

On-line measurements of $\delta^{15}\text{N}$ in biological fluids by a modified continuous-flow elemental analyzer with an isotope-ratio mass spectrometer

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A modified continuous-flow elemental analyzer coupled to an isotope-ratio mass spectrometer (modified EA-IRMS) was tested for on-line $\delta^{15}\text{N}$ measurement on urea solution and biological fluids (e.g. urine). The elemental analyzer configuration was adapted by adding a U-shaped cold trap and an X-pattern four-way valve for on-line trapping/venting of water from the liquid samples. Results indicate that the $\delta^{15}\text{N}$ ratios show little variation (standard deviation (SD) = 0.05‰) with a sample size above the equivalent N yield of 0.2 mg urea (0.092 mg N) when the mass spectrometer conditions were carefully optimized. By contrast, a significant logarithmic decrease in $\delta^{15}\text{N}$ with sample size was observed but this can be offset by applying a linearity correction or blank correction when the sample size is between equivalent N yields of 0.05 and 0.2 mg urea. The blank corrected $\delta^{15}\text{N}$ ratios give an overall precision of $\sim 0.16\%$ whereas the average precision for $\delta^{15}\text{N}$ corrected using combined linearity and shift correction is 0.05%. The relatively large variation in blank corrected $\delta^{15}\text{N}$ values may be attributed to the variability of the blank $\delta^{15}\text{N}$ in the sequence. Therefore, the blank correction should be carefully performed in routine measurements. As a result, the linearity range of a modified EA-IRMS can be extended to a minimum sample size of 0.023 mg N. In addition, the reproducibility of the new system is good, as indicated by the precision ($< 0.2\%$) for a set of standards and unknowns. The data show that fluids containing nitrogen can be successfully analyzed in the modified EA-IRMS. Copyright © 2008 John Wiley & Sons, Ltd.

Nitrogen isotope ratio measurements are used in many fields, such as agriculture,^{1,2} ecology,^{3–5} environmental pollution,^{6–8} paleoenvironment changes,⁹ and forensic studies.¹⁰ The samples used in most studies are in the solid state, e.g. soils, plant tissues and atmospheric aerosols. For these samples, nitrogen isotope ratios are now usually measured using an elemental analyzer coupled with an isotope-ratio mass spectrometer (EA-IRMS),^{11,12} or with some modified EA-IRMS.¹³ By contrast, analysis of $\delta^{15}\text{N}$ from biological fluids (e.g. plasma and urine) or water samples is more time-consuming and involves more complicated procedures, including off-line pretreatments such as chemical extraction,^{14,15} physical adsorption,¹⁶ or freeze drying,¹⁷ to produce a sample suitable for analysis using an EA-IRMS. Since the introduction of stable isotope analysis in dietetics and metabolism studies, more and more plasma and urine samples have been used in these fields, resulting in an increasing demand for an automatic system with a high daily

throughput for isotope measurements. This report summarizes the modification of an elemental analyzer coupled to an isotope-ratio mass spectrometer with the objective of producing reliable on-line measurements of $\delta^{15}\text{N}$ from liquid samples such as plasma and urine. The relative ease of the procedure coupled with the speed of the automated on-line isotope analysis provides reduced sample preparation time without sacrificing analytical precision.

EXPERIMENTAL

Instrumentation

In order to produce on-line measurement of $\delta^{15}\text{N}$ from liquid samples, we designed a new pretreatment unit, composed of a manually operated X-pattern four-way valve (74 series, Xiongchuan Corporation, Beijing, China) and a U-shaped stainless steel cold trap (i.d. 3/16"), and coupled it with the elemental analyzer (NA 1112, Thermo Electron, Milan, Italy). The arrangement of subparts within the pretreatment unit and the connection pattern of the designed unit with the EA are shown in Fig. 1. Briefly, the reduction unit of the EA was connected to the U-shaped stainless steel cold trap, subsequently to the X-pattern four-way valve and then to

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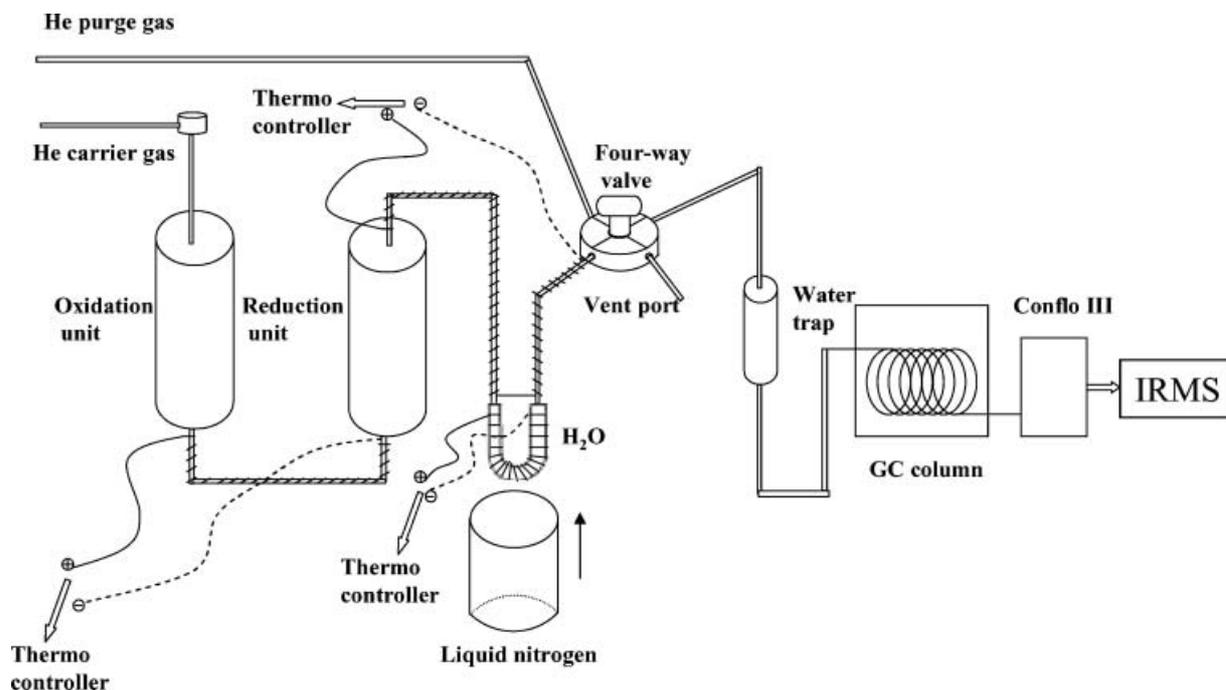


Figure 1. Functional diagram and running mode of the modified EA-IRMS system for on-line isotopic analysis of nitrogen in liquid sample (sample size up to 150 μL).

the water trap of the EA. Before the above-mentioned configuration was finally adopted, another arrangement had been tried where the U-shaped stainless steel cold trap and the X-pattern four-way valve were sequentially connected and set up between the oxidation and reduction unit of the EA. The reason for considering such an arrangement was that we wanted to prevent H_2O from entering the reduction unit of the EA in case extra Cu was consumed during the possible high-temperature (680°C) reaction of H_2O with Cu. However, the reproducibility of this configuration for $\delta^{15}\text{N}$ measurements was not very good due to the partial reaction of NO_x with water and the formation of nitric acids; the pH of the water vented from the system was up to 1. Therefore, the final version of the arrangement was as illustrated in Fig. 1. To facilitate the flow of water from the liquid sample through the system, all the stainless steel pipes, which link the different parts, such as combustion tube, reduction tube, U-shaped cold trap and X-pattern four-way valve, are wrapped with heating cords (Fig. 1) and heated to $>80^\circ\text{C}$ during the process of sample measurement.

In this modified system, the carrier gas and purge gas were connected to contiguous ports on the four-way valve and the water trap and vent pipe were connected to the other two ports. The purity level of the helium carrier and purge gas was always 99.999% and no gas purifier was connected. To guarantee the stability of the helium gas in the on-line system when changing the position of the four-way valve, we set both the carrier and purge gases at a flow rate of 90 mL/min. The reactor tubes were self-packed, one filled with chromium(III) oxide and silver cobalt oxide for combustion, and the other with copper to reduce nitric oxide compounds. The isothermal temperature values for the EA combustion tube and reduction unit were set to 960°C and 680°C , respectively.

During the process of nitrogen isotope measurement, the four-way valve was in the sampling position, which enables liquid samples to flow sequentially through the combustion tube, the U-shaped cold trap, the water trap tube and finally to the IRMS. At the same time, the U-shaped cold trap was immersed in liquid nitrogen to capture water. The separated gases were transferred via the ConFloIII™ interface (ThermoFinnigan, Bremen, Germany) to the IRMS (MAT 253, ThermoFinnigan). After finishing the nitrogen isotope determination, the four-way valve was switched to the venting function, which enables the carrier helium to flow through the U-shaped cold trap to the vent port and the purged helium through the water trap to the IRMS. Once the venting function was activated, the U-shaped cold trap was heated up to $>100^\circ\text{C}$ (e.g. 130°C), which enables the captured water to be easily flushed out by the carrier helium. When the captured water was completely removed, the U-shaped cold trap was again immersed in the liquid nitrogen. After 1–2 min of cooling, the four-way valve would be switched back to the sampling position and another 1 min standby makes the system ready for the next measurement. Generally, a complete analysis was performed within 22 min (Fig. 2).

The stable isotope data, expressed as delta values in parts per thousand, were calculated using the formula:

$$\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000, \text{ where } R = {}^{15}\text{N}/{}^{14}\text{N}.$$

In this study, each sample peak was individually calibrated with a reference N_2 gas pulse preceding the sample peak, and the reference gas was calibrated using IAEA-NO-3 ($\delta^{15}\text{N}_{\text{Vair-N}_2} = +4.7\%$) in advance (IAEA, Vienna, Austria).

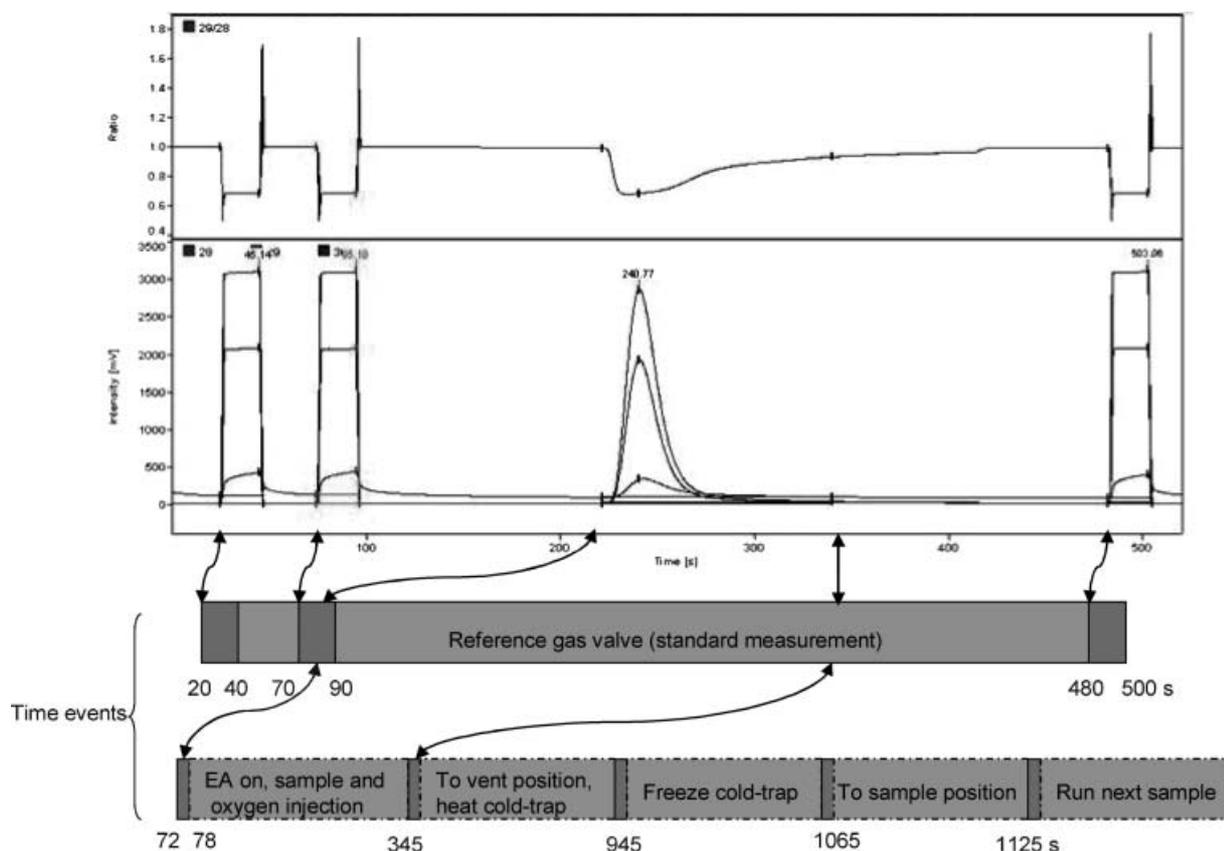


Figure 2. Chromatogram of nitrogen isotopic analysis on urea solution standard (100 μL , 6 mg/mL) together with the chart showing the time events occurring in the measuring process.

Materials and methods

To examine the practicability of this modified system, six liquid standard samples were prepared by dissolving urea in distilled water. The concentrations of the standards are 1, 2, 3, 4, 5 and 6 mg urea per mL water, corresponding to a nitrogen percentage (wt %) of 0.0463, 0.0927, 0.139, 0.185, 0.232 and 0.278%. The urea used here is the isotope working standard in our laboratory with the given $\delta^{15}\text{N}_{\text{VAir-N}_2}$ value of -1.3‰ , that was carefully calibrated using IAEA-NO-3 ($\delta^{15}\text{N}_{\text{VAir-N}_2} = +4.7\text{‰}$). Therefore, the urea solution can be used to calibrate $\delta^{15}\text{N}$ ratios of the unknowns to the international scale. Here the data sets from two different running sequences were compared, with the goal of defining: (1) the linearity and precision of the modified system; (2) the long-term stability of the system; (3) the reproducibility over and within two running sequences. In the first running sequence, the 6 mg/mL urea solution was used as a working standard and evenly distributed among the unknown urine samples for quality control. Meanwhile, six 6 mg/mL urea solutions with volumes ranging from 25 to 150 μL were measured twice to check the linearity. By comparison, in the second running sequence, both the 6 mg/mL urea solution and the urine sample (No. 2-18-9) were inserted after every eighth sample as working standards for quality control. At the same time, each of the six different concentration samples was measured twice for the nitrogen isotope ratio, and the average of two measurements was calculated and used to determine the linearity. The reproducibility was examined by the $\delta^{15}\text{N}$ of working standards from two running sequences. All the

samples were injected into the EA using a 250 μL syringe (Hamilton, Bonaduz, Switzerland) and the amount for each measurement was 100 μL except for those samples of different volumes that were used to check the linearity.

RESULTS AND DISCUSSION

Linearity of the modified system

For any newly established instrument, the linearity of it for certain measurements should be ascertained before it can be applied to routine measurement. The linearity of the modified system is discussed below.

Part A: linearity correction and shift correction

The results for the different volumes of 6 mg/mL urea solutions (the corresponding urea amounts are from 0.15 to 0.9 mg) and the 100 μL injection of samples with urea concentration between 1 and 6 mg/mL (the amount of urea is between 0.1 and 0.6 mg) are summarized in Fig. 3. Both the comparisons of sample weight versus peak area for the two running sequences indicate significant linear correlation (Fig. 3(a)). Figure 3(b) illustrates the change in uncorrected $\delta^{15}\text{N}$ values as a function of sample size for the two running sequences. With decreasing sample size, both the $\delta^{15}\text{N}$ values of the two running sequences decrease by about 1.2‰. However, the decreasing values change in a significant logarithmic fashion for both running sequences when the peak areas are less than 65 Volt seconds (Vs) (corresponding to the nitrogen yield from 0.6 mg urea, which is 0.276 mg N), allowing for a correction to be applied (Fig. 3(b)). This

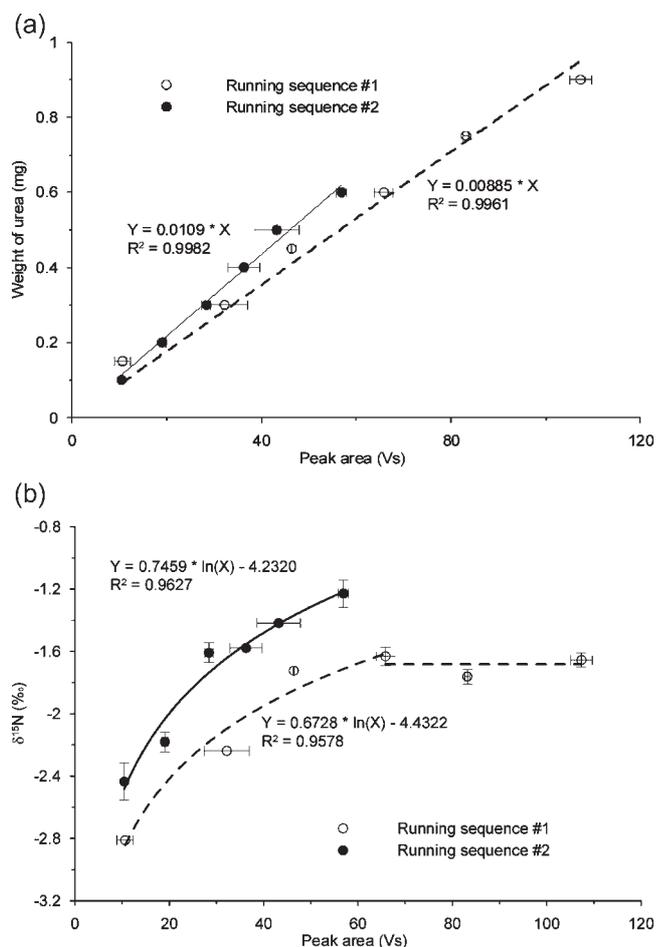


Figure 3. (a) Weight of urea contained in solution against area of the peak in the chromatogram for urea solution standards between 0.1 and 0.9 mg in size. The significant linear correlation allows for the determination of yields of N_2 from samples of unknown nitrogen content. (b) Changes in $\delta^{15}\text{N}$ values of urea solution standards less than 0.9 mg urea in size. Note the significant logarithmic decrease in values. This change in values can be corrected for, resulting in an average precision of 0.11 and 0.09‰, respectively, for running sequences #1 and #2 when samples are smaller than 0.6 mg urea equivalent yield. For samples larger than 0.6 mg, the $\delta^{15}\text{N}$ ratios show little variation with changes in size.

correction strategy is called the linearity correction and is calculated by using the peak area and the regression equations (y) in the following formula:

$$\begin{aligned} &\delta^{15}\text{N}_{\text{VAir-N}_2}(\text{sample, corr.}) \\ &= \delta^{15}\text{N}_{\text{VAir-N}_2}(\text{sample, raw}) - y \\ &+ \delta^{15}\text{N}_{\text{VAir-N}_2}(\text{Urea, ref.}), \end{aligned}$$

where $\delta^{15}\text{N}_{\text{VAir-N}_2}(\text{Urea, ref.}) = -1.3\text{‰}$.

By comparison, the $\delta^{15}\text{N}$ values show little variation when the peak areas are larger than 65 Vs (0.276 mg N yield). In this case, we employed another correction protocol for the systematic shift of the $\delta^{15}\text{N}$ values. The shift correction is applied using the following formula:

$$\begin{aligned} &\delta^{15}\text{N}_{\text{VAir-N}_2}(\text{sample, corr.}) \\ &= \delta^{15}\text{N}_{\text{VAir-N}_2}(\text{sample, raw}) - \delta^{15}\text{N}_{\text{VAir-N}_2}(\text{Urea, raw}) \\ &+ \delta^{15}\text{N}_{\text{VAir-N}_2}(\text{Urea, ref.}), \end{aligned}$$

where $\delta^{15}\text{N}_{\text{VAir-N}_2}(\text{Urea, ref.}) = -1.3\text{‰}$.

The corrected values for two running sequences are shown in Table 1. For the first running sequence, the raw data were divided into two groups, which have different correction criteria. The $\delta^{15}\text{N}$ values corrected using the sample-size method have an average precision of 0.11, whereas the $\delta^{15}\text{N}$ values corrected using the shift method display an overall precision of 0.07. For the second running sequence, all the $\delta^{15}\text{N}$ values were corrected using the linearity method and the corrected $\delta^{15}\text{N}$ values show an average precision of 0.09, which is comparable with that of the first running sequence (Table 1). The good agreement of the corrected $\delta^{15}\text{N}$ ratios between the two running sequences proves that this sample-size correction is practical due to the relatively constant function of $\delta^{15}\text{N}$ values vs. peak area. Therefore, this can be incorporated as part of the linearity correction that is performed, based on the relationship between the intensity of the sample peak and the raw $\delta^{15}\text{N}$ value of the sample in any one run. This is important because the reason for this relationship is not clear.

Part B: blank measurement and blank correction

Since the observed changing pattern of raw $\delta^{15}\text{N}$ values with peak areas of small-sized samples (Fig. 3(b)) is stable, there

Table 1. Sample sizes (mg), peak areas, raw data and corrected $\delta^{15}\text{N}$ values of the urea solutions for two different running sequences

		Running sequence #1			Running sequence #2			
Mass (mg)	Area (Vs)	$\delta^{15}\text{N}_{\text{raw}}$ (‰)	$\delta^{15}\text{N}_{\text{I-corr.}}$ (‰)	$\delta^{15}\text{N}_{\text{s-corr.}}$ (‰)	Mass (mg)	Area (Vs)	$\delta^{15}\text{N}_{\text{raw}}$ (‰)	$\delta^{15}\text{N}_{\text{I-corr.}}$ (‰)
0.15	10.62	-2.81	-1.27		0.1	10.43	-2.44	-1.25
0.3	32.18	-2.24	-1.44		0.2	19.03	-2.18	-1.45
0.45	46.37	-1.72	-1.17		0.3	28.38	-1.61	-1.17
0.6	65.82	-1.63	-1.32	-1.25	0.4	36.25	-1.58	-1.32
0.75	83.15	-1.76	—	-1.38	0.5	43.17	-1.42	-1.29
0.9	107.35	-1.66	—	-1.27	0.6	56.89	-1.23	-1.31
		Avg.	-1.30	-1.30			Avg.	-1.30
		SD	0.11	0.07			SD	0.09

Notes: $\delta^{15}\text{N}_{\text{I-corr.}}$ denotes the $\delta^{15}\text{N}$ values corrected using the linearity correction strategy, whereas $\delta^{15}\text{N}_{\text{s-corr.}}$ represents the $\delta^{15}\text{N}$ values corrected using the shift correction strategy. For shift correction, we adopted $\delta^{15}\text{N}_{\text{raw}}$ as $\delta^{15}\text{N}_{\text{VAir-N}_2}(\text{sample, raw})$ and the mean value of $\delta^{15}\text{N}_{\text{raw}}$ (e.g. -1.68‰ for running sequence #1) as $\delta^{15}\text{N}_{\text{VAir-N}_2}(\text{Urea, raw})$.

Table 2. The sizes and $\delta^{15}\text{N}$ values of two different types of blanks measured in running sequence #3 and blank corrected $\delta^{15}\text{N}$ values of working standard urea and IAEA-NO-3 obtained from repeated analysis of the standards and blank samples. Standard deviation (SD) and number of analyses (n) are given

Identifier	Peak area (Vs)	SD (Vs)	$\delta^{15}\text{N}_{\text{mean}}$ (‰)	SD (‰)	$\delta^{15}\text{N}_{\text{blk corr}}$ (‰)	SD (‰)	n
Blanks measured before real sample injection							
Empty blank	0	0	0	0	—	—	3
Distilled water blank	2.23	0.33	+6.27	0.33	—	—	3
Blanks measured between real sample injection							
Empty blank	2.13	0.52	-12.20	1.56	—	—	5
Distilled water blank	3.68	0.43	-4.30	1.01	—	—	5
*Calculated N blank from distilled water	1.55	—	+6.56	—	—	—	—
Standards measured between real samples injection							
Working standard urea	154.27	4.78	-1.32	0.06	-1.25	0.07	3
IAEA-NO-3	124.72	9.51	+4.33	0.07	+4.60	0.09	3

*The calculated N blank from distilled water is computed by the empty blank and distilled water blank measured between real samples injection in running sequence #3, using the following formula: $\delta^{15}\text{N}_{\text{VAir-N}_2}$ (distilled water, blank corr.) = $[\delta^{15}\text{N}_{\text{VAir-N}_2}$ (distilled water, raw) $\times A_{\text{distilled water}} - \delta^{15}\text{N}_{\text{VAir-N}_2}$ (blank) $\times A_{\text{blank}}]/(A_{\text{distilled water}} - A_{\text{blank}})$, where $A_{\text{distilled water}}$ is the measured peak area of distilled water, A_{blank} represents the peak area of empty blank, and $\delta^{15}\text{N}_{\text{VAir-N}_2}$ (blank) denotes the $\delta^{15}\text{N}$ of empty blank.

must be an inherent source of nitrogen in the system that is incorporated into the sample during the measurement. To locate the source of this nitrogen and elucidate the origin of non-linearity for $\delta^{15}\text{N}$ of small-sized samples, we designed another running sequence (running sequence #3), which included several blank measurements and a set of $\delta^{15}\text{N}$ measurements on urea solutions (working standard in our lab) and potassium nitrate (IAEA-NO-3) solutions.

For blank measurements, an empty blank (no sample injection) and a distilled water blank (only 100 μL distilled water injection) were examined. The sizes and nitrogen isotope composition of the blanks are shown in Table 2. Before the injection of a real sample, an empty blank was measured three times, followed by a distilled water blank check (also measured three times.). Since the combustion and reduction tubes were newly packed with clean reagents, no trace of a nitrogen peak was found during the empty blank measurements. The peak minimum height was set at 1 mV on the 'peak detection@N2' tab in the Isodat NT software. We made three injections of 100 μL distilled water to determine the blank N values from the water used to dissolve the urea standard. The detected N peak heights were all ~ 60 mV, the corresponding peak area was 2.23 ± 0.33 Vs and $\delta^{15}\text{N}$ was $+6.27 \pm 0.33\%$ (n = 3). This means there was a measurable N blank originating from the distilled water itself, so it is necessary to apply the blank correction to the distilled water.

For real sample measurements, 100 μL injections of urea solutions with 0.5, 1, 1.5, 2, 3, 4 and 6 mg urea per mL distilled water (the amount of urea is accordingly 0.05, 0.1, 0.15, 0.2, 0.3, 0.4 and 0.6 mg, respectively) and a 100 μL injection of potassium nitrate solution with 17.277 mg KNO_3 per mL distilled water (the amount of KNO_3 is 1.73 mg) were carried out. Each sample was measured three times and the average of three measurements was calculated. The results are shown in Fig. 4. The comparison of sample weight versus the peak area also indicates a significant linear correlation (Fig. 4(a)). However, the N yield per milligram of urea in running sequence #3 displays a greater increase relative to those in the first two running sequences because we had performed an

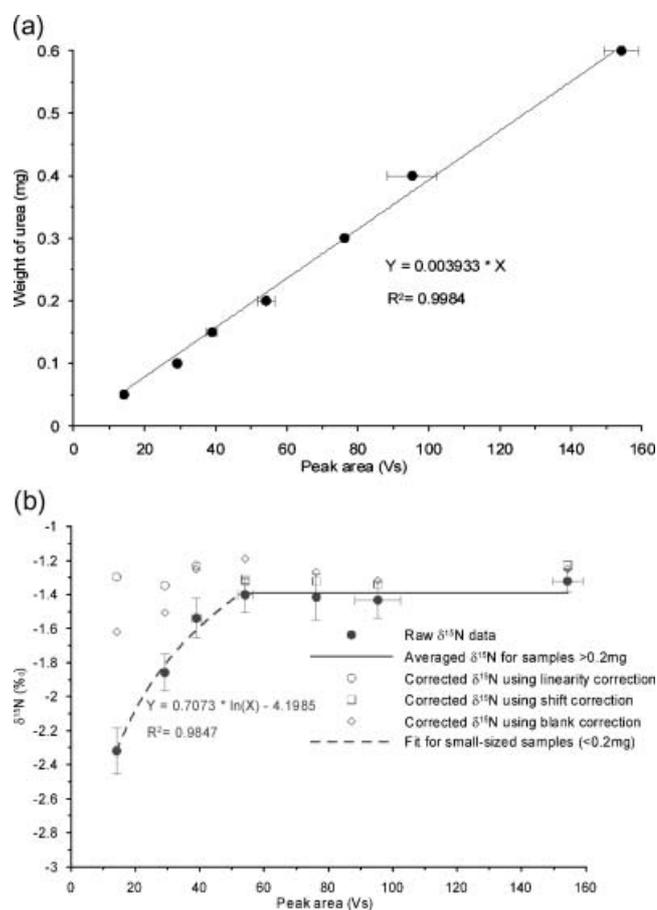


Figure 4. (a) Weight of urea contained in solution against peak area of the peak in the chromatogram for urea solution standards between 0.05 and 0.6 mg in size. The N_2 yield per mg urea in running sequence #3 greatly increased due to optimizing the condition of mass spectrometer. (b) Changes in $\delta^{15}\text{N}$ values of urea solution standards less than 0.6 mg urea in size, together with linearity, shift corrected and blank corrected $\delta^{15}\text{N}$ values. The standard deviation for corrected $\delta^{15}\text{N}$ using combined linearity and shift correction is 0.05‰ whereas that for blank corrected $\delta^{15}\text{N}$ is 0.16‰.

autofocus function to optimize the mass spectrometer conditions before the measurement started. Figure 4(b) illustrates the change in uncorrected $\delta^{15}\text{N}$ values as a function of sample size. With decreasing sample size, the $\delta^{15}\text{N}$ values decrease by about 1.1‰, showing the same pattern as the first two running sequences.

During the whole running sequence, both empty blank and distilled water blank samples were run after every other third sample. In total, five empty blanks and five distilled water blanks were measured. The empty blanks measured between real samples show 30–60 mV nitrogen peaks (mean peak area: 2.13 ± 0.52 Vs) with $\delta^{15}\text{N}$ of $-12.20 \pm 1.56\%$ ($n = 5$). This nitrogen apparently originates from previously measured real samples (e.g. urea solution) because no nitrogen peaks were observed on the empty blanks measured before the real sample injection. The N-containing liquid samples may leave some N residue in the combustion reactor of the EA due to the possible absorption of NO_x to granular chromium oxide in the presence of H_2O . Obviously, this portion of nitrogen would contribute a lot to the nitrogen isotope composition of small samples. As a result, it is also necessary to apply the 'empty blank' correction to improve the analytical precision for $\delta^{15}\text{N}$ measurements on real samples. The N blanks measured on distilled water between real samples in the sequence show higher peaks (mean peak area: 3.68 ± 0.43 Vs) and more negative $\delta^{15}\text{N}$ values ($-4.30 \pm 1.01\%$) than those measured before real sample injection. Calculation shows that these N blanks actually encompass both empty blank nitrogen and nitrogen from distilled water (see the calculated N blank from distilled water in Table 2, which has a $\delta^{15}\text{N}$ value comparable with that measured before). Therefore, a blank correction must be applied to the measured $\delta^{15}\text{N}$ of real samples. The blank correction is applied using the following formula:

$$\begin{aligned} \delta^{15}\text{N}_{\text{VAir-N}_2}(\text{sample, blank corr.}) \\ = [\delta^{15}\text{N}_{\text{VAir-N}_2}(\text{sample, raw}) \times A_{\text{sample}} \\ - \delta^{15}\text{N}_{\text{VAir-N}_2}(\text{blank}) \times A_{\text{blank}}] / (A_{\text{sample}} - A_{\text{blank}}), \end{aligned}$$

where A_{sample} is the measured peak area of sample, A_{blank} represents the peak area of blank, and $\delta^{15}\text{N}_{\text{VAir-N}_2}(\text{blank})$ denotes the $\delta^{15}\text{N}$ of blank.

Here the mean peak area ($=3.68$ Vs) and mean $\delta^{15}\text{N}$ ($=-4.30\%$) of the blanks were used for the correction since any single blank may not precisely represent the real blank in size and isotopic composition.

The blank corrected values for $\delta^{15}\text{N}$ of urine solutions measured in sequence #3 are shown in Fig. 4(b), compared with the corrected values using the linearity correction strategy. As can be seen, the blank correction can greatly improve the analytical precision of small samples. The mean value of all blank corrected $\delta^{15}\text{N}$ is -1.34% and the overall precision better than 0.16% (the open rhombuses in Fig. 4(b)). By contrast, the $\delta^{15}\text{N}$ values corrected using both linearity (for samples with peak area <54 Vs, the open circles in Fig. 4(b)) and shift correction (for samples with peak area >54 Vs, the open squares in Fig. 4(b)) show an average precision of 0.05% . The relatively large variation in blank corrected $\delta^{15}\text{N}$ values may be attributed to the variability of blank $\delta^{15}\text{N}$ in the sequence. In addition, the blank corrected

$\delta^{15}\text{N}$ values of both the working standard urea and IAEA-NO-3 (Table 2) show little difference from their given $\delta^{15}\text{N}$ values. This testifies to the high accuracy of blank corrected $\delta^{15}\text{N}$ values relative to the international scale. Therefore, the blank correction should be carefully performed during routine $\delta^{15}\text{N}$ measurements in order to improve the analytical precision, especially for small samples.

Thus, this modified system can produce good linearity when the sample size is larger than 0.6 mg for urea (0.276 mg N contained) in running sequences #1 and #2. For samples smaller than this, the correction on $\delta^{15}\text{N}$ could be applied by the established logarithmic function. This sample-size correction can help extend linearity down to a minimum of 0.1 mg for urea (corresponding to 0.046 mg N). After we had optimized the mass spectrometer conditions, good linearity could also be observed on samples of between 0.6 and 0.2 mg for urea as shown in running sequence #3. Moreover, a blank correction can help extend linearity down to a minimum of 0.05 mg for urea (corresponding to 0.023 mg N).

Reproducibility and long-term stability of the modified system

The reproducibility for the urea solution standard (with concentration of 6 mg/mL) and an unknown (human urine 2-18-9) of this modified system was tested by analyzing 35 urea solution standard samples in the first running sequence as well as 17 urea solution standard samples and 15 urine (2-18-9) samples in the second running sequence. Most of the standards and unknowns were evenly distributed among other unknown samples so that the working condition of this modified system in the two running sequences could be monitored from time to time. When the $\delta^{15}\text{N}$ ratio of the standard and the unknown began to differ from the mean values of previous samples, the working condition of this system had deteriorated. Therefore, we shut down the whole system and cleaned or repacked the combustion and/or reduction tube to make it ready for another set of samples. The changes in $\delta^{15}\text{N}$ of the urea solution standards and urine samples (2-18-9) along with analysis number are shown in Fig. 5. A total of 150 samples were analyzed in running sequence #1 and 175 samples in running sequence #2. The working condition for both runs worsened as indicated by the more negative $\delta^{15}\text{N}$ values after ~ 130 measurements (e.g. Nos. 17642–17773 for sequence #1; Nos. 17808–17940 for sequence #2) (Fig. 5). Of the 35 urea solution standards in sequence #1, the first 30 had an average $\delta^{15}\text{N}$ of -1.67% with a standard deviation (SD) of $\pm 0.16\%$. In sequence #2, the first 15 of the 17 standards had a mean $\delta^{15}\text{N}$ of -1.21% with an SD of $\pm 0.15\%$. In addition, the first 9 of 15 urine samples also showed a good SD of $\pm 0.19\%$ with a mean $\delta^{15}\text{N}$ of 8.79% . There is a $>0.4\%$ difference for the average $\delta^{15}\text{N}$ of standards between sequences #1 and #2, but it can be offset by a correction strategy since the difference is caused by a stable systematic shift of the working condition. In brief, the results show good reproducibility using this modified system for both standards and unknowns within both running sequences and thus testify to the long-term stability of the system for about 130 measurements in the sequence. This maximum number of measurements is less than that of more common EA-IRMS isotopic measurements on solid samples

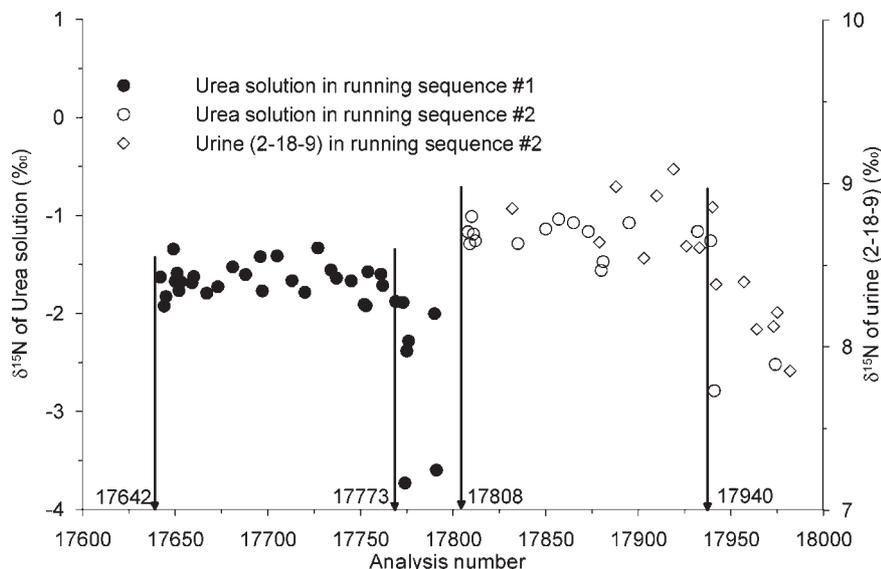


Figure 5. Chart showing the performance of the working standard sample (6 mg/mL urea solution) and unknown (2-18-9) for more than 130 measurements in two running sequences. Gaps are due to times when the mass spectrometer was used to run urine samples. The standard deviations are 0.16 and 0.15‰ for the standards in sequence #1 and #2, respectively, whereas for urine unknowns it is 0.19‰.

(e.g. about 400 measurements in one running sequence). This may be due to a memory effect caused by adsorption or reaction occurring in the complex matrix of NO_x , CO_x , Cr_2O_3 and water.

Future optimization of the modified EA-IRMS system

The procedure outlined above might be further optimized by: (1) using an autosampler to inject liquid samples; (2) using an automatic four-way valve triggered by compressed air from the EA rather than manual control; (3) programming the U-shaped cold trap to dip down into or lift out of the liquid nitrogen and to be heated during venting time instead of manual manipulation. In addition, the connections and steel pipes used to connect different parts of the modified EA (e.g. combustion and reduction tube, U-shaped cold trap and four-way valve) could be changed to being made of Teflon which is more resistant to acid and alkali attack so that the memory effect would be largely reduced to guarantee the long-term stability of this modified system.

CONCLUSIONS

In summary, the modified EA-IRMS system offers good precision ($<0.2\text{‰}$) and a wide linearity range (from 0.05 to 0.9 mg or more urea equivalent nitrogen yield if the blank correction was carefully performed in routine measurements) for on-line nitrogen isotopic measurements of liquid samples. The 22 min for one measurement can guarantee high sample throughput (e.g. 30 samples in ca. 10 h). If all the procedures during measurement could be automatically programmed by Isodat NT software, the daily sample throughput would be doubled. In addition to analyzing the $\delta^{15}\text{N}$ of urine samples to monitor metabolic status, the modified EA-IRMS system can also be employed to determine $\delta^{15}\text{N}$ of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in contaminated

groundwater, river or lake water for pollution source identification, or ecological studies.

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