# Formation of alkali-soluble fluoride on the surface of human dental enamel after treatment with fluoridated gels: influence of the pH variation and of the treatment time

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*The aim of this study was to quantify,* in vitro*, the formation of $CaF_2$ after the application of three fluoridated gels: one neutral, one acidulated and another highly acidulated, on a bovine enamel dental surface treated with a Dijkman's demineralizing solution (1990). 145 sections were utilized, obtained from 145 sound teeth and divided into seven groups: C (enamel without treatment); FN1 (enamel demineralized and treated with neutral gel for 1 minute); FN4 (enamel demineralized and treated with neutral gel for 4 minutes); FFA1 (enamel demineralized and treated with acidulated gel for 1 minute); FFA4 (enamel demineralized and treated with acidulated gel for 4 minutes); FAA1 (enamel demineralized and treated with highly acidulated gel for 1 minute) and FAA4 (enamel demineralized and treated with highly acidulated gel for 4 minutes). The formation of $CaF_2$ was analyzed by SEM and chemically by Caslavska's method (1975). The average and standard deviations from the groups studied were respectively: C-0.63; 0.38; FN1-23.06; 16.52; FFA1-54.11; 49.00; FAA1-43.87; 32.66; FN4-34.92; 23.00; FFA4-67.91; 42.36; FAA4-56.03; 38.96. (Mann-Whitney non-parametric test). The time of application did not interfere in the $CaF_2$ formation from the acidulated and highly-acidulated gels. A minor concentration of fluoride and amount of pH from highly-acidulated gel did not affect the higher formation from the $CaF_2$ in relation to the acidulated gel in both cases when the application was evaluated.*
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## INTRODUCTION

Fluoride is important in enamel demineralizing and remineralizing procedures and because it alters the ecology of the bacterial plaque, affecting the aciduric capacity of the bacteria and also their production of glucans.[2]

In a caries prevention program, an effort should be made to achieve a large formation of calcium fluoride in the shortest possible operating time, so as to reduce the risk of intoxication. Furthermore, the patient's capacity to accept fluoride must be considered, as well as the risk or the activity of the patient's caries disease, observing sugar intake frequency, oral hygiene and exposure to fluoride.[10]

In the case of topical fluoridated agents, various factors affect the capacity of enamel to absorb fluoride such as the type of fluoride, pH, temperature, vehicle, time of application and frequency of use.[3] Several studies have evaluated the absorption of fluoride by enamel, according to the application time, the concentration and the pH of the products.[2,4,11,15,18,19] Ögaard *et al*.[5] suggested that a fluoride product more acidified than those normally found in the market (acidulated phosphate fluoride with pH 4.3), would cause greater absorption of fluoride by the enamel. Consequently, a highly acidulated product was launched recently in Brazil with a pH of 1.9 ± 0.3.

Our purpose was to quantify the alkali-soluble fluoride formed after treating bovine enamel with three fluoridated gels: one neutral, one acidulated and one highly acidulated, varying the exposure times of the tooth surfaces to the products.

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## METHODS AND MATERIALS

One hundred and forty-five previously selected bovine incisors were resected with a steel disk coupled to a cooled low-rotation motor, in the facial-lingual direction, perpendicular to the incisal surface and approximately 2.00 mm from the incisal edge and at the level of the amelocementum junction, and perpendicular to proximal faces, about 1.5mm away. This resulted in a square block of enamel whose sides were approximately 4.0mm. All the samples, then had an enamel surface coming from the middle third of the buccal face of the bovine incisors and were divided into seven groups:

(a) Control group (C) without treatment, 25 samples.
(b) Group treated with 2% sodium fluoride (Flutop Gel, SS White Artigos Dentarios, Rio de janeiro, RJ, Brazil), 20 samples.
(c) Group treated with 2% sodium fluoride for four minutes (FN 4), 20 samples.
(d) Group treated with 1.23% acidulated phosphate fluoride (Nupro Acidulado Gel, Dentsply, Petropolis, RJ, Brazil) for 1 minute (FFA-1), 20 samples.
(e) Group treated with 1.23% acidulated phosphate fluoride for 4 minutes (FFA-4), 20 samples.
(f) Group treated with 0.6% highly acidulated fluoride (Fluor Super Acido, Hidroslabor, Belo Horizonte, MG, Brazil) for 1 minute (FAA-1), 20 samples.
(g) Group treated with 0.6% highly acidulated fluoride for 4 minutes (FAA 4), 20 samples.

With the exception of five samples of the control group, the fragments were demineralized using a solution recommended by Dijkman *et al*.[7] containing 3mM $Ca(CaCl_2)$ and 3 mM $P(KH_2PO_4)$ in 50 mM of acetic acid, at pH 4.5, for 60 hours at room temperature. Subsequently, the samples were removed from the demineralizing solution, washed with deionized water and dried with absorbent paper.

Fifteen samples from each group were chemically analyzed for quantifying the alkali-soluble fluoride in the form of $CaF_2$ using the method proposed by Caslavska *et al*.[1] A combined ion-selective electrode for fluoride was used, couple to an ion analyzer previously calibrated with standards of 0.1 to 1.0 or from 1.0 to 10.0 mgF/ml in KOH 2M, KOH 1M and TISAB II with HCl 1M, according to the fluoride concentration of the sample. The other five samples from each group were selected for analysis in the scanning electron microscope.

The measured fluoride values were analyzed statistically by the non-parametric Mann-Whitney test, using a significance level of 5%.

After the treatments, the forty samples previously selected for microscopic analysis of fluoride in the demineralized bovine enamel were stuck to metallic sample holders, coated with 300 Ω gold-palladium and were evaluated using the scanning electron microscope.

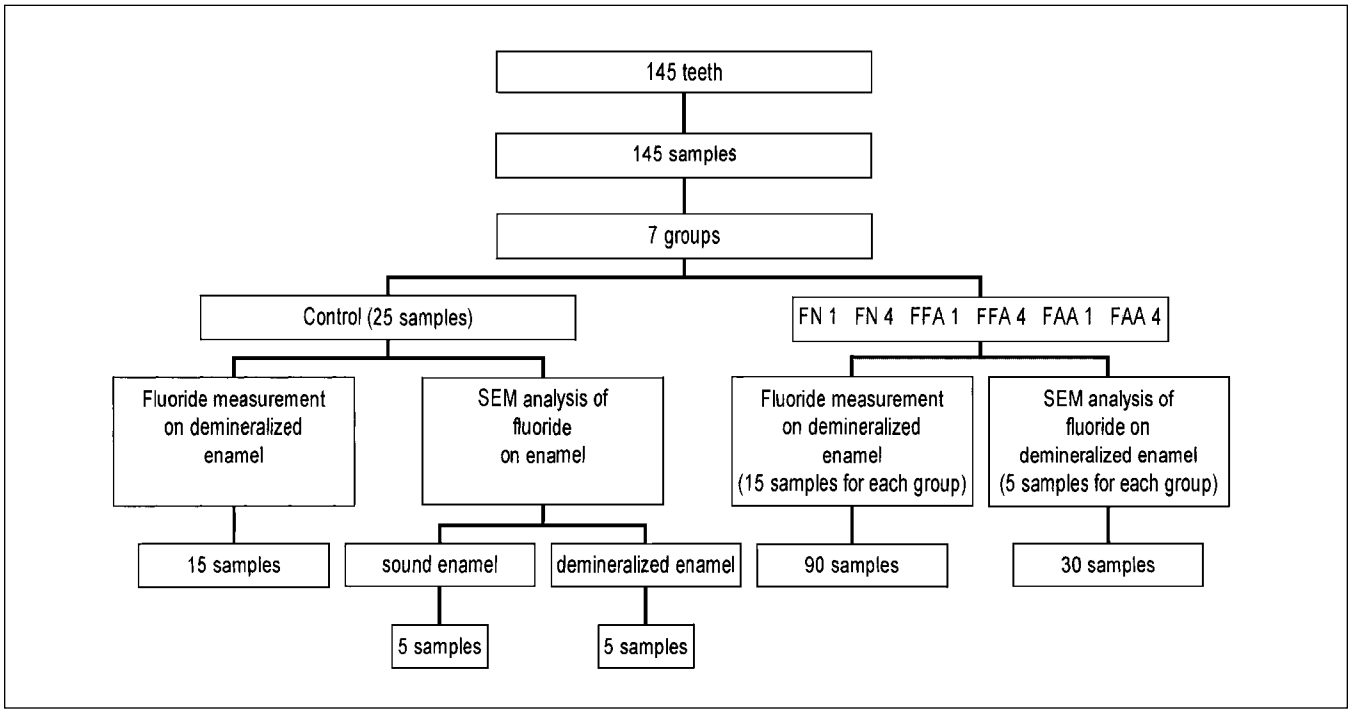


Figure 1. Experimental design of the Study.

Table 1. Concentration of alkali-soluble flouride CaF2 - (µF/cm²) formed on demineralized bovine dental enamel, treated with neutral, acidulated and highly acidulated flouridated gels.

| Groups | Control | FN 1 | FFA 1 | FAA 1 | FN 4 | FFA 4 | FAA 4 |
|---|---|---|---|---|---|---|---|
| 1 | 0.41 | 68.00 | 26.60 | 30.6 | 13.60 | 135.15 | 44.03 |
| 2 | 0.43 | 16.35 | 199.06 | 16.31 | 84.70 | 54.00 | 143.30 |
| 3 | 0.55 | 14.10 | 37.00 | 133.06 | 24.30 | 19.00 | 16.76 |
| 4 | 1.49 | 15.29 | 20.25 | 24.33 | 18.50 | 46.87 | 126.76 |
| 5 | 0.42 | 15.43 | 105.49 | 51.97 | 42.39 | 30.86 | 51.66 |
| 6 | 0.45 | 18.97 | 26.2 | 21.39 | 13.77 | 55.78 | 35.43 |
| 7 | 1.47 | 13.59 | 22.23 | 45.59 | 44.78 | 55.52 | 45.66 |
| 8 | 0.45 | 15.86 | 21.24 | 21.34 | 15.14 | 23.45 | 32.76 |
| 9 | 1.94 | 17.80 | 46.14 | 18.66 | 72.45 | 24.07 | 45.68 |
| 10 | 0.56 | 18.55 | 25.35 | 91.77 | 53.68 | 121.76 | 19.34 |
| 11 | 0.52 | 28.31 | 104.64 | 20.89 | 17.83 | 122.67 | 112.54 |
| 12 | 0.77 | 57.06 | 37.95 | 24.91 | 57.33 | 71.22 | 41.18 |
| 13 | 0.71 | 15.72 | 50.41 | 59.47 | 22.51 | 144.32 | 37.06 |
| 14 | 0.67 | 16.14 | 66.69 | 33.56 | 24.76 | 54.18 | 57.27 |
| 15 | 0.63 | 15.00 | 22.35 | 64.12 | 18.02 | 59.73 | 31.07 |
| Average | 0.63 | 23.08 | 54.11 | 43.87 | 34.92 | 67.91 | 56.03 |
| Standard Deviation | 0.38 | 16.52 | 49.00 | 32.66 | 23.00 | 42.36 | 38.96 |

Table 2. Levels of significance of crossovers of values of alkali-soluble flouride ($CaF_2$) formed before and after treatment with floridated gels for 1 and 4 minutes.

| Groups | FN1 | FFA 1 | FAA 1 | FN 4 | FFA 4 | FAA 4 |
|---|---|---|---|---|---|---|
| Control | p<0.01 | p<0.01 | p<0.01 | p<0.01 | p<0.01 | p<0.01 |
| | — | p<0.01 | p<0.05 | p<0.05 | p<0.01 | p<0.01 |
| | — | — | n.s. | n.s. | n.s. | n.s. |
| | — | — | — | n.s. | p<0.05. | n.s. |
| | — | — | — | — | p<0.05 | p<0.05 |
| | — | — | — | — | — | n.s. |

Note: n.s. = statistically not significant.

A descriptive analysis of the appearance of the surface of the bovine dental enamel treated with different fluoridated gels for one or four minutes, comparing the treated fragments with each other and with the control group (untreated), was then prepared. Figure 1 shows all the methodological steps.

## RESULTS

There was a statistically significant difference in the measured quantity of alkali-soluble fluoride, ($CaF_2$) between the control group and the other groups, between the groups treated with neutral gel and the acidulated gel during one minute, between the groups treated with neutral gel and the highly acidulated gel during one minute, between the groups treated with neutral gel during one minute and the acidulated gel during four minutes, between the groups treated with neutral gel during one minute and the highly acidulated gel during four minutes, between the groups treated with neutral gel and the acidulated gel during four minutes, between the groups treated with neutral gel during one and four minutes, between the groups treated with highly acidulated gel during one minute and the acidulated gel during four minutes and between the groups treated with neutral gel and the highly acidulated gel during four minutes (Tables 1 and 2).

The electromicrographs showed a larger formation of calcium fluoride crystals when acidulated gel was

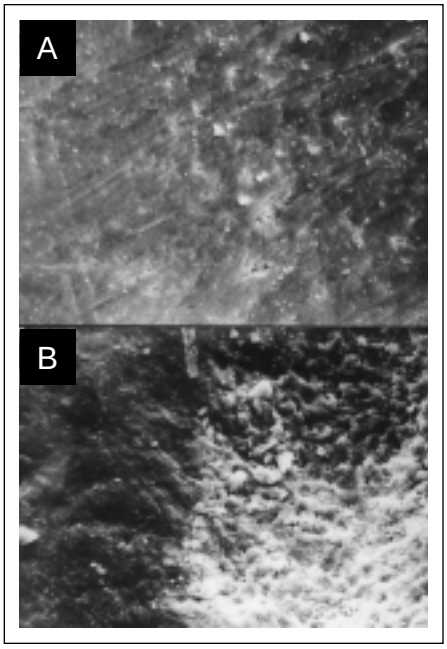


Figure2. Electromicrographs of bovine enamel surface before flouride treatment. (A) Sound enamel, 1,000 X. (B) Demineralized enamel, 1,000 X.

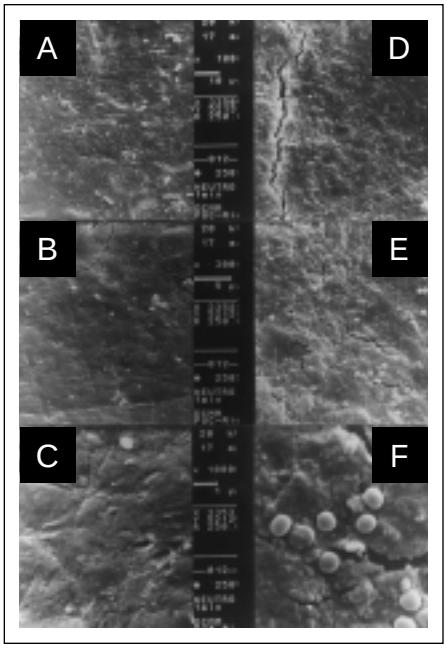


Figure 3. Electromicrographs of bovine enamel surface after flouride treatment with 2% sodium flouride. (A) FN1, 1,000 X. (B) FN1, 3,000 X. (C) FN1, 10,000 X. (D) FN4, 1,000 X. (E) FN4 3,000 X. (F) FN4, 10,000 X.

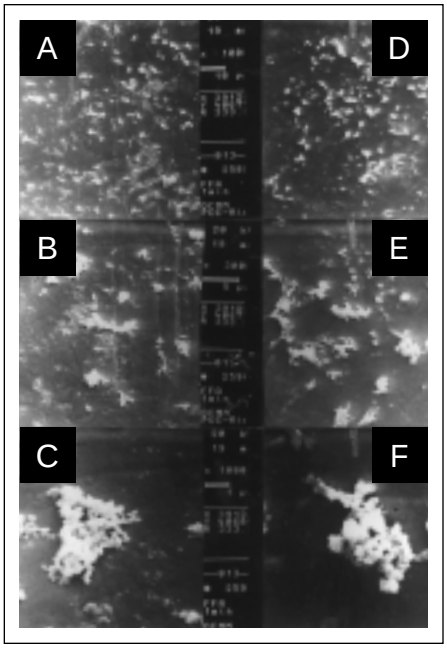


Figure 4. Electromicrographs of bovine enamel surface after flouride treatment with 1.23% acidulated phosphate flouride. (A) FFA1, 1,000 X. (B) FFA1, 3,000 X. (C) FFA1, 10,000 X. (D) FFA4, 1,000 X. (E) FFA4 3,000 X. (F) FFA4, 10,000 X.

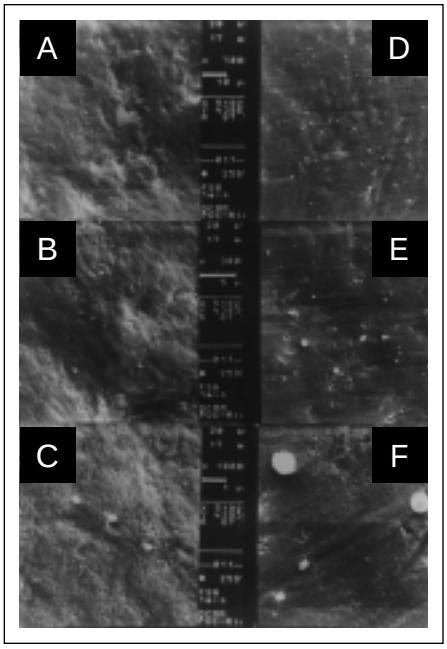


Figure 5. Electromicrographs of bovine enamel surface after flouride treatment with 0.6% highly acidulated flouride. (A) FAA1, 1,000 X. (B) FAA1, 3,000 X. (C) FAA1, 10,000 X. (D) FAA4, 1,000 X. (E) FAA4 3,000 X. (F) FAA4, 10,000 X.

used, for both the one and four minute periods. When the two treatment periods with neutral gel and the highly acidulated gel were analyzed, there was a larger formation of calcium fluoride crystals after four minutes of treatment. The formation of crystals appeared to be the same, when the bovine dental enamel was treated with the acidulated gel for one or four minutes and when the neutral gel and highly acidulated gel were compared after one or four minutes' treatment (Figures 2 to 5).

## DISCUSSION

The first important observation of this study was the verification of a significant difference between the untreated demineralized bovine enamel (control group) compared with the other groups that received any of the proposed types of treatment, confirming that any fluoridated gel causes a deposition of $CaF_2$. Campos *et al*[6] said that after a single topical application of acidulated phosphate fluoride gel there was an increase of fluoride on the surface, in the form of $CaF_2$, which even after immersion in artificial saliva, revealed a much higher residual amount of $CaF_2$ than that found in the untreated teeth.

In this experiment, when analyzing the three types of gels (neutral, acidulated and highly acidulated), comparing them with each other during one minute, the highly acidulated gel did not reveal a greater concentration of alkali-soluble fluoride formed in this period of time. The higher values were achieved by treating with the acidulated phosphate fluoride gel that has a higher pH. Modesto *et al*[19] quantified the alkali-soluble fluoride formed after applying fluoridated gels with different pH and concluded that the pH was decisive in increasing the acquisition of $CaF_2$, whereas the application time was not.

Delbem and Cury,[16] after using pH cyclings, chemically analyzed the enamel and micro-hardness, concluding that the acidulated gel was more effective than the neutral gel for limiting the progress of carious lesions. Reddy and Indushekar[8] found similar results by measuring the micro-hardness before and after the topical application of fluoride.

In this study, the acidulated phosphate fluoride gels were more effective than the neutral gel for the same periods of time. However, according to Rölla *et al*,[12] the lower the pH of the fluoridated agent the more alkali-soluble fluoride was deposited. This was not confirmed, because there was no statistically significant difference between the acidulated and highly acidulated gels in the times of one and four minutes. Nonetheless, one aspect that should also be considered is the fact that the highly acidulated gel shows a lower concentration of fluoride (7720 ppm average) than the acidulated gel (12080 ppm average).

That lower concentration would mean that there is less risk of intoxication for the patient. Even so, lowering the fluoride content and pH of the product did not achieve the desired effect, because neither the formation of $CaF_2$ exceeded nor even equaled that of the acidulated gel, when this methodology was used. Cruz and Rölla[13] observed, through scanning electron

microscopy, that the number and size of the crystals formed after applying the sodium fluoride solution on the enamel rose with the exposure time. Cruz and Rölla,[9] when using the sodium fluoride solution during thirty and sixty seconds, five and sixty minutes, found that, after the longer times, the amount of calcium fluoride formed was two and five times greater in relation to the time of thirty seconds. However, when the comparison is made between shorter times, such as one and four minutes, for example, several authors have shown that there is no significant difference between the results.[11,16]

Garcia-Godoy *et al*[14] analyzed the effect of applying acidulated phosphate fluoride gel for one and four minutes on the enamel with an artificial caries lesion, and found that there was a reduction of 37% to 40% in the depth of the lesion with the two times studied. Mendes[11] examined *in situ* the amount of alkali-soluble fluoride formed with the acidulated gel in the times of one and four minutes of treatment and also did not find significant differences. These results conform to Monte Alto *et al.,*[17] who did not find major differences between these times of treatment with the neutral and acidulated gels on deciduous teeth. Villena and Cury[18] observed that the anticariogenic effect of the acidulated phosphate fluoride is not influenced by the application time. Delbem and Cury[16] found that the acidulated gel was more effective than the neutral gel for limiting the progression of the caries lesion, although the time of one or four minutes of application did not influence this action.

Analyzing the photomicrographs (Figures 2 to 5), in spite of noting an apparently larger formation of $CaF_2$ for the time of four minutes in the acidulated gel and highly acidulated gel groups, the neutral gel group showed a notable difference in the times of one and four minutes.

## CONCLUSIONS

Based on the chemical and microscopic analysis of the bovine enamel samples, after treating them with the three fluoridated gels, a larger formation was found ($p<0.05$) of alkali-soluble fluoride ($CaF_2$) after applying neutral gel for four minutes compared with one minute, although there was a similar formation of $CaF_2$ for one or four minutes, in the case of the acidulated and highly acidulated gels, indicating that the time factor is not decisive for low pH gels.

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