Surface free energy of enamel treated with sodium hexametaphosphate, calcium and phosphate

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A B S T R A C T

Objective: This study evaluated the capacity of sodium hexametaphosphate (HMP) at different concentrations to alter the surface properties of dental enamel in order to increase calcium and phosphate adsorption.
Design: Bovine enamel blocks (4 mm × 4 mm, n = 144, 12/group) were divided: 0%; 0.25%; 0.5%; and 1% HMP, followed or not by application of solutions containing Ca or Ca-PO₄, totaling 12 groups. The treatments were performed for 2 min, and the surface free energy (mN/m) was calculated by measuring the contact angles of three probing liquids (deionized water, diiodomethane and ethylene glycol), which was used to determine the polar and nonpolar components of the enamel surface. Calcium (Ca), phosphate (PO_4) and HMP in the solutions treatment solutions were analyzed before and after treatment. The data presented normal and homogeneous distribution and then were subjected to ANOVA, followed by Student-Newman Keuls' test ($p < 0.05$).
Results: The higher the% of HMP in the solutions, the greater HMP adsorption and more electron-donor sites on enamel surface were achieved ($p < 0.05$). Also, Ca adsorption was higher with increasing% HMP in the solutions ($p < 0.05$), which in turn reduced electron-donor sites on enamel surface. Increased Ca and PO_4 adsorption occurred at 0.5% and 1% HMP after treatment with Ca-PO_4 solution, resulting in a less electron-donor sites on surface when compared to the other treatments ($P < 0.05$).
Conclusions: HMP leads to a more electron-donor sites on enamel surface, what promotes greater adsorption of Ca and PO_4 ions.

1. Introduction

As dental caries is the most common chronic oral disease worldwide (Clarkson, Watt, & Rugg-Gunn, 2010), enhancing the ability of fluoridated products against mineral loss can potentially improve its effect against dental caries. Sodium hexametaphosphate (HMP) is a cyclic inorganic phosphate (Kulaev, Vagabov, & Kulakovskaya, 2005, Ch. 1) that has been added to fluoride products as toothpastes (Camara, Miyasaki, Danelon, Sassaki, & Delbem, 2014; Camara et al., 2015, 2016) and gels (Conceição et al., 2015) with the purpose to increase the capacity of such products to reduce enamel demineralization and to enhance remineralization. The assumption would be that HMP would retain ionic species like CaF^+ and Ca^{2+}, leading to a reticular formation of HMP-Ca^{2+} layer on enamel, which in turn increases calcium availability during de- and re-mineralization processes (Camara et al., 2014, 2015, 2016; Conceição et al., 2015). However, the above-mentioned hypothesis still needs to be scientifically proven, given that both

HMP and tooth enamel are negatively charged (Choi, Wen, & Smith, 1993). Thus, the study of the interactions and chemical bonds among HMP, the enamel surface and a calcium and phosphate-rich medium might help to better understand the mechanisms surrounding the effects of HMP on enamel de- and remineralization.

Surface reactivity is assessed by surface free energy (SFE) (O'Brien, 2002, Ch. 5; Tsujimoto et al., 2016), which can be measured by determining the contact angles formed by different probing liquids on a solid surface (Neumann, Good, Hope, & Seipal, 1974; Owens & Wendt, 1969). Among the different methods to determine these parameters, the approach described by Van Oss, Fowkes and Good, and Chaudhury (Neumann et al., 1974; Owens & Wendt, 1969) allows the calculation of the dispersion values, acid-base Lewis parts, polar force and hydrogen bond force, which determine the Lifshitz–van der Waals acid–base equation (Chaudhury, 1996; Good, 1992). Although the acid-base components of surface free energy of some polymeric surfaces, such as polyvinyl chloride (PVC), can exhibit some anomalies when calculated

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by this method, (Della Volpe & Siboni, 1997), several researchers employed it to estimate the SFE on the surface of enamel (Imai et al., 2017). This method has the same foundation of hydrophobicity angle measurement, despite hydrophobicity is measured only by angles made with water. Therefore, the use of other probing liquids (with different physicochemical properties) (Carlen, Nikdel, Wennerberg, Holmberg, & Olsson, 2001; Sipahi, Anil, & Bayramli, 2001) in addition to water can provide further information on the interactions between treatment solutions and a given surface.

Thus, the aim of this study was to analyze the capacity of HMP to adsorb on enamel, as well as to determine enamel SFE after treatment with different concentrations of HMP, followed or not by the application of solutions containing calcium or calcium-phosphate. The null hypothesis was that HMP does not alter enamel SFE and does not influence calcium and phosphate adsorption on enamel.

2. Material and methods

2.1. Experimental design

Enamel blocks ($4 \times 4 \times 4$ mm, n = 144) obtained from buccal-cervical region of bovine incisors were stored in 2% formaldehyde solution for 30 days. The teeth were extracted from ~3 year old bovine and same race. The enamel surface of the blocks was sequentially polished using water-cooled silicon carbide paper disks (600, 800, and 1200 grit, Extec, Enfield, CT, USA) with a final polish using a felt disk (Extec) in a grinding polisher (VECTOR-PHOENIX BETA, Buehler, Lake Bluff, Il, USA). At the end of each polisher disk, the blocks were cleaned by ultrasound in deionized water (Unique USC 1400, Indaiatuba, SP, Brazil) during 20 min at room temperature. As inclusion criteria, blocks should be flat, with no risks, cracks or hypoplasia. They were divided into twelve groups (n = 12/group), according to the following treatment solutions: HMP at 0, 0.25, 0.5 and 1% HMP, followed or not by immersion in a solutions containing calcium (1.25 mmol/L Ca), or calcium and phosphate (1.25 mmol/L Ca and 3.5 mmol/L P). Surface free energy analysis was performed by determining the contact angles of three probing liquids on enamel surface. Calcium and phosphorus concentrations in the treatment solutions were determined before and after the treatments.

2.2. Formulation of the solutions and treatment

Solutions were prepared using HMP (CAS 68915-31-1, Aldrich Chemistry, UK), at concentrations of 0, 0.25, 0.5 and 1% (Camara et al., 2014, 2015). Solutions containing calcium (1.25 mmol/L CaCl$_2$·2H$_2$O) and Ca and PO$_4$ (1.25 mmol/L CaCl$_2$·2H$_2$O and 3.5 mmol/L NaH$_2$PO$_4$·2H$_2$O; Sigma, USA) were also prepared according to average values from human saliva (Edgar, Dawes, & O'Mullane, 2012, Ch. 5). Blocks (n = 36/group) were immersed in individual flasks containing 1 mL of HMP solutions under constant agitation (shaker table TE-420 Orbital Tecnal, Brazil) over a period of 2 min. Following, blocks were gently washed in deionized water for 30 s and air dried. Twelve blocks of each group were stored for posterior analysis, while the remaining specimens were immersed in individual flasks containing 1 mL of a Ca solution (12 blocks/group) or in a Ca-PO$_4$ solution (12 blocks/group), under constant agitation for 1 min.

2.3. Calcium and phosphorus analysis

Ca analysis was performed spectrophotometrically (Microplate Spectrophotometer EON, Biotek, USA), using the Arsenazo III method, at wavelength of 650 nm (Vogel, Chow, & Brow, 1983). Aliquots of 5 μL were taken from the samples and added to 50 μL of deionized water and 50 μL of Arsenazo. For calibration, standards containing 40–200 μg Ca/mL were used. Phosphorus was determined by the molybdate method, using aliquots of 20 μL of the solutions, added to a mixture of 50 μL

molybdate and 20 μL reactive reducer, as described by Fiske and Subbarow (1925). These analyses were performed before and after the treatments, and the adsorption to enamel calculated by subtracting the final concentrations from the initial values (Δ, μg). Phosphorus of the HMP solutions was determined after acid hydrolysis. Aliquots of 0.2 mL of the HMP solutions were added of 0.2 mL of hydrochloric acid (HCl) 1.0 mol/L and heated in water at 100 °C for 1 h (Tiveron, Delbem, Gaban, Sassaki, & Pedrini, 2013). The resulting solutions were then analyzed as described above.

2.4. SFE measurements

The physicochemical properties of the enamel surface were characterized by contact angle measurements, using the sessile drop method to determine the surface free energy. Measurements were performed by an automatic goniometer (DSA 100S, Krüss, Hamburg, Germany) using three probing liquids: diiodomethane, water and ethylene glycol. The treated blocks were air dried for 45 min in order to stabilize the pellicle formed (van der Mei et al., 2002). After, 0.5 μL of each liquid was automatically dispensed on a different quadrant of enamel surface of each block utilizing glass syringe (500 μL) and needle of 0.5 mm gage. Counted 1 s, the contact angles (right and left) were measured using the images captured by a CCD camera and Tangent method (Drop Shape Analysis DSA4 Software, version 2.0–01, Krüss). Each drop was measured 5-fold during 5 s at 20 °C (van der Mei et al., 2002) and relative humidity of 44% ± 6. Different parameters, such as acid (γ^+, receptor component), base (γ^-, donor component) and Lifshiz van der Waals (γ^{LW}, nonpolar component) of surface free energy (mN/m) were calculated according to the model of van Oss, Chaudhery and Good for the determination of the substrates free energy (Della Volpe & Siboni, 1997; Vogler, 1998).

2.5. Statistical analysis

The values of contact angle, SFE parameters, HMP, Ca and PO$_4$ adsorbed to enamel were considered as outcome measures and the% of HMP and post treatment Ca and Ca-PO$_4$ solutions, as the variation factors. The data presented normal (Kolmogorov-Smirnov) and homogeneous (Cochran) distributions and were submitted to two-way analysis of variance followed by Student-Newman-Keuls' test for multiple comparisons. Analyses were performed using the Sigma Plot statistical software (version 12.0, Systat Software Inc., San Jose, CA, USA) with a significance level set at 5 %.

3. Results

Treatment with HMP led to lower values of contact angles for the three probing liquids ($p < 0.001$) compared to HMP-free solutions (Table 1). Surface free energy (γ_S) was only significantly higher with 0.25 % HMP ($p = 0.002$). The values of apolar component (γ_S^{LW}) from enamel surface were higher after treatment with HMP ($p = 0.001$). The values of polar component (γ_S^{AB} = Lewis acid-base) became more negative with increasing% of HMP ($p = 0.017$) (Fig. 1). Among the parameters from γ_S^{AB}, γ_S^+ = eletron-acceptor (Lewis acid) and γ_S^- = eletron-donor (Lewis base), higher γ_S^- values were observed with increasing% of HMP ($p < 0.001$). The same was observed for HMP adsorption to enamel ($p < 0.001$) (Fig. 2A). Significant correlations were observed between HMP adsorption and γ_S^{AB} (Pearson's r = 0.600; $p < 0.001$) and γ_S^- (Pearson's r = −0.736; $p < 0.001$).

Enamel surface treated with the Ca solution only (i.e. not pretreated with HMP) presented higher values of contact angles for the three probing liquids ($p < 0.005$), as well as a reduction of γ_S values ($p = 0.013$) when compared to enamel without any treatment (Table 1). Furthermore, not alteration of the γ_S^{AB}, γ_S^+ and γ_S^- values ($p = 0.119$) and a slight reduction of γ_S^{LW} values ($p = 0.002$) were observed (Fig. 1). Treatment with Ca of enamel pretreated with HMP

Table 1

Means (SD) of the contact angles (θ °) of probing liquids and surface free energy (γ_S) after treatment of enamel surface with HMP and after treatment with Ca or Ca and PO4 solution on enamel surface pretreated with HMP solutions (n = 12).

Treatments		Water θ (°)	Diiodomethane θ (°)	Ethylene glycol θ (°)	γ_S (mN/m)
no-Ca and PO4	0%HMP	67.7[a] (4.1)	52.4[a] (5.5)	55.4[a*] (4.2)	28.8[a] (4.6)
	0.25%HMP	48.0[b] (4.4)	39.0[b] (5.1)	37.5[b] (5.7)	33.6[b*] (3.2)
	0.5%HMP	43.1[c] (3.8)	48.0[c*] (6.4)	40.8[b] (4.3)	27.5[a] (3.9)
	1%HMP	40.3[c] (8.6)	43.6[d] (5.4)	40.8[b] (5.1)	25.9[a*] (5.2)
Ca	0%HMP	73.6[a] (6.3)	57.6[a*] (2.6)	62.1[a] (3.7)	25.3[a*] (2.3)
	0.25%HMP	68.5[a*] (6.0)	55.8[a*] (3.9)	53.4[b*] (2.9)	31.5[b*] (3.4)
	0.5%HMP	63.7[b] (5.6)	50.7[b*] (3.4)	49.7[c] (4.3)	31.7[b*] (3.4)
	1%HMP	69.4[a*] (6.2)	54.2[a*] (3.5)	58.5[d] (3.5)	26.5[a*] (2.9)
Ca-PO4	0%HMP	58.4[a*] (3.1)	58.3[a*] (2.8)	54.7[a*] (3.2)	24.0[a*] (4.2)
	0.25%HMP	64.8[b*] (2.2)	55.0[a,b*] (2.3)	54.4[a*] (2.5)	27.3[b] (3.4)
	0.5%HMP	68.8[b,c] (3.1)	56.7[a,b] (3.1)	53.8[a] (3.3)	31.0[c*] (3.0)
	1%HMP	70.4[c*] (3.7)	53.4[b*] (2.4)	52.2[a] (3.1)	33.2[c] (2.3)

Distinct superscript letters indicate statistical significance among the groups in each analysis (two-way repeated measures ANOVA, Student-Newman-Keuls's test; P < 0.05). Values between parentheses indicate the standard deviation of the mean. Asterisk means there is no significant difference between groups with the same% HMP.

led to higher contact angles for the three probing liquids compared to enamel without Ca treatment. Also, γ_S values were higher for enamels treated with 0.25% and 0.5% of HMP (p = 0.002). Ca treatment led to less negative γ_S^{AB} values according to HMP content in the treatment solutions, and a reduction of 57% for γ_S^- values (Fig. 1B) for all% of HMP (p < 0.001). In addition, γ_S^{LW} values after treatment with Ca were significantly lower (Fig. 1A) in relation to its counterpart without Ca treatment (p = 0.013).

Treatment with Ca-PO4 solution of enamel pretreated with HMP promoted higher contact angles for the three probing liquids compared to enamel treated with HMP only (Table 1). The γ_S values were higher for enamel treated with 0.5% and 1% of HMP (p = 0.002). In addition, higher adsorption of HMP on enamel increased the absorption of Ca and PO4 after treatment with Ca-PO4 solution (Fig. 2B). A dose-response relationship between adsorption of HMP and Ca (Pearson's r = 0.601; p < 0.001) and PO4 (Pearson's r = 0.622; p < 0.001) adsorption on
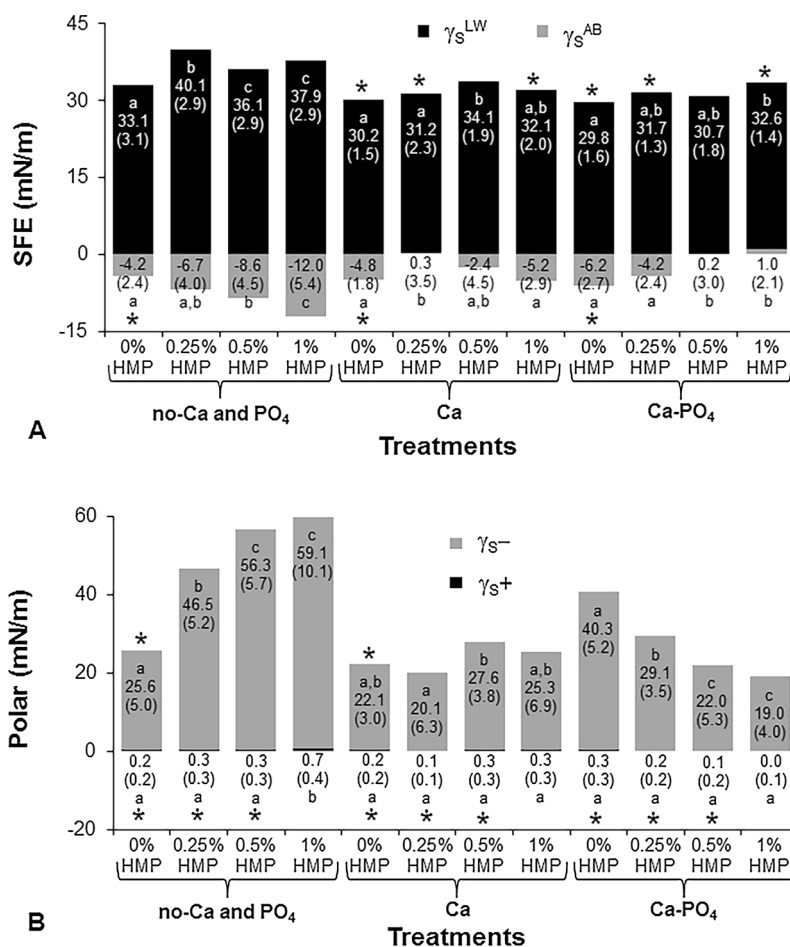
Fig. 1. (A) Surface free-energies and their components (γ_S^{LW}: Lifshitz-van der Waals surface tension component; γ_S^{AB}: Lewis acid-base interaction) with different enamel-surface treatments. (B) Influence of the treatments on the component polar of surface free energy on enamel surface: Lewis-acid (γ_S^+) and Lewis-base (γ_S^-). Values denote mean and standard deviation (n = 12). Distinct letters show significant differences among mean considering% HMP and treatment or not with Ca and Ca and PO4 solution (Student-Newman-Keuls, p < 0.05). Asterisk means there is no significant difference between groups with the same% HMP.
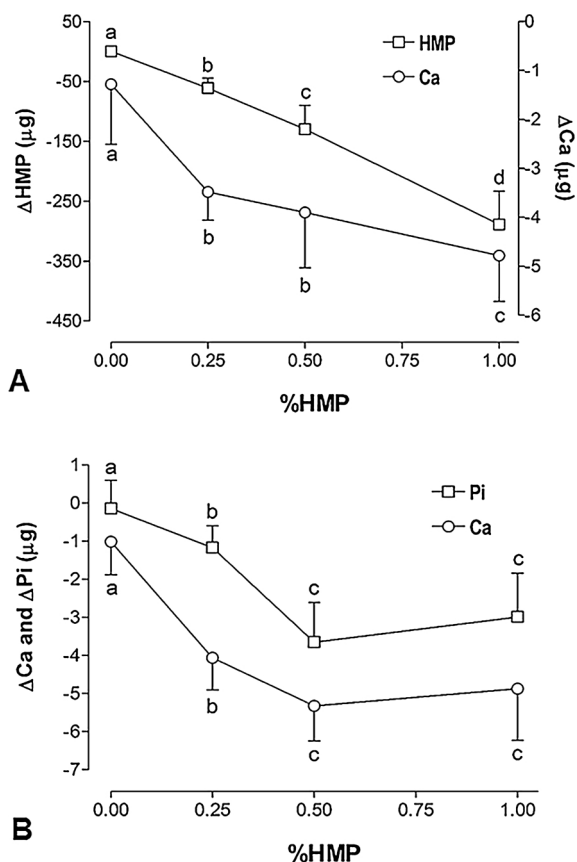
Fig. 2. Mean (SD) of HMP adsorbed (A) on enamel surface after treatment with different % of HMP solutions. Ca adsorbed (A) on enamel pretreated with different concentration of HMP after treatment with Ca solution. (B) Ca and Pi adsorbed on enamel pretreated with different concentration of HMP after treatment with solution containing Ca and PO_4. Distinct letters show significant differences between the% HMP for each adsorption analysis (Student-Newman-Keuls, p < 0.05).

enamel was observed. Furthermore, γ_S^{AB} values became positive with increasing Ca (Pearson's r = − 0.637) and PO_4 (Pearson's r = −0.621) adsorption on enamel. Also, γ_S^- values decreased (p < 0.002), showing a significant correlation with Ca (Pearson's r = 0.759; p < 0.001) and PO_4 (Pearson's r = 0.712; p < 0.001) adsorption on enamel.

4. Discussion

This study evaluated effect of HMP, followed or not by treatment with calcium or calcium/phosphate solutions, on the SFE of enamel. The results showed that the adsorption of HMP on enamel enhanced the adsorption of calcium and phosphate, so that the null hypothesis was rejected. A dose-response relationship was observed between the amount of HMP in the solutions and variables of adsorption, polar component (γ_S^{AB} = Lewis acid-base) and γ_S^- (Lewis base eletron-donor).

Considering only the contact angle with water (θ_W), it is possible to affirm that the treatment with HMP increases the hydrophilic character of enamel surface (Table 1), which makes it more liable to wetting, given that the contact angles were lower than 65° (Rüterman et al., 2011; van der Mei, White, & Busscher, 2004). Enamel not treated with HMP showed a hydrophobic characteristic and a lower probability of bacterial adhesion in as much as γ_S < 30 mN/m (Knorr, Combe, Wolff, & Hodges, 2005; van Oss, 1993) and γ_S^+ close to zero, as well as γ_S^- is less than 28.5 mN/m (van Oss, 1995). The adsorptions of HMP on enamel, on the other hand, occur leaving the surface with larger eletron-donor site (Fig. 1B). Despite γ_S^- values higher than 28.5 mN/m (what

characterizes a hydrophilic surface) (Olsson, van der Heijde, & Holmberg, 1992; van Oss, 1995), the γ_S remained unaltered because a negative γ_S^{AB} were higher and positive γ_S^{LW} values due the HMP adsorption (Fig. 1A). The increase of γ_S^{LW} values indicates hydrogen bonds between hydroxyl groups from enamel surface and phosphate groups from HMP. Although γ_S could suggest a hydrophobic surface, data from θ_W, γ_S^{LW} and γ_S^{AB} (γ_S^+; γ_S^-) effectively indicate a hydrophilic surface (Harnett, Alderman, & Wood, 2007; Quiryen & Bollen, 1995). However, the treatment with Ca and Ca-PO_4 solutions led to a more hydrophobic enamel surface when pre-treated with HMP (Table 1), since the θ_W values (Rüterman et al., 2011) and the γ_S values were close to 30 mN/m, and γ_S^- values (Fig. 1B) were less than 28.5 mN/m (Knorr et al., 2005; van Oss, 1993; van Oss, 1995).

As the enamel surface became with larger amount of electron-donor sites due to adsorption of HMP, the adsorption of calcium on enamel was enhanced in the same ratio as the γ_S^- parameter increases (Figs. 2A and 1B). Negative surfaces can be partly or entirely neutralized by pluivalent cations leading to a hydrophobic surface (Olsson et al., 1992). Apolar and polar components of the surface free energy were lower and close to enamel prior to treatment with HMP, which explains the previous studies about the reduction of enamel demineralization due the adsorption of HMP and its bond to calcium on enamel surface (Camara et al., 2014, 2015, 2016; Conceição et al., 2015). Enamel without treatment with HMP adsorbed around 200% less calcium that surfaces treated with HMP (Fig. 2A). This indicates no binding to phosphate ions on the hydroxyapatite surface (Harding, Rashid, & Hing, 2005; Vandiver, Dean, Patel, Bonfield, & Ortiz, 2005), since the values of polar component (γ_S^{AB}) and its parameters (γ_S^+; γ_S^-) did not change. The adsorption of calcium without changing the surface charge probably occurred by a displacement of two surfaces H^+ ions by a Ca^{2+} ion (Harding et al., 2005). Nevertheless, the surface became more hydrophobic because of higher θ_W and lower γ_S (as consequence of γ_S^{LW}) when compared to enamel prior to HMP or calcium. Thus, the formation of HMP layer covering the enamel provides more phosphate sites, thus increasing calcium retention. As consequence, a supersaturation of these ions close to the surface occurs, leading to a surface more prone to adsorption of phosphates ions. These data show the formation of HMP-Ca layer, therefore proving the hypotheses of previous studies (Camara et al., 2014, 2015, 2016; Conceição et al., 2015) that explain the effect of HMP on reducing mineral loss in the enamel.

The treatment of the enamel surface with calcium and phosphate solutions (not pretreated with HMP) turned the surface hydrophilic considering the θ_W < 65° and γ_S^- > 28.5 mN/m, even reducing γ_S and γ_S^{LW} values. As the calcium phosphate solution has the possibility to form ionic species with different charges and activities, enamel reactivity depends of the electrical charge created on the enamel surface. Utilizing the PHREEQC Interactive speciation software (version 2.18.3.5570) to calculate these species, it was possible to arrange in a descending order of reactivity, from $H_2PO_4^-$ > Ca^{2+} > HPO_4^{2-} > $CaH_2PO_4^+$ at 37 °C, at pH 5.92, and density of 1.0 g/cm³. The adsorption of Ca^{2+} and HPO_4^{2-} seems to explain the increase of the electron-donor sites (γ_S^- = 40.3 mN/m) on enamel surface prior to HMP faced with lower adsorption values (Fig. 1B). As the enamel surface present more electron-donor sites after the treatment with HMP, the reactivity with Ca^{2+} is greater leading to more adsorption of $H_2PO_4^-$ and $CaH_2PO_4^+$, resulting in lower γ_S^{AB} values and reduction in the γ_S^- parameter (Fig. 1). Also, the calcium and phosphate ions will be on the HMP-layer and not on enamel surface, maintaining the pores open and facilitating ions diffusion into the enamel (Camara et al., 2014). These differences might explain the higher ability to reduce the demineralization or enhance the remineralization of enamel adding HMP oral health products (Camara et al., 2014, 2015, 2016; Conceição et al., 2015). These data are in line with results of an in situ study (Camara et al., 2016) that showed a higher calcium concentration in enamel in the presence of HMP. Also, it showed the importance of ionic species as Ca^{2+}, $H_2PO_4^-$ and $CaH_2PO_4^+$ during de-remineralization process. Although it was possible to determine the influence of HMP on the adsorption of important ions in the process of

de-remineralization of enamel, the role played by fluoride in a such system has not yet been determined.

It was concluded that the adsorption of HMP on enamel increased the adsorption of calcium and phosphate. The polar component (γ_S^{AB}) of the surface free energy and its γ_S^{-} parameter from the enamel surface were higher with the HMP treatment.

Funding

Conflicts of interest

None.

Ethical approval

This study did not involve experiments with animals or humans, so that no Ethical Approval was needed.

Acknowledgments

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