The Effect of *ortho*-Phosphoric Acid Etching Application on Enamel Surface

ATR-FTIR and SEM studies

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The acid etching procedure represents a procedure that increases the bond strength between the etched enamel and the composite resin. The aim of this study was to evaluate the effect of ortho-phosphoric acid 37% on the morphology and composition of the enamel surface. The surfaces of four extracted teeth (two incisors and two premolars) were etched with 37% ortho-phosphoric acid solution for 15 seconds. The teeth were examined by ATR-FTIR spectroscopy and SEM microscopy in order to determine the degree of the enamel demineralization. A major change on teeth composition and morphology has been found. The results are discussed and compared with those of the effects of different concentrations of bleaching agent (carbamide peroxide) on the enamel surface. Etching with 37% ortho-phosphoric acid caused structural and morphological changes to the dental enamel surfaces.

Keywords: enamel, ortho-phosporic acid, SEM, ATR-FTIR

The acid etch procedure, a concept first proposed by Buonocore (1955), has been a usual clinical procedure that increases the bond strength between the composite resin and etched enamel [1]. Adhesion at the level of dental enamel imposes the use of 35-37% *ortho*-phosphoric acid which by demineralization leads to the appearance of micro-porosities, increasing the retention of the composite material on the enamel tissue [2]. When the enamel is treated with *ortho*-phosphoric acid of high concentration, there takes place an acid-base reaction with the formation of soluble salts of calcium phosphate thus producing an irreversible loss of hard substance. The substance loss between 5 and 12 μm, depends mostly on the concentration of acid used and the time of exposure [3]. Applied for more than 60 s, *ortho*-phosphoric acid can amplify the defects of structure and determine cracks in the enamel structure [4]. It is documented that acid-etched enamel can be remineralised, but the remineralisation is not complete [5].

On the other hand, the effect of bleaching agents on enamel has been evaluated *in vitro* studies which concluded that carbamide peroxide produces enamel surface morphological changes and mineral loss [6-8].

The aim of this *in vitro* study was to evaluate the morphological effects of 37% ortho-phosphoric acid on dental enamel surfaces using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) and Scanning Electron Microscope (SEM) techniques.

Experimental part

Material and method

The study was carried on 4 sound maxillary incisors and premolars, extracted for orthodontic and periodontal reasons, stored in 35% ethanol. Before the strat of the experiments the teeth were analyzed by Attenuated Total Reflectance (ATR) spectroscopy and Scanning Electronic Microscopy (SEM).

The 37% *ortho*-phosphoric acid was applied on the enamel surface for 15 s. Then the surface was continously washed with distilled water for 30 s. The teeth were dried in vacuum and analyzed using ATR-FTIR and SEM methods.

Measurements

Fourier Transform Infrared (FTIR) spectra were recorded using a Bruker Vertex 70 FTIR spectrometer equipped with a ZnSe crystal, in Attenuated Total Reflectance (ATR) 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 1200-600 cm^{-1}) were deconvoluted by a curve-fitting method, and the areas were calculated with a 50% Lorentzian and 50% Gaussian function. The curve-fitting analysis was performed with the OPUS 6.5 software. The procedure led to a best fit of the original curve with an error of less than 0.001. The differences in composition of the analyzed teeth (incisors and premolars) before and after etching of dental enamel surface with 37% *ortho*-phosphoric acid were highlighted by using the method of infrared spectral substraction of the samples. In this case the resulting substraction spectra will correspond to the differences between the spectrum of the treated teeth and the spectrum of the same teeth before etching of dental enamel. Spectra were processed by using Bruker OPUS 6.5 software.

The morphology of the teeth before and after etching procedure was investigated with the SEM type Quanta 200 (FEI Company), in low vacuum mode, at 20 kV. In order to conduct the treatment the samples were uncoated.

Results and discussions

The number of composite resin restorations has increased lately due to both their special esthetic qualities and the advancements in enamel and dentine adhesion. But, the excess of remained unpolymerized dimethacrylate monomer and the etching procedures may cause cytotoxic effects [9-11].

The results of our study show that, within the sensitivity of the applied method, the etching procedure significantly affected the dental enamel, in comparison with the

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bleaching method that used different concentrations of carbamide peroxide [12, 13]. The results of this study are in agreement with previous literature reports [14, 15]. Enamel has a heterogeneous composition which varies in mineral content, amount of organic matrix and chemical construction. Studies have demonstrated that the surface of the enamel is significantly affected by *ortho*-phosphoric acid, even for a short period of exposure [16].

ATR-IR spectroscopy

The advantage of ATR-FTIR spectroscopy as an analytical method for dental materials research is its ability to probe the structure of the tooth tissues and to establish the changes in composition after application of various treatments (bleaching, etching).

Combined with SEM method, ATR-FTIR supplies complete information of the enamel structure and its evolution during different treatments.

In our previous researches it was shown that the bleaching with different concentrations of carbamide peroxide (10, 16 and 35%) for 90 min, led to the redshift of the stretching mode of PO_4^{3-} suggesting a loosening of Ca-O bonds and a redistribution of the electron density to the adjacent P atoms and morphological changes on teeth surface. Therefore, the bleaching methods led to structural defects (erosion) of the enamel which become meaningful by increasing the concentration of the bleaching agent [12, 13].

In figure 1, are presented the substracted IR spectra of the incisors and premolars before and after etching procedure. It can be seen that the initial composition of the enamel (96 % minerals-hydroxyapatite, water and organic proteins) is changed, with a significant loss of specific vibrations bands for inorganic matrix at 600-700 cm^{-1} assigned to the bending mode of PO_4 tetrahedral, 833-845 cm^{-1} attributed to the bending mode of CO_3^{2-} in the structure of apatite and 900-1200 cm^{-1} characteristic for stretching modes of PO_4^{3-}Beside these, the IR subtracted spectra revealed a major demineralization process involving the organic protein matrix. The presence of the specific bands for amide I, II and III at 1728-1740 cm^{-1}, 1643-1550 cm^{-1} and 1230-1260 cm^{-1}, respectively clearly indicates the collagen structure in the dentine composition.

The spectral differences between the teeth that were treated or non-treated with *ortho*-phosphoric acid were studied by deconvolution of the IR spectra in the 1800-1200 cm^{-1} and 1200-600 cm^{-1} spectral region. The specific peak position and the corresponding area were compared and the results are presented in table 1.

In figures 2 and 3 can be seen the main changes in the IR spectra of the samples before and after etching procedure with *ortho*-phosphoric acid.

The structural changes in the surface of the teeth are highlighted by the disappearance of the specific vibration bands of enamel: inorganic carbonates at 1495-1410 cm^{-1}, 1090-1080 cm^{-1}, 885-870 cm^{-1}, 860-845 cm^{-1}, 715-695 cm^{-1}, PO_4^{3-} stretches at 1200-900 cm^{-1} and the appearance of those characteristic for protein matrix in the structure of dentine: amide I at 1700-1780 cm^{-1}, amide II at 1550 cm^{-1}, amide III at 1250 cm^{-1}. It can be seen a drastically decrease of the area specific for the stretching modes of phosphate and carbonate groups and an increase of the area specific for protein matrix. These modifications are associated with the demineralization process which occurs by application of the etching agent on the enamel surface of teeth. By etching procedure the degree of demineralization is higher than that resulting from bleaching treatments of teeth, as our previously studies revealed [12,13].

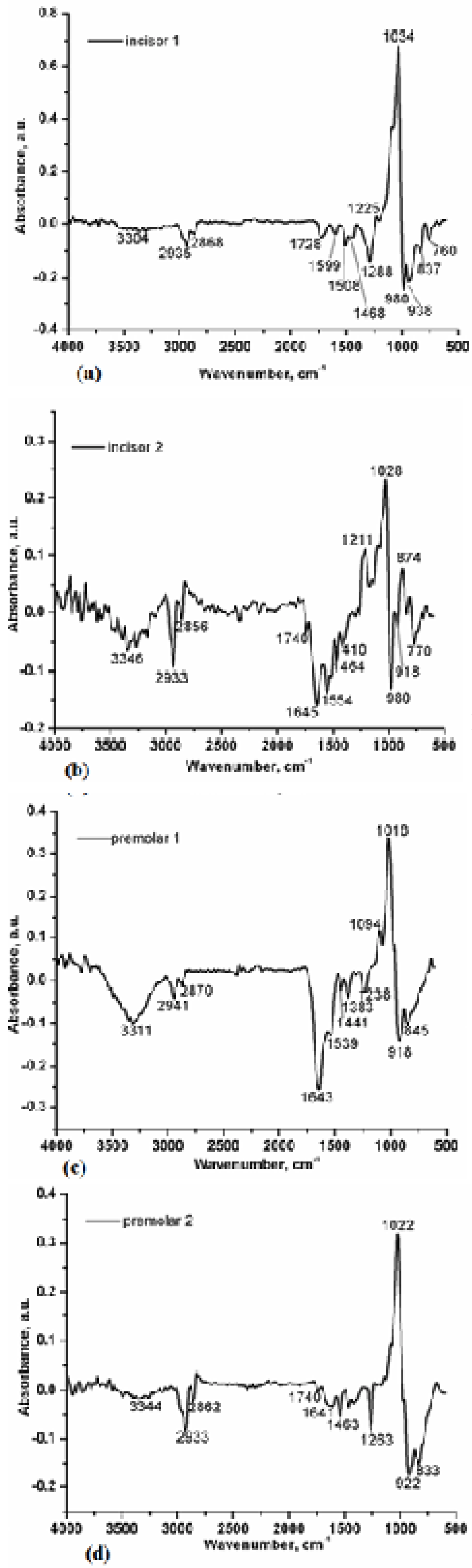

Fig. 1. Subtracted IR spectra of the incisors (a, b) and premolars (c, d) samples before and after etching procedure with *ortho*-phosphoric acid 37%

SEM technique

Figure 4 presents the initial surface of one incisor in comparison with its surface after the etching treatment with *ortho*-phosphoric acid. The changes of morphology of one premolar can be seen in figure 5.

As it can be seen from the SEM micrographs, the surface of the teeth changed completely after treatment, due to the demineralization of the enamel. The initial surface of the teeth was smooth, containing small defects due to mastication. As the demineralization process occurs, the enamel was destroyed, leaving a rough surface and

Sample	1800-1200 cm⁻¹ / Area		1200-600 cm⁻¹ / Area	
	Initially	After etching	Initially	After etching
Incisor 1	1722 / 6.99	-	-	1236/0.39
	1662 / 8.98	1693/1.34	-	1202/1.34
	1596 / 3.66	1650/1.20	-	1148/2.62
	1546 / 1.91	1551/0.59	1065/15.01	1097/17.87
	1510/5.38	1510/0.95	998/71.72	1027/60.75
	1461/18.07	1461/2.69	944/23.29	959/4.19
	1394/3.89	1414/0.87	910/4.25	-
	1328/7.84	-	876/18.76	873/2.16
	1286/12.61	1288/0.56	830/5.18	-
	1248/2.28	-	755/4.82	-
	1200/3.83	-	664/1.46	-
	1153/3.02	-		
Incisor 2	1738/1.23	1772/0.59	1091/5.32	1088/11.20
	1691/4.48	1713/0.81	1032/35.65	1018/65.80
	1651/11.12	1669/3.38	994/37.34	-
	1614/6.46	1627/1.60	949/31.36	948/43.12
	1557/6.65	1575/0.13	914/36.82	-
	1530/4.47	1551/0.15	870/1.37	877/32.27
	1509/3.11	1527/1.24	842/18.18	814/10.19
	1463/7.45	1478/2.42	766/4.87	-
	1418/6.54	1447/3.78	636/0.70	635/0.37
	1408/2.69	1415/1.81		
	1286/2.29	1281/0.34		
	1255/0.27	1239/1.33		
Premolar 1	-	1796/0.13	1085/9.43	1086/11.62
	-	1773/0.17	1034/33.79	1013/64.39
	-	1741/0.42	994/40.38	-
	1722/1.93	1700/0.72	952/26.19	956/38.79
	1683/7.53	1675/0.41	915/15.05	-
	1650/16.50	1650/0.99	870/18.32	873/6.63
	1615/11.85	1622/0.38	834/17.72	-
	1560/12.20	1555/1.87		
	1525/6.67	1507/1.75		
	1457/14.29	1463/3.94		
	1397/5.54	1417/3.60		
	1234/3.08	1283/0.33		
Premolar 2	1732/0.76	1780/0.27	1087/10.92	1089/10.64
	1655/4.65	1664/0.29	998/82.05	1012/75.65
	1604/3.06	1629/0.46	933/38.73	950/32.86
	-	1606/0.20	866/20.08	875/14.60
	1546/3.93	1571/0.51	826/3.72	-
	1466/1.13	1552/0.38	793/12.66	-
	1463/10.66	1491/1.15		
	1415/7.40	1418/4.29		
	1264/3.76	1272/0.59		
	-	1247/0.10		

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

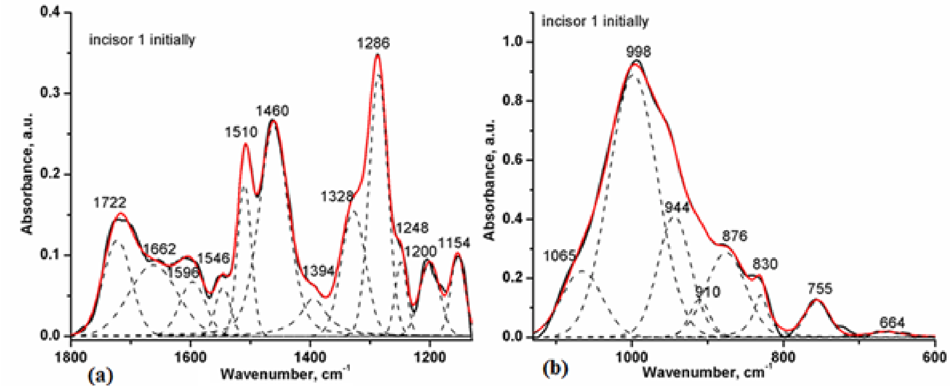

Fig. 2. ATR-IR deconvoluted spectra of the incisor 1 before (a, b) treatment with etching agent

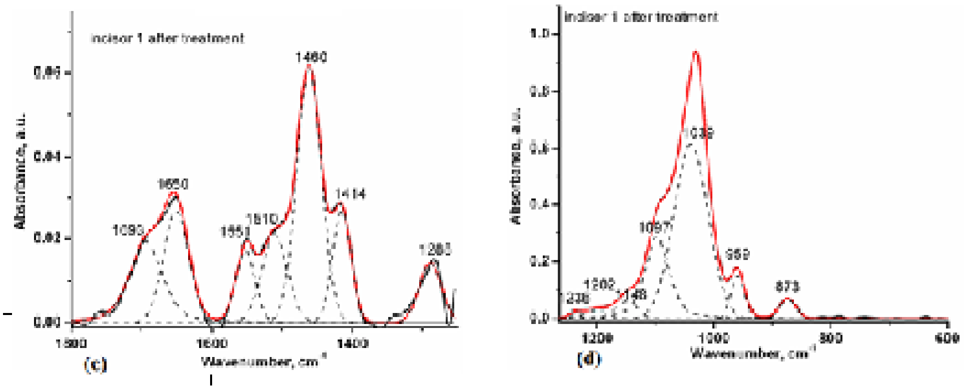

Fig. 2. ATR-IR deconvoluted spectra of the incisor 1 after (c, d) treatment with etching agent

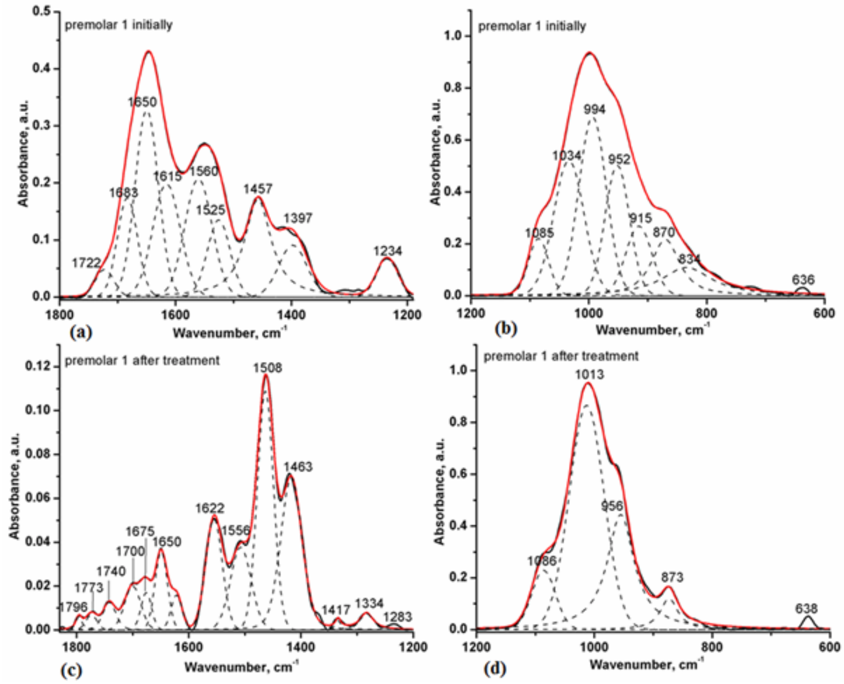

Fig. 3. ATR-IR deconvoluted spectra of the premolar 1 before (a, b) and after (c, d) treatment with etching agent

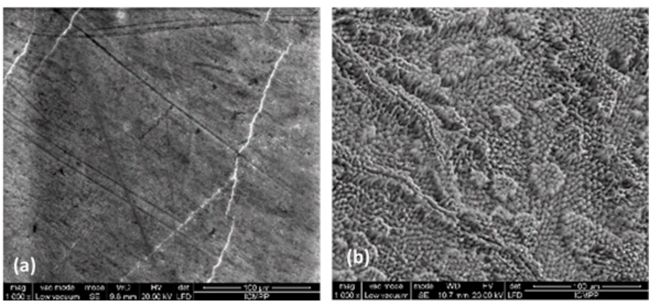

Fig. 4. Initial SEM micrographs of incisor 1 before (a) and after (b) treatment with the etching agent

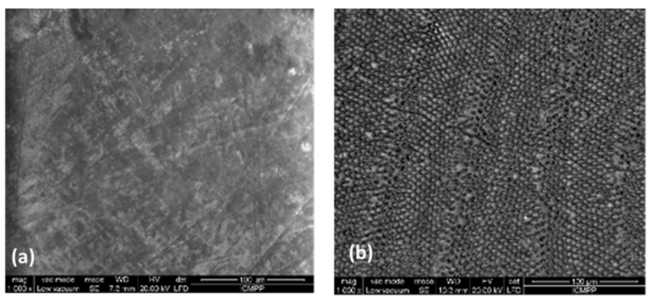

Fig. 5. SEM micrographs of premolar 1 before (a) and after (b) treatment with etching agent.

revealing the dentine underneath. In the case of the premolar, the demineralization is homogenous whilst the incisor's enamel is heterogeneously demineralized, exhibiting an uneven pattern. It is clear that in both cases the demineralization is severe, as we had expected.

In comparison with the bleaching treatments, on the teeth surface were observed holes and fractures varying in depth and width. The *ortho*-phosphoric acid demineralization process was total, the SEM images revealing the characteristic regular structure of dentin.

For these reasons, the latest researches in the field focused on the obtaining of some high performance adhesive systems (the 7th generation) with the increase of biocompatibility through the elimination of the acid impairment [17].

Conclusions

Etching with 37% *ortho*-phosphoric acid caused structural and morphological changes to the dental enamel surfaces as SEM and ATR-FTIR methods revealed. ATR-FTIR spectra revealed that the inorganic and organic components of dental enamel were removed from superficial and deeper enamel layers leading to a severe demineralization.

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