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INAA of enamel and dentine samples of a group of children and adults: A comparative study

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Instrumental neutron activation analysis (INAA) was applied to determine Ca, Cl, Mg, Mn, Na, Sr and Zn in dentine and enamel tissues from human permanent and deciduous teeth. Comparisons were made between the results obtained in these different dental tissues as well as those obtained in permanent and deciduous teeth. The findings obtained were also compared with the published data. The accuracy and the precision of the results were evaluated by analyzing certified reference material.

Introduction

There is a growing interest in determining trace elements in biological tissues in order to evaluate the roles they play in human organisms. Dental tissues are also analyzed since elements deposited in teeth during the mineralization process are retained to a large extent. Furthermore, tooth analyses are of particular interest to epidemiologists because the rate of growth is well defined together with a fixed temporal reference in deciduous teeth.[1]

The determinations from dental tissues have been performed to study dental health where trace elements have been correlated with the presence of dental caries.[2–4] Teeth have also been reported as an appropriate indicator of toxic element exposure and of eating habits.[5,6] Archaeologists use teeth to study diets and the age of skeletons found during excavation.[7]

There is also some interest in the field of dentistry to use animal teeth instead of human teeth in laboratory practice and chemical tests. Thus, this fact motivates the characterization of elements in human dental tissues to compare these results with those obtained for animals.

The purpose of this study was to verify the difference between trace element composition of permanent and deciduous teeth as well as between that of dentine and enamel tissues. There are analysis data for whole teeth but little existing data available concerning analyses of enamel and dentine separately. These determinations could be important to increase the current knowledge of the elemental composition in each tissue of human teeth and to establish a baseline of concentration values.

To determine trace element concentrations in teeth, several techniques, such as proton induced X-ray emission (PIXE),[1,8,9] inductively coupled argon plasma atomic emission spectroscopy (ICP-AES),[10,11] X-ray fluorescence (XRF),[12,13] neutron activation analysis (NAA)[13] and flame and electro-thermal atomic absorption spectrometry (FAAS and ETV-AAS)[11] are being used.

In this study instrumental neutron activation analysis (INAA) was applied to determine Ca, Cl, Mg, Mn, Na, Sr and Zn. INAA constitutes an advantageous method for dental tissues analysis as it enables simultaneous multi-element determinations and does not require sample dissolution.

Experimental

Samples

A total of 10 permanent and 8 deciduous teeth were supplied by dental clinics. These teeth were extracted for clinical purposes not related to this study. Permanent teeth were removed for orthodontic reasons while the deciduous teeth had been shed as result of physiological second dentition. The Ethics Committee, of the Instituto de Pesquisas Energéticas e Nucleares (IPEN) approved this research. All samples analyzed were from healthy teeth from individuals living in a coastal city of Santos, Brazil. After extraction each tooth was rinsed in tap water and then the soft tissue of gum was removed. The enamel was separated from the dentine mechanically by the dentist using appropriate tools. All the precautions were taken to avoid sample contamination.

After separating, each sample was placed in a Nalgene polyethylene vial for cleaning using purified water and under a shaker during 5 minutes. Then the sample was rinsed with acetone (p.a., Merck) to remove the water, allowing it to dry faster. Next the sample was placed over a filter paper and left for about 24 hours in an oven at 40 °C for drying.

For the analyses, each sample was ground to powder using an agate mortar. About 30 to 150 mg of each sample were weighed in clean polyethylene involucres and heat-sealed. These polyethylene involucres were manufactured using polyethylene foils previously cleaned using diluted nitric acid solution and purified water.

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Standards

Certified standard solutions of elements provided by Spex Certiprep, USA were utilized to prepare synthetic standards of elements. Aliquots of these solutions were pipetted onto small sheets of Whatman No. 40 filter paper, and after drying at room temperature, these sheets were folded and placed in clean polyethylene involucres and also heat-sealed. Elements Na and Br were detected at very low concentrations in the filter paper and their contributions in the synthetic standards could be considered negligible. The amounts of the elements used for irradiation were (in μg): Ca = 500; Cl = 500.0; Mg = 998.9; Mn = 10.04; Na = 100.0; Sr = 501.2 and Zn = 50.0.

Procedure for neutron activation analysis

Samples and synthetic standards of elements were irradiated at the IEA-R1 nuclear reactor. Fifteen-second irradiations under thermal neutron flux of $2 \cdot 10^{12}$ n·cm^{-2}·s^{-1} were used for Cl, Mg, Mn and Na determinations. Eight-hour irradiations under thermal neutron flux of $5 \cdot 10^{12}$ n·cm^{-2}·s^{-1} were carried out for Ca, Na, Sr and Zn determinations. Elements such as Fe, Rb and Se were not determined due to the interference of a large bremsstrahlung background from $^{31}P(n,\gamma)^{32}P$. The corresponding radioisotopes of these elements could not be detected in the gamma-spectra. After adequate decay times, the irradiated samples and standards were measured by a hyperpure Ge detector Model GEM20190 from EG & G Ortec coupled to a gamma-ray spectrometer. The resolution (FWHM) of the system was 0.90 keV for 122 keV gamma-ray peak of ^{57}Co and 1.87 keV for 1332 keV gamma-ray peak of ^{60}Co. Each sample and standards were measured at least twice for different decay times. Counting times from 200 to 50,000 seconds were used, depending on the half-lives or activities of the radionuclides considered. The gamma-ray spectra were processed using an appropriate computer program that evaluates peak area (counting rates) and gamma-ray energies. The radionuclides measured were identified according to their half-lives and gamma-ray energies. The concentrations of elements were calculated by a comparative method. The area under peaks corresponding to the gamma-rays of ^{47}Ca at 159 and 1296 keV, ^{38}Cl at 1642.7 keV, ^{27}Mg at 843.7 and 1014.4 keV, ^{56}Mn at 846.7 and 1810.7 keV, ^{24}Na at 1368.6 keV, ^{85}Sr at 514.0 keV and ^{65}Zn at 1115.6 keV were used.

The quality of the analytical results was evaluated by analyzing the certified reference material (CRM), NIST 1486 Bone Meal provided by the National Institute of Standards and Technology (NIST), USA. This reference material was analyzed by applying the same experimental conditions used in tooth analyses. The element concentrations of reference material were evaluated on a dry weight basis.

Results and discussion

Table 1 presents the results obtained in the analyses of certified reference material NIST 1486 Bone Meal together with its certified values.[14] The results agree the certified values with relative errors lower than 10.2% and they also presented good precision with relative standard deviations varying from 4.8 to 9.9%. The standardized difference or *Z*-score values[15] obtained are presented in Table 1 and they were $|Z\text{-score}|<2$, indicating that the results are satisfactory and agree with the certified values.

Results obtained in dentine and enamels from permanent healthy teeth are presented in Tables 2 and 3, respectively. In these tables the literature values are also listed for comparison's sake. For most of the elements of our results are of the same magnitude of published data with exception of Mn. Results obtained for Mn were slightly lower than the published values. Comparisons between the results obtained in dentine and enamel tissues of permanent teeth showed statistically significant difference for Ca, Mg and Na (Student's *t*-test, for $\alpha = 0.05$). Highest concentrations of these elements were found in the enamel.

Table 4 presents the results for dentine and enamel tissues of deciduous teeth. Unfortunately element concentrations in the literature for deciduous teeth are scarce and comparisons with our results were not possible. In the case of deciduous teeth, the Cl, Mg, Mn, Na and Zn concentrations in enamels were different of those obtained in dentine samples, while there were no significant differences for Ca and Sr (Student's *t*-test, for $\alpha = 0.05$).

Element concentrations in dentine tissues from permanent and deciduous teeth, as well as, in enamels from permanent and deciduous teeth were compared by the Student's *t*-test ($\alpha = 0.01$). There were no significant differences between element concentrations found in dentine of permanent and deciduous teeth except for Ca and Na. For enamel tissues the element concentrations of Cl, Na and Sr of permanent teeth presented differences from those obtained for deciduous ones.

Table 1. Analysis of certified reference material NIST 1486 Bone Meal

Element	Mean ± SD*	RSD, %	Er, %	*Z*-score	Values of certificate[14]
Ca, %	26.05 ± 1.26	4.8	2.0	–0.41	26.58 ± 0.24
Cl, μg·kg^{-1}	352 ± 34	9.6	–	–	–
Mg, %	0.494 ± 0.041	8.3	6.0	0.63	0.466 ± 0.017
Mn, mg·kg^{-1}	1.01 ± 0.10	9.9	–	–	(1)**
Na, %	0.46 ± 0.04	8.7	–	–	(0.5)
Sr, μg·kg^{-1}	291.1 ± 24.8	8.5	10.2	1.2	253.7 ± 19.2
Zn, μg·kg^{-1}	134.9 ± 8.1	6.0	8.2	–0.67	147 ± 16

* Mean ± SD = Arithmetic mean and standard deviation.
** Numbers in parentheses are informative values.
RSD = Relative standard deviation.
Er = Relative error.

Table 2. Element concentration in dentine tissues of permanent teeth

Element	This work			Literature values		
	Mean ± SD (*n*)*	Median	Range	CHAUDHRI and AINSWORTH[9]	CARVALHO et al.[16]	CARVALHO et al.[12]
Ca, %	21.40 ± 1.79 (10)	21.50	18.47–24.91	32.0	4.658–26.300	–
Cl, mg·kg^{-1}	798.9 ± 251.0 (10)	773.7	318.1–1193.7	2500–2600	779–4360	–
Mg, %	0.898 ± 0.457 (10)	0.82	0.28–1.75	0.300–0.800	–	–
Mn, mg·kg^{-1}	0.647 ± 0.184 (7)	0.60	0.42–1.01	5–10	–	2.4 ± 1.7
Na, %	0.532 ± 0.065 (10)	0.53	0.41–0.69	0.200–0.230	–	–
Sr, mg·kg^{-1}	174.2 ± 118.7 (10)	116.71	63.76–466.20	110–150	45–190	–
Zn, mg·kg^{-1}	174.0 ± 31.9 (10)	183.85	120.36–216.84	200–700	154–684	212 ± 24

* Arithmetic mean and standard deviation, *n* number of individuals.

Table 3. Element concentration in enamel of permanent teeth

Element	This work			Literature values		
	Mean ± SD (*n*)*	Median	Range	CHAUDHRI and AINSWORTH[9]	CARVALHO et al.[16]	CARVALHO et al.[12]
Ca, %	31.20 ± 4.76 (10)	31.48	22.59–41.16	35.70	13.960–29.700	–
Cl, mg·kg^{-1}	6572 ± 2347 (10)	6109	3675–11539	1600–2800	167–2890	
Mg, %	0.308 ± 0.194 (8)	0.267	0.059–0.600	0.100–0.110	–	
Mn, mg·kg^{-1}	1.490 ± 1.254 (10)	1.202	0.399–4.787	5–25	–	2.3 ± 2.1
Na, %	0.649 ± 0.084 (10)	0.663	0.464–0.815	0.170–0.260	–	–
Sr, mg·kg^{-1}	285.8 ± 181.7 (10)	212.5	72.7–731.0	100–150	54–140	
Zn, mg·kg^{-1}	202.6 ± 124.1(10)	172.1	123.0–550.9	50–150	124–332	209 ± 15

* Arithmetic mean and standard deviation, *n* number of individuals.

Table 4. Element concentration in dentine and enamel tissues of deciduous teeth

Element	Dentine			Enamel		
	Mean ± SD (*n*)*	Median	Range	Mean ± SD (*n*)*	Median	Range
Ca, %	25.0 ± 2.2 (8)	24.4	22.1–29.04	29.0 ± 5.2 (8)	29.2	19.7–37.8
Cl, mg·kg^{-1}	902 ± 557 (8)	682	333–2091	4297 ± 829 (8)	4722	2919–5119
Mg, %	0.90 ± 0.16 (8)	0.87	0.67–1.22	0.46 ± 0.11 (8)	0.46	0.32–0.70
Mn, mg·kg^{-1}	0.69 ± 0.24 (6)	0.73	0.30–0.95	1.49 ± 0.50 (8)	1.35	0.91–2.31
Na, %	0.41 ± 0.06 (8)	0.40	0.33–0.54	0.54 ± 0.05 (8)	0.56	0.46–0.65
Sr, mg·kg^{-1}	92.1 ± 25.4 (8)	80.6	63.2–127.5	84.8 ± 16.7 (8)	77.45	63.0–115.0
Zn, mg·kg^{-1}	155.9 ± 35.0 (8)	149.2	117.4–216.3	103.4 ± 11.2 (8)	100.6	87.4–118.6

* Arithmetic mean and standard deviation, *n* number of individuals.

Conclusions

In this preliminary study of tooth tissues analysis it was proved that there are differences between element concentrations present in permanent and deciduous teeth. Element composition of enamel tissues also differs from those of dentine.

The number of teeth analyzed in the present study was small due to the fact that it is difficult to obtain this type of sample in a short period of time. Further study is needed to define baseline values of element concentrations present in healthy teeth.

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