COMPARING METHODS FOR CO$_2$ PURIFICATION OF CREMATED BONE SAMPLES

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ABSTRACT. During the last two decades the radiocarbon (^{14}C) dating of hydroxyapatite archaeological cremated bones has become standard practice. Various pretreatment procedures exist among different laboratories of which some include fixation of SO$_2$ using "Sulfix" prior to CO$_2$ reduction. Recently it was reported that the use of Sulfix may cause the resulting ^{14}C age to be too old. Here we report on the use Sulfix at the Aarhus AMS Centre. Further, we report on an experiment designed to test alternatives for the use of Sulfix as a purification agent.

KEYWORDS: cremated bones, FTIR, pretreatment, radiocarbon.

INTRODUCTION

During the last two decades the radiocarbon (^{14}C) dating of hydroxyapatite archaeological cremated bones has become standard practice (Lanting et al. 2001; van Strydonck et al. 2005; Olsen et al. 2008; Zazzo and Saliege 2011; De Mulder et al. 2012; Quarta et al. 2014; Agerskov Rose et al. 2019; Annaert et al. 2020). The normal practice when considering bones for ^{14}C dating is to avoid the inorganic bone fraction (hydroxyapatite) because of the risk diagenetic carbon exchange with e.g., dissolved carbonate ions (Lee-Thorp et al. 1989; Krueger 1991; Munro et al. 2007). However, Lanting et al. (2001) demonstrated the that upon heating the crystallinity of the hydroxyapatite increases and as consequence hereof the hydroxyapatite becomes inert to diagenetic carbon exchange reactions. Thus for successful ^{14}C dating of cremated bones, it is of vital importance to ensure that the cremated bones are burned at temperatures high enough for the re-crystallization process to have taken place (Shipman et al. 1984; Stiner et al. 1995; Olsen et al. 2008). The crystallinity index (CI) representing a number between 3 (unburned bones) to 6–7 (bones burned at temperatures above ca. 600°C) can be measured using FTIR (Shipman et al. 1984; Weiner and Bar-Yosef 1990; Stiner et al. 1995). A more direct measure of the CI can be obtained using XRD (Person et al. 1995).

Carbon stable isotope (δ^{13}C) on hydroxyapatite from unburned bones lies typically around 15‰ representing the carbohydrate fraction of the diet (Lee-Thorp and van der Merwe 1991). Intriguingly, the hydroxyapatite δ^{13}C of cremated bones are much more ^{13}C depleted with values around 25‰. This has led to interesting experiments from which it is concluded that during the heating process a large fraction of the hydroxyapatite carbon is exchanged with CO$_2$ from the combustion fuel (Zazzo et al. 2009; Hüls et al. 2010; van Strydonck et al. 2010), i.e., measured ^{14}C originates from wood in an archaeological context. This represents a possible complication when ^{14}C dating cremated bones as the resulting ^{14}C results may be influenced by the so-called old-wood effect (Olsen et al. 2013).

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Various sample pretreatment methods exist for sample pretreatment of cremated bones (Lanting et al. 2001; De Mulder et al. 2007; Van Strydonck et al. 2009; Hüls et al. 2010). The original method suggested by Lanting et al. (2001) involves Sodium hypochlorite (NaOCl) to oxidize eventual organic material, then an Acetic acid (CH_3COOH) step to remove readily soluble calcite, absorbed carbonates as well as the less crystalline fractions of hydroxyapatite. Subsequently the sample is homogenized and CO_2 is produced by dissolving the cremated bone sample in phosphoric acid (H_3PO_4). As a last step the CO_2 is furthermore heated with "Sulfix" particles (consisting of Ag_2O and Co_3O_4, Waco Chemicals Ltd, LOT WAG 7038) to remove sulfur compounds. Some laboratories skip the organic matter oxidation step and use both hydrochloric acid (HCl) in combination with acetic acid and further re-combust the CO_2 using silver wool and cupric oxide (CuO) as an alternative to Sulfix (De Mulder et al. 2007; Van Strydonck et al. 2009; Hüls et al. 2010). A recent study evaluated the pretreatment methods for cremated bones and concluded that no differences could be observed between the different pretreatment methods (Agerskov Rose et al. 2019). However, the latter study pointed out that the use of Sulfix could be problematic and might lead to inaccurate ^{14}C results as also suggested by Zazzo et al. (2013). At Aarhus AMS Centre (AARAMS) we use the Lanting et al. (2001) protocol for sample pretreatment and the reporting of the possible problems with the usage of Sulfix was therefore disturbing news. As part of a larger project, JOINTIME, the laboratory have ^{14}C dated 31 cremated bones. We therefore decided to conduct a test comparing the CO_2 purification methods by selecting 10 samples on which (1) the Sulfix and (2) the silver wool and CuO purification methods were used. This paper will report on the results of this test.

METHODS

Out of the 31 cremated bone samples which were ^{14}C dated in the "JOINTIME: Connecting Bronze Age Europe: High-precision Radiocarbon Dating c.1700-1500 BCE" project 10 were selected for testing the CO_2 purification method. The JOINTIME project focused on Bronze Age finds from the Eastern Carpathian Basin with the purpose of creating a high-precision chronology for the 1700–1500 BCE period. In the project strict sampling criteria were implemented, and in the case of cremated bones an effort was made to only pick white-chalky ones with a distinct glassy sound. Additional samples were at least one bronze object in their funerary inventory were preferred. Furthermore, the 10 samples used for the present test were selected from various geographic regions of the Eastern Carpathian Basin, in order to make the results representative for the entire region.

Two grams of bone sample is soaked in a 1.5% sodium hypochlorite solution to dissolve remaining organic material (48 hr, 20ºC). The sample is then washed and submerged in 1 M acetic acid to remove post-depositional carbonates as well as less crystalline, soluble fractions of bio-apatite (24 hr, 20ºC). Subsequently, the sample is washed and dried (12 hr, 80ºC) with a bio-apatite yield of approximately 96% calculated as the weight of the pretreated sample divided with by the weight prior to any chemistry. The pretreated sample is crushed and 1.5 g is treated with 85% phosphoric acid (8 hr, 80ºC) to liberate CO_2. The CO_2 aliquot was then split into two samples; where one being combusted with Sulfix for 2 hr at 470ºC and the other were combusted with CuO and Ag for 3 hr at 900ºC. The Sulfix material is preheated to 500ºC for 2 hr prior to its use. The two differently combusted CO_2 aliquots were then reduced to graphite using hydrogen and Fe as a catalyst and pressed to targets for AMS analysis on the 1MV HVEE Tandetron accelerator (Olsen et al. 2017).

Figure 1 All samples were visually inspected for surface and interior color and burn cracks as exemplified by the selected images (AAR-31620, AAR-31626, and AAR-31627) of the samples used in this study.

FTIR spectra were obtained using an Agilent Technologies Cary 630 ATR-FTIR instrument. Scans were performed in the range from 1500–450 cm[1] with a resolution of 0.5 cm[1]. Each spectrum is an average of 64 scans. The spectra are baseline corrected and the C/P ratio is found using peak heights at 1415 cm[1] and 1035 cm[1], respectively. The CI is calculated after Olsen et al. (2008).

RESULT AND DISCUSSION

From the 10 selected samples two pairs, AAR-31653 and AAR-31654 and also AAR-31656 and AAR-31657, are double burials from two different burial grounds: Voiteni (groapa de împrumut lut, Romania) and Vojlovica (Rafineria nafte-nekropola II, Serbia), respectively (Bukvić 2000; Daróczi 2015; Szentmiklosi 2021). In both instances, the remains of the two cremated individuals were placed in separate urns and the two urns were deposited in a single pit, clearly recognisable by a homogenous, undisturbed fill during the excavation (Daróczi et al. 2023). Therefore, it can be expected that the ^{14}C ages of these samples are identical. Detailed information on all 10 sampled burials has been published, in which the archaeological context and relative chronological value of their inventories has been analysed (Daróczi 2021; Daróczi et al. 2022, 2023).

All samples were visually inspected for burn cracks and showed white interiors suggesting high temperature burning (Figure 1). The sample pretreatment yield ranged between 83.2 to 95.6% (Table 1). The calculated CI range between 5.6 and 7.4 with a mean and standard deviation of 6.1 ± 0.5 confirming that all bone sample were cremated at temperatures above 600ºC (Table 1; Figure 2). Furthermore, the C/P ratios ranging between 0.02 and 0.06 (mean C/P ratio: 0.04 ± 0.01) suggesting a significant loss of carbon compared to unburned bones with a C/P ratio around 0.23 (Garvie-Lok et al. 2004; Olsen et al. 2008).

The CO_2 generated from dissolution in 85% phosphoric acid was split into two aliquots one for CO_2 purification with CuO and Ag and another for CO_2 purification with Sulfix. Hence, if the CO_2 purification methods adds no further carbon to the sample, both CO_2 fractions are expected to be similar within measurement uncertainties. The overall difference between the

Table 1 Preparation yield, FTIR estimated C/P ratios and crystalinity index (CI) together with ^{14}C results with different CO_2 purification methods of either combustion with CuO and Ag or Sulfix. The ΔAge column denotes the difference in ^{14}C years between the purification methods as well as the calculated z-score. For ΔAge the mean difference and standard deviation is calculated to –16 ± 32 ^{14}C years, whereas the weighted mean difference is –13 ± 12 ^{14}C years. The mean and standard deviation of the z-scores is calculated to –0.4 ± 0.8. Further, a reduced χ^2 value of 0.9 is calculated with a χ^2 limiting value of 1.9 (95% confidence).

Lab ID	Name	Yield (%)	C/P	CI	With CuO + Ag ^{14}C age ^{14}C yr BP	With Sulfix ^{14}C age ^{14}C yr BP	ΔAge ^{14}C yr	z-score
AAR-31620	DM 11	83.8	0.05	6.0	3254 ± 32	3290 ± 27	–36 ± 42	–0.9
AAR-31626	DM 16	90.3	0.03	5.7	3043 ± 24	3023 ± 24	20 ± 34	0.6
AAR-31627	DM 17	83.4	0.05	6.5	3231 ± 32	3211 ± 29	20 ± 43	0.5
AAR-31641	DM 31	88.2	0.06	5.9	2820 ± 26	2806 ± 22	14 ± 34	0.4
AAR-31643	DM 33	89.5	0.04	5.7	3181 ± 31	3224 ± 32	–43 ± 45	–1.0
AAR-31653	DM 43	93.6	0.02	6.1	2964 ± 25	2979 ± 24	–15 ± 35	–0.4
AAR-31654	DM 44	83.2	0.05	5.9	2928 ± 25	2972 ± 24	–44 ± 35	–1.3
AAR-31655	DM 45	84.6	0.06	5.6	2993 ± 26	2970 ± 26	23 ± 37	0.6
AAR-31656	DM 46	95.8	0.03	7.4	2973 ± 30	3049 ± 29	–76 ± 42	–1.8
AAR-31657	DM 47	90.4	0.05	6.0	3071 ± 25	3089 ± 23	–18 ± 34	–0.5
							–16 ± 32	–0.4 ± 0.8
							(–13 ±12)	χ^2: 0.9 ≤1.9
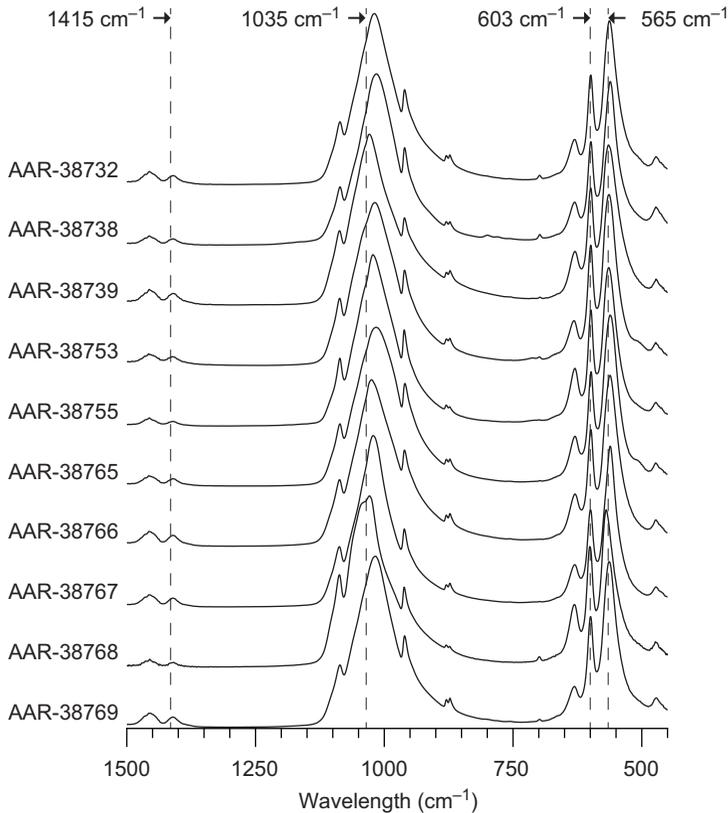
Figure 2   FTIR spectra of all samples used in this study. Marked are wavelength at 1415 and 1035 cm^{-1} representing the vibration bands of CO_3 and PO_4 respectively used to calculate the C/P ratio. The crystallinity index (CI) is a function of the extent of splitting of the two absorption bands at 603 and 565 cm^{-1}.

CuO and Ag and Sulfix CO_2 purification methods are 16 ± 32 ^{14}C years (Table 1; Figure 3). The weighted average difference is calculated to 13 ± 12 ^{14}C years. The differences pass a reduced a χ^2 test (reduced χ^2: 0.9 ≤1.9) suggesting no outliers even though sample AAR-31656 shows a large z-score of 1.8 (Table 1). The reduced χ^2 value of 0.9 further suggest the CO_2 purification method differences to be normally distributed. Hence, the purification test indicates a small but systematic difference between the two purification methods where the Sulfix method appears to be slightly older than the CuO and Ag purification method.

For the double burial (AAR-31653 with AAR-31654) the each of the purification methods can be combined to 2946 ± 18 ^{14}C years BP (χ^2: 1.0 ≤3.8, Cu and Ag) and 2976 ± 17 ^{14}C years BP (χ^2: 0.0 ≤3.8, Sulfix) respectively yielding a CO_2 purification method difference of 30 ±25 ^{14}C years (Table 2). For the double burial AAR-31656 and AAR-31657 the CuO and Ag fractions cannot be statistically combined as the χ^2 test fails (χ^2: 6.3 ≤3.8). However, the Sulfix fractions can be combined to yield a combined ^{14}C age of 3074 ± 18 ^{14}C years BP (χ^2: 1.2 ≤3.8). Considering all 4 ^{14}C ages of the double burial AAR-31656 and AAR-31657 then the CuO
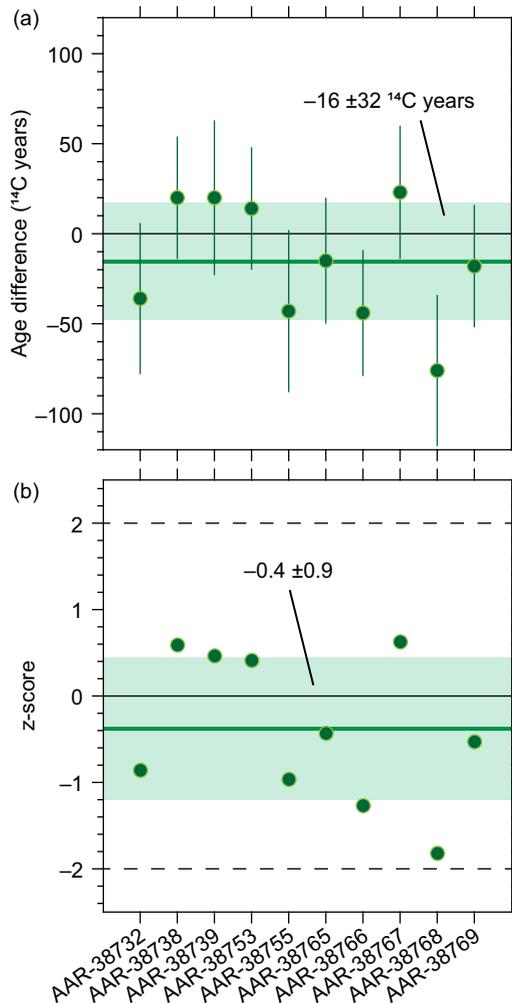
Figure 3   Panel A shows the difference in ^{14}C years between the CuO + Ag and Sulfix CO_2 purification methods. The mean difference between the two methods is calculated to 16 ± 32 ^{14}C years. Panel B shows the calculated z-scores of the difference between the CuO + Ag and Sulfix CO_2 purification methods. The z-scores mean is 0.4 ±0.9 and a χ^2 test indicates that the difference is normally distributed (reduced χ^2: 0.9 ≤1.9).

and Ag fraction of AAR-31656 appears much younger and may thus be considered an outlier. Removing the CuO and Ag of sample AAR-31656 then the double burial AAR-31656 and AAR-31657 yield a CO_2 purification method difference of 3 ± 31 ^{14}C years (Table 2). Using the updated CO_2 purification methods differences the overall difference is calculated to 4 ± 28 ^{14}C years with a weighted average difference of 5 ± 12 ^{14}C years (Table 2). The differences pass a reduced a χ^2 test suggesting no outliers (reduced χ^2: 0.5 ≤1.6). The updated purification test, where the ^{14}C ages of the double burials are combined, indicates a very small if not absent systematic difference between the two purification methods.

Table 2 CO_2 purification test where ^{14}C ages from double burials are combined.

Lab ID	With CuO + Ag	With Sulfix	ΔAge	z-score
	^{14}C age ^{14}C yr BP	^{14}C age ^{14}C yr BP	^{14}C yr	
AAR-31620	3254 ± 32	3290 ± 27	−36 ± 42	−0.9
AAR-31626	3043 ± 24	3023 ± 24	20 ± 34	0.6
AAR-31627	3231 ± 32	3211 ± 29	20 ± 43	0.5
AAR-31641	2820 ± 26	2806 ± 22	14 ± 34	0.4
AAR-31643	3181 ± 31	3224 ± 32	−43 ± 45	−1.0
AAR-31653	2964 ± 25	2979 ± 24		
AAR-31654	2928 ± 25	2972 ± 24		
	2946 ± 18 (χ^2: 1.0 ≤3.8)	**2976 ± 17** (χ^2: 0.0 ≤3.8)	−30 ± 25	−1.2
AAR-31655	2993 ± 26	2970 ± 26	23 ± 37	0.6
AAR-31656	2973 ± 30*	3049 ± 29		
AAR-31657	3071 ± 25	3089 ± 23		
	3071 ± 25	**3074 ± 18** (χ^2: 1.2 ≤3.8)	−3 ± 31	−0.1
		−4 ± 28 (−5 ±12)	−0.1 ± 0.8 χ^2: 0.5 ≤1.6	

CONCLUSION

Recently a study by Rose et al. (2019) pointed out that the use of Sulfix for CO_2 purification could be problematic and might lead to inaccurate ^{14}C results. At Aarhus AMS Centre (AARAMS) we use the Lanting et al. (2001) protocol for sample pretreatment and the reporting of the possible problems with the usage of Sulfix was therefore disturbing news. As part of a larger project, JOINTIME, the laboratory have ^{14}C dated 31 cremated bones. Presented here a test comparing the CO_2 purification methods by selecting 10 samples on which the Sulfix and silver wool and CuO purification methods are compared. Our CO_2 purification test suggest a small and systematic difference between the CuO and Ag and Sulfix CO_2 purification methods with the Sulfix purification method producing ^{14}C ages being on average 13 ±12 ^{14}C years older than the CuO and Ag method. The difference is small and for the majority within measurement uncertainties, i.e., the systematic differences are within ±1σ. Thus, we consider the ^{14}C ages obtained in the JOINTIME project to be valid. Taking into account that some of the chosen samples are double burials and therefore represents the same event. The ^{14}C ages of the double burials where combined and the CO_2 purification method difference were re-calculated yielding a purification method difference of 5 ± 12 ^{14}C years. Rose et al. (2019) reported ^{14}C age differences as large as 200 ^{14}C years, which was overcome by introducing a Sulfix precleaning step to remove the Sulfix carbon contamination. However, for future use we recommend to use a CO_2 purification using CuO and Ag in order to avoid the possible complications associated with Sulfix. Because the supply of the Sulfix material is discontinued by Waco Chemicals Ltd the test presented here shows that other laboratories using Sulfix may use CuO and Ag as an alternative way of removing sulfur compounds evolving during the H_3PO_4 dissolution.

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