Anticaries Potential of Commercial Fluoride Rinses as Determined by Fluoridation and Remineralization Efficiency

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Abstract

- **Objective:** The objective of this work was to compare the anticaries potential of several currently marketed fluoride-containing mouthrinse products using two *in vitro* approaches: 1) fluoride uptake studies of demineralized human enamel samples after exposure to rinse products; and 2) microhardness studies of sound enamel samples after exposure to the rinse products and demineralizing agents.
- **Methods:** Four currently marketed rinse products, formulated at 100 ppm F, were evaluated in fluoride uptake studies relative to a negative (water) rinse control (Study 1). The same rinse products were evaluated in microhardness studies (Study 2) against a positive control, ACT® Anticavity® rinse, which is formulated with 225 ppm F and carries the ADA Seal of Acceptance as an effective anticavity mouthrinse. Test products included ACT® Total Care® rinse (pH = 6.34), Listerine® Total Care rinse (pH = 3.57), Crest® Pro-Health for Me™ rinse (pH = 3.33), and Crest® Pro-Health™ Complete rinse (pH = 3.43).
- **Results:** Study 1—Samples treated with any of the fluoride-containing rinses showed significantly higher ($p < 0.05$) levels of fluoride uptake than the negative (water) control. Two of the products (Crest Pro-Health for Me and Crest Pro-Health Complete) showed significantly higher ($p < 0.05$) levels of fluoride uptake into demineralized enamel than the other marketed rinses (Listerine Total Care and ACT Total Care). Study 2—Samples treated with the same two rinse products (Crest Pro-Health For Me and Crest Pro-Health Complete) showed significantly lower mineral loss than the other rinse products, as well as the positive control.
- **Conclusion:** Results of these *in vitro* studies indicate that the Crest mouthrinse products evaluated here are capable of providing significantly better fluoridation of demineralized enamel, as well as significantly better protection against the initiation and progression of demineralization, compared to the other marketed fluoride-containing mouthrinse products tested.

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Introduction

Significant reductions in dental caries over the past several decades in many countries can largely be attributed to the use of fluoride-containing toothpastes.[1-7] In addition to toothpastes, fluoride-containing mouthrinses have also been demonstrated to be effective in the prevention of caries.[8,9] Although the exact mechanism for fluoride efficacy has been debated for many years, there is general agreement that the two primary mechanisms of action for fluoride are: 1) incorporation of fluoride into the enamel as a means to promote remineralization in carious enamel; and 2) the ability of fluoride to prevent demineralization of healthy enamel.[6,10-14] In order to assist in the prevention of demineralization or enhance remineralization, fluoride in the formulation must be available to be delivered to the tooth surface, the site of action for fluoride activity.[15]

The use of human clinical trials to evaluate the caries prevention efficacy of fluoride-containing products is expensive, and often requires a period of one to three years or more to detect significant differences between products of interest.[3,8,9,16] Shorter-term clinical models have been proposed, but these can also be expensive propositions.[17,18]

While clinical studies remain the gold standard for assessing efficacy, well-controlled *in vitro* models have been shown to be effective and efficient means for assessing potential anticaries efficacy.[12,19-25] The *in vitro* model designs used in the current studies have been previously confirmed to demonstrate dose response sensitivity, as well as to identify potential differences in performance and efficiency of fluoride-containing toothpastes.[12,19-25] These models, often referred to as pH cycling models, typically utilize human enamel samples that are subjected to treatment protocols specifically developed to reflect the changing pH conditions of the oral cavity and the impact of the product on demineralization and remineralization processes. After the treatment protocol is complete, the enamel specimens are analyzed for the amount of fluoride uptake,[12,19-21,23,25] the change in lesion depth,[24] or the change in surface microhardness.[12,22-24]

In vitro fluoride uptake studies are used to indirectly assess the relative levels of bioavailable fluoride in a series of products of differing formulations relative to a negative control. The amount of bioavailable fluoride in a toothpaste formulation has been shown to be a more effective predictor of potential anticaries efficacy than the level of total fluoride formulated into the commercial product.[21,23] Biological availability of fluoride from a toothpaste has been demonstrated to be highly dependent on the overall makeup of the formulation; certain product ingredients and conditions are known to reduce anticaries performance.[26-30] The bioavailability of fluoride from mounthrinse products is likely to be impacted by similar factors.

Several studies[13,21,23,28,31] have utilized surface microhardness evaluations in combination with fluoride uptake measurements to monitor the impact of fluoride incorporation (remineralization) on the integrity of tooth enamel as a result of product treatments. This combination approach provides additional information that allows the researcher to more completely and confidently assess and predict the relative anticaries potential of mouthrinse products across a broad range of product formulations.

The aim of this work was to utilize this combination of *in vitro* models to investigate the anticaries potential of various fluoride-

containing mouthrinses. The mouthrinses tested were Crest® Pro-Health For Me™ and Crest® Pro-Health™ Complete (Procter & Gamble Co. Cincinnati, OH, USA), ACT® Total Care® and ACT® Anticavity Rinse (Chattem, Inc, Chattanooga, TN, USA), and Listerine® Total Care (Johnson & Johnson Healthcare Products, New Brunswick, NJ, USA).

In the first study, artificially demineralized enamel specimens were subjected to a daily treatment protocol of saliva soak, product treatment, demineralization, product treatment, and saliva soak. After five days of treatment, specimens were subjected to fluoride uptake analysis in order to assess the relative ability of products to fluoridate and reverse demineralization.

In the second study, sound enamel specimens were subjected to a daily treatment protocol consisting of rinse, demineralization, rinse, and remineralization. After 14 days of treatment, specimens were subjected to cross-sectional microhardness analysis to assess the relative ability of the rinse products to inhibit the initiation and progression of damage.

Materials and Methods

Both *in vitro* pH cycling studies were carried out in the Advanced Enamel Care Laboratories and Caries Research Group of the Procter & Gamble Company, Mason, OH, USA. Mouthrinses evaluated are shown in Table I, along with the product ingredients as listed on their respective packages. All products included in the study were obtained from stores in the local Cincinnati, OH, USA area, and all products were used within the specified expiration dates on each package.

The coding and description of mouthrinses evaluated in each study are shown in Table II. The pH of each rinse was measured using a calibrated pH electrode (Thermo Electron Corp., Beverly, MA, USA) as shown in Table II. Four currently marketed mouthrinses were evaluated in both *in vitro* studies. The first study evaluated the test products, all formulated at 100 ppm F, relative to a negative control, deionized water. The second study evaluated the same four products relative to a positive control, formulated at 225 ppm F.

Table II
Coding and Description of Mouthrinses Evaluated

Test Group Code*	Preparation	Mouthrinse	Manufacturer	Measured pH
Study 1				
1	Neg. Control	Deionized Water	n/a	~7
2	Test Product	Crest Pro-Health For Me	Procter & Gamble Co.	3.33
3	Test Product	Crest Pro-Health Complete	Procter & Gamble Co.	3.43
4	Test Product	ACT Total Care	Chattem, Inc	6.34
5	Test Product	Listerine Total Care	J&J Healthcare Products	3.57
Study 2				
1	Test Product	Crest Pro-Health Complete	Procter & Gamble	3.43
2	Test Product	Crest Pro-Health For Me	Procter & Gamble	3.33
3	Pos. Control	ACT Anticavity Rinse	Chattem, Inc	6.21
4	Test Product	Listerine Total Care	J&J Healthcare Products	3.57
5	Test Product	ACT Total Care	Chattem, Inc	6.34

*Code was held until completion of studies by primary investigator.

Collection of Human Saliva for Study 1

Healthy volunteers were recruited to provide human saliva for Study 1 (remineralization medium). In Study 2, a prepared remineralization solution was used in place of human saliva (see details in Study 2, Solution Preparation). Saliva samples were collected from the volunteers each day of the study, pooled, and stored under refrigeration until use. All required precautions were in place to ensure proper handling of saliva from the point of collection to the ultimate use in the laboratory studies. Volunteers chewed paraffin wax, and expectorated any stimulated saliva generated into a plastic collection vessel over a period that averaged 30–40 minutes per volunteer per collection period. Saliva was collected early in the morning from each volunteer on each day of the study in order to maintain a relatively constant pool of saliva for use in the study. Once completed, collection

Table I
Ingredients of Test Products (Studies 1 and 2) and Positive Control (Study 2)
(In Order Listed on Packages)

Crest Pro-Health For Me	Crest Pro-Health Complete	Listerine Total Care	ACT Total Care	ACT Anticavity
NaF (100 ppm)*	NaF (100 ppm)*	NaF (100 ppm)*	NaF (100 ppm)*	NaF (225 ppm)*
Water	Water	Water	Blue #1	Ca-Na EDTA
Glycerin	Glycerin	Sorbitol	Ca-Na EDTA	Cetylpyridinium chloride
Propylene glycol	Flavor	Alcohol (22%)	Cetylpyridinium chloride	Disodium phosphate
Poloxamer	Cetylpyridinium chloride	Flavors	Disodium phosphate	Flavor
Polysorbate 80	Phosphoric acid	Poloxamer 407	Flavor	Green 3
Phosphoric acid	Methyl paraben	Sodium lauryl sulfate	Menthol	Menthol
Sodium benzoate	Sodium saccharin	Phosphoric acid	Methyl salicylate	Methyl salicylate
Potassium sorbate	Poloxamer 407	Disodium phosphate	Poloxamer 407	Poloxamer 407
Flavor	Propyl paraben	Sodium saccharin	Polysorbate 20	Polysorbate 20
Sodium saccharin	Disodium phosphate	Sucralose	Potassium sorbate	Potassium sorbate
Disodium phosphate	Red #33	FD&C Red #40	Propylene glycol	Propylene glycol
Green #3	Green #3	FD&C Blue #1	Sodium benzoate	Sodium benzoate
			Sodium phosphate	Sodium phosphate
			Sodium saccharin	Sodium saccharin
			Sorbitol	Sorbitol
			Water	Water
			Yellow #5	Yellow #5

*Fluoride level as stated on package.

vessels were pooled together, mixed and stored under refrigeration at approximately 5˚C until use. Saliva volunteers were restricted from using any toothpaste containing an antimicrobial agent, and were also restricted from using any mouthwash. Volunteers were not permitted to provide saliva if they were sick (fever, nausea, vomiting, etc.) or had any type of oral infections, irritations, or abrasions. No tobacco product use of any kind was permitted, including nicotine-containing gums. A two-week wash-out period (use of Crest® Cavity Protection toothpaste only, Procter & Gamble Co., Cincinnati, OH, USA) was required before returning to the panel after use of any of the following products or procedures: antibiotics, antihistamines, antivirals, tooth whitening products, dental cleanings.

Specimen Collection and Preparation

Enamel samples were prepared from human teeth for all studies. Teeth were obtained from local oral surgeons who collected the teeth after removing them, typically for orthodontic reasons. All required precautions were in place to ensure proper handling of tooth samples from the point of collection to the ultimate use in these laboratory studies. Available teeth were individually cleaned and checked for any visible surface cracks or other imperfections. Those with any visible imperfections were discarded. Teeth were stored prior to use under refrigeration (approximately 5˚C) in a 1% thymol solution.

Study 1: pH Cycling and Fluoride Uptake Evaluation

Sample Preparation. Cores of enamel with a diameter of approximately 4 mm were removed from human upper incisors. The enamel cores were embedded in cylindrical plastic rods using DuraBase™ methyl methacrylate (Reliance Dental Mfg. Co., Worth, IL, USA) so the enamel surface remained exposed. The enamel surface was treated with wet and dry Carbimet® silicon carbide abrasive paper (Buehler, Lake Bluff, IL, USA) to remove approximately 50 µm of the outer, naturally fluoride-rich enamel surface. The surface was then polished to a natural, mirror-like finish using a paste of Linde No. 3, AB Gamma Polishing Alumina® (Buehler, Lake Bluff, IL, USA) containing aluminium. Internal studies have shown this procedure results in the presentation of a renewed enamel surface that is essentially free of background fluoride.

Pretreatment of Samples. The prepared enamel samples were demineralized for 72 hours in a weak acid-containing solution (pH = 5.0) consisting of 0.1 M lactic acid and 0.2% Carbopol® C907 polyacrylic acid (B.F. Goodrich Co., Cleveland, OH, USA), 50% saturated with hydroxyapatite. This method produces lesions with a depth of approximately 50–80 µm, and has been reported in other publications using a similar pH cycling model.[19,21,23,32] Specimens were randomly assigned to four per treatment group.

Experimental Procedure. The human enamel samples were stored for a period of five days in closed vessels containing pooled human saliva. The saliva baths were refreshed three times each day as follows (Figure 1):

1) in the morning after the first treatment;
2) after the daily period of demineralization; and
3) at the end of each work day.

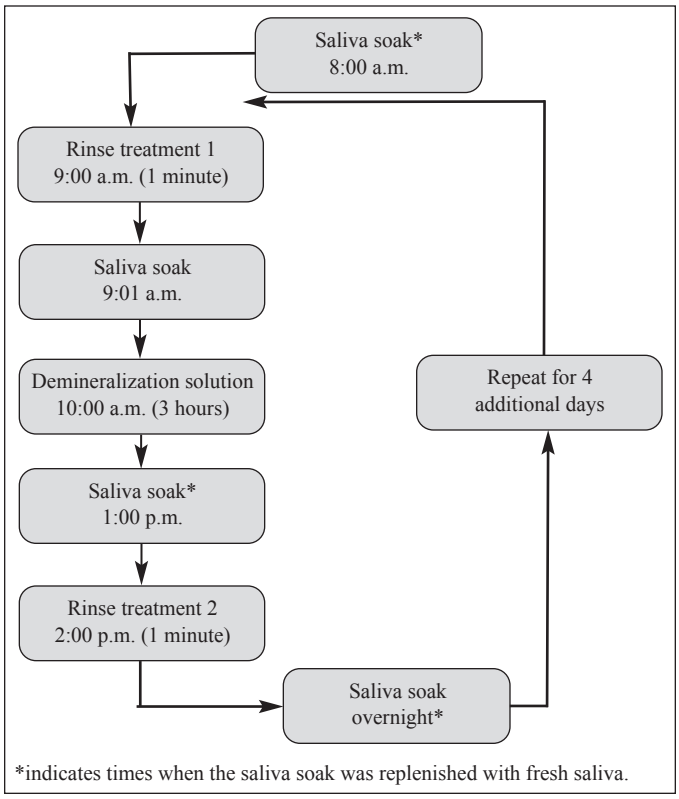

Figure 1. *Schematic of daily treatment protocol used in Study 1 prior to fluoride uptake analysis*

The saliva baths were continuously stirred with a mechanical magnetic system. Each group of four specimens was removed twice a day and treated with 10 mL of rinse according to the daily treatment schedule in Figure 1. Each treatment lasted one minute. Between the first and second treatments each day, each group of four samples was stored for three hours in a fresh volume (20 mL) of the demineralization solution (same recipe as that used for the initial demineralization of specimens).

Measurement of Fluoride Uptake. After the treatment period was completed, an enamel biopsy was taken from each specimen using the microdrill biopsy technique[6] to a depth of 100 µm. This ensured that the initial lesions were sampled to their full depth. The diameter of the enamel sample biopsy was measured using an Axio Scope™ (Carl Zeiss Ltd, Welwyn Garden City, Herts, UK) equipped with a stage micrometer and calibrated eyepiece. The enamel sample was removed and carefully collected, and then dissolved in a solution of three parts 0.5 M $HClO_4$: three parts TISAB II (Buffer): two parts 1.0N NaOH.

The fluoride content of each specimen solution was determined using an Orion® Model 96-09 fluoride ion-selective electrode (Thermo Electron Corp, Beverly, MA, USA) that had been calibrated using serial dilutions of NaF solutions of known concentrations. A polynomial fit was used to convert electrode responses to fluoride concentration (log ppm F). The fluoride concentration of each test solution was calculated and expressed as fluoride uptake in micrograms of fluoride per unit of surface area sampled ($\mu g/cm^2$). The mean and standard error in the mean were calculated for each treatment group, and differences in the means were tested using the Student's t-test.

Study 2: pH Cycling and Surface Microhardness Analysis

The general protocol for this study followed the pH cycling method of Featherstone, *et al.*,[33] specifically with respect to specimen preparation and analyses. In the Featherstone model, a prepared remineralization solution (see below) was used in place of human saliva, which was the remineralization medium used in Study 1.

Enamel Sample Preparation. Caries-free human crowns from molars and pre-molars were removed from the roots, brushed with warm detergent solution, the tooth surface gently abraded with 600 grit wet/dry Carbimet silicon carbide abrasive paper following the contour of the tooth surface, rinsed in deionized water, air dried, and painted with acid-resistant nail varnish leaving one exposed window (approximately 4.0 × 5.0 mm) on one enamel surface. Ten specimens were randomly assigned per treatment group.

Solution Preparation. Remineralization and demineralization solutions were prepared at least once a week as stock, according to the formulations listed below. Solutions were dispensed into individual treatment containers at the beginning of the week and used for two to three days. Unused stock solutions of the de-/remineralization solutions were stored in sealed containers at room temperature. At midweek, used solutions were discarded and fresh stock solution was dispensed into the individual containers for the remainder of the week. At the end of the week, fresh remineralization solution was prepared and dispensed for the weekend (two-day) remineralization period. The following week, stock solutions were again prepared and dispensed as described.

The demineralizing and remineralizing solutions were those used in previous studies by ten Cate, *et al*.[34] The demineralizing solution served as an acid challenge to simulate the acid challenge that is generated by plaque acids in the oral cavity. The composition of the demineralization solution was as follows:

Glacial Acetic Acid	75.0 mmol/L	CH_3COOH	mwt = 60.05	4.5083 g/L
Calcium, Phosphate	2.0 mmol/L	$CaHPO_4$	mwt = 136.06	0.272 g/L

The remineralizing solution served as the remineralization medium in all treatment regimens. The composition of the remineralization solution was as follows:

Potassium Phosphate	0.9 mmol/L	KH_2PO_4	mwt = 136.09	0.1225 g/L
Calcium Nitrate	1.5 mmol/L	$Ca(NO_3)_2.4H_2O$	mwt = 236.16	0.3542 g/L
Potassium Chloride	150.0 mmol/L	KCl	mwt = 74.55	11.2 g/L
Sodium Cacodylate	20.0 mmol/L	$NaC_2H_6AsO_2.3H_2O$	mwt = 214	4.28 g/L

Experimental Procedure. The teeth were suspended so that the treatment windows on the enamel samples were exposed to the test rinses at all times during the treatments and subsequent incubations in de- and remineralizing solutions (Figure 2). The treatments and incubations in de- and remineralization solutions, *i.e.*, pH cycling, were repeated daily for a total of 14 days, with two weekend interruption periods where specimens remained in remineralization solution for 48 hours at 37˚C. Treatment with mouthrinse products was carried out per the labeling instructions on the packages (twice daily). More specifically, in the morning specimens were treated with 40 mL of rinse for one minute, placed into demineralizing solution (see below) for six hours, treated with 40 mL of rinse for one minute, and then placed in remineralizing solution (see below) for 18 hours overnight.

The exception to this treatment regimen was the positive control formulated with 0.05% NaF (225 ppm F). Mouthrinses containing 225 ppm F are recommended for use by the FDA only once per day. As a result, samples were exposed to this product treatment only once per day, during the second treatment period in the daily treatment schedule.

Measurement of Surface Microhardness. At the end of the 14 days of pH cycling, specimens were cut in half through the lesion using a Dinar™ 6 × 0.020 × ½ radial diamond saw (National Diamond Lab, Los Angeles, CA, USA). One-half of each crown was embedded in epoxy resin so that only the cut section of the lesion and underlying sound enamel were exposed. The cut surface was then serially polished to a high luster.

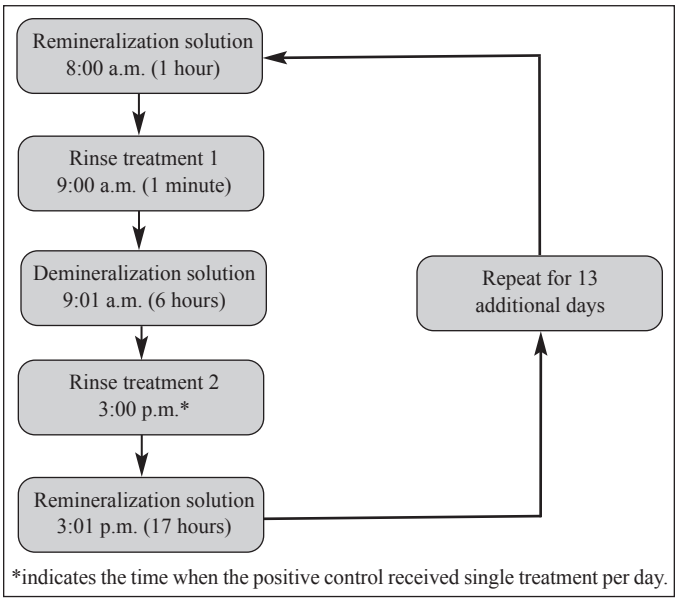

Figure 2. *Schematic of daily treatment protocol used in Study 2 prior to surface microhardness evaluations.*

Microhardness indentations were made on a line perpendicular to, and initiated at 12.5 µm from the anatomical surface of the lesion. The hardness indenting was continued to depths extending beyond the depth of the lesion, into the underlying sound enamel, at 12.5 µm intervals. Microhardness measurements were made using a standard microhardness indenter (Buehler, Lake Bluff, IL, USA) utilizing a Knoop diamond under a 10 or 50 gram load. The 10 gram load was used for the outer 12.5 µm measurements, while the 50 gram load was used for all subsequent measurements (25.0 µm intervals). The Knoop hardness numbers were subsequently converted to volume % mineral.[35] The individual volume % mineral values plotted versus depth permitted calculation of mineral loss, often referred to as Delta Z.[36] The mean Delta Z and standard error in the mean were calculated for each treatment group. Differences in the means were tested using the Student's t-test.

Results

Study 1

Findings show that treatment with all fluoride-containing mouthrinses evaluated resulted in significantly higher ($p < 0.05$) levels of fluoride uptake into demineralized enamel samples

than the negative water control (Table III). Two groups of samples (Groups 2 and 3) showed significantly higher ($p < 0.05$) levels of fluoride uptake than the other groups (Group 4 and 5) containing the same level of NaF. These fluoride uptake results appear to be independent of the product pH alone.

Table III
Study 1—Results of Fluoride Uptake Study by Treatment Group

Treatment Group	Test Product	Product pH	Mean Fluoride Uptake ± (SEM)*	Results of Statistical Analysis**
1	Deionized Water-negative control	~7	2.57 ± 0.11	e
2	Crest Pro-Health For Me	3.33	23.14 ± 1.11	a
3	Crest Pro-Health Complete	3.43	20.97 ± 0.24	b
4	ACT Total Care	6.34	14.88 ± 0.62	c
5	Listerine Total Care	3.57	12.65 ± 0.36	d

*Mean ± SEM (n = 4), expressed in micrograms of fluoride per unit area sampled (μg F/cm^2).
**Means with different letter designation are significantly different ($p < 0.05$) by the Student's t-test.

Study 2

Findings also show that treatment with the fluoride-containing mouthrinses evaluated resulted in significantly lower ($p < 0.05$) calculated Delta Z values for two treatment groups (Groups 1 and 2) than for the other treatment groups (Groups 4 and 5) and the positive control (Table IV). Lower Delta Z values indicate that the mouthrinses used to treat samples in Groups 1 and 2 protect the enamel better than the other rinses tested and the positive control against the onset and progression of demineralization. These findings also appear to be independent of the product pH alone.

Table IV
Study 2—Results of Microhardness Evaluations by Treatment Group

Treatment Group	Test Product	Product pH	Mean Delta Z ± (SEM)*	Results of Statistical Analysis**
1	Crest Pro-Health Complete	3.43	–44.26 ± 115.92	b
2	Crest Pro-Health For Me	3.33	–19.40 ± 217.82	b
3	ACT Anticavity—positive control	6.21	809.35 ± 150.76	a
4	Listerine Total Care	3.57	530.14 ± 152.85	a
5	ACT Total Care	6.34	510.11 ± 114.60	a

*Mean mineral loss expressed as Delta Z ± SEM (n = 10).
**Means with different letter designation are significantly different ($p < 0.05$) by the Student's t-test.

Discussion

Because fluoride is known to prevent demineralization of healthy enamel and to promote the remineralization of demineralized enamel,[10,11,13,14] it was appropriate for these studies to utilize a combination approach to assess the relative potential effectiveness of these fluoride-containing rinses.

Investigations of fluoride uptake into demineralized enamel, which represent active caries lesions, are known to be predictive of potential anticaries efficacy as measured in human clinical trials.[6] Clarkson, *et al.*[37] found higher levels of fluoride associated with smaller caries lesions. This is most likely due to the overall reversal of the lesions associated with the increased levels of fluoride and mineral within the body of the lesions.

The treatment protocol used in the second study is known to result in lesion advancement quantitatively similar to that obtained adjacent to orthodontic brackets following one month *in vivo* treatment.[38,39] This treatment protocol was found to be sensitive to fluoride dose, capable of distinguishing products known to have different caries protection benefits, and to mimic the progression of active caries processes. As a result, this was a particularly useful model for assessing relative product performance.

In the fluoride uptake studies, samples in Group 1 were treated with a deionized water negative control that did not contain fluoride, yet enamel samples showed a measurable level of fluoride uptake (Table III). This is attributed to low levels of fluoride in the pooled human saliva that was used to form an initial pellicle on the tooth specimens prior to the start of the study and used throughout the treatment protocol.

Mean fluoride uptake values from enamel samples treated with mouthrinse products containing 100 ppm F were found to differ significantly and to range between 12.7 and 23.1 μg F/cm^2, depending upon the rinse (Table III). It has been established that fluoride bioavailability and uptake are not directly related to the fluoride content in the formulation.[19,40] In addition, treatment with toothpastes containing the same fluoride content does not always result in the same level of fluoride uptake.[12,21,23] In fact, bioavailability of fluoride from a toothpaste is known to be highly dependent on the overall makeup of the formulation; certain product ingredients and conditions are know to reduce anticaries performance.[26-30] These findings indicate that the bioavailability of fluoride from mouthrinse formulations is also dependent upon the makeup of the formulation.

The highest fluoride uptake values measured here were from samples treated with two of the mouthrinses formulated at low pH (Groups 2 and 3), with the highest level of fluoride uptake delivered by the product with the lowest pH. These findings are consistent with previous work on the impact of formulation pH on fluoride delivery from toothpastes. In that work, a toothpaste formulated with 500 ppm F at low pH was compared with a similar formula containing 1100 ppm F at neutral pH. Olympio, *et al.*[41] found the lower pH toothpaste formulated at a lower level of F to be as effective at elevating salivary fluoride levels as the higher ppm F product at neutral pH. Brighenti, *et al.*[42] found the low pH toothpaste with lower levels of F to be as effective at enhancing mineralization as the higher ppm F product at neutral pH. Similarly, Vilhena, *et al.*[43] found the low pH toothpaste with lower levels of F to be as effective at preventing caries progression as the higher ppm F product at neutral pH.

The one exception to this trend in the current study was the mean fluoride uptake value obtained from samples treated with Listerine Total Care mouthrinse. This mouthrinse is formulated at low pH, but also includes an anionic surfactant, sodium lauryl sulphate (SLS), an ingredient absent from all other mouthrinses tested here (Table I). Studies by Barkvoll, *et al.,*[44] Pessan, *et al.,*[45,46] and Vogel, *et al.*[47] have demonstrated a reduction in fluoridating efficiency related to the presence of SLS in oral care formulations.

These results strongly suggest that the combination of low pH in an SLS-free mouthrinse, such as the Crest Pro-Heath for Me

and Crest Pro-Health Complete mouthrinses tested here, provide a particularly effective means to enhance the bioavailability and fluoridating efficiency of mouthrinse formulations. One concern with this formulation strategy might be that the low pH of the rinse product could lead to softening of the tooth enamel. Surface microhardness results from the second study clearly indicate this is not the case.

Mean Delta Z values calculated for enamel samples treated with the two low pH, SLS-free mouthrinses (Groups 1 and 2, Table IV) were lower than the other mouthrinses tested and the positive control. These results indicate that the low pH, SLS-free formulations completely inhibited the initiation and progression of enamel demineralization in this *in vitro* study. Interestingly, the other rinses tested, including the product that carries the ADA Seal of Acceptance used as a positive control, resulted in significantly more ($p < 0.05$) demineralization of the enamel than the SLS-free, low pH formulations. The combination of low pH, which enhances the performance of the fluoride active,[41-43] and the absence of SLS, which has the potential to interfere with fluoride activity,[44-47] enables these two products to deliver a high level of performance. As originally designed, the model utilized in this study generally demonstrates some level of demineralization with respect to the positive control.[33-35] A surprising effect in this study was that the use of the two SLS-free, low pH formulations effectively resulted in essentially no significant demineralization. Overall, results from the surface microhardness portion of the study strongly suggest that the combination of low pH in an SLS-free mouthrinse containing 100 ppm F might provide a particularly effective means to enhance the enamel strengthening properties of a mouthrinse formulation.

The highly significant increase in fluoride uptake delivered by the two low pH, SLS-free Crest mouthrinses compared to the other marketed formulations, coupled with the superior protection against the initiation and progression of enamel damage relative to the other rinses and the positive control, provides strong evidence that these low pH, SLS-free mouthrinses are capable of delivering high levels of anticaries benefits. Further *in vivo* evaluations of these Crest mouthrinses are recommended to demonstrate anticaries performance under in-use conditions.

Conclusions

These studies demonstrate that fluoride-containing mouthrinses formulated with the same level of NaF do not perform equally in well-controlled and accepted *in vitro* testing. Results from the fluoride uptake study strongly suggest the reactivity and bioavailability of fluoride in mouthrinses can be influenced by formulation pH, as well as the presence of sodium lauryl sulphate (SLS). Crest fluoride-containing mouthrinses, formulated at low pH and without SLS, delivered the highest levels of fluoride to demineralized enamel samples. Results from the surface microhardness study indicate that Crest mouthrinses formulated at low pH without SLS provided the highest level of protection against the onset and progression of demineralization. Taken together, these studies indicate that the two SLS-free Crest rinse products, Crest Pro-Health for Me and Crest Pro-Health Complete, formulated with 100 ppm NaF at low pH, not only provided the highest level of fluoride uptake compared to the other marketed products tested, but also protected the enamel significantly better than all the other products assessed, including a 225 ppm NaF rinse that carries the ADA Seal of Acceptance for anticaries efficacy.

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