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A faster plant stem-water extraction method

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Oxygen and hydrogen isotope ratios of stem water have been used by several studies which relate the ecophysiology of plants to their water source. Undoubtedly, there are several other applications and research areas which could use this type of analysis. However, the most often used methods of extracting stem water are slow, limiting the rate of sampling and consequently preventing a deeper understanding of spatial and temporal plant water source use. We have developed a faster batch method of stem-water extraction and compare it with the most commonly used online method of stem-water extraction. Samples are sealed in 18 cm long ampoules having their extremities placed sample end in a heating block and the condensing end in a cooling block, and allowed to distill overnight. Up to 72 samples can be distilled overnight and sealed the next morning. The isotope ratios of water distilled by the batch method introduced here compared with those from the online method were in excellent agreement. In addition to being faster, this method does not need the monitoring of hot water baths and liquid nitrogen traps during distillation and does not require a complex vacuum system. Copyright © 2006 John Wiley & Sons, Ltd.

Stable isotope analyses of stem water can contribute to our understanding of how plants use different water resources temporally and spatially.[1] This information leads to important correlations between water use and plant ecophysiological performance. For example, the long-standing conjecture that grasses and trees in savanna ecosystems partition water resources[2] has been confirmed by isotopic analyses of plant stem and soil water.[3] Other savanna ecosystems, however, have surprised us in showing that there is substantial competition between grasses and trees.[4,5] Stable isotope analyses of stem water have contributed to several other areas of ecophysiology and eco-hydrology such as: showing sea versus freshwater uptake by mangrove, hardwood hammocks and coastal sand dune plants in the Caribbean region,[6–8] determining the temporal partitioning of seasonal rainfall events in arid ecosystems,[9] partitioning between fog and soil water uptake by coastal redwoods in California,[10] and identifying the isotopic composition of transpired water.[11,12] Undoubtedly, there are many more applications for stem-water isotopic analyses. Separation of water from plant stems, however, is a slow process which limits our ability to process large number of samples and our understanding of water partitioning by plants.

Currently, there are three major ways of extracting water from stems: squeezing water,[13,14] online cryogenic distillation,[15,16] and azeotropic[17] distillation. All these methods offer their advantages and disadvantages. The first method of squeezing has the advantage of being a relatively quick method of separating water samples from stems in the field. However, this method cannot be easily adapted to the extraction of leaf and soil water. The second method, and the most commonly used, provides consistently high precision and accuracy for water distilled from several types of plant tissues and soil. This method, however, is slow, and it requires a somewhat complex vacuum system and the constant monitoring of the distillation process. The third method has the advantage of not needing a vacuum system. However, it requires expensive glassware and the number of samples that can be distilled per day is low. In addition, it uses a toxic substance (toluene), which requires a safe disposal method. The latter two methods require the preservation of the stem samples during transport from the field to the laboratory. In this study we show that the results from a batch method of stem-water extraction developed here, which overcomes many of the drawbacks of the above methods, are in agreement with those from the most commonly used online stem-water extraction method of cryogenic distillation. This method does not require a complex vacuum system and therefore can easily be adapted to facilities having low scientific resources.

EXPERIMENTAL

Distillation apparatus

Online distillation apparatus

The online distillation apparatus used here was similar to the one shown by West *et al.*[16] The apparatus consisted of eight distillation arms connected to a vacuum manifold. A vacuum of 10 mTorr was maintained with an oil diffusion pump and a rotary vacuum pump. Each distillation arm was isolated

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from the vacuum manifold with a vacuum stopcock (high vacuum, easy action plug; Ace Glass, Vineland, NJ, USA). A 9 mm collection tube was attached to one end of the distillation arm with a vacuum fitting (Ultra-Torr®; Swagelock, Solon, OH, USA). To the other end of the distillation arm a 7 mL Vacutainer® (BD, Franklin Lakes, NJ, USA) containing the stem sample was attached via a vacuum fitting (Ultra-Torr® reducing union; Swagelock).

Batch distillation apparatus

The batch apparatus consisted of a lower cooling and an upper heating pair of anodized aluminum blocks (VWR modular heating blocks for standard test tubes, Cat. # 13259-130; VWR, West Chester, PA, USA; Fig. 1). The bottom cooling pair of blocks was immersed in a tray containing anti-freeze liquid (VWR bath fluid Dynalene HC50, Cat. # 13272-034) and cooled with an immersion chiller (VWR immersion chiller, model # 1107, Cat. # 13271-500). The top heating pair of blocks was heated by a dry block heater unit (VWR analog dry block heater, Cat. # 12621-108) adjusted to a temperature of 100°C. In addition, 1.27 cm diameter wood dowels a few mm longer than 18 cm were placed in the two diagonally opposed corner wells of each block to prevent the top blocks and the additional weight of the heating unit from crushing the sealed ends of the sample tubes during the distillation procedure.

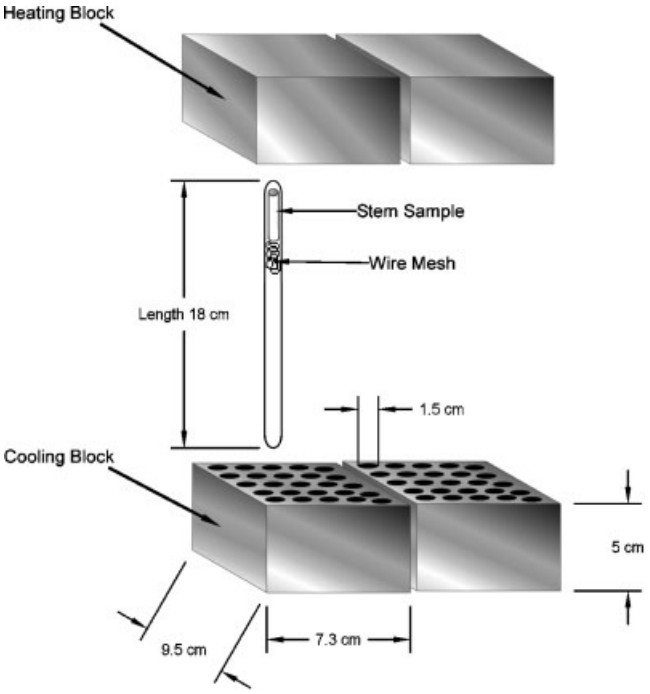

Figure 1. Apparatus for the batch distillation of plant stem-water samples consisting of aluminum heating blocks at the top and cooling blocks at the bottom. Also shown is an ampoule containing a stem sample for water extraction held up at the top of the vacuum sealed ampoule by a wire screen. Wood dowels to hold up the heating blocks and prevent weight stress in the sealed end of the ampoules are not shown.

Distillation procedure

Online distillation procedure

Distillation by the online method was carried out as previously described.[16] Stem samples in Vacutainers® and the collection tubes were connected to the distillation arms. Stem samples were then frozen with a liquid nitrogen bath and the distillation arms evacuated to 10 m Torr. After evacuation the distillation arms were isolated from the vacuum manifold by closing the isolation valves. Tubes containing stem samples were immersed in a boiling water bath, while the collection tubes were immersed in liquid nitrogen for a distillation period of 6 h Periodically, the water and liquid nitrogen baths were replenished and the distillation arms were heated with a torch to prevent the condensation of water on the inner surfaces of the glass arms. After the distillation, the collection tubes containing the frozen water were flame-sealed and kept until isotopic analysis.

Batch distillation procedure

For the batch method, 1.27 cm o.d. Pyrex tubes approximately 23 cm long were prepared by sealing one end and fire polishing the other end. Lines were marked approximately 4 cm and 18 cm from the bottom (the sealed end) as a guide to the length of the stem samples and the length of the sealed tube, respectively. Well-suberized stems approximately 4 cm long and 0.7 cm in diameter were cut, debarked and inserted into the tubes with a long glass rod and kept in place by insertion of a small roll of wire screening (Fig. 1). Glass tubes with stem samples were fitted into a vacuum line via a vacuum fitting (Swagelock Utra-Torr® reducing unit 1.3 cm to 0.9 cm) and stem samples at the bottom of the tube were frozen with liquid nitrogen. The tubes were then evacuated and flame-sealed at the 18 cm mark and placed sample side away from the lower cooling blocks (Fig. 1). Heating blocks were fitted on top of the tubes on the sample side and subsequently fitted into the heating unit. The distillation cycle took place during the afternoon and overnight. The cycle is initiated by heating the sample side without cooling the lower blocks for a period of 1 h beginning at approximately 15:00 h, after which the immersion chiller was turned on by a timer and the cooling bath temperature brought to about −25°C for a period of 4 h. The immersion chiller was then turned off and the cooling bath allowed to reach room temperature for a period of 8 h and subsequently cooled again for a period of 4 h. Tubes with the frozen water sample at the bottom were flame-sealed the next morning at the end of the second cooling cycle at approximately 8:00 h. The 8 h interruption of the cooling period increases the distillation efficiency by melting any frozen water blocking the path to the cooling block.

Experiment 1

In the first experiment we compared both distillation methods with water of a known isotopic composition. Three water samples with a known isotopic composition were prepared. Aliquots of 1 mL of water from each water sample were added to a small ball of quartz wool placed at the bottom of the batch and to the online method tube. Three replicates of each method for each water sample were

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prepared. We inserted the wire screening to hold the quartz wool up during distillation, and then sealed and distilled the samples according to each method.

Experiment 2

Initially this online method of distilling for an approximate period of 2 h yielded water which was isotopically lighter than the batch method for several species. We therefore tested to determine if insufficient time was given to the online distillation method. We collected long stems from a local tree (*Simauruba glauca*), debarked and cut them into 12 separate pieces. Water was distilled from four of the pieces by the batch method and water from the remaining eight pieces was distilled by the online method for periods of 2, 4, 6 and 8 h. For each period of distillation we used two replicates.

Experiment 3

We compared the distillation methods for several stem samples having water with a large range of isotopic composition (Table 1). Well-suberized stems were debarked and cut into six pieces three of which were placed in the batch method tubes and three of which were placed in the typical online method tubes. Samples were taken to the laboratory and distilled according to each method.

Isotopic analysis

Water samples were analyzed in a Multiflow system connected to an Isoprime mass spectrometer (GV, Manchester, UK). We used ~5 mg of platinum black powder (Sigma-Aldrich, St. Louis, MO, USA) to equilibrate hydrogen gas with water vapor for a 24 h period and analyzed the resulting equilibrated gas to derive the hydrogen isotope ratio of the water using a modification of the method of Prosser and Scrimgeour.[18] Water aliquots of 0.5 mL (including internal laboratory standards) were placed each in 5.9 mL vials (Exetainer® vials; Labco, High Wycombe, UK) together with the cuvettes containing the platinum black catalyst and sealed with screw-caps with a pierceable rubber septum (Exetainer® cap; Labco). Vials were placed in a 60-sample temperature-controlled rack of the Multiflow system and automatically and sequentially flushed for a period of 3 min with a 13% H_2/He (v/v) mixture. Samples were allowed to equilibrate at 25°C for a period of 24 h, whereupon a small aliquot of the H_2/He mixture was sequentially sampled by the Multiflow system, dried through a Nafion® membrane (Dupont, Wilmington, DE, USA), and injected into the mass spectrometer for hydrogen isotope analysis. The δD values of the water samples were then calculated using the appropriate fractionation for the equilibration reaction and further corrected with the internal laboratory standard.[18] After hydrogen isotope analysis, the above samples were flushed with a 5% CO_2/He (v/v) mixture for 3 min and allowed to equilibrate for a period of 48 h at 25°C. The CO_2/He mixture was then sampled as above and analyzed for the oxygen isotope ratios of the equilibrated CO_2. The δ^{18}O values of the water were then calculated using the appropriate equilibration fractionation factor and internal laboratory standards.[19] Isotopic ratios are expressed in δ units as:

$$\delta\text{D or } \delta^{18}\text{O} = \left[\frac{R_{Sample}}{R_{std}} - 1\right] \cdot 1000 \quad (1)$$

in which R_{sample} and R_{std} are D/H or ^{18}O/^{16}O ratios of the sample and the standard, respectively. The standard used here is Vienna mean standard water (vSMOW) and the precision of analysis is ±2.0‰ and ±0.2‰ for δD and δ^{18}O values, respectively.

Statistical analysis

We compared the isotopic composition of water extracted by both methods in experiments 1 and 3 by a one-way analysis of variance (ANOVA) for each particular water sample or species. We then adjusted the probabilities of significant differences for the experiment-wise error rate with a Bonferroni correction.[20]

RESULTS AND DISCUSSION

Experiment 1

Both methods of distilling water with a known isotopic composition yielded water with identical isotopic composition; i.e. no significant difference was observed between the methods and all data points fell close to a one-to-one line (Fig. 2). Distillation of the most enriched water sample yielded water that was on average 2.2‰ more enriched in deuterium than the original solution placed in the distillation tubes. Since the two distillation methods gave similar results with no significant difference for this particular water sample (Fig. 2), we conclude that the difference between the original solution and water from the distillation was caused by a possible contamination of residual water in the quartz wool.

Table 1. Average δ^{18}O and δD values (±σ) of water extracted from stems by the online and batch methods for several species collected in Florida, USA, and Belfast, UK. Also shown is the probability (*P*) that observed differences between the two water extraction methods for each species and isotope were by chance alone. According to the Bonferroni[18] correction, *P* must be less then 0.007 for a significant difference between the two methods

		Online method		Batch method		P	
Species	Location	δ^{18}O (‰)	δD (‰)	δ^{18}O (‰)	δD (‰)	δ^{18}O	δD
Eugenia confuse DC.	Florida, USA	−3.5 ± 0.2	−23.2 ± 1.6	−3.8 ± 0.3	−24.5 ± 2.5	0.145	0.510
Hamelia patens Jacq.	Florida, USA	−3.2 ± 0.2	−15.3 ± 1.2	−3.3 ± 0.1	−18.6 ± 2.8	0.600	0.140
Laguncularia racemosa L.	Florida, USA	−1.2 ± 0.1	−6.3 ± 1.4	−1.4 ± 0.1	−5.0 ± 2.3	0.126	0.992
Rhizophora mangle L.	Florida, USA	−0.9 ± 0.3	−7.2 ± 0.8	−1.5 ± 0.4	−10.4 ± 1.4	0.093	0.153
Betula pendula Roth	Belfast, UK	−5.6 ± 0.1	−45.1 ± 1.0	−6.4 ± 0.2	−44.0 ± 0.0	0.004	0.131
Castanea sativa Mill.	Belfast, UK	−6.0 ± 0.3	−40.9 ± 1.6	−6.4 ± 0.1	−43.5 ± 0.3	0.063	0.057
Taxus baccata L.	Belfast, UK	−4.0 ± 0.2	−32.5 ± 1.8	−4.3 ± 0.3	−35.5 ± 1.8	0.377	0.761

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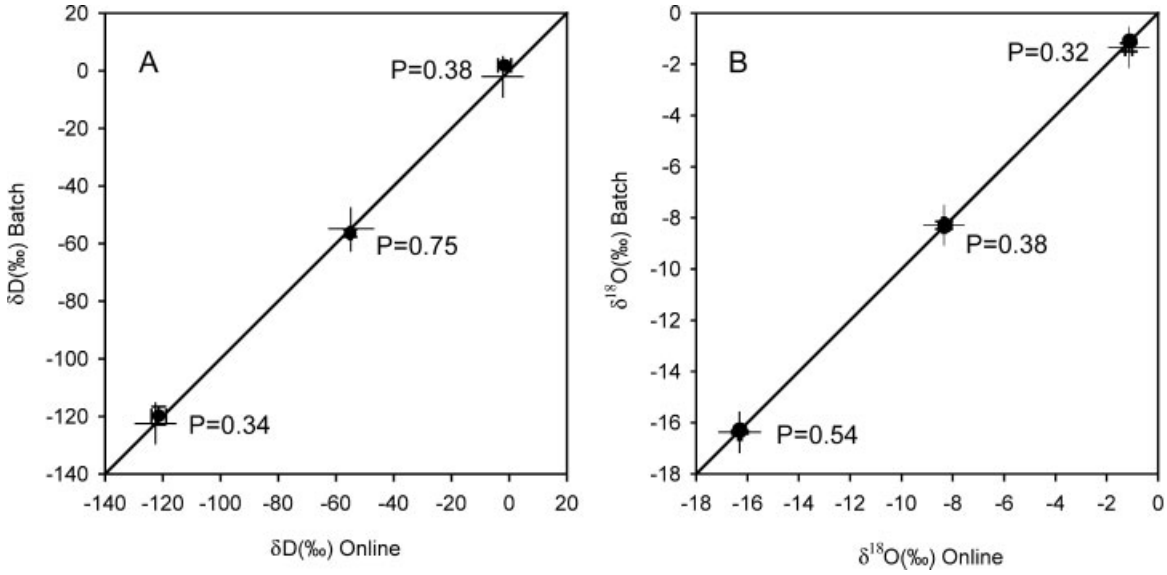

Figure 2. The isotopic composition of water extracted by the batch versus the online method for quartz wool samples soaked in water with known isotopic compositions. (A) and (B) show the δD and δ^{18}O values, respectively, for the water extracted by both methods (solid circles $\pm\sigma$). Crosses show the original isotopic composition of the water used in the soaking of the quartz wool. Solid line represents a one-to-one relationship. Also shown for each sample and isotope is the ANOVA calculated probability that the difference in isotopic composition for water distilled by both methods was by chance alone.

Experiment 2

A period of 6 to 8 h was needed to distill water completely from stem samples of *Simaruba glauca* (Fig. 3). We note that *Simaruba glauca* was the only species which during our preliminary distillation of 2 h consistently yielded water that was isotopically lighter than those from the batch distillation method. Our results indicate that a distillation time of several hours might be needed to achieve complete distillation for some species, in contrast to the 60 to 75 min of distillation for the stems collected by West *et al.*[16] There are three factors which might contribute to the discrepancy between their distillation times and ours. First, they noted that the time of distillation may be species-specific and it is possibly that *Simaruba glauca* might be unusual in this way. Secondly, the size of the stem sample (i.e. the quantity of water) might be an important determinant of the time that it takes to distill samples. Whereas their stem samples were of the order of 3 cm long, our stem samples tended to be 4 cm long and perhaps of a greater diameter. Thirdly, West *et al.*[16] precut their stem samples to 0.5 cm length allowing for a greater surface area and a more rapid distillation time, whereas our samples were placed whole in the glass ampoules.

Experiment 3

No significant difference was observed between the δD values of stem water distilled by both methods for all species (Table 1, Fig. 4(A)). Only one species (*Betula pendula*) yielded water which had a significantly higher average δ^{18}O value with the online method of distillation than that of water distilled by the batch method (Table 1). Nevertheless, the differences between the δ^{18}O values of water from the online and batch methods for this species were still within the variability encountered within a single stem sample (see, e.g., *P. edulis* in Fig. 2 of West *et al.*[16]). There was a tendency for the online method to yield water with slightly greater

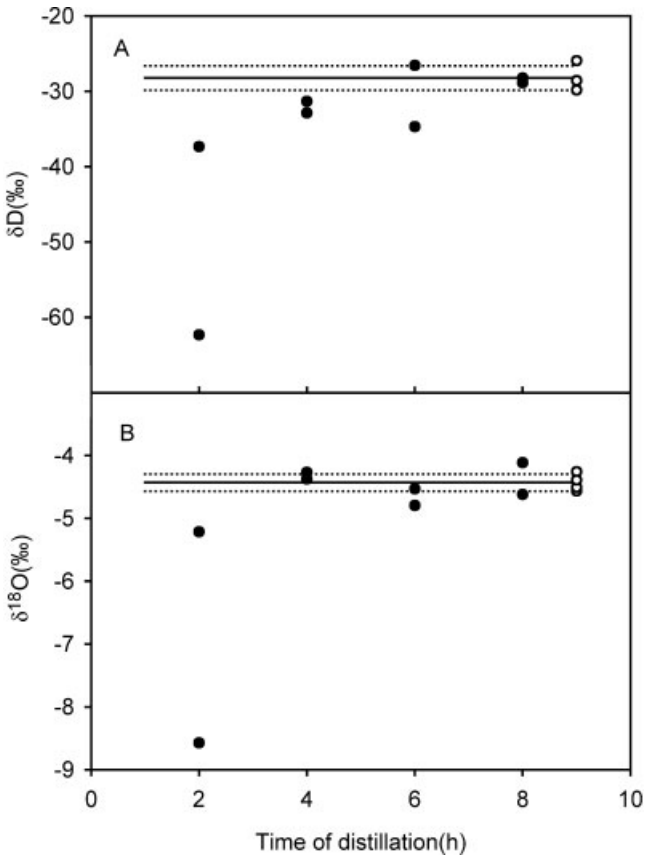

Figure 3. Oxygen and hydrogen isotope ratios of *Simaruba glauca* stem water extracted for various periods of time online (dark circles) compared with those extracted by the batch method (empty circles). Solid and stippled lines show the mean and standard deviation for samples extracted by the batch method.

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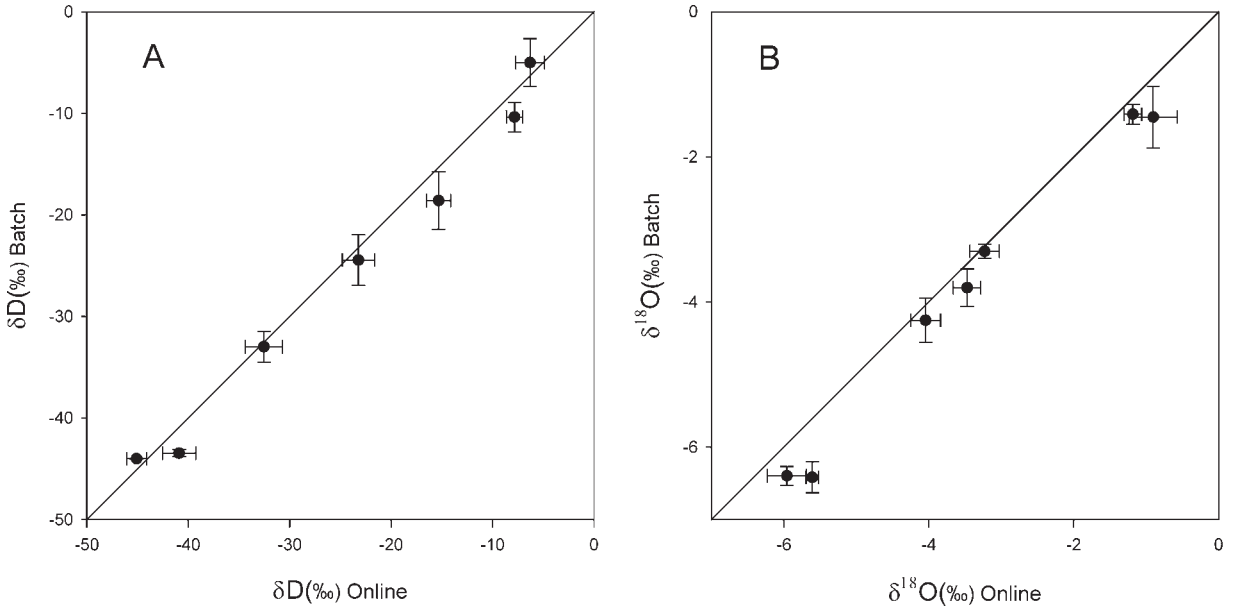

Figure 4. The relationship between the average δD and δ^{18}O values ($\pm\sigma$) of stem water extracted with the batch distillation (A) versus those by the online method (B). Solid line represents a one-to-one relationship.

δD and δ^{18}O values than the batch method (Table 1, Fig. 4) and this may be related to the condensation of isotopically heavier water on the glass walls between the heating and condensing ends for the batch method. No such condensation occurs in the online method because the whole distillation arm is heated periodically.

Overall comparison between the online and batch methods

The batch method presented here offers several advantages over the online method. Our apparatus as described above is capable of handling 36 samples at a time, therefore greatly increasing the daily sample output. The number of distillations can be increased to 72 if a dry block heating unit capable of accommodating four blocks, supplied by the above vendor, is used rather than the two block model used here. This method also offers the advantage of not needing constant supervision to assure that liquid nitrogen and water are replenished periodically. Samples are simply inserted into the dry block and the heating unit and timer for the cooling unit are initiated. Water samples are then sealed off on the following morning. This method can be easily adapted to the distillation of water from other solid materials such as leaves and soils. In the case of soils, oven-dried quartz wool plugs rather than wire screening can be used as a retainer for the solid material in the upper heating end of the distillation tubes. The limiting step here is how fast samples can be sealed off before and after distillation. With a manifold having eight outlets we estimate that we can seal 40 to 60 samples in 1 h. A similar rate of sealing samples can be accomplished on the following morning. The sealing of samples under vacuum before distillation does require some glass-blowing skills. Since the ampoules are approximately 1.3 cm in diameter and are under vacuum, the overheating of the glass may cause a rapid inward expansion of a glass bubble which eventually pops and causes a leaky seal. Therefore, the sealing should be done slowly and with a cool flame (low in oxygen). The seal, being of a fragile nature, should also be flame-annealed with a yellow flame after sealing off the sample. We observed an occasional sample loss caused by a leaky seal, which is surely compensated for by the large numbers of distillations possible by this method.

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