

Interfacial interaction of tartaric acid with hydroxyapatite and enamel

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The purpose of this study was to investigate the interfacial interaction of tartaric acid with hydroxyapatite and enamel. Hydroxyapatite particles were mixed with 15% (w/v) alcohol-aqueous (1:1) solution of tartaric acid for 72 h. After the mixture, the filtrate was separated from the solid. The filtrate, the solid, hydroxyapatite, and tartaric acid were dried for FTIR and XRD analysis. Enamel disks etched with tartaric acid for 60 s were analyzed by attenuated total reflectance (ATR). Enamel disks etched with 15% tartaric acid for 15, 30, 60, 120 s were analyzed by SEM. FTIR spectra showed carboxylate in the solid and in subtraction spectra. ATR spectra revealed carboxylate on the enamel surfaces. XRD data indicated the formation of calcium tartrate and calcium hydrogen phosphate after the reaction of tartaric acid with hydroxyapatite. SEM observations revealed typical decalcification of the periphery of the enamel rods due tartaric acid etching. It is concluded that tartaric acid can decalcify and chemisorb onto HA simultaneously, hence, tartaric acid could be used as etchant and ingredient of self-etching primers in adhesive dentistry.

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1. Introduction

In 1955, Buonocore reported that bonding of acrylic resin materials could be substantially increased by treatment of the enamel surface with 85% orthophosphoric acid [1], since then, acids, both inorganic and organic, have been studied to improve the bond strength of resin materials to enamel. The micro-mechanical interlocking at the tooth hard substance-resin interfaces is the main retentive force for bonding of the contemporary resin restorative materials [2–5].

Concerning the pulpal stimulation and irritation by phosphoric acid etching in the deep cavities, phosphoric acid is possibly replaced by diluted carboxylic acid solution, derivatives of carboxylic acids or the phosphoric acid esters used for enamel and dentin pretreatment [5–8]. Maleic acid has been used as a non-rinse conditioner and a functional ingredient of self-etching primers for bonding of resin restorations [9, 10]. Etching with 10% maleic acid revealed similar bond strengths as compared to etching with 37% phosphoric acid [5].

Chemical bonding does eliminate any unnecessary mechanical preparations at the sound tooth hard tissues in order to obtain the sufficient macro-mechanical re-

tention of the restoration [11]. Recently, Yoshida *et al.* [12] reported that carboxylic acids can decalcify and simultaneously adhere to the tooth hard tissues and hydroxyapatite (HA). Whether a carboxylic acid adheres to or decalcifies hydroxyapatite depends on the solution rate of the carboxylic salt in its own acid solution, regardless of the concentration and pH [12]. Therefore, it is essential to seek for suitable carboxylic acids that are able to decalcify and adhere to tooth hard tissues and hydroxyapatite.

In this context, tartaric acid might be an appropriate substance, since it contains two carboxyl as well as two hydroxyl groups. That could facilitate decalcification of and chemisorption to hydroxyapatite.

However, up to date, tartaric acid has rarely been investigated as an etchant for the tooth hard substance or an ingredient of priming agents in the adhesive dentistry [13]. The interfacial interaction of tartaric acid with hydroxyapatite and enamel has never been thoroughly studied.

The purposes of the present study were (1) to investigate the evidence of chemical bonding of tartaric acid to hydroxyapatite and enamel by Fourier Transformed

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Infrared Spectrometry (FTIR) and Attenuated Total Reflectance (ATR); (2) to analyze which substances are formed after the reaction of tartaric acid with hydroxyapatite by X-Ray Diffraction (XRD); and (3) to analyze the micro-morphologic surface pattern resulting from the etching with tartaric acid by SEM.

The specific hypothesis tested in this study was that (1) tartaric acid could decalcify and simultaneously chemisorb to the enamel surface, and that (2) calcium tartrate and calcium phosphate are produced by the reaction of hydroxyapatite with tartaric acid.

2. Materials and methods

2.1. Preliminary experiments

In the preliminary experiment, 100 mg of hydroxyapatite particles (High-molecular material's institute of Sichuan University, Lot #000124, Sichuan, China) with a particle size of 0.42 ± 0.12 μm were mixed with a 15% (w/v) DL-tartaric acid (Santuo Guanghua chemical factory, Lot #19951026, Santuo, China) alcohol-aqueous (1:1) solution (pH = 1.41). The molar ratios of hydroxyapatite to tartaric acid were 1:2, 1:8, 1:16, 1:24, 1:32, 1:40, 1:48, 1:56, 1:64, 1:72.

This preliminary experiment was performed in order to determine how many ml of alcohol-aqueous solution of the tartaric acid should be applied to HA in the following experiments.

2.2. Infrared spectrometry

Four hundred mg of HA particles was mixed with 15% (w/v) 11.95 ml alcohol-aqueous (1:1) DL-tartaric acid aqueous solution (pH = 1.41) at a 1:30 molar ratio of HA and tartaric acid as a result from the preliminary experiments. The mixed solution was filtered through two-layer filter paper in a glass funnel after the mixed solution was kept for 72 h at room temperature. The residual solid materials on the filter paper within the glass funnel were six times rinsed with 10 ml distilled water. The filtrate fluid was vaporized in vacuum over silica gel for 48 h. The residual solid materials were dried in vacuum over silica gel for 24 h.

Infrared spectra of KBr pellets were registered with Fourier Transformed Infrared Spectrometry (FTIR, NICOLET NEXUS 670, American) using the KBr-technique. The dried residual solid materials, the desiccated filtrates, the pure HA particles, and tartaric acid were milled in an agate mortar and subjected to spectroscopic determination for the further analysis. The subtraction technique was used. The spectrum of the dried solid materials was subtracted from that of HA at $\nu_{\text{P=O}}$ 1046 cm^{-1} as the base peak and the spectrum of the desiccated filtrate was subtracted from that of the corresponding stretching bands of the carbonyl group with the OMNIC ESP 5.2 software.

2.3. Enamel disks for attenuated total reflectance (ATR)

The occlusal surface of a freshly extracted human third molar tooth without visible caries was flattened by a grinding unit (Gripo 2v, Metkon Instruments Ltd. Bur-

bach, Germany) using #600 SiC paper (Buehler, Germany) under water spray. Then, the tooth was horizontally sliced with a diamond disk under water spray to obtain an occlusal enamel disk (approximate 1 mm thick). The debris on the enamel surface was washed away with water spray for 3 min. Each enamel disk was cut into two pieces. One enamel piece was not treated and served as control. The other enamel piece was treated with 15% tartaric acid for 1 min, then water-sprayed for 3 min and ultrasonically cleansed in distilled water for 1 min.

2.4. X-ray diffraction (XRD)

2.4.1. Calcium tartrate preparation

Fifty ml of 15% aqueous solutions of alcohol-aqueous (1:1) solution of tartaric acid were mixed with calcium hydroxide (1.48 g) at a 1:2.5 molar base to acid ratio. The mixed solutions were kept for 72 h at room temperature, and subsequently separated by filtration. The solid materials (calcium tartrate) were washed with 10 ml of de-ionized water each for 6 times, and then the solid materials were dried for analysis by a powder X-ray diffractometer (Rigaku, D/MAX—IIIB, Japan) using $\text{Cu K}\alpha$ radiation.

2.4.2. XRD analysis for reaction products of HA with tartaric acid

Four hundred mg of HA particles were mixed with 15% (w/v) 11.95 ml alcohol-aqueous (1:1) DL-tartaric acid aqueous solution (pH = 1.41) at a 1:30 molar ratio of HA to tartaric acid. The mixed solutions were kept for 72 h at room temperature, and subsequently vaporized in vacuum over silica gel until dry. The dried mixed reaction products of HA with tartaric acid, the dried self-prepared calcium tartrate and tartaric acid were kept dry until detection by XRD.

2.4.3. Scanning electron microscopic (SEM) observations

Enamel pieces were prepared as mentioned above. They were treated either with 15% alcohol-aqueous solution of tartaric acid, for 15, 30, 60, 120 s, respectively. All the specimens were dried, gold-sputtered and analyzed with a SEM (Toshiba, s-570, Japan).

3. Results

The information obtained in the preliminary experiment indicated that HA particles could not be completely dissolved in the aqueous-alcohol solution of tartaric acid.

3.1. Infrared spectra

3.1.1. Tartaric acid

The typical infrared spectrum of tartaric acid revealed the carbonyl stretching vibration $\nu_{\text{C=O}}$ at 1740 cm^{-1} , ν_{OH} at 3406 cm^{-1} , the deformation vibration for δ_{HO} and $\nu_{\text{C-O}}$ at 1308 and 1400 cm^{-1} , and the typical bands of $\nu_{\text{CH-OH}}$ 1088 cm^{-1} , 942 cm^{-1} stood for the deformational vibration (OH...O) of the dimer of carboxylic acid (no figure). Moreover, the subtraction spectrum

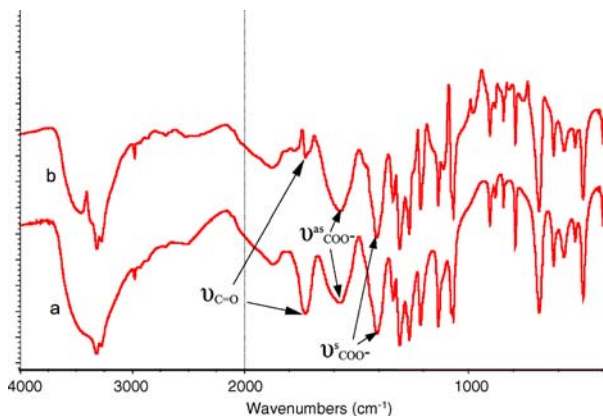


Figure 1 FTIR spectra. (a) Spectrum of the filtrate reaction product of tartaric acid with hydroxyapatite. (b) Subtraction spectrum of the filtrate reaction product of the HA and tartaric acid minus tartaric acid. Both of them reveal not only the typical carboxylic salt bands ($\nu_{\text{COO}^-}^{\text{as}}$ -1572 cm^{-1} , $\nu_{\text{COO}^-}^{\text{s}}$ -1411 cm^{-1}), but also the carbonyl group ($\nu_{\text{C=O}}$ at 1728 cm^{-1}). The carbonyl group did not disappear even after the subtraction (b), however, the peak intensity of carbonyl group was reduced significantly.

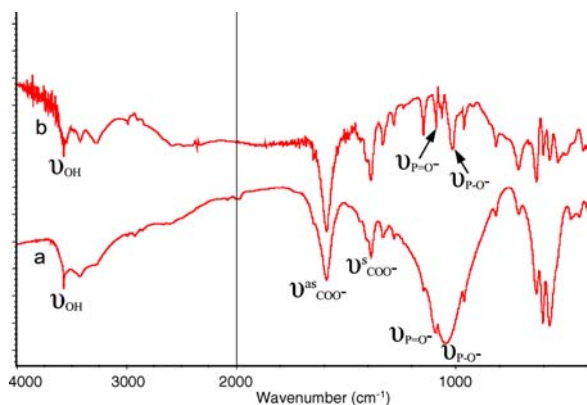


Figure 2 FTIR spectra: (a) Spectrum of the solid reaction products of hydroxyapatite and tartaric acid revealed the characteristic carboxylic salt bands ($\nu_{\text{COO}^-}^{\text{as}}$ at 1589 cm^{-1} , $\nu_{\text{COO}^-}^{\text{s}}$ at 1386 cm^{-1}), hydroxyl group (ν_{OH} at 3570 cm^{-1}), as well as the phosphoric groups for $\nu_{\text{P=O}}$ at 1089 cm^{-1} , $\nu_{\text{P-O}}$ at 1046 cm^{-1} . (b) Subtraction spectrum of the solid reaction products of the hydroxyapatite and tartaric acid minus hydroxyapatite reveal not only the typical carboxylic salt bands ($\nu_{\text{COO}^-}^{\text{as}}$ at 1589, $\nu_{\text{COO}^-}^{\text{s}}$ at 1386 cm^{-1}), but also the phosphoric groups and the disassociated hydroxyl group belonging to hydroxyapatite.

of the filtrate minus tartaric acid was similar to the spectrum of tartaric acid (Fig. 1(a) and (b)). Both of them revealed the typical carboxylic salt bands: $\nu_{\text{COO}^-}^{\text{as}}$ at 1572 cm^{-1} , $\nu_{\text{COO}^-}^{\text{s}}$ at 1411 cm^{-1} , as well as the carbonyl group ($\nu_{\text{C=O}}$ at 1728 cm^{-1}) (Fig. 1(a) and (b)).

The infrared spectrum of the solid reaction products of tartaric acid and HA revealed the characteristic carboxylic salts ($\nu_{\text{COO}^-}^{\text{as}}$ at 1589 cm^{-1} , $\nu_{\text{COO}^-}^{\text{s}}$ at 1386 cm^{-1}), the disassociated hydroxyl peaks (ν_{OH} at 3570 cm^{-1}), and the phosphoric groups for the $\nu_{\text{P=O}}$ at 1089 cm^{-1} , and the $\nu_{\text{P-O}}$ at 1046 cm^{-1} (Fig. 2(a)). In addition, the subtraction spectrum of the solid reaction products of HA and tartaric acid minus HA revealed not only the typical carboxylic salt bands, but also a few changes at the phosphoric groups (Fig. 2(b)). Furthermore, the carbonyl group did not appear in the solid reaction product spectra (Fig. 2(a) and (b)) and the disassociated hydroxyl peak did not disappear in the subtraction spectrum (Fig. 2(b)).

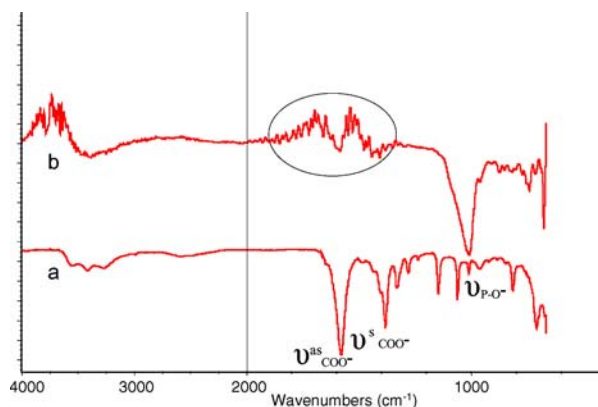


Figure 3 ATR Spectra. (a) The enamel disk was treated with 15% tartaric for 1 min and water-sprayed. The typical stretching bands ($\nu_{\text{COO}^-}^{\text{as}}$ at 1582 cm^{-1} , $\nu_{\text{COO}^-}^{\text{s}}$ -1385 cm^{-1}) of carboxylic salts are detected on the surface of enamel etched with tartaric acid. (b) The spectra of the untreated enamel disks reveal the phosphoric groups at 1011 cm^{-1} and water interference about at 1630 cm^{-1} that is marked with a circle.

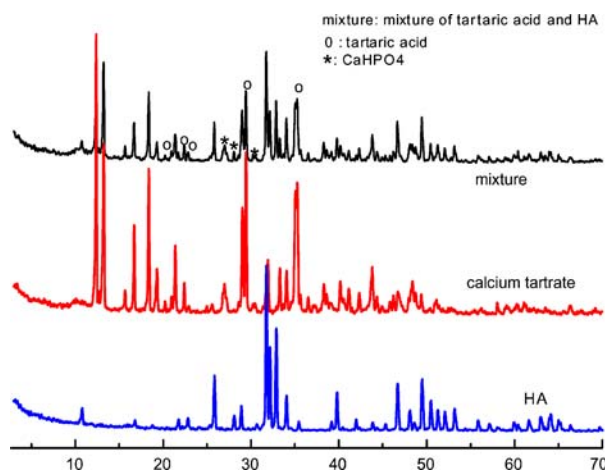


Figure 4 XRD spectra. The bottom line is the spectrum of hydroxyapatite. The middle line is the spectrum of self-prepared calcium tartrate. The top line is the spectrum of the reaction products of hydroxyapatite and tartaric acid. o: tartaric acid, *: CaHPO_4 .

3.1.2. ATR spectra

ATR spectrum of the enamel specimens etched with tartaric acid revealed the typical stretching bands of carboxylic salts and the phosphoric groups (Fig. 3(a)), while the control enamel disk did not show the typical stretching bands of the carboxylic salts (Fig. 3(b)).

3.1.3. Characterization by XRD

XRD patterns of the mixed reaction products of tartaric acid and HA revealed the calcium tartrate, calcium hydrogen phosphate (CaHPO_4) as well as the remaining tartaric acid and HA (Fig. 4).

3.1.4. SEM observations

The enamel etched with 15% tartaric acid for 15 s revealed a less distinct etching pattern—only a small amount of the periphery of the enamel rod was decalcified (Fig. 5(a)). Etching for 30, 60, and 120 s caused decalcification of the periphery of the enamel rods and an increase in surface roughness (Fig. 5(b)–(d)).

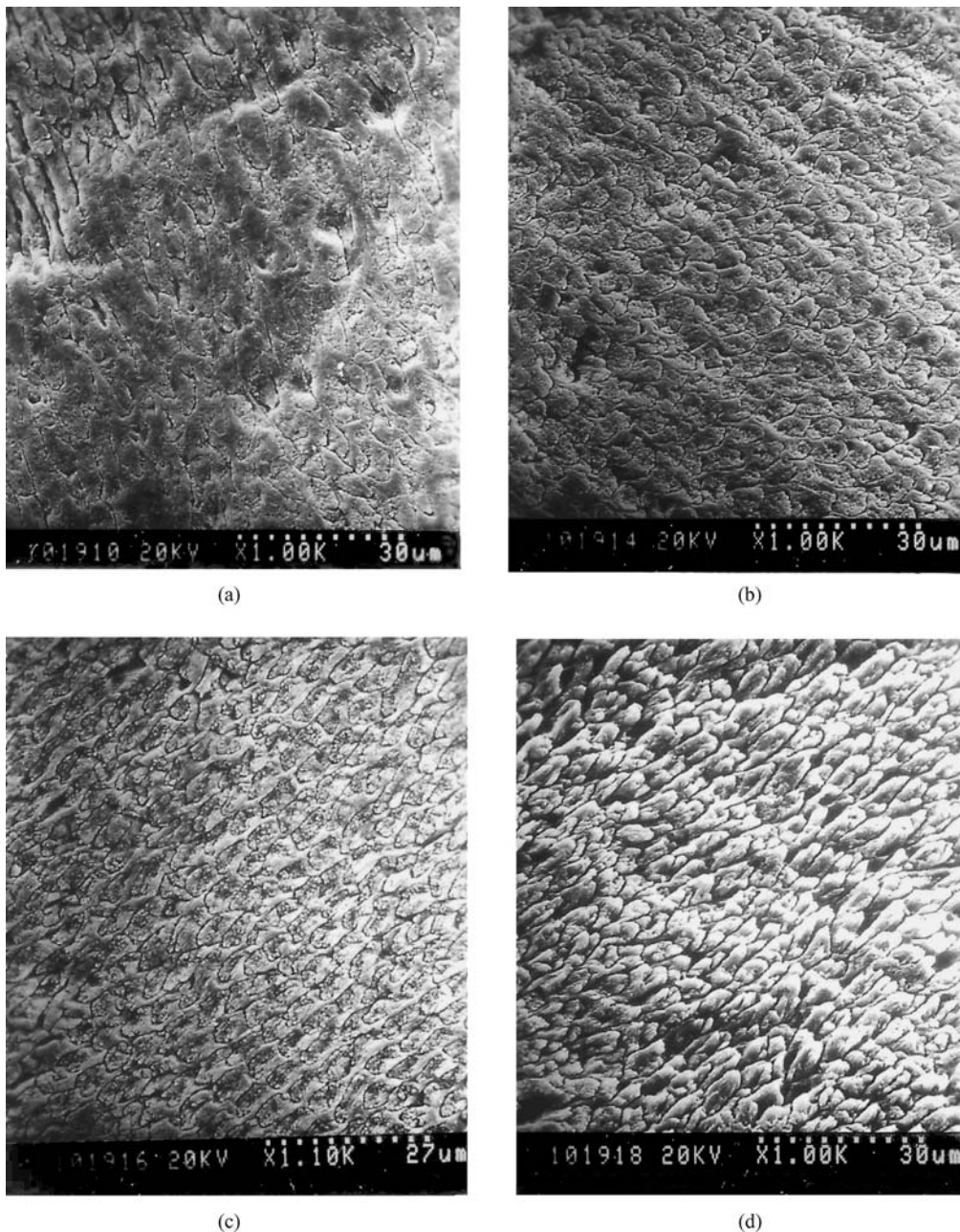


Figure 5 SEM observations. The enamel disks were etched with 15% tartaric acid for 15 s (a), 30 s (b), 60 s (c) and 120 s (d). Enamel etching for 15 s reveals a less distinct etching pattern (a). Etched for 30 s, or 60 s causes an increased decalcification of the periphery of the enamel rod (b, c), however, etching for 120 s decalcifies the periphery of the enamel rod deeply (d) (1000-fold magnifications. Dotted line = 30 μm).

4. Discussion

4.1. Chemical reaction and chemical bonding

FTIR has been used in surface analysis, in an attempt to prove the chemical bonding between bonding agents and dental hard tissues [14–16].

In the present study, tartaric acid remaining in the solid materials was easily washed away with water due to the high aqueous solubility. Although the pure reaction products (calcium tartrate and side products) could not be obtained in the present study, the pure spectrum of the reaction products (calcium tartrate and side products) could be approximately obtained by either the solid reaction product spectrum minus the HA spectrum or the filtrate spectrum minus tartaric acid spectrum.

FTIR findings revealed that there exist the typical bands of carboxylic salts after the reaction of HA with

tartaric acid. The carbonyl group was detected only in the desiccated filtrate separated from the mixture of HA and tartaric acid, but not in the solid materials. According to the present findings, one carboxylic group of tartaric acid reacts with the calcium of HA in the filtrate liquid, while two carboxylic groups react with the calcium of HA in the solid materials. These findings indicated that one type of calcium tartrate could be dissolved in its own acid solution.

Both spectra, ATR and FTIR, indicated the typical spectrum of the carboxylic salt after the enamel surface was etched with tartaric acid, and HA reacted with tartaric acid. Moreover, FTIR spectra indicated that the disassociated hydroxyl peak was found not only in the spectrum of the solid reaction products of hydroxyapatite and tartaric acid, but also in the subtraction spectrum of these reaction products minus

hydroxyapatite. This disassociated hydroxyl peak should belong to hydroxyapatite. These findings reveal that tartaric acid was chemisorbed onto the enamel/hydroxyapatite surfaces.

X-ray powder diffraction has been used to compare the spectra of the investigated materials with powder diffraction files for identification of unknown materials [17]. The XRD spectrum of calcium tartrate was not found in the powder diffraction file. Therefore, the calcium tartrate that resulted from the reaction of tartaric acid and calcium hydroxide is assumed as the standard XRD spectrum. Its three strongest peaks can completely match the spectrum of the reaction products of HA and tartaric acid. According to the XRD data, the calcium hydrogen phosphate (CaHPO_4) was found in the reaction products of HA with tartaric acid, but the three strongest peaks of calcium hydrogen phosphate hydrates ($\text{CaHPO}_4 \cdot \text{H}_2\text{O}$ or $\text{CaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) did not appear in these reaction products. These may be due to a quick proton exchange of CaHPO_4 and CaH_2PO_4 under acidic conditions so that the spectra of the calcium hydrogen phosphate hydrates could not be detected in XRD.

Based on the FTIR spectra and XRD data from the present study, the reaction of HA with tartaric acid produced calcium tartrate and calcium hydrogen phosphate.

The enamel etched with tartaric acid mainly revealed decalcification of the periphery of the enamel rods. Furthermore, the prolongation of the tartaric acid etching time caused an increased decalcification of the periphery of the enamel rods. According to the micro-morphology of the enamel etching pattern, it revealed that 30 s etching may be appropriate for resin-to-enamel bonding via micro-retentive interlocking.

4.2. Adhesion-decalcification concept (AD-concept)

If the solubility of the carboxylic acid is too low in a solvent, the acid-base reaction with HA hardly will take place. Inversely, if the calcium carboxylate such as calcium acrylate has very high solubility in water, the acid can decalcify HA and be washed away easily. Yoshida *et al.* postulated the adhesion-decalcification concept (AD concept) [12]: decalcification of and adhesion to HA are dependent on the solubility of the respective calcium carboxylate in the solution of the carboxylic acids themselves, regardless of concentration and pH.

According the solubility of HA in solution of tartaric acid, FTIR spectra and XRD data, the processes of the adhesion-decalcification can be described as following: The decalcification or demineralization (chemical reaction) immediately occurs after hydroxyapatite exposure to the solutions of tartaric acid. The over-saturated or

saturated solution is reached on HA surfaces after reaction of tartaric acid with HA. Hence, the dissolved materials might precipitate on HA surfaces again after the vaporization of the solvent. When the equilibrium of the chemical reaction is achieved, the chemical adsorption of tartaric acid onto HA surfaces takes place by means of ionic bond and hydrogen-bonding-groups (COOH , OH , etc.) [12, 15, 18, 19].

5. Conclusions

The present study for the first time indicates that tartaric acid can decalcify and adhere to hydroxyapatite and enamel simultaneously. Therefore, tartaric acid might be used as an etchant and functional ingredient in self-etching primers.

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