^{14}CHRONO Centre laboratory ^{14}C pre-treatment protocols

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Introduction

The following are standard protocols for sample pre-treatment in the laboratory. These may be modified depending on sample preservation or at the submitter's request. Pre-treatment of additional sample types may be possible. It is important to note any changes from standard procedure and why this was done on the sample sheets. Background (blank) samples are processed along with the samples. In general anthracite is used for organic samples, Icelandic spar calcite for carbonates and mammoth bones for bone sample backgrounds. However, for non-bone organic samples expected to be >20,000 BP we use a background more closely matched to the sample material type, e.g. MOIS7 kauri wood. For foraminifera samples, it is preferable to use forams of the same species that are from sediment older than 50,000 BP and ideally from the same core (Nadeau et al. 2001).

If a sample, such as bone, wood, or hair, has been subjected to a preservative, ink, glue, resin or petroleum product then this needs to be removed prior to commencing pre-treatment. We treat the sample selected for dating to a solvent extraction in a Soxhlet distillation apparatus using a minimum of two cycles of tetrahydrofuran, chloroform, petroleum spirit, acetone, methanol and lastly deionized water, similar to Bruhn et al. (2001). The thoroughness of the extraction of the contaminants by solvent extraction may then tested by analysing the treated sample using Fourier transform infrared spectroscopy (FTIR) (D'Elia et al. 2007).

AAA (acid-alkali-acid) pre-treatment for charcoal, wood, peat, charred plant macrofossils, organic residues on pottery, etc.

The sample is immersed in 0.1M hydrochloric acid and placed on a 70˚C hotplate for 20 minutes to remove carbonates, then rinsed with deionised water until neutralised. Humic acids are then removed from the sample through the addition of 0.25M sodium hydroxide. The sample is then warmed on a 70˚C hotplate for 20 minutes, followed by rinsing in deionised water until neutral. The neutralised sample is then acidified with 1M hydrochloric acid and heated on a 70˚C hotplate for 1 hour before the final rinses in deionised water. The pre-treated sample is then placed in an oven until dried.

Acid only pre-treatment (fabric, skin, hair, some macrofossils etc.)

In some cases, the AAA will remove material intrinsic to the material to be dated (e.g. skin, hair, fabric) and so an Acid only pre-treatment is used to remove potential carbonate contamination. For macrofossils which have been separated from sediment using alkali treatment an acid only treatment is sufficient. As for the third step of AAA, the sample is immersed in 1M hydrochloric acid. The contents of the beaker may be heated on a hotplate (60˚C for 30 min). The sample then receives subsequent washes with deionised water until neutral.

Bone Pre-treatment Procedure

Our routine bone pre-treatment procedure involves a simple AAA treatment followed by gelatinization (after Longin 1971) and ultrafiltration (Brown et al. 1988) using a Vivaspin® filter cleaning method below introduced by Bronk Ramsey *et al.* (2004b).

Using starting weight of 0.5–1 g of bone, the bone is crushed using a percussion mortar until small fragments are achieved (ideally 1-3mm or smaller) taking care not to produce only dust. Samples are sequentially treated with:

- 10 ml 0.5M Hydrochloric Acid *(3 or 4 rinses over ~18 hr)* or until no further acid/carbonate reaction is seen
- 10 ml 0.1M Sodium Hydroxide *(15 – 30 mins)*, and
- 10ml 0.5M Hydrochloric Acid *(15 – 30 mins)*

After the final HCL wash is rinsed, the crude collagen is gelatinized in pH2 – pH3 solution at 70°C for 15 hours. The resultant gelatin solution is then filtered using 'pre-baked' 7 µm and 12 µm glass fibre filters on a ceramic filter holder. The filtrate is transferred into a pre-cleaned ultrafilter (Vivaspin® Turbo 15–30 kD MWCO) and centrifuged until 0.5 – 1.0 ml of the >30 kD gelatin fraction remains. This gelatin is then removed from the ultrafilter with borosilicate Pasteur pipettes and ultrapure water before being freeze-dried. The collagen yield is calculated from the ratio of the final weight to the starting weight of the bone.

Measurement of C:N ratios, $\delta^{13}C$, and $\delta^{15}N$

For bone collagen samples %C, %N, $\delta^{13}C$ and $\delta^{15}N$ are also measured on a Thermo Delta V elemental analyser - isotope ratio mass spectrometer (EA-IRMS). Samples and standards are sealed into tin capsules and combusted in the elemental analyser which yields %C and %N values and C:N ratios are calculated from these. The EA is connected to the IRMS for measurement of the stable isotope ratios. Three blanks are measured at the start of the run followed by three standards of Nicotinamide (known values of 59.01% Carbon and 22.94% Nitrogen) for the % element calibration. The collagen samples are run in duplicate with standards IA-R041 L-Alanine ($\delta^{15}N$ -5.56; $\delta^{13}C$ -23.33), IAEA-N-2 Ammonium Sulphate ($\delta^{15}N$ = +20.3 ± 0.2) and IAEA-CH-Sucrose ($\delta^{13}C$ =-10.449 ± 0.033) interspersed throughout the run about every 10 samples to provide a two-point calibration for the two isotopes. An internal fishbone collagen sample is also analysed routinely.

Cremated bone pre-treatment procedure

Cremated bones are cleaned with a Dremel® tool and 2 – 5 grams of cleaned cremated bone are ground in a mortar and pestle. The sample is placed in 1.5% sodium hypochlorite solution and allowed to stand for 48 hours to remove any protein remaining in the sample. The sample is vacuum filtered on pre-combusted glass fibre filters, washed with deionised water and placed in 1M acetic acid for 24 hours. This removes contaminant carbonate in the sample. The sample is then vacuum filtered, washed with deionised water and placed in a drying oven at 60° overnight.

An aliquot of the sample is analysed by FTIR to check on the degree of cremation (crystallinity index or splitting factor) and only those samples with crystallinity above 5 (preferably 6 or 7) are radiocarbon dated (Olsen et al. 2011).

The sample is stored in a sealed vial prior to hydrolysis with stock (85%) orthophosphoric acid (15ml per g of bone).

Sediment Samples (various fractions)

Depending on the fraction chosen for dating (bulk, humin or humic acids) samples receive one or more of the following three steps. Humic acid and humin sample preparation follows Lowe et al. (2004).

Bulk (step 1)
The sample is placed in 0.5M HCl in a heating block at 80^0c for 2 hours, followed by rinsing in deionized water until neutral. This will remove any calcareous contamination along with fulvic acids. Alternatively, the acid fumigation protocol may be used for bulk dates (see protocol below for soil organic matter).

Humic acids (step 2)
The substrate from the step 1 is placed in warm 2M KOH for up to 1 hour to extract the humic acids that are subsequently precipitated by the addition of 1M HCl and separated by centrifuge in cleaned (10% HCl) polypropylene centrifuge tubes.

Humin (step 3)
The substrate remaining after the removal of the humic acids in step 2 must be acidified with 1M hydrochloric acid at 80°c for 1-2 hours, as in step 1. The substrate is again washed neutral and oven dried.

Soil organic matter

The pre-treatment follows a standard fumigation protocol (Harris et al. 2001; Komada et al. 2008). Spread a thin layer of soil on a watchglass and place in a desiccator with a vial of fuming HCl acid for a period of 10-24 hours under atmospheric pressure. If there is a large amount of carbonate in the sample, the soil can be moistened (with DDI) prior to fumigation. Do not seal the desiccator with grease as this will cause isotopic contamination of the sample (Schubert and Nielsen, 2000). The completion of the reaction is checked by testing a small amount of fumigated sample with a 2.5M HCl solution. If there is no bubbling/foaming, then the fumigation was successful. Dry the fumigated material and transfer it into a quartz tube for combustion (add an extra strip of silver ribbon to remove sulfur).

Shell samples (molluscs and other carbonates, except forams and corals)

Mollusc samples with aragonitic shells should generally be analysed by x-ray diffraction (XRD) to determine if they have been recrystallized to calcite before dating. This is not possible for molluscs with calcite shells and is not necessary for modern samples.

The sample is initially washed with Milli-Q® water in an ultrasonic bath to (remove surface dirt) then dried. A starting weight of 12 mg of shell is placed in a septa seal vial (exetainer) and 0.25M HCl (0.18 ml) is added to remove 25% of its mass (etching). The amount of acid is calculated according to the weight of sample. Following the etching stage samples are washed and rinsed using deionized water.

The samples are subsequently hydrolysed with orthophosphoric acid (H_3PO_4) in the exetainers on a heating block at 90°C to evolve carbon dioxide as described below (Santos et al. 2004).

The sample contained in the exetainer is evacuated by puncturing the septum with a hypodermic syringe attached to the vacuum line. When a good vacuum pertains, the sample is removed, and 2 ml

of orthophosphoric acid is introduced through the septum. The vacuum syringe is then carefully inserted into the rubber of the septum (without fully inserting through the septum) to evacuate everything down to the seal. The syringe is then fully inserted, and the CO_2 is drawn out under liquid nitrogen.

Corals

The method of coral pre-treatment follows Burr et al. (2004). As with mollusc shells, analysis with XRD should be undertaken prior to dating.

Preliminary cleaning and checking

A Dremel® tool is used to cut off small slabs of coral. If not previously done, XRD analysis is used to check for recrystallization from aragonite to calcite (carried out at an external laboratory at additional cost). These should be less than 1% calcite (approximate detection limit).

Slabs of coral are ultrasonically cleaned in Milli-Q® water and then a clean looking piece is cut out. This piece undergoes further ultrasonic washing with Milli-Q® water and is broken into pea-sized or smaller fragments. These fragments are again placed in the bath in Milli-Q® water and subsequently dried at 50 -60˚C.

Acid pre-treatment and hydrolysis

The coral is weighed into septa seal vials, aiming to obtain ca. 1 mg carbon if possible (allowing for 50-60% to be etched away before hydrolysis). $CaCO_3$ MW=100.09, sample size approximately 17 mg. An appropriate amount of ~0.1M HCl is added to etch away 50-60% of the sample. (This may take a day or two – when it stops bubbling it should be finished). The neutralized acid is decanted and rinsed repeatedly in Milli-Q® water.

While still damp, the vial is capped, placed on a syringe on the vacuum line, and evacuated. This prevents atmospheric CO2 from attaching to the coral. The vial can be gently heated to drive off the water. The vial is removed from the vacuum line and 0.5 ml of 85% orthophosphoric acid is added (an excess to dissolve 1mg sized sample), placed on a heating block at 90°C until coral is dissolved, then the gas is transferred to bomb or to reactor on graphite line. Vials should not be left standing any longer than necessary, as there is potential for leaks.

Foraminifera

Pre-treatment of the foraminifera specimens follows the approach by Nadeau et al. (2001). 6-12 mg of sample is weighed into a septa seal vial, 2ml of 5M hydrogen peroxide (H_2O_2) is added, and ultrasound for 3 minutes. The peroxide solution is removed using a fine syringe and rinsed with Milli-Q® water using a fine syringe to remove the water. While still damp, the septa seal vial is evacuated on a vacuum line. 1-2 ml of 85% orthophosphoric acid is added, depending on sample size, to hydrolyse the samples to CO_2. The vial is placed in heating block at 70°C until completely dissolved. The CO_2 gas is then transferred on the vacuum line.

Freshwater or groundwater carbon (DIC/DOC/POC)
any queries to Dr Evelyn Keaveney (e.keaveney@qub.ac.uk)

Field protocol:

1. Use acid-washed Nalgene® bottles. Bottles should be soaked in 2.5M HCl overnight before rinsing in Milli-Q® water. In an alkaline lake 60 ml of water will yield sufficient sample for AMS analysis. Rivers and less alkaline lakes will require a larger water sample (>1 L) or may not yield DIC/DOC for further analysis.
2. Rinse water bottles 2-3 times with the water from lake or river.
3. Fill water bottles under water. Samples should be taken from below the surface-air interface to prevent atmospheric contamination.
4. Close bottles under water to prevent contamination from atmospheric carbon.

Water DOC/POC

1. Water should be vacuum filtered with MF300 glass fibre filters (pore size (0.7µm).
2. What is left on the filter paper should be kept for a POC sample if the Submitter requests POC.
3. Filtered water should be rotary evaporated and decanted into progressively smaller vacuum flasks to reduce loss of solid material.
4. For lake samples, when the volume is < 15-20 ml water should be acidified with ~2 ml 1M HCl to remove carbonates (Note: the addition of acid may affect stable isotopes). For soil DOC samples collected with lysimeters this step may not be needed if pH is <6. Rotary evaporation should be resumed to reduce the final volume of liquid to be freeze-dried to 5-10ml.
5. Any material deposited on the walls of the flask can be re-suspended into the remaining liquid by briefly submerging the body of flask in the sonic bath (being careful not to contaminate the sample in the process) adding Milli-Q® water if necessary to assist re-dissolving solids.
6. If the submitter requests stable isotopes the acidification step is eliminated but the submitter should be asked for their preference on this. If both ^{14}C and stable isotopes are required, the water sample should be split before pre-treatment.
7. If there is a question over the quality of sample, 0.5M HCl should be used to acidify. Lakes with low terrestrial input (i.e. clear/green water) may require less acidification.
8. When the sample is sufficiently small, it can be lyophilised in the freeze drier and when dry, loaded into tubes as normal.

Water POC

1. POC can be obtained from the same sample bottle as DOC.
2. Water is vacuum filtered and what is left on the glass fibre filter paper is further pre-treated to obtain POC.
3. If there is sufficient sample to scrape off the filter paper the sample is rinsed with Milli-Q® water and then acidified with 0.5M HCl to remove carbonates. Higher concentrations of HCl may be required depending on the alkalinity of the lake.
4. Sample is then lyophilised and loaded for combustion as normal.
5. If the sample is small and has penetrated the glass fibre filter, then the entire filter paper must be acidified, rinsed with Milli-Q® water and then dried overnight in tin.
6. Ensure to cover the sample (tinfoil/petri dish) to avoid contamination from airborne particles.
7. The dried filter paper is then combusted.
8. The filter paper is loaded into quartz sample holder and then added to large tube for combustion and graphitisation as normal.

Water DIC

The submitter may add NaOH and $BaCl_2$ themselves; this should be noted on the submission in comments. The submitter is responsible for ensuring that atmospheric contamination is minimised during collection and shipping.

1. If the water has been sent untreated, enough NaOH should be added to push the pH to 11 (2-3 pellets) and a sufficient amount (usually 2-3g) of $BaCl_2$ depending on the lake alkalinity (see table 1). The precipitate formed is $BaCO_3$.
2. There is huge potential for contamination from the lab atmosphere from the time NaOH is added. To ensure that this is minimised/eliminated, the chemicals should be added without exposing the water.
3. The precipitate is filtered using vacuum filtration
4. The filter paper is rinsed with Milli-Q® water and remains slightly wet.
5. The entire filter paper is then hydrolysed. The filter paper containing the precipitate should **IMMEDIATELY** be transferred for hydrolysis, again to minimise contamination from the atmosphere.

Table 1: Volume of water and $BaCl_2.2H_2O$ required for sampling of ^{14}C in DIC (after IAEA 2004).

SO_4^{2-} (mg/L) →	0	50	100	200	400	500	600	800	1000	1500	2000	Volume of Sample (L) For 3 g C
Alkalinity HCO_3^{1-} (mg/L)	Minimum $BaCl_2 \cdot 2H_2O$ required per 50 L sample volume (g)											
10	1	4	7	14	24	32	38	50	65	93	133	1500
25	2.5	6	9	15	26	34	40	53	66	96	137	600
50	5	8	11	18	30	37	43	56	69	100	140	300
100	10	13	16	23	35	42	48	61	74	105	145	150
150	15	18	21	28	40	47	53	66	79	110	150	100
200	20	23	26	33	45	52	58	71	84	115	155	75
250	25	28	31	38	50	57	63	76	89	120	160	60
300	30	33	36	43	55	62	68	81	94	125	165	60
350	35	38	41	48	60	67	73	86	99	130	170	50
400	40	43	46	53	65	72	78	91	104	135	175	44
450	45	48	51	58	70	77	83	96	109	140	180	35
500	50	53	56	63	75	82	88	101	114	145	185	30
600	60	63	66	73	85	92	98	111	124	155	195	25
1000	100	103	106	113	125	132	138	151	164	195	235	15

Note that the above chart is for a volume of sample to produce 3 g C so the volume of water and $BaCl_2$ added should be adjusted for 1 -2 mg C.

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