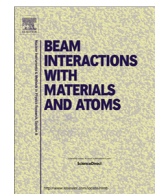




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journal homepage: www.elsevier.com/locate/nimbAMS- ^{14}C analysis of modern teeth: A comparison between two sample preparation techniquesC. Solis^{a,*}, E. Solis-Meza^a, M.E. Morales^a, M. Rodriguez-Ceja^a, M.A. Martínez-Carrillo^b, D. Garcia-Calderon^a, A. Huerta^a, E. Chávez^a^a Instituto de Física Universidad Nacional Autónoma de México, Avenida Universidad 3000, Circuito de la Investigación Científica S/N Ciudad Universitaria, 04510, Mexico^b Facultad de Ciencias Universidad Nacional Autónoma de México, Avenida Universidad 3000, Circuito de la Investigación Científica S/N Ciudad Universitaria, 04510, Mexico

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ABSTRACT

AMS- ^{14}C analysis of modern teeth has become important for forensic studies. ^{14}C content in human teeth reflects the ^{14}C atmospheric concentration during its formation and allows the calculation of the actual year of birth. Through AMS, it is possible to measure the ^{14}C concentrations in a tissue with high precision. However, there is a debate about which should be the best fraction for teeth carbon dating: collagen or enamel. This work focuses on the results obtained from enamel and collagen extracted from Mexican people in order to compare them. Collagen from dental pieces donated from people older than 60-years-old have been included to understand the turnover process and usefulness of collagen to determine the date of birth. Our results indicate that when a single dental piece is available, enamel method allows the determination of the tooth formation date. Dating collagen of the same tooth helps to discriminate if the formation date belongs to the left or the right side of the peak bomb, but also corroborates, the ages obtained through enamel analysis.

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1. Introduction

In Mexico, research in any of the forensic disciplines is extremely scarce. For this reason, forensic experts have frequently resorted to scientific knowledge generated abroad. However, this situation is changing and there is an increasing interest to apply the scientific methods available in Mexico to the study of materials found in criminal scenes, with the aim of having multidisciplinary tools to complement the expert studies and so contribute to the procurement and administration of justice in the prevention of offenses.

Analysis of ^{14}C content in human tissues such as teeth and bones has proved to be a useful way to establish the time elapsed since individual's birth and death. In forensic cases, the usefulness of modern tissues ^{14}C dating comes from the fact that ^{14}C is incorporated into tissues through metabolism. This exchange with the atmosphere stops when the organism dies, so ^{14}C contents within the tissue, reflects ^{14}C level that existed in the atmosphere [1,2].

Cosmic ray interactions with atmospheric ^{14}N produces ^{14}C , which oxidizes to CO_2 and distributes throughout the planet. Before 1950, the ^{14}C content in the atmosphere was more or less

stable [3]. During the so-called nuclear era, atmospheric ^{14}C content increased abruptly due to atmospheric nuclear weapon testing during the 50s and early 60s. From the 1963 peak, after the nuclear test banning, $^{14}\text{CO}_2$ levels have slowly decreased mainly due to exchange with carbon in the oceans and the biosphere [4]. These changes in atmospheric $^{14}\text{CO}_2$ levels are reflected in organisms born after 1950, who assimilate ^{14}C through food chain. Their tissues, which assimilate at different rates, reflect the atmospheric $^{14}\text{CO}_2$ levels, at the time of formation, being then possible to determine their formation date through ^{14}C content. Forensic application is based on the $^{14}\text{C}/^{12}\text{C}$ ratio (normalized to a ^{13}C value) which reflects the atmospheric isotopic ratio at the time when these tissues were formed. This method is called bomb/pulse dating [5]. Teeth are formed by organic and inorganic fractions. Inorganic teeth enamel contains approximately 2–3% of carbon while organic fraction, mainly collagen, has 30% of carbon. Because of their hardness and strength, teeth are frequently the unique useful remaining body parts to determine the date when a person was born. This can be an important step to establish the identity of victims that are classified as unknown because a lack of any other information.

Forensic studies have been focused on determine, through the analysis of ^{14}C in different dental pieces, the year-of-birth of an individual [6–10]. Analysis with Accelerator Mass Spectrometry

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(AMS) is the ideal technique for teeth because of its high precision and the possibility to measure this radionuclide in milligram or less-sized samples [11]. With the AMS facility (LEMA) installed in 2013 at the Physics Institute of the UNAM, it is now possible to perform high precision analyses of radionuclides [12].

Each dental piece has different development periods: crown dentine forms after the enamel and root dentine forms after crown dentine. The time span of the crown and root formation process is well known for each tooth, by what is a good candidate for determine the date of birth of a person [13,14]. How close is the ^{14}C contents in teeth related to that of the atmosphere, depends on the tooth type, if the formation was completed before or after the peak bomb, among other factors. However, there is a debate about which should be the best fraction to be used for dating: collagen or enamel.

Several studies show that, after being formed, enamel is not replaced and ^{14}C content is close to the atmospheric level at the time of crown formation [2,7–10]. Dentine collagen has also been used in a number of studies, since it accounts for a greater amount of carbon than tooth enamel. It also helps in distinguishing the side of the peak bomb where the sample belongs. Nonetheless, collagen's use to determine the year-of-birth seems to be controversial. Some authors base the use of collagen in the fact that racemization experiments have shown that there is no turnover [2,7,15]. However, some others have observed that collagen's carbon continues to recycle even when the tooth is already complete [9,16]. Wang et al. declare that collagen could capture ^{14}C even until the individual's death.

The aim of this work is to compare the dates coming from enamel and collagen analysis. With this purpose in mind, collagen or enamel was extracted from a set of dental pieces from individuals of known ages, in order to calculate the year of complete development. For enamel and collagen extraction from a single piece, third molars were chosen because their high carbon yield. A sample set of collagen from dental pieces donated from people older than 60-years-old whose teeth were extracted between 2014 and 2015, have been included to understand the turnover process and usefulness of collagen to determine the date of birth.

2. Material and methods

All dental pieces were supplied by a dentist and were extracted between 2014 and 2016. They belonged to Mexicans living in Mexico City and San Luis Potosi, two urban cities. Four teeth were chosen for enamel and collagen extraction, nine for collagen and seven for enamel. Each tooth was cleaned with distilled water in an ultrasonic bath and dried at 70 °C for 72 h. Four third molars were chosen for collagen and enamel extraction. Each molar was cut away lengthwise; one-half for enamel and the other half for collagen extraction. Collagen from crown and root was extracted from one tooth half. The other half was cut at the cervical line in order to

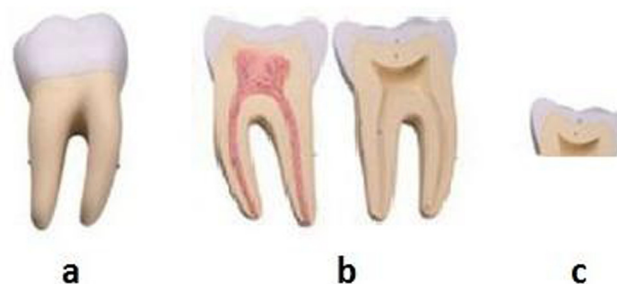


Fig. 1. Diagram showing the teeth fractions used: a) Whole tooth b) One half was used for collagen extraction; c) the crown of the other half was cut at the cervical line in order to extract the enamel.

extract the enamel from the crown (Fig. 1). Enamel was obtained by hydrolysis [7]. Every crown fraction was treated with 7 mL of 10 M NaOH for one week at 50 °C. The sample was placed in a sonic bath at 50 °C, NaOH was replaced every day before the sonic bath. After that week the samples were washed at least three times in 0.25 M HCl followed by several rinses with deionized water until pH = 7. Dentine and other tissues were removed by blunt dissection. Collagen was extracted with a Longin modified method with ultrafiltration. The corresponding half was pulverized by the mortar and pestle. Samples were demineralized with HCl (0.5 M) at 5 °C temperature over 24 h, then, rinsed 3 times. A further acid wash was carried out with HCl (0.2 M), at 76 °C overnight to obtain the gelatin. The supernatant was ultra-filtered and centrifuged and the fraction higher than 30KD (soluble collagen) was lyophilized [17].

Collagen samples containing 1 mg of C were converted to graphite in Automated Graphitization Equipment (AGE III Ion Plus), using a Peltier cooler (−5 °C) to retain the water produced in the reaction. Samples were processed for enamel extraction using a Carbonate Handling System (CHS Ion Plus), each sample reacted with orthophosphoric acid to obtain CO_2 which was graphitized in the AGE III. AMS ^{14}C analyses of graphite pressed in Al cathodes were performed in the HVEE LEMA-AMS system. Oxalic acid standard (NIST SRM4990C Oxalic Acid II) was employed for normalization and blanks (Phthalic acid ($\text{C}_8\text{H}_6\text{O}_4$) and marble (IAEA-C1) with no ^{14}C , were also processed and subtracted to correct for background. Graphite obtained from Phthalic acid gives a $F^{14}\text{C}$ close to 0.0030 ± 0.0001 .

Obtained $^{14}\text{C}/^{12}\text{C}$ ratios were converted to ^{14}C ages using a computer code developed at LEMA [11]. Calibrated ages were obtained with CALIBomb computer code, and the curve IntCal 13 and NHZ2 [18–20]. The ^{14}C results are expressed as a fraction modern carbon ($F^{14}\text{C} \pm 2\sigma$) [21]. In routine operation the normal precision obtained at LEMA for 1 mg of modern carbon is around 0.3%.

Table 1

Fraction modern carbon values in collagen and crown enamel from 4 third molars, calibrated date of formation ranges. Calculations were performed using CALIBomb.

LEMA ID		Year of birth	AFY*	$F^{14}\text{C}$	Calibrated date of formation range (2σ)	CFY**	AFY-CFY
273	Collagen	1936	1958 ± 4	1.107 ± 0.004	1957.8–1958.2	1958.0 ± 0.2	−0.5
273	Enamel	1936	1950 ± 2	0.975 ± 0.003	1934–1954	1944 ± 10	6
223	Collagen	1944	1966 ± 4	1.438 ± 0.007	1973.0–1974.9	1973.9 ± 0.9	−8.4
223	Enamel	1944	1958 ± 2	1.177 ± 0.005	1958.4–1959.1	1958.8 ± 0.4	−0.8
269	Collagen	1995	2014 ± 4	1.044 ± 0.005	≥ 2009.5	≥ 2009.5	
269	Enamel	1995	2009 ± 2	1.030 ± 0.006	2009.4–2009.5	2009.43 ± 0.03	−0.4
399	Collagen	1999	2021 ± 4	1.042 ± 0.004	≥ 2009.5	≥ 2009.5	
399	Enamel	1999	2013 ± 2	1.046 ± 0.004	≥ 2009.5	≥ 2009.5	

*AFY: Actual formation year.

**CFY: Calculated formation year.

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