Research Article

Measurement of extremely ^{2}H-enriched water samples by laser spectrometry: application to batch electrolytic concentration of environmental tritium samples

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RATIONALE: Natural water samples artificially or experimentally enriched in deuterium (^{2}H) at concentrations up to 10,000 ppm are required for various medical, environmental and hydrological tracer applications, but are difficult to measure using conventional stable isotope ratio mass spectrometry.

METHODS: Here we demonstrate that off-axis integrated cavity output (OA-ICOS) laser spectrometry, along with ^{2}H-enriched laboratory calibration standards and appropriate analysis templates, allows for low-cost, fast, and accurate determinations of water samples having δ^2H$_{VSMOW-SLAP}$ values up to at least 57,000 ‰ (~9000 ppm) at a processing rate of 60 samples per day.

RESULTS: As one practical application, extremely ^{2}H-enriched samples were measured by laser spectrometry and compared to the traditional ^{3}H Spike-Proxy method in order to determine tritium enrichment factors in the batch electrolysis of environmental waters. Highly ^{2}H-enriched samples were taken from different sets of electrolytically concentrated standards and low-level (<10 TU) IAEA inter-comparison tritium samples, and all cases returned accurate and precise initial low-level ^{3}H results.

CONCLUSIONS: The ability to quickly and accurately measure extremely ^{2}H-enriched waters by laser spectrometry will facilitate the use of deuterium as a tracer in numerous environmental and other applications. For low-level tritium operations, this new analytical ability facilitated a 10–20 % increase in sample productivity through the elimination of spike standards and gravimetrics, and provides immediate feedback on electrolytic enrichment cell performance. Copyright © 2016 John Wiley & Sons, Ltd.

Natural waters (^{1}H^{2}HO) artificially enriched in deuterium (^{2}H) well above natural abundance mass fractions are used as powerful tracers in medical, environmental, and hydrological applications. A widespread application of ^{2}H-enriched water is in doubly labelled water (DLW) used for energy expenditure testing of humans and animals.[1,2] Other applications include experiments of tissue turnover,[3,4] using deuterium as an artificial tracer in field and laboratory hydrogeological or diffusion experiments,[5,6] and for batch electrolytic enrichment of tritium in environmental water samples.[7,8] The high concentrations of ^{2}H compared to natural waters range from slightly above natural abundance mass fractions (~150 ppm) to extreme values potentially surpassing 10,000 ppm deuterium (e.g. δ^2H$_{VSMOW-SLAP}$ up to 60,000 ‰).

Hydrogen isotope (δ^2H) assays of liquid water samples are traditionally carried out using dual-inlet or continuous-flow isotope-ratio mass spectrometry (IRMS) using either H$_2$O$_{(water)}$/H$_{2(gas)}$ equilibration, or Zn/Cr/C high-temperature chemical reactor (HTC) reduction methods, via conversion into pure H$_2$ gas.[8–11] However, most IRMS laboratories are reluctant to measure waters extremely enriched in ^{2}H on IRMS instruments (minor collector not optimized), or on sample preparation apparatus routinely used for natural abundance waters (contamination). Some IRMS sample preparation devices suffer from considerable between-sample carryover, especially when measuring ^{2}H-enriched water samples that affect dozens of subsequent samples without the application of carryover correction models.[12] IRMS may suffer from large δ scale expansion at enriched ^{2}H concentrations when using H$_2$ gas. Moreover, most stable isotope laboratories do not have appropriate standards with δ^2H$_{VSMOW-SLAP}$ values of 1000 ‰ or more.[11] As a result, most stable isotope laboratories are unwilling to accept, or cannot measure, extremely ^{2}H-enriched water samples for any of the aforementioned applications.

In 2001, the first laser-based measurements of highly ^{2}H-enriched waters had a δ^2H$_{VSMOW-SLAP}$ limit of about 15,000 ‰, with demonstrably improved reduction in between-sample memory compared to contemporary IRMS methods, with sample measurement times on the order of 40 min.[13] Since 2009, low-cost, commercial water isotope laser spectrometers have overtaken IRMS as the primary means to measure δ^2H (and δ^{18}O) in natural waters. Requiring little water (<1000 nL) and few consumables, and with

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minimal training, laser-based water isotope measurements are at a stage of widespread adoption and affordability.[14,15] The first tests of ^{2}H-enriched DLW water by commercial cavity ring down (CRDS) laser spectrometry showed success with samples having $\delta^2H_{VSMOW-SLAP}$ values up to ~750 ‰, but required more than 20 sample injections to overcome significant between-sample memory; hence, only 15 samples per day could be measured.[16] However, with recent developments in laser spectrometry, the potential for modern liquid water isotope laser instrumentation has not been adequately explored for extremely ^{2}H-enriched water samples, which may be useful for the aforementioned applications, or in tracer or experimental studies.

The objective of this paper is twofold: (i) to demonstrate that commercial off-axis integrated cavity output laser spectrometry (OA-ICOS) can be used to rapidly obtain accurate and precise $\delta^2H_{VSMOW-SLAP}$ values for both natural abundance and water samples extremely enriched in deuterium up to 57,000 ‰, and (ii) subsequently to demonstrate the efficacy of using this analytical capability for laser-based ^{2}H-enrichment methods to improve the productivity of environmental tritium laboratories engaged in batch mode electrolytic enrichment of ^{3}H.

^{2}H METHOD FOR DETERMINING ELECTROLYTIC TRITIUM ENRICHMENT

Tritium is a popular radiotracer of short-term hydrologic and ground water residence times,[17] but exceedingly low concentrations in environmental waters nowadays are too low for direct decay counting. Thus water samples typically require pre-concentration of ^{3}H by electrolytic enrichment.[7,18] Methods for pre-concentrating tritium using 250–1000 mL water samples employ sets of mild-steel alkaline electrolysis cells, or polymer electrolytic membrane units.[9,19,20] All tritium enrichment units (TEUs) have three commonalities: (i) pre-distillation of samples to remove dissolved ions, (ii) electrolytic ^{3}H enrichment of the distilled samples to 8–60 mL final volume, and (iii) decay counting by liquid scintillation (LSC) or gas proportional counting (GPC) instruments. Depending on the ^{3}H concentration, starting and final sample volumes, electrolytic cell-type, and operational conditions, water samples may be enriched in ^{3}H by factors of 10–90 times or more[7] as needed for LSC or GPC. Notably, ^{2}H is correspondingly concentrated during electrolysis.

A critical requirement for any TEU is accurate determination of the tritium enrichment factor for each cell in order to correct each sample back to its original ^{3}H concentration in the environment (with appropriate decay correction). Most laboratories use the Spike-Proxy method, whereby 10–20 % of samples processed through the TEU are high-concentration tritium standards (spikes). The tritium recovery (R) is determined from pre- and post-electrolysis gravimetric H_2O recoveries ($V_{final}/V_{initial}$) and measurement of spike ^{3}H concentrations before and after electrolytic enrichment:

$$R = (T_f \cdot V_f)/(T_i \cdot V_i) \qquad (1)$$

where T is the tritium concentration of the spike (net counts per minute (CPM) after background subtraction) before (i) and after (f) electrolysis, and V is the water volume

(e.g. grams H_2O) of the spike sample before and after electrolysis. Initial tritium concentrations of unknown samples processed through the TEU are determined by rearrangement:

$$T_i = T_f/((V_i/V_f) \cdot R) \qquad (2)$$

The tritium recovery factor (R), unfortunately, can only be determined on spike cells. Hence spike recoveries and derived enrichment parameters are averaged and applied equally to all TEU cells containing unknown samples. Quantitative water recoveries and accurate weighing are critical in the Spike-Proxy method. Disadvantages are a significant reduction in sample throughput because of the spike requirement.

An alternative to the Spike-Proxy method is the ^{2}H-enrichment method,[8] which leverages the fact that ^{2}H (HDO) is correspondingly concentrated in a TEU electrolysis process, albeit to a lesser extent than tritium (HTO) due to different net isotope fractionation factors and vapor losses. Nevertheless, tritium (if present) and deuterium are very strongly correlated during electrolytic enrichment.[8,9] Because the electrolytically enriched ^{2}H sample can be measured as an independent variable, it provides a means for determining the ^{3}H-enrichment factors for each cell. This led to the concept of a cell constant (k) that correlates the ^{3}H- and ^{2}H-enrichment factors to each other:[19]

$$k = \ln(T_f/T_i)/\ln(D_f/D_i) \qquad (3)$$

where D is the final (f) and initial (i) sample ^{2}H concentration in ppm, and T is as above. Rearrangement allows determination of the initial unknown tritium concentration (T_i) of a sample by knowing k (for each, or by averaging identical cells), measuring ^{3}H in the electrolytically enriched sample (T_f), and measuring the initial and enriched ^{2}H concentrations:

$$T_i = T_f/(D_f/D_i)^k \qquad (4)$$

A key requirement of the ^{2}H method for determining tritium enrichment factors is accurate determination of the cell constant (k) for all TEU cells, by the ability to measure extremely ^{2}H-enriched liquid water samples. The cell constant is determined empirically by coupled ^{2}H and ^{3}H spike testing, along with careful gravimetric recoveries. The cell constant can be determined for individual cells, or averaged if identical behavior can be demonstrated for each TEU set.[19] An added benefit of the ^{2}H approach is elimination of laborious gravimetric weighing steps (see Eqn. (4)). While elimination of gravimetric weighing seems attractive, by maintaining it the deuterium recovery factor (R_d) for each cell can also be determined:

$$R_d = (D_f \cdot V_f)/(D_i \cdot V_i) \qquad (5)$$

The ^{2}H recovery factor facilitates immediate detection of electrolytic cell performance degradation, by not having to wait for weeks for spike LSC counting completion. To date, the ^{2}H method is employed by few laboratories, and in all cases highly ^{2}H-enriched samples are usually diluted by a factor of 1000 or more to the natural abundance range[7] and measured by traditional IRMS at considerable extra cost. The requisite large dilutions and isotope mass balance budgeting contribute significant error to the overall process.

EXPERIMENTAL

2H-enriched calibration standards

In order to measure extremely ^{2}H-enriched water samples, appropriate enriched calibration standards are needed that span the δ range of the samples.[11,12] Until recently, no primary reference waters highly enriched in deuterium were available. In 2015, the International Atomic Energy Agency (IAEA, Vienna, Austria) announced new ^{2}H- (and ^{18}O-) enriched primary HDO standards.[21] For the purposes of tritium enrichment, two of these standards (IAEA605, IAEA606) were useful for the development of ^{2}H-enriched laboratory calibration standards. The δ^2H$_{VSMOW-SLAP}$ values of the IAEA primary reference waters are summarized in Table 1. Owing to the small amount of reference materials provided (20 mL), we prepared 20 L of three ^{2}H-enriched laboratory standard waters for use in daily normalization; these laboratory standards were calibrated using assigned values for IAEA605 and IAEA606.

Three new laboratory standards were prepared gravimetrically[22] using distilled tap water (δ^2H$_{VSMOW-SLAP}$ = −77 ‰), and by adding 32–200 g of 99.9993 % deuterium oxide (Sigma-Aldrich, St. Louis, MO, USA) in order to span the δ^2H range for 250–2000 mL electrolytically enriched samples obtained in typical tritium operations (estimated to be in the 6000–60,000 ‰ range for δ^2H$_{VSMOW-SLAP}$). The laboratory standards were homogenized for 1 week prior to isotopic analysis and stored at 0.5 bar argon gas pressure in steel siphon-dispensing containers. All δ^2H$_{VSMOW-SLAP}$ calibrations were conducted by off-axis integrated cavity output laser spectrometry, as described below.

In order to obtain assigned δ^2H$_{VSMOW-SLAP}$ values for the laboratory standards, we conducted two-point data normalization using IAEA605 and IAEA606 as calibration standards, with the laboratory standards measured as unknowns. After calibration, δ^2H$_{VSMOW-SLAP}$ values were assigned to all three ^{2}H-enriched laboratory standards (IHL W-62, IHL W-63 and IHL W-68) and used to normalize the results of electrolytically enriched samples and spikes as measured by laser spectrometry. Each laboratory standard was measured several hundred times against IAEA605 and IAEA606. Notably, IHL W-68 was predicted to fall considerably outside the calibration range of the IAEA605 and IAEA606 reference materials.

Tritium enrichment unit test water samples

All the ^{3}H water samples for ^{2}H-enrichment testing were spike standards that were measured for volumetric mass balances and ^{3}H and ^{2}H concentrations, using 250 mL and 500 mL pre- and post-electrolytic TEU systems. A second set of test samples consisted of 500 mL low-level ^{3}H samples from the 2012 IAEA TRIC inter-comparison.[23] The TRIC samples allowed us to determine how well laser-based ^{2}H methods resulted in tritium enrichment factors that returned accurate and precise original low-level ^{3}H results.

Batch electrolytic enrichments were conducted using mild steel 24-cell TEU systems (250 mL, 500 mL) at the IAEA Isotope Hydrology Laboratory (IHL) which have been in use for over 20 years. The alkaline TEU system and its operation are fully described in the IAEA tritium standard operating procedure (SOP) available from the Isotope Hydrology Laboratory.[24] Briefly, the IHL uses the Spike-Proxy method where 3 of the 24 (13 %) samples in each TEU processing batch are spikes. These spike triplets are advanced by one position in each new electrolytic run. Thus, spikes are cycled through the TEU every 8 runs, and are used to determine enrichment parameters for all the remaining cells. For this test, 0.5 mL pre- and enriched spike samples were sampled opportunistically for ^{2}H from routine IHL sample processing operations. One discrete 500 mL, 24-sample, TEU analysis set contained triplicate samples of low-level TRIC test water samples. Deuterium sampling consisted of taking a 0.5 mL sub-sample of the pre-electrolysis spike (natural abundance, after pre-distillation) and a 0.5 mL sub-sample from the 10–12 mL final electrolytically enriched post-distilled sample (extremely ^{2}H-enriched). All the

Table 1. ^{2}H$_{VSMOW-SLAP}$ values of primary isotopically enriched reference waters, gravimetric estimates and laser spectrometric assay of three highly ^{2}H-enriched IHL laboratory water standards used in routine tritium processing

IAEA Primary Standards	δ^2H$_{VSMOW-SLAP}$ (‰)	^{2}H (ppm)		
VSMOW2	0	142.8		
SLAP2	−427.5	89.2		
IAEA605	5,997.9	1088.8		
IAEA606	15,993.6	2639.9		
Enriched Laboratory Standards	δ^2H$_{VSMOW-SLAP}$ (‰, gravimetric)	δ^2H$_{VSMOW-SLAP}$ (‰, measured)	N	^{2}H (ppm)
IHL W-62	9249–9064	**9173.7±3**	490	**1582.1**
IHL W-63	17,919–18,282	**18,064.2±6**	320	**2960.6**
IHL W-68	58,211–57,057	**56,842.8±10**	116	**8929.1**

[a]Gravimetric estimates were based on 20L (kg) of distilled tap water (δ^2H$_{VSMOW-SLAP}$=−77 ‰) and adding 32.00, 64.00 and 200.00g of 99.9993 deuterium oxide, assuming 1 % weighing error. Gravimetric estimates were made by mixing and isotope mass balance equations.[22]
[b]N=number of measurements made by laser spectroscopy. Boldface is mean±SEM, with assigned δ values used in the IHL laboratory.

distilled pre- and post-electrolysis samples for ^{2}H analysis were stored in tightly sealed 1.5 mL Teflon septum capped laser spectrometry vials until analysis. Laser analyses were conducted within 1 day to 1 week after collection.

Tritium measurements

Unenriched and enriched spike samples corresponding to the same ^{2}H sub-samples were measured by liquid scintillation counting (LSC) on a Quantulus 1220™ (Perkin Elmer, Akron, OH, USA) . The tritium-counting procedure is fully described in the IAEA SOP.[24] Briefly, 8 mL of pre- and post-enriched spikes were mixed with 12 mL of Ultima Gold uLLT™ (Perkin Elmer) scintillation cocktail and allowed to stabilize in the dark. The LSC template consisted of 24 unknown enriched samples including 3 enriched spikes, as well as 2 unenriched spikes, 2 calibration standards, and 4 background samples. The total counting time was 500 min/sample conducted over 10 counting cycles (approx. 12 days). Optimal operation of the Quantulus LSC has been described previously.[25] All spectral regions of interest were optimized for low-level ^{3}H beta counting in water to obtain the highest figure of merit (FOM). Net CPM values for pre- and post-enriched spikes were obtained by subtraction of the mean background CPM (~0.5 to 0.8 CPM) on a per run basis. For this study, we extracted net CPM data of pre-and post-spike data obtained from multiple Quantulus LSC runs covering the period from October 2014 to July 2015, and where deuterium measurements were conducted. All data processing was done using an Excel-based *Tritium Information Management System* (TRIMS) developed at the IHL. Error propagation was determined for all the processing steps as outlined in the IAEA SOP, and this was included in the final uncertainty reporting of the ^{3}H results.

Laser spectrometry for ^{2}H-enriched samples

Several off-axis integrated cavity output water isotope laser instruments from Los Gatos Research (Mountain View, CA USA) were tested for directly measuring highly ^{2}H-enriched waters. We tested 1st-3rd generation Los Gatos Research laser instruments and found they did not correctly report δ^2H values above ~12,000–15,000 ‰, despite apparently good isotopologue optical spectrometry, due internal software limitations in these older instruments (Doug Baer, Los Gatos Research, personal communication). In this study we therefore used the newest Los Gatos Research liquid water isotope analyzer model 912-0032 (4th generation).

For laser-based HDO measurements, all the pre- and enriched post-electrolysis samples and laboratory calibration standards were separated into three groups. The first comprised pre-enrichment samples having natural abundance ^{2}H levels. These were measured by routine laser spectrometry using natural abundance water standards, controls, and data-processing procedures using *LIMS for Lasers 2015*, as fully described elsewhere.[15,26] A batch of 24 TEU pre-enriched water samples and standards employed an analysis protocol of 8 injections, ignoring the first 4, at a rate of 11 min/sample.

For extremely ^{2}H-enriched water samples beyond the VSMOW-SLAP calibration scale, the same procedure was used but with some modifications. First, water samples were separated by TEU size and the expected degree of ^{2}H enrichment. For example, 250 mL cell and 500 mL cell

deuterium samples were grouped separately. For the 250 mL TEU system, we predicted δ values around 12,000 ‰ and therefore used a two-point calibration using laboratory standards IHL W-62 (low standard) and IHL W-63 (high standard); in the case of the 500 mL TEU or 1000 mL and higher experimental enrichments samples, we used IHL W-62 (low standard) and IHL W-68 (high standard), and IHL W-63 was used as a control (Table 1).

The only other minor modification for laser spectrometry compared with the natural abundance procedures was the number of injections per vial. Owing to the highly enriched ^{2}H levels, we empirically determined that 12 injections, ignoring the first 4, was sufficient to obtain accurate results and minimize between-sample memory corrections as quantified by *LIMS for Lasers 2015*. This resulted in an analysis rate of 16 min/sample for each enriched sample. For the routine TEU sets of 24 samples, this translated to only 504 injections per set on the laser instrument, well within high-performance septa specifications (Supelco Thermogreen LB-2, 6 mm disks; Supelco, Bellefonte, PA, USA). Including laboratory standards and controls, the processing rate was 60 unknown samples per day. If the laser instrument used for highly ^{2}H-enriched samples was subsequently needed for natural abundance water samples (pre-enriched samples, all other samples), approximately 50 sequential injections (approximately 1 h) of tap water was sufficient to flush away the residual memory of the ^{2}H-enriched water samples. In short, complete sets of pre- and post-electrolysis water samples could be analyzed by laser spectrometry in less than 48 h, thereby providing near immediate feedback on the batch TEU electrolytic enrichment performance.

All laser-based deuterium results (δ^2H$_{VSMOW-SLAP}$) were processed and reported in the ‰ notation relative to the VSMOW standard, and processed using *LIMS for Lasers 2015*. The δ values data were transformed into mass fractions deuterium (ppm) using:

$$D_{ppm} = (\delta^2 H_{VSMOW-SLAP} + 1000)/(\delta^2 H_{VSMOW-SLAP} + 1000 + 1000/0.00015576) \cdot 1000000 \qquad (6)$$

RESULTS AND DISCUSSION

^{2}H-enriched laboratory standards

Gravimetrically determined and laser-measured δ^2H$_{VSMOW-SLAP}$ values for three IHL laboratory standards measured relative to IAEA605 and IAEA606 are summarized in Table 1. In the case of IHL W-62 and IHL W-63, the laser spectrometry-measured δ^2H results fell within the δ range predicted by gravimetric determinations. For sample IHL W-68, the measured δ^2H value was only slightly lower than predicted by gravimetric calculations, probably because the sample was far outside the IAEA605 and IAEA606 reference calibration range, and due to possible effects of very slight isotopic δ-scale compression on the laser instrument. As a further check, we measured IHL W-63 as an unknown sample using assigned values of IHL W-62 and IHL W-68 as calibration standards. In this case, IHL W-63 returned a δ^2H$_{VSMOW-SLAP}$ value of +18,232 ± 33 ‰ (n = 38), close to that reported in Table 1, and within the range predicted by

gravimetric estimates. Without primary standards covering this higher δ range, the IHL W-68 value remains tentative, but for such a high enrichment was remarkably close to the predicted δ^2H value. For our purposes, we accepted the laser-measured δ^2H values in Table 1 as correct, and these δ values and their uncertainties were assigned for all subsequent routine ^{3}H electrolysis and data-processing operations. Sample δ value conversions into mass fractions (ppm) are listed in Table 1. For the primary RMs and laboratory standards, the mass fractions of deuterium ranged from 89.2 ppm (VSLAP2) to 2639.9 ppm (IAEA606), whereas the enriched IHL lab standards ranged from 1582.1 ppm (IHL W-62) to 8929.1 ppm (IHL W-68). These findings show that commercial laser spectrometry can be used to quickly and accurately measure water samples extremely enriched in ^{2}H, and having δ^2H values up to at least 57,000 ‰, and possibly even higher.

The enriched laboratory standard IHL W-68 was prepared in anticipation of electrolytic enrichment of large 1–2 L samples, which will be increasingly required as environmental ^{3}H levels decline to background levels. Indeed, the recent TRIC test revealed that only laboratories that enriched water samples 50× or higher were able to achieve accurate and precise results for low-level (<5 TU) tritium test samples.[23] Currently, few laboratories enrich samples greater than 500 mL (e.g. δ^2H$_{VSMOW-SLAP}$ of ~25,000 ‰) due to extra cost or the lack of a suitable TEU apparatus. The ability to directly measure ^{2}H at extreme concentrations by laser spectrometry provides a new way to quickly determine cell performance and ^{3}H-enrichment factors beforehand, without having to wait weeks for LSC spike-counting results.

Results of tritium spike standards

The results of ^{3}H and ^{2}H assays on spikes processed on 250 mL and 500 mL TEU systems between October 2014 and July 2015 are tabulated in Table 2. The ^{2}H and ^{3}H recoveries for each electrolytic cell were determined using Eqns. (1) and (5). The average deuterium recovery for the 250 mL TEU was 74.4 ± 1.6 %, with an overall range for individual cells of between 71 and 77 %. The average deuterium recovery on the 500 mL TEU was 77.0 ± 1.0 %, with a range for individual cells of between 75 and 79 %. Notably, cell #4 (excluded from summaries) was known to be performing badly, and was in the process of being reconditioned after chemical treatment. This TEU cell still indicated poor ^{2}H recovery of only 54.8 %. Thus, ^{2}H can also be used as an indicator for monitoring cell-enrichment performance prior to starting LSC counting. In our case, a threshold of 70 % for deuterium recovery appeared to be a reasonable lower bound to reveal cell degradation, upon which cell reconditioning may be warranted, and samples from degraded cells should not be used. Similarly, the mean ^{3}H recovery on the 250 mL TEU system was 86.3 ± 2.2 %, with an overall range for individual cells of 81–89 %. The mean ^{3}H recovery for the 500 mL TEU system was 92.4 ± 2.1 %, with an overall range for individual cells of from 90 to 97 %. Poorly performing cell #4 had a low ^{3}H recovery of only 81.0 %.

The cell ^{2}H-^{3}H constant (k) values for the 250 mL and 500 mL TEUs were determined using Eqn. (3) and the measured δ^2H and ^{3}H values from each triplicated spike and cell. The initial CPM of the unenriched spike sample for

each triplet was the mean of two measurements, summarized in Table 2. The individual cell constants were remarkably similar within and across both mild-steel TEU systems. For the 250 mL TEU system, the mean cell constant k was 1.0542 ± 0.0044, with an overall range for the individual cells between 1.0460 and 1.0620. For the 500 mL TEU system the mean cell constant was 1.0592 ± 0.0101, with the overall range for individual cells ranging from 1.0509 to 1.0670. A Student's t-test revealed that there was no significant difference between the mean cell constants of the 250 mL and 500 mL TEU systems ($p = 0.058$). The mean cell constant for the combined 250 and 500 mL TEU systems was therefore 1.0555 ± 0.0048. For all subsequent calculations, and due to the absence of long track records for individual cell constants, we used the mean cell constant and its uncertainty as applicable to the 250 mL and 500 mL IHL TEU systems.

To demonstrate how well using a cell constant k recovered the initial measured CPM for both IHL electrolysis systems, Eqn. (4) was used to determine the initial sample ^{3}H CPM values. In Table 2, a comparison of ^{3}H$_{init}$ (e.g. un-enriched spike) with the electrolytically enriched spike triplicates CPM, and application of the uniform cell constant, revealed excellent performance in determining the initial ^{3}H content (last column), despite the fact TEU sample enrichments were conducted over a 10-month period with two different TEU systems and using three different LSC counters. Notably, no gravimetric data was required with the ^{2}H method to determine the tritium enrichment factors or the initial sample ^{3}H content.

Poorly performing cell #4 showed a markedly different k constant (1.1219) from the good cells. Importantly, application of the measured cell constant yielded an inaccurate ^{3}H recovery (10.91 CPM vs actual 8.82 CPM), indicating that the ^{2}H-derived cell constants cannot be used blindly to remedy badly performing cells. Poorly performing cells should be removed from the TEU and reconditioned. Monitoring deuterium recovery factors is therefore an important control in evaluating TEU cell performance. Our data suggest that ^{3}H results obtained from cells with poor ^{2}H recoveries (e.g. <70 % on IHL TEU systems) should not be accepted. These ^{2}H control criteria will need to be developed for each unique laboratory TEU system. Ideally, over time it may be possible to obtain robust individual mean cell k constant criteria by maintaining regular or occasional spike testing. While the use of individual cell constants seems intuitively superior, it remains to be seen whether individual cell constants, versus averaged cell constants, provides demonstrably better ^{3}H results after all sources of uncertainty are factored in.

Evaluating the ^{2}H method for accuracy and precision

The final test consisted of applying the ^{2}H-^{3}H-determined mean cell constant k to a routine set of 24 samples processed by enrichment on the 500 mL TEU system (including poorly performing cell #4), and then comparing the final tritium (TU ± SD) outcomes with those obtained using the conventional Spike-Proxy method. This test set included triplicates of the IAEA tritium inter-comparison samples, which consisted of five low-level samples <8 TU having well-established ^{3}H values,[23] three spike standards (known TU), two unknown samples (one DI water placed in cell #4)

Table 2. Results of 250 mL and 500 mL TEU spike samples for determination of individual and average ^{2}H-^{3}H cell constants (k)

Electrolysis ID	Counter ID	Cell #	V_o	V_f	$^2H_{init}$	$^2H_{final}$	^2H Recovery %	$^3H_{init}$	$^3H_{final}$	^3H Recovery %	Cell constant	^3H Initial
			grams		ppm		Recovery %	cpm		Recovery %		
250 mL												
1405	Q3	10	249.4	11.4	143.5	2365.3	75.4	9.13	173.48	87.0	1.0508	9.00
1405	Q3	11	249.2	11.2	143.4	2398.6	75.0		177.31	87.1	1.0533	9.06
1405	Q3	12	249.2	11.0	143.5	2363.8	72.7		174.46	84.4	1.0531	9.06
1406	Q3	16	249.5	11.7	143.6	2303.9	75.3	8.91	165.53	87.1	1.0527	8.84
1406	Q3	17	249.3	11.3	143.7	2323.3	73.3		167.76	85.3	1.0546	8.89
1406	Q3	18	249.1	11.5	143.7	2300.9	73.9		167.28	86.7	1.0572	8.95
1407	Q2	19	249.3	11.7	143.5	2178.9	71.2	8.73	150.80	81.1	1.0475	8.54
1407	Q2	20	249.3	12.2	143.6	2208.2	75.3		153.73	86.2	1.0495	8.58
1407	Q2	21	249.1	11.0	143.6	2329.7	71.6		162.83	82.4	1.0500	8.59
1408	Q1	22	249.6	11.9	143.7	2204.3	73.1	9.21	167.28	86.6	1.0620	9.37
1408	Q1	23	249.4	12.0	143.7	2247.9	75.3		168.82	88.2	1.0577	9.26
1408	Q1	24	249.8	12.6	143.7	2102.3	73.8		158.12	86.6	1.0597	9.31
1409	Q1	1	249.6	12.6	143.6	2172.5	76.4	9.10	160.33	88.9	1.0560	9.11
1409	Q1	2	249.6	12.4	143.6	2207.4	76.4		163.61	89.3	1.0572	9.14
1409	Q1	3	250.0	12.9	143.6	2076.9	74.6		154.80	87.7	1.0607	9.23
1410	Q1	4	249.4	12.5	143.7	2164.1	75.8	9.01	158.21	88.4	1.0568	9.04
1410	Q1	5	249.5	12.7	143.7	2176.2	77.0		158.25	89.3	1.0545	8.98
1410	Q1	6	249.5	12.2	143.6	2186.2	74.2		160.29	86.7	1.0573	9.05
1411	Q1	7	249.4	11.8	143.5	2299.0	75.8	9.24	168.18	86.1	1.0460	8.99
1411	Q1	8	249.8	12.2	143.6	2177.3	74.1		161.22	85.2	1.0516	9.14
1411	Q1	9	249.6	11.7	143.7	2219.7	72.4		163.20	82.8	1.0490	9.07
500 mL												
2419	Q2	19	498.4	15.0	143.6	3754.3	78.4	8.66	271.96	94.2	1.0560	8.68
2419	Q2	20	498.6	16.1	143.4	3385.8	76.1		242.11	90.1	1.0535	8.61
2419	Q2	21	498.6	16.6	143.4	3251.0	75.6		233.05	89.7	1.0548	8.64
2420	Q1	22	498.5	16.4	143.5	3373.2	77.2	9.58	270.34	92.7	1.0579	9.65
2420	Q1	23	499.0	14.9	143.5	3676.4	76.6		294.93	92.0	1.0566	9.61
2420	Q1	24	498.7	15.4	143.4	3530.0	76.1		282.27	91.1	1.0563	9.60
2421	Q1	1	498.5	15.3	143.4	3578.4	76.5	9.63	282.85	90.1	1.0509	9.48
2421	Q1	2	498.9	16.3	143.5	3347.6	76.4		265.97	90.5	1.0538	9.57
2421	Q1	3	498.6	15.4	143.6	3639.1	78.1		288.30	92.3	1.0516	9.50
2422	**Q3**	**4**	**498.7**	**11.1**	**143.6**	**3548.0**	**54.8**	**8.82**	**322.03**	**81.0**	**1.1219**	**10.91**
2422	Q3	5	498.4	15.8	143.6	3526.0	77.7		261.80	93.9	1.0593	8.92
2422	Q3	6	498.0	15.5	143.5	3530.6	76.4		261.41	92.1	1.0583	8.90
2424	Q3	10	498.7	16.0	143.4	3454.7	77.3	8.80	252.06	91.9	1.0547	8.77
2424	Q3	11	499.0	15.9	143.5	3486.6	77.4		252.89	91.6	1.0528	8.72
2424	Q3	12	498.9	15.2	143.7	3634.1	77.1		266.23	92.2	1.0556	8.80
2425	Q2	13	499.5	16.8	143.5	3331.1	78.1	8.02	229.95	96.4	1.0671	8.32
2425	Q2	14	499.3	17.2	143.4	3292.4	79.1		226.48	97.3	1.0661	8.29
2425	Q2	15	499.5	15.3	143.4	3533.0	75.5		244.42	93.4	1.0664	8.31

Cell Constant	250 mL	500 mL	All
Mean	1.0542	1.0608	1.0555
SD	0.0044	0.0050	0.0048

[a]The mean cell constant k was used to determine $^3H_{initial}$ in the final column. Definitions and equations for recovery and the cell constant are given in the text. Q = Quantulus ID.
[b]Results are sorted by increasing date of analysis (October 2014 to July 2015) as spikes were rotated through each TEU system.
[c]Note underperforming Cell #4 in the 500 mL system is evident by poor ^{2}H and ^{3}H recoveries.

and one enriched background sample. The use of these inter-comparison samples allowed independent verification of the accuracy of performance of the ^{2}H and Spike-Proxy methods to obtain enrichment factors and produce accurate final TU results.

The electrolysis, ^{2}H and LSC counting test results are shown in Table 3, and give comparative outcomes using the laser ^{2}H-enrichment and Spike-Proxy methods. With the traditional Spike-Proxy method, three measured spikes shown in Table 3 were used to determine the mean tritium

Table 3. Results of a 500 mL, 24-sample, TEU run with spikes, TRIC and unknown samples, and an enriched background samples, comparing the tritium enrichment factors, ^{2}H recovery and the final TU plus uncertainties, comparing the ^{2}H-enrichment and Spike-Proxy methods

Cell #	Sample name	Lab No.	Sampling date	Enrichment	TU	Uncertainty	Recovery	Enrichment	TU	Uncertainty
					Spike method			^{2}H-Enrichment method		
1	**Spike**	2422	7/30/2014	29.7	537.86	5.38	76.5	29.8	535.34	3.34
2	**Spike**	2397	7/30/2014	27.8	539.16	5.16	76.4	27.8	540.42	3.57
3	**Spike**	2421	7/30/2014	29.5	550.90	5.61	78.1	30.3	536.51	3.49
4	Almendras July 2011	2454	7/15/2011	39.8	2.10	0.12	**64.1**	34.0	2.46	0.13
5	TRIC T25	2455	12/1/2012	28.3	7.56	0.12	77.5	28.7	7.45	0.10
6	TRIC T24	2449	12/1/2012	28.4	4.36	0.19	76.9	28.6	4.33	0.18
7	TRIC T23	2451	12/1/2012	29.0	2.88	0.10	76.5	29.1	2.88	0.09
8	TRIC T22	2509	12/1/2012	27.9	1.10	0.12	76.9	28.1	1.10	0.12
9	TRIC T21	2514	12/1/2012	30.2	0.45	0.11	77.9	31.0	0.44	0.11
10	TRIC T20	2510	12/1/2012	30.8	0.00	0.07	75.9	30.8	0.00	0.07
11	TRIC T25	2515	12/1/2012	29.6	7.38	0.26	76.6	29.8	7.34	0.25
12	TRIC T24	2511	12/1/2012	29.4	4.60	0.15	77.2	29.8	4.54	0.15
13	TRIC T23	2519	12/1/2012	28.8	2.88	0.10	78.0	29.5	2.82	0.10
14	TRIC T22	2520	12/1/2012	27.6	1.28	0.11	79.0	28.5	1.24	0.11
15	TRIC T20	2521	12/1/2012	31.6	0.30	0.09	74.9	31.2	0.31	0.09
16	TRIC T20	2516	12/1/2012	29.3	-0.12	-0.09	76.5	29.4	-0.12	-0.09
17	TRIC T21	2512	12/1/2012	29.2	0.65	0.11	75.5	28.9	0.65	0.11
18	TRIC T21	2517	12/1/2012	30.3	0.66	0.13	76.4	30.4	0.65	0.13
19	TRIC T22	2513	12/1/2012	30.4	1.18	0.09	79.7	31.9	1.12	0.09
20	TRIC T23	2518	12/1/2012	29.0	2.72	0.13	77.2	29.3	2.69	0.12
21	TRIC T24	350	12/1/2012	28.8	4.57	0.14	75.7	28.6	4.61	0.14
22	TRIC T25	250	12/1/2012	28.0	7.79	0.29	77.3	28.3	7.71	0.28
23	Sample TK-11	250	10/12/2014	30.2	1.53	0.06	76.7	30.5	1.52	0.06
24	Enriched Deadwater	250	1/26/2009	29.8	0.42	0.16	76.1	29.8	0.42	0.16

Table 4. Comparison of known-value IAEA TRIC inter-comparison samples, enriched and processed using the laser ^{2}H-enrichment and Spike-Proxy methods

TRIC sample	Known value	Spike method		^{2}H-Enrichment method		N
		Mean	SD	Mean	SD	
TRIC T25	*7.51*	7.58	0.21	7.50	0.19	3
TRIC T24	*4.37*	4.51	0.13	4.49	0.15	3
TRIC T23	*2.74*	2.83	0.09	2.80	0.10	3
TRIC T22	*1.12*	1.19	0.09	1.15	0.08	3
TRIC T21	*0.43*	0.59	0.12	0.58	0.12	3
TRIC T20	*0*	0.06	0.22	0.06	0.22	3

enrichment parameter, which was applied uniformly to all the sample cells, versus the ^{2}H method where we used the average cell constant as determined above for the TEU system. The reported final TU values and their propagated uncertainties were obtained by data processing with the IHL TRIMS software. Encouragingly, it was obvious from the results in Table 3 that the Spike-Proxy and laser ^{2}H-enrichment methods both returned nearly identical and accurate results for the laboratory spike (542.1 CPM) within the expected measurement uncertainty (10 %). For all cells in the 500 mL TEU system (except #4), the deuterium

recovery factors were above 75 %, indicating that the TEU cells were performing as well as expected. There was also excellent agreement among the ^{3}H-enrichment factors determined by the ^{2}H-enrichment and Spike-Proxy methods, with no significant difference in the mean tritium enrichment factors obtained using these methods (Student's *t*-test, $p = 0.388$). A summary of the international ^{3}H inter-comparison test sample results is given in Table 4. For the TRIC test samples, the ^{2}H-enrichment and Spike-Proxy methods both yielded accurate and precise outcomes compared with their well-established values. In short, the TRIC test sample results confirmed that the laser-based ^{2}H-enrichment method provided a faster and reliable way to obtain accurate ^{3}H-enrichment factors for all types of electrolytic enrichment cell systems.

CONCLUSIONS

The use of off-axis integrated cavity output laser spectrometry to accurately and directly measure extremely ^{2}H-enriched waters up to 57,000 ‰ (~9000 ppm deuterium) at a rate of 60 samples/day will facilitate the use of ^{2}H as a tracer in environmental and hydrological applications. The incorporation of the laser-based, ^{2}H-enrichment method into low-level tritium operations can facilitate a 10–20 % increase in sample throughput by elimination of replicated spike standards, and laborious gravimetrics, and by providing immediate feedback on the quality of electrolytic cell performance. Additional

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