

Trace Analysis of Released Metallic Ions in Static Immersion Test for Characterization of Metallic Biomaterials

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For characterization of corrosion resistance of metallic biomaterials, determination of trace amounts of metallic ions released from the materials in using static immersion into simulated body fluids (SBF) were investigated. In using a H₂SO₄-fume pre-treatment method, sensitive, precise and accurate determination of the trace metallic elements in SBFs could be performed by ICP-OES. For accurate analysis, it was necessary to employ a matrix-matched solution for the calibration. Moreover, usage of a PFA vessel in the static immersion test was recommended for the prevention of contamination. Thus, it was possible to determine $\mu\text{g dm}^{-3}$ (ppb) order of elements in SBFs and evaluate nano-gram order of the released metallic ions. [doi:10.2320/matertrans.MRA2007325]

(Received December 13, 2007; Accepted March 11, 2008; Published April 23, 2008)

Keywords: *metallic biomaterials, static immersion test, inductively coupled plasma-optical emission spectrometry, simulated body fluid*

1. Introduction

Metallic biomaterials have been widely employed by orthopedists or dentists in clinical practice. Each of the metallic biomaterials has been applied to appropriate parts of the human body. However, when the metallic biomaterials are implanted for a long time, metallic elements may be dissolved in the surrounding tissues. Accordingly, toxic influence of metals released from the metallic implants has been pointed out.¹⁻⁵⁾ It is recently reported that not only allergy but also several medical disorders have been caused by these metallic elements. Especially, allergy responses are sensitive to trace amounts of the metallic elements. Accordingly, metallic biomaterials, which have high corrosion resistance (high-safety for the human body and low metal release), are required; therefore, the evaluation of corrosion resistance is important for developing them.

The corrosion resistance of the metallic biomaterials is evaluated by using static immersion into a simulated body fluid (SBF) and then determining metallic ions in the SBFs. Therefore, trace metallic elements in the SBFs should be noted to investigate the properties of the metallic biomaterials. Moreover, in order to evaluate the trace elements released by the static immersion test, it is necessary to determine $\mu\text{g dm}^{-3}$ (ppb) level of trace metallic elements in the SBFs. Concentration of various elements in SBF solution samples has been determined by inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS).^{6,7)}

ICP-OES has analytical advantages such as simultaneous determination of elements having the wide dynamic range. However, the operating conditions of ICP are influenced by matrix elements included in a sample solution^{8,9)} or the viscosity of the solution. SBFs include a large amount of alkali metal salts (NaCl, KCl etc.) and/or organic compounds (glucose, amino acids etc.) and have a high viscosity. The signal intensities of analytes could be changed and thus it is difficult to obtain the precise and accurate results in analysis of elements in the SBFs. Accordingly, it is vital to select an

optimal sample pre-treatment method in analysis of elements by using ICP-OES. However, the sample pre-treatment method is not defined in detail by JIS T 0314.

On the other hand, contaminants attributed to reagents and/or experimental apparatuses in the sample solution are cause of errors in analytical values by using chemical procedures.¹⁰⁾ The contaminants are due to increase in a blank value in analysis. In determination of trace elements without considering influences of the contamination, the analytical signal describes addition of analyte content in sample and the blank value. When the blank value is high and variable, it is hardly to obtain sensitive and precise analytical values. Accordingly, it is important to evaluate influences of the contaminants derived from the reagents or the experimental apparatuses for the static immersion test on determination of trace elements in the SBFs.^{11,12)} Moreover, it is necessary to decrease the contamination.

In order to develop a simple and easy procedure for the high-sensitive, precise and accurate determination of trace metallic elements in the SBFs by ICP-OES, the authors have investigated a H₂SO₄-fume treatment method for pre-treatment.¹³⁾ By using this method, the viscosity of a sample solution for analysis by ICP-OES can be decreased and the solution can be stabilized in acidic condition. Moreover, the organic compounds contained in the SBFs can be removed. Thus, it is possible to determine ppb level of elements in the SBFs. In this paper, the authors investigated the influence of Na as the matrix element and contaminants during the experimental procedure of the static immersion test on determination of trace elements in the SBFs are investigated. Then, metallic materials were soaked into a lactic acid solution and the released elements were determined by ICP-OES in using the proposed method.

2. Experimental

2.1 Apparatus

The ICP-OES system is an IRIS Advantage-Duo (Thermo Fisher Scientific, Inc.). The operating conditions and the spectral lines employed are listed in Table 1. Analytes and Y (an internal standard element) were simultaneously measured

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Table 1 Operating conditions of instrument and spectral lines.

Instrument	IRIS Advantage DUO (Thermo Fisher Scientific, Inc.)								
Plasma conditions									
RF frequency	27.12 MHz								
RF power	1.15 kW								
Argon gas flow rate									
Plasma gas	15 L min ⁻¹								
Auxiliary gas	0.5 L min ⁻¹								
Nebulizer gas	1.9 × 10 ⁵ Pa								
Wavelength	Ca	II	393.366 nm	Mo	II	204.598 nm	Au	I	242.795 nm
	Ti	II	338.376 nm	Al	I	396.152 nm	Nb	II	316.340 nm
	Fe	II	238.204 nm	V	II	311.071 nm	Ta	II	240.063 nm
	Cr	II	283.563 nm	Zn	II	213.856 nm	Zr	II	343.823 nm
	Ni	II	231.604 nm	Pd	I	360.955 nm	Y	II	224.306 nm
	Co	II	238.892 nm	Pt	II	214.423 nm		II	371.030 nm
									(as Internal)

and the analytical values were corrected by using the intensity of Y (internal standard correction method). Y is generally used as the internal standard elements for analysis by using ICP-OES⁸⁾ and thus this method is adopted by an official analytical procedure.¹⁴⁾ An internal standard mixing kit (Thermo Fisher Scientific, Inc.) was employed for introducing the internal standard solution.¹⁵⁾ For the pre-treatment procedure, a quartz-glass beaker and a heater with a halogen lamp were used. For the static immersion test using SBFs, an incubator (Yamato Scientific Co. Ltd.) and a PFA vessel was used.

2.2 Reagents

H₂SO₄ and HNO₃ employed for the sample pre-treatment were electronics industry grade (Wako Pure Chemical Industries, Ltd.). Reagents for preparation of SBFs were guaranteed-grade reagents (Nacalai Tesque, Inc). Deionized water (Millipore Corp.) was used for all the preparation. Elemental standard solutions (1 g dm⁻³) were prepared by dissolution of several pure metals and compounds (more than 99.9%) in appropriate acids, respectively. These solutions were diluted before the use. SBFs (a Hanks' buffered saline solution (HBSS), a Phosphate buffered saline solution (PBS(-)), an Eagle' minimum essential medium solution (E-MEM), and a Lactic acid) were prepared by dissolution of these reagents in water before the use, respectively.¹³⁾

2.3 Testing materials for static immersion

A commercial SUS 316 (The Nilaco Corporation) and Ti-6Al-4V alloy (Sumitomo Metal Industries) were used for the static immersion test. The size of the specimen was 10.0 mm × 10.0 mm × 1.0 mm. These surfaces were polished with an emery paper (#1200) and ultrasonically washed in ethanol before the use.

2.4 Pre-treatment by heating to fume in sulfuric acid

The pre-treatment procedure is as follows: 25 cm³ of the SBF was exactly taken into a quartz beaker and then 2.5 cm³ of H₂SO₄ was added. In preparation for the E-MEM and the lactic acid solution, 10 cm³ of HNO₃ was also added for decomposition of organic compounds. The solution was

heated to fume. After cooling down to room temperature, the solution was exactly diluted to 25 cm³ with water. Finally, elements contained in the solution were determined by ICP-OES. The blanks containing no SBFs were prepared by the same procedure described above.

3. Results and Discussions

3.1 Influence of matrix element on measurement by ICP-OES

SBFs include high-concentration salts of NaCl, KCl and several alkali or alkali earth metals. The authors investigated influences of these matrix elements on the measurement by ICP-OES. Solutions containing analytes (2 mg dm⁻³) and NaCl (8 g dm⁻³) were prepared and the emission intensities of the analyte elements were measured by ICP-OES. Their intensities or the intensity ratios (Elements/Y) are shown in Fig. 1. Both the intensities and the intensity ratios are normalized as unity when containing no NaCl. When NaCl contains, the intensities for Al and Pd are more than 1 but the intensities for the other elements investigated are less than 1. The intensity ratios for Au, Ca, Cr, Ti, V and Zn were nearly 1. In this case, the internal standard correction method by using Y can be applied only for these elements; however, it is not effective for the other elements such Al and Pd. Accordingly, Na has a serious influence on the measurement of such elements by ICP-OES and it is thus necessary to prepare matrix matched standard solutions for calibration for the accurate determination.

3.2 Analytical results of elements in simulated body fluids

In order to evaluate amounts of elements contained in several SBFs, the authors determined the concentration of elements by ICP-OES with the H₂SO₄-fume treatment method. Al, Ca, Co, Cr, Fe, Mo, Ni, V and Zn in the SBFs were determined and the analytical results are shown in Fig. 2. The authors speculate that these elements determined in the SBFs were contaminants from water, reagents and/or an experimental environment in preparation of the SBFs. However, Au, Nb, Pd, Pt, Ta, Ti and Zr were not detected

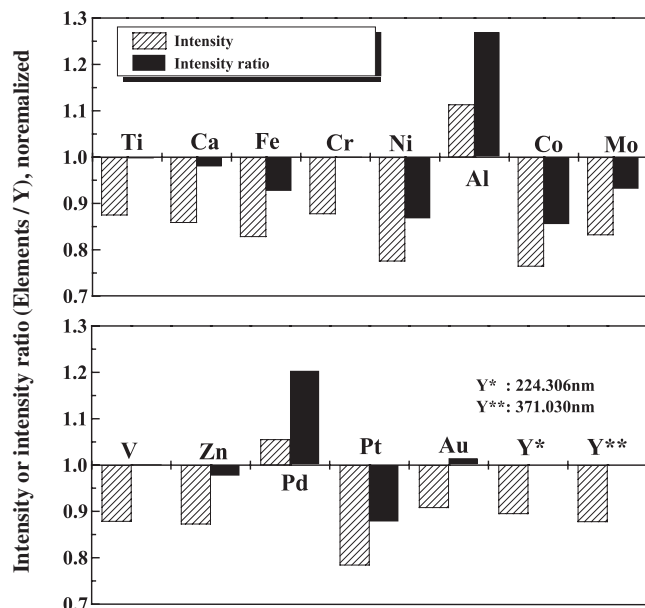


Fig. 1 Intensity and intensity ratio of analyte elements by ICP-OES in 8 g dm^{-3} NaCl solution: Both the intensities and the intensity ratios are normalized as unity when containing no NaCl.

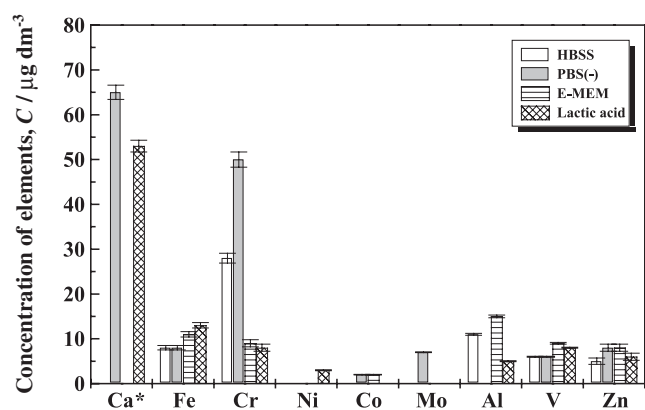


Fig. 2 Concentration of elements in SBFs: Ca* in HBSS and E-MEM is g dm^{-3} order.

in the SBFs. Therefore, it is necessary to control qualities of the reagents and the water and keep a cleansing of the experimental environment.

3.3 Influence of contaminants from experimental apparatuses on determination

In order to evaluate influence of contamination on difference of materials for the experimental apparatuses during experimental procedure, the authors determined the elements in the HBSS eluted from bottles made of several materials. In this work, four kinds of bottles made of the following materials were employed: (1) Borosilicate glass (Pyrex[®]), (2) Polypropylene (PP), (3) High-density Polyethylene (HDPE) and (4) Tetrafluoro Ethylene-Perfluoro Alkylvinyl Ether copolymer (PFA). The HBSS was added to a bottle made of these materials, respectively. Then, the bottles were sealed and left at 310 K for 20 days in ambient atmosphere. After leaving, elements eluted from the bottles into the HBSS were analyzed by the ICP-OES with the H_2SO_4 -fume treatment.

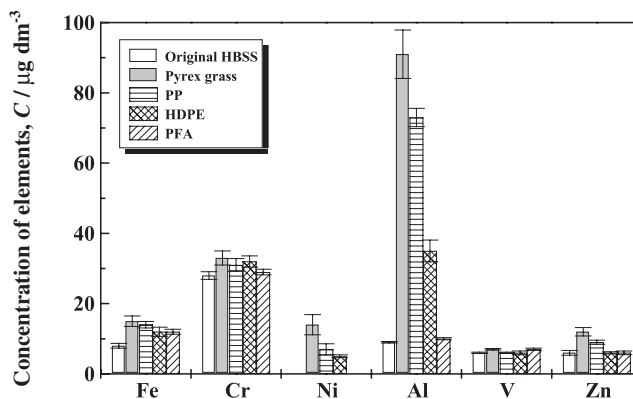


Fig. 3 Concentration of elements in HBSS after leaving.

The concentration of elements eluted in the HBSS after leaving is shown in Fig. 3. When using the bottles made of Pyrex, PP and HDPE, concentrations of Al, Cr, Fe, Ni and Zn in the HBSS were increased. The authors speculate that this phenomenon is contamination derived from the materials of bottles. Especially, Ni was not detected in the original HBSS (before leaving). However, ppb level of Ni was detected after leaving. Moreover, the contamination of Al from the Pyrex was serious. On the other hand, the contamination of the elements investigated was not found in the PFA bottle. Therefore, the authors recommend usage of a PFA bottle in order to prevent the contamination during the static immersion test.

3.4 Analytical results of elements released by static immersion

The authors determined elements released from SUS 316 or Ti-6Al-4V alloy by the static immersion test. These materials were respectively immersed into a lactic acid in a PFA bottle at 310 K for 20 days in ambient atmosphere. After leaving, the elements released from the materials into the lactic acid were determined by the ICP-OES with the H_2SO_4 -fume treatment. The concentrations of elements in the lactic acid after the static immersion test are shown in Fig. 4. It was possible to determine ppb level of the released elements by using the H_2SO_4 -fume treatment method.

4. Conclusion

The authors attempted to determine the concentration of metallic ions released into the SBF from the metallic biomaterials in using the static immersion test by the ICP-OES with the H_2SO_4 -fume pre-treatment. For sensitive, precise and accurate determination of ppb level of elements in the SBFs, it is necessary to consider the influence of Na which contains as a matrix element in SBFs on measurement by ICP-OES and the contamination from environment and the experimental apparatuses. In order to overcome these problems, the authors recommend preparation of matrix-matched standard solutions for calibration as well as a PFA bottle for the static immersion test. Thus, nano-gram level of the elements released by the static immersion test can be determined. Therefore, the proposed method can contribute to characterization of metallic biomaterials.

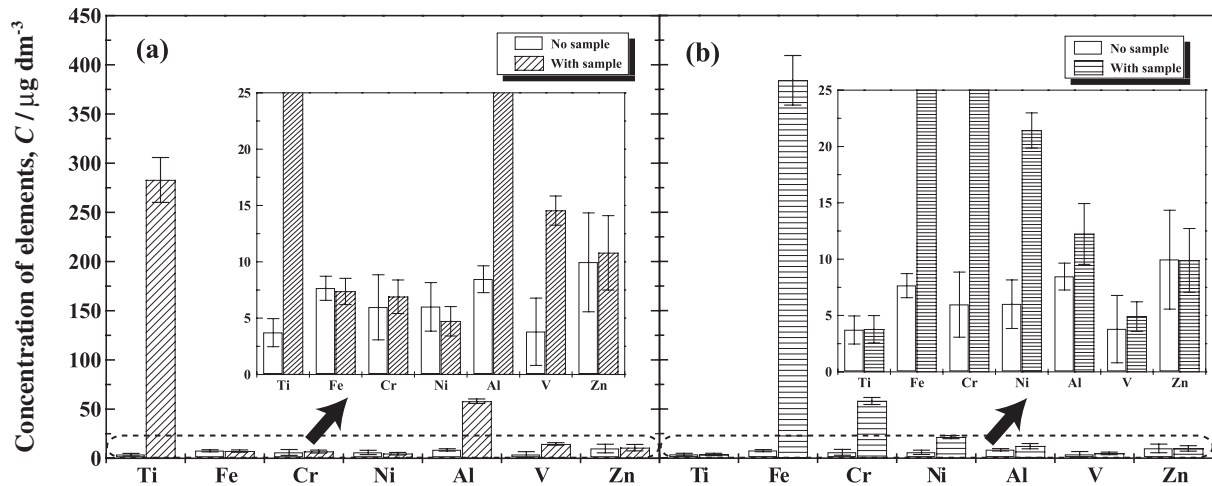


Fig. 4 Concentration of elements released in lactic acid after static immersion test (a) Ti-6Al-4V, (b) SUS 316.

REFERENCES

- 1) F. W. Sunderman Jr, S. M. Hopfer, T. Swift, W. N. Rezuke, L. Ziebka, P. Highman, B. Edwards, M. Folick and H. R. Gossling: *J. Orthop. Res.* **7** (1989) 307–315.
- 2) F. F. Hennig, H. J. Raithel, K. H. Scaller and J. R. Dohler: *J. Trace Elem. Electrolytes Health Dis.* **6** (1992) 239–243.
- 3) K. L. Waper: *Clin. Orthop. Relat. Res.* **271** (1991) 12–20.
- 4) Y. Okazaki, S. Rao, S. Asao, T. Tateishi, S. Katsuda and Y. Furuki: *Mater. Trans. JIM* **39** (1998) 1053–1062.
- 5) Y. Okazaki, E. Nishimura: *Mater. Trans. JIM* **41** (2000) 1247–1255.
- 6) Y. Okazaki and E. Gotoh: *Biomaterials* **26** (2005) 11–21.
- 7) Japan Industrial Standard (JIS) T 0304 (2002).
- 8) T. Ashino and K. Takada: *Anal. Sci.* **9** (1993) 737–739.
- 9) M. Ishikuro and K. Wagatsuma: *Bunseki Kagaku* **56** (2007) 933.
- 10) M. Rosner, R. L. Rower and A. Meixner: *Anal. Bioanal. Chem.* **382** (2005) 120–124.
- 11) T. Ashino, K. Takada, K. Wagatsuma and K. Abiko: *MATÉRIAUX & TECHNIQUES*, Numéro Hors Série-Décembre (2003) 31–36.
- 12) N. Ohtsu, T. Ashino and K. Asami: *Mater. Trans.* **45** (2004) 550–553.
- 13) N. Ohtsu, T. Ashino, K. Takada, H. Kimura and T. Hanawa: *J. Mater. Sci. Mater. Med.* **18** (2007) 429–433.
- 14) Japan Industrial Standard (JIS) G 1258 (1999).
- 15) M. Ishikuro: *Bunseki Kagaku* **54** (2005) 297–300.