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The Effect of Monofluorophosphate on the Acid Dissolution of Bovine Enamel

by

Richard Bryan Knight

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Oralfacial Sciences

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco

[redacted]

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Richard Knight

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surface. It was also shown that these MFP solutions contained about 70% of their fluoride from unhydrolyzed MFP, 30% of fluoride in the form of free fluoride ion, and an inconclusive concentration of phosphate. Additional studies are required to determine the effect of pure MFP on enamel dissolution.

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I dedicate this manuscript to my wife, Laurie, and my two children, Peyton and Aidan.

They remind me of what is important, over all other things.

Acknowledgement

The information within the following pages is the result of the combination of knowledge and hard work from several individuals:

Dr. John D.B. Featherstone, Chair of the Department of Preventative and Restorative Dental Sciences at the University of California, San Francisco, whose guidance and knowledge made this project possible.

Dr. Pamela K. DenBesten, Chair of Pediatric Dentistry at the University of California, San Francisco, and Dr. Joel M White, Professor of Preventative and Restorative Dental Sciences at the University of California, San Francisco, for guidance and support.

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INTRODUCTION

In 1955, sodium fluoride (NaF) and stannous fluoride were added to toothpastes to decrease the incidence of caries (Schmid et al. 1984). The free fluoride liberated from these species was shown to be responsible for this outcome. Unfortunately, it was demonstrated that calcium and magnesium abrasives within the dentifrice would react with the free fluoride resulting in less available fluoride, thereby reduced the overall anticariogenic effect (Hagen 1972; Pearce 1974; Stookey et al. 1985). This led some manufacturers to add monofluorophosphate (MFP) to their toothpaste as an alternate source of fluoride (Pearce and More 1975; White 1983).

In the MFP formulation, the fluoride is covalently bound to phosphorus, which eliminates its potential to react with other ions (i.e. Ca^{2+} and Mg^{2+}) (Ericsson 1963). Despite this, MFP dentifrices are able to maintain a significant anticaries effect (Pearce and More 1975; Mellberg and Mallon 1984). There is a range of scientific opinion on the comparative effectiveness of MFP and NaF. It has been shown that MFP has an efficacy that is less than (Pearce and More 1975, Mellberg 1983, White 1987), or even better than NaF (Ingram 1972; Nelson et al. 1992). Through meta-analysis of in-vivo comparison studies, it has been determined that MFP is equal to, or slightly less effective than NaF (Beiswanger and Stookey 1989; Proskin 1991; Stookey et al. 1993). Many researchers attribute the discrepancy in results to either impurities found within samples or to variations in experimental design (Pearce and More 1975; Eanes 1976; Arends et al. 1980; Bruun et al. 1984).

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With the introduction of less reactive silica abrasives in 1980, concerns regarding fluoride ions from NaF were diminished, prompting it to become a major additive to oral dentifrices once again (Bruun et al. 1984; Stookey et al. 1993). Today, MFP continues to be utilized in dentifrices and it is therefore important to understand the mechanism of action of both NaF and MFP (Cruz et al. 1994).

The effect of NaF on tooth structure

Sodium fluoride is a salt, with Na^+ and F^- ionically bound together. Upon entering an aqueous environment, this bond is broken and sodium and fluoride ions are liberated. Fluoride ions are then free to react with dental apatite, increasing remineralization and decreasing demineralization of tooth structure (Okazaki et al. 1981; Featherstone et al. 1982; Mellberg and Mallon 1984; White 1987; Shellis and Duckworth 1994).

The formula of a unit cell of carbonated apatite, the primary crystal of tooth mineral, is shown in equation 1. The carbonated apatite crystal is a variation of the more stable hydroxyapatite, shown in equation 2 (Okazaki et al. 1981; Shellis and Duckworth 1994, Nelson et al. 1983). In describing the effect of fluoride species on apatite, most literature available utilizes hydroxyapatite as the descriptive model.

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Equation 1: Carbonated apatite

$$[Ca^{2+}]_{10-x}[Na^{+}]_{x}[PO_4^{3-}]_{6-y}[CO_3^{2-}]_{z}[OH^{-}]_{2-u}[F^{-}]_{u}$$

Equation 2: Hydroxyapatite

$$[Ca^{2+}]_{10}[PO_4^{3-}]_{6}[OH^{-}]_{2}$$

Upon exposure to a solution containing fluoride ions, it has been demonstrated that fluoride replaces a hydroxide ion (OH^-) on the surface of the apatie crystal. (Equation 3) This exchange produces a less soluble, fluorapatite layer over the surface of the individual crystals (Shellis and Duckworth 1994; Featherstone 2000). Since fluoride is attracted to positively charged ions on the surface of the crystal, a fluoride rich environment is created immediately surrounding the mineral crystals. It is the presence of this fluoride which reduces demineralization and accelerates the remineralization of enamel (White 1987; Shellis and Duckworth 1994; Featherstone 2000).

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Equation 3: Fluorapatite

$$[Ca^{2+}]_{10}[PO_4^{3-}]_{6}\,[F^{-}]_{2}$$

Claims of fluorides effects are built on the observations that the solubility of enamel decreases after exposure to sodium fluoride (Shellis and Duckworth 1994; Featherstone 2000). These studies evaluate the effects of fluoride by exposing it to apatite for a given period of time prior to testing (e.g. exposure to acid). More dynamic methods involve observing the dissolution of enamel during an acid attack, while the apatite substrate is simultaneously being exposed to known concentrations of fluoride ion. In a study conducted by Featherstone and colleagues, 1990, it was determined that the addition of an initial dose of 0.1 mmol/L fluoride (equivalent to 1.9 mg/L or ppm) to an acetic acid solution (pH4.5) reduced the rate of dissolution of a synthetic carbonated apatite by 40%. Increasing fluoride concentrations thereafter, reduced the rate of dissolution to greater degrees (Featherstone et al. 1990).

Further analysis of the data produced by Featherstone and colleagues revealed that the rate of dissolution of the synthetic apatite decreased linearly in relation to the logarithm of the concentration of free fluoride (Featherstone et al. 1990). This reflects the fact that the reduction of solubility occurs through the adsorption of fluoride ions onto the crystal surface.

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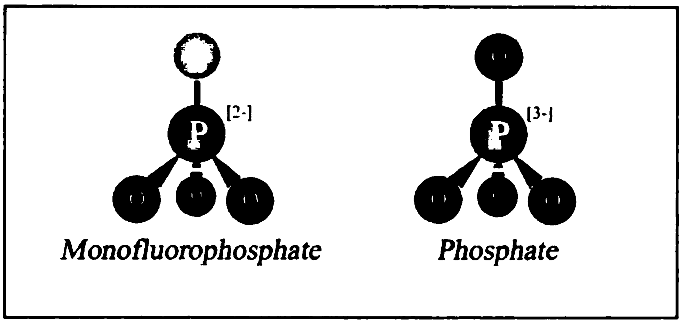
The effect of MFP on tooth structure

The MFP ion (FPO_3^{2-}) consists of fluoride covalently bound to phosphate (Figure 1). Unlike NaF, when placed in an aqueous solution free fluoride is not immediately available to produce its known anticariogenic effect (White 1988).

Figure 1: The structures of the monofluorophosphate (FPO_3^{2-}) and Phosphate (PO_4^{3-}).



Some researcher argued that the effect of MFP on enamel were the result of free fluoride present as an impurity of the sample (Pearce and More 1975; Eanes 1976; Arends et al. 1980; Bruun et al. 1984). However, after controlling for this initial impurity, MFP has still demonstrated favorable effects on tooth structure (Ingram 1972). In an MFP dentifrice, as much as 19% to 30% of the total fluoride concentration is in the form of free fluoride (Pearce and More 1975; Bruun et al. 1984). Two modes of actions of MFP have been proposed; Intra-oral hydrolysis of MFP to form free fluoride, and direct actions of intact MFP on enamel.

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Hydrolysis of MFP

Bruun and colleagues, 1984, conducted an experiment in which the levels of intra-oral fluoride were measured following exposure to MFP and NaF dentifrices. Within minutes the intra-oral concentration of free fluoride in those who had used an MFP dentifrice were similar to those using a NaF dentifrice (Bruun et al. 1984). This commonly cited study, demonstrates the intra-oral hydrolysis of MFP (Equation 4). The fluoride ions produced by this reaction can act identically to those originating from NaF.

Equation 4: MFP Hydrolysis

$$FPO_3^{2-} + H_2O \rightarrow HPO_4^{2-} + HF$$

It has been proposed that the apatite crystals in teeth catalyze the hydrolysis of MFP. This concept was supported by observations that the concentration of fluoride ion would increase in solutions containing MFP and apatite. Since the level of F^- would remain constant in MFP solutions lacking apatite, this seemed to be a reasonable conclusion (Gron et al. 1971; Eanes 1976). However, since more recent studies have not been able to reproduce such results (Pearce and More 1975; Duff 1983), apatite catalyzed hydrolysis of MFP is not widely accepted in the scientific community.

Nelson et al. proposed that acid in solution would initiate the breakdown of MFP (Nelson et al. 1992). To date, acid hydrolysis is a common method of experimentally determining how much MFP is in a solution. This is achieved by using strong acids, such as perchloric acid or concentrated hydrochloric acid (Gron et al. 1971; de Rooij et al. 1981; Duff and Stuart 1982; Jackson 1982). In solution with a pH in the range of 4-8, MFP has been shown to be stable and unlikely to undergo acid hydrolysis (Duff and Stuart 1982; Duff 1983). Because oral pH does not readily fall below this range, it is unlikely that this is how MFP is broken down in the oral cavity.

The primary mode of MFP hydrolysis in the oral cavity is through enzymes, known as phosphatases, which are found within saliva. Oral bacteria are the primary source of such oral phosphatases (Pearce and Jenkins 1977; Jackson 1982; Bruun et al. 1987; Saotome et al. 1987; Shellis and Duckworth 1994; Pearce and Dibdin 1995; Klimek et al. 1997). Furthermore, it has been demonstrated that those with high bacterial loads have an increased rate of MFP hydrolysis (Bruun et al. 1984; Ogaard et al. 1985; White 1987; Pearce and Dibdin 1995). Even though it seems reasonable that such individuals be exposed to greater levels of fluoride ion, it is unfortunate that those with low bacterial loads may have a lesser exposure to free fluoride when using MFP dentifrices (Pearce and Dibdin 1995). For these individuals, the direct actions of unhydrolyzed MFP may a play an important role in caries prevention.

Direct effects of MFP

It has been proposed that intact MFP can potentially reduce the solubility of apatite through direct interactions (Gron et al. 1971; Arends et al. 1980; Benton et al. 1980; de Rooij et al. 1981; Mellberg and Chomicki 1982; Duff 1983; Rolla 1983; Shellis and Duckworth 1994). Rather than replacing a hydroxide group of apatite, as fluoride ion does, MFP replaces a phosphate group (Equation 5). In 1972, Ingram observed that in the presence of hydroxyapatite, the concentration of MFP slowly decreased, while the concentration of phosphate slowly rose and the concentration of fluoride ion stayed the same (Ingram 1972). This was consistent with a phosphate/MFP exchange in, or on apatite crystals.

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Equation 5: Incorporation of MFP into hydroxyapatite

$$[Ca^{2+}]_{10}[PO_4^{3-}]_6[OH^-]_2 + FPO_3^{2-} \rightarrow [Ca^{2+}]_{10}[PO_4^{3-}]_{6-x}[FPO_3^{2-}]_x[OH^-]_2 + PO_4^{3-}$$

Erricsson and colleagues further complicated the sequence to include an intra-crystalline exchange of the fluoride from MFP and the hydroxide group within the apatite crystal (Ericsson 1963). Such an exchange would simply produce a fluorapatite crystal. However, the vast majority of literature cannot produce data to support such an intra crystalline exchange. Rather, Ericcson's observations may have been a product of free fluoride incorporation after MFP hydrolysis.

MFP incorporated apatite has been shown to have reduced solubility. In fact, the solubility of enamel treated with MFP has been reported to be superior when compared with NaF (Ingram 1972). Conversely, it has also been reported that MFP treated apatite is either equal, or inferior, when compared to those treated with NaF (Pearce and More 1975; Eanes 1976; ten Cate et al. 1981; White 1987; White 1988; Nelson et al. 1992; Shellis and Duckworth 1994). Such conflicting data has resulted in disagreement as to the effects of unhydrolyzed MFP on tooth structure. Analogous to the studies discussed with sodium fluoride, observations of the effect of specific concentrations of unaltered MFP during an acid attack may produce more pertinent results. These results would also allow for comparison with NaF at similar concentrations. To date, such observations with MFP have not been made.

Aims and hypothesis

The aim of this study was to determine the effect of MFP within an acetic acid solution (pH 4.5) on apatite dissolution, and to compare these results with those found with sodium fluoride and no fluoride. This study established whether MFP affects the acid dissolution of enamel, or whether hydrolysis is first required. With this knowledge, the scientific and dental community can better understand the function of monofluorophosphate on the dentition and improve methods of caries prevention.

The hypothesis tested in this study was that the acid dissolution of bovine enamel is decreased in the presence of MFP when compared to such dissolution rates without fluoride, and that such an effect is equal to that of sodium fluoride. These hypotheses were tested by measuring the dissolution rates at given concentrations of MFP, sodium fluoride, and no fluoride.

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MATERIALS AND METHODS

Preparation of bovine enamel

Bovine teeth were purchased from Tyson Products and sterilized through exposure to gamma radiation overnight as performed previously by White and associates (White et al 1994). They were sectioned into 2 mm deep blocks with a 3x3 mm flat enamel surface. To ensure that demineralization only occurred on the 9 mm^2 enamel surface, all other surfaces were coated with a methyl-methacrylate varnish. The samples were then bound to a Teflon disc with epoxy resin adhesive, which provided positional stability within the dissolution apparatus.

The surface of the enamel was prepared with a 600-grit silicon carbide paper, and polished with 6 and 3 μm diamond impregnated disks. After each treatment, the blocks were sonicated and washed with de-ionized water to remove impurities from the polishing sequence. Re-polishing the enamel surface allowed samples to be utilized more than once.

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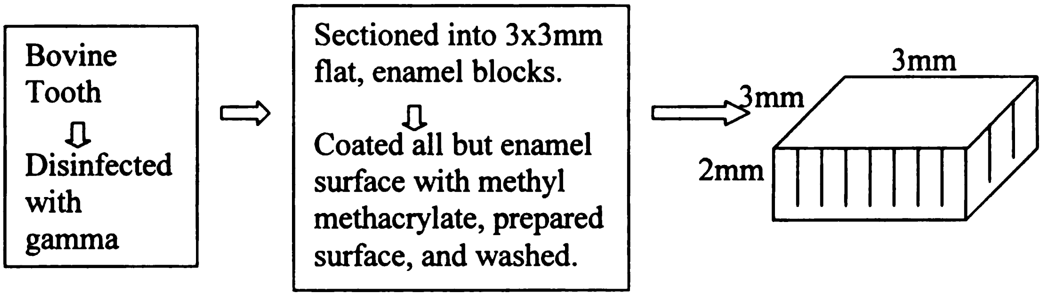
Figure 2: Preparation of bovine enamel



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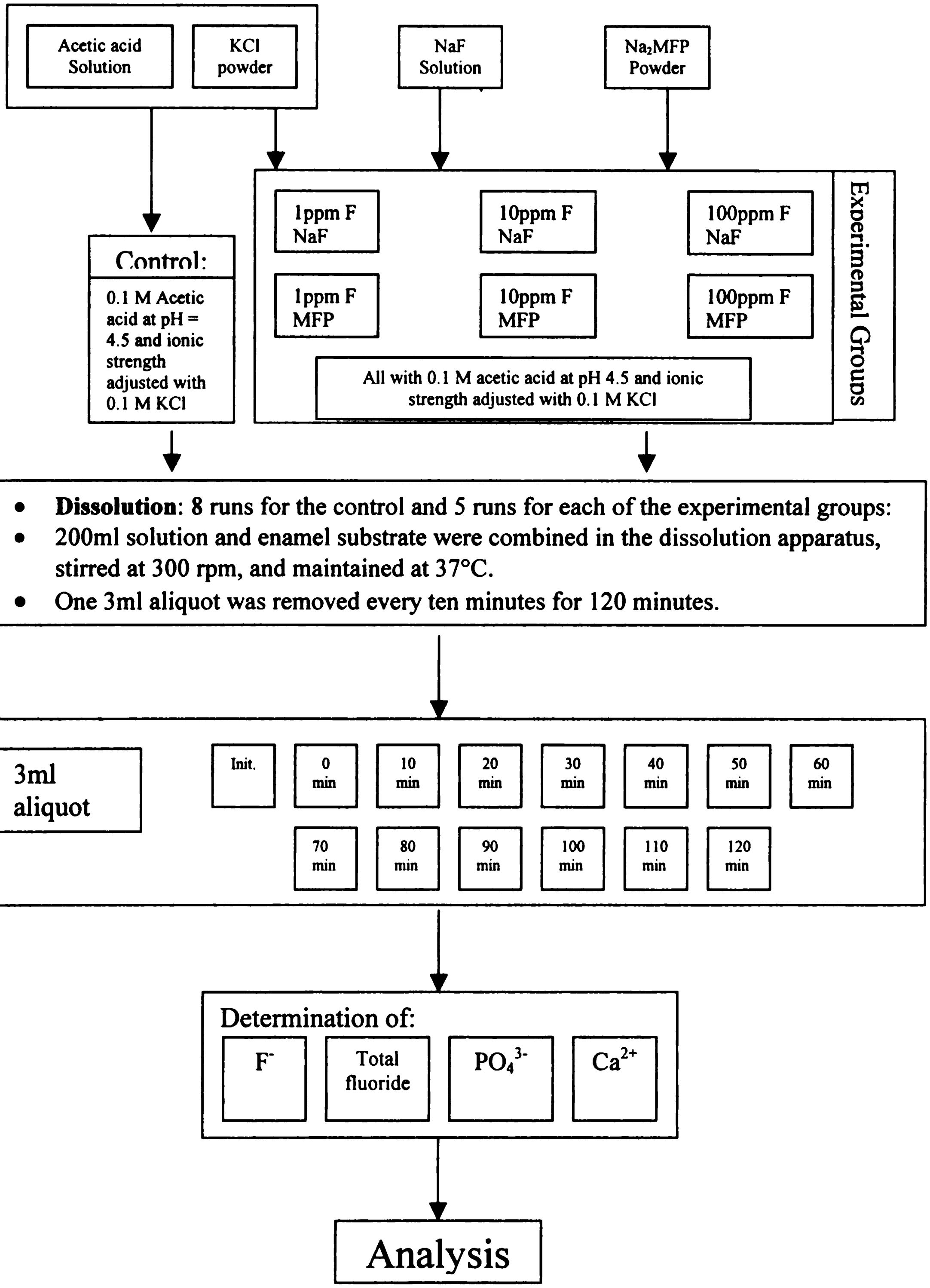
Dissolution experiment:

Figure 3: Summary of procedures



- **Dissolution**: 8 runs for the control and 5 runs for each of the experimental groups:
- 200ml solution and enamel substrate were combined in the dissolution apparatus, stirred at 300 rpm, and maintained at 37°C.
- One 3ml aliquot was removed every ten minutes for 120 minutes.

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Preparation of dissolution solution:

Dissolution solutions each contained 0.10 M acetic acid, 0.10 M KCl, and a specific concentration of either NaF or MFP. Measurements of NaF and MFP were such to produce solutions of total fluoride concentrations equivalent to 1 ppm, 10 ppm, and 100 ppm. The control dissolution solution contained no added fluoride.

A single concentrated 9 Liter solution was prepared, from which all other dissolution solutions were made. Amounts of acetic acid and KCl added were based on a 10 L solution. First, 57.190 ml Glacial acetic acid (17.4 N) was added to approximately 7 liters of double de-ionized water (DDW) and stirred. After approximately 10 minutes, 74.56 g KCl were added and stirred until completely dissolved. The solution was then diluted to 8.0 L and the acidity was measured with a pH meter. NaOH tablets were added to bring the pH of the solution to about 4.40, and the solution was volumetrically diluted to 9 Liters.

To make the final 1 L dissolution solutions, 900 ml were drawn from the above concentrated solution and initially diluted to about 950 ml. For the control solution, this initial dilution was accomplished with DDW alone. Dilution of the sodium fluoride dissolution solution involved the addition of both a specific volume of a 100 ppm or 0.1 M-fluoride standard solution and DDW; for the 1 ppm(F)NaF dissolution solution, 10.0 ml of a 100 ppm F standard was added, and for the 10 ppm(F)NaF and 100 ppm(F)NaF

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dissolution solutions, 5.264 ml and 52.64 ml (respectively) of a 0.10 M NaF standard were added. The 1 ppm(F)MFP, 10 ppm(F)MFP, and 100 ppm(F)MFP dissolution solutions involved the addition of .0076 mg, 0.0758 mg, and 0.7577 mg of sodium monofluorophosphate powder (Proctor and Gamble) respectively. Addition of the relatively small masses of MFP was accomplished by measuring larger masses with an analytical balance and performing successive volumetric dilutions with DDW prior to being added to the 900ml concentrated solution.

Dissolution solutions were diluted to about 950 ml (if needed) and the pH of each solution was adjusted to 4.50 with NaOH and HCl. The solution was then diluted volumetrically to 1000 ml. Each solution was then separated in five 200 ml volumetric flasks for use in each dissolution run. Solutions were made between 1 and 72 hours prior to their use.

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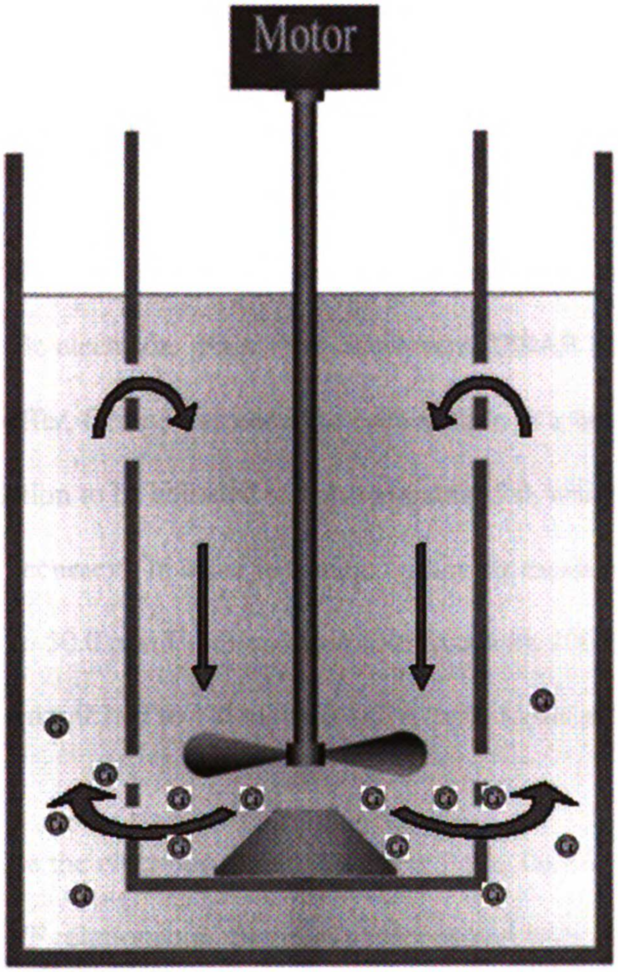
Dissolution experiment

Featherstone previously described the dissolution apparatus utilized in this study in 1983 (Featherstone et al. 1983). The device, as shown in figure 4, was fabricated to consist of two basic compartments; an inner functional compartment and an outer reservoir. The inner compartment contained the hydrated enamel sample, fixed in place directly beneath a motor-driven turbine. The turbine rotated at a constant rate of about 300 rpm and forced the dissolution solution over the enamel sample and then into the reservoir through several holes near the base of the inner compartment. Dissolution

solution within the reservoir could then flow into the top of the inner chamber through addition holes. The dissolution solution then resumed a cycle of flowing over the enamel, into the reservoir and back again, as propelled by the turbine. Nitrogen gas was slowly bubbled into the reservoir to limit carbon dioxide within the solution. The entire apparatus was located within a large water bath to maintain a constant temperature of 37°C.

Figure 4: Dissolution apparatus.



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Prior to each run, the given 200 ml dissolution solution was brought to 37°C in an incubator and was poured into the reservoir without the inner compartment in place. At that time, an initial 3ml aliquot was removed. After placing the inner chamber within the reservoir and starting the motor, another 3ml sample was taken from the reservoir. This was designated as the sample at time zero. Thereafter, a 3 ml sample was taken from the reservoir every 10 minutes for 2 hours. These 3 ml samples were then analyzed for free fluoride, total fluoride, phosphate and calcium. There were a total of 8 runs for the control and five runs for each experimental dissolution solution.

Free fluoride measurement

Fluoride ion from each sample was measured through the use of the Orion fluoride specific electrode. Prior to measurement, TISAB buffer (Total ionic strength adjustment buffer, Orion) was added to each sample in a ratio of 1:1. This allowed the pH of the solution to be adjusted to approximately 5.0, which was within range to allow for electrode accuracy. In order to remain within the measurable range of the electrode (0.020 ppmF to 50.0 ppmF), dissolution solutions with 100 ppm fluoride concentrations were diluted from 0.1ml to 1.0 ml with DDW prior to the addition of TISAB.

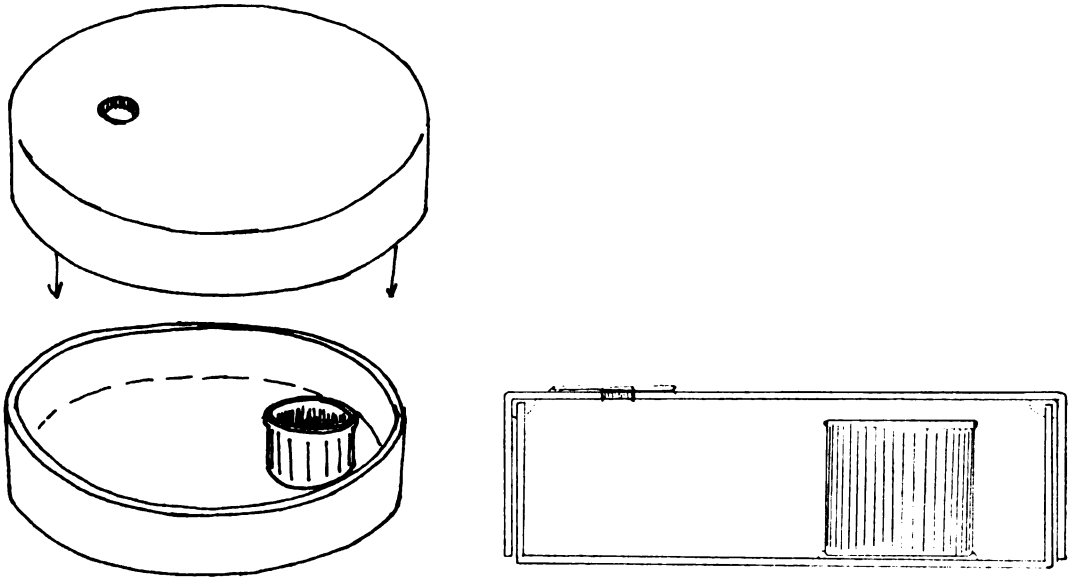
Because the electrode readings were in volts, fluoride standards were utilized to develop a volt/F relationship. Standards were mixed daily with TISAB in a ratio of 1:1 to mimic sample treatment. At least two standards above and two standards below the expected level of fluoride in the samples were utilized to develop the relationship. This was done at least once, between fluoride measurements for each run.

Total fluoride measurement:

The method of hydrolyzing the MFP and isolating the free fluoride product was a modification of a procedure previously described (Taves 1968). A diagram of the vessel used to hydrolyze and isolate the fluoride from MFP is shown in Figure 5. It is composed of a petri dish with a 2-3 mm hole in the lid, and an up-side-down cap affixed to the base of the dish. 1ml of sample, 1ml of TISAB, and 1ml of DDW were placed into the dish base. Note that the TISAB was added to the samples immediately after being collected to minimize the loss of fluoride in the form of HF gas. In addition, 100 ppm fluoride samples were diluted from 0.1ml to 1.0ml with DDW prior to the addition of TISAB.

Before placement of the petri dish lid, 0.1 ml of 1.65 M NaOH was placed in the up-side-down cap. Once the lid was in place, 1ml of 6N HCL was added to the 3ml of liquid through the hole in the lid, and the chamber was sealed with Vaseline. The HCl provided the acidity to hydrolyze the MFP into phosphate and free fluoride. At that point any free fluoride would turn to HF gas and re-dissolve in the NaOH inside the vile cap. Once the cycle was complete (12-18 hours), the cap was removed, its contents were dried, and the material was reconstituted in 0.66 M acetic acid. The fluoride concentration could then be measured directly using the fluoride ion electrode and identically treated fluoride standards.

Figure 5: Diagrams of diffusion chamber used to hydrolyze MFP and isolate total fluoride in sample.



Phosphate measurement

Phosphate was measured by the phospho-molybdate method using a UV/visible spectrophotometer (Chen and Warner 1956). The reagent used to prepare the sample, Reagent C, consisted of 2 parts double de-ionized water, 1 part of 6 N H_2SO_4, 1 part of 2.5% ammonium molybdate, and 1 part of 10% ascorbic acid. The dissolution samples for UV spectrophotometry were prepared by mixing 0.5 ml of the dissolution sample and 0.5 ml of Reagent C. Prior to the addition of reagent C, MFP samples were diluted to keep the final concentration of phosphorus under 2.5ppm; The 1 ppm(F)MFP, 10 ppm(F)MFP, and 100 ppm(F)MFP samples were diluted from 2 to 5, from 2 to 50, and from 1 to 100 respectively. The treated samples were then allowed to incubate at 37°C for 2-18 hours in the dark and were then analyzed with the spectrophotometer at a wavelength of 820 nm. Phosphorus standards were made and analyzed in an identical manner and were used to develop a standard curve to calculate phosphate concentrations (in terms of phosphorus = P) in the samples.

Calcium measurement

An atomic adsorption spectrophotometer was used to determine the adsorption from calcium in a nitrous oxide/acetylene flame. The unit automatically calculated the calcium concentration based on pre-measured standards. It is important to note that the adsorption/concentration relationship has been shown to be linear at concentrations below 5 ppm, which is where the calcium concentrations in this study were shown to lie. Five consecutive measurements were automatically obtained, and a mean concentration and standard deviation was generated for each sample. Because all samples contained 0.1 M KCl as part of the initial dissolution solution formula, which was required for atomic adsorption measurements, the samples were measured directly. The KCl suppresses the ionization of calcium in the hot flame. The hot flame breaks up any complexes formed between calcium and phosphate.

Statistical analysis:

Rates of concentration change within the dissolution solutions (e.g. rate of calcium dissolution) were determined by plotting concentrations of the species with respect to time. Linear regression was performed on the data and the slope of the resultant straight line represented the rate of change of concentration. When values for multiple runs were grouped together, each separate concentration at each time-point was utilized to determine the rate of change of that species (rather than means at each time-point). In such instances, the initial value of the given species in each run was subtracted from each subsequent sample in that run to allow for initial differences between runs.

Independent variables were the source and concentration of each fluoride species within each dissolution solution and the dependent variable was the rate of enamel dissolution based on calcium and phosphate. Comparison of data was performed via student-T test, ANOVA, and Student-Neuman-Keuls (SNK) multiple comparison test where appropriate. The specific use of each analysis is stated with the results. Significance was designated as $P<0.05$.

RESULTS:

Data obtained from the previous methods consisted of concentrations of free fluoride before hydrolysis, fluoride after hydrolysis (total fluoride), phosphate, and calcium. In reviewing the free fluoride and total fluoride data for the MFP groups, it can be seen that free fluoride is present prior to acid hydrolysis of the MFP dissolution solutions. The concentration of MFP in each sample was calculated as the difference between total fluoride and free fluoride. This data for each 3ml sample can be found in Table 11 through 41 in the appendix.

MFP hydrolysis in solution without enamel:

Fluoride data for each MFP sample was grouped by the day the procedure was performed, and the average values with the corresponding standard errors. Comparisons between days for a single dissolution solution group were performed via T-test. Because it was the stability of MFP that was of particular importance, this analysis only involved the MFP dissolution solutions. Mean values and the associated standard errors of free fluoride ion and MFP are shown in Table 1.

Table 1: Variation in the concentration of fluoride species between days for each group.

		Day1 Mean(SEM) [N] (ppm)	Day 2 Mean(SEM) [N] (ppm)	T-test (Day1=Day 2)
1 ppm(F) MFP	Free F	0.324 (0.001) [33]	0.320 (0.002) [26]	same
	MFP	0.688 (0.015) [29]	0.699 (0.016) [23]	same
10 ppm(F) MFP	Free F	2.54 (0.014) [26]	2.43 (0.023) [39]	Day1>Day2
	MFP	5.00 (0.046) [26]	5.96 (0.026) [34]	same
100ppm(F) MFP	Free F	31.58 (0.191) [38]	32.58 (0.304) [24]	Day1<Day2
	MFP	65.69 (1.297) [36]	75.41 (0.644) [12]	Day1<Day2

There was no significant decrease in MFP associated with an increase in free fluoride from day 1 to day 2. Thus, there was no significant hydrolysis of MFP between days. However, differences in free fluoride and MFP were observed. With the exception of data obtained on the second day for the 100 ppm(F)MFP samples, all data could be appropriately grouped together with respect to fluoride concentration and source, regardless of the day that enamel dissolution was performed. For reasons to be discussed, data obtained on the second day for the 100 ppm(F)MFP group was discarded from further analysis.

Hydrolysis of MFP in solution in the presence of enamel

Monitoring the concentration of free fluoride ion and MFP during the course of the dissolution runs determined if apatite catalyzed the hydrolysis of MFP. This was done by comparing the rates of change of the concentration of free fluoride and MFP within each dissolution solution group (Table 2). There was no statistical difference in any slope as demonstrated by ANOVA with 95% certainty. All fluoride components stayed the same during the course of the dissolution procedure, and apatite catalyzed hydrolysis of MFP did not significantly occur.

Table 2: Change in the concentration of each fluoride species with time in the presence of enamel

		Slope ([F]/min)	SEM
Ave. Total F (all groups)		0.00471	0.00266
1ppm(F) MFP	Free F	0.00008	0.00002
	MFP	0.00040	0.00028
10 ppm(F) MFP	Free F	0.00006	0.00043
	MFP	-0.00001	0.00065
100 ppm(F) MFP	Free F	0.01015	0.00487
	MFP	-0.00549	0.03561

Combined fluoride data:

Tables 3-5 show the average values of total fluoride, free fluoride and MFP for all dissolution solution groups. Fluoride values for the control solution were below the recommended range for the fluoride ion electrode as directed by the manufacturer (<0.020 ppmF). Free fluoride concentrations in the NaF dissolution solutions were not statistically different than the total fluoride within each group as shown with T-test (P>0.05). This was not true for MFP solutions, in which the total fluoride was a combination of free fluoride and MFP. For the purposes of analysis, groups were named based on desired concentrations, while actual fluoride concentrations were used for analysis.

Table3: Fluoride content of control dissolution solutions

	Total F (ppm) Mean(SEM)[N]	Free F (ppm) Mean(SEM)[N]
Control	<0.020 [113]	<0.020 [117]

Table 4: Fluoride content of NaF dissolution solutions

	Total F (ppm) Mean(SEM)[N]	Free F (ppm) Mean(SEM)[N]	% of Tot.
1 ppm(F)NaF	0.9695 (0.0047) [48]	0.9842 (0.0035) [50]	101.5%
10 ppm(F)NaF	10.21 (0.0559) [63]	10.73 (0.09993) [65]	105.1%
100 ppm(F)NaF	102.1 (0.0484) [59]	103.8 (0.5819) [64]	101.7%

(Free fluoride=Total fluoride for each group per T-test with 95% certainty)

Table 5: Fluoride content of MFP dissolution solutions

	Total F (ppm) Mean(SEM)[N]	Free F (ppm) Mean(SEM)[N]	% of Tot.	MFP (ppm) Mean(SEM)[N]	% of Tot.
1 ppm(F)MFP	1.017 (0.0100) [56]	0.3222 (0.0010) [59]	31.7%	0.693 (0.011) [52]	68.1%
10 ppm(F)MFP	8.459 (0.0265) [60]	2.480 (0.016) [65]	29.3%	5.978 (0.025) [60]	70.7%
100 ppm(F)MFP	97.81 (1.231) [37]	31.34 (0.191) [38]	32.0%	65.69 (1.297) [36]	67.2%

(Free fluoride≠MFP≠Total fluoride for each group per ANOVA/SNK with 95% certainty)

Phosphate Data:

As shown in Table 6, baseline concentrations of phosphorus in the control and NaF groups were minimal (0.02-0.05ppm P). Conversely, the 1 ppm(F)MFP, 10 ppm(F)MFP and 100 ppm(F)MFP solutions had phosphorus concentrations of 1.263 ppm, 14.33 ppm and 101.8 ppm respectively. (Note that concentrations of phosphate in the 10 ppm(F)MFP and 100 ppm(F)MFP groups were based on the average of all samples because of the high variation of initial concentrations). Figures 6, 7, and 8 assist in visualization of the high phosphate measurements seen in the MFP data.

Table 6: Baseline [P] in each dissolution solution group

	Baseline [P] (ppm) Mean (SEM) [N]
Control	^ 0.0456 (0.0291)[8]
1ppmNaF	^ 0.0262 (0.0034) [4]
10ppmNaF	^ 0.0396 (0.0200) [5]
100ppmNaF	^ 0.0187 (0.0022) [5]
1ppmMFP	^ 1.263 (0.005) [5]
10ppmMFP	* 14.33 (0.12) [64]
100ppmMFP	* 101.8 (0.6) [39]

Phosphate concentration based on
^= mean initial value, or *= mean of all values (estimate)

Figure 6: Total concentration phosphorus in the 1 ppm(F)MFP dissolution solution with time. (Data is displayed as Mean ± SEM at each time point)

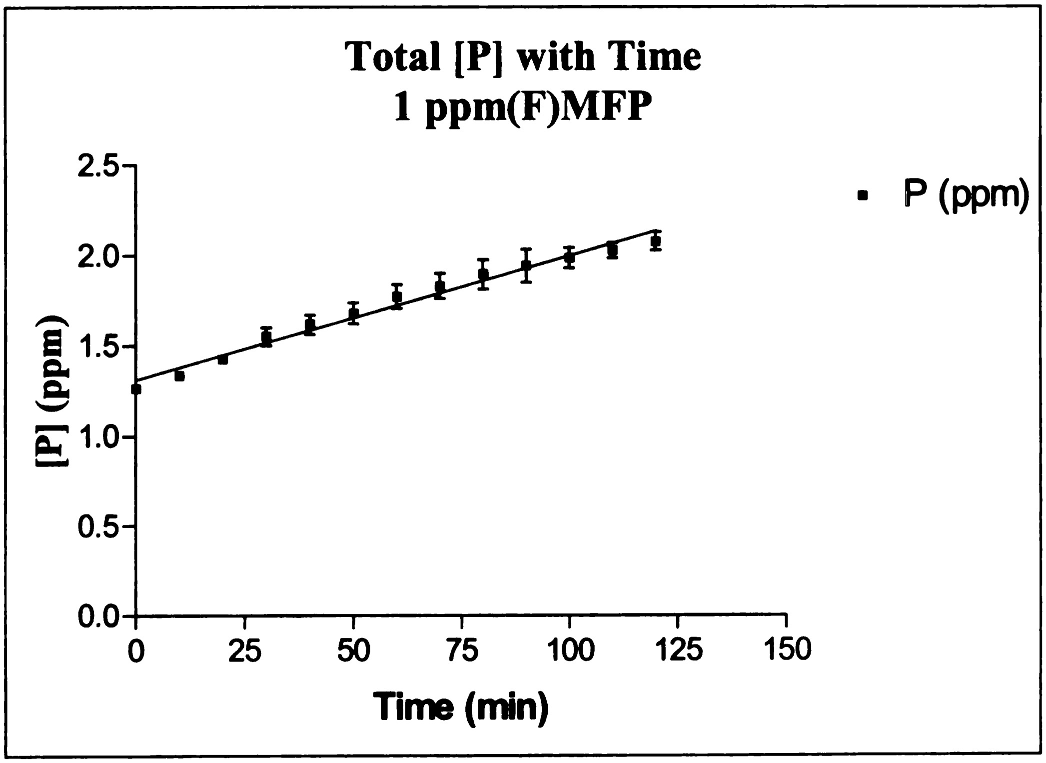


Figure 7: Total concentration phosphorus in the 10 ppm(F)MFP dissolution solution with time. (Data is displayed as Mean ± SEM at each time point)

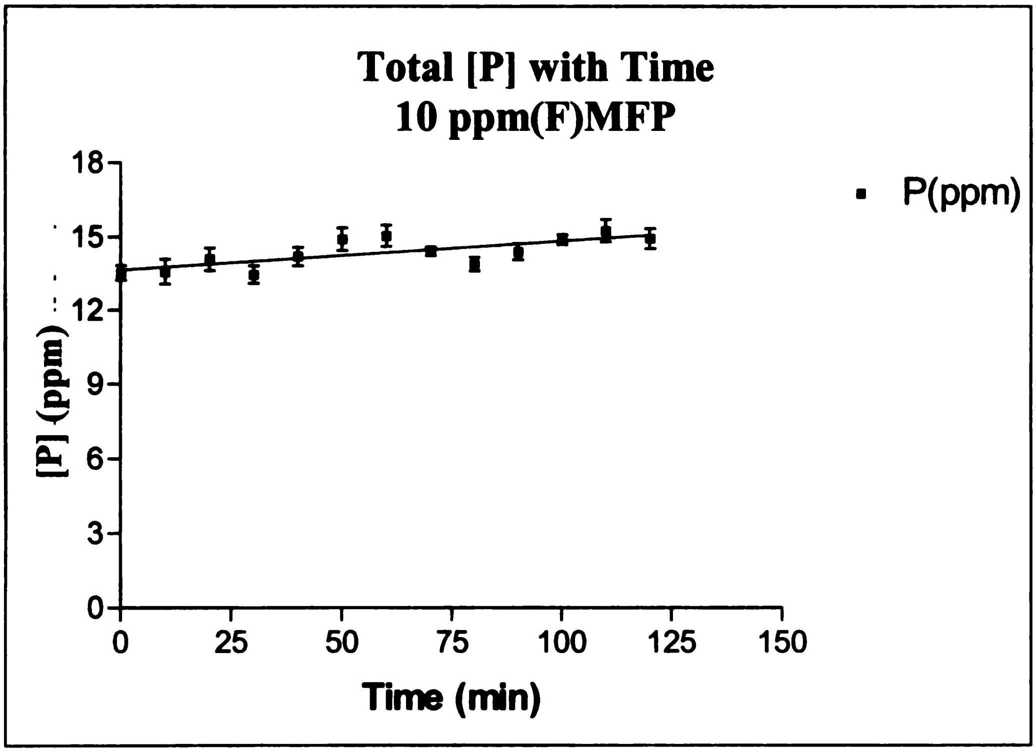
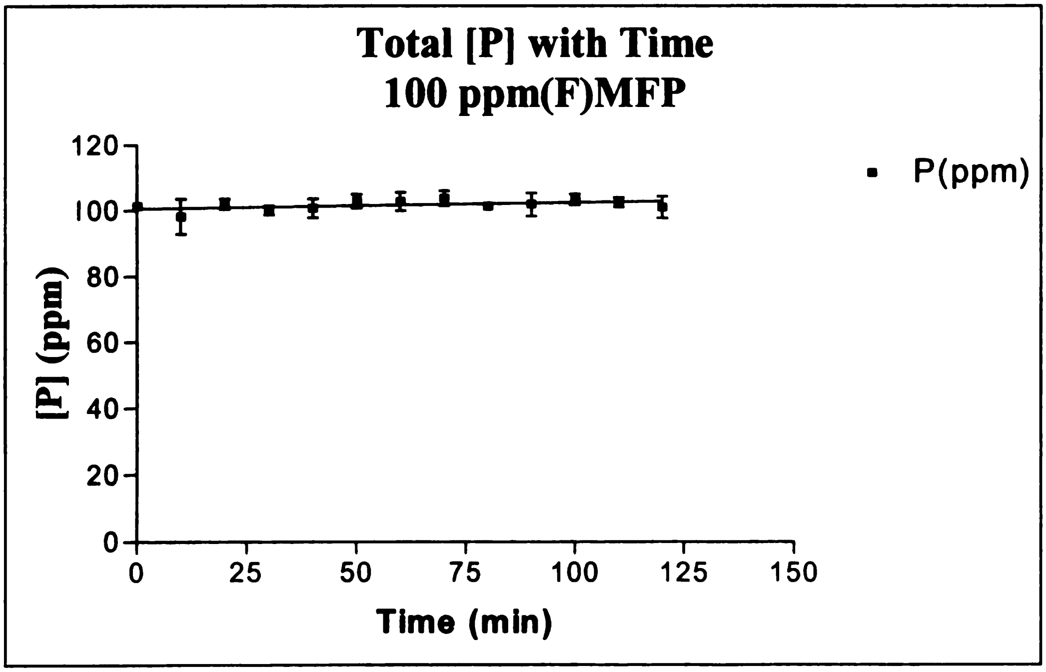


Figure 8: Total concentration phosphorus in the 100 ppm(F)MFP dissolution solution with time. (Data is displayed as Mean ± SEM at each time point)



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Figures 9 and 10 show the plots and regression lines used to determine the rates of dissolution as based on phosphate data. Rates were determined using the concentrations of phosphate for all samples with respect to time. Though each data point is shown as the mean ± SEM at each time point, all values for each run within the specific group were used to calculate these rates. To correct for initial levels of phosphate, concentrations of phosphate at time zero were subtracted from all subsequent measurements. All data for each dissolution solution source was then plotted with respect to time, and linear regression was performed on the data. The slopes of the resulting lines represented the rates of demineralization. (Table7)

As shown, there was a trend of decreasing dissolution rates with increasing concentration of fluoride from NaF. With the MFP dissolution solutions, there was an apparent increase in dissolution rates related to increasing total fluoride concentration. However, there was also a dramatic increase in variance of dissolution rates. Because of such high variation in the MFP dissolution groups, rates based on phosphate could not be used in the development of the conclusion.

Figure 9: [P] vs. time for NaF and control data. The value of [P] at time zero was subtracted from itself and all subsequent data in each run. (Data are displayed as Mean ± SEM at each time point)

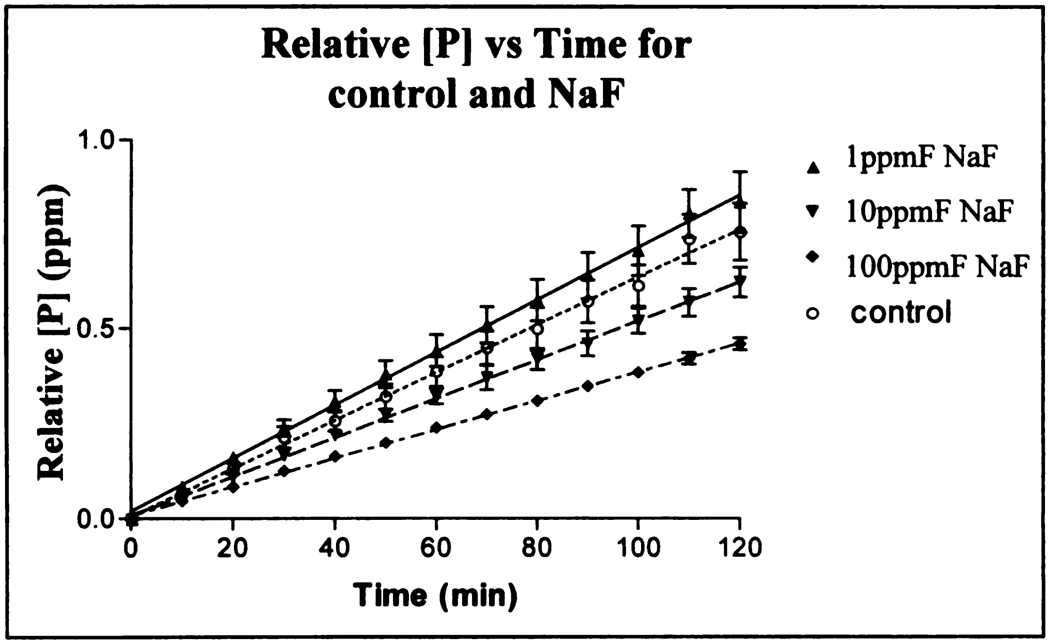


Figure 10: [P] vs. time for MFP and control data. The value of [P] at time zero was subtracted from itself and all subsequent data in each run. (Data are displayed as Mean ± SEM at each time point)

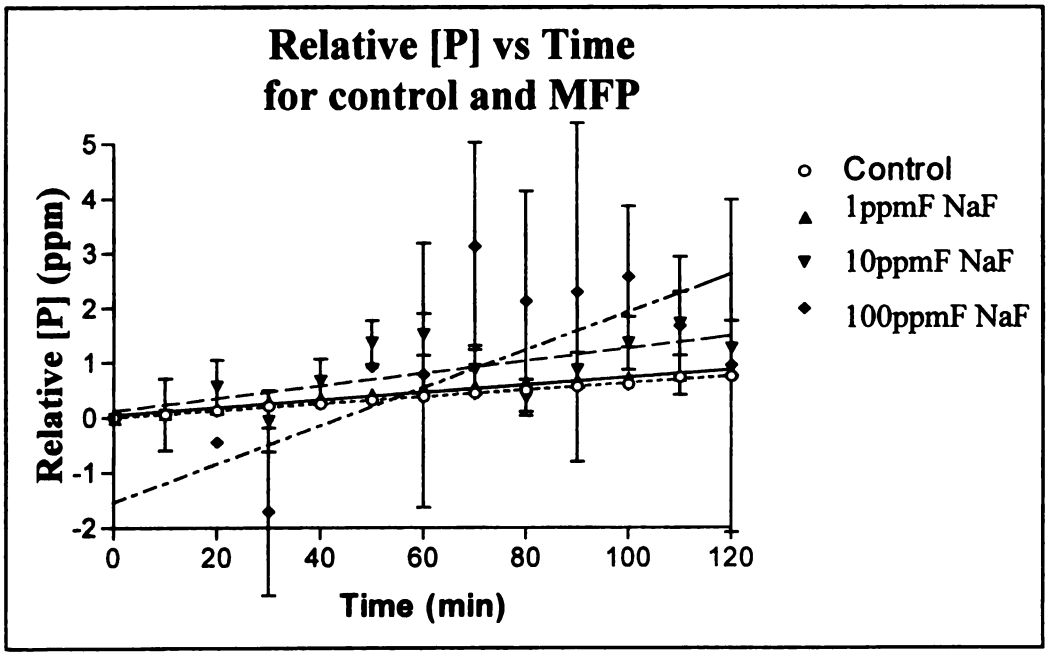


Table 7: Dissolution Rates Based on [P]

	Dissolution Rate (SEM) ([P]ppm/Min)
Control	0.00631 (0.00011)
1ppm(F)NaF	0.00695 (0.00009)
10ppm(F)NaF	0.00512 (0.00004)
100ppm(F)NaF	0.00377 (0.00003)
1ppm(F)MFP	0.00683 (0.00030)
10ppm(F)MFP	0.01141 (0.00320)
100ppm(F)MFP	0.01916 (0.01026)

Calcium Data:

For each dissolution solution group, the concentration of calcium for every sample was plotted against time. To correct for any enamel dissolution prior to the start time, the calcium values at time zero (which were all relatively similar for each group) were subtracted from all subsequent values in that dissolution run. Linear regression was then performed, resulting in a line with a slope that is representative of the rate of enamel dissolution with respect to calcium. These rates are listed in Table 8. With 95% confidence, ANOVA showed that a difference did exist within the data and Student Newman-Keuls multiple comparison pointed out those differences. These results are shown in table 8 and summarized in Figure 11. Graphical representation of the dissolution rates for NaF and MFP groups along with the control are located in figures 12 and 13.

Table 8: Dissolution rates based on calcium data

	Rate ppmCa/Min	**SD**	**Statistically sig groups**
control	0.01474	0.00041	**A**
1 ppm(F)NaF	0.01454	0.00061	**A**
10 ppm(F)NaF	0.01111	0.00041	**B**
100 ppm(F)NaF	0.00720	0.00020	**C**
1 ppm(F)MFP	0.01411	0.00044	**A**
10 ppm(F)MFP	0.00687	0.00025	**C**
100 ppm(F)MFP	0.00196	0.00005	**D**

Figure 11: Grouping of solutions based on dissolution rate with respect to calcium. (Fastest dissolution to slowest)

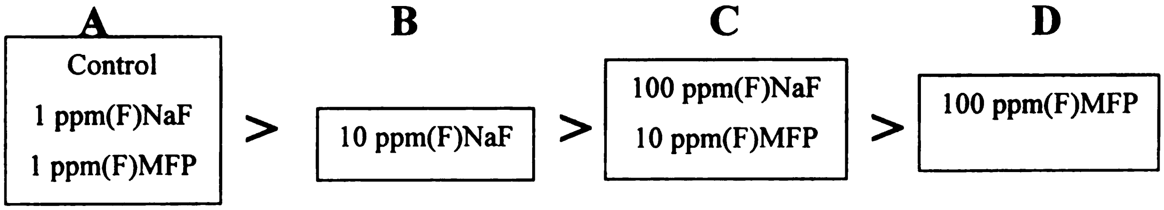


Figure 12: [Ca^{2+}] vs. time for NaF and control data. The value of [Ca^{2+}] at time zero was subtracted from itself and all subsequent data in each run. (Data are displayed as Mean ± SEM at each time point)

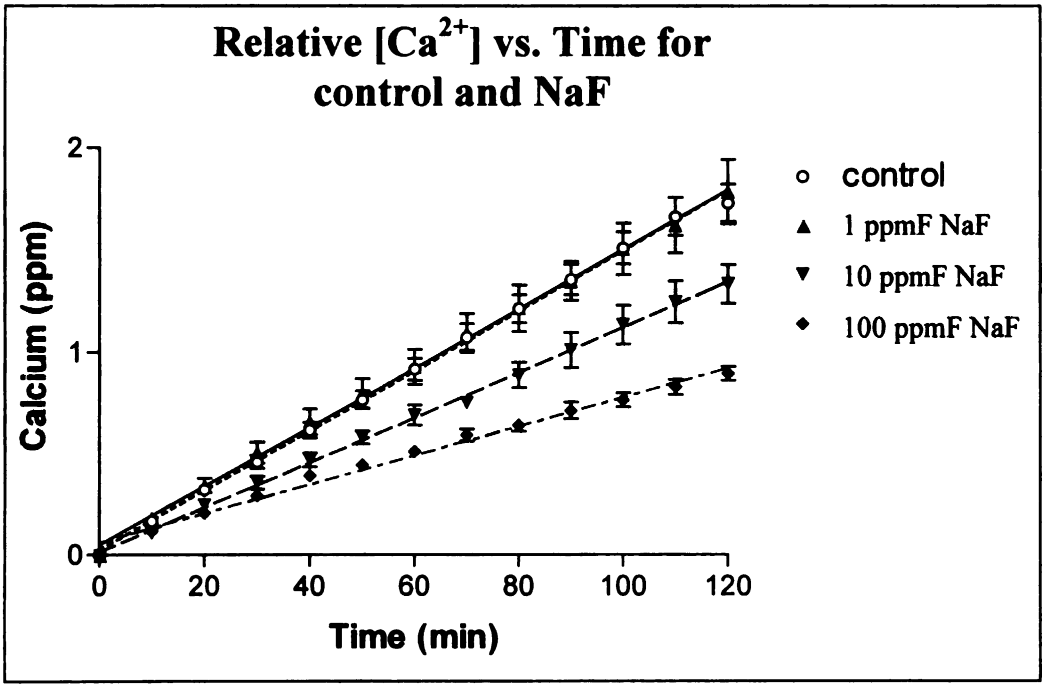
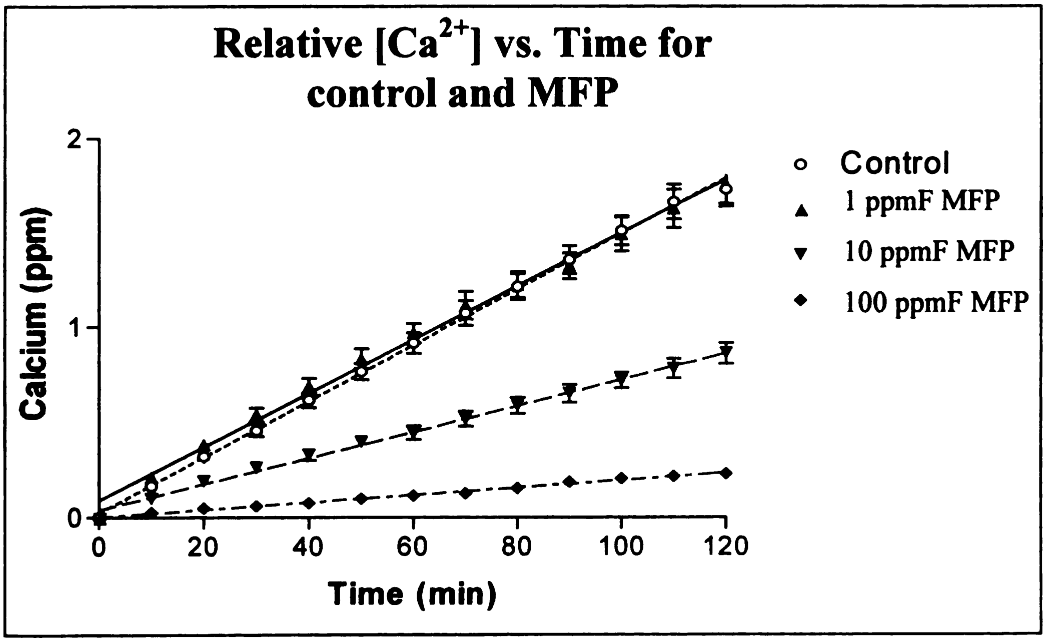


Figure 13: [Ca^{2+}] vs. time for NaF and control data. The value of [Ca^{2+}] at time zero was subtracted from itself and all subsequent data in each run. (Data are displayed as Mean ± SEM at each time point)



A more appropriate relationship of MFP and NaF can be drawn by plotting the dissolution rates against total fluoride concentration (Figure 14). Figure 15 illustrates that the relationship of the rate of dissolution for both NaF and MFP with respect to Log[F] was linear. The NaF relationship was represented with a line of slope -0.0036(0.0006) and an intercept of 0.0140(0.0007). With the MFP dissolution solution, the representative line had a slope of -0.0062(0.0006) and an intercept of 0.0137(0.0006). Correlation coefficients (r^2) for the NaF and the MFP samples were 0.7611 and 0.9299 respectively. The horizontal line represents the rate of dissolution with no fluoride as shown by the control solution, and has a slope of zero.

In comparing the three lines (control, NaF, and MFP) through ANOVA and SNK statistical analysis, it was demonstrated that all three lines were different with 95 % certainty. With respect to total fluoride concentration, the MFP solutions produced the greatest reduction of the rate of demineralization, followed by NaF. Both MFP and NaF significantly reduced the rate of dissolution compared to the control.

Figure 14: Rate of dissolution with respect to the total concentration of fluoride found within the dissolution solutions. (Data are displayed as Mean ± SEM of each run at the corresponding [F])

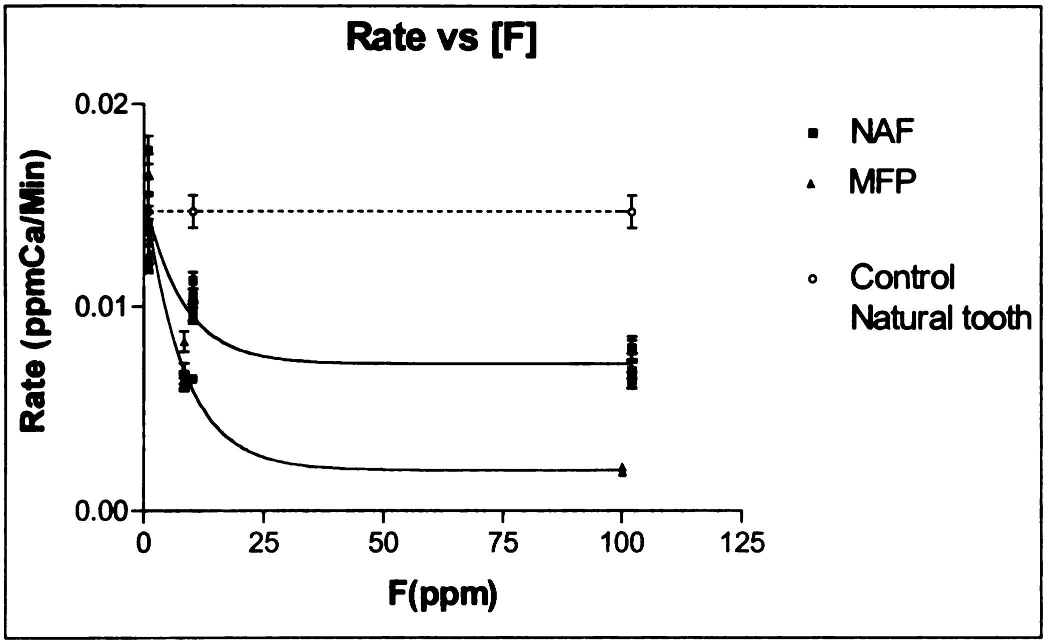
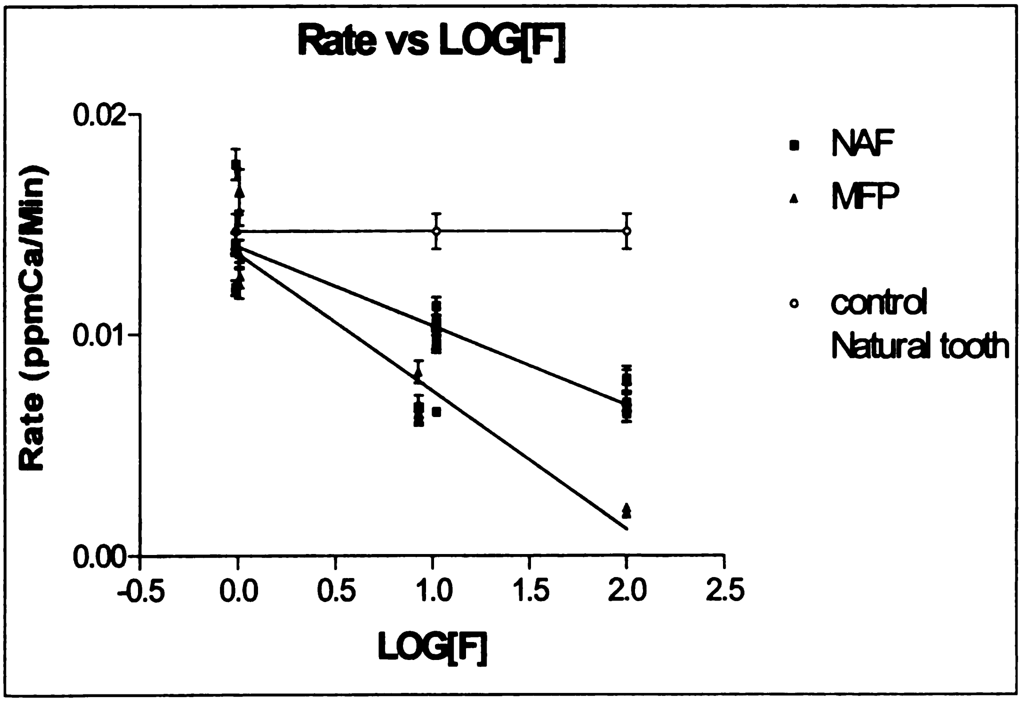


Figure 15: Rate of dissolution with respect to the logarithm of the total concentration of fluoride found within the dissolution solutions. (Data are displayed as Mean ± SEM of each run at the corresponding Log[F])



DISCUSSION:

Enamel substrate:

The substrate dissolved through the course of each dissolution procedure was bovine enamel. Bovine enamel has been deemed to be an acceptable alternative to human enamel for *in-vitro* dissolution procedures (Report 1986). Teeth were sectioned, sized, polished, and treated in such a way that ensured that a uniform enamel surface would be exposed to the dissolution solution for each sample (Report 1986; Featherstone et al. 2003). As the potentially high fluoride content on the enamel surface of the previously used samples was not likely to surpass the 100 micrometers thickness removed during re-polishing (Featherstone et al. 2003), enamel blocks were used multiple times.

Intrinsic fluoride, which may be present throughout the thickness of enamel, has been shown to produce no significant effect on the solubility of the enamel. (Nelson et al. 1992) It has also been demonstrated that such intrinsic fluoride does not significantly change the fluoride concentration in a 200ml solution surrounding the sample during enamel dissolution. (Featherstone et al. 1990)

Content of Dissolution solutions:

The dissolution solution in this study was designed to produce enamel demineralization without significant breakdown of MFP and to maintain a given ionic strength. To meet these requirements all dissolution solutions were composed of 0.1 M acetic acid and 0.1 M potassium chloride adjusted to pH 4.5. Solutions of 0.1 M acetic acid, adjusted to pH 4.5, have been utilized in prior studies and have been proven to be adequate in producing detectable rates of demineralization (Phan and Featherstone 1999; Featherstone et al. 2003). It is also note-worthy that the selected pH lies above the threshold of which MFP is likely to be hydrolyzed (Ingram 1972). Potassium chloride at 0.1M was chosen, not only to control ionic strength, but also because it is required for atomic adsorption measurements of calcium. Thus, raw samples of the dissolution solution could be drawn and measured for calcium directly.

Other than the fluoride content of the dissolution solutions, all factors were constant. Thus, any change in the rate of dissolution was considered to be a result of the fluoride source and concentration. Control solutions contained only background fluoride (below the lower threshold of the fluoride electrode) and experimental dissolution solutions contained specific added concentrations of either NaF or MFP. This range was chosen to reflect a logarithmic increase of the concentration of fluoride, while still remaining within the range of detectible change in dissolution rate as determined with NaF by Featherstone and colleagues (Featherstone et al. 1990).

The actual total fluoride concentrations for 1 ppm(F)NaF, 10 ppm(F)NaF, and 100 ppm(F)NaF dissolution solutions were shown to be 0.97ppm, 10.2ppm, and 102ppm respectively. These values were statistically similar ($P>0.05$) to the corresponding free fluoride concentrations as shown by the T-test. This confirmed that essentially 100% of the sodium fluoride in solution liberated fluoride ions, as expected from simple chemistry considerations. For these dissolution solutions, any change in the rate of dissolution can be attributed to the presence of fluoride ion alone.

The total fluoride concentrations in the 1,10, and 100 ppm F as MFP dissolution solutions were 1.02 ppm, 8.46 ppm, and 100.2 ppm F respectively. Free fluoride was measured to be about 32% of the total fluoride in the 1 and 100 ppm(F)MFP solutions, and 29% of the total fluoride for the 10 ppm(F)MFP solution. The observation that both free fluoride and MFP were present in dissolution solutions was in agreement with previous research (Pearce and More 1975; Eanes 1976; Arends et al. 1980; Bruun et al. 1984). The percentages of free fluoride in this study were consistent with Bruun and colleagues, who found that approximately 30% of fluoride in MFP dentifrices existed as free fluoride (Pearce and More 1975; Bruun et al. 1984).

The source of this free fluoride was not evident. That is, it was unknown if the free fluoride was present as a true impurity of the MFP powder, or if it was a product of MFP hydrolysis prior to initial fluoride measurements. Table 9 shows the concentration of phosphorus observed in the MFP samples, the equivalent fluoride concentration if all of the measured phosphorus was from MFP, and the concentrations of each fluoride component. Prior to discussing these results, it must be considered that the acidity of the Reagent C was likely to cause hydrolysis of MFP prior to measuring phosphate. Thus, it is expected that the total concentration of phosphate represent the combination of that which was present prior to analysis and that which was liberated during treatment. In the 1 and 10 ppm(F)MFP dissolution solutions, the concentration of fluoride equivalent to the observed phosphate was greater than the determined concentration of MFP. This indicates that at least part of the fluoride impurity was a product of MFP hydrolysis. In the 100 ppm(F)MFP sample only, was phosphate present in equal or greater concentrations compared to the total concentration of fluoride. This signifies that the free fluoride impurity is potentially completely from MFP hydrolysis. Because of the variation of findings, the degree of free fluoride originally present as MFP is unknown. Likewise, though the value is unknown, it can be assumed that some concentration of phosphate is initially present in the dissolution solution. Thus the presence of MFP, free fluoride, and phosphate in the MFP dissolution solutions must be considered in the development of the conclusion.

Table 9: [P] in MFP dissolution solution, its equivalent concentration of fluoride, and the actual concentration of each fluoride component in MFP dissolution solution in terms of free fluoride.

	[P] Mean (SEM) [N]	**Equivelent [F] (ppm F) if 100%P was from MFP**	**Total Fluoride (ppm F)**	**Free fluoride (ppm F)**	**MFP (ppm F)**
1 ppm(F)MFP	^ 1.263 (0.0051) [5]	0.77	1.02	0.32	0.69
10 ppm(F)MFP	* 14.33 (0.1207) [64]	8.79	8.46	2.48	5.80
100 ppm(F)MFP	* 101.8 (0.6432) [39]	62.42	97.81	31.34	65.69

Concentration of phosphate based on: ^ initial value or * average of all values

Hydrolysis of MFP in the dissolution solution with and without enamel.

Concern has been raised regarding the stability of MFP in water (Eanes 1976; Heidbuchel 1991). Because solutions were stored over the course of 2 to 3 days, the stability of the MFP dissolution solution was considered. Analysis of data showed that that there was not a significant degree of hydrolysis of MFP during this 2-3 day period. Specifically, there was no significant decrease of MFP associated with an increase in free fluoride. This confirms the observation by Ingram (1972) that MFP is stable in solutions above pH 4 and rejects the concerns made by Eanes (1976) and Heidbuchel (1991).

In reviewing the details of the results, differences in free fluoride and MFP between the first and second day in the 10 ppm(F)MFP and 100 ppm(F)MFP groups were observed (Table 1). In visualizing the trend of free fluoride measurements for the 10 ppm(F)MFP and 100 ppm(F)MFP groups on each individual day, it can be seen that the observed trends followed similar values and patterns (Figure 16 and 17). Differences in free fluoride measurements for the 10 ppm MFP and 100 ppm MFP dissolution solutions can therefore be disregarded. On the other hand, the increased MFP concentration on the second day for the 100 ppm MFP dissolution solution did not have similar values (figure 18) and reflected an unlikely significant increase in total fluoride as shown by T-test (Table 10). Likely, the perceived increase in total fluoride was a result of error in the process of fluoride measurement. In addition, only one run was performed on the second day, limiting its strength when considered alone. For these reasons, data for the 100 ppm(F)MFP dissolution solution on the second day was discarded from the analyses of dissolution rates.

Figure 16: Consecutive free-fluoride measurement made on two days for the 10 ppm(F)MFP dissolution solution.

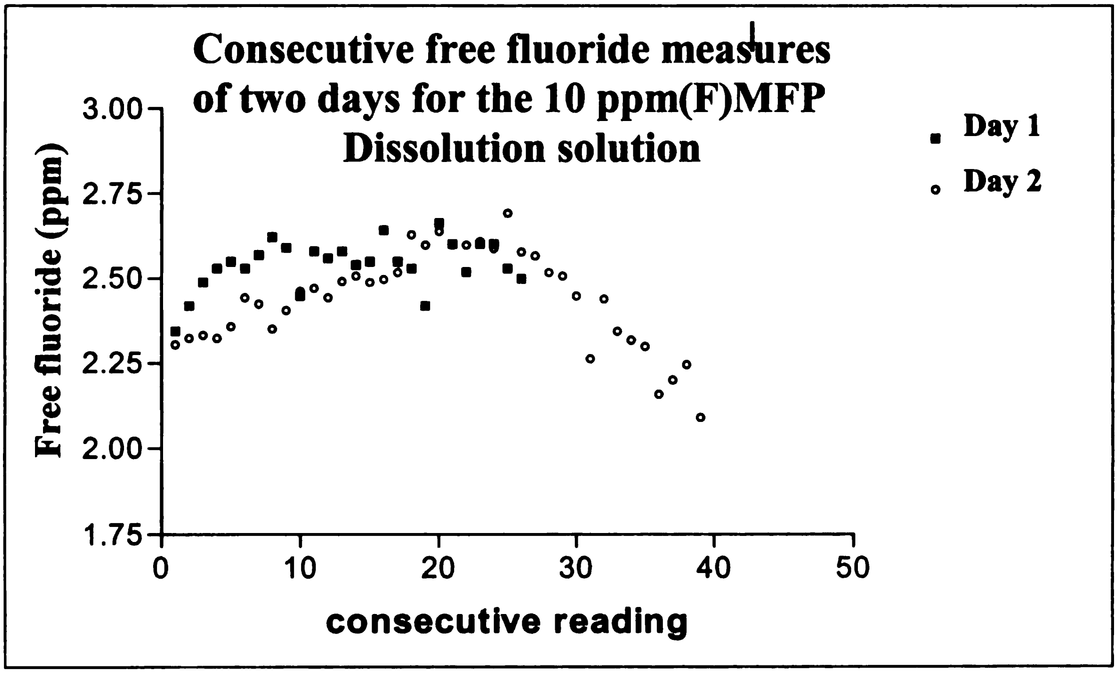


Figure 17: Consecutive free-fluoride measurement made on two days for the 100 ppm(F)MFP dissolution solution.

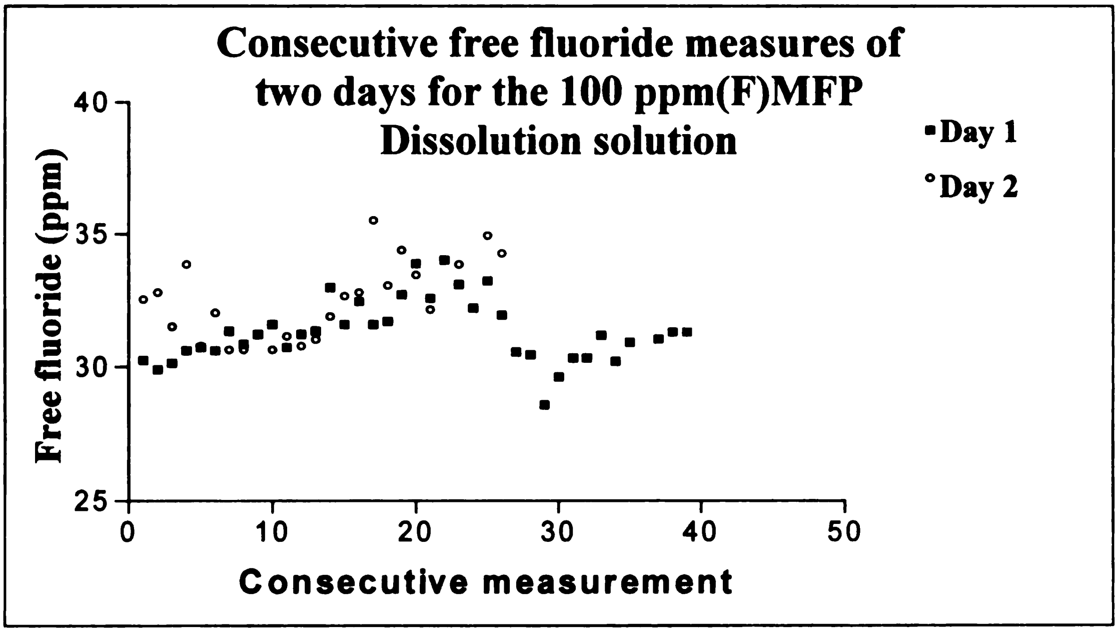


Figure 18: Consecutive MFP measurement made on two days for the 100 ppm(F)MFP dissolution solution.

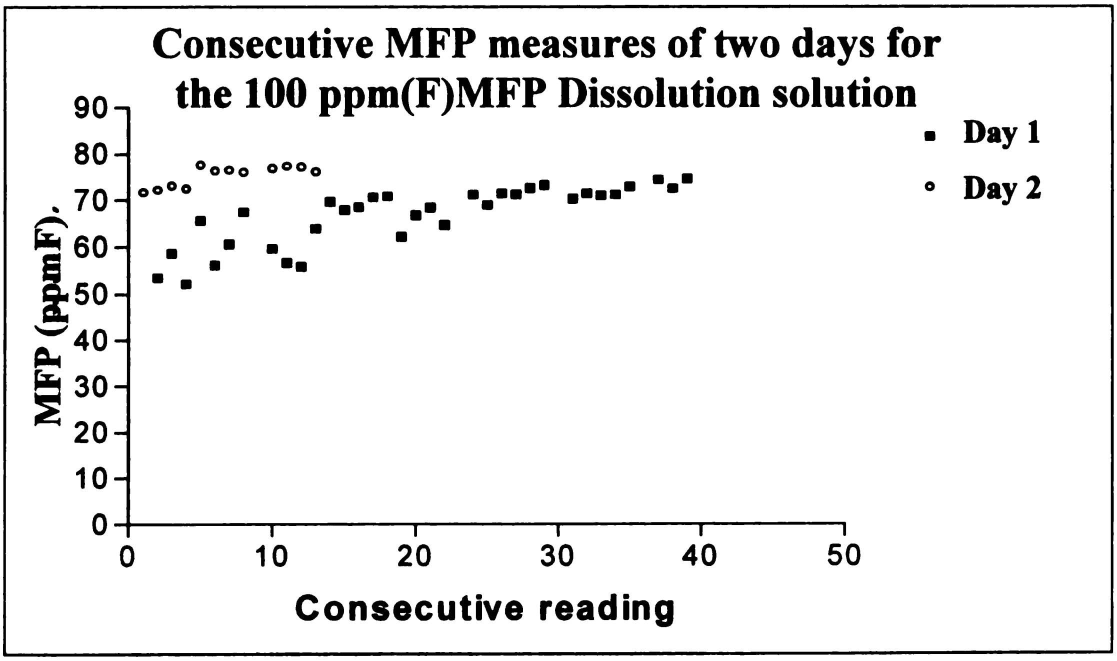


Table 10: Total fluoride measurement on day 1 and day 2 that 100 ppm(F)MFP dissolution group was run.

	Day1 Mean (SEM) [N]	**Day2** **Mean (SEM) [N]**	**T-test** **Day1=Day2**
100 ppm(F)MFP Total Fluoride	97.81 (1.231) [37]	107.1 (0.415) [13]	Day1<Day2 (Significant)

Additional concern arose with the proposal that apatite may be able to catalyze the hydrolysis of MFP (Gron et al. 1971; Eanes 1976). The majority of research has not been able to reproduce these findings and has disregarded such an occurrence. For completeness, this study entertained such a possibility. This was accomplished by comparing the rates of change in free fluoride to that of MFP during the course of enamel dissolution. As shown in table 2, in both the 10 ppm(F)MFP and 100 ppm(F)MFP groups, the change of free fluoride was positive and the change of MFP was negative. By comparing slopes, it was shown that this trend was not significant. Thus, there was no significant hydrolysis by apatite during the 120-minute dissolution.

What was of interest was an increasing concentration of total fluoride in almost all samples during the dissolution runs. When combining all total fluoride data, it was observed that the total fluoride rose at an average rate of 0.0047 ppmF/min. The only potential source for additional fluoride was from intrinsic fluoride within bovine enamel, which has previously been shown not to effect the concentration of fluoride in the surrounding solution (Featherstone et al. 1990, Nelson et al. 1992). Rather, the observed change can be considered to be an artifact produced by the progressive drop in millivolt readings with time by the fluoride electrode.

Rate of dissolution:

The rate of dissolution of enamel was determined as the rate of increase in calcium concentration in the dissolution solution with time. Because of the magnitude of variance observed with increased MFP concentrations, phosphate data was not used for the purpose of determining the rate of dissolution. Recall that phosphate was present in MFP samples after treatment with Reagent C in phosphate analysis. Because the spectrophotometer used in phosphate analysis is only accurate up to 2.5 ppm, MFP samples were diluted at ratios from 2:5 to 1:100. This dilution magnified variability within the spectrophotometer, and was likely the cause of the observed variance. Calcium data, which were all measured directly, lacked the drawbacks seen with phosphate.

Plotting the concentration of calcium with respect to time, and performing linear regression, determined the rate of demineralization. The first observation was that the rates of demineralization of enamel in the 1 ppm NaF and 1 ppm(F)MFP dissolution solutions were not different when compared to each other, nor to the control (ANOVA $p>0.05$). This signifies that 1ppm total fluoride from either NaF or MFP has no measurable effect on the dissolution of enamel during an acid attack comparable to caries activity. What may be more meaningful is that fluoride in concentrations equivalent to those of fluoridated water systems will have no effect on the rate of demineralization during a caries challenge. Thus, under these circumstances, there is no immediate benefit of fluoridated over non-fluoridated water with respect to reduction of demineralization. It is also made clear that fluoride in saliva, which can range between 0.005 ppmF and 0.1 ppmF (Zero 1992) will also play a negligible role in the inhibition of demineralization during an acid attack. Rather, the favorable effects of low concentrations of fluoride on the dentition are from long exposures between meals and snacks (Dijkman et al. 1983). This is of particular importance in young children who need to eat more frequently.

These results were different than the findings of Featherstone and colleagues, in which 1ppm fluoride significantly reduced the rate of demineralization of synthetic carbonated hydroxyapatite, with 3% carbonate content (Featherstone 1990). It is probable that the difference in substrate was enough to produce such a different effect. The synthetic carbonated apatite, although it had comparable carbonate content to dental enamel had much smaller crystals and a much higher dissolution rate than enamel. In addition, the specific surface area studied with synthetic apatite was about ten times that used in this study (Featherstone et al, 1990).

Beyond 1ppm fluoride, both MFP and sodium fluoride progressively reduced the rate of dissolution. Though a comparison of the rates of dissolution for each group was made, the results can be misleading. This is because the actual total concentrations of fluoride in each group are slightly different than that which was desired. For example, the 100 ppm(F)NaF group had a total fluoride concentration of 102.1 ppmF, while the

100 ppm(F)MFP group had a total fluoride concentration of 97.81 ppmF. To compare the rate of dissolution between the two is inappropriate. Rather, the dose dependent curves based on the actual total concentration of fluoride were evaluated.

In agreement with prior studies, the relationship of the rate of dissolution to the logarithm of the concentration of fluoride from NaF is linear (Featherstone et al. 1990). With synthetic apatite, it had previously been reported that the dissolution rate/Log[F] relationship to be represented by the line "Rate = -1.21log[F] + 3.69" for NaF. (Featherstone et al. 1990) This study showed the relationship to be, "Rate = -0.0036log[F] + 0.1401" for bovine enamel, confirming the much higher dissolution rate of the synthetic apatite crystals in the previous study.

In the MFP solutions, the relationship of rate of dissolution to Log of total fluoride (F_T) was represented by the line "Rate = -0.00624log[F_T] + 0.0137." The resulting decrease in the rate of dissolution with respect to total fluoride in MFP samples is significantly greater than that from NaF. It is more appropriate to report that, with respect to total equivalent fluoride concentration, the combination of about 70% fluoride from MFP, 30% fluoride as free fluoride, and an unknown concentration of phosphate (<101.8 ppmP) is more effective at reducing the rate of demineralization than 100% of fluoride as free fluoride ion. This finding can also be translated as MFP in solution, that is about 30% hydrolyzed, has a greater ability to reduce the rate of dissolution of enamel than if it were 100% hydrolyzed. This latter statement may not be completely correct, because it does not accurately take into consideration the effect of phosphate liberated from MFP hydrolysis. The effect of phosphate at these concentrations has not been measured in this model but it is likely there is some effect simply by the law of mass action. This question can only be resolved by further experimental work.

The linear trend observed in both NaF and MFP solutions demonstrates adsorption of fluoride onto the surface of the enamel apatite crystals, also known as Langmuir adsorption. This concept is based on the fact that specific sites over the surface of a crystal are capable of accepting ions such as fluoride. When fluoride is allowed to fill all of these sites, realizing that they exist in equilibrium with the environment, it is said to create a monolayer of fluoride. When simultaneously treated with an acid, it becomes more difficult to achieve this monolayer, and the adsorption characteristics of fluoride become more evident. Once a percentage of sites are filled, fewer total sites are available and the concentration of fluoride required to fill the remainder of sites increases exponentially.

Recall that a unit cell of hydroxyapatite has 6 phosphate groups and 2 hydroxide groups. Thus, more sites are available for an $\mathrm{MFP/PO_4^{3-}}$ exchange than a $\mathrm{F^-/OH^-}$ exchange. The fact that more sites are potentially available for MFP than free fluoride may be related to the greater reduction in rate of dissolution with the MFP solutions compared to the NaF solutions. Other factors may involve differences in affinity of MFP and free fluoride, or interactions near or within the crystal surface.

It must still be considered that the MFP samples in this study also contained free fluoride and phosphate. If MFP and phosphate both had no effect, then the decrease in the rate of demineralization would be expected to be less dramatic with the MFP solution than from NaF alone. Similarly, if MFP and phosphate had an equal effect compared to free fluoride, then the reduction in dissolution rate with the MFP dissolution solutions would be identical to that seen with the NaF dissolution solutions. As discussed, reduction of the rate of dissolution by the MFP solution was actually significantly greater than NaF. Therefore, it is reasonable to assume that unhydrolyzed MFP and phosphate are more effective than NaF alone. Based on data obtained in the current study, no quantitative conclusion could be made regarding the effect on the rate of dissolution of enamel by pure MFP.

Considerations

Error may arise from the presence of condensed phosphates. Such species include pyrophosphate ($P_2O_7^{4-}$), trimetaphosphate ($P_3O_{10}^{5-}$), and cyclic phosphate ($P_3O_9^{3-}$) (Ingram 1977). There is conflicting evidence regarding the interaction of condensed phosphates with apatite, and its role in MFP function. (Eanes 1976; Duff 1983) It has been proposed that such compounds, which have been shown to be in MFP samples, may add to its ability to reduce the solubility of enamel (Harris et al. 1965; Eanes 1976). However, condensed phosphates may also inhibit the uptake of MFP by competing for the same site on apatite (Eanes 1976; Ingram 1977; Duff 1983).

In a similar manner to condensed phosphates, the presence of cyclic-MFP may also contribute to the reduction in the rate of dissolution. (Gron et al. 1971; Duff 1983) Cyclic-monofluorophosphate, which has been shown to be present in acidic solutions, can potentially bind to apatite. This would reduce the overall incorporation of MFP. Its presence, however, is believed to primarily affect the measurement of the total MFP in solution rather than the solubility of the apatite. (Duff 1983)

Another impurity with the potential to skew MFP findings is acid phosphate (HPO_4^{2-}). In order for an MFP phosphate exchange to occur, it is likely that a surface phosphate group is protonated. (Equation 6) This allows an MFP/Phosphate exchange of ions with equal charges. (Ingram 1973; de Rooij et al. 1981) Such a mechanism implies that the presence of acid-phosphate in solution may have the potential to compete with the exchange of MFP. (Duff 1983) It has been demonstrated that adjusting the pH of the solution from 7.4 to 6.5, acid phosphate drops from 60% to about 16% of the total phosphate concentration. (Ingram 1973) Thus, solutions with a lower pH, as were used in this study, are likely to be less effected by acid phosphate.

Equation 6: Detailed MFP/phosphate exchange

$$\begin{gathered}
[Ca^{2+}]_{10}[PO_4^{3-}]_6[OH^-]_2 + \mathbf{H^+} \\
\downarrow \\
[Ca^{2+}]_{10}[PO_4^{3-}]_5\mathbf{[HPO_4^{2-}]}_1[OH^-]_2 + \mathbf{FPO_3^{2-}} \\
\downarrow \\
[Ca^{2+}]_{10}[PO_4^{3-}]_5\mathbf{[FPO_3^{2-}]}_1[OH^-]_2 + \mathbf{HPO_4^{2-}}
\end{gathered}$$

Equation 6 illustrates the circumstance that most research utilized the formula of hydroxyapatite to describe the incorporation of MFP. Since the natural enamel is carbonated apatite, the above mechanism may not be the only mechanism that occurred. It is also possible that MFP would replace the more loosely bound, and equally charged CO_3^{2-} ion. This MFP not only adds a fluoride compound to the crystal, but also helps reduce the percentage of carbonate. Thus, treating carbonated apatite with MFP may be more effective at reducing the rate of demineralization than treating hydroxyapatite with MFP.

Significance

The MFP sample, in which about 30% of the total fluoride existed as free fluoride ion, reduced the rate of demineralization of bovine enamel to a greater degree than equivalent concentrations of free fluoride. This also signifies that MFP samples that are 30% hydrolyzed are more effective at inhibiting enamel dissolution than if the same sample were 100% hydrolyzed. Because similar ratios of free fluoride to MFP have been found in dentifrices, the MFP sample was clinically relevant. (Bruun et al. 1984) This should not be misinterpreted as signifying that the daily use of an MFP dentifrice would be more effective than a NaF dentifrice. Recall that in the oral cavity, MFP is likely to be hydrolyzed into free fluoride and phosphate. Therefore, only limited concentrations of MFP will come into contact with tooth structure. (Bruun et al. 1984; Ogaard et al. 1985; White 1987; Pearce and Dibdin 1995)

From this data, it would seem reasonable that if the hydrolysis of MFP were prevented in the oral cavity, its effects could be maintained. However, the circumstances in which MFP had the favorable effect in this study are not likely to occur in life situations. The oral cavity is rarely, if at all, exposed to high concentrations of either free fluoride or MFP during an acid attack. At best, 1ppm fluoride might be present as a result of drinking fluoridated water, which had no significant direct effect on the rate of dissolution of enamel.

Perhaps the incorporation, and effects of MFP would be maximized when given with a weak acid during a professional fluoride treatment. Other modifications might involve the development of an MFP-like species, which resist hydrolysis. Thus, the favorable effects of MFP could be maximized.

Though the immediate clinical relevance is limited, insight has been made in regards to the dynamic interactions between monoflurophosphate and apatite. First, a linear relationship of rate of dissolution with respect to Log[F] was seen. This indicates that the mechanism by which both NaF and MFP affect enamel is through adsorption onto the tooth mineral crystal surfaces. The fact that the rate of dissolution was decreased to a greater extent in the presence of MFP than NaF might indicate that the adsorption of MFP produces a more stable crystal than the adsorption of free fluoride. The exact mechanism for this is not known. It may be that MFP has a higher affinity to enamel than does fluoride ion. It may also be that, because there are more potential binding sites for MFP in apatite, more MFP is incorporated.

In summary the initial hypothesis was disproved; the MFP dissolution solutions did have a greater effect on the rate of dissolution of MFP than no fluoride, however its effect was greater than that of NaF. Though, it must be considered that the MFP solution also contained free fluoride as an impurity and potentially phosphate, and the reduction in dissolution rate was significantly greater than that of the free fluoride present, strongly indicating an effect of MFP in concert with fluoride. No precise quantitative conclusion regarding the effect of pure MFP can be made without further experimental work involving relevant phosphate concentrations.

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APPENDIX

Table 11: Control Free fluoride (ppm F)

	5/10/04	5/25/04A	5/25/04B	6/8/04C	6/8/04D	7/9/04F	7/15/04G	8/14/04H
Initial	0.00534	0.00526	0.00591	0.01392	0.01658	0.0033	0.00747	0.00967
0 min	0.00431	0.00588	0.00521	0.01221	0.01431	0.00454	0.00633	0.0103
10 min	0.00509	0.0061	0.00594	0.01443	0.01671	0.00478	0.00975	0.00938
20 min	0.00458	0.00594	0.00542	0.01495	0.01781	0.0051	0.01048	0.01026
30 min	0.00446	0.00638	0.00552	0.01426	0.01645	0.006	0.00963	0.01043
40 min	0.00428	0.00655	0.00533	0.01538	0.01685	0.00598	0.01066	0.01075
50 min	0.00414	0.00562	0.00528	0.01495	0.01671	0.00603	0.01052	0.01088
60 min	0.00382	0.0056	0.00516	0.01626	0.01705	0.00629	0.01155	0.01126
70 min	0.00486	0.00613	0.00526	0.01507	0.01726	0.00701	0.01066	0.01052
80 min	0.00428	0.00596	0.00523	0.01678	0.01692	0.00689	0.01284	0.01088
90 min	0.00493	0.00686	0.00487	0.01692	0.01658	0.00692	0.01295	0.01121
100 min	0.00446	0.00673	0.00478	0.01705	0.01678	0.00701	0.01215	0.0114
110 min	0.0044	0.00664	0.00505	0.01581	0.01645	0.00713	0.01174	0.0114
120 min	0.00486	0.00667	0.00547	0.01556	0.01839	0.00745	0.01052	0.01102

Table 12: Control total fluoride (ppm F)

	5/10/04	5/25/04A	5/25/04B	6/8/04C	6/8/04D	7/9/04F	7/15/04G	8/14/04H
Initial	0.00460	0.00828	0.01125	0.01111	0.00751	0.00692	0.01031	0.00577
0 min	0.001416	0.00595	0.01287	0.01071	0.00925	0.01205	0.01346	0.00495
10 min	0.00759	0.0058	0.01685	0.0088	0.00959	0.00808	0.02005	0.00443
20 min	0.00909	0.00798	0.01303	0.01271	0.01111	0.00827	0.0147	0.00521
30 min	0.00781	0.00724	0.00798	0.01007	0.01058	0.00765	0.01302	0.0051
40 min	0.04881	0.00724	0.00715	0.00971	0.01097	0.00955	0.01422	0.00537
50 min	0.00989	0.00959	0.01167	0.00936	0.0121	0.00874	0.01331	0.00521
60 min	0.01074	0.0124	0.01491	0.00818	0.01196	0.01031	0.01273	0.00589
70 min	0.00935	0.01491	0.01196	0.00733	0.01256	0.00987	0.0109	0.00626
80 min	0.00792	0.01225	0.01071	0.00971	0.01196	0.01055	0.01205	0.0051
90 min	0.012011	0.01335	0.01084	0.00838	0.00925	0.00845	0.00976	0.00589
100 min	0.01209	0.01368	0.04772	0.00959	0.00724	0.01078	0.00817	0.00456
110 min	0.011602	0.0121	0.01097	0.01287	0.00818	0.00987	0.01218	0.00632
120 min	0.01009	0.01509	0.06807	0.01097	0.0102	0.0102	0.01487	0.00639

Table 13: Control phosphate (ppm P)

	5/10/04	5/25/04A	5/25/04B	6/8/04C	6/8/04D	7/9/04F	7/15/04G	8/14/04H
Initial	0.01968	0.17309	0.17050	0.00286	0.00286	-0.0192	-0.0192	-0.0192
0 min	0.03376	0.17567	0.17697	0.00756	0.01225	-0.0169	-0.0097	-0.0145
10 min	0.12768	0.21060	0.22225	0.05688	0.08741	0.04008	0.05908	0.07808
20 min	0.22630	0.24553	0.26752	0.11089	0.16726	0.10420	0.13982	0.14694
30 min	0.30378	0.28175	0.31410	0.16256	0.24241	0.32267	0.20869	0.22768
40 min	0.38831	0.31539	0.35808	0.21892	0.31521	0.22056	0.27755	0.30843
50 min	0.47518	0.35549	0.40336	0.27294	0.38802	0.27755	0.34642	0.39629
60 min	0.56206	0.39301	0.44605	0.33165	0.45847	0.34642	0.42241	0.48178
70 min	0.62780	0.43829	0.48874	0.38802	0.53128	0.39154	0.51028	0.56727
80 min	0.73111	0.47322	0.54437	0.45143		0.44616	0.54827	0.64801
90 min	0.81329	0.50944	0.57412	0.503	0.67219	0.50553	0.61714	0.72875
100 min	0.73346	0.54825	0.61681	0.57590	0.74499	0.55065	0.68126	0.80474
110 min	0.98234		0.65950	0.61582	0.83424	0.60527	0.75013	0.8854
120 min	1.06452	0.62587	0.69702	0.67454	0.9	0.65276	0.81424	0.96860

Table 14: Control calcium (ppm Ca++)

	5/10/04	5/25/04A	5/25/04B	6/8/04C	6/8/04D	7/9/04F	7/15/04G	8/14/04H
Initial	0.3	0.2	0.17	0.14	0.13	0.1	0.09	0.01
0 min	0.42	0.32	0.26	0.2	0.23	0.16	0.12	0.17
10 min	0.66	0.47	0.43	0.32	0.38	0.36	0.27	0.33
20 min	0.88	0.63	0.63	0.44	0.55	0.43	0.42	0.49
30 min	1.01	0.77	0.79	0.5	0.73	0.54	0.56	0.67
40 min	1.18	0.91	1.01	0.68	0.9	0.65	0.69	0.81
50 min	1.37	1.03	1.16	0.82	1.04	0.78	0.84	0.99
60 min	1.52	1.24	1.32	0.9	1.21	0.89	0.97	1.18
70 min	1.75	1.34	1.53	1.06	1.37	1	1.09	1.35
80 min	1.91	1.5	1.63	1.18	1.51	1.13	1.21	1.52
90 min	2.05	1.64	1.78	1.29	1.68	1.21	1.39	1.68
100 min	2.19	1.88	1.95	1.44	1.81	1.34	1.49	1.86
110 min	2.39	2.06	2.16	1.55	1.97	1.43	1.6	2.02
120 min	2.35	2.13	2.13	1.68	2.16	1.54	1.73	2.19

Table 15: 1 ppm(F)NaF Free fluoride (ppm F)

	8/25/04A	8/25/04B	8/25/04C	8/26/04D
Initial	0.99227	0.97699	1.00779	0.94691
0 min	0.99227	0.97699	0.99613	0.94322
10 min	1.00779	0.96944	0.98843	0.9807
20 min	1.01171	0.96569	0.97699	0.93226
30 min	1.02355	0.95451	0.97321	0.96554
40 min	0.97699	0.96569	0.98079	1.0039
50 min	1.01171	0.96195	0.9846	0.96931
60 min	1.01564	0.95451	0.98079	0.98838
70 min	1.00389	0.95082	0.99227	0.98453
80 min	1.03956	0.95451	0.96195	1.01176
90 min	1.01171	0.96195	0.9846	
100 min	1	0.96195	0.97321	1
110 min	1.03553	0.96944	0.97321	1.01176
120 min	1.01564	0.9846	0.93981	1.01176

Table 16: 1 ppm(F)NaF total fluoride (ppm F)

	8/25/04A	8/25/04B	8/25/04C	8/26/04D
Initial	0.98614	0.90842	0.96215	0.95821
0 min	1.01073	0.95429		0.95821
10 min	0.94261	0.94649	0.96215	0.92726
20 min	0.96215	0.95038	0.97008	0.8827
30 min	1.00659	0.90842	0.97808	0.89731
40 min	0.96215	1.00247	0.99836	0.95038
50 min	0.97808	0.97407	0.99836	0.94649
60 min	0.9821	0.9902	0.9349	
70 min	0.96611	0.96215	0.99836	
80 min	0.92726	0.97008	0.96215	
90 min	0.98614	0.97808	1.01073	0.97407
100 min	0.9821	0.9902	0.9902	0.90842
110 min	1.00659	1.00659	1.00659	0.97008
120 min	0.98614	0.9902	1.04876	0.93875

Table 17: 1 ppm(F)NaF phosphate (ppm P)

	8/25/04A	8/25/04B	8/25/04C	8/26/04D
Initial	0.015874	0.015874	0.018145	0.020416
0 min	0.022686	0.022686	0.03631	0.022686
10 min	0.124866	0.106701	0.102159	0.108971
20 min	0.220234	0.183903	0.168009	0.181633
30 min	0.320143	0.252023	0.229317	0.254294
40 min	0.41097	0.317872	0.290624	0.315602
50 min	0.501796	0.383722	0.347391	0.388263
60 min	0.58354	0.442759	0.404158	0.442759
70 min	0.672096	0.508608	0.465465	0.497255
80 min	0.75384	0.563104	0.519961	0.565375
90 min	0.837854	0.631224	0.567645	0.624412
100 min	0.917327	0.697073	0.622141	0.690261
110 min	1.014966	0.758381	0.808336	0.740216
120 min	1.089898	0.819689	0.733404	0.801524

Table 18: 1 ppm(F)NaF calcium (ppm Ca++)

	8/25/04A	8/25/04B	8/25/04C	8/26/04D
Initial	0.11	0.09	0.1	0.1
0 min	0.14	0.14	0.2	0.12
10 min	0.37	0.34	0.32	0.3
20 min	0.56	0.51	0.45	0.46
30 min	0.77	0.66	0.58	0.62
40 min	0.96	0.81	0.71	0.76
50 min	1.13	0.96	0.85	0.86
60 min	1.3	1.07	0.95	1
70 min	1.48	1.23	1.08	1.19
80 min	1.66	1.36	1.19	1.25
90 min	1.73	1.47	1.33	1.46
100 min	1.99	1.6	1.44	1.59
110 min	2.11	1.74	1.51	1.72
120 min	2.35	1.9	1.65	1.84

Table 19: 10 ppm(F)NaF Free fluoride (ppm F)

	9/15/04F	9/15/04G	9/15/04H	9/17/05I	9/17/04J
Initial	7.93344	10.324	10.569	10.9363	11.3781
0 min	8.98029	10.6543	11.1617	11.067	11.5598
10 min	9.34662	10.5708	10.8191	11.0233	11.3781
20 min	9.27218	10.5708	11.0751	11.2438	11.6057
30 min	9.19833	10.7809	10.6517	11.3781	11.698
40 min	9.65041	10.7809	10.3651	11.3332	11.7444
50 min	9.57355	10.8661	9.85299	11.3781	11.8378
60 min	10.0441	10.7809	8.97315	11.4686	12.0746
70 min	10.1653	10.4056	10.9463	11.3332	11.8848
80 min	10.2879	10.4879	10.777	11.5141	11.7444
90 min	10.0843	10.7385	10.3651	11.4686	11.698
100 min	10.3705	10.5292	10.165	11.7444	11.6518
110 min	10.6649	9.57997	9.81466	11.4686	11.1993
120 min	10.4121	9.54233	9.32978	11.6057	10.6795

Table 20: 10 ppm(F)NaF total fluoride (ppm F)

	9/15/04F	9/15/04G	9/15/04H	9/17/05I	9/17/04J
Initial	10.1443	10.066	9.10205	8.32653	11.1321
0 min	10.5448	10.2628	9.75902	9.13736	9.98839
10 min	10.1443	10.0271	10.3827	9.10205	10.3426
20 min	10.3026	10.2231	10.4634	9.13736	10.5448
30 min	10.3026	10.0271	10.5857	9.38839	9.91134
40 min	10.3426	5.37591	10.6267	10.4634	10.2628
50 min	10.1836	9.91134	9.75902	12.5033	10.5448
60 min	9.75902	10.3827	10.7509	10.504	10.2628
70 min	10.4634	10.504	10.7926	10.5857	10.1443
80 min	10.504	9.60905	10.4634	10.1443	9.91134
90 min	10.1443	10.1443	10.2231	10.3426	9.87304
100 min	10.423	10.1443	10.5857	10.423	11.3059
110 min	10.4634	9.91134	10.668	9.68375	10.961
120 min	10.504	10.2628	9.83489	10.7093	9.06687

Table 21: 10 ppm(F)NaF phosphate (ppm P)

	9/15/04F	9/15/04G	9/15/04H	9/17/05I	9/17/04J
Initial	0.015632	0.016765		0.016765	0.119619
0 min	0.021296	0.01903	0.01903	0.01903	0.119619
10 min	0.075668	0.082465	0.071137	0.073403	0.176257
20 min	0.127775	0.139103	0.123244	0.123244	0.235161
30 min	0.182148	0.193475	0.173086	0.168555	0.312188
40 min	0.235387	0.242184	0.220662	0.213865	0.38242
50 min	0.290893	0.290893	0.263706	0.259175	0.450385
60 min	0.338469	0.340734	0.309017	0.299955	0.51382
70 min	0.391708	1.872225	0.352062	0.338469	0.578387
80 min	0.431355	0.435886	0.397372	0.386044	0.646353
90 min	0.438151	0.485727	0.442682	0.429089	0.701858
100 min	0.531038	0.537834	0.487993	0.4744	0.763027
110 min	0.580879	0.588808	0.528772	0.517898	0.824196
120 min	0.63072	0.635251	0.571817	0.580879	0.892161

Table 22: 10 ppm(F)NaF calcium (ppm Ca++)

	9/15/04F	9/15/04G	9/15/04H	9/17/05I	9/17/04J
Initial	0.14	0.1	0.09	0.1	0.12
0 min	0.18	0.2	0.15	0.14	0.16
10 min	0.27	0.33	0.23	0.25	0.3
20 min	0.38	0.48	0.41	0.38	0.42
30 min	0.48	0.59	0.46	0.46	0.63
40 min	0.6	0.69	0.58	0.56	0.76
50 min	0.71	0.82	0.7	0.66	0.85
60 min	0.82	0.91	0.76	0.76	1.04
70 min	0.92	1.03	0.88	0.86	0.15
80 min	1.02	1.08	0.94	0.95	1.29
90 min	1.09	1.23	1.04	1.03	1.5
100 min	1.21	1.4	1.15	1.11	1.64
110 min	1.37	1.5	1.25	1.17	1.77
120 min	1.44	1.55	1.32	1.34	1.85

Table 23: 100 ppm(F)NaF Free fluoride (ppm F)

	10/06/04K	10/6/04L	10/06/04M	10/08/04N	10/08/04O
Initial	99.6038	96.4901	100.95	101.884	98.0676
0 min	100	92.3671	106.673	101.488	97.3051
10 min	104.05	97.2593	100.553	102.679	94.314
20 min	102.005	99.6038	100.95	100.702	103.575
30 min	104.464	102.005	106.673	103.884	98.836
40 min	103.638	105.715	111.836	104.695	99.2225
50 min	103.638	105.296	110.087	104.695	101.178
60 min	104.879	103.227	113.612	107.166	100.391
70 min	106.136	105.296	102.553	101.884	92.8531
80 min	107.407	102.411	107.516	102.679	91.7724
90 min	111.757	106.558	107.941	104.695	100.391
100 min	109.997	107.407	109.655	102.281	102.369
110 min	108.264	107.835	106.253	99.5345	
120 min	108.264	109.561	109.655	101.095	109.391

Table 24: 100 ppm(F)NaF total fluoride (ppm F)

	10/06/04K	10/6/04L	10/06/04M	10/08/04N	10/08/04O
Initial	106.892	105.219	101.951	99.9606	101.15
0 min	106.471	101.951	104.805	99.5672	96.0954
10 min	103.164	109.02	103.164	99.9606	99.5672
20 min	106.892	101.15	102.354	99.1753	99.5672
30 min	100.752	103.164	103.164	103.164	10.3531
40 min	106.471	106.052	99.5672	101.15	94.2192
50 min	104.392	98.0089		99.5672	93.8484
60 min	104.392	98.0089	103.164	102.758	93.8484
70 min	103.164	100.356	103.164	96.8563	106.052
80 min	106.052	104.805		96.4751	98.3962
90 min	106.892	103.572	103.981	92.7447	103.164
100 min	106.052	105.219		101.15	
110 min	106.471	106.052	106.892	103.572	105.219
120 min	102.758	100.752	103.164	101.55	

Table 25: 100 ppm(F)NaF phosphate (ppm P)

	10/06/04K	10/6/04L	10/06/04M	10/08/04N	10/08/04O
Initial	0.016412	0.016412	0.016412	0.016412	0.016412
0 min	0.029883	0.018657	0.018657	0.018657	0.018657
10 min	0.074787	0.088258	0.063561	0.056825	0.05458
20 min	0.112955	0.112955	0.103974	0.103974	0.092748
30 min	0.158981	0.157858	0.142142	0.146632	0.128671
40 min	0.189291	0.198271	0.182555	0.187045	0.164594
50 min	0.227458	0.24093	0.218478	0.222968	0.198271
60 min	0.265626	0.283588	0.252155	0.263381	0.238684
70 min	0.299304	0.324001	0.290323	0.299304	0.265626
80 min	0.330736	0.368904	0.324001	0.335227	0.299304
90 min	0.366659	0.411563	0.357678	0.37115	0.335227
100 min	0.400337	0.454221	0.391356	0.409317	0.368904
110 min	0.447485	0.492389	0.422789	0.44524	0.400337
120 min	0.50586	0.528312	0.458711	0.476673	0.434014

Table 26: 100 ppm(F)NaF calcium (ppm Ca++)

	10/06/04K	10/6/04L	10/06/04M	10/08/04N	10/08/04O
Initial	0.12	0.11	0.1	0.1	0.09
0 min	0.18	0.17	0.25	0.35	0.23
10 min	0.28	0.31	0.38	0.47	0.33
20 min	0.37	0.4	0.47	0.56	0.42
30 min	0.43	0.51	0.55	0.66	0.5
40 min	0.5	0.61	0.72	0.74	0.57
50 min	0.55	0.68	0.7	0.83	0.65
60 min	0.63	0.74	0.75	0.91	0.72
70 min	0.68	0.83	0.881	0.98	0.78
80 min	0.73	0.88	0.89	1.05	0.84
90 min	0.76	0.98	0.994	1.12	0.9
100 min	0.86	1.02	0.99	1.2	0.95
110 min	0.91	1.1	1.06	1.25	1.02
120 min	1.01	1.17	1.11	1.3	1.08

Table 27: 1 ppm(F)MFP Free fluoride (ppm F)

	10/27/04A	10/27/04D	10/27/04P	10/29/04Q	10/29/04C
Initial	0.32111	0.33314	0.30036	0.31363	0.38075
0 min	0.32238	0.32066	0.32365	0.3124	0.30398
10 min	0.32111	0.32066	0.31735	0.31363	0.30866
20 min	0.3262	0.3134	0.33137	0.32238	0.30984
30 min	0.31985	0.31944	0.3262	0.31985	0.31102
40 min	0.31486	0.31701	0.33137	0.32365	0.31581
50 min	0.32492		0.32492	0.32492	0.31701
60 min	0.3262		0.32492	0.3186	0.32684
70 min	0.32365		0.33268	0.3161	0.32189
80 min	0.32365		0.33007	0.31985	0.32684
90 min	0.3262	0.30514	0.33662	0.32749	0.32436
100 min		0.32312	0.3353	0.33007	0.32935
110 min		0.31701	0.32878	0.34061	0.32066
120 min	0.32365	0.32066	0.3262	0.33007	0.3146

Table 28: 1 ppm(F)MFP total fluoride (ppm F)

	10/27/04A	10/27/04D	10/27/04P	10/29/04Q	10/29/04C
Initial	1.00265	1.06509	0.79938	0.89526	1.0334
0 min	0.9951	1.0813	0.89526	1.0334	1.02562
10 min	0.97649	1.10191	0.88518	0.92272	1.04912
20 min	0.98762	1.08539	0.89865	0.92972	1.00265
30 min	1.0334		0.92272	0.88853	1.07722
40 min	1.09775	1.08539	0.92272	0.94031	15.549
50 min	1.10608	1.00644	0.94386	0.98389	1.10608
60 min		1.08539	0.94386	0.96185	1.11446
70 min	1.11026	1.10191	0.96185	0.96549	1.14
80 min	1.00644			0.96915	15.549
90 min	1.0334	1.10608	0.92621	0.97281	1.13142
100 min	0.97281	1.11026	0.94744		1.0895
110 min		1.09775		0.97649	1.06509
120 min	1.04123	1.0895	0.94031	0.99135	1.10608

Table 29: 1 ppm(F)MFP true MFP (ppm F)

	10/27/04A	10/27/04D	10/27/04P	10/29/04Q	10/29/04C
Initial	0.68154	0.73195	0.49902	0.58163	0.65265
0 min	0.67272	0.76064	0.57161	0.721	0.72164
10 min	0.65538	0.78125	0.56783	0.60909	0.74046
20 min	0.66142	0.77199	0.56728	0.60734	0.69281
30 min	0.71355		0.59652	0.56868	0.7662
40 min	0.78289	0.76838	0.59135	0.61666	
50 min	0.78116		0.61894	0.65897	0.78907
60 min			0.61894	0.64325	0.78762
70 min	0.78661		0.62917	0.64939	0.81811
80 min	0.68279			0.6493	
90 min	0.7072	0.80094	0.58959	0.64532	0.80706
100 min		0.78714	0.61214		0.76015
110 min		0.78074		0.63588	0.74443
120 min	0.71758	0.76884	0.61411	0.66128	0.79148

Table 30: 1 ppm(F)MFP phosphate (ppm P)

	10/27/04A	10/27/04D	10/27/04P	10/29/04Q	10/29/04C
Initial	1.305949	1.233625	1.26376	1.26376	1.287868
0 min	1.257733	1.26376	1.251706	1.260746	1.281841
10 min	1.348138	1.330057	1.299922	1.330057	1.372246
20 min	1.426489	1.44457	1.414435	1.426489	
30 min	1.480732	1.516894	1.486759	1.525934	1.751947
40 min	1.516894	1.56511	1.568123	1.62538	1.818244
50 min	1.607299	1.631407	1.583191	1.676609	1.896595
60 min	1.655515	1.848379	1.643461	1.721812	1.993027
70 min	1.691677	1.902622	1.697704	1.80619	2.053297
80 min	1.800163		1.779068	1.86646	2.131648
90 min	1.80619		1.836325	1.920703	2.209999
100 min	1.902622		1.908649	1.987	2.143702
110 min	1.980973	2.017135	1.92673	2.032202	2.173837
120 min	1.956865	2.04727	1.993027	2.179864	2.209999

Table 31: 1 ppm(F)MFP calcium (ppm Ca++)

	10/27/04A	10/27/04D	10/27/04P	10/29/04Q	10/29/04C
Initial	0.1	0.1	0.11	0.1	0.12
0 min	0.18	0.18	0.32	0.18	0.18
10 min	0.39	0.38	0.5	0.42	0.42
20 min	0.51	0.54	0.67	0.61	0.64
30 min	0.65	0.68	0.81	0.77	0.84
40 min	0.8	0.83	0.94	0.93	1
50 min	0.94	0.97	1.05	1.07	1.19
60 min	1.06	1.13	1.17	1.22	1.31
70 min	1.14	1.27	1.32	1.4	1.49
80 min	1.31	1.36	1.38	1.49	1.62
90 min	1.4	1.5	1.53	1.72	
100 min	1.5	1.61	1.62	1.81	1.95
110 min	1.62	1.73	1.73	1.99	2.1
120 min	1.77	1.86	1.85	2.1	2.21

Table 32: 10 ppm(F)MFP Free fluoride (ppm F)

	11/10/04E	11/10/04	11/11/04G	11/11/04H	11/11/04I
Initial	2.45978	2.50948	2.26205	2.50832	2.4886
0 min	2.34449	2.53977	2.30623	2.50832	2.56843
10 min	2.42073	2.54995	2.32415	2.4886	2.51824
20 min	2.48948	2.64343	2.33315	2.49844	2.50832
30 min	2.52963	2.54995	2.32415	2.51824	2.44962
40 min	2.54995	2.52963	2.36039	2.62998	2.26369
50 min	2.52963	2.42073	2.44402	2.59902	2.43997
60 min	2.57044	2.66467	2.42518	2.64038	2.34554
70 min	2.62237	2.60147	2.35128	2.59902	2.31793
80 min	2.59108	2.51953	2.40649	2.59902	2.29971
90 min	2.44996	2.60147	2.463	2.6093	2.15897
100 min	2.58074	2.60147	2.47255	2.58878	2.202
110 min	2.56017	2.52963	2.44402	2.693	2.24589
120 min	2.58074	2.49946	2.49176	2.57859	2.09187

Table 33: 10 ppm(F)MFP total fluoride (ppm F)

	11/10/04E	11/10/04	11/11/04G	11/11/04H	11/11/04I
Initial	7.89875	8.24782	8.24782	8.02394	8.34568
0 min	8.68031	8.31293	8.24782	8.41155	8.44469
10 min	8.74883	8.41155	8.24782	8.41155	8.44469
20 min	8.34568	8.47795	8.41155	8.28031	13.8583
30 min	8.54487	8.47795	13.8583	8.61232	13.8583
40 min	8.51135	8.31293	8.37855	13.8583	8.21546
50 min	8.74883	8.28031	8.18323	8.64625	8.41155
60 min	8.7145	8.34568	13.8583	8.57853	8.51135
70 min	8.9225	8.51135	8.41155	8.51135	8.41155
80 min	8.88749	8.31293	8.41155	8.64625	8.51135
90 min	8.78329	8.34568	8.41155	8.51135	8.54487
100 min	8.81789	8.44469	8.44469	8.51135	8.28031
110 min	8.99293	8.44469	8.37855	8.51135	8.24782
120 min	8.28031	8.37855	7.99246	8.47795	7.83689

Table 34: 10 ppm(F)MFP true MFP (ppm F)

	11/10/04E	11/10/04	11/11/04G	11/11/04H	11/11/04I
Initial	5.43897	5.73834	5.98577	5.51562	5.85708
0 min	6.33582	5.77316	5.94159	5.90323	5.87626
10 min	6.3281	5.8616	5.92367	5.92295	5.92645
20 min	5.8562	5.83452	6.0784	5.78187	11.34998
30 min	6.01524	5.928	11.53415	6.09408	11.40868
40 min	5.9614	5.7833	6.01816	11.22832	5.95177
50 min	6.2192	5.85958	5.73921	6.04723	5.97158
60 min	6.14406	5.68101	11.43312	5.93815	6.16581
70 min	6.30013	5.90988	6.06027	5.91233	6.09362
80 min	6.29641	5.7934	6.00506	6.04723	6.21164
90 min	6.33333	5.74421	5.94855	5.90205	6.3859
100 min	6.23715	5.84322	5.97214	5.92257	6.07831
110 min	6.43276	5.91506	5.93453	5.81835	6.00193
120 min	5.69957	5.87909	5.5007	5.89936	5.74502

Table 35: 10 ppm(F)MFP phosphate (ppm P)

	11/10/04E	11/10/04	11/11/04G	11/11/04H	11/11/04I
Initial	11.55422	12.11551	13.85552	13.85552	11.94713
0 min	12.95745	13.74326	14.08004	14.13617	12.67681
10 min	15.1465	12.00326	13.57488	13.85552	13.29423
20 min	14.75359	12.50842	14.69746	14.92198	13.51875
30 min	14.41682	12.73294	14.02391	12.56455	13.51875
40 min	14.69746	12.95745	14.69746	14.86585	13.68714
50 min	13.85552	13.79939	15.82005	15.98844	14.97811
60 min	15.70779	14.24843	15.42714	16.04457	13.74326
70 min	14.64133	14.41682	14.52907	13.68714	14.69746
80 min	13.85552	13.18197	13.74326	14.92198	13.68714
90 min	14.86585	13.79939	14.47295	15.31489	13.46262
100 min	15.09037	15.03424	14.36069	14.47295	15.42714
110 min	15.31489	13.74326	16.38134	14.86585	15.87618
120 min		14.75359	16.04457	14.08004	14.80972

Table 36: 10 ppm(F)MFP calcium (ppm Ca++)

	11/10/04E	11/10/04	11/11/04G	11/11/04H	11/11/04I
Initial	0.15	0.14	0.15	0.16	0.13
0 min	0.19	0.29	0.25	0.25	0.33
10 min	0.26	0.39	0.32	0.42	0.43
20 min	0.35	0.48	0.4	0.54	0.51
30 min	0.42	0.56	0.51	0.59	0.56
40 min	0.5	0.63	0.53	0.69	0.62
50 min	0.55	0.67	0.64	0.75	0.71
60 min	0.59	0.76	0.62	0.83	0.76
70 min	0.65	0.79	0.73	0.93	0.82
80 min	0.71	0.89	0.79	1	0.88
90 min	0.73	0.95	0.87	1.07	0.96
100 min	0.87	1.01	0.93	1.13	0.98
110 min	0.9	1.05	0.97	1.24	1.07
120 min	0.97	1.17	1.02	1.32	1.15

Table 37: 100 ppm(F)MFP Free fluoride (ppm F)

	1/20/05K	1/20/05M	1/20/05N	1/21/05O
Initial	30.9879	33.1121	31.0716	32.6832
0 min	30.2712	32.9832	30.5843	32.5536
10 min	29.9191	31.5981	30.4637	32.8134
20 min	30.1534	32.4726	28.5972	31.5349
30 min	30.6275	31.5981	29.6326	33.8734
40 min	30.7472	31.7216	30.3435	30.7919
50 min	30.6275	32.7269	30.3435	32.0402
60 min	31.3526	33.896	31.1946	30.6698
70 min	30.8673	32.5995	30.2239	30.6698
80 min	31.2306	34.0285	30.949	410.902
90 min	31.5981	33.1121		30.6698
100 min	30.7472	32.2203	31.0716	31.1612
110 min	31.2306	33.2415	31.3181	30.7919
120 min	31.3526	31.97	31.3181	31.0376

Table 38: 100 ppm(F)MFP total fluoride (ppm F)

	1/20/05K	1/20/05M	1/20/05N	1/21/05O
Initial	80.2861	102.336	104.768	106.838
0 min	101.538	102.737	101.936	104.359
10 min	83.4908	99.5704	103.14	105.179
20 min	88.8865	101.142	101.936	104.768
30 min	82.8398	102.336		106.421
40 min	96.501	102.737	100.746	108.524
50 min	86.8234	95.0019	101.936	108.524
60 min	92.0734	100.746	102.336	107.257
70 min	98.4081	101.142	101.538	106.838
80 min	75.4125	98.794	103.951	108.524
90 min	91.3554	83.1647	104.768	107.678
100 min	87.5057	103.545	105.591	108.609
110 min	87.1639	102.336	103.951	108.1
120 min	95.3745	103.545	106.005	107.257

Table 39: 100 ppm(F)MFP true MFP (ppm F)

	1/20/05K	1/20/05M	1/20/05N	1/21/05O
Initial	49.2982	69.2239	73.6964	74.1548
0 min	71.2668	69.7538	71.3517	71.8054
10 min	53.5717	67.9723	72.6763	72.3656
20 min	58.7331	68.6694	73.3388	73.2331
30 min	52.2123	70.7379		72.5476
40 min	65.7538	71.0154	70.4025	77.7321
50 min	56.1959	62.275	71.5925	76.4838
60 min	60.7208	66.85	71.1414	76.5872
70 min	67.5408	68.5425	71.3141	76.1682
80 min	44.1819	64.7655	73.002	-302.378
90 min	59.7573	50.0526		77.0082
100 min	56.7585	71.3247	74.5194	77.4478
110 min	55.9333	69.0945	72.6329	77.3081
120 min	64.0219	71.575	74.6869	76.2194

Table 40: 100 ppm(F)MFP phosphate (ppm P)

	1/20/05K	1/20/05M	1/20/05N	1/21/05O
Initial	139.5653	107.076	105.0594	94.52834
0 min	99.90589	101.4743	102.3706	105.0594
10 min	101.2503	87.80641	105.9556	103.0428
20 min	99.00963	104.1631	103.0428	100.8022
30 min	101.0262	102.1465	97.55322	101.2503
40 min	95.20054	104.3872	102.8187	96.54492
50 min	98.78557	105.2834	104.9473	103.4909
60 min	107.3	97.88931	103.4909	103.2669
70 min	104.3872	107.5241	99.90589	109.5407
80 min	100.8022	100.5781	102.8187	113.1257
90 min	106.1797	94.97647	104.8353	112.0054
100 min	104.8353	100.354	105.7316	108.1963
110 min	104.1631	99.68183	104.1631	107.5241
120 min	107.5241	99.68183	96.32086	109.0925

Table 41: 100 ppm(F)MFP calcium (ppm Ca++)

	1/20/05K	1/20/05M	1/20/05N	1/21/05O
Initial	0.14	0.13	0.11	0.12
0 min	0.21	0.21	0.19	0.17
10 min	0.24	0.24	0.21	0.16
20 min	0.26	0.25	0.25	0.19
30 min	0.28	0.27	0.25	0.21
40 min	0.3	0.41	0.26	0.24
50 min	0.31	0.3	0.3	0.27
60 min	0.33	0.32	0.31	0.3
70 min	0.35	0.33	0.32	0.32
80 min	0.38	0.36	0.34	0.36
90 min	0.41	0.4	0.37	0.4
100 min	0.44	0.4	0.39	0.45
110 min	0.45	0.42	0.4	0.46
120 min		0.44	0.43	0.5

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