Infrared Absorption Bands of Enamel and Dentin Tissues from Human and Bovine Teeth

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ABSTRACT

The chemical components present in the hard tissue such as water, phosphate, carbonate and organic material strongly absorb infrared radiation. The research in dentistry area has a fundamental interest in identifying the chemical origin of all the bands present in the infrared region to study the chemical and thermal action on the hard dental tissues. To improve this technique it is important to identify chemical alterations in the tissues and characterize their properties in a more natural form. Transmission and reflection spectra in the infrared range from $2.5\,\mu m$ to $25\,\mu m$ of the enamel and dentin tissues from human and bovine teeth were acquired. Sliced samples were used for the transmission spectra, polished blocks and natural surfaces for the reflection spectra. Tissue spectra were registered between 4000 and $400\,cm^{-1}$ in a FTIR Spectrometer. No differences were found between the mineral matrix of human and bovine tissues, only differences in intensity among bands were observed. Regarding the organic matrix, the bands observed in the dentin were not observed in the enamel tissue and, as the same, the bands observed in the enamel tissue were not observed in the dentin tissue with a significant intensity. Using infrared spectroscopy of sliced samples or blocks forms it is possible to characterize the chemical and optical alterations produced in the dental hard tissues by either thermal or chemical treatment, or an association of the two treatments, Therefore this technique can be used in dental research that study the effect of laser irradiation on hard tissues and the acid attack produced by intraoral bacteria.

Key Words: Enamel; Dentin; FTIR spectroscopy; Hydroxyapatite; Collagen.

I. INTRODUCTION

The enamel and dentin tissues are composed of an organic matrix, a mineral matrix and water. The percentage values of each component are

Table 1. Percentage values of the organic matrix, mineral matrix and water present in human enamel and dentin tissue.[1]

	Enamel		Dentin	
	Weight (%)	Volume (%)	Weight (%)	Volume (%)
Mineral matrix	95	87	75	62
Organic matrix	1	2	20	38
Water	4	11	5	Not considered

showed in Table 1 by tissue weight and volume. The precise identification of chemical components depends on some details such as the sample preparation or how old the subject are, but despite these variables the values remain very similar.[1–3] The values for the water depend on the form how this component is found in the tissue: free or bound water. In Lit.[1] presents for the dentin tissue values between 13 and 5 wt%, respectively, for free and bound water. The values showed in Table 1 for dentin in weight and volume do not consider the water present in the tissue.

Mineral matrixes of the enamel and dentin tissues are mainly composed of crystals of carbonated hydroxyapatite. These crystals are different in size and quantity for each tissue and for different areas in the tooth. The organic matrix in the enamel tissue is smaller than the organic matrix in the dentin, which is basically formed by collagen besides other components in small quantity.[4] Differences in the chemical composition can occur among different areas in the same tissue,[5,6] between inner and external areas of the tooth Surface,[7] deciduous or permanent teeth[8] and different individuals or animals.[9]

The identification of optical properties such as absorbance, reflectance and the chemical composition of the tissues have a great importance in dentistry research. The chemical components present in the hard tissue such as water, phosphate, carbonate and organic material absorb the infrared radiation strongly. It is important to identify the chemical origin of all the bands present in the infrared region, because this spectra information is like a "finger-print" which identifies the tissue. This infrared information is possible by the use of infrared spectroscopy in the transmission and reflection mode. To improve this technique to identify chemical alterations in the tissue is necessary to characterize their properties in a more natural form. To characterize spectroscopically the tissue in its natural form it is necessary to be careful with some details in the sample preparations, because the tissue chemical composition can undergo some

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alterations. Therefore it is necessary to use samples in slices or blocks to keep the natural structure of the tissue, mainly the junction of the organic-mineral matrix and the water, which can show alterations after the grinding process.

II. OBJECTIVE

The purpose of this work is to study the transmission and reflection spectra in the infrared range from $2.5\,\mu m$ to $25\,\mu m$, of the enamel and dentin tissues, from human and bovine teeth when using sliced, polished blocks and natural samples.

III. MATERIALS AND METHODS

The samples used in this work are bovine incisors and human third molars teeth; the human teeth were extracted by orthodontic reasons. In order to maintain the tissue physical and chemical properties, the samples were kept in a solution of sodium chloride at $0.9\,wt\%$, since the extraction until the sample preparation and the infrared experiment. It is necessary to obtain very thin slices for the transmission experiment. Initially the teeth were cut in slices $0.5\,mm$ thick with a diamond disc and than sanded until thicknesses under $100\,\mu m$. The final slices were $100\,\mu m$ for the human enamel tissue and $80\,\mu m$ for the bovine enamel; for the dentin tissue the slices were thinner, $30\,\mu m$ for the human dentin and $40\,\mu m$ for the bovine dentin. Samples in two different forms were used for the reflection spectra acquisition: blocks with polished surfaces until granulometry of $3\,\mu m$ and natural surfaces cleaned with a toothbrush. The selected natural surfaces were buccal enamel and cervical cementum. The tissue spectra were registered in transmission and reflection modes. The analyzed region was between 2.5 and $25\,\mu m$ or in wavenumber between $4000\,cm^{-1}$ and $400\,cm^{-1}$. A Fourier Transform Infrared Spectrometer—FTIR (Magna-IR System 850 Series II, Nicolet, Wadison, United States of America) was used. To minimize some noise in the spectra signal from the water vapor and carbon dioxide gas present in the air, the spectra were acquired after the interior of spectrometer was maintained with dry air for 20 min. For the reflection experiment with the buccal enamel and the cervical cementum, the tooth was positioned so that the detector showed the major reflected infrared signal.
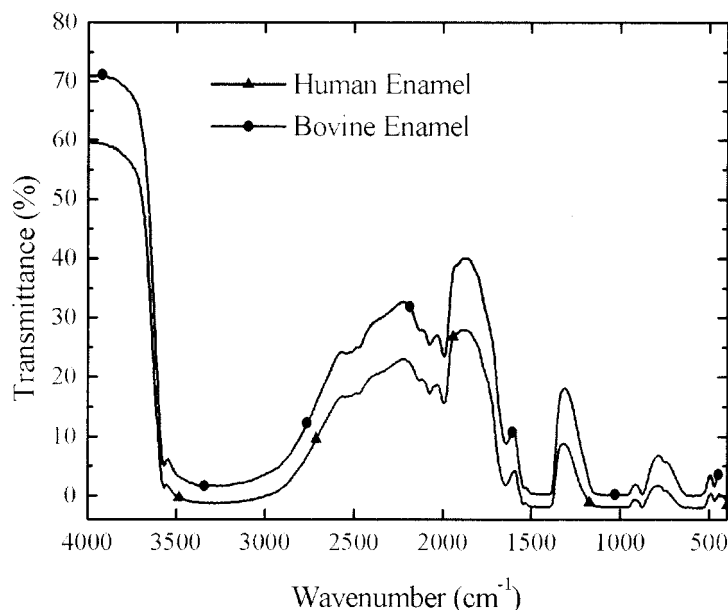
Figure 1. Transmission spectra for the human and bovine enamel between $4000\,cm^{-1}$ and $400\,cm^{-1}$.

IV. RESULTS

The enamel slices have thickness greater than the dentin slices, consequently the infrared signal of stronger bands such as the water and phosphate are more intense. The transmission spectra can be seen in Fig. 1 for the human and bovine enamel; the reflection spectra for the polished bovine enamel and bucal enamel in Fig. 2. For the human and bovine dentin the transmission spectra can be visualized in Fig. 3(a) for all range and in Fig. 3(b) between 1800 and $400\,cm^{-1}$; the reflection spectra for the polished bovine dentin and cervical cementum in Fig. 4. Both human and bovine enamel have strong absorption bands between 3500 and $3000\,cm^{-1}$, at $1660\,cm^{-1}$ associated to water bands; between 1100 and $1000\,cm^{-1}$, at $600\,cm^{-1}$, associated to the phosphate radical. The dentin samples are thinner and do not show saturated absorption bands in the region that absorbs the water and phosphate radicals, despite the bands being present with high intensity. In Table 2, the absorption bands for the human and bovine dentin and enamel tissue are listed. These values were extracted from the transmission spectra presented in Figs. 1, 3(a), and 3(b).

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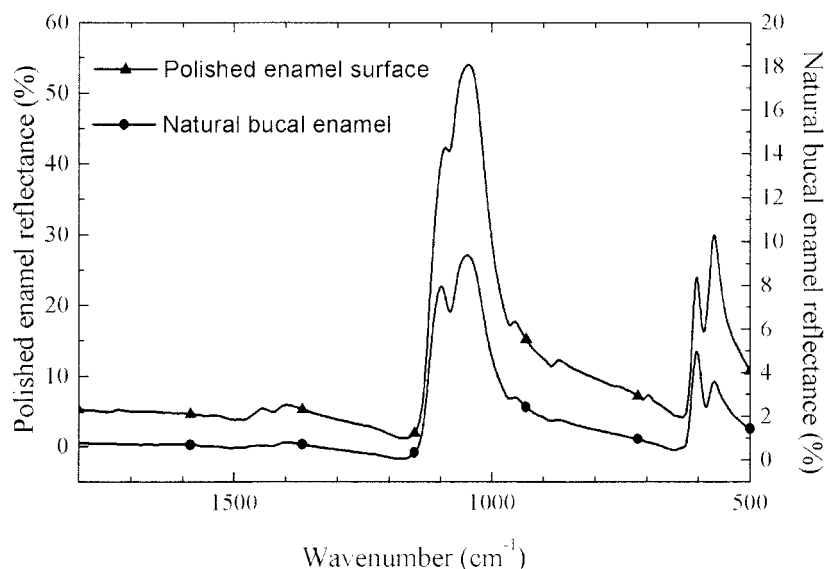
Figure 2. Reflection spectra for the polished surface and buccal bovine enamel between $1800 \, \text{cm}^{-1}$ and $400 \, \text{cm}^{-1}$.

V. DISCUSSION

The absorption spectra of the tissues are composed of the sum of the spectra of their primary components. Therefore the dental hard tissues are constituted by a sum of the mineral matrix composed of phosphate, carbonate and hydroxyl bands,[10–12] organic matrix composed of collgen or non-collagen proteins with bands originated from chemical compounds as C–H, C=O, C–N, N–H, NCO$^-$, NCN^{2-} [13–15] and the water present in the tissue.[16,17]

A. Water

The water can be present in the biological tissues in two forms: weakly bounded to the tissue, called adsorbed water and strongly bounded to the structure, called structural water.[16] Between 4000 and $400 \, \text{cm}^{-1}$ four absorption bands associated to the water molecule are observed.[17] For this region the most intense band can be observed at $3398 \, \text{cm}^{-1}$, associated to a sum of v_1, $2v_2$ and v_3 modes; another band it is observed at $1643 \, \text{cm}^{-1}$, associated to v_2 mode. Due to the high intensity
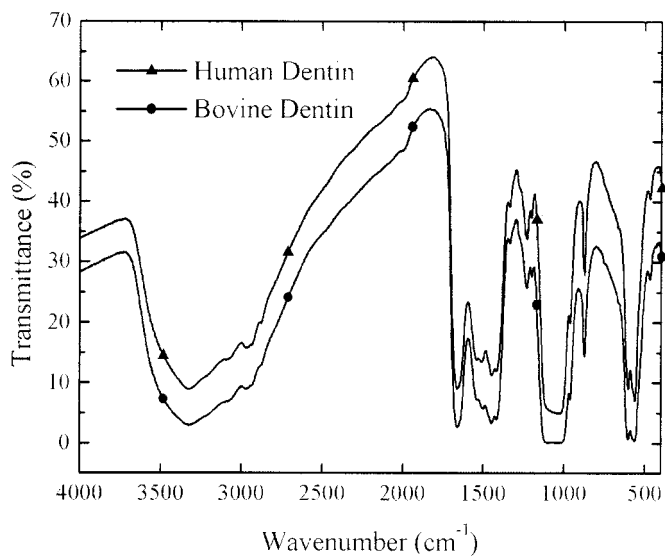
Figure 3a. Transmission spectra for the human and bovine dentin between $4000\,cm^{-1}$ and $400\,cm^{-1}$.
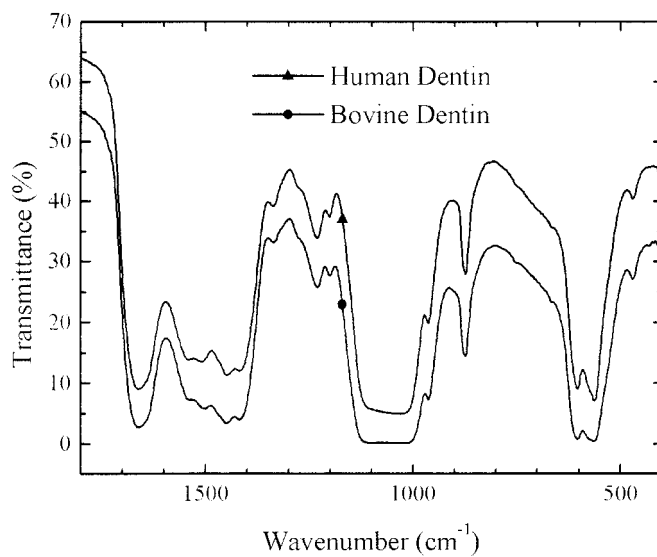
Figure 3b. Transmission spectra for the human and bovine dentin between $1800\,cm^{-1}$ and $400\,cm^{-1}$.

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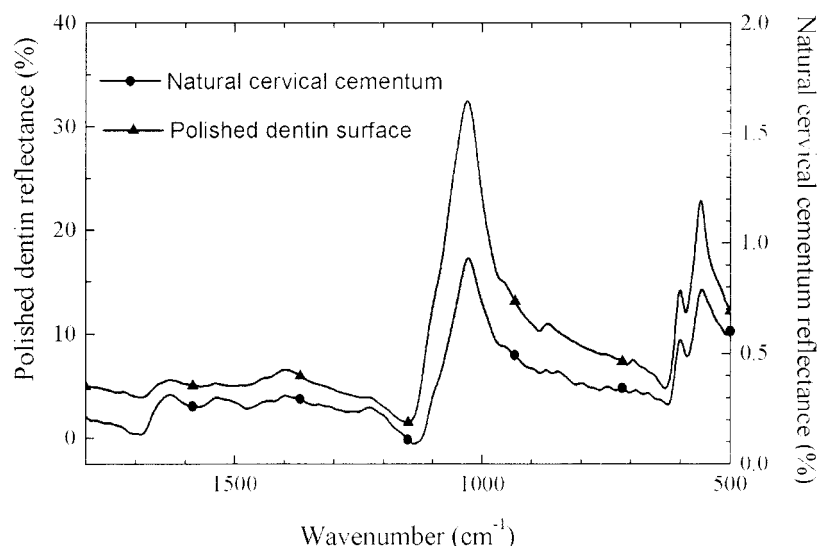
Figure 4. Reflection spectra for the natural cervical cementum and polished surface dentin of bovine teeth between $1800\,cm^{-1}$ and $400\,cm^{-1}$.

of this band is possible to observe its presence in the enamel and dentin tissues. Another band occurs around $2123\,cm^{-1}$ with low intensity and it is not visible in the tissues spectra. In this work a broad band between $3500\,cm^{-1}$ and $2900\,cm^{-1}$ is recorded for the enamel tissue in human and bovine teeth. In other region the v_2 mode is observed at $1642\,cm^{-1}$ for the enamel and at $1660\,cm^{-1}$ for the dentin tissue. This band in dentin tissue is overlapped by the collagen band. As a consequence, a shift in the band position occurs.

B. Mineral Matrix

The mineral matrix is composed in its majority of crystals of carbonate hydroxyapatite[18] and the absorbed components in the infrared region are the hydroxyl (OH^-), carbonate (CO_3^{2-}) and phosphate radical (PO_4^{3-}). In the enamel tissue, two infrared modes from the hydroxyl radical can be observed at $3570\,cm^{-1}$ associated to the stretching mode and at $749\,cm^{-1}$ associated to the librational mode.[19] These two modes are observed only in the enamel tissue, which has a high degree of crystalinity, while in the dentin tissue, which has a small crystalinity, these bands appear very weakly.

Table 2. Absorption bands of chemical components present in the dentin and enamel tissues.

Chemical components	Absorption bands (cm^{-1})			
	Human enamel	Bovine enamel	Human dentin	Bovine dentin
OH$^-$	3570	3570	Not observed	Not observed
H$_2$O	3550–2900	3550–2900	3327	3320
Organic material	Not observed	Not observed	3115–2860	3115–2860
	2570–2490	2570–2490	Not observed	
	2472	2474		
	2132	2125		
	2073	2073		
	1992	1992		
H$_2$O and organic material	1642	1642	1660	1660
CO$_3^{2-}$ and organic material	1544	1538	1541	1539
	1530–1380	1530–1380	1508	1502
			1470	1470
			1448	1450
			1419	1417
Organic material	Not observed		1336	1336
			1320	1320
			1280	1280
			1232	1232
			1201	1201
PO$_4^{3-}$	1170–930	1170–930	1130–1000	1130–1000
			964	964
CO$_3^{2-}$	876	874	880–870	880–870
OH$^-$	749	749	752	752
PO$_4^{3-}$	650–520	650–520	603	603
			575	575
			563	563
PO$_4^{3-}$	469	472	471	471

The phosphate radical shows four vibrational modes in the infrared region (4000–400 cm^{-1}), the v_1 and v_2 modes are inactive in infrared spectroscopy and only the v_3 and v_4 are observed. The v_4 vibrational mode has three absorption bands, in the enamel sample the bands appear between 650 and 520 cm^{-1} and in the dentin, where the bands are more resolved, three bands are present at 603, 563 cm^{-1} and a shoulder around 575 cm^{-1}. The v_3 mode is very intense and it is observed with a broad shape in both, enamel and dentin tissues. Despite the band being more saturated in the enamel tissue, the absorption region occurs between 1200 and 900 cm^{-1}. Using deconvolution

techniques is possible to determine more precisely the intensity and position of these bands.

The carbonate radical has four vibrational modes and only two are important to infrared spectroscopy, the v_2 and v_3 modes. The v_1 mode is not so intense and is overlapped by the stronger v_3 mode from the phosphate radical; the v_4 mode is a very weaker band.[20] The carbonate radicals are present in the apatite crystals substituting the phosphate or hydroxyl radicals. When the carbonate substitutes the hydroxyl radical it is designed type A and when it substitutes the phosphate radical it is designed type B. Absorption bands assigned to the carbonate radical are observed in two regions, both in enamel and in dentin tissues. The v_3 mode appears in enamel tissue at $1544\,cm^{-1}$ and $1538\,cm^{-1}$, respectively, for human and bovine tissues, and between 1530 and $1380\,cm^{-1}$ for both human and bovine tissues. In the dentin tissues the carbonate bands absorb from $1540\,cm^{-1}$ to $1420\,cm^{-1}$, in the same region organic material is absorbed. In this region the bands appear with some variations between human and bovine tissues. For the v_2 vibrational mode two bands occur between 890 and $860\,cm^{-1}$, the positions of these bands are very near so it is necessary to acquire spectra with high resolution to distinguish them. In human enamel tissue for the v_3 carbonate mode the literature assigned the bands at 1545, 1450, and $890\,cm^{-1}$ to type A, substituting the hydroxyl; and the bands at 1465, 1412, and $873\,cm^{-1}$ to type B, substituting the phosphate. For the v_2 carbonate mode, a band at $879\,cm^{-1}$ is assigned to type A and other at $873\,cm^{-1}$ to type B.

C. Organic Matrix

The organic matrix of the enamel tissue is very small when compared with the matrix of the dentin tissue. Besides this difference there are others in the chemical composition between both matrixes. The dentin organic matrix is composed basically of collagen and other components in lower quantity such as citric acids, lipids, mucopolysacharide, and insoluble proteins, citric acid, peptides, proteins, keratin and a little fraction of collagen compose the enamel.[4] These differences can be observed in the infrared spectra of the two tissues, the bands observed in the enamel are not so intense in the dentin spectra and the bands from the organic matrix at the dentin tissue are not observed in the enamel tissue with great intensity.

In the enamel tissue bands between 2570 and $1990\,cm^{-1}$ can be observed, bands with low intensity between 2570 and $2490\,cm^{-1}$ and other bands with more intensity, between 2490 and $1990\,cm^{-1}$ are present

for both human and bovine tissues; the specific position values can be seem in Table 2. In this region, between 2570 and 1990 cm^{-1} infrared activity from the cyanate (NCO$^-$) and cyanamide (NCN^{2-}) ions[13] is predicted. Besides this region others are predicted at 2200 cm^{-1} for the v_3 vibrational mode of the cyanate ion, at 1200 cm^{-1} assigned to v_1 vibration and between 600 and 640 cm^{-1} to the v_2 vibration mode. The cyanamide ion bands are observed in the same region where the cyanate ion bands are mentioned, excepting for the v_1 vibration mode. The presence of bands in the dentin tissue assigned to the organic matrix was more intense. Starting from greater wavenumbers in the dentin spectra, the first band groups between 3115 and 2860 cm^{-1} can be observed. In this region, a lot of bands appear, masked by the broad water band; these bands are assigned to amida A, amida B, and CH stretching mode.[14] When these results are compared with the enamel spectra, these bands are not observed, despite a shoulder being noted in the water band of the enamel spectra.

A second region where absorption bands are assigned to the organic matrix in the dentin spectra, starting at 1660 cm^{-1} and finishing at 1200 cm^{-1}. In this region some overlapping happens with bands other chemical components, such as water and carbonate. Near the water band predicted at 1645 cm^{-1} another band at 1655 cm^{-1} assigned to amide I[15] was observed too, resulting in a common band positioned at 1660 cm^{-1}. Concerning smaller wavenumbers, carbonate bands were observed next to bands assigned to amide II, present at around 1525 cm^{-1}, and CH bending modes at around 1445 cm^{-1}.[21] In a third region, bands assigned to the amide III were observed at 1240 cm^{-1} and CH bending mode at 1310 cm^{-1}, where overlapping with other bands did not occur.

D. Bands Positions and Intensities

Despite the infrared spectroscopy being a qualitative technique for chemical analysis, the absorption band identification by this technique can be useful to analyze the chemical composition of the dental tissues. For the tissues analyzed in this work, no changes was observed as to the presence or absence of bands between the two human and bovine tissues. On the other hand, there was a changes in the relative intensity of the bands. Between the two enamel tissues the bands values were saturated, making difficult the comparison of the bands intensities.

When human and bovine dentin tissues were compared, some variations were observed in the intensity values between the bands centered at 1550 cm^{-1} and 1380 cm^{-1}, assigned respectively to the carbonate

radical and the organic material. These variations can be due to a difference either in the quantity of carbonate or the organic matrix in the two tissues. Some differences were observed in the bands values between 1400 and 1100 cm^{-1}, where only bands from the organic matrix occurred; the intensity values variation between the bands were found at 1336, 1232, and 1201 cm^{-1}. Analyzing the bands originated from the mineral matrix in the dentin and enamel tissues, there were no differences as to the presence or absence of bands. There were differences in the intensity as a consequence of the differences in the crystalinity of the two tissues.

Regarding the organic matrix of the dentin and enamel tissues a great difference was observed between them. Comparing the spectral region of the organic matrix, from 2570 cm^{-1} to 1990 cm^{-1} of the enamel tissues and from 1400 cm^{-1} to 1200 cm^{-1} of the dentin tissues, it could be observed that the bands present in these two regions did not occur in both tissues with a significant intensity. The bands between 2570 to 1990 cm^{-1} of the enamel tissues are not present in the same spectral region of the dentin tissues; and the bands between 1400 to 1200 cm^{-1} of the dentin tissues are not present in the same spectral region of the enamel tissues. The infrared signal of the polished samples in the reflected mode is more intense than the natural tissues. With the weak infrared signal of the natural tissues, some noise signal will be more significant, mainly between 1000 and 500 cm^{-1}.

Infrared spectroscopy of biological sliced hard tissues samples has a great importance to some research areas rather than measurements of powder samples. When the samples are turned into powder, the water is the first chemical component lost to the ambient. This component is released from the tissue or adsorbed during the grinding process because the dentin sample is hydrophilic and has the ability of adsorbing the water from the environment.[1] As the same, the granulometry of the powder can influence the intensity of the absorption bands values in the infrared region here analyzed.[22] Using samples of dental hard tissues in thin slices it is possible to evaluate the chemical composition of the tissues by infrared spectroscopy, mainly components affected by the grinding process like water. In this work no differences were found between the mineral matrix of human and bovine tissues. Concerning the presence of absorption bands in human and bovine tissues, only a change in the intensity between bands was observed. Regarding the organic matrix, the bands observed in the dentin tissue were not observed in the enamel tissue; as the same, the bands observed in the enamel tissue were not observed in the dentin tissue with a significant intensity in the same regions. Using infrared spectroscopy of sliced samples in transmission mode, polished block samples and natural surfaces of the tooth in reflection mode, it is possible to characterize the chemical and optical

alterations produced in the dental hard tissues. This can be done by laser irradiation, chemical treatment or an association of the two. Thus, this technique can be used in dentistry research areas studying the effect of laser irradiation on hard tissues and the effect of acid attack produced by the intraoral bacteria.

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