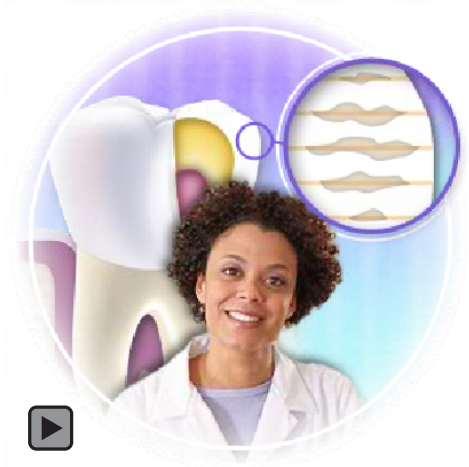# Anticaries Potential of Commercial Dentifrices as Determined by Fluoridation and Remineralization Efficiency

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Abstract

Aim: The aim of this *in vitro* study was to investigate fluoride uptake in human enamel after use of commercially available toothpastes containing different fluoride compounds, or combinations of fluoride actives formulated into a single product, as a means of determining the efficiency of each formula for delivering caries preventing fluoride to demineralized (caries active) enamel.

Methods and Materials: Four test dentifrices and two controls were assessed and placed in groups as follows: Group 1: Lacer® (Spain); Group 2: Positive control-USP Reference Standard 1100 ppm F; Group 3: Fluocaril® Bi-Fluoré 250 (France); Group 4: Colgate Fluor Active (Denmark); Group 5: Elmex® (France); and Group 6: A placebo (formulated the same as the USP Reference Standard toothpaste with the exception that it contained < 1 ppm F). Cores 3 mm in diameter were removed from erupted human enamel specimens (extracted by local oral surgeons for orthodontic reasons) and stored in 1% Thymol solution prior to use. They were ground and polished to remove the natural fluoride rich enamel layer, then exposed to a demineralization solution, and assessed for surface microhardness to enable randomization for use in the study. Each group of five specimens underwent a daily pH cycling procedure that involved exposure to pooled human saliva (refreshed three times daily). The groups were then exposed to dentifrice slurries four times daily for one minute per exposure and to a demineralization solution for three hours. The cycling procedure was repeated for five days. Specimens were again analyzed for surface microhardness and fluoride uptake upon completion of five days of treatment.

Results: Average surface hardness: Groups 2 and 3 showed a statistically significant greater ($p<0.05$) change indicating greater remineralization compared to all other groups. The average change was 23.45 for Group 2 and 22.65 for Group 3. All other groups had changes ranging from 4.25-8.62. No other statistically significant differences were observed between groups. Fluoride uptake results: Groups 2 and 3 showed statistically significantly greater fluoride uptake versus all other groups ($p <0.05$). Groups 1 and 5 were significantly different from Group 6. No other statistically significant differences were observed for either analysis.

Conclusions: Of the marketed products included in the study, the Fluocaril® Bi-Fluoré 250 product formulation provided both the highest level of fluoride uptake and mineralization to the demineralized enamel. The clinical significance of these *in vitro* results is the confirmation Fluocaril® Bi-Fluoré 250 is effective at remineralizing enamel caries lesions.

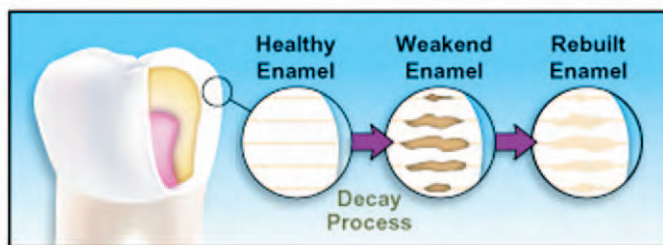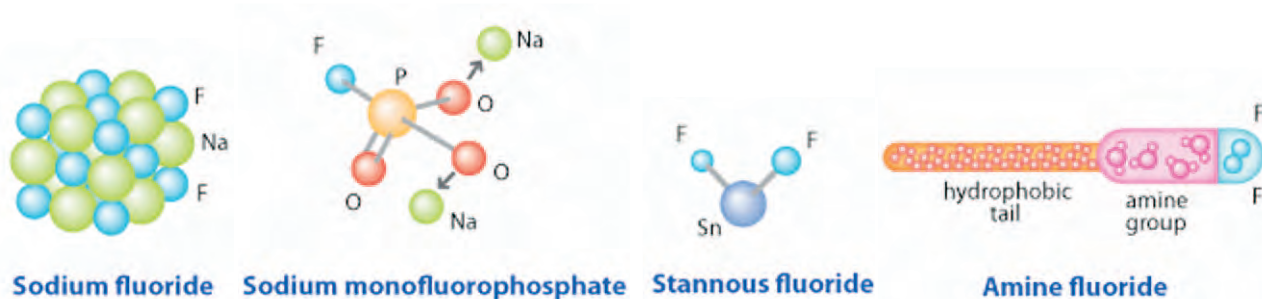**Keywords:** Fluoride, dentifrice, remineralization, caries

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.[1-12] Although the exact mechanism for fluoride efficacy has been debated for many years, there is general agreement the two primary mechanisms of action for fluoride are: (1) its ability to prevent demineralization of healthy enamel and (2) incorporation of fluoride into the enamel as a means to promote remineralization in carious enamel.[13-17]

In order to assist in the prevention of demineralization or enhance remineralization fluoride must first be delivered efficiently to the enamel surface which is the site of action for fluoride activity.[18] Clinical trials for measuring caries prevention efficacy of toothpastes are expensive and often require a period of one to three years to detect significant differences between products of interest.[9,19] Shorter term clinical models have been proposed although even these can be quite expensive propositions.[20,21] While clinical studies remain the gold standard for assessing efficacy, well controlled *in vitro* models can provide a valuable and efficient means for assessing potential anticaries efficacy. The *in vitro* model design used in this study has been previously confirmed to demonstrate dose response sensitivity as well as identify potential differences in product performance and efficiency.[22,23] *In vitro* models have demonstrated the amount of bioavailable fluoride in a toothpaste formulation is a more efficient predictor of potential anticaries efficacy than simple measures of total fluoride incorporated into a commercially available product.[24-26] There have been several experiments concerning how much fluoride is taken up in caries-free or experimentally demineralized enamel samples after use of various toothpastes with different fluoride compounds. Biological availability of fluoride is highly dependent on the

From: An Update on Demineralization/Remineralization, ME Jensen, http://www.dentalcare.com/soap/conteduc/index.htm

Sodium fluoride   **Sodium monofluorophosphate**   **Stannous fluoride**   **Amine fluoride**

overall makeup of the toothpaste with certain ingredients and conditions being capable of reducing or at least minimizing potential anticaries performance.[24,27-32] Other studies have utilized surface microhardness, or combinations of fluoride uptake and microhardness, as a means to demonstrate not only how much fluoride incorporates into a tooth during treatment but also to reflect mineral changes (remineralization) that have occurred within the tooth as a function of treatments.[15,16,23,33] Almost all of these studies compared products containing single sources of fluoride. Globally, both single fluoride sourced products (NaF, SnF_2, AmF, or SMFP) as well as dual fluoride active products (NaF+SMFP, AmF+SnF_2) are commercially available.

The aim of this *in vitro* study was to investigate fluoride uptake and remineralization of human enamel after use of commercially available toothpastes containing different fluoride compounds, or combinations of fluoride actives formulated into a single product, as a means of determining the efficiency of each formula for preventing or reversing the development of caries lesions in enamel.

Methods and Materials
The investigations were carried out in the Advanced Enamel Care Laboratory of the Procter & Gamble Company, Mason, OH, USA as a joint collaboration among all of the authors. Before the start of the study, the toothpastes to be tested were put into neutral (blank, white) packaging and coded by one of the external collaborators. The codes were only broken by the external collaborator once the investigation was complete.

Products Investigated
Four commercially available toothpastes containing different fluoride compounds were investigated (Table 1). The USP Reference Standard for anticaries efficacy (1100 ppm F as NaF/silica abrasive) and a fluoride free placebo (prepared the same as the USP Reference Standard, with the exception that it contained <1 ppm F) were used as controls. Commercially available products included in the study were obtained from France, Spain, and Denmark in July, 2007.

Experimental Procedure
The enamel samples were stored for a period of five days in closed vessels containing pooled human saliva, continuously collected over that same period of time from a panel of ten healthy volunteers. 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. Fresh, paraffin-stimulated saliva was collected from each of the individuals who participated on the panel each day of the study and stored under refrigeration until use. The saliva baths were refreshed three times each day as follows:

1. In the morning after the first treatment.
2. After the daily period of demineralization.
3. At the end of each work day.

The saliva baths were continuously stirred with a mechanical magnetic system. Each group of specimens was removed four times a day and treated with a slurry consisting of three parts (15 g) of fresh pooled human saliva to one part (5 g) toothpaste. Slurries were prepared fresh for each treatment and were mixed with a mechanical magnetic stirring system to make slurries homogenous for a period of about four minutes prior to the actual treatment of demineralized samples. Each treatment lasted one minute. Between the second and third treatments each day, each group of five samples was stored for three hours in a fresh volume (~18 ml) of the demineralization solution (Figure 1).

Sample Preparation

Thirty-three cores of enamel with a diameter of approximately 3 mm were removed from extracted human upper incisors. Teeth were collected by local oral surgeons who removed the teeth primarily for orthodontic reasons and then stored them in a 1% Thymol solution until use. All required precautions were in place to ensure proper handling of specimens 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 to ensure a consistent source of specimens. The enamel cores were embedded in cylindrical plastic rods using methylmethacrylate (Dura Base, Reliance Mfg. Co., Worth, IL, USA) so the enamel surface remained exposed. The enamel surface was treated with wet and dry abrasive paper (Silicon carbide 600 grit) to remove approximately 50 μm of the outer, naturally fluoride-rich enamel surface. The surface was then polished with a paste containing aluminum (Linde No. 3, AB Gamma Polishing Alumina) to a natural, mirror-like finish. Internal studies have shown this procedure results in the presentation of a renewed enamel surface that is essentially free of background fluoride.

Day 1	TIME
Saliva soak	8:00pm
Treatment 1	8:00am
Saliva soak*	
Treatment 2	9:00am
Saliva soak	
DEMINERALIZATION	10:00am
Saliva soak*	1:00pm
Treatment 3	2:00pm
Saliva soak	
Treatment 4	3:00pm
Saliva overnight*	3:30pm

Repeat 4 Additional Days

*Indicates saliva soak was replenished with fresh saliva

Figure 1. Daily treatment schedule.

Procedures From *In Vitro* Study

Human Enamel Chips | 96-hr Carbopol Lesion | Slurry Preparation | Slurry Treatment of Specimen | Microdrill Biopsy Technique

Pretreatment of Samples
The prepared enamel samples were demineralized for 96 hours in a weak acid containing solution (pH = 5.0). The demineralization solution consisted of 0.1 M lactic acid and 0.2% polyacrylic acid (Carbopol C907, B.F. Goodrich Company, Cleveland, OH, USA), 50% saturated with hydroxyapatite, and prepared according to the Carbopol method of White.[34] This method produces lesions with a depth of approximately 50-80 μm, and use of this lesion has been reported in other publications using a similar pH cycling model.[22,23,35]

Determination of Surface Microhardness on Human Enamel Pre-pH Cycling
Each specimen was analyzed for surface microhardness with a Buehler microhardness tester. Using a Vickers diamond at a weight of 200 g and dwell time of 15 seconds, hardness indentations were made three times on each surface by essentially dividing the specimen into three equal, pie shaped parts and taking an indentation within each piece of the pie. Hardness numbers were recorded for each of these three indentations, and the number averaged for each specimen. In our laboratory we have chosen to focus on the use of Vickers hardness as a preferred method for assessing surface microhardness, whereas Knoop hardness is routinely employed for cross-sectional micro-hardness assessment. The Vickers diamond gives the ability to measure the impact of the diamond in two directions, which in our technical judgment provides a more useful measure on enamel surfaces than the single length measurement of the Knoop diamond. The average surface micro-hardness value (after demineralization) was calculated for each specimen (Table 2). Using these values, specimens were placed five to a group in such a way that the initial surface hardness of each group of specimens was not significantly different (Table 3). The remaining three specimens with the highest microhardness values (specimens 6, 17, and 25) were discarded. This procedure ensured the baseline level of demineralization was consistent across all groups at the start of the study.

Each specimen was re-analyzed for surface microhardness with a Buehler Micromet® microhardness tester (BUEHLER, Lake Bluff, IL, USA). Hardness numbers were again recorded three times on each specimen in an area adjacent to each of the original indentations using a Vickers diamond at a weight of 200 g and dwell time of 15 seconds. The average hardness numbers were calculated for each specimen (Table 4).

Determination of Soluble Fluoride for Dentifrice Formulations
Soluble fluoride measurements were taken for each test product. Analyses were done using both an aqueous dilution and a pooled human saliva dilution of products. Ten grams of product were measured into a 50 ml beaker and 30 grams of deionized, distilled water were added. Each beaker of solution was mixed thoroughly for four to five minutes. Slurries were then transferred to centrifuge tubes and placed in a centrifuge for ten minutes at 10,000 rpm. Next, 1 ml of supernatant was removed and added to 1 ml of Tisab II buffer. Sample solutions were analyzed by reading the millivolt potential with a calibrated fluoride ion specific electrode (Orion, Model 96-09, Thermo Fisher Scientific, Waltham, MA, USA). Fluoride concentration was determined from a calibration curve obtained on the same day of the analysis (Table 5, Figure 2).
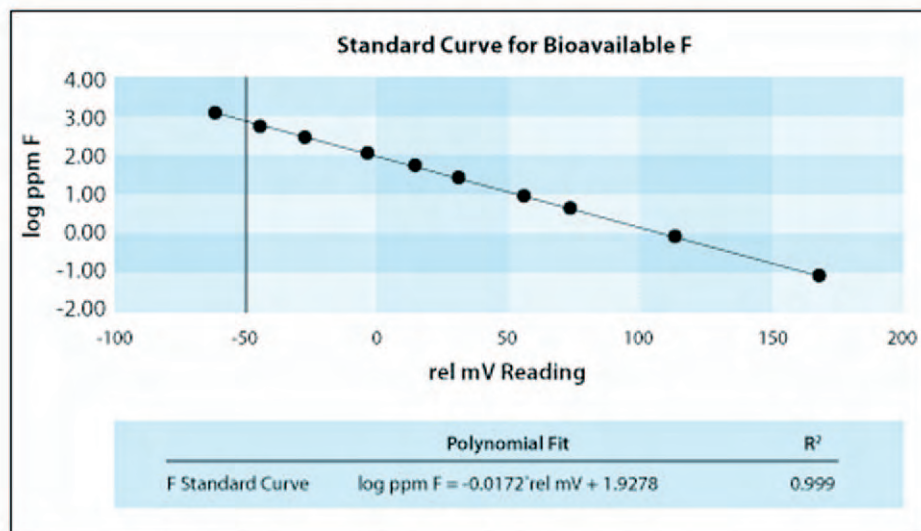
Figure 2. Standard curve used to convert relative millivolt readings into fluoride concentration (ppm F).

Fluoride Uptake Measurement

Once the treatment of the enamel samples with the toothpaste-saliva mixture was completed, an enamel biopsy was taken from each sample of enamel using the microdrill biopsy technique to a depth of 100 μm.[11] This ensured the initial lesions were sampled to their full depth. The diameter of the enamel sample biopsy was measured. The enamel sample was removed and carefully collected, and then dissolved in a solution of 3 parts 0.5 M $HClO_4$: 3 parts TISAB II: 2 parts 1.0N NaOH. The fluoride content of the solution was determined using the Orion, Model 96-09 ion-selective electrode. The fluoride concentration related to the surface removed was calculated and expressed as fluoride uptake in micrograms of fluoride per unit of surface area sampled (μg/cm^2).

Results

Average Surface Hardness

Groups 2 and 3 showed a statistically significant greater ($p<0.05$) change, indicating greater remineralization, compared to all other groups (Table 6). The average change was 23.45 for Group 2 and 22.65 for Group 3. All other groups had changes ranging from 4.25-8.62. No other statistically significant differences in surface microhardness were observed between groups.

Fluoride Uptake Results

Groups 2 and 3 showed statistically significantly greater fluoride uptake versus all other groups

($p<0.05$). Groups 1 and 5 were significantly different from Group 6. No other statistically significant differences in fluoride uptake were observed (Table 7). Although cross-sectional microhardness or quantitative, transverse microradiography would have provided greater information regarding the overall performance of each product tested in the study, these measures were not included in this phase of the study.

Discussion

Since fluoride not only prevents demineralization of healthy enamel but also promotes remineralization of demineralized enamel,[13-17] it is inappropriate to focus the measure of fluoride uptake from fluoride-containing preparations on healthy enamel. In the absence of mineral deficiency in healthy enamel, there is essentially no place on or in the crystal structure for fluoride to incorporate. Investigations using demineralized enamel, which represent active caries, are thus more predictive of potential anticaries efficacy. The importance of fluoride uptake in demineralized enamel has also been noted by Clarkson et al.[36] in which he found the smaller the lesions, the more fluoride they contained. This effect 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 mean fluoride uptake values measured after treatment of the demineralized enamel samples with a mixture of saliva and toothpaste

were between 3.6 and 40.4μg F/cm^2, depending upon the toothpaste (Table 7). Although the negative control paste (Group 6) did not contain any fluoride, enamel samples treated with this paste had a low yet measurable level of fluoride uptake. This can be attributed to fluoride contained in the pooled saliva that was mixed with all the toothpastes before treatment of the enamel samples. Since pooled saliva was used for treatment of all samples in this study, it can be concluded the baseline fluoride uptake of all samples was 3.6 μg/cm^2. In this study, the highest fluoride uptake of any of the European market products was achieved after use of the toothpaste containing a combination of NaF and SMFP (Table 7).

Previous studies have demonstrated the differences in fluoride uptake by enamel cannot necessarily be attributed simply to the fluoride content of the toothpastes for the highest fluoride uptake does not always result after use of the toothpaste with the highest fluoride concentration.[22,37] In addition, toothpastes with roughly the same theoretical fluoride content do not always produce the same level of fluoride uptake.[23,24]

The fluoride uptake values of 34.9 to 40.4 μg/cm^2 for Groups 2 and 3, respectively, did not differ significantly. Groups 1, 4, and 5 all yielded significantly lower fluoride uptake than preparations 2 and 3; ($p < 0.05$ by the Tukey-Kramer HSD test), in spite of the fact all of

the products tested had higher total fluoride concentrations than Group 2 (1100 ppm F as NaF). The only Group depositing a higher level of fluoride into the teeth than the 1100 ppm F control was Group 3, formulated with 1500 ppm F as NaF + 1000 ppm F as SMFP (Fluocaril® BiFluoré 250). Although this value was not significantly different from the positive control dentifrice, the strong directional increase in fluoride uptake beyond that provided by the positive control indicates the product is effective at delivering fluoride to caries lesions. Further comparison of these products in situ is recommended in order to determine if there is a greater difference in performance under *in vivo* conditions of use.

Fluoride uptake values measured for Groups 4 and 5, both of which are formulated exclusively with 1400 ppm F as amine fluoride (AmF), provided less than one-half of the fluoridating efficiency of the USP Reference Standard toothpaste formulated with 1100 ppm F as NaF (corrected for placebo effect). Fluoride uptake for Group 1, which contained 2500 ppm F as SMFP, also provided less than one-half of the amount of fluoride provided by the NaF based positive control.

Results for the AmF based products (Groups 4 and 5) appear to be at odds with the experiments of Klimek[38] who showed no significant difference in uptake of fluoride in demineralized enamel in vitro between the fluoride compounds NaF, AmF, and NaMFP. However, in contrast to the present study, Klimek used pure fluoride solutions rather than commercially formulated toothpastes. In addition, the samples were not treated with fresh human saliva but with artificial saliva. Similarly, Newby et al.[33] demonstrated relatively high fluoride uptake values for a marketed 1400 ppm F (AmF) formulation relative to results presented for an 1100 ppm F (NaF) control. In this study, the authors diluted product with water rather than with saliva, a condition that does not occur in the mouth. The bioavailability of fluoride can be affected not only by the composition of the toothpastes[9,23,32,37] but also by interactions with human saliva.28 AmF, in particular, has a relatively low pH when diluted with water rather than saliva (Table 8). When *in vitro* studies utilize water instead of saliva as the product diluent, results for AmF are generally more favorable with the aqueous dilution.[28,33] This effect is generally

considered to be an artifact of study design rather than product effectiveness. *In vivo* product is always diluted with saliva rather than water. Thus, in order to gauge potential effectiveness of an AmF based formulation, saliva should always be considered a necessary requirement for dilution of product in *in vitro* studies. Use of salivary dilution is also recommended for testing SMFP based formulations as well as salivary enzymes assist in the hydrolysis of the covalently bound SMFP to release free F ions.[1] As the model used for this study includes both salivary dilution of product as well as multiple daily freshening of pooled, human saliva baths between treatments, the model is well suited to assess the relative differences in potential performance for not only NaF based products but for assessing performance for AmF and SMFP based formulations as well.

Differences in fluoride uptake between AmF-containing toothpastes in Groups 4 and 5 is somewhat puzzling, especially considering the fact that both products are reportedly formulated with 1400 ppm F and pH values of both products taken with either aqueous or salivary dilution as well as available (soluble) fluoride appear to be roughly similar (Table 8, Table 9). However, in a previous report,[22] the authors also found a similar effect in comparing results of two AmF based formulations that also performed at levels of efficiency different from initial predictions. In that study, however, the authors discovered the two formulations in question were somewhat different in constituents. In this study, the authors noted a particular difficulty in dispersing both of these toothpastes in saliva (Groups 4 and 5) with the magnetic stirring system, in spite of additional efforts to fully disperse product using the aid of a spatula. Further evaluation of these products is advised to determine whether this issue of dispersion is also present in the mouth as poor dispersion has the potential to result in a lowered effectiveness of a product *in vivo*.

Sättler et al.[25] demonstrated in the presence of human saliva the bioavailability of fluoride from NaF-containing toothpastes is significantly greater than from NaMFP-containing toothpastes. The results of some *in situ* studies, in which demineralized enamel samples were worn by volunteers, also showed fluoride uptake was less from NaMFP-containing toothpastes than from NaF- or AmF-containing toothpastes.[27,37] The most effective Group tested in this study (Fluocaril® Bi-Fluoré 250) contained a combination of NaF and SMFP and a total F level of 2500 ppm F. This particular combination of fluoride actives coupled with a compatible silica based abrasive system provides a high level of fluoridating efficiency.

Surface microhardness values taken at the end of the cycling experiment provides an excellent indication of changes that have occurred in the underlying enamel structure. Positive changes in surface microhardness over the course of a study are indicative of significant degrees of remineralization, while negative values would indicate further demineralization. In this study, all of the products tested including the placebo, resulted in a net increase in surface microhardness. However, the level of remineralization demonstrated for Test Groups 2 (1100 ppm F as NaF) and 3 (1500 ppm F as NaF + 1000 ppm F as SMFP) was significantly greater than that resulting from treatment with Test Groups 1, 4, 5, and 6. Importantly, the 2500 ppm F (SMFP) product (Group 1) and the two products formulated with 1400 ppm F as AmF (Groups 4 and 5) failed to demonstrate any significant change in surface microhardness relative to the placebo (Group 6) control (Table 6).

Although Groups 1, 4, and 5 all provided greater levels of fluoride to the enamel over the course of the study than the placebo control, the level of F delivered from these particular formulations appears to be insufficient to enhance the rebuilding of mineral structure within the demineralized zone under the conditions of this study. In contrast, Groups 2 (USP Clinically Proven Reference Standard) and 3 (Fluocaril® Bi-Fluoré 250) both provided statistically significant levels of remineralization.

Conclusions
The results of the present study clearly demonstrate the ability of in vitro experiments to reveal relative efficiencies in the ability of various toothpaste formulations to deliver fluoride to the teeth. It is insufficient to simply measure the fluoride concentration in the toothpaste itself as an indication of potential efficacy. From detailed studies under conditions simulating the human oral cavity, the bioavailability of fluoride is heavily

influenced by interaction with human saliva. The inclusion of human saliva as a diluent for product to simulate *in vivo* treatment is a critically important aspect to consider for any *in vitro* study and most importantly when testing either AmF or SMFP containing formulations.

The results of this study confirm the Fluocaril Bi-Fluoré 250 product formulation provided the highest level of fluoride uptake and mineralization to the demineralized enamel compared to the other marketed products included in the study. The combination of NaF and SMFP in a compatible silica based abrasive system provided over twice the level of added fluoride to the demineralized enamel compared with either the 2500 ppm F (SMFP) or either of the 1400 ppm F (AmF) products.

Table 1. Names and coding of toothpastes tested.

Test Group Code*	Preparation	Toothpaste	Manufacturer	Country of Origin
1	Test Product	Lacer®	Lacer, S.A.	Spain
2	Positive Control	USP Reference Standard	Procter & Gamble Co.	USA
3	Test Product	Fluocaril® Bi-Fluoré 250	Procter & Gamble Co	France
4	Test Product	Colgate Fluor Active	Colgate-Palmolive Co.	Denmark
5	Test Product	Elmex®	GABA International AG	France
6	Negative Control	Placebo	Procter & Gamble Co.	USA

Table 2. Initial Surface Hardness – Individual Specimens: Hardness numbers using the Vickers' hardness scale at 200 gram weight, dwell time of 15 seconds.

Sample Number	Surface Hardness Measurement			Average Surface Microhardness Value	Std Dev
	1	2	3		
1	18.50	15.90	20.90	18.43	2.5
2	9.00	12.90	11.10	11.00	2.0
3	15.30	19.80	12.20	15.77	3.8
4	22.40	31.60	21.90	25.30	5.5
5	14.60	10.10	11.50	12.07	2.3
*6	28.70	21.90	47.90	32.83	13.5
7	17.60	10.50	13.50	13.87	3.6
8	9.80	11.20	13.00	11.33	1.6
9	24.10	10.00	19.10	17.73	7.1
10	13.00	23.00	24.70	20.23	6.3
11	5.90	13.80	12.30	10.67	4.2
12	9.30	13.20	15.00	12.50	2.9
13	15.20	14.60	11.60	13.80	1.9
14	10.20	12.00	10.10	10.77	1.1
15	31.30	17.00	21.40	23.23	7.3
16	15.10	17.00	16.20	16.10	1.0
*17	32.30	23.00	35.00	30.10	6.3
18	28.20	18.00	26.00	24.07	5.4

Table 2. Continued

19	11.80	13.20	12.90	12.63	0.7
20	17.40	15.30	15.50	16.07	1.2
21	18.50	22.40	20.60	20.50	2.0
22	19.00	14.60	14.40	16.00	2.6
23	24.60	26.00	23.10	24.57	1.5
24	9.60	23.00	9.20	13.93	7.9
*25	32.80	23.60	23.50	26.63	5.3
26	10.10	12.60	10.50	11.07	1.3
27	19.10	16.20	18.30	17.87	1.5
28	20.90	14.70	18.20	17.93	3.1
29	15.20	13.30	17.10	15.20	1.9
30	19.10	18.80	16.80	18.23	1.3
31	11.40	12.70	15.60	13.23	2.2
32	11.80	15.40	11.90	13.03	2.1
33	19.00	12.80	8.40	13.40	5.3

* Total of 30 specimens were required for the study. Specimens with three highest initial surface microhardness values were not included in the randomization of specimens to be included in this study.

Sample Number: Unique number assigned to each chip.

Hardness Number: Vickers hardness number calculated from indent length for each measurement.

Table 3. Average initial surface hardness by group.

Test Group	$VHN_{initial}$ (S.D.)
1	15.85 (3.76)
2	16.18 (5.40)
3	16.12 (4.72)
4	16.36 (5.03)
5	15.64 (3.73)
6	15.95 (5.45)

Table 4. Surface microhardness values after completion of pH cycling.

Sample Number	Surface Hardness Measurement			Average Surface Microhardness Value	Std Dev	Treatment Group
	1	2	3			
11	31.6	19.5	30.6	27.23	6.7	1
20	14.6	23.0	28.0	21.87	6.8	1
7	27.0	15.0	21.3	21.10	6.0	1
1	24.8	23.6	24.1	24.17	0.6	1
10	21.0	38.4	24.6	28.00	9.2	1
14	51.9	33.7	33.8	39.80	10.5	2
33	47.0	30.9	32.5	36.80	8.9	2
24	31.8	37.8	44.0	37.87	6.1	2
30	39.1	43.5	39.7	40.77	2.4	2
23	44.1	45.9	38.8	42.93	3.7	2
2	34.5	26.9	33.4	31.60	4.1	3

Table 4. Surface microhardness values after completion of pH cycling.

31	42.6	45.8	49.7	46.03	3.6	3
29	31.1	53.5	41.1	41.90	11.2	3
28	35.7	22.3	36.2	31.40	7.9	3
15	52.5	46.8	29.4	42.90	12.0	3
26	12.8	20.2	11.5	14.83	4.7	4
32	25.7	25.4	12.4	21.17	7.6	4
3	20.9	33.9	22.8	25.87	7.0	4
27	16.9	20.3	19.9	19.03	1.9	4
18	28.0	32.6	42.4	34.33	7.4	4
8	9.5	12.1	17.6	13.07	4.1	5
19	9.5	17.4	22.4	16.43	6.5	5
22	22.4	17.0	23.2	20.87	3.4	5
9	16.1	27.8	14.1	19.33	7.4	5
21	33.8	29.7	25.7	29.73	4.1	5
5	21.0	18.8	22.7	20.83	2.0	6
12	33.8	16.1	14.5	21.47	10.7	6
13	22.0	19.7	21.8	21.17	1.3	6
16	15.6	17.9	23.6	19.03	4.1	6
4	26.1	38.3	24.5	29.63	7.5	6

Table 5. Fluoride Electrode Calibration – Standard Curves: Solutions containing known fluoride concentrations were diluted, in the same manner as the samples, and measured to generate a curve to which the samples could be compared.

ppm F	log ppm F	rel mV
1000	3.00	-63
500	2.70	-45.4
250	2.40	-27.7
100	2.00	-4.3
50	1.70	13.7
25	1.40	31.3
10	1.00	54.8
5	0.70	72.3
1	0.00	113.2
0.1	-1.00	168

Table 6. Change in average surface hardness by group.

Test Group	$VHN_{initial}$	VHN_{final}	VHN_{change}	Student t*	Tukey-Kramer HSD*
2	16.18	39.63	23.45	a	a
3	16.12	38.76	22.65	a	a
1	15.85	24.47	8.62	b	b
4	16.36	23.05	6.69	b	b
6	15.95	22.43	6.47	b	b
5	15.64	19.89	4.25	b	b

*Means with different letter designation are significantly different ($p < 0.05$) by the Student t Test as well as Tukey-Kramer HSD test.

Table 7. Fluoride Uptake Results: Means and standard errors of the means were calculated for Fluoride Uptake. Means were ranked (largest to smallest) and differences in the means were tested using JMP 5.1 Statistical Discovery Software (Tukey-Kramer Honestly Significant Difference Test).

Treatment Group	Test Product	Mean Fluoride Uptake ± (SEM)*	
3	Fluocaril® Bi-Fluoré 250 (2500 ppmF: 1500ppm F as NaF+1000ppm F as NaMFP)	40.4 ± 2.65	a**
2	USP Reference Standard NaF (1100ppmF/silica)	34.9 ± 4.87	a
5	Elmex® Caries protection (1400ppmF as AmF)	17.7 ± 1.41	b
1	Lacer® (2500ppmF as NaMFP)	15.6 ± 2.28	b
4	Colgate Fluor-Active (1400ppmF as AmF)	9.6 ± 0.76	b,c
6	Placebo (0ppm F)	3.6 ± 0.58	c

*Mean ± SEM (n = 5), 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 Tukey HSD test.

Table 8. pH values using water and saliva as diluents for each treatment group.

Treatment Group	Batch Reference	Test Product	pH value	
			Water Dilution	Saliva Dilution
1	7086 11 2011	Lacer® (NaMFP 2500ppmF)	8.52	8.31
2	HCS4143-080	USP Reference Standard NaF (1100ppmF/silica)	7.19	7.32
3	26146-04 2009	Fluocaril® Bi-Fluoré 250 (2500ppm F: 1500ppm F as NaF + 1000ppm F as NaMFP)	7.45	7.66
4	7187H3	Colgate Fluor-Active (1400ppmF as AmF)	4.96	6.37
5	6465H2	Elmex® Caries protection (1400ppmF as AmF)	4.79	6.22
6	HCS4723-095	Placebo	7.08	7.30

Table 9. Soluble fluoride analysis of test products.

Test Group	Water Mass (grams)	Dentifrice Mass (grams)	rel mV	log ppm F	ppm F
1	30.01	10.04	0.6	1.917	82.70
2	30.03	10.01	-30.0	2.444	277.84
3	30.01	10.00	-27.3	2.397	249.67
4	30.00	10.02	-29.5	2.435	272.40
5	30.01	10.05	-30.0	2.444	277.84
6	30.04	10.06	149.6	-0.645	0.23

Trt: treatment group
Rel mV: Fluoride ion selective electrode reading (after 1 ml of diluent is added to 1 ml of Tisab II buffer)
log ppm F: calculated from the fluoride standard curve
ppm F: log ppm F (calculated from standard curve) converted to ppm F ($10^{\log ppm F}$ = ppm F)

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