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Investigation for analytical procedure for determination of trace metallic ions in simulated body fluids by inductively coupled plasma atomic emission spectrometry (ICP-AES)

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Abstract Influences of matrix elements and high viscosity in three kind of simulated body fluids (SBFs) on determination of trace metallic elements (Co, Cr, Ni, Al and V) by inductively coupled plasma atomic emission spectrometry (ICP-AES) were investigated. In addition, decreases of these effects were attempted by H_2SO_4 fume treatment. Calibration lines of the elements were constructed by the standard solutions made of elemental solutions and HCl or the SBFs. Gradients of calibration lines constructed by the each standard solution were different. Therefore, for accurate determination, calibration curve must be constructed by the elemental standard solution and the analytical solution. Limit of detection (LOD) of each element in the solutions was measured by a blank test. Although LODs of $\mu g \cdot L^{-1}$ (ppb) order were nominal instrumental data, because of influences of the matrix elements and the high viscosity, the measured LODs of the elements in the SBFs were higher than those. However, the LODs were lowered by employing the H_2SO_4-fume treatment and approached to the nominal instrumental data. Therefore, H_2SO_4-fume treatment is extremely effective treatment in order to reduce the influences.

1 Introduction

Metals and alloys have been widely employed as biomedical materials because of their good mechanical properties. Each of the metallic biomaterials has their characteristic advantage and is applied to their appropriate part of human body. For example, Co-Cr-Mo alloy is used as artificial joint and bone head because of its high wear resistance [1]. Ti and Ti-6Al-4V alloy are widely used as bone fixation, dental implants and so on [1]. However, after implanting in human body for a long period, metal ions released from the implants are often detected in the surrounding tissues. It has been pointed out that these released metal ions might be toxic for human body. For example, V in Ti-6Al-4V is identified as a toxic element [2–4]. Ni ions are considered to be one of the causative agents of allergic reaction [5–7]. Therefore, in order to guarantee the safety of metallic biomaterials in human body, corrosion resistance is one of the important properties.

Corrosion resistances of metallic biomaterials are evaluated by electrochemical analysis and static immersion test. These procedures are specified as Japan Industrial Standard (JIS) T0302 and T0304, respectively [8, 9]. The electrochemical analysis, anodic polarization measurement is a simple method [1, 8]. In this method, corrosion resistances of the materials are evaluated by the anodic polarization curves obtained in a simulated body fluid (SBF). On the other hand, in the static immersion test, the materials are immersed in SBFs for allotted periods, and corrosion resistances are evaluated by the amount of the metal ions released from the materials into the SBF [9]. Therefore, for precise evaluation of the corrosion resistance, accurate determinations of the elements dissolved into the SBFs are indispensable.

Amounts of various elements in sample solutions are determined by an inductively coupled plasma atomic emission spectrometry (ICP-AES), an inductively coupled plasma

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mass spectrometry (ICP-MS) and atomic adsorption spectroscopy (AAS) [4, 9]. Each of analytical method has its own advantages and disadvantages. Although analytical sensitivity of ICP-MS is the highest, pretreatment of sample solutions is complicated. AAS is effective for determinations of specified elements, *e.g.* As, Se and Bi. Although analytical sensitivity is inferior to that of ICP-MS, ICP-AES can measure almost all elements without any complex pretreatment [10]. Thus, ICP-AES is widely used as a general analytical method. In ICP-AES, sample solution is nebulized and analytical elements are introduced into inductively coupled plasma (ICP), and intensities of atomic emission are detected by a photomultiplier or a semiconductor detector. The concentration of each element is measured by comparison with a calibration curve made by measurement of standard solutions. ICP-AES can be adopted for $\mu g \cdot L^{-1}$ (ppb) order of analysis. However, weakness of analysis in using this instrument is as follows: Since condition of ICP is changed by matrix elements including in sample solution, signal intensities derived from analytical elements might be influenced [11]. Next, in analysis of high-viscosity solution, injection volume of sample solution is not constant. Therefore, it is difficult to obtain reproducible analytical results, and limits of detection (LOD) might be higher. Since SBFs include a large quantity of NaCl and organic compounds and have high viscosity, the influences of matrix elements on the ICP-AES analysis are not negligible. Therefore, investigation of these influences must be indispensable for accurate, sensitive and reproducible determination.

The aim of this study was to investigate the influences of matrix elements and high viscosity in the three kinds of SBFs on analytical results of the trace metallic ions (Co, Cr, Ni, Al and V) by ICP-AES. Moreover, we attempted to reduce the influence of matrix elements and high viscosity of the SBFs by heating to fume in H_2SO_4.

2 Experimental procedure

2.1 Preparation of SBFs

We employed the following three kinds of SBFs as sample solutions. Hanks' balanced salt solution (HBSS) was prepared by dissolving NaCl, KCl, $CaCl_2$, $MgSO_4 \cdot 7H_2O$, $Na_2HPO_4 \cdot 2H_2O$, KH_2PO_4 and $NaHCO_3$ in ion exchanged water. Phosphate buffered saline solution (PBS(-)) was prepared by dissolving NaCl, KCl, NaH_2PO_4 and KH_2PO_4 in ion exchanged water. Eagle's minimum essential medium solution (E-MEM) was prepared by dissolving NaCl, KCl, $CaCl_2$, $MgSO \cdot 7H_2O$, $Na_2HPO_4 \cdot 2H_2O$, $NaHCO_3$ and Glucose in ion exchanged water. Guaranteed-grade regents (Nacalai Tesque, Inc) were used for all SBFs. The concentrations of regents in the SBFs are summarized in Table 1.

Table 1 Concentrations of regents in SBFs ($g \cdot L^{-1}$)

Regents	PBS (-)	HBSS	E-MEM
NaCl	8.0	8.0	6.8
KCl	0.2	0.4	0.4
$CaCl_2$	—	0.14	0.2
$MgSO_4 \cdot 7H_2O$	—	0.2	0.2
$Na_2HPO_4 \cdot 2H_2O$	—	0.06	0.15
NaH_2PO_4	1.15	—	—
KH_2PO_4	0.2	0.06	—
Glucose	—	—	1.0
$NaHCO_3$	—	0.35	2.0

2.2 Influence of the matrix elements on analytical results

In order to investigate the influences of the matrix elements and the high viscosity, the differences of the amount of the atomic emissions from Co, Cr, Ni, Al and V dissolved into the SBFs and HCl were evaluated. The relationships between the amount of the atomic emissions and the concentrations of the elements are represented by calibration curves. Thus, the calibration curves of each individual element in HCl solution and the SBFs were constructed and compared.

The calibration curves were constructed as follows: Stock solutions of the elements were prepared by ordinary methods. From 0- to 4-$mg \cdot L^{-1}$ (ppm) standard solutions were stepewisely prepared by diluting proper amounts of stock solutions. Then, the standard solutions were adjusted to 0.24 $mol \cdot L^{-1}$ HCl solution. Generally, the amounts of atomic emissions from the elements dissolved into HCl solution are similar to that into water. Moreover, the standard solutions were prepared by diluting with the three kinds of SBFs.

The employed ICP-AES was a Thermo Elemental IRIS Advantage Duo, which has a CID detector. For observing the ICP condition, 20 $mg \cdot L^{-1}$ Yttrium solution was simultaneously injected into the ICP as an internal standard solution [9, 12]. Spectra lines for measurement were listed in Table 2.

Table 2 Spectra lines for measurements

Elements	Spectra lines (nm)
Co	Coll 238.892
Cr	Crll 283.567
Ni	Nill 231.604
Al	All 396.152
V	Vll 311.071
Y (Internal line for Cr, Al and V)	Yll 371.030
Y (Internal line for Co and Ni)	Yll 224.306

2.3 Attempts to reduce the influence of matrix elements and high viscosity by H_2SO_4-fume treatment

The aim of H_2SO_4-fume treatment is; (1) to vaporize Cl^- and CO_3^{2-} ions, (2) to adjust the pH values of SBFs to low and (3) to decompose organic compounds. We also expected that the viscosity of the SBFs decreased by this treatment. In addition, by decreasing the pH values of SBFs, it is possible to avoid formation of precipitates. 25 mL of SBF was exactly measured and 2.5 mL of H_2SO_4 was added. In the E-MEM, in order to decompose organic compounds, 10 mL of HNO_3 was also added. Then, the solution was heated to fume on a hot plate at about 523 K. After cooling at room temperature, the solution was exactly diluted to 25 mL with ion-exchanged water.

Changes in the influences of the matrix elements and the high viscosity before and after this treatment were examined by the comparison of the calibration curves and of the LODs of each element. The calibration curves in SBFs with the treatment were constructed by the same methods as the above-mentioned method. LODs were measured by a blank test. Determinations of each element in the solutions were repeated five times, and then, standard deviation (SD) was calculated. We defined ten times of SD as the LOD.

3 Results and discussions

3.1 Comparison with calibration curves in SBFs

Figure 1 shows the calibration curves of Ni and Co in the HCl solution and the PBS(-) with and without H_2SO_4-fume treatment. In all of analyzed solutions, good correlations between concentration of elements and intensities of atomic emission were obtained in the range from 0 to 4 $mg \cdot L^{-1}$. However, gradients of the calibration lines differed with the solutions. In the Ni calibration lines, the intensities of atomic emission were small in this order: HCl solution, PBS(-) with the treatment and PBS(-) without the treatment. The calibration lines of Co were similar tendency to that of Ni. These results indicate that the intensities of atomic emission are changed by the influences of the matrix elements and the high viscosity of PBS(-). Thus, when concentration of Ni in PBS(-) is determined, accurate results may not be obtained by using the calibration curve prepared by elemental standard solutions and HCl.

In the determinations of amounts of Co, Cr, Ni, Al and V in the SBFs, good correlations between concentration of the elements and intensities of atomic emission were obtained in the range of analyzed concentration, too. Gradient-ratios of the all calibration lines are summarized in Table 3. The gradients for each individual element in the SBFs are normalized by those in HCl solution. The gradients in SBFs without H_2SO_4-fume treatment were different from those in HCl solution. These results indicate that the intensities of atomic emissions from all elements might be changed by the influences of the matrix elements and the high viscosity of SBFs.

After H_2SO_4-fume treatment, the gradients were similar to those in HCl compared to those in the SBFs. In particular, the gradients of Cr and V were almost equal to those of HCl. Thus, it is considered that the influences of the matrix elements and the high viscosity of the SBFs on analytical results can be decreased by using H_2SO_4-fume treatment. However, the gradients of Co, Al and Ni were still not agreed with those in the HCl solutions. Therefore, in the determination of elements in the SBFs with and without H_2SO_4-fume treatment, it is necessary to employ the calibration curve constructed by elemental standard solution and the analyzed solution. Furthermore, the gradients of each element in PBS(-), HBSS

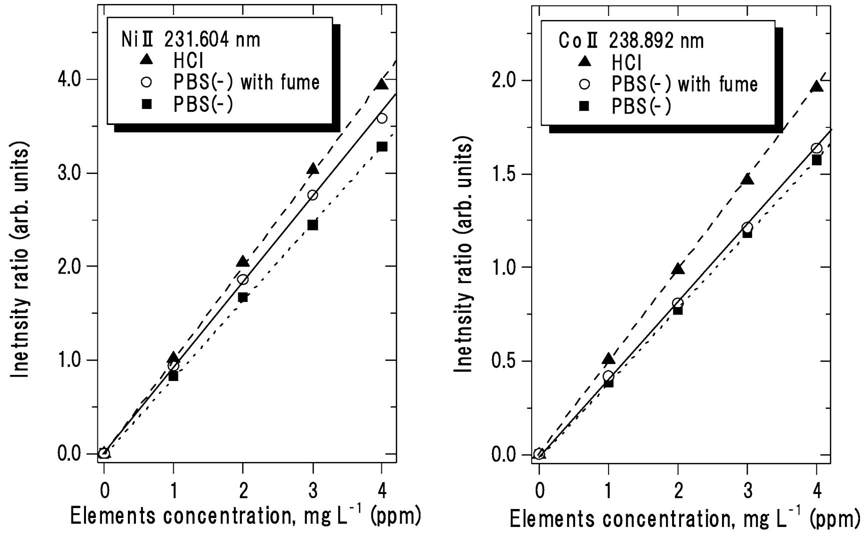

Fig. 1 Calibration curves of Ni and Co in the HCl and PBS(-) with and without H_2SO_4-fume treatment. Intensities are corrected by using internal standard of Yttrium.

Table 3 The ratio of gradients of all calibration lines

Elements	PBS(-)	PBS(-) with fume	HBSS	HBSS with fume	E-MEM	E-MEM with fume
Co	0.81	0.83	0.82	0.82	0.81	0.83
Cr	0.90	0.99	0.92	1.0	0.92	1.0
Ni	0.83	0.90	0.82	0.91	0.81	0.91
V	0.93	1.0	0.89	1.0	0.93	1.0
Al	0.96	1.1	0.92	1.1	0.89	1.1

All gradients for each element in the SBFs with and without H_2SO_4-fume treatment are normalized by that in HCl solution.

Table 4 Analytical results of Co, Cr, Ni, Al and V in SBFs with and without H_2SO_4-fume treatment, $(\mu g \cdot L^{-1})^{*}$

Elements	PBS(-)	PBS(-) with fume	HBSS	HBSS with fume	E-MEM	E-MEM with fume
Co	<30	2	<10	<3	<30	2
Cr	<60	50	<30	28	<50	36
Ni	<6	<3	<7	<2	<10	<1
Al	<30	<2	10	11	<60	<5
V	<10	6	<20	6	<20	9

These values were determined by the average of five measurements.
$^{*}\mu g \cdot L^{-1} =$ ppb.

Table 5 LODs of Cr, Ni, Al and V of nominal instrumental data of the used apparatus

Elements	LOD $^{*}(\mu g \cdot L^{-1})$
Cr	1
Ni	2
Al	2
V	1

$^{*}\mu g \cdot L^{-1} =$ ppb.

and E-MEM with the H_2SO_4-fume treatment were almost agreed. This result suggests that elements in these SBFs can be determined by using only one calibration curve.

3.2 LODs of Co, Cr, Ni, Al and V in the SBFs

Analytical results of Co, Cr, Ni, Al and V in the SBFs with and without H_2SO_4-fume treatment determined by a blanks test are listed in Table 4. Amounts of the elements were evaluated by using calibration curves constructed by the elemental standard solutions and the analyzed solutions and were determined by the averages of five-time measurements.

LODs were defined as the standard deviation of these five measurements. LODs of these elements in the solutions are also shown in Fig. 2. For comparison, LODs of these elements of nominal instrumental data are also listed in Table 5. LODs of the elements in the SBFs without the treatment elevated compared with the nominal data. For example, LOD of Cr in

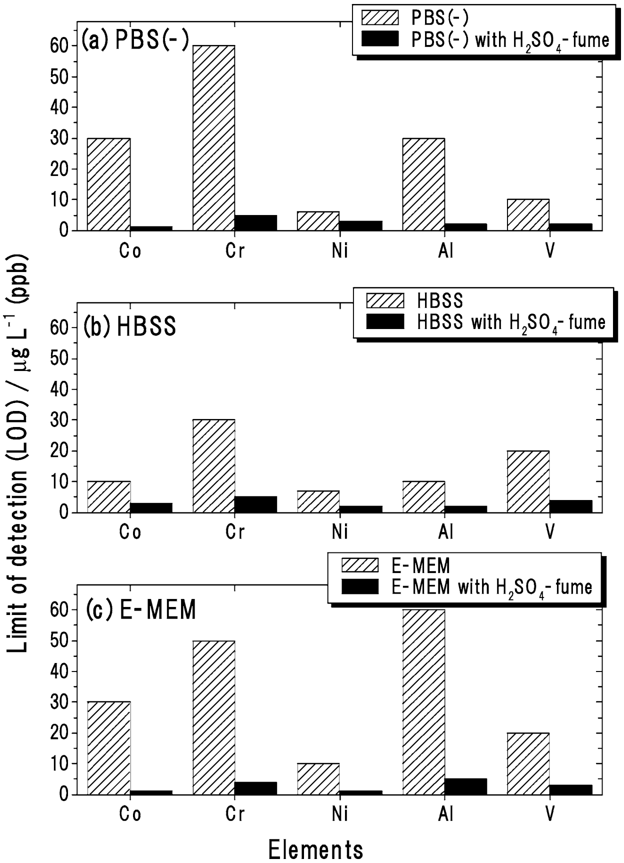

Fig. 2 Limit of detection (LOD) of Co, Cr, Ni, Al and V in the SBFs. LODs are defined as 10 times of standard deviation.

PBS(-), in HBSS and in E-MEM were 60, 30 and 50 times of the nominal data, respectively. These increases should be due to the influences of the matrix elements and the high viscosity of the SBFs. Generally, analytical results below the LODs might lack accuracy and reproducibility. Thus, for accurate and reproducible determination of trace elements in SBFs, LODs of each individual element in the analyzed solutions must be measured, and it is necessary to examine whether the analytical results of the elements are below their LOD.

After H_2SO_4-fume treatment, all LODs of the elements were drastically decreased compared with those in the SBFs before the treatment. These decreases should be due to the decreases of the matrix elements and the viscosity of the solution. Thus, H_2SO_4-fume treatment was extremely effective in the decreases of the influences. As the result of the decrease of the LOD, lower and reproducible analytical results of these elements in PBS(-), HBSS and E-MEM were obtained.

4 Conclusions

Calibration curves of Co, Cr, Ni, Al and V in the simulated body fluids were linier in the analytical range. However, different gradients according to the differences of standard solutions were obtained. The matrix elements and the high viscosity of SBFs change the amounts of atomic emissions from the each element. Therefore, in order to accurately determine the contents of trace metals, analytical results must be evaluated by the calibration curve constructed by the elemental solution and the analyzed solution.

In the SBFs without any treatments, LODs were higher than the nominal instrumental data. These LODs decreased by employing the H_2SO_4-fume treatment and approached to the nominal data. Therefore, H_2SO_4-fume treatment is extremely effective in decreasing the influences of the matrix elements and the viscosity. By employing the H_2SO_4-fume treatment, sensitivity and reproducibility of the determination of the trace metals in $\mu g \cdot L^{-1}$ (ppb) order in the SBFs are improved.

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References

1. T. HANAWA, In “Recent Research Developments in Biomaterials” edited by Y. Ikada (Reseach Simpost, Kerala, 2002) p. 12.
2. F. W. SUNDERMAN JR, S. M. HOPFER, T. SWIFT, W. N. REZUKA, L. ZIEBKA, P. HIGHMAN, B. EDWARDS, M. FOLICK and H. R. GOSSLING, *J. Orthop. Res.* **7** (1989) 307.
3. F. F. HENNIG, H. J. RAITHEL, K. H. SCALLER and J. R. DOHLER, *J. Trace. Elem. Electrolyte. Health Dis.* **6** (1992) 239.
4. Y. OKAZAKI and E. GOTOH, *Biomaterials* **26** (2005) 11.
5. K. L. WAPER, *Clin. Orthop. Relat. Res.* **271** (1991) 12.
6. Y. OKAZAKI, S. RAO, S. ASAO, T. TATEISHI, S. KATSUDA and Y. FURUKI, *Mater. Trans. JIM.* **39** (1998) 1053.
7. Y. OKAZAKI and E. NISHIMURA, *Mater. Trans. JIM.* **41** (2000) 1247.
8. Japan Industrial Standard (JIS) No. T 0302, 2000.
9. Japan Industrial Standard (JIS) No. T 0304, 2002.
10. T. ASHINO, K. TAKADA, K. WAGATSUMA and K. ABIKO, METÉRIAUX & TECHNIQUES –N HORS SÉRIE-DÉCEMBRE (2003) 31.
11. T. ASHINO and K. TAKADA, *Anal. Sci.* **9** (1993) 737.
12. M. ISHIKURO, *Bunseki Kagaku* **54** (2005) 297.