Bound Energy of Water in Hard Dental Tissues

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ABSTRACT

Enamel and dentin are composed, respectively, of 3 wt% and 10 wt% of water, which exhibits different features in the tissues: loosely and tightly bound water. The objective of this study is to clarify by infrared spectroscopy, the different features of the water in heated (100–1000°C) hard dental tissues (enamel and dentin). The water band between $3800\,cm^{-1}$ and $2500\,cm^{-1}$ was analyzed by infrared spectroscopy. The area dependence of the water band with temperature was compared with the Arrhenius equation in two regions (100–400°C and 700–1000°C).

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The activation energy was determined for these two regions, and similar values were observed for both tissues. For enamel we obtain -4.1 ± 0.2 kJ/mol at $100-400\,^{\circ}$C and -63 ± 9 kJ/mol at $700-1000\,^{\circ}$C; for dentin -4.1 ± 0.2 kJ/mol at $100-400\,^{\circ}$C and -60 ± 11 kJ/mol at $700-1000\,^{\circ}$C. The water loss changes the color of the tissues, hydroxyapatite crystallographic parameters, and produce ESR signals. These changes were discussed and compared with the results observed in this work and after laser irradiation. We conclude that these two activation energies could be assigned to the adsorbed (loosely bound) and trapped (tightly bound) water.

INTRODUCTION

The presence of water in enamel and dentin plays a significant role in the properties of these tissues. The literature suggests that water in enamel may exist in two states, loosely bound water and tightly bound water.[1−4] These two types are named also as adsorbed water and structural water, respectively. The elimination of water can introduce changes in the crystallographic parameters,[4] color,[5,6] and birefringence,[7] and can produce paramagnetic signals in hard dental tissues.[8] The adsorbed water is reversible, thermally unstable below $200\,^{\circ}$C and does not produce changes in the hydroxyapatite lattice parameter. On the other hand, the structural water is irreversible, thermally unstable between $200\,^{\circ}$C and $400\,^{\circ}$C and its presence changes the lattice parameters.[4] The enamel is composed of 3 wt% water, $1-2$ wt% organic material and 95% inorganic material; while the dentin is composed of 10 wt% water, 20 wt% organic material, and 70 wt% inorganic material.[9]

For a clinical procedure with laser irradiation, safe parameters must be selected to avoid a temperature rise greater than $5.5\,^{\circ}$C in order to preserve the pulp tissue.[10] Despite the low temperature at the pulp tissue, the first sub-surface layers of the irradiated surface tissue undergoes high temperatures that can change, locally, the tissue structure and chemical composition. These changes would affect the tissue color,[5] chemical composition,[11] and crystallographic structure,[12] and consequently can affect the caries resistance. The temperature rise during the tissue irradiation, due to highly absorbed laser wavelengths, is dependent on the laser parameters and tissue properties. At the enamel ablation threshold, an Er:YAG ($2.94\,\mu$m) laser produces a maximum surface temperature rise of about $300\,^{\circ}$C, the Er:YSGG ($2.79\,\mu$m) laser about $800\,^{\circ}$C, and the CO_2 ($9.6\,\mu$m) laser about $1000\,^{\circ}$C.[13]

High intensity laser irradiation can produce changes in all components present in hard dental tissues: water, mineral, and organic matrices.[14]

The structural and compositional changes, observed after highly absorbed wavelengths, are roughly dependent on the residual energy deposited in the remaining tissue. The residual energy deposited into the tissue will determine the temperature rise and consequently the degree of tissue changes. Therefore, the component that is more thermally unstable will be changed or lost initially, as loosely bound water and organic matrix. These changes are observed after laser irradiation with different wavelengths: Er:YAG,[11,15] Nd:YAG,[16] and CO_2—10.6 μm.[16,17] The mineral matrix is more stable thermally, but changes are also observed: carbonate loss after CO_2—10.6 μm irradiation;[18] OH increases after CO_2 (10.6 μm) irradiation;[17] crystal growth after Nd:YAG (1.064 μm) irradiation;[19] and formation of new crystallographic phases after Ho:YLF (2.065 μm),[20] CO_2 (9.3 μm),[12] and CO_2 (10.6 μm) irradiation.[21]

The enamel and dentin components (water, organic, and mineral matrices)[9,22] produce several infrared absorption bands between 2.5 and 25 μm that can be observed by Fourier transform infrared spectroscopy.[23] Therefore, this technique can be applied to monitor the chemical composition of hard dental tissues after thermal treatment, laser irradiation, and other physical or chemical treatment. The objective of this study is to clarify different features of the water in hard dental tissues heated at different temperatures (100–1000°C) and analyzed by infrared spectroscopy. The thermal stability of water will be determined and compared with the two different kinds of water proposed in the literature: adsorbed water (loosely bound) and structural water (tightly bound/trapped).

MATERIALS AND METHODS

For this experiment, we use bovine incisor teeth free of dental diseases. In the first step, using a diamond blade system, the teeth were cut longitudinally into slices of 0.5 mm thick. From the slices, enamel and dentin were separated mechanically and each tissue was ground manually with a mortar and pestle. The powder was sieved and particle sizes between 25 and 38 μm were selected. The final amount of enamel and dentin powder was divided into 56 samples with 5 mg each: 28 dentin and 28 enamel samples. The results from dentin sample heated at 375°C were rejected because the measured spectrum showed noise and irregular signals. Each 5 mg tissue sample is mixed with 100 mg of KBr. To produce a tablet with diameter of 12.7 mm, the samples were compressed under 4 ton. The spectral acquisition was conducted in a Fourier transform infrared spectrometer (MB-Series, Bomem Hartmann & Braun, Quebec, Canada) with a resolution of 0.5 cm^{-1}. The thermal treatment was conducted with temperatures in the range of

100–1000°C during 30 min. The infrared spectra was acquired ~15 min after the thermal treatment. The analyzed area corresponds to the total area between 3800 and 2500 cm^{-1}, minus the additional areas observed in the same range and assigned to OH$^-$ and CH stretch.[23]

RESULTS

The infrared spectra were registered between 4000 and 400 cm^{-1}. In this region, the intense water band is observed between 3800 and 2500 cm^{-1}. The water lost in heated enamel and dentin exhibits a similar behavior for both the tissues. The band shape for the room temperature enamel and the enamel heated to temperatures of 100, 400, 700, and 1000°C can be visualized in Fig. 1. These spectra at selected temperature were chosen to show the water band behavior with increasing temperature, and the area under the water band
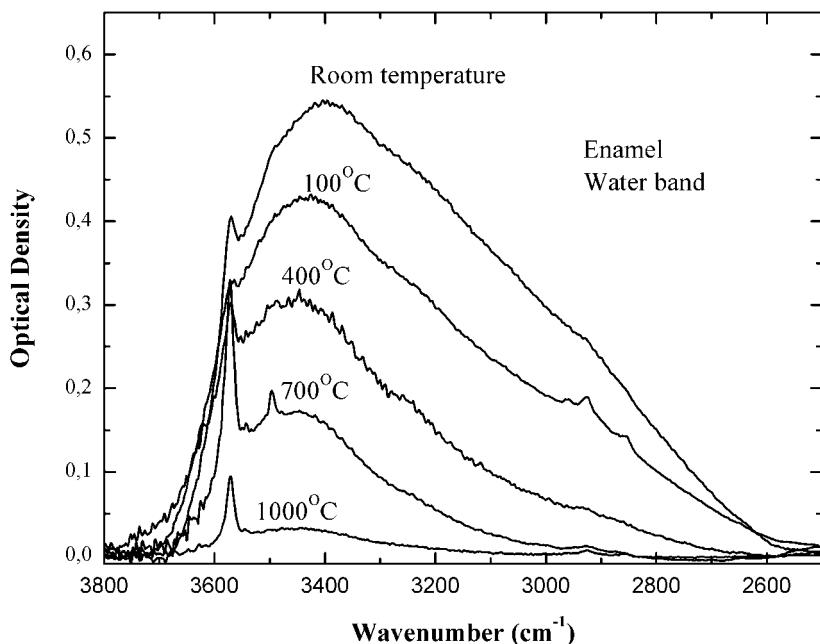

Figure 1. Infrared spectra of natural enamel, and enamel heated during 30 min at 100, 400, 700, and 1000°C were observed between 3800 and 2400 cm^{-1}. The OH$^-$ band at 3570 and 3496 cm^{-1}; and C–H bands between 3000 and 2800 cm^{-1} were also observed in the spectra.

for all analyzed temperatures can be observed in Fig. 2. For the dentin, the band shape for the same selected temperature values, as described for enamel, can be observed in Fig. 3, while the area under the water band for all analyzed temperatures can be observed in Fig. 4.

The area of the water band in heated tissues was compared with the Arrhenius equation, which can be written as:

$$A = C \exp\left(-\frac{E_a}{R}\frac{1}{T}\right)$$

where A is the area of the water band; R, the gas constant (8.314 J/Kmol), C, a constant; and E_a is the activation energy obtained from the fit to the
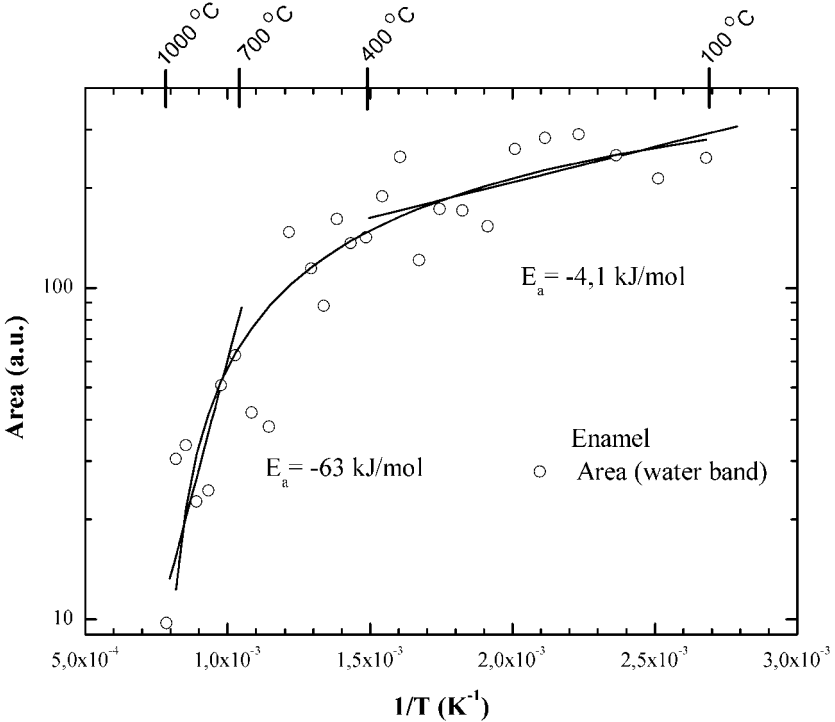

Figure 2. The band area of the remained water in the enamel heated between 100 and 1000°C were observed; below 500°C (1.3×10^{-3} K^{-1}) the area decreases slowly with the applied temperature, and above 500°C the area decrease faster. For the experimental data two temperature regions (100–400°C and 700–1000°C) can be approximated to linear functions, and the respective activation energies for these two regions were obtained: -4.1 ± 0.2 and -63 ± 9 kJ/mol.
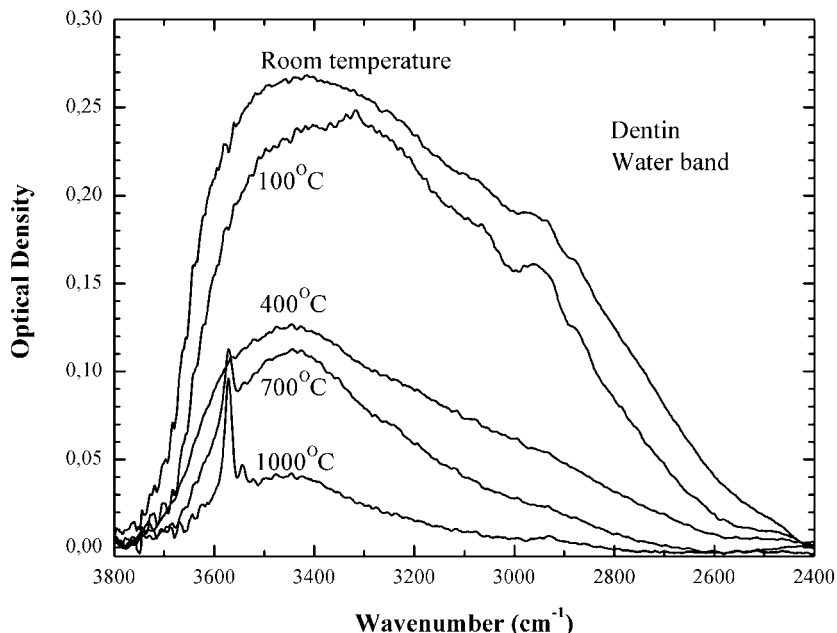
Figure 3. Infrared spectra of natural dentin, and dentin heated during 30 min at 100, 400, 700, and 1000°C were observed between 3800 and 2400 cm^{-1}. The OH^{-} band at 3570 cm^{-1} and C–H bands between 3000 and 2800 cm^{-1} were also observed in the spectra.

experimental data. The semi-log graph in Fig. 2 (enamel) and Fig. 4 (dentin) show two linear regions (100–400°C and 700–1000°C). In these two regions the Arrhenius equation was fitted to the experimental data. From the best fit in the mentioned regions, the activation energy was obtained. For the enamel heated between 100°C and 400°C the calculated activation energy was −4.1 ± 0.2 kJ/mol, and between 700°C and 1000°C the activation energy was −63 ± 9 kJ/mol. For the heated dentin between 100°C and 400°C the activation energy was −4.1 ± 0.2 kJ/mol, and between 700°C and 1000°C the activation energy was −60 ± 11 kJ/mol. The uncertainties for the activation energy were obtained from the standard deviation between linear fit and experimental data. The interme-diated region (400–700°C) is probably a composition of the two linear functions, i.e., a temperature region where the two different reactions occur simultaneously, and therefore was not possible to adjust the linear function.
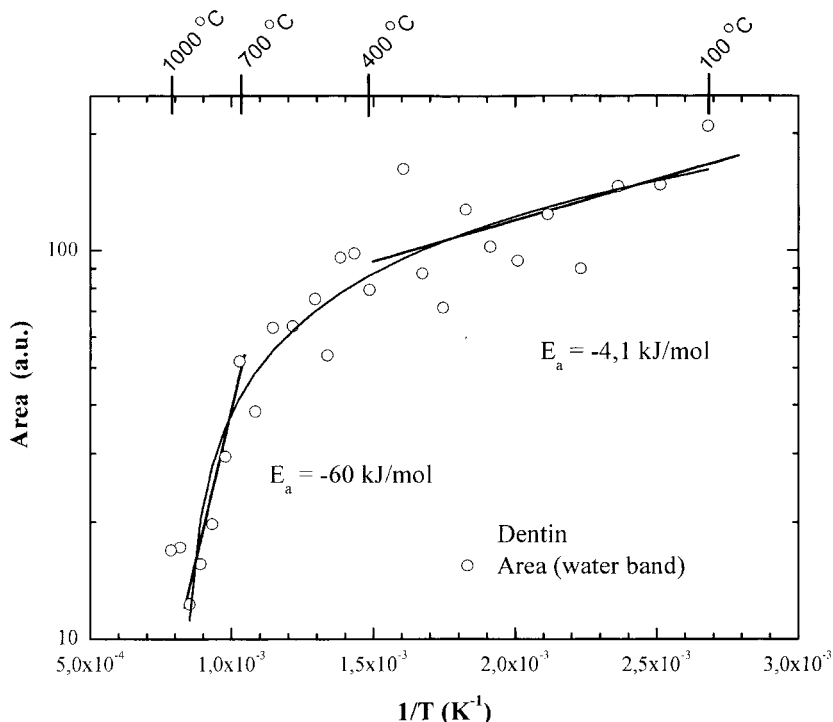
Figure 4. The band area of the remained water in the heated dentin between 100°C and 1000°C were observed; below 500°C (1.3×10^{-3} K^{-1}) the area decreases slowly with the applied temperature, and above 500°C the area decrease faster. For the experimental data two temperature regions 100–400°C and 700–1000°C) can be approximated to linear functions, and the respective activation energies for these two regions were obtained: -4.1 ± 0.2 and -60 ± 11 kJ/mol.

DISCUSSION

Very similar values for the activation energies were observed for both enamel and dentin. The activation energy was determined for two temperature ranges: 100–400°C and 700–1000°C. For the first region the energy was -4.1 kJ/mol for both the tissues; and for the second region was -63 kJ/mol for the enamel and -60 kJ/mol for the dentin. The minus sign corresponds to bound state of the water molecule. The similarity of the values is indicative that these two kinds of water show similar bound characteristics in both the tissues. For the samples heated under 400°C the water

molecule may be bound to a substrate (chemical structure) present in both mineral and organic matrix; and for samples heated above 400°C the water would be bound only to the mineral matrix. The specificity above 400°C originated because the organic matrix is completely lost at 350°C.[24−26]

To a first approximation, the electrostatic interaction of water within the substrate (organic or mineral matrix) can be compared with the electrostatic interaction observed between water molecules.[27−29] In water, the hydrogen atom is covalently bound to the oxygen by an energy of 492 kJ/mol,[27] and an additional attraction of about 23.3 kJ/mol[28] is observed between the water molecule and other neighboring oxygen atoms. This attraction is far greater than the van der Waals interaction that is about 5.5 kJ/mol.[29] The measured energy (4.1 kJ/mol) assigned to the water elimination between 100°C and 400°C is quite similar to the predicted van der Waals bound energy: 5.5 kJ/mol.[29] Otherwise, the electrostatic attraction between a water molecule and a oxygen atom of a neighbor water molecule, 23.3 kJ/mol,[28] is lower than the bound energy determined between 700°C and 1000°C: 60−63 kJ/mol.

In our system (hard dental tissues), this electrostatic attraction can occur with oxygen present in the mineral matrix (carbonate and phosphate), organic matrix, or other radical formed with the temperature, as cyanate or CO_2.[30] Considering that the proposed model for the loosely and tightly bound water present in the enamel[3,4] is well established, the question about the site where the water is bound is not settled. If we consider the samples heated to temperatures above 400°C, the organic matrix is lost and our complex system can be reduced only to the mineral matrix. At this temperature region, we can list the proton acceptors present in the mineral matrix that can produce an electrostatic attraction with the hydrogen atom of the water molecule (proton donor). For the mineral matrix we found P–O and P=O in the phosphate, C–O and C=O in the carbonate, O–H in the hydroxyl, C=O in the carbon dioxide, and N–O and N=O in the nitrogen compounds as cyanamide and cyanate. All the described radicals can attract the hydrogen atoms of water electrostatically, and therefore the bound water can be dependent with the presence of these radicals.

The thermal stability of radicals present in the tissues will be discussed in the following paragraphs. The phosphate radical is the most stable chemical radical of the mineral matrix, while the hydroxyl and carbonate undergo changes after thermal treatments above 100°C. The carbonate loss starts when the enamel is heated at 100°C, showing a maximum loss at about 800°C, and it is totally eliminated from the tissue at 1100°C.[30] The hydroxyl radical increases and shows a maximum intensity at about 400°C and then decreases again falling to zero. The cyanate radical, produced with temperature shows a maximum intensity at 700°C; the carbon dioxide is also

produced with temperature and its maximum is observed at 400°C. Therefore, if the water molecule is absorbed eletrostatically to oxygen atoms from one of these radicals, the loss of this radical can produce also the loss of water. The more suitable radical is the carbonate, because its loss from the tissue occurs after thermal treatments with temperatures above 100°C, and its maximum loss is observed at about 800°C. Two sites in hydroxyapatite are predicted for the carbonate radical: at hydroxyl site (carbonate type A) and at phosphate site (carbonate type B). The carbonate type A decreases slightly between 100°C and 300°C, then increases up to a maximum at 750°C, and it is totally eliminated at 1100°C.[30] Otherwise, the carbonate type B is eliminated linearly with the applied temperature between 100°C and 1100°C.

For the temperature region between 100°C and 400°C, both organic and mineral matrices are suitable for changes, and can influence the water loss. As described previously, the carbonate radical and organic material are lost, and carbon dioxide and hydroxyl are formed. The dentin organic matrix is 20 wt% of the tissue and is mainly composed of collagen protein; while the enamel organic matrix is only 1 wt% of the tissue and is mainly composed of non-collagen proteins.[9] Therefore, it is very difficult to evaluate the water environment in the tissues, but the energy determined between 100°C and 400°C can be roughly associated with the van der Waals attraction between the water molecules and the organic matrices.

The water elimination by heating can introduce changes in the crystallo-graphic parameters[4] and color,[5,6] or produce paramagnetic signals in enamel and dentin.[8] These changes are temperature dependent and can help us to identify the source of the water during heating. The crystallographic parameters of heated enamel are not altered for temperatures below 200°C, and between 200°C and 400°C a contraction of the unit-cell is observed, while between 500°C and 900°C an expansion occurs. The contraction in the a-axis is assigned to the loss of lattice water and the expansion to the loss of carbonate.[4] The color of the tissues can change with water loss; the enamel translucency is reduced after tissue dehydration with air jet or desiccation,[6] and after heating in oven up to 200°C the enamel becomes totally opaque.[5] Water, observed between the crystals, has a refractive index of 1.33 and the enamel prisms at about 1.7.[7,31] When the water is replaced by air ($n = 1.00$[6]), the refractive index of the surrounding medium between the crystals is changed. This value increases the difference between the refractive index of enamel and its surrounding regions; increasing the light scattering, reducing the translucency, and for that the enamel become opaque. The samples thermally treated between 100°C and 300°C produce ESR signals in the dentin; below 200°C the signals are unstable and decay 6 months after the thermal treatment.[8] The formation of the ESR signals in this temperature range is associated to the water loss and organic

material denaturation, and its disappearance after storage is assigned to water incorporation.

Similar changes observed after conventional heating of enamel and dentin in oven are also observed after laser irradiation for wavelengths that are highly absorbed by the tissues. As can be seen in the following paragraphs, similar changes are observed in both treatments (heating in oven or laser irradiation): loss of water, organic material, and carbonate; increase of hydroxyl; and formation of new compounds such as cyanate, carbon dioxide, or crystallographic phases. In Fig. 5 the thermal stability observed for the radical that compose the hard dental tissues are summarized, the temperature ranges in this figure is only an approximation and does not represent the exact range for the radical thermal stability. The temperature ranges in this figure are obtained from the references that conducted thermal analysis experiments with hard dental tissues and synthetic hydroxyapatite.

The water loss can be observed in enamel desiccated with an air jet at room temperature,[6] while its complete elimination occurs only at 1300°C.[3] For laser irradiated enamel, the water loss is observed with CO_2—10.6 μm laser (10^4 J/cm^2).[17] In irradiated dentin the water loss is observed with a CO_2—10.6 μm laser (0.5 W),[11] with an Er:YAG laser using 0.4 W,[11] or $0.365-1.94$ J/cm$^{2[15]}$ and after irradiation with a Nd:YAG laser ($571-2857$ J/cm^2).[16] The organic matrix of enamel composes
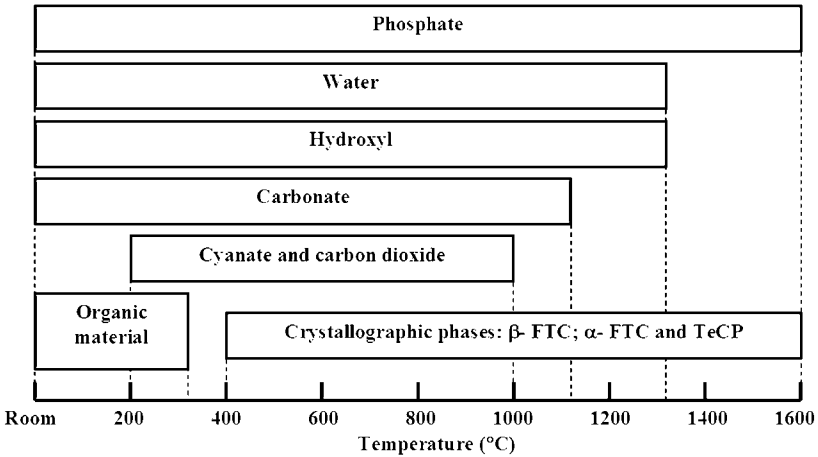

Figure 5.   Diagram that represents the thermal stability of the chemical compounds in the enamel and dentin. The temperature range for each radical was selected from different references that conduct experiments with hard dental tissues or synthetic hydroxyapatite.[4,7,24,30,32–35]

only 1–2 wt% of the tissue, therefore its characterization after thermal treatment is very difficult and the following results will concern only the dentin organic matrix. The organic material loss occurs after heating dentin, and at about 350°C when a maximum loss is observed.[24] The laser irradiation shows changes (degradation) in the organic matrix of the dentin with Er:YAG laser using 4 W[11] or fluences between 0.365 and 1.94 J/cm^2,[15] with CO_2—10.6 μm laser using 0.5 W[11] or 10^4 J/cm^2[17] and after irradiation with Nd:YAG laser (571–2857 J/cm^2).[16]

Carbonate loss in heated enamel starts at 100°C and the maximum loss rate occurs at about 800°C; finally, at 1100°C, all carbonate is eliminated.[30] The carbonate loss is observed in enamel irradiated with CO_2 laser using different wavelengths (9.6, 9.3, and 10.6 μm) and fluences.[12,17,18] Hydroxyl radical increases about 70% in heated enamel and a maximum is observed near 400°C,[30] over this temperature the radical content decrease again. In irradiated enamel with CO_2—10.6 μm laser (10^4 J/cm^2) a hydroxyl reduction of about 30% is observed;[17] and in irradiated dentin with Er:YAG laser (0.365–1.94 J/cm^2) an increase of the hydroxyl content is observed.[15]

The cyanate radical (NCO^-) was observed in enamel heated between 325°C and 950°C and in dentin heated between 250°C and 950°C.[32,36] In dentin irradiated with Nd:YAG and with CO_2—10.6 μm laser (688–2857 J/cm^2), two absorption bands at 2015 and 2200 cm^{-1} assigned to cyanmide and cyanate were observed.[16] After the irradiation of enamel with CO_2—10.6 μm laser (10^4 J/cm^2) the cyanate radical was also observed.[17] The carbon dioxide is formed in heated enamel and shows a maximum intensity after thermal treatment at 400°C,[30] above this temperature the CO_2 is lost and at 1100°C is totally eliminated from the tissue. The carbon dioxide formation is observed in irradiated enamel with CO_2—10.6 μm laser using fluence of 10^4 J/cm^2.[17]

The mineral phase (hydroxyapatite) of the hard dental tissues shows a melting point at about 1300°C, but partial fusion was found above 1000°C.[25] After laser irradiation, a melting surface is observed in enamel and dentin irradiated with Nd:YAG,[16] Ho:YAG,[19] Ho:YLF,[20,37] and after CO_2 irradiation with different wavelengths.[12,21] Natural enamel heated in conventional and then slowly cooled down to room temperature develops three new crystalline phases.[33−35] One of them (β-TCP; β-$Ca_3(PO_4)_2$) appears at 400°C and is present at least up to 1200°C and then is converted to α-TCP; (α-$Ca_3(PO_4)_2$). The third phase (TetCP; $Ca_4(PO_4)_2O$) appears at about 1100°C. In irradiated tissues the β-TCP is observed in enamel after Nd:YAG irradiation (829–2487 J/cm^2);[38] and the TetCP is observed in enamel irradiated with Ho:YLF laser (600–800 J/cm^2 [20]) and with CO_2—9.3 μm laser (50 J/cm^2).[12]

The similarities observed between conventional heating in an oven and laser treatment are assigned to similar temperature effects. Differences between the two treatments could be also observed because the fast temperature rise and cooling process of the irradiated tissue can change the structure and chemical composition as not observed after the slow oven treatment. The kind of water, which could be eliminated from the tissue after laser irradiation depends on the temperature generated at the irradiated spot and surrounding regions, i.e., the water with high bound energy will be eliminated only at regions near the irradiated region, while the water with lower bound energy will be eliminated at more distant regions.

CONCLUSION

The activation energy of about $4.1 \, kJ/mol$ for the temperature range between $100°C$ and $400°C$ could be assigned to the adsorbed water that is loosely bound to the tissue and the higher activation energy $\sim 60-63 \, kJ/mol$ for the temperature range between $700°C$ and $1000°C$ could be assigned to the structural water that are tightly bound to the tissue. From our data, we conclude that these values are valid for both dentin and enamel. The different kinds of water elimination depend on the maximum temperature rise produced during the laser irradiation.

ACKNOWLEDGMENTS

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