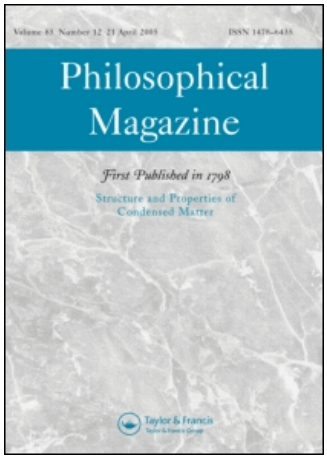

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Thermal degradation of dentin collagen evaluated with ESR, infrared and optical spectroscopy

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Thermal degradation of dentin collagen evaluated with ESR, infrared and optical spectroscopy

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Heating dentin to temperatures <300°C produces tissue browning and electron spin resonance (ESR) radicals. This study reveals the origin of these effects and relates them to conformational changes in collagen molecules and water content in the tissue. Bovine dentin was analyzed by (i) Fourier transform infrared spectroscopy to determine collagen conformation and water content, (ii) ESR spectroscopy operating at the X band to determine the paramagnetic species and (iii) an optical spectrometer in transmission mode to determine changes in the visible spectral absorbance. After heating the tissue to temperatures between 100 and 300°C, some water is eliminated and the hydrogen bonds, which determine collagen alpha-helix structure stabilization, are lost. After elimination, the collagen matrix is changed and electrons are probably trapped, giving rise to ESR signals and absorption bands in the ultraviolet–visible spectral range.

1. Introduction

Heat treatment of dentin at temperatures <300°C causes water loss [1, 2], collagen conformational changes [3], a rise of ESR (electron spin resonance) signals [4, 5] and tissue browning [6, 7]. Organic matrix degradation occurs at 175–200°C [3] and organic material elimination from the tissue at ~310°C [8, 9]. The origin of the effects prior to organic matrix degradation (<175°C) is not totally understood. ESR signals in heated hard and soft dentin tissue have been observed [10, 11], but the origin of these signals is yet to be established. Tissue browning after heating is precisely determined [12] but its origin is also not established. We can assign the origin of these changes to the organic matrix because, in this temperature range (100–300°C), the inorganic matrix remain mainly unaffected although one third of structural water is lost when enamel is heated at 250–300°C [13]. This water loss produces a contraction of the crystal lattice parameter. The observation has been reported in enamel but not extended to the inorganic matrix of the dentine, as discussed in this study.

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We reveal the origin of ESR signals and tissue browning in heat-treated dentin and relate both to conformational changes in collagen molecules.

2. Materials and methods

Samples were from bovine incisor teeth extracted and stored in 0.9 wt% sodium chloride solution. Fourier transform infrared (FTIR) spectroscopy was used to determine collagen conformation and water content. ESR spectroscopy operating at X band was used to determine the paramagnetic species and an optical spectrometer in transmission mode to determine changes in the visible spectral absorbance.

For FTIR spectroscopy, thin samples of root dentin were cut longitudinally at thickness of ~500 μm using a diamond blade. The slices were reduced with carbide abrasive to a thickness of ~50 μm and then polished with 0.3 μm diamond paste. To minimize undesirable thermal effects during cutting and polishing, these procedures were conducted with water irrigation. Spectral transmission was obtained in a Fourier transform Infrared spectrometer (MB-Series, Bomem Hartmann & Braun, Quebec, Canada), in the range of 4000–400 cm^{-1} (2.5–25 μm) with a resolution of 2 cm^{-1}.

To detect paramagnetic signals, a piece of dentin root of $2 \times 2 \times 10$ mm in size was used. ESR measurements were performed using an ESR spectrometer (Varian E-4) with a rectangular resonant cavity (TE–102) operating in the X band.

Samples for optical spectroscopy were fabricated as described for the infrared spectroscopy above, with a thickness of ~100 μm. Optical absorbance was obtained with a transmission spectrometer (Cary–17, USA) in the spectral region 350–750 nm.

3. Results

The infrared absorbance spectrum of natural dentin, shown in figure 1, is composed of bands arising from water molecules, the organic matrix (collagen) and the mineral matrix (hydroxyapatite) [14]. After heat treatment at temperatures between 100 and 300°C, we observed loss of water and degradation of the collagen structure, identified at region A and B, respectively, in figure 1.

Loss of water with heating is correlated with degradation of the collagen structure (figure 2). Heat treatment was carried out at temperatures between 80 and 160°C and the correlation factor was found to be 0.90.

Figure 3 presents the FTIR spectra from region B in figure 1. The absorption bands are assigned to the collagen molecule and are sensitive to molecular conformation [2, 15]. The spectra of natural dentin and dentin heat-treated to 200°C for 30 min are shown. The area of the bands between 1350 and 1150 cm^{-1} decreases with temperature, as shown in figure 4. The band area is reduced to 50%

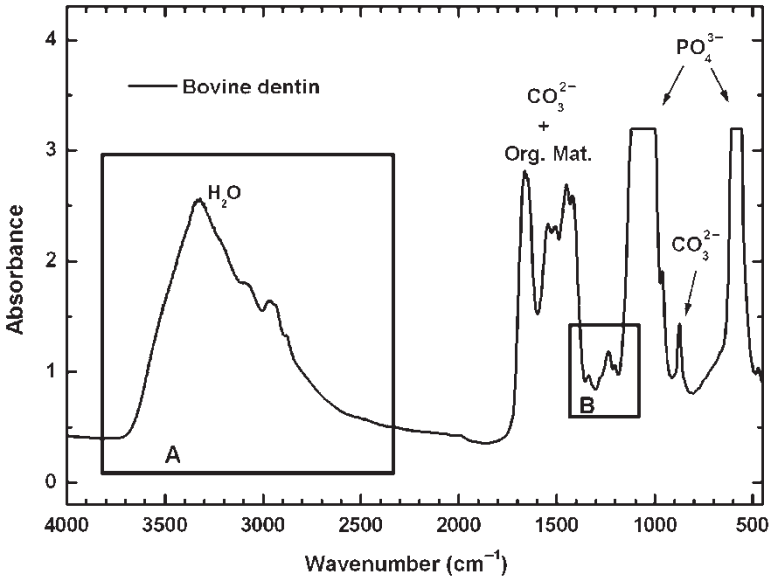

Figure 1. Infrared absorption spectrum of bovine dentin between 4000 and 400 cm^{-1} with a broad water band near 3700–2500 cm^{-1} (region A) and organic matrix bands near 1400–1100 cm^{-1} (region B). The band areas of both water and collagen decrease as treatment temperature increases. Figure 2 displays the correlation of thermally induced water loss and collagen conformation change. The phosphate bands show a saturated absorbance value of 3.

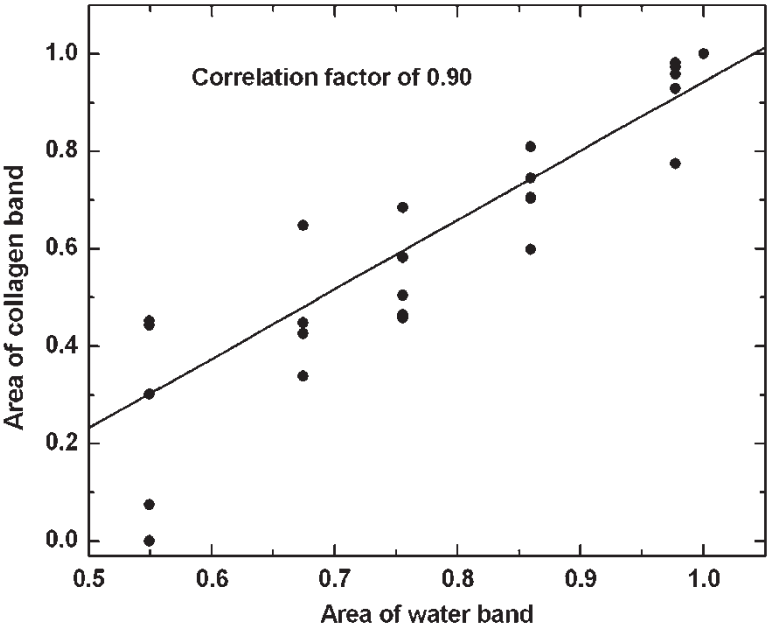

Figure 2. Correlation of water and collagen absorption bands after dentin heat treatment at different temperatures. As water is eliminated from the tissue, collagen conformation changes by unwinding. The values are normalized by the band areas of untreated samples.

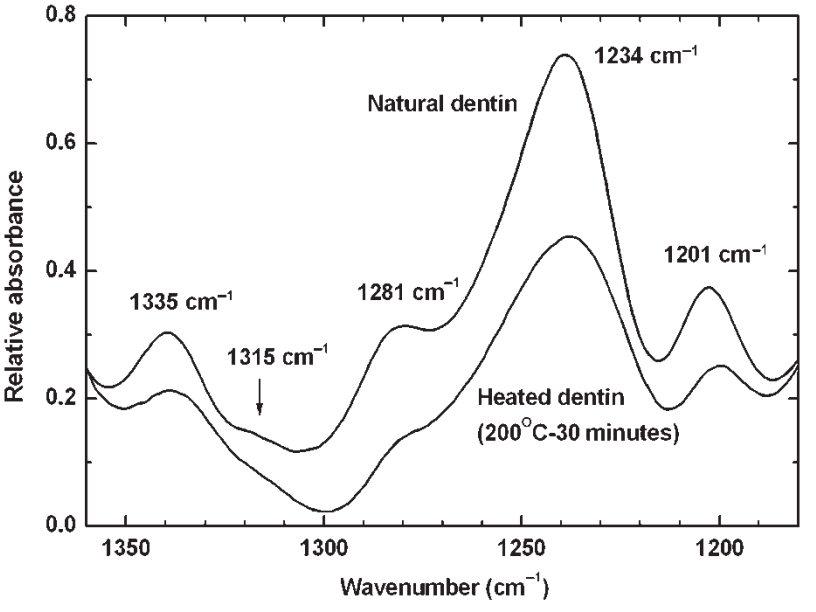

Figure 3. Infrared absorption bands of the collagen molecule in natural dentin and dentin heat-treated at 200°C for 30 min. The bands are assigned to several chemical bonds in collagen that are sensitive to molecule conformation; for more details, see [2].

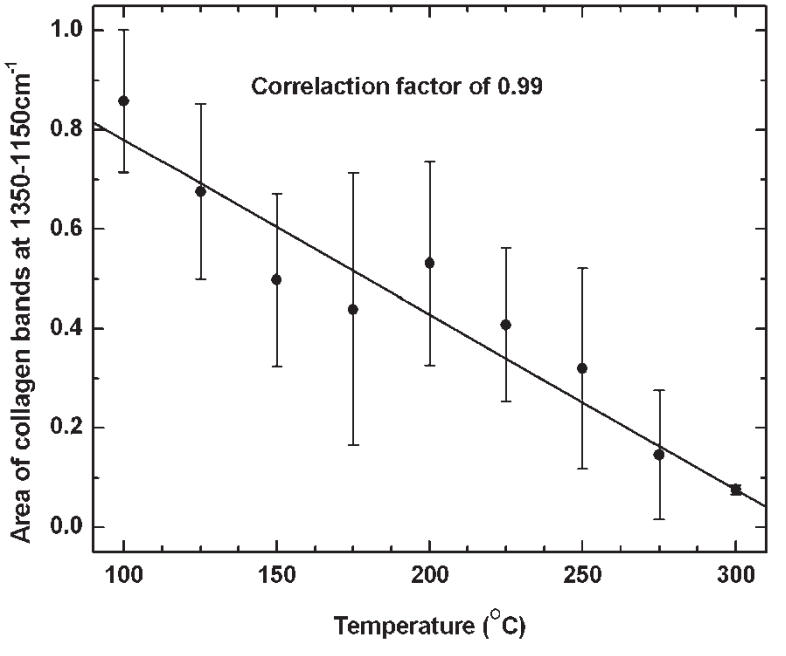

Figure 4. Normalized area of the infrared bands (1400–1100 cm^{-1}) in dentin heated between 100 and 300°C. Data were normalized by area of natural dentin and sample thickness. The area is correlated with temperature at 0.99.

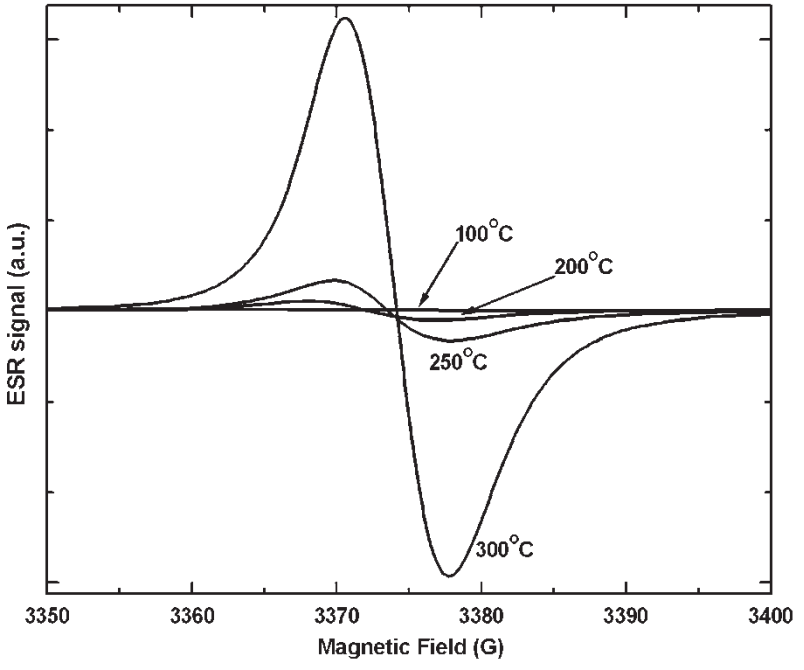

Figure 5. ESR signal of dentin heat-treated for 30 min at 100, 200, 250 and 300°C. The paramagnetic species produce a signal that increases with temperature. The amplitude of all samples heated between 100 and 300°C is visualized in figure 6.

after heat treatment of the tissue at 150°C and to near 0% after heat treatment at 300°C.

Heat treatment of dentin in the same temperature range (100–300°C) increased ESR signals, as shown in figure 5. The intensity is temperature-dependent and is visualized in figure 6. The shape of the signal is unaltered with temperature, except for a slight reduction in width. The intensity in the figure 6 is normalized by weight and expressed in a logarithmic scale.

Over the temperature range 100–300°C, the dentin becomes brown as the temperature increases. Absorbance in the visible spectral range is shown in figure 7; these data are relative absorbance, determined from the difference between absorbance of the heated and unheated sample. To measure tissue browning, a power law (absorbance $= a\lambda^{-b}$) it was adjusted to the experimental data [16], where a and b are constants obtained from the adjustment to the experimental data. This exponent will measure the strength of browning. Figure 8 shows the exponent values in dentin heat-treated to temperatures between 100 and 300°C.

4. Discussion

Over the evaluated temperature range (100–300°C) collagen degradation (figure 4) is accompanied by the formation of a paramagnetic species (figure 6) and tissue

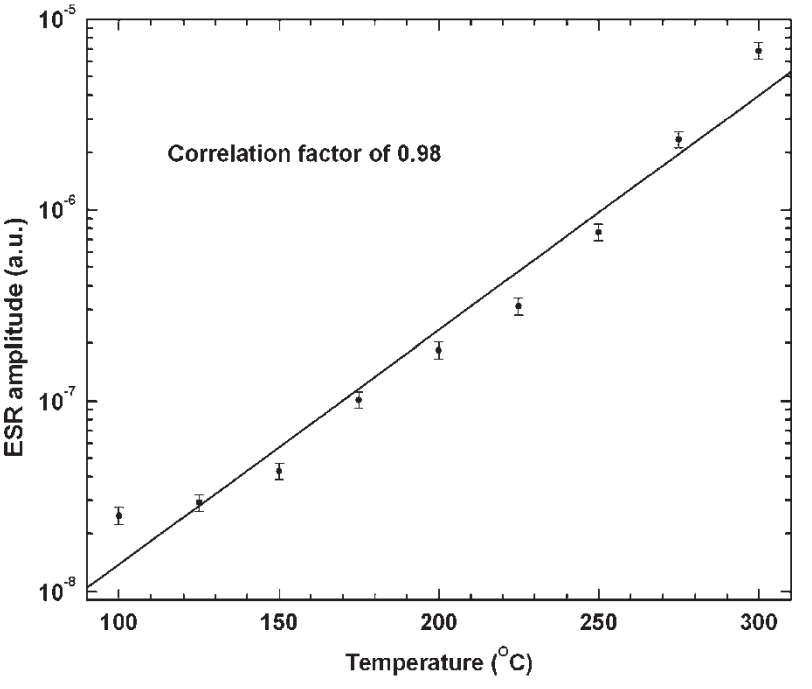

Figure 6. ESR signals formed in dentin heated (100–300°C) for 30 min at each temperature. Amplitude is normalized by sample mass. The amplitude is correlated with temperature at 0.98.

browning (figure 8). Figure 9 details the correlation of these results during heating. The correlation factors are as follows: between collagen structure and tissue browning −0.87, between collagen structure and ESR species −0.94 and between ESR species and tissue browning 0.92. These results are also associated with water elimination during heating, as shown in figure 2, where water loss is accompanied by collagen structure degradation.

Water is the first chemical compound released from the tissue upon heating. Literature reports show that adsorbed water is completely released from enamel tissue at 200°C [17]; a similar results was observed for dentine tissue [3]. The remaining water is bonded to the tissue structure and is released only when the tissue is heated to between 400 and 1300°C [18, 19]. The thermal stability of water is attributed to different bond energies between the molecule and its site in the tissue [1].

The organic matrix is mainly composed of collagen molecules type I and it is assume for our model that dentin is filled with collagen and hydroxyapatite, as described for bone tissue [20]. The collagen molecule consists of three polypeptide chains, each twisted around an axis with a pitch of about 9.3 Å. It has three amino acid units per turn [21]. Each twisted chain is twisted again with a longer pitch of about 28.6 Å with 10 amino acids per turn. Finally, the three polypeptide chains,

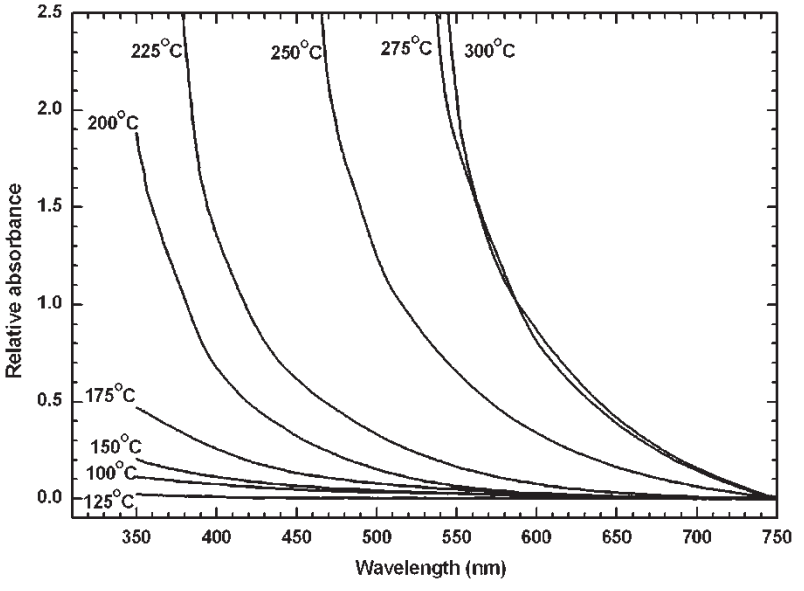

Figure 7. Relative absorbance of dentin heated between 100 and 300°C. After heating, the absorbance is higher for shorter wavelengths. Values are normalized by the absorbance of unheated tissue and sample thickness. A power law (absorbance $= a\lambda^{-b}$) was used to measure the browning of heated tissue [16]. The temperature dependence of exponent b is visualized in figure 8.

with a twisted phase of 120° between each chain, build the collagen 'super-helix'. Polypeptide chain proximity in the molecule allows the formation of inter-chain weak hydrogen bonds or strong covalent bonds. Though the hydrogen bonds are very weak, their presence in the polypeptide chain occurs at each three amino acids, i.e. collectively, they are sufficiently strong to hold the three chains together. Additional covalent cross-links, also present in collagen, increase the structural stabilization [22].

According to other authors who propose the unwinding and shrinkage of the collagen triple helices after thermal denaturation [23], the hydrogen bonds, which determine collagen alpha-helix structural stabilization, are lost with water loss from heat-treated dentin.

To correlate water loss and collagen conformation to the paramagnetic species and tissue browning, an electrostatic model proposed for the collagen fibril [24] is employed. According to this model, the amino acid sequence in the collagen molecule has charges along the axis of the molecule. The natural collagen fibril is neutral because a positive charge of one molecule is neutralized by a negative charge of a neighbour molecule or other molecules of water or hydroxyapatite. After heating, the unwound structure has a different charge distribution and the molecules are no longer neutral, giving rise to charges or trapped electrons. Therefore, the tissue displays

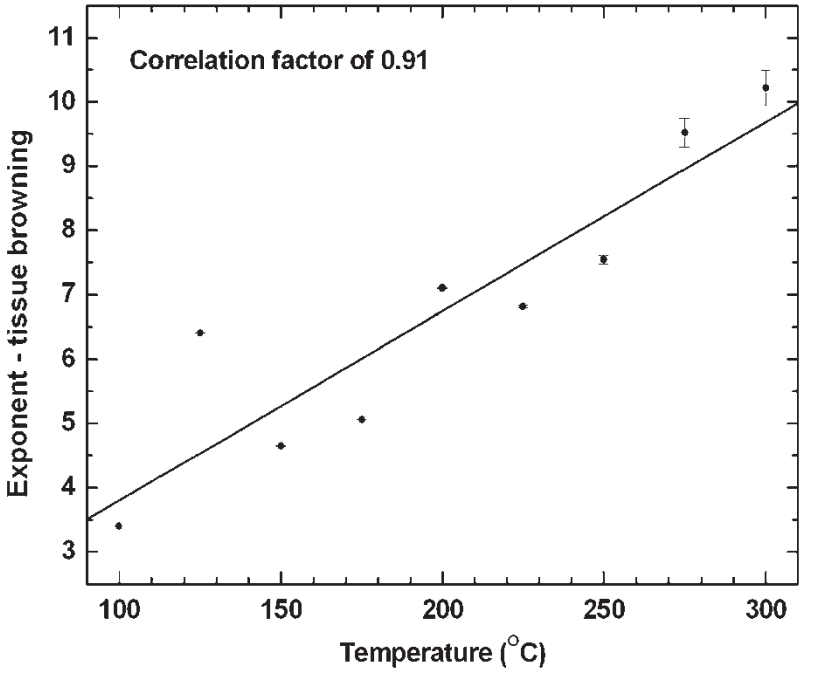

Figure 8. Exponent of the power law (absorbance $= a\lambda^{-b}$) of natural dentin and dentin heated between 100 and 300°C. The exponent value was determined by adjusting the function to the experimental data; the exponent is correlated with temperature at a factor of 0.91.

paramagnetic signals and modified optical properties, such as the tissue browning observed in this work. Although localization of the charges in the tissue is not determined, they are probably located near the hydrogen bond sites.

To reinforce the correlation between water loss and collagen structure degradation with the formation of paramagnetic species and tissue browning, the reversibility of all changes in samples heat treated at temperatures below 200°C were observed. Literature reports indicate that only the adsorbed water, which is eliminated after heating the tissue to 200°C, can be reincorporated [17]. This agrees with our results, which show restoration of collagen structure, paramagnetic species and tissue browning only for samples heat-treated below 200°C.

According to our model, the incorporation of water molecules during the hydration of heat-treated tissue ($T < 200$°C) re-establishes the hydrogen bonds. Consequently, these bonds reverts the collagen to its natural structure observed before treatment and the charge distribution is reversed. As ESR signals and tissue browning originate from trapped electrons, both effects disappear after tissue hydration.

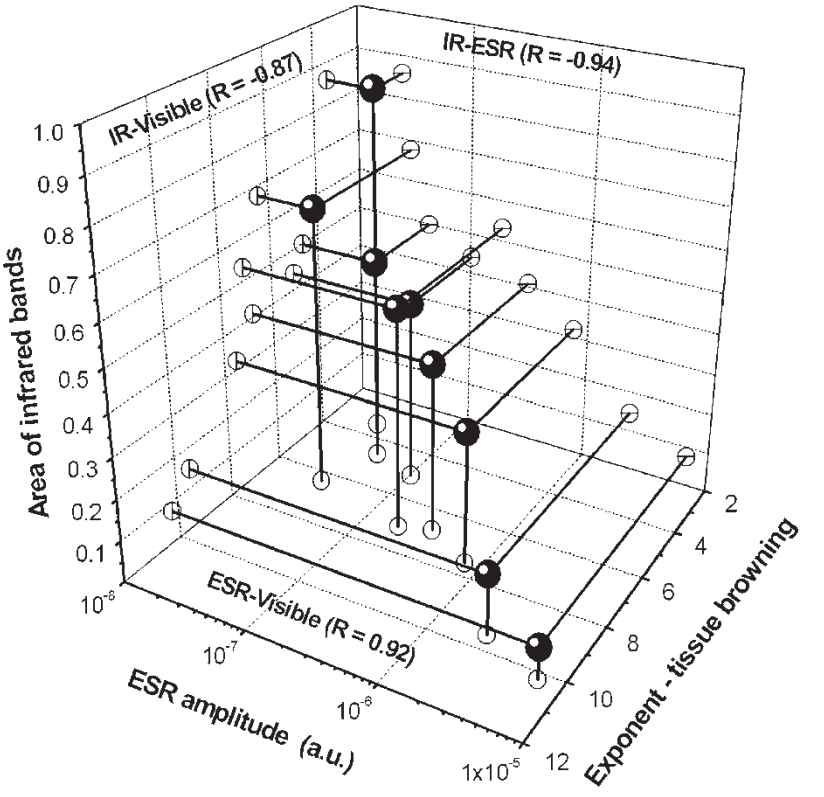

Figure 9. Correlation of ESR signals, tissue browning and infrared absorption bands of heated dentin (100–300°C). The ESR signals are correlated with infrared band degradation at a factor of −0.94, with tissue browning (exponent value) at 0.90 and the infrared bands are correlated with tissue browning at −0.82. These results showed the appearance of ESR signals and tissue browning as the collagen structure is deformed. As showed in figure 2, water loss is also correlated to collagen structure at a factor of 0.90, indicating that the four variables: water loss, collagen degradation, ESR signals and tissue browning are interrelated, indicating the effect of a particular phenomenon.

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