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Multielement analysis of micro-volume biological samples by ICP-MS with highly efficient sample introduction system

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ABSTRACT

A method for multielement analysis of micro-volume biological sample by inductively coupled plasma mass spectrometry (ICP-MS) with a highly efficient sample introduction system was presented. The sample introduction system was the combination of (1) an inert loop injection unit and (2) a high performance concentric nebulizer (HPCN) coupled with a temperature controllable cyclone chamber. The loop injection unit could introduce 20 μL samples into the carrier liquid flow of 10 μL min^{-1} producing a stable signal for 100 s without any dilution. The injection loop is continuously washed with 0.1 M HNO_3 carrier solution during the measurement, thereby much improving sample throughput. The HPCN is a triple tube concentric nebulizer, which can generate fine aerosols and provide a stable and highly measurement sensitivity in ICP-MS at a liquid flow rate less than 10 μL min^{-1}. With the combination of the chamber heating at 60 °C, the sensitivity obtained with the proposed sample introduction system at the liquid flow rate of 10 μL min^{-1} was almost the same as that with a common concentric nebulizer and cyclone chamber system at the liquid flow rate of 1 mL min^{-1}, though the sample consumption rate of the HPCN was two orders of the magnitude lower than that of the common nebulizer. The validation of the proposed system was performed by analyzing the NIST SRM 1577b Bovine Liver. The observed values for 12 elements such as Na, P, S, K, Ca, Mn, Fe, Co, Cu, Zn, Mo, Cd were in good agreement with their certified values and information value. Satisfactory analytical results for 14 elements such as Na, Mg, P, S, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Y, Ba in *Escherichia coli* sample were also obtained. The proposed sample introduction system was quite effective in the cases when only micro-volume of biological sample is available.

1. Introduction

Inductively coupled plasma mass spectrometry (ICP-MS) is a powerful technique for trace and ultra-trace element analyses in various research fields [1,2]. Nowadays, its applications extend to a low volume sample analysis especially in clinical [3] and biological [4–7] research fields, in which the available sample volume is limited. However, a typical sample introduction system consisting of conventional nebulizers and spray chambers are not suited for these low volume sample analyses due to their large sample consumption, which lead to a poor absolute detection limit [8,9]. Conventional nebulizers consume 1–5 mL of sample for multielement measurement, because they are designed to work at sample introduction rate of 0.1–2 mL min^{-1}. In addition, only 1–10% aerosols are introduced into the plasma, because coarser aerosol droplets should be cut off by a spray chamber to maintain the stability of the plasma. To overcome these drawbacks, various low consumption sample introduction systems have been proposed [10–20].

Low sample consumption nebulizers are quite effective tools for limited amount and highly efficient sample introduction. There are two types of nebulizers; one is a direct injection type nebulizer such as a direct injection nebulizer (DIN) and a direct injection high efficiency nebulizer (DIHEN) [10–13]. The DIN types are directly mounted inside the plasma torch by replacing the center tube of the torch. The DIN types provide 100% aerosols transport into the plasma, but suffer from the following disadvantages; it is fragile and complicated handling, the nozzle is often clogged with total dissolved solids (TDS) which easily deposit inside the nozzle by heating with the plasma. The other one is a microflow type nebulizer with a narrow bore liquid capillary such as the micro-concentric nebulizers (nDS-200 [14], nDS-5[15], CEI-100[16]), the

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high efficiency nebulizer (HEN) [17], and the parallel path nebulizer (AriMist) [18]. The performance of an aerosol generation strongly influences the sample introduction efficiency into the plasma and also the sensitivity of ICP-MS. In addition, a high TDS tolerance is also needed for application to trace element analysis of a small amount of biological samples, since these samples contain large amount of alkaline and alkaline earth elements. However, an efficient aerosol generation and a high TDS tolerance are incompatible for the above nebulizers. Indeed, the HEN can generate fine aerosols and provides a high sensitivity in ICP-MS [19], but shows a poor TDS tolerance due to a narrow gas annulus area [8,9]. On the contrary, the AriMist exhibits a high TDS tolerance, because a liquid flow does not touch the nebulizer gas orifice in their bodies, but its structure is not suitable for a fine aerosol generation.

We developed a high performance concentric nebulizer (HPCN) for less than 10 μL min^{-1} flow rate sample introduction to ICP-MS [20–22]. The HPCN has a demountable triple tube concentric structure consisted of a concentric type nebulizer body and a tapered fused silica glass capillary mounted in the center of the nebulizer body. By the unique structure, a microthread liquid flow is formed inside the nebulizer nozzle by a capillary flow focusing. This phenomenon gives both a fine aerosol generation and a high TDS tolerance [20]. The HPCN is applicable to quantification of trace elements in a small amount of biological sample, but the following challenges still remain for its application. First is a throughput of the measurement. At a liquid flow rate of less than 10 μL min^{-1}, a replacement of solution in liquid flow line is time consuming, which restricts the throughput of analysis [23]. Second is a transport efficiency of aerosols into the plasma. A part of aerosols is lost by the impact against the wall of a chamber [24,25]. Therefore, the transport efficiency would be further improved by prevention of the impact loss.

As response to the above challenges, in this study, we propose a high throughput sample introduction system consisting of (1) an inert loop injection unit and (2) the HPCN-heating chamber unit. The loop injection unit can reduce sample consumption as well as realize high throughput analysis. When a 20 μL sample solution was introduced by the loop injection into the carrier liquid flow of 10 μL min^{-1}, it can be measured for 2 min without any dilution. The injection loop can be washed with the carrier solvent during the measurement without any solution replacement, thereby much improving sample throughput. The HPCN can generate fine aerosol; over 90% of the primary aerosols generated by the HPCN are below the diameter of 10 μm [20]. The heating of the chamber could contribute to not only improve the aerosol transport towards the plasma but also enhance the plasma thermal characteristics with a vapor of water [25,26]. Therefore, a high sensitivity and stability in ICP-MS could be realized with the combination of the HPCN and the heating chamber. In this study, the analytical performance of the sample introduction system was evaluated by the analysis of a reference material, and then applied to multielement analysis of a small amount of *Escherichia coli* sample.

2. Experimental

2.1. Sample introduction system

The high-efficient sample introduction system was combination of an inert loop injection unit and a HPCN-heating chamber unit. A schematic illustration of the sample introduction unit is shown in Fig. 1.

The loop injection unit (KP-11 model, Ogawa. Co. Ltd., Japan) was constructed of an inert double plunger pump and an inert micro injection valve equipped with a 20 μL PEEK sample loop. A

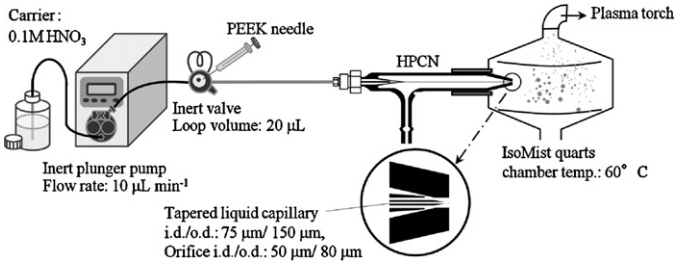

Fig. 1. Schematic illustration of the proposed sample introduction system.

polypropylene micro syringe attached with a PEEK needle was used for the sample loading. A carrier solution was 0.1 M HNO_3.

The HPCN was constructed of a customized concentric type nebulizer body (ONIZUKA Glass/ST JAPAN, Tokyo, Japan) and a tapered liquid capillary (straight line i.d./o.d.: 75 μm/150 μm, tip orifice i.d./o.d.: 50 μm/80 μm) mounted in the center of the nebulizer body. The tapered capillary was made from a fused silica capillary (75 μm i.d./150 μm o.d., 12 μm polyimide coating, GL Sciences, Japan). The procedure of the tapering was similar as described in the Ref. [20]. In brief, a certain part of the polyimide coating was stripped with a fire, and the stripped part was burned off with an arc discharge under a mechanical tension. The burned end of the capillary was polished with an alumina lapping film (#8000, Sumitomo 3M, Japan). The tapered capillary was set at the proper position recessed by 100 μm from the nebulizer nozzle tip. The capillary and the nebulizer body were fixed with a 1/16 FEP sleeve and a 1/8 to 1/16 PTFE joint adapter. The loop injection unit was connected to the PTFE joint adapter with a 150 mm of a 1/16 PTFE tube (i.d. 130 μm, Glass Expansion, Australia).

A temperature controllable cyclone chamber unit was IsoMist (Quarts chamber type, Glass Expansion, Australia), which can control the chamber wall temperature between −20 °C and 60 °C. The HPCN was attached with a default adapter and was set at the position where a maximum sensitivity of ICP-MS was obtained.

2.2. Instrumentation

The ICP-MS used was ELEMENT 2 (Thermo Fisher Scientific, Bremen, Germany). The operating conditions were summarized in Table 1. A conical nebulizer (Glass expansion) and a cyclone chamber without any temperature control were used as a conventional sample introduction system for a comparison. The operating conditions were optimized to obtain a maximum signal intensity of $^{89}Y^{+}$. At the optimum conditions, the oxide formation ratio YO^{+}/Y^{+} was 0.5% with the proposed system and 1.5% with the conventional system. An internal standard correction using Rh and Re was applied in the determination, where Re was used as the internal standard for Ba and Pb, and Rh was used for the other elements.

2.3. Reagent, sample and equipment

Working standard solutions of 17 elements (Na, Mg, P, S, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, Ba, and Pb) and internal standard elements (Rh and Re) were prepared by diluting proper amounts of their inorganic standard solutions (1000 mg L^{-1} each, Kanto Chemical Industries, Ltd., Japan). Nitric acid and hydrogen peroxide were of ultrapur grade (Kanto Chemical). Pure water prepared by a Milli-Q water purification system (resistivity 18 MΩ cm, Nihon Millipore Kogyo, Tokyo, Japan) was used throughout the experiments. Luria-Bertani (LB) agar and NaCl used for cultivation of *E. coli* were purchased from Wako Pure Chemical (Japan).

The standard reference material 1577b bovine liver was purchased from National Institute of Standards Technology (NIST,

Table 1
Operating conditions of the ELEMENT2.

Plasma conditions	
Rf frequency	27.12 MHz
Incident Rf power	1.5 kW
Reflected power	<2 W
Outer gas flow rate	16 L min^{-1}
Intermediate gas flow rate	0.90 L min^{-1}
Carrier gas flow rate	1.05 L min^{-1} for HPCN 0.95 L min^{-1} for conical nebulizer
Sampling conditions	
Sampling depth	−2 mm
Sampling and skimmer cone	Platinum cones
Proposed sample introduction system	
Nebulizer	HPCN
Solution flow rate	10 μL min^{-1}
Spray chamber	IsoMist set at 60 °C
Conventional sample introduction system (for comparison)	
Nebulizer	Conical nebulizer
Solution flow rate	1 mL min^{-1} (natural aspirate)
Spray chamber	Cyclone chamber at room temperature (i.e. 27 °C)
Mass resolution setting	Medium resolution m/Δm 4000
Data acquisition	
Scanning mode	E-Scan
Integrated mass window	50%
Data points	20 points/peak
Dwell time	10 ms/point
Integration	5 times
Repetition	5 times
Measured m/z	$^{23}Na^+$, $^{26}Mg^+$, $^{31}P^+$, $^{32}S^+$, $^{39}K^+$, $^{44}Ca^+$, $^{52}Cr^+$, $^{55}Mn^+$ $^{56}Fe^+$, $^{59}Co^+$, $^{60}Ni^+$, $^{63}Cu^+$, $^{66}Zn^+$, $^{89}Y^+$, $^{95}Mo^+$, $^{111}Cd^+$, $^{137}Ba^+$, $^{208}Pb^+$ Internal standard elements: $^{103}Rh^+$, $^{185}Re^+$

USA). *E. coli* strain MG1655 was kindly donated by Ajinomoto Co., Inc., Japan.

All equipments such as digestion vessels, micropipette tips, sample tubes, injection syringe were soaked in 2 M HNO_3 for 3 days, then rinsed with the Milli-Q water and dried in a clean box until use.

2.4. Sample preparation procedure

The SRM 1577b were digested with a microwave digestion system (ETHOS E, Milestone General, Italy). Approximately 100 mg of the SRM1577b was weighted into a PTFE digestion vessel, into which 1.5 mL of HNO_3 and 0.5 mL of H_2O_2 were added. The temperature program of a microwave irradiation was ramped to 200 °C for 30 min and hold for 10 min. After cooling the vessels, proper amount of the internal standard solution contained Rh and Re was added, and then the solution in the vessel was transferred into a poly-propylene bottle and diluted with MilliQ water up to 20 g. A blank was also prepared in the same manner.

The *E. coli* cells were plated onto LB agar culture plates and incubated at 37 °C for 24 h. The cells were suspended with 10 mL of physiological salt solution (0.9%, g/g NaCl), followed by centrifugation at 4500 × *g* for 5 min at 4 °C. These procedures were repeated two times. ca. 10 mg of the centrifuged cells was weighted into the digestion vessel, into which 1.45 mL of HNO_3, 0.5 mL of H_2O_2, and 50 mg of the internal standard solution (20 μg L^{-1} of Rh and Re) were added. The temperature program of a microwave irradiation was the same as the bovine liver. After cooling, 0.5 mL of Milli-Q water was added, and then the mixture was measured as the sample solution for Cr, Mn, Ni, Cu, Zn, Y, and Ba. For Na, Mg, P, S, K, Ca, and Fe, 1 mL of the mixture was further diluted with Milli-Q water up to 10 mL.

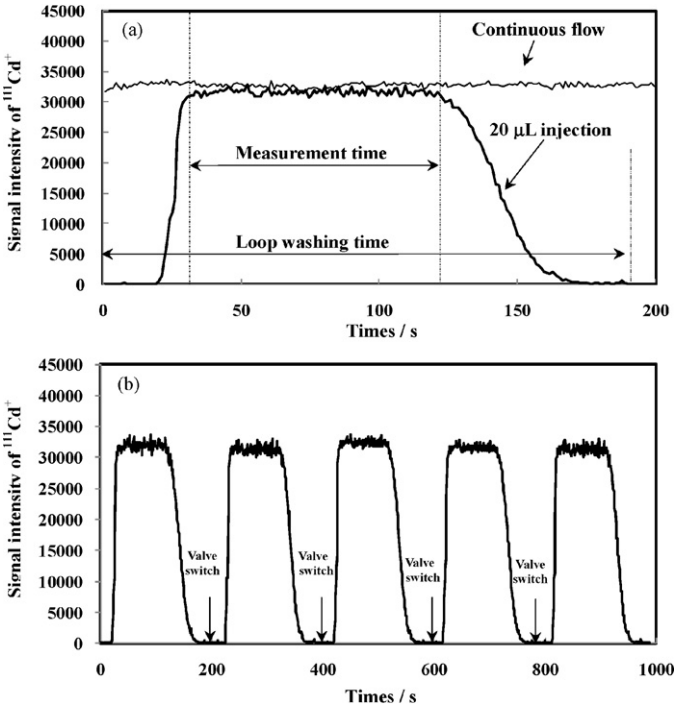

Fig. 2. Measurement profiles of 10 μg L^{-1} Cd: (a) the profiles for the continuous flow and 20 μL injection and (b) the profile for back to back injection of 20 μL.

3. Results and discussion

3.1. Performance of the loop injection unit

Fig. 2(a) shows the measurement profiles of 10 μg L^{-1} Cd for 20 μL sample injection in the 10 μL min^{-1} carrier solution flow, and for the continuous sample introduction at the flow rate of 10 μL min^{-1}. A stable flat-top-signal was obtained for the 20 μL injection. The time of the steady state signal was ca. 100 s, which is adequate time to measure fifteen elements. The signal stability at the steady state was 1.5%, which was the same level as the signal stability obtained by the continuous sample introduction (1.2%). It is clearly seen that the signal intensity at the steady state was almost the same intensity obtained by the continuous sample introduction. The dispersion coefficient (D) in the loop injection was calculated by dividing an average of the signal intensity of the continuous sample introduction (C^0) with highest signal intensity (steady state signal) obtained by the loop injection unit (C^{max}). The D value was 1.02, which means that only <2% of the injected sample diluted with the carrier solution during the flow towards plasma.

The loop injection has an advantage for a high throughput measurement. In a continuous sample introduction method, a replacement of solution and a washing flow line are time consuming. When the conventional system is used in a continuous sample introduction method, typically 3 min are additionally required between the measurements for the replacement of solution and the washing flow line. Almost the same time was required when a continuous sample introduction to the HPCN nebulizer at a liquid flow rate of less than 10 μL min^{-1}. In contrast, the replacement and washing time can be greatly reduced with the loop injection method, because the sample loop and flow line to the nebulizer is continuously washed with the 0.1% HNO_3 carrier solution flow during the measurement. Thus, a back to back injection can be carried out. Fig. 2(b) shows the profile for the back-to-back injection of 20 μL into the 10 μL min^{-1} carrier solution flow. Five sample injections can be measured during 1000 s (ca. 16.7 min) and thus

one measurement requires ca. 3.3 min, which is about a half of the time required for the continuous sample introduction method. In addition, the repeatability of the steady state signal obtained by the five replicate injections was 1.4%. The repeatability would be improved by using an internal standard correction method. These results indicate that the loop injection unit can not only reduce sample consumption but also achieve high throughput analysis without loss of sensitivity and precision.

3.2. Effect of the spray chamber temperature on analytical sensitivity

In order to determine the influence of the spray chamber wall temperature on the analytical sensitivity, the signal intensity obtained with the loop injection-HPCN-IsoMist system at 10 μL min^{-1} liquid flow rate was measured at the various chamber wall temperatures from 15 °C to 60 °C. Fig. 3 shows relative intensities of P, S, Fe, Cu, Zn and Cd at four different chamber wall temperatures, where the relative intensity means the ratio of the signal intensity obtained with the loop injection-HPCN-IsoMist system at each chamber wall temperature to the signal intensity obtained using the conventional sample introduction system at the liquid flow rate of 1 mL min^{-1}. It was observed that the relative intensity increased with the increase in the chamber temperature from 15 °C to 45 °C, and it tends to be stable from 45 °C to 60 °C. It should be noted here that an adequate precision of signal intensity was also achieved at 60 °C (relative standard deviation, RSD < 2%). The relative intensities at 60 °C were in the range of 0.97–1.25, it means that the sensitivity with the loop injection-HPCN-IsoMist system was almost the same as than that with the conventional system at the liquid flow rate of 1 mL min^{-1}, though the sample consumption rate of the HPCN was two orders of the magnitude lower than that of the common concentric nebulizer. The oxide formation also increased with the increase in the chamber temperature due to increase in the vapour loading amount into the plasma. The oxide formation ratio YO^+/Y^+ at 15 °C was 0.3%. Although the ratio was up to 1.5% at 60 °C, it is still acceptable level for quantitative analysis of biological trace elements. Consequently, the spray chamber temperature was set at 60 °C for further experiments.

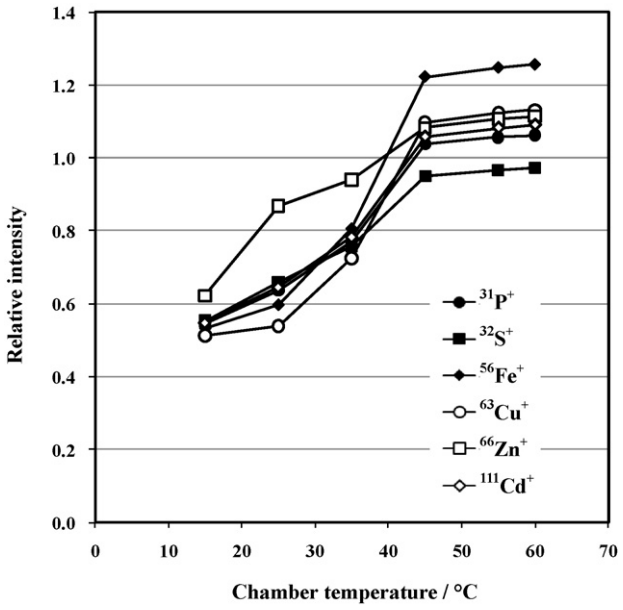

Fig. 3. Effect of the chamber temperature on the signal intensities in ICP-MS. The relative intensity means the ratio of the signal intensity obtained with the loop injection-HPCN-IsoMist system at the liquid flow rate of 10 μL min^{-1} to the signal intensity obtained with the conventional sample introduction system at the liquid flow rate of 1 mL min^{-1}.

3.3. Analytical figures of merit

Concentration detection limits obtained with the proposed sample introduction system and the conventional sample introduction system were summarized in Table 2. The concentration detection limits obtained with the proposed system are almost the same levels of those obtained with the conventional system, though the sample consumption of the proposed system was two orders of the magnitude lower than that of the conventional system. Therefore, lower absolute detection limits, multiplying the concentration detection limits by the sample consumption amounts, were obtained with the proposed system, as is seen in Table 2. The ratios of the absolute detection limits for the proposed system to those

Table 2
Comparison of the concentration and absolute detection limits.

Element	Concentration detection limit (μg L^{-1})		Absolute detection limit (pg)		Improvement factor[c]
	Proposed system[a]	Conventional system[b]	Proposed system[a]	Conventional system[b]	
Na	0.2	0.5	4	1000	250
Mg	0.07	0.14	1.4	280	200
P	0.012	0.014	0.2	28	117
S	0.3	0.2	6	400	67
K	11	8	220	16000	73
Ca	0.3	0.2	6	400	67
Cr	0.0014	0.0009	0.03	1.8	64
Mn	0.003	0.0012	0.06	2	40
Fe	0.02	0.02	0.4	40	100
Co	0.0008	0.0008	0.016	1.6	100
Ni	0.02	0.011	0.4	22	55
Cu	0.007	0.006	0.14	12	86
Zn	0.02	0.02	0.4	40	100
Y	0.0012	0.0011	0.02	2	92
Mo	0.0012	0.0014	0.02	3	117
Cd	0.0006	0.0007	0.012	1.4	117
Ba	0.0014	0.002	0.03	4	143
Pb	0.004	0.003	0.08	6	75

[a] 20 μL loop injection + HPCN-IsoMist (60 °C), 20 μL sample consumption/measurement.
[b] Conical nebulizer (1 mL min^{-1})-cyclone (room temperature, i.e. 27 °C), 2 mL sample consumption/measurement.
[c] Ratio of the absolute detection limits, proposed system/conventional system.

Table 3
Comparison of relative and absolute detection limits with the literature values.

Element	Concentration detection limit ($\mu g\,L^{-1}$)				Absolute detection limit (pg)			
	Present system	Micronebulizer/ disolvator system [7]	d-DIHEN [27]	Micronebulizer/ TISIS [28]	Present system	Micronebulizer/ disolvator system[a]	d-DIHEN[b]	Micronebulizer/ TISIS[c]
Cr	0.0014	0.004		0.0015	0.03	4		0.3
Mn	0.003	0.0042	0.015	0.0041	0.06	4	1.5	0.8
Fe	0.02	0.21		0.0036	0.40	210		0.7
Co	0.0008	0.0011	0.0056	0.0016	0.016	1.1	0.6	0.3
Ni	0.02	0.068	0.037		0.40	68	4	
Cu	0.007	0.079		0.005	0.14	79		1.0
Zn	0.02	0.069		0.037	0.40	69		7
Cd	0.0006	0.0029		0.0019	0.012	3		0.4
Pb	0.004	0.08	0.015	0.0008	0.08	80	1.5	0.16

[a] 1000 μL of sample consumption/measurement.
[b] 100 μL of sample consumption/measurement.
[c] 200 μL of sample consumption/measurement.

Table 4
Analytical results of NIST SRM 1577b bovine liver.

Element	Measured value ($mg\,kg^{-1}$)	RSD (%)	Certified value ($mg\,kg^{-1}$)	Coincident factor[b]
Na	2620 ± 30	1.0	2420 ± 60	108%
P	11400 ± 100	0.9	11 000 ± 300	104%
S	7820 ± 70	0.8	7850 ± 60	100%
K	9700 ± 120	1.2	9940 ± 20	98%
Ca	108 ± 2	2.1	116 ± 4	93%
Mn	10.9 ± 0.3	2.9	10.5 ± 1.7	104%
Fe	192 ± 5	2.5	184 ± 15	104%
Co	0.243 ± 0.011	4.6	0.25[a]	
Cu	172 ± 5	2.9	160 ± 8	107%
Zn	137 ± 3	2.4	127 ± 16	108%
Mo	3.84 ± 0.11	2.9	3.5 ± 0.3	110%
Cd	0.553 ± 0.002	0.3	0.50 ± 0.03	111%

[a] Information value.
[b] Ratio of the measured value to the certified value.

for the conventional system were calculated as improvement factors. The absolute detection limits were 40- to 250-fold improved by using in the proposed system.

Comparison of the detection limits by using the proposed system with the literature values obtained with other micro sample introduction systems [7,27,28] were summarized in Table 3. The concentration detection limits obtained with the proposed system were 5–10 fold better than those obtained with a micronebulizer/disolvator system [7] and a demountable direct injection high efficiency nebulizer (d-DIHEN) [27], and were almost the similar levels obtained with a micronebulizer/torch integration sample introduction system (TISIS) [28], although the sample consumption amount with the proposed system (20 μL) was one to two orders of magnitude smaller than those (100 μL to 1000 μL) with the literature sample introduction systems [7,27,28]. As a result, one to two orders of magnitude better absolute detection limits could be obtained with the proposed system, compared with the literature sample introduction systems.

In order to assess the reliability of the proposed system, multielement analysis of the NIST SRM 1577b (bovine liver) was performed. The analytical results are summarized in Table 4. The analytical results were quite good agreement with the certified values, except for Co, and information value for Co. The relative standard deviations of the results, except for Co, were in the range of 0.3% to 2.9%. The relative standard deviation of Co was relatively worse than those of the other elements. However, it might reflect the variance in the sample digestion and/or the in-homogeneity of Co in the SRM, because the measurement precision of the Co standard solution was below 2% and the Co value is provided as the information value. These results indicate that the proposed system is reliable enough for the determination of multielements in a small amount of biological sample. In comparison to the conventional sample introduction system, the proposed system achieved a drastic downsizing of sample consumption without sacrificing accuracy and precision.

3.4. Application to multielement analysis in *E. coli*

E. coli is widely used by many researches as a model organism because of its low toxicity along with the convenience for cultivation. In this study, *E. coli* was also used to demonstrate the applicability of the proposed system for a small amount of biological sample.

Fourteen elements in *E. coli* sample were successfully determined as shown in Table 5. For the measurement of Na, Mg, P, S, K, Ca, and Fe, the digested solutions were 10-fold diluted, because the concentrations of Na, Mg, P, S, K, Ca, and Fe were much higher than

Table 5
Analytical results of *E. coli*.

Element	Measured value ($mg\,kg^{-1}$)	RSD (%)
Na	260 000 ± 3000	1.2
Mg	8510 ± 130	1.5
P	102 000 ± 4000	3.9
S	16 800 ± 600	3.6
K	22 400 ± 700	3.1
Ca	2220 ± 210	9.5
Cr	48.1 ± 1.5	3.1
Mn	44.7 ± 1.2	2.7
Fe	49 100 ± 2000	4.1
Ni	393 ± 22	5.6
Cu	31.1 ± 0.9	2.9
Zn	468 ± 25	5.3
Y	3.8 ± 0.5	12.1
Ba	720 ± 11	1.5

those of Cr, Mn, Ni, Cu, Zn, Y, and Ba. All the elements examined by the proposed system could be measured using only 20 μL of the sample solution and 20 μL of the diluted sample solution with the analysis time of about 3 min for the sample solution and 3 min for the diluted sample solution.

4. Conclusion

High efficiency sample introduction system of ICP-MS, which allows a drastic downsizing of sample volume (20 μL) and a high throughput analysis, was developed. The utilization of a loop injection unit and a laboratory-made HPCN-heating chamber unit as a sample introduction system provides a versatile technique for the multielemental analysis of limited volume of biological samples with satisfied results. In addition, excellent absolute detection limit (sensitivity) accompanied with high accuracy and precision could be attributed to the proposed system.

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