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Fourier Transform Infrared Spectroscopy Measures $^1\text{H}/^2\text{H}$ Ratios of Native Water with a Precision Comparable to that of Isotope Ratio Mass Spectrometry

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Summary: A fast and easy method is described which uses *Fourier* transform infrared spectroscopy (FT-IR) to measure the $^1\text{H}/^2\text{H}$ ratio of aqueous samples of less than 100 μl with high precision (± 0.2 – 0.5% in the range of 89 to 2680 $\mu\text{l/l}$). Using a thermostat-controlled CaF_2 cell, low resolution absorption spectra (8 cm^{-1}) are measured. The integral of absorption in the range of 2600 and 2460 cm^{-1} (O^2H vibration) is used to analyse the ^2H content of the sample. For measurements at low enrichment five standards are used (SLAP: 89.00 $\mu\text{l/l}$, GISP: 126.3 $\mu\text{l/l}$, V-SMOW: 156.0 $\mu\text{l/l}$, all from the International Atomic Energy Agency, Vienna, Austria, standard 1: 183.3 $\mu\text{l/l}$, standard 2: 222.5 $\mu\text{l/l}$, both prepared by weighing and controlled by isotope ratio mass spectrometry (IR-MS)). For measurements at high enrichment three standards are used (standard 2: 222.5 $\mu\text{l/l}$, standard 3: 1323 $\mu\text{l/l}$, standard 4: 2680 $\mu\text{l/l}$, all prepared by weighing and controlled by IR-MS). Measured and reported ^2H concentrations coincide very well, two samples for quality control (145 and 1612 $\mu\text{l/l}$) were measured with a precision of 0.3 and 0.4% corresponding to ± 0.5 and 5.9 $\mu\text{l/l}$.

Introduction

In bioscience and medical research, measurements of $^1\text{H}/^2\text{H}$ ratios of biological samples are used to investigate total body water, body composition (1–7), water turnover (8–12), and drug complicity (13). An example of repeated measurements of total body water and water turnover is given in figure 1 which shows the ^2H concentrations in saliva samples obtained from one participant during the German Medical Karakoram Expedition 1991. Total body water is calculated from the increase of ^2H concentration after oral load with deuterium oxide. Water turnover is calculated from the subsequent log-linear decay of tracer concentration and distribution volume. In geosciences, measuring the $^1\text{H}/^2\text{H}$ ratio of a water sample may help to identify its origin. The ^2H content of a river mainly fed by glacial water may be lower than of one mainly fed by superficial rain water. The ^2H

content of a river of mainly glacial origin is lower in summer than in winter, whereas the ^2H content of a water sample coming from a river of rainy regions is higher in summer than in winter. $^1\text{H}/^2\text{H}$ ratios of aqueous solutions may be determined by different methods. The most precise measurements are performed using isotope ratio mass spectrometry (IR-MS) yielding an error of ± 0.20 – $0.50\text{ }\mu\text{l/l}$ (14, 15). However, this method requires expensive equipment and maintenance, as well as an elaborate technique of sample preparation and well trained personnel. When IR-MS is used for the analysis of $^1\text{H}/^2\text{H}$ ratios in biological samples the amount of $^2\text{H}_2\text{O}$ given to a subject or animal may be as low as 0.05–0.10 ml/kg body weight, leading to $^2\text{H}_2\text{O}$ concentrations in body water of 80–150 $\mu\text{l/l}$ above the natural level (corresponding to 220–300 $\mu\text{l/l}$) at equilibrium. Using NMR for the measurement of $^1\text{H}/^2\text{H}$ ratios, no sample

Fig. 1. Time course of $^2\text{H}_2\text{O}$ concentrations in saliva used five times to measure total body water and water turnover in one participant of the German Medical Karakorum Expedition 1991: Total body water is calculated from the increase of ^2H enrichment after oral load with deuterium oxide. Water turnover is calculated from the log-linear decay and total body water.

preparation is required which makes analysis faster and easier (16, 17). The precision, however, is limited to about $\pm 30\text{--}40\text{ }\mu\text{l/l}$. As a consequence, larger doses of $^2\text{H}_2\text{O}$ must be given if body water is to be measured with a precision of about 1%. Typical values are 1–2 ml/kg body weight which will lead to $^2\text{H}_2\text{O}$ concentrations of 1700 to 3200 $\mu\text{l/l}$.

Infrared spectroscopy using dispersive gratings has been used mostly to determine $^1\text{H}/^2\text{H}$ ratios in body fluids. The analysis is fast, but the precision is only slightly better than that of NMR. Therefore doses of 1–2 ml/kg body weight must be used to keep the error in the estimation of body water below 1%. Moreover, sample preparation is required to avoid absorption from components other than H_2O and $^2\text{H}_2\text{O}$, and many of these preparation procedures are complicated and time consuming (18–21). Recently, we published a method of sample preparation and IR analysis which is precise (mean random error of 25 $\mu\text{l/l}$) and fast (40–60 samples in 8 hours) (22).

Based on general toxicological considerations, the $^2\text{H}_2\text{O}$ concentration of body fluids should not exceed 5000 $\mu\text{l/l}$. Due to the biological half-time of five to ten days in humans, repeated measurements of total body water, body composition or water turnover are limited to about two applications of $^2\text{H}_2\text{O}$ per week when using dispersive IR spectroscopy with doses of 1–2 ml/kg body weight. This problem may be overcome by the use of Fourier transform IR spectrometers (FT-IR). In biochemical sciences, FT-IR spectrometers have generally replaced the conventional dispersive

spectrometers during the last years, because of their higher speed and sensitivity. In the present paper, we describe a procedure using FT-IR which allows the determination of $^2\text{H}_2\text{O}$ concentrations with a random error of 0.2–0.5 $\mu\text{l/l}$, representing a precision at least 50 times greater than that of instruments with dispersive gratings.

Materials and Methods

The $^2\text{H}_2\text{O}$ concentration of a native water sample or a purified biological sample (serum, urine, saliva) is determined by measuring the absorption of the O^2H -bond at 2510 cm^{-1} . A PC-controlled Fourier transform infrared (FT-IR) spectrometer (Bruker, Karlsruhe, Germany, type IFS 66) is used which is equipped with an InSb detector cooled by liquid nitrogen (Bruker, part no. D413). The software used is OPUS, version 1.3. The instrument housing is purged with compressed dry air at a rate of at least $10\text{--}20\text{ l}\cdot\text{min}^{-1}$. A CaF_2 cell with a path length of 0.1 mm and a volume of 60 μl (Philips, Kassel, Germany, part no. 942325716751) is mounted to a heatable water jacket (Philips, part no. 942325702831). The temperature of the cell is kept constant at $22.0 \pm 0.01\text{ }^\circ\text{C}$ by a thermostat-controlled water bath (Haake, Karlsruhe, Germany, type F3-C). Temperature equilibration of the equipment is started at least 30 minutes before the measurements.

Absorption of the O^2H -bond at 2510 cm^{-1} is used to measure the $^2\text{H}_2\text{O}$ content of a sample. The solution is placed in the cell, then respiratory CO_2 is removed from the spectrometer by purging the instrument with dry air for 300 seconds; during this period the temperature of the solutions is allowed to equilibrate. Then, five subsequent measurements are made, each requiring 800 single scans. Table 1 shows the instrument parameters for data acquisition and Fourier transformation which — after performing long-term experiments — were chosen to obtain highest accuracy. After Fourier transformation each absorption spectrum is integrated in the range from 2680 to 2460 cm^{-1} and the median of the five measurements is used. The solution is subsequently removed by a vacuum pump and the cell is rinsed with distilled water and acetone (quality for UV spectroscopy) while a plastic shield covers the cell to avoid contamination by micro water drops. Analysis is performed in blocks measuring first the reference (demineralized water) and then six samples (five unknown and one calibration sample). Two blocks (2 reference solutions, 12 samples) are measured within 150 minutes. Table 2 shows the complete algorithm for routine measurements.

For quantitative analysis at low enrichment levels we use three international water standards (Standard Light Arctic Precipitation, 89.0 $\mu\text{l/l}$ $^2\text{H}_2\text{O}$ (SLAP); Greenland Ice Sheet Precipitation, 126.4 $\mu\text{l/l}$ $^2\text{H}_2\text{O}$ (GISP); Vienna-Standard Mean Ocean Water, 156 $\mu\text{l/l}$ $^2\text{H}_2\text{O}$ (V-SMOW); all purchased from the International Atomic Energy Agency, Vienna, Austria) and two laboratory standards (standard 1: 183.3 $\mu\text{l/l}$ $^2\text{H}_2\text{O}$; standard 2: 222.5 $\mu\text{l/l}$ $^2\text{H}_2\text{O}$) prepared by weighing (see tab. 3). For measurements at high enrichment levels we use three internal standards prepared by weighing (standard 2: 222.5 $\mu\text{l/l}$ $^2\text{H}_2\text{O}$, standard 3: 1323 $\mu\text{l/l}$ $^2\text{H}_2\text{O}$, standard 4: 2680 $\mu\text{l/l}$ $^2\text{H}_2\text{O}$, see tab. 3). All standard and unknown samples are measured against demineralized water as reference solution. $^2\text{H}_2\text{O}$ concentrations of a new charge of internal laboratory standards are evaluated once using isotope ratio mass spectrometry (Gesellschaft für Strahlen- und Umweltforschung (GSF), Section of Hydrology, Munich, Germany) as well as using FT-IR with the international water standards SLAP, GISP and V-SMOW. A calibration curve is calculated using a least-square fitted linear regression.

Tab. 1. Instrument settings for data acquisition and Fourier transformation

<i>Optic conditions</i>	
Aperture:	0.25 mm for align mode; 1.00 mm for sample mode;
Resolution:	8.0 cm^{-1} ;
Mirror velocity:	9; 60 kHz;
Gain switch gain	off
Signal gain	automatic
Number of reference scans:	1000;
Number of sample scans:	800.
<i>Acquisition conditions</i>	
Acquisition mode:	double sided fast return
Lower spectral bandwidth:	0 cm^{-1} ;
Upper spectral bandwidth:	7800 cm^{-1} .
<i>Fourier transform conditions</i>	
Start frequency limit for spectrum	4000 cm^{-1}
End frequency limit for spectrum	2000 cm^{-1}
Apodisation window:	4-term Blackmann-Harris;
Zero filling factor:	2.0;
Phase correction mode	Power

Results

Figure 2 shows the single channel spectrum of natural water (Spectrum 1) in the region of 3000 to 2200 cm^{-1} . Spectra 2 and 3 show a magnified plot of two solutions containing 145 and 222.5 $\mu\text{l/l}$ $^2\text{H}_2\text{O}$ in the region of 2700 and 2500 cm^{-1} . Spectrum 4 shows the resulting absorption spectrum representing 77.5 $\mu\text{l/l}$ ^2H enrichment. Beside the CO_2 absorption at 2350 cm^{-1} the vibration of the O^2H -bond is seen at 2510 cm^{-1} .

Figure 3 shows the absorption spectra of the five low enrichment standards measured against natural water (145 $\mu\text{l/l}$) as reference solution: No. 1 SLAP (89.0 $\mu\text{l/l}$), No. 2 GISP (126.4 $\mu\text{l/l}$), No. 3 V-SMOW (156.0 $\mu\text{l/l}$), No. 4 standard 1 (183.3 $\mu\text{l/l}$), No. 5 standard 2 (222.5 $\mu\text{l/l}$). The spectra of SLAP and GISP show a negative absorption due to the lower $^2\text{H}_2\text{O}$ content as compared with the reference solution.

Figures 4a and b show typical examples of the high (4a) and low (4b) enrichment calibration curves established on one day.

Table 4 shows precision and reproducibility of the method. Standard solutions and two solutions for quality control (sample 1: distilled water for low enrichment, sample 2: 1612 $\mu\text{l/l}$ $^2\text{H}_2\text{O}$ for high enrichment) were measured on ten different days. The measured $^2\text{H}_2\text{O}$ concentration of the standards was found to be very close to the reported values. The absolute random error of the measurement was about ± 0.5 $\mu\text{l/l}$ for low enrichment and about ± 5.0 $\mu\text{l/l}$ for high enrichment measurements (corresponding to a relative error of about 0.2 to 0.7%). The $^2\text{H}_2\text{O}$ content of

Tab. 2. Algorithm for the routine measurements

Adjust interferometer mirrors once each day before measuring

1. Mount empty CaF_2 cell
2. Set instrument as listed in table 1
Set aperture to 0.25 mm
3. Execute "auto align" for mirror adjustment

Measurement of samples (one block)

1. Set aperture to 1.0 mm
2. Measurement of reference samples
 - a. Fill in reference solution (demineralized water)
 - b. Wait for 300 s for equilibration
 - c. Record one spectrum with 1000 scans
3. Subsequent measurement of five unknown and one calibration sample
 - a. Place unknown sample in cell
 - b. Wait for 300 s for equilibration
 - c. Record 5 spectra with 800 scans
 - d. Place a second unknown in cell
 - e. Repeat step 3.b. and 3.c.
 - f. Place in third unknown in cell
 - g. Repeat step 3.b. and 3.c.
 - h. Place one calibration sample in cell
 - i. Repeat step 3.b. and 3.c.
 - j. Place a fourth unknown in cell
 - k. Repeat step 3.b. and 3.c.
 - l. Place a fifth unknown in cell
 - m. Repeat step 3.b. and 3.c.
 - n. Continue with Step 2

Tab. 3. Solutions used for calibration for measurements at low and high enrichment

Sample	^2H concentration	
	[δ per mil]	[$\mu\text{l/l}$]
<i>Low enrichment</i>		
SLAP	-429.1	89.0
GISP	-189.7	126.4
SMOW	0.0	156.0
Standard 1	+175.1	183.3
Standard 2	+426.5	222.5
<i>High enrichment</i>		
Standard 2		222.5
Standard 3		1323
Standard 4		2680

distilled water (sample 1) was found to be 143.6 $\mu\text{l/l}$ measured with an interassay reproducibility of 0.5 $\mu\text{l/l}$ (corresponding to a relative error of 0.3%). The $^2\text{H}_2\text{O}$ content of sample 2 was found to be close to the expected value (1619 versus 1612 $\mu\text{l/l}$) measured with an interassay reproducibility of 5.9 $\mu\text{l/l}$ (corresponding to a relative error of 0.4%).

The amplitude of noise is smaller than 0.0001 absorption units; in absolute terms of transmission this corresponds to 0.00005 (0.005%), which is mainly limited by the properties of the detector used.

Fig. 2. Spectrum 1: Single channel spectrum of natural water in the region of 3000 to 2200 cm^{-1} . Spectra 2 and 3: Magnified plot of two solutions (145 and 222.5 $\mu\text{l/l}$ $^2\text{H}_2\text{O}$) in the region of 2700 and 2500 cm^{-1} . Spectrum 4: Resulting absorption spectrum representing 77.5 $\mu\text{l/l}$ ^2H enrichment (CO_2 absorption at 2350 cm^{-1} , O^2H absorption at 2510 cm^{-1}).

Fig. 3. Absorption spectra of the five low enrichment standards measured against natural water (145 $\mu\text{l/l}$) as reference solution.

No. 1: SLAP (89.0 $\mu\text{l/l}$), No. 2: GISP (126.4 $\mu\text{l/l}$), No. 3: V-SMOW (156.0 $\mu\text{l/l}$), No. 4: standard 1 (183.3 $\mu\text{l/l}$), No. 5: standard 2 (222.5 $\mu\text{l/l}$).

The spectra of SLAP and GISP show a negative absorption due to the lower $^2\text{H}_2\text{O}$ content as compared to the reference solution.

Fig. 4. Typical example of a high (a) and a low (b) enrichment calibration curve established on one day.

Tab. 4. Random error of the method at low and high ^2H enrichment established on ten different days

Sample	Reported ² H concentration [μl/l]	Measured ² H concentration [μl/l]	Random error	
			absolute [μl/l]	relative [%]
<i>Low enrichment</i>				
SLAP	89.0	89.45	0.45	0.5
GISP	126.4	126.71	0.67	0.7
V-SMOW	156.0	155.88	0.67	0.4
Standard 1	183.3	180.95	0.58	0.3
Standard 2	222.5	222.11	0.83	0.4
Sample 1 (quality control)				
Distilled water		143.6	0.52	0.3
<i>High enrichment</i>				
Standard 2	222.5	215	3.3	1.6
Standard 3	1323	1335	4.3	0.3
Standard 4	2680	2674	6.6	0.2
Sample 2 (quality control)				
	1612	1619	5.9	0.4

Discussion

When Fourier-transform infrared spectroscopy (FT-IR) is used to measure the $^1\text{H}/^2\text{H}$ ratio of aqueous samples, an analytical precision may be achieved which is close to that of isotope ratio mass spectrometry (IR-MS). Additionally, the use of FT-IR offers the following advantages:

1. The costs of an FT-IR spectrometer and its maintenance are considerably lower than that of an IR-MS (up to a factor of five) and considerably more FT-IR spectrometers are available than IR-MS. FT-IR spectrometers usually are found in departments of chemistry, pharmacology or toxicology.
2. The measurement procedure is easy to learn and to perform; skilled personnel (as required for the IR-MS) are not absolutely necessary.
3. The analysis is fast; without the use of automatic sample changers, up to 50 samples may be measured during 10 hours.
4. The dynamic range of the reported method is large, samples with $^1\text{H}/^2\text{H}$ ratios of up to 3000 $\mu\text{l/l}$ may be analysed with the same precision without an error-producing dilution step.

Even less expensive and more simply constructed FT-IR spectrometers with a lower spectral resolution (below 1 cm^{-1} , e. g. the IFS-48 of Bruker) may achieve the reported precision, because the resolution need not be better than 8 cm^{-1} . We prefer to use the more sensitive InSb detector compared with the standard MCT detector, because of the higher sensitivity in the range of 4000 to 2000 cm^{-1} . We would like to em-

phasize that a plastic shield must cover the CaF_2 cell during the entire procedure of sample changing to prevent contamination of the windows by the rinsing solutions. Even very small contaminations will influence the absorption measured and thus alter the precision. It would be desirable to use an automatic sample changer with two CaF_2 cells for the reference and the sample solution in order to save the time which is used for equilibration of the reference solution. Unfortunately, it is impossible to have two cells with nearly identical pathlengths. Even very small differences between the pathlengths cause considerable baseline distortions and lower the analytical precision of the measurement.

The analytical procedure described in this paper enables the determination of body water, body composition or water turnover with high precision (mean random error smaller than 1%) after applying small $^2\text{H}_2\text{O}$ doses of only 0.1 ml/kg body weight. As a next step we intend to develop a procedure which allows accurate and fast purification of biological samples such as serum, urine or saliva.

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