Water enriched in the rare stable isotopes

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Chapter 1

General Introduction

This chapter will provide a general introduction to isotope concepts and analytical techniques: mass spectrometry and infrared laser spectroscopy for stable isotope ratio measurements will be introduced. A brief introduction to the research topics in the following chapters and the structure of the thesis is also presented.

1.1 Introduction

Variations in the natural abundance of stable isotopes are used to study a wide variety of phenomena occurring in several scientific disciplines. In this thesis, the focus is on the stable isotopes of water, both in natural water and in water with artificially changed isotope concentrations. The latter are used in biomedical studies, for instance for the assessment of the total body water of an animal (or human), for which the concentration of only one of the (normally rare) isotopes is increased (referred to as the singly-labelled water (SLW) method) and for the energy expenditure measurements of humans and animals, for which the concentrations of two isotopes is increased (hence the doubly-labelled water (DLW) method). In general, the accuracy and precision of these two methods depends on the accuracy of stable isotope measurements on body water samples collected for this purpose, as well as on calibration of those measurements.

The natural stable isotope composition of water is not constant; but small and distinct differences/changes occur over the globe. For example, for precipitation this composition depends on the latitude and altitude. This natural variability of the isotopic composition of water is used, as said, in a multitude of applications, mostly in some way or another as tracer for water transport and/or phase transitions. One of the consequences of this variability is that tap water varies slightly in its isotopic composition across the globe. Outside of the isotope community, such variations are often not known, and mostly not important. Sometimes, however, these variations do matter, as for instance in the case of the realization of the triple point of water. This triple point is used to define the unit of temperature, the kelvin, in the International System of Units. The triple point of "water", however, depends on its isotopic composition, as the temperature metrology community learnt over the years.

This thesis will focus on the above subjects. Key elements are the development of a laser spectrometer for water isotope measurements in biomedical research, the production of new well-characterized isotope-enriched reference waters for calibration of isotope measurements such as SLW and DLW measurements (and others), and the production of another set of natural and slightly enriched reference waters for which the temperature shifts of the triple point have been determined. This chapter continues with general introductions to the isotope concepts and measurement techniques for determining the isotopic ratios of a water sample.

1.2 Isotopes

Isotopes are atoms of one element with the same number of protons and electrons but with a different number of neutrons. These atoms thus have different atomic masses. Most elements have two or more stable isotopes (see for a complete overview the website and publications of the Commission on Isotopic Abundances and Atomic Weights [1]). Although the natural abundances of the stable (non-radioactive) isotopes of elements are almost constant all over the earth, small changes can be observed, as a result of the fact that most chemical and physical processes are slightly isotope-specific. This is called isotopic fractionation. There are two main causes: firstly, the heavier isotopes generally form stronger bonds than lighter isotopes, thus more energy is needed to break the bonds that contain heavier isotopes. This results in slower reaction rates of the heavier isotopes. Secondly, molecules containing a heavier isotope have a lower velocity and thus lower mobility than the lighter ones. This leads for example to lower diffusion and also lower reactivity.

Fractionation can occur in either reversible equilibrium reactions or irreversible unidirectional kinetic reactions. Kinetic fractionation can be seen as a change in isotopic composition by transition of a compound from one state to another (for example, the evaporation of water with immediate removal of the water vapour from further contact with the liquid water), or into another compound (for example, in photosynthesis in which the carbon atom of CO_2 is transferred into plant organic carbon). Equilibrium isotopic fractionations can be understood in terms of isotope-exchange reactions that involve the transfer of isotopes between two compounds in chemical equilibrium (for example, dissolved bicarbonate and carbon dioxide) or in physical equilibrium (for example, liquid water and water vapour in close contact with each other)[2]. Variations in isotopic abundances due to physical and chemical processes are small, but measurable, thus allowing the use of stable isotopes as tracers to study complex processes.

1.3 Delta notation

In practice, instead of absolute isotope abundances, isotope abundance ratios are measured; defined as R= abundance of rare isotope/ abundance of abundant isotope. For instance, for oxygen-18 in water:

$$^{18}R(H_2O)=\frac{[H_2{}^{18}O]}{[H_2{}^{16}O]} \quad (1)$$

a superscript before the ratio symbol R refers to the isotope under consideration. These ratios can be measured with high precision, but not with high accuracy due to isotope fractionation effects that occur in the measurement system itself. Therefore, the isotope abundance ratio of a sample is compared to that of a reference material that is treated in an as identical as possible way, such that all fractionation effects occur in the same way in both sample and reference material, and thus cancel. The measurement result is usually expressed in the so-called delta notation:

$$\delta = \frac{R_{\text{sample}}}{R_{\text{reference}}} - 1 \quad (2)$$

As differences between reference material and sample are mostly small, they are usually expressed in parts per thousand (‰ or per mille). If the δ value is positive, the sample is enriched with respect to the reference, whereas a negative δ value indicates the sample is depleted with respect to the reference. The international standard used for the determination of stable oxygen and hydrogen isotope abundance ratios in water is Vienna Standard Mean Ocean Water (VSMOW) [3]. Another water reference SLAP (Standard Light Antarctic Precipitation), considerably depleted in heavy isotopes with respect to VSMOW, was prepared from South Pole firn in 1967 as an anchor for the lower level of the isotopic scale [4]. It has been recommended by the Advisory Group Meeting on Stable Isotope Reference Samples [3] that the δ^2H and $\delta^{18}O$ values of all water samples be expressed relative to the scale spanned by both VSMOW and SLAP, thus making use of their well-known (and actually defined) difference. This procedure was recommended to eliminate instrumental bias and thus improve inter-laboratory comparison of test results.

Due to the exhaustion of the original VSMOW and SLAP reference waters, they are now superseded by VSMOW2 and SLAP2 [5]. VSMOW2 and SLAP2 were prepared at the IAEA by mixing of several carefully distilled natural water samples and carefully calibrated natural water samples from the Antarctic, respectively, in order to obtain isotopic compositions as close as possible to the predecessor materials. The reference values were assessed from the measurement results by three laboratories in direct comparison to those of VSMOW and SLAP. Table 1 shows

the recommended δ^2H and $\delta^{18}O$ values for VSMOW2 and SLAP2 with their combined uncertainties. The latter have been evaluated from the measurement uncertainties in results of laboratories and isotopic homogeneity tests of the prepared ampoules of VSMOW2 and SLAP2. All water samples are then normalized to the VSMOW/ SLAP scale by assigning δ^2H and $\delta^{18}O$ values (as in Table 1) to VSMOW2 and SLAP2.

Table 1. δ^2H and $\delta^{18}O$ reference values for VSMOW2 and SLAP2 and their associated combined standard uncertainties (1-σ) [5].

IAEA name	$\delta^2H_{(VSMOW/SLAP)}$ (‰)	Combined standard uncertainty $\delta^2H_{(VSMOW/SLAP)}$ (‰)	$\delta^{18}O_{(VSMOW/SLAP)}$ (‰)	Combined standard uncertainty $\delta^{18}O_{(VSMOW/SLAP)}$ (‰)
VSMOW2	0.0	0.3	0.00	0.02
SLAP2	–427.5	0.3	–55.50	0.02

However, these reference materials have been prepared in limited quantities, and are made available to laboratories over the globe in small amounts. Daily-use reference waters (or in-house reference waters) that are carefully calibrated against the VSMOW-SLAP international scale must be used to normalize the measured isotopic results of samples. A general formula for normalization of isotope measurement results using "true" (that is well-calibrated against VSMOW-SLAP) and measured values for two laboratory standards (LS1 and LS2) can be expressed as:

$$\delta_{true(sample)} = \delta_{true(LS1)} + \left(\delta_{raw(sample)} - \delta_{raw(LS1)}\right) \times \left(\frac{\delta_{true(LS1)} - \delta_{true(LS2)}}{\delta_{raw(LS1)} - \delta_{raw(LS2)}} \right) \qquad (3)$$

The procedure is generally called "normalization", with the last term within bracket being the normalization factor.

1.4 Stable Isotope Analysis Techniques

1.4.1 Isotope Ratio Mass Spectrometry

Isotope ratio mass spectrometry (IRMS) is the conventional method for precise and accurate stable isotope ratios measurements, including $^{12}C/^{13}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$, and $^{18}O/^{16}O$. The mass spectrometer has three main sections: an ion source, a mass analyzer, and ion collection assembly. Prior to entering the ion source of an IRMS, samples are converted into a single non-condensable gas (e.g., H_2 for hydrogen, CO_2 or CO for carbon and oxygen, and N_2 for nitrogen) depending on the isotopic composition of the sample and the isotopes of interest. In the ionization source, the gas molecules are ionized through interaction with a focused electron beam (at energy of about 70 eV) in a high vacuum (electron ionization). These ions are repelled from the ion source and are focussed and accelerated through an electric field (of several kV) and then pass through a strong magnetic field, where the Lorentz force causes their separation according to their mass (more correctly according to their mass-to-charge ratio *m/z*, where z is mostly 1). The ions are collected by a collector array, which generally consists of three (sometimes up to eight) collectors, called Faraday cups. The IRMS is tuned such that the different masses of the isotopologues (thus the same molecules with different isotopic composition) are collected by the Faraday cups, which makes their currents proportional to the isotope abundances of the sample.

There are two common sample introduction techniques for the IRMS: Dual-inlet isotope ratio mass spectrometry (DI-IRMS) and Continuous-flow isotope ratio mass spectrometry (CF-IRMS). DI determines isotope ratios of pure gases by alternately introducing a sample gas and a reference gas (of known isotopic composition) into an IRMS via the inlet system. This system consists of several valves and two gas bellows; one contains the gas sample and one contains the reference gas and they are alternatively introduced into the ion source of the IRMS by switching the valves of the inlet system. In order to obtain high precision the bellows are adjusted such that sample and reference gas are kept at identical pressures; together with the carefully balanced capillaries through which the gases flow into the ionization source. This assures that the isotopic fractionation effects that inevitably occur are identical to both sample and reference.

In the DI technique the sample preparation is done offline which is usually time-consuming. In contrast, in the CF technique the sample preparation is done immediately before introduction into the IRMS. The CF technique uses a carrier gas (usually helium) to carry the gas into the ion

source of IRMS. A pulse of reference gas is introduced into the IRMS just before or after the introduction of the gas sample.

The CF has largely taken over stable isotope analysis for most scientific fields due to its major advantage: the possibility of coupling a large variety of analytical chemical analysis instrumentation "on-line" for isotope analysis. Examples are "elemental analyzers": automatic sample combustion systems that deliver N_2, CO_2 and sometimes also SO_2 in helium, time-separated using a gas chromatographic column, pyrolysis sytems and complex GC separation systems for compound-specific isotopic analysis. All these combinations lead to highly automated sample preparation and measurement, along with the possibility of dealing with small sample size. The only advantage that DI still has is its higher accuracy, which is nevertheless crucial for some applications. Brand has provided a comprehensive review of DI-IRMS and CF-IRMS for analyzing stable isotope ratios [6].

1.4.1.1 Pretreatment systems

IRMS instrumentation is unsuitable for condensable gases like water (vapour), as they can condense (with accompanying isotopic fractionation) throughout the whole inlet system onto the high vacuum ion source. Consequently, some chemical preparation is required to transfer the isotope signature of water first to a gas that can be analyzed by the IRMS (Even if the direct measurement of H_2O using IRMS would be possible, due to mass overlaps of isotopologues $H^2H^{16}O$ and $H_2{}^{17}O$ it would thus be impossible to determine either of the two accurately.)

In the case of water, the technique classically used for the ^{18}O isotope is equilibrating the water first with CO_2 of known isotope composition at constant temperature and then analyzing the ^{18}O of the CO_2 in a DI IRMS [7]. At the CIO we have such a system in use.

The first step in this procedure is removing dissolved gases from the water sample. The water sample (typically 0.6 ml) is frozen using dry ice (-89°C), after which any gas present in the vial is pumped away. Second, the water is melted and the remaining dissolved gases in the water are transferred to the gas phase due to the low pressure. Next, the water is refrozen and the vial once more evacuated. Then, 0.2 mmol of CO_2 gas with known isotopic composition is added to the still frozen water sample. Finally, the batch with 16 of those vials is brought into a water bath, where the isotopic equilibrium process begins. The oxygen atoms of the water and CO_2 gas through the bicarbonate reaction will exchange until an isotopic equilibrium is reached.

Equilibrium is attained after several hours and accurate temperature stabilization is required in order to quantify the oxygen isotopic fractionation associated with the bicarbonate reaction.

In our (static) setup, complete isotopic equilibrium is only reached after >36 hours, and the CO_2 gas and the water sample are kept at constant temperature (25.00 ± 0.02 °C) for 36-48 hours. After this period the CO_2 gas is introduced into the IRMS, after its collection in a trap at liquid nitrogen temperature installed just before the inlet system of the IRMS, while a water trap at ~ -60 °C in between prevents water vapour from entering the IRMS. The whole system functions automatically, and can deal with up to 80 samples in sequence.

The IRMS is equipped with a standard triple collector for *m/z* 44, 45, and 46, which corresponds to the ions of the isotopologues ($^{12}C^{16}O_2$), ($^{13}C^{16}O_2$, $^{12}C^{17}O\ ^{16}O$) and ($^{12}C^{18}O^{16}O$, $^{13}C^{17}O^{16}O$, and $^{12}C^{17}O_2$) respectively. Both mass 45 and 46 show mass interference, and a correction must be applied to correct for the contribution by ^{17}O-containing isotopologues [8, 9]. As in our case we know the $\delta^{13}C$ value of the original CO_2 equilibrium gas, we can use the mass-45 signal (of which about 6% stems from $^{12}C^{17}O^{16}O$) to determine the $\delta^{17}O$ value, albeit with less precision.

The classical procedure for δ^2H measurement of water has been off-line reduction of water to hydrogen gas, H_2, at an elevated temperature using a variety of metal surfaces such as hot uranium [10], or zinc [11]. In modern CF-IRMS, applications of this and similar reduction methods ([12-14]) have been miniaturized, and connected on-line to the IRMS. In our lab, we also possess such a CF-IRMS based on Chromium reduction [12]. A water sample (of typically 0.4 µl) is injected into an oven that contains chromium powder heated to ~ 1050 °C, where it is reduced to hydrogen gas.

The hydrogen gas is driven through a gas chromatographic column (GC) to separate the produced H_2 gas from possible minor contaminants, and to produce reproducible peak shapes, which is important for high quality analysis. It is then transferred into the IRMS with helium as a carrier gas. The IRMS is tuned to measure ion currents of *m/z* 2 and 3 which correspond to the ions of the isotopologues $^1H_2$ and $^1H^2H$, respectively. An electrostatic deflector in front of the m/z=3 cup prevents interference by the tail of the overwhelming *m/z* = 4 signal of ionized helium.

Like all (preparation) systems dealing with water, this system suffers from memory effects, and each sample gas has to be measured several times. In order to reduce the memory effect, the

syringe is flushed with the water sample before injecting, the injection block is heated to 140 °C and the oven is replaced regularly. Furthermore, a memory correction algorithm is applied [15].

For several applications, like biomedical doubly labelled water (DLW) studies [16] and in the determination of the so-called deuterium excess parameter used in ice-core research [17], it is a disadvantage to have two entirely separate sample preparation processes for δ^2H and $\delta^{18}O$ measurements. Alternatively, the use of a high temperature pyrolysis of water in combination with a CF-IRMS allows simultaneous δ^2H and $\delta^{18}O$ measurements in water [18]. A water sample of typically 0.12 µl is injected through a heated septum into a glassy carbon reactor at a temperature higher than 1300°C, where it is converted into H_2 and CO gases. The gaseous products are separated by a gas chromatographic column and carried by helium to the IRMS, where first δ^2H is analysed in the produced H_2 gas, and subsequently $\delta^{18}O$ in the produced CO (using a triple collector for m/z 28, 29 and 30). The latter method can deal with very small sample sizes and provides a high throughput; however, the accuracy of the method is lower, especially for $\delta^{18}O$ when compared to the equilibrium technique. Furthermore, but this is generally true for all IRMS applications, the instrumentation is quite bulky and power consuming and cannot be used in the field for performing real-time measurements. The only way to measure water isotopes directly, avoiding the need of chemical sample preparation for each measurement, is the isotope ratio infrared spectrometry (IRIS) method where the isotopic composition is derived from the absorption spectrum of water in the gas phase [19]. Nowadays, the IRIS has been recognized as a valid alternative to the IRMS.

1.4.2 Laser Spectrometry

Optical measurement techniques, in particular laser-based techniques, have been successfully used to determine isotope ratios in numerous applications. An overview of early demonstrations is given in [20, 21]. The near- and mid-infrared spectral region is well suited for isotopic ratio determination, as many small molecules exhibit a characteristic isotope-dependent signature associated with rotational-vibrational transitions in this region. Isotopic substitution affects the frequencies of the rotational motion, as well as the vibrational modes of a molecule, and thus each isotopologue has its own ro-vibrational spectrum. Different isotope ratio infrared spectrometry (IRIS) methods have been developed. The instrument of which the development is described in the next chapter is based on the detection technique known as Optical Feedback

Cavity Enhanced Absorption Spectroscopy (OF-CEAS). However, before describing this technique in next chapter, it is worthwhile to review briefly a few other important techniques here.

1.4.2.1 Direct Absorption Spectroscopy Technique

Direct absorption spectroscopy (DAS) is probably the most straightforward laser-based absorption method. In DAS a light beam is sent through a gas sample and the transmitted intensity is measured. When the light frequency matches an atomic or molecular transition, the light is absorbed and the transmitted intensity decreases. The absorbance $A(\nu)$ can be calculated from the transmitted light $I(\nu)$ and incident light $I_0(\nu)$ intensities by the Lambert-Beer law (see, for example, Demtröder, 1982)[22]:

$$A(\nu) = \ln\left(\frac{I_0(\nu)}{I(\nu)}\right) = \alpha(\nu)L = n\sigma(\nu)L \qquad (4)$$

where $\alpha(\nu) = n\sigma(\nu)$ is called the absorption coefficient of the (isotopic) species with number density n (usually expressed in molecule/cm^3) and the frequency dependent absorption cross section σ(ν) (cm^2/molecule). L is absorption path length (cm). The integrated absorbance can be written as:

$$A = \int A(\nu)d(\nu) = nL\int \sigma(\nu)d\nu = nLS\int g(\nu-\nu_0)d\nu = nLS \qquad (5)$$

with g the normalized line shape function ($\int g(\nu-\nu_0)d\nu = 1$) and S = S(T) the temperature-dependent molecular line absorption intensity (cm/molecule), which depends on the specific ro-vibrational transition that is excited. The HITRAN database tabulates a large number of transitions for most small molecules of interest [23] and allows one to recalculate line intensities at different temperature than the standard temperature of 296 K.

The isotope ratio then can be calculated as:

$$R = \frac{n_r}{n_a} = \frac{A_r}{A_a}.\frac{S_a}{S_r} \qquad (6)$$

where the subscript *r* refers to the rare isotopic species and the subscript *a* refers to the abundant isotopic species. Direct absorption spectroscopy is a simple technique for delivering relative concentrations. However, direct absorption spectroscopy suffers from a low sensitivity. The

minimum absorption that can be measured with the direct absorption technique is typically 10^{-3}, which is not sufficient for many applications, such as in the field of trace gas detection. It can be seen from the Lambert-Beer law (Equation (4)) that an increase in the absorption path length results in a stronger absorption signal. Several techniques have been established to extend the effective absorption path length for achieving high sensitivity. Over the past two decades, particularly powerful detection techniques have been developed that exploit the properties of high finesse optical cavities. Recently, Romanini et al. presented an extensive historical overview and general review of such techniques [24]. Following Romanini et al., the ensemble of such techniques will be referred to as Cavity Enhanced Absorption Spectroscopy (CEAS). However, CEAS will also be used in a narrower sense, when the intracavity absorption is deduced from a measurement of the average light intensity transmitted by the cavity, as opposed to the technique of cavity ring down spectroscopy (CRDS) in which a measurement is made of the time-dependence of the light transmission.

1.4.2.2 Cavity Ring Down Spectroscopy

Figure 1 gives a schematic and simplified representation of CRDS and the detected light intensity at the output mirror, following either pulsed light injection or an abrupt shut-down of continuous wave (CW) injection into the cavity. In a CRDS instrument, light enters the cavity through one of the mirrors and is reflected back and forth inside the cavity, while the light transmitted through the other mirror is recorded by a detector as a function of time. The technique is based on the measurement of the rate by which this transmitted light (which is proportional to the light intensity in the cavity) decreases (either at the end of the laser pulse, or after a fast shut-down of a CW laser). This decrease is exponential, and characterised by a cavity decay rate or ring down time (see below). There are various ways by which the cavity decay rate can be determined from the observed signal at the detector, the simplest being an exponential fit to the light intensity decay transient.

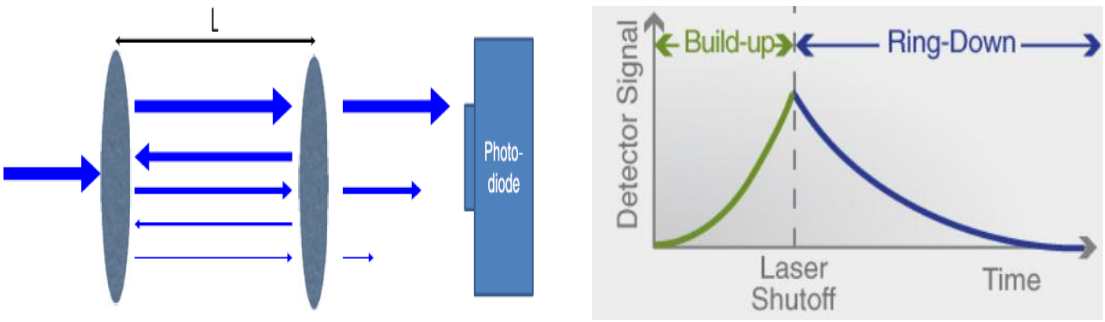

Figure 1. CRDS operation and detected radiation at output mirror (Figure from [25]).

As said, CRDS can be performed with both pulsed and CW lasers, depending strongly on the application. CRDS using a pulsed laser source has a simpler set-up; however, CW lasers made compact cavity ring-down spectrometers possible with a higher sensitivity and higher resolution, as explained later.

The light intensity inside the cavity at each round trip will decay by a factor of R^2 because of mirror transmission T and losses P (T+P=1-R), and a factor of $\exp(-2\sigma(\nu)nd)$ following the Lambert-Beer law due to the absorbing species in the cavity with density n and absorption cross section $\sigma(\nu)$ extending over a length d within the cavity. One finally obtains [24, 26]:

$$I(t) = I_0 e^{-tc\left(\frac{-\ln(R)+n\sigma(\nu)d)}{L}\right.} = I_0 e^{\frac{-t}{\tau(\nu)}} \qquad (7)$$

where τ(ν) is the decay time constant of the cavity or ring-down time. Since the reflectivity of the mirrors $R \approx 1$, one can safely approximate $\ln(R) \approx$ -(1-R). The decay rate is then given by:

$$\frac{1}{\tau(\nu)} = \frac{1}{\tau_0} + c\alpha(\nu)\frac{d}{L} \qquad (8)$$

with $\frac{1}{\tau_0} = c\frac{(1-R)}{L}$ the decay rate for the empty cavity ($\alpha(\nu)$ =n× $\sigma(\nu)$ =0).

The minimum detectable absorption coefficient (limit of detection (LOD) with CRDS in the limit of $\tau \rightarrow \tau_0$, $\alpha_{\min}$, is obtained as follows [26]:

$$\alpha_{\min} = \frac{L}{cd}\left(\frac{1}{\tau_0 - \Delta\tau_{\min}} - \frac{1}{\tau_0}\right) = \frac{L}{cd\tau_0}\frac{\Delta\tau_{\min}}{\tau_0} = \frac{1-R}{d}\frac{\Delta\tau_{\min}}{\tau_0} \qquad (9)$$

where $\Delta\tau_{\min}$ is the minimum measurable difference between τ and τ_0.

The LOD (cm^{-1}) of an absorption measurement as an indicator of instrument performance is often expressed as the noise equivalent absorption (NEA) with units of cm^{-1} $Hz^{-1/2}$; this is defined as the smallest variation of the absorption coefficient one can detect for a single spectral element during a 1-s measurement interval. If *f* spectra are collected in 1 s by fast frequency tuning, it is usual to normalize the limit of detection (LOD) of an experiment by $\frac{1}{\sqrt{f}}$ [24]:

$$\text{NEA} = \frac{1}{\sqrt{f}} \frac{L}{d} \frac{\Delta\tau_{\min}}{c\tau_0^2} \qquad (10)$$

It follows from equation (10) that the spectrometer sensitivity can be optimised by either using very high reflectivity mirrors or by increasing *d*, as well as by minimizing the ratio $\Delta\tau_{min}/\tau_0$ which corresponds to the relative error of the cavity ring-down time measurements. This ratio would be minimum for a single exponential rather than a multi-exponential decay, which can occur for simultaneous excitation of different cavity modes. Multiple longitudinal/ transverse mode excitation, as achieved with pulsed lasers, cause multi-exponential decays; as each mode will have its own characteristic ring down time [26]. NEA sensitivities in the range of 10^{-6}-10^{-9} cm^{-1} $Hz^{-1/2}$ have been demonstrated using simple experimental CRDS systems. Several schemes were developed to optimize cavity injection with a CW laser for achieving higher sensitivity and precision [27-29]. A detection limit of 5×10^{-13} cm^{-1} by averaging spectra over several days has been reported with CW-CRDS [30].

1.4.2.3 Cavity Enhanced Absorption Spectroscopy

Cavity enhanced absorption spectroscopy (CEAS), in the strict sense as defined above, is also known as cavity transmission spectroscopy or integrated cavity output spectroscopy. In this method, the time-averaged transmission through the cavity as a function of frequency is recorded, rather than light intensity decay as in CRDS. Different approaches of CEAS have been reported to optimize the cavity injection [31, 32]. In the next chapter the optical feedback cavity enhanced absorption spectroscopy (OF-CEAS) approach invented by Morville et al. [33, 34] will be presented in detail. This approach has been used for the instrument used and studied in this thesis for simultaneous isotope ratio measurements in enriched waters for biomedical research. OF-CEAS has been used in a variety of applications: trace gas detection [35, 36], isotope ratio analyses [37], aerosol studies [38, 39], and other applications [40, 41].

1.5 Production and Characterization of Stable Isotope labelled water: applications in this thesis

An important part of this thesis deals with waters with artificially increased concentrations of the isotopes ^{2}H, ^{17}O and/or ^{18}O. Those are commonly called "labelled" waters. Preparing labelled waters with accurately known isotopic composition lies at the basis of chapters 4-7 of this thesis. There are, however, two distinctly different fields of application, which I will introduce below.

1.5.1 The Doubly labelled water (DLW) method

The doubly labelled water method is an isotope-based technique for measuring whole-body CO_2 production as an index of total energy expenditure in free-living subjects [16]. The main advantage of this technique over traditional methods for energy expenditure measurement, (such as direct calorimetry (DC) by assessing heat exchange between body and environment and indirect calorimetry (IC) by measuring oxygen consumption and/or carbon dioxide production for a certain time period), is that this technique can be employed without encumbering subjects. The subjects are free to behave normally in their natural environment while the assessment of the energy expenditure is in progress. The technique uses two stable isotopes of water, ^{2}H and ^{18}O, as tracers. It is based on the measurement of the different turnover rates of both isotopes in the body. After administration of a dose of ^{2}H and ^{18}O enriched water, ^{2}H leaves the body as water, whereas ^{18}O leaves the body both as water and CO_2. Therefore, the difference between the two turnover rates is proportional to the CO_2 produced, which in its turn is a direct measure of energy expenditure. The principle of this technique was invented by Lifson and collegues in the early 1950's [42] and was validated in their subsequent studies [43-47]. However, for a long time, this technique was not used widely, and only on small animals due to the high costs of the method; firstly the extremely high costs of the ^{18}O stable isotope and secondly the costs related to the IRMS measurements, which were cumbersome prior to the development of CF-IRMS and laser spectrometry. Advances in the automation, and related to that the better precision and accuracy of the IRMS technology made it economically feasible in large studies [48-50]. Ironically, the large increase of the demand for ^{18}O (used for the production of ^{18}F used in PET scans) initially threatened the use of ^{18}O due to the increasing prices, but gradually led to much larger production of ^{18}O and thus lower prices (as well as a more stable supply situation). The advent of

continuous-flow pyrolysis-IRMS, for simultaneous measure of ^{2}H and ^{18}O, paved the way further for increased sample processing numbers. This technique and, more recently, laser spectroscopy [51-54] are commonly used for isotope analysis of enriched samples. In the next chapter, we report on the development of an infrared laser spectrometer for isotope measurements of enriched waters of the DLW method. As the DLW method uses a wide range of isotopic enrichments, an extensive set of well-characterized enriched reference waters over the whole range of enrichment is required. IAEA had two reasonably characterized reference waters available, but these are exhausted. In chapter 4, we report on our production of international reference waters with unprecedented quality for the calibration of isotope measurements of DLW-related samples.

1.5.2 Triple point of water (TPW)

The triple point of water is the temperature at which all the three phases of water (solid-liquid-vapour) coexist in thermodynamic equilibrium. The kelvin, the unit of the thermodynamic temperature, is defined as the 1/273.16 fraction of the TPW temperature. The TPW is also one of the most important defined fixed-points of the International Temperature Scale of 1990 (ITS-90) [55] for calibration of platinum resistance thermometers in the range of 13.8033-1234.93 K. The triple point of water can be realized in a so-called triple point cell, which is loaded with pure water in high vacuum, then frozen to well below the triple point temperature, and then gradually thawed until the triple point is spontaneously reached. Just like all natural water, the fresh waters with which triple point cells are filled show natural variability in their isotopic composition. The influence of these variations on the triple point temperature of water exceeds the state-of-the-art precision of the realization of the TPW. This effect was first identified in 1996 [56], thereafter several studies have been performed to quantify this effect [57-59], such that corrections could be applied. However, the accuracy and reliability of the correction was still not satisfactory. Therefore, we carried out a detailed experimental investigation of the TPW dependence on both the hydrogen and oxygen isotopic composition, using carefully prepared labelled waters with different isotopic compositions. The Dutch Metrology institute, the NMi-VSL (Nederlands Meetinstituut- van Swinden Laboratorium) manufactured triple point cells with the above-mentioned water samples and the corresponding triple point temperatures were measured. The isotopic measurements were performed using the laser spectrometer developed in chapter 2 and

IRMS techniques explained in this chapter. The analysis of the results led to the accurate determination of the coefficients that express the dependence of the triple point temperature on the isotope abundances. We deal with this subject in detail in Chapter 6 and 7.

1.6 Outline of the thesis

This thesis consists of eight chapters. Following this chapter 1 with the general introduction and background, chapter 2-7 present the main results of the thesis. Chapter 2 reports on the development of a prototype gas analyzer based on Optical Feedback Cavity Enhanced Absorption Spectroscopy (OF-CEAS) for more sensitive and precise measurement of the $^{2}H/^{1}H$, $^{17}O/^{16}O$, and $^{18}O/^{16}O$ isotope ratios in enriched waters. In chapter 3, we characterize the OF-CEAS liquid phase spectrometer and show preliminary results. Chapter 4 details production and certification of a new set of singly (^{2}H) and doubly (^{2}H and ^{18}O) stable isotope labelled reference waters for biomedical and other isotope-labelled research. The prepared reference waters are now distributed by the International Atomic Energy Agency (IAEA) as international reference materials (IAEA-604 to IAEA-609). These supplies are estimated to last for over 15 years.

In Chapter 5, we present a thoroughly validated spreadsheet for calculating isotopic abundances (^{2}H, ^{17}O, ^{18}O) for mixtures of waters with different isotopic compositions. We developed this calculation tool connected to the work described in chapter 4. The calculation procedure, validation and some applications of the spreadsheet are described.

Chapter 6 and 7 cover the dependence of the tripe point of water on the isotopic composition of the water. Here we benefitted from our own set of enriched reference waters. This work presents a complete, detailed, and accurate experimental study on this subject. The result of this research is an improved realization of the triple point of water, the international temperature standard and basis of the SI definition of the kelvin.

Finally, chapter 8 gives the conclusions of this work and recommendations for future research.

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