Chemical composition of human enamel and dentin. Preliminary results to determination of the effective atomic number

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Abstract. The theoretical or practical dosimetry involving radiation interactions in humans needs the reliable elemental composition data of body tissues. The object of this research was to obtain the characterization dental hard tissues and to determine its effective atomic number. An analytical research of inorganic composition, from 30 intact human molars, extracted for periodontal reasons, was performed by Neutron Activation Analysis (NAA), ICP/AES, Thermogravimetric (TG) and Differential Thermal Analysis (DTA). The coronal dentin and enamel were separated by two techniques: (1) - mechanically by chipping and breaking by chirurgic hammer, allowed to dry in an electric oven for 5 hours at 160^0C. (2) - through by high-running round steel burs. The samples were thoroughly cleaned with distilled deionizer water and sent for analysis in CDTN/CNEN laboratories, Belo Horizonte, Minas Gerais, Brazil. The results showed concentrations of 11 elements measured in dentin and enamel. The five elements of the higher concentration by neutron activation analysis and ICP/AES were Ca, P, Na, Mg and Al. Thermogravimetric analysis of enamel showed a loss of water of hydroxyapatite to 500^0C. Thermogravimetric analyses of dentin showed tree temperatures at which mass loss occur. These processes are related to superficial water loss (100^oC); organic decomposition and water liberation from hydroxyapaptite (100^oC to 600^oC); and the beginning of hydroxyapatite decomposition (600^oC to 850^oC). Differences, in mineral concentration, were found between enamel and dentin, with higher concentrations in enamel. The two techniques proposed to separated dentin and enamel, no presented differences in elements concentration, statement that the high-running round steel burs technique didn't affected the samples.

KEYWORDS: Dental materials, Dentin, enamel, Thermal analysis, nuclear analysis, dosimetry

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1. Introduction

With the evolution diagnostic process by image, the development of the quality assurance programmers is today, the best expressive of the evolution and effectiveness in the radiological area, possibiliting a more precise and accurate diagnostic formulation. Certain liquid and solid materials have been used in experimental radiation for investigation radiation effects within and around irradiated human tissues. These materials have been called “tissue-equivalent materials” and for a material to be acceptable as a tissue substitute for photons and electrons the radiation absorption and scattering obtained with a given thickness or mass of the material must be the same as that experienced in a similar thickness or mass of tissue (WHITE, 1978). A convenient way of comparing the radiation characteristics of a tissue and tissue equivalent material is to consider either photon mass attenuation coefficient and mass energy absorption coefficient or the effective atomic number (KUMAR e REDDY, 1997).

Evaluation of the effective atomic number of a mixture/compound involves of the cross section and finding out the equivalent element that has the same cross section at that energy (WHITE, 1978). The accurate data on photoelectric cross sections as well as scattering cross sections of individual elements are available and this method gives effective atomic numbers to an accuracy of about 1% (KUMAR e REDDY, 1997). Such investigations have been carried out by RAO et al. (1985) and PARTHASARADHI et al. (1992) in the low energy region and PARTHASARADHI et al. (1989) in the high energy region.

The theoretical or practical dosimetry involving radiation interactions in humans needs the reliable elemental composition data of body tissues. The object of this research was to obtain the characterization dental hard tissues and to determine its effective atomic number.
Enamel is the hardest biological substance of human body and comprises 92% hidroxyapatite, 6% water and 2% organic matter, while dentin composition is 47% Hidroxyapatite, 30% organic matter and 23% water. According to OKTAR (2007) few early investigations focused in the understanding of basic proprieties of enamel’s hidroxyapatite with the aid of X-ray diffraction or thermal analyses, or in the determination of important features for plasma spraying, etc.

According to SMITH et al., 1984, there have been a few reports of multiple-element analysis of human teeth and none involves the separate analysis of dentin and enamel. Your study was undertaken to develop a method for separating the enamel and dentin of human teeth before analysis for trace metals or fluoride, and to minimize the risk of sample contamination. The sample teeth were weighed and dissolved the various layers of the tooth with the 0.6M “suprapur” nitric acid. The analysis of metals and boron used an ARL ICPQ-137 inductively-coupled plasma spectrometer under standard operating procedures.

Analysis of elements in normal human dentin was determined by SOREMARK and SAMSAHL, in 1962, by Gamma-Ray Spectrometric. According to authors, this method has been found to be very sensitive and quite adequate for many kinds of biologic materials. The coronal dentin was separated from enamel mechanically by chipping and breaking by means of glass and polythene- covered instruments. The samples were allowed to dry in an electric oven for 48 hours at 105^{o}C. The reproducibility of the methods used was very good and reported the concentration of eleven trace elements in dentin.

The concentrations of twelve different elements of primary and permanents teeth, collected in six different localities in Finland were related by LAKOMAA e RYTÖMAA, 1977. The crowns were ground to a fine powder in an agate mortar, and enamel and dentin were separated by flotation method. The Na, Cl, Al, Mn and Ca were determined by neutron activation analysis in a Triga Mark II reactor at Otaniemi (Reactor Laboratory, Technical Research Center of Finland). μ-activity was measured with an Ortec Ge(Li)-detector.

HOLAGER, 1970, used the themogravimetry analyze to structure dental investigation. Enamel and dentin were separated by grinding with a diamond wheel and then were pulverized in a steel mortar.

Two samples of enamel and three samples were used. The termobalance, according the authors, represent a tool for qualitative and quantitative examination of some components of human dental enamel and dentin, particularly of the carbonates. This analyze generally gives an automatic, continuous recording of weight of solid matter during thermal reaction. Within the range of the present termobalance, about 20 to 1050^{o}C, three distincts slopes had been found which represent water, protein and eventually crystalline water and an unknown carbonate phase, respectively.

In 1972, HOLAGER realized an investigation for to check the results of the before study because there was a doubt about the carbonate phase. The termobalance used gave results about tooth material that agree with an earlier experimental with water, water from crystallization, protein and a possible new phase at about 750^{o}C.

2. Materials and Methods

2.1 Samples tooth

The material analyzed consisted of 30 human molars (intact, impacted third molars) extracted for periodontal reasons. The teeth were cleaned thoroughly distilled deionizer water and kept in the freezer at 18^{o}C negative. The coronal dentin and enamel samples were weighed and sealed in polythene bags to minimize the risk of sample contamination. The coronal dentin and enamel were separated by two techniques: (1) - mechanically by chipping and breaking by chirurgic hammer, allowed to dry in an electric oven for 5 hours at 160^{0}C, according SÖREMARK & SAMSAHL, 1962 and study of OKTAR, 2007; (2) - through by high-running round steel burs (KG Sorensen) according, 1970, 1972. Then the samples were pulverized in a glass mortar; contamination of these two substances was avoided.

The samples were cleaned with distilled deionizer water and sent for analysis in CDTN/CNEN laboratories, Belo Horizonte, Minas Gerais, Brazil.

2.2 Methods of Analysis

The analytical research of inorganic composition was performed by Neutron Activation Analysis (NAA), Inductively-coupled plasma spectrometer (ICP/AES), Thermogravimetric (TG) and Differential Thermal Analysis (TGA/DTA).

The technique applied Instrumental Neutron Activation Analysis (INAA) was used to determine the chemical composition of the samples. This technique is based on the bombarding with reactor neutrons a sample and its chemical elements are then identified and analyzed by measurement of the gamma radiation emitted by radio nuclides formed in the (n,γ) reaction. INAA does not require any chemical processing either during preparation or during the determination of the elemental concentration. Currently is used the k_0-method (De CORTE, 1986; MENEZES et al., 2003) the most powerful instrumental method, were comparators or monitors are used to calculate the analytical concentrations instead of standards of the analyzed elements.

An Inductively-coupled plasma spectrometer (ICP/AES) was used, under standard operating procedures. The calibration curve used Ca, P and Mg elements. The Ca and P concentrations in the calibration curve were 100, 200, 300, 400 e 450 mg/L. The Mg concentration in the calibration curve were 1, 2, 3, 5 e 10 mg/L. The wave length used was 318.12 nm (Ca); 213.61 (P) and 285.21 (Mg).

The termogravimetry analyze is continuous recording of the weight of solid matter during thermal reaction. The equipment TA instruments, model SDT 2960, was used in the analyze. The temperature range is approximately 20 to 900^{o}C in 10mg of the sample. By examination of the registered curve (where loss is a function of temperature or time) conclusions were draw.

3. Results

Concentrations of different elements in enamel and dentin of teeth are given in Table 1 and 2. The mean values computed for the whole samples were compared with element concentrations reported by other authors.

In the neutron activation analyze, the samples were simply weighed in suitable polyethylene vials and irradiated in the research nuclear reactor TRIGA MARK I IPR-R1, located at CDTN/CNEN, 100kW power. Under an average thermal neutron flux of 6.6 x 10^{11} neutrons cm^{-2} s^{-1}, the samples were irradiated for 5 minutes, which objective was to determine Al, Na, Cl, W which radionuclide is a short half-life radionuclide. The samples were re-irradiated for 4 hours and for 8 hours, to determined medium half-life radionuclides – Ca, Na, W - and long half-lives – Ta, Co, Ca and Zn - respectively. After irradiation and decay time, the gamma spectrometry was carried out using a gamma detector HPGe with 15% nominal efficiency. The elemental concentration was performed using the k_0-method.Quantitative data on the concentrations ± standard deviation were tabulated.

The quantitative data obtained from analyze with ICP/AES determined the concentration of Ca, Mg and P. In the TGA/DTA identical qualitative results were found for enamel and dentin; in both there are three definite slopes at about 100, 400 and 800^{o}C.

Table 1: Experimental results and consulted in the literature

	Elements	Present study* (%)						Literature (%)				
		INAA		ICP/AES		TGA/DTA		INAA		ICPQ-137	TGA	
	Tecnics	1	2	1	2	1	2	Lakamaa and Rytomaa (1977)	Soremak (1962)	Smith et al. (1984) (mg/g)	Holager (1972) Flow	
											Air	CO_2
Dentin	Ca	23 ±1	24,8 ±1	26.35 ±0,5	23.10 ±0,5			25.1 ±1.1	28.2 ±1.2			
	Na	0.616 ±0.01	0.599 ±0.01					0.54 ±0.05	0.75 ±0.21	4870		
	Mg			0.55 ±0,03	0.69 ±0,03			0.85 ±0.06		4670		
	Al	0.026 ±0.001	0.019 ±0.001					0.00015 ±0.005				
	Cl	0.041 ±0.01	0.036 ±0.01					0.072 ±0.022	0.39 ±0.11			
	Zn	0.018 ±0.001	0.013 ±0.001					0.000126 ±21	199ppm ±78.1	100		
	Ta	0.005 ±0.0001	0.003 ±0.0001									
	Co	0.004 ±0.0001	0.016 ±0.0001									
	W	0.016 ±0.001	0.062 ±0.001						2.6ppm ±1.1			
	P			12.65 ±0,5	10.98 ±0,5				13.5 ±2.8			
	(Ca:P)					1.95	2.18	1.80 ±0.19				
	H_20					4.24	4.09				9.65	8.25
	CO_2					5.67	4.16				3.35	5.05
	PO_4					47.40	53.82					
	Mat Org					18.02	13.98				21.3	16.1

*****mean of 4 samples

Table 2: Experimental results and consulted in the literature

	Elements	Present study* (%)						Literature (%)			
		INAA		ICP/AES		TGA/DTA		INAA	ICPQ-137	TGA	
	Tecnics	1	2	1	2	1	2	Lakamaa and Rytomaa (1977)	Smith et al. (1984) (mg/g)	Holager (1972)	
										Flow	
	Ca	35 ±1	35 ±1	36.8 ±0,5	36 ±0,5			34 ±2.0		Air	CO_2
	Na	0.77 ±0.01	0.75 ±0.01					0.69 ±0.03	5490		
	Mg			0.2 ±0,01	0.2 ±0,01			0.28 ±0.07	1670		
	Al	0.03 ±0.001	0.02 ±0.001					0.00024 ±80			
	Cl	0.33 ±0.01	0.41 ±0.01					0.32 ±0.01			
	Zn	0.02 ±0.001	0.02 ±0.001					0.00018 ±40	104		
Enamel	Ta	0.003 ±0.0001	0.0002 ±0.0001								
	Co	0.001 ±0.0001	0.02 ±0.0001								
	W	0.005 ±0.001	0.07 ±0.001								
	P			18.2 ±0,5	17.5 ±0,5			18.6 ±0			
	(Ca:P)					1.97	2.03	2.01			
	H_20					0.22	0.80			1.7	1.4
	CO_2					1.42	1.48			2.6	1.6
	PO_4					58.32	58.18				
	Mat Org					4.14	4.04			3.45	2.5

*mean of 4 samples

4. Discussion

Comparison with earlier studies

The concentrations of most elements were found to be higher in enamel than dentin, mainly Ca, Na, Cl, Zn and P which are in agreement with data previously reported in the literature, in studies of SOREMARK and SAMSAHL (1962), LAKOMAA and RYTOMAA (1977) and SMITH et al. (1984). This fact is not surprising due the high organic content of dentin, evidently because the minor ionic components are often incorporated into the apatite crystal structure.

In agreement with those studies SOREMARK and SAMSAHL (1962), LAKOMAA and RYTOMAA (1977), the enamel and dentin present not much different in mineral composition. The Mg concentration in dentin is higher than enamel in agreement with a study of LAKOMAA and RYTOMAA (1977) and SMITH et al. (1984). According to authors this data is due because Mg does not fit easily into the lattice of hidroxyapatite and the dentin is more porous than enamel, thus enabling the systemic introduction of elements. Mg is able to substitute easily for calcium in the structure of the tooth, which is primarily hidroxyapatite.

The high concentration of W, Ta and Co can so far not be explained. SOREMARK and SAMSAHL (1962) related to the presence of W in their study. Maybe, procedures of preparing the samples and biological variations can be associated with these present elements.

A comparison between the present study and the other authors showed that the qualitative results were similar in TDG/DGA analyze and there were three definite slopes within the range at about 20 to 900^{o}C. The first slope of both samples, about 100^{o}C is considered evaporating water. In enamel, the weight loss measured from 20 to 200^{o}C is 0.22 % (tecnics 1) and 0.80 % (tecnics 2). The literature gives 1.7% and 1.4% (HOLAGER, 1970, 1972). In dentin, the weight loss measured 4.24% (tecnics1) and 4.09 % (tecnics 2). The literature gives 9.65% and 8.25% (HOLAGER, 1970, 1972). The second slope of both samples, about 300^{o}C is considered to represent decomposition of organic matter and crystalline water. In enamel, the weight loss measured from 200 to 600^{o}C is 4.14 % (tecnics 1) and 4.04 % (tecnics 2). The literature gives 3.45% and 2.5% (HOLAGER, 1970, 1972). In dentin, the weight loss measured 18.02 % (tecnics1) and 13.98 % (tecnics 2). The literature gives 21.3% and 16.05% (HOLAGER, 1970, 1972). The third slope of both samples, about 800^{o}C represents a carbonate phase. In enamel, the weight loss measured from 600 to 900^{o}C is 1.42 % (tecnics 1) and 1.48 % (tecnics 2). The literature gives 2.6% and 1.6% (HOLAGER, 1970, 1972). In dentin, the weight loss measured 5.67 % (tecnics1) and 4.16 % (tecnics 2). The literature gives 3.35% and 5.05% (HOLAGER, 1970, 1972).

5. Conclusion

The analyses through INAA, ICP/AES and TGA/D allowed for the identification of the element composition present in dentin and enamel. These analyses are a tool for the quantitative and qualitative determination of components of human dental which are necessary for the evaluation of the effective atomic number.

The results of this study show the advantages in analyzing the enamel and dentin separately. It was possible to identifying the composition of these portions in human teeth and the difference in the mineral and organic composition.

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