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Salivary fluoride concentration and retention after rinsing with 0.05 and 0.2% sodium fluoride (NaF) compared with a new high F rinse containing 0.32% NaF

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ABSTRACT

Objective: To compare salivary fluoride (F) concentration and F retention after rinsing with a new 0.32% sodium fluoride (NaF) rinse and conventional 0.05 and 0.2% NaF rinses.

Methods: Seventeen subjects (aged 22–26 years), with normal salivary secretion rates, participated in a double blind, cross-over study. In three separate sessions with a minimum washout period of 48 h, they rinsed for 1 min with 10 ml of 0.05, 0.2 or 0.32% NaF mouthrinse. Unstimulated whole saliva was collected before (baseline: 0 min) and after 1, 3, 5, 10, 20, 30, 45 and 60 min. The F concentration was plotted against time, and the area under the curve (AUC) calculated. Salivary F concentration and F retention for the three mouthrinses were compared by a randomized block test, followed by Tukey's test and a paired 2-tailed test.

Results: There was a clear dose–response for AUC 3–60 min; 0.32% > 0.2% > 0.05% ($p < .05$). The mean F retention was 0.25 mg for 0.05% NaF, 0.86 mg F for 0.2% Na and 1.31 mg F for 0.32% NaF, ($p < .05$).

Conclusions: The higher salivary F concentration over time and the higher F retention after rinsing with an 0.32% NaF solution suggests a potential application in prevention of caries and dental erosion.

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Fluoride; mouthrinse solutions; retention; unstimulated whole saliva

Introduction

There is a dose–response relationship between the fluoride (F) concentration in toothpaste and the caries preventive effect [1]. Thus, the more F, the better effect. Higher F concentrations in the paste (5000 vs. 1450 ppm F) give higher levels of F in the oral biofilm [2,3], as well as in the oral mucosa and saliva [4]. There is strong evidence to support the use of high F toothpastes (5000 ppm) in teenagers at greater risk for caries, e.g. those brushing only once a day [5,6]. Regular use of dentifrices containing 5000 ppm F may also be of special interest for prevention of root caries [7,8]. However, in younger children the F concentration must be balanced against the risk of dental fluorosis.

In many countries, F mouthrinse solutions are recommended as a supplement to F toothpaste for patients at risk for caries and dental erosion. The most commonly used concentration for adults and the elderly is 0.2% sodium fluoride (NaF) (900 ppm F) for daily rinsing [9,10]. Lower concentrations (0.05% NaF; 225 ppm F) however, are recommended for children <12 years and this is the standard concentration in many countries [11,12].

Post-brushing rinsing for control of dental caries was recently discussed at a consensus meeting of experts from Europe and USA, as to what advice should be given to patients [13]. One of the statements of the group was that

'mouthrinses containing F can be used after brushing with F toothpaste'. It could be argued that the minimum concentration of NaF solution to be used as a post-brushing rinse should be 0.2% – in order not to dilute the F concentration after brushing with 0.32% NaF toothpaste. This is in accordance with the study by Mystikos et al. [14] showing that post-brushing mouthrinse solutions exert a 'wash-out' effect if the concentration of F is too low, i.e. $\leq 0.05\%$ NaF.

With respect to the role of F in prevention of erosion, Huysman et al. [15] concluded that 'solutions and rinses with high F concentrations' are most promising. In this context, it was of interest to evaluate a recently introduced high F mouthrinse solution, containing 0.32% NaF (1450 ppm F), i.e. the same concentration as in most F toothpastes today.

The aim of the present study was to compare salivary F concentration in the hour following rinsing with 0.05, 0.2 and 0.32% NaF and also to measure the retention, i.e. how much F is potentially swallowed.

Materials and methods

Study subjects and design

Twenty healthy students, with subjectively normal salivary secretion, were recruited at the Faculty of Dentistry in Malmö, Sweden, by means of a flyer. The volunteers were

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given verbal and written information before the study and gave written consent to participation. The study protocol was approved by the Ethics Committee, EPN, in Lund, Sweden, (DNR 2018/180). Three students chose not to enter the study.

The following inclusion criteria were applied: good general health and a minimum of 24 teeth, 6 in each quadrant, without extensive restorations. The criteria for exclusion were: gingivitis, periodontitis or active caries, orthodontic appliances or removable oral prostheses, pregnancy, and participation in any other study during the past month.

The design was a randomized, double-blind, cross-over study, with three treatment sessions.

Experimental protocol

Subjects were instructed not to brush their teeth after 11 p.m. the night before the test session. All subjects were asked to use a toothpaste containing 1450 ppm F (NaF), 'Pepsodent Long Active Intensive Cleaning' (Unilever, Solna, Sweden), which was provided free of charge.

At baseline, at the start of the first session, unstimulated saliva samples were collected for 5 min, to determine the secretion rate and to measure the baseline salivary F level.

At three sessions, separated by a minimum wash-out period of 48 h, the subjects were given one of three mouthrinses with different F concentrations. The following products were used: 1) Dentan[®] mint 0.05% NaF, containing 225 ppm F (Meda AB, Solna, Sweden), 2) Dentan[®] mint 0.2% NaF, containing 900 ppm F, (Meda AB) and 3) Maximum Fluoride 0.32% NaF, Brilliant Smile[™], containing 1450 ppm F, (Brilliant Smile Sweden AB, Hisingsbacka, Sweden). The products were purchased from a pharmacy and the experimental supervisors and subjects were blinded to the contents. The table of contents, declared by the manufacturers, is presented in Table 1.

At each session, the subjects rinsed with 10 ml of the test solution for 1 min and then expectorated the saliva and mouthrinse solution into an empty pre-weighted plastic container (Container 25 ml 54 × 27PS + Cap NAT, Sarstedt, Helsingborg) (0-min sample). Whole resting saliva samples were then collected at 1, 3, 5, 10, 20, 30, 45 and 60 min post-rinse, by passively letting saliva flow into a plastic container for one minute. The container was then sealed with a tightly fitting screw lid. The first sample (0 min) was used to calculate the oral F retention.

During the test session, held in a quiet room, the subjects were not allowed to eat, drink or talk. The samples were stored in a freezer (−20 °C) until analysed.

Fluoride determination

To determine the F concentration in the saliva samples, an ion-specific electrode, ISE, was used (PerfectION[™] F and Mettler Toledo[™], SevenCompact S220 Basic, pH/Ion benchtop metre, Mettler Toledo[™], Mettler-Toledo AB, Stockholm, Sweden). The ISE was calibrated according to the manufacturer's instructions by using a series of F-containing standard solutions.

The available F concentrations of the three mouthrinses being tested were measured by the ISE, and were estimated to 243, 958 and 1464 ppm respectively. Ten milliliters of the rinse solution contained 2.4 mg, 9.6 mg and 14.6 mg F for each of the 0.05, 0.2 and 0.32% NaF rinses, respectively.

Prior to measurement, the saliva samples were heated to 25 °C in an incubator. For each saliva sample, an amount of 200 µl was mixed with 20 µl Total Ionic Strength Adjustment Buffer (TISAB III, Mettler Toledo[™]) and vortexed for 10 s before being placed on a plastic Petri dish, as a 100 µl drop, in which the surface of the ISE was placed, in close contact with the solution, to measure the F concentration (ppm).

If the amount of saliva in a sample was less than 200 µl, it was diluted 1:2, with deionized water, before adding TISAB III in proportions 1:10. The concentration was then measured as described above.

F retention was calculated as follows: the concentrations in the rinse solutions were measured by the ISE and the amount of F was determined by multiplying with the volume of 10 ml. The expectorated saliva was weighed (1 mg equals 1 ml), the concentration measured, and the amount of F calculated. This value was subtracted from the amount in the mouthrinse solution.

Data analysis

The primary efficacy variable was the integrated area under the curve (AUC) for F concentration in saliva as a function of time. The AUC for the three mouthrinses, 0.05%, 0.2% and 0.32% NaF, were compared by a randomized block design. If a significant difference was found, a post hoc test, Tukey's test, followed.

The mean values for F retention of the three mouthrinses were calculated and compared by a paired 2-tailed *t*-test.

Table 1. Table of contents for the three mouthrinses, according to manufacturers' product label.

0.05% NaF	0.2% NaF	0.32% NaF
Sodium fluoride 0.5 mg/ml	Sodium fluoride 2 mg/ml	Aqua hydrogenated starch hydrolysate
Xylitol 30 mg	Xylitol 30 mg	Glycerine
Methylparahydroxybenzoate	Methylparahydroxybenzoate	Potassium nitrate
Peppermint oil	Peppermint oil	Propylene glycol
Water	Water	PEG-40-Hydrogenated castor oil
Other excipients	Other excipients	Sodium fluoride
		Sodium methylparaben
		Aroma
		Menthol
		Sodium saccharin

Results

Seventeen of the 20 volunteers participated in the test sessions: 13 women and 7 men, aged 22–26 years. In all, 358 samples were analysed, whereof two were diluted prior to measurement of F concentration. The mean resting salivary flow rate was 0.3 ml/min.

All samples which were too small in volume and therefore impossible to analyse, were excluded from the study. It was assumed that the change in F concentration over time in these subjects followed a linear pattern when calculating AUC. If a subject produced more than two salivary samples of very low volume for a certain mouthrinse, all samples of the said mouthrinse collected from this subject were excluded. The number of subjects on which the final calculations for AUC 3–60 min were based, was 14 for the 0.05% solution, 12 for the 0.2% solution and 15 for the 0.32% solution.

The F concentration in the expectorated saliva was plotted against time from 1 to 60 min, and the area under the curve (AUC) was calculated for each of the three different mouthrinses. Clearance curves are shown in Figure 1(a,b) (both as logarithmic and non-logarithmic values). The inter-group differences are more pronounced during the first half of the clearance time period (Figure 1(a)).

As shown in Figure 2, Tukey's test, after pairwise comparison, disclosed a significant difference in AUC 3–60 min. This applied to all three concentrations of F rinse.

The median and mean values for the F retention for the three different mouthrinses are shown in the boxplot in Figure 3. As expected, the 0.32% NaF mouthrinse had the highest level of retention (1.31 mg), followed by 0.86 and 0.25 mg for the mouthrinses containing respectively 0.2% and 0.05% NaF ($p < .05$).

Discussion

A mouthrinse containing a high concentration of NaF (0.32%, 1450 ppm F) has recently been introduced to the Swedish market, as an over-the-counter (OTC) product. The aim of

this study was to compare this product with two commonly used OTC products available in several countries, containing either 0.2% (900 ppm F) or 0.05% NaF (225 ppm F). After a single, one minute rinse with 10 ml solution, two parameters were evaluated: 1) the salivary F concentration and 2) F retention in the oral cavity.

There was a clear dose–response effect with respect to the salivary F concentration of the three products. The results correspond well with those of our earlier studies, i.e. the higher the F concentration in the mouthrinse solution, the higher salivary F levels throughout the clearance period [16,17]. These data are also in agreement with another study, comparing 1–10 ml rinsing solution with 250–2500 ppm F [18], in which the authors concluded that ‘the applied F concentration is a more important factor than the applied F amount per se in determining the elevation of oral F levels following topical F use’. In the present study, a volume of 10 ml was selected, being the dosage recommended by most manufacturers and dental societies. However, if there is a risk that the patient might swallow too much of the solution, this volume could be reduced, for example, to 5 ml, probably without any loss of effect.

There may be several advantages of mouthrinsing with a F concentration as high as 1450 ppm, used either as a post-brushing rinse, or on a separate occasion between brushings. Mystikos et al. [14] found that a post-brushing mouthrinse solution with 900 ppm F might be suitable for patients at high risk for caries, especially for those who rinse extensively after brushing. For this group of patients – and in light of the results of the present study – a mouthrinse solution containing 1450 ppm F might be preferable even to 900 ppm F and certainly preferable to 225 ppm F.

With respect to availability of F in the oral cavity, rinsing solutions may have some advantages over a dentifrice or a brush-on gel [19]. Thus, a solution which is swished around the dentition for one minute with active cheek and lip movements, may easily penetrate the dental biofilm and also reach the approximal tooth surfaces. This is supported by an experimental *in situ* study, showing that F rinsing achieved

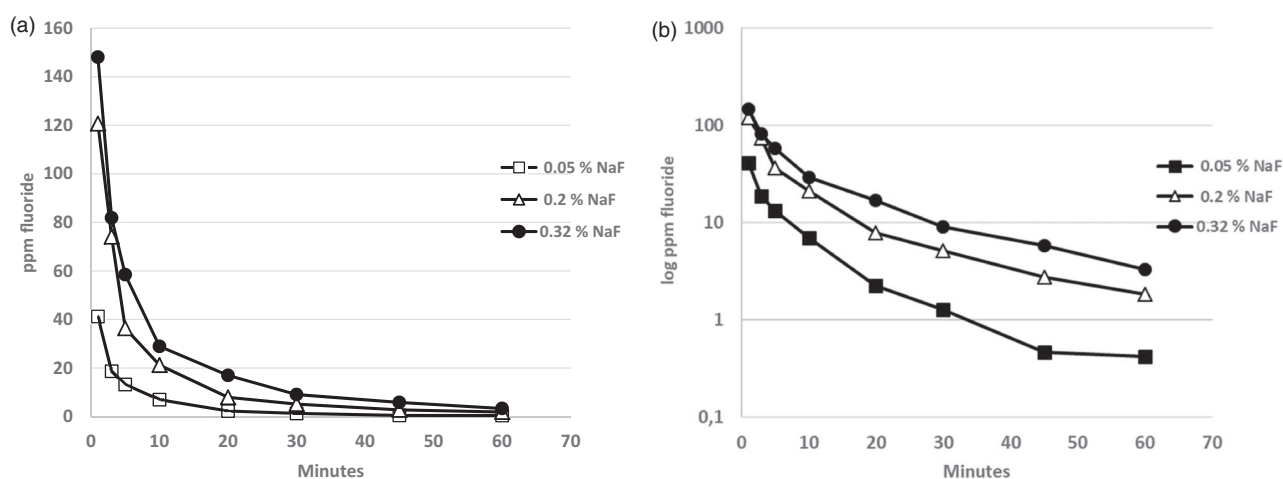


Figure 1. (a, b) Mean salivary fluoride concentration (non-logarithmic (a) and logarithmic (b)) as a function of time in expectorated saliva at 1, 3, 5, 10, 20, 30, 45 and 60 min after one minute's single, supervised use of 10 ml of the indicated mouthrinse.

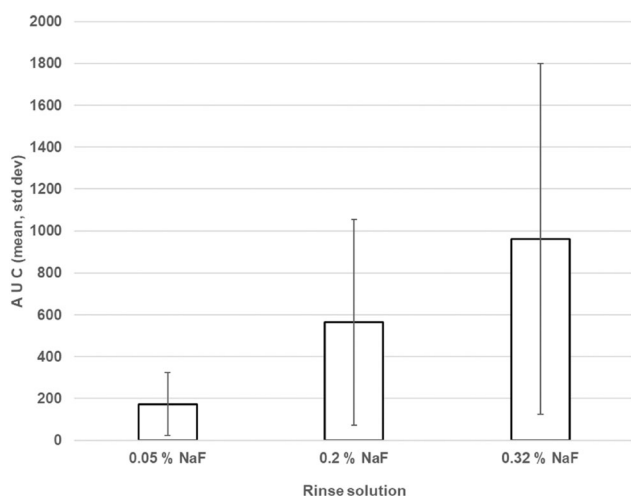


Figure 2. Mean values \pm SD for AUC 3–60 min after rinsing with 0.05, 0.2 and 0.32% sodium fluoride (NaF) ($p < .05$, Tukey's test).

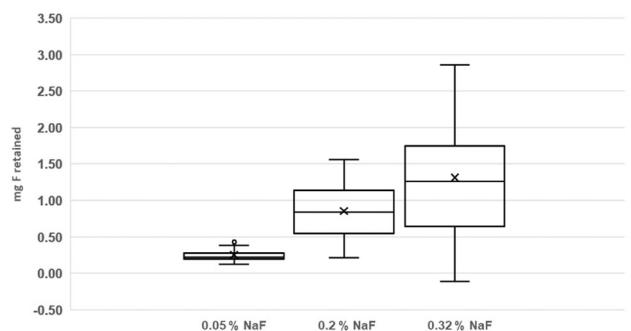


Figure 3. Box-plot salivary fluoride retention \pm SD after rinsing with 0.05, 0.2 and 0.32% sodium fluoride (NaF) ($p < .05$, two-tailed t -test).

greater remineralization of demineralized enamel and dentine specimens located approxiamally than buccally [20].

Recently, Parkinson et al. [21], using an *in situ* caries model, showed that brushing with a 1150 ppm F dentifrice, followed by a 225 ppm F mouthrinse, is effective in promoting remineralization of an enamel caries lesion. They concluded that 'F mouthrinses provide advantages for F delivery by maintaining elevated intra-oral F concentrations following F dentifrice use'. The specimens were flush with the surface of the buccal flange of the participant's partial denture. It may be speculated that the additional effect of the rinsing solution would have been even more pronounced if a 900 or 1450 ppm F solution had been used instead of 225 ppm F and if the specimens had been sited approxiamally instead of buccally.

There are a substantial number of clinical studies showing the additional caries preventive effect of F rinsing to complement daily use of F toothpaste [11,12]. For example, a 3-year randomized controlled trial of school-based F mouthrinsing (0.2% NaF) among 13- to 16-year-olds ($n = 622$), using toothpaste at home, was carried out in Sweden 1999–2003 [22]. The main conclusion was that rinsing with F at school reduces caries: the preventive effect varied between 30 and 59% [22]. Apart from clinical trials, there are also *in situ* studies demonstrating the additional effect of F rinsing as an adjunct to F toothpaste for prevention of both dental

erosion [23] and caries [21]. In this context, a recent study, using micro-computed tomography, has shown that in combination with twice daily brushing with a NaF dentifrice, bed-time rinsing with a 0.05% NaF solution increased remineralization of an incipient caries-like lesion *in situ* [19].

Root caries is an increasing problem among elderly people and there is a need for simple preventive measures. This has recently been discussed in a review by Magalhães [24]: 'Among the self-applied products, there is strong evidence that 5000 ppm F toothpaste is more effective in arresting root caries lesions and in preventing new lesions compared to 1100–1450 ppm F toothpastes. Wierichs and Meyer-Lueckel [7], in a systematic review of non-invasive treatment of root caries lesions, support regular use of dentifrices containing 5000 ppm F. Both these authors however also point out that a previous review by Marinho et al. [11], revealed a lower coronal caries incidence associated with use of a F rinse in addition to brushing with a F dentifrice. Wierichs and Meyer-Lueckel [7] conclude, "since the findings for coronal caries showed a rather good efficacy, it seems plausible to recommend the daily use of NaF rinses to reduce not only the initiation of coronal caries lesions but also the initiation of root caries lesions". Among the reviewed self-applied topical fluoride methods, daily use of a 0.2% sodium fluoride (NaF) mouth rinse is most likely to be the most effective' [25]. Based on the literature reviews and the results of the present study, daily mouthrinsing with 900 ppm F, or preferably with 1450 ppm F, as a supplement to daily use of F toothpaste, has potential as a preventive measure for elderly people at risk of root caries.

Beside dental caries, a high F solution may also be useful for prevention of dental erosion. In a recent review by Huysmans et al. [15], the authors discuss various strategies to prevent dental wear. Although stannous fluoride (SnF_2) has received increasing attention as a promising anti-erosive agent, the use of high concentrations of NaF in dentifrices and mouthrinses is also discussed. To date, there are no long-term clinical studies on the effects of F solutions in preventing erosion of enamel and dentine. A high F solution such as the 0.32% NaF solution tested in the present study, may have potential application as a daily rinse in patients at risk of erosion. In an *in situ* erosion remineralization model, Maggio et al. [23] studied the combination of NaF toothpaste (1450 ppm F) and NaF mouthrinse (450 ppm F). There was a significant effect on hardening of incipient erosive lesions of enamel and increased resistance to a second erosive challenge. In future studies it would be of interest to evaluate the effect of a 1450-ppm F rinse in this model.

Finally, with reference to the second aim of the present study, i.e. F retention in the oral cavity: increasing the F concentration from 900 to 1450 ppm F results in a difference of the order of 1.61. This is in accordance with the results in Figure 3, showing that for the 0.32% NaF solution, mean F retention was 1.31 mg, compared to 0.86 mg F for the 0.2% NaF ($1.31/0.86 \approx 1.52$). The large range reflects individual variations in retention, especially for the 1450 ppm F solution. If the products are used twice daily, the estimated ingestion is around 2.6 mg and 1.7 mg F, respectively, which is half the

recommended F intake from water and food (3–4 mg F/day) [26]. Thus, from a toxicological point of view, neither 0.2% NaF nor 0.32% NaF rinses are of concern.

A shortcoming of this study was that some saliva samples were too small to be analysed. However, most of the samples were measurable and the statistical analysis disclosed significant inter-group differences.

In this study, standard procedure was followed, i.e. 10 ml solution was evaluated by expectorating once. Both the 900 and the 1450 ppm F rinsing solutions should be used only by children >12 years and by adults and elderly people who have no problem in spitting out the solution. There are several means of reducing F retention in the oral cavity and thereby the amount of F swallowed. One way is to reduce the volume of the solution, as mentioned above, from 10 ml to 5 ml. Alternatively instead of spitting out only once, spitting out twice in quick succession could be advised, or more vigorous spitting out.

For future research, it would be of interest to determine salivary F concentration and F retention after rinsing with 5 ml instead of 10 ml, in order to reduce F retention. Another topic to be investigated could be the above-mentioned parameters in subjects using the 0.32% mouthrinse as an adjunct to brushing with high F toothpaste (5000 ppm F).

In conclusion, the overall aim of the present investigation was to evaluate a mouthrinse with high NaF concentration, recently introduced to the Swedish market. It contains 0.32% (1450 ppm F) and is sold over-the-counter (OTC). The product was compared with two commonly used OTC products available in many countries for daily or weekly rinsing, containing either 0.2% (900 ppm F) or 0.05% NaF (225 ppm F). The results disclosed a dose–response effect. The mouthrinse solution with 0.32% NaF gives both higher salivary F concentrations over time and higher F retention than a 0.2% NaF solution and much higher retention than a 0.05% NaF solution. It is concluded that this high F mouthrinse has potential benefit for prevention of caries and erosion.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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