LIENSs Stable Isotope Facility

Sample preparation and shipment procedures for analysis of %C, %N, $\delta^{13}C$ and $\delta^{15}N$

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1. Sample encapsulation

1.1. Sample weight

The sample weight is not strictly necessary to get the stable isotope ratio values. However, these values are obtained with a high precision and confidence within a certain weight range, and if the measurements of the %C and %N have to be determined, the sample weight has to be precisely known (± 1 µg).

The mass spectrometers available at the LIENSs stable isotope facility are fitted with the Smart-EA option, which allows an individual adjustment of the sample gas to the level of the corresponding reference gas peak. Even if that configuration allows the analysis of samples within a wide weight range with the same precision, it is better to try to have sample weights within the optimal range. Below the minimal values of this range, measurements are less precise, and above maximal values, saturation of the ion collectors can occur.

The following table gives the minimal, maximal and optimal weights for various types of samples (as mg DW) for C and N isotope ratio measurements. For sediments, tests on few samples must be done to determine the optimal weight, if no data on their C and N content is available. For filters, the weight is not useful since it includes an unknown quantity of glass fibers relative to the organic matter: experience is needed to know how much organic matter has to be scrapped from the filter. %C and %N calculations are done relative to the filtered volume, if the entire filter can be analyzed.

Sample type	Minimum	Optimal	Maximum
Animal tissue	0.1 mg	0.4 mg	1.5 mg
Micro- or macroalgae	0.2 mg	0.6 mg	1.8 mg
Marine phanerogams	0.3 mg	0.7 mg	2.0 mg
Vascular plants	0.3 mg	0.7 mg	2.0 mg
Sediment (high content in organic matter)	0.2 mg	0.6 mg	1.8 mg
Sediment (low content in organic matter)	1.0 mg	-	> 30.0 mg

1.2. Capsule selection

The facility provides the 96-well trays and the capsules (tin or silver) for the packing of samples (shipping by post mail). Please indicate us the number and the type (acidified or not) of sample you want to analyze, so we can send you the right amount of material. For

animal or plant samples, the analyzed quantity is very small, 8 x 5 mm capsules are convenient (although smaller capsules can be used, they may rise to problems with our automatic sampler if they are too much pressed and then too small). The same capsules can be used for sediment and filters but it can be necessary to use larger ones.

Tin capsules are the ones commonly used, but they can be rapidly damaged by acidified samples of sediment (especially if they are not stored under vacuum). To avoid it, analyses should be performed very quickly after weighing (maximum 8 to 10 days), or samples should be first packed in silver capsules, themselves packed in a second tin capsule to ensure a better combustion. In any case, samples should be kept in a desiccator.

1.3. Closing the capsules

Place the capsule vertically in the working zone on the aluminum plate. Close it by pressing firmly its top 1 or 1.5 mm using a flat ended plier (Fig. 1 A) then fold this pressed part down (Fig. 1 B). Fold again in the perpendicular direction (Fig. 1 C), then press the capsule using 2 flat ended pliers to end in a packed capsule in a shape of a sphere (max. diameter 2.5 mm for animal or plant tissues).

For sediments and filters, packing down to that size is difficult, sample volume being generally more important. In that case packed capsules should never be larger than 7 mm in diameter.

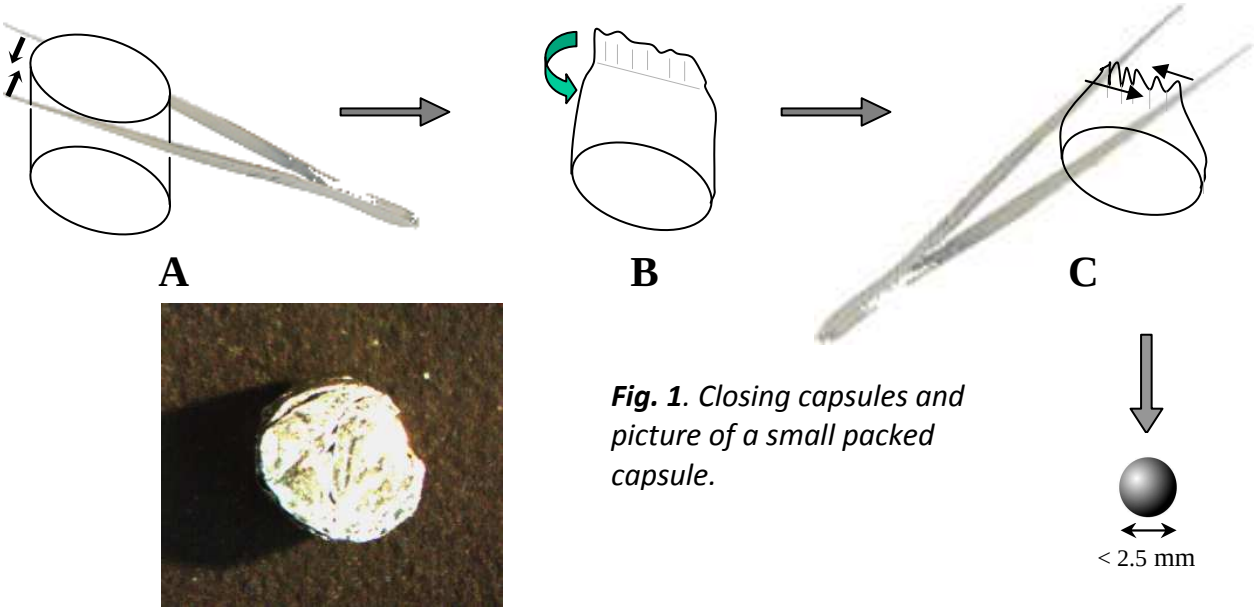

***Fig. 1**. Closing capsules and picture of a small packed capsule.*

<u>Note 1:</u> It is preferable to make small packed capsules, that can be used with the 100-positions tray on the automatic sampler, which allows analyzing about 30% more samples per day.

<u>Note</u>: It is very important to respect the recommendations concerning the shape and size of the packed capsules. Badly packed capsules (Fig 2A) can either leak if they are not well sealed (sample lost and contamination of other samples), or get caught in the well of the automatic sampler tray (and being not analyzed), or even jam the automatic sampler (with, in worst cases, loss of all of the samples in the tray).

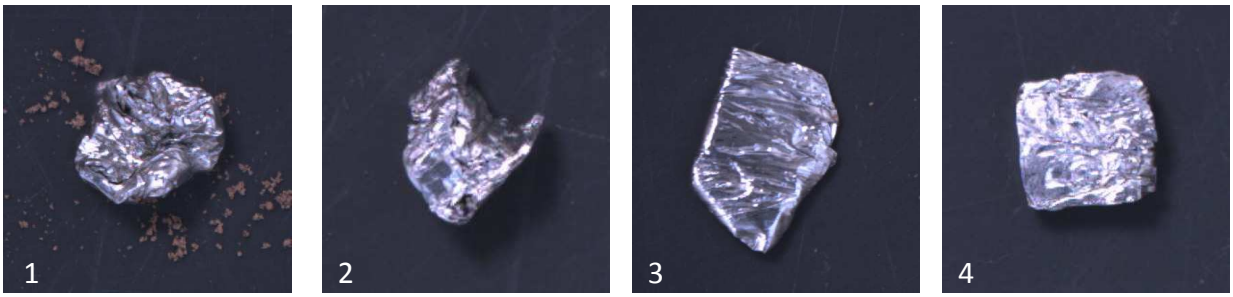

***Fig. 2A**. Examples of badly packed capsules: 1) Badly sealed: loss of part of the sample; 2) Not enough spherical: risk to get caught in the tray; 3) Too flat: risk of jamming and blocking the automatic sampler; 4) Cubical: risk to get caught in the tray.*

***Fig. 2B**. Examples of well packed capsules: 5-6) Small capsules (animals and plants); 7) Big capsule (sediment and filters).*

1.4. Analysis of filters

The sizes of the wells of the automatic sampler trays available at LIENSs SIF (3 mm and 7 mm) do not allow to analyze capsules containing a full 25 mm filter (and even more so 47 mm filters). Several other ways may be used:

- scrapping the filter surface to collect the organic matter: In any case, the collection cannot be guaranteed as quantitative. Minimizing the quantity of glass fibers is important since it hampers the combustion and increases the ash volume. This way of collecting organic matter rises also the problem of the heterogeneity of the sample and the non-representativity of the collected part.
- cutting out a filter fraction: Parts of filters can be cut out using a punch, that allows through a surface ratio to estimate the total quantity of material on the filter, in case it is needed. However, distribution of the organic matter on the filter is generally heterogeneous and for both the quantitative estimation of the %C & %N, and isotope ratios, this protocol may give a higher variability.
- use of mini-filters: filtration can be performed using filters of 12 mm in diameter (to cut out from larger filters) thanks to 13 mm filter holders (e.g., Sweenex). After cutting out of the white external part (very easy simply by strong tightening of the holder just after filtration when the filter is still wet) and after sample treatment (see § 1.3), peel the back side of the filter, that does not hold any organic matter, as much as possible. The rest can easily be contained in a small capsule. This protocol allows obtaining a value for the entire filtered sample, it works well, but needs to have a minimal load of suspended organic matter.

2. Storage, preservation and sample shipment

2.1. Storage

Packed capsules are stored in 96-well trays (Fig. 3), identified by rows (A to H) and columns (1 to 12). Sample list forms (Excel files) are organized to correspond to this storage box (see § 3).

- **Group samples of similar material or similar sizes** (small capsules: animals/plants together and large capsules: sediment/filters together). Putting a large capsule among small ones forces to analyze the whole series with the large well tray of the automatic sampler, which does not allow analyzing more than 30 samples at a time instead of 80.
- **Never left empty wells between series!!**

96-well tray cover must be hold in place using **reusable laboratory tape**. It is indeed necessary to open the trays many times to load or remove capsules, this should be easily done.

- ***Never use office adhesive tape to maintain the covers! There is a high risk of sudden opening, of mixing or knocking over of the capsules (glue traces are also a problem).***

Each 96-well tray must be clearly identified by noting some indications on a piece of **reusable laboratory tape put on the cover**. The indications must allow the right association of the sample tray and of the Excel file of the sample list form. It is therefore necessary to indicate on the cover at least the name of this file and the name of the sender (Fig. 3). **The best is to give the name of the file as reference for the tray**.

- ***Please do not write directly on the cover but on a piece of reusable laboratory tape! Trays are re-used after cleaning.***

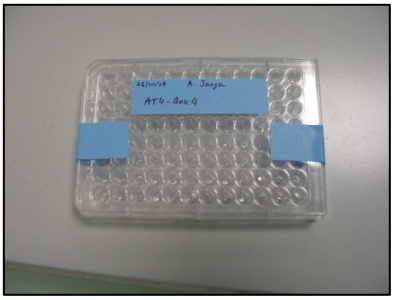

***Fig.3**. 96-well tray used for storage and shipping of the samples. Notice the 2 pieces of reusable tape to hold the cover and the one to note the identification information.*

2.2. Preservation

Except for acidified sediment in tin capsules, that should be quickly analyzed, other capsules can be stored during long periods, better under vacuum in a desiccator (at room temperature) than in a freezer where samples can get some humidity.

2.3. Sending samples to LIENSs SIF

96-well trays can be sent by postage services, enclosed in a bubble wrapped envelope. To avoid that capsules, and in peculiar the smallest ones, get out the wells, **place an aluminum foil (in 2 or 3 layers) over the tray before securing the cover**. **Never use Parafilm or adhesive tape or any type of paper (that may release fibers) to cover the wells of the tray.** For shipment, it is generally enough to hold the cover with 2 pieces of reusable laboratory tape (see Fig. 3). However, to better secure the cover, wrap the tray within an aluminum foil.

3. Filling in the sample list form

Each tray must be associated to a separate sample list form (submitted as an Excel file). Please to not send us the forms as a paper version with your samples, send them to the following address: irms-lienss@univ-lr.fr. The form includes an information sheet, that includes various fields for the administration, and a sample list sheet.

Samples must be identified following the instructions below:

- The tray reference must be indicated in the upper right field (Fig. 4) and should be the same as indicated on the tray itself and the same as file name of the sample list form. This reference should be very specific (avoid generic terms as Box 1, Tray 3 ..).
- The name of the sender must be indicated in the upper left field.

Sender	P Richard		Project : FoodWeBio	Tray ref. :	FWB 4
Prepared by	MZ		Date : 14/01/09		

Well #	Sample reference	Weight (mg)	Type	Comment
A 1	FWB-LR-M4	0.346	muscle	
A 2	FWB-LR-M5	0.309	muscle	
A 3	FWB-LA-M1	0.441	muscle	
A 4	FWB-LA-M2	0.288	muscle	
A 5				

Fig. 4. *Sample list sheet*

- Each reference of a sample must be unique, even for replicates that should be separately identified. It must have more than one character and not be only made of numbers. Avoid generic references as 'Sample 1', 'Sample 2' or 'A1', 'A2'…
- Do not use any special characters like: . , / ; ( ) ?+ @ # $ % ^&*. However spaces and hyphens (-) can be used. Avoid accented letters.
- Save the Excel file under the same filename that the tray reference.

4. Enriched samples

Stable isotopes can be used in experimentations based on enrichments from products that can have > 95% of the heavy isotope.

ⓘ **The highest caution must be taken at any step of the preparation of enriched samples: any contamination may have disastrous effect on measurements at the level of natural abundance.**

Users of enriched products must consider the following points before submitting enriched samples to LIENSs SIF:

- The abundance of the heavy isotope (^{13}C, ^{15}N…) must be below **3% for carbon and below 1% for nitrogen**. In case of higher enrichment, as it is the case of labelled prey in grazing experiments, samples must be diluted with the same but non-labelled sample whose natural abundance is measured apart and taken into account in the calculations. Any other compound can also be used but the corrections should then include the relative proportions of the element (C or N).

- For enriched samples, arrange samples from low to high enrichments to avoid possible memory effect. Place non-enriched samples ahead of enriched ones. Use separate trays for enriched samples and samples at natural abundance.

<u>Warning:</u> Higher than 1% of enrichment, a "memory effect" may be found on samples following a sample with a high enrichment. Samples must be arranged from low to high enrichments, and separating samples with high enrichment from non-enriched compounds should be considered.

<u>Warning:</u> It is of the responsibility of customers to be sure that the level of enrichment respects these rules. Higher enrichments may lead to an irremediable contamination of the reactors (and even of the GC column) whose replacement may be charged to the customer.

Enriched samples in a tray must be indicated on the tray and in the sample list forms!