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Technical note

A novel device for batch-wise isolation of α-cellulose from small-amount wholewood samples

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Abstract

A novel device for the chemical isolation of α-cellulose from wholewood material of tree rings was designed by the Potsdam Dendro Laboratory. It allows the simultaneous treatment of up to several hundred micro samples. Key features are the batch-wise exchange of the chemical solutions, the reusability of all major parts and the easy and unambiguous labelling of each individual sample. Compared to classical methods labour intensity and running costs are significantly reduced.

Keywords: Cellulose; Extraction; Isolation; Dendroclimatology; Stable isotopes

1 Introduction

Most environmental studies based on the analysis of stable isotope ratios of carbon and oxygen in tree rings use α-cellulose (McCarroll and Loader, 2004; Boettger et al., 2007). The reason for that lies in different isotopic signatures of individual wood constituents, e.g., cellulose, lignin and their varying contents along a radial ring sequence (Wilson and Grinsted, 1977). Thus, tree-ring isotope time series of wholewood exhibit a biologically induced bias which is normally unwanted in environmental studies. Other arguments for the use of α-cellulose are its local and temporal fixation and its high relative abundance within the wood (see McCarroll and Loader, 2004).
Isolating α-cellulose without alteration of its original isotopic composition requires the application of a labour intensive chemical method. Frequently, scientists need to process large numbers of samples, especially, if annual or even sub-annual resolution and high population density are desired for climate reconstructions or ecophysiological studies. While the scientific questions have become more complex and the isotope mass spectrometry has advanced, the chemical isolation techniques have not. Hence, a technical system is needed that does not change well-adopted chemical procedures but allows for treating large batches of small samples simultaneously in order to reduce labour times and costs. Here, we present a solution which meets the requirements in an innovative and contemporary way.

2 State of the art

Presently, there are two technical approaches for the isolation of α-cellulose. The first utilises a variety of parts made of borosilicate glass. Up to 50 modified Büchner funnels (glass thimbles equipped with a sintered glass disc) containing the wood sample and the reagents are suspended over the rim of a water-filled crystallising dish to be heated up to 70 °C. A hot plate placed underneath the crystallising dish keeps the water at reaction temperature. Different reagents have to

be applied and changed repeatedly during the isolation procedure of α-cellulose. In order to achieve this each funnel has to be accessed and emptied individually by a tube connected to a vacuum pump. The second well known method uses porous bags which contain the wood samples. The samples can be processed simultaneously in a soxhlet apparatus and/or a beaker glass. A variety of bag materials, e.g., Polytetrafluoroethylene (PTFE), fibre glass are utilised. After the bags have been filled with the samples they are sealed. Individual encoding of the bags is complicated since they cannot be simply inscribed with a pencil or felt-tipped pen. Therefore, they are either labelled by cutting out bits and pieces in different shapes from their borders or expensive pre-numbered bags are used. After one time application bags need normally to be replaced.

3 Technical description of the Multiple Sample Isolation System for Solids (MSISS)

The MSISS (Fig. 1) consists of the drainage module, the filter funnel (after Büchner), the water bath and a heating plate.

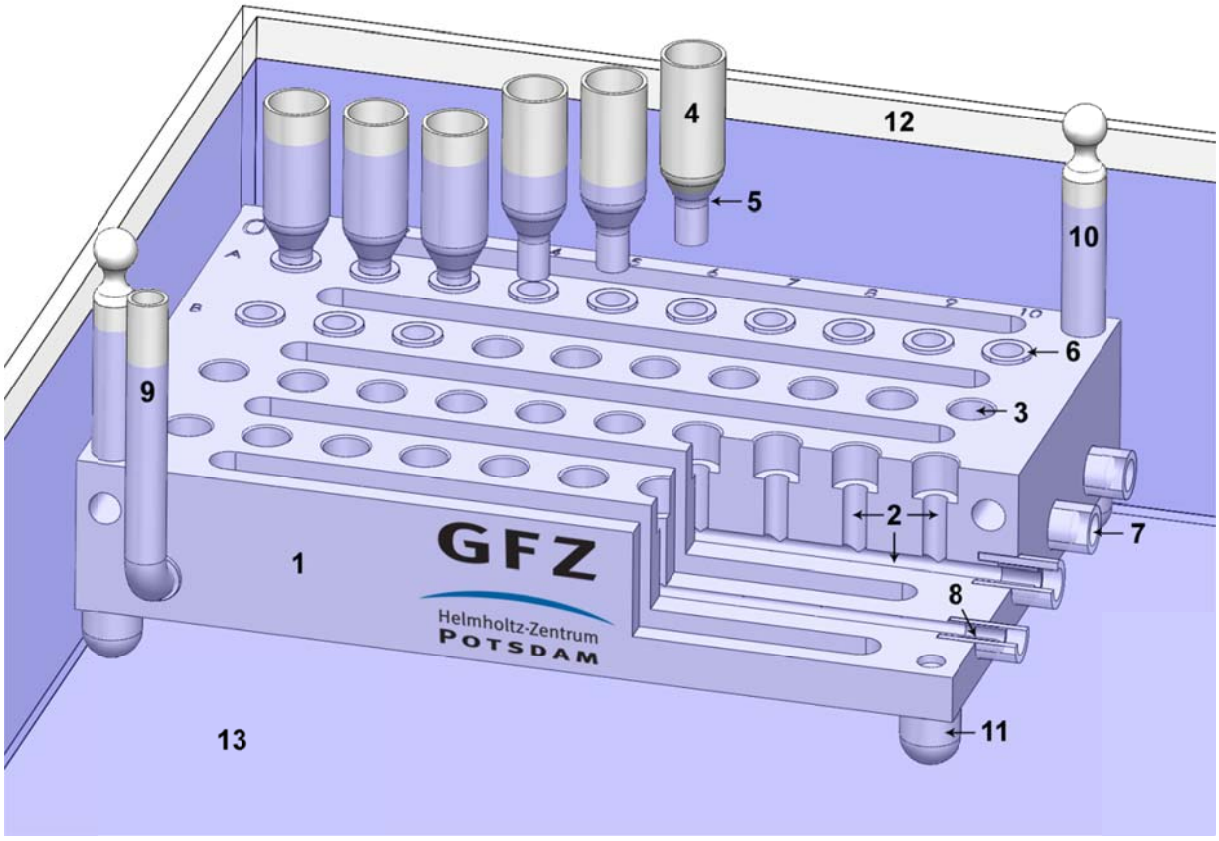

Figure 1 Multiple Sample Isolation System for Solids: 1 = drainage module, 2 = network of channels, 3 = access point, 4 = Büchner funnel, 5 = sintered glass disc, 6 = piece of silicon hose pipe, 7 = opening, 8 = interconnection assembly, 9 = PTFE tube as port for the aspiration pump, 10 = handhold, 11 = feet, 12 = water bath, 13 = hot water. For details see text.

The drainage module is made of a massive PTFE block which encases a network of channels. It was produced at the GFZ Potsdam workshop for fine mechanics by means of a CAD application (SolidWorks 2009, Dassault Systèmes SolidWorks Corp., Concord, Massachusetts, USA) and CNC milling/drilling machines (DMU 50T, DECKEL MAHO Pfronten GmbH, Pfronten, Germany; LDA-1, Braungart Präzisionsmaschinen GmbH, Villingen-Schwenningen, Germany). 40 access points, arranged in 4 rows (labelled from A to D) and 10 columns (labelled from 1 to 10), on top of the module hold the desired number of commercially available Büchner funnels (e.g., Sigma Aldrich) in

upright position. The funnels are made of borosilicate glass and equipped with a sintered glass disc acting as a filter near the bottom of a funnel. This allows for a subsequent treatment of solid sample material with various chemical solutions. In order to avoid clogging of the filters it is important to ensure that the grain size of the sample material is larger than the porosity of the filter.
The funnels are tightly connected to the module by means of silicon hose pipes (approx. 1 cm in length) which prevent chemicals from leaking out of the system. It is recommended to establish the connection by initially placing the silicon gaskets into the access points and subsequently pressing the Büchner funnels with the neck first into each of the 40 positions. This procedure avoids wearing out of the access points over time. Inactive ports can be closed with commercially available rubber plugs. At three sides of the module the four rows have openings providing easy access to the channel system for cleaning purposes and the interconnection of multiple modules. The modules are inter-connected by commercially available PTFE tubes (about 1.5 cm in length) enclosed by silicon hose pipes to ensure impermeability and stability.
One opening or access point of the drainage module connects the inter-linked channel system to a vacuum aspirator pump by means of a PTFE tube that is attached to the module as described above. This assembly allows simultaneous emptying of all funnels. Additionally, the drainage module comes with two PTFE handholds to lift it out of the water bath. Four PTFE feet and slots within the module enable effective circulation of the warming water and homogeneous temperature distribution within the water bath. The water bath container made of borosilicate glass was found to be appropriate for applying temperatures of up to 100 °C. It is heated from underneath by a portable ceramic heating plate providing constant temperature conditions for the reaction processes. The water bath is filled up to a level slightly below the upper rim of the funnels and it is consequently independent from the chemical cycle.

4 Chemical isolation procedure

4.1 Introduction

Different stable isotope laboratories worldwide apply various methods for the isolation of α-cellulose which normally only deviate from another in details like reaction temperature, application timing as well as the concentration of the solvents (Sohn and Reiff, 1942; Green, 1963; Brenninkmeijer, 1983; Loader et al., 1997). A couple of them are described in early publications and have been modified individually without documenting this in publications. Boettger et al. (2007) compare carbon and oxygen isotope compositions of α-cellulose from six oak and pine wholewood standards (Quercus and Pinus species) isolated by five European laboratories whose methods differ significantly. The deviations they find are mostly but not entirely within the error limits of the isotope measurement method employed. It is, nevertheless, desirable to develop and establish a standard isolation method in order to minimize errors before the actual measurements start and to achieve α-cellulose truly comparable across all laboratories.
The method described below is used at the Potsdam Dendro Laboratory and was originally developed by Sohn and Reiff (1942). After several initial arrangements it now comprises three to four consecutive steps to obtain α-cellulose from wholewood samples.

4.2 Getting started

At first, it must be assured that the wholewood material is sufficiently sized and homogenised for the chemical treatment by either slicing massive chunks into slim shavings of approx. 0.5–0.8 mm

thickness or grinding them. Grinding is usually accompanied with sample loss and especially affects small sample amounts. Furthermore, samples can be contaminated if cleaning of the mill is not done carefully. After grinding, 2.5–50 mg of the wood sample is transferred into a filter funnel. It is important that the relative composition of a sub-sample is the same as that of the whole sample, e.g., whole tree-ring, earlywood or latewood. For this reason grinding is usually the favoured method of homogenisation if sub-samples need to be taken. Because the glass filters have a porosity of 40–100 µm, the particle size needs to be >100 µm and it is recommended to stay over 500 µm in order to maintain the optimum permeability of the filters. The Potsdam Dendro Laboratory uses an ultracentrifugal mill (ZM200, Retsch GmbH, Haan, Germany; mesh size of the sieve: 500 µm). Once the funnels are loaded with the appropriate sample material they are plugged into the drainage module as described above. As soon as all idle openings are closed the module is placed into the water bath, which is then heated up to 60 °C.

4.3 Chemical treatment

Table 1 outlines the three step isolation procedure applied at the Potsdam Dendro Laboratory. Traditional methods use different organic solvents for pre-extracting fatty acids and resins from wood (Green, 1963). However, Rinne et al. (2005) proved that this step is not necessary for the isolation of α-cellulose from the resin-rich wood of Scots pine (Pinus sylvestris L.). The necessity of this very initial step should always be tested whenever new sites or tree species (e.g., tropical species) are being processed.

Table 1 Chemical procedure for α-cellulose isolation of the Potsdam Dendro Laboratory. For details see text.

	Step 1: First alkaline extraction	**Step 2: Chlorination**	**Step 3: Second alkaline extraction**
Chemical solution (concentration)	NaOH (5%)	Acidified $NaClO_2$ (7%), adjustment at pH 4-5 with acetic acid (96%)	NaOH (17%)
Duration per addition	2h	10h	2h
Number of repetitions	2	4	1
Water temperature	60°C	60°C	Room temperature

Filling up the funnels with the appropriate liquids and emptying them is best achieved with a wash bottle and by vacuum aspiration, respectively. Every step is followed by a rinsing procedure with hot de-ionised water removing chemical remnants and extracts from each funnel. From our experience rinsing the samples at least three times in a row with almost boiling de-ionised water is sufficient. The effectiveness of rinsing can be checked by measuring the pH of the water inside the funnels reaching the pH of the de-ionised water after completing the washing procedure. However, the test works less well after the second step as the pH of the chemical solution at this point is pretty close to that of ordinary de-ionised water. The washing process can be simplified when the samples are flushed with HCL (1%) after the third step prior to rinsing.

The whole isolation process is performed within a well-ventilated fume cupboard as toxic ClO_2–gas is being generated during the second step and protective clothing (laboratory coat, rubber gloves and safety glasses) is obligatory.

4.4 Final steps

The resulting α-cellulose is finally transferred from the filter funnels into 2 ml vials (No. 0030 120.094; Eppendorf Vertrieb Deutschland GmbH, Hamburg, Germany) and covered with 1 ml de-ionised water. This is followed by ultrasonic homogenisation (Laumer et al., 2009) and freeze drying. Dried samples are then weighed for the determination of their yield and finally measured by isotope ratio mass spectrometry.
Since the Büchner funnels are reusable containers they need to be cleaned thoroughly. Small sample fractions frequently adhere to their filters and, therefore the funnels must be baked in a muffle furnace setting at a temperature of approximately 500 °C. After the funnels have cooled down they are kept in HCl (5%) for 24 h before a final thorough flush with de-ionised water, which ensures the removal of all potentially remaining sample material prior to the reuse.

5 Conclusion

The improved α-cellulose isolation device described comprises three important advantages. With the current set-up at the Potsdam Dendro Laboratory, 320 samples can be treated simultaneously in a fume cupboard, i.e., the chemical solvents can be removed from batches of up to 8 MSISS sets of 40 individual samples. Since the solvents need to be exchanged several times during the isolation procedure a couple of hours per week can be saved by utilising the new device.
All major parts of the system are composed of either PTFE or borosilicate glass. Both materials are chemically stable as well as heat resistant and thus long-lived. Hence, costs are confined to the initial acquisition of the system and the replacement of broken glass parts, whereas, e.g., porous bags need to be replaced after each application.
A challenge during the isolation with bags is not to mix them. The methods available comprise placing numbered tags alongside the wood shavings, writing inscriptions onto the bag-closing cable-tie-buckle when sample identification from outside, e.g., for weighing purposes is desired (see Leavitt and Danzer, 1993) or using expensive pre-numbered bags and heat-seal them (Cullen and MacFarlane, 2005). The MSISS on the other hand is permanently and easy to read because it is marked with a codification that has no contact to corrosive chemicals and is not altered easily.
Because of its chemical and physical properties the device might be well suited for other isolation and extraction tasks.

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7 References

Boettger, T., Haupt, M., Knoller, K., Weise, S.M., Waterhouse, J.S., Rinne, K.T., Loader, N.J., Sonninen, E., Jungner, H., Masson-Delmotte, V., Stievenard, M., Guillemin, M.-T., Pierre, M., Pazdur, A., Leuenberger, M., Filot, M., Saurer, M., Reynolds, C.E., Helle, G., Schleser, G.H., 2007. Wood cellulose preparation methods and mass spectrometric analyses of $\delta^{13}C$, $\delta^{18}O$, and nonexchangeable $\delta^{2}H$ values in cellulose, sugar, and starch - An interlaboratory comparison. Analytical Chemistry 79 (12), 4603-4612.

Brenninkmeijer, C.A.M., 1983. Deuterium, oxygen-18 and carbon-13 in tree rings and peat deposition in relation to climate. Ph.D. Thesis, Groningen.

Cullen, L.E., MacFarlane, C., 2005. Comparison of cellulose extraction methods for analysis of stable isotope ratios of carbon and oxygen in plant material. Tree Physiology 25 (5), 563-569.

Green, J.W., 1963. Wood cellulose, in: Whistler, R.L., Green, J.W., BeMiller, J.N., Wolfrom, M.L. (Eds.), Methods in carbohydrate chemistry: Cellulose, 3. Academic Press, New York, 9-21.

Laumer, W., Andreu, L., Helle, G., Schleser, G.H., Wieloch, T., Wissel, H., 2009. A novel approach for the homogenization of cellulose to use micro-amounts for stable isotope analyses. Rapid Communications in Mass Spectrometry 23 (13), 1934-1940.

Leavitt, S.W., Danzer, S.R., 1993. Method for batch processing small wood samples to holocellulose for stable-carbon isotope analysis. Analytical Chemistry 65 (1), 87-89.

Loader, N.J., Robertson, I., Barker, A.C., Switsur, V.R., Waterhouse, J.S., 1997. An improved technique for the batch processing of small wholewood samples to α-cellulose. Chemical Geology 136 (3-4), 313-317.

McCarroll, D., Loader, N.J., 2004. Stable isotopes in tree rings. Quaternary Science Reviews 23 (7-8), 771-801.

Rinne, K.T., Boettger, T., Loader, N.J., Robertson, I., Switsur, V.R., Waterhouse, J.S., 2005. On the purification of α-cellulose from resinous wood for stable isotope (H, C and O) analysis. Chemical Geology 222 (1-2), 75-82.

Sohn, A.W., Reiff, F., 1942. Natriumchlorit als Aufschlussmittel. Der Papier-Fabrikant 1/2, 1-7.

Wilson, A.T., Grinsted, M.J., 1977. $^{12}C/^{13}C$ in cellulose and lignin as palaeothermometers. Nature 265 (5590), 133-135.