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# Effects of Fluoride on in vitro Enamel Demineralization Analyzed by $^{19}$F MAS-NMR

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**Key Words**
$^{19}$F MAS-NMR · Caries · Demineralization · Fluorapatite · Fluoride · Strontium

**Abstract**

The mechanistic action of fluoride on inhibition of enamel demineralization was investigated using $^{19}$F magic angle spinning nuclear magnetic resonance (MAS-NMR). The aim of this study was to monitor the fluoride-mineral phase formed on the enamel as a function of the concentration of fluoride ions [$F^-$] in the demineralizing medium. The secondary aim was to investigate fluorapatite formation on enamel in the mechanism of fluoride anti-caries efficacy. Enamel blocks were immersed into demineralization solutions of 0.1 M acetic acid (pH 4) with increasing concentrations of fluoride up to 2,262 ppm. At and below 45 ppm [$F^-$] in the solution, $^{19}$F MAS-NMR showed fluoride-substituted apatite formation, and above 45 ppm, calcium fluoride ($CaF_2$) formed in increasing proportions. Further increases in [$F^-$] caused no further reduction in demineralization, but increased the proportion of $CaF_2$ formed. Additionally, the combined effect of strontium and fluoride on enamel demineralization was also investigated using $^{19}$F MAS-NMR. The presence of 43 ppm [$Sr^{2+}$] in addition to 45 ppm [$F^-$] increases the fraction of fluoride-substituted apatite, but delays formation of $CaF_2$ when compared to the demineralization of enamel in fluoride-only solution.

Topical applications of fluoride (e.g. fluoride toothpastes, gels, varnishes and mouthwashes) are considered a more effective measure for caries prevention than systemic use of fluorides [Ripa, 1991]. Fluoride incorporated into the enamel apatite structure has been shown to enhance the resistance of the tooth to acidic challenges and thus reduce lesion development [ten Cate and Featherstone, 1991; Takagi et al., 2000]. In addition, the anti-caries effect of topically applied fluoride has also been attributed to calcium-fluoride-like deposits formed on dental hard tissues, which are thought to act as a protective barrier on the surface and serve as a reservoir for fluoride [ten Cate, 1997].

Recently, there is a perception that 'the more the better' and thus a number of products incorporate very high

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levels of fluoride up to 15,000 ppm F⁻ [Tavss et al., 1997; Newbrun, 2010]. These products range from various dentifrices giving short-term fluoride exposure to fluoride-containing gels and varnishes exhibiting longer-term fluoride release and exposure. Tooth restorative materials, in particular glass ionomer (polyalkenoate) cements, have properties that enable relatively long periods of fluoride release [ten Cate and van Duinen, 1995; Mitra et al., 2011]. Given the large number of caries-preventative dental products available containing highly variable fluoride concentrations, it is perhaps surprising that few experimental studies have been undertaken to establish the effects of increasing fluoride concentration on the resulting mineral phases formed on enamel.

In this study, a less common approach was undertaken using $^{19}$F magic angle spinning nuclear magnetic resonance (MAS-NMR) to characterize the solid phase precipitated on enamel as a function of fluoride concentration used during exposure to an in vitro demineralization system. $^{19}$F MAS-NMR has been previously used to characterize fluoride-hydroxyapatite interactions. Yesinowski and Mobley [1983] demonstrated the ability of this technique to distinguish between fluorapatite ($Ca_{10}(PO_4)_6F_2$, FAp), fluorohydroxyapatite ($Ca_{10}(PO_4)_6(OH)_{2-x}F_x$, FHAp) and calcium fluoride ($CaF_2$) both in the bulk phase and on hydroxyapatite surfaces. Advantages of $^{19}$F MAS-NMR are the following: (1) It selectively probes the local environment of only the fluorine atoms in the sample, permitting direct identification of the possible structural forms in which F⁻ may exist within the enamel. (2) It detects all fluorine present, whether crystalline, amorphous or adsorbed. The range of $^{19}$F chemical shifts and the sensitivity of $^{19}$F chemical shifts to the local fluorine environment are extremely high. (3) It can measure very low concentrations of fluoride in the order of 0.1%. Fluorine-19 is a spin 1/2 nucleus and at 100% natural abundance, all F atoms in a sample can be detected [White et al., 1988].

Although the potential of $^{19}$F MAS-NMR as a spectroscopic tool for studying the fluoridation of apatitic surfaces was recognized over two decades ago, there have been few studies applying this technique to determine fluoride-enamel interactions in dental research. A reason for this could be that a specialized fluorine-free MAS-NMR probe is required as fluorine present in the probe introduces additional signals into the spectra, potentially disguising any fluorine-19 signals generated from the sample. Such probes are not widely accessible [Brauer et al., 2009].

White et al. [1988] first addressed the reaction products formed on powdered dental enamel (thus increasing surface area for reaction) under conditions of mineral fluoridation at neutral pH in 1988, and later studied these effects using high-resolution NMR [White et al., 1994]. They reported a peak which was ascribed to 'non-specifically adsorbed fluoride' that was suggested to be fluoride which is hydrogen-bonded to the phosphate protons on the apatite surface. Furthermore, a relatively small peak for FHAp was reported, although no peak for $CaF_2$ was observed.

The aim of the present study was to use $^{19}$F MAS-NMR to investigate the effects of varying fluoride concentrations (0.45–2,262 ppm [F⁻]), used typically in topical applications, on the fluoride-enamel interactions under acidic conditions using bulk enamel blocks rather than powder. This method mimics the oral environment more closely as the changes occurring in the enamel are limit-ed to the interactions at the natural enamel surface with fluoride. The $^{19}$F MAS-NMR results were corroborated by measuring the calcium and phosphorus ions released from the enamel during these reactions using inductively coupled plasma optical emission spectroscopy (ICP-OES) and by measuring the changes in weight of the enamel blocks.

Further, the synergistic action of fluoride and strontium on enhancing enamel remineralization has been reported [Thuy et al., 2008]. When incorporated together in synthetic carbonated apatites, fluoride and strontium improved apatite crystallinity and reduced acid reactivity [Featherstone et al., 1983]. In the present study, the combined effects on the resulting mineral phases formed on enamel of both ions (~45 ppm of [F⁻] and [$Sr^{2+}$] respectively) present in a demineralizing solution were investigated using $^{19}$F MAS-NMR.

## Materials and Methods

### *Preparation of Human Enamel Blocks*

Caries-free permanent molars were used (with approval from Queen Mary Research Ethics Committee QMREC 2011/99) to cut enamel blocks (~5 × 5 mm) with a maximum thickness of ~1 mm using an annular diamond blade (Microslice 2, Malvern Instruments, UK). The location of cut in each tooth was recorded. The linear dimensions and weight of each block were also recorded.

### *Demineralization of Specimens – Fluoride*

A series of F⁻-containing demineralizing solutions were prepared from analytical grade reagents. A 10-liter batch of demineralizing solution of 0.1 M acetic acid buffered with NaOH to pH 4.0 was prepared with deionized water and subsequently divided into 1-liter stock reservoirs. NaF was added to the stock solutions to prepare concentrations containing 0, 0.45, 7, 11, 29, 45, 136, 452, 656, 1,357 and 2,262 ppm [F⁻], selected to include concentrations of topically available fluoride.

### *Demineralization of Specimens – Strontium*

Further, the simultaneous effects of strontium and fluoride ions were investigated at 45 ppm [F$^-$] and 43 ppm [Sr$^{2+}$]. Sr(Ac)$_2$ was used to prepare a Sr$^{2+}$-containing solution.

### *Mineral Weight Loss*

Each enamel block was placed in a container with 50 ml of demineralizing solution at 37°C in a shaking incubator (KS 4000i control, IKA, UK) at 60 rpm for 24 h. Subsequently, each sample was immersed in 50 ml of a F$^-$-containing demineralizing solution at 37°C for 96 h. Samples were dried and weighed using a balance accurate to ±0.0001 g (Mettler HK, Switzerland) before and after immersion to calculate the percent mineral weight loss of each sample after 96 h.

### *$^{19}$F MAS-NMR*

Each sample was dried and ground to a fine powder for solid-state $^{19}$F MAS-NMR analysis. $^{19}$F MAS-NMR was carried out using a 600 MHz (14.1 T) spectrometer (Bruker, Germany) at a Larmor frequency of 564.5 MHz under spinning conditions of 15 kHz in a 2.5 mm rotor. The spectra were acquired using a low-fluorine background probe in a single-pulse experiment of 30 s recycle duration. The $^{19}$F chemical shift scale was referenced using the –120 ppm peak of 1 M NaF solution, with a secondary reference of CFCl$_3$. Typically spectra were acquired for 10–24 h depending on the fluoride level and are an accumulation of between 600 and 1,440 scans.

### *ICP-OES*

After reaction with enamel, the fluoride-containing demineralizing solutions were diluted by a factor of 1:20 to lower the background Na levels (for analysis of phosphorus and calcium), with an acidification of 1% (0.1 ml of 69% nitric acid in 10 ml) and quantitatively analyzed by ICP-OES (ICP; Varian Vista-PRO, Varian Ltd., Oxford, UK). Each measurement was replicated. Calibration solutions were demineralizing solutions (0.1 M acetic acid, pH 4.0) with the same ionic strength as a sample reaction solution, but diluted by a factor of 1:20 and also with an acidification of 1% using 69% nitric acid. Calcium and phosphorus standards were used in the concentration range 0.1–10 ppm. The instrumentation error was determined by repeated measurements of 1.0 ppm calcium- and 1.0 ppm phosphorus-containing standard solutions.

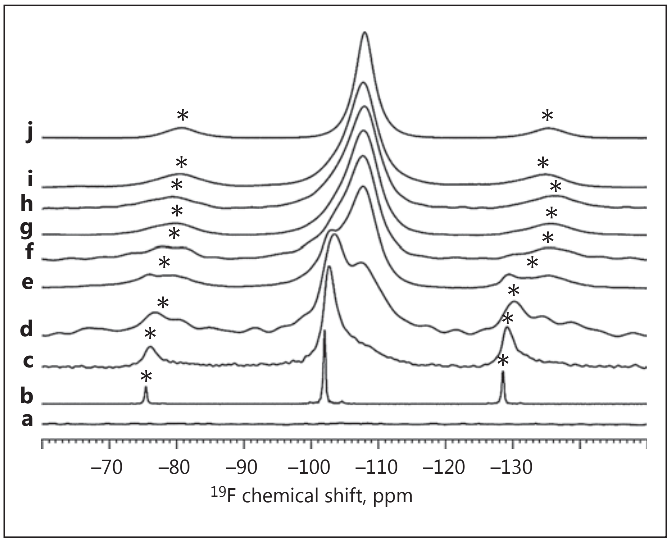

**Fig. 1.** $^{19}$F MAS-NMR spectra of enamel samples immersed in increasing concentrations of fluoride in the demineralizing solutions for 96 h. **a** Control enamel block not exposed to fluoride. **b** FAp reference. **c** Enamel block in 11 ppm [F$^-$]. **d** Enamel block in 45 ppm [F$^-$]. **e** Enamel block in 136 ppm [F$^-$]. **f** Enamel block in 452 ppm [F$^-$]. **g** Enamel block in 656 ppm [F$^-$]. **h** Enamel block in 1,357 ppm [F$^-$]. **i** Enamel block in 2,262 ppm [F$^-$]. **j** CaF$_2$ reference. Asterisks mark spinning side bands.

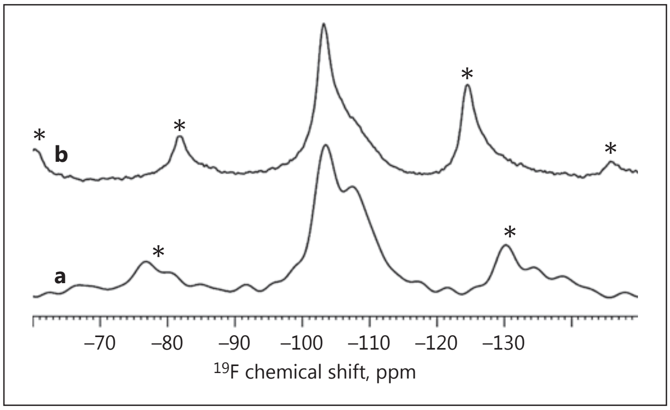

**Fig. 2.** $^{19}$F MAS-NMR spectra of enamel blocks immersed in 45 ppm of fluoride for 96 h. **a** Without presence of strontium. **b** With an additional 43 ppm of Sr$^{2+}$ (recycle delay 5 s) showing decrease in CaF$_2$ at –108 ppm. Asterisks mark spinning side bands.

## Results

### *$^{19}$F MAS-NMR*

Figure 1 shows a series of $^{19}$F MAS-NMR spectra of the enamel demineralized in the presence of a range of [F$^-$]. $^{19}$F MAS-NMR spectra obtained for the enamel section exposed to no F$^-$ exhibited a flat baseline with no detectable fluoride present. The FAp reference spectra showed a characteristic peak at –102 ppm corresponding to the triangles F-Ca(3) in the apatite structure, whilst the CaF$_2$ reference showed a characteristic peak at –108 ppm corresponding to the F-Ca(4) site. For the samples demineralized in the presence of F$^-$, $^{19}$F chemical shifts were identified by comparison with reference spectra, and formation of fluoride-substituted apatite ($Ca_{10}(PO_4)_6F_{2-x}(OH)_x$, F$_s$-HAp) and CaF$_2$ was observed from the peaks. Signals from both the F$_s$-HAp and CaF$_2$ environment are clearly observed in approximately equal

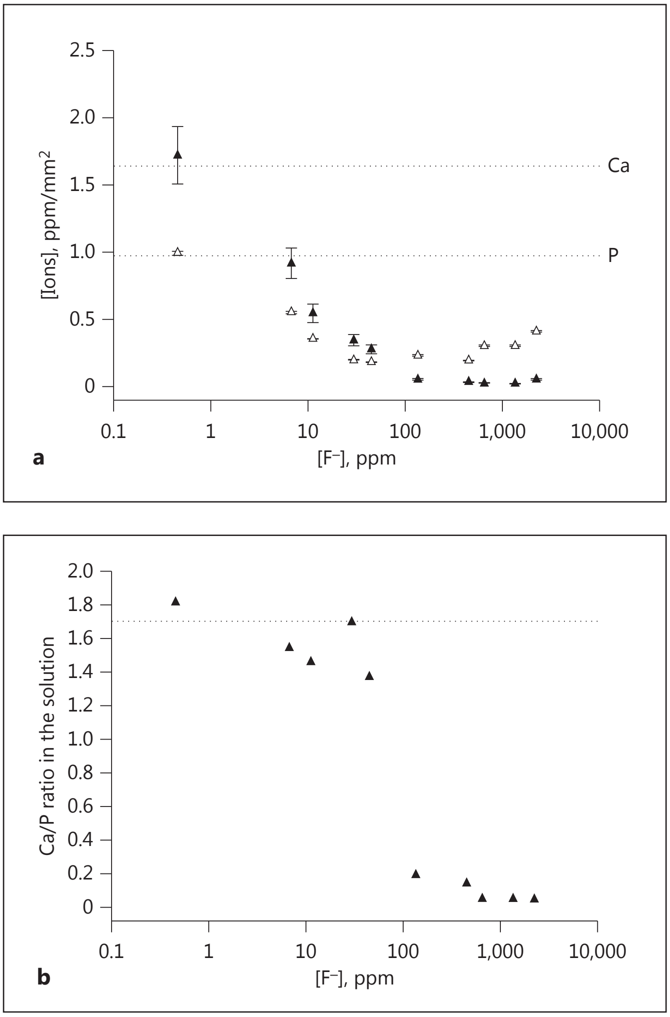

**Fig. 3. a** ICP measurements of the calcium and phosphorus detected in reaction solutions containing increasing [$F^-$]. △ = Phosphorus ions; ▲ = calcium ions. The dashed lines show the calcium and phosphorus in solutions under control conditions. The error bars show the instrumentation error. **b** Ca/P ion ratio in the solution as a function of [$F^-$]. The dashed line shows the Ca/P ion ratio under control conditions.

proportions in the spectrum of the 45 ppm [$F^-$] solution. At [$F^-$] above 45 ppm, less $F_s$-HAp forms and instead there is an increased $CaF_2$ signal. For [$F^-$] above 136 ppm, the spectra are nearly identical and demonstrate mostly a $CaF_2$-like environment.

Figure 2 demonstrates the effect of $F^-$ (45 ppm) demineralizing solution with additional strontium ions $Sr^{2+}$ (43 ppm). The $CaF_2$ signal is clearly decreased in the presence of both $Sr^{2+}$ and $F^-$ compared to that from the enamel exposed to 45 ppm [$F^-$] only solution.

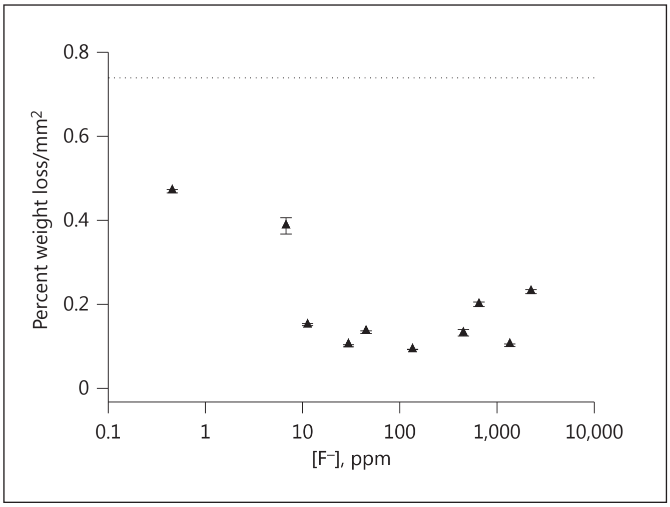

**Fig. 4.** Percent enamel weight loss/$mm^2$ of samples with increasing [$F^-$]. The dashed line shows the percent enamel weight loss/$mm^2$ under control conditions. The error bars show the instrumentation error.

**Table 1.** Percent enamel weight loss/$mm^2$ of samples and calcium and phosphorus ion release with increasing [$F^-$]

| [$F^-$] (ppm) | % weight loss | [Ca] (ppm) | [P] (ppm) | Ca/P | $-\log(T_{Ca}\times T_P)$ |
|---|---|---|---|---|---|
| 0.00 | 41.0±0.5 | 90.5±11.2 | 54.3±0.82 | 1.29 | 5.4 |
| 0.45 | 31.9±0.3 | 116.8±14.4 | 67.5±1.02 | 1.34 | 5.2 |
| 6.79 | 15.0±0.8 | 35.4±4.38 | 21.3±0.32 | 1.29 | 6.2 |
| 11.31 | 16.2±0.2 | 58.4±7.22 | 37.9±0.57 | 1.19 | 5.7 |
| 29.41 | 9.6±0.2 | 32.3±4.00 | 18.4±0.28 | 1.36 | 6.3 |
| 45.25 | 12.2±0.3 | 25.3±3.13 | 16.9±0.25 | 1.16 | 6.5 |
| 135.74 | 10.6±0.1 | 5.9±0.73 | 26.6±0.40 | 0.17 | 6.9 |
| 452.47 | 8.9±0.5 | 2.1±0.26 | 12.9±0.19 | 0.13 | 7.7 |
| 656.09 | 14.4±0.4 | 1.8±0.22 | 22.0±0.33 | 0.06 | 7.5 |
| 1,357.42 | 9.0±0.2 | 2.0±0.24 | 26.7±0.40 | 0.06 | 7.4 |
| 2,262.37 | 16.1±0.3 | 3.8±0.47 | 28.7±0.43 | 0.10 | 7.1 |

The $-\log(T_{Ca}\times T_P)$ was calculated from the molar concentration of calcium and phosphorus in solutions and substitutes for a solubility of biomineral.

### *ICP-OES*

Figure 3a shows the $Ca^{2+}$ and $PO_4^{3-}$ released into the reaction solution following demineralization of enamel samples. The $Ca^{2+}$ and $PO_4^{3-}$ released into the demineralizing solutions decreased markedly as the [$F^-$] increased from 0 to 45 ppm. Further, the $PO_4^{3-}$ release was lower than the $Ca^{2+}$ release. Above 100 ppm [$F^-$], there was substantially lower $Ca^{2+}$ release. Above 452 ppm [$F^-$], no further decrease in $Ca^{2+}$ release was detected, however $PO_4^{3-}$ release increased as the $F^-$ in the demineralizing solution increased in the range 136–2,262 ppm. Figure 3b shows the Ca/P ratio calculated from the ion release data.

*Mineral Weight Loss*

Mineral loss was measured by comparing the weight loss per unit area of enamel samples before and after immersion into demineralizing solutions. Figure 4 and table 1 show that for the control sample with no additional $F^-$ there is a weight loss of 0.74%/mm$^2$. However, the addition of 0.45 ppm $[F^-]$ reduced mineral loss to 0.47%/mm$^2$. For the solution with 11 ppm $[F^-]$, demineralization further decreased down to 0.15%/mm$^2$ and did not change substantially up until 452 ppm $[F^-]$. However, the percent mineral weight loss increased above 136 ppm $[F^-]$.

## Discussion

The $^{19}F$ MAS-NMR spectra (fig. 1) show the formation of $CaF_2$ and $F_s$-HAp as the main chemical species formed on the enamel samples that were demineralized with the addition of varying fluoride concentrations. Below 45 ppm $[F^-]$, fluoride is predominantly present as $F_s$-HAp with smaller amounts of $CaF_2$ present. At 136 ppm $[F^-]$ and above, $CaF_2$ is the predominant phase. Larsen and Jensen [1994] reported that in solutions saturated with enamel apatite, $CaF_2$ formation was initiated with as little as 100 ppm $[F^-]$ present in the solution at low pH conditions, in agreement with this study. The spectra are identical for all concentrations of fluoride above 136 ppm, demonstrating a $CaF_2$-like environment. Deconvolution of the spectra showed that there may be a variety of $F_s$-HAp phases present and further work is required to support this. In the present study the type of $F_s$-HAp could not be identified.

Previous $^{19}F$ MAS-NMR studies [White et al., 1988] reported the formation of $F_s$-HAp in powdered enamel at neutral pH and another signal ascribed as a 'non-specifically adsorbed fluoride' in the presence of fluoride. Note that the formation of $CaF_2$ was not observed. Their use of powdered enamel increased the surface area, which allows for greater ionic interactions between fluoride and apatite, whereas in the present study bulk enamel samples were used, subjected to longer equilibration periods (96 h) and under demineralizing conditions (pH 4). This could explain why $CaF_2$ precipitation was observed in this study with increasing $[F^-]$.

ICP-OES analysis showed a decrease in $PO_4^{3-}$ and $Ca^{2+}$ release with increasing $[F^-]$ in the demineralizing solution (fig. 3a). Above 45 ppm, $Ca^{2+}$ release was lower than $PO_4^{3-}$ release. At lower $[F^-]$ (≤45 ppm) (fig. 3b), the Ca/P ratio decreased from 1.8 to 1.4, suggesting incongruent dissolution of the mineral. However, at $[F^-]$ above 45 ppm, this ratio decreases sharply to below 0.2, which is due to calcium being used to form $CaF_2$. A decrease in demineralization rate was also seen from the weight loss data with increasing $[F^-]$ (fig. 4). In the present study, $[F^-]$ above 136 ppm did not substantially decrease demineralization further, and in fact a slight increase in demineralization was observed. This change in behavior is consistent with the ICP-OES data which show an increase in $PO_4^{3-}$ released for $[F^-]$ of 136 ppm and above (fig. 3a). This suggests that at higher $[F^-]$, there is preferential release of phosphate from the apatite structure, i.e. a slight increase in the rate of enamel demineralization. The $^{19}F$ MAS-NMR spectra show that $CaF_2$ is formed in greater proportions relative to FAp with ≥45 ppm $[F^-]$ in the demineralizing solutions, but does not alter significantly with further increases in $[F^-]$. ICP-OES shows a reduction in $Ca^{2+}$ release into the solution with increasing $[F^-]$ due to $CaF_2$ formation. As the $Ca^{2+}$ released into the solution decreases due to the precipitation of $CaF_2$, $PO_4^{3-}$ is unable to react to form apatite and is therefore released into the solution.

Previous studies have reported that the formation of $CaF_2$ may exhibit anti-caries effects by forming a physical barrier on the enamel surface, thereby slowing the demineralization process, as well as serving as a reservoir for fluoride [Saxegaard and Rölla, 1988; Ganss et al., 2007]. However, the $^{19}F$ MAS-NMR, ICP-OES and weight loss results of this study demonstrate that the formation of $CaF_2$ is potentially detrimental to the structural integrity of enamel as its formation reduces the available $Ca^{2+}$ required for remineralization of apatite. This in turn causes loss of $PO_4^{3-}$, thereby decreasing the mineral content in the tooth. The present findings suggest that the formation of a fluoridated-apatite phase is favorable as this retains the apatitic structure lowering enamel solubility [Featherstone, 1999].

In this study, the ICP-OES and weight loss data (fig. 3a, 4) showed that dissolution of enamel was increasingly inhibited up until 136 ppm of $[F^-]$, above which little further inhibition of demineralization was observed, and the $^{19}F$ MAS-NMR data showed that $CaF_2$ was the predominant species formed. This suggests that any benefit associated with the formation of $CaF_2$ at $[F^-]$ >136 ppm is offset by the disturbances caused to the structural integrity of the enamel. Although a static rather than a cycling model was used in this study, these conditions could mimic salivary conditions at night after brushing with a fluoride-containing dentifrice where the salivary flow rate is low and therefore elevated fluoride levels may persist for longer time periods. It would be interesting to perform a further study using a dynamic $F^-$ cycling system, with additional $Ca^{2+}$ and $PO_4^{3-}$ to more closely model in vivo conditions.

It has been proposed that $CaF_2$ formed on dental hard tissues, and within plaque, may act as a reservoir providing fluoride release under acidic conditions [Fischer et al., 1995]. $CaF_2$ has a very low solubility product constant and is relatively stable under low-pH conditions [Ogaard et al., 1983]. However, it appears that the terminology '$CaF_2$-like' has been implemented in the literature to overcome the point that $CaF_2$ is, in fact, not very soluble. The term $CaF_2$-like is sometimes referred to as a phosphate-containing $CaF_2$ [Rølla and Saxegaard, 1990], which these authors suggest is more soluble than pure $CaF_2$ and may thus release fluoride at a higher rate than pure $CaF_2$ [Christoffersen et al., 1988]. It has also been suggested that $CaF_2$ formed at low pH contains less internal phosphate and is therefore less soluble [Ogaard, 2001]. In the presence of salivary $PO_4^{3-}$ it is possible that a $CaF_2$-like material is formed in the biofilm. However Vogel et al. [2010] reported that either $CaF_2$-like deposits did not form in plaque after exposure to fluoride (228 ppm $F^-$), or if formed they were rapidly dissolved. Therefore, it may be that these $CaF_2$-like materials are nanocrystals of fluoridated apatite, which are more soluble than $CaF_2$ at low pH. This would explain the release of fluoride from these $CaF_2$-like materials at low pH conditions. It has also been suggested that phosphate-containing $CaF_2$ is formed on enamel during the treatment of enamel with acidified solutions of high fluoride content, which would then act as a reservoir for fluoride at low pH [Christoffersen et al., 1988; Rølla et al., 1993].

The 136 ppm 'threshold' in the present study may be particularly relevant to the case of fluoride applied topically from 'standard' (i.e. <1,500 ppm fluoride) dentifrices and mouthrinses. During studies where a fluoride mouthrinse was used, and whose concentration simulated salivary fluoride concentration during brushing with fluoride dentifrices, Vogel and co-workers reported that plaque fluid concentrations were more than an order of magnitude lower than 136 ppm [e.g. Vogel et al., 2000a, b], even following acidification and concomitant fluoride release [Vogel et al., 2000a]. Ekstrand [1997] reported that when 912 ppm fluoride (as both sodium fluoride and sodium monofluorophosphate) mouthrinses were used, plaque fluid fluoride concentrations did not approach 136 ppm. Thirty-minute values for all subjects were less than 15 ppm. These authors did not report plaque fluid fluoride concentrations immediately after the application of fluoride, which would almost certainly have been higher than, for example, the 30-min values. However, these 30-min values do make it very difficult to imagine values close to 136 ppm immediately following application. Regardless, the available data suggest that for most of the day, concentrations will be more than an order of magnitude lower.

Fluoride is added to various dentifrice products at concentrations up to 15,000 ppm with the aim of reducing caries formation [Preston et al., 1999] (although concentration will be somewhat lower in the mouth due to dilution effects). The current study suggests that there is an optimum range of local fluoride concentrations for inhibiting enamel demineralization, and further work is required to assess its effects on enamel structure after exposure to high fluoride-containing products, especially those above 5,000 ppm.

The present study demonstrates that the addition of fluoride produces $F_s$-HAp as a major chemical species only at low concentrations of fluoride. There is overwhelming evidence that low fluoride levels found in saliva can significantly reduce enamel demineralization, and those found in plaque have the potential to remineralize, even at pH values typically regarded as demineralizing [Fox et al., 1983; Lynch et al., 2006]. The potential of enamel remineralized in the presence of fluoride to both resist a caries challenge and to release fluoride into the oral fluids during dissolution has been somewhat ignored of late. The former effect is potentially quite important in terms of enamel solubility [Koulourides et al., 1980], while the latter effect may be sufficient to tip the 'caries balance' in their favor in low caries risk individuals. The speculated slow dissolution of $CaF_2$-like deposits is viewed as a potential 'slow-release vehicle' for the widespread availability of fluoride in the oral fluid. FHAp may also be an important fluoride reservoir, but previously its importance has been considered mainly in the context of reduced solubility when compared with HAp [Moreno et al., 1974].

The presence of $Sr^{2+}$ and $F^-$ both at ~45 ppm in the demineralizing solutions causes the formation of $CaF_2$ to be suppressed relative to the apatite phase (fig. 2). The present findings demonstrate the potential for strontium ions to offset or inhibit the formation of $CaF_2$, which in the context of this study behaves as an inhibitor of apatite remineralization, though this could also be explained by the formation of a strontium fluoride precipitate. Conversely, there was no difference between the NMR spectra of enamel samples demineralized at high concentrations of both fluoride and strontium in the solutions (452 ppm) compared to the spectra of enamel demineralized in fluoride only (452 ppm) solution. The synergistic effects of $Sr^{2+}$ and $F^-$ on the mechanism of enamel demineralization have to be clarified, and further work is required to assess whether strontium displays these effects at even higher concentrations of both ions in the demineralizing solutions.

In conclusion, this study confirms that the cariostatic effect of fluoride is due to the formation of $F_s$-HAp and $CaF_2$, depending on the $[F^-]$ in the solution. Below 45 ppm, the $F^-$ from the solution mostly substitutes into the lattice of the enamel mineral as $F_s$-HAp. At 45 ppm $[F^-]$, nearly equal amounts of $F_s$-HAp and $CaF_2$ phases form, whereas above 45 ppm $CaF_2$ is the main phase formed on the enamel surface.

The simultaneous presence of both fluoride and strontium ions may offset the formation of $CaF_2$ under higher fluoride concentrations when compared to the presence of fluoride alone in the demineralizing acidic medium.

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## Disclosure Statement

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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