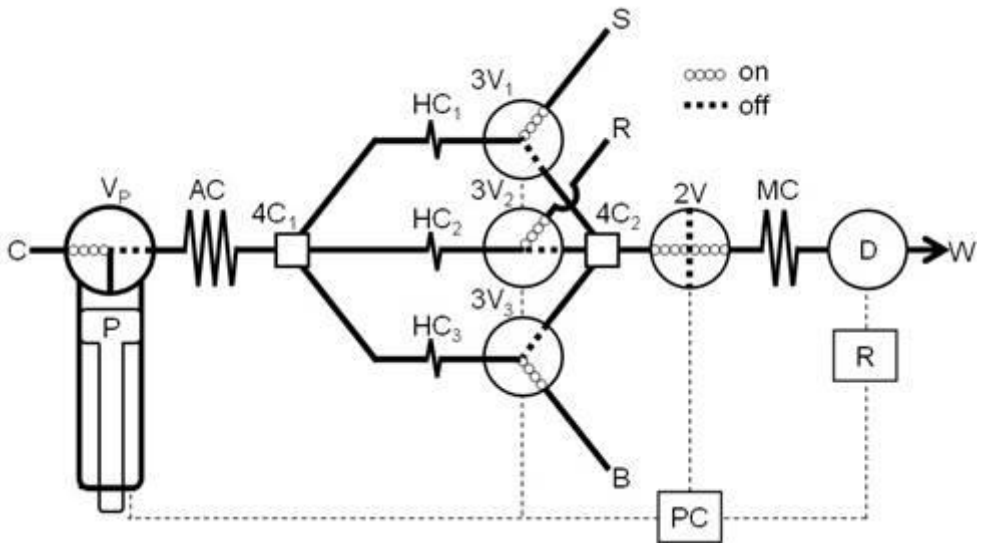

Fig. 4-1. Schematic diagram of the SIEMA for Pd determination. C, water; P, syringe pump; V_p, 2-way solenoid valve atop P; AC, auxiliary coil (2 mm i.d., 0.65 m long); $4C_1$ and $4C_2$, 4-way cross connector; HC_1, HC_2, and HC_3, holding coils; $3V_1$, $3V_2$, and $3V_3$, 3-way solenoid valves; S, standard/sample; R, $3.0x10_{-4}$ mol L^{-1} 5-Br-PSAA; B, 0.2 mol L^{-1} acetate buffer (pH 4.5); 2V, 2-way solenoid valve, MC, mixing coil (0.8 mm i.d., 0.6 m long); D, spectrophotometer (612 nm); R, recoder; PC, computer; W, waste.

4.2.3. Procedure

The temporal operation and each function of the SIEMA system are given in Table 4-1. A palladium standard/sample, 5-Br-PSAA and acetate buffer (100 µL each) were simultaneously aspirated into each holding coil (HC_1, HC_2, and HC_3) via each 3-way solenoid valve ($3V_1$, $3V_2$, and $3V_3$). While aspirating them, the 2-way valve (2V) was turned off for selective aspiration with accurate volume via 3-way solenoid valve. Then, all solutions were simultaneously dispensed to be merged at a confluence point ($4C_2$). After the confluence point, an effective mixing condition was obtained, and therefore complex formation took place successfully in the mixing coil. The absorbance of the colored product was measured with a spectrophotometric detector at 612 nm.

Table 4-1. Temporal operation of the SIEMA system.

Step	V_p	$3V_1$	$3V_2$	$3V_3$	2V	Flow rate/ µL s^{-1}	Function
1	OFF	ON	ON	ON	OFF	50	Aspiration of palladium(II) sample, 5-Br-PSAA and acetate buffer into HC_1, HC_2 and HC_3, respectively
2	ON	OFF	OFF	OFF	OFF	300	Aspiration of carrier into syringe
3	OFF	OFF	OFF	OFF	ON	50	Dispensing all aspirated zones to detector simultaneously

Abbreviations (V_p, $3V_1$, $3V_2$, $3V_3$, 2V, HC_1, HC_2, HC_3) as in Fig. 4-1.

4.3. Results and discussion

4.3.1. Optimization

In preliminary experiments of this study, sequential aspiration of the sample and reagents was performed using a syringe of 2.5 mL capacity. Although a reduction in the reagent consumption was achieved, in order to dispense the all aspirated zones to the detector, aspiration of carrier to a full volume of the syringe (2.5 mL) was required and the waste amount was larger compared with FIA. Therefore, a syringe volume of 1.5 mL was selected for further reduction of waste. The operation conditions were studied as is described below.

4.3.1.1. Effect of mixing coil length

The length of the mixing coil was varied in the range of 0.3 - 1.2 m. Results are shown in Fig. 4-2. Although, a longer mixing coil increases the net absorbance, it is difficult to push the solution to the end of the flow line; 0.6 m length was selected.

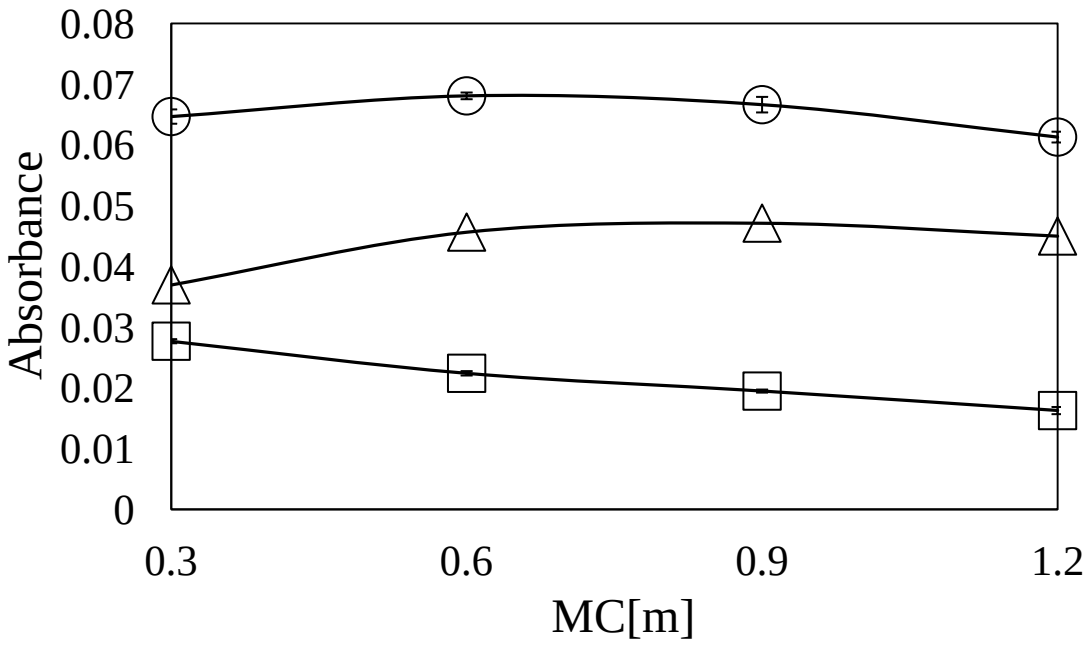

Fig. 4-2. Effect of mixing coil. ○: complex; △: net; □: blank

4.3.1.2 Effect of aspiration volume

Each volume can be aspirated simultaneously, it was changed in the range of 150 - 600 μL. Results are shown in Fig. 4-3. Meanwhile increasing the amount of aspiration, the absorbance of net increases, but because the baseline rises was selected 300 μL.

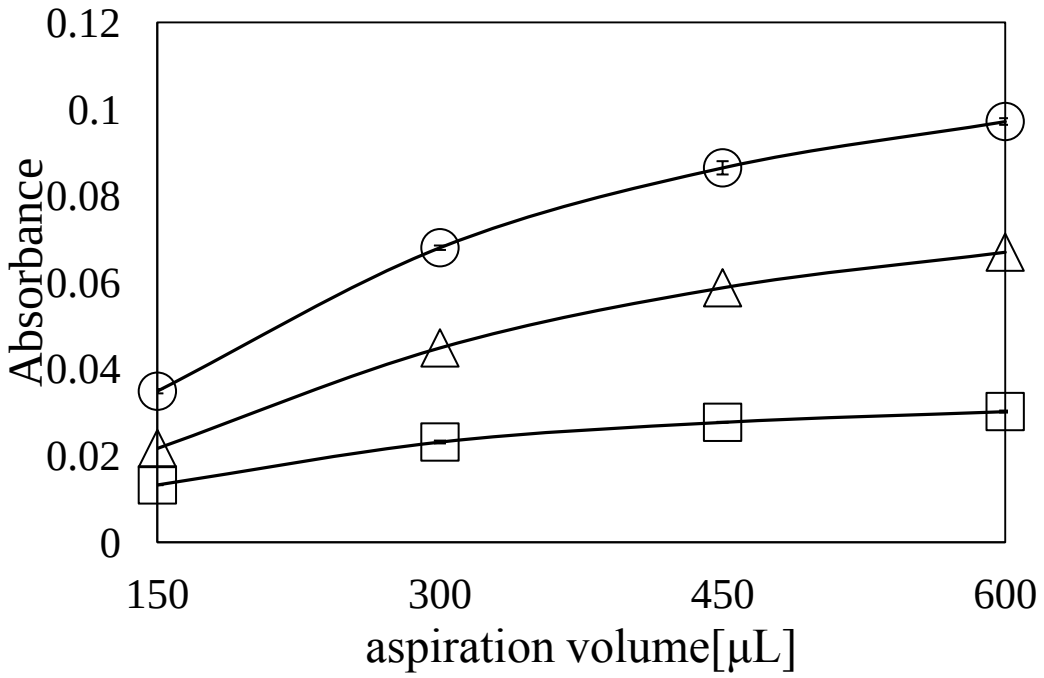

Fig. 4-3. Effect of aspiration volume. ○: complex; △: net; □: blank.

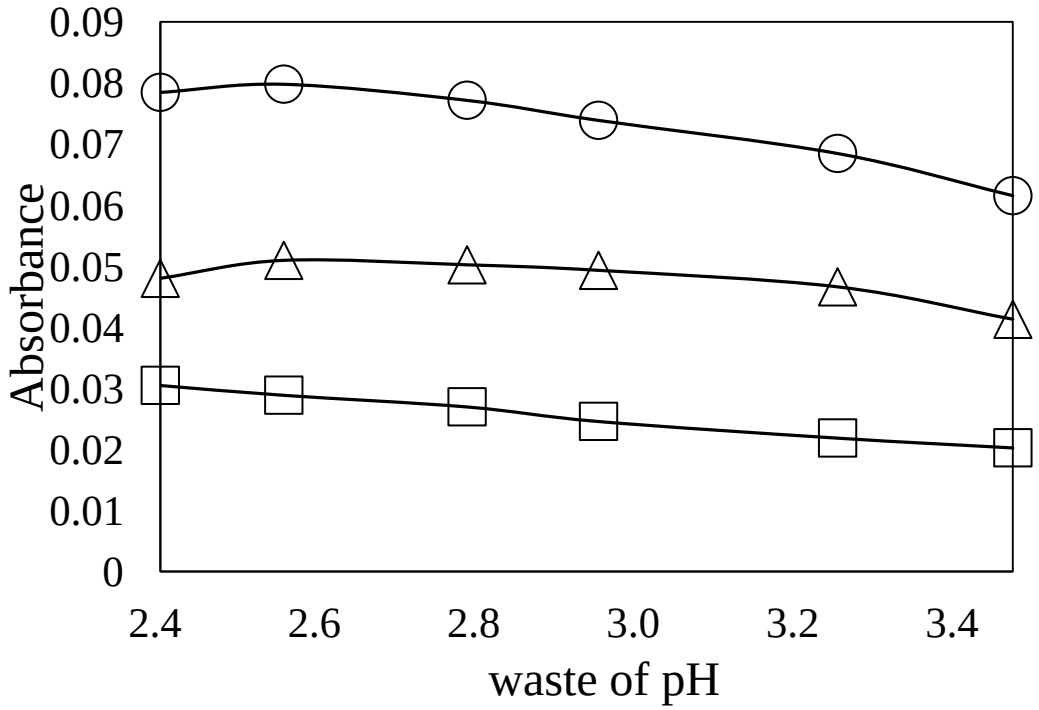

Fig. 4-4. Effect of pH. ○: complex; △: net; □: blank.

4.3.1.3. Effect of pH

The pH of the buffer solution was changed to make the pH of the waste to be 2.4 to 3.5. Results are shown in Fig. 4-4. The net absorbance became substantially flat in the pH range of 2.6 to 3.0. The absorbance of the blank was lower as the pH was increasing. Therefore, pH of 3.1 was selected to obtain a high net absorbance.

4.3.1.4. Effect of 5-Br-PSAA concentration

The concentration of 5-Br-PSAA was varied in the range of 1.5 - 5x10^{-4} mol L^{-1}. Results are shown in Fig. 4-5. Further than 3.0x10^{-4} mol L^{-1}, net absorbance change was moderate. In this study, 3.0x10^{-4} mol L^{-1} was chosen.

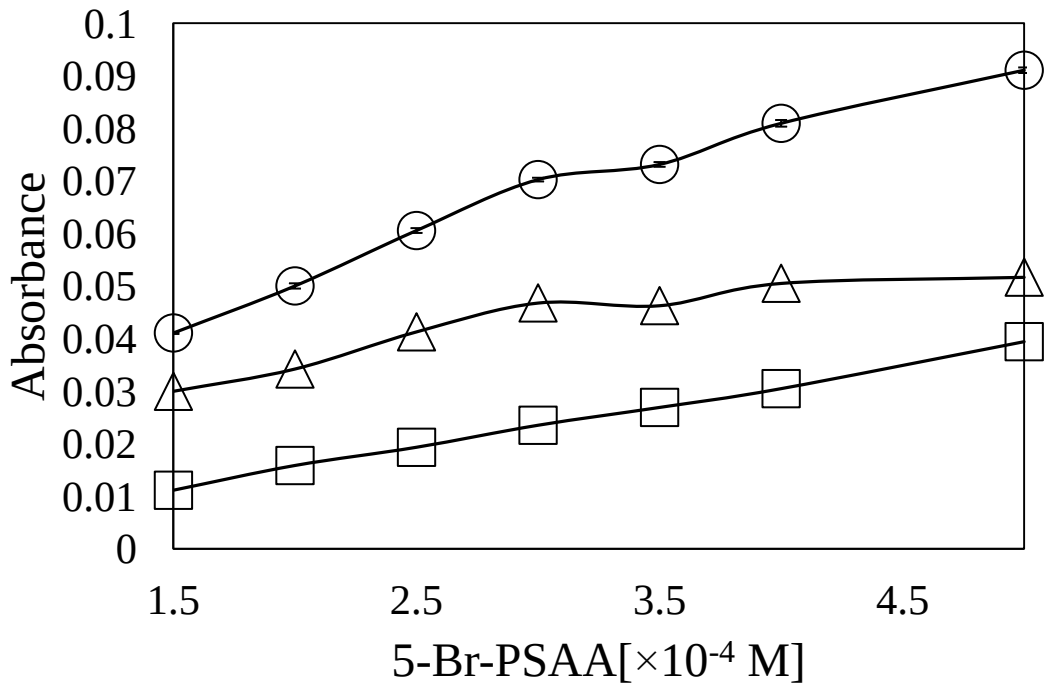

Fig. 4-5. Effect of 5-Br-PSAA concentration. ○: complex; △: net; □: blank.

4.3.2. Analytical performances

The analytical signals for obtaining a calibration curve in a range of 0 – 1.0 mg L^{-1} are shown in Fig. 4-6. A very good linearity was showed by its linear equation (y = 0.101x) with a correlation coefficient (r^2 = 0.998). In a previous paper,[103] chemical and physical parameters for the complex formations of palladium with 5-Br-PSAA have been optimized in the flow system. Thus, the analytical performance of the proposed flow system was compared with the FIA.[103] As summarized in Table 4-2, the sensitivity and repeatability between these two methods are comparable. The time taken per one analysis by SIEMA is shorter than that of FIA. Furthermore, the 5-Br-PSAA consumption of the proposed system is less than in FIA method, hence SIEMA could save reagent consumption.

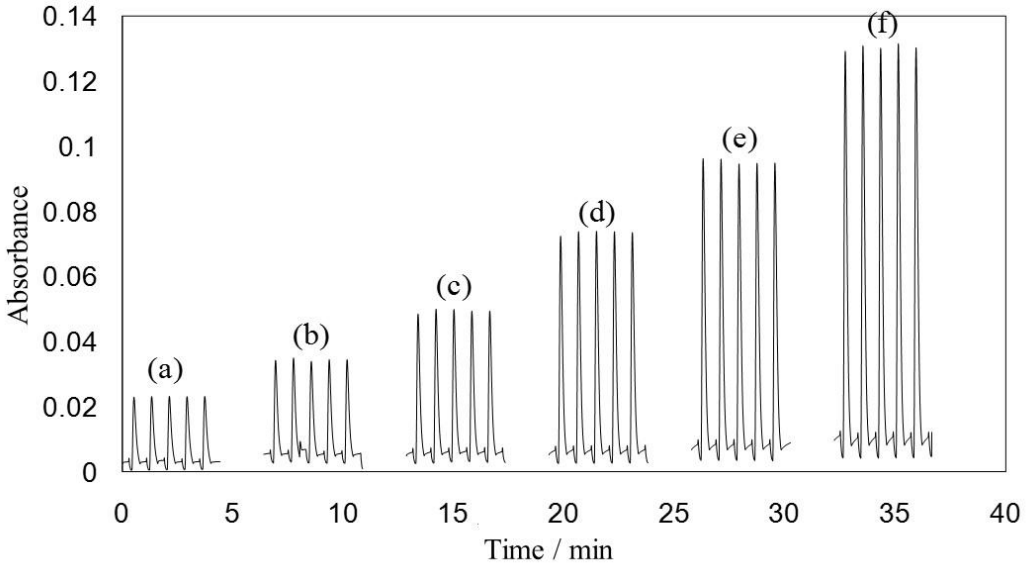

Fig. 4-6. System output response to standard palladium (II) concentrations in mg L^{-1}. (a), 0; (b), 0.10; (c), 0.25; (d), 0.50; (e), 0.70; (f), 1.00.

Table 4-2. Comparison of the analytical performances between this work and FIA.[103]

Parameter	SIEMA	FIA
Reagent consumption / one determination in mol	$3.0x10^{-8}$	$1.6x10^{-7}$
Waste volume / ten determinations in mL	15	21
Time taken / one determination in sec	53	64
Linear range in mg L^{-1} palladium	0 - 1.0	0 – 0.1
Linear regression equation	y = 0.101x	y = 0.181x
Regression coefficient	0.998	0.999
3σ limit of detection in μg L^{-1}	1.8	2.0
Repeatability (RSD %) for 0.1 mg L^{-1}	1.0[a]	0.6[b]

a, n = 5; b, n = 10

4.3.3. Interferences

Different ions were added to a 0.5 mg L^{-1} palladium standard solution. The effects of the coexisting substances was considered tolerable within ±5%. The results are shown in Table 4-3. Cu(II) showed the largest interference in the complex formation with 5-Br-PSAA. However, Pd contained in samples that do not contain Cu, can be quantified without problems.

Table 4-3. Tolerance limits on the determination of 0.5 mg L^{-1}.

Tolerance / mg L^{-1}	Ion added
500	Cd(II), Pb(II), Se(IV), Br^-, Cl^-, NO^-
200	Fe(III)
100	Ni(II), Zn(II)
50	Al(III), In(III)
20	Rh(III)
10	Fe(II)
5	Ag(I), Co(II)
0.5	Au(III), Pt(IV)
0.02	Cu(II)

4.3.4. Determination of palladium in alloys

The proposed SIEMA was applied to the determination of palladium in dental alloy and palladium carbon. The pretreatment of the samples before their injection into the flow system was carried out through their acid digestion with nitric acid for dental alloy and aqua regia for palladium carbon, respectively. The results obtained with the proposed method and by ICP-MS are summarized in Table 4-4 and 4-5. The recovery rates of spiked palladium concentrations in both samples, an average of 101% for dental alloy (for three spiked palladium concentrations of 0.1, 0.25 and 0.5 mg L^{-1}) and an average of 99% palladium carbon (for two spiked palladium concentrations of 0.1 and 0.25 mg L^{-1}), obtained with the proposed method are close to 100%. As well, the palladium content values obtained of 9.6% for dental alloy and 10.2% for palladium carbon, are in agreement the labeled value of palladium content in sample of 10%. Furthermore, the comparison of the results in the obtained by SIEMA and inductively coupled plasma mass spectrometry (ICP-MS) showed a good agreement.

Table 4-4. Determination of palladium in dental alloy[a].

Added/ mg L^{-1}	Proposed method			ICP-MS	
	Found/ mg L^{-1}	Recovery, %	Pd content in sample, %	Found/ mg L^{-1}	Pd content in sample, %
0	0.191±0.004	-	9.5	0.200±0.003	9.9±0.2
0.1	0.291±0.003	100	9.5		
0.25	0.440±0.003	100	9.4		
0.5	0.702±0.004	102	10		
			Ave. 9.6±0.3		

a. Labeled value of Pd content is 10%

Table 4-5. Determination of palladium in palladium carbon[a].

Added/ mg L^{-1}	Proposed method			ICP-MS	
	Found/ mg L^{-1}	Recovery, %	Pd content in sample, %	Found/ mg L^{-1}	Pd content in sample, %
0	0.213±0.004	-	10.3	0.200±0.003	9.7±0.2
0.1	0.313±0.003	101	10.3		
0.25	0.459±0.003	99	10.1		
			Ave. 10.2±0.1		

a. Labeled value of Pd content is 10%

4.4. Conclusions

The proposed SIEMA shows good accuracy in the determination of palladium in dental and palladium carbon alloys. Furthermore, the system reduces the reagent consumption and waste generation compared with a conventional FIA. The SIEMA system can be applied to other chemical analysis.

Chapter 5 Advanced Stopped-in-Loop Flow Analysis with Reagents-Merging Zones Technique for Catalytic Successive Determination of Vanadium and Iron[104]

5.1. Introduction

Recently, vanadium has attracted attention because an oxidized form of vanadium appears to have an insulin-like action.[70] It was reported that the consecutive administration of drinking water including vanadate resulted in a significant reduction of blood glucose levels in human diabetes.[71] However, its biological functions have not been clearly established. Iron plays an important role in several biological functions such as hemoglobin and myoglobin generation and cell proliferation. Nevertheless, excessive intake of iron can increase the risk of developing type 2 diabetes.[73] Nowadays, bottled mineral water including small amounts of vanadium and/or iron is on the market for the benefit to health. Therefore, a detection method with a low detection limit is needed for the simultaneous monitoring of vanadium and iron for water quality control.

Many reports have been published on flow injection spectrophotometric methods for the determination of vanadium, based on its catalytic action.[79-81,105-107] Also, flow injection spectrophotometric methods for the determination of iron, based on catalytic,[82-84,108] redox,[109] and complexation[110-112] reactions. However, the number of papers on flow injection spectrophotometry for simultaneous/successive determination of two different metal ions (including two oxidation states of a metal) in the same sample is limited. For instance, binary mixtures of iron(III)/vanadium(V),[113] iron(III)/chromium(VI),[113] and vanadium(IV)/vanadium(V),[114] were selectively determined using the redox reactions, and also catalytic methods for iron/copper[115,116] and vanadium/iron[67,88] were proposed.

In recent years, our research group has proposed alternative concepts for flow injection analysis (FIA) systems, called stopped-in-loop flow analysis (SILFA)[3] and stopped-in-dual-loop flow analysis (SIDLFA)[98] to reduce the reagent consumption compared with conventional FIA. Furthermore,

simultaneous injection effective mixing flow analysis (SIEMA) system having advantages in terms of simple automation, rapid analysis and high sensitivity was proposed.[96,97,117]

In a previous study,[67] the SILFA concept was applied to the successive catalytic determination of vanadium and iron in water samples, developing an automated system with better analytical performance compared with the results obtained with conventional FIA systems. However, the SILFA concept still has a drawback, *i.e.*, when reagent(s) and sample solutions are loaded into the stopped-in-loop, a part of the solution is discarded into a waste bottle in order to exchange the solution inside the loop with the newly loaded solution completely. However, the amounts of discarded reagent(s) and sample can be minimized by coupling a reagents-merging zones technique to a SILFA system.

The aim of this work is to develop an advanced SILFA system, which can reduce reagent consumption and waste generation, is proposed for successive catalytic determination of vanadium and iron in drinking mineral water samples.

5.2. Experimental

5.2.1. Reagents

All the reagents were of analytical grade. Deionized water used to prepare solutions was obtained from an Aquarius GSH-210 apparatus (Advantec, Tokyo).

Common reagents. Commercially available 1000 mg L^{-1} vanadium(V) and 1000 mg L^{-1} iron(III) standard solutions (Wako, Osaka) were used as stock solutions. Each working solutions were prepared by serial dilution of the standard solutions with 0.01 mol L^{-1} nitric acid (Sigma-Aldrich Japan, Tokyo). A 0.25 mol L^{-1} *p*-anisidine stock solution was prepared by dissolving 6.16 g of *p*-anisidine (Alfa Aesar, US) in 200 mL of 3 mol L^{-1} hydrochloric acid (Sigma-Aldrich Japan). A 4 mol L^{-1} acetate buffer solution was made from solutions of acetic acid (Sigma-Aldrich Japan) and sodium acetate trihydrate (Wako).

Vanadium determination. A 0.5 mol L^{-1} Tiron solution was prepared daily by dissolving 3.93 g of 1,2-dihydroxy-3,5-benzenedisulfonic acid disodium salt monohydrate (Tokyo Chemical Industry, Tokyo) in 25 mL of water. A mixed reagent solution of 0.06 mol L^{-1} *p*-anisidine, 1 mol L^{-1} acetate buffer, and 0.08 mol L^{-1} Tiron was daily prepared by mixing the stock solutions and adjusting the pH to 2.9 with 10 mol L^{-1} sodium hydroxide (Wako). The mixed solution was delivered from RS_1 shown in Fig. 5-1, when the optimization study with an experimental design technique (see "5.3.1. Optimization" section) has been carried out. A 0.1 mol L^{-1} diphosphate stock solution was prepared by dissolving 4.46 g of sodium diphosphate decahydrate (Wako) in 100 mL of water. The addition of diphosphate as a masking agent for iron in RS_1 allowed us to improve the selectivity in the vanadium determination. Eventually, a mixed solution (pH 2.9) of 0.06 mol L^{-1} p-anisidine, 1 mol L^{-1} acetate buffer, 0.08 mol L^{-1} Tiron, and 0.015 mol L^{-1} diphosphate was therefore delivered from RS_1. A bromate solution of 0.09 mol L^{-1} was daily prepared by dissolving 0.301 g of potassium bromate (Wako) in 20 mL of water and delivered from RS_2.

Iron determination. A 0.01 mol L^{-1} 1,10-phennanthroline (phen) solution was prepared by dissolving 0.0991 g of 1,10-phennanthroline monohydrate (Tokyo Chemical Industry) in 50 mL of 0.1 mol L^{-1} hydrochloric acid. A mixed solution of 0.05 mol L^{-1} p-anisidine, 1 mol L^{-1} acetate buffer, and 5×10^{-4} mol L^{-1} phen was prepared daily, and the pH of the solution was adjusted to 3.8 with 10 mol L^{-1} sodium hydroxide. The mixed solution was delivered from RS_3. A 0.1 mol L^{-1} hydrogen peroxide solution (delivered from RS_4) was prepared from a 1 mol L^{-1} stock solution, which was made by 10-fold dilution of 30% hydrogen peroxide solution (Wako) with water.

5.2.2. Apparatus and procedure

A schematic representation of the proposed system in this work is shown in Fig. 5-1. The system mainly consisted of a syringe pump (SP), a syringe pump valve (SPV), five three-way solenoid valves (SV_1–SV_5), and two six-port switching valves (SWV_1, SWV_2). All these components were controlled

by a personal computer in order to perform the analytical operations shown in Table 5-1. So long as the same standard/sample was repeatedly determined, the steps 1–7 were repeated. When a standard/sample solution was replaced with another standard/sample solution, the sampling tubing connected to the SWV_1 was washed by the aspiration of an appropriate volume of another solution and sent to the waste. The same heater device described in a previous paper[67] was used for heating the reaction solution filled in the loop. Spectrophotometric measurements were made using an Ocean Optics USB2000 UV-VIS detector equipped with a z-shaped flow-through cell (28 μL, 15 mm path length). Absorbance was measured at 510 nm for both analytes. The detector output signal was recorded with a SpectraSuite (Ocean Optics, US) software. The manifold was built using 0.8 mm inner diameter Teflon tube, and only the auxiliary coil and the back pressure coil were of 2.0 mm and 0.25 mm inner diameter Teflon tube, respectively.

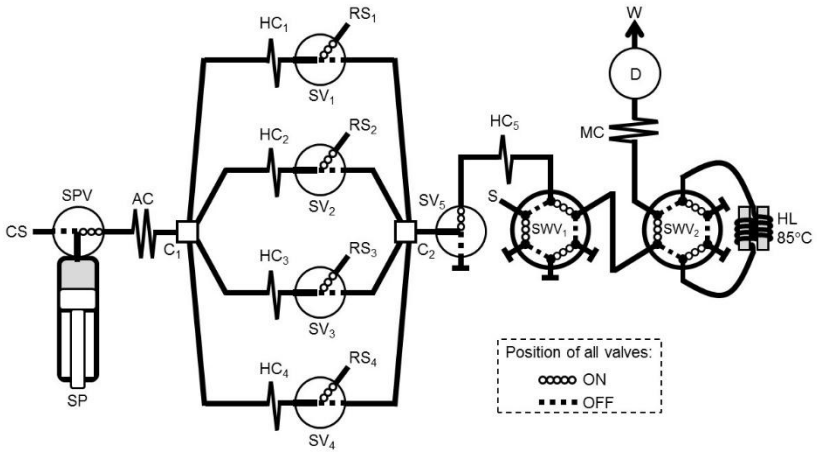

Fig. 5-1. Schematic representation of the proposed hybrid system. CS: 0.01 mol L^{-1} HNO_3 carrier solution; RS_1: 0.06 mol L^{-1} *p*-anisidine + 1 mol L^{-1} acetate buffer (pH 2.9) + 0.08 mol L^{-1} Tiron + 0.015 mol L^{-1} diphosphate; RS_2: 0.09 mol L^{-1} $KBrO_3$; RS_3: 0.05 mol L^{-1} *p*-anisidine＋1 mol L^{-1} acetate buffer (pH 3.8)＋5×10^{-4} mol L^{-1} 1,10-phenanthroline; RS_4: 0.1 mol L^{-1} H_2O_2; S: V or Fe standard (or sample); SP: syringe pump; SPV: syringe pump valve; AC: auxiliary coil; C_1, C_2: five-way cross connector; HC_1, HC_2, HC_3, HC_4, HC_5: holding coil; SV_1, SV_2, SV_3, SV_4, SV_5: solenoid valve; SWV_1, SWV_2: six-port switching valve; HL: heated loop; MC: mixing coil; D: detector (510 nm); BPR: back pressure restrictor; W: waste.

Table 5-1. Operation sequence of the analytical method for vanadium or iron determination[a].

Step	SPV	Motion direction of SP	SV_2	SV_2	SV_3	SV_4	SV_5	SWV_1	SWV_2	Description
1	ON	↓	ON[b] OFF[c]	ON[b] OFF[c]	OFF[b] ON[c]	OFF[b] ON[c]	OFF	ON	ON	Aspiration of reagents into "HC_1 and HC_2" [b] or "HC_3 and HC_4".[c]
2	ON	↑	OFF	OFF	OFF	OFF	ON	ON	ON	Dispense reagents to HC_5 for contacting the sample.
3	ON	↓	OFF	OFF	OFF	OFF	ON	OFF	ON	Aspiration of sample into HC_5.
4	ON	↑	OFF	OFF	OFF	OFF	ON	ON	OFF	Loading reagents and sample into HL.
5	ON	↑	OFF	OFF	OFF	OFF	ON	ON	ON	Reaction in HL, and washing the main line.
6	OFF	↓	OFF	OFF	OFF	OFF	ON	ON	ON	Reaction in HL, and loading CS into syringe.
7	ON	↑	OFF	OFF	OFF	OFF	ON	ON	OFF	Signal monitoring for peak.

a. Abbreviations (SPV, SP, SV_1–SV_5, SWV_1, SWV_2, HC_1–HC_5, HL, and CS) as in Fig. 5-1.

b. For vanadium determination.

c. For iron determination.

5.3. Results and Discussion

5.3.1. Optimization

In preliminary experiments, the optimal operational conditions involving the stopped-in-loop step (stopping time, reaction temperature, loop volume, and mixing coil length) were set in order to

improve the analytical signal. The stopping time of 180 seconds, the reaction temperature of 85°C, the loop volume of 500 μL, and the mixing coil length of 50 cm were selected.

Table 5-2. Optimal operation conditions obtained by an experimental design.

Variable	Optimal Value
Common [a]	
Sample volume (μL)	100
Reagents volume (μL) [b]	400
Flow rate (μL s^{-1})	200
Vanadium	
p-anisidine (mol L^{-1})	0.06
$KBrO_3$ (mol L^{-1})	0.09
Tiron (mol L^{-1})	0.08
pH	2.9
Iron	
p-anisidine (mol L^{-1})	0.05
H_2O_2 (mol L^{-1})	0.1
1,10-phennanthroline (mol L^{-1})	5×10^{-4}
pH	3.8

a. These common experimental valuables were optimized using the iron-catalyzed reaction.

b. The total aspiration volume (400 μL) from RS_3 and RS_4: 200 μL of each reagent was aspirated. The optimized total aspiration volume was also used in the vanadium-catalyzed reaction. In this case, 200 μL of each reagent was aspirated from RS_1 and RS_2.

The optimal condition of each variable (shown in Table 5-2) to produce the best possible analytical response was determined by performing a Box-Behnken response surface experimental design with the model 2k, which allows the efficient estimation of their first and second order

interactions.[118,119] A computer statistics package, Minitab 15 (Minitab, US), was used to build the response surface experimental design, achieving a total of 69 experimental runs. The effects of individual factors and their second order interactions were thus investigated. We finally obtained the optimized conditions summarized in Table 5-2.

In order to improve the selectivity of the vanadium determination, we examined the effect of diphosphate concentration as a masking agent for iron (we did not used the experimental design with a statistical procedure in this study). In the presence of 0.015 mol L^{-1} diphosphate in the RS_1, 400 μg L^{-1} iron was tolerable for the determination of 1 μg L^{-1} vanadium.

5.3.2. Analytical characteristics

Linear calibrations graphs were obtained for vanadium and iron in the ranges of 0 – 5 μg L^{-1} (slope = 0.0336, r^2 = 0.997) and 0 – 50 μg L^{-1} (slope = 0.0053, r^2 = 0.999), respectively. The 3σ limits of detection of the proposed method (0.67 μg L^{-1} for vanadium, 2.20 μg L^{-1} for iron) were higher than those obtained with previous FIA methods (*e.g.*, 0.01 μg L^{-1} for vanadium[80] and 0.05 μg L–1 for iron[82]) and with a previous SILFA method[67] (0.052 μg L^{-1} for vanadium and 0.55 μg L^{-1} for iron). The RSD values (n = 5) of the present work were 2.4% for 5 μg L^{-1} vanadium and 2.8% for 50 μg L^{-1} iron, respectively. The repeatability was comparable to the previous SILFA method,[67] whose RSD values (n = 5) were 2.2 for 1 μg L^{-1} vanadium and 2.3% for 10 μg L^{-1} iron, respectively. However, the sensitivities of the proposed method for the successive determination of vanadium and iron were better than a catalytic spectrophotometric method for the simultaneous determination of mg L^{-1} levels of vanadium and iron.[88] The sample throughput of this method was 13 samples h^{-1} (275 s per one determination of vanadium or iron). The throughput was better than that of the previous method[67] 10 samples h^{-1} (345 s per one determination of vanadium or iron). Furthermore, the total volume of reagents and sample consumption per analysis (500 μL) was dramatically reduced, compared with

that of the SILFA method (approximately 10 mL),[6 7] achieving a greener and more economic method. Also, the smaller size of the proposed hybrid flow analysis system allowed more miniaturization the FIA and SILFA systems. The proposed system was fully automated and controlled by a personal computer providing an easier interface with the final user.

Table 5-3. Tolerance limits of foreign ions on the determination of 1.0 μg L^{-1} vanadium or 10 μg L^{-1} iron.

Tolerance limit / μg L^{-1}	Ion added [a]	
	Vanadium	Iron
50000	Cl^-, Co(II), K(I), Mg(II), Na(I), Pb(II)	NO_3^-, SO_4^{2-}
30000	Ba(II), Ca(II), Cd(II), Mn(II), Ni(II), NO_3^-, Se(IV), SO_4^{2-}	Ca(II), Cl^-, K(I), Na(I)
10000	Si(IV)	Cd(II)
5000		Se(IV)
3000	Zn(II)	Si(IV)
2000		Mg(II)
1000	Al(III), Cr(VI)	Ba(II), Mo(VI), Zn(II)
600		Pb(II)
400	Fe(III), Mo(VI)	
200		Co(II), Ti(IV)
100	Cu(II), Ti(IV)	Mn(II), V(V)
50		Al(III), Cu(II), Cr(VI), Ni(II)

a. An error of ±5% or less was considered to be tolerable.

5.3.3. Interferences

Cu(II) and Ti(IV) showed the largest interference at concentration of over 100 μg L^{-1} in the determination of 1 μg L^{-1} of vanadium. For determination of 10 μg L^{-1} of iron, Al(III), Cu(II), Cr(VI), and Ni(II) gave remarkable interference at the concentration of over 50 μg L^{-1}. However, the presence of these ions is not expected in such concentrations in drinking mineral water samples, allowing the application of this method. Table 5-3 summarizes the tolerance limits of foreign irons on the determination of vanadium or iron.

5.3.4. Determination of vanadium and iron in water samples

The proposed system was validated by the analysis of three certified water samples: JSAC 0302-3b (river water) issued by Japan Society for Analytical Chemistry, SLRS-5 (river water), and SLEW-3 (estuarine water) issued by National Research Council of Canada. Typical system outputs for vanadium and iron in certified reference material of river water (JSAC 0302-3b) are shown in Fig. 5-2.

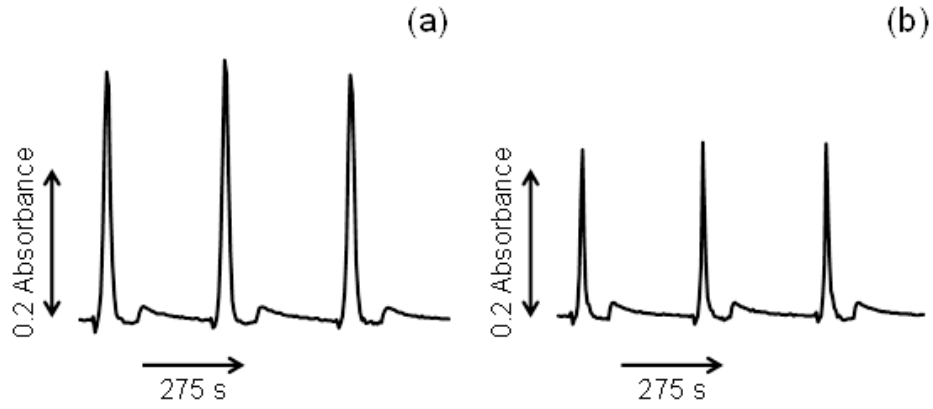

Fig. 5-2. Typical system outputs for (a) vanadium and (b) iron in certified reference material of river water (JSAC 0302-3b).

Table 5-4 shows the results obtained with the hybrid flow system and their comparison with the certified values for vanadium and iron (we determined the concentration of vanadium in the JSAC 0302-3b by GFAAS, because there is no certified value of vanadium). There were no significant differences between the analytical values and the certified/reference values, although some concentrations were lower than the LOD of the proposed method.

Table 5-4 Determination of vanadium and iron in certified reference materials.

Sample	Proposed method / μg L^{-1}		Certified (or reference) value / μg L^{-1}	
	V	Fe	V	Fe
JSAC 0302-3b [a]	7.44±0.01	59.1±0.005	7.43±0.007 [d]	59.6±1.5
SLRS-5 [b]	< LOD	90.8±0.006	0.317±0.033	91.3±5.8
SLEW-3 [c]	2.71±0.04	< LOD	2.57±0.31	0.568±0.059

a. Certified reference material of river water issued by Japan Society for Analytical Chemistry. The sample was diluted to 2-fold before measurements for both vanadium and iron.

b. Certified reference material of river water issued by National Research Council of Canada. The sample was diluted to 4-fold before measurement for iron.

c. Certified reference material of estuarine water issued by National Research Council of Canada.

d. Reference value obtained by GFAAS in our lab.

Four commercially available drinking mineral waters were analyzed for vanadium and iron contents by the proposed system. As shown in Table 5-5, the analytical results of vanadium were in agreement with the labeled values. Furthermore, satisfactory results were obtained for the recovery of spiked iron.

Table 5-5. Determination of vanadium and iron in drinking mineral water.

Sample No. [a]	V Labeled / μg L^{-1}	Added / μg L^{-1}		Found / μg L^{-1}	
		V	Fe	V	Fe
1	30	0	0	$30.3 \pm 0.0_1$	<LOD
		0	20.0	–	$21.7 \pm 0.0_1$
2	70	0	0	$69.9 \pm 0.0_2$	<LOD
		0	20.0	–	$21.5 \pm 0.0_1$
3	90	0	0	$90.1 \pm 0.0_1$	<LOQ
		0	20.0	–	$20.0 \pm 0.0_0$
4	160	0	0	$160 \pm 0._0$	<LOQ
		0	20.0	–	$20.0 \pm 0.0_1$

a. For vanadium determination, samples 1–3 were diluted to 20-fold, and sample 4 was diluted to 40-fold before measurement.

5.4. Conclusions

The proposed advanced stopped-in-loop flow analysis allows the determination of vanadium and iron in drinking mineral waters, offering the advantages of low consumption of sample and reagents, a minimal waste generation, optimum sensitivity and good reproducibility. The proposed technique shows a good accuracy in its working range, making it suitable for drinking water samples.

Chapter 6 Automatic On-Line Solid-Phase Extraction-Electrothermal Atomic Absorption Spectrometry Exploiting Sequential Injection Analysis for Trace Vanadium, Cadmium and Lead Determination in Human Urine Samples[120]

6.1. Introduction

Several metals and their compounds have long been recognized as important toxic agents, causing acute and chronic poisoning cases in environmental high-exposure situations. On the other hand, there are some metals, like vanadium, which are essential for the living organisms at low concentrations, but can cause a number of problems to human health when the uptake is too high. Among toxic heavy metals, cadmium and lead in urine, with low-to-moderate chronic exposure, reflect an integrated exposure over time and a total body burden.[121] According to the "Agency for Toxic Substances and Disease Registry" (ATSDR), normal cadmium and lead concentrations in human urine in the general population (≥ 6 years of age) are 0.185 and 0.677 μg L^{-1}, respectively, while the normal vanadium concentration in human urine is 0.5 μg L^{-1}.[122]

Atomic spectrometric (AS) techniques, such as flame absorption atomic spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) as well as mass spectrometry (ICP-MS), have been extensively employed for metal determination in various types of samples. However, direct trace metal determination is usually difficult due to the low analyte concentration and matrix complexity. Therefore, a preconcentration and/or separation step prior to the final measurement is usually required. The combination of on-line solid phase extraction (SPE) with ETAAS has proved to be of considerable interest, providing increased sensitivity and selectivity as well as higher recovery.[123-125] As a result, it has become one of the most commonly used and greenest alternative sample pretreatment techniques due to its simplicity, low cost, reduced sample and reagents consumption.[126] In this context, sequential injection (SI) analysis is a well-established automated-pretreatment (Auto-Pret) platform, offering great advantages in the automation and miniaturization of the analytical methods.[2,30,127-129]

Over the last few decades, research in the field of solid phase extraction has focused on the synthesis and study of new sorbent materials with improved characteristics,[130] like co-polymeric beads Oasis HLB,[131,132] carbon nanotubes,[133-135] magnetic particles,[126,136] lysine-modified mesoporous silica (Fmoc-SBA-15),[137] polytetrafluoroethylene (PTFE),[138] polyether–ether–ketone (PEEK),[139] since the selection of the appropriate one is of prime importance for the sensitivity and selectivity of an analytical method. Nobias chelate PA-1, first introduced by Sakamoto *et al.*[140] and Yamamoto *et al.*,[141] is a chelating adsorbent consisting of a hydrophilic methacrylate polymer backbone, which is chemically modified by amino and carboxylic groups. In the batch mode, this type of chelating adsorbent has been successfully employed for the ICP-MS determination of trace metal ions in environmental water samples (seawater, tap water)[140-153] and food samples.[154] On the other hand, the use of Nobias chelate PA-1 as an adsorbent for on-line column preconcentration is a new task in the field of on-line sample preparation with limited applications of metal determination in water samples[155] and leached solutions from ceramic ware.[127]

The aim of this work was to develop a fully automated SI system for the on-line column preconcentration and determination of trace metal ions in biological samples using Nobias chelate PA-1 resin as the sorbent material. The proposed method was demonstrated for vanadium, cadmium and lead determination by ETAAS in human urine. As far as we are concerned, this is the first reported application of the above-mentioned resin in a minicolumn format for on-line V(V), Cd(II) and Pb(II) preconcentration and determination in biological samples. All major chemical and hydrodynamic factors affecting the analyte adsorption and elution, such as sample acidity, eluent type and its concentration, loading and elution flow rates as well as the ETAAS operating conditions were systematically studied and optimized. The accuracy of the proposed method was evaluated by analyzing a certified reference material and a spiked urine sample.

6.2. Experimental

6.2.1. Instrumentation

A polarized Zeeman graphite furnace atomic-absorption spectrometer, Z-2700 (Hitachi High-Technologies, Tokyo, http://www.hitachi-hitec.com/global/science/index.html), with a pyrolitically coated graphite tube platform cuvette was used throughout the measurements. Hitachi High-Technologies single element hollow cathode lamps (HCL) for vanadium, cadmium and lead operated at 10, 7.5 and 7.5 mA respectively, were used as light sources. The wavelength was set at 318.4, 228.8 and 283.3 nm for vanadium, cadmium and lead, respectively, and the monochromator spectral bandpass (slit) was set at 1.3 nm for each metal. The integrated absorbance (peak area) was used for signal evaluation throughout the study. The graphite furnace temperature/time program for each metal determination is summarized in Table 6-1.

Table 6-1. Graphite furnace temperature program for vanadium, cadmium and lead determination in 50 μL of eluent

Step	Temperature / ^{o}C			Ramp	Hold	Argon flow rate
	V	Cd	Pb	time / s	time / s	/ mL min^{-1}
Drying	120	120	140	100	100	200
Pyrolysis	900	300	700	20	0	200
Atomization	2700	1500	2400	0	5	0
Cleaning	2800	2800	2500	0	10	200

An Auto-Pret SI system, programmable by a personal computer, reported previously by the authors, [127] was used for the automatic process of the proposed method. The SI system, as shown in Fig. 6-1, consisted of a 3-port syringe pump, equipped with a 5 mL glass syringe barrel (Hamilton PSD/4, USA, http://www.hamiltoncompany.com/home.php), an 8-port selection valve (Hamilton, USA) and a 6-port 2-position injection valve (Hamilton, USA). The pump and valves were computer controlled by a

program written by Visual Basic software. A trigger switch was placed next to the graphite furnace and, which was turned on by the movement of the autosampler arm of the graphite furnace (GF) for synchronization of the Auto-Pret-SI-SPE system with ETAAS.

PTFE tubing was used for the flow lines (0.5 mm i.d.) and the holding coil (1.5 mm i.d.). The length of tubing used for the delivery of the eluent into the graphite tube was kept as short as possible in order to minimize the dispersion and the dead volume in the proposed system.

A Horiba (Kyoto, Japan, http://www.horiba.com/) pH-meter was used for pH measurements.

6.2.2. Sorbent material-column preconcentration

Nobias chelate PA-1® resin (Hitachi High-Tech Fielding, Tokyo) is a reversed-phase solid-phase extraction sorbent material comprising of a hydrophilic poly(hydroxy-methacrylate) based porous resin functionalized with ethylenediaminetriacetic acid (EDTriA) and iminodiacetic acid (IDA) compounds acting as chelating groups. This type of resin selectively adsorbs transition and alkaline earth metals via chelation with the polyamino-polycarboxylic sites in a suitable pH range. In addition, Nobias chelate PA-1 can also retain oxyanions, such as V(V), Cr(VI) and U(VI) (but not As(III)).[143] In a pH 4 – 8, V(V) is probably retained as the vanadate anion $H_2VO_4^-$, having a similar sorption behavior with other chelating resins containing iminodiacetate groups, like Chelex 100;[156] namely there would be an applicable interaction between such oxyanions and protonated amino groups from the resin at a pH around 6 (we chose this pH for retention). It should be mentioned that Na, K, Ca and Mg ions are rarely adsorbed at a pH of less than 7,[140] which is a significant advantage for urinary analysis.

An amount of 40 mg of Nobias chelate PA-1resin was packed firmly into a methacrylate (40 mm length, 2.6 mm i.d.) on-line preconcentration column (M&G CHEMATechs JAPAN) with glass-wool frits at both ends for sorbent immobilization. The minicolumn was attached to the 6-port injection

valve (IV) between ports 3 and 5, as shown in Fig. 6-1. As it was revealed from the whole experimental study, the performance of the proposed minicolumn was consistent for at least 1000 preconcentration cycles without any decrease in the integrated absorbance. This is an essentially important advantage for keeping the process cost down in routine analysis.

6.2.3. Reagents and samples

All chemicals were of analytical reagent grade. Ultra-pure quality water, produced by an Elix 3/Milli-Q Element System (Nihon Millipore, Tokyo, Japan), was used throughout all experiments. All metal standard solutions were prepared by appropriate stepwise dilution of 1000 mg L^{-1} V(V), Cd(II) and Pb(II) stock standard solutions in 0.5 mol L^{-1} HNO_3 (Wako Pure Chemical Industries, Osaka) prior to use. The working standard solutions and samples were adjusted to pH 6.0 using an ammonium acetate buffer solution. An ammonium acetate buffer solution at a concentration level of 0.1 mol L^{-1} was prepared by dissolving an appropriate amount of solid ammonium acetate (≥ 97%, analytical grade, Sigma-Aldrich Japan, Tokyo) in water, and being adjusted to pH 6.0 with a 10% (v/v) nitric acid solution. The eluent was a 1.0 mol L^{-1} HNO_3 aqueous solution prepared from concentrated nitric acid (60% (v/v) HNO_3, analytical grade, Sigma-Aldrich Japan). Laboratory glassware was rinsed with ultra-pure water and decontaminated overnight in 10% (v/v) HNO_3.

The accuracy of the developed method was estimated by analyzing the following standard reference material (CRM): Seronorm™ Trace Elements Urine Level 1, containing trace elements in urine. Moreover, a urine sample was taken from a healthy adult and digested, using concentrated nitric acid. The digestion procedure was carried out at 130 – 140ºC, using the START D microwave digestion system (Milestone General K.K., Kawasaki, Japan) under the recommendations of the manufacture.

6.2.4. Analytical procedure

A schematic diagram of the flow system for trace metal determination by ETAAS is presented in Fig. 6-1, and the operation sequences of the analytical method are summarized in Table 6-2. Each analytical cycle starts with the aspiration of a small volume (50 μL) of air into a holding coil (HC) in order to eliminate any dispersion of aqueous solutions with the solution of the carrier (water). Initially, conditioning of the minicolumn takes place by delivering 1000 μL of an ammonium acetate buffer solution (pH 6) in order to activate the sorbent material. In steps 4 and 5, a volume of 4500 μL of the standard or sample is aspirated into the HC and, consecutively, dispensed through the minicolumn (C) at a flow rate of 100 μL s^{-1} for analyte preconcentration. Thereafter, valve IV is switched to the "elution" position, and the minicolumn is evacuated by a segment of 700 μL of air. In the following steps (8, 9 and 10), 1000 μL of the eluent (1.0 mol L^{-1} HNO_3), 50 μL of air and 500 μL of 1.0 mol L^{-1} HNO_3 are aspirated sequentially into the HC for elution purposes. The elution is performed in the reverse direction than that of the loading procedure in order to avoid any analyte dispersion into the segment of the eluent. Next, 470 μL are dispensed through the minicolumn in order to elute the retained analyte and transport the front portion of the HNO_3 segment up to the edge of the nozzle of the autosampler. After that, the arm of the autosampler of ETAAS moves towards the dosing hole of the graphite tube, and presses the trigger switch, which is placed at an appropriate position next to the furnace. Once the trigger switch is pressed (step 12), the front 50 μL portion of the eluent segment, containing the highest analyte amount, is dispensed into the graphite tube for atomization and measurement. The atomization program of ETAAS runs in parallel, and is synchronized with the program of Auto-Pret-SI. During the following steps (13, 14 and 15), a thorough cleaning of the minicolumn, syringe and tubing is accomplished so as to eliminate any possible sample carryover. Five replicate measurements are made in all instances.

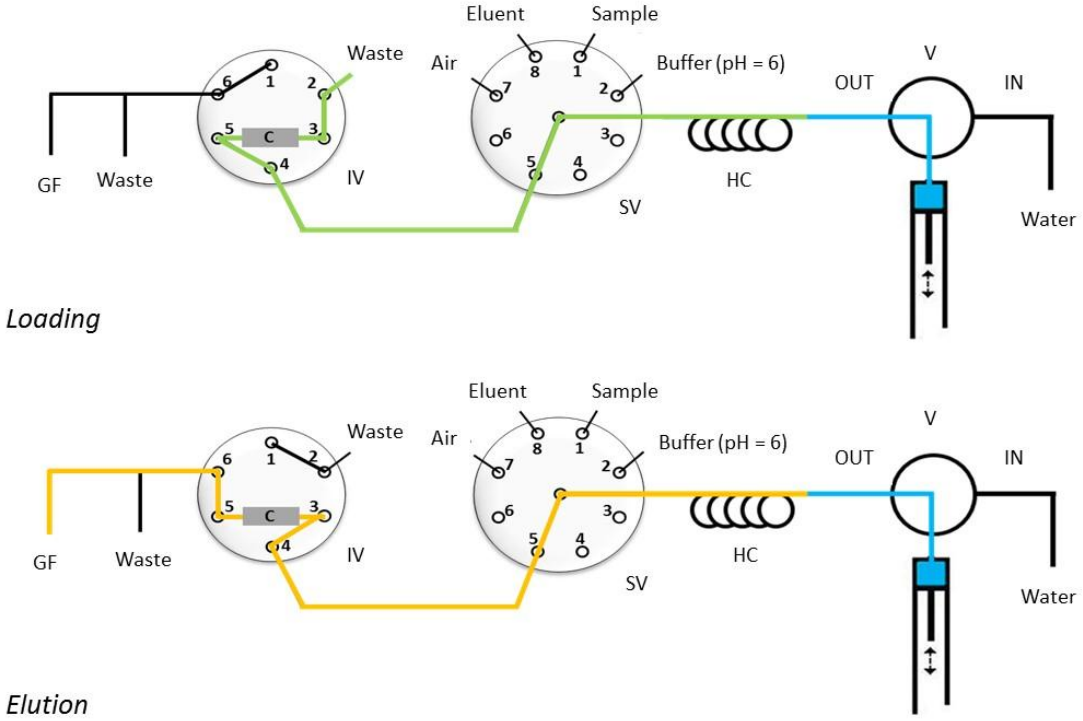

Fig. 6-1 Schematic diagram and the operation sequences (step 5: loading and step 11: elution) of the Auto-Pret-SI-SPE-ETAAS system for metal determination. IV, injection valve; SV, selection valve; V, valve; HC, holding coil; GF, graphite furnace; C, minicolumn packed with Nobias chelate PA-1.

Table 6-2. Operation sequences of the analytical method for metals determination.

Step	Position			Operation	Flow rate	Volume	Comment
	V	SV	IV		/ μL s^{-1}	/ μL	
1	OUT	7	L	Aspirate	50	50	Aspiration of air segment
2	OUT	2	L	Aspirate	250	1000	Aspiration of buffer solution
3	OUT	5	L	Dispense	100	1000	Conditioning of the minicolumn
4	OUT	1	L	Aspirate	200	4500	Aspiration of sample solution
5	OUT	5	L	Dispense	100	4500	Loading of sample /preconcentration
6	OUT	7	E	Aspirate	250	700	Aspiration of air segment
7	OUT	5	E	Dispense	100	700	Evacuation of the minicolumn
8	OUT	8	E	Aspirate	250	1000	Aspiration of eluent segment
9	OUT	7	E	Aspirate	50	50	Aspiration of air segment
10	OUT	8	E	Aspirate	50	500	Aspiration of eluent segment
11	OUT	5	E	Dispense	50	470	Elution and transportation of the eluent up to the edge of the nozzle
				Autosampler arm into the hole of graphite tube			
12	OUT	5	E	Dispense	10	50	Injection of 50 μL of the eluent into the graphite tube
				Autosampler arm into standby position			
13	OUT	5	E	Dispense	100	1080	Evacuation of the minicolumn
14	IN	5	E	Aspirate	200	1000	Aspiration of water
15	OUT	5	E	Dispense	200	1000	Cleaning of the minicolumn and the tubing

6.3. Results and discussion

6.3.1. Optimization study

The chemical and hydrodynamic parameters associated with the loading and elution procedures of the proposed method were investigated, so that optimum analytical conditions could be reached. Standard aqueous solutions of V(V), Cd(II) and Pb(II) at 2.0, 0.050 and 0.3 μg L^{-1} concentration levels, respectively, at a fixed loading sample (each standard solution) volume of 3.0 mL were used for the optimization study.

6.3.1.1. Effect of the pH

The pH value of the sample solution plays a key role to the quantitative adsorption of target metal ions on the surface of the chelating resin, controlling the preconcentration process. The effect of the pH on the integrated absorbance values for V(V), Cd(II) and Pb(II) was studied in the range of 3.0 to 9.0 by adjusting it by adding either a 10% (v/v) HNO_3 solution or 0.1 mol L^{-1} ammonium acetate as a buffer solution. The obtained results are presented in Fig. 6-2. The absorbance increased with an increase of the pH up to 5.0, while higher sensitivity was achieved at between 5.0 and 7.0, where the absorbance was leveled off for all metals. At higher pH values the signals decreased due to possible hydrolysis, which leads to the precipitation of metal hydroxides, as has been reported previously.[127, 140] The low signals at lower pH values are attributed to the protonation of EDTriA and IDA functional groups, resulting in inefficient complex formation. Consequently, a pH value of 6.0 ± 0.2 was selected for all subsequent experiments.

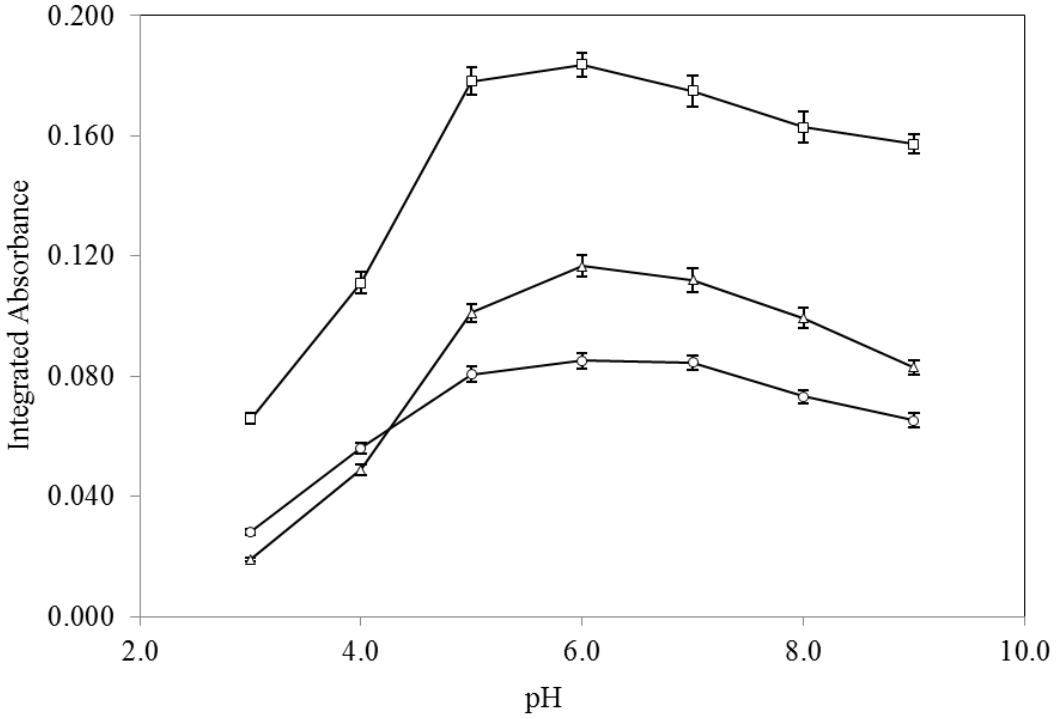

Fig. 6-2. Effects of the pH on the integrated absorbance values of 2.0 μg L^{-1} V(V) (△), 50.0 ng L^{-1} Cd(II) (□) and of 0.3 μg L^{-1} Pb(II) (○). Volume of a loaded standard solution: 3.0 mL; all other experimental parameters as in Table 6-2.

6.3.1.2. Effect of the eluent

The quantitative desorption of the retained analytes from the surface of a sorbent material strongly depends on the eluting agent and its concentration. An ideal eluent should offer fast and sufficiently strong elution ability in a volume as small as possible so as to achieve a high preconcentration ratio. As revealed from preliminary experiments, comparing nitric and hydrochloric acids, a solution of 500 μL of 1.0 mol L^{-1} HNO_3 could effectively elute the retained metals. Thus, nitric acid was adopted as the eluent. The effect of the nitric acid concentration on the sensitivity of the method was studied in the range 1.0–2.5 mol L^{-1}. The obtained results showed that the absorbance was not influenced by the nitric acid concentration in the studied range for all metals. Therefore, 1.0 mol L^{-1} HNO_3 solution was

selected as the eluent for subsequent experiments, while considering the fact that higher nitric acid concentrations might reduce the lifetime of the graphite tube of ETAAS.

6.3.1.3. Effect of the loading flow rate

In on-line sorbent extraction systems, the loading flow rate affects the preconcentration efficiency as well as the time of analysis through complex formation, and its retention on the sorbent surface. Both the exchange kinetics and the back-pressure of the column depend on the speed that the sample solution passes through the minicolumn. The effect of the loading flow rate on the absorption of the analytes was studied in the range between 10.0 and 100.0 μL s^{-1}. The experimental results showed that the sample loading flow rate had no significant effects on the integrated absorbance values for all metals in the studied area, indicating that each sorption was very fast within the studied range. Thus, a loading flow rate of 100 μL s^{-1} was selected for the preconcentration step, considering not only the time of analysis and the sampling frequency, but the sensitivity as well.

6.3.1.4. Effect of the elution flow rate

The effect of the elution flow rate on the sensitivity of the method was examined within the range of 10.0 – 100 μL s^{-1}. As is shown in Fig. 6-3, a higher absorbance for all metal ions was achieved at flow rates of between 10.0 and 50.0 μL s^{-1}. The reduced analytical signals for values higher than 50.0 μL s^{-1} are attributed to the short contact time of the eluent with the sorbent material, possibly due to the slow elution kinetics and the higher dispersion of the analytes into the segment of eluent. Therefore, a flow rate of 50.0 μL s^{-1} was adopted for further experiments as a compromise between the sensitivity and the time of analysis.

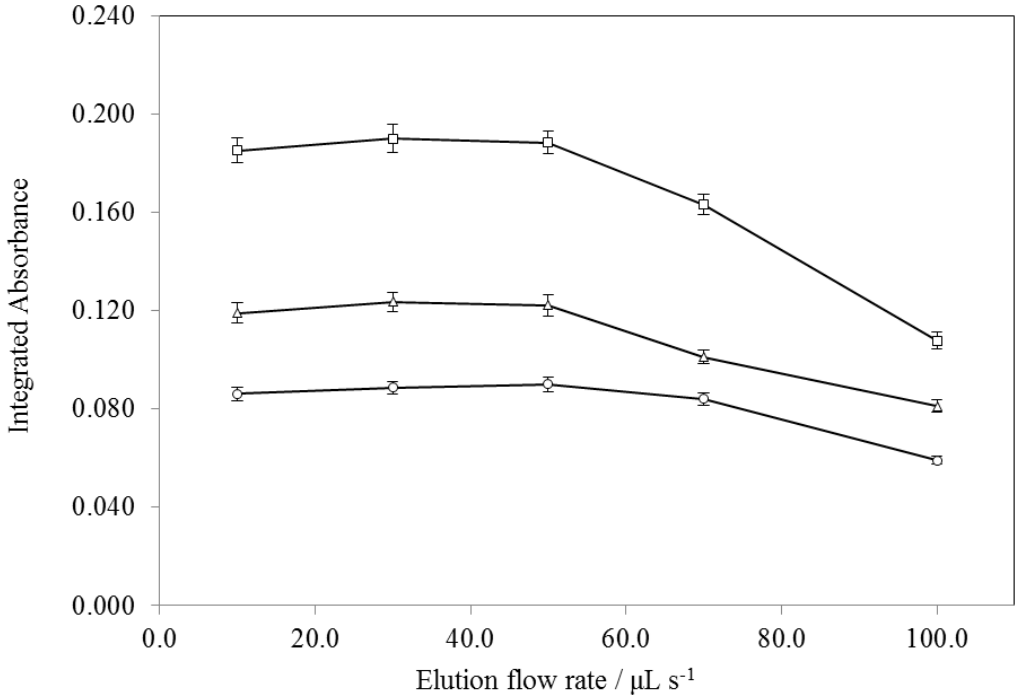

Fig. 6-3. Effects of the elution flow rate on the absorbance values of of 2.0 μg L^{-1} V(V) (△), 50.0 ng L^{-1} Cd(II) (□) and of 0.3 μg L^{-1} Pb(II) (○). Volume of a loaded standard solution: 3.0 mL; all other experimental parameters as in Table 6-2.

6.3.1.5. Effect of the injection volume of eluent into the graphite furnace

Since the ETAAS signal depends on the analyte mass, there is an effective degree of control on the recorded absorbance by controlling the injected sample volume. Generally, a larger injected volume of sample contains a greater absolute amount of the analyte available for atomization, which results in a higher sensitivity of the method. For very low concentrations, the maximum volume of the analyte can be used, while for higher concentrations the sample volume can be reduced. On the other hand, the maximum volumes of the sample that can be used depend on the graphite tube configuration. Without a graphite platform, sample volumes at up to 100 μL can be used, while with the platform in place, a volume of up to 50 μL is recommended.

In the proposed method the first portion of the zone of the eluent is injected into the graphite tube, as presented in the analytical procedure section. Taking into account that the concentration of the analytes along the zone of the eluent is varied, and a higher concentration is in the front side, the effect of the injection volume of the eluent on the absorbance values for the three analytes was studied in the range of 10 – 50 μL. A volume of 50 μL gave a higher sensitivity, and was used throughout the experiments.

6.3.1.6. Effect of the sample volume

An efficient and sensitive volume-based on-line preconcentration system largely depends on the sample volume, which is directly related to the amount of analyte retained on the surface of the sorbent material during the preconcentration procedure. The influence of the sample volume on the sensitivity of the method was studied in the range between 0.5 and 4.5 mL under the optimized conditions. The experimental results confirmed a practically proportional increase of the recorded signal with the increase of the sample volume up to at least 4.5 mL. Therefore, a sample volume of 4.5 mL was selected for the proposed method as a compromise between the sensitivity and low consumption of the sample as well as the analysis time. On the other hand, for higher sensitivity and lower detection limits, a larger sample volume could be adopted by increasing the total time of the analysis.

6.3.2. Interferences

The interferences of commonly ions existing in biological fluids, like urine, as well as some heavy metals were examined regarding their competition for the active sites on the sorbent, for the determination of 1.0 μg L^{-1} V(V), 15.0 ng L^{-1} Cd(II) and 1.0 μg L^{-1} Pb(II) using the optimized procedure. A variation in the recovery greater than ± 5 % was considered to be interference. The metal ions of Al(III), Cr(VI), Fe(III), Mn(II) and Zn(II) can be tolerated at least up to 2.0 mg L^{-1} for

vanadium, cadmium and lead determination, as was revealed from the experimental results. Moreover, for each analyte, the presence of the other two metals can be tolerated up to 1.0 mg L^{-1}. In addition, common cations, such as Na(I), K(I), Ca(II) and Mg(II) do not interfere up to a concentration level of 500 mg L^{-1}.

6.3.3. Analytical characteristics

The analytical performance characteristics of the developed Auto-Pret-SI-SPE-ETAAS method for V(V), Cd(II) and Pb(II) determination under the optimal conditions are summarized in Table 6-3. For a sample consumption of 4.5 mL, the sampling frequency was 27 h^{-1} and the enhancement factors (calculated by the ratio of the slopes of the calibration curves obtained with and without preconcentration using ETAAS) were 21, 37 and 12 for vanadium, cadmium and lead, respectively. The detection limits (c_L), based on the 3*s* criterion (according to IUPAC[157]), were found to be 3.0, 0.06 and 2.0 ng L^{-1} for vanadium, cadmium and lead determination, respectively, while the precision of the method, evaluated as the relative standard deviation (RSD), was between 1.9 and 3.7%. The analytical performance of the Auto-Pret-SI-SPE-ETAAS method for cadmium determination with ICP-MS in terms of the detectability and sample consumption is better[127,158] than and comparable[148,155] to others reported in the literature. In addition, the proposed method presents a 3-orders of magnitude better detection limit for cadmium determination than the previous reported one using a similar SI-SPE-ETAAS system.[127]

The accuracy of the proposed method was estimated by analyzing the standard reference material, Seronorm™ Trace Elements Urine Level-1, containing trace elements in urine. The student *t*-test was employed to examine any possible statistically significant differences between the obtained and certified values of the determined metals.[159] The t_{exp} values were calculated by the following equation:

$$t_{\text{exp}} = (\bar{x} - \mu)\frac{\sqrt{n}}{s},$$

where $\bar{x}$ is the sample mean value, μ is the certified value, s is the sample standard deviation of the sample values and n is the replicate determinations (n = 3). In this case, there are two degrees of freedom (n – 1), and therefore the t value obtained from the table of student t-destribution, t_{crit}, is 4.303 at the 95% probability level. The analytical values and t_{exp} values for V, Cd and Pb determinations in the above CRM are given in Table 6-4. Since all t_{exp} values are lower than, t_{crit} = 4.303, no statistically significant differences were found at the 95% probability level, indicating the applicability of the developed method in urine type samples.

Table 6-3. Analytical performance characteristics of the analytical method for vanadium, cadmium and lead determination under the optimized conditions.

	Vanadium[a]	Cadmium[b]	Lead[a]
Sample volume / mL	4.5	4.5	4.5
Sampling frequency, f / h^{-1}	27	27	27
Enhancement factor	21	37	12
Linear range	0.011 – 3.0	0.20 – 40.0	0.007 – 2.0
Detection limit, c_L / ng L^{-1}	3.0	0.06	2.0
Precision, RSD, % (n = 10)	2.2 % (at 1.0 µg L^{-1})	1.9 % (at 10.0 ng L^{-1})	3.7 % (at 0.8 µg L^{-1})
Regression equation ([Pb], [V] in µg L^{-1}, [Cd] in ng L^{-1}, n = 9)	A = (0.0925 ± 0.0015) [V] + (0.0014 ± 0.0019)	A = (0.0057 ± 0.0000) [Cd] + (0.0015 ± 0.0019)	A = (0.1272 ± 0.0049) [Pb] + (0.0016 ± 0.0051)
Correlation coefficient (r)	0.9996	0.9997	0.9989

a. Concentration in µg L^{-1}, b. Concentration in ng L^{-1}.

Table 6-4. Determination of vanadium, cadmium and lead in CRM, Seronorm™ Trace Elements Urine L-1.

Analytes	Certified value / μg L^{-1}	Found[a] / μg L^{-1}	Relative error, %	t_{exp}
V	0.66 ± 0.08	0.63 ± 0.04	4.5	1.299
Cd	0.20 ± 0.04	0.194 ± 0.01	3.0	1.039
Pb	0.66 ± 0.13	0.62 ± 0.06	6.1	1.155

a. Mean value ± standard deviation based on three replicates.

t_{crit} = 4.303 at 95 % probability level.

6.3.4. Analytical applications

The method was applied for the determination of V, Cd and Pb in urine samples collected by an adult healthy person. The analysis was completed under the optimum conditions of the proposed method and the recovery estimated by the standard addition. The results are presented in Table 6-5. The recoveries of the three metals varied within the range of 94.0 – 98.0%, showing that the proposed method can be successfully applied to the simultaneous determinations of V, Cd and Pb in urine samples with good performance.

Table 6-5. Analytical results of cadmium, lead and vanadium determination in urine sample by the Auto-Pret-SI-SPE-GFAAS method

Analyte	Added[a]	Found[a, b]	Recovery, %
V	-	0.45 ± 0.02	-
	0.50	0.94 ± 0.04	98.0
	1.00	1.42 ± 0.05	97.0
Cd	-	21.42 ± 0.65	-
	10.00	30.85 ± 1.10	94.3
	15.00	35.92 ± 1.20	96.7
Pb	-	1.05 ± 0.05	-
	0.50	1.52 ± 0.05	94.0
	1.0	2.02 ± 0.07	97.0

a. Concentration in μg L^{-1} for V and Pb determination and in ng L^{-1} for Cd determination.

b. Mean value ± standard deviation based on three replicates.

6.4. Conclusions

A new on-line Auto-Pret-SI-SPE method has been developed for metal determination by ETAAS. The use of Nobias chelate PA-1resin as an adsorbent in a handmade minicolumn format has been demonstrated for the first time for the on-line column preconcentration of vanadium, cadmium and lead in urine samples. The chemical stability, large surface area and fast kinetics of the proposed sorbent material as well as its long lifetime (> 1000 analytical cycles) make it very attractive for on-line SPE systems. The proposed method is considered to be a simple, rapid, accurate and low-cost approach for the routine monitoring of trace-level concentrations of metal species in biological samples.

Chapter 7 Conclusions

Nowadays, the application of simple, rapid and sensitive methods is required for trace analysis. As well, a reduction and sample consumption and waste generation is desirable. In this study, automated flow analysis systems with the characteristics mentioned above were developed.

In the chapter 2, a multiparametric automated monitoring system was developed for sulfate, nitrite and nitrate in drinking and wastewater. The turbidimetric method was employed for sulfate detection. Meanwhile, nitrite was detected with a Griess-Illosvay modified reaction. Nitrate was reduced to nitrite by the action of a copperized cadmium reduction column followed by its detection as total nitrite and calculated by difference of nitrite concentration without and with pass through the reduction copperized cadmium column. These chemical reactions were introduced to a sequential injection analysis obtaining a robust system with high precision. This method is capable of monitoring drinking and wastewater. Also, this method can be applied to another chemical analysis and its application *in situ* could be plausible.

In the chapter 3, an automated stopped-in-loop flow analysis system for monitoring of trace vanadium and iron in drinking water was proposed. The detectable reaction for vanadium is based on its catalytic effect on the oxidation of *p*-anisidine by bromate in the presence of Tiron as activator. Meanwhile, the catalytic effect of iron on the oxidation of *p*-anisidine by hydrogen peroxide in the presence of 1,10-phenanthroline as activator was used. The catalytic reaction was accelerated in the loop located on the six-way valve, meanwhile all the pumps were stopped during the heating step. This procedure leads to diminish reagent consumption and waste generation.

In chapter 4, a simultaneous injection effective mixing analysis system was introduced aiming for an enhancement in the mixing of the reagent(s) and sample to obtain a better analytical performance. This system was small compared with flow injection analysis system. Furthermore, this proposed method reduces drastically the reagent consumption and waste generation compared with a conventional FIA. The SIEMA system can be applied to other chemical analysis.

In the chapter 5, taking advantage of the main analytical performance characteristics of SIEMA and SILFA an advanced stopped-in-loop flow analysis with reagents-merging zones technique for catalytic successive determination of vanadium and iron. The same chemical reactions described in chapter 3 aiming for low consumption of sample and reagents and a minimal waste generation were introduced to this system. Therefore, this method was environment-friendly. This proposed technique shows a good accuracy in its working range, making it suitable for drinking water samples.

In the chapter 6, a multiparametric fully automated solid-phase extraction coupled with an electrothermal atomic absorption spectrometry exploiting sequential injection analysis was developed. A handmade minicolumn packed with chelating resin (NOBIAS CHELATE PA1) was used for the pre-concentration of vanadium, cadmium and lead. All protocol for the on-line SPE method was controlled by a home-made software. A trigger switch placed aside the graphite furnace was employed to synchronize the software with built-in one in the GFAAS. The proposed system showed high sensitivity comparable to ICP-MS. Vanadium, cadmium and lead were detectable even at ppt levels in human urine samples by this method.

The proposed flow-base techniques have big advantages over conventional methods, such as simplicity, rapidity and sensitivity. As well, these flow-based system are expected to be applied to different environmental and biological samples or in quality tests.

It is necessary and important to preserve our environment for future generations, therefore, the development of high technology on trace analysis and speciation is required. The evolution to environmental-friendly flow-based analysis techniques can be achieved.

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List of publications

1. Alejandro Ayala, Luz Olivia Leal, Laura Ferrer, Victor Cerda,
   Multiparametric automated system for sulfate, nitrite and nitrate monitoring in drinking water and wastewater based on sequential injection analysis, *Microchem. J.*, **2012**, *100*, 55–60.

2. Georgia Giakisikli, Alejandro Ayala Quezada, Junpei Tanaka, Aristidis N. Anthemidis, Hiroya Murakami, Norio Teshima, Tadao Sakai,
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3. Alejandro Ayala Quezada, Hiroya Murakami, Norio Teshima, Tadao Sakai, Shoji Motomizu,
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4. Alejandro Ayala Quezada, Keisuke Ohara, Nuanlaor Ratanawimarnwong, Duangjai Nacapricha, Hiroya Murakami, Norio Teshima, Tadao Sakai,
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5. Alejandro Ayala Quezada, Daisuke Noguchi, Hiroya Murakami, Norio Teshima, Tadao Sakai,
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