

# Effect of mouthrinses with different active agents in the prevention of initial dental erosion

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## ABSTRACT

**Introduction:** Hydrochloric acid (HCl) from the gastric juice is the only source of intrinsic acid, which can reach the oral cavity in cases of gastroesophageal reflux or chronic vomiting, enhancing the risk of dental erosion.

**Aim:** Compare the effects of mouthrinses with different active agents in the prevention of initial dental erosion caused by HCl.

**Subjects and Methods:** Casein (CAS at 0.2%), sodium hexametaphosphate (HMP at 0.02%), titanium tetrafluoride (TiF<sub>4</sub> at 0.34%), and stannous fluoride (SnF<sub>2</sub> at 0.87%) were individually added to an experimental mouthrinse. The mouthrinse without additives was used as the negative control (C) and a commercially available mouthrinse for erosion (ELM – Elmex®) as the reference product. Enamel specimens were exposed to human saliva and randomly assigned to 6 experimental groups (*n* = 8). Specimens were submitted to erosion in HCl for 10 s, followed by to the experimental mouthrinses for 30 s, and artificial saliva for 60 min. This cycle was repeated 3 times. The total amounts of calcium and phosphorus released by the specimens in the 2<sup>nd</sup> and 3<sup>rd</sup> erosive challenges were evaluated by atomic emission spectrometry. Statistical analysis used Shapiro–Wilks and Hartley tests, followed by one-way ANOVA and Tukey tests.

**Results:** When compared with C, ELM and HMP presented significantly less calcium in solution, with no difference between them. All the groups showed similar and significantly less phosphorus than C, except CAS.

**Conclusions:** HMP was the only agent that could match the protection against initial erosion of the commercially available mouthrinse in both analyses.

**Key words:** Calcium, enamel erosion, fluoride, mouthrinse, prevention

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Dental erosion is the chemical dissolution of the tooth surfaces caused by nonbacterial acids of extrinsic and intrinsic origin.<sup>[1,2]</sup> While extrinsic acids are usually provided by the diet, the hydrochloric acid (HCl) from the gastric juice is the only source of intrinsic acid, which can reach the oral cavity in cases of gastroesophageal reflux or chronic vomiting.<sup>[3,4]</sup> Due to the high acidity of this acid (pH ranging from 0.9 to 1.5),<sup>[5]</sup> patients with such

conditions are not only at greater risk for dental erosion,<sup>[2,6]</sup> but the severity of their erosive lesions was also found to be 5.5 times higher.<sup>[7]</sup>

The initial stage of dental erosion is characterized by a superficial dissolution of the tooth surface, resulting in a demineralized softened layer, which can be remineralized by saliva and other agents as fluoride. With the progression, continuous dissolution of the enamel crystals leads to a permanent bulk loss of structure.<sup>[8]</sup> The irreversible nature of the advanced stage of this process, combined with its undesirable clinical

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consequences, such as loss of tooth anatomy and dentin hypersensitivity, emphasizes the importance of early diagnosis and implementation of preventive/therapeutic measures. For patients with dental erosion of intrinsic origin, reduction of acid exposure requires causal therapy, involving reference to a physician.<sup>[9]</sup> Regular application of topic fluoride agents has also been recommended.<sup>[9,10]</sup>

Sodium fluoride is the most common source of fluoride present in oral hygiene products;<sup>[10]</sup> however, previous investigations have shown that it has only a limited protective effect against dental erosion.<sup>[11,12]</sup> Improved benefits were found with the use of titanium tetrafluoride (TiF<sub>4</sub>) and stannous fluoride (SnF<sub>2</sub>),<sup>[13-16]</sup> which have been tested in different concentrations and forms of application.<sup>[13-17]</sup> Besides providing fluoride to increase enamel resistance, the stannous and titanium ions can also form a coating on the enamel surfaces and incorporate into its structure, both of which are thought to contribute for their additional protection.<sup>[18,19]</sup> Some food proteins and phosphate polymers such as casein (CAS) and sodium hexametaphosphate (HMP) have also demonstrated anti-erosive potential. In a similar manner, these compounds can reduce erosion by depositing a protective layer on the tooth surfaces.<sup>[20-23]</sup> Since positive results were observed with these agents in previous investigations, it would be also interesting to test their effects against the initial stages of intrinsic erosion.

Mouthrinses have shown to be a suitable vehicle for the delivery of the preventive agents cited above.<sup>[24-27]</sup> They could be used immediately after vomiting episodes by patients suffering from eating disorders, as well as by patients with reflux before bedtime, with the purpose of preventing the action of acid regurgitation during the night. In this study, it was hypothesized that some active agents, when added to a mouthrinse formulation, would have different protective effects on initial erosion caused by HCl. Thus, the aim of this investigation was to compare, *in vitro*, the effects of experimental mouthrinses and a reference product in the prevention of enamel erosion of intrinsic origin.

## SUBJECTS AND METHODS

This study was conducted after approval by the Ethics Committee in Research of the School of Dentistry, University of São Paulo (CEP/FOUSP 111/11).

This investigation tested 1 experimental factor, mouthrinses, at 6 levels such as C: Experimental mouthrinse without active agents as negative control; TiF<sub>4</sub>: Experimental mouthrinse with 0.34% of TiF<sub>4</sub>; SnF<sub>2</sub>: Experimental mouthrinse with 0.87% of SnF<sub>2</sub>; HMP: Experimental mouthrinse with 0.02% of sodium HMP; CAS: Experimental mouthrinse with 0.2% of CAS; and ELM: Commercially available reference product for erosion, in an erosion-remineralization cycling model using bovine enamel specimens (*n* = 8). Before the cycling

phase, the specimens were exposed to human pooled saliva to allow the formation of the acquired pellicle. The total amounts of calcium and phosphorus in the acid solution after the first, second, and third erosive challenges were determined with atomic emission spectrometry, and the sum of values found for each compound in the second and in third challenges were considered the response variable. All the analyses were performed in random sequence and in blind conditions.

Bovine incisors were sectioned in a microtome (Isomet 1000 - Buehler, Lake Bluff, IL, EUA) to obtain enamel slabs with 6 mm × 6 mm × 2 mm dimensions. The slabs were embedded in acrylic resin and polished with a sequence of 400, 600, 800, 1200, and 4000 - granulation silicon carbide discs, followed by felt with diamond paste to obtain flat and smooth surfaces (Buehler, Lake Bluff, IL, USA). Specimens with cracks or any other anomaly were discarded.

The active ingredients used in this study were: CAS (Labsynth, Produtos para Laboratórios Ltda, Diadema, SP, Brazil), sodium HMP (Sigma-Aldrich Co., St. Louis, MO, USA), TiF<sub>4</sub> (Sigma-Aldrich Co.) and SnF<sub>2</sub> (Sigma-Aldrich Co.). The concentrations of the agents and the experimental groups are shown in Table 1. These concentrations were chosen based on the promising results against dental erosion obtained with these agents in previous investigations.<sup>[20,28,29]</sup> All experimental fluoride-containing solutions had equimolar fluoride concentration of 0.1 M.

All the agents were added to the same mouthrinse base, manipulated without any active ingredients (Fórmula and Ação, São Paulo, SP, Brazil). A commercially available mouthrinse for erosion, Elmex Erosion® (Gaba Laboratoires, Lorrâch, Germany), was used as the reference product. The compositions of the mouthrinse base and of the reference product are shown in Table 2. Immediately after the mixture of the reagents, the pH of the mouthrinses was measured with a pH meter (Quimis Scientific Instruments Ltd., Diadema, SP, Brazil). To avoid interference in the properties of the agents, the pH was not adjusted [Table 1].

**Table 1: Experimental groups and their codes, active ingredients of the rinses, their concentrations and final pH of the mouthrinse solutions**

Experimental groups	Active ingredients and their concentration	pH
Base mouthrinse (C)	None	5.94
Elmex Erosion® (reference product-ELM)	Stannous chloride (800 ppm Sn), sodium fluoride and amine fluoride (500 ppm F)	4.45
Base mouthrinse + CAS	0.2% casein	5.05
Base mouthrinse + Sodium HMP	0.02% sodium HMP	6.88
Base mouthrinse + TiF <sub>4</sub>	0.34% TiF <sub>4</sub> (approximately 1900 ppm F)	1.99
Base mouthrinse + SnF <sub>2</sub>	0.87% (approximately 1900 ppm F)	3.13

SnF<sub>2</sub>=Stannous fluoride, TiF<sub>4</sub>=Titanium tetrafluoride, HMP=Sodium Hexametaphosphate, CAS=Casein, C=Control

Human saliva was collected from seven volunteers, free of any oral and systemic diseases to create a salivary pool. Saliva was kept frozen until its use. Before cycling, the specimens were immersed in the pooled saliva for 2 h, at 37°C, under gentle agitation to allow the formation of the acquired pellicle.<sup>[15]</sup> It was used only for obtain the acquired pellicle. For erosive challenge, it was used an artificial saliva with known composition that was already used in other erosive works.<sup>[17,30,31]</sup>

For the erosive challenge, 0.01 M HCl (pH 2.4) was used to simulate the intrinsic erosion,<sup>[11,13]</sup> and artificial saliva (0.213 g/l of CaCl<sub>2</sub> 2H<sub>2</sub>O; 0.738 g/l of KH<sub>2</sub>PO<sub>4</sub>; 1.114 g/l of KCl; 0.381 g/l of NaCl; and 12 g/l of Tris buffer) for remineralization. A complete erosion-remineralization cycle consisted of: (1) 10 s immersion in 20 ml of HCl, (2) 60 s immersion in 20 ml of artificial saliva, (3) 30 s exposure to 20 ml of the respective test mouthrinse, and (4) 60 min immersion in 20 ml of artificial saliva. This cycle was repeated three times during the same day. The acid solutions used in the last two demineralization periods were individually stored and kept frozen until the chemical analyses to avoid contamination.

The amounts of calcium and phosphorus in the acid solution used in 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> erosive challenges were determined with 700 Series Introducing Coupled Plasma – Optical Emission Spectrometry, Agilent Technologies Inc., Wilmington, USA.<sup>[32]</sup> For the analysis, it was used 2.5 ml of the acid solution. The parameters used to determine the chemical elements were established using a pilot wavelength of 393.366 nm to 177.434 nm for calcium and phosphorus. The analysis was also performed in a sample of the acid that was not in contact with the specimens. The quantification of ions in this sample was then used as reference values for the determination of the actual amounts of calcium and phosphorus released from the specimens.

Data were processed on a scale of ppm (parts per million) by ICP Expert II Software (Agilent Technologies Inc., Wilmington, USA). For both analyses, the concentration of calcium and phosphate were expressed in nmol/mm<sup>2</sup>. As the 1<sup>st</sup> erosive challenge, the specimens had not been treated with experimental solutions yet, Ca and P evaluation were performed only to verify whether the specimens were releasing similar amounts of calcium and phosphorus. The sum of the calcium and phosphorus released in the 2<sup>nd</sup> and the 3<sup>rd</sup> erosive challenges was calculated for each group and considered the response variable.

Statistical analysis was carried out by the statistical software Sigma Plot 11.0 (Systat Software Inc., Chicago, IL, USA) with a significance level of 5%. Homoscedasticity and normal distribution of the data were checked by Shapiro–Wilks and Hartley tests, respectively. Comparisons

among groups were performed with one-way ANOVA and Tukey tests.

## RESULTS

The means (standard deviation [SD]) of calcium and phosphorus released by the specimens in the first erosive challenge, for all groups, are shown in Table 3. The means (SD) of the total calcium and phosphorus released by the specimens (in nmol/mm<sup>2</sup>) in the 2<sup>nd</sup> and the 3<sup>rd</sup> erosive challenges, for all the groups, are presented in Figures 1 and 2, respectively. In the 2<sup>nd</sup> and 3<sup>rd</sup> erosive challenges, ELM and HMP presented significantly less total calcium in solution when compared with the control ( $P < 0.039$  and  $P = 0.047$ , respectively) and they did not differ from each other. In the phosphorus analysis, all the groups showed significantly less total P in solution than the control ( $P < 0.05$ ), except CAS ( $P = 0.409$ ). CAS did not significantly differ from HMP, SnF<sub>2</sub>, TiF<sub>4</sub>, and ELM ( $P > 0.05$ ).

## DISCUSSION

The hypothesis of this study stating that the active agents would show different protective effects against the initial stage of intrinsic erosion had to be accepted, as sodium HMP was able to reduce the dissolution of both tooth minerals, calcium and phosphorus, similar to the commercially available mouthrinse for erosion (ELM). Although SnF<sub>2</sub> and TiF<sub>4</sub> also reduced total phosphorus loss, such effect was not observed with calcium.

**Table 2: Composition of the base mouthrinse and the reference product**

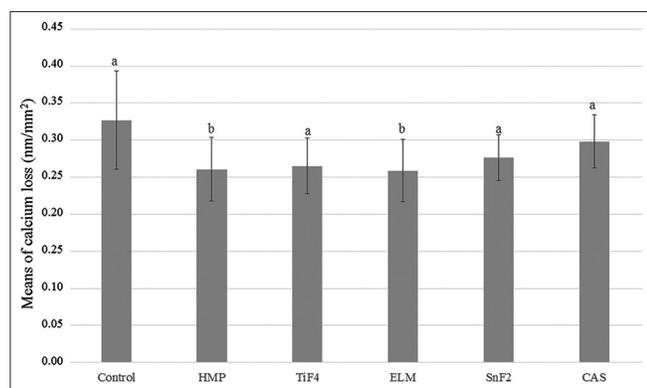
Product	Composition
Base mouthrinse (C)	Mint flavor, cinnamon flavor, saccharin sodium, tween 20, sorbitol, glycerin, methyl salicylate, and nipagim
Elmex Erosion® (ELM)	Water, glycerin, sodium gluconate, hydrogenated castor oil PEG-40, olaflur, flavoring, stannous chloride (800 ppm Sn), sodium fluoride, cocamidopropyl betaine, sodium saccharin, and hydrochloric acid. Contains two fluorine derivatives (500 ppm F) amine fluoride and sodium fluoride

C=Control

**Table 3: Means (SD) of calcium and phosphorus (in nmol/mm<sup>2</sup>) released by the specimens of each group in the 1<sup>st</sup> erosive challenge**

Groups	Means (SD) of calcium loss	Means (SD) of phosphorus loss
Control	0.15 (0.03)	0.02 (0.01)
HMP	0.12 (0.02)	0.02 (0.01)
TiF <sub>4</sub>	0.14 (0.02)	0.02 (0.01)
ELM	0.15 (0.02)	0.02 (0.01)
SnF <sub>2</sub>	0.13 (0.02)	0.01 (0.00)
CAS	0.16 (0.05)	0.03 (0.01)

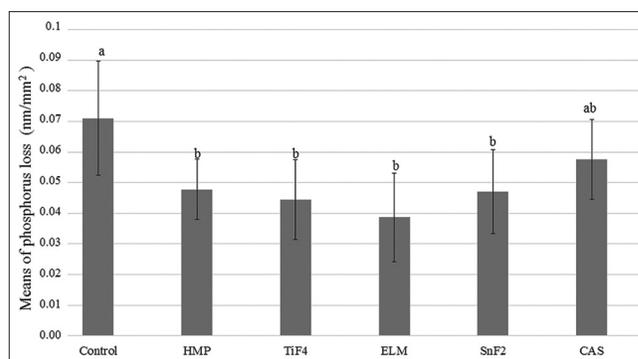
SnF<sub>2</sub>=Stannous fluoride, TiF<sub>4</sub>=Titanium tetrafluoride, HMP=Sodium Hexametaphosphate, CAS=Casein, SD=Standard deviation



**Figure 1:** Means (standard deviation) of total calcium (in nmol/mm<sup>2</sup>) released by the specimens of each group. Same letters imply no significant difference among groups in both experimental times ( $P > 0.05$ )

It is not clear why different outcomes were obtained in both analyses, but this could be related to the ability of titanium and stannous ions to complex the phosphate groups present in the hydroxyapatite, thereby forming a complex binding with the tooth surfaces.<sup>[16,33,34]</sup> Thus, in view of the short-term erosive challenge used in this study, it can be speculated that more calcium was released into the solution in comparison to phosphate. This lack of reduction in calcium loss observed with the TiF<sub>4</sub> and SnF<sub>2</sub> mouthrinses is, however, in contrast to the observations of a previous study, where significant reduction in calcium dissolution was observed after treating enamel with solutions containing both compounds, at identical concentrations.<sup>[28]</sup> These different outcomes may be in part related to the more aggressive erosive challenge used in the present investigation, which was performed with 0.01 mol/l HCl versus the 0.01 mol/l citric acid used previously, both for the duration of 1 min. It can be suggested that the more aggressive challenge may have dissolved to a greater degree the coating of titanium and stannous oxides, as well as the fluoride deposits, therefore reducing TiF<sub>4</sub> and SnF<sub>2</sub> protection. Perhaps increased in concentration of both compounds would be necessary for improved results, as observed in other reports.<sup>[16,35,36]</sup>

The mouthrinse with HMP could significantly reduce the amounts of calcium and phosphate dissolved after cycling, matching the protection of the commercially available mouthrinse for erosion. HMP is a phosphate polymer of P-O-P chain,<sup>[37]</sup> which is expected to adsorb onto the surface of the hydroxyapatite through both electrostatic and covalent bonding,<sup>[38]</sup> thereby reducing demineralization and the total area for dissolution.<sup>[22]</sup> The effect of HMP against dental erosion is consistent with the investigation of Hooper *et al.*,<sup>[22]</sup> where an experimental HMP-containing fluoride toothpaste showed improved erosion protection when compared to a conventional benchmark product. Based on the results of the present study, HMP also stands out as a viable anti-erosive agent to be added to mouthrinses. It is clear that the next step would be to evaluate behavior of this agent in the presence of fluoride and in more relevant



**Figure 2:** Means (standard deviation) of total phosphorus (in nmol/mm<sup>2</sup>) released by the specimens of each group. Same letters imply no significant difference among groups in both experimental times ( $P > 0.05$ )

clinical conditions. It should also be mentioned that HMP may experience hydrolytic degradation to orthophosphates over time,<sup>[37]</sup> therefore, in prolonged acid challenges, its protection can be reduced. Moreover, in this scenario, the phosphorus analysis may not be as accurate as calcium to evaluate enamel dissolution, once the phosphorus resulting from the HMP hydrolysis may act as a confounding factor in the evaluation.

Although some studies have demonstrated the anti-erosive effects of CAS,<sup>[20,21,39]</sup> in this study, no significant reduction in enamel erosion was observed with the addition of this protein to the mouthrinse. These conflicting results may be related to the fact that the adsorption mechanism of CAS to the tooth surface is driven by its concentration and by the duration of the application. The time required for adsorption and rearrangement of the protein layer may be high when the CAS concentration is low and vice versa.<sup>[40]</sup> Thus, considering that the above-mentioned studies used prolonged exposure times to CAS when compared with the present investigation, it can be speculated that increased application times would be necessary for significant protection.

The reference product (ELM) used in this study is a commercially available mouthrinse for erosion, and it was chosen because of its promising efficacy, as demonstrated in previous investigations.<sup>[26,31]</sup> This product provides the combined effect of two fluoride compounds (NaF and AmF; total F concentration of approximately 0.03 M) and the stannous ion (as SnCl<sub>2</sub>; approximately 6.70 M of Sn). Interesting, more erosion protection was observed with this mouthrinse when compared to the experimental mouthrinse with SnF<sub>2</sub>, which had concentrations of fluoride (0.10 M) and stannous (55 mM) three and eight times higher, respectively. Although further testing on both formulas was not made, we may suppose that the differences in composition, pH and availability of the active agents between both solutions may have accounted for the results observed.

The erosion-remineralization cycle used in this study was an attempt to simulate, *in vitro*, the initial stage of dental erosion caused by recurrent vomiting. The exposure time of 30 s for the mouthrinse treatment was chosen to be consistent with the recommendations of the commercially available product. Some of the experimental mouthrinses presented very low pH after the addition of the active agents. Although low pH values can lead to enamel demineralization, acidic mediums might be desirable for the fluoride compounds to maintain their protective capacity.<sup>[41]</sup> Thus, in this study, we decided not to adjust the pH of the solutions. The salivary pellicle plays an important protective role against dental erosion. It is composed of proteins and lipids, which can interact with the enamel surface and serve as a barrier against erosive acids.<sup>[16,42]</sup> Therefore, in this study, exposure of the specimens to human saliva was performed before the acid challenges to simulate the clinical situation more closely.<sup>[15,16]</sup> It is worth noting that the cycling was performed with artificial saliva. Since it is known that artificial saliva has a greater remineralization potential than human saliva, caution should be taken when transporting the results of this study to the clinical scenario.

## CONCLUSIONS

Considering the limitations of this investigation, we can conclude HMP was the only agent that could match the protection against initial erosion of the commercially available mouthrinse in both, calcium and phosphorus analyses.

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## Conflicts of interest

There are no conflicts of interest.

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