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Development of a procedure for measuring nitrogen by cold neutron prompt gamma-ray activation analysis

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An instrument for cold neutron prompt gamma-ray activation analysis (CNPGAA) has been used for the nondestructive determination of nitrogen. The samples were analyzed in an evacuated box to minimize background from neutron capture by atmospheric nitrogen. The system features lower background and lower detection limits than obtainable with the University of Maryland–National Institute of Standards and Technology (NIST) thermal neutron PGAA instrument. CNPGAA has been used to measure nitrogen in standard reference materials which included biological materials and soils; the results are in agreement with certified values. The detection limit for nitrogen in most biological and geological samples is near 1000 mg kg^{-1}.

The nitrogen content of materials is difficult to determine, especially when the element is present at low concentrations. Nitrogen is usually determined using the Kjeldahl procedure, which requires destruction of the sample and does not always yield total nitrogen. Thermal neutron prompt gamma-ray activation analysis (PGAA) has been used for the nondestructive measurement of total nitrogen in biological materials, however, the sensitivity is generally poor.

The introduction of cold neutrons and neutron guide tubes has greatly improved the applicability of PGAA.[1,2] The use of cold neutrons yields higher neutron capture rates for most elements. Furthermore, since the neutron beam is virtually free of reactor gamma-rays and fast neutrons, less shielding is needed, and a more compact detector to sample geometry is possible. The net result is increased sensitivities and lower detection limits for most elements than is achieved with most thermal neutron PGAA instruments. An instrument for cold neutron PGAA, located in the NIST Cold Neutron Research Facility (CNRF),[3–6] has been operational since 1991. Because of its hydrogen-free construction and compact geometry, the instrument offers better detection limits for hydrogen, nitrogen and other elements than the University of Maryland–NIST thermal PGAA facility.[7,8] In addition to the advantages introduced by cold guided neutrons, the (CN)PGAA instrument includes an atmosphere-controllable sample chamber, allowing samples to be analyzed in vacuum. Gamma-ray background due to neutron capture by atmospheric nitrogen is nearly eliminated, resulting in further improvement in detection limits.

Despite the advantages gained with cold neutrons, CNPGAA has not often been used in the analysis of biological materials, primarily because of difficulties caused by neutron scattering. Scattering by hydrogen (σ_s = 80 b, δ_s = neutron scattering cross section, b = barns) affects neutron capture rates by altering the average distance that neutrons travel within a target. In thermal neutron (TN)PGAA this leads to an increase in neutron capture rates in thin hydrogenous targets.[9–12] Hence in hydrogenous targets thinner than they are wide, element sensitivities (counts s^{-1} mg^{-1}) are enhanced by up to 10% over sensitivities in nonhydrogenous targets. Analytical bias introduced by neutron scattering in TNPGAA may be minimized by carefully matching sample and standard matrix and geometry,[13,14] or by the use of spherical or near-spherical targets which mitigate the effects of neutron scattering.[9–12] The effects of neutron scattering on element sensitivities in CNPGAA are more complicated. Scattering of cold neutrons in a room temperature target leads to an increase in average energy of neutrons within the target, and a corresponding decrease in neutron capture rate.[15] Hence element sensitivities in hydrogenous targets more than a few mm thick are decreased, sometimes by over 50%, relative to sensitivities in nonhydrogenous targets of the same thickness. Studies have shown, however, that the variation of element sensitivities with hydrogen content is minimized if the target thickness is kept between 1 and 2 mm.[15] The effects of neutron scattering are also minimized if element ratios are measured.[16]

A method for the determination of nitrogen by CNPGAA has been developed and evaluated through the analysis of Standard Reference Materials. Uncertainties in the analyses of hydrogenous materials are minimized by measuring the ratio of nitrogen to hydrogen. The method is capable of measuring nitrogen mass fractions below 1% in most materials.

Experimental

Samples analyzed were NIST Standard Reference Materials; which included biological materials (in powdered form) and soils. Biological samples were dried over $Mg(ClO_4)_2$ in a desiccator for five days, and were then pressed into 12.7 mm diameter disks using a stainless steel die and a hydrolic press. In order to prevent contamination of one reference material by another, the die was thoroughly cleaned with deionized water and ethanol and dried after each sample pressing. The thickness of each disk was measured using a caliper. Disk thicknesses for the biological samples ranged from 1.5 mm to 2.25 mm, with an average disk mass of about 300 mg. Soil samples were pressed into 1–2 mm thick disks, weighing from approximately 290 to 500 mg.

Nitrogen standards were prepared to cover a range of hydrogen mass fractions. Disks, ranging in thickness from 1 to 2.5 mm were prepared from urea (CH_4N_2O), deuterourea (CD_4N_2O), mixtures of urea and deuterourea, and tris-(hydroxymethyl)aminomethane ($C_4H_{11}NO_3$, abbreviated as THAM). Urea–deuterourea mixtures were prepared using a mixer mill, as described in a previous paper.[15] The mass, thickness, and hydrogen mass fraction of each standard disk is given in Table 1.

Samples and standards were sealed into Teflon bags and analyzed for nitrogen using the NIST cold neutron prompt

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gamma-ray spectrometer. Targets were irradiated inside an evacuated magnesium sample chamber. Standards were irradiated for about 2 h each, while samples were irradiated for at least 12 h (overnight). Nitrogen background was determined by irradiating an empty Teflon bag overnight in the evacuated chamber. Compton suppressed and unsuppressed spectra up to 11 MeV were taken on a modular MCA (multichannel) linked by Ethernet to a VAX analyzer) station.† Changes in neutron fluence rate throughout the course of the measurements were monitored by irradiating a titanium foil (12.7 mm diameter, 147.5 mg) at regular intervals. Nitrogen count rates were normalized to the average Ti flux monitor count rate (at 1381 keV) measured on that day.

Nitrogen was determined using the 10831 keV gamma-ray photopeak and the corresponding single and double escape peaks at 10320 keV and 9809 keV in the unsuppressed spectrum. Because the Compton background is low above 10 MeV, better detection limits are achieved than with lower energy peaks. Peak integration was performed using the SUM4 code written at NIST.[17] The total nitrogen count rate for each sample was determined from the sum of the counts in the full energy, single escape, and double escape peaks. Counting uncertainties from the individual peaks were added in quadrature to obtain the total counting uncertainty. Relative (1σ) uncertainties from counting statistics were $<1.5\%$ for standards, $<3\%$ for biological samples, and 10–20% for soils. Nitrogen count rates ranged from 0.05 to 0.5 counts s^{-1} for biological samples, and 0.005 to 0.03 counts s^{-1} for soils. The nitrogen background count rate, from the empty Teflon bag irradiation, was determined to be 0.0029 ± 0.0004 counts s^{-1} (1σ uncertainty). This background nitrogen count rate may be due to neutron capture by residual air in the bag, or by nitrogen in the air outside the evacuated sample chamber. Since the entire beam path is not evacuated, some nitrogen in air is irradiated. Although this air is outside the field of view of the lead-shielded detector, it is likely that a small fraction of the nitrogen 10 MeV gamma-rays may penetrate the lead shield.

Results and discussion

As mentioned in the introduction to this paper, the primary difficulty with the determination of elements in hydrogenous materials by CNPGAA is the variation of element sensitivities with the neutron scattering power of the target. When even a small change in H concentration or pellet thickness leads to significant change in element sensitivity, calibration of sensitivities can be laborious. Fig. 1 illustrates the magnitude of the problem. Nitrogen sensitivities measured in all standards are plotted against hydrogen count rate. Sensitivities measured in urea disks decrease by nearly 20% with an increase of pellet thickness from 1 to 2.5 mm. Furthermore, sensitivities measured in urea and THAM disks of comparable thickness do not fall on a single line, making calibration even more difficult.

Table 1 Nitrogen standards used in this investigation

Disk identity/composition	H mass fraction (%)	Disk mass/mg	Disk thickness/mm
Urea A	6.67	200.20	1.12
Urea B	6.67	250.81	1.42
Urea C	6.67	307.50	1.73
Urea D	6.67	359.33	2.03
Urea E	6.67	429.70	2.44
Deuterourea A	0.55 ± 0.05[c]	216.20	1.22
Deuterourea B	0.55 ± 0.05[c]	309.33	1.68
Deuterourea C	0.55 ± 0.05[c]	427.20	2.29
75% Urea–25% Deuterourea[a]	5.18 ± 0.05	312.76	1.82
50% Urea–50% Deuterourea[a]	3.51 ± 0.05	319.92	1.82
25% Urea–75% Deuterourea[a]	2.02 ± 0.05	327.47	1.84
THAM[b] A	9.15	262.50	1.68
THAM B	9.15	334.20	2.08

[a] Urea–deuterourea mixture. [b] Tris(hydroxymethyl)aminomethane: $C_4H_{11}NO_3$. [c] Estimated from H count rate and H sensitivities determined from urea disks. Uncertainties are 2σ, based on counting statistics, background subtraction, and the uncertainty in the hydrogen sensitivity.

Since the ≈300 mg disks of urea, deuterourea, and the three urea–deuterourea mixtures span the range of H count rates measured in the samples and are of the same approximate thickness as the sample disks (1.5–2 mm), an attempt was made to calibrate the nitrogen sensitivity using these five disks as standards. Nitrogen sensitivities measured in the five disks are plotted as a function of H count rate in Fig. 2. The plot shows that for urea–deuterourea disks of 1.5–2 mm thickness, nitrogen sensitivity remains constant over the range of hydrogen concentrations measured. An average nitrogen sensitivity, calculated from the five values, was then used in the determination of nitrogen in the biological samples. The results are given in Table 2. Nitrogen mass fractions measured in this manner are 5–10% higher than certified values, even for samples with thicknesses comparable to the standards.

A more reliable approach to determining nitrogen by CNPGAA is to measure the ratio of nitrogen to an element of known concentration.[16] The method is based on the observation

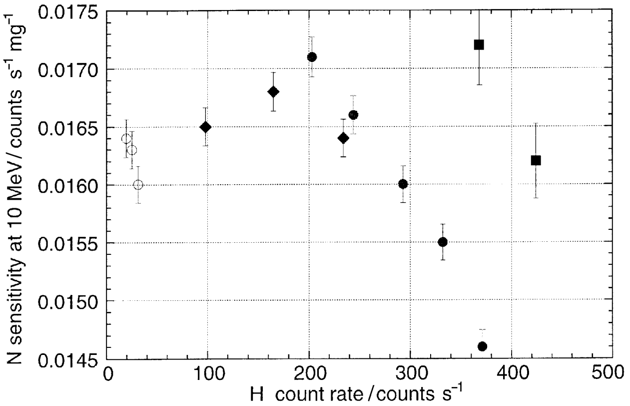

Fig. 1 Nitrogen sensitivity at 10 MeV *vs.* H count rate for nitrogen standards. Symbols represent urea (●), deuterourea (○), urea–deuterourea mixtures (◆), and THAM (■). Error bars are 1σ, based on counting statistics.

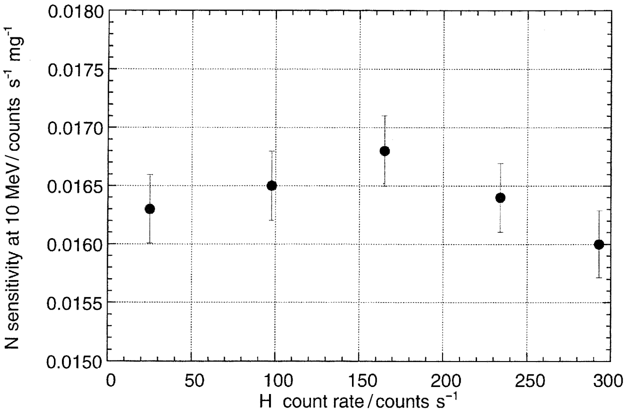

Fig. 2 10 MeV nitrogen sensitivities for ~300 mg urea, deuterourea, and urea–deuterourea disks are plotted *vs.* hydrogen count rate. Urea–deuterourea mixtures are, from lowest to highest H concentration, 0–100, 25–75, 50–50, 75–25, 100–0. Error bars are 1σ, based on counting statistics.

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† Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedures in adequate detail. Such identification does not imply recommendation by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

that at cold neutron energies ($\lambda > 0.4$ nm), the neutron capture cross sections for most elements vary inversely with neutron velocity (*i.e.* they exhibit '1/*v*' behavior).[18] Therefore sensitivities for all '1/*v*' elements are affected equally by neutron scattering and self shielding, and the effects of these processes should be minimized when element ratios are measured. Consequently, although element sensitivities in CNPGAA tend to decrease with increasing H concentration or target thickness, ratios of sensitivities remain constant. This is illustrated in Fig. 3. The ratios of nitrogen to hydrogen sensitivity for urea, urea–deuterourea, and THAM disks are plotted *vs.* H count rate. Sensitivity ratios for all targets are constant within the stated uncertainties. Using the ratio approach, nitrogen mass fractions for biological samples were calculated using nitrogen and hydrogen count rates measured in the samples, the mean N/H sensitivity ratio measured in the standards (calculated as 0.00113 ± 0.000015), and hydrogen mass fractions previously measured using thermal neutron PGAA. Since thermal neutrons are not subject to energy change during interaction with the target, the calibration of hydrogen sensitivity with neutron scattering power is relatively straightforward.[13] The results of the analyses are given in Table 3. The mean nitrogen value for each SRM is in agreement with the certified value within the stated uncertainty. Final nitrogen values measured by CNPGAA and expanded uncertainties are given in Table 3. A best or final nitrogen value for each SRM is given in Table 3 along with an expanded uncertainty. These final values are based only on mass fractions calculated using the ratio method. Expanded uncertainties, based on counting statistics, background subtraction, target positioning and neutron flux variation, sensitivity calibration and normalization to hydrogen, were calculated as described by Taylor and Kuyatt.[19]

Table 2 Nitrogen mass fractions measured in biological standard reference materials by CNPGAA using the nitrogen sensitivity calibration method. Values are 5–10% greater than certified values

Sample	N mass fraction (%)	Mean	*s*	Certified value
SRM 1515	2.39			
Apple leaves	2.35			
	2.40			
	2.42			
	2.34	2.38	0.03	2.25 ± 0.19
SRM 1547	3.16			
Peach leaves	3.05			
	3.05			
	3.13	3.10	0.05	2.94 ± 0.12
SRM 1570a	6.40			
Spinach leaves	6.37			
	6.49			
	6.37			
	6.37	6.46	0.12	5.90 ± 0.25
SRM 1573a	3.31			
Tomato leaves	3.35			
	3.37			
	3.17			
	3.15	3.27	0.11	3.03 ± 0.15
SRM 1575	1.18			
Pine needles	1.23			
	1.17	1.19	0.04	1.2[a]
SRM 1577	11.4			
Bovine liver	11.3	11.3	0.05	10.6 ± 0.6

[a] Information value only. Certified values are given with expanded uncertainties.

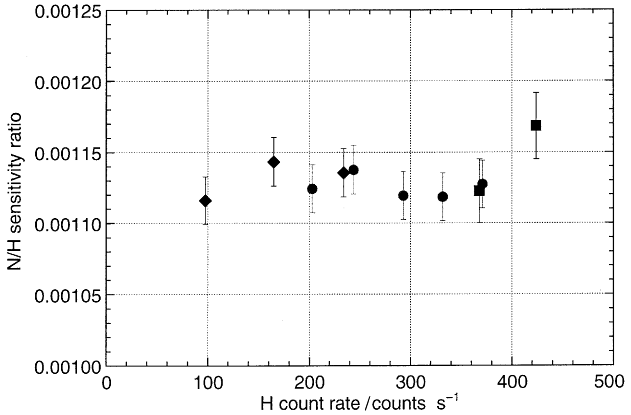

Fig. 3 N sensitivity (at 10 MeV)/H sensitivity (at 2.223 MeV) *vs.* H count rate for nitrogen standards. Symbols represent urea (●), urea–deuterourea mixtures (◆), and THAM (■). Error bars are 1σ, based on counting statistics.

Because no hydrogen data were available for the soil SRMs, the ratio method was not used for the soils. Instead nitrogen mass fractions in these materials were calculated using nitrogen sensitivities determined in the deuterourea disks, which best match the soil samples in hydrogen content. Since the H content of the soils is low, the variation in N sensitivity with disk thickness is not as marked. Nitrogen results for soil samples are given in Table 4. Because the nitrogen count rates yielded by the soil samples were low, care was taken during analysis to ensure that the nitrogen being measured was actually in the samples, rather than in air which might be trapped inside the Teflon bags. This was done by measuring the nitrogen background yielded by a Teflon bag irradiated in vacuum, as described in the Experimental section. As an additional test of whether the nitrogen measured was actually in the sample, three samples of each soil SRM were analyzed: a disk weighing ≈290 mg and two ≈500 mg disks. For each SRM, nitrogen measured in the disk of lesser mass was in agreement with the nitrogen mass fractions measured in the two heavier disks. If residual nitrogen in the bag were being measured, the smaller sample would probably yield a higher apparent nitrogen content.

Table 5 gives limits of detection for nitrogen, determined for various materials. Detection limits were calculated using the method outlined by Currie,[20] for an optimum-size sample for CNPGAA. For biological, soil, and rock samples, the optimum size was determined as the amount of sample needed to form a 2 mm thick, 13 mm diameter disk; for titanium metal the sample size must be kept smaller in order to keep the total count rate manageable and to avoid large corrections for dead time and neutron self-shielding.

Conclusions and future plans

Cold neutron prompt gamma-ray activation analysis is capable of measuring nitrogen mass fractions down to ≈0.1% in biological samples, soils, and other materials. And because, unlike the Kjeldahl method, CNPGAA relies on a nuclear rather than chemical reaction, the results do not depend on the chemical form of nitrogen in the sample. Furthermore, because the analysis is nondestructive, valuable specimens may be saved for further study. The method is expected to prove useful for the certification of nitrogen in biological materials and in soils, particularly at mass fractions below 1% where thermal neutron PGAA measurements have yielded large uncertainties. And because the Cold Neutron Research Facility at NIST is a national users facility, open to the public through submission of proposals, the cold neutron PGAA instrument may prove valuable for measurement of nitrogen by scientists worldwide.

Because the analysis of hydrogenous materials is hampered by the effects of neutron scattering, at present the most reliable method for measuring element mass fractions in biological materials by CNPGAA is to measure the ratio of the element of

Table 3 Nitrogen mass fractions measured in biological standard reference materials by CNPGAA using the ratio method. The nitrogen/hydrogen mass ratio was measured by CNPGAA, then multiplied by hydrogen mass fraction (previously determined by thermal neutron PGAA) to obtain the nitrogen value

Sample	H mass fraction (%)[a]	N mass fraction (%)	Mean	*s*	Final value by CNPGAA[b]	Certified value
SRM 1515		2.23				
Apple leaves		2.23				
		2.21				
		2.17				
	6.10 ± 0.15[c]	2.20	2.21	0.02	2.21 ± 0.10	2.25 ± 0.19
SRM 1547		2.97				
Peach leaves		2.92				
		2.87				
	6.28 ± 0.15[c]	2.92	2.92	0.04	2.92 ± 0.14	2.94 ± 0.12
SRM 1570a		6.10				
Spinach leaves		6.10				
		6.19				
		6.32				
	5.60 ± 0.17[c]	6.25	6.19	0.10	6.19 ± 0.32	5.90 ± 0.25
SRM 1573a		3.17				
Tomato leaves		3.09				
		3.16				
		2.95				
	5.20 ± 0.13[d]	2.99	3.07	0.10	3.07 ± 0.23	3.03 ± 0.15
SRM 1575		1.12				
Pine needles		1.14				
	6.13 ± 0.15[c]	1.08	1.11	0.03	1.11 ± 0.07	1.2[e]
SRM 1577		10.3				
Bovine liver	6.84 ± 0.18[c]	10.5	10.4	0.15	10.4 ± 0.6	10.6 ± 0.6

[a] Hydrogen mass fractions determined by thermal neutron PGAA. [b] Expanded uncertainties based on counting statistics, background subtraction, target positioning and neutron flux variation, sensitivity calibration, and normalization to hydrogen. [c] D. L. Anderson, unpublished data. [d] D. L. Anderson and W. C. Cunningham, submitted for publication. [e] Information value only.

Table 4 Nitrogen mass fractions measured in soil samples by CNPGAA

Sample	N mass fraction (%)	Mean	*s*	Final value by CNPGAA[a]
SRM 2709	0.11			
San Joaquin soil	0.11			
	0.11	0.11	0.003	0.11 ± 0.03
SRM 2710	0.31			
Montana soil	0.26			
	0.32	0.29	0.03	0.29 ± 0.04

[a] Expanded uncertainties based on counting statistics, background subtraction, target positioning and neutron flux variation, and sensitivity calibration.

Table 5 Nitrogen detection limits for various materials, irradiated in vacuum, and counted for 24 h. Detection limits were calculated using the nitrogen 10 MeV gamma-ray peaks and equations given by Currie

Matrix	Detectable N mass fraction (%)
Biological (SRM 1573a, tomato leaves, 300 mg sample)	0.13
Soil (SRM 2710, Montana soil, 500 mg sample)	0.05
Rock (USGS GSP-1, 500 mg sample)	0.12
Titanium (SRM 354, unalloyed, 200 mg)	0.7

interest to an element of known concentration. More research is needed to fully understand the effects of cold neutron scattering on element sensitivities so that sensitivities may be calibrated absolutely. A long range solution may be to analyze targets in a cryogenic sample chamber in order to bring targets into thermal equilibrium with cold neutrons.[21] The effects of neutron scattering would then be comparable to those observed in thermal neutron PGAA, and could be mitigated by the use of spherical or near-spherical targets.

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