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Structural aspects of bleaching and fluoride application on dental enamel

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To my family

Table of Contents:

Abstract

Tooth bleaching has become a popular modality to whiten discolored teeth. It occurs via the oxidation reactions between oxidizing agents and chromogenes deposited into dental hard tissues. Bleaching products are divided into two groups according to the type of the active oxidizing agent: peroxide-based and non-peroxide-based substances. Although various products have been widely used, the impact of bleaching on the structural aspects of dental enamel remains unclear so far. In addition, contradictory findings on the effect of fluoride application on enamel erosion have been reported. Therefore, this *in vitro* study focused on the effect of different whitening agents and fluoride reagents on the inorganic crystal chemistry of enamel using attenuated total reflectance infrared spectroscopy (ATR IR), combined with scanning electron microscopy (SEM), Raman spectroscopy, x-ray diffraction (XRD), electron probe microanalysis (EPMA), flame atomic absorption spectroscopy (FAAS) and total reflection x-ray fluorescence (TXRF).

In the first part of this thesis, three peroxide-based bleaching products: Opalescence Xtra Boost, Opalescence PF 20% and sodium perborate were studied. No significant difference was observed between unbleached and bleached samples by different analysis techniques, which indicated no structural and chemical changes in enamel apatite due to peroxide-based bleaching treatment. However, a comparison between neutral and acidic aqueous hydrogen peroxide (HP) solutions suggested that a low pH value can modify the dental enamel apatite.

Secondly, enamel alteration caused by a non-peroxide-based, “over-the-counter” bleaching product (Rapid White) was studied. Rapid White consists of a sodium chlorite-containing “accelerator” and a citric acid-containing “whitening gel”. The separate investigation of each component of Rapid White revealed that the citric acid-containing “whitening gel” rather than the sodium chlorite-containing “accelerator” substantially impacted on dental enamel. Enamel was affected at several levels: (i) the organic component was removed from superficial and deeper enamel layers and remnants of the bleaching gel were embedded in the emptied voids; (ii) cracks and chemical inhomogeneities with respect to Ca and P occurred on the surface; (iii) within a submicron layer of enamel, the Ca-O bond strength in apatite decreased, thus enhancing calcium leaching from the bleached dental enamel. Additional studies on aqueous citric acid solutions indicated that the structural modification of enamel apatite increased with the increase of the citric acid concentration and the number of treatments.

To study the protective potential of fluoride reagents against citric acid-induced erosion, enamel and, for comparison, geological hydroxyapatite samples were treated with 0.1 mol/l citric acid aqueous solutions and sodium fluoride of different concentrations ranging from 0.5 to 2.0%, respectively. The two chemical agents were applied either simultaneously or consecutively. The application of sodium fluoride alone did not suppress the atomic-level changes in apatite exposed to acidic agents. The admixture solutions containing citric acid and sodium fluoride led to the formation of CaF_2 and considerably reduced the changes in the apatite P-O-Ca framework. However, the CaF_2 globules deposited on the enamel surface seem to be unable to prevent the alteration of the apatite structure during further exposure to acidic agents. No evidence of fluoride-induced recovery of the modified apatite structure was found.

Abbreviations

Abbreviation	Meaning
ATR IR	Attenuated total reflectance infrared spectroscopy
ClAP	Chlorapatite
CP	Carbamide peroxide
EPMA	Electron probe microanalysis
FAAS	Flame atomic absorption spectroscopy
FAP	Fluorapatite
HA	Hydroxyapatite
HP	Hydrogen peroxide
OTC	Over-the-counter
SEM	Scanning electron microscopy
SP	Sodium perborate
TXRF	Total reflection x-ray fluorescence
XRD	X-ray diffraction

1 Introduction

In the past decade, tooth bleaching has become the fastest growing field in esthetic dentistry [Haywood, 2000; Attin et al., 2005]. It has been reported that 28% of adults in the UK are dissatisfied with their current tooth color [Qualtrough et al., 1994] and in the United States the number reaches 34% [Odioso et al., 2000]. As a more simple, less invasive and less expensive means available to lighten discolored teeth when compared to bonding veneers, crowns and laser bleaching [Haywood, 1992; Sulieman et al., 2004; Matis et al., 2005], tooth bleaching has shown favorable clinical long-term results and high patient satisfaction [Attin et al., 2005].

Tooth bleaching, also called tooth whitening, refers to a technique where chemical bleaching materials are applied to brighten discolored teeth. Tooth bleaching materials usually contain a strong oxidizing agent and the ability to whiten teeth is chiefly due to oxidation reactions [Gregus and Klaassen, 1995; Attin et al., 2005]. Hydrogen peroxide (HP) or one of its precursors (carbamide peroxide or sodium perborate) is a popular oxidizing agent used to whiten teeth [Tredwin et al., 2006]. Bleaching occurs because of the decomposition of peroxide into free oxygen radicals, which can break down large pigmented molecules deposited in teeth into smaller, less pigmented molecules [Haywood, 1992]. In recent years, an “over-the-counter” bleaching product (Rapid White) based on sodium chlorite ($NaClO_2$) has been introduced onto the market [Attin et al., 2004; Ünlü et al., 2004]. Sodium chlorite can be used as a tooth bleaching agent resulting from the generation of chlorine dioxide (ClO_2) in the presence of acid [Attin et al., 2004; Zantner et al., 2007]. The oxidation capability of chlorine dioxide (ClO_2) has been confirmed in water treatment and in paper pulp industry [Tzanavaras et al., 2007].

The population’s increasing interest in whitening their teeth has prompted many manufacturers to develop products for use either in the dental office or at home. It was estimated that bleaching has been performed on more than one million patients in the dental office [Watts and Addy, 2001]. The fact that more than 35 million tooth whitening kits have been sold worldwide from May 2001 to March 2005 [SCCP, 2005] points out that a large number of people have used self-administered home bleaching products. The effect of peroxide-based bleaching products on dental hard tissues has been investigated [Seghi et al., 1992; Leonard et al., 1997; Tam, 1999; Potocnik et al., 2000; Dahl, 2003; Park et al., 2004; Ünlü et al., 2004; Attin et al., 2005]. However, the results are conflicting and, therefore, claims that all bleaching systems are safe cannot be generally accepted [Sulieman et al., 2004; Added et al., 2006; Duschner et al., 2006].

A limited number of studies investigated the influence of non-peroxide-based bleaching materials on dental hard tissues [Attin et al., 2004; Zantner et al., 2006]. These peroxide-free bleaching materials can be obtained "over-the-counter" and applied without the dentist's supervision. Citric acid contained in Rapid White "whitening gel" normally exists in a variety of fruits and vegetables and can cause enamel demineralization [Hughes et al., 2000; Eisenburger et al., 2004; Newby et al., 2006]. The erosive potential of citric acid on dental enamel implies that Rapid White may lead to enamel erosion. The effect of fluoride application on enamel erosion was controversially discussed [Ganss et al., 2001; Larsen, 2001], even though fluoride has been successfully used to inhibit dental caries [Rølla et al., 1993; Ten Cate, 1997]. Therefore, it could also be questioned whether or not the application of fluoride may prevent or reverse enamel erosion due to dental bleaching treatment.

In summary, further studies on bleaching-induced changes in the enamel chemistry and structure, as well as the role of fluoride reagents in preserving the apatite structure, are required.

2 Literature review

2.1 Dental enamel

Enamel structure

Dental enamel is the outer layer of the anatomical crown of a tooth (Figure 2.1). It is a hard, thin and translucent layer of a calcified substance that envelops and protects the dentine (the main portion of the tooth volume). Enamel is the hardest substance in the body [Ten Cate, 1994; Reitznerova et al., 2000] and contains no collagen and no cells. Mature enamel consists of approximately 96-98% mineral by weight, the rest is water and organic materials [Cuy et al., 2002].

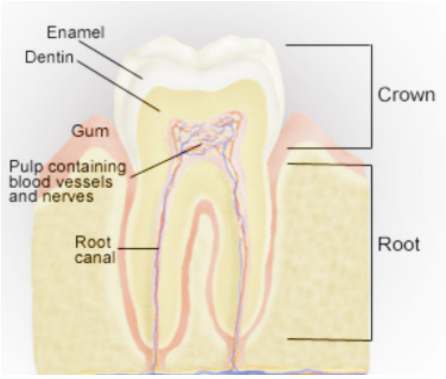


Figure 2.1 A schematic illustration of the components of a molar tooth.

Enamel mineral is mainly composed of calcium phosphate salts in the form of nanoscale hexagonal hydroxyapatite (HA) crystallites that are both carbonated and defective. The enamel crystallites elongate in their c-axis directions and bundle to needle-like crystal rods or prisms which are tens of microns long (up to 100 μm) but sometimes only 50 nm wide [Miake et al., 1993]. Enamel rods or enamel prisms, the basic units of enamel, run about parallel to each other and project perpendicularly from the dentino-enamel junction (DEJ) to the surface of the tooth [Anderson and Elliott, 2000]. The crystallites that are around a rod are named interprismatic or interrod (shown in yellow in Figure 2.2). The principal distinction between enamel rod and interrod lies in the crystallites orientation. In the case of rods, the long c-axes of crystallites are essentially parallel to the long axis of the rod, while their a- and b-axes may be at any angle. Interrod crystallites, however, tilt about 40 to 65° relative to the direction of the rod [Meckel et

al., 1965; Cevc et al., 1980]. Between the rod and interrod enamel is rod sheath (shown in blue in Figure 2.2), a thin organic matrix containing no crystallites [Wakita et al., 1981].

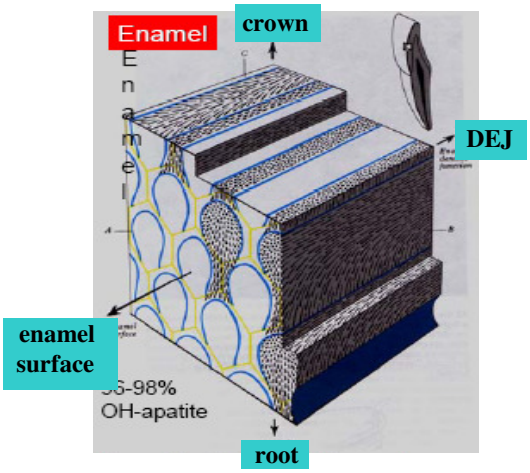


Figure 2.2 Illustration of an enamel cross-section.

Enamel mineral is described as a non-stoichiometric carbonated calcium hydroxyapatite [Young, 1974; Elliott et al., 1985]. The closest structural model for enamel apatite is stoichiometric calcium hydroxyapatite (HA). The unit cell of calcium hydroxyapatite has the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, which belongs to the hexagonal system (space group $P6_3/m$) with lattice parameters of a = b = 0.942 Å, c = 0.688 Å, $\alpha = \beta = 90°$, $\gamma = 120°$ [Simmer and Fincham, 1995; Hairul Nizam et al., 2005]. The structure is depicted in Figure 2.3 and Figure 2.4.

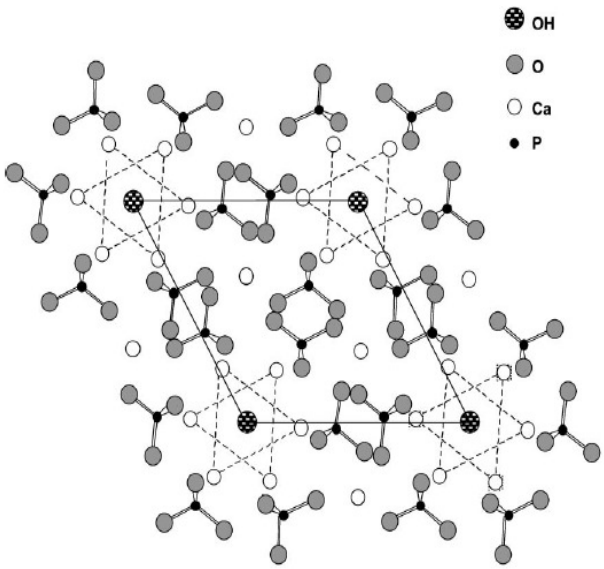


Figure 2.3 Sketch of the hydroxyapatite (HA) structure. View along the c-axis showing PO_4 tetrahedral ionic groups, Ca-ions and "channel ions". Parallelogram indicates HA unit cell. Six Ca atoms form a six-fold site (indicated by dashed lines) in which the channel ions reside (OH^- in the case of HA). These channels are oriented along the viewing direction.

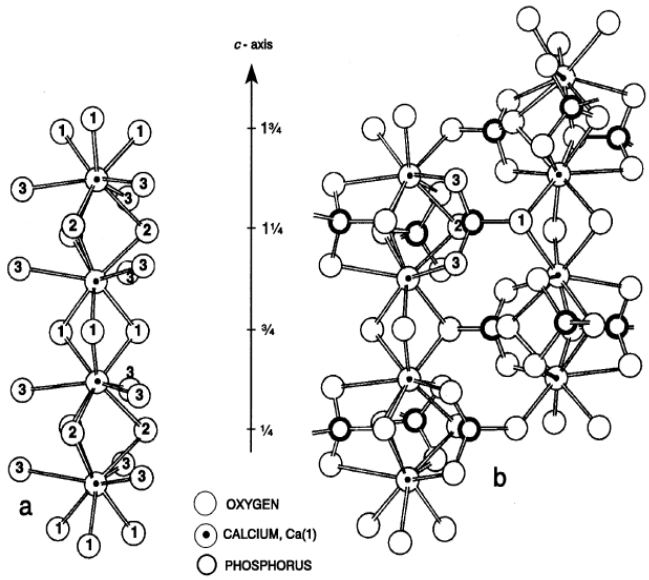


Figure 2.4 (a) Oxygen coordination of columnar Ca1 ions in apatite. (b) Columns linked via PO_4 tetrahedra. The oxygen atoms in (a) and in one tetrahedron in (b) have been numbered, and positions of the horizontal mirror planes at ¼, ¾ etc. are marked on the c-axis [Beevers and McIntyre, 1946].

The 10 Ca^{2+} ions occupy two crystallographically different symmetry sites, 4f and 6h. Four Ca^{2+} ions (4f) are located in columns along the three-fold axes at atomic coordinates 1/3, 2/3, 0 and 2/3, 1/3, 0 separated by approximately one-half of the c-axis. These are commonly referred to as Cal (or column Ca). Ca1 is coordinated by nine O atoms, with six shorter bonds that define an approximate trigonal prism and three longer bonds capping the prism faces. The Ca-[O_9] polyhedra share the trigonal faces to form chains parallel to the c-axis. The remaining six Ca^{2+} ions (6h sites, referred to as Ca2 or triangular Ca) form two triangular sets at z = 1/4 and 3/4 on the mirror planes (arranged in such a way that they form a channel along the c-axis, the so-called anion-channel). The Ca2 ions are seven-coordinated, with six O atoms and one OH^- ion. The six PO_4^{3-} ions occupy 6h positions similar to the Ca2 ions in expanded triangular positions. Adjacent Cal and Ca2 polyhedra are linked via O atoms of the PO_4 tetrahedra. The channel site is occupied by two OH^- ions per unit cell. Because of the crystallographic mirror symmetry imposed by the space group, each OH^- ion has to be considered at statistically disordered positions (4e) both above and below the mirror planes at z = 1/4 and 3/4.

Enamel mineral differs from ideal HA since it incorporates HPO_4^{2-}, CO_3^{2-}, K^+, Mg^{2+}, Na^+, Cl^- and other ions in its apatite lattice [Curzon and Featherstone, 1983]. The variations in chemical composition are systematic and are related to both developmental stage and location within the tissue [Robinson et al., 2004]. Such substitutions are likely to change the structure of a mineral and often have critical effects on mineral properties, such as solubility, hardness, brittleness, strain, thermal stability and the optical birefringence [Elliott, 2002]. Among such substitutions,

CO_3^{2-} is the most prevalent impurity in the order of 3-4% by weight in dental enamel apatite. Approximately 10-15% of the CO_3^{2-} replaces OH^- (type-A), the remaining 85-90% replaces PO_4^{3-} (type-B) [Elliott et al., 1985]. It has been shown that the incorporation of CO_3^{2-} into HA increases its solubility [LeGeros and Tung, 1983] and alters its physical properties, in terms of crystallinity and crystal size and shape [LeGeros et al., 1967]. The so-called channel site can be occupied not only by OH^-, but also by the substituting ions F^- or Cl^- [Mathew and Takagi, 2001]. In fact, among these anions, F^- is the best one to fit into the channel site, because its ionic radius is small enough to permit F^- in the most symmetric position in the channel (i.e. on mirror planes perpendicular to the c-axis) (Figure 2.5). Thus, fluorapatite (FAP) is more stable than HA and chlorapatite (ClAP), and results in a significant decrease in the enamel erosion susceptibility. Therefore, the surface layer of enamel (50 nm), where F^- might be incorporated into the HA crystal lattices, is more resistant to the enamel demineralization [Dijkman et al., 1982; Wefel and Harless, 1982].

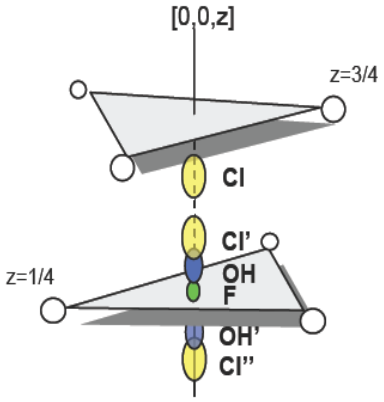


Figure 2.5 Possible anion positions in the channel inside the HA unit cell.

Enamel matrix proteins are present during the process of the enamel formation and are known to be essential for proper enamel development [Simmer and Fincham, 1995]. Once enamel attains its final hardened form, the matrix proteins are almost completely removed. There is only a small quantity left in the mature enamel. The organic portion of the mature tooth enamel contains 60% proteins and 40% lipids [Girija and Stephen, 2003]. The organic matrix is concentrated at the rod sheath but it is also present in smaller amounts among crystallites (Figure 2.6) [Poole et al., 1961; Travis and Glimcher, 1964]. These organic layers make enamel a semi-permeable membrane [Jansen and Visser, 1950] and small molecules can pass freely through intact enamel to the pulp. It has long been determined that peroxide solutions flow freely through enamel and dentine, due to the relatively low weight of the peroxide molecule (30 g/mol) [Goldstein et al., 1989]. Hence, bleaching material enters the tooth regardless of whether there are cracks present in the teeth and will bleach the entire tooth. *In vitro* experiments have demonstrated that the penetration of peroxide into the pulp chambers of extracted teeth after exposure time of 15-30 minutes occurred from a range of peroxide products and solutions [Thitinanthapan et al., 1999]. On the other hand, during tooth bleaching procedure, whitening agents may result in disruption of the enamel matrix

proteins, with subsequent loss of the embedded enamel crystallites, which in return fosters the penetration of bleaching agents deeper into the tooth [Albers, 1991]. In summary, enamel organics will act as channels for bleaching agents to penetrate enamel and play a significant role in the dental bleaching process [Miranda et al., 2005].

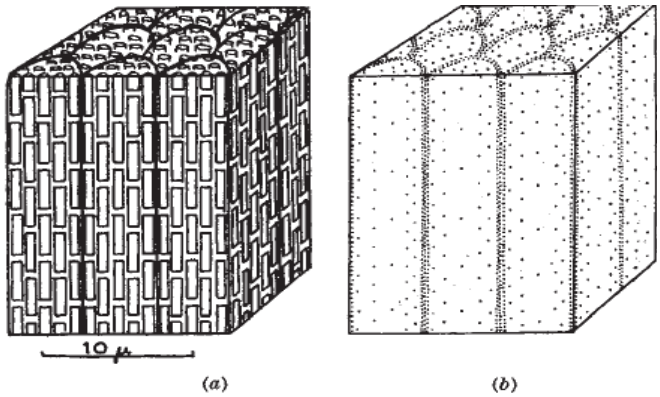


Figure 2.6 Diagrams illustrating the structure of normal tooth enamel. (a) Arrangement of mineral elements; (b) Distribution of organic elements. Proteins are concentrated around the margins of the prisms, but smaller amounts occur throughout.

The pH value and enamel erosion

Dental erosion is the process whereby dental hard tissues are destroyed generally by the action of acid on the teeth without the involvement of microorganisms [Eccles and Jenkins, 1974]. The acid may be of endogenous (from within the body) or exogenous (from outside the body) origin. Compared to the caries process, enamel erosion involves a more widespread and rapid dissolution and leads to progressive loss of the enamel surface over a long period of time [Larsen, 1973; Arends and Ten Cate, 1981]. Larsen (1974) pointed out that in a certain pH region HA may dissolve while FAP can precipitate forming a subsurface lesion, i.e. caries. If the conditions of the demineralizing solution are such that also FAP is undersaturated, then an erosive defect is formed rather than a subsurface lesion. Hence, after erosive mineral loss, only a thin partly demineralized and softened surface layer is left to provide the structure for remineralization [Zentner and Duschner, 1996]. Therefore, erosion is primarily a surface phenomenon, while caries generally begins as a subsurface demineralization of the enamel structure that eventually leads to a pit in the tooth surface.

Enamel erosion normally occurs at a low pH value and is influenced by many factors. When HA is in contact with water, the following reaction occurs [Dawes, 2003]:

Precipitation ↔ Dissolution

$$Ca_{10}(PO_4)_6(OH)_2 \leftrightarrow 10Ca^{2+} + 6PO_4^{3-} + 2OH^-$$

Solid ↔ Solution

A small amount of HA dissolves and releases Ca^{2+}, PO_4^{3-} and OH^- ions. This process continues until the water is saturated with respect to HA. At that point, the rate of the forward reaction (mineral dissolution) is equal to the rate of the backward reaction (mineral precipitation). The solubility product (Ksp) for a solution saturated with respect to HA is $[Ca]^{10}[PO_4]^6[OH]^2$. Strictly speaking, the values within brackets represent the activities (effective concentrations) of the component ions rather than their actual concentrations. Although Ksp is a constant, the concentrations of each of the three component ions in a saturated solution can vary, provided that their product remains equal to Ksp. Thus, in a more acidic solution where the hydroxyl concentration is reduced, the concentrations of the calcium or phosphate ions (or both) would have to increase to maintain the saturation. In fact, the solubility of HA depends on the pH value due to two reasons. First, the hydrogen ions remove hydroxyl ions to form water, as follows:

$$H^+ + OH^- \leftrightarrow H_2O$$

The product of $[H^+][OH^-]$ in water always equals 10^{-14} $(mol/l)^2$. Therefore, as $[H^+]$ increases in an acid solution, $[OH^-]$ will decrease in a reciprocal manner. Second, the inorganic phosphate in any fluid such as saliva is usually present in four different forms, namely H_3PO_4, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}, and the proportions depend entirely on the pH value. Figure 2.7 illustrates how the proportions of the four phosphate species vary with the pH value when the total phosphate concentration is 5×10^{-3} mol/l, as is typical of saliva [Dawes, 2003]. The lower the pH value, the lower the concentration of PO_4^{3-} which is the only species that contributes to the ion product of HA. Thus, as any solution is acidified, the concentrations of both OH^- and PO_4^{3-} are reduced although the Ca concentration is unaffected. It has been reported that enamel demineralization

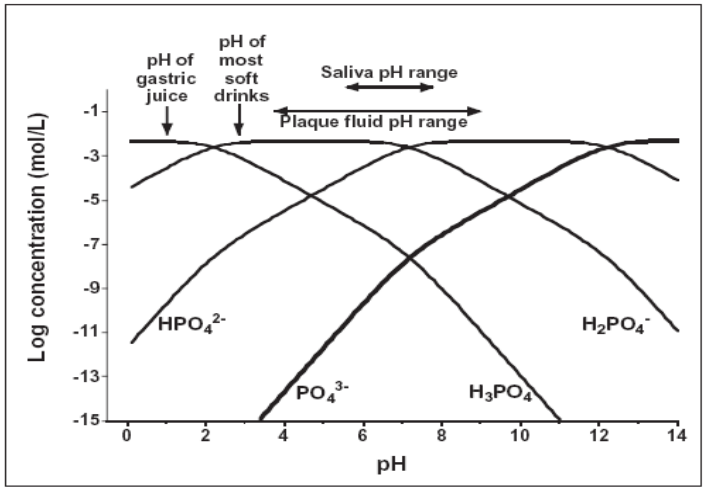


Figure 2.7 The effect of pH on the concentrations of the various inorganic phosphate species in saliva containing a total phosphate concentration of 5×10^{-3} mol/l. There is a marked fall in the concentration of PO_4^{3-} (thick line) as the pH value is reduced [Dawes, 2003].

occurs at a pH value lower than 5.5 [Lagerlöf, 1983; Driessens et al., 1986]. If the pH of a solution is less than this critical pH value of enamel, the solution is unsaturated by HA and enamel mineral will tend to dissolve until the solution becomes saturated.

Fluoride and enamel erosion

The profound effect of fluoride on reducing the incidence of caries is well documented in clinical studies [Volpe et al., 1995]. It is suggested that when a low concentration of fluoride is applied to dental enamel, F^- ion can replace OH^- ion in hydroxyapatite (HA) crystals and such doped crystals are sometimes referred to as fluor-hydroxyapatite ($Ca_{10}(PO_4)_6(F,OH)_2$) (Figure 2.8) [McCann, 1953; Leach, 1959] which is more resistant to enamel demineralization than HA [Elliott, 1997]. When a high concentration of fluoride is used, a calcium fluoride (CaF_2) layer is formed on the enamel surface (Figure 2.8) [Rølla et al., 1993; Ten Cate, 1997]. The phosphate- and/or protein-containing CaF_2-like materials have been proven to be the genuine products after the topical application of fluoride [Saxegaard and Rølla, 1988]. These observations and the reported delay of the dissolution of CaF_2 [Bruun et al., 1983] suggest that CaF_2-like deposition may serve as a storage of F^- ion which induce the reprecipitation of the mineral in the form of fluorapatite or fluor-hydroxyapatite interfering with further demineralization for considerable periods, i.e. months [Fejerskov et al., 1981].

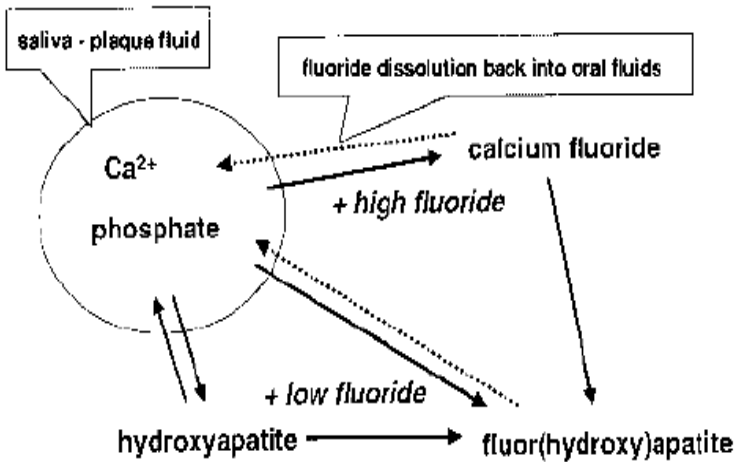


Figure 2.8 Chemical reactions relevant to the caries process involving fluoride [Ten Cate, 1999].

The question can be raised if fluoride can be effective in reducing the formation of erosive dental lesions [Larsen, 2001]. Attin *et al.* (2001) and Larsen and Richards (2002) concluded that

moderate fluoride concentrations such as those commonly used in toothpaste could not prevent enamel erosion, while a slight protective effect after applying topical fluoride was found by Van Rijkom *et al.* (2003) and Hughes *et al.* (2004). In a study by Wiegand and Attin (2003), promising results were obtained with a high concentrated fluoride application which can form CaF_2-like precipitation on the enamel surface. Erosion in enamel and dentine was found to be decreased. Subsequently, concentrated fluoride was recommended to reduce tooth erosion [Amaechi and Higham, 2005; Lagerweij et al., 2006]. SEM studies give evidence that the CaF_2-like precipitate is deposited in the form of small globules with diameters in the submicron range [Nelson et al., 1983]. The quality and quantity of CaF_2-like globules depend on various factors such as the concentration and the pH value of the fluoride reagents, application duration and tooth surface characteristics [Saxegaard and Rølla, 1988]. The higher the concentration and the more frequent the application, the more CaF_2-like compounds are deposited on the enamel surface and, therefore, the more prevention of erosion there is [Curz et al., 1992; Duschner et al., 1997; Attin et al., 2000; Petzold, 2001]. Acidulated fluoride agents have also been suggested to be more effective in preventing erosion when compared to fluoride gels with neutral pH value because of the formation of a denser and thicker CaF_2-like layer [Delbem and Cury, 2002]. It is thought that moderate acidic pH values slightly accelerate the dissolution of enamel apatite, which increases the Ca concentration in the solution near the surface and facilitates the deposition of CaF_2-like globules [Larsen and Jensen, 1986]:

$$Ca_5(PO_4)_3OH + 7\ H^+ \rightarrow 5\ Ca^{2+} + 3\ H_2PO_4^- + H_2O$$

$$Ca^{2+} + 2\ F^- \rightarrow CaF_2$$

In neutral solutions, the pH value rises due to the release of OH^- caused by the dissolution of enamel apatite according to the reaction scheme below:

$$Ca_5(PO_4)_3OH + 3\ H_2O \rightarrow 5\ Ca^{2+} + 3\ HPO_4^{2-} + 4\ OH^-$$

the released OH^- ions and the increased pH quickly end the dissolution of apatite and limit the CaF_2-like layer formation. In addition, artificially eroded enamel is believed to retain more fluoride as compared to sound enamel due to the increased surface area, which offers a higher number of possible retention sites for the fluoride [Attin et al., 2000]. Hence, the increased amount of adsorbed fluoride should enhance the formation of CaF_2-like material. Bruun and Givskov (1993) measured the formation of the CaF_2-like layer on sound enamel and artificial caries lesions after 1 minute exposure to 2.0% NaF solution. CaF_2-like precipitation on the enamel surfaces with caries-like lesions amounted to 30 µg F cm^{-2} whereas KOH-soluble fluoride on sound enamel amounted to 1.0 µg F cm^{-2} only.

2.2 Tooth color

Natural tooth color

The color of a tooth is determined by the combination of its optical properties. When light encounters a tooth, four phenomena associated with the interactions of the tooth with the light flux have to be considered [Jahangiri et al., 2002]: (1) specular transmission of the light through the tooth, (2) specular reflection at the surface, (3) diffuse light reflection at the surface and (4) absorption and scattering of light within the dental tissues. Tooth color has been shown to result from the volume scattering of light, i.e. the illuminating light follows a highly irregular light paths through the tooth before it emerges at the surface and reaches the eye of the observer [O'Brien et al., 1990].

The normal color of enamel varies from light yellow to grayish white. Since enamel is semitranslucent, the clinically perceived shade of teeth is determined by the outmost layer of enamel and the inherent dentine and pulp shades [Fondriest, 2003].

Tooth discoloration

Over the lifetime of the tooth, there are numerous factors that affect both the color and the brightness of teeth, and it is to a certain degree inevitable that tooth discoloration is caused. Tooth discolorations are divided into three categories: extrinsic stains, intrinsic stains and age-related stains.

Extrinsic stains

Extrinsic stains are caused by the deposition of chromogenic materials on the tooth surface. It is generally believed that the dietary chromogens or the pre-chromogens adhere onto the tooth surface via saliva proteins. Figure 2.9 shows the adhesion mechanism in the case of typical tannin chromogens causing e.g. tea, coffee and red wine stains [Nathoo, 1997]. Saliva proteins are selectively bonded onto the enamel surface via calcium bridges, thus forming the pellicle. At the early stage of staining, chromogens themselves are assumed to interact with the pellicle via

hydrogen bridges. At this stage, food stains can be removed by tooth brushing. However, over a longer period of time, food stains will darken and become more tenacious. Chemical analysis of aged stains of foods and beverages revealed the presence of furfurals and furfuraldehyde derivatives due to the Millard reaction (nonenzymatic browning reaction), which is a series of chemical rearrangements and reactions between sugars and amino acids [Viscio et al., 2000]. Tenacious surface stains are highly amenable to bleaching [Goldstein and Garber, 1995]. Binding of colorless materials to teeth with subsequent reactions to chromogens may also result in extrinsic stains. For example, colorless stannous fluoride is prone to reduction to tin by sulfuridyl groups of pellicle proteins causing dark external metallic stain. Another example is brown stain caused by redox reactions of chlorhexidine. Removing stains of antimicrobial agents requires bleaching with oxygenating agents [Nathoo, 1997].

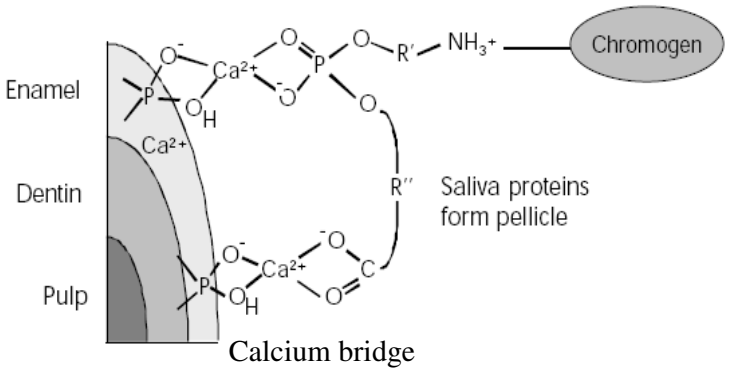


Figure 2.9 Adhesion mechanisms of chromogens onto the tooth surface, e.g. tannin.

Intrinsic stains

Intrinsic stains are due to the incorporation of chromogenic materials within enamel or dentine. They can be divided into two groups: the pre-eruptive due to intake of certain drugs and the post-eruptive due to e.g. trauma or aged dental materials. One of the most common types of pre-eruptive stains is fluorosis due to excessive fluoride ingestion during tooth development. Antibiotic tetracycline has the ability to chelate calcium ions and to be incorporated into the tooth during tooth calcification and even prenatally [Sadan and Lemon, 1998]. Fully formed teeth in adults may be also stained from taking the tetracycline minocycline for acne. This discoloration is malformation of dental tissues associated with inherited conditions (e.g. amelogenesis imperfecta and dentinogenesis imperfecta) can also cause pre-eruptive dental stains [Nathoo, 1997; Viscio et al., 2000]. Blood penetrating into the dentine tubuli and metals released from dental restorative materials will cause post-eruptive stains. Another source of

intrinsic stains is aged dental materials like root-filling and other restorations materials [Glockner et al., 1997]. Intrinsic stains are not removed by brushing or any abrasive process, but can be reduced by bleaching with agents penetrating enamel and dentine to oxidize the chromogens. However, extended treatment time and higher concentration of the bleaching agent are needed to remove persistent intrinsic stains [Glockner et al., 1997; Haywood, 2000].

Age-related stains

It is unavoidable that a person's teeth will darken with age. One of the reasons is the cumulative effect of extrinsic and intrinsic stains. However, the more important reason is that dental enamel becomes thinner with age because of enamel attrition or abrasion, therefore, allowing the yellow dentine to show through and be visible through the enamel. Normally, unless the enamel is badly worn, bleaching is an efficient technique to lighten teeth of older patients.

2.3 Bleaching

History

Many attempts to find an effective tooth whitening method have been made throughout the history of dentistry. The earliest attempts focused on using chloride of lime to whiten non-vital teeth [Dwinelle, 1850]. Up to the late nineteenth century, numerous other bleaching agents were also successfully employed on non-vital teeth [Haywood, 1992], including cyanide of potassium [Kingsburg, 1861], oxalic acid [Bogue, 1872], sulphurous acid [Kirk, 1889], aluminum chloride [Harlan, 1891], sodium hypophosphate [Harlan, 1891], pyrozone [Atkinson, 1892], hydrogen dioxide (hydrogen peroxide or perhydrol) and sodium peroxide [Kirk, 1893]. All these substances were considered as either direct or indirect oxidizers acting on the organic portion of the tooth, except for sulphurous acid which was a reducing agent [Kirk, 1889]. Later on, hydrogen peroxide was used in combination with hydrochloric acid to remove "brown stain from mottled teeth" due to a chronic endemic dental fluorosis [McInnes, 1966]. Cohen and Parkins (1970) verified that the mechanism of tooth whitening using hydrogen peroxide involves penetration to the dentine. In 1976, Nutting and Poe introduced the walking bleaching technique,

which uses 35% hydrogen peroxide and sodium perborate for non-vital teeth bleaching. This technique is still being used.

Vital teeth were whitened with oxalic acid as early as 1868 [Latimer, 1868], and later with pyrozone [Atkinson, 1892] or hydrogen peroxide [Fisher, 1911]. By 1910, concentrated hydrogen peroxide solutions had been an accepted method in dental clinics [Fisher, 1911]. The current in-office bleaching technique typically uses 35% hydrogen peroxide with rubber dam isolation [Haywood, 2000]. At-home bleaching began in 1968 when an American orthodontist, Dr. Bill Klusmier, instructed his patients to use an "over-the-counter" oral antiseptic, Gly-oxide (Marion Merell Dow), containing 10% carbamide peroxide at night to resolve inflammationory issues. Dr. Bill Klusmier noticed that Gly-oxide not only improved the gingival health but also whitened teeth [Haywood et al., 1990]. Later, Proxigel (a mixture of 10% carbamide peroxide, water, glycerine and carbopol) replaced Gly-Oxide in patients' orthodontic positioners, due to its slow release of carbamide peroxide. In 1988, the University of North Carolina clinically ascertained the effectiveness of the Proxigel technique. One year later, Haywood and Heymann (1989) published their article, "Nightguard and Vital bleaching" and an at-home bleaching product "White and Brite™" (Omnii International) was marketed. The two events marked the beginning of "modern day" tooth bleaching. "Over-the-counter" (OTC) bleaching agents were first launched in the United States in the 1990s. This bleaching system contains lower concentration of hydrogen peroxide or carbamide peroxide compared to medical practice bleaching products and is sold directly to consumers in stores for unsupervised home use [Greenwall, 2001]. Since then, products and techniques made claims for whitening teeth and were introduced.

The effectiveness of bleaching depends upon the ability of oxidizing agents to enter the dental hard tissues and decompose the pigment molecules. Bleaching agents are expected to permeate into the tooth structure deeply enough and remain there long enough to modify deep stains [Goldstein, 1997]. The first-generation tooth bleaching materials are in a liquid form. These materials do not remain in the trays for a long time and need more replenishment over time. The second-generation materials are more viscous and are gelled to prolong the release of active bleaching agents. More inactive ingredients are included in the third-generation bleaching systems to increase effectiveness, enhance security and facilitate application. In general, quality control by the manufacturers has improved, together with changes in the packaging and patient instruction, to make them more patient-friendly [Fasanaro, 1992; Blankenau et al., 1999].

Methods of tooth bleaching

According to the vitality status of the tooth to be treated, techniques to whiten tooth can be classified into vital and non-vital tooth bleaching.

Vital tooth bleaching

Vital teeth are bleached by the application of whitening substances on the external surface of the teeth. The whitening procedure can be carried out in the dental office or at home by patients or by a combination of both techniques.

In-office bleaching products contain a high percentage of 30-38% of hydrogen peroxide and are used under the supervision of the dentist while patients are seated in the dental chair. The procedure involves the isolation of the teeth with rubber dam or alternatives [Powell and Bales, 1991] and the application of bleaching gel directly onto the tooth surface for up to 1 hour per appointment, with or without the activation by heat, light or laser. Several appointments may be necessary to achieve the desired result.

At-home bleaching, which was first reported in the dental literature by Haywood and Heymann (1989), "Nightguard and Vital bleaching", is the most popular whitening method performed. This technique involves the application of a mild bleaching agent (10-20% carbamide peroxide, equaling 3.5-6.5% hydrogen peroxide) onto the teeth by wearing a custom fabricated tray overnight or during the day at home. Treatment is carried out by patients themselves, but the process is monitored by the dentist during recall appointments. Compared to the in-office technique, at-home tooth whitening is more cost-effective but it depends upon the patients' compliance and requires a longer treatment time because of the comparatively lower strength of the oxidizing agent [Russell et al., 1996]. The required treatment time depends on the concentration of the carbamide peroxide used. The 10% carbamide peroxide is recommended to be used for 8 hours per day, and the 15-20% carbamide peroxide for 3-4 hours per day.

The use of a combination of in-office and at-home bleaching techniques produces the most effective result in the shortest period of time. Brightening of the tooth by in-office bleaching

treatment can be further preserved and enhanced by follow-up treatments with an at-home bleaching system.

Sales of "over-the-counter" (OTC) tooth bleaching products have sharply increased in recent years because of their convenient use. Most of the OTC bleaching agents contain a small percentage of carbamide peroxide (≤ 6%) in a certain mode of delivery, e.g. whitening dentifrices, pre-fabricated trays, whitening strips and toothpastes [Zantner et al., 2007]. An alternative active bleaching agent used in this type of product is sodium chlorite ($NaClO_2$) in combination with citric acid, which was launched several years ago. OTC products are inexpensive and convenient to use, and represent the fastest growing segment of the dental market [Kugel, 2003]. However, many retail products have not undergone rigorous and objective clinical testing before the introduction onto the market and tooth whiteners are not regulated by the Food and Drug Administration. Hence, these bleaching agents may be of highly questionable efficiency and safety [Polydorou, 2004].

Non-vital tooth bleaching

Non-vital or internal bleaching is used to lighten a discolored tooth that has had traumatic damages of the pulp, pulp necrosis or endodontic treatment. Sodium perborate mixed with water, or 30% hydrogen peroxide solution alone or mixed with sodium perborate are common formulations for non-vital tooth bleaching. The bleaching agent is supplied into the prepared pulp chamber or/and the coronal portion of the root canal to remove tooth discoloration in two different methods: 1. "in-office bleaching": the bleaching agent is left inside the tooth for a period of time, and then removed before leaving the office. This may be done once or several times, depending on the discoloration of the tooth; 2. "walking bleaching": the bleaching agent is sealed into the endodontic access cavity. The patient returns to the dentist to have the bleaching agent renewed until the satisfied shade change or the maximum effect is achieved. Root resorption is suggested to be the potential adverse effect and can lead to the loss of the tooth [Goon et al., 1986].

The mechanism of tooth bleaching

Bleaching is a chemical process which is widely applied to whiten materials mostly using oxidizing compounds, e.g. hydrogen peroxide, chlorine or sodium hypochlorite. Although bleaching processes are a complex sequence of chemical reactions, the underlying principle of the vast majority is the stepwise oxidation of dyes to decompose them. Total oxidation of organic chromogens via several intermediates ends up in the final products carbon dioxide (CO_2) and water (H_2O). In dentistry, according to oxidizing compounds, modern bleaching materials can be classified into two groups: peroxide-based and non-peroxide-based.

Peroxide-based bleaching agents

Peroxide-based bleaching agents are widely used even though the mechanism by which teeth are whitened is not fully understood [Sulieman et al., 2004]. These products generally contain hydrogen peroxide or one of its precursors (carbamide peroxide or sodium perborate) as an active oxidizing agent [Hägg, 1969].

Hydrogen peroxide (H_2O_2, HP) has strong oxidizing properties and, therefore, is a powerful bleaching agent in whitening teeth. When bleaching materials are applied onto the tooth surface, HP will be released and permeate into the enamel and dentine (Figure 2.10) due to the relatively low molecular weight of the peroxide molecule (30 g/mol) [Goldstein et al., 1989]. Hence, not only extrinsic but also intrinsic chromogens can be oxidized to form colorless products.

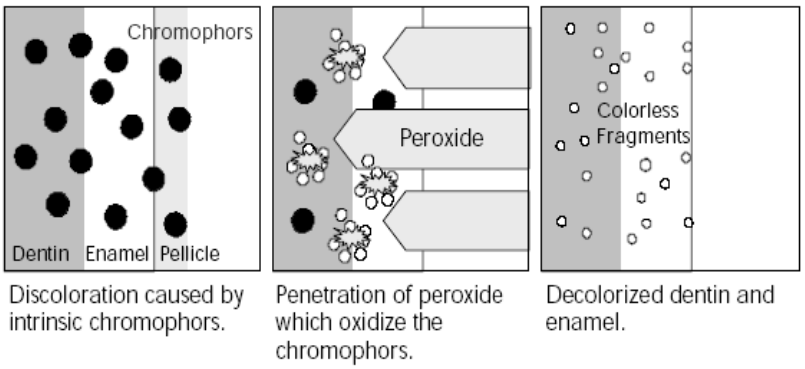


Figure 2.10 Schematic illustration of the bleaching mechanism with peroxides [Dahl, 2003].

The oxidation process of staining organic compounds by HP is a complex series of reactions, based on the formation of free radicals like hydroxyl ($HO^{\bullet}$), perhydroxyl radicals ($HO_2^{\bullet}$) and superoxide anions ($O_2^{\bullet-}$) (A) [Gregus and Klaassen, 1995], reactive oxygen molecules {O} that are unstable and transform to oxygen (B) and hydrogen peroxide anions (HOO^-) (C) [Cotton and Wilkinson, 1972] through:

(A) $H_2O_2 \rightarrow 2\ HO^{\bullet}$

$HO^{\bullet} + H_2O_2 \rightarrow H_2O + HO_2^{\bullet}$

$HO_2^{\bullet} \leftrightarrow H^+ + O_2^{\bullet-}$

(B) $2\ H_2O_2 \leftrightarrow 2\ H_2O + 2\ \{O\} \leftrightarrow 2\ H_2O + O_2$

(C) $H_2O_2 \leftrightarrow H^+ + HOO^-$

These reactive molecules can react with many compounds with unsaturated double bonds. Typically, staining organic chromogens are characterized by conjugated double bonds in the molecule, which is the structural reason for the color [Nathoo, 1997]. Via several intermediate steps, the long-chained, dark-colored chromophore molecules are split into smaller and less colored molecules with higher transport properties. This is schematically illustrated in Figure 2.11. As bleaching proceeds, teeth continually lighten. When all chromophors are converted to colorless molecules, the so-called saturation point is reached. Ideally, this is the point at which whitening should be terminated. If the degradation process continues, i.e. overbleaching, there is further decomposition of the organic matrix, which can lead to complete oxidation with the generation of carbon dioxide (CO_2) and water (H_2O). Overbleaching bears the risk to oxidize matrix proteins of the enamel and dentine which may cause significant alteration of the enamel and dentine structure. However, it would not increase the whitening effect [Goldstein and Garber,

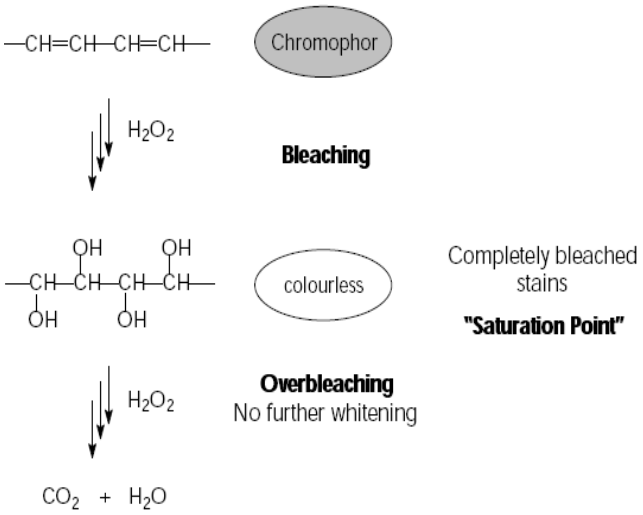


Figure 2.11 Illustration of the chemical oxidation reaction of dye molecules comprising unsaturated double bonds with HP to colorless molecules. After conversion of all stains to colorless products the saturation point is reached. Further bleaching would merely cause degradation to carbon dioxide (CO_2) and water (H_2O) but would not increase the whitening effect (overbleaching).

1995]. Therefore, the application of bleaching, e.g. treatment procedures, the amount of agents and duration of treatment, must be in accordance with the manufacturers' recommendations.

Carbamide peroxide ($CH_6N_2O_3$ or $CH_4N_2O•H_2O_2$, CP), also called urea peroxide, is introduced as an alternative to traditional HP and its use has become widespread. This agent is very unstable and in the presence of water immediately breaks down into urea and HP at a ratio of about 6.5 urea to 3.5 HP:

$$CH_4N_2O •H_2O_2 \rightarrow CH_4N_2O + H_2O_2$$

This means that a 10% CP gel is equivalent to a 3.5% HP gel in terms of its bleaching effectiveness [Haywood, 1992]. HP further decomposes to water and oxygen, while urea breaks down to ammonia and carbon dioxide. The release of ammonia and carbon dioxide elevates the pH value of the bleaching agent in the oral cavity during the bleaching process [Leonard et al.,1994]. Moreover, adding urea to HP stabilizes the formula and improves the taste [Christensen, 1997]. Commercial CP products normally contain either a carbopol or a glycerine base. The carbopol base slows down release of HP without changing the efficiency of the bleaching treatment [Haywood et al., 1990]. CP bleaching materials are suggested to have a slightly acidic pH value to extend shelf life [Goldstein and Garber, 1995].

Hydrogen peroxide *vs* **carbamide peroxide**

An important difference between HP and CP lies in the rate that each releases peroxide [Haywood, 2005]. The urea stabilizes CP and makes CP break down more slowly than straight HP. CP releases about 50% of its peroxide in the first 2 hours, then the remainder over the next 6 hours [Haywood, 2005]. Hence, CP is a time-release approach to bleaching. HP breaks down almost immediately when heat, sodium hydroxide or light is applied, releasing its peroxides entirely within the first hour [Haywood, 2005]. It is thought that due to this immediate bombardment of peroxides on the pulp, HP produces more sensitivity than CP of a comparable concentration.

Sodium perborate ($Na_2[B_2(O_2)_2(OH)_4]$, SP) is the safest and most easily controlled material for internal bleaching when prepared with HP or distilled water. When fresh, the pH value of the distilled water-based SP paste is 9.9 [Rotstein and Friedman, 1991]. SP is stable when it is dry, but in the presence of acid, warm air or water it decomposes to form sodium borate and HP.

Non-peroxide-based bleaching agents

Recently, tooth bleaching materials based on sodium chlorite ($NaClO_2$) activated by citric acid have been investigated [Attin et al., 2004; Zantner et al., 2006]. The bleaching process consists of two steps. The first step is to moisten the tooth surface using Rapid White "accelerator" which is made of sodium chlorite solution; and the second is to apply Rapid White "whitening gel" which contains citric acid and other inactive ingredients onto the moistened tooth surface to form an interface between "accelerator" and "whitening gel". Sodium chlorite, an inexpensive oxidizing agent, has been extensively used in water treatment, paper, pulp and textile industries due to the generation of chlorine dioxide (ClO_2) in the acid circumstances [Geng et al., 2005; Zantner et al., 2007]:

$$5\ ClO_2^- + 4\ H^+ \rightarrow 4\ ClO_2 + 2\ H_2O + Cl^-$$

As an oxidant, chlorine dioxide (ClO_2) readily attacks reducing substances such as organic materials primarily by a one-electron pathway [Gallagher et al., 1994]:

$$ClO_2 + e^- \leftrightarrow ClO_2^-$$

Likewise, the tooth is whitened resulting from the presence of chlorine dioxide (ClO_2) in the interface between "accelerator" and "whitening gel".

Composition of commercial bleaching agents

Bleaching products are based on an oxidizing agent, e.g. HP or chlorine, as active ingredient. However, in order to improve working efficiency of the active agent and enhance patient comfort, some other ingredients are included. The major inactive ingredients of tooth whiteners may include carbopol, glycerin, surfactant and pigment dispersant, preservative and flavoring.

1) Thickening agents

Carbopol (carboxypolymethylene), a high molecular weight polyacrylic acid polymer, is usually present at a 0.5 to 1.5% concentration. It is the most common thickening agent in the tooth bleaching formula and helps to prolong the release of active bleaching ingredient. It has been found that carbopol can extend the active oxygen-releasing time of the bleaching solution by up to 4 times [Rodrigues et al., 2007]. Carbopol also enhances the viscosity of the bleaching materials. The thixotropic nature of carbopol allows better retention of the slow releasing gel in

the tray. Thus, less bleaching material is required for treatment. The viscosity also improves adherence to the tooth.

2) Carrier

The carrier can retain moisture, impart sweetness and help to suspend or dissolve other ingredients. Examples of common commercial carriers for active bleaching agents are glycerin and propylene glycol. These carriers are considered non-toxic and convenient because of their compatibility with desirable additives, e.g. thickening agents, preservatives and flavorings.

3) Surfactant and pigment dispersant

The surfactant, as a surface wetting agent, allows the active bleaching ingredient to diffuse across the gel-tooth boundary. A pigment dispersant keeps pigments in suspension (as in commercial water softeners). Gels with surfactant or pigment dispersants may be more effective than those without them [Feinman et al., 1991].

4) Preservative

Preservatives, e.g. methyl, propyl paraben or sodium benzoate, are included to prevent bacterial growth in bleaching products. Moreover, these preservatives can sequestrate transitional metals, e.g. iron, copper and magnesium, which accelerate the breakdown of HP.

5) Flavoring

Flavorings are used in bleaching materials to enhance the patients' acceptance of the product. Common flavorings are peppermint, spearmint, wintergreen, sassafras and anise. Additionally, sweeteners, e.g. saccharin, are added to further improve the taste of bleaching products.

2.4 Effects of tooth bleaching

Peroxide-based bleaching agents

Effects on soft tissues

Gingival irritation is a common side effect caused by bleaching treatment [Weitzman et al., 1986; Tam, 1999]. Occasionally, gastrointestinal mucosal irritation, e.g. a burning palate and throat,

and minor upsets in stomach or intestine, are also reported by patients [Howard, 1992; Pohjola et al., 2002]. In most cases, soft tissues irritation results from an ill-fitting mouthpiece tray rather than from the tooth bleaching agent itself. These symptoms are temporary and usually disappear within a few days after finishing the treatment [Schulte et al., 1994].

Effects on enamel

Surface morphology

In the past two decades, effects of peroxide-containing bleaching agents on the surface morphology of tooth enamel have been evaluated [Haywood et al., 1990; Covington et al., 1990; Hunsaker and Christensen, 1990; Bitter and Sanders, 1993; Ernst et al., 1996; Josey et al., 1996; Gürgan et al., 1997; Hegedüs et al., 1999]. Some studies showed that bleaching did not significantly affect the enamel surface [Haywood et al., 1990; Covington et al., 1990; Ernst et al., 1996]. However, other investigations demonstrated morphological alterations in the bleached enamel surface: depressions, porosity and increased depth of enamel grooves [Bitter, 1998; Josey et al., 1996; Hegedüs et al., 1999], indicating an erosive process due to tooth bleaching.

In a scanning electron microscopy (SEM) analysis, Haywood *et al.* (1990) reported no morphological change in the enamel surface after 10% CP bleaching. Ernst *et al.* (1996) drew the same conclusion based on an investigation with a concentrated bleaching agent, 35% HP. On the contrary, McGuckin *et al.* (1992) observed that surface morphological patterns of the bleached enamel were similar to type II acid etching. This type II acid-like pattern did not become more severe after a longer bleaching period of approximately 6 months [Leonard et al., 2001]. The results of profilometric analyses are also conflicting. Titley *et al.* (1992) observed a slight increase in the surface roughness, whereas Hunsaker and Christensen (1990) and Gürgan *et al.* (1997) reported that no modification of the surface roughness occurred. Using atomic force microscopy (AFM), Hegedüs *et al.* (1999) observed changes in the enamel surface after 28 hours of bleaching with 10% CP and 30% HP, and found that the samples surface became more irregular and surface grooves became deeper after treatment.

Mechanical properties

Microhardness changes are related to a loss or gain of mineral phases (demineralization or remineralization) of the dental structure [Featherstone et al., 1983]. Microindentation has been used to determine slight changes in the surface microhardness after bleaching treatment, using Knoop hardness number (KHN) [Murchison et al., 1992; Basting et al., 2003; Joiner, 2004] or Vickers hardness number (VHN) [Seghi and Denry, 1992; Potocnik et al., 2000; Park et al., 2004; Ünlü et al., 2004]. Nanoindentation is emerging as a new technique. It is more sensitive to early erosive processes [Barbour and Rees, 2004] and has been applied in recent dental bleaching studies [Hairul Nizam et al., 2005; Chng et al., 2005]. Different results on enamel microhardness changes due to bleaching have been published. Seghi and Dendry (1992) showed that bleaching enamel specimens with 10% CP for 12 hours did not alter the surface microhardness values. Potocnik *et al.* (2000) came to the same conclusion even though the bleaching period was extended to 336 hours. However, Cimilli and Pameijer (2001) measured the VHN of enamel after applying 10% CP on teeth (6 hours per day, 10 days) and found the demineralized enamel up to 110 μm below the surface. Using a bleaching agent with higher concentration (30% HP), Lewinstein *et al.* (1994) reported a decrease in the enamel microhardness after a treatment for 15 minutes. Even if samples were stored in artificial saliva, a 47% reduction in KHN of enamel exposed to 10% CP (8 hours per day, 42 days) was observed by Rodrigues *et al*. (2001).

Seghi and Denry (1992) and McCracken and Haywood (1996) reported an increased fracture susceptibility of the enamel following bleaching. In an *in vitro* study, Rotstein *et al.* (1992) found a loss of strength and a high solubility of enamel after bleaching. Tensile strength and Young's modulus of enamel were also proven to be significantly decreased by bleaching [Cavalli et al., 2004; Hairul Nizam et al., 2005].

Chemical composition

The effect of bleaching agents on dental hard tissues was also investigated by measuring the change of constituent enamel elements [McCracken and Haywood, 1996; Rotstein et al., 1996; Potocnik et al., 2000; Cimilli and Pameijer, 2001; Duschner et al., 2006; Lee et al., 2006; Added et al., 2006; Tezel et al., 2007]. In a study by McCracken and Haywood (1996), teeth exposed to commercial products based on 10% CP for 6 hours lost an average of 1.06 $\mu g/mm^2$ of Ca. Using electron probe microanalysis, Potocnik *et al.* (2000) reported that 10% CP decreased the

concentration of Ca and P and the Ca:P ratio. In addition, Ca and P were found in the bleaching gel after use. Lee *et al.* (2006) demonstrated that the amount of Ca loss from teeth after 120 hours of bleaching treatment was similar to the amount of Ca loss from teeth exposed to a soft drink or a juice for a few minutes. These studies concluded that changes in the chemical composition of enamel were slight and not clinically significant. However, Rotstein *et al.* (1996) and Tezel *et al.* (2007) verified that the concentrated bleaching agent caused a significant loss of Ca from the enamel surface.

Crystal structure

Several research groups investigated the crystal structure of bleached enamel. In the study of Cimilli and Pameijer (2001), Infrared (IR), Fourier transform infrared spectrophotometer (FT-IR) and X-ray diffraction (XRD) established a change from hydroxyapatite (HA) to primary calcium ortho phosphate [$Ca(H_2PO_4)_2$] for all experimental groups (15% Opalescence, 10 and 16% Nite White Excel) except for 10% Opalescence. Oltu and Gürgan (2000) observed that the shift of the IR absorption peaks happened only when enamel was treated with 35% CP. 10 and 16% CP caused no adverse effect on the crystal structure. However, using a Fourier transform Raman spectrophotometry (FT-Raman), Park *et al.* (2004) and Goo *et al.* (2004) reported no significant influence of bleaching with 30% HP or 10% CP on dental enamel, respectively. Tezel *et al.* (2007) drew the same conclusion using micro-Raman spectroscopy.

Effects on dentine

Hunsaker and Christensen (1990) and Zalkind *et al.* (1996) revealed changes in the dentine surface morphology using SEM. Pecora *et al.* (1994) found a decreased microhardness values after applying a 10% CP agent on dentine for 72 hours. In a study by Basting *et al.* (2005), not only 10% CP but also the thickening agent (carbopol and/or glycerin) caused a decrease in the microhardness of dentine. Rotstein *et al.* (1992) reported a loss of strength and higher solubility of dentine after bleaching. They observed a significant reduction in the mineral content after immersing dentine in different bleaching agents for 7 days [Rotstein et al., 1996]. In a recent study, direct exposure to 10% CP caused a significant decrease in the flexural strength and flexural modulus of bovine dentine [Tam et al., 2005]. Dentine bonding also may be altered after bleaching [Della Bona et al., 1992], which suggests that adhesive dentistry should be delayed for

2 weeks post-bleaching [Powell and Bales, 1991]. On the other hand, Nathoo *et al.* (1994) found no microhardness changes in the dentine surface.

Effects on restorations

Studies evaluating the effects of bleaching agents on restorative materials give conflicting results. Data from laboratory studies documented an increased mercury release from dental amalgams exposed to CP solutions for periods ranging from 8 hours to 28 days [Hummert et al., 1993; Rotstein et al., 1997]. The amount of mercury released varied with the type of amalgam and the type of bleaching agent and it ranged from 4 to 30 times higher than in saline controls. Swift and Perdigão (1998) suggested that bleaching may increase the solubility of glass-ionomer and other cements. In a study by Dishman and Baughan (1992), the bond strength between enamel and resin-based fillings was reduced in the first 24 hours after bleaching. After 24 hours, there was no difference in the strengths of dental composite resin cement bonds to bleached and unbleached enamel [Homewood et al., 2001]. Lai *et al.* (2002) suggested that HP residuals in the enamel inhibited the polymerization of resin-based materials and thus reduced bond strength. Therefore, tooth bleaching agents should not be used prior to restorative treatment with resin-based materials. It was also demonstrated that bleaching treatment significantly caused shade changes, cracks occurred and microhardness reduced in the surface of restoration materials [Kao et al., 1991; Bailey and Swift, 1992]. However, in some cases, no adverse effect of dental bleaching on porcelain, resin composite, amalgam or gold restorations was observed [Hunsaker and Christensen, 1990; Haywood and Heymann, 1991].

Non-peroxide-based bleaching agents

The effect of "over-the-counter", non-peroxide-based bleaching products on dental enamel has been investigated only recently. Zantner *et al.* (2007) compared enamel bleached with different products and found a significant decrease in the surface microhardness for agents containing sodium chlorite ($NaClO_2$) in combination with citric acid. Attin *et al.* (2005) demonstrated that the decrease in the microhardness was not limited to the superficial enamel layer and subsurface softening was also particularly pronounced for samples treated with Rapid White [Attin et al., 2004], a popular peroxide-free bleaching material. Moreover, these studies showed that

non-peroxide-based bleaching products were more aggressive to dental enamel than peroxide-containing products. Citric acid contained in non-peroxide-based bleaching products to active sodium chlorite might lead to enamel erosion [Attin et al., 2005; Zantner et al., 2007]. Citric acid is a tribasic carboxylic acid and may dissolve enamel by reaction of hydroxyapatite (HA) with acid [Dorozhkin, 1997]:

$$Ca_{10}(PO_4)_6(OH)_2 + 2\ H^+ \leftrightarrow 10\ Ca^{2+} + 6\ PO_4^{3-} + 2\ H_2O$$

Besides, the citrate ion $(C_6H_5O_7)^{3-}$ (cit) is a calcium-chelating ligand and can form a soluble Ca-cit complex, promoting further enamel dissolution [Koulourides et al., 1961; Rhee and Tanaka, 1999]. Barbour *et al.* (2003) found a significant decrease in the enamel microhardness and elastic modulus after human enamel was exposed to citric acid solutions with $2.30 \leq pH \leq 6.30$. In a study by Attin *et al.* (2003), the effect of a mineral supplement to citric acid on bovine enamel was evaluated. The results showed that calcium, phosphate and fluoride exerted a significant protective effect with respect to dental erosion caused by citric acid. However, enamel dissolution was not completely prevented by the application of minerals of low concentrations. Citric acid can be regarded as a potential erosive substance to enamel [White et al., 2001]. Hence, it is of vital importance to better understand the effects of such sodium chlorite-based bleaching materials on dental enamel.

2.5 Aims of this study

The objective of the present *in vitro* study was to investigate the structural aspects of bleaching and fluoride application on dental enamel utilizing different test methods. Firstly, the effect of different bleaching formulae (peroxide-based and non-peroxide-based) on the physicochemical properties of dental enamel was studied. The changes in the surface morphology, chemical composition and crystal structure of enamel treated with commercial peroxide-based bleaching reagents (Opalescence Xtra Boost, Opalescence PF 20% and sodium perborate) and a non-peroxide-based bleaching product (Rapid White) were observed by different techniques: scanning electron microscopy (SEM), attenuated total reflectance infrared spectroscopy (ATR IR), Raman spectroscopy, x-ray diffraction (XRD), electron-probe microanalysis (EPMA) and electron-probe element mapping, as well as flame atomic absorption spectroscopy (FAAS) and total reflection X-ray fluorescence (TXRF). Subsequently, the role of fluoride reagents in preserving the apatite structure against bleaching-induced deterioration was studied using ATR IR spectroscopy and complementary SEM imaging. In addition, the impact of citric

acid-containing bleaching agents or aqueous solutions on enamel apatite was compared with that on single-crystal hydroxyapatite (HA) of geological origin.

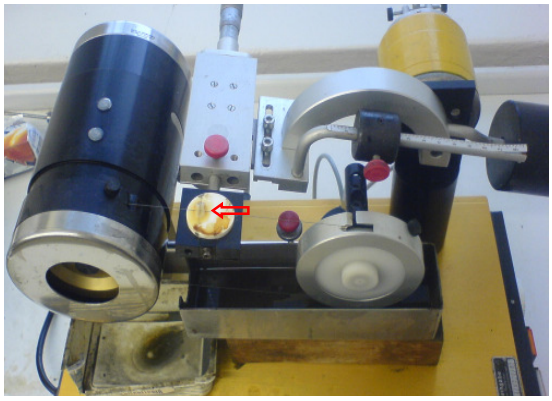
3 Materials and methods

3.1 Sample preparation

1) Enamel samples

Extracted human molars were stored in distilled water at room temperature prior to the experiment. Sound molar teeth with no caries, enamel defects or crazing/cracks were selected in this study. The soft tissues of the teeth were cleaned and the roots were removed, approximately to the cemento-enamel junction, with a water cooled high-speed hand piece.

For electron microscopic, XRD and vibrational spectroscopic experiments one-side polished enamel slabs were used. Slabs sized ~ 5×3×2 mm were cut from the middle one third of the enamel surface. The cuts were parallel to the enamel-dentine junction, i.e. approximately perpendicular to the six-fold symmetry axis of hydroxyapatite (HA). Specimens were mounted in autopolymerizing acrylic resin so that their external surfaces were exposed. After the resin was left to polymerize for 24 hours, the molds were removed and the external surface of the enamel fragments was leveled with a water-cooling mechanical grinder. 1200-grit silicon carbide paper discs were used to produce parallel surfaces for following measurements. Samples surface was polished using 0.1 µm-sized colloidal-silica suspensions under continuous water cooling. To avoid any uncertainties and misinterpretation of the experimental data due to tooth-to-tooth variability and slight variations in specimen orientation, each enamel slab was further divided into several segments as shown in Figure 3.1: one segment was kept untreated to be used as a reference sample and the remaining segments were subjected to different bleaching treatments. Finally, samples were cleaned ultrasonically for 3 minutes.



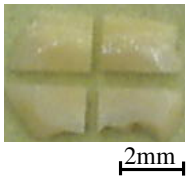


Figure 3.1 Sample preparation. (Left) red arrow is pointing at the enamel slab. (Right) divided enamel slab and magnification scale.

For FAAS and TXRF analyses the teeth were fixed into small polypropylene tubes (Figure 3.2), thus allowing only crown enamel to be exposed to water and bleaching products and preventing the tooth roots from any contact with the treatment reagents.

Figure 3.2 Sample in polypropylene tube for Ca leaching analysis.

2) Geological hydroxyapatite (HA) samples

In order to understand the fundamental chemical aspects that are implicit in the applications of bleaching materials and fluoride to enamel, the interaction of these bleaching agents and fluoride reagents with geological hydroxyapatite (HA), the structural prototype for the principal inorganic crystalline constituent of teeth, was studied as well. Therefore, one-side polished samples of geological hydroxyapatite (HA) were cut from a monolithic single crystal. The cuts were also perpendicular to the six-fold axis of symmetry and sized ~ 5×5×2 mm. We used an original mineral specimen from Snarum, Buskerud, Norway, a traditional location for hydroxyapatite (HA). Crystal structure and chemistry were verified by XRD, Raman scattering and infrared transmittance spectroscopy.

3.2 Experimental materials

Commercial bleaching agents

1. Opalescence® Xtra® Boost™ (Ultradent Products Inc, South Jordan, UT, USA)

The Opalescence® Xtra® Boost™ tooth whitening system is an in-office bleaching material based on HP. This chemically activated product consists of two syringes. One barrel contains a

chemically activated chemical and the other contains 38% HP (pH ～ 7.0). The mixture is performed just prior to application (Figure 3.3) and then an approximately 1.0-mm thick layer of the resultant gel is applied onto the enamel surface.

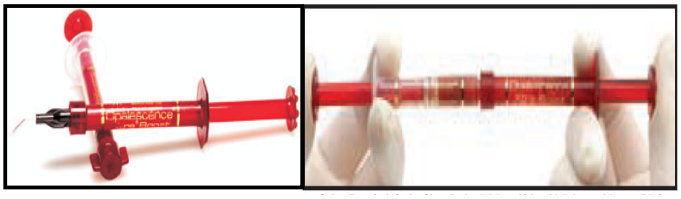


Figure 3.3 Opalescence® Xtra® Boost™ and activation procedure.

2. Opalescence® PF 20% (Ultradent Products Inc, South Jordan, UT, USA)

Opalescence® PF 20% is a syringe delivered, at-home bleaching material (pH ～ 6.5) (Figure 3.4). 20% CP is equal to 7.5% HP. The PF stands for fluoride (0.11%) and potassium nitrate (3.0%). Both ingredients are added to reduce tooth hypersensitivity by occluding dentine tubules [Absi et al., 1995] or by blocking nerve conduction [Peacock and Orchardson, 1999]. During the bleaching treatment, the tooth to be treated will be covered with a 1.0-mm thick layer of bleaching gel according to the manufacturer's recommendations.

Figure 3.4 Opalescence® PF 20%.

3. Sodium perborate (Borax, Apotheke UKE)

Sodium perborate (SP) is used alone or in combination with distilled water or HP to whiten non-vital tooth. It was found that there is no difference in whitening of teeth after exposure to sodium perborate mixed with 30% HP, 3% HP and H_2O [Ari and Üngör, 2002]. While compared to the solutions containing HP, perborate-water solution might cause less toxic to cells [Kinomoto et al., 2001]. Therefore, in the current study, SP powder was mixed with distilled water to form a thick paste and was then applied onto the enamel surface. The pH value of the water-based SP paste is reported to be approximately 9.9 [Rotstein and Friedman, 1991].

4. Rapid White (Rapid White Products, USA)

Rapid White is a sodium chlorite-based, "over-the-counter" bleaching material (Figure 3.5). The bleaching procedure with Rapid White is two-step: (i) a treatment for 5 seconds with a $NaClO_2$-containing liquid material, the so-called "accelerator", and (ii) a treatment for 10 minutes with a 1.0-mm thick layer of "whitening gel" which is composed of citric acid, aqua, glycerine carbomer 974P, aroma, sodium hydroxide and methylparaben. The pH value of "whitening gel" is 3.7. The recommended treatment time to achieve tooth whitening is 10 days, twice daily for 10 minutes, while the recommended maximal treatment time per day is 4 times daily for 10 minutes.

Figure 3.5 Rapid White.

Aqueous solutions

1. Hydrogen peroxide (HP) solutions (Borax, Apotheke UKE)

Four hydrogen peroxide (HP) solutions at different concentrations and pH values were investigated: acidic 30% HP (pH = 2.93), neutral 10, 20 and 30% HP (pH = 7.0). Sodium peroxide was used to adjust the pH value of the solutions.

2. Citric acid (CA) solutions (Sigma-Aldrich Chemie GmbH, No 27488)

Aqueous solutions of citric acid were prepared by dissolving an appropriate amount of powdered citric acid in distilled water. The pH value of the solutions was measured as follows (Table 3.1):

Table 3.1 Characteristics of the aqueous citric acid (CA) solutions

Aqueous solution	0.01mol/l CA	0.03mol/l CA	0.05mol/l CA	0.1mol/l CA	0.5mol/l CA
pH	2.82	2.54	2.43	2.23	1.89

3. Sodium fluoride (NaF) solutions (Merck, Darmstadt)

Aqueous solutions of NaF were prepared by dissolving an appropriate amount of powdered NaF in distilled water. The pH value of the solutions was measured as follows (Table 3.2):

Table 3.2 Characteristics of the aqueous NaF solutions

Aqueous solution	0.5% NaF	1.0% NaF	2.0% NaF
pH	9.53	10.05	10.30

4. Admixture solutions containing citric acid and sodium fluoride

The admixture solutions contain 0.1 mol/l citric acid and NaF of different concentration. The pH value of the solutions was measured as follows (Table 3.3):

Table 3.3 Characteristics of the admixture solutions containing citric acid (CA) and NaF

Aqueous solution	1		2		3	
	0.1mol/l CA	0.5% NaF	0.1mol/l CA	1.0% NaF	0.1mol/l CA	2.0% NaF
pH	4.15		4.88		5.24	

3.3 Treatment procedures

Bleaching treatment of dental enamel

(1) Sample treatment for SEM, EPMA, XRD and vibrational spectroscopic measurements

In this *in vitro* study, two series of samples were used corresponding to peroxide-based and non-peroxide-based bleaching products. Bleaching procedure is listed in Table 3.4 (peroxide-based materials) and in Table 3.5 (non-peroxide-based materials), respectively. The bleaching treatment with commercial agents was performed at 37 ºC and 100% humidity. At the end of each bleaching treatment, the bleaching substances were carefully removed from the sample surface with a soft toothbrush under running tap water and then the samples were stored in distilled water.

Table 3.4 Bleaching procedure of the peroxide-based bleaching materials used in the study according to the manufacturers' recommendations as well as for increased number of treatment

Sample	Bleaching product	Treatment
S1_0	None	Stored in distilled water
S1_1	Opalescence Xtra Boost	Bleaching gel was applied 3 times for 15 minutes.
S1_2	Opalescence Xtra Boost	Bleaching gel was applied 12 times for 15 minutes, totally 3 hours.
S1_3	Opalescence PF 20%	Bleaching gel was applied 4 hours per day, 10 days.
S1_4	Sodium perborate	The fresh prepared water-based paste was applied 7 hours per day, 10 days.
S1_5	30% HP aqueous solution (pH = 2.93)	Samples were immersed into 10 ml solution, which was changed every 24 hours.
S1_6	Neutral 10% HP aqueous solution (pH = 7.0)	The same as S1_5
S1_7	Neutral 20% HP aqueous solution (pH = 7.0)	The same as S1_5
S1_8	Neutral 30% HP aqueous solution (pH = 7.0)	The same as S1_5

Table 3.5 Bleaching procedure of the non-peroxide-based bleaching materials used in the study according to the manufacturers' recommendations

Sample	Bleaching product	Treatment
S2_0	None	Stored in distilled water
S2_1	Rapid White "accelerator"	The "accelerator" was applied directly onto the sample surface twice daily for 5 seconds, 10 days
S2_2	Rapid White "whitening gel"	1.0-mm layer of "whitening gel" was applied twice daily for 10 minutes, 10 days.
S2_3	Rapid White	Step 1: moistening the sample surface with the liquid "accelerator"; step 2: covering the moistened sample surface with a 1.0-mm layer of the "whitening gel". The procedure was conducted twice daily for 10 days.
S2_4	Rapid White	Step 1: moistening the sample surface with the liquid "accelerator"; step 2: covering the moistened sample surface with a 1.0-mm layer of the "whitening gel". The procedure was conducted 4 times per day, 10 days.
S2_5	0.5 mol/l citric acid aqueous solution	Samples were immersed into 10 ml solution, which was applied for 10 minutes in a single-step process.
S2_6	0.1 mol/l citric acid aqueous solution	The same as S2_5
S2_7	0.05 mol/l citric acid aqueous solution	The same as S2_5
S2_8	0.03 mol/l citric acid aqueous solution	The same as S2_5
S2_9	0.01 mol/l citric acid aqueous solution	The same as S2_5

(2) Sample treatment for the calcium leaching experiment

The calcium leaching experiments were performed at room temperature. Solutions were prepared in a clean bench of class 100. First, each tooth was moistened with 20 µL distilled water and carefully rinsed to ensure that all calcium leached from the enamel surface was collected. The solutions prepared in such a way were used as a reference for the corresponding bleaching procedure. Then, the same tooth was subjected to a bleaching procedure. This study compared the degree of bleaching-induced Ca leaching for teeth treated with Rapid White and with Opalescence Xtra Boost. Both whitening products were applied following the protocol specified by the manufacturers: (i) for Rapid White the tooth was first moistened with 20 µL of "accelerator" and then coated with 40-50 mg of "whitening gel"; (ii) for Opalescence Xtra Boost the two components of the bleaching agent were mixed and 30-50 mg of the resultant mixture was applied on the tooth surface. Additionally, we prepared solutions from teeth treated with "accelerator" alone for 5 seconds and with "whitening gel" alone for 10 minutes to investigate the effect of each component. After 10 minutes of treatment with the corresponding reagent the tooth surface was washed with distilled water to remove the agent and finally rinsed to collect Ca leached from enamel. The collected aqueous solutions from both unbleached and bleached enamel surfaces were subsequently filled up with distilled water to 2000 mg to be further analyzed by FAAS and TXRF. To each solution 20 µl of a 0.01 g/l standard solution were added as an internal standard for the TXRF experiments.

Sodium fluoride treatment of dental enamel

Enamel samples treated according to the following procedures were investigated: (i) treated first with a 0.1 mol/l citric acid solution and then with aqueous NaF solutions of different concentrations; (ii) treated first with aqueous NaF solutions of different concentrations and then with 0.1 mol/l citric acid solution; and (iii) treated with aqueous solutions containing both citric acid and NaF. The exact concentrations and the pH values of the corresponding solutions have been shown in Tables 3.1-3.3.

To check if fluoride can recover the modified structure of enamel caused by citric acid, samples of Series 1 were subjected to a multi-step treatment, where a single-step process consisted of immersion of the sample into 10 ml citric acid solution for 10 minutes and subsequent rinsing with distilled water, and then immersion of the sample into 10 ml NaF solution for 10 minutes

and subsequent rinsing with distilled water. The concentration of citric acid was chosen to be 0.1 mol/l because a pilot study showed that this concentration was sufficient to alter the structure of enamel apatite after a 10 minute-treatment. ATR IR spectra were measured after the treatment with citric acid and after the treatment with NaF (Figure 3.6), respectively. After 14 cycles of citric acid-NaF treatment, samples were immersed into NaF solutions for 48 hours to ensure the detection of any structural recovery processes if such occur.

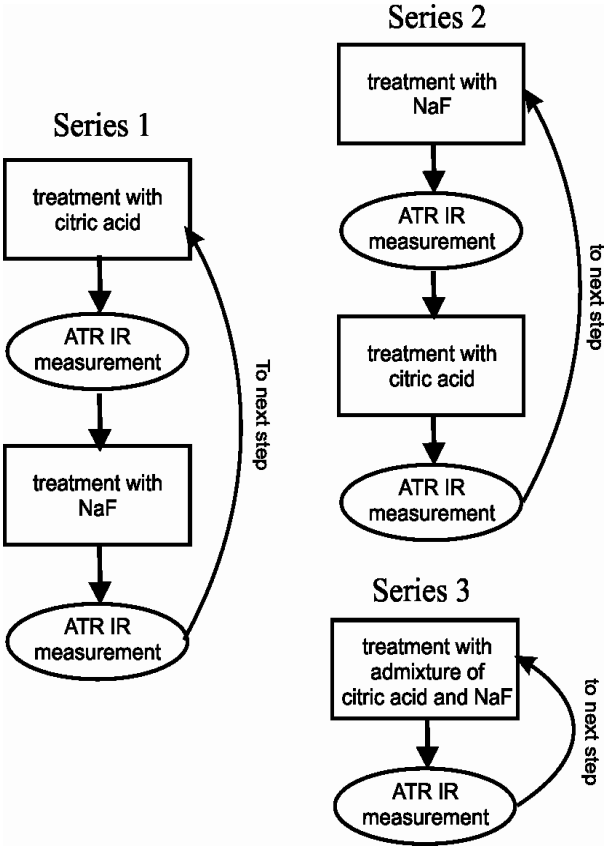


Figure 3.6 Sketch of a single step treatment for Series 1, 2 and 3, respectively.

To explore the ability of fluoride to protect the enamel from erosion induced by citric acid, samples of Series 2 were prepared similarly to the samples of Series 1 but the order of treatments was reversed (NaF prior to citric acid). ATR IR spectra were measured after the treatment with NaF and after the treatment with citric acid (Figure 3.6), respectively. Additionally, to ensure the detection of any protective effects due to fluoride, the following experiment was performed: enamel samples were immersed first in NaF solutions for 60 hours and then in 0.1 mol/l citric acid solution.

Finally, to investigate the effect of reagents containing fluoride and citric acid, samples of Series 3 were exposed to a multi-step treatment for 10 minutes in 10 ml admixture solutions containing both citric acid and NaF and then rinsed with distilled water (Figure 3.6). The pH values of the used admixture solutions correspond to mild acidic conditions (pH ~ 4.2-5.2), which according to previous studies [Petzold, 2001; Larsen, 2001] are favorable for the formation of CaF_2 globules on the enamel surface. ATR IR spectra were measured after 1, 2, 4, 6, 8, 10 and 14 cycles of treatment. In addition, to check the ability of the deposited CaF_2 globules to protect the structure of enamel apatite from further acidic impact, we exposed the sample treated for 14×10

min with (0.1 mol/l citric acid + 0.5% NaF) solution to 0.1 mol/l citric acid solution for 10 minutes.

All the immersion processes were performed at room temperature in static liquids. For all samples the time between the two steps of acidic treatment was approximately 12 hours. The samples were stored in distilled water between the treatments.

Bleaching and sodium fluoride treatment of geological hydroxyapatite

For comparison a series of geological hydroxyapatite (HA) single crystal samples were subjected to 1 of 3 treatment agents: two-step Rapid White, 0.1 mol/l citric acid solution and the admixture solution of (0.1 mol/l citric acid + 2.0% NaF). The treatment procedure was identical to the one previously described for human teeth.

3.4 Methods of analysis

Scanning electron microscopy (SEM)

The scanning electron microscope (SEM) is a type of electron microscope capable of producing high-resolution images of a sample surface. Due to the manner in which the image is created, SEM images have a characteristic three-dimensional appearance and are useful for judging the surface topology of the sample. During SEM inspection, a beam of electrons is focused on a spot volume of the specimen, resulting in the transfer of energy to the spot. These bombarding electrons, also referred to as primary electrons, dislodge

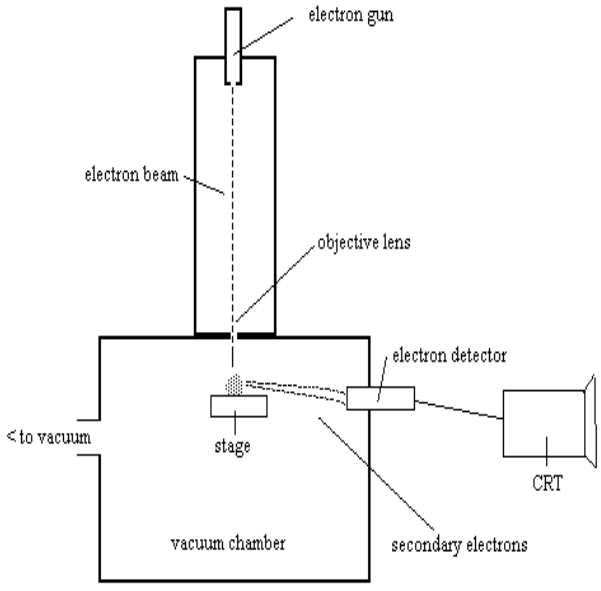


Figure 3.7 Schematic drawing of a generic SEM.

electrons from the specimen itself. As a result, secondary electrons and other electrons which are produced from the specimen are attracted and collected by a positively biased grid or detector and then translated into a signal. To produce the SEM image, the electron beam is swept across the area being inspected, producing many such signals. These signals are then amplified, analyzed and translated into images of the topography being inspected. Finally, the image is shown on a cathode ray tube (CRT) (Figure 3.7).

The specimens examined by SEM must be able to withstand the strong electric currents produced by the electron beam. Samples that do not conduct electricity can be damaged by the charges that can build up. Non-conductive specimens must first be coated with a thin layer of conductive material. This coating is accomplished using a sputterer. A sputter coater produces a nanometer thickness of conductive material on the surface through a cold plasma process that retains the contours of the specimen.

In this study, the morphology of the tooth surface was studied by SEM imaging performed with a JEOL 5600LV scanning electron microscope operating at 12 kV and using different magnifications up to 20 000× (Figure 3.8). Prior to the SEM measurements, the surfaces of the examined samples were sputtered with gold.

Figure 3.8 JEOL 5600LV scanning electron microscope.

Electron probe microanalysis (EPMA)

EPMA is an analytical technique that is used to establish the composition of small areas on specimens. EPMA is one of several particle-beam techniques. Particular, although not unique, to

EPMA is bombardment of the specimen with a beam of accelerated electrons. The electron beam is focused on the surface of a specimen using a series of electromagnetic lenses, and these energetic electrons produce characteristic x-rays within a small volume (typically between one and nine cubic microns) of the specimen. The characteristic x-rays are detected at particular wavelengths, and their intensities are measured to determine concentrations. All elements (except hydrogen, helium, and lithium) can be detected because each element has a specific set of x-rays that it emits. The analysis of the characteristic x-radiation can yield both qualitative identification and quantitative compositional information of micro-volumes (roughly one to several hundred cubic micrometers). The minimum detection limits are below 0.1% in the best cases and typically less than 1.0%. Therefore, this analytical technique has a high spatial resolution and sensitivity, and individual analyses are reasonably short, requiring only a minute or two in most cases. Because it is relatively non-destructive of the analyzed surface, and requirements for sample preparation are minimal, EPMA has been a valuable tool for analyzing both inorganic and organic materials.

The chemical composition of enamel in the present study was determined by EPMA using a Cameca Microbeam SX100 electron microscope equipped with a wavelength- and energy-dispersive system (Figure 3.9). Prior to the experiment, the samples were dehydrated under vacuum for 48 hours to remove any unbound water and subsequently coated with a thin carbon layer in a vacuum evaporator to minimize surface charging during the analysis. The experiments were carried out with an acceleration voltage of 15 kV, a beam current of 20 nA, an electron beam diameter on the sample surface of 10 μm and averaging over 50 spatial points. Thus the maximum penetration depth in chemical composition analyses was approximately 10 μm. In addition, back-scattered electron (BSE) imaging as well as mapping of P- and Ca-content was performed to probe the lateral chemical inhomogeneity with a step size of 0.1 μm. The

Figure 3.9 Cameca Microbeam SX100 electron microscope.

chemical-element mapping was conducted with an electron-beam spot diameter on the sample surface of 1.0 μm, which consequently reduced the probe-radiation penetration depth.

Vibrational spectroscopy (ATR IR and Raman)

Vibrational spectroscopy involves the use of electromagnetic radiation to probe the vibrational behaviour of molecular systems via an absorption or a light scattering experiment. The vibrational energy range of molecules and crystals is approximately between 0-5000 cm^{-1}, which corresponds to the infrared region of the electromagnetic spectrum. As shown in Figure 3.10, infrared spectroscopy (IR) studies the direct absorption of light by molecular vibrations. Raman spectroscopy or Raman scattering studies the energy changes of the incident laser light beam due to the inelastic interaction between the incident light beam and the vibrational excitation. Some vibrational bands observable by the IR absorption technique also appear in the Raman spectrum. Other bands, however, are unique to either one of the two techniques because of the difference in the selection rules governing the two different processes (light absorption and Raman emission). When both techniques are combined, more bands can be identified. Therefore, the IR and Raman techniques are regarded to be complementary and are applied qualitatively and quantitatively to analyze the structural or molecular group or phase in a sample in chemistry, physics, mineralogy material science and many other scientific branches as well as in industry.

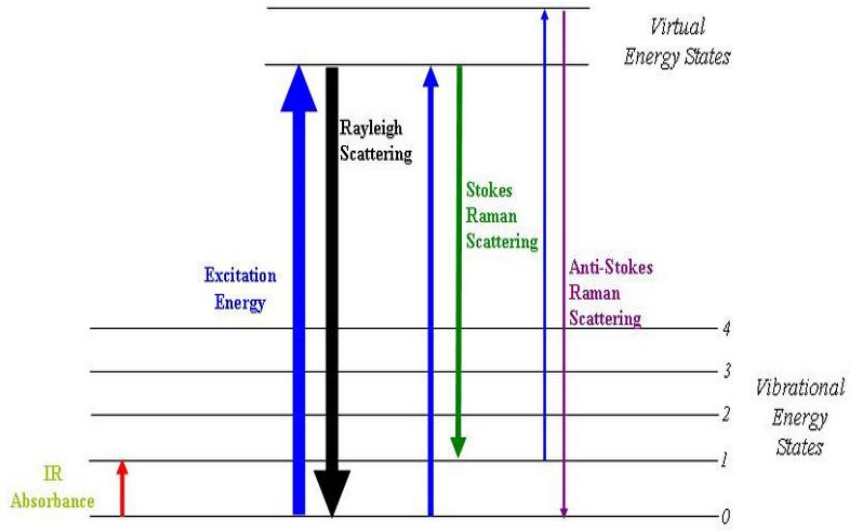


Figure 3.10 Differences in mechanism of infrared and Raman spectroscopy.

Attenuated total reflectance infrared (ATR IR)

Traditional IR spectrometers have been used to analyze solids, liquids and gases by means of transmitting the infrared radiation directly through the sample. For the classical transmission technique a solid sample was powderized and kept in a transparent matrix material. Where the sample is in a liquid or solid form the intensity of the spectral features is determined by the thickness of the sample and typically this sample thickness cannot be more than a few tens of microns. The technique of attenuated total reflectance (ATR) has in recent years revolutionized solid and liquid sample analyses because it combats the most challenging aspects of infrared analyses, namely sample preparation and spectral reproducibility.

Attenuated total reflectance (ATR) is a spectroscopic method frequently used in conjunction with infrared spectrometers and to analyze the surface of materials. The method involves placing a solid or liquid sample surface in close physical contact with a crystal of a high refractive index such as ZnSe or Ge. When the IR radiation from the spectrometer enters the crystal, it reflects through the crystal and penetrates "into" the sample a finite amount with each reflection along the top surface via the so-called "evanescent" wave (Figure 3.11). This evanescent wave protrudes only a few microns (0.5-5 μ) beyond the crystal surface and into the sample. Consequently, there must be good contact between the sample and the crystal surface. In regions of the infrared spectrum where the sample absorbs energy, the evanescent wave will be attenuated or altered. The attenuated energy from each evanescent wave is passed back to the IR beam, which then exits the opposite end of the crystal and is passed to the detector in the IR spectrometer.

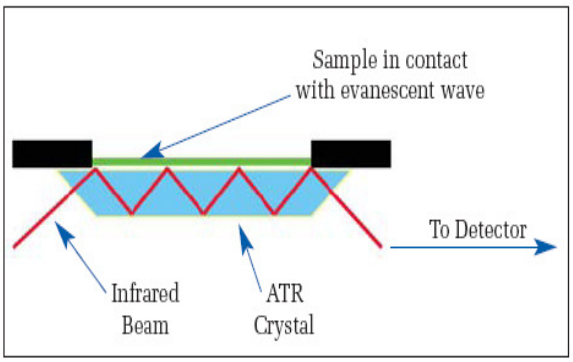


Figure 3.11 A multiple reflection ATR system.

For this technique to be successful, the following two requirements must be met:

1) the sample must be in direct contact with the ATR crystal, because the evanescent wave only extends beyond the crystal 0.5-5 μ.

2) to obtain internal reflectance, the angle of incidence must exceed the so-called "critical" angle. This angle is a function of the real parts of the refractive indices of both, the sample and the ATR crystal:

$$\theta_c = \sin^{-1}(n_2/n_1)$$

where n_2 is the refractive index of the sample and n_1 is the refractive index of the ATR crystal. The evanescent wave decays into the sample exponentially with distance from the surface of the crystal over a length in on the order of microns. The depth of penetration of the evanescent wave d is defined as the distance from the crystal-sample interface where the intensity of the evanescent decays to $1/e$ (37%) of its original value. It can be given by:

$$d = \lambda / \{2\pi n_1 [\sin^2\theta - (n_2/n_1)^2]^{1/2}\}$$

where λ is the wavelength of the IR radiation. For instance, if a ZnSe crystal (n_1 = 2.4) is used, the penetration depth for a sample with the refractive index of 1.5 at 1000 cm^{-1} is estimated to be 2.0 µm when the angle of incidence is 45°. If a Ge crystal (n_1 = 4.0) is used under the same condition, the penetration depth is about 0.664 µm. The depth of penetration and the total number of reflections along the crystal can be controlled either by varying the angle of incidence or by the selection of ATR crystals because different crystals have a different refractive index.

The ATR IR spectroscopic measurements were performed using a Bruker Equinox 55 FTIR spectrometer equipped with a Pike MIRacle ATR accessory and a contact sample area of 1.8 mm in diameter (Figure 3.12). The spectra were recorded with an instrumental resolution of 4 cm^{-1}, averaging over 512 scans. The error in determining the peak positions was $\pm$ 2 cm^{-1}. A Ge ATR crystal was used and, consequently, the characteristic penetration depth of the ATR IR experiments was approximately 700 nm. Compared to a ZnSe ATR crystal, Ge crystal has a higher refractive index and, hence, a shorter penetration depth, revealing that the use of Ge as a refractive element ensures the collection of ATR spectra with negligible contribution of specular reflection. Thus, the ATR IR spectra presented here possess no heavily modified peaks due to the dispersion of the refractive index across an absorption band and/or due to a strong dependence of the effective thickness. The spectra were measured in the spectral range 500-4000 cm^{-1} and subsequently normalized to a constant penetration depth.

Figure 3.12 Bruker Equinox 55 FTIR spectrometer equipped with a Pike MIRacle ATR accessory.

The structural state of the organic-mineral system of dental enamel was probed by Raman spectroscopy. Raman scattering was excited with near-infrared rather than with a visible laser source in order to assure luminescence-free spectra. The Raman measurements were accomplished using a Bruker IFS 66 FTIR spectrometer equipped with a Bruker FT-Raman module FRA 106 (Figure 3.13), a liquid N_2-cooled Ge detector, and a Nd:YAG laser emitting at 1064 nm. The spectra were collected with an output laser power of 280 mW and a spectral resolution of 2 cm^{-1}, averaging over 500 scans. The lower limit of laser penetration depth for the Raman experiments was approximately 500 µm.

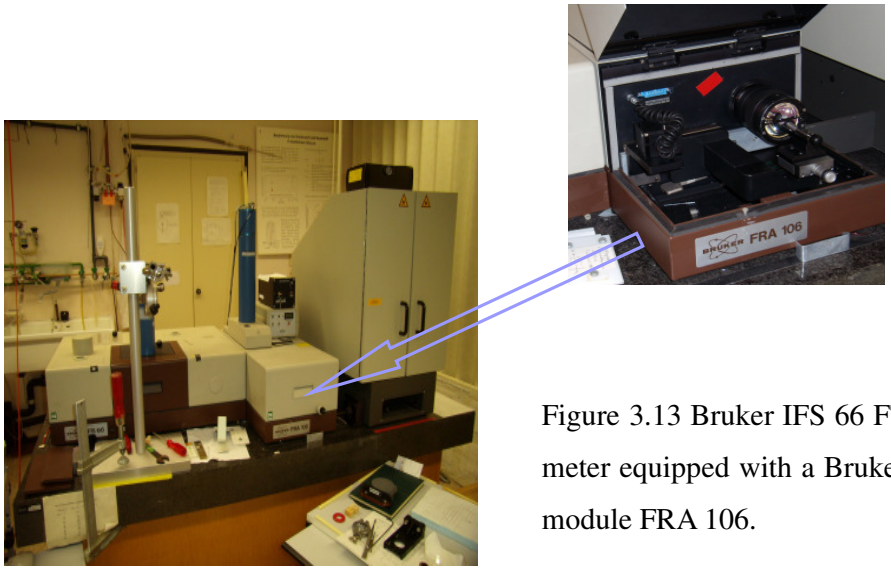


Figure 3.13 Bruker IFS 66 FTIR spectrometer equipped with a Bruker FT-Raman module FRA 106.

X-ray diffraction (XRD)

X-ray diffraction (XRD) is a versatile, non-destructive technique that reveals detailed information about the chemical composition and crystallographic structure of natural and manufactured materials. A crystal lattice is a regular three-dimensional symmetrical arrangement of atoms. These are arranged so that they form a series of parallel planes separated from one another by a distance *d*, which varies according to the local nature of the material. For any crystal, planes exist in a number of different orientations - each with its own specific *d*-spacing. When a monochromatic x-ray beam with wavelength λ is projected onto a crystalline material at an angle θ, Bragg-diffraction occurs only when the distance travelled by the rays reflected from successive planes differs by an integer number *n* of wavelengths (Figure 3.14). By varying the angle θ, the Bragg's Law conditions are satisfied by different *d*-spacings in polycrystalline materials. Plotting the angular positions and intensities of the resulting diffracted peaks of radiation produces a pattern, which is characteristic of the structure of the sample. Where a mixture of different phases is present, the resulting diffractogram is formed by superposition of the individual patterns. Based on the principle of x-ray diffraction, structural, physical and chemical information about the material investigated can be obtained. A host of application techniques for various material classes is available, each revealing its own specific details of the sample studied.

In the present study, samples were powdered by grinding with an agate mortar and then were analyzed in the 2θ range 5-80º using a Philips X'pert diffractometer with $CuK_{\alpha}1$ (λ = 1.5405 Å) radiation (Figure 3.15).

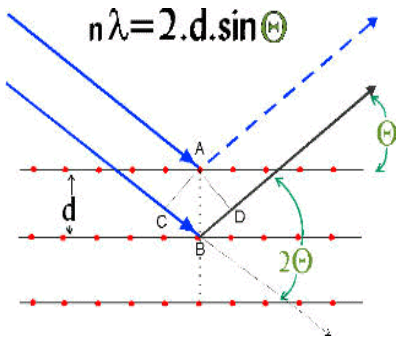


Figure 3.14 Bragg's Law.

Figure 3.15 Philips X'pert diffractometer.

Flame atomic absorption spectrophotometer (FAAS) and total reflection x-ray fluorescence (TXRF)

Atomic absorption spectrometry (AAS) is an analytical technique that measures the concentrations of elements. Atomic absorption is so sensitive that it can measure down to parts per billion of a gram (μg dm^{-3}) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. They correspond to the energies needed to promote electrons from one energy level to another higher energy level. Atoms of different elements absorb characteristic wavelengths of light. Analyzing a sample to see if it contains a particular element means analyzing light from that element. For example with lead, a lamp containing lead emits light from excited lead atoms that produce the right mix of wavelengths to be absorbed by any lead atoms of the sample. In AAS, the sample is atomized, i.e. converted into ground state free atoms in the vapor state, and a beam of electromagnetic radiation emitted from excited lead atoms is passed through the vaporized sample. Some of the radiation is absorbed by the lead atoms in the sample. The greater the number of atoms there is in the vapor, the more radiation is absorbed. Hence, the amount of light absorbed is proportional to the number of lead atoms. A calibration curve is constructed by running several samples of known lead concentration under the same conditions as the unknown. The amount of the standard absorption yields a calibration curve and this enables the calculation of the lead concentration in the unknown sample. Consequently, an atomic absorption spectrometer needs the following three components: a light source, a sample cell to produce gaseous atoms and a device to measure the specific light absorbed. In flame atomic absorption spectroscopy (FAAS) a liquid sample is aspirated and mixed as an aerosol with combustible gasses (acetylene and air or acetylene and nitrous oxide). The mixture is ignited in a flame with a temperature ranging from 2100 to 2800 ºC (depending on the fuel gas used). During combustion, atoms of the element of interest in the sample are reduced to the atomic state. A light beam from a lamp whose cathode is made of the element being determined is passed through the flame into a monochronometer and detector. Free, unexcited ground state atoms of the element absorb light at characteristic wavelengths. This reduction of the light energy at the analytical wavelength is a measure of the amount of the element in the sample.

Total reflection x-ray fluorescence spectrometry (TXRF) is a well-established technique for metallic-trace analysis on Si wafers. A primary beam of x-rays impinges on the wafer substrate at grazing incidence and under conditions of total reflection (i.e. incidence angle below a critical

angle at the order of a few millirad or tenths of a degree). This results in the excitation of the top few nanometers of the substrate from which the fluorescence is detected using a Si (Li) detector positioned normal to the substrate. The typical TXRF geometry (subcritical-angle grazing incidence and perpendicular detection) allows three-orders-of-magnitude-improved detection limits compared to conventional XRF equipment (Figure 3.16). As such, TXRF offers a multielement-analysis capability with detection limits in the order of 10^{10}-10^{11} at/cm^2 for most elements. The combination with the preconcentration method vapor phase decomposition-droplet collection (VPD-DC) allows detection limits of 10^{8}-10^{9} at/cm^2. In the VPD-DC method, the SiO_2 from the surface of the wafer is etched in the VPD step ending on a hydrogenated Si surface. This surface has hydrophobic characteristics, which enables the collection of the metallic contaminants by scanning the surface with a microdroplet of an aqueous mixture. Thus, enrichment (proportional to the scanned wafer area) in the metallic-trace concentration is resulting in improved detection limits as well as in a facilitated one-measurement analysis procedure. Besides these analytical features, TXRF has a number of practical advantages: the design of the equipment is relatively simple; the operation can be fully automated and is robust; the calibration is easily applied using external wafer standards; the spectral simplicity allows facile quality control of the analytical results and easy data management.

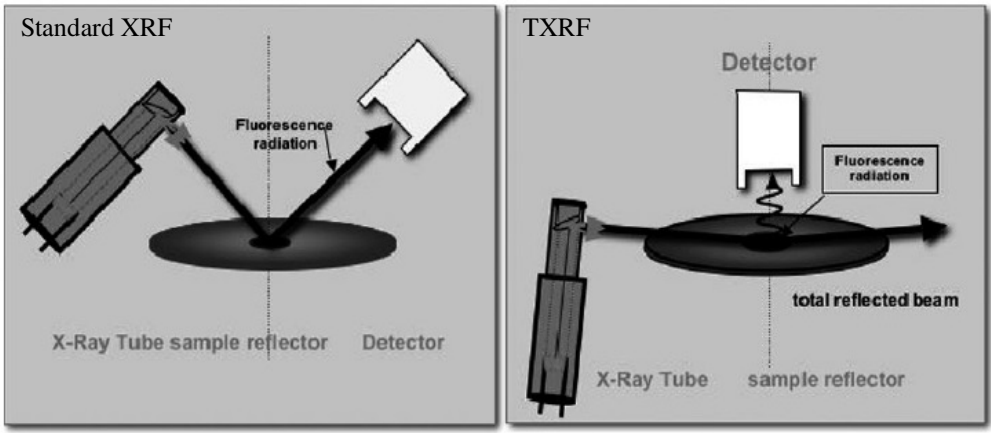


Figure 3.16 Comparison between conventional (left) and total reflection mode of excitation (right).

In this study, the concentration of the leached calcium was determined using a FAAS Spectrophotometer S series from a Thermo (Figure 3.17) and a TXRF Atomica 8030C instrument. In the TXRF experiments the Ca K_α line was used for quantification. In the atomic

absorption experiments, absorption on the Ca 422.7 nm line was analyzed. For both methods the achieved detection limit of Ca in aqueous solutions was found to be 0.2 lg/m.

Figure 3.17 Flame atomic absorption spectrometer (FAAS).

4 Results

4.1 Effects of bleaching treatment on dental enamel

SEM analysis

SEM micrographs of the samples surface treated with two different bleaching formulae (peroxide-based and non-peroxide-based) are shown in Figure 4.1. It can be seen that the sodium chlorite-based bleaching agent (Rapid White) caused a much stronger modification of the enamel surface than the peroxide-based products. The unbleached enamel (S1_0) surface shows a uniform flat layer due to polishing procedure. Some small and irregular deposits might result from insufficient cleaning procedure. No or only subtle morphological changes are observed for samples S1_2, S1_3 and S1_4 treated with Opalescence Xtra Boost (3h), Opalescence PF 20% and sodium perborate, respectively, whereas the formation of cracks and noticeable roughening is clearly seen in the surface of sample S2_4 bleached with sodium chlorite-based Rapid White.

XRD and IR analyses on powders

The powder XRD patterns of the reference group (S2_0) and the two-step Rapid White group (S2_4) are compared in Figure 4.2. The powders were collected from the outer surface of the samples. There are no substantial differences in the positions, intensities and widths of the Bragg reflections between two samples. Further, the sample powders measured by XRD were analyzed by IR transmittance spectroscopy using the KBr-pellet technique. As can be seen in Figure 4.3, the IR absorption spectrum of the bleached sample resembles that of the unbleached one, which is in accordance with the XRD data. However, the expected structural changes of the superficial enamel are within a micron- or sub-micron-thick layer. The use of powders does not allow probing the structure within such a layer. Therefore, surface-sensitive spectroscopic methods as ATR IR were further utilized to analyze if treatments by various bleaching agents impact the atomic structure and bonding of the outermost enamel.

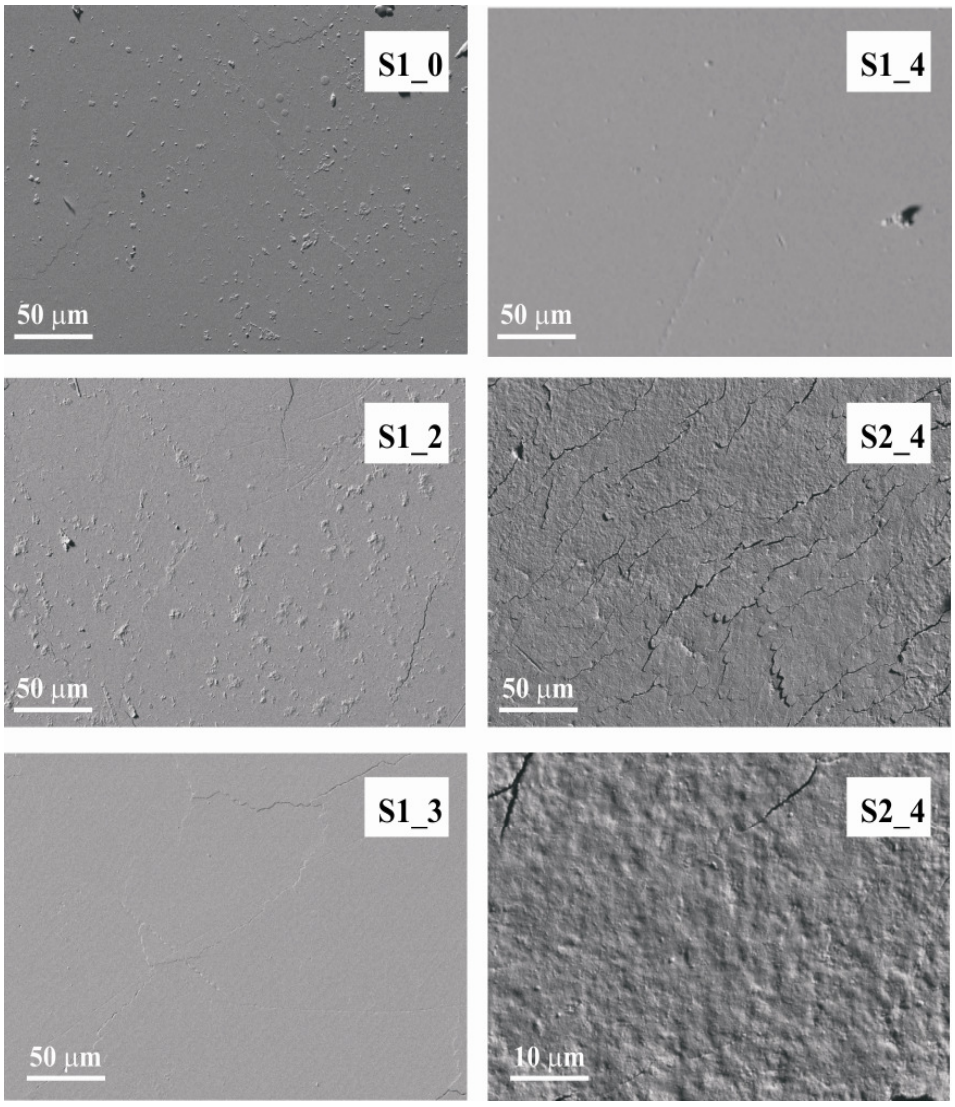


Figure 4.1 SEM micrographs of samples S1_2, S1_3, S1_4 and S2_4 treated with Opalescence Xtra Boost (3h), Opalescence PF 20%, sodium perborate and sodium chlorite-based Rapid White, respectively. For comparison the SEM micrograph of unbleached enamel (S1_0) is also included; the micrograph of S2_4 is given in two different magnifications (see scale bars).

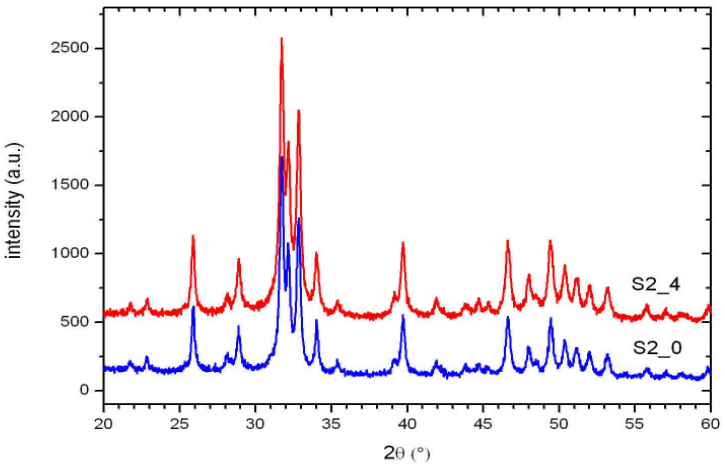


Figure 4.2 XRD patterns of the control group (S2_0) and two-step Rapid White (S2_4).

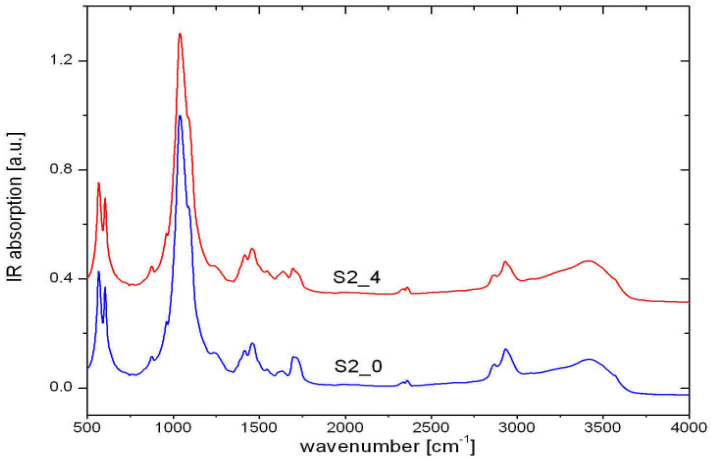


Figure 4.3 IR absorption spectra of the control group (S2_0) and two-step Rapid White (S2_4).

ATR IR analysis

Peroxide-based bleaching agents

Figure 4.4 displays the ATR IR spectra of series S1_0-S1_4 collected in the frequency range of 550-4000 cm^{-1}. The spectra are analyzed in four frequency ranges. These ranges are between 550-700 cm^{-1}, 830-1165 cm^{-1}, 1200-2000 cm^{-1} and 2000-4000 cm^{-1}. All the spectra are normalized to the intensity of the band centered around 1010 cm^{-1}, which is stemming from the ν_3 PO_4 excitation. As can be seen, the spectra of samples bleached with peroxide-based agents (S1_1, S1_2, S1_3 and S1_4) are identical to the spectrum of the control sample S1_0, whether the exposure time is recommended by the manufactures (S1_1, S1_3 and S1_4) or is prolonged (S1_2). The band between 550 and 700 cm^{-1} gives information about the ν_4 bending mode of PO_4

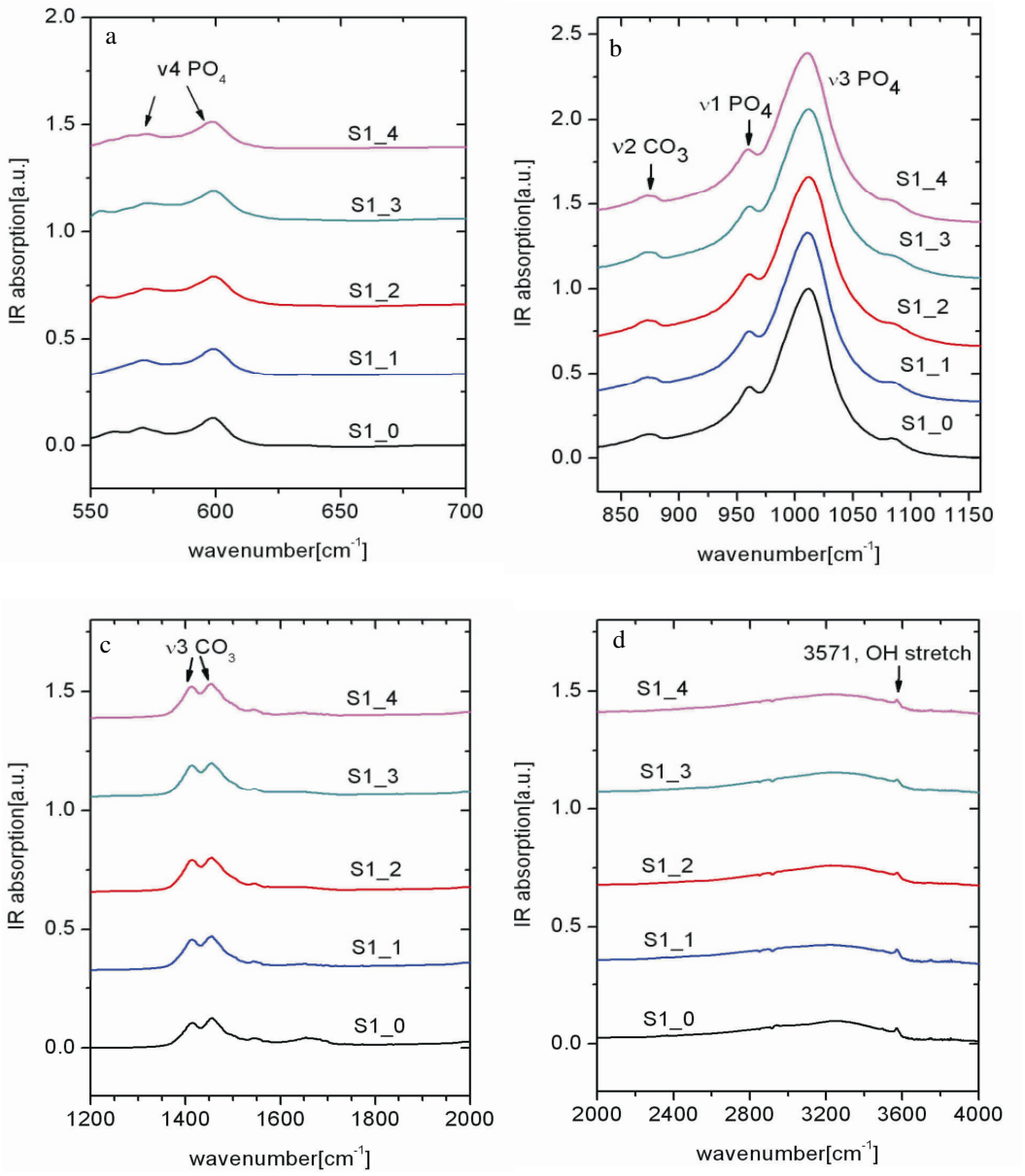


Figure 4.4 ATR IR spectra of samples treated with Opalescence Xtra Boost for recommended exposure time (S1_1) and prolonged time (S1_2), Opalescence PF 20% (S1_3) and sodium perborate (S1_4). For comparison the spectrum of unbleached enamel (S1_0) is also included. (a) spectra in the range of 550-700 cm^{-1}, (b) spectra in the range of 830-1165 cm^{-1}, (c) spectra in the range of 1200-2000 cm^{-1} and (d) the spectra range of 2000-4000 cm^{-1}.

tetrahedra. The peak at about 875 cm^{-1} results from the ν_2 bending mode of the CO_3^{2-} groups, existing in the apatite structure. The spectra in the 900-1200 cm^{-1} region are dominated by the ν_1, ν_3 stretching modes of PO_4 tetrahedra. The band positions of the ν_3 anti-symmetrical stretching

modes of the CO_3^{2-} occur near 1410-1550 cm^{-1}. The signal at 3571 cm^{-1} is due to the OH stretching mode.

HP is the active oxidizing agent in the peroxide-based bleaching materials. To investigate the effect of the oxidizing agent itself on enamel, the aqueous HP solutions were specifically studied. The specimens were entirely flooded by a 30% HP solution (pH = 2.93) (Borax, Apotheke UKE) at room temperature for 7 days totally. The ATR IR spectra were collected at 10 minutes, 3 hours, 1 day and 7 days after the beginning of the treatment. The solution was changed every 24 hours. Figure 4.5 shows the spectral range from 830 to 1165 cm^{-1} after HP solution-bleaching treatment, in which the vibrational modes of the PO_4 tetrahedral groups contribute. The shifts of the peak position occurred after a 3 hour-treatment and were independent of the exposure time once the maximum shift position ($\approx$ 6 cm^{-1}) was reached.

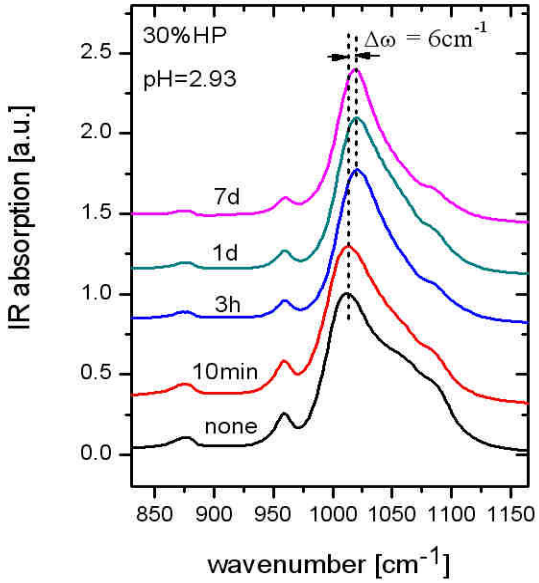


Figure 4.5 ATR IR spectra of samples before (none) and after 10 minutes, 3 hours, 1 day and 7 days treated with 30% HP solution (pH = 2.93).

The pH value of peroxide-based products ought to be above 5.5 [Price et al., 2000], which is the critical value of dental enamel demineralization [Driessens et al., 1986]. In order to avoid enamel demineralization due to an acidic environment, the pH values of aqueous HP solutions of different concentration (10, 20 and 30%) were adjusted to 7.0 using NaOH. Figure 4.6 shows the ATR IR spectra of samples S1_6, S1_7, S1_8 treated with 10, 20 and 30% neutral HP solutions, respectively. After neutral HP solutions treatment, no detectible changes in the ATR IR spectra of samples were observed.

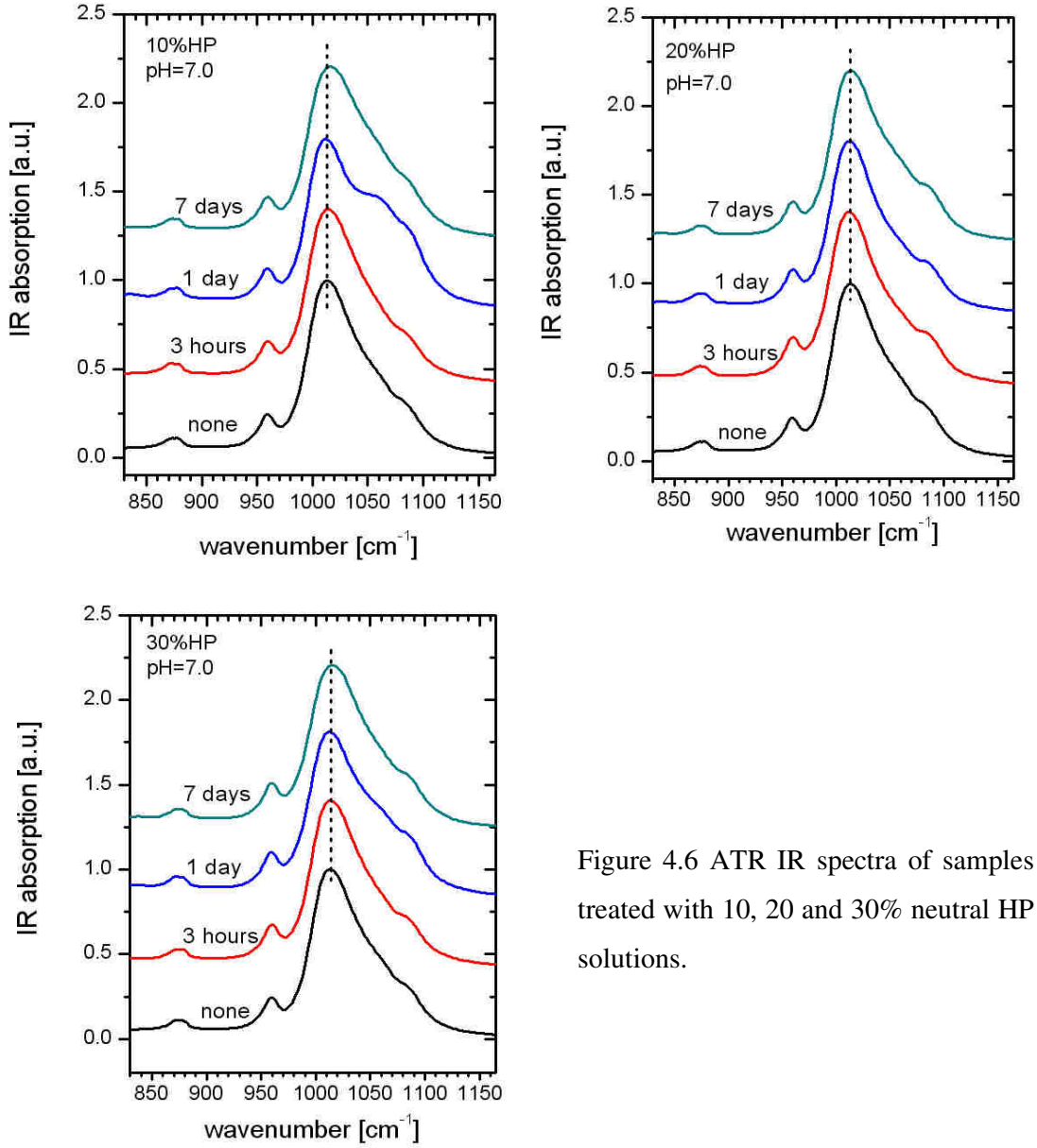


Figure 4.6 ATR IR spectra of samples treated with 10, 20 and 30% neutral HP solutions.

Non-peroxide-based bleaching agents

In this series, considering the two-step bleaching process of Rapid White, samples were treated with “accelerator” (S2_1), with “whitening gel” (S2_2) and with two-step Rapid White for 10 days, twice (S2_3) or 4 times (S2_4) daily for 10 minutes. The ATR IR spectra of those samples together with the spectra of the control sample (S2_0) and the “whitening gel” itself are plotted in Figure 4.7. The spectrum of the sample treated with “accelerator” (S2_1) resembled that of the reference sample (S2_0), while samples treated with “whitening gel” (S2_2) and two-step Rapid

White (S2_3 and S2_4) exhibited a shift ($\approx$ 12 cm^{-1}) of the main peak arising from the ν_3 anti-symmetrical mode of PO_4 tetrahedra to higher wavenumbers.

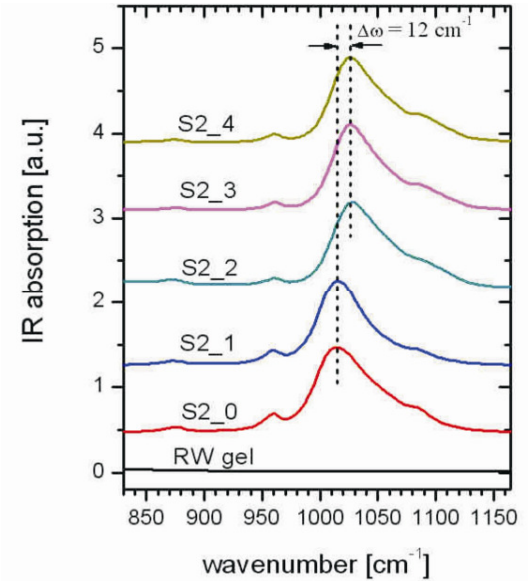
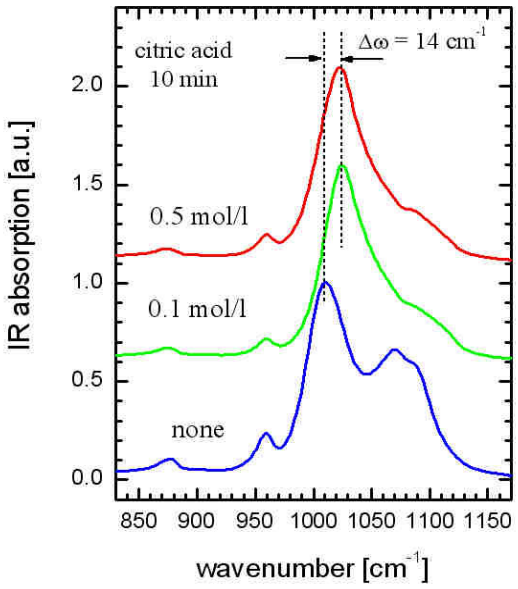


Figure 4.7 ATR IR spectra of Rapid White "whitening gel" itself, unbleached enamel (S2_0), enamel treated with "accelerator" (S2_1), with "whitening gel" (S2_2), with two-step Rapid White twice daily (S2_3) and 4 times daily (S2_4), respectively.

Citric acid in Rapid White "whitening gel" is suggested to cause enamel erosion [Attin et al., 2005; Zantner et al., 2006]. To analyze the effect of citric acid itself, in a preliminary experiment, samples S2_5 and S2_6 were immersed into the aqueous solutions of 0.5 and 0.1 mol/l citric acid for 10 minutes, respectively. Comparing the ATR IR spectra of before and after 10 minute-treatment, a peak shift and, therefore, alteration of atomic bonding in superficial enamel apatite was observed (Figure 4.8). The repetition of the citric acid 10 minute-treatment did not

Figure 4.8 ATR IR spectra of enamel treated with 0.1 and 0.5 mol/l citric acid solutions for 10 minutes.



lead to further spectral changes, which indicates that these concentrations of acidic molecules during a single treatment of 10 minutes is high enough to affect the apatite atomic bonding in the whole available surface.

The use of citric acid solutions at lower concentrations (< 0.1 mol/l) enabled us to follow the citric acid-induced changes in the enamel surface with repeated acid treatment. Figure 4.9 shows the ATR IR spectra measured after each treatment step with citric acid solution of 0.01, 0.03 and 0.05 mol/l. For the lowest concentration (0.01 mol/l) a single step treatment for 10 minutes did not induce a detectible peak shift, while for concentrations of 0.03 and 0.05 mol/l the shift was 4 and 8 cm^{-1}, respectively. When the cycle of treatments was increased to 4, the peak shift approached 14 cm^{-1}, which is the value observed for a single-step treatment with high-concentra-

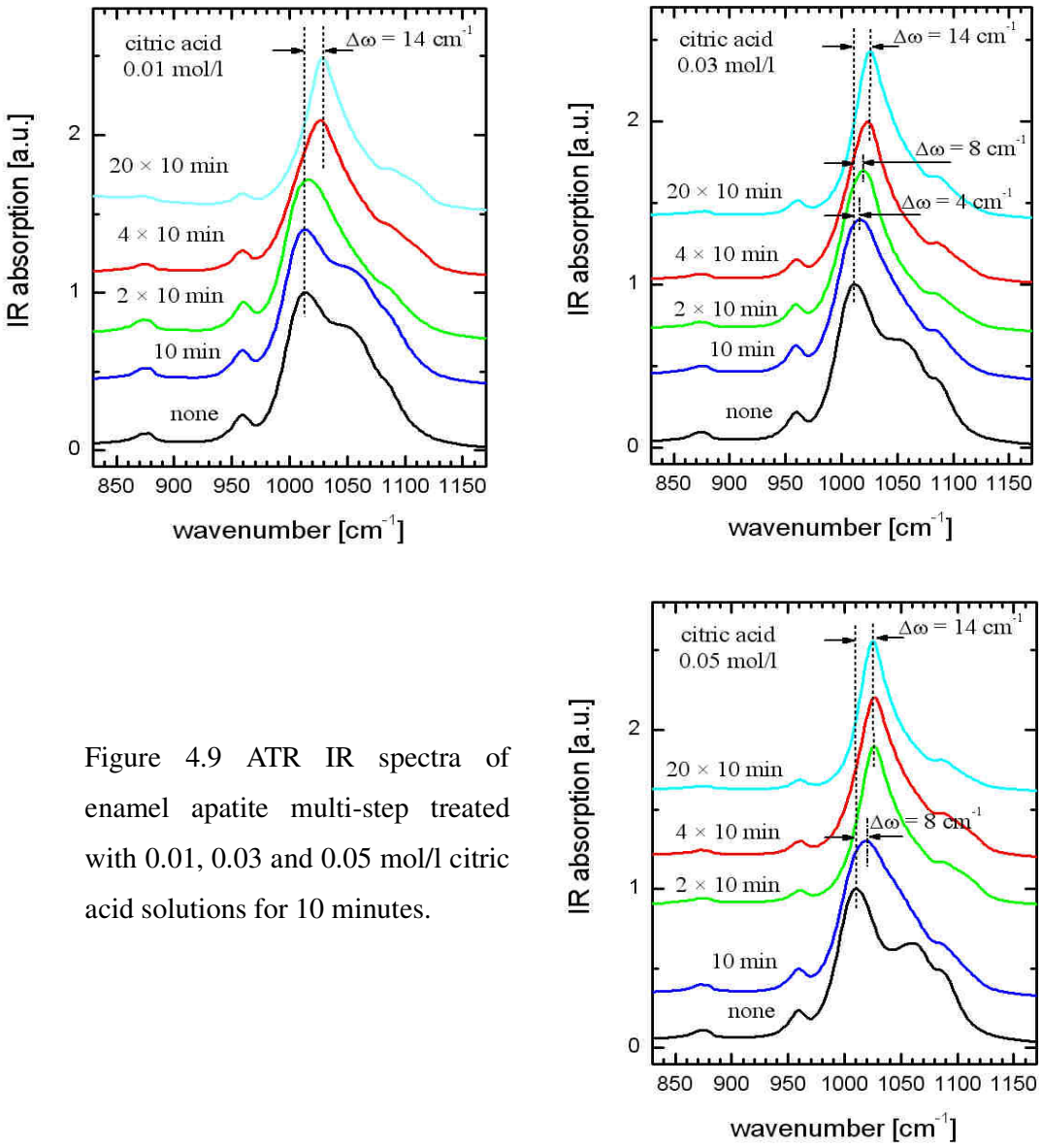


Figure 4.9 ATR IR spectra of enamel apatite multi-step treated with 0.01, 0.03 and 0.05 mol/l citric acid solutions for 10 minutes.

tion (0.1 and 0.5 mol/l) solutions of citric acid, and during further acid treatment the peak shift did not change within the experimental error (Figure 4.10). A closer examination of the ATR IR spectra of the samples exposed to low-concentration citric acid solutions (Figure 4.9) showed that the main peak broadened after a single treatment. After repeating the acid treatment several times, the peak reached its final position and became narrower again. The phenomenon is best seen for the sample treated with 0.05 mol/l citric acid solution.

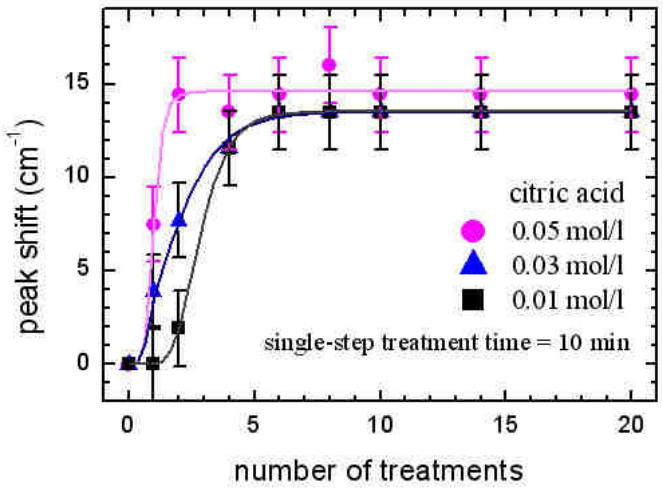


Figure 4.10 Peak shift versus number of 10 minute-treatment with 0.01, 0.03 and 0.05 mol/l citric acid.

Raman scattering spectroscopy analysis

Further, the unbleached sample (S2_0), samples treated with "accelerator" (S2_1), with two-step Rapid White twice daily (S2_3) and 4 times daily (S2_4) were examined by Raman scattering spectroscopy. The spectra are shown in Figure 4.11. No changes in the Raman bands near 430, 580, 960 and 1050 cm^{-1}, which arise from PO_4 vibration modes, were detected because the characteristic penetration depth of the Raman experiments in the current study is approximately 500 µm and thus the signal is dominated by the Raman scattering from the bulk rather than from the submicron surface layer. However, there was an apparent difference between the samples bleached with two-step Rapid White (S2_3 and S2_4) and the unbleached sample (S2_0) in the Raman scattering generated from organics. As can be seen in Figure 4.11, all the Raman peaks that are typical of dental organics (near 1270, 1450, 1672 and 2940 cm^{-1}) [Fattibene et al., 2005] disappeared with two-step Rapid White bleaching. Moreover, in the range 2800-3050 cm^{-1}, which is characteristic for C-H bond stretching modes, the shape of the Raman scattering from sample treated two-step Rapid White (S2_3 and S2_4) resembled that from the "whitening gel"

itself. These data indicate that the non-peroxide-based bleaching procedure has a strong impact on the organic component of enamel. Apparently, bleaching with Rapid White results in not only the decomposition of the dye molecules embedded in dental enamel, but also the destruction of the basic organics in teeth. Besides, some remnants of the bleaching gel are incorporated into the emptied inter- and intra-rod voids of dental enamel. Since the penetration depth of the Raman experiments about 500 µm, it can be stated that the bleaching treatment affects the organic component of enamel deeply inside the dental tissues. The Raman spectrum of the sample "accelerator" (S2_1) is the same as that of the unbleached (S2_0). This result is in agreement with the ATR IR data that "accelerator" alone does not damage the dental enamel tissues.

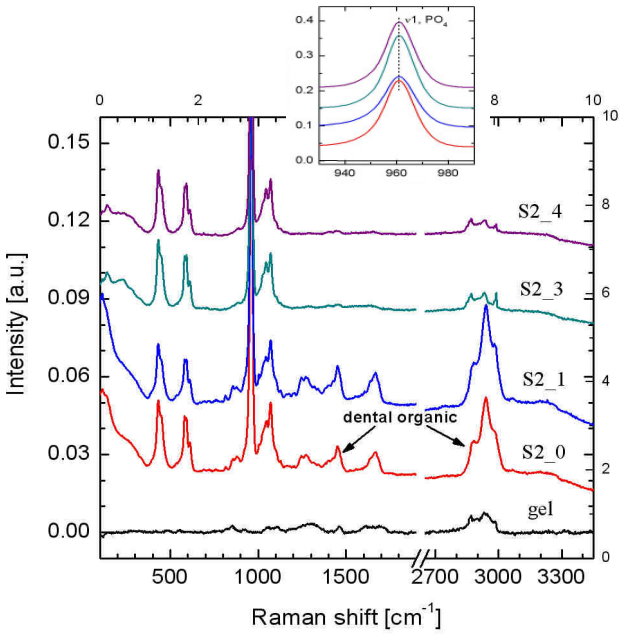


Figure 4.11 Raman spectra of samples treated with the non-peroxide-based bleaching agent (Rapid White) and the unbleached reference sample: S2_0, unbleached; S2_1, treated with "accelerator"; S2_3, with two-step Rapid White twice daily and S2_4, with two-step Rapid White 4 times daily. The insert shows details of the ν_1 PO_4 mode.

EPMA analysis

BSE imaging on the unbleached sample (S2_0), samples treated with Opalescence Xtra Boost (S1_2), with Rapid White "accelerator" (S2_1), with Rapid White "whitening gel" (S2_2), with two-step Rapid White (S2_3) and aqueous 0.1 mol/l citric acid solution (S2_6) for 7 days, 2x10 min per day was performed (Figure 4.12). The micrographs of S2_0, S1_2 and S2_1 are practically the same and reveal the keyhole-like morphology typical of an enamel cross section parallel to the enamel-dentine junction. However, BSE images of S2_2, S2_3 and S2_6 show an etched-like surface. There are numerous cracks, which appear in the micrograph as black irregular fringes, in the surface of S2_3. The cracks are related to the morphology of the enamel

apatite rods, which implies that the destruction of organics in the inter-rod voids, as detected by Raman spectroscopy, facilitates the decomposition of apatite in the vicinity of rod walls within several microns in depth. S2_2 and S2_6 samples were derived from different teeth which may be responsible for variation in erosion in the sample surfaces. The regular prismatic structure of enamel was destroyed, with partly eroded intrarod and even interrod. Shallow scratch lines shown in S2_0, S1_2 and S2_1 sample surfaces arise from the polishing procedure.

The chemical composition of S2_0, S2_1 and S2_3 determined by EPMA is given in Table 4.1. The total amount of elements for unbleached enamel is less than 100 wt% because of the existence of hydrous species in the structure, which are out of the range of sensitivity of the method. Again, the sample treated with “accelerator” (S2_1) shows the same data as the control sample (S2_0), whereas the sample bleached with two-step Rapid White (S2_3) exhibits a slight decrease in the totals. The change in the chemical composition is almost within the experimental error; however, a 10% reduction of the totals was measured for enamel bleached with Rapid White (S2_3), which confirms the tendency. These data indicate a decrease in the mineral component of enamel due to the formation of cracks after bleaching. Besides, bleaching treatment causes a decrease in the average concentration of P on account of a higher concentration of Si (Table 4.1). The preformed electron-probe mapping on P- and Ca-content showed that bleaching with Rapid White “whitening gel” (S2_2), two-step Rapid White (S2_3) and 0.1 mol/l citric acid solution (S2_6) induced a substantial lateral chemical inhomogeneity in the surface (Figure 4.13). The surface erosion of apatite appears to be stronger in the core of the enamel rods. Opalescence Xtra Boost (S1_2) did not influence the enamel surface and showed a similar chemical homogeneity as the unbleached sample (S2_0).

Table 4.1 Chemical compositions in wt% of unbleached enamel (S2_0) and enamel treated with Rapid White “accelerator” (S2_1) and with two-step Rapid White following the instructions of the manufacturer (S2_3).

	Ca	P	Na	K	Mg	Si	F	Cl	O	total
S2_0	36.3±0.3	17.5±0.1	0.63±0.07	0.019±0.002	0.22±0.02	0.10±0.04	3.38±0.04	0.32±0.07	36.1±0.2	94.5±0.5
S2_1	36.3±0.2	17.6±0.1	0.68±0.03	0.019±0.002	0.20±0.02	0.08±0.01	3.40±0.03	0.29±0.04	36.2±0.2	94.7±0.3
S2_3	36.2±0.4	16.8±0.3	0.48±0.05	0.011±0.002	0.18±0.01	0.62±0.05	3.17±0.06	0.34±0.03	35.8±0.3	93.7±0.7

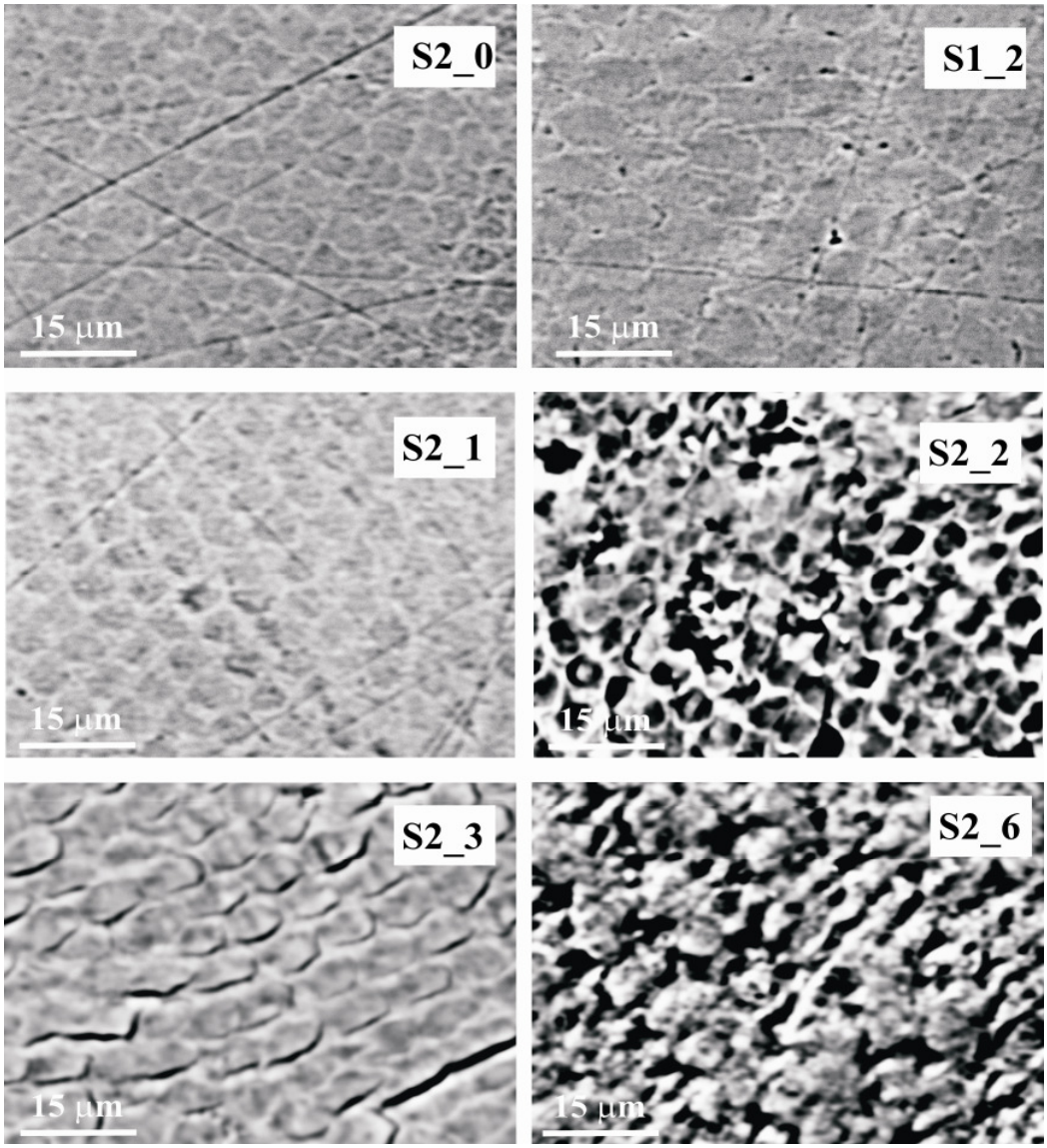


Figure 4.12 BSE images of enamel unbleached (S2_0), treated with Opalescence Xtra Boost (S1_2), with Rapid White "accelerator" (S2_1), with Rapid White "whitening gel" (S2_2), with two-step Rapid White (S2_3) and 0.1 mol/l citric acid solution (S2_6) for 7 days, 2x10 min per day.

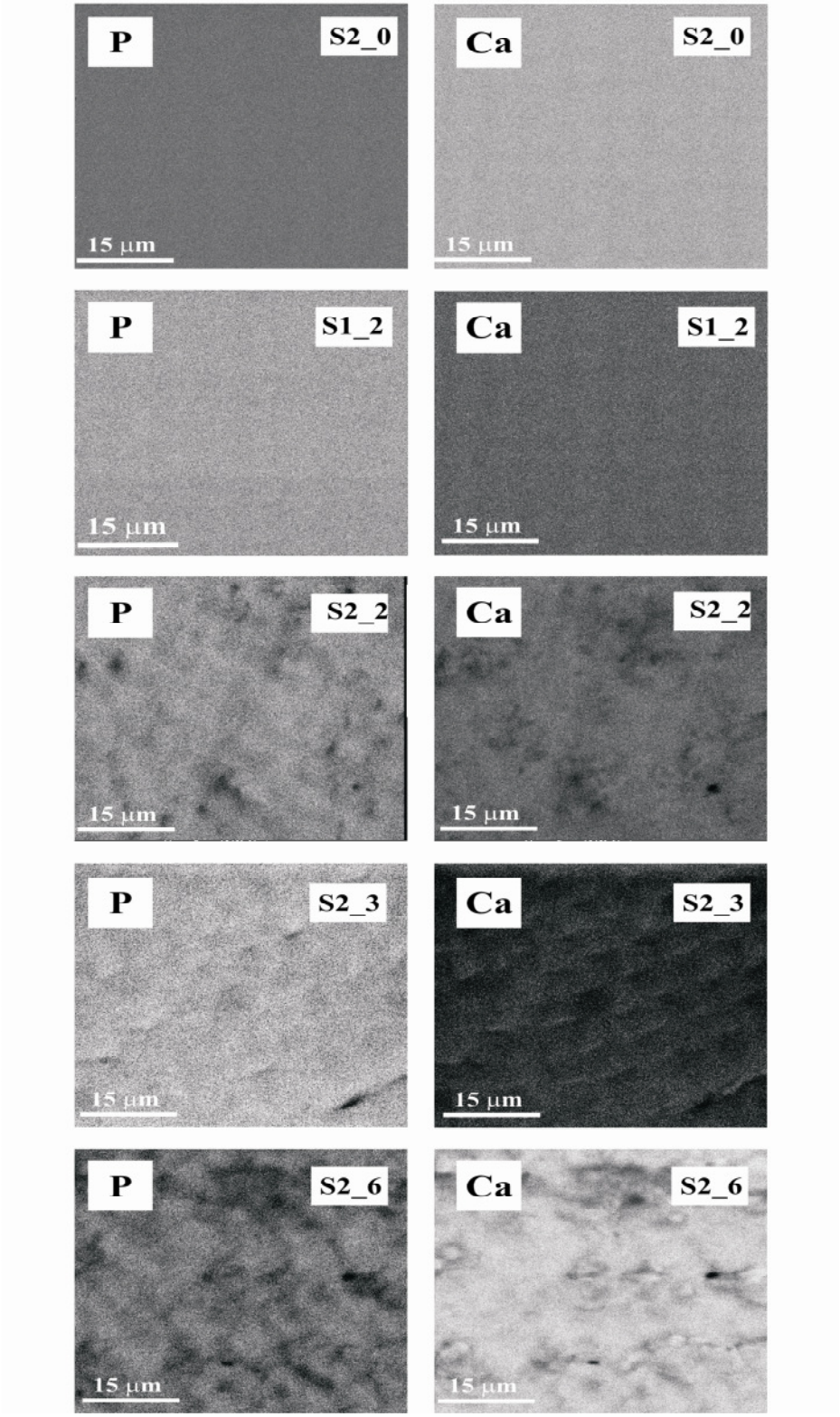


Figure 4.13 Electron-probe P- and Ca-mapping of enamel unbleached (S2_0), treated with Opalescence Xtra Boost (S1_2), with Rapid White "whitening gel" (S2_2), with two-step Rapid White (S2_3) and 0.1 mol/l citric acid solution (S2_6) for 7 days, 2x10 min per day.

FAAS and TXRF analyses

Ca leaching from enamel caused by dental bleaching agents was studied using FAAS and TXRF techniques. From Figure 4.14 it can be seen that, within the sensitivity of the methods, FAAS and TXRF analyses gave similar results for the content of Ca in the aqueous solutions collected from teeth treated with a single component or a combination of components of Rapid White. In the case of peroxide-based-bleached teeth the presence of K in the collected solutions substantially impacted the detection limit of TXRF and, hence, the Ca leaching for those samples was measured only by FAAS. The diagram in Figure 4.14 clearly shows that: (i) treatment with the sodium chlorite- containing Rapid White "accelerator" alone has no significant effect on the degree of Ca leaching; (ii) treatment with the citric acid-containing Rapid White "whitening gel" considerably increases the Ca leaching and the level of Ca loss approaches that after treatment with two-step Rapid White for single or multiple bleaching; and (iii) the peroxide-based bleaching agent does not significantly affect the degree of Ca leaching, compared to the unbleached sample.

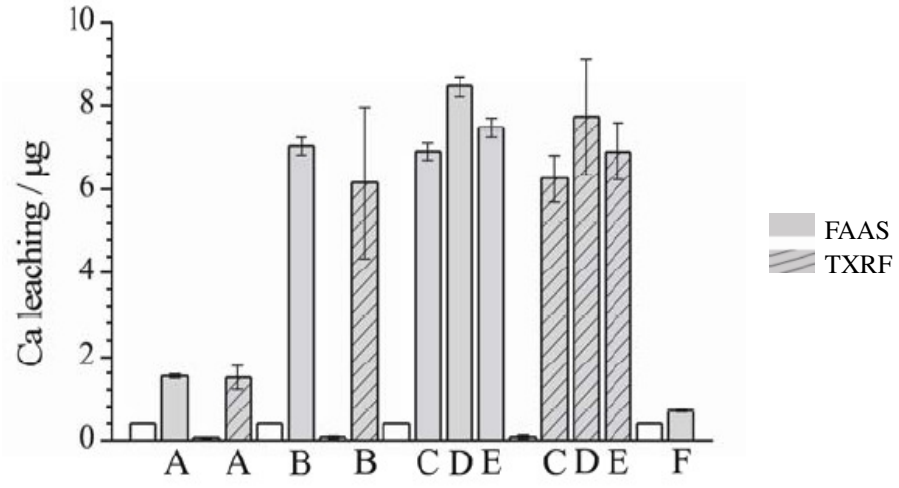


Figure 4.14 Calcium leaching determined by FAAS and TXRF from enamel before (white bars) and after (gray bars) treatment: **A**. a single treatment with the Rapid White "accelerator" for 5 seconds; **B**. a single treatment with the Rapid White "whitening gel" for 10 minutes; **C**. a single two-step treatment, consisting of an "accelerator" treatment for 5 seconds and a "whitening gel" treatment for 10 minutes; **D**. two cycles of the two-step treatment; **E.** three cycles of the two-step treatment; **F**. a single treatment with Opalescence Xtra Boost (38% HP) for 10 minutes.

4.2 Effects of sodium fluoride treatment on enamel

Enamel erosion-reversing effect

Figure 4.15 displays the ATR IR spectra of enamel samples of Series 1, in which at each step the treatment with 0.1mol/l citric acid was followed by a treatment with an aqueous NaF solution with concentrations of 0.5, 1.0 and 2.0%. The results clearly show that fluoride treatment has no effect on the altered structure of enamel. After a single treatment for 10 minutes with 0.1 mol/l citric acid solution the peak shifted to higher wavenumbers by approximately 10 cm^{-1} and remained at the same position after subsequent immersion in NaF solutions, regardless of the concentration. Besides, the fluoride treatment did not reverse or diminish the peak shift during further treatment with citric acid and after a multi-step treatment the peak shift approached a maximum value. Even prolonged, 48 hour-immersion of the acid-affected enamel samples in NaF solutions did not reverse any changes in the ATR IR spectra.

Enamel erosion-preventing effect

Experiments with enamel samples firstly treated with NaF solution and then with 0.1 mol/l citric acid solution (Series 2) confirm that a single 10 minute-treatment with fluoride solutions does not prevent the enamel apatite structure from citric acid-induced alteration. As can be seen in Figure 4.16, a previous immersion in NaF solution did not suppress the peak shift induced by citric acid treatment, i.e. did not protect enamel apatite against destruction by citric acid. The ATR IR spectra of enamel firstly immersed in NaF solutions for 60 hours and then exposed to 0.1 mol/l citric acid solution for 10 minutes are shown in Figure 4.17. It is apparent that a complete alteration of the apatite structure occurred after a single 10 minute-treatment of citric acid, exactly as in the case of a direct exposure to citric acid without any pretreatment (Figure 4.8). Therefore, the resistance of enamel apatite to acid erosion was not improved even after a prolonged (60 hours) pretreatment with NaF.

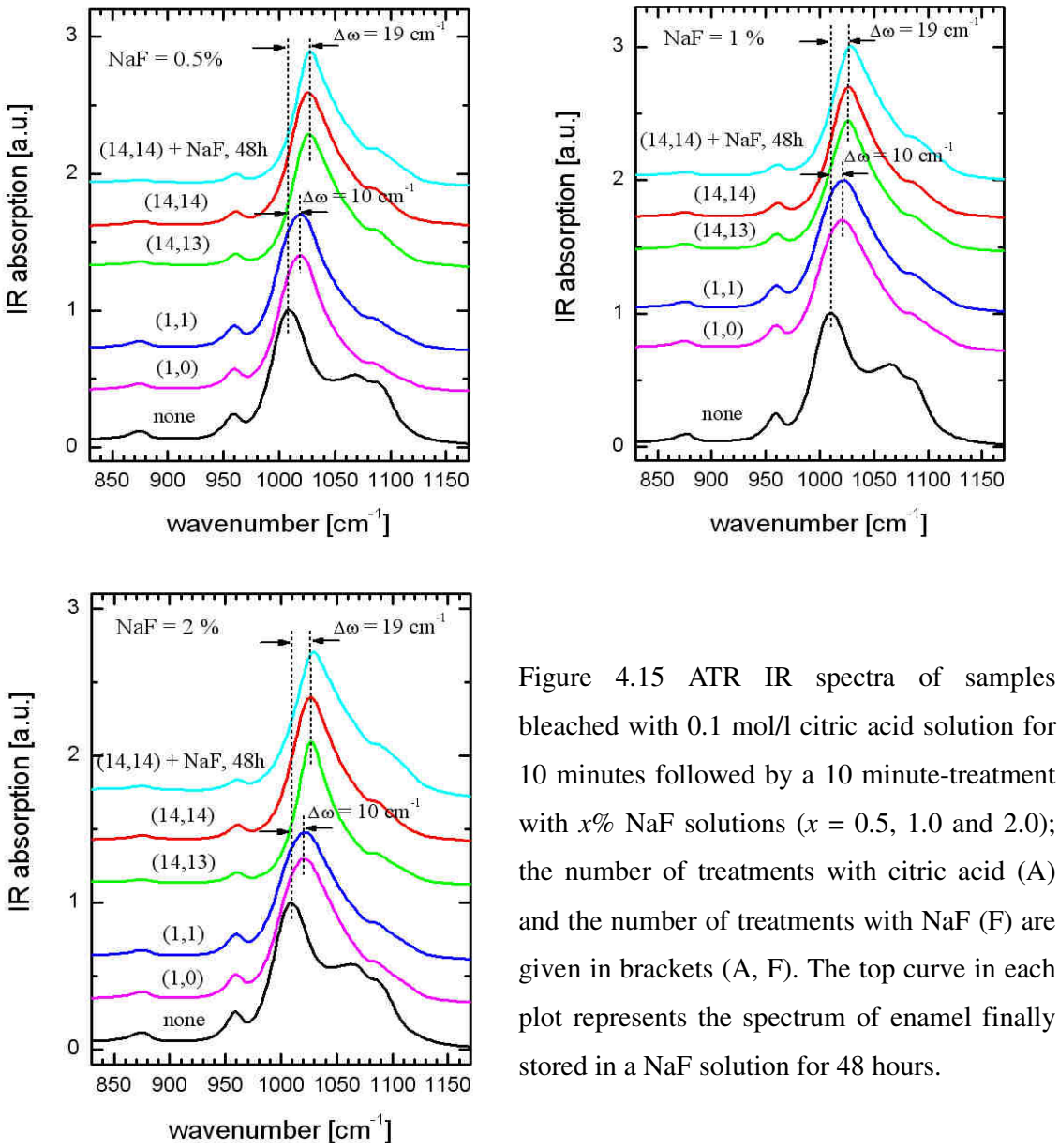


Figure 4.15 ATR IR spectra of samples bleached with 0.1 mol/l citric acid solution for 10 minutes followed by a 10 minute-treatment with *x*% NaF solutions (*x* = 0.5, 1.0 and 2.0); the number of treatments with citric acid (A) and the number of treatments with NaF (F) are given in brackets (A, F). The top curve in each plot represents the spectrum of enamel finally stored in a NaF solution for 48 hours.

The combined application of sodium fluoride and citric acid

Figure 4.18 shows the ATR IR spectra of enamel samples of Series 3 treated with admixture solutions containing citric acid and NaF. In contrast to the results obtained for Series 1 and Series 2, the citric acid-induced peak shift is considerably smaller than that caused by citric acid solution alone (Figure 4.8). For the three admixture solutions of (0.1 mol/l citric acid + *x*% NaF, *x* = 0.5, 1.0 and 2.0) no shift of the major peak was detected during the first 2 cycles of 10 minute-treatment. After 14 cycles of acid treatments for 10 minutes, the peak shift approached 4

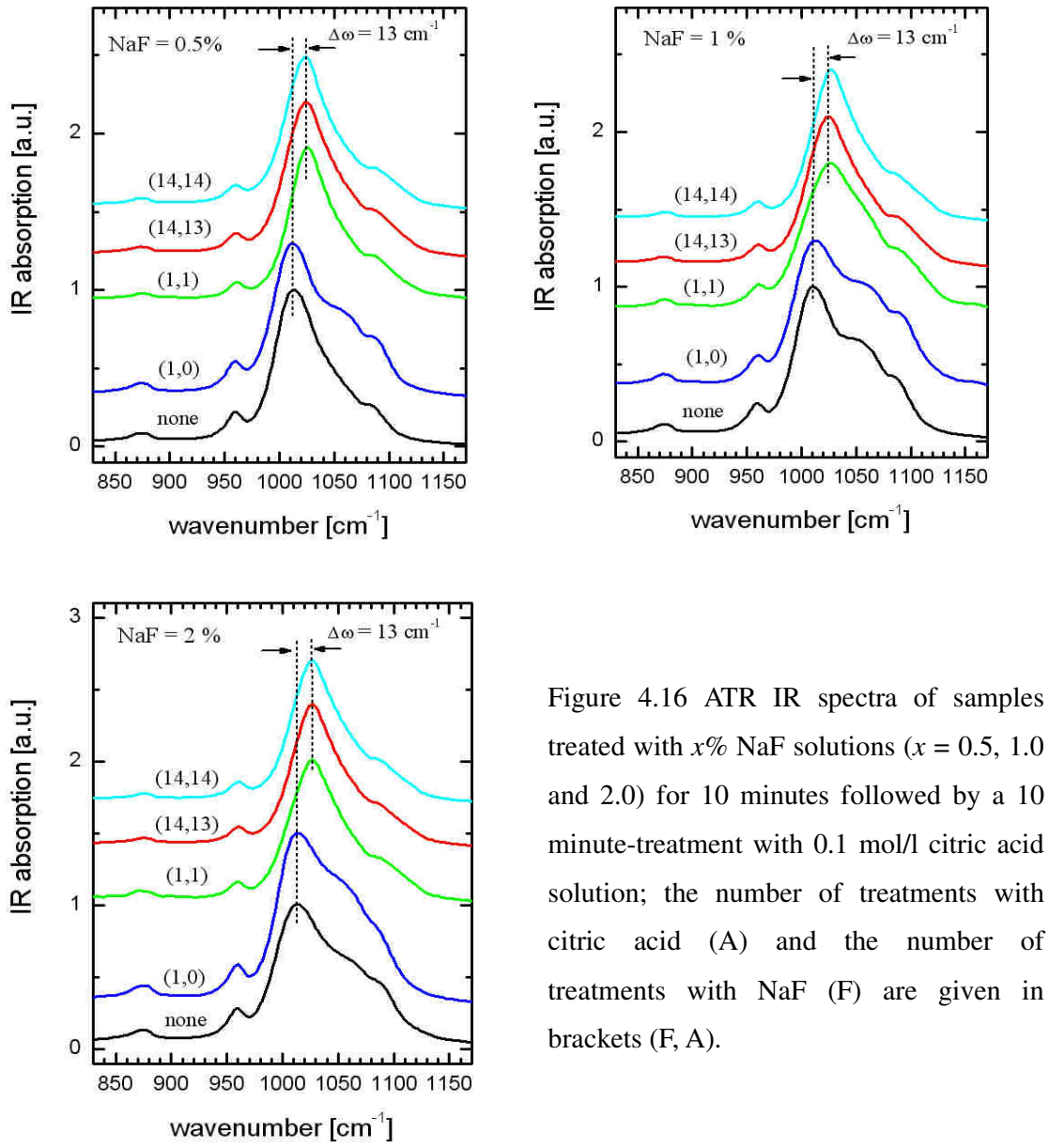


Figure 4.16 ATR IR spectra of samples treated with *x*% NaF solutions (*x* = 0.5, 1.0 and 2.0) for 10 minutes followed by a 10 minute-treatment with 0.1 mol/l citric acid solution; the number of treatments with citric acid (A) and the number of treatments with NaF (F) are given in brackets (F, A).

and 8 cm^{-1} for the samples treated with (0.1 mol/l citric acid + 0.5% NaF) and (0.1 mol/l citric acid + 2.0% NaF) solutions, respectively. Interestingly, for the sample treated with (0.1 mol/l citric acid +1.0% NaF) solution the peak shift was negligible even after 14 treatment period. Note that the three samples treated with admixture solutions were cut from the same tooth.

Due to the specific pH values of the treatment solutions, the formation of a surface CaF_2 layer was expected for the three samples of Series 3. To compare the degree of CaF_2 deposition we conducted SEM imaging from the sample showing no peak shift and that having a peak shift of 8

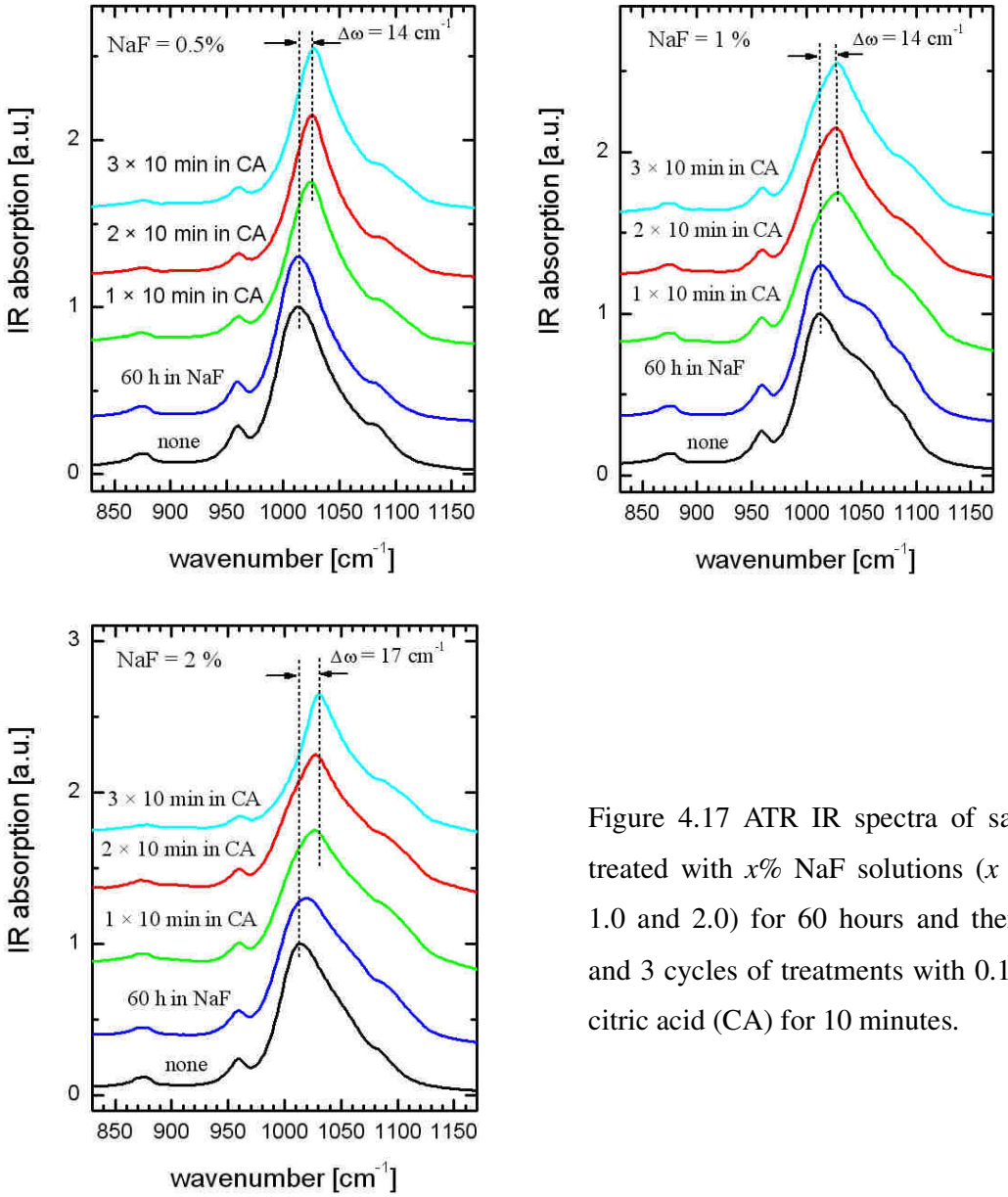


Figure 4.17 ATR IR spectra of samples treated with *x*% NaF solutions (*x* = 0.5, 1.0 and 2.0) for 60 hours and then 1, 2 and 3 cycles of treatments with 0.1 mol/l citric acid (CA) for 10 minutes.

cm^{-1}, while the sample exhibiting a medium value of the peak shift (4 cm^{-1}) was kept for additional ATR IR measurements.

Figure 4.19 shows the SEM micrographs of untreated enamel and enamel treated with admixture solutions of (0.1 mol/l citric acid + *x*% NaF, *x* = 1.0, 2.0) for 14 cycles of 10 minutes. Compared to the untreated sample (Figure 4.19 a and b), a dense layer of CaF_2 globule-shaped aggregates with a mean diameter of 250 nm is observed for the sample immersed in (0.1 mol/l citric acid + 1.0% NaF) solution (Figure 4.19 c and d). This is in a good accordance with the ATR IR spectra, revealing no peak shift for this sample. The sample treated with a (0.1 mol/l citric acid + 2.0%

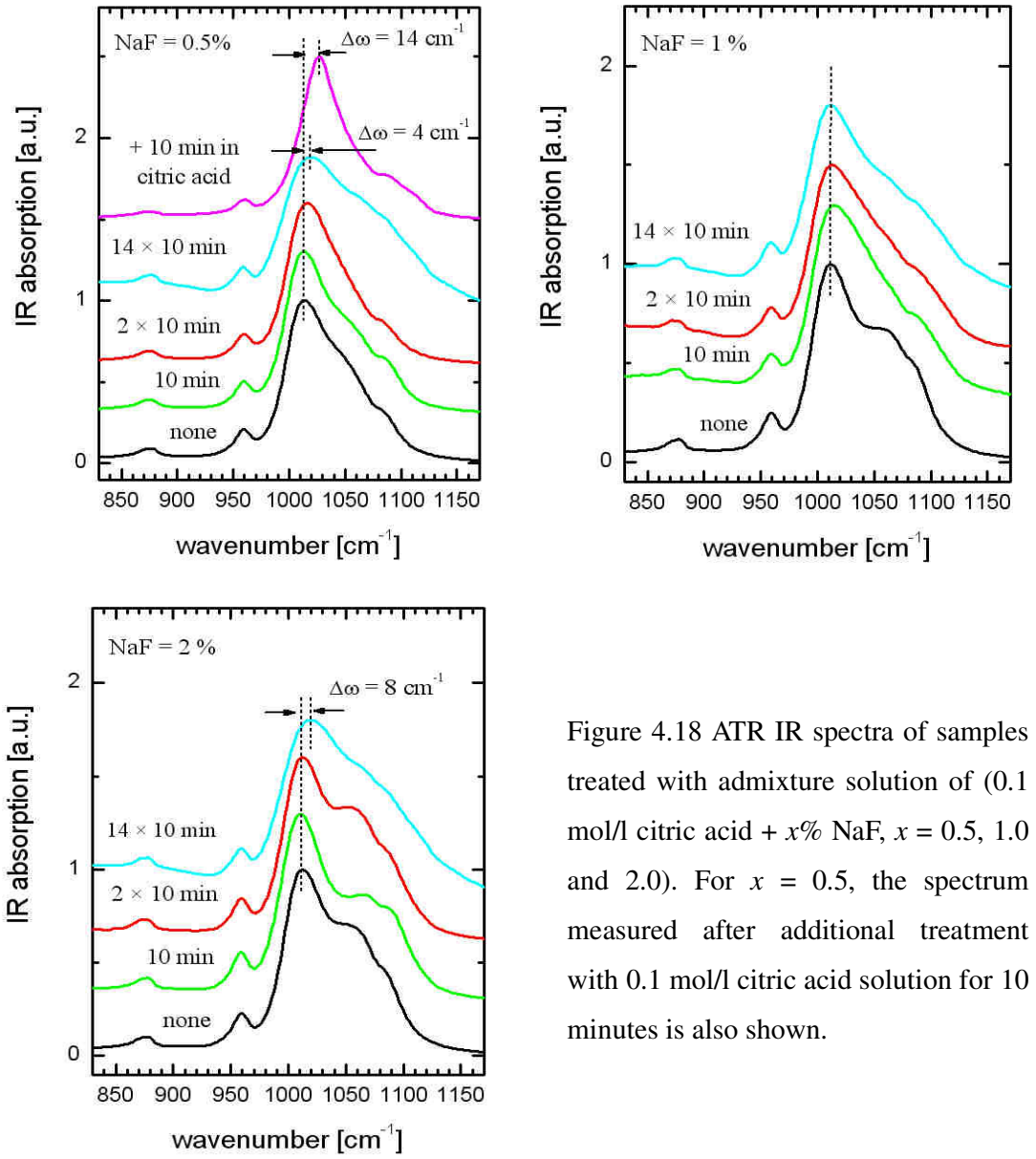


Figure 4.18 ATR IR spectra of samples treated with admixture solution of (0.1 mol/l citric acid + *x*% NaF, *x* = 0.5, 1.0 and 2.0). For *x* = 0.5, the spectrum measured after additional treatment with 0.1 mol/l citric acid solution for 10 minutes is also shown.

NaF) solution exhibits only partial covering with CaF_2 globules, which are substantially smaller in size (Figure 4.19 e and f). Besides, cracks and enamel areas of altered morphology can be seen in the SEM image of this sample. Apparently, the peak shift observed in the ATR IR spectrum results from the contribution of the uncovered apatite areas, which are affected by citric acid.

Figure 4.18 also displays the ATR IR spectrum of the sample firstly treated with the admixture solution of (0.1 mol/l citric acid + 0.5% NaF) for 14×10 min and then exposed to 0.1 mol/l citric acid solution for 10 minutes (the top line in the left-hand-side plot). The final immersion of the sample in citric acid solution induced a peak shift of 14 cm^{-1}, which is similar to the peak shift of

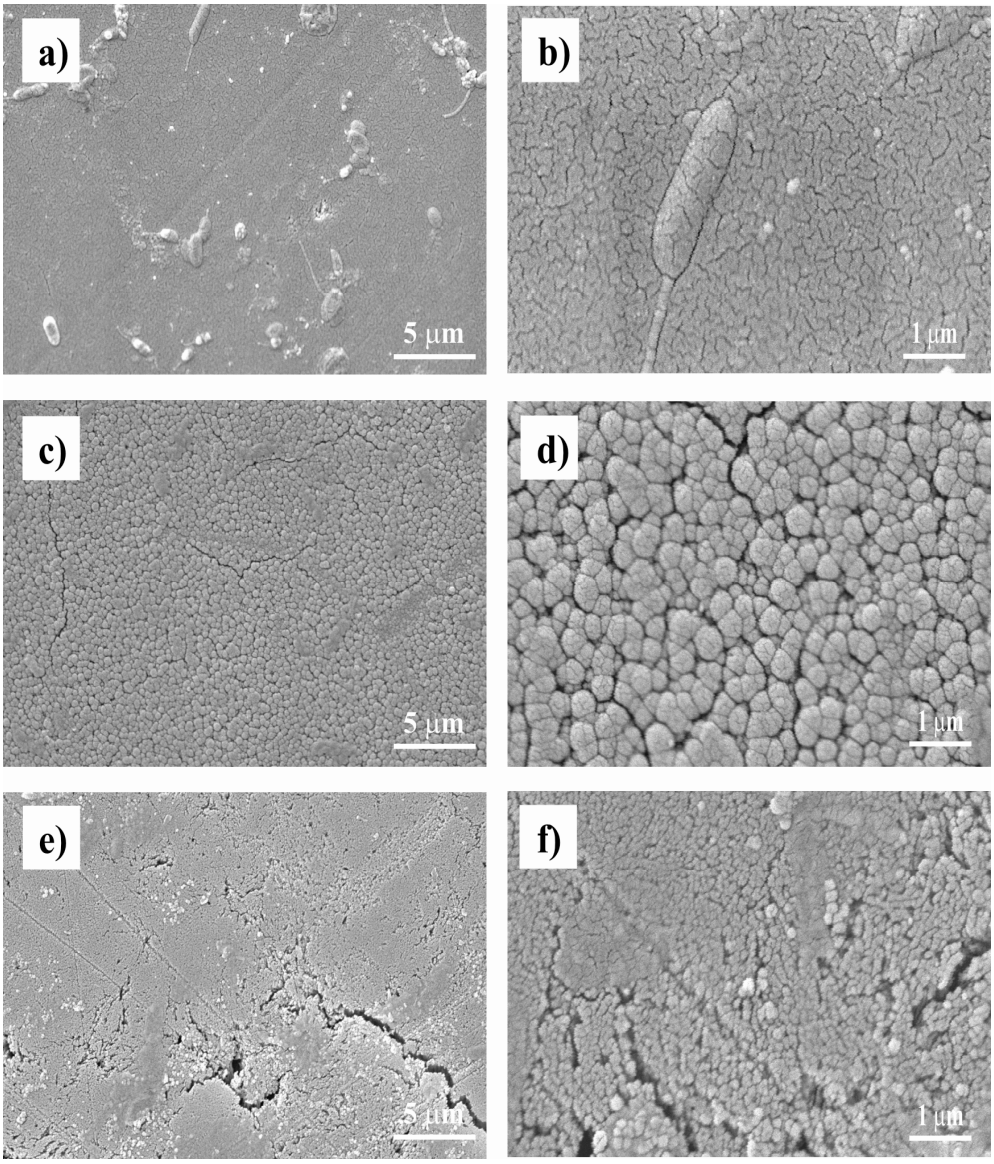


Figure 4.19 SEM micrographs of untreated enamel (a, b), enamel treated for 14 cycles of 10 minutes with (0.1 mol/l citric acid + 1.0% NaF) solution (c, d) and treated for 14 cycles of 10 minutes with (0.1 mol/l citric acid + 2.0% NaF) solution (e, f). The magnification for (a), (c) and (e) is 5 000×, while for (b), (d) and (f) it is 20 000×.

enamel apatite treated directly with 0.1 mol/l citric acid for 10 minutes (Figure 4.8). Therefore, CaF_2 formed on the enamel surface did not reduce the citric acid-induced erosion. Figure 4.20 shows SEM micrographs of citric acid-treated enamel with or without previous immersion in fluoride-containing admixture solution. The SEM imaging reveals similar morphological changes related to erosion caused by citric acid in the surface of two samples (Figure 4.20 a and c).

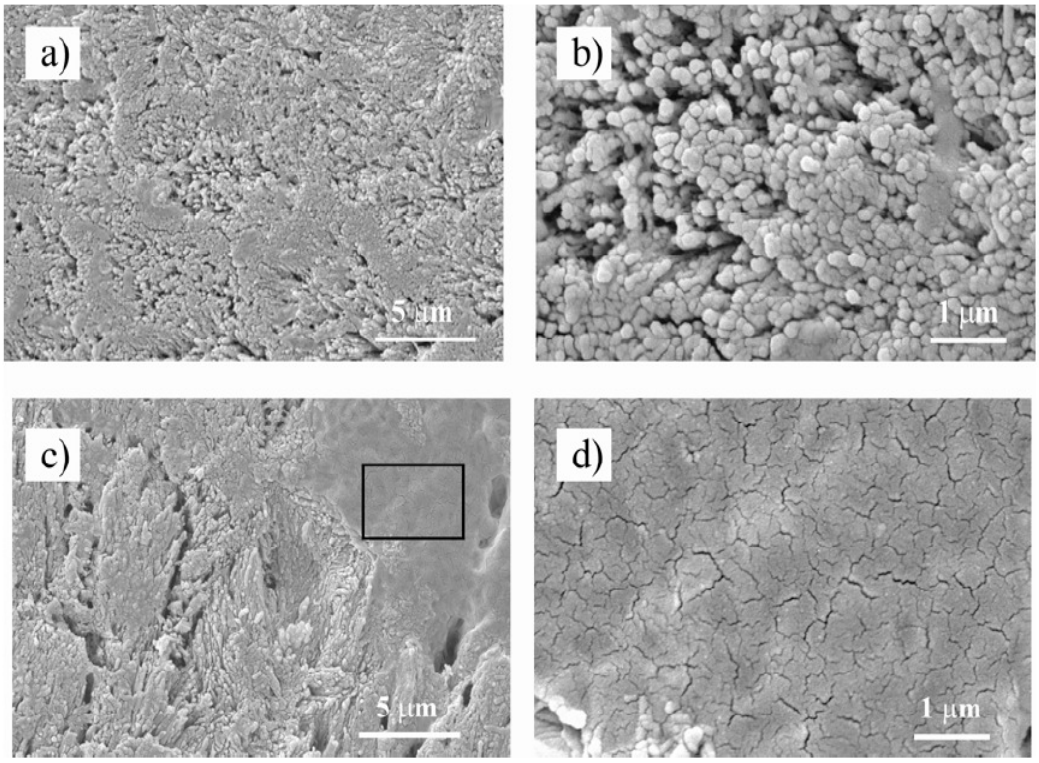


Figure 4.20 SEM micrographs of enamel treated with 0.1 mol/l citric acid solution for 10 minutes (a, b) and enamel firstly treated with (0.1 mol/l citric acid + 0.5% NaF) solution for 14×10 min and then immersed in 0.1 mol/l citric acid solution for 10 minutes (c, d). The magnification for (a) and (c) is 5 000×, while for (b) and (d) it is 20 000×; the rectangular frame in (c) marks the area from which the micrograph in (d) was taken.

The results agree with the ATR IR data. In addition, the high magnification images taken from less affected areas in the sample previously treated with (0.1 mol/l citric acid + 0.5% NaF) solution (Figure 4.20 d) reveal no remnants of CaF_2 globules. At high magnification heavily affected areas from both samples exhibit similar morphology and are shown in Figure 4.20 b, revealing severe alteration of the dental enamel exposed to citric acid.

4.3 Effects of different treatments on geological hydroxyapatite

The impact of Rapid White, 0.1 mol/l citric acid as well as the (0.1 mol/l citric acid + 2.0% NaF) admixture solution on the vibrational modes of single crystals of geological hydroxyapatite (HA) is depicted in Figure 4.21. Untreated geological hydroxyapatite (HA) exhibits sharper and better

resolved ATR IR peaks at 1070 and 1090 cm^{-1} as compared to the untreated enamel apatite. These peaks, similarly to the major peak near 1017 cm^{-1}, are related to internal stretching modes of PO_4 tetrahedra. From Figure 4.21, it can be seen that citric acid solutions and the citric acid-containing bleaching product (Rapid White) broaden the peaks, especially that near 1070 cm^{-1}. Similarly to enamel apatite, the undesired citric acid-induced structural alteration of geological hydroxyapatite (HA) is suppressed if NaF is added to the solution (Figure 4.21), while no significant influence is found after the separate treatment with citric acid and NaF (Figure 4.22).

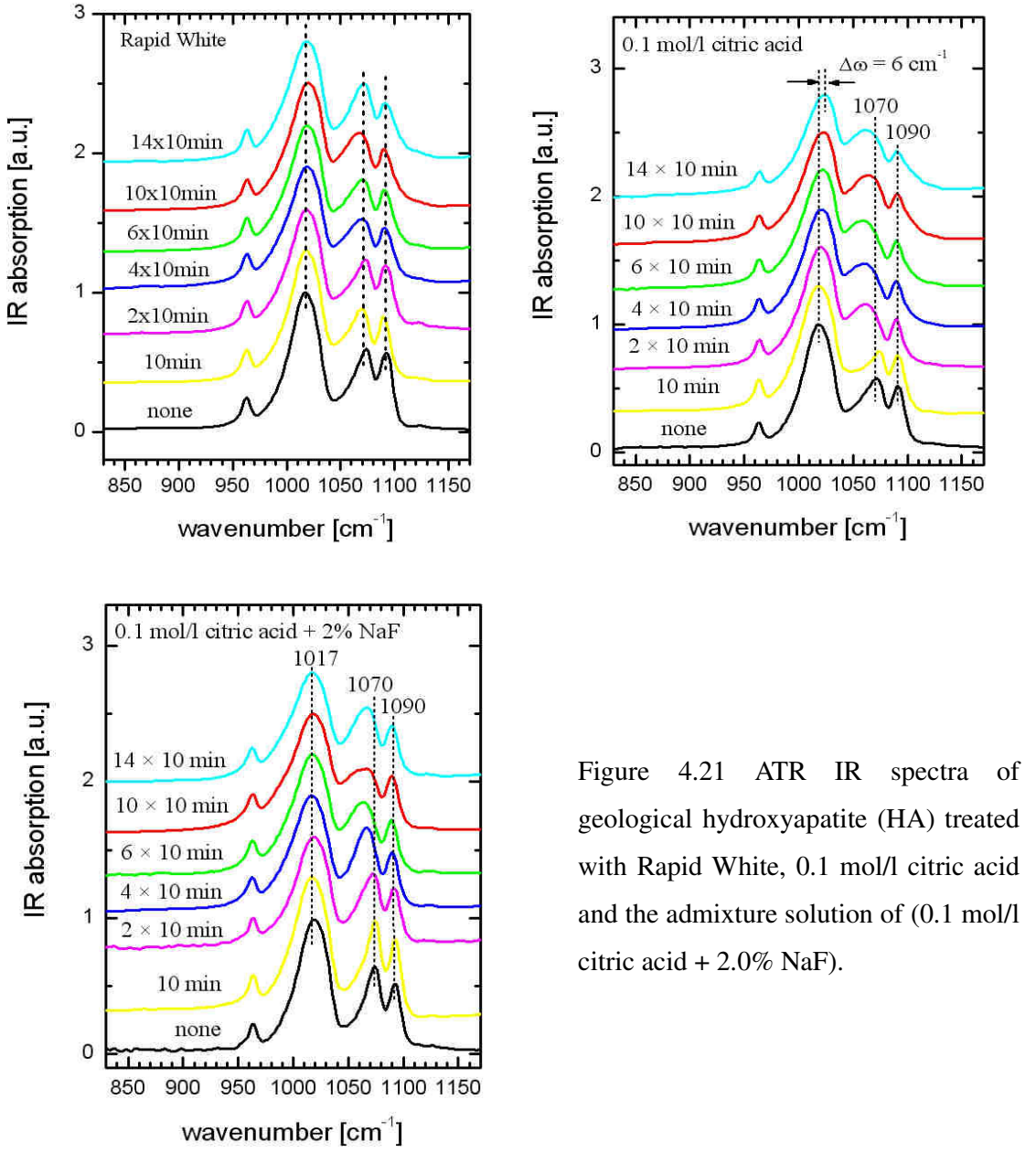


Figure 4.21 ATR IR spectra of geological hydroxyapatite (HA) treated with Rapid White, 0.1 mol/l citric acid and the admixture solution of (0.1 mol/l citric acid + 2.0% NaF).

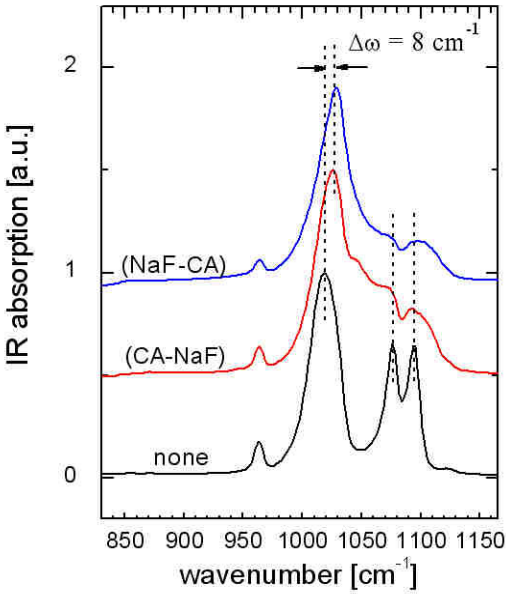


Figure 4.22 ATR IR spectra of geological hydroxyapatite (HA) treated with 0.1 mol/l citric acid solution for 10 minutes followed by a 10 minute-treatment with 1.0% NaF solution (CA-NaF), and with 0.1 mol/l citric acid solution for 10 minutes after a 10 minute-treatment with 1.0% NaF solutions (NaF-CA). Both treatments were repeated 14 cycles.

5 Discussion

5.1 Effects of peroxide-based bleaching agents on enamel

In the current *in vitro* study, the effects of peroxide-based agents on enamel were investigated using three commercial products (Opalescence Xtra Boost, Opalescence PF 20% and sodium perborate). Results reveal that within the sensitivity of the applied methods none of the investigated peroxide-based products significantly affected dental enamel.

Opalescence Xtra Boost containing 38% HP is a highly concentrated bleaching product and, therefore, most likely to cause damage to enamel if dental enamel is indeed susceptible to HP. However, following bleaching, no significant changes in the enamel surface were observed, whether surface morphology, crystal structure or chemical composition, even when samples were exposed to Opalescence Xtra Boost for a long-term treatment period. An increased number of treatment was applied to simulate conditions for patients receiving prolonged bleaching treatments, e.g. for tetracycline stains. This corresponds to previous investigations [Monaghan et al., 1992; Cullen et al., 1993; Basting et al., 2003; Dietschi et al., 2006].

Basic results of this comparative study are in agreement with previous reports [Potocnik et al., 2000; Cimilli and Pameijer, 2001; Goo et al., 2004; Lee et al., 2006]. On the other hand, studies have demonstrated that enamel surface is significantly affected by peroxide-based bleaching products. McGuckin *et al.* (1992) and Josey *et al.* (1996) found morphological alteration of the enamel surface after applying a low and high concentration of HP, respectively. In a study by Oltu and Gürgan (2000), the impact of 35% CP on the inorganic structure of enamel was verified using XRD and IR techniques. A decreased Ca concentration and Ca:P ratio in the enamel surface were also reported [Rotstein et al., 1996; Tezel et al., 2007]. The discrepancy between different studies may be due to the difference in the samples. Oltu and Gürgan (2000) used unerupted molars, while Cimilli and Pameijer (2001) selected maxillary bicuspids extracted for orthodontic reasons. Enamel is a heterogeneous material and it varies in mineral content, amount of organic matrix and chemical construction [Goldberg et al., 1983]. Variation occurs between individuals and teeth, and age also changes enamel surface characteristics, permeability and color [Ten Cate, 1994]. The difficulty to obtain enough background information pertaining to the tooth origin allows for variation in dental enamel due to age and health conditions. Therefore, it is difficult to compare the structural changes of enamel samples in different teeth. This study

examined a number of samples and observed slight differences in the ATR IR peak positions, in the ratio between the organic and apatite Raman peaks as well as in the degree of Ca leaching for different unbleached tooth samples. Thus, in order to prevent misinterpretation of results, in the present study special attention was paid to sample preparation and comparison was limited to samples from the same tooth.

The comparison between the acidic and neutral HP solutions confirms that HP itself does not affect dental enamel, while low pH values have an enamel erosive potential. In order to ensure enamel stability, many commercial bleaching systems are kept at low pH values. When the pH value is lower than 5.5, the critical value of enamel demineralization [Lagerlöf et al., 1983; Driessens et al., 1986], enamel erosion is expected to take place. Thickening agents are widely used in commercial products to slow down the release of active bleaching agent. It was observed *in vitro* as well as *in situ* that treatment with carbopol might cause a decrease in the enamel microhardness [McCracken and Haywood, 1996; Basting et al., 2003]. However, in an *in vitro* study by Rodrigues *et al.* (2007), no significant reduction in the enamel microhardness was found after enamel samples were treated with 2.0% carbopol and carbowax for 6 hours daily. This conclusion is supported indirectly by the findings in the present study that thickening agents have no significant effect on enamel. Additionally, the present investigation did not give evidence that the presence of urea caused the detrimental effects on dental enamel. The morphology and crystal structure of samples treated with Opalescence PF 20%, a urea-containing agent, resembled those treated with bleaching agents which do not release urea. However, fluoride and KNO_3 included into Opalescence PF 20% may be helpful to remineralize the eroded enamel [Basting et al., 2003]. Thus, additional investigations should be performed to more thoroughly assess the role of urea in enamel erosion processes.

5.2 Effects of non-peroxide-based bleaching agents on enamel

A variety of experimental techniques used in this study proved that the structure and chemistry of dental enamel were substantially affected by an “over-the-counter”, sodium chlorite-based whitening product (Rapid White). Through separate investigation of the single component of Rapid White, it was revealed that the substance responsible for the bleaching-induced deterioration of enamel is citric acid-containing “whitening gel” rather than sodium chlorite-containing “accelerator”.

The technique of attenuated total reflectance infrared (ATR IR) spectroscopy has recently been demonstrated to be a nondestructive and easy handling surface-sensitive method for studying biomaterials [Simon et al., 2006; Klocke et al., 2007]. The advantage of ATR IR spectroscopy as an analytical method for dental materials research is its ability to probe the structure of outermost layers of the tooth tissues within micron and submicron thickness. In the present study, being combined with Raman, ATR IR supplies complete information of the apatite structure in the enamel surface within a thickness of ~ 700 nm, the characteristic penetration depth of ATR IR experiments. Additionally, this technique permits repeated analyses of the sample surface at sequential stages of chemically induced transitions. Therefore, ATR IR is favorable for the determination of time lapsed chemical changes of enamel surfaces exposed to bleaching agents or NaF solutions. The concentration of Ca in the bleaching gel after use was measured using flame atomic absorption spectrometry (FAAS) and total reflection x-ray fluorescence (TXRF). Both techniques allow to determine Ca at low concentrations. As the two techniques are based on different physical processes, the use in combination can give an accurate measure of the Ca concentration.

ATR IR spectra showed a bleaching-induced shift of the anti-symmetrical ν_3 PO_4 stretching mode to higher wavenumbers, indicating that the corresponding O atoms are bonded more strongly to P atoms. 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 (Figure 5.1). 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. Those local structural defects are not necessarily periodically arranged. The overall spectrum profile and number of observed peaks is preserved, which points out that the average apatite structure is conserved. However, the occurrence of a peak shift indicates that the local structure is heavily affected. The occurring atomic-scaled local structural changes explain the well known bleaching-induced softening of enamel. The inorganic structural modifications due to Rapid White bleaching

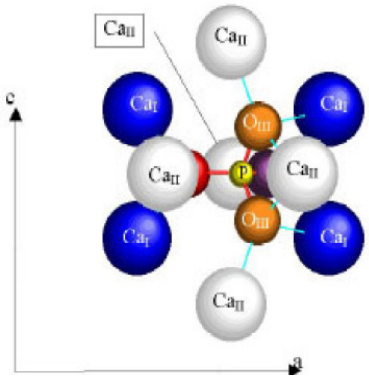


Figure 5.1 The PO_4^{3-} ion and its local environment.

treatment occur in the surface submicron layer (~ 700 nm) of enamel, even after the shortest treatment recommended by the manufacturer. Additionally, Raman spectra indicated that the Rapid White bleaching procedure affected the enamel organic component as well. The whitening capabilities of bleaching agents are due to the oxidation reaction between oxidizing substances and unsaturated organic pigments inside dental hard tissues. However, these oxidizing substances are not specific and can potentially react with dental organic structures. Previous investigations demonstrated that the breakdown of the organic enamel matrix might remove mineral crystals embedded within the enamel matrix [Albers, 1991]. Since the penetration depth of these Raman experiments is about 500 µm, it can be stated that bleaching affects the organic component inside the dental tissues. Attin *et al.* (2005) demonstrated that the decrease in the microhardness was not limited to the superficial enamel layer and subsurface softening was particularly pronounced for samples treated with Rapid White. The reason for the decrease in the subsurface microhardness is likely to be related to the decomposed enamel organic and the accompanying mineral loss. Hence, the main reasons for bleaching-enhanced Ca leaching, determined qualitatively and quantitatively by EPMA, FAAS and TXRF, should be (i) the weakening of the Ca-O bonds inside the apatite crystallites and (ii) the destruction of the basic organic component which leads to occurrence of emptied intra- and inter-rod voids. The release of Ca from the enamel apatite may be realized mostly via atomic diffusion through the apatite channels along the crystallographic c-axis and the inter-crystallites and inter-rod special voids with openings on the surface.

Further experiments investigated the effect of aqueous citric acid solutions of different concentrations on enamel. The exposure time to citric acid solutions was fixed at 10 minutes to simulate the Rapid White bleaching treatment. Results showed that for a short-term exposure the degree of alteration of enamel apatite depends on the concentration of citric acid solutions. A single treatment for 10 minutes with ≥ 0.1 mol/l citric acid was sufficient to affect the atomic P-O-Ca linkages in the whole available enamel surface. A repetition of the acid treatment did not induce any further spectral changes. The variations in the peak shape and width when low concentration of citric acid was used (Figure 4.9) indicate that a short-term treatment leads to the development of an intermediate stage: spatial regions of altered enamel within a non-altered matrix. The observed broadening of the major peak is a result of the existence of two overlapping components, which are positioned at two different wavenumbers corresponding to non-altered and altered enamel. The two components contribute with different weight coefficients to the infrared absorption in the range 1010-1030 cm^{-1}, which manifests as a shift of

the resultant ATR IR peak. After a sufficient cycle of treatments the structure in the whole probed volume is altered. Consequently, the component related to the non-altered enamel vanishes, thus leading to the observation of one narrower peak shifted to a higher wavenumber. For citric acid of low concentrations (≤ 0.05 mol/l) an approximately linear dependence of the enamel modification on the concentration was found for a single treatment period of 10 minutes. As the citric acid concentration increased, a continuous, time-dependent increase in the modification of the enamel structure occurred during the first 4 cycles of 10 minute-treatment (4×10 min). However, after this exposure time, the alteration in the enamel structure probed by ATR IR spectroscopy reached its final, saturated stage and became independent of the concentration. This indicates that, regardless of the concentration of citric acid, after a certain cycle of acid treatment, the modification of the apatite atomic structure occurs within the whole surface layer with a thickness of ~ 700 nm. The impact of citric acid on dental enamel has been well documented as the main acid in many fruit drinks and juices and factors such as its pH value and exposure time have been investigated. The results in this study on short-term treatment are in accordance with already published data that the rate of enamel dissolution increases with decreasing pH [Hughes et al., 2000; Barbour et al., 2003]. The critical pH value below which demineralization of dental enamel occurs was found to be in the vicinity of 5.5 [Lagerlöf, 1983; Driessens et al., 1986]. The pH values of aqueous citric acid solutions prepared in the current study are well below the critical pH value (Table 3.1) and, hence, results on multi-step treatments are in agreement with this conclusion.

It is worth noting that grinding and polishing procedures may change the physical and chemical properties of enamel surface by removing the outer layer from enamel surface, which is more resistant to demineralization [Dijkman et al., 1982; Wefel and Harless, 1982]. However, to allow positioning of samples in the test devices, in this study, samples had to be ground and polished to obtain a flat and smooth surface. Therefore, in the intraoral environment, enamel erosion caused by citric acid in Rapid White might be less pronounced. Further *in vivo* and clinical studies are necessary.

5.3 Effects of sodium fluoride on enamel

Protocols utilizing pH-cycling have served as a powerful means for providing mechanistic insight into the caries process and preventive measures. A similar protocol has also been used in

different *in situ* and *in vitro* studies on the erosive potential of commercially available soft drinks [West et al., 1998]. In recent years, fluoride uptake measures have been included as a subset analysis within this pH-cycling protocol [White, 1995]. In this study, three different models were built to comprehensively investigate the impact of fluoride on enamel erosion: 1) citric acid-NaF cycle; 2) NaF-citric acid cycle; 3) admixture solutions containing NaF and citric acid.

The most popular fluoride agents are sodium fluoride (NaF), acidulated phosphate fluoride (APF), stannous fluoride (SnF_2) and amine fluoride. The degrees of caries inhibition by all of the currently used fluoride compounds appear similar [Ripa, 1981, Vieira et al., 2005], despite differences in the pH value and in the amounts of CaF_2 they produce on the enamel surface [Larsen and Jensen, 1986; Duschner and Uchtmann, 1988; Saxegaard and Rølla, 1988]. NaF was applied as a remineralization agent in this study because the simple and straightforward formulation makes NaF an ideal vehicle to observe the effect of fluoride application on dental erosion *in vitro* [Damato et al., 1990; Proskin et al., 1992].

It has been suggested that a treatment with fluoride reagents can improve the resistance of enamel to acid-induced erosion because of two processes: (i) “structural” – F atoms enter the channels of the apatite crystal structure, thus stabilizing and even recovering the Ca-O-P atomic framework of apatite; and (ii) “chemical” – F atoms from the solution and Ca atoms from enamel apatite form a CaF_2 layer on the enamel surface, which might have a protective effect against the acidic impact. The separate treatment procedure with citric acid and NaF reveals that the application of fluoride solution alone cannot neither reverse nor prevent enamel erosion progression by a 10 minute-citric acid treatment. No suppression of the major peak shift was observed for Series 1 and 2, respectively. The multi-step treated samples of Series 1 exhibited an even larger peak shift when compared to samples treated with citric acid alone. However, the variation in the maximum value of the peak shift between different series will not be further discussed, because it might be partially due to differences in sample origin (cuts from different teeth). The ATR IR spectra of Series 1 and 2 unambiguously demonstrates that the application of NaF alone does not stop the undesired changes in the apatite crystal structure caused by a 10 minute-citric acid treatment and, in particular, it does not lead to a structural recovery of apatite even after a prolonged treatment in NaF solutions. Therefore, the ATR IR spectroscopic studies on samples exposed to NaF solutions indicate that the occurrence of “structural” processes due to exogenous treatments with fluoride should be ruled out.

The treatment with admixture solutions containing citric acid and NaF suppresses the alteration of the apatite structure for both enamel and geological hydroxyapatite (HA). However, it has to be emphasized that the pH values of these admixture solutions, as compared to the pH values of citric acid solutions, are closer to the critical pH value of 5.5 [Lagerlöf, 1983; Driessens et al., 1986]. Hence, the favorable effect of the admixture of fluoride to citric acid solutions is indirect, of "chemical" character, rather than related to any modification of apatite atomic structure. The protective effectiveness of such fluoride-containing acidic solutions, which can be considered as acidified fluoride solutions, has been related to the fact that the mild acidic pH values considerably enhance the deposit of CaF_2 on the enamel surface [Nelson et al., 1984; Bruun and Givskov, 1991]. The CaF_2 layer has been shown to play an important role in caries prevention [Dijkman et al., 1983; Rølla and Saxegaard, 1990] and fluoride solutions with a pH value of 4.5 or slightly higher have been advocated [Kidd and Joyston-Bechal, 1980; Strübig and Gülzow, 1986]. The present study indicates that favorable effects of acidified fluoride solutions may be related to the formation of CaF_2 globules on the enamel surface. The treatment with an admixture solution of (0.1 mol/l citric acid + 1.0% NaF) (pH ≈ 4.9) yielded a dense, homogeneous CaF_2 layer and no peak shift was observed in the ATR IR spectrum. Within the sensitivity of the ATR IR technique, the amount of Ca leached from enamel apatite to form CaF_2 globules on the enamel surface seems to be insignificant for the stability of apatite structure. In this *in vitro* study the pH value of 4.9 was found to be the optimal pH value to deposit a sufficient amount of CaF_2 without causing severe changes in the apatite crystal structure. However, slight variations of the pH value of the admixture solutions may hinder the CaF_2 deposit and, thus, lead to undesired apatite alteration. The present study also shows that the CaF_2 globules deposited on the enamel surface are not resistant to further aggressive acidic action and, consequently, may have a limited protective effect. The results presented are consistent with the fact that Ca can form a strong complex with citrate [Koulourides et al., 1961], which implies that the CaF_2-like layer deposited can be dissolved during the subsequent acidic treatment. Present conclusions are also in accordance with the results of Larsen and Richards (2002), who measured the dissolution rate of CaF_2 and lesion depth of enamel exposed to acidic soft drinks and found a rather limited protective effect of the CaF_2 layer.

5.4 Comparison of treated enamel and geological hydroxyapatite

The untreated geological hydroxyapatite (HA) samples showed better-resolved ATR IR peaks related to the anti-symmetrical ν_3 PO_4 mode of apatite. The reason for this observation is that geological hydroxyapatite (HA) is a monolithic single crystal, whereas enamel apatite is an inorganic/organic nanocomposite built up of small mineral nanorods, i.e. it is a polycrystalline material. Besides, bioapatite has a lower Ca/P ratio than 1.67, the typical value of geological hydroxyapatite (HA). Thus, a lower degree of point defects, as well as the absence of grain boundaries and individual, slightly misaligned crystallites for geological hydroxyapatite (HA) lead to a difference in the intensity ratios and narrower peaks.

The broadening of the ATR IR peaks induced by citric acid indicates that citric acid-containing materials can enhance the structural disorder and polycrystallize the surface layers of geological hydroxyapatite (HA). The multi-step treatment with citric acid induces a shift of the major peak to higher wavenumbers, as in the case of enamel apatite, while no detectible peak shift is observed after Rapid White treatment. The difference in peak shifts caused by citric acid and Rapid White might be due to the pH value. The pH value of Rapid White (3.7) is higher than that of aqueous 0.1 mol/l citric acid solution (2.23) and, therefore, it is comprehensible that in a short-term period citric acid solution induced more severe alteration of geological hydroxyapatite (HA) than Rapid White.

In summary, the structural alteration induced by citric acid-containing products is considerably less for geological hydroxyapatite (HA) than for enamel apatite, which is also due to the fact that the former is a monolithic single crystal. Citric acid destroys the organics that cover mineral rods in enamel, which increases the liquid-solid interface available for interaction between citric acid and apatite. As a consequence, after a certain period of citric acid treatment enamel exhibits more pronounced structural modification than geological hydroxyapatite (HA).

6 Conclusions

Summarizing the results of the effect of two bleaching formulae (peroxide-based and non-peroxide-based) and fluoride application on the structural modification of enamel, the following conclusions can be gained:

(1) The investigation of three peroxide-based bleaching agents containing different HP concentration showed that surface morphology, crystal structure and Ca leaching of the bleached samples were identical to that of the unbleached sample. However, an acidic bleaching environment might cause enamel erosion.

(2) Rapid White, a sodium chloride-based, "over-the-counter" product was applied to evaluate the influence of non-peroxide-based bleaching agents on enamel. Raman spectra reveal that the organic component of dental enamel is removed from superficial and deeper (≈ 500 μm) enamel layers and remnants of the bleaching gel are embedded in the emptied voids. The formation of cracks was found in the bleached tooth surface, indicating an erosion of the mineral component in the surface. ATR IR spectra showed that the atomic structure of enamel apatite was impacted in a submicron layer (≈ 700 nm). The loosening of Ca-O bonds facilitates the leakage of Ca from the enamel apatite which was confirmed in the following FAAS and TXRF analyses. Citric acid in "whitening gel" is responsible for the enamel erosion by Rapid White bleaching treatment. The structural modification of the enamel is dose-dependent. The severity of enamel alteration by citric acid treatment increases with an increase in the concentration and the number of treatments. In summary, significant side effects on enamel are expected with Rapid White bleaching treatment and, therefore, this non-peroxide-based material cannot be recommended.

(3) The effect of NaF on enamel erosion was observed by applying citric acid and NaF simultaneously or consecutively. ATR IR and SEM were used to analyze the changes in the crystal structure and surface morphology of enamel. Results demonstrated that the alteration of the enamel crystal structure caused by citric acid is not reduced by the application of NaF alone before or after citric acid treatment. The admixture solutions containing NaF and citric acid change the pH to values which favor the formation of CaF_2 globules on the enamel surface and have a considerably less pronounced erosive effect on the apatite crystal structure. For *in vitro* treatment suppression of enamel apatite erosion is most efficient for an admixture solution

having a pH value of 4.9. The CaF_2 globules deposited on the enamel surface are not sufficient to prevent apatite structural alterations due to further treatment with strong acidic agents.

(4) Geological hydroxyapatite (HA) is more resistant to acidic erosion than enamel apatite because the former is monolithic, while the latter is organic-inorganic nanocomposite.

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