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Challenges and successes in the use of neutron activation analysis procedures for value assignment of animal serum and bovine liver Standard Reference Materials

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Abstract Analyses for value assignment in the renewal Standard Reference Materials® SRM 1598a Animal Serum and SRM 1577c Bovine Liver included extensive characterization by neutron activation analysis (NAA). Conventional instrumental NAA procedures were complemented by pre-irradiation chemical separations for the determination of Al, V, Mn, and Cu, radiochemical separations for the determination of Ag, As, Cd, Cr, Cu, Mo, Sb, and Se, and the use of (anti-) coincidence gamma-ray spectrometry systems for the instrumental determination of Ag, Cr, and Hg. The previous materials, SRMs 1598, 1577, and 1577b, were analyzed together with the new materials for quality control.

Keywords Chemical separation · Radiochemistry · Gamma-ray spectrometry · Low level trace elements

Introduction

The National Institute of Standards and Technology (NIST) has prepared a fresh-frozen animal serum SRM and a freeze-dried powdered bovine liver tissue SRM to renew the supply of two benchmark materials, SRM 1598 Bovine Serum and SRM 1577b Bovine Liver, respectively. The analytical approach to the value assignment in the certification process of the renewals of benchmark SRMs 1598 Bovine Serum and 1577b Bovine Liver had to address the challenges of very low levels of trace elements typically present in uncontaminated biological tissues, the changes in available analytical techniques compared with those available for use in the certification of the original serum and bovine liver SRMs, and the addition of new elements of interest.

The Centers for Disease Control and Prevention (CDC) have identified ten elements of interest in their efforts to monitor the population health and nutritional status (National Health and Nutrition Examination Survey) which together with a selection of elements with known toxicological effects formed the target for the analytical characterization. When reviewing the elements targeted for characterization, the selection of time-proven NAA techniques became central to the characterization effort. Instrumental NAA (INAA), NAA with radiochemical separations (RNAA), and pre-irradiation separation NAA (PNAA) were used in this work.

Most elements discussed in the context of this paper are present at levels near or below a few μg/kg in the investigated samples, a challenge for most analytical techniques. Recent laboratory proficiency testing for analysis of liver and blood samples showed only a fraction of the laboratories provided highly acceptable results for a number of these elements in SRM 1598a [1]. For example, results reported for Ag, As, Cd, or Sb spanned an order of magnitude and a significant number of laboratories reported detection limits at least one order of magnitude above the target values. Factors affecting analytical techniques, including some NAA applications, are blank contributions in dissolution and to the analytical signal, losses of analyte in chemical processes and during analysis, low signal to background ratios, and possible interferences from matrix and other elements in the detection of the very low level elements.

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The elements discussed here are Ag, Al, As, Cd, Cr, Hg, Mo, Se, and V. Other elements, among them the major electrolytes and some essential trace elements such as Co, Fe, Mn, Rb, Zn will not be highlighted in this paper; only Cu is included as part of the PNAA procedure. The determinations of the latter elements can be considered routine by NAA, as well as by a number of different techniques. This paper highlights the application of NAA procedures that were chosen for the certification process specifically for their ability to determine trace elements at very low levels.

Experimental

Sample preparation

The sample preparation steps for NAA were designed to minimize contamination. All preparations were done in clean air environments, substrates and implements were from non-contaminating materials such as Ti and pre-cleaned Teflon, quartz, and polyethylene containers [2]. Irradiations in quartz vials made from high purity synthetic quartz (Suprasil AN, Heraeus, Darmstadt, Germany), with subsequent counting of the samples in these vials, were used for the serum and bovine liver samples. The animal serum was dispensed after thawing in 1-mL portions directly into the quartz vials. The serum was then frozen and freeze-dried. This step was repeated with an additional portion to arrive at about a 2-mL serum aliquot dried in the vial. Subsequently the vials were sealed.

The bovine liver powder was scooped into the quartz vials without use of additional implements. The vials were sealed after weighing. Each bovine liver sample consisted of approximately 300 mg as sampled; the dry mass basis was determined using separate portions. For INAA bovine liver was also prepared by forming 100 mg pellets from the powdered material. The pellets were sealed in pouches of high-density linear polyethylene (LPE) film for irradiation. The separation columns from the PNAA procedure were encapsulated in 5 mm diameter polyethylene vials. These column samples were removed from their containers after irradiation and repacked and sealed in polyethylene pouches for counting.

NAA procedures

INAA was carried out in sequential steps with the pelletized samples as described elsewhere [3]. The samples in quartz vials were first assayed similarly to the INAA procedure used for the assay of the pelletized samples for intermediate- and long-lived nuclides, and then the samples were submitted to additional longer (3 d to 5 d) counts in close geometry positions on high efficiency detectors for the determination of Cr and Hg, and/or the coincidence counting system for the determination of Se and Ag [4]. In the latter instances the amount of Zn and Co determined in the initial counts using conventional counting geometry for each sample was used to derive correction factors for sample-to-sample differences in the counting geometry. The contributions measured in blank quartz vials were corrected by an average efficiency based on the Zn corrections from all samples.

RNAA of bovine liver followed the previously published procedure for the determination of Ag, As, Cr, Cu, Mo, Sb, and Se [5]. The previously developed RNAA approach for the sole determination of Cr in the serum samples [6] did not yield results for all investigated samples because it was carried out after completion of the INAA assays and consequently lacked sensitivity because of too long decay times.

PNAA was carried out with the serum samples and control samples by modifying a previously developed procedure [7]. Modifications included the use of microwave sample digestion and smaller sample sizes, which resulted in a corresponding decrease in the amount of resin and reagents used. Higher sensitivity obtained through high-rate counting techniques [8] in the INAA portion of the determinations permitted use of these smaller sample sizes. Only one sample of the previous SRM 1598 was available for quality control; additional quality control was achieved by including samples of SRM 1643e Trace Elements in Water.

The serum was allowed to thaw and the entire contents ($\approx$5 g) of each vial was transferred by pouring into a clean TFM Teflon microwave vessel. Five grams of high-purity nitric acid, (Seastar Chemicals, Sidney, BC, Canada) were added to all serum samples, control materials and process blanks, and the vessels processed in a commercially available microwave oven for 40 min at 500 W power using a multi-step procedure. The vessels were heated in a class 10 clean fume hood to evaporate the nitric acid. Residues were rinsed once with high purity water and were dissolved in 2.5 mL of 2 mol/L ammonium acetate.

Samples, controls and procedure blanks were separated with Chelex 100 resin using the materials and parameters listed in Table 1. Solutions were prepared in pre-cleaned PFA Teflon bottles from concentrated high purity nitric acid, ammonium hydroxide, and glacial acetic acid (Seastar Chemicals) diluted with high purity water (sub-boiling, quartz-distilled in-house). Cleaned, plastic Pasteur pipettes were used to add reagents and samples. Metals are retained by the Chelex resin and the matrix elements are removed with 2 mol/L ammonium acetate, followed with high purity water. The columns were allowed to sit uncovered on a class 10 clean bench for 3 weeks until the resin bed dried

Table 1 Pre-irradiation separation procedure using Chelex 100 for the determination of Al, Cu, Mn, and V by INAA

Step	Description
Pack column	Chelex-100 (Bio-Rad, Richmond, CA) 200–400 mesh in the Na form loaded as water slurry (0.6 cm × 2.8 cm). Plastic columns and frits (0.6 cm i.d. × 5.5 cm, Alltech, Deerfield, Il) were pre-cleaned by soaking 4 days in 20% HCL followed by 20% HNO_3
Clean/Condition (perfom twice)	5 × 1 mL, 2.5 mol/L HNO_3
	4 × 1 mL, high purity H_2O
	4 × 1 mL, 2 mol/L NH_4OH
	3 × 1 mL, high purity H_2O
	4 × 1 mL, 2 mol/L NH_4COOCH_3 at pH 5.5
Load Sample	3 × 0.8 mL, 2 mol/L NH_4COOCH_3 at pH 5.5
Separate	12 × 1 mL, 2 mol/L NH_4COOCH_3 at pH 5.5
	3 × 1 mL, high purity H_2O
Dry	In columns, uncovered on class 10 clean bench for 3 weeks
INAA	Transfer dried Chelex-100 to irradiation vials Irradiate one vial each with standard and Ti flux monitor
	Transfer to unirradiated pouch and count 3 times

completely. When dry, the resin shrank into a solid plug that could be easily removed.

INAA for determination of short-lived nuclides was carried out using the following conditions: 300 s irradiation of individual samples and standards with Ti flux monitors at 1.03E14 cm^{-2} s^{-1} neutron flux followed by counting for 300 s after 120 s decay, 600 s after 500 s decay, and for 0.5 h after approximately 3 h decay. Standards consisting of portions of a solution prepared from high purity metals (Al, V, Mn, Cu) dried on filter papers were formed into pellets to provide consistent irradiation and counting geometries. The standards were counted following the sample counts.

Results and discussion

Value assignment for certification of an SRM in general includes measurements carried out at NIST [9]. The overall contributions of NAA to the value assignment of SRM 1598a and SRM 1577c have been significant, contributing values for 16 of 23 elements reported in SRM 1598a [10] and for 24 of 31 elements reported in SRM 1577c [11]. The majority of these values have been obtained by conventional INAA procedures. The results from the NAA procedures highlighted in this paper are shown in Tables 2 and 3 together with other results that are combined to form the assigned values for the certificate of analysis. The certified values are either derived from primary methods (PRI), or from combining results from several techniques using the "Bound on Bias" (BOB) method [12] or the Ruhkin-Vangle Maximum Likelihood" (RVML) [13] calculations (see "Mode" column in Tables 2 and 3).

Two elements determined by INAA with anti-coincidence counting need consideration in the context of this discussion: Cr and Hg. As mentioned above, both elements required the encapsulation of the sample in quartz vials for irradiation. This adds uncertainty to the assay because of less defined irradiation and counting geometries compared to conventional INAA. In particular, counting in the anti-coincidence system is subject to geometry errors and counting losses. However, these were successfully corrected by using Zn as an internal standard.

The largest uncertainty component is added for Cr in SRM 1598a by a variable low blank in the batch of quartz used in this work that can only be determined in separate vials and not directly in the sample vials. Its variation between "0", i.e., not detectable, and an amount approaching the amount of Cr found in the serum samples is the cause for the high expanded uncertainty shown in Table 2. It was essential to obtain the data provided by inductively coupled plasma mass spectrometry (ICP-MS) to confirm a reasonable level of homogeneity for the element. The determination of Hg has large uncertainties due to the still significant, although reduced, contribution of Se to the 279 keV-line of Hg. In SRM 1577c the Se blank contribution in quartz is negligible, the value was confirmed by three RNAA determinations after an additional assay with the anti-coincidence system.

PNAA was the only NAA method suitable for the determination of Al since it provides a significant reduction of P in the sample. However, the corrections for blank contributions from the pre-irradiation procedure add uncertainty to the result. With the reference given by PNAA it was possible to reduce the blanks in the sample preparations for graphite furnace atomic absorption spectrometry (GFAAS) and add this method to the value assignment in SRM 1598a and 1577c. The use of PNAA for the characterization of Al and V in SRM 1577c is pending. The additional elements gained in these determinations have been covered by other techniques in the certification process of SRM 1598a. The PNAA results provided valuable confirmation by an independent method. Mn had to be excluded from this evaluation because of the higher mobility of this element on the columns and consequently possible loss in the process optimized to separate the other metals from the P and Na matrix.

The details of the RNAA determinations of SRM 1577c, including the determinations in the previous materials,

Table 2 Contributions of determinations by PNAA and INAA with anti-coincidence counting to the value assignment in SRM 1598a (mass fractions in μg/L)

	PNAA			Other			Assigned value		
Element	Value	1s*	Exp. U	Value	Exp. U.	Method	Value	Exp. U.	Mode
Al	2.4	0.82	1.3	2.01	0.24	GFAAS	2.3	0.6	BOB
				2.57	0.65	GFAAS			
Cu	1595	72	325	1575	75	ICP-MS (2)	1580	90	BOB
						ICP-OES			
Mn	(1.4)			1.76	0.30	ICP-MS (3)	1.79	0.32	BOB
				1.85	0.21	RNAA			
V	1.93	0.04	0.39	1.82	0.13	ICP-MS	1.88	0.11	BOB
	INAA			Other			Assigned value		
Element	Value	1s*	Exp. U	Value	Exp. U.	Method	Value	Exp. U.	Mode
Cr	0.34	0.065	0.20	0.300	0.010	GFAAS	0.33	0.08	BOB
				0.34	0.20	RNAA			
				0.362	0.023	ICP-MS			
Hg	0.48	0.26	0.30	0.240	0.12	ICP-MS (2)	0.32	0.19	BOB

* Typical values representing the 1s uncertainty associated with counting statistics

Table 3 Contributions of determinations by RNAA and INAA to the value assignment in SRM 1577c (mass fractions as indicated)

	RNAA			Other			Assigned value		
Element	Value	1s*	Exp. U.	Value	Exp. U.	Method	Value	Exp. U.	Mode
Ag (μg/kg)	5.73	0.53	0.28	4.87	0.22	ICP-MS	5.6	1.7	BOB
				5.48	0.37	INAA			
				7.79	1.33	ICP-MS			
As (μg/kg)	19.6	2.5	1.4	19.7	N.A.	CCQM	19.6	1.4	PRI
Cd (μg/kg)	96.1	4.5	2.2	97.10	0.81	ID ICP-MS	97.1	1.2	PRI
Cu (mg/kg)	268	2	4	273.31	0.85	ICP-OES	272.5	4.6	RVML
Mo (mg/kg)	3.11	0.05	0.19	3.05	0.86	ICP-MS	3.30	0.11	BOB
				3.22	0.15	INAA (2)			
				3.434	0.047	ICP-MS			
Sb (μg/kg)	3.06	0.4	0.30	3.20	0.33	INAA	3.13	0.31	BOB
Se (mg/kg)	1.94	0.012	0.04	2.070	0.042	INAA	2.032	0.052	BOB
						ID ICP-MS			
	INAA			Other			Assigned Value		
Cr (μg/kg)	53.0	5.3	17	51.4	N.A.	CCQM	53	14	PRI

* Typical values representing the 1s uncertainty associated with counting statistics

have been discussed elsewhere [10]. These results confirmed the consistent performance of RNAA over 30 years of analyses at NIST. Here it should be noted that the combination of RNAA with advanced INAA procedures carried out at NIST substantiated the important role of NAA in low-level determinations. Table 3 illustrates for example the agreement found in Ag between the RNAA results and the ones obtained by INAA with coincidence counting [4]. In this instance the two contributing ICP-MS laboratories were relatively far apart in their mean values and showed similar divergence in the results for SRM 1577b.

The exceptional value of independent NAA determinations at the low mass fraction levels remains without substitution in NIST's certification program. For the other elements in Table 3, ICP-MS, which was also the most-used technique in the quoted values of the CCQM (Consultative Committee for Amount of Substance) key

comparison [14] shows very solid performance, and when combined with isotope dilution is the primary method for value assignment. RNAA and INAA results serve as independent confirmation for these procedures.

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