



Fluoride bioavailability on demineralized enamel by commercial mouth rinses

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The ability of mouth rinses, available in the international market, to form reaction products on demineralized enamel (bioavailability test) was evaluated *in vitro*. Nine mouth rinses purchased in Chile were evaluated; eight formulated with NaF (one containing 100 µg F/mL and seven containing 226) and one with Na₂FPO₃ (226 µg F/mL as ion F). Demineralized enamel slabs (n=15 per mouth rinse) were sectioned; one half was subjected to the assigned mouth rinse treatment for 10 min and the other half was used to obtain baseline data. Loosely bound and firmly bound fluoride formed on enamel were determined with an ion-specific electrode and the values were expressed in µg F/cm². The concentration of fluoride and the pH of the mouth rinses were previously determined. Concentrations of loosely bound and firmly bound fluoride formed on enamel were independently analyzed by ANOVA and Tukey's test (α=5%). The loosely bound and firmly bound fluoride concentrations (µg F/cm²) formed ranged from 3.2 to 36.2 and 0.4 to 1.7, respectively. Loosely bound fluoride formed on enamel was significantly more effective in discriminating the effect of different commercial mouth rinses than firmly bound fluoride. Mouth rinses with 226 ppm F as NaF and low pH presented significantly greater bioavailability of fluoride on enamel than those with higher pH or lower NaF concentration. The mouth rinse with Na₂FPO₃ showed low reactivity. Although further studies are necessary, the findings showed that commercial fluoride-containing mouth rinses have important variations in enamel fluoride bioavailability, which may result in differences on anticaries efficacy.

Introduction

According to the best available scientific evidence, mouth rinses with sodium fluoride (NaF) 0.05% (226 ppm F) for daily use or with 0.2% (900 ppm F) for weekly use are effective for caries control (1). Aqueous solutions of 0.2% NaF are still used in community programs to control caries (2), but most of the commercial products are formulated with 0.05% NaF. Commercial fluoride mouth rinses are products with a cosmetic rather than a therapeutic use. These products contain complex formulations with several ingredients to make the product attractive to the consumer. Some of these substances can positively or negatively interfere with the anticaries mechanism of action of fluoride (3).

The anticaries mechanism of action of fluoride mouth rinses involves an increase in the fluoride concentration in the oral cavity during rinsing, followed by retention of varying levels of fluoride in the oral cavity over time (4). Fluoride retention occurs by the enrichment of dental biofilms and the formation of reaction products with enamel (5-7). While the diffusion of fluoride throughout the biofilm is a simple phenomenon that depends only on fluoride solubility in the mouth rinse (6), other ingredients of the formulation, such as detergents, preservatives, and some additives (8-11), might influence the formation of reaction products on clean dental surfaces.

When fluoride reacts with enamel two reaction products are formed, one that is soluble in alkali and another only solubilized by acids. As these products were not still characterized chemically, they are respectively named in the literature as loosely bound (or CaF₂-like) and firmly bound (or FAp-type). Loosely bound fluoride is the main product of reaction in terms of concentration (>90%) and is currently considered the most important product regarding anticaries efficacy of topical fluorides (12), mainly professional fluoride application (13-14). The formation of fluoride reservoirs on enamel by fluoridated products depends directly on the concentration of fluoride applied and inversely to the pH of the product (15). It also depends on the type of fluoride salt used in the commercial product. Thus,

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when compared among the fluoride salts usually used in dental hygiene products; sodium fluoride (NaF), stannous fluoride (SnF₂), amine fluoride (AmF), and sodium monofluorophosphate (Na₂FPO₃), Na₂FPO₃ is the least reactive, because fluoride ion is bound to the phosphate moiety and is not available to react with enamel upon rinsing. Therefore, it is expected that a mouth rinse containing Na₂FPO₃ is less effective than a NaF-based one. In addition, different ingredients in hygiene products, such as pyrophosphate and sodium lauryl sulphate, reduce the reactivity of fluoride with enamel (8-9,16). So, a validated test of fluoride reactivity with enamel (bioavailability test) (17) could be used to differentiate fluoride mouth rinses available in the market.

Thus, considering the high variety of mouth rinses currently in the market (18-19) with different fluoride concentrations (10,18-22), different pH (19) and different ingredients (antimicrobials, oxidizing agents, analgesic agents, flavorings, preservatives, among others), we evaluated if it would be possible to differentiate commercial mouth rinses by their ability to form reaction products on enamel. We used a previously validated protocol (17) in terms of the dose-response effect of fluoride concentration and reaction time to form products of reaction on demineralized enamel implicated on the anticaries mechanism of fluoride (12). The null hypothesis of this study is that commercial mouth rinses can not be differentiated by the ability to form reaction products on enamel.

Material and methods

Experimental Design

Nine fluoridated mouth rinses were purchased in different pharmacies and supermarkets from Talca, Chile (Table 1). These mouth rinses are among the five most sold in Chile. Since multinationals company manufactures them, they are also found worldwide. Eight were formulated with sodium fluoride (NaF), one containing 100 ppm F (µg F/mL) and the others containing 226 ppm F. One product was formulated with sodium monofluorophosphate (Na₂FPO₃) at a concentration of 226 ppm as total fluoride. As controls, two solutions were prepared, one with NaF (Merck, lot1064497002) and the other with Na₂FPO₃ (BK Giulini, lot M#06565), both containing 226 ppm F; the pH of these solutions was not adjusted. For the analyses, letters encoded the mouth rinses and the control solutions. Table 1 shows information of the mouth rinses tested. The concentration of fluoride and pH found in the commercial mouth rinses and the controls were determined as previously described (19).

Table 1. General information of the mouth rinses, total fluoride (TF) concentration (µg F/mL) expected and found (as F⁻ or FPO₃²⁻; mean±SD), and pH of the mouth rinses evaluated and the controls.

Code	Treatments	*Manufacturer	*Lot	*Fluoride salt	*Expected	TF (µg F/mL)		pH
						as F ⁻	as FPO ₃ ²⁻	
A	Colgate Plax Ice Infinity	Colgate	7321BR121AH1106	NaF	225	222.4 ± 0.6	-	5.28
B	Colgate Plax Kids	Colgate	8024BR121AH1340	NaF	225	227.6 ± 1.9	-	5.27
C	Colgate Plax Fresh Mint	Colgate	7267BR122AH1515	NaF	225	224.6 ± 0.0	-	5.22
D	Colgate Plax Soft Mint	Colgate	5335BR121AH0915	NaF	225	238.1 ± 1.3	-	5.24
E	Listerine Anticaries Zero Alcohol	Johnson&Johnson	2547COC184084	NaF	220	210.6 ± 2.9	-	4.28
F	Listerine Cuidado Total Zero	Johnson&Johnson	3011COC184789	NaF	100	97.5 ± 1.3	-	4.36
G	Vitis Orthodontic	Dentaid	L1020	NaF	225	229.9 ± 0.0	-	4.63
H	Vitis Sensible	Dentaid	L1004	Na ₂ FPO ₃	226	3.0 ± 0.2	221.6 ± 0.0	5.75
I	Oral B Complete	Procter & Gamble	7178852522	NaF	226	220.2 ± 1.2	-	5.79
J	NaF (control)	-	-	NaF	226	226.2 ± 1.2	-	6.19
K	Na ₂ FPO ₃ (control)	-	-	Na ₂ FPO ₃	226	4.0 ± 0.0	223.6 ± 0.6	6.70

NaF= Sodium fluoride; Na₂FPO₃= Sodium monofluorophosphate; F⁻= ion fluoride; FPO₃²⁻= ion monofluorophosphate

*Declared in the label by the manufacturer

For the reactivity test (17, 23), hemi-slabs of bovine enamel with induced caries lesions were used (n=15/treatment). One hemi-slab was used for baseline data (negative control) and the other was subjected to specific treatment (mouth rinse or control solutions). The net loosely bound fluoride and firmly bound fluoride concentrations formed ($\mu\text{g F}/\text{cm}^2$) were determined, subtracting the values found in the treatment from the respective baseline. Loosely bound and firmly bound fluoride data were statistically analyzed as further described.

Preparation of Demineralized Enamel Slabs

Enamel slabs (4x4x2 mm) were obtained from bovine teeth as previously described by Noronha et al. (24). Surface hardness (SH) was determined and 226 slabs presenting SH of $323.9 \pm 18.4 \text{ Kg}/\text{mm}^2$ were selected. Slabs were immersed (2 mL solution per mm^2 of enamel) in 0.1 M acetate buffer pH 5.0 containing 1.28 mM Ca, 0.74 mM Pi and $0.03 \mu\text{g F}/\text{mL}$, during 16 h at 37°C , to create caries-like lesions (23, 25). The SH was again determined and 165 slabs with a mean SH of $4.64 \text{ Kg}/\text{mm}^2$ (SD 0.99) were selected for the reactivity test. All demineralized slabs were randomly distributed into the 11 treatments (n=15 per group). For the reactivity test, the slabs of each treatment were coded, sectioned through the middle and the exposed area was calculated. The 15 control hemi-slabs and the 15 treatment hemi-slabs were mounted on wax plates for each specific treatment (Figure 1).

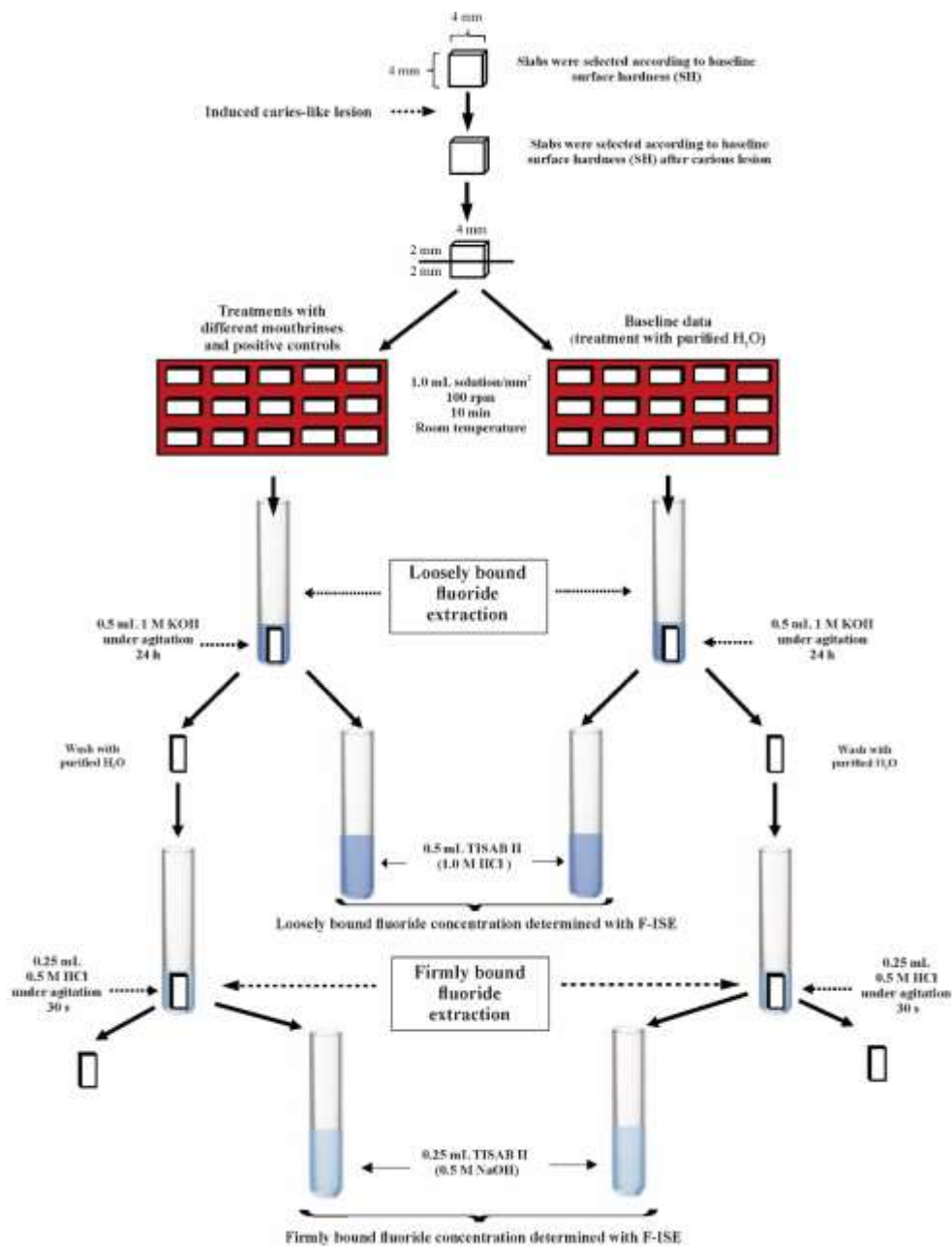


Figure 1. Flow chart of the experimental design

Reactivity Test (Enamel Bioavailability Test)

For the assessment of the fluoride reactivity from the mouth rinses, a validated protocol described by Arthur et al. (17) was used and is illustrated in Figure 1. This protocol presents dose-response effect to fluoride concentration of the treatment, at a standardized time of 10 min of reaction, and the concentration of fluoride products formed in demineralized enamel. The applicability of this protocol was showed because it was successfully used to evaluate commercial mouth rinse formulation before launching in the market (23). Each wax plate was placed in the assigned treatment mouth rinse or in the control fluoride solutions at a volume of 1.0 mL/mm² of enamel area. The wax plate with the control hemi-slabs were placed in purified H₂O. After 10 min at room temperature and under agitation (100 rpm), the wax plates were removed from the treatment and the enamel slabs were rinsed with purified water during 1 min. The hemi-slabs were removed from the wax plates and their surfaces were isolated with wax and placed in individual encoded tubes, leaving the active enamel surface uncovered.

Immediately after the reactivity test, each hemi-slab was individually immersed in 0.5 mL of 1.0 M KOH for loosely bound fluoride extraction (26). After 24 h at room temperature and under agitation (100 rpm), 0.5 mL of TISAB II (1.0 M acetate buffer, pH 5.0, 1.0 M NaCl, 0.4% CDTA, containing 1.0 M HCl) was added to each tube to neutralize and buffer the extract. The hemi-slabs were removed and washed for 30 s with purified water. After loosely bound fluoride extraction, each hemi-slab was immersed in 0.25 mL of 0.5 M HCl for 30 s under agitation, for the extraction of firmly bound fluoride formed in the enamel. The extract was neutralized and buffered with the same volume of TISAB II pH 5.0, containing 0.5 M NaOH (27). Fluoride concentrations in the alkali and acid extracts were determined with fluoride ion-specific electrode (F-ISE).

Fluoride Analysis

To quantify fluoride content extracted from enamel, a F-ISE (Orion 96-09, Thermo Scientific Orion, Boston, MA, USA) coupled to an ion analyzer VersaStar (Thermo Scientific Orion) were used. For loosely bound fluoride quantification, the electrode was calibrated in triplicate with fluoride standard solutions ranging from 0.125 to 8.0 µg F/mL prepared from NaF (Sodium fluoride 99.99%, Sigma-Aldrich, lot 215309, St Louis, MO, USA) in 0.5 M KOH and TISAB II (containing 1 M HCl) at 50% (v/v). The variation coefficient of the triplicates was 0.4% ($r^2 = 1.000$). The accuracy of the calibration was checked with a fluoride standard solution Orion 940907 (Thermo Scientific). For firmly bound fluoride determination, the electrode was calibrated in the same way but with fluoride standard solutions ranging from 0.0625 to 4.0 µg F/mL prepared in 0.25 M HCl and TISAB II (containing 0.5 M NaOH) at 50% (v/v). The variation coefficient of the triplicates was 0.5% ($r^2 = 0.9998$). The readings in mV of the sample solutions were transformed into fluoride concentration using the software Microsoft Office Excel. The net results of fluoride formed on enamel (µg F/cm²) was calculated subtracting the values found in each treated hemi-slab from its control hemi-slab (baseline values).

Statistical Analysis

The assumption of equal variances and normal distribution were verified, and the loosely bound fluoride data were transformed to log₁₀(X) and firmly bound fluoride to square root (\sqrt{x}) (28). These transformed variables were analyzed by ANOVA followed by the Tukey test. The analyses were made with the software SAS (SAS Institute Inc., Version 8.01, Cary, N.C., USA), with a significance level of 5%. The correlation between pH and concentration of loosely bound fluoride was determined by linear regression using the Microsoft Office Excel.

Results

Table 1 shows that the mouth rinses presented the expected fluoride concentrations based on the information declared by the manufacturers, with a pH ranging between 4.28 to 6.70.

The data from enamel bioavailability (reactivity test) (Table 2) showed that the mouth rinses formed more loosely bound fluoride products on enamel than firmly bound fluoride. These findings were confirmed by the results found with the fluoride control solutions prepared.

Table 2. Mean (\pm SD; n) of fluoride concentration ($\mu\text{g F/cm}^2$) formed in demineralized enamel as Loosely bound and Firmly bound fluoride according to the treatments and positive controls

Code	Treatments	$\mu\text{g F/cm}^2$	
		Loosely bound fluoride	Firmly bound fluoride
A	Colgate Plax Ice Infinity	19.0 ± 4.5^C (n=15)	1.0 ± 0.7^b (n=14)
B	Colgate Plax Kids	23.3 ± 2.6^B (n=15)	$1.3 \pm 0.4^{a,b}$ (n=15)
C	Colgate Plax Fresh Mint	23.9 ± 3.7^B (n=15)	1.0 ± 0.3^b (n=15)
D	Colgate Plax Soft Mint	23.3 ± 3.4^B (n=15)	1.1 ± 0.7^b (n=15)
E	Listerine Anticaries Zero Alcohol	36.2 ± 4.2^A (n=15)	1.7 ± 0.8^a (n=13)
F	Listerine Cuidado Total Zero	18.3 ± 3.0^C (n=15)	$0.8 \pm 0.5^{b,c}$ (n=15)
G	Vitis Orthodontic	34.7 ± 3.9^A (n=15)	1.1 ± 0.5^b (n=15)
H	Vitis Sensible	3.2 ± 0.7^E (n=15)	$0.4 \pm 0.24^{c,d}$ (n=15)
I	Oral B Complete	$19.7 \pm 2.7^{B,C}$ (n=15)	0.9 ± 0.4^b (n=15)
J	NaF (Positive control)	13.4 ± 2.3^D (n=15)	$0.7 \pm 0.4^{b,c}$ (n=15)
K	Na ₂ FPO ₃ (Positive control)	3.2 ± 0.6^E (n=15)	$0.5 \pm 0.41^{c,d}$ (n=15)

Treatments, within columns, followed by distinct letters statistically differ ($p < 0.05$).

Values presented in the table are not transformed; Loosely bound fluoride values were transformed to \log_{10} and Firmly bound fluoride to square root for statistical analysis.

Differences among the mouth rinses on fluoride bioavailability on enamel were better distinguished by loosely bound than by firmly bound fluoride. Hence, when using loosely bound fluoride, commercial mouth rinses could be separated into four groups (E, G>B, C, D>A, F>H). Conversely, if the products were compared based on their firmly bound fluoride, only two groups (E>A, C, D, G, I) could have resulted ($p < 0.05$). This better performance of loosely bound is confirmed by the effect of the positive control solutions prepared. Thus, the NaF standard solution (J) is more reactive than the Na₂FPO₃ solution (K), in terms of loosely bound fluoride formation on enamel ($p < 0.05$), but the reactivity of these fluoride salts is similar when compared by firmly bound fluoride, without statistical differences ($p > 0.05$).

Among the commercial mouth rinses, Listerine Anticaries Zero Alcohol (E) and Vitis Orthodontic (G) presented greater bioavailability of loosely bound fluoride than most of the other products. The product with Na₂FPO₃ (H) presented the lowest concentration of loosely bound fluoride ($p < 0.01$), in comparison with all the other commercial mouth rinses.

Furthermore, the findings (Table 1 and 2) also suggested that products with lower pH formed more loosely bound fluoride on enamel. In fact, Figure 2 shows the correlation found between these

variables ($r^2=-0.62$; $p=0.0039$). The correlation between pH and firmly bound fluoride was not significant ($r^2=-0.0015$; $p=0.752$).

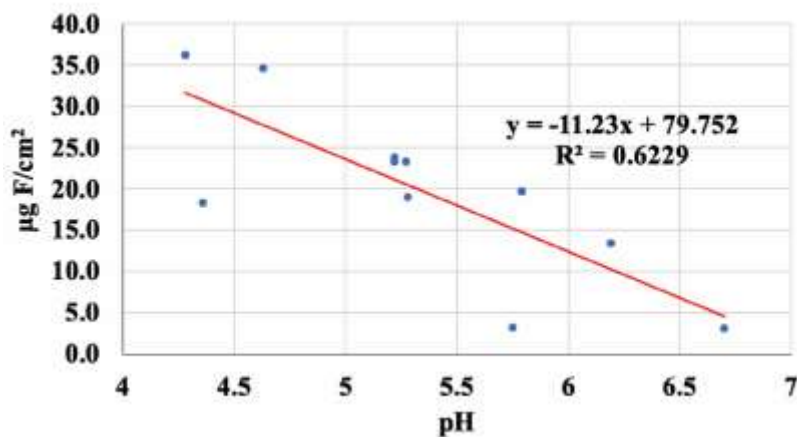


Figure 2. Linear regression analysis between the concentrations of loosely bound on enamel and pH of the treatments. ($p=0.0039$).

Discussion

Our results reject the null hypothesis formulated because they clearly showed that the commercial mouth rinses evaluated were different regarding fluoride bioavailability on enamel, providing new knowledge about these products. The results may be explained by the composition of the mouth rinses tested, whose type of fluoride salt and the fluoride concentration were confirmed, and their pH were also checked (Table 1) to give support to our findings. This discussion will be focused on loosely bound fluoride formed on enamel rather than firmly bound fluoride because the former: *i*) is considered more important for the anticaries effect of topical fluorides than the firmly bound fluoride formed (12); *ii*) is able to differentiate the effect of fluoride salts from control solutions (treatment J vs. F); and *iii*) is able to separate the mouth rinses into four groups ($E,G > B,C,D > A,F > H$) compared with only two groups ($E > A,C,D,G,I$) if data of firmly bound fluoride were considered (Table 2).

Thus, the mouth rinses E and G were the two more reactive commercial products in terms of loosely bound fluoride formation on enamel (Table 2). These mouth rinses have in common NaF as the fluoride salt, high fluoride concentration (>200 ppm F) and low pH (<5.0) when compared to the other commercial products (Table 2). Our data show that the effect of fluoride concentration is important for the concentration of loosely bound fluoride formed on enamel, because mouth rinses E and F have similar pH (4.28 and 4.36), but, E was 2-fold more reactive than F as its fluoride concentration is 2.15-fold greater. However, the effect of pH seems to be more important than fluoride concentration (29), because NaF mouth rinses A, B, C, D, and I have similar concentration than E (around 220 ppm F), but they were 1.7 times less reactive than E (Table 2), although the pH of E is only 1.1 units lower than the mean pH of these five mouth rinses (Table 1). Indeed, we showed that there is a significant inverse correlation ($r^2=-0.62$; $p=0.0039$) between the pH of the mouth rinses tested and loosely bound fluoride concentration formed on demineralized enamel (Figure 2), but not for firmly bound fluoride ($r^2=0.0015$; $p=0.752$).

The effect of pH on loosely bound fluoride formation on enamel is very well known for products intended for professional application (15) and dentifrice (30). For the other hand, it is less known for fluoridated mouth rinses (29), but we clearly showed that it also occurs with mouth rinses. Loosely bound fluoride forms upon acid dissolution of the enamel crystal because calcium ion becomes available to react with free ionic F from the mouth rinse, precipitating according to the degree of supersaturation reached. On the other hand, when mouth rinses A, B, C, and D, that present the same NaF concentration and pH greater than 5.0, are compared (Table 2), A was less reactive than B, C or D ($p<0.05$). The lowest reactivity of A may be due to the interference of other ingredients in the formulation, which is very well known (8-11, 16).

The lowest reactivity found for the commercial mouth rinse H can be explained by the formulation with Na_2FPO_3 (Table 1). This type of fluoride is usually used in toothpastes containing calcium as abrasive (6). It has been long recognized that the reactivity of Na_2FPO_3 with enamel is due to the residual free ionic fluoride released from FPO_3^{2-} moiety rather than from FPO_3^{2-} (31). As this mouth rinse had only 1.3% of total fluoride as free fluoride, it presented low bioavailability with enamel. Based on the comparison with the Na_2FPO_3 pure solution prepared in the laboratory as control, low reactivity was not due to pH or interferences of this commercial mouth rinse (Table 2), as commercial and control did not show statistical differences ($p>0.05$). Mouth rinses formulated with Na_2FPO_3 could be effective on caries control if the mechanism of action of fluoride from these commercial products is not their reactivity, but the simple diffusion to dental biofilms, where MFPases hydrolyze Na_2FPO_3 and fluoride ion is released to interfere with the caries process (32).

Interestingly, there was a lower activity of the NaF control solution (J) prepared (Table 1), compared with the commercial mouth rinses, albeit both presented pH values higher than 5.0 and similar NaF concentrations (Table 2). Thus, the concentration of loosely bound fluoride formed on enamel by the mouth rinses A, B, C, and D was statistically greater than J solution ($p<0.05$). The lower reactivity could be simply explained by the higher pH (6.19) of this solution compared to lower pH of the mouth rinses (mean 5.36), but this was not the case. We have shown previously (10-11, 33-34) that the initial low pH of fluoride mouth rinses is important on the reaction with enamel, but the maintenance of the pH during the time of the reaction is more relevant. Therefore, commercial mouth rinses with buffer capacity to maintain the low pH during the reaction might be able to form more loosely bound fluoride products on enamel.

To the best of our knowledge, this is the first publication showing that fluoride commercial mouth rinses can be compared by their ability to react with demineralized enamel, but the limitations of the findings should be clearly stated considering: *i*) the experimental model used and *ii*) the importance of loosely bound products formed on enamel related to the anticaries effect of fluoride mouth rinse.

First of all, there is a model able to simulate the real conditions that occurs in the oral cavity during the use of fluoride products. On the other hand, every model is valid only if it shows dose-response effect between fluoride concentration and the variable response under study. The model used (17) was validated in terms of dose response-effect to fluoride (NaF) concentration using fluoride aqueous solutions and the products of reaction formed on demineralized enamel. Sound enamel could also be used, but dose-response effect is rather found with demineralized enamel and one of the effects of fluoride is to repair early caries lesions. The time of reaction does not simulate the time of fluoride rinse in the mouth, but it was stated because the chemical reaction between fluoride at concentration around 250 ppm F with enamel is time dependent and stabilizes in 10 min. The reaction was made in the absence of saliva, because the proportion of mouth rinse: saliva during the rinse is 10:1, what makes saliva effect of low relevance in the amount of products formed on enamel. Considering that loosely bound fluoride (CaF_2 -like) is the main product of reaction formed and saliva even not diluted is undersaturated regarding to CaF_2 , the only relevant source of Ca for the reaction are minerals from enamel. Irrespective of these limitations, the model used is able to differentiate the effect of fluoride concentration, pH and the type of fluoride salt, present in the formulation, which makes our results scientifically relevant.

The findings found are promising but should not be used to claim anticaries superiority of any product. Thus, our data clearly showed that loosely bound products are mainly formed on enamel rather than firmly bound (Table 2). The clinical relevance of these data is another limitation of our study because there is no research showing that for the anticaries effect of fluoride from mouth rinse it is the most relevant product. For professional fluoride application, there is consensus that the anticaries effect of fluoride is attributed to loosely bound fluoride formed on enamel (12), and in addition, dose-response effect was experimentally showed (14). In fact, loosely bound fluorides are expected to be the main products of reaction formed on enamel by mouth rinse, because the concentration in these products are greater 50 ppm F, and from that the formation of CaF_2 -like products is favored (35). Thus, further studies will be necessary to show that loosely bound fluoride products formed either on clean sound enamel surfaces or surfaces with early caries lesions would be relevant, respectively for the “preventive” or the “therapeutic” effect of fluoride, as it has been showed for other fluoride products (36-37).

In conclusion, the present findings of fluoride bioavailability in demineralized enamel showed that commercial fluoride mouth rinses can be differentiated by this test, but further studies are necessary to confirm if there is a dose-response effect between loosely bound fluoride products formed on enamel by commercial mouth rinses and reduction of demineralization or enhancement of remineralization.

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Resumo

Os enxaguatórios bucais comerciais fluoretados diferem na concentração e tipo de sal de fluoreto, no pH e têm alguns ingredientes que podem interferir na reatividade do fluoreto com o esmalte desmineralizado. A capacidade de enxaguatórios bucais comerciais de formar produtos de reação em esmalte desmineralizado (teste de biodisponibilidade) foi avaliada *in vitro*. Nove enxaguatórios bucais adquiridos no Chile foram avaliados, oito formulados com NaF (um contendo 100 µg F/mL e sete contendo 226) e um com Na₂FPO₃ (226 µg F/mL como íon F). Os blocos de esmalte desmineralizados (n=15 por grupo) foram seccionados, uma metade foi submetida ao tratamento com o enxaguatório designado por 10 min e a outra metade foi usada para dados *baseline*. Fluoreto fracamente e firmemente ligados no esmalte foram determinados com um eletrodo íon-específico e os valores foram expressos em µg F /cm². A concentração de fluoreto e o pH dos enxaguatórios foi previamente determinada. As concentrações de fluoreto tipo fluoreto fracamente ligado e fortemente ligado formadas no esmalte foram analisadas independentemente por ANOVA e teste de Tukey (α=5%). As concentrações de fluoreto fracamente ligado e fortemente ligado formados variaram de 3,2 a 36,2 e 0,4 a 1,7, respectivamente. O fluoreto fracamente ligado formado no esmalte foi significativamente mais eficaz para discriminar o efeito dos diferentes enxaguatórios bucais comerciais do que o fluoreto firmemente ligado. Enxaguatórios bucais com 226 ppm F na forma de NaF e baixo pH apresentaram significativamente maior biodisponibilidade de fluoreto no esmalte do que aqueles com maior pH ou menor concentração de NaF. O enxaguatório com Na₂FPO₃ apresentou reatividade muito baixa. Embora mais estudos sejam necessários, os resultados mostraram que os enxaguatórios bucais comerciais contendo fluoreto apresentam variações importantes na biodisponibilidade do fluoreto, o que poderia resultar em diferenças na eficácia anticárie.

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