José Guilherme Neves

ENERGIA LIVRE DE SUPERFÍCIE DO ESMALTE DENTÁRIO TRATADO COM HEXAMETAFOSFATO DE SÓDIO, CÁLCIO E FOSFATO

Araçatuba – SP
2016

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Dissertação apresentada à Faculdade de Odontologia da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Araçatuba, para obtenção de título de Mestre em Ciência Odontológica - Área de Concentração: Biomateriais.

Orientador: Prof. Titular Alberto Carlos Botazzo Delbem
Coorientadora: Profª. Drª. Marcelle Danelon

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Epígrafe

José Guilherme Neves

"Não tenho palavras pra agradecer Tua bondade

Dia após dia me cercas com fidelidade

nunca me deixes esquecer

Que tudo o que tenho

Tudo o que sou

O que vier a ser

Vem de Ti Senhor"

(Diante do Trono)

Resumo

NEVES, J. G. **Energia livre de superfície do esmalte dentário tratado com Hexametafosfato de sódio, Cálcio e Fosfato.** 2016 64f. Dissertação (Mestrado em Ciência Odontológica, área de concentração Biomateriais) - Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2016.

Objetivo: Esse estudou avaliou a capacidade de adsorção do hexametafosfato de sódio (HMP) em diferentes concentrações no esmalte dentário. Desenho: Blocos de esmalte bovino (4 mm x 4 mm, n=144, 12/grupo) foram selecionados e divididos em 12 grupos: 0%; 0,25%; 0,5%; e 1% HMP, e essas concentrações de HMP seguida da aplicação em solução contendo Ca ou Ca-PO_4 Os tratamentos foram realizados por 1 min (1 mL/bloco), e a seguir expostos ao ar para que houvesse a formação de película. A energia livre de superfície (mN/m) foi calculada pela medida dos ângulos de contato de três líquidos sondas: água deionizada, diiodometano e etileno glicol; determinando os componentes polar e apolar da superfície do esmalte. Analisaram-se as concentrações de cálcio (Ca), fosfato (PO_4) e HMP nas soluções, antes e após os tratamentos. Os dados apresentaram distribuição normal (Kolmogorov-Smirnov) e homogênea (Cochran) e a seguir foram submetidos a ANOVA seguido pelo teste Student-Newman Keuls ($p<0,05$). Resultado: Quanto maior a % de HMP nas soluções maior a adsorção de HMP e a eletronegatividade na superfície do esmalte ($p<0,05$). A adsorção de Ca foi maior com o aumento da % de HMP na solução ($p<0,05$) reduzindo a eletronegatividade na superfície do esmalte. Maior adsorção de Ca e PO_4 ocorreu com 0,5% HMP e 1% HMP após o tratamento com solução Ca-PO_4, deixando a superfície menos eletronegativa quando comparado aos demais tratamentos ($p<0,05$). Conclusão: Conclui-se que o HMP promove uma superfície

de esmalte mais eletronegativa favorecendo uma maior adsorção dos íons Ca e PO_4.

Palavras-chave: Fosfatos, Esmalte dentário, Energia.

Abstract

José Guilherme Neves

NEVES, J.G. **Surface free energy of enamel treated with sodium hexametaphosphate, calcium and phosphate.** 2016 64f. Dissertação (Mestrado em Ciência Odontológica, área de concentração Biomateriais) - Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista, Araçatuba 2016.

Objective: This study evaluated the sodium hexametaphosphate (HMP) capacity adsorption at different concentrations in the dental enamel. Design: Bovine enamel blocks (4 mm x 4 mm, n = 144, 12 group) were selected and divided into 12 groups: 0%; 0.25%; 0.5%; and 1% HMP, and these HMP concentrations followed by the application solution containing Ca or Ca-PO_4. The treatments were performed for 1 min (1 ml /block), and then exposed to air so that there was the formation of a pelicle. The surface free energy (mN/m) was calculated by measuring the contact angles of liquids three probes: deionized water, diiodomethane and ethylene glycol; determining the polar and nonpolar components of the enamel surface. It was analyzed the calcium (Ca), phosphate (PO_4) and HMP in the solutions, before and after treatment. The data were normally distributed (Kolmogorov-Smirnov) and homogeneous (Cochran) and then were subjected to ANOVA followed by Student-Newman Keuls test ($p < 0.05$). Results: The greater HMP % in solutions, the greater adsorption of HMP and electronegative surface enamel ($p<0.05$). The Ca adsorption was higher with HMP % increasin in the solution ($p<0.05$) reducing enamel surface electronegativity. Increased adsorption of Ca and PO_4 occurred in 0.5% and 1% HMP and HMP after treatment with Ca-PO_4 solution, leaving the less electronegative surface when compared to the other treatments ($P<0.05$). Results: The higher HMP% solutions was greater HMP adsorption and

electronegative surface enamel ($p<0.05$). The Ca adsorption was higher with increasing HMP % in the solution ($p<0.05$) in reducing electronegativity enamel surface. Increased adsorption of Ca and PO_4 occurred in 0.5% and 1% HMP HMP after treatment with Ca-PO_4 solution, leaving the less electronegative surface when compared to the other treatments ($P <0.05$). Conclusion: The HMP promotes more electronegative in enamel surface, achieving greater adsorption of Ca and PO_4 ions.

Keywords: Phosphates, Dental enamel, Energy.

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Figure 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.

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Lista de Abreviatruras

LISTA DE ABREVIATURAS

ANOVA Análise de variância

Ca^{2+} íon cálcio

$CaCl_2$ Cloreto de Cálcio

CaF^{+} íon fluoreto de cálcio

HMP Hexametafosfato de sódio

HCl Ácido Clorídrico

H_2O Água

LWAB Equação Ácido-base Lifhitz van der-Walls

mm Milímetro

mmol Milimol

ml Mililitro

min Minuto

mN/m MiliNilton por metro

NaOH Hidróxido de sódio

n Numero da amostra

sec Segundos

µL Microlitros

γ^{+} Componente receptor

γ^{-} Componente doador

γ^{LW} Componente van der Walls

γ^{AB} Componente Ácido-Base de Lewis

γ^{L} Energia Livre de Superfície Total

Sumário

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Artigo

José Guilherme Neves

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

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Short title: Surface free energy of enamel treated with sodium hexametaphosphate

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***De acordo com as instruções aos autores do periódico Caries Research. (Anexo A)**

ABSTRACT

Objective: This study evaluated the sodium hexametaphosphate (HMP) capacity adsorption at different concentrations in the dental enamel. Design: Bovine enamel blocks (4 mm x 4 mm, n = 144, 12/group) were selected and divided into 12 groups: 0%; 0.25%; 0.5%; and 1% HMP, and these HMP concentrations followed by the application solution containing Ca or Ca-PO_4. The treatments were performed for 1 min (1 ml/block), and then exposed to air so that there was the formation of a pelicle. The surface free energy (mN/m) was calculated by measuring the contact angles of liquids three probes: deionized water, diiodomethane and ethylene glycol; determining the polar and nonpolar components of the enamel surface. It was analyzed the calcium (Ca), phosphate (PO_4) and HMP in the solutions, before and after treatment. The data were normally distributed (Kolmogorov-Smirnov) and homogeneous (Cochran) and then were subjected to ANOVA followed by Student-Newman Keuls test ($p < 0.05$). Results: The greater HMP % in solutions, the greater adsorption of HMP and electronegative surface enamel ($p<0.05$). The Ca adsorption was higher with HMP % increasin in the solution ($p<0.05$) reducing enamel surface electronegativity. Increased adsorption of Ca and PO_4 occurred in 0.5% and 1% HMP and HMP after treatment with Ca-PO_4 solution, leaving the less electronegative surface when compared to the other treatments ($P<0.05$) Results: The higher HMP% solutions was greater HMP adsorption and electronegative surface enamel ($p<0.05$). The Ca adsorption was higher with increasing HMP % in the solution ($p<0.05$) in reducing electronegativity enamel surface. Increased adsorption of Ca and PO_4 occurred in 0.5% and 1% HMP HMP after treatment with Ca-PO_4 solution, leaving the less electronegative surface when compared to

the other treatments (P<0.05). Conclusion: The HMP promotes more electronegative in enamel surface, achieving greater adsorption of Ca and PO_4 ions.

Keywords: Phosphates, Dental enamel, Energy.

INTRODUCTION

Cyclophosphates have been added to fluoride products as toothpastes [da Camara et al., 2014; da Camara et al., 2015; de Castro et al., 2015; Danelon et al., 2015], gels [Danelon et al., 2013; Danelon et al., 2014; Pancote et al., 2014; Conceição et al., 2015], varnishes [Moretto et al., 2013; Manarelli et al., 2013; Manarelli et al., 2014] and mounthrinses [Manarelli et al., 2011; Favretto et al., 2013] with propose to increase the products capacity to reduce teeth demineralization and enhance their remineralization. As dental caries is the most common chronic oral disease in worldwide [Clarkson, 2010], increase the ability of fluoride products in reduce the mineral loss can improve the effect against dental caries. Sodium hexametaphosphate (HMP) is one of cyclic inorganic phosphate [Kulaev et al., 2005] that reduces enamel desmineralization [da Camara et al., 2014; da Camara et al., 2015; Conceição et al., 2015; da Camara et al., 2016] due to its ability to adsorption on enamel surface and increase permselectivity, facilitating the diffusion of cations into the enamel [McGaughey and Stowell, 1977; van Dijk et al., 1980]. The assumption would be that the HMP would retain ionic species like CaF^+ and Ca^{2+}, leading to a reticular formation of HMP-Ca^{2+} layer on enamel, which increases calcium availability during de- and

re-mineralization processes [da Camara et al., 2014; da Camara et al., 2015; Conceição et al., 2015]. However, this hypothesis needs to be proven as HMP is a negatively charged cyclic phosphate [Choi et al., 1993] as well as the enamel [McGaughey and Stowell, 1977; van Dijk et al., 1980]. Thus, the study of the interactions and chemical bonds among HMP, enamel surface and rich medium in calcium phosphate can help us to understand and to prove the above hypothesis.

The surface reactivity is evaluated through the surface free energy (SFE) [O`Brien, 1997] measuring the contact angle formed by different liquids on a solid surface [Zisman, 1964]. Among the different methods to achieving these parameters [Zisman, 1964; Owens, 1969; Neuman, 1974; Baier, 1992], the approach described by Van Oss, Fowkes and Good and Chaudhury provides the most complete SFE description. It allows calculating the dispersion values, acid-base Lewis parts, polar force and hydrogen bond force, determining the Lifshitz–van der Waals acid–base equation (LWAB) [Fowkes, 1987; van Oss, 1987; van Oss, 1988; Good, 1992; Chaudury, 1996]. The dispersion force discusses interactions values of nonpolar molecules, the polarity force shows the interactions of metal molecules and the hydrogen bond strength reports the interaction of water and hydroxyl groups [Comyn, 1992; Ueta, 2016]. This method has the same foundation of hydrophobicity angle measurement, but hydrophobicity is measured only by angles made with water, different from this proposal where is used at least three liquids with different physico-chemical properties [Blunden, 1994; Carlen, 2001; Sipahi, 2001].

Therefore, the aim of this study was to analyze the HMP adsorption and the SFE of the enamel after treatment with different concentrations of HMP. Also,

if the HMP treatment would interfere in the adsorption of calcium phosphate on enamel. The null hypothesis was that the HMP is not adsorbed and do not alters the SFE of the enamel and the adsorption of calcium phosphate.

MATERIAL AND METHODS

Experimental design

Enamel blocks (4 x 4 mm, n= 144) of bovine incisors were stored in formaldehyde solution 2% for 30 days. The enamel surfaces of blocks were sequentially polished, and as inclusion criteria the blocks should be flat, with no risk, cracks or hypoplasia. They were divided in twelve groups (n = 12/group) and immersed in the solutions content: free of HMP (0%), 0.25% HMP, 0.5% HMP and 1% HMP with or without posterior treatment with calcium (1.25 mmol/L Ca) or calcium and phosphate solutions (1.25 mmol/L Ca and 3.5 mmol/L P). Surface free energy analysis was performed trough contact angles, and of calcium and phosphorus concentrations determined before and after treatments.

Formulation of the solutions and treatment

Solutions were prepared using microparticulate HMP (Aldrich Chemistry, UK), in concentrations of 0%, 0.25%, 0.5% and 1%. Solutions containing calcium (1.25 mmol/L $CaCl_2 \cdot 2H_2O$) and calcium and phosphate (1.25 mmol/L $CaCl_2 \cdot 2H_2O$ and 3.5 mmol/L $NaH_2PO_4 \cdot 2H_2O$; Sigma, USA) also was prepared [Fejerskov, 2003]. Blocks (n = 12/group) were immersed in individual flasks containing 1 mL of solutions of HMP under constant agitation (shaker table TE-420 Orbital Tecnal,

Brazil) over a period of 2 min. After this treatment, the blocks were washed with deionized water for 30 seconds and dried. Twelve blocks of each group were stored to posterior analysis, another 12 were immersed in an individual flask containing 1 mL of a calcium solution and the last 12 blocks immersed in a calcium/phosphate solution, under constant agitation for 1 min.

Calcium and phosphorus analysis

The Ca analysis was performed by a spectrophotometric (Microplate Spectrophotometer EON, Biotek, USA), with a wavelength of 650 nm by adopting a colorimetric method Arsenazo III [Vogel et al., 1983]. Aliquots of 5 µL were taken from the samples and 50 µL of deionized water and arsenazo were added. For calibration, standards containing 40 to 200 µg Ca/mL were used. The phosphorus was colorimetric measured by the molybdate method through an aliquot of 20 µL from the samples and in sequence, added to a mixture of 50 µL of molybdate and 20 µL reactive reducer, as described by Fiske and Subbarow (1925). This analysis was performed before and after the treatments and the adsorption to enamel calculated from the initial concentrations of these compounds in the solutions and the concentration after treatment (Δ, µg). The phosphorus of the HMP solutions was dosed 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 hour [Tiveron et al., 2013].

SFE Measurements

The physico-chemical 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 liquid contained within of probes: diiodomethane, water and ethylene glycol (Table 1). The treated blocks were exposed for 45 min in the air in order to stabilize the formed pellicle [van der Mei et al., 2002], after this 0.5 µL of each liquid has been dispensed on the blocks surface, and the contact angles were measured using images captured by a CCD camera. For each specimen 5 measurements were performed at 20° C [Harnett et al., 2007; van der Mei et al., 2002]. Different parameters, such as acid (γ+, donor component), base (γ-, receptor 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 interaction free energy substrates [Van Oss et al., 1990; Della Volpe, 1997].

Statistical Analysis

The data presented a normal (Kolmogorov-Smirnov) and homogeneous (Cochran) distribution and were submitted to analysis of variance (two-way) followed by Student-Newman-Keuls test for multiple comparisons. Analyses were performed using the Sigma Plot statistical software (version 12.0) with a significance level set at 5 %. The values of contact angle, SFE parameters, HMP, calcium and phosphate were considered as outcome measures and the % of

HMP and post treatment with calcium and calcium/phosphate solutions as the variation factor.

RESULTS

Treatments with HMP led to lower values of contact angles for three probes liquids ($p<0.001$) compared to no-HMP (Table 2), which means that HMP effectively affects the surface composition. Surface free energy (γ_S) was only 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 the increases of %HMP ($p<0.017$) (Figure 1). Among the parameters from γ_S^{AB}, γ_S^{+} = eletron-acceptor (Lewis acid) and γ_S^{-} = eletron-donor (Lewis base), the γ_S^{-} values got greater with the increases of %HMP ($p<0.001$). The same was observed with the adsorption of HMP on enamel ($p<0.001$) (Figure 2). There was a correlation between values of HMP adsorption and γ_S^{AB} (Pearson's $r = 0.600$) and γ_S^{-} (Pearson's $r = -0.736$).

The enamel surface without pretreatment with HMP but treated with calcium solution presented higher values of contact angles for probes liquids ($p<0.005$) as well as a reduction of γ_S values ($p=0.013$) when compared to enamel without any treatment (Table 2). Furthermore, there was not alteration in the γ_S^{AB}, γ_S^{+} and γ_S^{-} values ($p>0.119$) and slightly reduction of γ_S^{LW} values ($p=0.002$). The treatment with calcium on enamel surface pretreated with HMP gives greats contact angles for probes liquids compared to enamel without

calcium treatment. The γ_S values were higher for enamels treated with 0.25% and 0.5% of HMP ($p<0.002$). Calcium treatment led to less negatives γ_S^{AB} values according to HMP content and a reduction of 57% for γ_S^- values for all % of HMP ($p<0.001$). The values of γ_S^{LW} post treatment with calcium were lower in relation to its correspondent % of HMP without calcium treatment ($p<0.013$).

The treatment with Ca-PO_4 solution on enamel surface pretreated with HMP showed higher contact angles for probes liquids compared to enamel treated solely with HMP. The γ_S values were higher for enamel treated with 0.5% and 1% of HMP ($p<0.002$). Larger adsorption of HMP on enamel increased absorption of calcium and phosphate after treatment with Ca-PO_4 solution. There was dose-response relationship between adsorption of HMP and Ca^+ (Pearson's $r = 0.601$) and PO_4 (Pearson's $r = 0.622$) adsorption on enamel. As consequence, the γ_S^{AB} values became positive with the increases of calcium (Pearson's $r = -0.637$) and phosphate (Pearson's $r = -0.621$) adsorption on enamel. Also, γ_S^- values decreased ($p<0.002$) showing correlation with calcium (Pearson's $r = 0.759$) and phosphate (Pearson's $r = 0.712$) adsorption on enamel.

DISCUSSION

This study evaluated the SFE alteration on enamel after treatment with HMP and the calcium and phosphate interference. The results showed that the adsorption of HMP on enamel enhanced the adsorption of calcium and phosphate. Thus, 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, which makes it more liable to wetting, given that the contact angles were lower than 65° [Vogle, 1998]. The surface free energy (γ_S) and its Lifshiz van der Waals (γ^{LW}) and Lewis acid-base (γ^{AB}) components also indicate that enamel surface treated with HMP are more susceptible to bacterial adhesion [Rüttermann et al., 2011; Rüttermann et al., 2014; Tiznado-Orozco et al., 2015].

The enamel prior to HMP treatment showed hydrophobic character and lesser probability to bacterial adhesion inasmuch as γ_S < 30 mN/m [Knorr et al., 2005; Rüttermann et al., 2011] and γ_S^+ close to zero, as well as γ_S^- is less than 28.5 mN/m [van Oss, 1993; van Oss, 1995]. Nevertheless, attractive interaction to biological species, compounds and cells was present but weaker than in enamel treated with HMP because γ_S^{LW} was higher than 22 mN/m [van Oss, 1993; van Oss, 1995]. In this case, γ_S is lower because of lower γ_S^{AB} values indicating a surface less susceptible to cell adhesion [Knorr et al., 2005; Lee et al., 2009]. The adsorption of HMP on enamel, on the other hand, occur leaving the surface negatively charged. Despite γ_S^- values higher than 28.3 mN/m that characterizes a hydrophilic surface [van Oss, 1993; van Oss, 1995], the γ_S remained the same because a negative γ_S^{AB} even with higher and positive γ_S^{LW} values due the HMP adsorption. Although γ_S could suggest a hydrophobic surface less susceptible to

cell adhesion, data from θ_W, γ_S^{LW} and γ_S^{AB} (γ_S^+; γ_S^-) effectively indicate a surface that favors the adherence of biofilms [Olsson et al., 1992; Quirynen et al., 1989].

As the surface enamel became negatively charged due HMP the adsorption of HMP, the adsorption of calcium on enamel is enhanced in the same ratio as the γ_S^- parameter increases. Negatively surfaces can be partly or wholly neutralized by plurivalent cations leading to a hydrophobic surface [van Oss, 1995]. Apolar and polar components from the surface free energy were lower and close to enamel prior to HMP, which confirms previous studies about the reduction of enamel demineralization due the adsorption of HMP and its bond to calcium on enamel surface [da Camara et al., 2014; da Camara et al., 2015; Conceição et al., 2015]. Enamel without treatment adsorbed around 200% less calcium that surfaces treated with HMP. It indicates no binding to phosphate ions on the hydroxyapatite surface [Harding et al., 2005; Vandiver et al., 2005] since the values of polar component (γ_S^{AB}) and its parameters (γ_S^+; γ_S^-) did not change. To calcium be adsorbed without changing the surface charge probably there was a displacement of two surface 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 gives more phosphates sites increasing the retention of calcium. As consequence, it occurs a supersaturation these ions close to surface with possibility of higher adsorption of phosphates ions.

The treatment of the enamel surface prior to HMP with calcium phosphate solution turned the surface hydrophilic once the $\theta_W < 65°$ and $\gamma_S^- > 28.5$ 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, pH 5.92, and density of 1.0 g/cm^3. The adsorption of Ca^{2+} and HPO_4^{2-} seems to explain the increase of the negative charges (γ_S^- = 40.3 mN/m) on enamel surface prior to HMP faced with lower adsorption values. As the enamel surface present negative charges 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 higher γ_S^{AB} values and reduction in the γ_S^- parameter. These differences might explain the higher ability to reduce the demineralization or enhance the remineralization of enamel adding HMP oral health products [da Camara et al., 2014; da Camara et al., 2015; Conceição et al., 2015]. Although it was possible to determine the influence of HMP in the adsorption of important ions in the process of des-remineralization of enamel, the role played by fluorite in a such system still undetermined.

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.

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REFERENCES

1. Baier RE. Principles of adhesion. Oper Dent 1992; 5: 1-9.
2. Blunden RE, Oliver RG, O'Kane CO. Microbial growth on the surfaces of various orthodontic bonding cements. Br J Orthodont 1994; 21:125-132.
3. Carlen A, Nikdel K, Wennerberg A, Holmberg K, Olsson J. Surface characteristics and in vitro biofilm formation on glass ionomer and composite resin. Biomaterials 2001; 22: 481-487.
4. Chaudhury MK. Interfacial interaction between low energy surfaces. Mater Sci Engng 1996; 16:97-59.
5. Choi IK, Wen WW, Smith RW. Technical note the effect of a long chain phosphate on the adsorption of collectors on kaolinite. Miner Eng 1993; 6:1191–1197.
6. Clarkson J, Watt RG, Rugg-Gunn AJ et al. Proceedings. 9th World Congress on Preventive Dentistry (WCPD): "Community Participation and Global Alliances for Lifelong Oral Health for All," Phuket, Thailand. Adv Dent Res 2010; 22: 2–30.
7. Comyn J. Contact angles and adhesive bonding. Int J Adhes Adhesives 1992; 12:145–149
8. Conceição JM, Delbem ACB, Danelon M, Camara DM, Wiegand A, Pessan JP. Fluoride gel supplemented with sodium hexametaphosphate reduces enamel erosive wear in situ. J Dent 2015; 43: 1255–1260.
9. da Camara DM, Miyasaki ML, Danelon M, Sassaki KT, Delbem AC. Effect of low-fluoride toothpastes combined with hexametaphosphate on in vitro enamel demineralization. J Dent 2014; 42:256–262

10. da Camara DM, Pessan JP, Francati TM, Santos Souza JA, Danelon M, Delbem AC. Synergistic effect of fluoride and sodium hexametaphosphate in toothpaste on enamel demineralization in situ. J Dent 2015; 43:1249-1254.
11. da Camara DM, Pessan JP, Francati TM, Souza JA, Danelon M, Delbem AC. Fluoride toothpaste supplemented with sodium hexametaphosphate reduces enamel demineralization in vitro. Clin Oral Investig 2016 Jan 13.
12. Danelon M, Pessan JP, Neto FN, de Camargo ER, Delbem AC. Effect of toothpaste with nano-sized trimetaphosphate on dental caries: In situ study. J Dent 2015; 43:806-13.
13. Danelon M, Takeshita EM, Peixoto LC, Sassaki KT, Delbem. Effect of fluoride gels supplemented with sodium trimetaphosphate in reducing demineralization. Clin Oral Invest 2014; 18:1119–1127.
14. Danelon M, Takeshita EM, Sassaki KT, Delbem ACB. In situ evaluation of a low fluoride concentration gel with sodium trimetaphosphate in enamel remineralization. Am J Dent 2013; 26:15-20.
15. de Castro LP, Delbem AC, Danelon M, Passarinho A, Percinoto. In vitro effect of sodium trimetaphosphate additives to conventional toothpastes on enamel demineralization. Clin Oral Investig 2015; 1683-1687
16. Della Volpe C; Siboni S. Some Reflections on Acid–Base Solid Surface Free Energy Theories. J Colloid Interf Science 1997; 195:121–136
17. Favretto CO, Danelon M, Castilho FC, Vieira AE, Delbem AC. In vitro evaluation of the effect of mouth rinse with trimetaphosphate on enamel demineralization. Caries Res 2013;47:532-8
18. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus J. Biol. Chem 1925; 66:375–400
19. Fowkes FM. Wetting, SCI monograph. London. Society of Chemical Industry; 1967
20. Good RJ: Contact angle, wetting, and adhesion: a critical review. J Adhes Sci Technol 1992; 6:1269-302.
21. Harding IS, Rashid N, Hing KA. Surface charge and the effect of excess calcium ions on the hydroxyapatite surface. Biomaterials 2005; 26: 6818-6826

22. Harnett EM; Alderman J; Wood T. The surface energy of various biomaterials coated with adhesion molecules used in cell culture. Colloids and Surfaces 2007; 55:90–97
23. Kulaev IS, Vagabov VM, Kulakovskaya TV. The biochemistry of inorganic polyphosphates. 2nd ed. John Wiley & Sons 2005; pp. 294.
24. Knorr SD, Combe EC, Wolff LF, Hodges JS. The surface free energy of dental gold-based materials. Dental Materials 2005; 21: 272–277.
25. Lee SP, Lee SJ, Lim BS, Ahnd JS. Surface Characteristics of Orthodontic Materials and Their Effects on Adhesion of Mutans streptococci. Angle Orthodontist 2009; 79: 353-360.
26. Manarelli MM, Vieira AE, Matheus AA, Sassaki KT, Delbem AC. Effect of mouth rinses with fluoride and trimetaphosphate on enamel erosion: an in vitro study. Caries Res. 2011; 45:506-9
27. Manarelli MM, Delbem ACB, Lima TMT, Castilho FCN, Pessan JP. In vitro remineralizing effect of fluoride varnishes containing sodium trimetaphosphate. Caries Res 2014; 48:299–305.
28. Manarelli MM, Moretto MJ, Sassaki KT, Martinhon CCR, Pessan JP, Delbem ACB. Effect of fluoride varnish supplemented with sodium trimetaphosphate on enamel erosion and abrasion. Am J Dent 2013; 26:307-312.
29. McGaughey C, Stowell EC. Effects of polyphosphates on the solubility and mineralization of HA: relevance to a rationale for anticaries activity. J Dent Res 1977; 56: 579–587.
30. Moretto MJ, Delbem ACB, Manarelli MM, Pessan JP, Martinhon CCR. Effect of fluoride varnish supplemented with sodium trimetaphosphate on enamel erosion and abrasion: An in situ/ex vivo study. Arch Oral Biol 2013; 41:1302-1306.
31. Neumann AW, Good RJ, Hope CJ, Seipal M. Equation of state to determine surface tensions of low energy solids from contact angles. J Colloid Interf Sci 1974; 49:291-304.
32. O'Brien WJ. Dental materials and their selection, 2nd ed. Chicago. Quintessence Publishing Co; 1997. p. 37.

33. Olsson J, van der Heijde Y, Holmberg K. Plaque formation in vivo and bacterial attachment in vitro on permanently hydrophobic and hydrophilic surfaces. Caries Res 1992; 26: 428-33.
34. Owens DK, Wendt RC. Estimation of the surface free energy of polymers. J Appl Polym Sci 1969; 13:1741-
35. Pancote LP, Manarelli MM,Danelon M, Delbem ACB. Effect of fluoride gels supplemented with sodium trimetaphosphate on enamel erosion and abrasion: In vitro study. Arch Oral Biol 2014; 59: 336-340.
36. Quirynen M Bollen CML. The influence of surface roughness and surface-free energy on supra-and subgengival plaque formation in man: a review of the literature. J Clin Periodontol 1995; 22: 1-14.
37. Rüterman S, Trellenkamp T, Bergmann N, Raab WHm, Ritter H, Janda R. A new approach to influence contact angle and surface free energy of resin-based dental restorative materials. Acta Biomaterialia 2011; 7: 1160-1165.
38. Rüterman S, Beickler T, Janda R. Contact angle and surface free energy of experimental resin-based dental restorative materials after chewing simulation. Dental Materials 2014; 30: 702-707.
39. Sipahi C, Anil N, Bayramli E. The effect of acquired salivary pellicle on the surface free energy and wettability of different denture base materials. J Dent 2001; 29:197-204.
40. Tiveron AC, Delbem AC, Gaban G, Sassaki KT, Pedrini D. Effect of resin composites with sodium trimethaphosphate with or without fluoride on hardness, ion release and enamel desmineralization. Am J Dent 2013; 26:201-6.
41. Tiznado-Orozco GE, Reys-Gasga J, Elefterie F, Beyens C, Maschke U, Brès EF. Wettability modification of human tooth surface by water and UV nad eléctron-beam radiation. Materials Science and Engineering C 2015; 57: 133-146.
42. Ueta H, Tsujimoto A, Barkmeier WW, Oouchi H, Sai K, Takamizawa T, Latta MA, Miyazaki M. Influence of an oxygen-inhibited layer on enamel bonding of dental adhesive systems: surface free-energy perspectives. Eur J Oral Sci 2016; 124:82-

43. van der Mei HC1, White DJ, Kamminga-Rasker HJ, Knight J, Baig AA, Smit J, Busscher HJ. Influence of dentifrices and dietary components in saliva on wettability of pellicle-coated enamel in vitro and in vivo. Eur J Oral Sci. 2002 110:434-8
44. van Dijk JW, Borggreven JM, Driessens FC. The effect of some phosphates and a phosphonate on the electrochemical properties of bovine enamel. Arch Oral Biol 1980; 25: 591–595.
45. Vandiver J, Dean D, Patel N, Bonfield W, Ortiz C. Nanoscale variation in surface charge of synthetic hydroxyapatite detected by chemically and spatially specific high resolution force spectroscopy. Biomaterials 2005; 26: 271-283.
46. van Oss CJ. Aspecific and specific intermolecular interactions in aqueous media. J Mol Recognit. 1990; 3:128-36
47. van Oss CJ, Chaudhury MK, Good RJ. Monopolar surfaces. Adv Colloid Interf Sci 1987; 28:35-64.
48. van Oss CJ, Good RJ, Chaudhury MK. Additive and nonadditive surface tension components and the interpretation of contact angles. Langmuir 1988; 4:884-9
49. van Oss CJ. Hydrophobicity of biosurfaces – origin, quantitative determination and interaction energies. Colloids and Surfaces B: Biointerfaces 1995;5: 91-110.
50. van Oss CJ. Acid-base interfacial interactions in aqueous media. Colloids and Surfaces A: Physicochemical and Engineering Aspects 1993 78: 1-49.
51. Vogel GL, Chow LC, Brow WL. A microanalitical procedure for the determination of calcium, phosphate and fluoride in enamel biopsy samples Caries Res 1983; 17: 23–31.
52. Vogler EA. Structure and reactivity of water at biomaterial surfaces Advances in Colloid and Interface Science 1998; 74 69-117.
53. Zisman WA. Contact angle, wettability and adhesion. Advances in Chemistry series, vol. 43. Washington, DC: American Chemical Society 1964; 1-51.

Table legend

Table 1. Surface free energy and component values in test liquids

Table 2. 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 PO_4 solution on enamel surface pretreated with HMP solutions (n = 12)

Figure legends

Figure 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 PO_4 solution (Student-Newman-Keuls, $p < 0.05$).

Figure 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, $p < 0.05$).

Table 1. Surface free energy and component values in test liquids

Liquid	CAS.	Manufacturer	γ_L	γ_L^{LW}	γ_L^{AB}	γ_L+	γ_L-
Deionized water			72.8	21.8	0.00	25.50	25.50
Diiodomethane	75116	Sigma-Aldrich	50.80	50.42	0.38	0.00	0.00
Ethileneglycol	107211	Sigma-Aldrich	48.0	29.0	18.9	3.0	30.10

γ_L, surface free energy; γ_L^{LW}, nonpolar component; γ_L^{AB} , polar component; γ_L^{+}, donor component ; γ_L^{-}, receptor component . Values are given in mN/m.

Table 2. 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 PO_4 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 PO_4	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-PO_4	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.

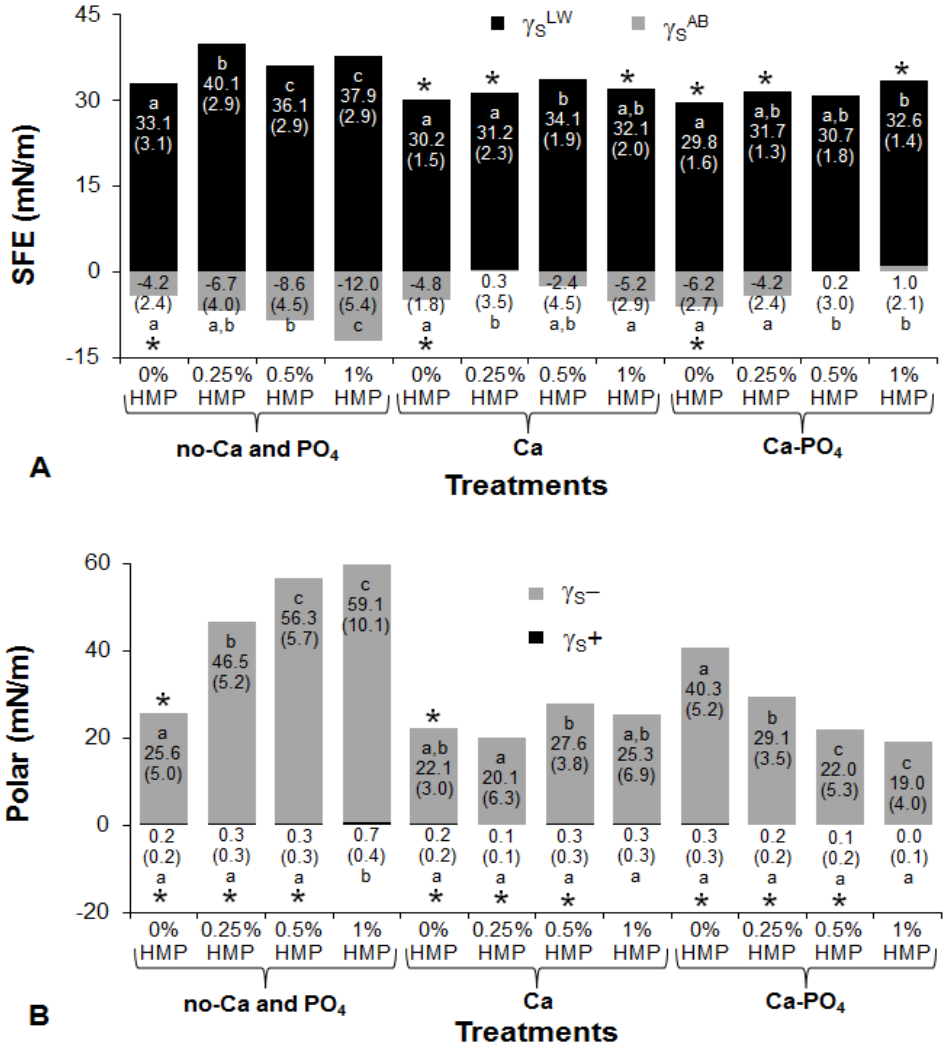


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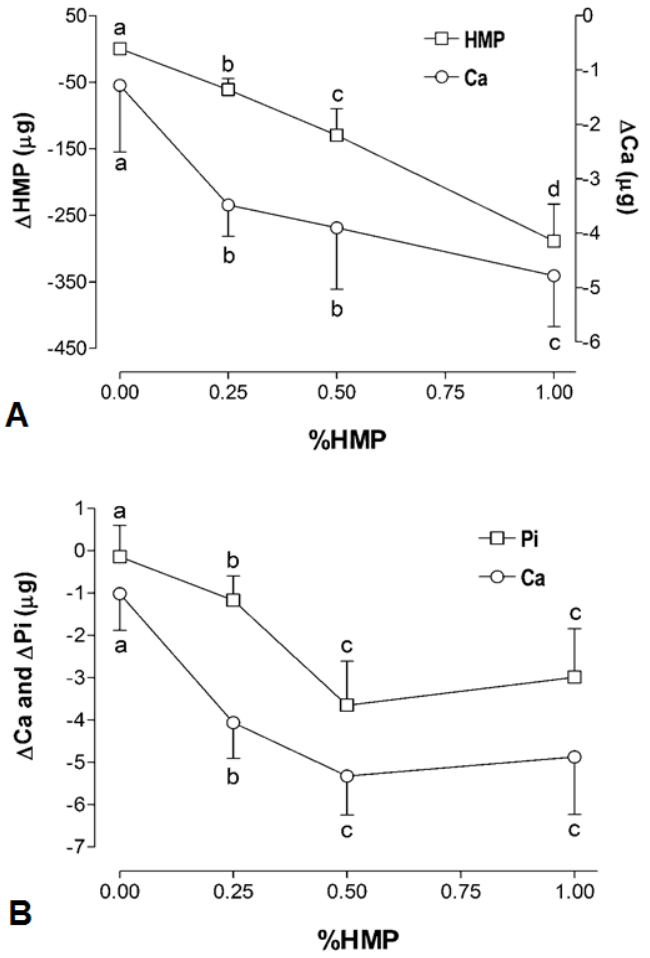


Figure 2. Mean (SD) of HMP adsorbed (A) on enamel surface after treatment with different % of HMP solutions. Ca adsorbed (A) on enamel pretreated with different concentration of HMP after treatment with Ca solution. (B) Ca and Pi adsorbed on enamel pretreated with different concentration of HMP after treatment with solution containing Ca and PO_4. Distinct letters show significant differences between the % HMP for each adsorption analysis (Student-Newman-Keuls, $p < 0.05$).

Anexo

ANEXO A

INSTRUÇÕES AOS AUTORES

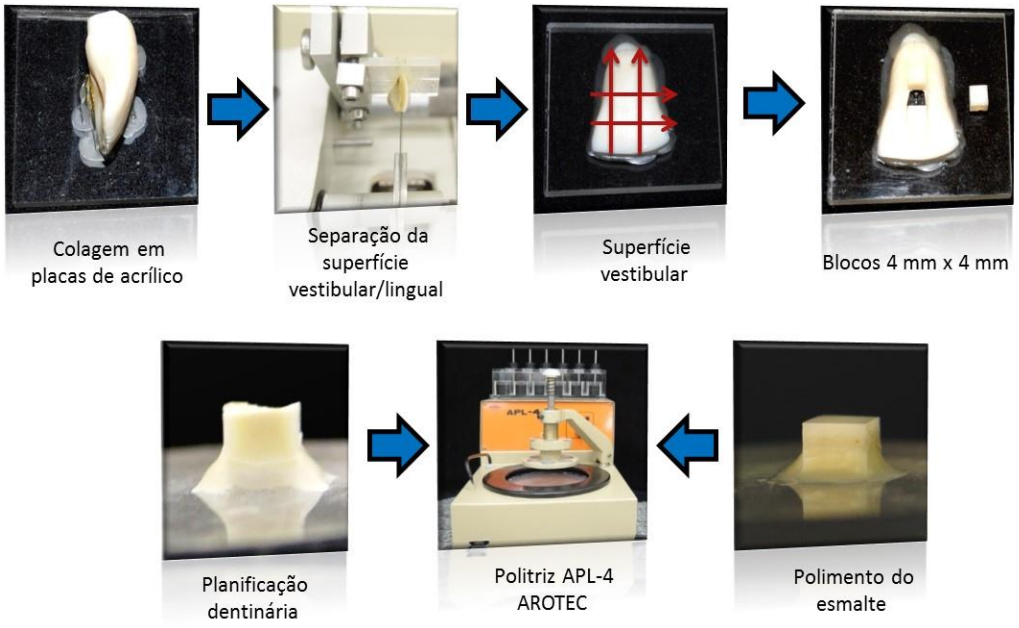
Caries Research

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ANEXO B

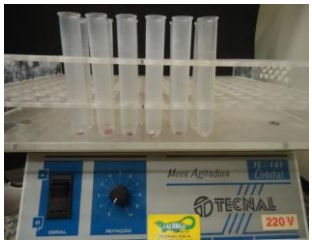
PREPARO DOS BLOCOS DE ESMALTE



ANEXO C

TRATAMENTO DOS BLOCOS DE ESMALTE COM SOLUÇÕES EXPERIMENTAIS

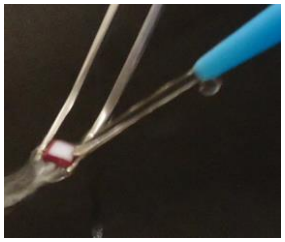




1 min

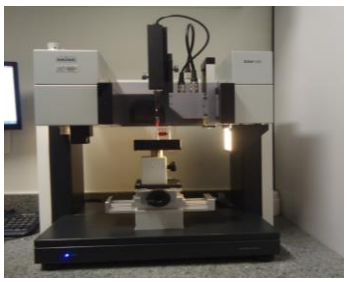


Mesa Agitadora
TE-420 Orbital
Tecnal, Brasil

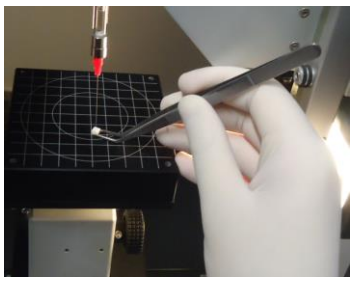


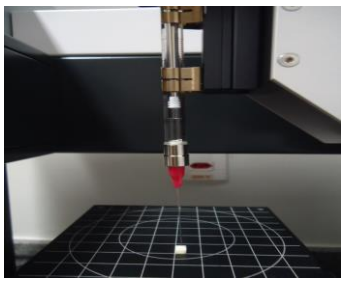
ANEXO D

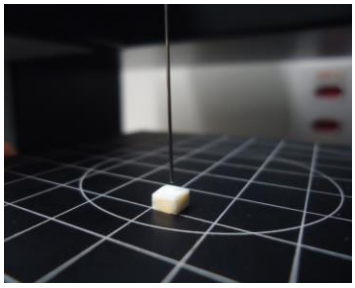
ANÁLISE DA ENERGIA LIVRE DE SUPERFICIE DO ESMALTE DENTÁRIO



Goniômetro Automático DAS110S, Kruse, Germany

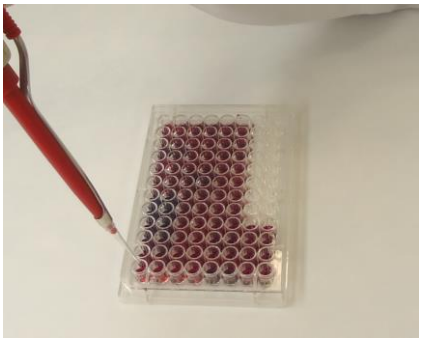




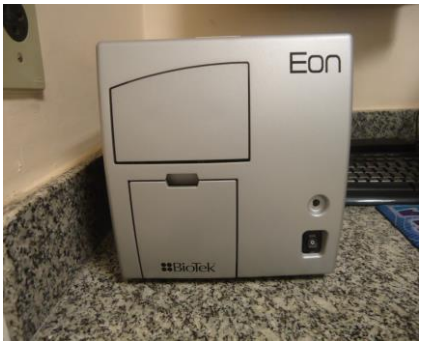


ANEXO E

DOSAGEM DE CÁLCIO NAS SOLUÇÕES EXPERIMENTAIS



5 µL da amostra,
50 µL de Água deionizada,
50 µL de Arsenazo III

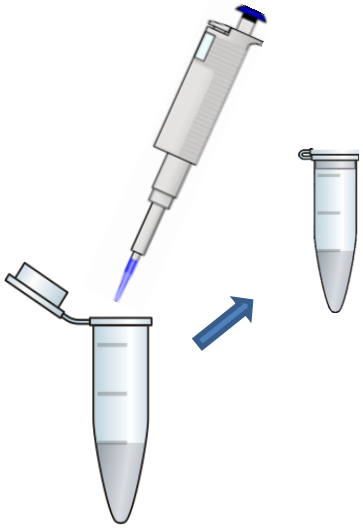


Espectrofotômetro de microplaca EON, Biotek, USA



ANEXO F

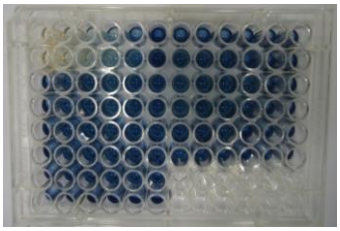
DOSAGEM DE HMP NAS SOLUÇÕES EXPERIMENTAIS



200 µL da solução HMP
+
200 µL de HCl 1 M



Banho Térmico TE-054 MAG, Tecnal



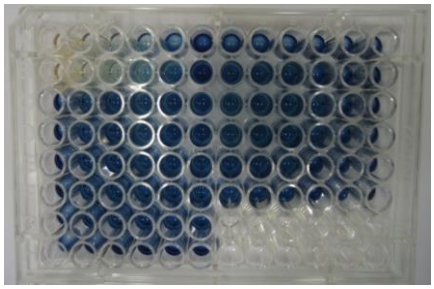
20 µL da amostra
+
50 µL de molibidato
+
20 µL de reativo redutor



Espectrofotômetro de microplaca EON, Biotek, USA

ANEXO G

DOSAGEM DE FÓSFORO NAS SOLUÇÕES



20 µL da amostra
+
50 µL de molibidato
+
20 µL de reativo redutor



Espectrofotômetro de microplaca EON, Biotek, USA