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Determination of tantalum from tantalum oxide nanoparticle X-ray/CT contrast agents in rat tissues and bodily fluids by ICP-OES

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Electronic Supplementary Information available: sample digestion reagents, instrument calibration standards, tantalum oxide nanoparticle contrast agent digestion procedures and results, within-run recovery and precision for each biological material per digestion method, and *in vivo* retention case study results.

Accurate and precise means for quantifying Ta in tissues, bodily fluids, and bone is critical in understanding anticipated safety-profiles for tantalum oxide (TaO) nanoparticle-based X-ray/CT diagnostic imaging agents and has prompted the development of three digestion methods which are the focus of this work. Spike recovery studies were employed to evaluate bias, precision, and sample matrix effects in the quantification of Ta in (1) liver, blood and femur by microwave-assisted digestion, (2) urine by open-beaker digestion, and (3) carcass, liver and feces by dry-ash digestion. All analyses were performed with inductively coupled plasma optical emission spectrometry (ICP-OES). Spike recoveries were 98.5–102.3% for all biological matrices except femur (91.6%); however, a modified version of the original microwave digestion procedure improved the recovery of Ta in femur to 103.8%. Precision of spike recovery reported as one standard deviation ranged from 0.1 to 3.8% for within-run and from 0.5 to 3.3% for overall recovery depending on the tissue type and digestion method. Limit of detection (LOD) was 0.006 to 6 µg Ta/g and limit of quantification (LOQ) was 0.02 to 20 µg Ta/g depending on the method. The presented methods were applied to the determination of Ta in liver, kidney, spleen and carcass from an *in vivo* TaO nanoparticle retention study, and the results for percent injected dose (% ID) of Ta retained are given.

Introduction

Tantalum oxide nanoparticles (TaO NPs) are being investigated as the next generation of medical imaging X-ray/computed tomography (CT) contrast agents and have shown improved solution properties (viscosity and osmolality), pharmacokinetics, imaging efficacy and dose requirement over the current industry standard, iodinated contrast media.[1] The performance of TaO NPs as X-ray/CT contrast agents, either alone or in combination with other molecules for multimodal imaging, has been studied for imaging the vascular system, cells, bone, cartilage, and lymph nodes.[1,2,3] The *in vitro* uptake or *in vivo* distribution of TaO NPs is typically assessed by evaluating the change in image contrast over time but has also been investigated using fluorescence imaging via fluorophore tags or by taking advantage of the fluorescent properties of secondary molecules present.[2a,b,d]

In addition to clinical applications of tantalum and tantalum oxide as NP contrast agents, these materials are already in use in regenerative medicine, orthopedics and dentistry.[4] Although the determination of Ta by ICP-OES or ICP-mass spectrometry has been reported for various tantalum compounds and minerals,[5] niobium compounds,[6] tungsten carbide and cemented carbide,[7] lead and lead-based alloys,[8] and gold,[9] details regarding the quantitation of Ta in biological matrices by ICP-OES are limited.[3,10] Thus, precise and accurate methods for the determination of Ta content in tissues and bodily fluids are needed to advance our understanding of the fate of Ta in biological systems.

In this study we present three digestion methods for the quantitative determination of Ta in rat tissue and bodily fluids by inductively coupled plasma optical emission spectrometry (ICP-OES) which were developed in support of X-ray/CT contrast agent research. Limited details of our early in-house methods are given in previous publications[3] but a thorough investigation of method performance was not conducted at that time. The methods have been modified since then and the current versions are reported here. Blood and tissue aliquots of approximately 1 g or less are prepared by microwave-assisted acid digestion. Carcass, feces, intestines, tail, and any tissue aliquot greater than approximately 1 g are processed by dry-ashing as an effective way to decompose organic materials of high mass or high fat content, and open-beaker digestion is applied for the determination of Ta in urine.

The primary objectives of the present study were to: (1) assess method bias for quantitation of Ta in rat biologicals resulting from TaO NP agent with these three methods, (2) estimate the within-run and overall variation for each method and biological matrix, and (3) determine method LOD and LOQ by ICP-OES. It is well known that the best approach to assessing method bias is through the measurement of certified reference materials of similar matrix to the unknown samples;[11] however, none of the available biological reference materials are certified for Ta content. As an alternative, method performance was evaluated through spike recovery studies in which known quantities of NP agent were added to different tissue types at high and low concentrations. Each digestion method is described in detail and the corresponding spike recoveries, precision and LOD and LOQ are presented. During this investigation we also identified and corrected poor Ta recovery from sample matrices with high Ca and Mg content (carcass, feces and femur). Lastly, an *in vivo* study was conducted to assess the retention of a new TaO NP agent, and the microwave-assisted and dry-ash digestion methods were used to determine the Ta content in liver, kidney, spleen and carcass from animals in the retention study.

Experimental

Materials and reagents

Core-shell TaO NP contrast agents of the carboxybetaine (CZ) class were synthesized in-house according to previously published procedures. The reference NP agent, CZ3-TaO[3d] was provided as an aqueous stock solution of suspended nanoparticles and was subsequently diluted w/w with deionized water to create three additional solutions for sample spiking studies. NP agent CZ2-

TaO[3e] used in the *in vivo* retention study was provided as an aqueous stock solution of suspended nanoparticles which was further formulated for *in vivo* injection depending on the measured osmolality of the supplied solution. Both imaging agents are comprised of TaO nanoparticulate cores (prepared identically) functionalized with structurally similar zwitterionic ligand shells (Figure 1). The average particle size ($Z_{effective}$), as measured by dynamic light scattering (DLS), was found to be 3.7 nm for CZ3 and 3.1 nm for CZ2. DLS measurement was performed on the particles in water at 25 °C using a ZetaPALS analyzer (Brookhaven Instruments Corporation, Hotsville, NY). Sample digestion reagents, instrument calibration standards, and TaO NP agent spiking-solution digestion and quantitation procedures may be found as Electronic Supplementary Information (ESI).

Biological samples for spike recovery studies

Tissue and fluid samples for spiking experiments were acquired from BioreclamationIVT (Westbury, NY, USA) and all samples were collected from male Lewis rats 200–300 g in size. Whole blood was supplied in 1 mL portions and shipped frozen and stored at -20 °C until use. Livers were shipped cold in PBS and sectioned manually in-house as needed per experiment design. Livers were stored at -20 °C after sectioning until use. Individual femur bones were shipped and stored at -20 °C until use. Whole carcasses were shipped frozen then thawed and sectioned manually in-house to fit into 50 mL polypropylene centrifuge tubes for storage at -20 °C until use. Care was taken to divide each head into two sections to facilitate destruction of organic matter during ashing. Urine was supplied as 15 pooled lots of 20 mL, which were subdivided and frozen into 10 mL portions for shipment. Feces was supplied frozen in 10 g portions, each of a different lot. Urine and feces were stored at -20 °C until use.

Biological sample digestion

The acid amounts and heating conditions utilized in the biological sample digestion methods are modified from our previous TaO NP publications[3] and were developed based on previously reported procedures for the dissolution of Ta_2O_5[5b] and the decomposition of biological material.[12] Each sample type was spiked with NP agent at a low and high concentration similar to what has been found previously in authentic in-house *in vivo* samples[3] and digestion replicates were prepared at each spike concentration. In order to evaluate between-run variation, replicate digestions at either the high or low spike level were prepared on three separate occasions. The Ta concentration observed most often in past *in vivo* studies for a particular sample type was used to determine which spike level would be replicated for that tissue type. The analyst, digestion equipment, and ICP instrument were the same for each run; however, a unique set of calibration standards was utilized for each instance. Thus, run replication captures the variation which occurs when conducting the analyses over a time span of approximately three months using different sets of calibration standards and reagent lots and under typical laboratory environmental variation.

Microwave-assisted acid digestion of biologicals, Method 1a. Liver (0.5 g, wet-weight), individual femur bone (1.5 g), or whole blood (0.2 g) was placed in a TFM vessel from CEM Corporation (Matthews, NC, USA) and the designated amount of NP agent was added to each sample for spiking studies. The spiked sample was kept at room temperature for 30 to 60 min to allow the agent time to bind with the sample material prior to the addition of 0.5 mL HF, 1 mL HNO_3, 2 mL H_2O_2, and 2 mL deionized water. Sample pre-digestion occurred at room temperature and standard atmospheric pressure for 30 to 60 min. During the pre-digestion time, the femur softened such that each piece could be submerged below the solution level prior to initiating the heating cycle. Microwave digestion was conducted using either a CEM Mars 5 or Mars X-Press unit, and the operating parameters were ramp to temperature (180 °C) in 30 min followed by a 30 min hold at temperature. The maximum permitted pressure was 600 psi and the maximum power output was 1600 W for the Mars X-Press or 1200 W for the Mars 5. The vessels cooled to less than 50 °C before depressurization. The dissolution solutions were transferred to polypropylene centrifuge tubes and 2 mL of 100 µg/mL Nb internal standard was added to each tube. The solutions were diluted with deionized water to 20 mL to contain 2.5% HF, 5% HNO_3, and 10 µg/mL Nb. Ten portions of unspiked liver were processed for the determination of method LOD and LOQ values, and additional unspiked femur, unspiked whole blood, and procedural blanks were carried through the digestion process alongside spiked samples.

Microwave-assisted acid digestion of biologicals, Method 1b. One set of five femur bone samples was digested following the procedure outlined as Method 1a except that: (1) the solution of 100 µg/mL Nb internal standard (2 mL) was added to the TFM vessel prior to the start of pre-digestion, and (2) the amount of deionized water added was reduced to 1 mL to keep the total volume unchanged. All other steps were carried out as described.

Open-beaker acid digestion, Method 2. Urine (10 g) was placed in a 100-mL capacity PTFE beaker (Fisher Scientific) and the designated amount of NP agent added for spiking experiments. All solids present in the urine as-received were included in the digestion. Mixtures were kept at room temperature for 30 to 60 min to give the agent opportunity to bind with the solids present in the urine before 0.5 mL HF, 2 mL HNO_3, and 0.5 mL H_2SO_4 was added. Beakers were heated by hot plate at the solution boiling point until the volume reduced to ca. 0.5 mL and the solution became dark brown or black. HNO_3 (0.5 mL) was added in 5- to 15-min increments until NO_x gas formation was no longer observed. H_2O_2 (2 mL) was added drop-wise followed by an additional 1 mL HNO_3 and deionized water to wash residue from the beaker walls. The volume was reduced with hot plate heat set at 275 °C until ca. 0.5 mL of clear and nearly colorless solution remained and white fumes of SO_3 were observed. The heat was reduced to 100 °C before 2.5 mL HF, 2.5 mL HNO_3, and 5 mL deionized water was added. The solutions were heated 5 to 10 min before transfer to polypropylene tubes and dilution to 50 g with deionized water. Five-fold or fifty-fold secondary dilutions were prepared to contain 1% HF, 2% HNO_3, 0.2% H_2SO_4, and 10 µg/mL Nb. Ten portions of unspiked urine were processed for the determination of method LOD and LOQ values, and procedural blanks were carried through the digestion process alongside spiked samples.

Dry-ash digestion, Method 3. Liver (10 g), feces (10 g), or carcass (50 g) was placed in a 100-mL capacity platinum dish (Alfa Aesar). All sample masses are on a wet-weight basis. The designated amount of NP agent was added for spiking experiments followed by 5 mL of 100 µg/mL Nb internal standard solution. Samples were kept at room temperature for 30 to 60 min before ashing in a Fisher Scientific Isotemp muffle furnace (model 126) according to the sequence in Table 1. Samples were heated first at low temperature for controlled evaporation of all water before increasing the temperature for destruction of organic matter. Following ashing, 0.4 mL HF, 1 mL HNO_3, and 1 mL H_2SO_4 (1.5 mL for feces samples) was added and the sample heated by hot plate until the volume was reduced to ca. 0.5 mL. The hot plate temperature was reduced to less than 100 °C before adding 2 mL HF, 2 mL HNO_3, and 5 to 10 mL deionized water. The mixtures were heated 5 to 10 min before

transfer to 50 mL polypropylene tubes. The solutions were further diluted with deionized water to contain 4% HF, 4% HNO_3, 1% H_2SO_4, and 10 µg/mL Nb. Ten portions of unspiked carcass were processed for the determination of method LOD and LOQ values. Additionally, unspiked feces and procedural blanks were carried through the digestion process alongside spiked samples.

Prior to conducting the spike recovery study described above, exploratory studies were conducted in which 5 mL of 100 µg/mL Nb internal standard was added (1) after acid dissolution and transfer to polypropylene tubes, (2) after ashing but before acid dissolution, and (3) before ashing. Recoveries closest to 100% were obtained when the internal standard was added before ashing, so that is the procedure used in the method-evaluation work presented here.

In Vivo retention study

Prior to injection, the osmolality of CZ2-TaO was measured using a Vapor Pressure Osmometer, VAPRO 5520 (Wescor, Inc., Logan, UT, USA). The agent solution was determined to be hyperosmolar and was formulated using deionized water (800µL total) until the osmolality was measured to be 285–300 mmol/kg. Eight-week-old male Lewis rats (Charles River Laboratories, Wilmington, MA) were used for this study. The study was approved by the IACUC at the General Electric Global Research Center (Niskayuna, NY), which is AAALAC and OLAW accredited. Rats were provided food and water *ad libitum* and were maintained on a 12-hr light and 12-hr dark cycle. Rats were injected intravenously (tail vein) with CZ2-TaO at a dose of 400 mg Ta/kg body weight. At day 7 post-injection, rats were euthanized by CO_2 inhalation, and liver (0.5–0.8 g), kidney (0.6 g), spleen (0.5–0.6 g), and carcass (190–220 g) samples were collected for ICP determination of Ta content. All samples were stored in 50 mL polypropylene centrifuge tubes at -20 °C and were thawed in the refrigerator immediately prior to digestion.

Instrumentation

Quantification of Ta was carried out with an ARCOS SOP ICP-OES (SPECTRO Analytical Instruments, Kleve, DE) equipped with a CETAC ASX-520 AutoSampler (Omaha, NB, USA). ICP-OES sample introduction components and operating parameters are given in Table 2. The spray chamber, torch, and injector are from Glass Expansion (Pocasset, MA, USA) and the nebulizer is from Burgener (Mississauga, Ontario, CA). The instrument was calibrated at the beginning of each run with a set of working standards prepared in the same acid matrix as the samples according to the digestion procedure applied (additional details are provided in ESI). A new set of working calibration standards was prepared from a 1000 µg/mL Ta stock standard for each analysis run. Nb internal standard solution was accurately and precisely added to all working standards for the normalization of Ta intensity to Nb intensity.

Statistical Analysis

ANOVA calculations, variance equality testing and significance testing were conducted with Minitab version 17 (Minitab Inc., PA, USA). P-values less than 0.05 were considered statistically significant.

Results and discussion

Although many different types of tissues and bodily fluids may be submitted for analysis from an *in vivo* NP retention study, it was not practical in this investigation to assess the figures of merit for every individual sample type. As a concession, liver was selected as a proxy for kidney, spleen, heart, brain, lung and skin in both the microwave-assisted acid digestion and dry-ash methods. Nevertheless, we do acknowledge the possibility that bias and precision for the recovery of Ta in these other matrices might be better or worse than what is observed for liver according to the degree of mismatch between the matrices.[11b] Recognizing the considerably different physical and/or compositional properties of bone, carcass, feces, urine and blood versus liver, each of these materials was included for the evaluation of recovery and precision unique to that material. In the presented work, not all sample types are processed by all three methods. The microwave digestion method is conveniently applied to the majority of samples generated in our typical *in vivo* studies (blood and tissue portions of 1 g or less). Urine is treated with open-beaker digestion so that the entire volume can be processed as received. The dry-ash digestion method is applied to biological materials where subsampling or homogenization is not practical or desired (e.g. carcass, feces, and some whole organs).

Bias

In lieu of biological reference materials with certified Ta content, method bias was assessed by spiking liver, blood, femur, carcass and feces with known quantities of NP agent where the form of Ta in the NP agent spiking solution is the same as that in the NP agent injected *in vivo*. Tissue and bodily fluid spiked with NP agent in the described recovery studies function as surrogates to NP agent incurred in live animals upon injection.[11] In this situation, the observed recovery might be an overestimation of the true recovery because NP agent may not be incorporated into the biological material to the same degree as NP agent injected into a live animal.[11,13] Even though the added NP agent spike is not likely to be in full equilibrium with the tissue material prior to digestion, it has been noted that surrogate and incurred components will be in balance when complete destruction of organic matter is applied via strong reagents, such as in the measurement of metals in organic matrices.[11b] It follows then that surrogate recovery in this study should be a reasonable reflection of incurred TaO NP constituent recovery.

Bias is expressed as relative spike recovery, R,[13] and the equation can be found in ESI. Overall R and precision is given in Table 3 as the grand mean and standard deviation calculated per tissue type and digestion method using all replicate digestions from both spike levels. Full details of within-run R and Ta spike mass are presented in Tables S1–S3 of the ESI. Overall R is between 98.5 and 102.3% for all experiments except femur which is 91.6% by method 1a. Although one-sample t-tests of R for liver (method 3) and urine (method 2) indicate that R is statistically different than 100%, the recoveries obtained are satisfactory for our purposes.

One-sample t-test confirms that R for femur by method 1a is not equal to 100%, and the low recovery is likely related to the significant amount of insoluble calcium fluoride present following microwave digestion of femur due to the use of hydrofluoric acid.[14] Since recoveries from liver and blood by method 1a are near 100% for similar Ta concentrations and insoluble fluoride material is not visible in these matrices following digestion, it is surmised that the low recovery in femur is due to the loss of Ta within calcium fluoride formed during digestion. In the original procedure (method 1a), niobium is added as an internal standard to the sample solutions at the end of the preparation procedure for correction of instrument-related variations in the final result. Because of its known similarity in chemical behavior to Ta,[15] we expected that Nb would track with Ta during the sample preparation process and could also be used to correct for Ta loss during sample digestion as

a methodological internal standard.[11b,16] To test this hypothesis, five new replicates of femur were digested by method 1b in which the Nb internal standard was added prior to performing the digestion cycle (instead of after). The mean relative recovery for this set is 103.8% with precision comparable to before. Although the recovery by method 1b appears to be biased slightly high, possibly due to subtle differences in the behavior of Nb compared to Ta,[15] the result is closer to 100% and is preferable to the nearly 10% low bias with method 1a.

Knowing that a large quantity of similar insoluble fluoride material was observed in past dry-ash preparation of carcass and feces samples,[14] exploratory recovery tests were conducted with carcass prior to conducting the full spike recovery study to determine if internal standard added earlier in the dry-ash process would impact recoveries. Our legacy in-house dry-ash method detailed that the internal standard was to be added at the end of the digestion sequence, just before final dilution, but this resulted in the lowest recovery in carcass at 72.3 ± 9.9% (n=3). Ta recovery was 91.0 ± 6.0% (n=3) when the internal standard was added to carcass after ashing but before acid dissolution. Similar to femur digestion by method 1b, the internal standard added to carcass prior to ashing resulted in recoveries closest to 100% (96.2 ± 3.9%, n=3), and thus is the protocol applied for dry-ash digestion in this spike recovery study.

General liner model ANOVA was used to evaluate trends in *R* with respect to run number and spike level per tissue type and digestion method. Run number was not found to be a significant factor in the recovery of Ta with the exception of urine by method 2, but additional urine analysis is needed to confirm the run-number trend. Spike level was also not found to be significant regardless of the tissue type or digestion approach applied. Still, since demonstrating low agent retention is an important safety performance metric,[1,3a,3b] further study of low-concentration spike recovery will be valuable as NP agent clearance properties are improved and the mass of Ta retained in tissue is reduced in future *in vivo* studies.

Precision

Precision is calculated per method and spike level as the standard deviation of *R* from replicate digestions performed. Method 1a precision at low and high spike levels for blood, liver and femur are 0.5/0.2%, 0.7/0.7%, and 2.4/0.4% (low/high) respectively. Method 2 precision for urine is 0.6/0.5% (low/high), and method 3 precision for carcass, feces and liver are 3.2/0.4%, 2.5/0.4%, and 2.2/1.1% (low/high) respectively. Full details of within-run precision are presented in Tables S1–S3 of ESI. Significance testing for equal variances indicates that no statistical difference is observed between low/high spike-level precision for liver, blood or urine but that precision is not equal for low/high spike recovery pairs for femur, carcass and feces. This could be related to the formation of insoluble calcium and magnesium fluoride during digestion of femur, carcass and feces which does not occur in the other matrices examined.

Considering first the liver, blood and urine matrices, overall precision given in Table 3 shows that the smallest standard deviation is achieved with microwave digestion. This is not unexpected as this procedure has the least number of process steps. Considering next the matrices which form a significant amount of visible insoluble fluoride material during digestion, we see that precision of Ta recovery from carcass was improved from 9.9% to 3.9% (exploratory dry-ash study) when the internal standard addition was made prior to digestion. On the other hand, precision of Ta recovery from femur was similar whether internal standard was added before (2.4%) or after (2.2%) digestion for the same spike concentration. Precision of *R* for feces was determined only with internal standard added to the sample prior to digestion, so the corresponding comparison of internal standard performance in feces cannot be made. Additional investigation of precision at lower Ta levels and at concentrations nearing each method LOD will be beneficial to improve our understanding of method performance in this range, particularly for matrices with high Ca and Mg content. A rigorous assessment of precision under reproducibility conditions – including multiple analysts, various microwave digestion unit designs, multiple ICP instruments, and a longer time span – would likely show a higher and more realistic estimate of method imprecision but was beyond the scope of this study.

LOD and LOQ

Ten independent portions of unspiked liver, urine and carcass were processed by methods 1a, 2 and 3 respectively alongside spiked samples. The LOD was calculated as three times the standard deviation and LOQ as ten times the standard deviation of the ten replicate digestions plus the mean sample blank concentration. If the mean blank concentration was a negative value, it was taken to be zero in the LOD and LOQ calculations. Instrument LOD and LOQ were estimated using the standard error, slope and intercept of the 10 calibration curves generated in this study.[17] The results are given in Table 4, and multiple LOD and LOQ are estimated for each digestion method to demonstrate the range possible depending on the sample weight that might be processed or dilution factor used.

Additionally, three replicates each of blood, femur and feces were analyzed to check for detectable Ta background for the calculation of *R* and to estimate the LOD for these matrices. The background in blood and femur were less than the expected LOD estimated using the liver replication standard deviation, yet the background in feces was calculated to be 0.09 µg Ta/g (higher than the expected LOD of 0.03 µg/g) due to spectral interference from Mn on the Ta 268.517 nm wavelength.[18] Ta wavelength 240.063 nm was considered as an alternative for the quantification of Ta in feces since no interferences were observed on this wavelength from feces matrix elements; however, the LOD determined for feces by 240.063 nm is 0.15 µg/g due to a significantly higher standard deviation of the replicate blank measurements. As such, Ta 268.517 nm remained the preferred wavelength for feces samples.

By digesting the blank samples in the same set as spiked samples, we mimic the practical application of the method where blank, low and high concentration samples are processed simultaneously. Hence, any carry-over or cross-contamination caused by samples with high Ta content will be reflected in the method LOD result. This was observed with the blank urine set where three of the ten urine blanks showed slightly elevated Ta over the other seven indicating cross-contamination may have occurred. Beakers were placed close together on the hot plates and covers were not utilized; so, it is conceivable that sample solution could splatter from a high-spike sample beaker into a neighboring beaker of blank urine and result in an elevation of the Ta background in the blank urine. Use of covers is advisable when processing samples of very low concentration in order to minimize cross-contamination but will increase the time needed for the volume-reduction step of the digestion process. The degree of cross-contamination observed in the LOD sample set is minimal and, although it may be responsible for the increased LOD, it does not appear to negatively impact the measured recoveries at the spike concentrations examined.

Method application – an *in vivo* retention case study

A seven-day retention study was conducted to evaluate the performance of a new TaO NP agent, CZ2. Contrast agent

performance is evaluated in several ways, and one key safety metric is tissue exposure. Tissue exposure is assessed as a combination of percent injected dose (% ID) retained per organ and speed of clearance, and safety risk is minimized when total body retention is less than 1.5% ID within 48 hours of injection.[3a,3b] Portions of liver, kidney and spleen were processed by method 1a and the carcass was processed by method 3. Using these methods, we were able to quantitate Ta in each organ and carcass and subsequently show that the total body % ID Ta retention was 1.13 ± 0.08 (mean ± SD, n=4 rats). Individual organ and carcass results are provided in ESI Table S4. From Table S4, the % ID retained (mean ± SD) of CZ2 at seven days (0.29 ± 0.03 in kidneys and 0.07 ± 0.01 in liver) was similar to that of a clinically-used iodine-based contrast agent (0.50 ± 0.05 in kidneys and 0.20 ± 0.08 in liver).[19]

Conclusions

Tissue exposure is a fundamental criterion in understanding NP X-ray/CT imaging agent safety. Since tissue exposure is a function of concentration, an accurate and precise means for quantifying Ta in tissues and bodily fluids over a wide range of concentrations is critical in guiding the development, optimization, and implementation of TaO NP imaging agents. In the present work, we describe three accurate and precise digestion methods with low LOD for the determination of Ta in a variety of tissues and bodily fluids. Acceptable accuracy was initially challenging in calcium-containing matrices such as femur and carcass but this was overcome with a minor adjustment to the sample preparation protocol by adding the internal standard prior to digestion. Additional recovery studies with femur are needed to fully assess bias and precision afforded by method 1b for bone matrix; also of interest is whether or not liver and blood processed by method 1b will show high recovery bias similar to what was observed for femur.

Ultimately, near-unity spike recovery and low imprecision was achieved in all sample matrices at the Ta concentrations examined. The greatest disparity in bias and precision was found for the dry-ash digestion method, but this is not surprising considering the aggressive nature of the preparation procedure, long duration of exposure to the environment, and, in the case of carcass, the great variety of biological material within a given sample. Yet, of the three methods evaluated in this work, it is the most practical approach for the digestion of entire carcass or other samples of high mass. Microwave-assisted digestion produced data with the least bias and imprecision for matrices which are not high in Ca; however, it is the most limited of the three methods in maximum sample size which can be processed per digestion vessel. Adequate bias, precision, and LOD was achieved for urine processed by open-beaker digestion; however, it would be advantageous to assess the recovery of Ta in urine by microwave digestion as open-beaker digestion requires the most hands-on time, has the greatest potential for cross-contamination, and utilizes the most reagents of the three methods. Uncertainty due to subsampling of authentic retention-study tissues was not examined in this study but could be of interest in future investigations.

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14 Solids remaining following femur, carcass and feces digestion were collected, washed with deionized water, dried, and pressed into pellets. A semi-quantitative analysis was performed for boron through uranium with a Rigaku ZSX 100e WD-XRF. The primary constituents present at >0.1% by weight are Ca, F, O and Mg in femur, Ca, F, O, S, Mg, Na, K, and Fe in carcass, and F, Ca, O, S, Mg, Al, Fe, K, and Na in feces. The elements are listed in order from highest to lowest concentration.

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Figure 1 Graphical representations of CZ2-TaO and CZ3-TaO nanoparticle contrast agents with TaO core and ligand shell

Table 1 Furnace operating parameters for ashing of liver, feces, and carcass by dry-ash digestion method

Sequence Step	Target Temperature, °C	Ramp Rate, °C/min	Hold Time at Temperature, min
1	80	1	30
2	120	1	30
3	250	1	30
4	350	1	30
5	450	1	30
6	550	1	30
7	650	1	60
8	750	10	120
9	25	5	-

Table 2 ICP-OES configuration and operating parameters

Spray Chamber	Tracey PTFE cyclonic
Nebulizer	PTFE Mira Mist
Torch	D-Torch with ceramic outer tube
Injector	Tapered alumina, 1.8 mm
Rf Power (W)	1450
Coolant gas flow (L/min)	14.5
Auxiliary gas flow (L/min)	1.30
Nebulizer gas flow (L/min)	0.82
Measure time strategy phase 1, 2, 3, 4, and 5 settings[a] (sec)	0.5, 1, 5, 10, 0
Number of measurements per analysis	3
Element wavelengths (nm)	Ta 268.517, Nb 295.088

[a] Technical term of the measurement setting within the Spectro Arcos ICP software. *PTFE* = polytetrafluoroethylene

Table 3 Relative percent recovery (*R*) grand mean and precision (SD)

Sample	n	Method[a]	*R* ± SD %
Blood	12	1a	100.0 ± 0.5
Liver	12	1a	100.3 ± 0.8
Femur	12	1a	91.6 ± 2.5
Femur	5	1b	103.8 ± 2.2
Urine	20	2	98.5 ± 1.1
Carcass	12	3	98.7 ± 3.3
Feces	12	3	100.8 ± 1.2
Liver	9[b]	3	102.3 ± 1.8

[a] Methods 1a and 1b are microwave-assisted acid digestion, 2 is open-beaker acid digestion, and 3 is dry-ashing digestion.
[b] The third set of liver "low spike" digestions by dry-ash was not completed (by accident), thus n=9 for liver by method 3.

Table 4 LOD and LOQ for each digestion method

Digestion Method[a]	Material Examples	Sample Mass g[b]	Sample Dilution Factor	LOD_{method} µg Ta/g Sample	LOQ_{method} µg Ta/g Sample
1a	Blood[c]	0.2	100	0.6	2
	Liver, kidney, spleen	0.5	40	0.3	0.9
	Femur[c]	1.5	13	0.09	0.3
2	Urine	10	25	0.6	2
	Urine	10	250	6	20
3	Liver[c]	10	5	0.03	0.1
	Feces[c]	10	5	0.1	0.2
	Carcass	50	1	0.006	0.02
Instrument			1	0.004	0.01

[a] Method 1a is microwave-assisted acid digestion, 2 is open-beaker acid digestion, and 3 is dry-ashing digestion.
[b] Wet-weight of tissue or fluid at time of collection.
[c] The presented LOD and LOQ are estimated from the standard deviation of n=10 replicates of unspiked liver or carcass and are adjusted to account for the difference in sample mass between sample types. In addition, the feces LOD and LOQ estimates include the measured Ta background due to interference from Mn on the reported Ta wavelength.