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AMS RADIOCARBON DATING OF BONES AT LSCE

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ABSTRACT. In this paper, we explain our routine pretreatment of bone for radiocarbon dating by accelerator mass spectrometry (AMS), based on the specific reaction between amino acids and ninhydrin described by Nelson (1991). The values and uncertainties of the total system background are presented as a function of the carbon sample mass and the reliability of this method is discussed.

INTRODUCTION

Since the first ^{14}C dates were obtained (Arnold et al. 1951), radiocarbon laboratories have developed many methods of bone pretreatment. Usually, these methods are based on the extraction of the bone organic matter. The extract can consist of the whole collagen (Longin 1971; Brown et al. 1988; Hedges et al. 1989; Law et al. 1989; Kretschmer et al. 1998), a mixture of collagen amino acids (Gillespie et al. 1986; Gurfinkel 1987; Long et al. 1989; Redvers-Newton et al. 1994), specific individual amino acids (van Klinken et al. 1990), or non-collagenous proteins (Ajie et al. 1992). These extracts are oxidized to CO_2, then reduced to graphite and dated.

For more than 10 years at the Laboratoire des Sciences du Climat et de l'Environnement (LSCE), we have prepared bone by the method described by Nelson (1991), based on a chemical reaction that extracts CO_2 from carboxylic groups of proteinaceous molecules. This chemical treatment is preceded by elemental analyses (%N, %C, C/N) in order to quantify the bone collagen, and, consequently, to determine if the bone is datable.

In this paper, we describe the protocol of bone preparation. We present the blank values obtained on bones from 2 sites, Sclayn and Gerde. Finally, we discuss the reliability of the ^{14}C ages obtained by this method by comparing some of them to other ^{14}C dates available for the same archeological layers. These samples are either charcoal, burnt bones, or bones treated differently.

MATERIAL AND METHOD

Material

On the basis of porosity, bone may be classified as cortical bone (also known as compact bone) or trabecular bone (also called cancellous or the spongy part). The cortical bone, which is much denser and less porous than the cancellous bone, is preferred for ^{14}C dating since it is generally less altered by diagenesis.

The fossil bones used to estimate the degree of contamination introduced by our protocol come from 2 sites. Five bones were collected in Scladina Cave (Sclayn, Belgium), under a stalagmitic floor in layer 4A, which was dated by thermoluminescence to approximately 100,000 yr ago (Debenham 1998). Another bone comes from layer 2b of Carrière cave (Gerde, France), which is below a stalagmitic floor and dated to 52,500 yr ago by U/Th (Clot 1987).

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Method

The protocol used for the bone treatment is summarized in Figure 1.

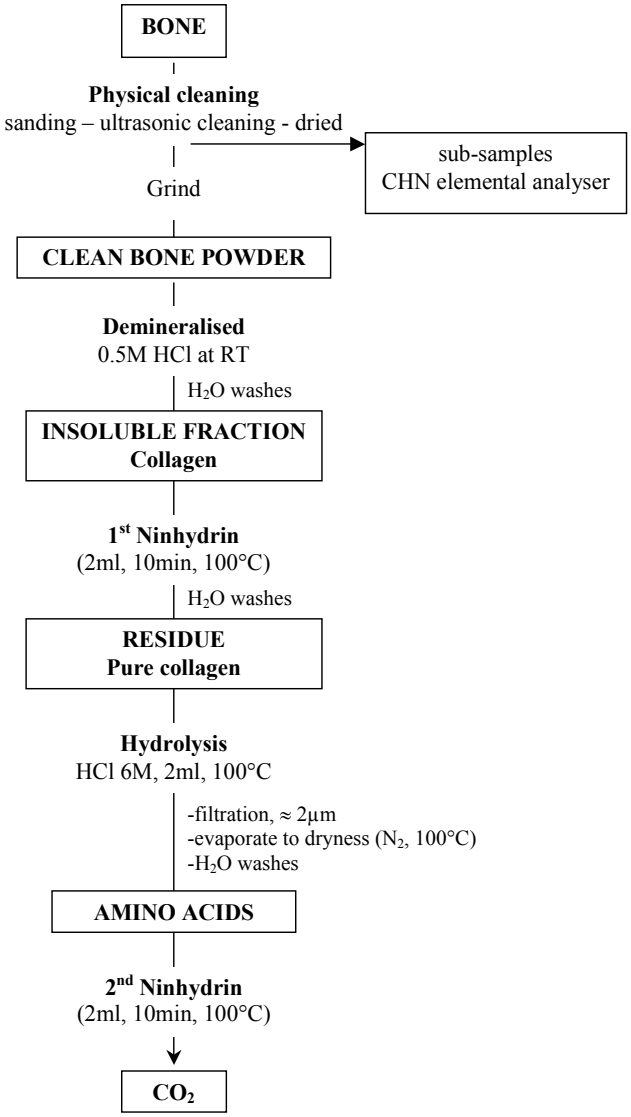


Figure 1 Diagram showing pretreatment steps of bones for AMS ^{14}C dating

Mechanical Treatment and Elemental Analyses

A piece of bone sample (~1–2 cm) is cleaned mechanically with an airbrasive system with 27 µg aluminum oxide to remove superficial contaminants (roots, glue) and the spongy part, which is considered to be the most contaminated.

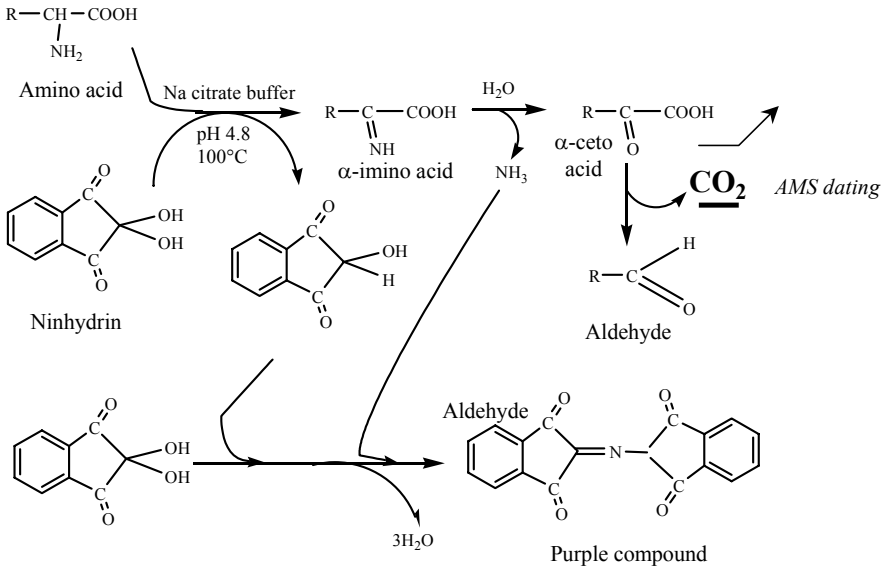
A sub-sample of approximately 5 mg is drilled out and subjected to elemental analysis. It is introduced in a tin capsule into a Carlo-Erba NA 1500 elemental analyzer.

The other part of the mechanically cleaned bone (1–3 g)—if it contains enough collagen—is ultrasonically rinsed in Milli-Q water to remove aluminum oxide and dried at 45 °C. This bone is then finely ground in a planetary micro-mill composed of bowls and balls of zirconium oxide (ZrO_2).

Chemical Treatment

The powdered bone is repeatedly treated with 0.5M HCl and stirred at room temperature to remove carbonates, phosphates, and fulvic acids until the residue becomes colloidal. The acid-insoluble collagenous residue is then rinsed with Milli-Q water until neutral pH is reached.

Next, 50 mg of ninhydrin (2,2-dihydroxy–1,3-indandione) in a 2-ml sodium citrate buffer (pH = 4.8) is added to the residue, which is heated at 100 °C for 10 min. The ninhydrin reacts specifically with the free amino acids, which come from either degraded collagen or contaminants. The ninhydrin reacts with the α-NH_2 of amino acids to give α-imino acids, which react with water to give α-ceto acids (Moore et al. 1950). These α-ceto acids are unstable and release CO_2 from the α-carboxyl group:



The CO_2 is not collected and the residue is rinsed until the solution is decolorized.

Next, this "pure" residue is hydrolyzed to free amino acids with hot acid (HCl 6M at 100 °C overnight). The solution of free amino acids is filtered on a precleaned glass filter and collected in a glass reactor. This filtrate is evaporated at 80 °C under nitrogen. The free amino acid residue is rinsed 5 times with Milli-Q water, which is then evaporated at 80 °C under nitrogen.

The reactor is connected to a vacuum line (Figure 2) and heated to 100 °C with heating coils. Once the vacuum reaches ≈2.10^{-4} mb after ~2 days, 2 ml of ninhydrin solution is injected through a septum. The released CO_2 is dried by passing through 2 "water traps" (–78 °C, mix of dry ice and ethanol), trapped in a liquid nitrogen trap (–196 °C), quantified into the calibrated volume, and then collected in a glass vial. The entire treatment and the CO_2 transformation take more than 8 days.

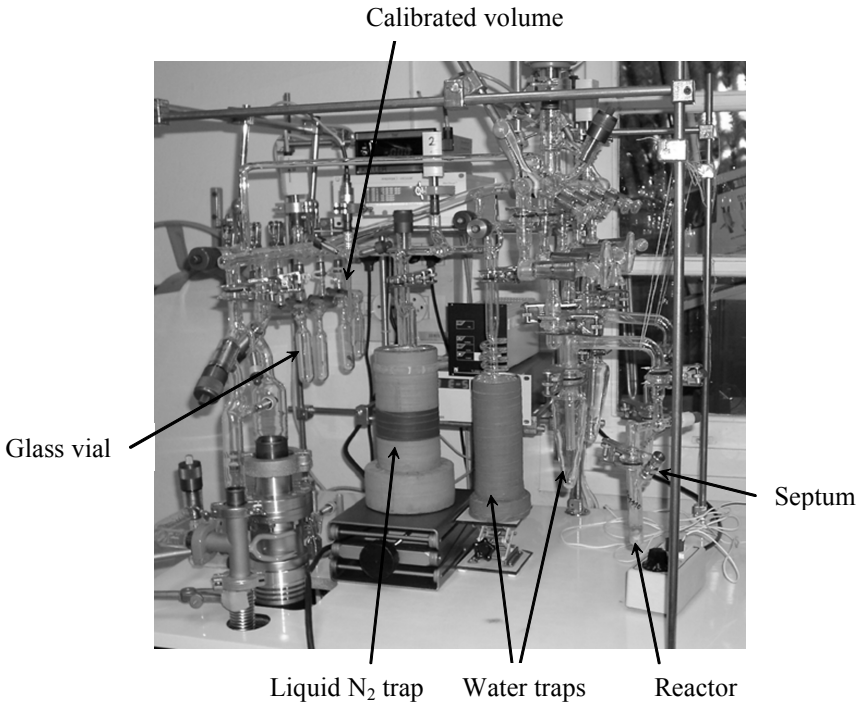


Figure 2 Photograph of the vacuum line

The CO_2 was reduced to graphite (Arnold et al. 1989) and the ^{14}C ages were obtained by accelerator mass spectrometry (AMS) at the Gif-sur-Yvette Tandetron Facility (UMS 2004).

RESULTS AND DISCUSSION

Blanks

Results of Elemental Analyses

Several sub-samples were removed from different parts of the Sclayn and Gerde bones for elemental analyses (%N, %C, C/N). The results are reported in Table 1.

Nitrogen concentrations in the Sclayn bones range from 0.57–2.17%wt, in agreement with previous measurements (Bocherens et al. 1997), and in the Gerde bones from 1.21–2.5%wt. These nitrogen concentrations in the whole bone give some idea of the quantity of collagen (Hedges et al. 1992; Bocherens et al. 1997; Gillespie et al. 1984; Ambrose 1990; Hedges et al. 1995). Indeed, the quantity of nitrogen ranges from about 4% in a fresh bone (Stafford et al. 1988; Ambrose 1993) to below 0.2% in poorly preserved bone, which cannot be dated by the ninhydrin method. With nitrogen amounts ranging from 0.5–2.5%wt, the Sclayn and Gerde bones contain enough collagen for AMS dating.

The scatter of the nitrogen measurements shows that the diagenesis of the organic matter is not homogeneous within any one bone. The C/N ratio of the whole bone can help to estimate the degree of diagenetic alteration. High values (i.e., >5) indicate extensive diagenesis (deamination) or a high proportion of exogenous carbon (humics). For the Sclayn bones, the C/N ratios are statistically similar with a mean value of 4.55 ± 0.4 (n = 14; χ^2; $P_{0.05}$ = 9.95/22.40), excluding the value of the

Table 1 Nitrogen and carbon concentrations in bone, expressed as % of bone weight (%wt) and the atomic C/N ratio of the Sclayn (SC91-) and Gerde blank bones. The results of the underlined line correspond to the analyses carried out on the spongy part of the bone.

Sample	Nitrogen (%wt ± Δ)	Carbon (%wt ± Δ)	C/N ratio
SC91-500G30	1.33 ± 0.14	6.6 ± 0.3	5.0 ± 0.8
	0.67 ± 0.07	3.9 ± 0.2	5.9 ± 0.9
SC91-450F28	0.69 ± 0.07	3.1 ± 0.2	4.4 ± 0.7
	0.57 ± 0.06	3.0 ± 0.2	5.3 ± 0.8
	1.33 ± 0.14	5.2 ± 0.3	3.9 ± 0.6
	1.02 ± 0.11	4.5 ± 0.2	4.4 ± 0.7
SC91-503G30	0.59 ± 0.06	3.0 ± 0.2	5.1 ± 0.8
	0.60 ± 0.06	3.3 ± 0.2	5.5 ± 0.9
	0.60 ± 0.06	5.4 ± 0.2	9.0 ± 1.3
SC91-588F27	2.17 ± 0.22	8.2 ± 0.4	3.8 ± 0.6
	0.65 ± 0.07	3.3 ± 0.2	5.0 ± 0.8
	1.07 ± 0.11	4.6 ± 0.2	4.3 ± 0.7
SC91-619F30	1.12 ± 0.12	4.7 ± 0.2	4.2 ± 0.6
	1.21 ± 0.13	4.9 ± 0.2	4.0 ± 0.6
	0.79 ± 0.08	4.0 ± 0.2	5.1 ± 0.8
Gerde	1.21 ± 0.14	4.7 ± 0.3	3.9 ± 0.7
	2.50 ± 0.29	8.4 ± 0.4	3.4 ± 0.6
	1.66 ± 0.19	6.0 ± 0.3	3.6 ± 0.6

spongy part of sample SC91-503G30/3 (C/N = 9). This high C/N ratio is attributed to the addition of humic contaminants since the nitrogen concentrations are similar within the compact and the spongy parts; the high C/N ratio confirms the importance of removing this porous part of the bone. This mean C/N ratio of Sclayn bones is approximately equal to the fresh bone (5) and shows the good preservation of these bones. For the Gerde sample, the mean C/N ratio is equivalent to 3.58 ± 0.6 (n = 3, χ^2; $P_{0.05}$ = 0.4/5.99), and is slightly lower than the C/N value in Sclayn. This lower value can be explained by a loss of inorganic carbon (decalcification) during burial. The Gerde bone seems less well preserved than the Sclayn bones.

The nitrogen concentrations show that the Sclayn and Gerde bones contain enough collagen for AMS datings and the C/N ratios show their degree of preservation and their non-contamination.

AMS ^{14}C Results

The ^{14}C values of the Sclayn and Gerde bones (Table 2) are presented as a function of the carbon mass in Figure 3. These blank values take into account the chemical pretreatment, the conversion into CO_2, the graphitization, and the machine background contaminants. They increase from 0.10 pMC to 0.80 pMC as the carbon sample size decreases from 2400 µg to less than 300 µg.

Table 2 ^{14}C results of the Sclayn (SC91-) and Gerde bones, reported in pMC

Sample	Mass (μg)	Fraction modern pMC ± 1 σ
SC91-500G30	280	0.60 ± 0.07
	290	0.31 ± 0.03
	950	0.15 ± 0.02
	1760	0.31 ± 0.07
SC91-450F28	1040	0.32 ± 0.03
SC91-503G30	460	0.16 ± 0.02
	460	0.18 ± 0.02
	970	0.66 ± 0.06
	1240	0.37 ± 0.04
	1270	0.24 ± 0.03
SC91-588F27	2390	0.16 ± 0.02
SC91-619F30	390	0.79 ± 0.05
	660	0.28 ± 0.03
	1300	0.17 ± 0.03
	1345	0.16 ± 0.03
	1575	0.19 ± 0.03
	1630	0.22 ± 0.03
	1740	0.10 ± 0.02
	1790	0.18 ± 0.02
	2050	0.10 ± 0.02
Gerde	290	0.42 ± 0.06
	580	0.41 ± 0.05
	990	0.30 ± 0.04

The data indicate a statistically significant mass dependence relationship, as previously reported by several studies of ^{14}C background (Vogel et al. 1987; Kirner et al. 1995; Brown et al. 1997; Schleicher et al. 1998; Tisnérat-Laborde et al. 2001). By using the least-squares method, the best fit between ^{14}C concentrations and the inverse carbon mass is obtained by:

$$y = 119.68\ (\pm\ 28.59)\ /\ x + 0.1295\ (\pm\ 0.0486)$$

where y = ^{14}C concentration (pMC) and x = carbon mass (μg). This increase of the ^{14}C background is due to the addition of 1.3 μg of modern carbon (100 pMC) per mg of sample during the whole process.

All the blank values from Sclayn and Gerde (Figure 3) are consistent, although the C/N analyses showed a lower preservation for the Gerde sample. Such agreement indicates the reliability of our protocol.

These blank values are higher than those obtained from the Carrara marble IAEA C-1, which range from 0.06–0.14 pMC as the size decreases from 2400–300 μg (Tisnérat-Laborde et al. 2001). The contamination during the chemical treatment and the conversion of bone into CO_2 may be responsible for the high blank values since the graphitization and the machine processing are the same for

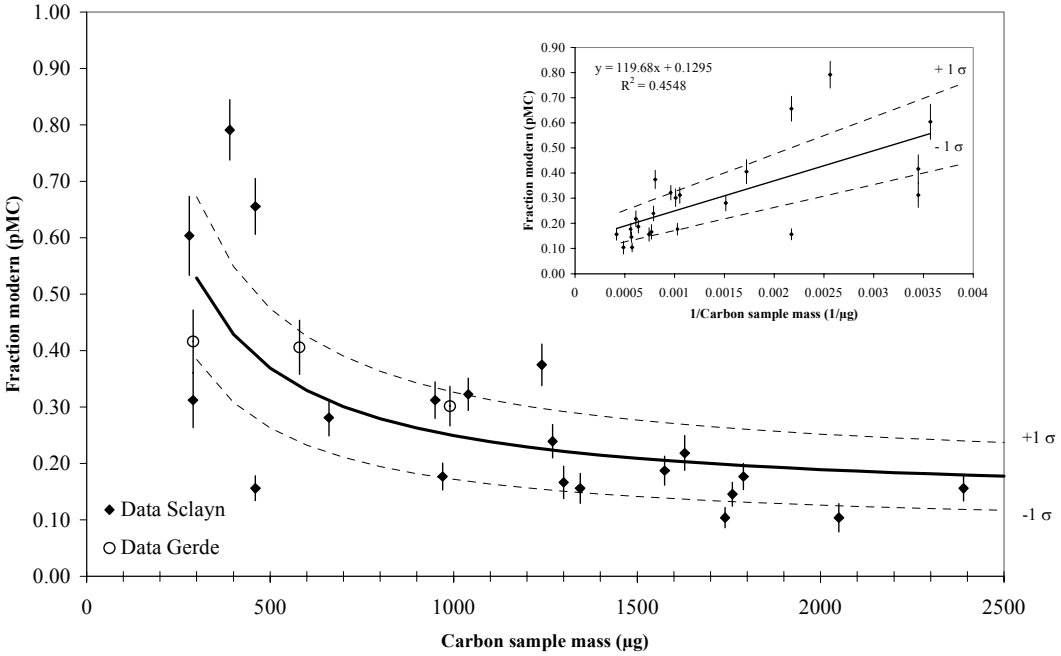


Figure 3 ^{14}C concentration (pMC) as a function of carbon sample mass (µg). The error bars are shown as ±1 σ (68% of overall confidence). The inset small figure is the relation between the inverse carbon sample weight and the ^{14}C concentration (pMC). Dashed lines correspond to the 1 σ error.

these 2 types of sample. We also reject the intrinsic contamination because the same results were obtained for different bones and different sites. We suspect the vacuum line processes before the conversion stage of the amino acids to CO_2 to be responsible for this level of contamination for the following 2 reasons:

1. The pumping is less effective for the bone than for the carbonate (by ~1 order) because the residue quickly becomes pasty under the vacuum;
2. Atmospheric CO_2 may have been introduced during the addition of ninhydrin, either in the form of dissolved CO_2 in the ninhydrin or when the septum was perforated.

The ^{14}C ages of bone are calculated using the blank values determined from the mass dependent equation. From this equation, the age limit is about 50,000 BP (0.2 ± 0.08 pMC) for a carbon mass of 1500 µg, and about 45,000 BP (0.37 ± 0.1 pMC) for a carbon mass of 500 µg.

Reliability of the Method

The ^{14}C ages of bones treated by the ninhydrin method are compared to those obtained for the same archeological layer on associated organic materials (charcoal, burnt bones) and bones treated by other methods. We use the Chi-squared test statistic to check consistency of these determinations (Ward et al. 1978).

In the first test of reliability, 3 sites (Trois-Frères Cave, Laugerie Haute, and Kozarnika) allowed the comparison of ^{14}C dates of ninhydrin-treated bones with those of associated charcoals or burnt bones. These charcoals or burnt bones underwent the classical AAA treatment. The ^{14}C results of the 3 archeological sites are reported in Table 3 and Figure 4. At Trois-Frères Cave and Laugerie-

Haute (Delpech et al. 2001; Roque et al. 2001), the burnt bones and bones have consistent ^{14}C ages. The Kozarnika site (Fontugne et al. 2002) reveals the ^{14}C age of the bone to be slightly older than those of the 2 charcoals, but, nevertheless, statistically in agreement.

Table 3 Comparison between ^{14}C dates from bones (ninhydrin method) and from associated charcoals or burned bones (AAA treatment). All ages are given in ^{14}C yr BP (before 1950). Statistical errors are given at 1 σ.

Site	Lab nr	Material	Age (yr BP)	Error (1 σ)	Chi-squared test	Reference
Trois-Frères Cave	GifA 99552	bone	14210	110	$6.21/\chi^2_{6;\,0.05} = 11.1$	
	GifA 99555	bone	13930	110		
	GifA 99550	burnt bone	14060	110		
	GifA 99551	burnt bone	13980	120		
	GifA 99553	burnt bone	14210	110		
	GifA 99554	burnt bone	14200	120		
		average	**14100**	**50**		
Laugerie Haute	GifA 100634	bone	19550	340	$0.75/\chi^2_{5;\,0.05} = 9.49$	Delpech & Riguad 2001
	GrN-4442	bone	19600	140		Roque et al. 2001
	GrN-4495	bone	19740	200		
	Ly-1173 (OxA)	burnt bone	19525	155		
	GifA 100630	burnt bone	19600	200		
		average	**19600**	**80**		
Kozarnika	GifA 99662	bone	39310	1000	$3.37/\chi^2_{3;\,0.05} = 5.99$	Fontugne & Tisnérat-Laborde, in press
	Gif/LSM-10994	charcoal	38700	1400		
	GifA 101050	charcoal	37170	700		
		average	**38000**	**530**		

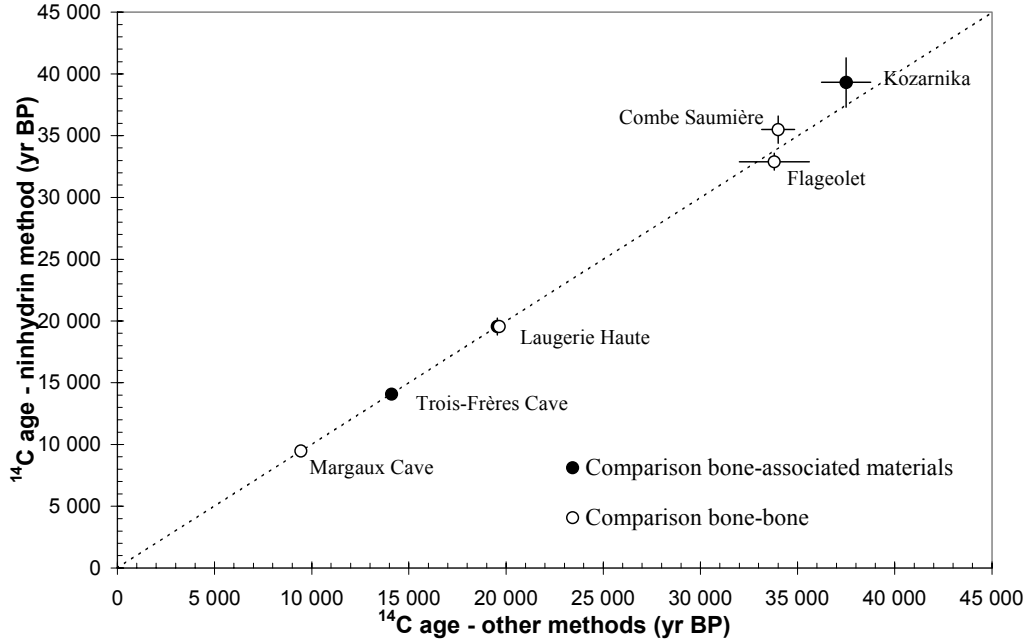


Figure 4 From a same archeological level, the average ^{14}C dates obtained with the ninhydrin method are plotted versus the average ^{14}C ages obtained with the Oxford bone method (open circles) or associated materials (solid circles). The ages are expressed as yr BP. The error bars are shown as ± 1 σ (for 5 of the data points, these error bars are smaller than the symbols). The dotted line is the 1:1 correlation line.

In the second test, we compared the ^{14}C dates of bones treated by different chemical methods. The results are reported in Table 4 and Figure 4. A comparison with the Oxford method (Hedges et al. 1989; Law et al. 1989) may be made for 3 sites: Margaux Cave, Flageolet, and Combe Saumière (Delpech et al. 2001) (Table 4). A comparison with the Groningen procedure can be done for the site of Laugerie Haute (Table 3). In all cases, the ^{14}C ages are similar whatever the chemical treatment, as previously noted by Nelson (1991).

Table 4 Comparison of ^{14}C ages of bones pretreated by the method used by the Oxford Radiocarbon Laboratory (OxA) and by the ninhydrin method (LSCE, GifA). All ages are given in ^{14}C yr BP (before 1950). Statistical errors are given at 1 σ.

Site	Lab nr	Material	Age (yr BP)	Error (1 σ)	Chi-squared test	Reference
Margaux Cave	GifA 92354	bone	9590	110	$5.76/\chi^2_{5;\,0.05} = 9.49$	
	GifA 92355	bone	9530	110		
	GifA 92362	bone	9260	120		
	OxA-3533	bone	9530	120		
	OxA-3534	bone	9350	120		
		average	**9460**	**50**		
Flageolet	GifA 95538	bone	32040	850	$2.87/\chi^2_{3;\,0.05} = 5.99$	Delpech & Riguad 2001
	GifA 95559	bone	34300	1100		
	OxA-598	bone	33800	1800		
		average	**33000**	**630**		
Combe Saumière	GifA 96768	bone	35500	1100	$1.16/\chi^2_{2;\,0.05} = 3.84$	Delpech & Riguad 2001
	OxA-6507	bone	34000	850		
		average	**34560**	**670**		

The 2 tests show the good correlation between the ^{14}C ages of the ninhydrin-treated bones and those of samples collected at the same archeological level (Figure 4) for time intervals ranging between 9000–45,000 yr BP. All these comparisons confirm the reliability and accuracy of the method for dates up to 45,000 yr BP.

CONCLUSION

In this paper, we described our routine protocol of bone pretreatment for AMS ^{14}C dating. This routine is applied to fossil bones containing more than 0.2% of nitrogen in whole bone.

The blank level is a function of the mass of the carbon sample. According to the equation ($y = 119.68 / x + 0.1295$), the blank value is equal to 0.20 ± 0.08 pMC (≥ 50,000 yr BP) for a sample mass of 1500 µg and 0.37 ± 0.10 pMC (≥ 45,000 yr BP) for a sample mass of 500 µg. The contamination by modern carbon is attributed either to the difficulty of degassing the sample or to the introduction of atmospheric CO_2.

The validity of the method and the protocol is tested by comparing the ^{14}C ages obtained on bones by this method and those obtained by other methods (Oxford and Groningen bone methods or associated materials). The satisfactory results of these comparisons and the good estimation of the blank level show the reliability and accuracy of the method for dates up to 45,000 yr BP.

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