The stable isotope analysis of saline water samples on a cavity ring–down spectroscopy instrument

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ABSTRACT: The analysis of the stable hydrogen and oxygen isotope composition of water using cavity ring-down spectroscopy (CRDS) instruments utilizing infrared absorption spectroscopy have been comprehensively tested. However, potential limitations of infrared spectroscopy for the analysis of highly saline water have not yet been evaluated. In this study, we assessed uncertainty arising from elevated salt concentrations in water analysed on a CRDS instrument and the necessity of a correction procedure. We prepared various solutions of mixed salts and separate solutions with individual salts (NaCl, KCl, MgCl₂ and CaCl₂) using deionised water with a known stable isotope composition. Most of the individual salt and salt mixture solutions (some up to 340 g L⁻¹) had δ -values within the range usual for CRDS analytical uncertainty (0.1‰ for $\delta^{18}\text{O}$ and 1.0‰ for $\delta^2\text{H}$). Results were not compromised even when the total load of salt in the vaporiser reached ~38.5 mg (equivalent to build up after running ~100 ocean water samples). Therefore, highly saline mixtures can be successfully analysed using CRDS, except highly concentrated MgCl₂ solutions, without the need for an additional correction if the vaporiser is frequently cleaned and MgCl₂ concentration in water is relatively low.

KEYWORDS: stable isotope; water; salt; CRDS; Picarro;

INTRODUCTION

Analysing the stable hydrogen and oxygen isotope composition ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) of saline water, in contrast to fresh water, can be analytically challenging. High salt concentrations can be a serious obstacle to direct stable isotope analyses of water and the obtained results may require correction in order to improve the analytical accuracy¹. The analytical problem arises for highly saline water due to substantial fractionation occurring between free water molecules and those associated with the hydration sphere of the cations in water solution^{1,2,3}. Consequently, stable hydrogen and oxygen isotope results for waters can be reported on two scales: 1) concentration scale – mean stable isotope composition of all water in sample; 2) activity scale – only “free” water not including water associated within cations hydration sphere. The “activity correction”, as defined by Sofer and Gat^{2,3}, allows recalculation from the activity scale to a concentration scale, and is a function of molalities of respective chemical compounds having a large influence on the measured water stable isotope composition. The original correction was defined by simplified equations (Eq. 1, Eq. 2)^{2,3}:

$$\delta^2\text{H} = m\text{NaCl} \times (-0.4) + m\text{MgCl}_2 \times (-5.1) + m\text{CaCl}_2 \times (-6.1) + m\text{KCl} \times (-2.4) \quad \text{Eq. 1}$$

$$\delta^{18}\text{O} = m\text{MgCl}_2 \times (1.11) + m\text{CaCl}_2 \times (0.47) + m\text{KCl} \times (-0.16) \quad \text{Eq. 2}$$

where m is the salt concentration of the respective ions given in molalities (mol kg^{-1}), the value of $\delta^2\text{H}$ or $\delta^{18}\text{O}$ are required “activity corrections” (the δ -value on the concentration scale minus that on the activity scale).

The original “activity” correction factors based on salt concentrations defined by Sofer and Gat^{2,3} were later revised^{4,5,6} as well as optimised for specific analytical techniques^{7,8}. Hence,

while the definition of the concentration scale is unambiguous, the activity scale can be defined in two different ways: 1) in relation to free water fluxes in the natural environment^{2,9} or 2) in relation to indirect measurement of the stable isotope composition using equilibration methods under laboratory conditions^{7,8}.

Traditionally, water samples were distilled prior to stable hydrogen isotope analyses using uranium¹⁰ or chromium¹¹ methods but stable oxygen isotope composition was analysed using CO₂ – H₂O equilibration^{7,12,13} followed by IRMS measurement. Therefore, in most of the historical data sets hydrogen is reported on a concentration scale while oxygen is reported on an activity scale. The complete extraction of water (100 % extraction efficiency) was assumed to be achievable by high temperature distillation (250-800°C). Thus, results were considered to be on the concentration scale regardless what subsequent method was utilised for stable isotope analyses of distilled water. In contrast, if water is not distilled prior to the stable isotope analysis then results can be either on a concentration or activity scale depending on the analytical method applied. Methods such as 1) used in vacuum systems: uranium¹⁰, chromium¹¹ or zinc reduction¹⁴ of water in order to obtain H₂ for the stable hydrogen isotope analysis or 2) used in continuous flow thermal conversion methods¹⁵ (e.g., TC/EA – Thermal Conversion Elemental Analyser, reduction to H₂ and CO at 1400-1430°C and analyses on IRMS), allow extraction and analysis of all water from a sample regardless of its salinity. Therefore, the results obtained using vacuum systems or continuous flow thermal conversion methods are on the concentration scale. Other methods, which are used in both dual inlet and continuous flow systems, are based on equilibration of head space gas (CO₂ and H₂) with water^{7,8}. Therefore, the results of equilibration methods reflect the stable isotope composition of exchangeable water (“active water”) and are on

the activity scale^{7,8}. Thus, an “activity correction” is required in order to convert them to a concentration scale.

Recently, a novel and low cost alternative to traditional dual inlet techniques or continuous flow systems for stable hydrogen and oxygen isotope analyses of water has been introduced¹⁶⁻¹⁸. This new method is the cavity ring-down spectroscopy (CRDS), frequently also referred as IRIS (isotope ratio infrared spectroscopy) or LAS (Laser Absorption Spectroscopy) depending on instrument version and manufacturer. Two instruments are widely commercially available: 1) wavelength-scanned cavity ring-down spectroscopy (WS-CRDS by Picarro, Sunnyvale, CA, USA)¹⁹ and 2) off-axis integrated cavity output spectroscopy (OA-ICOS by Los Gatos Research, Mountain View, California)²⁰. CRDS as an analytical method has its own limitations, and has therefore, been extensively tested in respect to: 1) precision^{16,21}; 2) memory effect²² and especially 3) interference due to organic contaminants²²⁻²⁵. Consequently, analytical protocols and corrections have been proposed in order to improve analytical accuracy and precision^{26,27}. Recently a unified analytical protocol has been developed (LIMS for lasers – Laboratory Information Management System)²⁸ by the International Atomic Energy Agency (IAEA) in cooperation with United States Geological Survey (USGS). However, the influence of various salt contents in water samples on CRDS performance has not yet been reported in the scientific literature, despite the large interest of the scientific community in using CRDS systems for the analysis of saline ground, surface and sea water samples.

In contrast to other techniques, the measurement in CRDS systems is performed directly on vapour yielded from a water sample (see description in Lis et al.¹⁶). Therefore, the results are on the concentration scale. The water sample undergoes rapid evaporation under vacuum conditions in a vaporiser operating at relatively low temperature (110 or 140°C). Water vapours are then

flushed by a stream of dry air or nitrogen to a laser analyser chamber. The evaporation process under vacuum in the vaporiser is similar to that occurring in off-line vacuum distillation of more traditional methods, however cryogenic traps are not used. Therefore, the potential influence of incomplete distillation of saline water samples on measured δ -value should be considered for CRDS, as it is for “traditional” vacuum distillation^{1,9}. However, evaporation in CRDS vaporisers and off-line vacuum distillation lines occur under different conditions. The principal differences are: 1) temperature, 110 or 140°C (CRDS) instead of a few hundred degrees Celsius using a heat gun or gas torch (off-line distillation) and 2) in the sample volume ~2 μ L (CRDS) instead of 10-25 μ L (off-line distillation)^{10,11}; 3) water vapour in a CRDS vaporiser is not cryogenically condensed, like in vacuum lines, but is instead flushed from the vaporiser to the analyser in a dry gas stream. Moreover, since the volume of water is small, it is rapidly evaporated in the vaporiser forming a cloud of salt sprayed around the injection point. Crucially, if part of the water sample remains trapped with the salt precipitated in the vaporiser, the results will not necessarily be on the concentration scale and will not reflect the mean isotope composition of all water in the sample.

Differences between δ -values of various saline solutions (prepared using the same deionised water) obtained on a CRDS instrument may reflect the combined influence of three major factors that in-turn influence the precision and accuracy of CRDS. These factors are:

- 1) an effect related to incomplete extraction/evaporation of water from individual samples – in such case the stable isotope composition of saline solution will vary depending on salt type and concentration in a water sample;
- 2) a progressive increase of isotope fractionation due to water reabsorption on salt and other chemical reactions during distillation leading, e.g., to the formation of NaOH or HCl^{1,29}; this

effect likely will escalate as salt load in the vaporiser increases (in such a case, a fresh water sample with similar stable isotope composition, injected between saline water samples, would have progressively different δ -values from original stable isotope composition depending on salt load in the vaporiser at the time of the injection);

3) a memory effect due to water absorption on accumulated salt, however, without necessarily significant isotope fractionation (in such a case the background would influence δ -value of subsequently analysed samples if its stable isotope composition will be significantly different from previously analysed samples). The isotope fractionation of water samples via absorption onto salt accumulated in the vaporiser could be expected, since the process of water bonding in salt crystals could lead to similar fractionation as those observed for saline water solutions where water in the cation hydration sphere have different a stable isotope composition compared to free water².

In this study, we experimentally tested for the influence of these three major effects on the stable isotope analysis of saline water on a CRDS instrument.

EXPERIMENTAL

Sample preparation for Experiment #1. For experiment #1 we chose to test the general range of ratios between different salt concentrations similar to those observed in the Indian Ocean at the Western Australian coast³⁰ and saline groundwater from north-western Western Australia³¹. Deionised water (DI) was prepared, well mixed and left overnight in a 5 L container to ensure water sample homogeneity. The following day, 100 mL of the DI water was transferred into 14 sealed polyethylene vials (volume 120 mL, Sarstedt, Ingle Farm, Australia,

#75.9922.421.). One vial was left with pure DI water. Four different salts, recognised by Sofer and Gat^{2,3} as those contributing the most to analytical uncertainty during stable isotope analyses, were added to the remaining 13 vials (Eq. 1 and 2). The concentrations ranged from 0 to 305.40 g L⁻¹ for NaCl, from 0 to 30.50 g L⁻¹ for KCl, from 0 to 5.01 g L⁻¹ for MgCl₂ and from 0 to 3.60 g L⁻¹ for CaCl₂ (for details see Tab. S1). Consequently, the total dissolved solids (TDS) in the prepared solutions varied from 0 to 339.4 g L⁻¹. Salts used in preparing the solutions were analytical grade: NaCl (99.9%, Ajax, Taren Point, Australia, #465–500G), KCl (99.8%, Ajax, Taren Point, Australia, #383–500G), MgCl₂ (99.0% hexahydrate, Merck, Kilsyth, Australia, #10149.4V) and CaCl₂ (78.0% dihydrate, Ajax, Taren Point, Australia, #127–500G). No treatments or drying were applied to any of the salts. As CaCl₂ and MgCl₂ salts are hygroscopic, we minimized the time of atmospheric exposure of the contents as much as possible; however, we did not use a glove box for this experiment. The advantage of using hydrate salt, comparing to anhydrous salt, is that dissolution does not lead to a significant exothermic effect which may compromise the original stable isotope composition of water. However, extra water absorbed in hydrate salt during the chemical production process is added to the samples with salt, which may influence original stable isotope composition of water in solutions. The salt solutions were well mixed with DI water (Table 1) and left overnight in tightly capped vials at 23°C in an air-conditioned laboratory in order to ensure complete dissolution and dissociation of salts. The following day, samples were transferred into 1.5 mL glass vials (Grace–Davison, Melbourne, Australia, #98133) with septa (Grace–Davison, Melbourne, Australia, #98144) for stable isotope analyses.

Sample preparation for Experiment #2. For experiment #2 we prepared various solutions of individual salts and salt mixtures following the same principles as for experiment #1. However, we used only brand new anhydrous chemicals and weighed them in glove box purged with dry nitrogen gas. Salts were of analytical grade from Sigma–Aldrich (Sydney, Australia): NaCl (ACS 99.8%, #31434–500G–R), KCl (ACS 99.0–100.5%, #P3911–500G), MgCl₂ (98.0% anhydrous, #M8266–100G) and CaCl₂ (96.0% anhydrous, #C4901–500G). The dissolution process of anhydrous MgCl₂ and CaCl₂ leads to a significant exothermic effect, therefore we added salts in small portions to water chilled to 5°C. The procedure requires closing and opening of vials a few times which may result in the condensation of moisture from air on the walls of vials. Moreover, a change in temperature following the addition of each portion of chemicals may potentially affect the initial stable isotope composition of DI water used for solutions due to evaporation. The advantage of using anhydrous salt, comparing to hydrate salt, is that no additional water is added with salt to prepared solutions.

Stable isotope analysis. All stable isotope analyses of prepared saline solutions were performed utilising an Isotopic Liquid Water Analyser Picarro L1115–i with V1102–I vaporiser (Picarro, Santa Clara, California, USA) operating at 140°C. Each sample was injected using 10 µL syringe (10F–C/T–GT–5/0.47C (Teflon tipped), SGE, Melbourne, Australia, #002977). After each injection the syringe was washed three times with DI water and once with acetonitrile (BDH, AnalR 99.5%, Poole, England). The signal levels for all samples were in the required range for the instrument and varied between 19,000 and 21,000 ppmv.

The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of samples were normalized to the VSMOW scale (Vienna Standard Mean Ocean Water), following a three-point normalization³² based on three laboratory standards

(Perth Ocean Water POW $\delta^2\text{H} = -1.8\text{‰}$ and $\delta^{18}\text{O} = -0.41\text{‰}$, Polish spring water POL $\delta^2\text{H} = -71.9\text{‰}$ and $\delta^{18}\text{O} = -10.34\text{‰}$, Canadian spring water CAD $\delta^2\text{H} = -143.2\text{‰}$ and $\delta^{18}\text{O} = -18.26\text{‰}$), each replicated twice and reported in per mil (‰) following the principles of multipoint normalization as summarised by Skrzypek³³. All laboratory standards were calibrated against international reference materials using the VSMOW–SLAP scale³⁴, provided by the International Atomic Energy Agency (for VSMOW2 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of equal 0‰ and for SLAP equal $\delta^2\text{H} = -428.0\text{‰}$ and $\delta^{18}\text{O} = -55.50\text{‰}$). The long-term analytical (one standard deviation) uncertainty of our instrument was determined to be 0.8‰ for $\delta^2\text{H}$ and 0.06‰ for $\delta^{18}\text{O}$ for deionised waters (calculations as by Gröning²⁶), which is consistent with the precision reported by the manufacturer for this version of the instrument (1.0‰ for $\delta^2\text{H}$ and 0.10‰ for $\delta^{18}\text{O}$).

Samples of saline solution that showed a significant departure from the stable isotope composition of DI water, as measured on CRDS instrument, were re-analysed using a Thermal Conversion technique (TC/EA) in an independent laboratory, the Reston Stable Isotope Laboratory (RSIL) of the U.S. Geological Survey (Reston, Virginia, USA). Samples were analysed using the silver–tubing method and a TC/EA system (Thermo Finnigan, Bremen, Germany) equipped with a Costech Zero–Blank 50–position autosampler (Costech, Valencia, CA, USA) at 1430°C³⁵. The samples were normalized to VSMOW scale based on direct analyses of VSMOW and GISP international reference materials¹⁵.

RESULTS AND DISCUSSION

Experiment #1 – salt mixtures solutions. Measurement of the stable isotope composition of the solutions of mixed salts using CRDS from experiment #1 varied in a narrow range (Tab. S1,

Fig. 1). The δ -values of DI water and water of saline solutions were different by no more than 0.09‰ for $\delta^{18}\text{O}$ and 0.90‰ for $\delta^2\text{H}$. The observed standard deviations for all saline solutions pooled together (0.04 for $\delta^{18}\text{O}$ and 0.4‰ for $\delta^2\text{H}$) were in the range of the expected reproducibility for this version of the instrument for repetitive analyses of the same fresh water sample. Therefore, even though the addition of salt resulted in a departure of the stable isotope composition of water from its original values; the difference in δ -values over the studied concentration range and salt types (TDS 0 to 339.4 g L⁻¹) was negligible when considering the expected analytical uncertainty of this instrument.

In general, the δ -values of water progressively decreased as the concentration of salts in water increased (Fig. 1). However, there was no trend observed in δ -values for low salinity samples (0 to 13.1 g L⁻¹, n=7) either in relation to total salt concentration (TDS) or to particular types of salt. All relationships were not significant and weak (r from <0.01 to 0.28).

Figure 1. The difference in the stable hydrogen and oxygen isotope composition of mixed salt solutions and the DI water used for their preparation, as measured by CRDS. For details see Table. 1. Grey areas represent analytical uncertainties as per manufacturer specification (one standard deviation).

However, there was a statistically significant trend between TDS and $\delta^{18}\text{O}$ for samples with TDS between 117.8 and 339.4 g L⁻¹ (n=5, r = 0.89, p = 0.04). In contrast, the relationship

between TDS and $\delta^2\text{H}$ was not significant for this range of salinity ($n=5$, $r=0.70$, $p=0.19$). The observed differences in $\delta^2\text{H}$ were in the range of analytical uncertainty and were therefore considered to be negligible. The observed decrease in δ -values could be associated either with the actual “salt effect” resulting from insufficient distillation in the CRDS vaporiser or a laboratory artefact due to the introduction of additional water with hydrous salts (CaCl_2 and MgCl_2) or absorption of moisture by highly hygroscopic salts during the preparation of solutions. The results of CRDS analyses of stable hydrogen and oxygen isotope compositions of solutions in the range of 0–305.4 g L⁻¹ NaCl, 0–3.6 g L⁻¹ CaCl_2 , 0–30.5 g L⁻¹ KCl and 0–5.0 g L⁻¹ MgCl_2 are identical (within analytical uncertainty) to those of the distilled water with which the solutions were prepared.

Experiment #2 – individual salt solutions. In order to assess the possible influence of individual salts at high concentrations on δ -values, individual salt solutions were prepared and differences between δ -values of DI water used for the preparation of solutions and δ -values of water in saline solution were calculated (Tab. S2). The DI water used for the preparation of each solution had $\delta^{18}\text{O}$ $-1.48 \pm 0.03\text{‰}$ and $\delta^2\text{H}$ $-4.0 \pm 0.7\text{‰}$ as analysed on the CRDS system (where $\pm 0.03\text{‰}$ and 0.7‰ are standard deviation of four replicates as in Tab. S2). The δ -values of DI water were confirmed using a TC/EA method³⁵ and similar values were obtained: $-1.57 \pm 0.05\text{‰}$ for $\delta^{18}\text{O}$ and $-5.3 \pm 1.2\text{‰}$ for $\delta^2\text{H}$ (where $\pm 0.05\text{‰}$ and 1.2‰ are standard deviation of five replicates). All results for NaCl, CaCl_2 , and KCl solutions varied within $\pm 0.10\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 1.0\text{‰}$ for $\delta^2\text{H}$ in relation to the δ -values of DI water used for the preparation of the solutions (with one exception 1.5‰ for $\delta^2\text{H}$ in 5.2 g L⁻¹ solution of CaCl_2). The results for MgCl_2 still exhibited similar variation, within $\pm 0.10\text{‰}$ for $\delta^{18}\text{O}$ for solutions up to 49.7 g L⁻¹. The obtained

$\delta^{18}\text{O}$ value was 1.21‰ more negative than the δ -value of DI water for a solution of MgCl_2 at a concentration of 150.5 g L^{-1} (Fig. 2, Tab. S2). Similarly, $\delta^2\text{H}$ of MgCl_2 solutions was within a range of $\pm 1.0\%$ in relation to DI water δ -value for low concentrations ($4.7 - 9.3 \text{ g L}^{-1}$). For higher concentrations (49.7 and 150.5 g L^{-1}) $\delta^2\text{H}$ was 1.2 and 1.5‰ higher than DI water. Dissolution of anhydrous MgCl_2 led to a significant exothermic effect and a characteristic fizzing sound occurred even for dissolution of a small portion of salt. Therefore, in order to ensure that the solution preparation procedure itself does not lead to significant changes of the stable isotope composition, we reanalysed two samples with the highest MgCl_2 concentrations using a TC/EA technique. However, these data confirmed that the stable isotope composition of water in solution ($\delta^{18}\text{O} -1.58 \pm 0.08\%$ and $\delta^2\text{H} -5.4 \pm 1.1\%$) was very close to the DI water used for solution preparation ($\delta^{18}\text{O} -1.59 \pm 0.08\%$ and $\delta^2\text{H} -5.5 \pm 0.3\%$). Hence, the stable isotope composition of waters with high MgCl_2 concentrations analysed on CRDS is neither accurate nor precise. Similar isotope fractionation was reported earlier by Koehler et al.²⁹ for water extracted from small samples of MgCl_2 solutions on a manually-operated vacuum line. However, the range of precision was lower than observed for CRDS, despite higher distillation temperature (250°C) and cryogenic water condensation on liquid nitrogen traps (-196°C)²⁹. Different energy is required for dehydration of different chemical compounds to immobilise different types of water bound in hydrous salts. MgCl_2 forms one of hydrates ($\text{MgCl}_2 \cdot n\text{H}_2\text{O}$, $n = 1, 2, 4, 6, 8$ or 12) and it is particularly difficult to dehydrate as bonds with H_2O are forming a $\text{Mg-Cl}_2\text{-O}_2\text{-H}_2$ system, where magnesium is bound not only to chlorine but also to hydrogen and oxygen^{36,37}. The final dehydration of MgCl_2 requires high temperature, and even at 555°C MgOHCl is converted to MgO and HCl , bonding part of the oxygen from the hydration sphere. Therefore, temperatures over 1000°C are used for production of fully anhydrous MgCl_2 . At lower temperatures not all

water is yielded from salt precipitated during distillation.³⁶ This complex process likely leads to isotope fractionation observed during both IRMS and CRDS analyses. The fractionation mechanism during salt crystallisation is not well understood; however, it depends on the stable isotope composition of water and salt concentration¹⁻⁶ and is negligible for low MgCl_2 concentrations²⁹. The isotope fractionation is observed to lesser extent in CaCl_2 solutions as confirmed also by Koehler et al.²⁹ due to slightly different chemical properties of the bound with water.³⁷

Figure 2. The difference in the stable hydrogen and oxygen isotope compositions between individual salt solutions and DI water used for their preparation, as measured by CRDS. Artificial mixtures of various salts: SW x1 is as ocean water, SW x2 twice concentration as in ocean water, GW_{max} groundwater. For details see Table S2. Grey areas represent analytical uncertainties as per manufacturer specification of one and two standard deviations.

In order to replicate experiment #1 at higher concentrations of mixed salts, especially MgCl_2 , additional mixed salt solutions for experiment #2 were prepared (Tab. S2). The solutions containing up to 225 g L^{-1} of salt (about seven times ocean water), including up to 30.3 g L^{-1} of MgCl_2 had δ -values close to DI water within 1.0‰ for $\delta^2\text{H}$ and within 0.09 to 0.15‰ for $\delta^{18}\text{O}$. However, all mixed salt solutions with $\text{MgCl}_2 \geq 47.6 \text{ g L}^{-1}$ exhibited a significant departure of $\delta^{18}\text{O}$ from DI water from -0.51 to -2.52‰ . TC/EA analyses confirmed that the actual $\delta^{18}\text{O}$ for these saline water samples was not different from DI water by more than 0.20‰ and therefore for these samples the stable isotope composition measured on CDRS was neither precise nor accurate. These results confirm that highly saline water samples can be successfully analysed on CRDS system, excluding samples with extremely high concentration of MgCl_2 ($>30 \text{ g L}^{-1}$).

Salt accumulation in the vaporiser test #1. In the final test we evaluated if the salt accumulation in the CRDS vaporiser has a significantly increases analytical uncertainty either due to increasing isotope fractionation in subsequent samples as the salt load increases or due to a memory effect, when subsequently analysed samples with very different δ -values are influenced by the background from a previous sample.

Eighty two samples of ocean water ($\text{TDS } 35 - 40 \text{ g L}^{-1}$) were analysed after vaporiser cleaning (sequential flushing with DI water, following the manufacturer instructions, Vapouriser Cleaning Procedure, Picarro, Santa Clara, California, USA). Among these analysed ocean water samples four TAP water samples were analysed (place in the sample sequence no #1, #20, #64 and #86; Fig. 3A). Consequently, the last TAP water sample was analysed after approximately 410 injections of $2\mu\text{L}$ of ocean water. These TAP water samples were treated as unknown and were not used for any drift nor memory correction. Despite the increasing load of salt in the

instrument vaporiser caused by evaporation of the saline samples, the measured isotopic composition of TAP water did not vary (1 standard deviation was 0.03‰ for $\delta^{18}\text{O}$ and 0.6‰ for $\delta^2\text{H}$) above the range of expected analytical uncertainty for this version of CRDS. Moreover, the obtained stable isotope composition of the TAP water was not correlated with the position in the injection sequence. This test confirmed that stable isotope fractionation due to increasing salt load in the vaporiser is negligible and it is within the expected range of analytical uncertainty.

Figure 3. The variability in the stable hydrogen and oxygen isotope composition of a freshwater standard (TAP water) injected between saline water samples, reflecting the influence of salt load in the vaporiser: A – injection of 82 natural ocean water samples with TDS 35 – 40 g L⁻¹ did not influence the δ -values of the TAP standard, B – after 310 highly saline water as in experiment #2 (Tab. S1) the obtained δ -values of the TAP standard significantly departed from original values: the injection of a highly concentrated solution of MgCl₂ (marked on the figure with dashed line) was more important than the number of high salinity samples that were analysed, C – the photo of a vaporiser septa after injection of 65 mg of salt.

All ocean water samples had $\delta^{18}\text{O}$ in a range between -0.5 and +1.0‰, and $\delta^2\text{H}$ between -5 and +5‰ and these δ -values likely would be determining the background, if the memory effect due to salt load is significant. Therefore, we additionally tested the presence of a “memory effect” by analysing a freshwater laboratory standard with contrasting δ -values ($\delta^{18}\text{O}$ -10.32‰ and $\delta^2\text{H}$ -71.8‰) after analysed 82 ocean water samples

However, a large memory effect was not evident in the final measurement of the laboratory standard. The measured values only differed by 0.03‰ in $\delta^{18}\text{O}$ and 0.5‰ in $\delta^2\text{H}$ from the true δ -values of the standard and the memory effect observed was therefore not higher than usual for this version of CRDS. The outcomes of this test confirmed that the measurement of stable isotope composition was not compromised by the accumulation of salt in the vaporiser following the injection of ~82 sea water samples, which equates to approximately 29–33 mg of salt added to vaporiser (82 samples $\times$ 2 μL of water $\times$ 5 injections $\times$ 35–40 g L⁻¹). However, as a matter of precaution, we recommend that the vaporiser should be cleaned after analysing every ~80 highly saline samples to avoid influence of salt accumulation on the measured stable isotope compositions of subsequent samples.

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356
357 **Salt accumulation in the vaporiser test #2.** The salt accumulation test #1 was replicated after
358 vaporiser cleaning but with higher loads of salts in the vaporiser. Instead of ocean water samples,
359 highly saline solutions with a known concentration of each salt were analysed. Eight TAP water
360 samples were subsequently analysed after every few saline solution samples during the run (Fig.
361 3B). After finalizing experiment #2, the vaporiser septa was not changed and several more highly
362 saline solutions were injected aiming for extreme salt accumulation prior to the final injection of
363 two TAP water samples. The final samples were injected, literally, through a crust of salt
364 accumulated on the inner side of the septa (Fig. 3C). During normal operation of a CRDS
365 instrument part of salt is removed from the vaporiser after each change of septa. In general, the
366 saline water solutions were analysed starting from the lowest salinity to the highest.

367 The variability in obtained results for TAP water was higher compared to the test #1 that used
368 ocean water samples, but also the load of salt in the vaporiser was significantly higher. For salt
369 loads of up to 38.5 mg, most results were within one standard deviation, with a few within 2
370 standard deviations (Fig. 3B). Even with a large salt load accumulated within the vaporiser (6.0
371 mg of MgCl_2 , 38.5 mg total salt in vaporiser); the results were not compromised. However,
372 despite similar precision, the measurement accuracy was lower. The results were not
373 significantly impacted until after several injections of solutions with MgCl_2 concentrations close
374 to saturation (see last three points on Fig. 3B). These results confirm that the stable isotope
375 fractionation due to salt accumulation is negligible up to ~ 6.0 mg of MgCl_2 and ~ 38.5 mg of total
376 salt in vaporiser. The results became incorrect when highly saline solutions with very high
377 MgCl_2 concentrations exceeding almost ten times ocean water were analysed (≥ 47 mg L^{-1}).

The DI water used for preparation of solutions in experiment #2 had $\delta^{18}\text{O}$ -1.48‰ and $\delta^2\text{H}$ -4.0‰. If the salt accumulation effect would result in water absorption from subsequent samples without significant isotope fractionation these δ -values would determine the instrument background. Therefore, it is very likely δ -values of these saline solutions and TAP water ($\delta^{18}\text{O}$ -1.89‰; $\delta^2\text{H}$ -7.4‰) were too close to each other to detect a memory effect. In order to evaluate progressively increasing memory effect due to high salt load, six SLAP standards with very different values from expected background ($\delta^{18}\text{O}$ -55.5‰ and $\delta^2\text{H}$ -428‰)³⁴ were injected. A significant difference from true δ -values in the last two SLAP injections was not observed when the load of salt in the vaporiser reached ~38.5 mg (including 6 mg of MgCl_2). The obtained δ -values were in the range of expected analytical uncertainty and typical for the instrument memory; $\delta^{18}\text{O}$ was different from true value by 0.02‰ and $\delta^2\text{H}$ by 0.90‰. The difference between obtained and true δ -values was high and exceeded the expected analytical uncertainty when SLAP was reanalysed when the salt load in the vaporiser reached ~65.3 mg (including 14 mg of MgCl_2), after recently injected samples with a MgCl_2 concentration close to the saturation point. The results for SLAP were very different from true δ -values by 1.06‰ for $\delta^{18}\text{O}$ and 7.5‰ for $\delta^2\text{H}$. The memory effect seems to be negligible up to ~38.5 mg of salt load especially when MgCl_2 contribution is low (≤ 6 mg). Higher salts loads with particularly high loads of MgCl_2 may significantly impact instrument precision, therefore care should be taken to minimize salt accumulation in the vaporiser through regular cleaning.

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SUPPORTING INFORMATION

Two tables related to Experiment #1 and #2 – showing differences in stable hydrogen and oxygen isotope compositions of various salt solutions prepared from deionised water are included in supporting information (Table S1 and S2). This information is available free of charge via the Internet at <http://pubs.acs.org>.

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