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Oxygen Isotope Analysis of Cellulose: An Interlaboratory Comparison

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Oxygen isotope analysis of tree ring cellulose is hindered by the lack of a fast and reliable technique to measure $\delta^{18}\text{O}$ at natural abundance levels. Most of the existing off-line techniques are not conducive to rapid generation of oxygen isotope data. Further, a comparison between oxygen isotope values of a cellulose sample measured using the different off-line techniques resulted in a discrepancy of 1.3‰. This discrepancy is a result of inconsistencies inherent to these techniques and the lack of a reliable cellulose oxygen isotope standard. We addressed these problems by analyzing a number of cellulose samples by three different analytical procedures. These include two relatively well established off-line methods (pyrolysis in nickel tubes and decomposition using mercury(II) chloride) and a recently proposed on-line method based on pyrolysis in the presence of glassy carbon in an elemental analyzer and the subsequent isotope analysis of carbon monoxide. We were successful in improving the reproducibility of the on-line method ($\sigma_{n-1} \approx 0.2\text{‰}$) to the extent that natural variations can be resolved and found a good agreement between the on-line and off-line methods, although the cellulose samples extracted from tree rings are subject to more scatter than the commercial cellulose samples. The on-line method may even allow determination of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of a cellulose sample in a single run (from 29/28 and 30/28 ratios of CO).

Variations of the oxygen isotope ratio in natural systems are widely used as a proxy indicator of past climatic and environmental conditions. The method is well established for water stored in ice sheets and carbonates in marine sediments,¹ as well as for cellulose extracted from tree rings.^{2–5} Nevertheless, the potential of oxygen isotope analysis of tree rings has not yet been fully

exploited, and this field is currently regarded as a very promising area of climatic research.⁶ Tree rings can provide climatic information in continental areas at an annual resolution—information which otherwise might be difficult to assess.⁷ A reason for the limited application of $\delta^{18}\text{O}$ in tree rings is the tedious nature of the techniques involved. Unlike carbon isotope analysis, there is no generally accepted and easy method for oxygen isotope analysis in cellulose,⁸ and the existing off-line methods appear to be “operator dependent”.⁹ This is reflected by the fact that the measurement of a proposed standard cellulose by different laboratories yielded a range of 1.3‰,¹⁰ whereas the natural variations should be resolved at a level of about 0.2‰ (although some of this range may be due to sample inhomogeneity). Recently, attempts have been made to establish an on-line method for the determination of the oxygen isotope ratio in organic matter.^{11–13} The existence of a rapid on-line method would, no doubt, enhance the application of oxygen isotope analysis of tree rings and would allow us to better determine the climate–isotope relationship through the generation of long time series of $\delta^{18}\text{O}$.

The technique by Werner et al.¹¹ is based on the pyrolysis of the organic material in the presence of glassy carbon with the isotope ratio determination on the carbon monoxide evolved. This on-line method makes use of an elemental analyzer coupled to an isotope ratio mass spectrometer, thereby using a configuration which is already in operation in many laboratories for routine carbon and nitrogen isotope analysis. Using glassy carbon instead of graphite or diamonds reduces memory and blank problems which are usually associated with the pyrolysis in quartz tubes and which has, in the past, made the Unterzaucher procedure difficult to use for the determination of oxygen-18.¹⁴ In this study,

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we evaluate the procedure proposed by Werner et al. to find out whether the reproducibility for the $\delta^{18}\text{O}$ measurement of cellulose can be improved from 0.6‰. We selected a range of commercial cellulose samples as well as cellulose extracted from tree rings, because differing structures or impurities may influence the results. Further, we compare the results of the on-line method with those of the two most commonly used off-line methods: the nickel tube method⁷ and the mercury(II) chloride method.¹⁵

EXPERIMENTAL SECTION

Isotope Analysis. On-Line Method. An elemental analyzer (Carlo Erba 1108, Milan, Italy) is linked to an isotope ratio mass spectrometer (Delta-S, Finnigan MAT, Bremen, Germany) via an open split interface (Conflo II, Finnigan MAT). The quartz pyrolysis tube in the elemental analyzer is filled to half of its length with glassy carbon (Sigradur G; 3150–4000 μm grit; HTW GmbH, Thierhaupten, Germany) and is kept at a temperature of 1080 °C. It is not recommended to use a smaller grain size of the glassy carbon, as this might increase the retention time of the gases and reduce the overall precision.¹⁶ The samples (~1.5 mg) are loaded in tin capsules and dropped into the pyrolysis furnace by an autosampler (without addition of oxygen). After the pyrolysis, the oxygen in the sample is mainly found in the form of CO (less than 5% in the form of CO_2). The resultant gases are swept by the helium carrier gas (99.9999% pure; flow rate 80 mL/min) through a water trap (magnesium perchlorate), a CO_2 trap (Elemental Microanalysis, Devon, U.K.), and a separation column (Poropak QS) at 40 °C and are then allowed to enter the mass spectrometer, where the 29/28 and 30/28 ratios of the carbon monoxide are measured. Care has to be taken that no traces of N_2 (e.g., from a leak) interfere with the measurement of the CO sample peak. The CO_2 trap prevents the formation of CO^+ ions from dissociation of CO_2 in the ion source. A relatively long period (1200 s) should be allowed between consecutive samples, because impurities may evolve slowly from the column. The first two or three samples in a series of measurements usually had too low 30/28 values and were discarded.

The isotope ratios are calculated from the time integrals of the peak areas of the ion intensities m/z 30 and 28 (i.e., $^{12}\text{C}^{18}\text{O}$ and $^{12}\text{C}^{16}\text{O}$). For every sample, the 30/28 ratio is related to the 30/28 ratio of a CO standard gas which is introduced to the mass spectrometer about 30 s before the pyrolysis of the sample. Whereas the sample gas flows into the ion source through the capillary from the Conflo interface, the standard gas is introduced via a capillary from the dual inlet. The isotope ratio of the CO standard gas (99.97% pure; Linde, Unterschleissheim, Germany) was found to be close to that of the CO from the pyrolysis of the cellulose samples (see Figure 1).

Safety Considerations: Please note that CO is toxic and should be handled accordingly!

Isotope ratios are defined as $\delta^{18}\text{O}_{\text{sample}} = [(^{18}\text{O}/^{16}\text{O}_{\text{sample}})/(^{18}\text{O}/^{16}\text{O}_{\text{VSMOW}}) - 1] \times 1000$. The calibration vs VSMOW is hampered by the absence of an international CO standard; thus, the on-line method must be cross-calibrated against a method that generates CO_2 . For this purpose, a standard cellulose is used (Merck cellulose), the $\delta^{18}\text{O}$ of which has been determined on CO_2

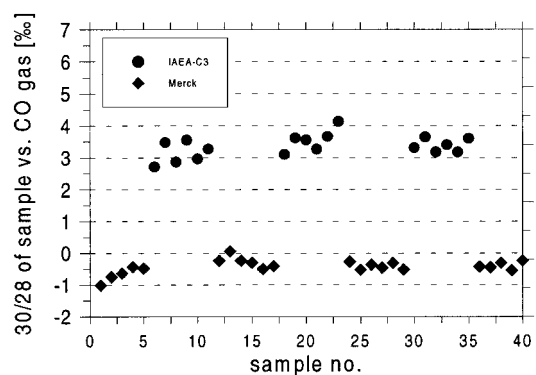


Figure 1. 30/28 ratio of CO produced from Merck cellulose and IAEA-C3 cellulose calculated vs the 30/28 ratio of the CO reference gas, i.e., $[(30/28_{\text{sample}})/(30/28_{\text{CO-gas}}) - 1] \times 1000$ (‰). Forty consecutive measurements were done, where the analysis of one sample takes 20 min.

produced by the nickel pyrolysis method. The calibration of the CO_2 (vs VPDB) is based on CO_2 obtained from the calcite reference material NBS-19 by treatment with 95% phosphoric acid at 50 °C. The values relative to VPDB are converted to VSMOW using the expression $\delta_{\text{VSMOW}} = 1.0415\delta_{\text{VPDB-}\text{CO}_2} + 41.5$ (where $\delta_{\text{VPDB-}\text{CO}_2}$ is the $\delta^{18}\text{O}$ of CO_2 obtained from VPDB).¹⁷ The CO standard gas is measured against the Merck cellulose, and, from this difference, the oxygen isotopic composition of the CO standard gas can be calculated. The calibration has to be checked regularly, as the difference in $\delta^{18}\text{O}$ between standard CO gas and cellulose is not a constant but depends on system parameters of the elemental analyzer (e.g., the carrier gas flow rate, the age of the catalyst in the pyrolysis tube etc.). The influence of the interfering isotope ^{17}O is discussed below in the section Isotopic Corrections for CO. No blank correction was applied because the amount of CO produced by the pyrolysis of empty tin capsules was small (less than 0.1% compared to the CO produced by a sample of 1.5 mg). Accordingly, no improvement of the reproducibility was achieved by the use of silver capsules.

Principally, the 29/28 mass ratio of the CO produced should reflect the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample (provided that the carbon in the CO is originating from the sample and not from the glassy carbon). To evaluate the possibility to determine $\delta^{13}\text{C}$ from the 29/28 ratio of CO, we remeasured samples that had previously been analyzed with a conventional off-line technique for $\delta^{13}\text{C}$ (combustion in sealed and evacuated quartz tubes with excess copper(II) oxide).¹⁸

Nickel Tube Pyrolysis. This method is based on the observation that hydrogen diffuses rapidly in nickel at temperatures above ~1000 °C.¹⁹ Therefore, the pyrolysis of cellulose in a nickel tube yields only CO and CO_2 , as all the hydrogen has diffused through the nickel walls. In a second step, CO is usually converted to CO_2 , either by disproportionation in a high-voltage electric discharge or by catalytic conversion on nickel powder at 400 °C.²⁰ Edwards et al.²¹ found that the disproportionation step can be

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omitted because the minimal fractionation caused by CO oxygen can be corrected for. The oxygen isotope ratio is determined on the CO₂. We used a technique similar to that of Brenninkmeijer and Mook²⁰ and obtained a standard deviation of 0.2‰ for replicate analysis of standard cellulose. For details of the method, see ref 7.

Mercury(II) Chloride Method. In this method, cellulose is pyrolyzed with excess mercury(II) chloride. The high temperature causes the mercury(II) chloride to dissociate into mercury and chlorine. The cellulose is oxidized by the chlorine, with the mercury acting as the catalyst. The oxygen may be found in both carbon monoxide and carbon dioxide. The hydrogen chloride evolved prevents oxygen from being removed as water but must be separated from the other gases, because its presence adversely affects the isotope ratio measurement. This was originally done with an organic base such as quinoline or 5,6-benzoquinoline.²² However, as these compounds are toxic, they have been replaced with zinc²³ or a macroreticular ion-exchange resin such as Amberlyst A-21.¹⁵ If carbon monoxide is present, fractionation may occur. Carbon monoxide may be quantitatively converted to carbon dioxide by means of a high-voltage electric discharge.²⁴ This approach is adopted here, with Amberlyst A-21 used to absorb the hydrogen chloride.¹⁵ $\delta^{18}\text{O}$ values were determined on the combined carbon dioxide, and the precision for a standard cellulose was better than $\pm 0.2\text{‰}$.

Isotopic Corrections for CO. The isotopic corrections necessary to deduce isotope ratios from the measurement of CO₂ are well known.²⁵ In an analogous way, we present equations which allow the determination of $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios from the measurement of CO. This correction is important mainly for $\delta^{13}\text{C}$.

The isotope ratios of a sample are defined as $R^{13} = ^{13}\text{C}/^{12}\text{C}$, $R^{17} = ^{17}\text{O}/^{16}\text{O}$, and $R^{18} = ^{18}\text{O}/^{16}\text{O}$, and S^{13} , S^{17} , etc. for a standard. For $m/z = 29$ and 30 , we have

$$R^{29} = \frac{^{13}\text{C}^{16}\text{O} + ^{12}\text{C}^{17}\text{O}}{^{12}\text{C}^{16}\text{O}} = R^{13} + R^{17} \quad (1)$$

and

$$R^{30} = \frac{^{12}\text{C}^{18}\text{O} + ^{13}\text{C}^{17}\text{O}}{^{12}\text{C}^{16}\text{O}} = R^{18} + R^{13}R^{17} \quad (2)$$

and the respective δ values are given by $\delta^x = R^x/S^x - 1$, with $x = 13, 17, 18, 29$, and 30 (to simplify the calculations, we omit the 1000 factor in the δ definition in this section). To calculate δ^{13} and δ^{18} from the measured δ^{29} and δ^{30} , the ratios of the (working) standard S^{13} , S^{17} , and S^{18} have to be known, and an assumption has to be made for the relationship between R^{17} and R^{18} . This relationship is usually taken as²⁵

$$(R^{17}/S^{17})^2 = R^{18}/S^{18}$$

which leads to

$$2\delta^{17} \approx \delta^{18} \quad (3)$$

From eq 1 and the definition of δ , we find $(\delta^{29} + 1)S^{29} = (\delta^{13} + 1)S^{13} + (\delta^{17} + 1)S^{17}$. This reduces to $\delta^{29}S^{29} = \delta^{13}S^{13} + \delta^{17}S^{17}$ and results in $\delta^{13} = (S^{29}/S^{13})\delta^{29} - (S^{17}/S^{13})\delta^{17}$. Substituting δ^{17} using eq 3 and taking $\delta^{18} \approx \delta^{30}$ in the correction term, we get

$$\delta^{13} \approx (S^{29}/S^{13})\delta^{29} - (S^{17}/2S^{13})\delta^{30} \quad (4)$$

As a numerical example, we insert the standard ratios of VPDB-CO₂ ($S^{13} = 0.011\,2372$, $S^{17} = 0.000\,3800$, $S^{18} = 0.002\,0790$): $\delta^{13} \approx 1.0338\delta^{29} - 0.0169\delta^{30}$. Equation 4 is an approximation only but is accurate within 0.01‰ when sample and standard do not differ by more than 50‰.

To calculate δ^{18} , we start with $(\delta^{30} + 1)S^{30} = (\delta^{18} + 1)S^{18} + (\delta^{13} + 1)S^{13}(\delta^{17} + 1)S^{17}$, which leads to $\delta^{18} = (S^{30}/S^{18})\delta^{30} - (S^{13}S^{17}/S^{18})\delta^{13} - (S^{13}S^{17}/S^{18})(1 + \delta^{13})\delta^{17}$. The term with $\delta^{13}\delta^{17}$ is neglected and δ^{13} is substituted by eq 4, which results in $\delta^{18} \approx (S^{30}/S^{18})\delta^{30} - (S^{17}S^{29}/S^{18})\delta^{29} - (S^{13}S^{17}/S^{18})(1 + S^{17}/S^{13})\delta^{17}$. We take $(1 + S^{17}/S^{13}) \approx 1$, use $2\delta^{17} \approx \delta^{18}$ and $S^{30} = S^{18} + S^{13}S^{17}$, and finally get

$$\delta^{18} \approx \left(1 + \frac{S^{13}S^{17}}{2S^{18}}\right)\delta^{30} - \frac{S^{17}S^{29}}{S^{18}}\delta^{29} \quad (5)$$

The respective correction factors are rather small: $\delta^{18} \approx 1.0010\delta^{30} - 0.0021\delta^{29}$. This means that, by simply taking $\delta^{18} \approx \delta^{30}$ (i.e., not applying any correction), the error is not more than about 0.1‰ for natural abundance samples.

Materials. We have selected several types of cellulose samples which differ in their oxygen isotope ratio and which are either obtained from commercial suppliers or extracted from tree rings. The commercial samples may differ from the manually extracted samples mainly regarding the purity, the degree of polymerization, and the hygroscopic properties. The commercial samples include IAEA-C3 cellulose,¹⁰ Merck cellulose (cellulose native no. 2351, grain size $< 20\,\mu\text{m}$, Merck, Darmstadt, Germany), Sigma α -cellulose (approximately 99.5% pure, reference 92 F 0243, Sigma Chemical Co., Dorset, England), and Whatman CF-11 (a fibrous purified cellulose, Whatman Chemical Separation Ltd., Maidstone, England).²⁶ The samples extracted from tree rings include the following:

(i) α -cellulose samples extracted from tree rings of beech (*Fagus sylvatica* L.),⁷ referred to as "tree rings PSI". The samples were milled, and the cellulose was extracted in the following steps: Soxhlet extraction with toluene-ethanol to remove soluble constituents, and delignification by repeated treatment with sodium chlorite at 70 °C, which yields holocellulose (a mixture of polysaccharides, comprising hemicellulose and cellulose). The cellulose is obtained by dissolution of the hemicellulose in 4% sodium hydroxide at 70 °C.²⁶

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Table 1. Mean $\delta^{18}\text{O}$ Values (‰ vs VSMOW) of the Different Cellulose Samples (Mean $\pm \sigma_{n-1}$; n = Number of Measurements) As Determined with Three Techniques^a

sample	method		
	mercury(II) chloride	nickel tube	on-line
IAEA-C3	32.14 \pm 0.20 (n = 6)	32.20 \pm 0.11 (n = 7)	32.52 \pm 0.33 (n = 21)
Merck	28.63 \pm 0.30 (n = 8)	28.67 \pm 0.19 (n = 25)	(28.67) \pm 0.22 (n = 54)
Sigma	27.04 \pm 0.14 (n = 10)		27.54 \pm 0.16 (n = 6)
Whatman CF-11	26.70 \pm 0.19 (n = 6)		27.57 \pm 0.28 (n = 6)
Tucson	34.15 \pm 0.35 (n = 6)		33.83 \pm 0.51 (n = 6)

^a For calibration purposes, the mean on-line value of the Merck cellulose is set to equal the value determined by the nickel tube method. The value for the IAEA-C3 material determined with the mercury(II) chloride method in the Cambridge Laboratory differs from the value determined earlier from a different sheet of the IAEA-C3 material (32.7 \pm 0.13).¹⁰

(ii) α -cellulose samples extracted from the tree rings of oak (*Quercus robur* L.), referred to as "tree rings Cambridge".²⁷ The cellulose was extracted in the following steps: a 12-mm-diameter core was cut into slivers approximately 40 μm thick. The latewood samples were placed into individual Soxhlet extraction thimbles. These thimbles were transferred to a beaker and placed in an ultrasonic bath. Delignification was achieved by repeated treatment with acidified sodium chlorite at 70 °C. The α -cellulose was obtained by dissolution of the hemicelluloses in 10% (w/v) sodium hydroxide at approximately 80 °C, followed by 17% (w/v) sodium hydroxide at room temperature.²⁸

(iii) Holocellulose extracted from a large branch of a eucalyptus tree (*Eucalyptus polyanthemus* Schauer), referred to as "Tucson holocellulose". The sample was ground to 20 mesh in a Wiley mill. The resins were removed with a 12-h Soxhlet extraction using a 2:1 toluene–ethanol azeotrope, followed by a 12-h Soxhlet extraction with ethanol. Delignification was achieved by repeated treatment with acidified sodium chlorite at 70 °C.²⁹

RESULTS

Oxygen Isotope Analysis. In Figure 1, a series of measurements of the Merck and the IAEA-C3 cellulose samples is shown. The results are given as raw data, i.e., no correction and no calibration was applied. Each data point represents the deviation of the 30/28 ratio of the cellulose data peak from the CO standard gas peak in per mil (i.e., δ values with the CO standard gas set to zero). While the first two or three samples are affected by an increasing trend of about 0.5‰, the standard deviation is good for the subsequent samples (0.2–0.3‰). The increasing trend at the beginning of the series points to a conditioning of the glassy carbon or the separation column taking place and may also be related to the small memory effect (see below). The Merck cellulose is about 0.5‰ more depleted than the CO standard gas, whereas the IAEA-C3 is about 3.5‰ enriched. The difference between the two cellulose materials is the same as the difference measured with the nickel tube and the mercury(II) chloride methods (Table 1). This result clearly indicates that the 30/28 ratio of the CO produced during the pyrolysis is, indeed, reflecting the $^{18}\text{O}/^{16}\text{O}$ ratio of the cellulose and, thus, its $\delta^{18}\text{O}$ value.

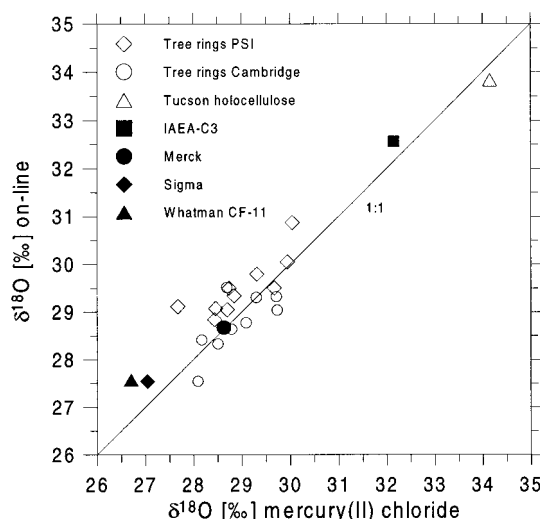


Figure 2. $\delta^{18}\text{O}$ values of the different cellulose samples determined with the on-line method, plotted against the values determined with the mercury(II) chloride method. The filled symbols represent the commercial cellulose samples, whereas the open symbols show the values of the samples extracted from tree rings. The solid line is the 1:1 line (for a perfect match between the two methods all data points should be on this line).

Additionally, the results of Figure 1 show that the memory effect between samples is small for the range of values investigated here (4‰ difference between samples). As the standard deviation for repeat analyses is about 0.3‰, the detection limit for the memory effect is about 7.5% ($=0.3\text{‰}/4\text{‰}$). The mean of the first IAEA sample of each series is $3.04\text{‰} \pm 0.30\text{‰}$ (n = 3), whereas the mean of the last three measurements in each series is $3.44\text{‰} \pm 0.34\text{‰}$ (n = 9). The difference between the first sample analyzed and the last three measurements was found to be insignificant ($p > 0.05$) for both IAEA and Merck samples. Yet, we cannot exclude that, for samples with larger isotopic differences ($> 4\text{‰}$), the memory could exceed the analytical uncertainty.

We selected a range of commercial cellulose samples and cellulose extracted from tree rings and measured the oxygen isotope composition of these samples with both the on-line and the mercury(II) chloride methods (Figure 2). When all the data are pooled, the correlation between the results of the two methods is highly significant ($r^2 = 0.83$; $p < 0.001$), and the slope is 0.85 ± 0.08 (whereby we have defined the mercury(II) chloride method as the reference method). That is, the slope appears to be slightly below unity. The $\delta^{18}\text{O}$ value for the IAEA-C3 cellulose is $32.14\text{‰} (\pm 0.20\text{‰})$ measured with the mercury(II) chloride method and

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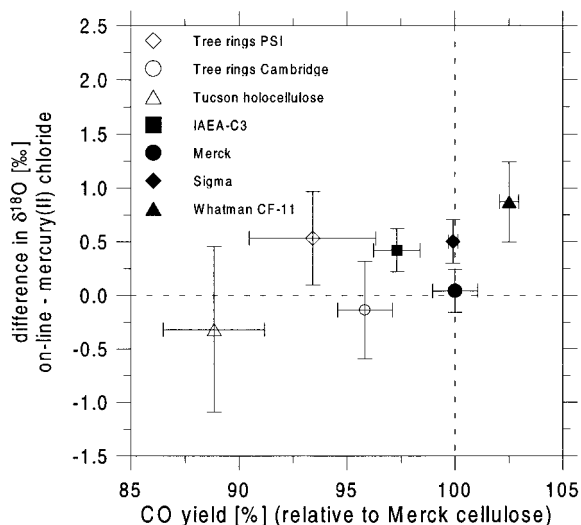


Figure 3. Difference in $\delta^{18}\text{O}$ between the on-line method and the mercury(II) chloride method plotted versus the CO yield of the on-line method (for the definition of the CO yield, see text). The CO yield of the Merck cellulose is set to 100%.

32.52‰ ($\pm 0.33\%$) measured with the on-line method. Both values (as well as the value determined with the nickel tube method, Table 1) are in the range of values reported in Buhay et al.¹⁰ for the IAEA-C3 material (31.3‰–32.7‰). Further, the deviations from the 1:1 line are greater for the samples extracted from tree rings. This corresponds to the finding that the standard deviation for the repeat analysis of the Tucson cellulose (which is also manually extracted) is significantly higher than the standard deviation for the IAEA-C3 cellulose ($\sigma_{\text{Tucson}} = 0.51\%$, $n = 6$; $\sigma_{\text{IAEA-C3}} = 0.33\%$, $n = 21$; $p < 0.05$ according to *F*-test) when measured with the on-line method (see a summary of the standard deviations for the different materials in Table 1). The standard deviation for the Tucson cellulose is also higher compared to that for the IAEA-C3 when determined with the mercury(II) chloride method, but the difference is not significant ($\sigma_{\text{Tucson}} = 0.35\%$, $n = 6$; $\sigma_{\text{IAEA-C3}} = 0.20\%$, $n = 6$; $p = 0.12$ according to *F*-test).

There are several potential reasons for the differential behavior of commercial cellulose and cellulose extracted from tree rings when measured with the on-line method:

(i) The tree ring samples might not be homogeneous; indeed, relatively large fibers are found in these samples.

(ii) Impurities in the tree ring samples could affect the isotope ratio measurement. We suspect that impurities could reduce the CO yield, as we found that the CO yield tends to be lower for the tree ring samples. The CO yield is defined as the ratio of the peak area of the masses 28 + 30 to the sample weight, given as a percentage of the CO yield of the Merck cellulose (which is arbitrarily defined as 100%). The CO yield cannot easily be determined absolutely because the split ratio in the Conflo interface is not known exactly. The CO yield, defined in such a way, appears to have a small influence on the difference in $\delta^{18}\text{O}$ values determined with either the on-line or the mercury(II) chloride method, as can be seen from Figure 3. However, the correlation between the CO yield and the difference between the methods (using the mean values from Figure 3) is not significant according to *F*-test ($r^2 = 0.43$; $p > 0.05$). Moreover, we found that there is a small amount of nitrogen in the samples (deter-

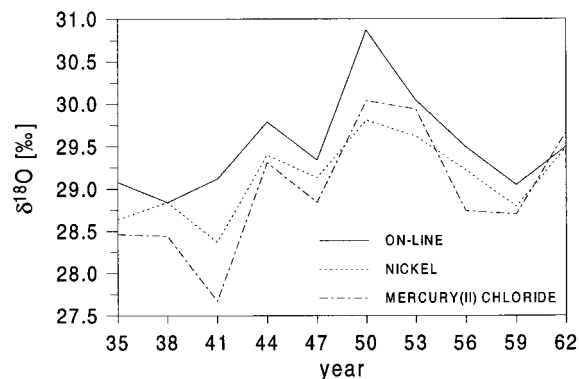


Figure 4. Tree ring sequence measured with (1) the on-line, (2) the mercury(II) chloride and (3) the nickel methods.

mined with the elemental analyzer set to C/N analysis). Thus, N_2 produced during pyrolysis from these samples could interfere with isotope ratio measurement on CO. A particularly high nitrogen content was found for the Tucson holocellulose ($0.027\% \pm 0.001\%$), whereas the other samples are in the range from 0.009% (Merck cellulose) to 0.018% (Sigma α -cellulose). Nevertheless, there is no direct relation between the N content and the deviation between the on-line and the mercury(II) chloride methods. The use of a GC column that separates N_2 and CO (e.g., molecular sieves, 5 Å) probably would reduce problems associated with impurities in the sample.¹¹

(iii) The water content of the samples could affect the measurement. During the off-line method, water is removed prior to the analysis because the sample is in a vacuum system. On the other hand, it is difficult to remove the water in the on-line method. Cellulose is hygroscopic and takes up moisture within seconds after drying when exposed to air. For practical reasons, we decided to measure all samples in equilibrium with air moisture. The water problem is somewhat reduced for our on-line system (pyrolysis with glassy carbon) because the glassy carbon does not act as a major carbon source (in contrast to other types of pyrolysis using elemental carbon),^{12,13} and, therefore, the oxygen of water is not significantly transferred into CO. We confirmed this by the pyrolysis of water, which gave minor CO yields. The fact that the 29/28 ratio of CO can be used to infer $\delta^{13}\text{C}$ values of the cellulose (see below) is another indication that the glassy carbon is quite inert. Nevertheless, the $\delta^{18}\text{O}$ value of oven-dried Merck cellulose was $29.13\% \pm 0.33\%$ ($n = 7$), which is significantly higher ($p < 0.01$) than the value of $28.67\% \pm 0.22\%$ ($n = 54$) determined from Merck cellulose in equilibrium with air moisture. This shift is consistent with the expectation that the low $\delta^{18}\text{O}$ values of water compared to organic matter should result in an underestimation of $\delta^{18}\text{O}$ when measuring moist cellulose. We did not correct our results for this difference because samples and standard were treated equally. Yet, water is, indeed, a serious problem for the on-line methods. This interference could be more thoroughly evaluated by equilibrating cellulose with isotopically labeled water.

Finally, we measured a tree ring series obtained from cellulose extracted from tree rings with the on-line, the mercury(II) chloride, and the nickel tube methods (Figure 4). The results obtained with the on-line and the nickel tube methods are not completely independent in as far as we have used the $\delta^{18}\text{O}$ value

Table 2. Regression Analysis for the Crosswise Correlation between Tree Ring $\delta^{18}\text{O}$ Values from Figure 4 Determined by the Three Methods^a

	nickel tube			on-line		
	r^2	slope $\pm \sigma_{\text{em}}$	F	r^2	slope $\pm \sigma_{\text{em}}$	F
mercury(II) chloride	0.93	0.59 ± 0.06	100.6	0.66	0.65 ± 0.16	15.8
nickel tube				0.72	1.10 ± 0.24	20.4

^a The correlation coefficients (r^2), the slopes of the regression lines (\pm standard error of the estimate), and the F -test values are indicated. All correlations are significant ($p < 0.01$).

of the Merck cellulose determined by the nickel tube method as a calibration standard for the on-line method. As this corresponds to a one-point calibration, we nevertheless can compare the trends of the two methods. However, the values from the mercury(II) chloride method are completely independent. The general trends in Figure 4 are similar for all three methods, whereas the values from the on-line method appear to have a systematic offset. The correlation between the nickel tube and the mercury(II) chloride methods is very good ($r^2 = 0.93$; Table 2). The correlation coefficients between the values of the nickel tube and the mercury(II) chloride methods with values of the on-line method are $r^2 = 0.72$ and $r^2 = 0.66$, respectively. Obviously, the on-line method is introducing more scatter, but all the correlations are highly significant ($p < 0.01$).

The sensitivity of the nickel tube method appears to be smaller compared to that of the mercury(II) chloride method: the slope of the respective correlation is 0.59 ± 0.06 and, thus, significantly below unity. This means that, for this type of samples, the nickel tube method will yield only about half the difference in $\delta^{18}\text{O}$ between two samples compared to the mercury(II) chloride method! A possible explanation could be that the measurements with the nickel tube method are affected by a memory effect. Indeed, a reexamination of standard measurements indicated that oxygen deposited on the nickel tube walls from the previous sample can influence the $\delta^{18}\text{O}$ value of the subsequent analysis. On the other hand, the sensitivities of the on-line and the nickel tube methods are similar (the respective slope is 1.10 ± 0.24).

Carbon Isotope Analysis. The 29/28 ratio of CO produced during the pyrolysis should reflect the $^{13}\text{C}/^{12}\text{C}$ ratio of the cellulose, provided that no carbon from the catalyst is introduced. We found that the 29/28 ratio of replicate analysis of a standard cellulose is strongly dependent on sample size. This points to a fractionation taking place during pyrolysis. Nevertheless, the correlation between sample weight and 29/28 ratio usually was very high ($r^2 > 0.95$). Accordingly, when the 29/28 values are linearly corrected to an arbitrarily defined constant weight, the corrected $\delta^{13}\text{C}$ values have a small standard deviation ($\approx 0.1\text{‰}$). This encouraged us to remeasure a number of tree ring samples which had been analyzed before with a conventional off-line technique.¹⁸ The 29/28 ratios of the samples are corrected to constant weight using the relationship found for the (Merck) standard cellulose, and the results are shown in Figure 5. There is a good agreement between the values determined by the two methods ($r^2 = 0.98$), but the slope is significantly below unity (0.89 ± 0.02). We measured the $\delta^{13}\text{C}$ value of the glassy carbon

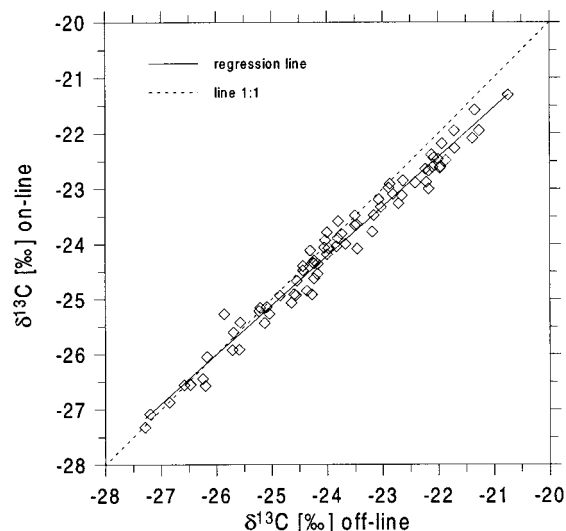


Figure 5. $\delta^{13}\text{C}$ values of tree ring cellulose samples measured with the on-line method (i.e., inferred from the 29/28 ratio of CO) plotted against the $\delta^{13}\text{C}$ values as determined with an off-line method on CO_2 (combustion in sealed quartz tubes). The solid line is the regression line, and the dotted line is the 1:1 line.

by combustion (with the elemental analyzer set for C analysis). The carbon yield was only 6.5%, which, again, shows that the glassy carbon is quite inert. The $\delta^{13}\text{C}$ value of $-30.3 \pm 0.2\text{‰}$ for the glassy carbon is depleted compared to the range of values for the samples (-21‰ to -27‰). A contribution of this isotopically distinct carbon source (e.g., by a reaction of CO_2 with glassy carbon to produce CO) could be the cause of the reduced sensitivity, i.e., the slope being smaller than 1.

CONCLUSIONS

The pyrolysis with glassy carbon in an elemental analyzer, as presented in this study, provides a fast and reliable technique to measure $\delta^{18}\text{O}$ variations of tree ring cellulose at the natural abundance level. Possibly, this on-line method might even allow us to determine the carbon and oxygen isotope ratio of a sample in a single run. For $\delta^{18}\text{O}$, the comparison of every sample CO peak to a standard CO gas introduced by the dual-inlet system may be the main reason for the improvement of the reproducibility from 0.6‰, reported by Werner et al.,¹¹ to approximately 0.2‰. Further, the use of very pure helium carrier gas may be important. We find glassy carbon to provide a good catalytic surface for the thermal degradation of cellulose. Also, when the CO yield is not 100%—because some CO_2 is produced—the results for the determination of $\delta^{18}\text{O}$ on CO are reliable. The conversion step from CO to CO_2 , as often recommended,²⁶ appears not to be necessary. A disadvantage of the on-line method presented here may be that it cannot be used to determine the $\delta^{18}\text{O}$ of both organic material and water, in contrast to other techniques.^{12,13,30} Yet, the simplicity of the proposed on-line method does compensate well for this disadvantage.

We consider it as the minimum requirement for testing a new technique that two isotopically distinct materials with well-established isotopic composition be measured. This requirement

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is not so easy to fulfill (and was often neglected in the past) due to the lack of standard materials for $\delta^{18}\text{O}$. We find a good agreement between the on-line and the off-line methods, although the cellulose extracted from tree rings may produce more scatter in the $\delta^{18}\text{O}$ results than the commercial cellulose. We recommend measuring every sample twice with the on-line method—the analysis time is still less than a single measurement with an off-line method, and smaller samples are required for analysis. The two well-accepted off-line methods (pyrolysis with mercury(II) chloride and nickel tube pyrolysis) show a different sensitivity in $\delta^{18}\text{O}$ for a number of tree ring samples (though the correlation between the methods is very high). This makes it clear that there is an urgent need for interlaboratory comparison runs and that the work initiated by the IAEA¹⁰ should be continued and expanded. This should result in the establishment of at least two isotopically distinct cellulose standards for use in intercomparison studies, in a manner similar to the VSMOW and SLAP standards established for natural waters.³¹

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