APPLICATION NOTE

ICP - Mass Spectrometry

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New Frontiers in Biomonitoring: Total and Single Particle Analysis of Titanium in Serum

Introduction

For many years, ICP-MS has been the tool of choice for the trace analysis of elements like lead (Pb), arsenic (As), mercury (Hg), and copper (Cu) in body fluids such as urine, blood, serum, saliva and also in tissues and bones. Single elements, or panels of toxic or nutritional elements, are run in the proper matrices providing doctors with comprehensive views on patient conditions. Recently, due to the popularity of implants, elements like titanium (Ti), cobalt (Co), and vanadium (V) have been added to the common list of tested analytes.

Titanium (Ti) is a metal of choice in all kinds of prosthetics, such as artificial hip joints (Figure 1), knee, dental implants and also as medical clips, screws, etc. The artificial joints are actually made from various Ti alloys, usually with a small addition of aluminum and vanadium. These alloys are known as "medical" titanium. There are several reasons why Ti is used. First, it is considered the most biocompatible metal due to its resistance to corrosion from body fluids and one of only a few metals which can be integrated into bone (osseo-integration). In addition, medical Ti is non-toxic, non-ferromagnetic, and has flexibility and elasticity similar to bones.[1]

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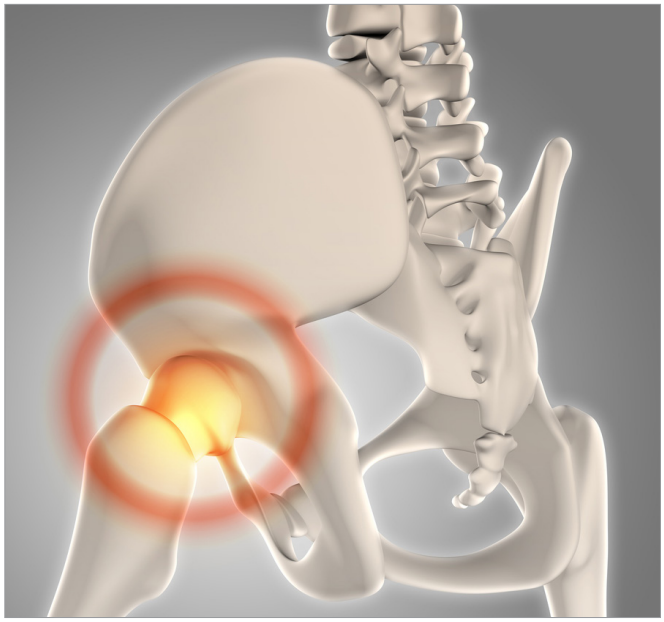

Figure 1. Illustration showing an artificial hip replacement joint made from medical titanium.

However, because the hip ball-and-socket joint undergoes extensive use, Ti could slowly wear away over time and enter the blood stream. Even though Ti is nontoxic, its levels can give medical providers information on the level of implant degradation. Therefore, it is important to monitor the presence of Ti in the serum or hip aspirate of people who have received artificial hips.

It is somewhat challenging to accurately measure Ti in serum due to a number of factors, including low concentrations, many potential spectral interferences, and complex body fluid matrix. This research will explore the analysis of Ti in serum, looking at instrumental parameters and techniques to overcome the challenges. This work is intended solely for research purposes and is not meant for diagnostic evaluation.

Experimental

Samples and Sample Preparation

The serum certified reference material (CRM) used in this work was Trace Elements in Serum – normal level (UTAK® Laboratories Inc., Valencia, California, USA). This CRM was not certified for Ti, but it served as a base serum matrix for experiments. In addition, patient samples (serum and hip aspirate) were obtained from University Hospital Saint-Louis - Lariboisière - Fernand-Widal, Paris, France.

For all analyses, serum and aspirate were diluted 20 times in 0.2% HNO_3 (Optima Grade, Thermo Fisher Scientific, Waltham, Massachusetts, USA). The diluted nitric acid was required to keep Ti stable in solution, but a low concentration was used to prevent precipitation of proteins and cell debris.

For total Ti analysis, the method of standard additions (MSA) was used, where the calibration curves were made in serum diluted 20 fold.

For analysis of Ti nanoparticles, NIST 1898 TiO_2 nanoparticles were used for feasibility testing. NIST SRM 8013 (60 nm Au nanoparticles) was used for transport efficiency (TE) determination. A dissolved calibration was prepared in 20-times-diluted serum.

Instrumental Parameters

All analyses were performed on a PerkinElmer NexION® 2000 ICP-MS using the conditions listed in Table 1. Because of many potential interferences on the Ti isotopes, ammonia was used as a reaction gas to convert Ti to the Ti-NH_3 cluster [$TiNH(NH_3)_4$] at m/z 131. To maximize signal, the Quadrupole Ion Deflector (QID) voltage was set to transmit m/z 48, the most abundant Ti isotope (73.8%). All analyses were performed with the same sample introduction components using similar conditions with the exception of the dwell time: while total Ti measurements used a 50 ms dwell time, the SP-ICP-MS analysis of Ti used a dwell time of 50 µs. Dwell times of less than 100 µs are critical for SP-ICP-MS analysis to reduce the probability that two nanoparticles will be detected in the same analysis time window. All analyses and data processing were done with Syngistix™ for ICP-MS software and the Syngistix Nanoparticle Application Module.

Table 1. NexION 2000 ICP-MS Instrumental Conditions for Ti Analysis.

Parameter	Value
Sample Introduction Rate	≈ 0.3 mL/min
Nebulizer	MEINHARD® Type C
Spray Chamber	Glass cyclonic
Neb Gas Flow	≈ 1 L/min
RF Power	1600 W
QID Voltage	Set for maximum transmission of $^{48}Ti^+$
Dwell Time	50 ms (total) 50 µs (SP-ICP-MS)
Cell Gas	NH_3 at 1.0 mL/min
RPq	0.25

Results and Discussion

Analysis of Total Titanium in Serum

Previous work[2] has shown the ability to measure Ti in the reaction – mass shift mode as an ammonia cluster at m/z 131. During current work, after the NH_3 flow optimization (Figure 2), a scan was performed to confirm the presence the Ti-NH_3 cluster at m/z 131 (Figure 3).

In this work, the QID voltage was set for maximum transmission of m/z 48 (the major Ti isotope), while the analytical quadrupole was set to transmit m/z 131, the Ti-NH_3 cluster. By decoupling the QID voltage and quadrupole transmission mass, the analytical signal can be optimized when analyzing reaction products while simultaneously discriminating against potential interferences.

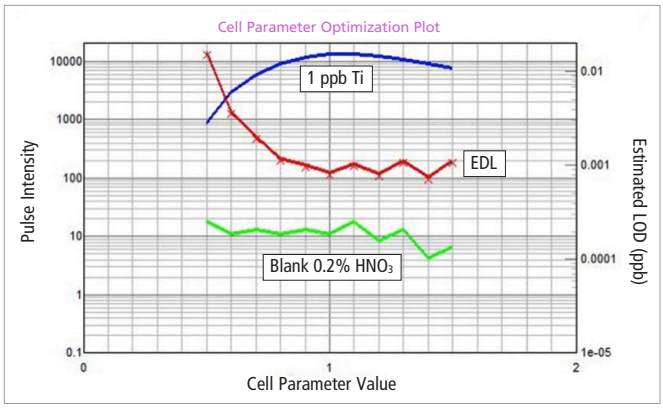

Figure 2. Optimization of NH_3 flow (mL/min) for creation of the Ti cluster at m/z 131. The estimated Ti detection limit (EDL) is 1 ppt.

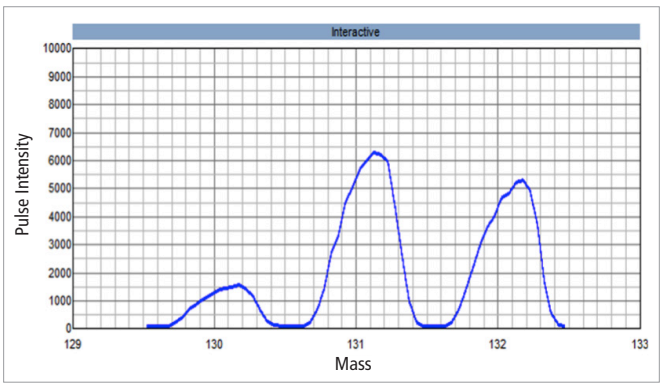

Figure 3. Scan of Ti-NH_3 clusters in 130-132 mass range. Concentration of Ti is 1 ppb.

Although Xe also has isotopes at m/z 130, 131 and 132, Xe reacts rapidly with NH_3 via charge transfer, resulting in backgrounds of single digit cps at these masses.

Analysis of total Ti in samples was done based on an MSA calibration curve with 0.5, 1, and 2 µg/L Ti standards in UTAK serum diluted 20x. Figure 4 shows the calibration curve with a correlation coefficient of 0.99996.

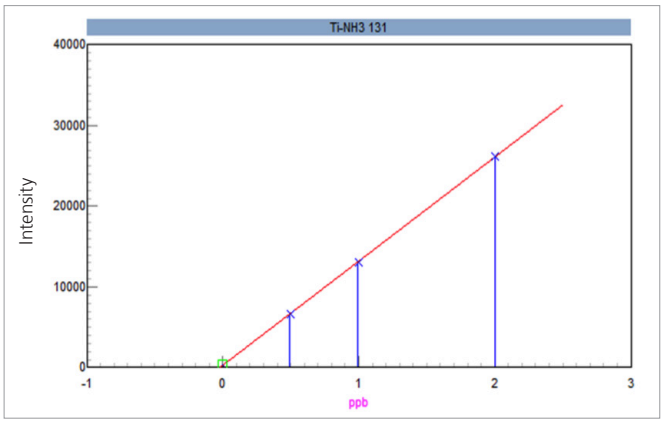

Figure 4. Ti calibration curve at m/z 131 using MSA in 20x diluted serum.

Three samples were analyzed: a serum reference material (UTAK, not certified for Ti) and two samples from a patient with a hip implant – one serum and one hip aspirate (a bodily fluid taken from around the implant). The results for all three samples (diluted 20x) are shown in Table 2. The Ti level in the UTAK (Normal Serum) reference material is not certified, but the result is low and an estimated DL in 20x diluted serum is approximately 0.004 ug/L.

A 0.5 µg/L Ti spike was added to the UTAK serum and recovered at 98%, demonstrating the accuracy of the methodology. The patient samples show higher levels of Ti, especially in the serum.

Table 2. Results for Ti Analysis in 20x Diluted Samples.

Sample	Ti Concentration (µg/L)	% RSD
UTAK Serum	0.008	9.2
UTAK Serum + 0.5 ug/L	0.498	1.3
Patient Serum	1.22	2.0
Patient Aspirate	0.109	1.9

Next, 1 µg/L of TiO_2 nanoparticles (that corresponds to 0.6 µg/L Ti) were added to the three samples and measured again. The results in Table 3 show an average recovery of 71%, but more notable is that the RSDs were much higher than for the serum samples without the addition of TiO_2 NPs (Table 2). This raises the possibility that the elevated RSDs observed during Ti analysis in serum may be an indicator of the presence of Ti nanoparticles in the sample.

Table 3. Results for Ti Analysis in Serum after Addition of 1 µg/L Ti Nanoparticles.

Sample	Ti Concentration (µg/L)	% Recovery	% RSD
UTAK + Ti NPs	0.430	72	67
Serum + Ti NPs	1.65	72	11
Aspirate + Ti NPs	0.413	69	35

Single Particle Analysis of Ti Particles in Serum

To confirm the ability to measure Ti nanoparticles at m/z 131 as $TiNH(NH_3)_4^+$, TiO_2 NPs were suspended in 0.2% HNO_3 at a concentration of 1 µg/L and analyzed. Figure 3 shows the resulting real-time signal vs. time plot and the size vs. frequency histogram, indicating that the most frequent particle size corresponds to 80 nm, which closely matches values shown in the NIST certificate of 71-83 nm.

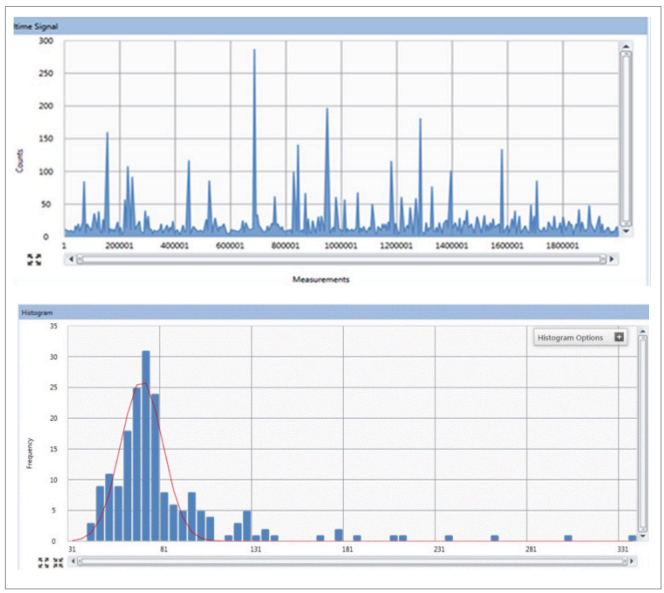

Figure 5. TiO_2 NPs measured as $TiNH(NH_3)_4^+$ (top) and corresponding TiO_2 particle size distribution (bottom).

Next, UTAK CRM serum, patient aspirate and patient serum were evaluated for Ti NPs and dissolved Ti.

Table 4 shows the SP-ICP-MS results for three separate analyses of UTAK serum. Almost no particles were detected, which is expected in the reference material. The results are consistent between all measurements.

Table 4. SP-ICP-MS for Ti in UTAK Serum.

Sample	Most Frequent Size (nm)	No. of Peaks	Particle Conc. (Part/mL)	Dissolved Conc. (µg/L)
1	111	15	192	0.027
2	105	13	166	0.025
3	119	10	128	0.027
Average	**111**	**13**	**162**	**0.026**

Finally, the samples from a patient with an artificial hip were analyzed. As shown in Table 5, there are significantly more particles in the patient samples than the reference material, most likely the result of Ti wearing from the artificial hip. In addition, the dissolved Ti concentration for patient serum is very similar to what was measured as total Ti analysis.

Table 5. SP-ICP-MS Results for Patient Serum Samples.

Sample	Most Frequent Size (nm)	No. of Peaks	Particle Conc. (Part/mL)	Dissolved Conc. (µg/L)
Serum-1	61	123	2151	1.28
Serum-2	87	121	2116	1.23
Serum-3	51	119	2081	1.27
Average	**66**	**121**	**2116**	**1.26**
Aspirate-1	53	67	1172	0.03
Aspirate-2	82	64	1119	0.02
Aspirate-3	87	67	1172	0.02
Average	**74**	**66**	**1154**	**0.02**

Conclusion

This work demonstrates the ability to accurately measure low levels of Ti in serum leveraging the NexION 2000 ICP-MS' ability to do reaction – mass shift analysis. Using NH_3 as a cell gas, Ti^+ reacts efficiently to form $TiNH(NH_3)_4^+$ at m/z 131 which is far away from the common interferences on the Ti^+ isotopes. Furthermore, this reaction proved to be effective for measuring Ti nanoparticles in serum. Taken together, these results show that Ti was present in both the dissolved and particulate forms in samples taken from a patient with an artificial hip. Further research is required to determine if SP-ICP-MS can be used for early detection of implant wear, but the initial research is promising.

References

1. Wikipedia, Search - Titanium biocompatibility.
2. "*The Advantages of the NexION 300D/350D ICP-MS for the Determination of Titanium in Serum*", Application Brief, PerkinElmer 2014.

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