A REASSESSMENT OF THE ROUTINE PRETREATMENT PROTOCOL FOR RADIOCARBON DATING CREMATED BONES

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ABSTRACT. In the late 1990s, it was demonstrated that reliable radiocarbon dates could be obtained directly from cremated bone. Many ^{14}C laboratories have since used a protocol for pretreating cremated (calcined) bones that consists of consecutive treatments with bleach and acetic acid to remove organic matter and extraneous or diagenetic carbonate, respectively. In most instances, the bleach used is sodium hypochlorite, although in recent years the Oxford Radiocarbon Accelerator Unit (ORAU) has used acidified sodium chlorite instead. However, properly calcined (white) bones should not contain any organic material; hence, the bleach treatment is potentially unnecessary. This article describes studies investigating the effectiveness of bleach (and the specific bleach used) during pretreatment of calcined bone, and demonstrates that ^{14}C dates on six cremated bone samples are statistically indistinguishable whether or not the initial bleach step is applied.

KEYWORDS: pretreatment, cremated bone, bleach.

INTRODUCTION

Cremated bone is found in a wide range of archaeological contexts but has, for a long time, been excluded from radiocarbon dating. Indeed, once fully calcined (i.e. cremated), bone is visibly very white and does not retain any organic matter, leaving no collagen for ^{14}C dating. However, Lanting and Brindley (1998), and subsequently Lanting et al. (2001), demonstrated that reliable ^{14}C dates could be obtained from the small carbonate fraction remaining within the bioapatite of cremated bone fragments, as long as the fragments are fully calcined and white (i.e. not gray or black). Charred bone (gray and black) is not a good candidate for dating as it still contains significant amounts of organic matter and is more prone to postburial alterations due to its lower crystallinity (Lebon et al. 2010; Snoeck et al. 2014a).

As part of the Fifth International Radiocarbon Intercomparison (VIRI) exercise, six radiocarbon laboratories undertook dating of six cremated bones (including two duplicate pairs), with ^{14}C ages spanning approximately 1500–2800 BP (Naysmith et al. 2007). Five of the laboratories used pretreatments based on that proposed by Lanting et al. (2001), consisting of 1.5% sodium hypochlorite (NaOCl) to remove any remaining organic matter, followed by 1M acetic acid (CH$_3$COOH) to remove any adsorbed or diagenetic carbonates. However, the Belgian Royal Institute for Cultural Heritage (KIK-IRPA) developed and applied another pretreatment protocol whereby the samples were treated with 1% HCl until 50% of the material is leached (Van Strydonck et al. 2005, 2009). All six laboratories subsequently reacted the pretreated material with phosphoric acid in sealed vessels to liberate CO$_2$ for accelerator mass spectrometry (AMS) ^{14}C dating.

The rationale for the inclusion of the sodium hypochlorite bleach step is to remove any remaining organic matter. However, once fully calcined, no organic matter should remain in the sample, and such pretreatment is possibly superfluous. Furthermore, any soil organic matter deposited on the bone during burial should be physically removed prior to chemical pretreatment (e.g. by air-abrasion). Pretreating the sample with acetic acid only should be sufficient, and phosphoric acid should not react with any potential remaining organic matter anyway.

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Removing the initial bleach step would save time and money and potentially limit the amount of sample loss, as well as reducing the possibility of introducing additional contaminants.

Furthermore, at ORAU, the 1.5% NaOCl (pH ~10) step was replaced with acidified (pH 3) 1.5% sodium chlorite (NaO_2Cl) (Brock et al. 2010) at some point after the VIRI intercomparison, although the reason for this change is not recorded. However, NaO_2Cl is not as strong an oxidizing agent as NaOCl, and may not be as efficient at removing organic matter from cremated bones prior to dating.

The experiments designed here were aimed at answering the following three questions: (1) How efficiently do NaOCl and NaO_2Cl remove organic matter from calcined bone? (2) Does the presence of organic matter impact on the isotope composition of the carbon dioxide emitted during the reaction of carbonates with phosphoric acid? (3) Does the bleach stage have any statistically significant impact upon the ^{14}C dates produced (and therefore could the bleach step be removed from routine pretreatment of cremated bones for ^{14}C dating)?

MATERIALS AND METHODS

Testing the Efficacy of Organic Matter Removal by NaOCl and Acidified NaO_2Cl

To compare the efficacy of NaOCl and acidified NaO_2Cl at removing organic matter, 2 aliquots of an unburned modern sheep bone were pretreated. Since cremated bone does not contain organic matter, testing the efficacy of NaOCl and NaO_2Cl on such samples would have been difficult, hence the use of modern bone (containing significant amounts of collagen). The first bone aliquot was treated with 1% NaOCl and the second with 1.5% NaO_2Cl at pH 3. Both samples were pretreated for 72 hr before thorough rinsing with ultrapure (Milli-Q™) water. (The standard ORAU pretreatment for cremated bones is only for 48 hr.) Both pretreated samples, as well as a fraction of untreated sheep bone, were then crushed using a pestle and mortar.

The pretreated samples and untreated material were analyzed by Fourier transform infrared spectroscopy with the attenuated total reflectance mode (FTIR-ATR; Agilent Technologies Cary 640 FTIR with GladiATR™ from Pike Technologies). Each sample was measured three times. The background was subtracted and a baseline correction was carried out using Agilent Resolution Pro software. The water-amide on phosphate index (WAMPI) can be calculated from the infrared spectra. This evaluates the amount of organic matter present in bone by comparing the amide I band of collagen at $1650\,cm^{-1}$ and the phosphate band at $605\,cm^{-1}$ (Roche et al. 2010). Structural water also absorbs around $1650\,cm^{-1}$ (Praprotnik and Dušanka Janežič 2005; Yoder et al. 2012), making it difficult to detect small amounts of organic matter.

To measure the amount of nitrogen (i.e. protein, and therefore organic matter) present in the untreated and treated unburned modern bone samples, approximately 5 mg of powder was placed in a tin capsule and analyzed by mass spectrometry (Sercon Geo 2022 IRMS coupled to Sercon Europa EA–GSL running in continuous-flow mode with helium carrier gas at 80 mL/min). The limit of detection is 2 μg nitrogen.

Testing the Influence of Organic Matter on Carbon Isotopic Composition

To assess the impact of the presence of organic matter on the isotopic composition of the carbon dioxide released during the phosphoric acid treatment, 20 mg individual aliquots of the IAEA-C01 Carrara marble standard ($\delta^{13}C = 2.42 \pm 0.33$‰ relative to PDB[1]) were each contaminated with

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[1]As reported in the IAEA reference sheet for quality control materials ref RS-IAEA-C1 to IAEA-C9.Rev01/2014-03-24.

10 and 50% of collagen (by weight) of known carbon isotope composition ($\delta^{13}C_{coll} = -21.8 \pm 0.3\text{‰}$; $n = 28$). These, as well as 2 aliquots of uncontaminated marble, were then reacted with 85% phosphoric acid under vacuum, without any additional pretreatment. The carbon dioxide emitted by the reaction was trapped cryogenically and subsequently reinjected into a coupled EA/IRMS system as above, and $\delta^{13}C$ values measured. These experiments were carried out in duplicate.

Pretreatments of Calcined Bone for Radiocarbon Dating

Finally, to show that pretreatments with NaO_2Cl are superfluous when dating fully calcined bone, two archaeological calcined bone fragments (one from the Neolithic court tomb of Annaghmare, Co. Armagh, Ireland and one from a Neolithic/Bronze Age house in Ness of Gruting 7, UK) were pretreated using both the routine pretreatment protocol at ORAU (Brock et al. 2010) and the same protocol without the initial NaO_2Cl treatment. Furthermore, four of the six archaeological calcined samples used in the VIRI interlaboratory comparison study (Naysmith et al. 2007) were prepared following the ORAU routine protocol but without NaO_2Cl, having each been previously dated in duplicate at ORAU as part of the VIRI exercise. The calcined bones were reacted with phosphoric acid (85%) under vacuum, and the CO_2 released was trapped cryogenically. As above, the CO_2 collected was reinjected into a coupled EA/IRMS system, $\delta^{13}C$ values were measured, and the CO_2 was then converted into graphite and pressed into aluminium targets prior to AMS ^{14}C dating on the ORAU HVEE tandem AMS system (as detailed by Bronk Ramsey et al. 2004; Brock et al. 2010).

RESULTS AND DISCUSSION

Efficiency of NaO₂Cl

The FTIR and nitrogen elemental analyses of the unburned modern sheep bone study (Table 1 and Figure 1) show that when NaO_2Cl is used, almost no organic matter is removed. However, no detectable amount of organic matter remains after pretreatment with NaOCl. The small peak observed at $1650\,cm^{-1}$ in the infrared spectrum of the sample pretreated with NaOCl is most likely due to structural water remaining in the sample after pretreatment. This confirms that NaO_2Cl is much less efficient than NaOCl at removing organic components. However, the use of NaOCl induces the adsorption of atmospheric carbon dioxide (Zazzo et al. 2006; Snoeck and Pellegrini, 2015), which could have a significant impact on the ^{14}C dates. (The switch to acidified NaO_2Cl at ORAU may, at least in part, be linked to this observation.) The subsequent pretreatment with acetic acid should remove these adsorbed atmospheric carbon contributions. Nevertheless, each additional step in the pretreatment process could affect the ^{14}C dates and limiting pretreatment to only what is necessary could improve the ^{14}C dates obtained.

Reactivity of Organic Matter with Phosphoric Acid

The $\delta^{13}C$ values of contaminated Carrara marble standard samples show limited variation (Table 2), within the accepted range of 2.42‰ with a standard deviation of 0.33‰. This suggests a very limited impact of organic matter on the $\delta^{13}C$ values of the carbon dioxide emitted during the

Table 1 Infrared and nitrogen elemental analyses for the unburned modern bone samples, untreated and pretreated with NaO_2Cl and NaOCl (WAMPI is the water-amide on phosphate index).

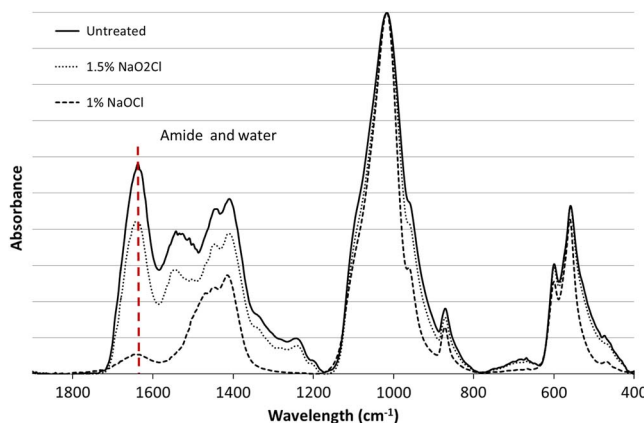|	WAMPI	%N (wt.%)
Untreated	1.85	4.8
1.5% NaO_2Cl at pH 3	1.43	4.7
1% NaOCl	0.21	below level of detection

Figure 1 Infrared spectra of modern bone untreated (solid line), pretreated with 1.5% NaO_2Cl at pH 3 (dotted line) and 1% NaOCl (dashed line); the band at 1650 cm^{-1} corresponding to amide (collagen) and water is highlighted.

Table 2 $\delta^{13}C$ values of carbonate standard samples contaminated with known amounts of collagen.

Organics content (%wt.)	$\delta^{13}C$ (‰PDB)
0% (20 mg marble)	2.44
0% (20 mg marble)	2.43
10% (20 mg marble + 2.2 mg collagen)	2.28
10% (20 mg marble + 2.2 mg collagen)	2.10
50% (20 mg marble + 20 mg collagen)	2.31
50% (20 mg marble + 20 mg collagen)	2.22

reaction of carbonates and phosphoric acid. The absence of variation in $\delta^{13}C$ values strongly suggests that no variation would be observed in the ^{14}C dates measured on calcined bone samples containing small amounts of remaining organic matter. This, once again, highlights that it is unnecessary to use a bleaching agent when dating properly calcined bone as the amount of organic matter remaining is well below 10%.

Furthermore, even if some organic carbon were to be released during the reaction, it could only have originated from endogenous carbon from the body, fuel, or atmospheric carbon (Hüls et al. 2010; Van Strydonck et al. 2010; Zazzo et al. 2012; Snoeck et al. 2014b). At the time of cremation, if the body and fuel are contemporary (which is the underlying assumption for ^{14}C dating of cremated bone, though there are some exceptions, e.g. Olsen et al. 2012; Van Strydonck et al. 2013), then all three carbon sources are identical from the ^{14}C perspective.

Comparison of the Traditional and Updated Pretreatments

While the presented results emphasize that a pretreatment with only acetic acid should be sufficient, it is necessary to test this on archaeological samples. Here, two samples have been dated after having undergone both pretreatments with and without acidified sodium chlorite. Furthermore, four samples (out of the six) from the VIRI intercomparision exercise (Naysmith et al. 2007) were redated using the updated pretreatment. The dates obtained are statistically identical (i.e. pass a χ^2 test at 95% confidence) with both pretreatments (Figure 2, Table 3),
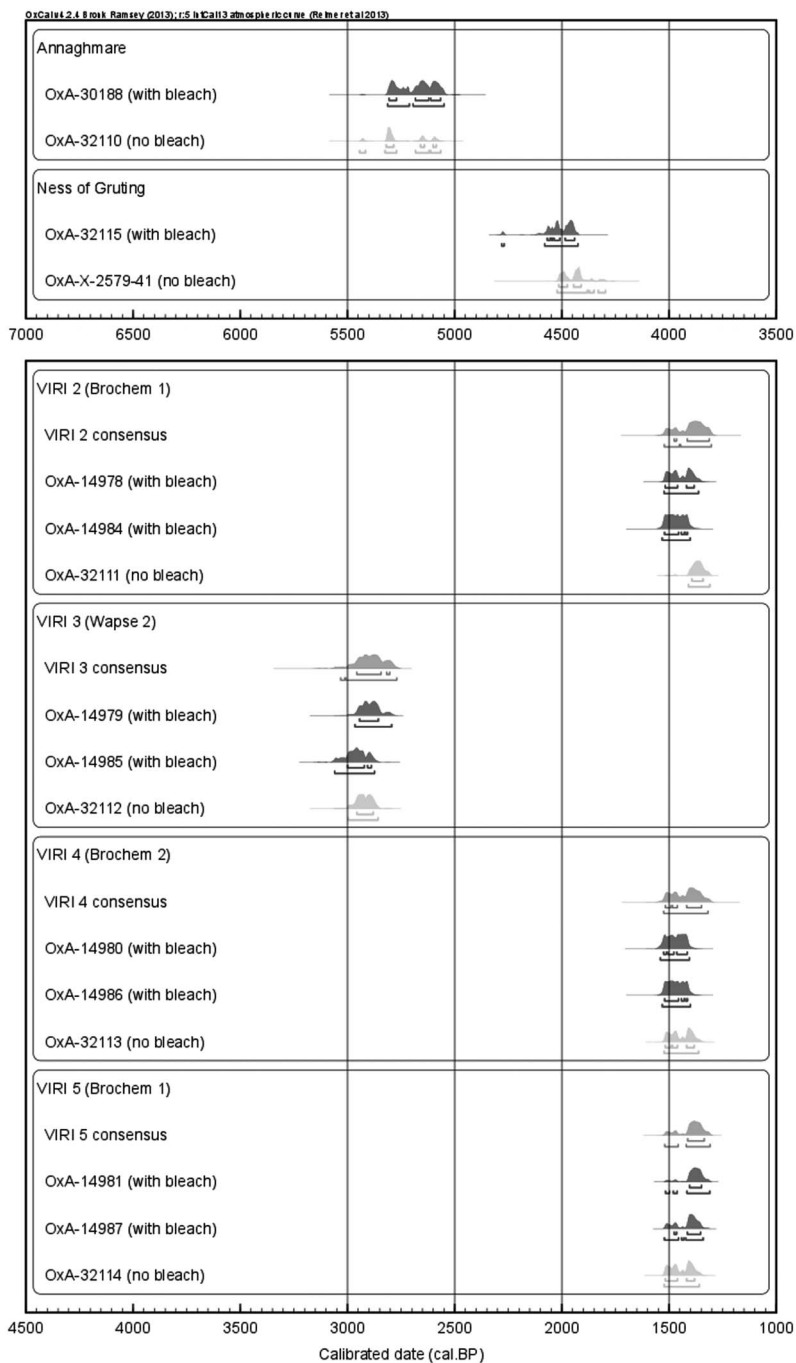
Figure 2 Calibrated ages of the six cremated bone samples, with and without the application of a bleach pretreatment stage. Additionally, the consensus values of the four VIRI samples (Naysmith et al. 2007) are plotted in pink. The horizontal bars below each probability density function indicate the 68.2% and 95.4% highest probability density ranges, respectively. The IntCal13 calibration curve was applied (Reimer et al. 2013), using OxCal v 4.2.4 (Bronk Ramsey 2013).

Table 3   Radiocarbon and stable isotopic results for archaeological samples.

Sample/Site	VIRI consensus (median) date (BP)[a]	VIRI mean date (BP) (±1σ)[a]	Mean δ13C (‰ PDB)[a]	ORAU pretreatment	ORAU laboratory numbers*	Conventional 14C age BP (±1σ)	δ13C (‰)	χ2 test
Annaghmare	—	—	—	No bleach / With bleach	OxA-32110 / OxA-30188	4572±28 / 4532±36	−20.4 / −20.5	df(ORAU) =1, T = 0.8 (5% 3.84)
Ness of Gruting	—	—	—	No bleach / With bleach	OxA-X-2579-41 / OxA-32115	3964±31 / 4043±28	−27.6 / −27.6	df(ORAU) =1, T = 3.6 (5% 3.84)
VIRI 2 (Brochem 1)	1515	1496±54	−25.47	No bleach / With beach[a]	OxA-32111 / OxA-14978 / OxA-14984	1484±26 / 1537±29 / 1571±27	−25.7 / −25.8 / −25.3	df(ORAU) =2, T = 5.49 (5% 5.99) df(VIRI) =1, T = 0.04 (5% 3.84)
VIRI 3 (Wapse 2)	2802	2790±52	−23.45	No bleach / With bleach[a]	OxA-32112 / OxA-14979 / OxA-14985	2823±27 / 2794±31 / 2848±30	−20.1 / −21.3 / −20.7	df(ORAU) =2, T = 1.57 (5% 5.99) df(VIRI) =1, T = 0.32 (5% 3.84)
VIRI 4 (Brochem 2)	1521	1518±47	−26.00	No bleach / With bleach[a]	OxA-32113 / OxA-14980 / OxA-14986	1536±26 / 1581±29 / 1570±27	−22.9 / −27.4 / −26.2	df(ORAU) =2, T = 1.51 (5% 5.99) df(VIRI) =1, T = 0.11 (5% 3.84)
VIRI 5 (Brochem 1: duplicate of VIRI 2)	1511	1500±36	−24.47	No bleach / With bleach[a]	OxA-32114 / OxA-14981 / OxA-14987	1535±27 / 1496±29 / 1518±27	−25.6 / −23.1 / −22.5	df(ORAU) =2, T = 0.97 (5% 5.99) df(VIRI) =1, T = 0.60 (5% 3.84)

*Samples dated at ORAU are routinely issued with OxA- laboratory codes. The Ness of Gruting sample with the revised pretreatment was issued an OxA-X- code due to low AMS target current; [a]data from Naysmith et al. (2007). Mean quoted error for consensus values is 32 yr. Note that Naysmith et al. (2007) reported considerable scatter in δ13C values for individual samples, with ranges from 4–10‰, attributed to variation in methods of measuring δ13C values and also uneven cremation temperatures; df(ORAU) compares all samples pretreated at ORAU with different pretreatments while df(VIRI) compares the date obtained after the updated pretreatment with the consensus values of the VIRI intercomparison exercise; df = degrees of freedom; T = the test statistic.

confirming that the additional NaO$_2$Cl pretreatment stage is unnecessary. Therefore, this step has now been removed from the ORAU standard pretreatment protocol for cremated bones (as of OxA-32109), and the pretreatment process will consist of only treatment with 1M acetic acid over 24 hr and subsequent water rinses with ultrapure Milli-Q water prior to reaction with phosphoric acid, as detailed by Brock et al. (2010).

CONCLUSION

The results presented here highlight that (1) NaO$_2$Cl is not very efficient at removing organic matter, (2) the presence of organic matter has a very limited impact (if any) on the isotopic composition of the carbon dioxide released during the reaction of carbonates with phosphoric acid, and (3) archaeological calcined bone samples pretreated with both the traditional and updated pretreatments return statistically identical ^{14}C dates. The updated, simpler, and shorter pretreatment of several washes with 1M acetic acid over 24 hr proves to be sufficient prior to ^{14}C dating of calcined bone. No pretreatment with NaO$_2$Cl or NaOCl is required. Calcined bone will, from OxA-32109, be pretreated using the updated protocol when ^{14}C dated at ORAU.

ACKNOWLEDGMENTS

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