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Hydrogen Isotope Determination for Small-size Water Samples Using an Equilibration Technique

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The hydrogen/deuterium/oxygen (H/D/O) equilibration technique for measuring isotope ratios has proved to be of great interest for a number of applications in addition to the doubly labelled water (DLW) method, which measures energy expenditure in free living subjects. However, the limiting factor of the sample volume represents a common problem in many of these methodologies. By utilizing a fully automated sample preparation device, water samples with different volumes were analysed with respect to the $^2H/^1H$ isotope ratios. The sample volume ranged from 4–0.25 ml. It was demonstrated that high precision was obtainable over the entire range. Although fractionation did seem to occur, it remained insignificant. Therefore, subsequent mass correction is possible and isotope ratios can be reliably obtained from water samples as small as 0.25 ml. This implies that the fully automated H/D/O equilibration technique is a valuable and useful tool for an extended range of research areas, in particular where the sample volume is limited. © 1998 John Wiley & Sons, Ltd.

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INTRODUCTION

Many techniques used in physiological and biological research rely on the accurate determination of $^2H/^1H$ isotope ratios in water. The predominant sample preparation technique for deuterium-enriched aqueous samples is a reduction method employing zinc reduction or continuous-flow uranium furnaces. A different approach, the development of the H_2/H_2O equilibration method using a platinum catalyst to determine the $^2H/^1H$ isotope ratio of water samples has been reviewed by Kirshenbaum.[1] Horita *et al.*[2] improved this technique by automating the sample analysis and employing a newly developed platinum catalyst. They reported that this approach is limited to samples of 1 ml and larger. A fully automated H/D/O equilibration technique for measuring $^2H/^1H$ and $^{18}O/^{16}O$ isotope ratios has been described recently,[3] in which the sample volume was reported to be 5 ml. The same H/D/O sample preparation device has been used for assessing total energy expenditure by the doubly labelled water (DLW) method.[4] The results reported were obtained with a sample volume of 5 ml. This volume is easy to obtain when, for example, urine is sampled from adults subjects. However, when for experimental reasons saliva or plasma is required for analyses, the volume obtainable may represent a limiting factor. The same holds true in plant and geological studies where the sample volume is limited by nature. This paper discusses the problem of conducting measurements with water samples smaller than 5 ml and proposes a correction algorithm for water samples as small as 0.25 ml.

EXPERIMENTAL

The experimental set-up has been described in detail elsewhere.[4] Briefly, 20 ml glass bottles are filled with sample fluid ranging from 4–0.25 ml and a hydrophobic platinum catalyst is added before screwing the bottles to the sample rack. Each sample bottle is connected via a short capillary to a manifold with a pneumatically actuated valve. The sample rack holding 24 glass bottles is mounted on a sled to enable the bottles to be immersed and shaken in a temperature-controlled water-bath. The temperature is held constant at $19 \pm 0.05\,°C$. After pumping out the sample bottles are filled with equilibration gas. The pressure is 0.4 bar, resulting in an appropriate pressure in the inlet system of the isotope ratio mass spectrometer after equilibration (150 min) and transfer of the sample gas.

The isotope ratio measurements were carried out on a Delta S instrument (Finnigan MAT, Bremen, Germany) equipped with an H/D collector. The H_3^+ factor was found to be 4.36 ± 0.02 ppm nA^{-1}. The contribution of H_3^+ to the mass 3 ion current was then

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quantitatively corrected. Measurements were carried out at a mass 2 signal of 6.5 nA, corresponding to an analyser pressure of 1×10^{-7} mbar. Interfering masses (= impurities in the sample as measured through the inlet capillary in the mass spectrometer), e.g. ion currents at *m/z* 14 and 18, were measured for each sample in order to check for air leaks and H_2O vapour.

RESULTS AND DISCUSSION

Since the equilibration technique has proved to be of great interest for a number of investigators in different research areas, we discuss the performance of this technique with respect to small sample volumes and provide a simple mathematical approach for correcting results obtained from small water samples. Changing the water volume and keeping the other variables constant result in changes in the measured $^2H/^1H$ isotope ratios.[5] This occurs because, after equilibration, the isotope ratio of the sample gas depends on the H_2/H_2O mole ratio, the isotope ratio of the equilibration gas and the isotope ratio of the water sample. The volume of the vials is 20 ml and the sample amount varies from 4–0.25 ml. Therefore, the amount of H_2 can be treated as a constant only as a first approximation. A simple mass balance correction, however, is appropriate for calculating the true δ_D of the water samples. $\delta_{measured}$ is δ_{H_2} times the H_2 molar contribution plus δ_{H_2O} times the H_2O molar contribution:

$$\delta_{measured} = \delta_{H_2} M_{H_2}/(M_{H_2} + M_{H_2O}) + \delta_{H_2O} M_{H_2O}/(M_{H_2} + M_{H_2O}) \quad (1)$$

Hence

$$\delta_{measured} = \delta_{H_2} + M_{H_2O}/(M_{H_2} + M_{H_2O})(\delta_{H_2O} - \delta_{H_2}) \quad (2)$$

Therefore, a plot of $\delta_{measured}$ *vs.* the H_2/H_2O molar ratio should give a straight line. This is shown in Fig. 1.

Table 1 shows the measured raw data (δ_{HD/H_2}) and the development to the data corrected *vs.* SMOW/SLAP. Column 2 represents δ_{HD/H_2} according to the following equation:

$$\delta_{g/l} = \left(\frac{R_{Sa}}{R_{St}} - 1\right) \times 1000 \quad (3)$$

where subscripts g and l represent gas and liquid, respectively, R_{Sa} is the isotope ratio of the sample and R_{St} is the isotope ratio of the standard. The data in column 3 were obtained by applying Eqn (4) and yields the raw δ of the sample expressed *vs.* SMOW (δ_{HD/H_2} *vs.* $SMOW_{raw}$):

$$\delta^{i.St}_{Sa} = \delta^{w.St}_{Sa} + \delta^{i.St}_{w.St} + \left(\frac{\delta^{w.St}_{Sa}\,\delta^{i.St}_{w.St}}{1000}\right) \quad (4)$$

where the superscript i.St is the international standard, e.g. SMOW, and w.St is the working standard (−267.3‰ *vs.* SMOW).

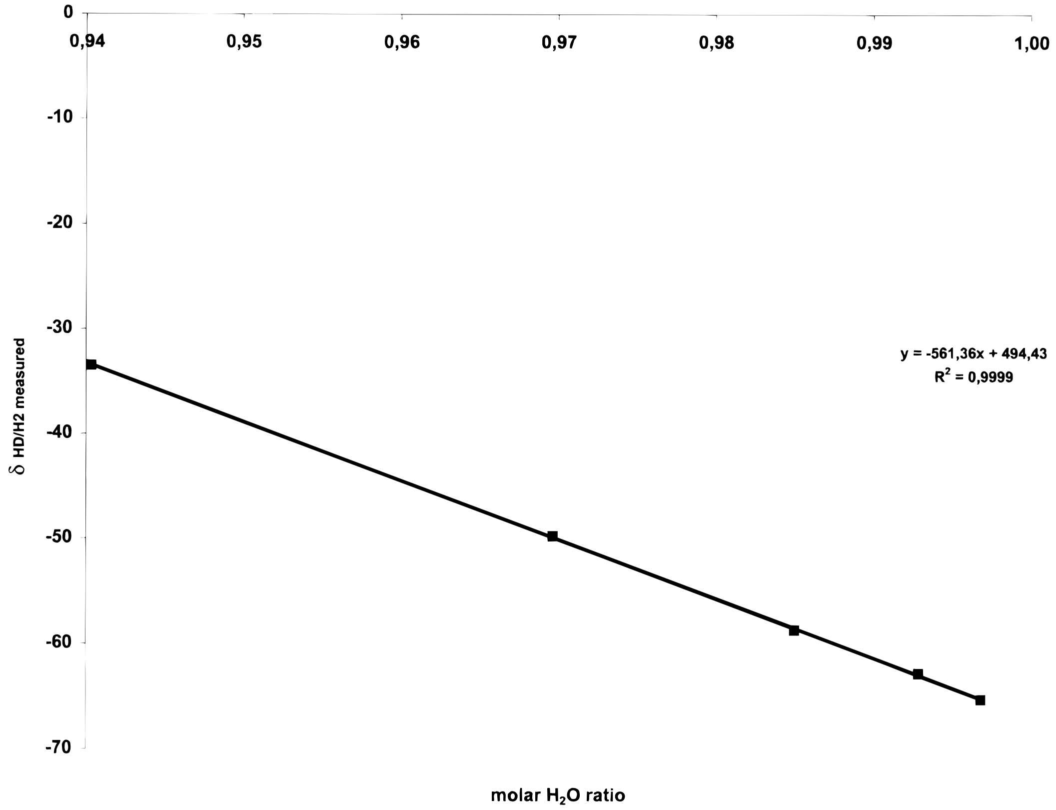

Figure 1. Plot of $\delta_{measured}$ against the molar ratio of the water. There are no significant deviations from the regression line, suggesting that fractionation is negligible.

Table 1. Measured raw data and the development of final data expressed *vs.* SMOW/SLAP

Volume of H_2O (ml)	δ_{HD/H_2} (‰)	δ_{HD/H_2} *vs.* $SMOW_{raw}$ (ml)	δ_{HD/H_2} *vs.* $SMOW_{time\ corr}$ (‰)	Inverted value (‰)	Sample *vs.* $SMOW_{corr}$ (‰)	Sample *vs.* SMOW/SLAP (‰)
4.00	−682.89	−770.41	−754.36	3071.05	−65.32	−65.21
2.00	−682.02	−769.79	−754.34	3070.72	−62.86	−62.76
1.00	−680.63	−768.78	−754.34	3070.72	−58.75	−58.65
0.50	−677.62	−766.60	−754.36	3071.00	−49.83	−49.94
0.25	−672.07	−762.58	−754.35	3070.74	−33.52	−33.47

The next step is a time correction (linear regression) based on the value of $SMOW_l$, which has been measured in the same run (column 4). This results in δ_{HD/H_2} *vs.* $SMOW_{time\ corr}$. By calculating

$$\delta_{l/g} = \left[\left(\frac{\delta_{HD/H_2}\ vs.\ SMOW_{time\ corr}}{1000} + 1 \right)^{-1} - 1 \right] \times 1000 \tag{5}$$

the inverted SMOW value is obtained, which is given in column 5.

Applying the formula

$$\left(\frac{\delta^{i.St}_{Sa}\, \delta_{l/g}}{1000} \right) + \delta^{i.St}_{Sa} + \delta_{l/g} \tag{6}$$

results in $\delta_{g/l}$ *vs.* $SMOW_{corrected}$ (column 6).

The last step involves the scale correction of δ_{HD/H_2} *vs.* SMOW for the accepted SLAP value (−428‰). The value obtained is given in column 7.

The system was calibrated by using working standards with a known relationshp to V-SMOW in different volumes so that the measured δ_{HD/H_2} could be normalized to V-SMOW as proposed elsewhere.[6] δ_{HD/H_2}(‰) increases with decreasing volume (Table 2). This is due to the initial isotopic composition of the added hydrogen gas. From the regression line in Fig. 1, +493.62‰ was obtained for the added hydrogen *vs.* hydrogen gas equilibrated with V-SMOW. This corresponds to −634.1‰ on the V-SMOW scale. In absolute terms, the deuterium contents of the liquids and gases in the study were as follows: V-$SMOW_l$, 156; V-$SMOW_g$, 38.2; working reference, 114.3; added hydrogen, 57.1; $sample_l$, 145.4; and $sample_g$, 35.6 ppm.

The precision of the final δ_{HD/H_2} (column 7 in Table 2) was 0.12‰. Note that such a precision was obtained on the basis of a deuterium content of only 35.6 ppm virtually independent of sample size. This precision is very good and sufficient for studies of natural variations. The straight line in Fig. 1 suggests that the possible fractionation error due to water loss during equilibration is negligible. By applying Eqns (1) and (2), the described approach has the potential to serve as a minimal model for measuring water samples in the range 4–0.25 ml, and measuring smaller sample volumes seems possible. The constant molar ratio of sample volume and equilibration gas must be emphasized. A promising approach could be to use smaller sample bottles. This, however, is limited by the amount of gas transferred to the isotope ratio mass spectrometer, resulting in an appropriate pressure in the inlet system of the mass spectrometer.

CONCLUSIONS

We have demonstrated a high level of precision in the determination of $^2H/^1H$ isotope ratios in small size water samples. When tested for linearity, fractionation due to water loss seems likely. It was shown that the fractionation error is small and exerts little influence on the mathematical correction approach. Therefore, it is concluded that the automated H/D/O equilibration technique is a useful tool for investigations with limited sample volumes for the determination of $^2H/^1H$ isotope ratios in small-size water samples, e.g. with sample volumes ranging from 4–0.25 ml.

Great care needs to be taken in order reliably to avoid fractionation and ensure known H_2O/H_2 molar ratios. Furthermore, it needs to be established whether this system is equally useful for $^{18}O/^{16}O$ isotope analysis in small-size water samples.

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Table 2. Observed δ_{HD/H_2} and corrected δ_{HD/H_2corr} for working standard of different volumes

Volume of H_2O (ml)	H_2O (mol)	Volume of H_2 (ml)	H_2 (mol)	Molar H_2O ratio, $M_{H_2O}/(M_{H_2O}+M_{H_2})$	δ_{HD/H_2} (‰)	δ_{HD/H_2corr} (‰)
4.00	0.222 222	16.00	0.000 714	0.996 796	−65.21	−67.01
2.00	0.111 111	18.00	0.000 804	0.992 820	−62.76	−66.79
1.00	0.055 556	19.00	0.000 848	0.984 962	−58.65	−67.10
0.50	0.027 778	19.50	0.000 871	0.969 613	−49.94	−66.80
0.25	0.013 889	19.75	0.000 882	0.940 307	−33.47	−66.98
Mean ± SD						−66.93 ± 0.12

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