

# ***In vitro* Study Using ATR-FTIR Methode for Analyze the Effects of the Carbamide Peroxide on the Dental Structure**

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*This study aims to analyze the effect produced by carbamide peroxide solution used in the teeth whitening process on the enamel and dentin structure through the Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) technique. To carry out the in vitro study, we used three different types of teeth, namely the incisor, premolar and molar, as well as three different concentrations of peroxide carbamide, namely 10%, 16% and 35%, for 90 min. We measured and compared the frequency bands as phosphate ( $PO_4^{3-}$ ) and carbonate ( $CO_3^{2-}$ ) by FTIR spectroscopy method both before and after whitening. The study showed that FTIR method is useful for the analysis of the organic and inorganic structure of enamel and dentin, the highest modifications being noticed when using the 35% peroxide carbamide concentration by the reduction of the mineral component especially carbonate and phosphate anions.*

**Keywords:** ATR-FTIR, carbamide peroxide, dental structure

Teeth whitening represents a method for the elimination of pigments from the surface of enamel by using chemical substances such as hydrogen peroxide or carbamide peroxide at different concentrations varying between 10% for the products used at home and 40% for those applied by the dentist in the dental room [1]. The effect of bleaching agents on enamel has been evaluated using *in vitro* study in three aspects: mineral loss, morphological integrity and alteration of surface microhardness [2, 3]. As for the loss of minerals from enamel structure, mainly calcium and phosphate, there are controversial data: some authors such as Mc Cracken shows in a study carried out in 1996 that there is a risk of alteration of the dental structure regardless of the whitening agent concentration [4]. Others affirm that deteriorations in the enamel structure may occur only at high concentrations of carbamide peroxide [5]. On the contrary, other researchers consider that peroxide carbamide does not have any effect on the enamel structure [6].

Quantitative analyzing using infrared spectroscopy (IR) is based on the intensity of IR absorption being proportional to the magnitude of the change in the dipole moment of a bond during vibration [7]. Peak intensity (height) and the area are most commonly used measurements [7]. Since it is a nondestructive method, it has been used successfully in *in vitro* studies in different fields, from chemistry to physics, geology and archeology up to various medical and pharmaceutical domains [8-11].

The purpose of this study *in vitro* was to analyze the structural modifications of the enamel and dentine, before and after bleaching, using Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) technique, with the hypotheses that the modification in enamel is proportional to hydrogen peroxide concentration.

## **Experimental part**

For this study we used sound teeth extracted for orthodontic and periodontal reason (incisor, premolar and molar), treated with 10% (incisive), 16% (premolar) and 35% (molar) carbamide peroxide (44% Teeth Whitening Gel, WGS44-10, United States) solutions, respectively. The

pH of the carbamide peroxide solutions was 7.4 (measured in laboratory). The bleaching treatments were performed for each tooth at room temperature in closed dishes for 30 min/day over three months; the total treatment time was 90 min. We measured each tooth, before and after bleaching procedure, using the spectroscopy method, Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR)-FTIR.

Fourier transform infrared (FTIR) spectra were recorded using a Bruker Vertex 70 FTIR spectrometer equipped with a ZnSe crystal, in ATR (Attenuated Total Reflectance) mode in the range 600-4000  $cm^{-1}$  at room temperature with a resolution of 4  $cm^{-1}$  and accumulation of 32 scans. The spectral regions (1800-1200 and 1200-770  $cm^{-1}$ ) corresponding to the protein matrix and mineralized tissue (enamel) were deconvoluted by a curve-fitting method, and the areas were calculated with a 50% Lorentzian/50% Gaussian function. Like other methods, (dilatometer analysis), in FTIR method, the curve-fitting analysis was performed with the OPUS 6.5 software [12]. The procedure led to a best fit of the original curve with an error of less than 0.001.

## **Results and discussions**

In the IR spectra of the teeth before whitening treatment can be seen the characteristic vibration bands corresponding to the mineral component of enamel and dentine (hydroxyapatite  $Ca_{10}(PO_4)_6(OH)_2$  - 95% in enamel and 70% in dentine tissue): the phosphate ( $PO_4^{3-}$ ) at 1020  $cm^{-1}$  (incisor), 1001  $cm^{-1}$  (premolar) and 1030  $cm^{-1}$  (molar) and carbonate ( $CO_3^{2-}$ ) at 874-878  $cm^{-1}$ . The frequencies and the ration between these bands depend on the local environment and vary depending on the modifications of the crystallinity degree. It can be observed in figure 1 that the most intense band in the spectra of the mineralized tissues is the phosphate one. The carbonate band and the amide I-III bands can be observed at 1450-1462  $cm^{-1}$  and 1547-1645  $cm^{-1}$  (amide II and I) and 1240  $cm^{-1}$  (amide III), respectively [13, 14].

After the whitening treatments ATR-IR spectra of the analyzed teeth revealed a bleaching-induced shift of the

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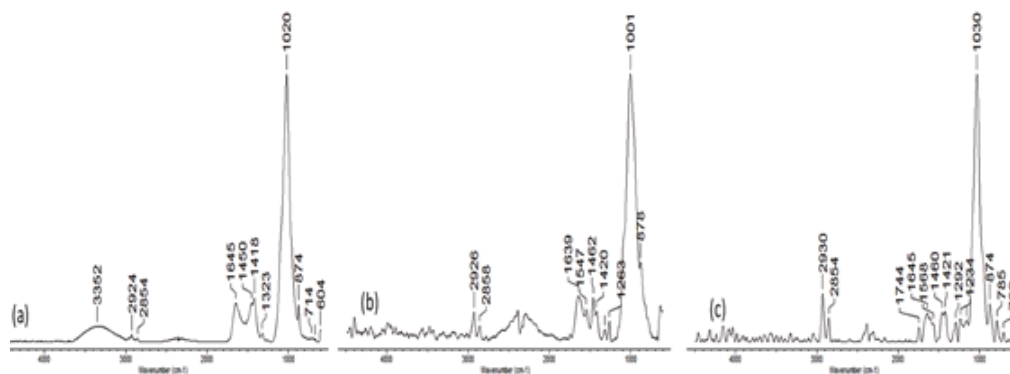


Fig. 1. ATR-FTIR spectra of the incisor (a), premolar (b) and molar (c) before the bleaching treatments.

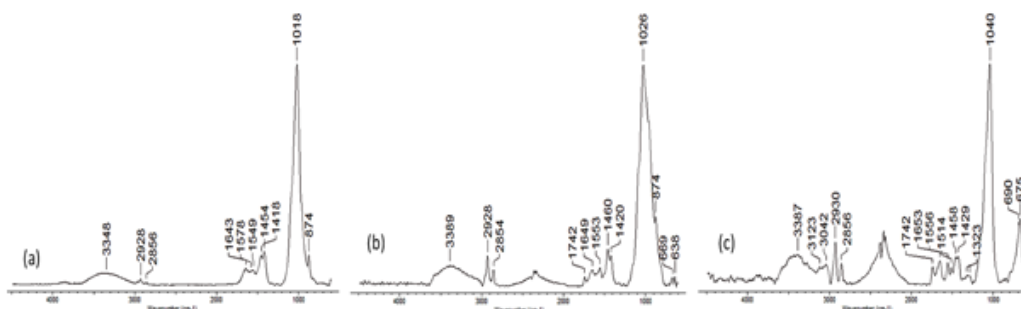


Fig. 2. ATR-FTIR spectra of the incisor (a), premolar (b) and molar (c) after the bleaching treatments

Sample	1200-770 cm <sup>-1</sup> / Area		1800-1200 cm <sup>-1</sup> / Area	
	Before bleaching	After bleaching	Before bleaching	After bleaching
Incisor	1080 / 27.40	1073 / 26.90	1649 / 7.51	1649 / 4.59
	1017 / 50.82	1015 / 45.81	1574 / 7.68	1576 / 3.75
	954 / 22.49	954 / 21.13	1456 / 8.86	1456 / 9.20
	872 / 2.55	872 / 2.33	1411 / 6.29	1414 / 3.77
			1321 / 0.53	1259 / 0.04
Premolar	1093 / 16.28	1091 / 32.72	1638 / 14.51	1740 / 0.97
	1006 / 86.61	1028 / 55.05	1543 / 9.79	1652 / 5.17
	942 / 22.97	961 / 62.34	1460 / 5.36	1560 / 5.87
	872 / 21.68	867 / 24.11	1415 / 4.57	1460 / 10.62
			1319 / 1.36	1416 / 3.79
Molar			1263 / 1.89	1324 / 0.16
	1087 / 17.94	1158 / 4.45	1742 / 1.86	1738 / 2.78
	1028 / 73.57	1102 / 21.00	1653 / 10.30	1660 / 7.99
	952 / 18.39	1062 / 25.64	1594 / 7.82	1558 / 4.70
	872 / 7.24	1033 / 20.71	1558 / 2.49	1512 / 2.58
			1513 / 0.73	1462 / 4.76
			1467 / 5.52	1423 / 7.05
		1421 / 24.90	1318 / 3.39	
		1307 / 3.80		

**Table 1**  
THE RESULTS OF THE DECONVOLUTION OF FTIR SPECTRA AND THE AREA FOR THE CHARACTERISTIC PEAKS OF THE ANALYZED SAMPLES

phosphate stretching vibration to higher wavenumbers for premolar and molar samples suggesting a strongly bonded O atoms to the phosphorous ones (fig. 2). In the apatite structure each P atom is linked to four Ca atoms via a shared O atom, i.e. the framework is composed of P-O-Ca atomic bridges. Hence, tightening of P-O bonds means in fact loosening of the adjacent Ca-O bonds due to the redistribution of the electron density of states in the vicinity of the bridging oxygen. Therefore, the bleaching-induced peak shift points indirectly to a decrease in the Ca-O bond strength, i.e. changes in the local structure. The weakening of Ca-O bonds means that the ability of Ca to be released from the structure should be increased during the bleaching process [15].

Teeth whitening treatments attempt to lighten a teeth's color by chemical or mechanical action. Working chemically, a bleaching agent, in our case carbamide peroxide, was used to carry out an oxidation reaction in the enamel which can influence its structure and composition and can affect the dentin integrity. The observed spectral differences between the intensity and absorption bands characteristic for enamel and dentin

before and after bleaching procedures were analyzed by deconvolution of the spectra in the 1800-1200 cm<sup>-1</sup> and 1200-770 cm<sup>-1</sup> spectral region and are reflected by their specific areas (table 1 and figs. 3-6).

The IR spectra recorded in the 1200-770 cm<sup>-1</sup> and 1800-1200 cm<sup>-1</sup> spectral regions showed the characteristic bands of stretching vibrations of the following groups: 1015-1080 cm<sup>-1</sup> (orthophosphate), 954 cm<sup>-1</sup> (disordered phosphate), 872 cm<sup>-1</sup> (ordered phosphate), 1259-1263 cm<sup>-1</sup> (amide III, C-N valence vibration and deformation of N-H groups), 1540-1600 cm<sup>-1</sup> (amide II band-deformation vibration of N-H group and valence vibration of C-N groups), 1400-1460 cm<sup>-1</sup> (carbonate bands), 1630-1660 (amide I band), 1738-1742 cm<sup>-1</sup> (acids/esters formed in the organic matrix) [16].

The main changes in the IR spectra of the samples before and after bleaching procedures depend on the concentration of the carbamide peroxide. The structural modifications of enamel can be observed by the changes in the area of the 1015-1030 cm<sup>-1</sup> and 1540-1560 cm<sup>-1</sup>, while those of the dentine tissue by the area associated with organic compounds (amides I, II, and III) (fig. 3 and 4).

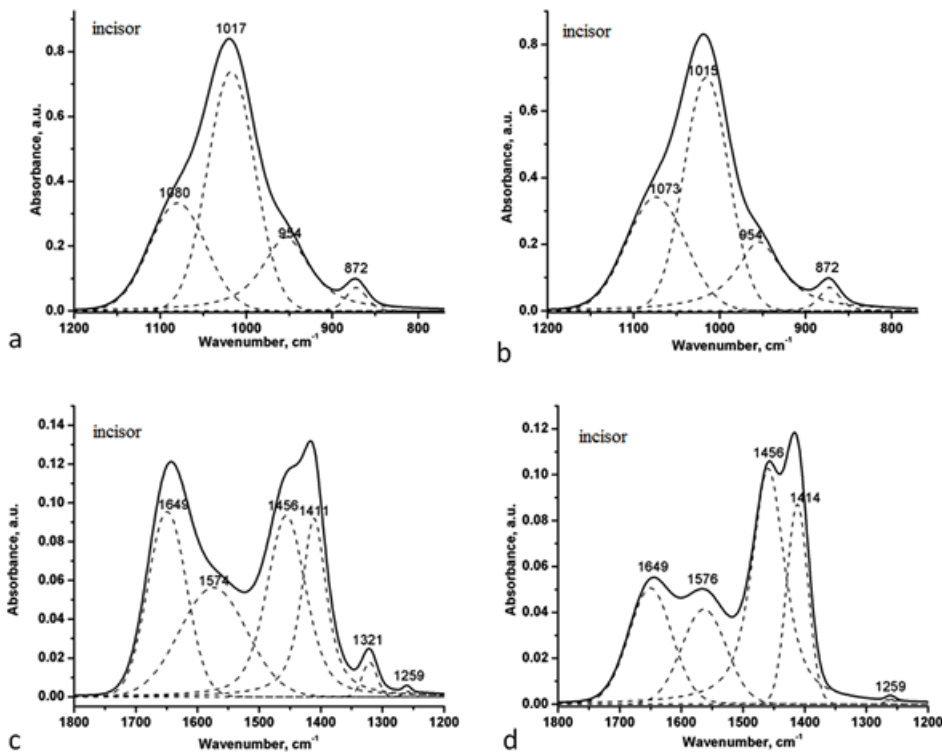


Fig. 3. Curve-fitting results in the 1800-770  $\text{cm}^{-1}$  stretching region of the incisor before (a, c) and after (b, d) 90 min bleaching treatment with 10% carbamide peroxide solution

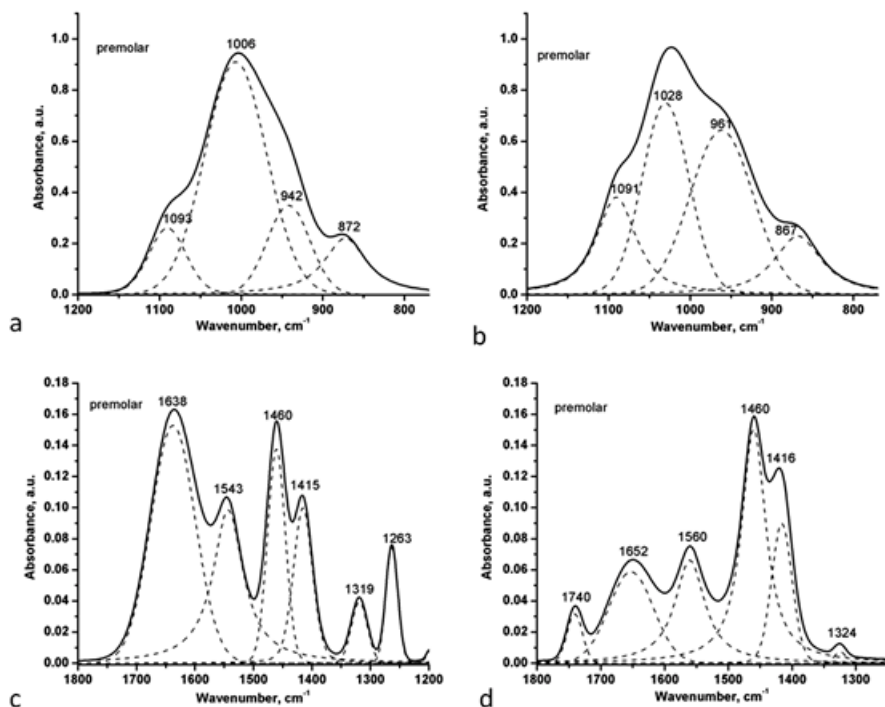


Fig. 4. Curve-fitting results in the 1800-770  $\text{cm}^{-1}$  stretching region of the premolar before (a, c) and after (b, d) 90 min bleaching treatment with 16% carbamide peroxide solution

It can be seen that the most important changes occur in molar structure, where the bands at 872 and 952  $\text{cm}^{-1}$  assigned to the carbonate and phosphate anions disappeared and the specific areas of the bands at 1087 and 1028  $\text{cm}^{-1}$  dramatically decreased, suggesting a destruction of the enamel tissue (fig. 5). The concentration and the exposure time of the treatment also affect the dentine tissue. The specific areas of the peaks assigned to the organic matrix in the dentine structure revealed this observation. In the other cases (incisor and premolar samples) the effect of the concentration of carbamide peroxide solutions on the enamel and dentine structure led to an alteration in the band position and its area specific for the mineral and organic contents.

Our results are in agreement with those of other studies that demonstrated alteration in enamel structure using ATR-FTIR method. Bistey et al. found that bleaching agents are capable of causing alteration in enamel at low and high concentrations as well [17]. Soares et al. using 10% and 16% of carbamide peroxide gels concluded that both concentrations reduced the mineral content, especially, Calcium and Phosphate [18]. According Tezel, higher concentrations of bleaching agents cause more Calcium loss than lower concentrations. The contact time of high concentrated bleaching agents may also be an important factor for Calcium loss [19].

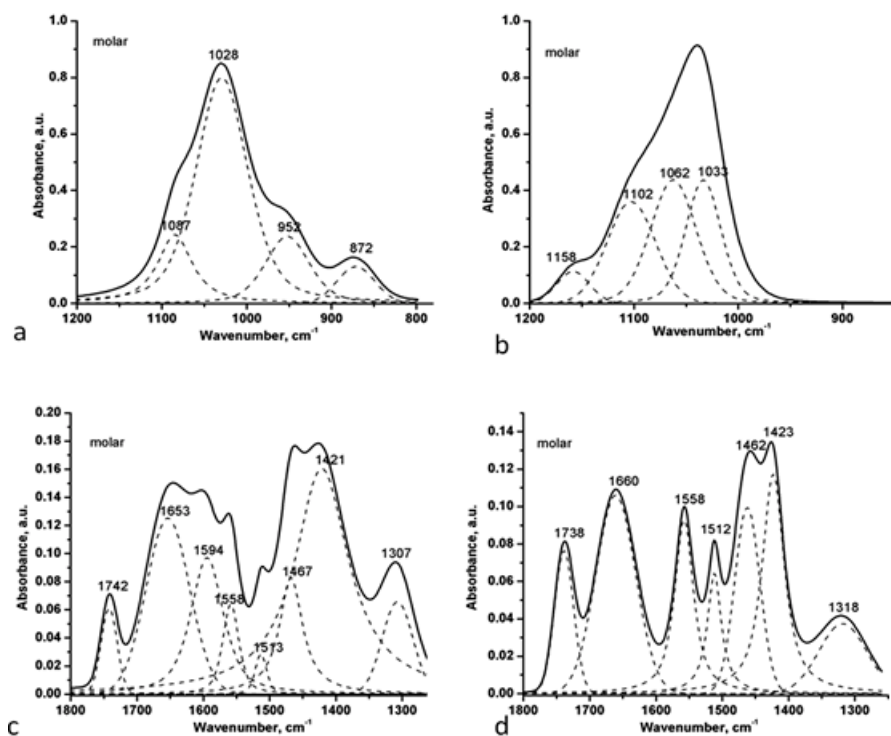


Fig. 5. Curve-fitting results in the 1800-770  $\text{cm}^{-1}$  stretching region of the molar before (a, c) and after (b, d) 90 min bleaching treatment with 35% carbamide peroxide solution

## Conclusions

We observed spectral differences between the intensity and absorption bands characteristic for enamel and dentin before and after bleaching procedures. The results of our investigation may confirm the hypothesis that the concentration and the exposure time of the treatment affect the dental tissue, the most important changes occur with higher concentration of 35% peroxide carbamide. FTIR spectra showed that the total amount of phosphate and carbonate mineral contents decreased significantly.

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