Vertical Scanning Interferometry: A New Method to Quantify Re-/De-Mineralization Dynamics of Dental Enamel

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Abstract

Remineralization and demineralization are processes that compete in the oral environment. At this time, numerous therapeutic agents are being developed to promote remineralization (precipitation) or suppress demineralization (dissolution). To evaluate the relative efficacy of such treatments, there is a need for non-invasive, real-time, high-resolution quantifications of topographical changes occurring during demineralization and remineralization. Vertical scanning interferometry (VSI) is demonstrated to be a quantitative method to assess reactions, and topographical changes occurring on enamel surfaces following exposure to demineralizing, and remineralizing liquids. First, the dissolution rate of enamel was compared to that of synthetic hydroxyapatite (HAP) under acidic conditions (pH = 4). Second, VSI was used to compare the remineralization effects of F⁻-based and CCP-ACP agents. The former produced a remineralization rate of ≈ 349 nm/h, similar to simulated body fluid (SBF; concentration 4.6x) while the latter produced a remineralization rate of ≈ 55 nm/h, corresponding to 1.7x SBF. However, the precipitates formed by the CCP-ACP agent are found to demineralize 2.7x slower than that produced by its F⁻-counterpart. Based on this data, a remineralization factor (RF) and demineralization (DF) factor benchmarked, respectively, to 1x SBF and the demineralization rate of human enamel are suggested as *figures of merit* of therapeutic performance of dental treatments. Taken together, the outcomes offer new insights that can inform clinicians and researchers on the selection of remineralization strategies.

Keywords: teeth, demineralization, remineralization, vertical scanning interferometry, fluoride toothpaste, CPP-ACP toothpaste

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1.0 Introduction

Remineralization and demineralization are dynamic processes that compete in oral environments. While caries is associated with bacterial activity in dental plaque [1,2], erosion involves intrinsic and extrinsic acidic substances [3–9]. Both conditions result from imbalances that favor tissue demineralization. Demineralized hydroxyapatite (HAP) surfaces are more susceptible to other damage, e.g., abrasion, and the combination of acid dissolution and mechanical stresses promotes further enamel removal by exposing underlying tissues to successive acid attack [10–12]. As a result, there is significant interest in favoring remineralization and preventing demineralization. Besides enforcing dietary changes to reduce acidic food intake and hence demineralization [13], the routine use of topical agents can enhance remineralization [9,14–27].

Demineralization and/or remineralization of dental tissues has been widely studied using scanning electron microscopy [28], transmission electron microscopy [29], atomic force microscopy [30], indentation [31], microradiography [32], electron probe microanalysis [33], light induced fluorescence [34], secondary ion mass spectroscopy [7], confocal microscopy [35], electrochemical impedance spectroscopy [36], ultrasonic measurements [37], iodide permeability test [38] and contact profilometry [39]. However, microscopy techniques only offer qualitative 2D means for assessment [40]. Other techniques, e.g. microradiography and transmission electron microscopy are destructive and do not provide real-time evaluation. Further, the experimental conditions can also be limiting – e.g. in the case of microradiography the alignment and geometry of the X-ray beam can limit imaging precision at the sample edge [40]. While methods such as atomic force microscopy offer nanoscale resolution, the measurements are time-consuming [7] and of limited statistical relevance, as only small lateral areas can be scanned in realistic durations.

Recently, non-contact profilometry (e.g., laser scanning or white light interferometry, WLI) has been proposed as a new method to study dental tissues [10,14–17,41–45]. By building on past studies, this paper establishes procedural details for full-field quantitative assessments of reactions (i.e. dissolution or precipitation) occurring on dental tissue surfaces in contact with a solution using vertical scanning interferometry (VSI). VSI allows quantifications of surface topographies with a vertical resolution of ≈ 0.1 nm and a lateral resolution of around 500 nm. VSI uses interferometric objectives consisting of an objective lens, a reference mirror and a beam-splitter (see Figure 1). The most common interferometric objectives differ in terms of the mirror location, such that the mirror can be located between the objective lens and the beam-splitter (Mirau objective) or it can be placed in another position because of its larger size (a Michelson objective). In both cases, a source directs a light beam onto the sample surface through the interferometric objective, where the beam-splitter separates the light into two beams. One beam is reflected back by the reference mirror, while the other travels along the optical axis and interacts with the sample. This latter beam is reflected by the sample's surface. This results in an optical path difference between the two light beams and, a pattern of interference fringes forms when the beams are recombined.

This interference pattern is composed of light and dark bands: when the two beams are in phase their amplitudes are summed and a light band forms, whereas when the beams are out of phase their amplitudes are subtracted and a dark band of zero amplitude results. The interference fringes are sampled by a CCD (charge-coupled device) sensor and the signal is digitized and

processed to obtain 3D topographical maps of the sample's surface. VSI offers distinct advantages over other techniques, including: (i) non-destructive evaluation, (ii) nanoscale resolution of surface profiles (≈ 0.1 nm in the z-direction) with the ability to scan large lateral areas on the order of 10s of mm^2 , in real time, and (iii) the ability to render statistically relevant quantifications of true reaction (dissolution or precipitation) rates, while duly accounting for the exposed (surface) area of the solid. Therefore, this paper uses VSI to quantify the dissolution rate of enamel and synthetic HAP at pH 4. Further, the remineralizing effects of fluoride and CPP-ACP toothpastes are measured with subsequent analysis of demineralization behavior of the remineralized enamel surfaces.

The first aim of this study is to establish VSI as a quantitative tool to evaluate demineralization and remineralization rates of dental tissues. The main motivation is to use real-time, continuous, high-resolution, quantitative data to rationalize the contradictory observations in the literature. For instance, Prabhakar et al. [19] reported fluoride-based toothpastes to be a superior remineralizing agent compared to CCP-ACP treatments, while Somasundaram et al. [20] noted opposite findings. Elsayad et al. [46] stated that fluoride-based and CCP-ACP toothpastes can have a “synergistic effect” on remineralization if used together; while Huang et al. and Balan et al. [47,48] reported no significant difference in the effectiveness of these toothpastes. Besides minor differences in the treatment protocol, the studies also relied on analytical techniques that did not provide real time quantitative demineralization/remineralization rates. The second aim of this paper is to identify and benchmark therapeutic treatments that can mitigate acid attack of hard dental tissues.

2.0 Material and methods

2.1 Sample preparation

Caries-free, extracted human molars were collected from local oral surgery clinics. The teeth were sectioned axially using a precision slow speed diamond saw (IsoMetTM 1000, Buehler Inc.). The sections comprised the buccal, lingual, mesial and distal walls of the tooth and had a size on the order of 5 mm x 5 mm x 5 mm. After sectioning, the specimens were embedded in a cold-

cured, inert, epoxy resin (EpoxiCure Resin, PN 203430128: Buehler Inc.), with the enamel portion facing outward and polished to a uniform smoothness using SiC abrasive sheets of grit levels ranging from 400-to-1200. The polished sections were fixed to a glass slide using an inert adhesive (double sided tape 3M 950, supplied by Uline) to facilitate handling. It should be noted that the samples are polished flat to remove natural variations in the surface of tooth sites that may result in different responses to acid attack [49,50].

Powdered synthetic hydroxyapatite (HAP: $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$; molecular weight of 502.31 g/mole) was sourced from Acros OrganicsTM (CAS 1306-06-5). For dissolution analysis, powder particles with median diameter $d_{50} = 6.5 \mu\text{m}$ were fixed on a glass slide using inert double sided tape (3M 950, supplied by Uline) prior to further analysis.

2.2 De-/Re-mineralizing treatment protocol

VSI was used to monitor the enamel surfaces over three steps: (Step 1) demineralization, (Step 2) remineralization, and (Step 3) demineralization of the newly remineralized surface (Figure 2).

Step 1: Demineralization is carried out at room temperature ($25^\circ \pm 3^\circ\text{C}$) using a pH = 4 buffer solution (Fisher Scientific CAS 50-00-0: water 98.91 %, 1,2-Benzenedicarboxylic acid, monopotassium salt 1.0 %, formaldehyde 0.05 %, methyl alcohol 0.02 %, fluorescein, 2',4',5',7'-tetraiodo, disodium salt 0.02 %, all percentages written as mass %). A few drops of buffer solution (50-75 μL) were applied on the sample surface using a micropipette to obtain a liquid-to-solid ratio (l/s, by mass) between 50000-75000 to approximate the dilute limit. This elevated ratio hindered solution saturation with ions and prevents precipitation, if any. After 20 minutes of solvent contact, the sample is rinsed using distilled water and gently dried with compressed air. The dry sample is analyzed by VSI over fifteen cycle's demineralization cycles resulting in a solution contact time of 5 hours per sample. For comparison, the dissolution rate of synthetic hydroxyapatite (HAP) was also determined under the same experimental conditions.

Step 2: Enamel remineralization was carried out at room temperature using 2 commercial agents: (i) PreviDent5000 (Colgate-Palmolive, New York, USA) containing 1.1% NaF, and (ii) MI Paste (GC Corporation, Tokyo, Japan) whose active ingredient is RecaldentTM (CPP-ACP: casein phosphopeptide, CPP, and amorphous calcium phosphate, ACP). Each toothpaste was applied on the enamel surface for a contact time of 60 minutes. This procedure was repeated 9 times (i.e., for a total application duration of 9 hours). Between each remineralization treatments, the samples were rinsed thoroughly and then dried prior to VSI analysis.

Besides comparing the toothpastes' remineralization efficacy against each other, it would be more beneficial to compare them to standard benchmark remineralization agents. Simulated body fluid (SBF) was selected as a benchmark agent because of its two advantages over artificial saliva:

(i) SBF is widely used and its composition (and ionic strength) can be closely controlled, and,
(ii) For the purposes of this study, i.e., to demonstrate the ability to detect varying degrees of mineralization, it is important to select an easily reproducible protocol that can be replicated by other investigators around the world.

SBF is a well-established agent (ISO 23317) that is suitable for use in international standardized protocols to evaluate biomineralization [52]. In contrast, it is difficult to replicate the unique

properties of human saliva because this complex fluid is a mixture of fluids secreted by several salivary glands. Each of these individual saliva producers make highly variable saliva in terms of viscosity, ionic strength, etc. according to the time of day, diet, and many physiologic factors. For example, Gal. et al [53] investigated several different artificial saliva formulations and found that several were arbitrary formulations which yielded highly variable properties. While some of the more recent artificial saliva formulations contain similar ions as SBF, they do not allow simple additions of calcium and phosphates species to alter ionic strength, without producing insoluble precipitants. On the other hand, SBF is a well-designed system that allows easy manipulation of ionic strength without precipitation in most hands.

Therefore, three SBF concentrations (1x, 2x and 5x) were prepared, and their remineralization rates were used to provide data against which the commercial toothpastes could be compared. After demineralization at pH 4 (Step 1), remineralization is carried out by contacting the enamel surface with 50-75 μ L of SBF for 30 minutes. After each SBF application, the sample is rinsed with distilled water and dried with compressed air prior to VSI analysis. The remineralization step was repeated 14 times for a total contact time of 7 hours.

Step 3: Similar to Step 1, acid exposure (pH = 4) was carried out to ascertain the demineralization rates of the “deposited or remineralized” tissue surfaces, as noted in Step 2.

Figure 2. A schematic illustration of the procedure used in the demineralization and remineralization treatments.

2.3 Vertical Scanning Interferometry (VSI)

A Zygo NV 8200 vertical scanning interferometer fitted with a 50x Mirau objective (N.A. = 0.55) was used in the analyses. This setup permitted visualization of a scanning area of 152.27 μ m x 141.86 μ m per image field. Since images are recorded in stitched mode, using a P x Q grid (P rows x Q columns) composed of PQ tiles, the full-image field is on the order of (152.27·P) μ m x (141.86·Q) μ m wherein P and Q values ranged between 2 to 5. A back-scan length of 145 μ m was used, which allows peak-to-valley height differences up to 290 μ m to be sampled. The

acquisition time of the tiled image field, depending on grid size, ranged between 300-to-1200 seconds.

For any given sample, a scanning area was selected to monitor the topography of the surface (i.e., enamel or HAP), while establishing the embedment resin (for the enamel) or the polymer substrate (for the HAP), as a reference plane. Both the resin and the polymer used are inert to the solutions applied (see Section 3.1). After the images are captured, a data processing procedure consisting of several steps is applied:

(i) *filtering* is carried out to include or exclude specific characteristics of the surface. For the data presented herein, a median filter is chosen to reduce noise.

(ii) *Form removal* is carried out, if needed, to minimize alignment errors and to remove any background tilt that could mask details of the surface. To perform such leveling, mathematical functions are chosen to describe a geometrical form (e.g., a plane) that can be fit to, and then subtracted from the acquired data.

(iii) *Masking* is used to select and analyze a specific region in detail, while excluding (masking) the rest of the image field from the analyses. With the use of multiple masks placed over different regions of a given image-field, topographical parameters spanning a large area can be acquired, and, as such, a statistically significant analysis can be performed.

(iv) *Topographical parameters* are calculated using the Mx software (Ver. 6.1.0.4, distributed by Zygo Corporation) to quantify the changes in surface height. The parameter used in this study is the area averaged height (h) that is computed by averaging the height profile over $M \times N$ pixels as follows [54]:

$h = \frac{1}{MN} \sum_{x=0}^{M-1} \sum_{y=0}^{N-1} Z(x, y) - \bar{Z}$	(1)
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where, M and N are the number of pixels along the x and y directions, Z is the height of the sample (nm) at the location of the pixel located at (x, y) , and $\bar{Z}$ is the height of the reference plane (i.e., XY plane with respect to fixed origin in 3D space; in this case the epoxy in which the enamel is embedded). The difference between Z and $\bar{Z}$, therefore, is a measure of the height of a given feature on the sample with respect to the reference plane.

(v) The *demineralization or remineralization rates* (D_R , or R_R) is computed as:

$D_R \text{ (m/s)} = \Delta h / \Delta t$	(2)
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where, $\Delta h = h(i) - h(i+1)$ is the change in the average height (nm) of the exposed material with respect to the reference plane between step (i) and its successive step $(i+1)$ measured over a period of time Δt (seconds). The highest uncertainty in dissolution and precipitation rates, quantified in this manner is on the order of 10% [54–56]. The reproducibility of the experimental results is examined by repeating each experiment three times under the same conditions.

2.4 Scanning Electron Microscopy (SEM)

SEM images of select enamel samples were obtained before and after demineralization and after subsequent remineralization. Imaging was carried out using secondary electrons (SE) using a FEI Nova NanoSEM 230 (10 kV, 80 pA). The images allow for qualitative comparison of the pristine

enamel surfaces before the application of any treatment, and following demineralization (Step 1, $t = 5$ h) and remineralization (Step 2, $t = 14$ h).

2.5 X-Ray Diffraction (XRD)

XRD patterns were obtained on demineralized enamel surfaces treated with a F-based and CPP-ACP toothpastes using a Bruker D8 diffractometer with θ - θ “Bragg-Brentano” geometry, using Cu-K α radiation ($\lambda = 1.5406$ Å), 40 kV accelerating voltage, and 40 mA beam current. The samples were scanned on a rotating stage over 5° - 75° 2θ range with a scan step of 0.02° (2θ) and a step time of exposure time of 0.15 second per step.

3. Results and discussion

3.1 Method of analysis

To quantify the dissolution (demineralization) or precipitation (remineralization) rates, the height of the enamel surface was measured. During demineralization treatments (Step 1 and 3) the height progressively decreases, whereas during remineralization treatment (Step 2) the height increases (Figure 3). The change in height of the surface are calculated relative to the height of the reference plane $\bar{Z}$ (Eq. 1), which remains fixed throughout the experiment. The stability of the reference plane (i.e., epoxy resin) was confirmed by several experiments, wherein after prolonged exposure to acidic or basic solutions (for $4 \leq \text{pH} \leq 12$), its change in height (i.e., of the resin) was found to be less than 0.1 nm; which is within the measurement's resolution [54–56]. It should also be noted that additional pilot experiments carried out which involved the submersion of enamel and resin in water confirmed that no volume changes occurred; following gentle drying for ≤ 1 minute.

Figure 3. Representative topographical maps obtained from the VSI analysis. The isometric images show

the pristine, demineralized and remineralized enamel sections. (a) The top-left corner represents the resin reference, whereas the remainder shows the exposed enamel surface. (b) Shows a retreat in the spatially averaged height of the enamel surface (due to valleys and pits formed on the enamel surface; bottom-right corner of image), with respect to the resin reference due to demineralization. (c) Shows the formation of precipitates (see re-emergent peaks of material) following remineralization of the enamel surface. Each data point shown features an uncertainty of $\pm 14\%$.

The demineralization or remineralization rates are computed using Eq. (2) by averaging over different regions within a larger scanning area to obtain a statistically relevant value, which duly accounts for both exposed surface area and roughness. Therefore, the values of D_R (dissolution rate, nm/h) and R_R (remineralization rate, nm/h) are independent of the presence or the absence of heterogeneities which are randomly distributed across the surface.

3.2 Dissolution of enamel and synthetic hydroxyapatite

The dissolution rate of enamel was compared with that of hydroxyapatite (HAP), because HAP is a dominant inorganic constituent of teeth. The dissolution of HAP under acidic conditions has been studied to understand the evolution of pathologies such as osteoporosis, dental caries and erosion. Nevertheless, the dissolution of hydroxyapatite has not been fully clarified, and even now, several models and competing mechanisms continue to be proposed to describe its progress [57] including: (i) diffusion control, (ii) surface control, (iii) polynuclear model, (iv) self-inhibition, (v) congruent or incongruent dissolution; (vi) chemical control; (vii) etch pit formation, (viii) ion exchange and (ix) the hydrogen catalytic model.

The dissolution rate of synthetic HAP at low pH ($\text{pH} \leq 4$) was previously compared to that of enamel [58,59] indicating that the latter is faster dissolving than the former. But our results suggest that enamel dissolves slower than HAP at pH 4 (Figure 4). This difference is thought to arise because dissolution rates can be affected by chemical substitutions in the HAP crystallite structure of dental enamel. For example, it is well-known that fluoride substitutions reduce the dissolution rate of HAP (i.e., in enamel) while the inclusion of carbonate ions destabilizes the HAP structure, thus, increasing its tendency to dissolve [60,61]. Significant fluoride ion uptake in the enamel, e.g., resulting from the use of fluoridated treatments can result in suppressing enamel dissolution rates vis-à-vis synthetic (pure) HAP.

Figure 4. A comparison of the measured dissolution rates of enamel and synthetic HAP in acidic conditions ($\text{pH} = 4$). The uncertainty in the measured dissolution rate is $\pm 14\%$.

It should be noted that while the VSI technique can analyze erosive surface lesions, it cannot detect subsurface changes which can be produced by cyclic demineralizing regime. However, the type of lesion formed depends on the cause of destruction. Dietary acids and bases can induce surface erosion, as can many combinations of bacteria in dental plaque. For example, while *Streptococcus mutans* alone, and the combination of *S. mutans* and *Lactobacillus acidophilus* tend to induce subsurface lesions, the combination of *S. mutans* and *L. casei*, or *S. mutans*, *L. acidophilus*, and *L. casei*, has been shown to produce surface cavitations [51]. Nevertheless, the application of VSi for analysis of surface erosion is relevant, independent of the cause/origin of such erosion.

3.3 Remineralization treatments

Previous studies on the effectiveness of topical dental therapies lack consensus. Several studies have suggested that fluoride toothpastes reduce demineralization and enhance remineralization [14,17,24]. Others claimed that fluoride treatments do not prevent demineralization [62] and that intensive fluoridation can cause oral mucosa irritation [63]. On the other hand, the application of CCP-ACP toothpastes is recommended elsewhere [20,50,64], although the effectiveness of both fluoride and CCP-ACP-based treatments has been questioned by Huang et al. [47], who noted no differences following their application of these treatments vis-à-vis standard oral hygiene.

To resolve these contradictions, the rates of remineralization of a variety of treatments were quantified using a consistent protocol. The rates of remineralization were quantified using SBF (simulated body fluid; [52]) prepared at different strengths (concentrations) as a benchmark (see Figure 5). It was noted that the SBF remineralization rates follow a natural log function in relation to strength. When compared to the SBF treatments, it is noted that PreviDent5000 and the MI Paste induce remineralization at rates that would correspond to SBF strengths of 4.6x and 1.7x respectively. Using the remineralization rate of SBF 1x as a reference, the efficacy of MI Paste and PreviDent5000 were benchmarked by a remineralization factor (RF, unitless) noted in Eq. (3).

$RF = \frac{\text{Remineralization rate of toothpaste}}{\text{Remineralization of SBF (1x)}}$	(3)
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Figure 5. (a) The remineralization rates of enamel surfaces following their exposure to SBF prepared at three strengths (1x, 2x, 5x; circles) and two commercially available treatments: a fluoride toothpaste (PreviDent5000; square) and a CPP-ACP toothpaste (MI Paste; diamond). By using SBF remineralization rates as a reference, a method is established to compare quantitative remineralization rates of other non-

SBF treatment. (b) The remineralization factors (RF) of the two toothpastes. The uncertainty in the remineralization rates is $\pm 14\%$.

Such analysis revealed that PreviDent5000 has a higher remineralizing capability, with RF = 15.2, as compared to MI Paste, which yields RF = 2.4. While this may explain the outcomes of previous studies that suggest that fluoride-based toothpastes are superior remineralizing agents [17,19,65–68], it should be emphasized that RF is only a kinetic indicator of remineralization, and it does not predict the stability of the deposited precipitates, which can be determined by subjecting this new precipitant to dissolution. The remineralization effect of fluoride-based treatments is attributed to the rapid formation of NaF precipitates, that protect enamel surfaces by “sacrificial action” and that constitute a F⁻-ion reservoir [16,18,22,23,69]. Conversely, the remineralization kinetics of MI Paste are known to be lower than other treatments [19,50,65], in agreement with our results.

3.4 Dissolution of new precipitants (stability of remineralized surface)

To determine the stability of the remineralized surface against acid attack, a second sequence of demineralization is carried out to assess the stability of the newly formed precipitant. In this case, the rate of demineralization is quantified by a demineralization factor (DF, unitless) which describes the kinetic resistance to dissolution, of the precipitated phases, vis-à-vis pristine dental enamel, where:

$DF = \frac{\text{Demineralization rate after toothpaste treatment}}{\text{Demineralization rate of untreated enamel}}$	(4)
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Figure 6 shows that the F⁻ treated sample demineralized faster (DF = 1.6) than the pristine enamel, whereas the CPP-ACP paste treated sample demineralized slower than pristine enamel (DF = 0.6). This difference can be explained by the fact that the NaF bearing deposits (i.e., identified by X-ray diffraction, Figure 7) that formed during PreviDent5000 application, served as a sacrificial material that “preferentially dissolves” before the underlying enamel is attacked by acid [23]. This sacrificial behavior is likely on the account of the soluble nature of these deposits, which dissolve faster than crystalline HAP that constitutes dental tissues. It is also important to highlight that the XRD pattern (Figure 7) shows low intensity peaks at 47° 2 θ and 28.3° 2 θ , which suggests the presence of CaF₂. However, given the much larger intensities of the principal NaF peaks vis-à-vis CaF₂ peaks; NaF exists as the dominant precipitate formed.

(a)	(b)
<p>Figure 6. (a) A comparison of the demineralization rates of pristine enamel (Step 1), and the demineralization rates of previously demineralized enamel surfaces that are treated with a fluoride-based and CPP-ACP toothpaste (Step 3). (b) The demineralization factors (DF) of both commercial toothpastes.</p>	

It should be noted that the NaF deposits formed on F⁻ treated samples appear globular (2000x, see Figure 8d). On the contrary, the SEM images of pristine teeth showed elongated hydroxyapatite crystals within the typical honeycomb structure of teeth (Figures 8b, c). The instability of the NaF deposits was also noted by Hove et al. [15], who reported that these deposits are likely effective only for a limited period, and while they may provide resistance against acid attack initially, this resistance declined progressively after repeated and prolonged acid exposure – as the NaF deposits are progressively removed. The rapid dissolution of the NaF deposits at low pHs is also confirmed by [48,62] – an explanation of why fluoride-based toothpastes do not prevent dental erosion at longer time scales; unless repeated, and regular toothpaste applications are carried out.

Figure 7. The XRD patterns of demineralized enamel surfaces treated with a fluoride-based toothpaste (PreviDent5000). The peaks at 39° 2θ and 56.3° 2θ indicate NaF precipitation.

The precipitate formed during remineralization by the CPP-ACP toothpaste is far more resistant (from a kinetic perspective) to acid attack than even pristine enamel [20,50,70] (Figure 6). Thus, even if the CPP-ACP toothpaste did not result in significant remineralization (in terms of rate and hence the thickness of the precipitated layer), the deposited material provided excellent protection against dental erosion. This protective action is a result of CPP-ACP's behavior which increased the number of Ca²⁺-binding sites, decreased diffusion, and reduced calcium loss (i.e., by leaching) from enamel during acid exposure [71,72]. Casein phosphopeptide (CCP) is also thought to stabilize amorphous calcium phosphate (ACP) on enamel surfaces, thereby buffering calcium and phosphate ion activities, preserving their supersaturation, i.e., in the proximity of enamel, thereby retarding the latter's demineralization [50,73,74]. Zhang et al. [75] also attributed the better acid resistance of the CCP-ACP remineralization product to the formation of nano-size particulates which are homogeneously arranged and more tightly packed, than those formed by the application of fluoride toothpaste. This resulted also in the formation of physical barriers in the porous zones of enamel making it more resistant to future demineralization.

Taken together, the discussion above suggests that the application of PreviDent5000 is better suited when prescribed for regular and repeated application, over shorter durations. On the other hand, MI Paste is likely a better treatment in the long term, but an extended duration of application may be needed (e.g., using a dental tray) for substantial benefit to be noted. VSI reveals the quantitative differences in the behavior and performance of fluoride and CPP-ACP toothpastes, and highlights the relative strengths and weaknesses for experts in the field to debate. Moreover, the results also suggest that the application of NaF + CPP-ACP treatments may be synergistic – because while the former rapidly forms a precipitate, the latter forms a protective layer that envelopes the otherwise readily-soluble NaF deposit – as a result, “synergistic” benefits are expected.

4.0 Conclusions

This study demonstrates the application of vertical scanning interferometry (VSI) as a quantitative, rapid and high-resolution methodology to study solid-solution reactions of dental tissues. The nanoscale resolution of the VSI method is exploited to study the kinetics of both, demineralization and remineralization of dental enamel. Special focus is placed on quantifying the kinetics of these processes and the effectiveness of commercially available remineralization treatments. The VSI quantification form a basis to propose a demineralization (DF) and remineralization (RF) factor as *figures of merit* to compare quantitatively, the performance of two remineralizing agents, e.g. PreviDent5000 and MI Paste. Analysis of these factors and imaging of the hard tissue surfaces allowed for better understanding of how these treatments

work, and resolution of inconsistencies previously noted in their healing performance. The results unambiguously indicate that while PreviDent5000 offers fast remineralization rates ($\approx 6.3\times$ higher than MI Paste), the precipitates formed following its application dissolved $2.7\times$ faster than those formed by MI Paste.

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