Influence of Surfactants and Fluoride against Enamel Erosion

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Abstract

This study investigated the effect of surfactants associated with sodium fluoride (NaF) on enamel erosion prevention, using an erosion-remineralization in vitro model. Sodium lauryl sulfate (SLS), polysorbate 20 (P20), and cocoamidopropyl betaine (CAPB) were tested, at concentrations of 1.0 and 1.5%, and associated or not with NaF (275 ppm). The control groups were distilled water and the NaF solution. Bovine enamel samples ($n = 12$) were prepared and submitted to a 5-day cycling model: acid challenge (0.3% citric acid, pH 2.6, 4×/day), human saliva (2 h, 4×/day), and the treatment solutions (2 min, 2×/day). The protective potential of the agents against initial erosion was assessed by microhardness and the surface loss by profilometry. Enamel surface wettability was determined by goniometry, protein adsorption was measured by spectroscopy (FTIR), and the KOH-soluble fluoride was quantified. Goniometry showed that SLS and CAPB increased enamel wettability. No differences were found among the surfactants regarding protein adsorption. Microhardness showed that SLS reduced NaF protection. P20 (1 and 1.5%) and CAPB 1.5% presented a protective effect, but lower than the NaF solution. Profilometry showed that CAPB protected enamel, but no agent associated with NaF promoted a higher protection than the NaF solution alone. KOH-soluble fluoride analysis showed that all surfactants reduced the fluoride adsorption on the enamel surface. Therefore, the surfactants tested (except for P20) changed the enamel surface energy. The SLS decreased the protective potential of NaF on initial erosion, but no tested agent interfered with the protective effect of NaF on enamel erosive wear.

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Dental erosion is a multifactorial process characterized by the chemical demineralization of tooth structures promoted by non-bacterial intrinsic or extrinsic acids and chelating agents [Lussi and Carvalho, 2014]. Before coming into contact with the enamel, the acid must diffuse through the acquired pellicle [Featherstone and Lussi, 2006]. The enamel acquired pellicle (EAP) is a film, free from bacteria, that covers dental tissues and is com-

posed of several proteins, such as mucins, glycoproteins, and proline-rich proteins [Hannig et al., 2005; Buzalaf et al., 2012]. It acts as a diffusion barrier or semipermeable membrane that prevents the direct contact between the acids and the tooth surface [Lussi et al., 2011], thus protecting enamel against demineralization [Buzalaf et al., 2012; Vukosavljevic et al., 2014].

Oral hygiene products, such as dentifrices and mouthwashes, are commonly used to remove organic material from tooth surfaces and act over the biofilm control [Veeregowda et al., 2011]. Surfactants present in these products' composition can influence the adsorption of salivary proteins [van der Mei et al., 2002; Veeregowda et al., 2011], potentially affecting the EAP formation. Also, these oral hygiene products often contain fluorides, such as sodium fluoride, which presents a protective effect against dental erosion [Ganss et al., 2008; Jager et al., 2013]. This protective action is attributed to the interaction of fluoride with calcium [Magalhaes et al., 2009, 2011], decreasing the enamel dissolution rate. However, previous findings speculate that the presence of surfactants, such as sodium lauryl sulfate (SLS), can influence the fluoride ions' availability and their binding to tooth structures [Barkvoll et al., 1988b; Zero, 2006]. Additionally, detergents can interact with hydroxyapatite [Barkvoll et al., 1988a] and also modulate the abrasiveness of dentifrices [Moore and Addy, 2005].

Although SLS is the most common surfactant found in dentifrices, it is usually related to alterations in oral mucosa, affecting epithelial surfaces [Neppelberg et al., 2007]. Therefore, some products contain other surfactants, such as cocoamidopropyl betaine (CAPB) [Rantanen et al., 2002].

The influence of surfactants and their interaction with fluoride during erosive events have not been well established so far. Thus, the aim of this study was to evaluate the effect of surfactants in different concentrations over the enamel surface energy and their influence on the acquired pellicle formation and fluoride protection under erosive situations. The null hypotheses tested were that: (a) the surfactants do not change the enamel wettability and (b) do not interfere with fluoride protection during erosive challenges on enamel.

Materials and Methods

Experimental Design
The 3 experimental factors tested were: (1) the surfactant type: SLS, CAPB and polysorbate 20 (P20 – also known as Tween 20); (2) the surfactant concentration: 1.0 and 1.5% v/v; (3) the presence of sodium fluoride (with or without NaF – 275 ppm). The study variables were surface wettability characterized by contact angle, absorbance of proteins on enamel by Fourier transformed infrared (FTIR) spectroscopy, surface microhardness (SMH) for initial erosion analysis, surface loss (μm) measured by contact profilometry for erosive wear assessment, and fluoride adsorption on enamel (KOH-soluble fluoride).

Sample Preparation
For the surface energy analysis, 35 crowns from freshly extracted bovine incisors were embedded in acrylic resin (Extec Fast Cure Acrylic, Extec Corp., Enfield, CT, USA) using a cylindrical silicone matrix. After curing, the specimens were flattened with SiC abrasive paper #1,200 (FEPA P, Struers, Ballerup, Denmark) under irrigation at a polishing machine (DP-10, Panambra Industrial e Técnica SA, São Paulo, Brazil) until an area of 9 mm^2 on the labial surface was exposed.

For the spectroscopy and erosion analysis, cylindrical enamel specimens ($n = 12$) with a 3-mm diameter were obtained from the labial surface of the bovine crowns using a custom-made diamond-coated trephine mill. The specimens' heights were standardized to 2 mm by wearing down the dentin surface with water-cooled SiC abrasive papers (#1,200 grit, FEPA P, Struers).

For the erosion analysis, the cylindrical specimens were embedded in self-curing acrylic resin using a silicone mold (6 mm in diameter and 3 mm in depth), leaving a 0.1-mm enamel projection as previously described [Ávila et al., 2017]. The specimens were then ground flat and polished with water-cooled sequential SiC abrasive papers (#1,200, #2,400, and #4,000 grit, FEPA P, Struers) coupled in a polishing machine at 300 rpm for 30, 60, and 120 s, respectively. Specimens were sonicated in deionized water for 10 min after each paper grit change. The specimens presenting cracks and imperfections under the stereomicroscope examination (×20; Stemi 2000, Carl Zeiss, Oberkochen, Germany) were replaced. After preparation, the specimens were stored in relative humidity at 4°C to avoid dehydration.

Solution Preparation
The following surfactant agents were tested: SLS (Synth, Diadema, Sao Paulo, Brazil), CAPB (Emfal, Belo Horizonte, Minas Gerais, Brazil), and P20 (Kotilen V1, Kolb, Moerdijk, the Netherlands). The concentrations tested were 1 and 1.5%. The solutions were prepared by diluting the surfactants in water, under agitation, until achieving the concentration in volume for each group. Table 1 shows the chemical properties of all the surfactants.

For the groups containing fluoride, 275 ppm of NaF was added to the solution in order to simulate a dentifrice with 1,100 ppm. This fluoride concentration was used to simulate the 1:3 dilution that occurs with the saliva during brushing [Duke and Forward, 1982].

Surface Wettability – Contact Angle Characterization
The polished crown specimens were divided into 7 groups ($n = 5$) according to the surfactants (SLS, CPB, and P20) and the concentrations (1 and 1.5%) used. For the contact angle analysis, the solutions were tested without fluoride. The control group was ultrapure water. The specimens were immersed in each group's respective solution for 2 min under agitation (60 rpm – Kline shaker, TS2000A, Biomixer), then placed on absorbent paper to dry before the measurements. This was performed due to the presence of wa-

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Table 1. Chemical properties of the surfactants

	SLS anionic	CAPB amphoteric	P20 nonionic
Chemical formula	$C_{12}H_{25}SO_4Na$	$C_{19}H_{38}N_2O_3$	$C_{58}H_{114}O_{26}$
Molar mass, g/mol	288.4	342.5	1,227.5
Density, g/cm^3	1.05	1.05	1.10
Solution pH	10.5	5.1	5.3
CMC, mM	8.2	0.28	0.08
CMC according to tested concentration, mM	1%: 36.4	1%: 30.6	1%: 8.9
	1.5%: 546	1.5%: 45.9	1.5%: 13.4

CMC, critical micellar concentration.

ter that might possibly increase the wettability of solid surfaces [Zhang et al., 2015].

The contact angle of the enamel surfaces was measured using the sessile drop analysis system (Theta Lite, Biolin Scientific, Stockholm, Sweden), with ultrapure water used as the probe's liquid. After dropping the water, a 20-s pause was given to let the drop rest. Pilot investigations showed that this time is required for achieving equilibrium between the water and the surface, with little change in the contact angle. After the rest period, a dedicated software (One Attension, Biolin Scientific, Stockholm, Sweden) was used to measure the right and left contact angles for 10 s, and those measurements were then averaged.

Spectroscopy Analysis – FTIR Assessment

For the spectroscopy analysis, the polished enamel specimens ($n = 12$) were divided into the same 7 groups described for the contact angle analysis. The initial absorbance was obtained from all specimens using a FTIR spectrometer (Frontier; Perkin Elmer, MA, USA). The equipment was calibrated to 32 spectra per second with a resolution of 4 cm^{-1}, and the spectra of the specimens were obtained from 600 to 4,000 cm^{-1}.

After the initial measurements, the specimens were immersed in 2 mL of each group's respective solution for 2 min under agitation. Then, they were immersed in human saliva for 120 min for EAP formation. The saliva was previously collected from 20 donors (University Ethics Committee approval, protocol 367.946) under stimulation by chewing Parafilm M (Bemis Company Inc., Neenah, WI, USA), 2 h after breakfast and toothbrushing with a fluoride-free dentifrice. The donors were selected according to their salivary flow (stimulated flow higher than 0.8 mL/min and unstimulated flow higher than 0.2 mL/min), had no health disease, and were not pregnant. Saliva was collected in cooled Falcon tubes, then mixed to form a pool, centrifuged (10,000 rpm/4°C, 5 min) [Masson et al., 2013] and frozen at –80°C until the moment of use [Cheaib and Lussi, 2011].

Immediately after EAP formation, the specimens were placed on absorbent paper to dry with the enamel facing up, and the final spectra were obtained with the same parameters described for the initial ones. The adsorbed proteins were quantified by the comparison of the amide I (1,648 cm^{-1}) and amide II (1,517 cm^{-1}) bands, which corresponds to the β-sheet and α-helix structures, respectively, found in proteins such as α-amylase, albumin, mucin,

and proline-rich proteins [Caetano Junior et al., 2015]. The 980 cm^{-1} band corresponds to the phosphate found in hydroxyapatite and was used as the internal control. The spectra were submitted to baseline correction and normalized (between 0 and 1). After that, the absorbance increase was calculated using the following formula: $\%A = ([A_f/A_i] - 1) \times 100$, in which %A refers to the absorbance increase, A_f refers to the final values, and A_i to the initial values.

Erosion Analysis – Initial Microhardness and Profilometry

The cylindrical embedded enamel specimens were submitted to the initial microhardness (SMH_i) measurements using a Knoop microhardness tester (FM-700, Future-Tech, Tokyo, Japan) adjusted to a 50-g load for 10 s. Three indentations were performed 100 μm apart from each other and the values averaged. The mean microhardness value for all specimens was obtained (326.8 ± 34.7), and those presenting a value difference higher than ±10% were replaced.

The baseline profiles of all specimens were obtained using a contact profilometer (MaxSurf XT 20, Mahr-Göttingen, Germany). Two parallel grooves were made at the resin, close to the specimen, in order to produce orientation guides during the profile readings. The profilometer diamond stylus moved from the first reference area in the acrylic resin to the second one (4.2 mm long) with a constant speed of 0.5 mm/s. Three profile measurements were performed for each specimen at intervals of 0.25 mm.

After the initial microhardness and profile measurements, the specimens were divided into 14 groups ($n = 12$) according to the surfactant type (SLS, CAPB, P20), concentration (1 and 1.5%), and the presence or not of NaF. The negative control group was ultrapure water, and the positive control was a NaF solution (275 ppm).

Erosive Cycle

The erosive cycle consisted of demineralization/remineralization events, with 4 immersions in 0.3% citric acid (pH 2.6 – 5 min each), 4 immersions in human saliva (120 min each), and 2 immersions in the surfactant solutions (2 min each). Figure 1 shows a schematic drawing of the erosive cycle. The cycle was repeated for 5 consecutive days, always initiated by immersion in human saliva to form the acquired pellicle (red dotted line in Fig. 1). The specimens were kept under 100% relative humidity overnight. The immersions in the surfactant solutions were made after the first and
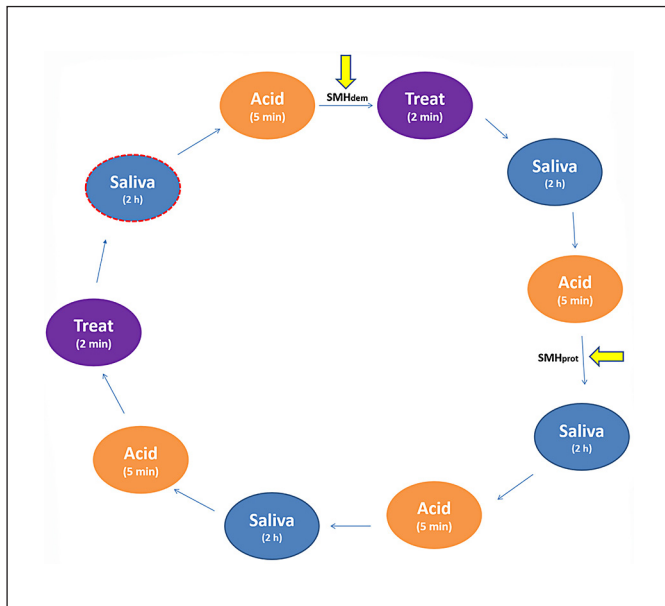
Fig. 1. Schematic chart of the erosive cycle. The red dashed line refers to the beginning of the erosive cycle each day. SMH_{dem} refers to the microhardness after the first acid challenge. SMH_{prot} refers to the microhardness after the second acid challenge. SMH_{dem} and SMH_{prot} were obtained only on the first day of the cycle.

Table 2. Mean and standard deviation (in degrees) for the contact angle in all groups

	1%	1.5%	p (t test: concentration)
SLS	41.3±7.0	30.7±9.2	0.608
CAPB	20.0±10.6	30.5±6.8	0.405
P20	50.0±6.4*	45.0±12.1*	0.244
Water (control)		58.6±7.5*	

The asterisk shows values similar to that of the control group.

the last acid challenge of the day, for 2 min. The human saliva was used for EAP formation and remineralization, and was collected as previously described.

Final Microhardness and Profilometry

The initial erosion was evaluated by microhardness, which was assessed after immersing the specimens in the first acid challenge (SMH_{dem}) to evaluate the demineralization potential of the citric acid. Then, microhardness was assessed again after the second acid immersion (SMH_{prot}) to evaluate the ability of the solutions to promote protection against a new erosive challenge (Fig. 1). All 3 microhardness data were obtained using the same parameter previously described. Microhardness data were presented in terms of relative percentage ($\%SMH_{dem}$ and $\%SMH_{prot}$) using the formulas: $\%SMH_{dem} = (SMH_{dem}/SMH_i) \times 100$ and $\%SMH_{prot} = SMH_{prot}/SMH_i) \times 100$.

The enamel loss was assessed by profilometry after the fifth day of the cycle. The specimens were positioned in a custom-made setting device in order to guarantee the exact replacement of the specimens after the experimental assessments. The final profiles were obtained with the same parameters previously described for the baseline ones. The surface loss (μm) was calculated by the subtraction of the final profiles from the baseline profiles, with the previously described grooves as guides, using a dedicated software (MarSurf XCR 20 4.50-07 SP3, 2011).

KOH-Soluble Fluoride Determination

The amount of KOH-soluble fluoride was assessed after the profilometric analysis, using a previously described method [Caslavska et al., 1975]. Therefore, the specimens were individually stored in plastic containers with 0.5 mL of 1 M KOH solution, under agitation and at room temperature for 24 h. After this, 0.25 mL of the solution was transferred to another container with 0.25 mL of 1 M HClO$_4$, and to this solution, 0.5 mL of TISAB II buffer was added.

The fluoride content was determined by an ion-selective electrode (Perfection, Mettler Toledo, Schwerzenbach, Switzerland), using a standard calibration curve prepared from solutions with a known concentration to define the fluoride concentration in the samples. The concentration of fluoride was expressed in μg/cm^2 (μg F$^-$/cm^2 enamel), and was measured using the formula $Cf = F^-/A$, in which F^- refers to the fluoride content in μg and A refers to the specimen area ($A = \pi \times r^2$, in which π is equal to 3.14 and r is the specimen radius defined in 0.15 cm).

Statistical Analysis

Normality and homoscedasticity assumptions (Shapiro-Wilk and Levene tests) were checked for all data analysis. The contact angle data were submitted to 1-way ANOVA ($p < 0.05$). Dunnett's test was performed to check the differences between the surfactant and the control group, and the Student t test was applied to check for differences between the concentrations. The microhardness and profilometry data were submitted to 2-way ANOVA ($p < 0.05$), followed by the Dunnett test to compare the surfactant solutions with both control groups (water and NaF solution). The Sidak test was also applied to surface loss data. The comparison between the surfactant concentrations was made by the Student t test. Fluoride data and absorbance values failed the normality test, and the Kruskal-Wallis test was applied ($p < 0.05$), followed by the Dunn test.

Results

The 1-way ANOVA performed for the contact angle characterization data showed significant differences among the groups tested ($p < 0.0001$). The Dunnett test revealed that SLS and CAPB in both concentrations decreased the contact angle between water and the enamel surface, while P20 presented values similar to the control (Table 2). Regarding the different concentrations, the

Fig. 2. Box plot distribution of absorbance increase (%) for all groups tested at amide I and amide II regions.
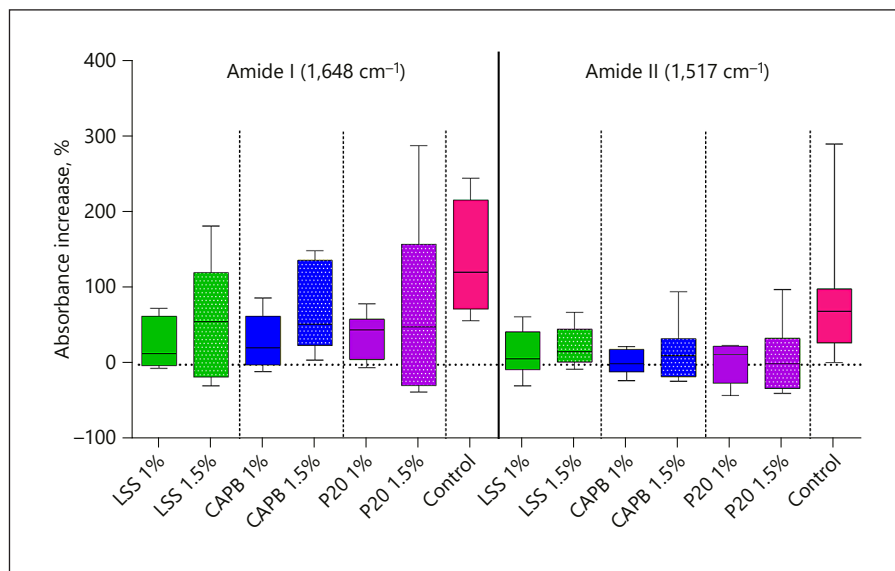

Fig. 3. a Water spectra showing peaks between 2,900 and 3,600 cm^{-1}. **b** An example of initial (blue) and final (red) spectra from a specimen of the control group with the phosphate (PO_4^{3-}), carbonate (CO_3^{2-}), and organic compound peaks (CH_2/CH_3). The star symbol indicates an absorbance increase both in the water peak (**a**) and in the enamel after pellicle formation (**b** – red line).
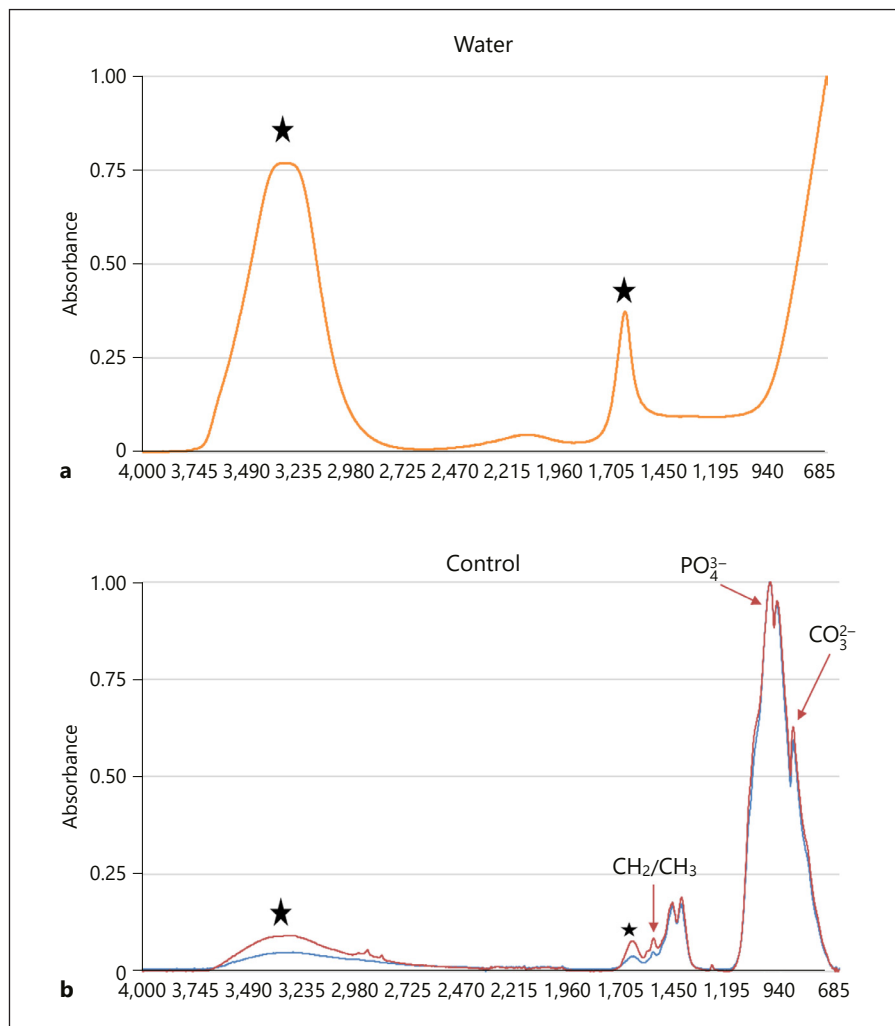
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Table 3. Mean and standard deviation for SMH$_{dem}$, SMH$_{prot}$ (relative percentage, measured after erosion), and surface loss (µm) for all groups tested

		%SMH$_{dem}$		%SMH$_{prot}$		Surface loss	
		without NaF	with NaF	without NaF	with NaF	without NaF	with NaF
SLS	1.0%	80.0 (6.0) Aa	78.9 (4.6) Aa	47.4 (3.5) Aa¥	51.3 (5.0) Ba	3.12 (0.87) Aa	3.13 (0.68) Aa
	1.5%	83.3 (4.7) Aa	79.3 (4.9) Aa	56.3 (5.0) Aa	53.0 (9.9) Ba	3.22 (1.04) Aa	3.40 (1.17) Aa
CAPB	1.0%	81.8 (7.7) Aa	77.8 (6.4) Aa	55.8 (4.8) Aa	60.0 (3.5) Aa	2.91 (0.82) Ba	2.68 (0.91) Aa
	1.5%	80.0 (5.6) Aa	81.6 (3.9) Aa	59.3 (7.0) Ba	63.2 (5.3) Aa	2.96 (0.76) Ba	2.37 (0.62) Aa
P20	1.0%	80.3 (5.4) Aa	75.7 (8.0) Aa	58.5 (3.0) Aa	56.2 (6.4) Aa	2.27 (0.7) Ba	2.12 (0.59) Aa
	1.5%	79.1 (6.4) Aa	79.8 (8.0) Aa	56.2 (6.4) Aa	59.6 (6.5) Aa	3.19 (0.79) Aa	3.13 (0.85) Aa
Control	–	76.4 (7.1) Aa	76.2 (5.8) Aa	52.3 (6.4) Aa	58.8 (7.5) Aa	3.86 (0.76) Aa	2.59 (0.66) Ab

Uppercase letters show differences within columns for all surfactants and the control group (Dunnett test). Lowercase letters show differences within lines (presence of NaF; Tukey test). The ¥ symbol shows difference between the concentrations for each surfactant (Student t test).

Student t test showed no differences between 1 and 1.5% for all surfactants tested (Table 2).

For the FTIR analysis, the Kruskal-Wallis test revealed no differences among the groups for both bands: amide I, $p = 0.053$ and amide II, $p = 0.059$. Figure 2 shows the box plot distribution for all groups. Despite the statistical results, a significant increase in the absorbance between 3,400 and 3,600 cm^{-1} (Fig. 2) was observed. This increase, associated with the increase at 1,648 cm^{-1}, could indicate the presence of water in the specimen, which could have interfered with the final absorbance analysis (Fig. 3).

Regarding the erosion analysis, 2-way ANOVA showed no differences among the surfactants ($p = 0.403$) for microhardness values (SMH$_{dem}$), regardless of the presence or not of NaF, as shown in Table 3 that presents the relative percentage of microhardness.

For the SMH$_{prot}$ values, the Student t test revealed that the concentration factor showed significant differences only for SLS without fluoride, with lower concentration presenting lower microhardness values. The 2-way ANOVA showed differences among the surfactants ($p < 0.0001$) and for the presence of NaF ($p < 0.0001$). The comparison test adopted was Dunnett (all groups without NaF compared with the water control group, and all groups with NaF compared with the NaF solution group). It was observed that 1.5% CAPB presented higher microhardness values than the control group (Table 3). The SLS associated with NaF, in both concentrations, presented lower values than the NaF solution group. Regarding the presence and absence of NaF, the Tukey test revealed that there were no differences between presence and absence of fluoride for all tested groups (Table 3).

For the enamel loss profilometry analysis, 2-way ANOVA showed significant differences for the surfactant ($p = 0.019$) and for the presence of NaF ($p < 0.0001$). The Dunnett test revealed that CAPB in both concentrations and 1% P20 presented a lower surface loss than the control group. For the groups associated with fluoride, the Dunnett test showed no differences with the NaF solution. Regarding the protective effect promoted by the fluoride, the Sidak test showed that only the NaF solution presented a lower surface loss than the control group. All surfactants associated with fluoride presented similar values with the NaF solution group. Table 3 shows the mean and standard deviation for surface loss after 5 days.

Finally, the KOH-soluble fluoride content was assessed in the solutions containing NaF. The Kruskal-Wallis test revealed significant differences among the groups ($p < 0.001$). The Dunn test showed that all surfactant solutions presented a lower fluoride concentration than the NaF solution group, except for 1.5% P20, which presented similar values (Table 4). The statistical analysis was performed only in the groups containing fluoride, as the values obtained from the groups without fluoride were frequently below the detection level of the electrode used and therefore would lead to false results.

Discussion

The contact angle analysis allowed the rejection of the first null hypothesis, since the enamel surface energy changed after the treatment with surfactants, with SLS and CAPB in both concentrations increasing the enamel

Table 4. Mean and standard deviation for KOH-soluble fluoride ($\mu g/cm^2$) on enamel surface

		KOH-soluble fluoride
SLS + NaF	1.0%	0.247±0.354 A
	1.5%	0.858±0.104 A
CAPB + NaF	1.0%	0.647±0.448 A
	1.5%	0.926±0.179 A
P20 + NaF	1.0%	0.705±0.431 A
	1.5%	0.941±0.126 B
NaF (positive control)	–	1.597±0.253 B

Uppercase letters indicate significant differences among the groups ($p < 0.05$).

surface wettability. The concentrations used in this study were chosen based on previous studies [Fisher et al., 2002; Strand, 2011; Masters et al., 2012] and were higher than the critical micellar concentration of the surfactants (Table 1). The critical micellar concentration is defined as the maximum concentration in which the surfactants can be used without forming micelles [Bergstrom, 2015]. When surfactants are used in concentrations higher than their critical micellar concentration, the molecules do not find free surfaces on which to bind, thus they start aggregating between each other forming spherical or cylindrical structures, called micelles. In aqueous systems, the conformation of these micelles tends to present a core composed of the hydrophobic hydrocarbon chains (such chains turn to the center, repelled by water), with the exterior formed by the polar head of the molecule [Bruce et al., 2002]. The head is composed of sulfate ions in SLS, quaternary nitrogen ions in CAPB, and ethylene oxide ions in P20.

The increase in surface wettability promoted by SLS might be explained by the interactions of sulfate and sodium ions with the calcium from hydroxyapatite, as shown in other studies [Barkvoll et al., 1988a, b; Jones et al., 2014]. Even though the micelles from SLS tend to expose the hydrocarbon chains (hydrophobic) on its external surface, due to the lower amount of the sulfate radical, it has been shown that they allow the entrance of water into its interior [Chun et al., 2015].

Although no studies were found showing that the CAPB binds to hydroxyapatite, it might be speculated that the quaternary nitrogen ions from CAPB micelles bind to phosphate sites and also interact with water, increasing enamel wettability. Regarding P20, the results show no change on the enamel surface, indicating that strong chemical interactions between this agent and hydroxyapatite are unlikely to happen.

Although previous studies have reported an influence of SLS on the enamel pellicle, causing a delay in its formation and also leading to a thinner film [Rykke et al., 1990; Veeregowda et al., 2011], the analysis of adsorbed proteins on enamel in this study did not reveal an influence of SLS, or the other surfactants tested, over EAP formation. However, this result must be carefully analyzed, since the presence of water inside the enamel prisms (and in the pellicle) might have influenced the absorbance patterns, leading to false-positive results (star symbol in Fig. 3). Additional analysis should be conducted to better assess the effect of surfactants over the pellicle.

The initial erosion analysis showed that, after the second erosive challenge (SMH_{prot}), the SLS in both concentrations presented similar values to the negative control group, indicating that it does not affect the pellicle formation. Thus, the second null hypothesis was denied. Although previous studies have shown that SLS causes a delay in the pellicle formation [Rykke et al., 1990] and also produces a thinner film, which is less able to protect enamel from acids [Veeregowda et al., 2011], this was not observed for the short-duration erosive situation.

However, the association of SLS with NaF led to a reduction in the enamel microhardness when compared to the NaF solution group. This indicates that the SLS competes with the fluoride for the calcium binding sites, avoiding or decreasing the amount of NaF that can interact with hydroxyapatite, thus reducing its protective effect. It has been previously shown that SLS decreases the sodium fluoride deposition on enamel and increases the solubility of the calcium fluoride (CaF_2) precipitates formed [Barkvoll et al., 1988b]. The formation of CaF_2 precipitates on the enamel surface is the main component responsible for the NaF protection in erosive situations [Magalhaes et al., 2009, 2011]. It is believed that these precipitates work as a mineral barrier to be dissolved during acid challenges [Ganss et al., 2001], therefore, the understanding of the interaction between surfactants and NaF is important for erosion control treatments. However, it must be highlighted that the CaF_2 formed depends on the fluoride concentration, pH, and application time [Petzold, 2001; Scaramucci et al., 2013]. In the tested concentration (275 ppm) and short application time (2 min), it is unlikely that CaF_2 precipitates are formed; instead, it can be speculated that a nonspecific fluoride phase adsorbed onto the enamel surface. This is believed to make the fluoride ion available to reduce the hydroxyapatite demineralization rate during erosive situations [Scara-

mucci et al., 2013]. Nevertheless, even with the fluoride protection decrease associated with SLS, and the lower KOH-soluble fluoride values found in both concentrations on the enamel surface, the profilometry results showed that this potential deleterious effect was not maintained with successive erosive situations. The enamel surface loss after a 5-day cycle was similar to the control group in both concentrations tested and associated or not with fluoride. Thus, the presence of SLS does not seem to jeopardize the protection provided by the pellicle and the fluoride in long-lasting erosive situations.

Regarding the effects of CAPB on initial erosion, it was observed that it was able to protect enamel when used at 1.5%, enhancing the possibility of chemical interactions between this agent and hydroxyapatite. When associated with NaF, this surfactant was also able to protect enamel in both concentrations tested, as it did not differ from the NaF group (positive control). As CAPB presents a positive radical (sodium ions), it does not compete with fluoride for calcium sites and, therefore, does not influence the fluoride protective effect. With the persistency of the erosive condition, CAPB was the only surfactant able to protect the enamel per se, promoting lower structural wear than the negative control group (water), and with similar results to the positive control group (NaF solution). The decrease in KOH-soluble fluoride on the enamel surface found for CAPB (Table 4) supports the hypothesis that it alone exerts a protective effect. The results from CAPB indicate the possible deposition of this agent on the enamel, preventing direct contact of the acid with the surface, thus promoting the enamel's protection.

Regarding P20, a significant protective effect was not observed in both concentrations tested for the initial erosion; however, differently from SLS and CAPB, this agent showed no evidence of chemical interaction with hydroxyapatite (contact angle analysis). Also, the association of P20 with NaF did not show protective ability for initial erosion. The persistence of the erosive situations showed that the protection of P20 only happened when used in the lower concentration (1%). No studies were found evaluating the relation of this surfactant with enamel; thus, the protective effect can only be speculated and might have happened due to its high molecular weight (Table 1). This may have promoted its deposition over the enamel surface, without chemical interactions, functioning as a temporary protective layer against acids.

Since the erosive process occurs in two distinct phases, enamel surface softening followed by structure loss with erosive situation persistence [Ganss et al., 2014], an in vitro study model that mimicked everyday situations was sought [West et al., 2011]. The adopted model consisted of 2 daily applications of the surfactant solutions (totaling 4 min), 6 daily erosive challenges (totaling 30 min), and 4 daily applications of human saliva (totaling 8 h per day) for enamel remineralization and pellicle formation. Citric acid is commonly used to simulate extrinsic erosion [Wiegand et al., 2008; Aykut-Yetkiner et al., 2014] and was used in this model. The model was repeated for 5 days to promote a measurable deleterious effect on the enamel surface [Barbour et al., 2011]. Considering that the intraoral pH after an acidic attack is reduced for up to several minutes, and the total erosion time in the present study lasted for only 2.5 h, the erosive cycling in this study can be classified as relatively mild [Zanatta et al., 2017]. Future studies should aim to investigate the chemical interactions between CAPB and P20 to hydroxyapatite, and also the interaction of the surfactants with other fluoride compounds, such as stannous fluoride. In addition, as surfactants are commonly found in dentifrices, future studies should include abrasion cycles.

Therefore, it must be concluded that SLS and CAPB in both concentrations tested (1 and 1.5%) increased enamel wettability, while P20 promoted no alteration on the enamel surface contact angle. SLS decreased the fluoride protection in the initial erosion, but this deleterious effect did not persist with the maintenance of the erosive cycle. CAPB in both concentrations and 1.0% P20 protected the enamel against erosive structure loss, but this effect was not maintained when these surfactants were associated with fluoride.

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Disclosure Statement

The authors declare no conflict of interests.

Author Contributions

Conception and design of the experiments were done by R.F.Z. and A.B.B. Performing the experiments were R.F.Z., D.M.S.A., and K.M.M. Analyzing the data was done by R.F.Z., C.R.G.T., and A.B.B. Writing the manuscript was done by R.F.Z., C.R.G.T., and A.B.B.

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