*Brazilian Dental Journal (2013) 24(3): 253-257*
*http://dx.doi.org/10.1590/0103-6440201302116*

ISSN 0103-6440

# *In Vitro* Enamel Remineralization by Low-Fluoride Toothpaste with Calcium Citrate and Sodium Trimetaphosphate

[1]Center for Biological and Health Sciences, UNIOESTE - State University of West of Paraná, Cascavel, PR, Brazil
[2]Department of Community and Pediatric Dentistry, Araçatuba Dental School, UNESP - Univ Estadual Paulista, Araçatuba, SP, Brazil

Correspondence: Prof. Dr. Alberto Carlos Botazzo Delbem, Rua José Bonifácio, 1193, 16015-050 Araçatuba, SP, Brasil. Tel. +55-18-3636-3314. e-mail: adelbem@foa.unesp.br, adelbem@pq.cnpq.br

Edo Hirata[1], Marcelle Danelon[2], Isabelle Rodrigues Freire[2], Alberto Carlos Botazzo Delbem[2]

The objective of this study was to evaluate *in vitro* the effect of a low fluoride toothpaste (450 µgF/g, NaF) combined with calcium citrate (Cacit) and sodium trimetaphosphate (TMP) on enamel remineralization. Bovine enamel blocks had the enamel surface polished sequentially to determine the surface hardness. After production of artificial carious lesions, the blocks selected by their surface hardness were submitted to remineralization pH cycling and daily treatment with dentifrice suspensions (diluted in deionized water or artificial saliva): placebo, 275, 450, 550 and 1,100 µgF/g and commercial dentifrice (positive control, 1,100 µgF/g). Finally, the surface and cross-section hardness was determined for calculating the change of surface hardness (%SH) and mineral content (%ΔZ). Fluoride in enamel was also determined. The data from %SH, %ΔZ and fluoride were subjected to two-way analysis of variance followed by Student-Newman-Keuls's test ($p<0.05$). The mineral gain (%SH and %ΔZ) was higher for toothpastes diluted in saliva ($p<0.05$), except for the 450 µgF/g dentifrice with Cacit/TMP ($p>0.05$). The 450 Cacit/TMP toothpaste and the positive control showed similar results ($p>0.05$) when diluted in water. A dose-response was observed between fluoride concentration in toothpastes and fluoride present in enamel, regardless of dilution. It was concluded that it is possible to enhance the remineralization capacity of low F concentration toothpaste by of organic (Cacit) and inorganic (TMP) compounds with affinity to hydroxyapatite.

Key Words: dentifrices, fluoride, calcium, phosphate, dental caries prevention.

## Introduction

From low to high concentrations of fluoride have been tested, but the beneficial effect of fluoride has its limits. One cannot indefinitely increase the concentrations of fluoride dentifrice at risk of adverse effects such as dental fluorosis, even without losing their cariostatic effect (1). A review confirms the benefits of using fluoride toothpaste for caries prevention in children and adolescents when compared with placebo, but significant only for fluoride concentrations of 1,000 ppm and above (2). There is strong evidence that higher levels of fluoride (1,000 ppm or more) in toothpaste are associated with an increased risk of fluorosis if given to children under 5 to 6 years of age (3). Considering that most toothpastes available to the children present more than 1,000 ppm F (4), it would be interesting to use dentifrice with lower fluoride concentration (5), while maintaining the effectiveness of a commercial standard dentifrice (1,100 ppm F). The acidifying (6,7) or addition of organic or inorganic salts (8-10) can improve the effectiveness of fluoride toothpastes.

Combination of active agents with ability to link on enamel can enhance the effect of fluoride. The inorganic phosphates, such as sodium trimetaphosphate (TMP), have great affinity with hydroxyapatite (HA) and great action against enamel demineralization (8-12). Similar to TMP, the calcium citrate (Cacit, organic salt) can also be adsorbed to HA and influence the demineralization process (10). In *in vitro* studies, the association of TMP and Cacit to toothpastes improved the effect against caries with only 450 µgF/g (10). However, the effect of Cacit/TMP on remineralization has not been tested yet.

It should be noted that in these studies the dentifrice was diluted in water (6-10). However, in *in situ* and *in vivo* studies with both materials, calcium and phosphate, are available from the saliva, reducing the difference between toothpastes (13). As Cacit, TMP and fluoride have affinity to hydroxyapatite, they can interfere on the saliva mainly in the remineralization process. Thus, the aim of this experiment was to evaluate *in vitro* the ability of a low fluoride toothpaste (450 µgF/g) containing Cacit/TMP to promote enamel remineralization using saliva and water as dilution means.

## Material and Methods

### *Study Design*

Enamel blocks (4x4 mm) were obtained from bovine incisors stored in a solution of 2% neutral formalin for 30 days at room temperature (10). The blocks had their enamel surface polished sequentially to determine the initial surface hardness (SH) (325.6 to 348.0 kgf/mm$^2$).

Enamel blocks with artificial carious lesions presenting surface hardness ($SH_1$) between 22.8 to 130.8 kgf/mm$^2$ were selected. They were assigned to twelve groups (n=10), according to the concentration of fluoride in dentifrice (placebo, 275, 450, 550 and 1,100 µgF/g) and the dilution media (deionized water and artificial saliva). A commercially available dentifrice was used as positive control (1,100 µg F/g, Crest™, Procter & Gamble, Cincinnati, OH, USA). The blocks were subjected to six pH cycling regimens twice a day to assess the ability of dentifrices to promote remineralization and treatment with dentifrice diluted in water or artificial saliva. After pH cycling, the surface ($SH_2$) and cross-sectional hardness were determined as well as the fluoride concentration in enamel. The fluoride concentration range of dentifrices and the dilution were considered as factors while hardness and fluoride in enamel as variables.

### Determination of Fluoride in Toothpaste

Toothpastes were prepared (Sara Lee Household and Body Care Research, Utrecht, The Netherlands) with the following composition: cellulose gum, methyl p-hydroxybenzoate sodium saccharinate sodium, peppermint oil, propylene glycol, glycerin, sorbitol, sulfonated olefins, hydrated silica, titanium dioxide, trisodium phosphate and water. Sodium fluoride (275, 450, 550 and 1,100 µg F/g) was added to all dentifrices except for placebo. Cacit (0.25%) and TMP (0.25%) were added to toothpastes with 450 µgF/g (450 Cacit/TMP).

Determination of fluoride in toothpaste was made in triplicate, according to Delbem et al. (14). After dilution in deionized water, samples of the suspension (0.25 mL) were hydrolyzed by adding 0.25 mL of HCl 1 M/L and heating at 45ºC for 1 h for total fluoride measurement (TF). For the determination of ionic fluoride (IF), the suspension (0.25 mL) was centrifuged (906 g, 20 min) and added 0.25 mL of HCl 1 M/L. An amount of 0.5 mL TISAB II ("total ionic strength adjustment buffer", Orion Research Inc., Beverly, MA, USA) combined with NaOH (20 g/L) was added to the TF and IF solutions. The reading was performed with the Orion 9609 (Orion Research Inc.,) fluoride ion specific electrode and 720 A+ Orion (Orion Research Inc.) ion analyzer, calibrated with standards containing 0.125 to 4.0 µgF/mL.

### Induction of Subsurface Enamel Demineralization

The enamel blocks were completely isolated with a thin layer of nail polish except for the enamel surface (area=16 mm$^2$). The blocks (n=120) were placed individually in demineralizing solution (1.3 mM/L $Ca(NO_3)_2 \cdot 4H_2O$ and 0.78 mM/L $NaH_2PO_4$ $H_2O$ in 0.05 M/L acetate buffer, 0.03 µgF/mL, pH 5.0, 32 mL/block) for 16 h at 37°C to produce subsurface enamel demineralization (15,16). The blocks were assessed for surface hardness after demineralization ($SH_1$), with indentations distanced 100 µm from the initial impressions.

### pH Cycling and Treatments with Toothpastes

Enamel blocks were submitted to pH cycling simulating the process of remineralization for six days at 37ºC, based on Vieira et al. (17). The blocks were kept in a remineralizing solution (1.5 mM/L $Ca(NO_3)_2 \cdot 4H_2O$, 0.9 mM/L $NaH_2PO_4$ $H_2O$, 0.15 M/L KCl in cacodylate buffer 0.02 M/L, pH 7.0, 0.03 µgF/mL, 0.25 mL/mm$^2$) for 22 h and demineralizing solution (2.0 mM/L $Ca(NO_3)_2 \cdot 4H_2O$ and $NaH_2PO_4$ $H_2O$ in acetate buffer 0.075 M/L, pH 4.7, 0.04 µg F/mL, 0.75 mL/mm$^2$) for 2 h. Toothpastes were diluted (1:3 in weight) in artificial saliva (1.5 mM/L $Ca(NO_3)_2$ $4H_2O$, 3.0 mM/L $NaH_2PO_4$ $H_2O$ and 7.5 mM/L $NaHCO_3$, 0.05 µgF/mL, pH 7.0) and deionized water. The treatment was performed twice a day (before and after a demineralization) under agitation (2 mL/block). The pH cycling solutions were changed daily.

### Determination of Surface and Cross-Sectional Hardness

Surface and cross-sectional hardness were determined using a microhardness tester (HMV-2000; Shimadzu, Tokyo, Japan) under load of 25 g and for 10 s. For the initial surface hardness (SH) five Knoop indentations were made 100 µm distant from each other in the center of the block, as described by Vieira et al. (17). After induction of subsurface enamel demineralization ($SH_1$) and pH cycling ($SH_2$), five indentations were made spaced 100 µm from each other and from the baseline. The percentage of surface hardness recovery (%SH) was calculated [$\%SH = ((SH_2 - SH_1)/(SH - SH_1)) \times 100$].

To assess the cross-sectional hardness, the blocks were sectioned in the center and one-half included in acrylic resin and polished sequentially. Three sequences of eight indentations at distances of 10, 30, 50, 70, 90, 110, 220 and 330 µm from the outer surface of enamel were made in the central block, and two 100 µm above and below. The averages were calculated for each distance and the values converted into mineral content ($\%\ vol.\ min. = 4.3*(\sqrt{KHN})+11.3$), according to Featherstone et al. (18). Integrated area (% vol. min. x µm) for sound enamel (Z), post demineralization enamel ($Z_1$) and after pH cycling ($Z_2$) was calculated by the trapezoidal rule (GraphPad Prism, version 3.02; GraphPad Software, Inc. La Jolla, CA, USA) and the percent mineral recovery (%ΔZ) determined [$\%\Delta Z = ((Z_2 - Z_1)/(Z - Z_1)) \times 100$].

### Determination of Fluoride Concentration in Enamel

Blocks (4x2 mm) were obtained from one of the halves of the longitudinally sectioned blocks. The outer enamel surface (84.5 [13.9] µm) was removed by immersing the enamel blocks in 0.5 M/L HCl for 90 s under agitation (10). The same volume of TISAB II (total ionic strength adjustment buffer) modified with NaOH (20 g/L) was added to the solution. Fluoride measurements were made using a fluoride specific electrode Orion 96-09 and an Orion 720 A+ ion analyzer, previously calibrated with standards containing 0.125 up to 2.0 µgF/mL and 0.25 up to 4.0 µgF/mL.

### *Statistical Analysis*

After proof of homogeneity, %SH, mineral gain (%ΔZ) and fluoride concentration in enamel (µgF/cm²) data were subjected to two-way ANOVA followed by Student-Newman-Keuls's test. Two-way ANOVA was used to analyze the data of mineral volume as a function of depth in each dilution (water and saliva). A correlation test (Pearson's test) was applied to verify relationship between the variables. Analyses were performed using SigmaPlot version 12.0 and the level of significance was set at 5%.

## Results

The mean (SD) of total and ionic fluoride concentration (µg F/g) in the placebo, 275, 450 Cacit/TMP, 550, 1,100 µg F/g and positive control are described in Table 1. The average fluoride concentrations of all the toothpastes were within the expected values, showing a variation of less than 14%. The toothpastes diluted in saliva presented higher values ($p<0.001$) of %SH and %ΔZ when compared with water, except for the toothpaste with 450 µgF/g and Cacit/TMP (Table 2). There was correlation between the results for dentifrices diluted in water and saliva for %SH (Pearson's $r=0.941$, $R^2=0.885$, $p<0.001$) and %ΔZ (Pearson's $r=0.974$, $R^2=0.948$, $p<0.001$). With the increase of fluoride concentration in toothpastes there was a dose-response relation to %SH (Pearson's $r=0.965$, $R^2=0.931$, $p<0.001$) and %ΔZ (Pearson's $r=0.970$, $R^2=0.942$, $p<0.001$), regardless of dilution. The toothpaste with 450 µgF/g and Cacit/TMP presented the best results when diluted with water ($p<0.05$). Profile of cross-sectional hardness showed higher mineral gain at 10-30 µm of depth when diluted in saliva (Fig. 1B).

The dilution of fluoride toothpastes with saliva did not improve the presence of fluoride in the enamel (Table 2). A dose-response between fluoride concentration in toothpastes and fluoride in enamel (Pearson's $r=0.861$, $R^2=0.742$, $p<0.001$) was observed. The toothpastes with 550 and 450 µgF/g presented similar fluoride concentration in enamel ($p>0.05$), regardless of dilution. There was correlation between fluoride in enamel and %SH (Pearson's $r=0.870$, $R^2=0.757$, $p<0.001$) and %ΔZ (Pearson's $r=0.864$, $R^2=0.747$, $p<0.001$).

Table 1. Mean (SD) concentration of total (TF) and ionic (IF) fluoride in the formulation according to the toothpastes (n=3)

| Toothpastes | TF, µg/g | IF, µg/g |
|---|---|---|
| Placebo | 17.4 (3.3) | 17.2 (1.4) |
| 275 µgF/g | 235.9 (12.2) | 235.8 (9.6) |
| 550 µgF/g | 557.9 (21.3) | 558.2 (5.9) |
| 1,100 µgF/g | 1,109.6 (31.4) | 1,105.8 (11.1) |
| Positive control | 1,115.1 (71.2) | 1,109.4 (29.2) |
| 450 Cacit/TMP | 449.9 (36.8) | 411.9 (12.8) |

## Discussion

Studies show that phosphate compounds (inorganic or organic) have caries-protective activity related with their ability to interact with enamel (8-11,19,20). As Cacit, TMP and fluoride have affinity to enamel and may compete for the same sites of linkage, it is important to determine how their association could improve the enamel remineralization. Based on this, one concern in this study

Table 2. Mean (SD) of percentage of surface hardness recovery (%SH), mineral recovery (%ΔZ) and fluoride (µg F/cm²) in enamel according to toothpastes and dilution (n=10)

| Toothpastes | %SH | | %ΔZ | | µg F/cm² | |
|---|---|---|---|---|---|---|
| | Water | Saliva | Water | Saliva | **Water** | **Saliva** |
| Placebo | 6.1 (1.6)$^{a,A}$ | 11.4 (6.2)$^{a,B}$ | 5.9 (4.7)$^{a,A}$ | 16.2 (5.9)$^{a,B}$ | 6.3 (1.0)$^{a,A}$ | 7.7 (1.2)$^{a,B}$ |
| 275 µgF/g | 22.6 (4.7)$^{b,A}$ | 27.7 (5.3)$^{b,B}$ | 23.6 (3.9)$^{b,A}$ | 30.3 (3.2)$^{b,B}$ | 7.9 (0.9)$^{b,A}$ | 9.0 (2.2)$^{b,A}$ |
| 550 µgF/g | 39.9 (5.0)$^{c,A}$ | 46.0 (4.3)$^{c,B}$ | 41.9 (2.5)$^{c,A}$ | 50.4 (4.3)$^{c,B}$ | 15.7 (2.1)$^{c,A}$ | 17.0 (2.1)$^{c,A}$ |
| 1,100 µgF/g | 66.7 (3.6)$^{d,A}$ | 69.3 (6.1)$^{d,A}$ | 72.3 (3.6)$^{d,A}$ | 78.4 (4.8)$^{d,B}$ | 19.5 (5.6)$^{d,A}$ | 21.2 (3.9)$^{d,A}$ |
| Positive control | 65.7 (2.4)$^{d,A}$ | 70.3(4.5)$^{d,B}$ | 74.6 (2.4)$^{d,A}$ | 76.1 (1.8)$^{d,A}$ | 21.2 (4.2)$^{d,A}$ | 20.1 (4.4)$^{d,A}$ |
| 450 Cacit/TMP | 64.5 (8.0)$^{d,A}$ | 58.4 (5.2)$^{e,B}$ | 75.2 (2.9)$^{d,A}$ | 71.8 (2.5)$^{e,B}$ | 14.5 (3.7)$^{c,A}$ | 14.8 (1.6)$^{c,A}$ |

Means followed by distinct letters are statistically different (Student-Newman-Keuls, $p<0.05$). Distinct superscript lowercase letters indicate statistically significant difference among toothpastes. Distinct superscript capital letters indicate statistically significant difference between dilutions (water and saliva).

was to compare two means of dilution (water and saliva). Diluting the dentifrice in artificial saliva, the experimental model introduces a further condition of the oral cavity, i.e., sources of calcium and phosphate ions. The adsorption of Cacit and TMP on enamel may enhance or not the ion diffusion into the enamel lesion when combined with fluoride.

Although the results for toothpastes diluted in water and saliva present correlation, the data show that the availability of calcium and phosphate in the medium promotes higher remineralization, even in an *in vitro* study. This is evident in the placebo group when it provided an increase of 86% (%SH) and 174% (%ΔZ) in enamel remineralization. Added to this, the presence of fluoride in the enamel has not been influenced by the dilution. However, fluoride not only increased the ability of remineralization of toothpastes, but also reduced the difference between the dilution means. In toothpastes with higher fluoride concentrations this difference does not exist (Table 2).

The association of Cacit/TMP with 450 μgF/g improved the effectiveness of the dentifrice. Two data support this statement: (A) enhancing the remineralization ability by 62% (dilution in water) compared to 550 μgF/g dentifrice and (B) similar fluoride concentration in enamel for both groups. This supports the hypothesis of the capacity of Cacit and TMP of binding to the enamel surface and favor the diffusion of ions through the lesion (8-11,19-22). The present study showed that Cacit and TMP are able to bind on the enamel surface and to remain bound for a longer time. After toothpaste applications, the enamel blocks were always washed with deionized water. As there were improvements in the remineralizing capacity, the addition of Cacit/TMP led to greater diffusion of calcium and phosphate ions from pH cycling solutions, since the fluoride concentration did not differ between the toothpastes with 550 and 450 μgF/g.

As described above, the saliva dilution produces a large increase in the capacity of remineralizing toothpastes whereas the suspension remains in contact with the enamel for 1 min only. However, in the saliva dilution, only 450 Cacit/TMP toothpaste showed reduction of remineralization ability. One hypothesis is that the adsorption of Cacit and TMP on enamel could: (A) reduce the reaction of calcium and phosphate from saliva during the treatment; or (B) induce a high precipitation of these ions on the enamel surface, blotting the pores and reducing the diffusion into the lesion. Calcium citrate is an unionized soluble salt, which can interact with surface of hydroxyapatite by two carboxylate groups via hydrogen and calcium sites of HA (21,22). In the presence of a calcium- and phosphate-rich solution (saliva). Cacit can increase the precipitation with respect to HA (22). The cross-sectional profile shows a lower remineralization in the depth of 30-50 μm when compared with 1,100 μgF/g toothpastes (Fig. 1B). The results were reversed when diluted in water (Fig. 1A). This



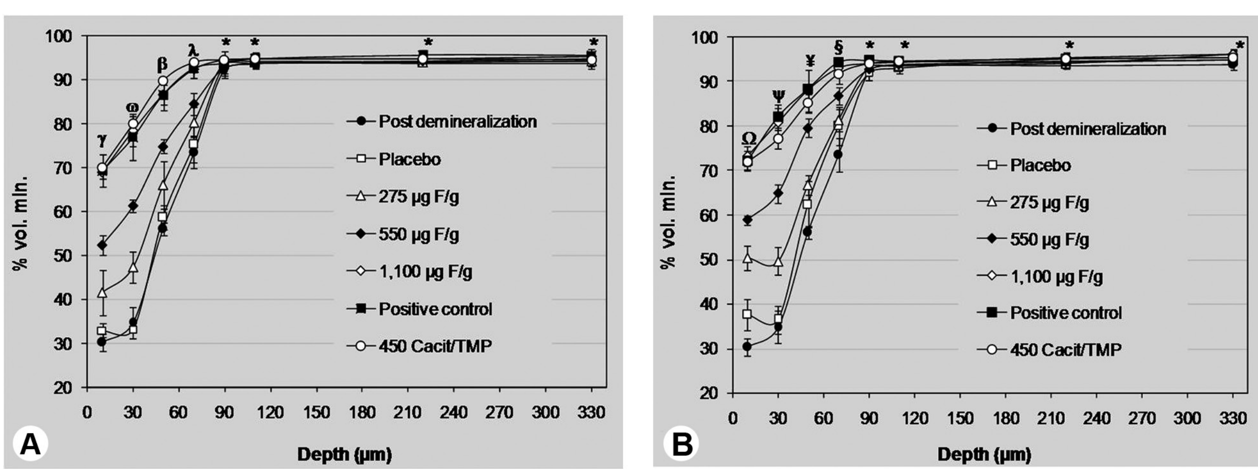


Figure 1. Profile of cross-sectional hardness according to the groups. A: Toothpastes diluted in water. B: Toothpastes diluted in saliva. Symbols indicate equality among groups in each depth. The other groups not identified by the symbols are different among them (Student-Newman-Keuls, p<0.05). Vertical bars indicate the standard deviations of means. *Indicates no statistically significant difference among groups. (A)γ: indicates equality between the groups healthy, positive control, 1,100 μgF/g and 450 Cacit/TMP (p≥0.485). ω: indicates equality between the groups healthy, post demineralization and placebo (p=0.223), 1,100 μgF/g and 450 Cacit/TMP (p=0.099), and 1,100 μgF/g and positive control (p=0.194). β: indicates equality between the groups healthy, 1,100 μgF/g and positive control (p=0.980). λ: indicates equality between the groups healthy, post demineralization and placebo (p=0.051), 1,100 μgF/g, positive control and 450 Cacit/TMP (p≥0.202). (B) Ω: indicates equality between the groups healthy, positive control, 1,100 μgF/g and 450 Cacit/TMP (p≥0.284).: indicates equality between the groups healthy, post demineralization and placebo (p=0.058), and 1,100 μgF/g and positive control (p=0.158). ¥: indicates that the healthy groups, 1,100 μgF/g and positive control are similar statistically (p=0.809). §: indicates equality between the groups healthy, placebo and 275 μgF/g (p=0.168), 1,100 μgF/g and 450 Cacit/TMP (p=0.154), and 1,100 μgF/g and positive control (p=0.187).

confirms a lower diffusion of calcium and phosphate ions in the depth of the lesion as the fluoride concentration was not different between the dilutions.

Under the experimental conditions, it was concluded that it is possible to increase the remineralization efficacy of a dentifrice with low-F concentration by organic (Cacit) and inorganic (TMP) compounds with affinity to hydroxyapatite. The calcium and phosphate-rich dilution medium was shown to be important, especially when associated with other active substances that may influence the diffusion and precipitation of ions into enamel.


## Resumo

O objetivo do presente trabalho foi avaliar *in vitro* o efeito de um dentifrício com reduzida concentração de fluoreto (450 μgF/g, NaF) associado ao citrato de cálcio (Cacit) e trimetafosfato de sódio (TMP) na remineralização do esmalte. Blocos de esmalte bovino tiveram sua superfície de esmalte polida seqüencialmente para determinação da dureza de superfície. Após o desenvolvimento de lesões artificiais de cárie, os blocos selecionados através da dureza de superfície foram submetidos a ciclagem de remineralização e tratamento diário com suspensões de dentifrícios (diluição em água deionizada ou saliva artificial): placebo, 275, 450, 550 e 1.100 μgF/g e com dentifrício comercial (controle positivo, 1.100 μgF/g). Ao término, determinou-se a dureza de superfície e em secção longitudinal, para cálculo da variação da dureza de superfície (%SH) e do conteúdo mineral (%ΔZ). O fluoreto presente no esmalte também foi determinado. Os dados de %SH, %ΔZ e fluoreto foram submetidos a análise de variância a dois critérios seguido pelo teste de Student-Newman-Keuls (p<0,05). O ganho mineral (%SH e %ΔZ) foi maior para os dentifrícios diluídos em saliva (p<0,05), exceto para os dentifrícios 450 μg F/g com Cacit/TMP (p>0,05). Os dentifrícios 450 Cacit/TMP e controle positivo apresentaram resultados semelhantes (p>0,05) quando diluídos em água. Uma relação dose-resposta foi observada entre a concentração de fluoreto nos dentifrícios e o fluoreto presente no esmalte, independente da diluição. Concluiu-se que é possível melhorar a capacidade de remineralização de dentifrícios com reduzida concentração de fluoreto pela adição de compostos orgânico (Cacit) e inorgânico (TMP) com afinidade a hidroxiapatita.


## References


1. Davies RM, Ellwood RP, Davies GM. The rational use of fluoride toothpaste. Int J Dent Hyg 2003;1:3-8.
2. Walsh T, Worthington HV, Glenny A, Appelbe P, Marinho VCC, Xin S. Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents. Cochrane Database Syst Rev In: The Cochrane Library 2010;7:CD007868.
3. Wong MCM, Glenny AM, Tsang BWK, Lo ECM, Worthington HV, Marinho VCC. Topical fluoride as a cause of dental fluorosis in children. Cochrane Database Syst Rev 2010;1: CD007693.
4. Cury JA, Oliveira MJL, Martins CC, Tenuta LMA, Paiva SM. Available fluoride in toothpastes used by Brazilian children. Braz Dent J 2010;21:396-400.
5. Moraes SM, Pessan JP, Ramires I, Buzalaf MAR. Fluoride intake from regular and low fluoride dentifrices by 2-3-year-old children: influence of the dentifrice flavor. Braz Oral Res 2007;21:234-240.
6. Brighenti FL, Takeshita EM, Sant'ana CO, Buzalaf MAR, Delbem ACB. Effect of low fluoride acidic dentifrices on dental remineralization. Braz Dent J 2013;24:35-39.
7. Alves KM, Pessan JP, Brighenti FL, Franco KS, Oliveira FA, Buzalaf MA et al.. *In vitro* evaluation of the effectiveness of acidic fluoride dentifrices. Caries Res 2007;41:263-267.
8. Takeshita EM, Castro LP, Sassaki KT, Delbem AC. *In vitro* evaluation of dentifrice with low fluoride content supplemented with trimetaphosphate.Caries Res 2009;43:50-56.
9. Takeshita EM, Exterkate RA, Delbem AC, ten Cate JM. Evaluation of different fluoride concentrations supplemented with trimetaphosphate on enamel de- and remineralization *in vitro*. Caries Res 2011;45:494-497.
10. Delbem ACB, Bergamaschi M, Rodrigues E, Sassaki KT, Vieira AEM, Missel EMC. Anticaries effect of dentifrices with calcium citrate and sodium trimetaphosphate. J Appl Oral Sci 2012;20:94-98.
11. Barbour ME, Shellis RP, Parker DM, Allen GC, Addy M. An investigation of some food-approved polymers as agents to inhibit hydroxyapatite dissolution. Eur J Oral Sci 2005;113:457-461.
12. Barbour ME, Shellis RP, Parker DM, Allen GC, Addy M. Inhibition of hydroxyapatite dissolution by whole casein: the effects of pH, protein concentration, calcium, and ionic strength. Eur J Oral Sci 2008;116:473-478.
13. Biesbrock AR, Faller RV, Bartizek RD, Court LK, McClanahan SF. Reversal of incipient and radiographic caries through the use of sodium and stannous fluoride dentifrices in a clinical trial. J ClinDent 1998;9:5-10.
14. Delbem ACB, Sassaki KT, Vieira AEM, Rodrigues E, Bergamaschi M, Stock SR, et al.. Comparison of methods for evaluating mineral loss: hardness x synchrotron microcomputed tomography. Caries Res 2009;43:359-366.
15. Queiroz CS, Hara AT, Leme FP, Cury JA. pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- and remineralization. Braz Dent J 2008;19:21-27.
16. Spiguel MH, Tovo MF, Kramer PF, Franco KS, Alves KMRP, Delbem ACB. Evaluation of laser fluorescence in the monitoring of the initial stage of the de/remineralization process: an *in vitro* and *in situ* study. Caries Res 2009;43:302-307.
17. Vieira AE, Delbem AC, Sassaki KT, Rodrigues E, Cury JA, Cunha RF. Fluoride dose response in pH-cycling models using bovine enamel. Caries Res 2005;36:514-520.
18. Featherstone JDB, ten Cate JM, Shariati M, Arends J. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. Caries Res 1983;17:385-391.
19. Anbar M, Farley EP, Denson DD, Maloney KR. Localized fluoride release from fluorine-carrying polyphosphonates. J Dent Res 1979;58:1134-1145.
20. Grenby TH, Bull JM. Chemical studies of the protective action of phosphate compounds against the demineralization of human dental enamel *in vitro*. Caries Res 1980;14:210-220.
21. López-Macipe A, Gómez-Morales J, Rodríguez-Clemente R. The role of pH in the adsorption of citrate ions on hydroxyapatite. J Colloid Interfac Sci 1998;200:114-120.
22. Martins MA, Santos C, Almeida MM, Costa ME. Hydroxyapatite micro- and nanoparticles: nucleation and growth mechanisms in the presence of citrate species. J Colloid Interface Sci 2008;318:210-216.


*Received November 16, 2012*
*Accepted January 24, 2013*